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THÈSE

Présentée par

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Pour l'obtention du grade de

DOCTEUR EN SCIENCES

Filière : Biologie
Option : Sciences Alimentaires
Thème

Etude de techniques de séchage et d'extraction par microondes et ultrasons des substances bioactives de quelques plantes de la région de Bejaia

Soutenue le : /09/2017 **Devant le Jury composé de :**

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THESIS

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Mme; BOUAOUDIA-MADI Nadia

Thesis Title

Study of drying and extraction technique of bioactive substances by microwave and ultrasound of *Myrtus communis plant.*

Supported on: /09/2017. **In front of the jury:**

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Scientific Publications

1. Nadia Bouaoudia-Madi[1], Lila Boulekbache-Makhlouf[1], Nabil Kadri[1,2*], Farid Dahmoune[1, 2], Hocine Remini [1], Sofiane Dairi[1,3], Sonia Oukhmanou–Bensidhoum[1] and Khodir Madani[1] : **Phytochemical analysis of *Myrtus communis* plant*:* Conventional versus microwave assisted-extraction procedures.** Journal of Complementary and Integrative Medicine

2. Farid Dahmoune, Hocine Remini, Sofiane Dairi, Omar Aoun, Kamal Moussi, Nadia Bouaoudia-Madi**,** Nawel Adjeroud, Nabil Kadri, Khalef Lefsih, Lhadi Boughani, Lotfi Mouni, Balunkeswar Nayak, Khodir Madani: ***Ultrasound assisted extraction of phenolic compounds from P. lentiscus L. leaves: Comparative study of artificial neural network (ANN) versus degree of experiment for prediction ability of phenolic compounds recover***. Industrial Crops and Products 12/2015; 77:251-261.

Communications

1. **MADI Nadia ,** ALOUI Salima , MADANI Khodir : Traitement des rejets laitiers par éléctroflottation . Le 24ème Forum des Sciences Biologiques et de Biotechnologie Hôtel El Mouradi Club Kantaoui à Sousse Tunisie , du 25 au 28 mars 2013

2. Hocine Remini, Farid Dahmoune, Sofiane Dairi, Hayate Haddadi-Guemghar, Nadia Madi-Bouaoudia, Omar Aoun, Amine Belbahi, Khodir Madani: ***Recent Kinetic degradation modelling of anthocyanins and ascorbic acid in stored blood orange juice***. Quatrième Colloque International de Chimie (CIC-4), Université de Batna (Algeria); 11/2014

3. Hocine Remini, Sabiha Achat, Hayate Haddadi-Guemghar, Lila Boulekbache-Makhlouf, Sonia Oukhmanou–Bensidhoum, Nadia Bouaoudia-Madi, Lamia Haddache, Sofiane Dairi, Farid Dahmoune, Khodir Madani: ***Physicochemical parameters and anthocyanins kinetic degradation modelling from blood orange juice as affected by ascorbic acid fortification during pasteurisation.*** International Conference on Food Chemistry and Technology (FCT-2015), November 16-18, 2015• San Francisco, USA; 11/2015

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General Introduction

General Introduction-Thematic research presentation

For thousands of years mankind is using plant source to alleviate or cure illnesses. Plants constitute a source of novel chemical compounds which are of potential use in medicine and other applications. Plants contain many active compounds such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids which are deposited in their specific parts such as leaves, flowers, bark, seeds, fruits, root, etc. The beneficial medicinal effects (antioxidant, antibacterial, of plant materials typically result from the combination of these secondary products (Wong and Chye 2009), Due to these countless beneficial characteristics for human health, researches have been intensified aiming to find fruits, vegetables, plants, agricultural and agro-industrial residues as sources of bioactive phenolic compounds**.** ***Myrtus communis L***. (Family - Myrtaceae) is one of the important aromatic evergreen perennial shrub or small tree with small foliage and deep fissured bark. It is native to Southern Europe, North Africa and West Asia. It is distributed in South America, North western Himalaya and Australia and widespread in the Mediterranean region (Wannes, Mhamdi et al. 2010).

For the recovery of high-added value compounds from plant materials, they must be firstly separated from their original plant matrix which requires many long and costly steps, such as extraction, isolation and identification still hampering industrial development. The objective of extracting phenolic compounds from their plant sources is to release these compounds from the vacuolar structures where they are found, either by rupturing plant tissue or by a diffusion process (Escribano-Bailon and Santos-Buelga 2003). Classical techniques for the solvent extraction of bioactive substances from plant matrices are based on the choice of solvent coupled with the use of agitation and/or heat. They include maceration, Soxhlet extraction, and percolation; however, they are often time-consuming, require relatively large

quantities of solvents, and the active compounds sometimes degrade (De Castro and Garcıa-Ayuso 1998)The fact that one single plant can contain up to several thousand secondary metabolites, makes the need for the development of high performance and rapid extraction methods an absolute necessity in the direction of "Green chemistry" and eco-friendly processes (Nyiredy 2004). Novel extraction methods including ultrasound assisted extraction (UAE), microwave assisted extraction (MAE), supercritical fluid extraction (SCFE), pressurized solvent extraction (PSE) have drawn significant research attention in the last decade(Mandal, Mohan et al. 2007).

Compared with the traditional methods, microwave- assisted extraction (MAE) has many advantages, such as reducing analysis time, simplified manipulation and work-up and higher purity of final product(Ganzler, Salgo et al. 1986, Kingston and Jassie 1988). However one of the disadvantage of MAE was the higher temperature witch can caused the degradation of Phenolic compounds(Grigonis, Venskutonis et al. 2005).

Ultrasound Assisted Extraction (UAE) is particularly attractive for its simplicity, low cost of equipment, efficiency in extracting analytics from different matrices, low energy requirement and reduced solvent- and time-consumption. The enhancement of the extraction process by ultrasounds is attributed to the disruption of the cell walls, reduction of the particle size and the increase of the mass transfer of the cell content to the solvent caused by the collapse of the bubbles produced by acoustic cavitation (Chemat, Grondin et al. 2004).

In the other hand microwave and ultrasound technology were used in drying plants to improve extraction yield (Zhang, Tang et al. 2006). Among emergent new technologies ultrasonic dehydration as pretreatment is very promising because the effects of power ultrasound are more significant at low temperature which reduces the probability of food degradation. In addition, ultrasound permits the removal of moisture content from solids without producing a liquid phase change. (Gallego-Juarez, Rodriguez-Corral et al. 1999**)**

Within this context, the objective of this thesis work is to contribute to the technological Advances by:

1. Comparing the effects of MAE and CE on the extraction efficiency from different myrtle parts (in terms of TPC, flavonoids, anthocyanins and condensed tannins), and to estimate the recovery and the antioxidant capacity of the extracts.

2. Optimization of UAE process parameters using an RSM, including ethanol concentration, extraction time, irradiation amplitude and liquid-to-solid ratio, in order to maximize the content of myrtle pericarp extract. Than The yield of phenolic compounds and antioxidant activity in the *M. communis* extract obtained under the optimum setting parameters (UAE-OPT extract) were compared with those obtained by MAE and CSE methods. Then, the individual phenolic compounds present in the optimized extract were identified by UHPLC-DAD-ESI-MS^n.n;

3. Developing low-cost drying processes based on the combination of more cost-effective innovative technologies with a higher extraction yield.

To address the above objectives, this PhD thesis is organized according to the following structure:

1) A cognitive part (Chapter I): presents a knowledge on microwave and ultrasounds assisted drying and extraction of phenolic natural products in addition some details about extraction by microwaves, ultrasounds, the mechanism, some applications, and environmental impacts. MAE and UAE separation is a research topic which affects several fields of modern chemistry.

2) An application part (Chapter II, III and IV): Initially (chapter II), Microwave assisted extraction (MAE) of total phenolic compounds (TPC) from myrtle leaf, stems, pericarp, and seeds was studied and the results were compared with those of the conventional method

extraction (CME) in terms of extraction time, extraction yield/efficiency, and antioxidant activity which was measured using radical scavenging assay ($DPPH^{\bullet}$) and reducing power. In the Chapter III, Response surface methodology (RSM) was used to optimize extraction conditions of total phenolic compounds (TPC), and antioxidant activity of myrtle pericarp, with ultrasound-assisted extraction (UAE). Results were compared with those obtained by microwave-assisted extraction (MAE) and conventional solvent extraction (CSE) methods. The individual phenolic of the optimized extract were then identified by means of ultrahigh performance liquid chromatography coupled with diode array detection and electrospray ionization mass spectrometry (UHPLC-DAD-ESI-MS^n). All the reported applications have shown that MAE and UAE can be a valid alternative to conventional techniques for food and natural products. The last chapter, discusses the investigation and development of microwave drying -assisted ultrasound pretreatment (MD-AUP) of *Myrtus communis* pericarp by compared with microwave and conventional drying in term of recovery of phenolic compounds and its antioxidant activity. Therefore, the aim of this study was to evaluate the influence of pre-treatments on water loss, total phenol content and their antioxidant activity were analyzed. The comparison between the microwave drying assisted by ultrasound pretreatment, conventional and microwave drying was also investigated.

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First Part I
Literature Review

Chapter I

Myrtus communis

An Overview

Introduction

Myrtus, the Greek name for Myrtle and *communis* means common plant growing in groups. The common Myrtle was introduced into Britain in around 1597 and was described by Linnaeus in 1753. The distribution of myrtle is a common part of typical Mediterranean flora. Myrtle is native to southern Europe, North Africa and west Asia. It is also distributed in nouthern America, northwestern Himalaya and Australia. In Algeria, the genus Myrtus is represented by only one species, *M. communis*, which grows wild in the coastal areas, the internal hills, and the forest areas. In Italy, it grows along the coasts and on the internal hills and it is abundant especially on the islands, where it represents one of the most characteristic species (Cannas, Molicotti et al. 2013). In Portugal, myrtle grows wild mainly in the central and southern parts of the country. The genus *Myrtus*, in Tunisia, is represented by only one species, *M. communis* L., which grows wild in the coastal areas, the internal hills, and the forest areas of northern Tunisia. Two myrtle varieties are described in old local Tunisian flora: *M. communis* var. italica L. and *M. communis* var. baetica L. (Pottier Alapetite 1979), which possesses the same vegetative characters. The morphological difference between the two varieties regards to size of fruits and leaves ; (Mahmoud, Gharaibeh et al. 2010, Berka-Zougali, Ferhat et al. 2012);

I.1 Botany

M. communis L is an evergreen shrub that grows to a height of about 1-5 m. It has upright stem, 2.4-3 m high, its branches form a close full head, thickly covered with evergreen leaves. The stem of the plant is branched and dark green leaves are glossy, glabrous, coriaceous, opposite, paired or whorled, ovate to lanceolate with stiff structure, aromatic, entire margined, acuminate and 2.5-3.8 cm long, glands absent in the lamina. It has axillary white flowers on slender peduncles, medium sized about 2 cm in diam., stiff having yellow anthers. The petals are pure white with glands, they give off a sweet fragrant smell. The berries are pea sized,

orbicular or ovoid-ellipsoid, blue-black or white with hard kidney shaped seeds. They are of varying sizes (0.7-1.2 cm) and shapes. The developed fruit is initially pale green, then turns deep red and finally becomes dark indigo when fully mature. They are bitter when unripe, sweet when ripe. It is highly drought tolerant and needs only little to moderate water. Soil should be allowed to dry in-between watering. It can grow in damp places, shades as well as full sun up to 800 m altitudes (Mahmoud, Gharaibeh et al. 2010). (Table. I.1).

Table. I.1: The morphological description of different parts of *Myrtus communis* plant.

	Description	**Traditional application**	**Photographs**	**References**
Plant	Shrub of 1 à 3m from height to sheets persistent and dense, with pennate nervation			(Quezel 1963) Govaerts et Lucas, 2008.
Flowers	They are large (10-15mm), white, Hermaphrodites. Flowering this fact in summer (June at July)	Medicine –against varicose veins and for preparing capillary lotions for external use		(Messaoud, Laabidi et al. 2012)
Fruit	Spherical bays dark crimsons (diameter: 5mm) with many seeds, appears from November – December	Food: preparation flavoring meat and sauces; Medicine, used orally for infectious disease(diarrhea, dysentery).and externally for skin diseases and wound healing		(Messaoud, Laabidi et al. 2012)

Leaves	Ovoid lanceolate, 2 to 3 times longer than broad, pennate persistent, opposed nervation, with very short petiole, coriaces and of a brilliant green	Food preparation Perfume and cosmetic; hair tonic and stimulant; orally used as antiseptic, anti-inflammatory agent, laxative, analgesic		(Messaoud, Laabidi et al. 2012) (Chalchat, Garry et al. 1998)

1.2. Chemical composition

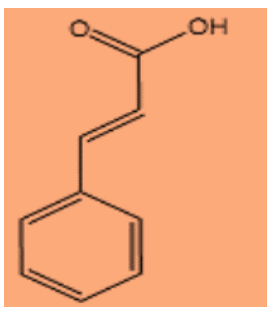
Previous studies on Myrtle, aerial parts have revealed the presence of several specific chemical compounds, for example, the essential oils, phenolic acids, flavonoids and tannins in leaf and flowers (Messaoud, Zaouali et al. 2005, Wannes, Mhamdi et al. 2010)**;** and anthocyanin, fatty and organic acids in berries (Martín, Rubio et al. 1999)**,** (Messaoud, Laabidi et al. 2012**).** The fruit of myrtle plant is rich in fibers and contains considerable quantities of proteins, reducing sugars and essential oils (Aydın and Özcan 2007).

I.2.1. Phenolic compounds

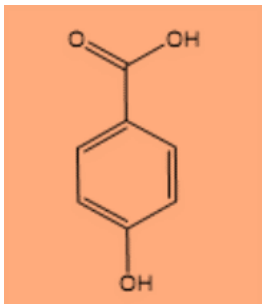
Phenolic compounds are secondary metabolites, ubiquitous widely exist in nature and food-industry by-products. They are differentiated from one another by their structure and molecular weight, and the resulting physicochemical and biological properties. Due to this enormous variety, there are reports of more than 10000 phenolic molecules and the list continues expanding (Vázquez, González et al. 1997)**.** The total phenol content of myrtle leaf and berry extracts ranged between 9.0 and 35.6mg GAE per g extract (Amensour, Sendra et al. 2009), for each solvent (methanol, ethanol, water), leaf extracts contained significantly higher amount of total phenolic compounds than berry extracts.

I.2.1.1. Phenolic acids

The predominant phenolic acids in fruits and vegetables are acidic hydroxybenzoic and hydroxycinnamic: hydroxybenzoic acids (C1–C6) and hydroxycinnamic acids (C3–C6) (Fig.1). Phenolic acids are commonly present under two principal forms in all plant-derived foods: a free and a bound form. The latter is found more frequently and occurs in the form of esters, glycosides and bound complexes (Agostini-Costa, Vieira et al. 2012).



(a)



(b)

Figure. I.1: Structure of hydroxybenzoïc acid (a), and hydroxycinnamic acid (b) (Agostini-Costa, Vieira et al. 2012)

I.2.1.2. Flavonoids

Flavonoids represent a large group of phenolic compounds found in plants that are synthesized from both the shikimate and acetate–malonate pathways involving numerous enzymatic steps. Flavonoids are thought to perform a variety of functions in plants including protection from UV radiation, defense against pathogens, pollinator attraction, pigmentation, and playing an essential role in reproduction (Li, Ou-Lee et al. 1993).

Flavonoids also contribute to the quality characteristics of fresh and processed food products including, texture, taste and color. Because of their biological importance, flavonoid

biosynthesis-related genes have been isolated from many plant species and have been extensively investigated at the molecular level (Hahlbrock and Scheel 1989, Winkel-Shirley 2001).

1.2.2. Essential oil

Essential oil composition of *Myrtus communis* varied with plant parts and varieties. Essential oil yield aried in leaves, fruits and stems (Wannes, Mhamdi et al. 2010). So, in leaves, it was 0.5% for var. italica and 0.3% for var. baetica and was higher than in fruits and stems with respectively 0.1% and 0.04% for italica and 0.07% and 0.03% for baetica. Essential oil composition was characterized by a high percentage of monoterpene hydrocarbons in leaves, largely due to a-pinene with 51.3% for italica and 27.7% for baetica; 1,8-cineole, the alone compound of ether class, was predominant in fruits and stems with respectively 31.6% and 34.7% for italica and 19.8% and 25.8% for baetica.

I.3. Medicinal proprieties

Many authors announced that the myrtle plant and its essential oils have a great potential like plants medicinal, with hypoglycemic (Fellah, Berger et al. 2003)**,** anti-inflammatory (Amira, Behija et al. 2012), anti-ulcerous (Sumbul, Ahmad et al. 2010), anti-mutagen (Hayder, Bouhlel et al. 2008) (Mimica-Dukić, Bugarin et al. 2010)**)** and antioxidant proprieties(Montoro Rios, Luque Martinez et al. 2006);(Wannes, Mhamdi et al. 2010). *Myrtus communis* L has long been used by locals for its culinary and medicinal properties (Ghasemi, Ni et al. 2014), **t**here are ample references to myrtle in ancient Egyptian medical texts as a remedy for urinary disorders, pain, heartburn, swelling, stiffness of the limbs, cough and to remove mucus from the chest**.** Soranus (a Greek physician from Ephesus, 1st-2nd century) mentions myrtle under the title of contraception and recommends smearing the cervix with a paste of myrtle oil with

white lead to block the passage of sperm. In addition the large application in the perfume, cosmetic, food, and pharmaceutical industries, these berries are widely used in industrial formulation of sweet liqueur (Aidi Wannes and Marzouk 2013).

I.3.1. Antibacterial activity

All extracts prepared from myrtle leaves liquid-liquid extraction (LLE) with different solvents were found to be very rich in polyphenols and to have high antioxidant activity (Aidi Wannes and Marzouk 2013). In particular, hydroalcoholic extracts contain galloyl-glucosides, ellagitannins, galloylquinic acids and flavonol glycosides; ethylacetate extract and aqueous residues after LLE are enriched in flavonol glycosides and hydrolysable tannins (galloyl-glucosides, ellagitannins, galloyl-quinic acids), respectively. Addition of these extracts did not affect the basal oxidation of human LDL but dose-dependently decreased the oxidation induced by copper ions. These results suggested the myrtle extracts to have a potent antioxidant activity mainly due to the presence of galloyl derivatives.

The richness of myrtle in phenolic compounds (flavonoids and tannins) and essential oil is at the origin of its antibacterial activity, *Escherichia coli* and *Staphylococcus aureus* is the germs most sensitive. Myrtucommulone A and B and semimyrtucommulone are responsible for this activity compared to that of penicillin and streptomycine (Montoro Rios, Luque Martinez et al. 2006, Yadegarinia, Gachkar et al. 2006, Amensour, Sendra et al. 2009). The plant extract can inhibit the growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* (Masoudi, Miraj et al. 2016).

I.3.2.Antidiabitic activity

Experiment was carried out rabbit's reaches diabetes by the administration of 50 mg/kg of the extract of *Myrtus communis* each day during one week. A diminution of 51% of the concentration of glucose in blood was observed, without affecting the rate of insulin, as well as

a diminution on the rate of blood triglyceride of 14%. That would be explained by the inhibiting activity of the myrtle extract of the sweats alpha glycosidase and the stimulation of the glucokinase which is a key enzyme of glycolysis (Sepici, Gürbüz et al. 2004).

I.3.3. Anti-inflammatory activity

Myrtus cummunis (MC), semimyrtucommulone (S-MC) and nonprenylated acylphloroglucinols present in the leaves of *M. communis*, potently suppress the biosynthesis of eicosanoids by direct inhibiting cyclooxygenase-1 and 5-lipoxygenase *in vitro* and *in vivo*. Their ability to suppress typical pro-inflammatory cellular responses suggests their therapeutic use for the treatment of diseases related to inflammation and allergy (Feißt, Franke et al. 2005)

I.3.4. Induction of apoptosis in cancer cells

Myrtus communis is reported to induce cell death of different cancer cell lines with characteristics of apoptosis, visualized by the activation of caspase-3, -8 and -9, cleavage of poly (ADP-ribose) polymerase (PARP), release of nucleosomes into the cytosol, and DNA fragmentation. It caused loss of the mitochondrial membrane potential in MM6 cells and evoked release of cytochrome c from mitochondria (Tretiakova, Blaesius et al. 2008).

I.3.5. Protective effect on cholesterol and human low density lipoprotein (LDL)

Myrtus communis have significant protective effect on LDL from oxidative damage, remarkable protective effect on the reduction of polyunsaturated fatty acids and cholesterol and inhibiting the increase of their oxidative products. Both the compounds have been suggested as natural dietary antioxidants with potential anti atherogenicity (Rose, Hoy et al. 2008)

I.3.6. Antioxidant activity

Myrtus communis L. is a rich source of antioxidant compounds and possesses strong antioxidant properties (Dairi, Galeano-Díaz et al. 2015). The myrtle is employed like remedy to treat the diseases related to the oxydative stress for its richness in antioxidant compounds such as myrtucommulone and semimyrtucommulone which can stop the formation of the

oxygenated reagents and of the peroxides which are responsible of the initiation and the maintenance of the inflammatory activity. The essential oils of this plant presents also an antioxidant character (Rotstein, Lifshitz et al. 1974); (Montoro Rios, Luque Martinez et al. 2006); (Yadegarinia, Gachkar et al. 2006) .

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Chapter II
Microwave, Ultrasound assisted – drying and separations

Introduction

For the recovery of high-added value compounds from plant materials, and to introduce bioactive plant extracts in pharmaceutical and cosmetic formulations, pharmaceutical industries and food industries are challenged to find new drying and extraction technologies in order to reduce energy consumption, to meet legal requirements on emissions, product/process safety and control, and for cost reduction and increased quality as well as functionality.

Recently, the evolving concept of green extraction demands for the development and utilization of techniques with highly efficient approach for reducing energy consumption and utilization of solvents with reduced generation of hazardous wastes. Extraction of components by extensively used operations like solvent extraction or leaching is usually enhanced by assistance of different processes like mechanical agitation, pressing, or heating which along with generation of large amount of heat and wastes, also results in degradation of sensitive components. These short comings have led to considering the use of new techniques in the extraction of natural substances, which typically use less solvent, energy, and automated methods have been recently used, e.g. supercritical fluid extraction (SFE), pressurized liquid extraction (PLE) ,microwave-assisted extraction (MAE) and ultrasound assisted extraction (UAE) (Liazid, Palma et al. 2007). The similarity between closed these techniques is the possibility of working at elevated temperatures and pressures, which drastically improves the peed of the extraction process.

These innovative techniques are also used for drying of such perishable fruits (Myrtle) for food preservation, which can save time and energy and minimize quality degradation with low price. In addition the traditional naturel drying has many disadvantages due to the inability to handle the large quantities and to achieve consistent quality standards, contamination problems and long drying times (Soysal 2004). The long drying times at relatively high temperatures during the falling rate periods often lead to undesirable thermal

degradation of the finished products, consume more energy, and yield low drying efficiency (Ozkan, Akbudak et al. 2007). Owing to these reason, development of new methods of drying The new technologies may be applied in a continuous or intermittent way alone or combined (Jumah 2005).

1. Microwave Technology - An Overview

The initial surge in microwave technology development was driven by the military needs of the world war (Meredith, 1998). The tremendous effort that went into development of radar during World War II generated a great body of knowledge on the properties of microwaves and related technologies (Stein et al., 1994). From a commercial standpoint the microwave oven was first developed in 1951 when a large floor standing model was produced by the Raytheon Company of North America (Osepchuck, 1984). For domestic purposes ovens become available in the early 1960's and from then a mass market was initiated Microwaves were produced using various apparatus; Albert Hull, a researcher at General Electric's research laboratories invented the simple two-pole magnetron, or split-anode magnetron. Application of microwave power to numerous food processes has been investigated on a laboratory scale, and a few successful industrial processes are presently in operation (Bengtsson and Risman 1971). The processes studied include drying; (Rzepecka, Hamid et al. 1972); (Suzuki and Oshima 1973), freeze-drying (Decarreau 1985); preheating, thawing (Phan, Allaire et al. 1977), sterilization (Kenyon 1971); (Lin and Li 1971)), enzyme inactivation (Goldblith 1968), meat tempering (Meisel 1973); (Schiffmann 1973), blanching (Avisse and Varoquauxt 1977);(Chen, Briggs et al. 1971), and cooking (Goldblith 1968) (Nykvist and Decareau 1976) . One of the main advantages using MAE is waves. This can mainly be attributed to the difference in heating performance employed by the microwave technique and conventional heating. In conventional,

heating a finite period is needed to heat the vessel before the heat is transferred to the solution, while microwaves heat the solution directly (Dahmoune, Nayak et al. 2015).

1.1. Microwaves theory

Microwaves belong to the portion of the electromagnetic spectrum with wavelengths from 1 mm to 1 m with corresponding frequencies between 300 MHz and 300 GHz (fig. II.2). within this portion of the electromagnetic spectrum, there are frequencies that are used for cellular phones, radar, and television satellite communications. For microwave heating, two frequencies, reserved by the Federal Communications Commission (FCC) for industrial, scientific, and medical (ISM) purposes are commonly used for microwave heating. The two most commonly used frequencies are 0.915 and 2.45 GHz. Recently, microwave furnaces that allow processing at variable frequencies from 0.9 to 18 GHz have been developed for material processing (Bassyouni, Abu-Bakr et al. 2012).

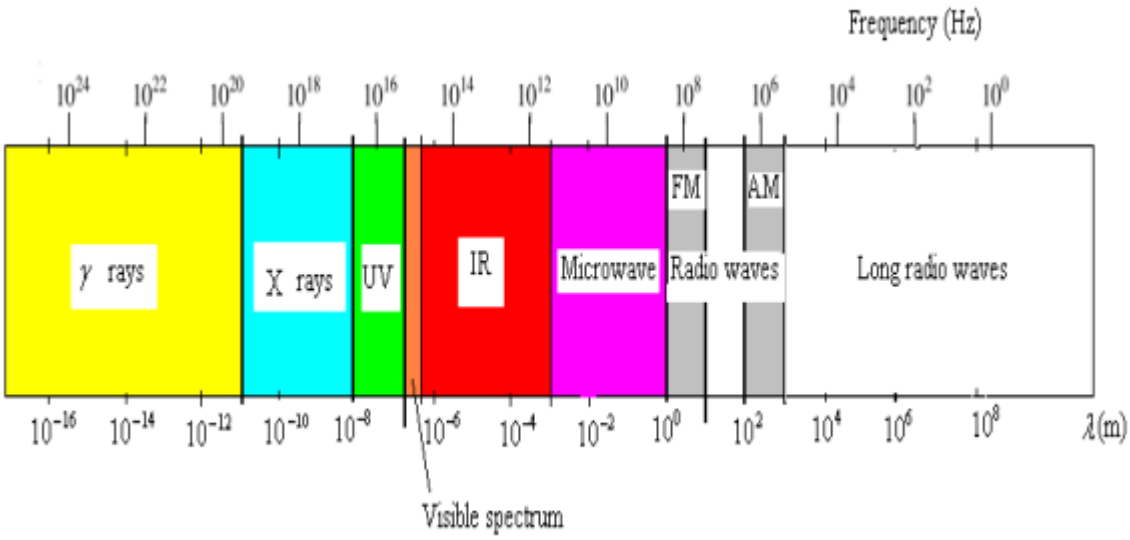

Figure II.2: Electromagnetic spectrum and frequencies used in microwave(Ali 2010)

1.2. Electromagnetic theory

Several distinctions between types of microwave ovens can be made. Microwave ovens can have monomode or multimode cavity. The monomode cavity (Fig. II.3a) can generate a frequency which excites only one mode of resonance. The sample can be placed on the maximum of the electrical field as the distribution of the field is known. The multimode cavity is large (Fig. II.3b) and the incident wave is able to affect several modes of resonance. This superimposition of modes allows the homogenization of field. Homogenization systems such as rotating plate are added(Letellier and Budzinski 1999). Microwave ovens can be operated under pressure (with or without regulation) or at atmospheric pressure.

There are two types of commercially available MAE systems: closed extraction vessels and focused microwave ovens. The former performs extraction under controlled pressure and temperature. The latter is also named as focused microwave assisted Soxhlet or solvent extraction (FMASE), in which only a part of the extraction vessel containing the sample is irradiated with microwave. However, both the above-mentioned systems are available as multimode and single- mode or focused systems (Chemat-Djenni, Hamada et al. 2007). The first studies of organic compound extraction have been performed in domestic oven (multimode cavity) in closed vessels without pressure control(Letellier and Budzinski 1999). Even a modified multimode domestic microwave oven operates as an open vessel extraction system(Mandal, Mohan et al. 2007) (fig. II.4). Microwave systems consist of three major components: the source, the transmission lines, and the applicator. The microwave source generates the electromagnetic radiation, and the transmission lines deliver the electromagnetic energy from the source to the applicator. In the applicator, the energy is either absorbed or reflected by the material (Mandal, Mohan et al. 2007):

1.2.1. Microwave source

These are the tubes used in conventional microwave ovens found in almost every home and in industrial ovens. The magnetron **(**microwave source) is the major player in a class of tubes termed 'cross field' so named because the basic interaction depends on electron motion in electric and magnetic field that are perpendicular to one another and thus 'crossed' (Stein 1994). In Magnetron, a cylindrical electron emitter or a cathode is surrounded by a cylindrical structure, or anode, at high potential and capable of supporting microwave fields. Magnets are arranged to supply a magnetic field parallel to the axis and perpendicular to the anode cathode electric field. The interaction of electrons traveling in the cross field supplied by the anode causes a net energy transfer from the applied DC voltage to the microwave field. The interaction occurs continuously as the electron transverse the cathode anode region. The magnetron is the most efficient microwave tubes, with efficiencies of 90% having been achieved (Stein 1994) with 70-80% efficiencies common.

1.2.2. Transmission lines

The transmission lines couple the energy of the microwave source to the applicator. In low power systems, the transmission lines are often coaxial cables, which are similar to cables that are used on televisions. At high frequencies and output power, the losses that occur in coaxial cables are significant, and waveguides are often the transmission line of choice in microwave heating systems. Waveguides are hollow tubes in which the electromagnetic waves propagate. The most commonly used cross-sections are rectangular (Thostenson and Chou 1999).

1.2.3. Microwave applicators

In simple term, microwave applicators are devices that are designed to heat a material by exposing it into a microwave field in a controlled environment. The objective is to cause a

controlled interaction between the microwave energy and the material to occur under safe, reliable, repeatable, and economic operating conditions (Meredith 1998). Microwave energy may also be combined inside the applicator with other energy sources, such as hot air, infrared and steam in order to achieve special results (Stein 1994).The applicator used in a microwave processing system often depends on the materials to be processed. Commercially available single mode, multi-mode, and variable frequency multi-mode processing systems are all used for microwave processing research, (Dahmoune, Nayak et al. 2015).

1.2.3.1. Single mode

Single mode or focused systems allow focused microwave radiation on a restricted zone where the sample is subjected to a much stronger electric field than in the previous case. In the microwave applicator, or cavity, theoretical analysis can be performed to describe the response of microwaves. Given the geometry of the applicator, it is often possible to solve the Maxwell equations analytically or numerically with the appropriate boundary conditions(Mandal, Mohan et al. 2007).

1.2.3.2. Multi-mode applicators

A multimode system allows random dispersion of microwave radiation within the microwave cavity, so every zone in the cavity and ample it contains is evenly irradiated. Applicators that are capable of sustaining a number of high order modes at the same time are known as multimode cavities. This type of applicator is used in home microwave ovens(Mandal, Mohan et al. 2007) .

However, the applicator in case of multi- mode system can be a closed cavity inside which microwaves are randomly dispersed. Uniform distribution of microwave energy inside the cavity can be achieved by using beam reflectors or turntable that makes heating of the sample

independent of the position. In focused microwave systems, the extraction vessel is however kept directly in a microwave waveguide and that acts as the applicator.

The advantages of closed-vessel systems can be summarized as follows.(Mandal, Mohan et al. 2007)

- They can reach higher temperatures than open vessel systems because the increased pressure inside the vessel raises the boiling point of the solvents used. The higher temperatures in turn decrease the time needed for the microwave treatment.
- Loss of volatile substances during microwave irradiation is virtually completely avoided.
- Less solvent is required. Because no evaporation occurs, there is no need continually to add solvent to maintain the volume. Also, the risk of contamination is avoided as a result there is little or no risk of airborne contamination.

By contrast, closed-vessel systems are subject to several shortcomings, as follows.

- The high pressures used pose safety (explosion) risks.
- The amount of sample that can be processed is limited

Atmospheric pressure (open-vessel) microwave sample preparation can be even more effective than closed-vessel methods. The use of atmospheric pressure provides substantial advantages over pressurized vessels, as follows.

- Increased safety results from operating at atmospheric pressure with open vessels
- The ability to add reagents at any time during the treatment.
- The ability to use vessels made of various materials, including PTFE, glass and quartz.

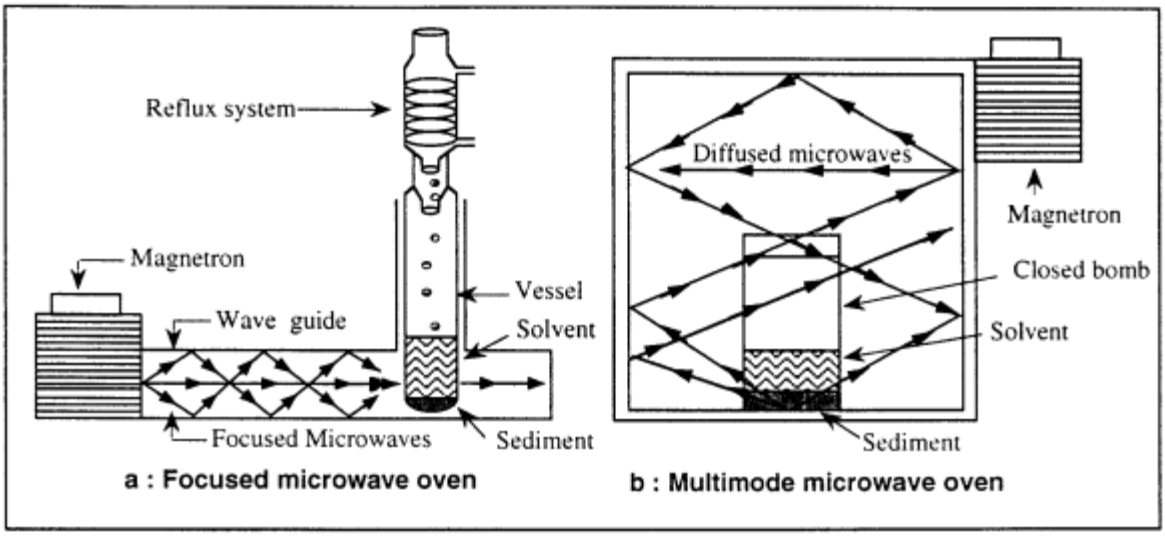

Figure II .3: Schematic view of focused microwave oven (a) and multimode microwave oven (b) (Letellier and Budzinski 1999).

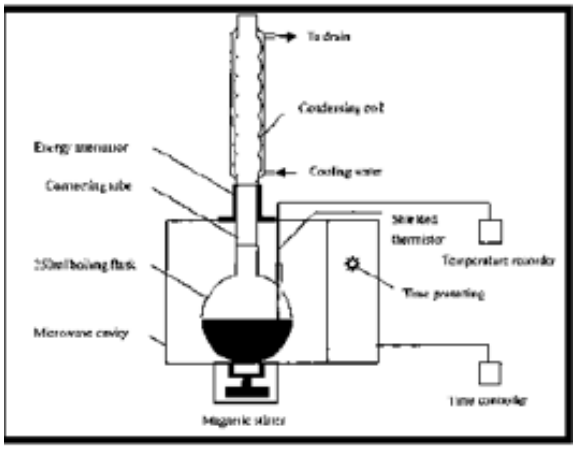

Figure II.4: Scheme of a modified domestic microwave oven (open vessel extraction) (Letellier and Budzinski 1999) .

1.3. Application of Microwave Heating

Interest in microwave heating applications started towards the end of World War II when major manufacturers of microwave tubes, such as Westinghouse, General Electric and Raytheon, started to look into alternative uses for microwave tubes, and filed many patents to make use of microwave heating for industrial applications, such as drying of tires, textiles, and wood; commercial

processing; and treatment of food. Microwave heating is used most commonly for the heating of food due to the good microwave susceptibility of water molecules in the food and has been increasingly applied for processing of polymers, ceramics, metals, minerals, chemicals, composites and biological subjects. For food processing, microwave heating has been applied to drying of potato chips and pasta, meat tempering, cooking of bacon, blanching of fruits and vegetables, *etc.* (Wong and Gupta 2015).

1.3.1. Fundamentals of Microwave Heating

One of the earliest description of the characteristics of microwave heating was in a Scientific American article in 1943 where it was mentioned that heat was generated from within the object and involves no transfer of heat to it (Wong and Gupta 2015).

The principle of heating using microwave energy is based on the direct effect of microwaves on molecules by ionic conduction and dipole rotation. In many applications these mechanisms take place simultaneously. Ionic conduction is the electrophoretic migration of ions when an electromagnetic field is applied. The resistance of the solution to this flow of ions will result in friction and, thus, heat the solution. Dipole rotation means realignment of dipoles with the applied field. A t 2450 MHz, which is the frequency used in commercial systems, the dipoles align and randomize 4.9310 times per second and this forced molecular movement results in heating (Ganzler, Salgo et al. 1986). Three principal microwave heating mechanism exist (Whittaker 1997): Dipolar polarisation, conduction mechanisms, interfacial polarisation.

1.3.1.1. Dipole polarization

Dipolar polarization is a polarization that is particular to polar molecules. In polar molecules, the different electronegativites of individual atoms results in the existence of a permanent electric dipole on the molecule (Figure. II.5).The dipole is sensitive to external electric fields, and will attempt to align with them by rotation, the energy for this rotation being

provided by the field. This realignment is rapid for a free molecule, but in liquids instantaneous alignment is prohibited by the presence of other molecules (Whittaker 1997). A limit is therefore placed on the ability of the dipole to respond to a field, which affects the behavior of the molecule with different frequencies of electric field.

Under low frequency irradiation, the dipole may react by aligning itself in phase with the electric field. Whilst some energy is gained by the molecule by this behavior, and some is also lost in collisions, the overall heating effect is small. Under the influence of a high frequency electric field, on the other hand, the dipoles do not have sufficient time to respond to the field, and so do not rotate. As no motion is induced in the molecules, no energy transfer takes place, and therefore, no heating (Whittaker 1997).

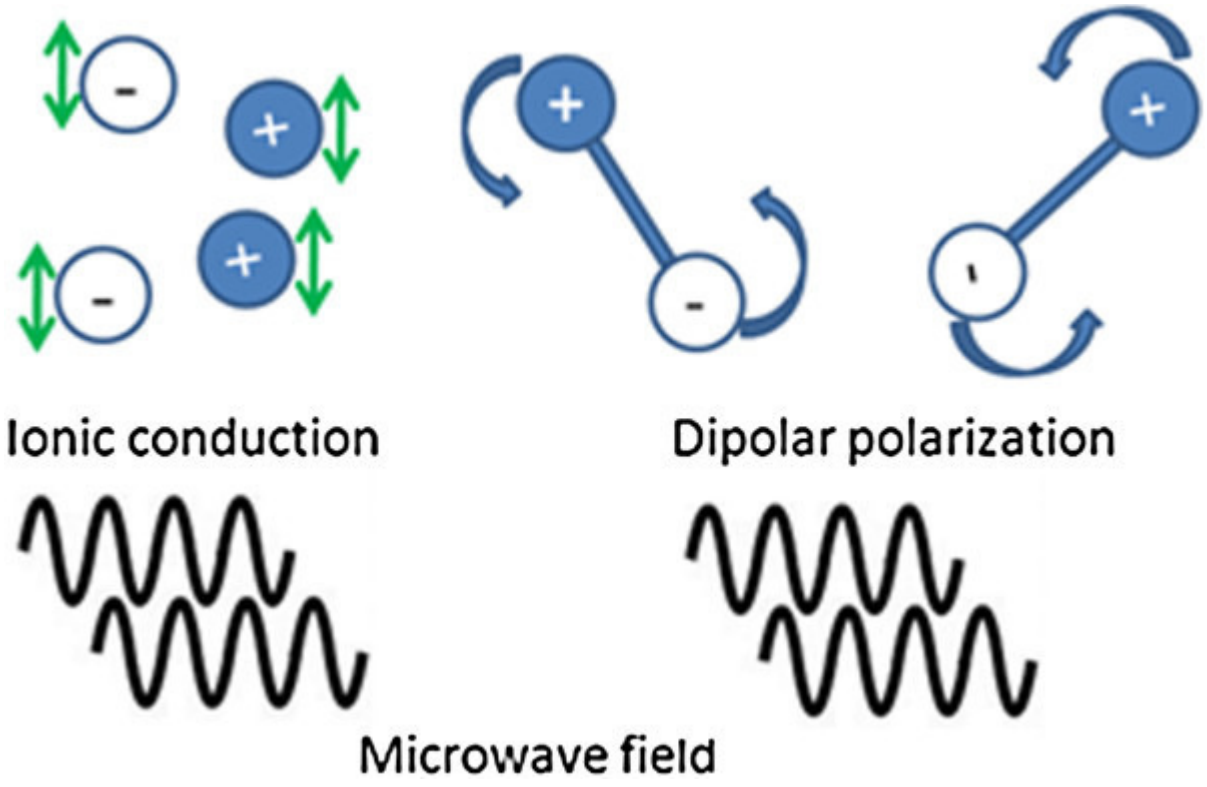

Figure. II.5: Realignment of a dipole in an electromagnetic field

1.3.1. 2. Conduction Mechanisms

When the microwave irradiated sample is an electrical conductor, the charge carriers (electrons, ions, etc.) are moved through the material under the influence of the electric field resulting in a polarisation. These induced currents will cause heating in the sample due to any electrical resistance. For a very good conductor, complete polarisation may be achieved in approximately 10-18 seconds, indicating that under the influence of a 2.45 GHz microwave, the conducting electrons move precisely in phase with the field (Whittaker 1997). When the conductivity of the material is very large (typical for metal-like material), the fields attenuate rapidly toward the interior of the sample due to skin effect.

1.3.1.3. Interfacial Polarisation

Interfacial polarization is most easily viewed as a combination of the conduction and dipolar polarisation effects. This mechanism is important for systems comprised of conducting inclusions in a second, non-conducting material. An example would be a dispersion of metal particles in sulphur. Sulphur is microwave transparent and metals reflect microwaves yet, curiously, the combination forms an extremely good microwave absorbing material (Whittaker 1997).

1.4. Conventional Heating Methods versus Microwave Heating

In conventional thermal processing, energy is transferred to the material through convection, conduction, and radiation of heat from the surfaces of the material. In contrast, microwave energy is delivered directly to material through molecular interaction with the electromagnetic field Fig. II.6. The difference in the way energy is delivered can result in many potential advantages to using microwaves for processing of materials. As microwaves can penetrate materials and deposit energy, heat can be generated throughout the volume of the

material. The transfer of energy does not rely on diffusion of heat from the surfaces, and it is possible to achieve rapid and uniform heating of relatively thicker materials. In traditional heating, the cycle time is often dominated slow heating rates that are chosen to minimize steep thermal gradients that result in process-induced stress. There is a balance between processing time and product quality in conventional processing (this is also true of microwave heating). As microwaves can transfer energy throughout the volume of the material, the potential exists to reduce processing time and enhance overall quality (Thostenson and Chou 1999). Due to the penetrative nature of microwaves, heat is generated from within the object through the absorption of microwave energy directly by the object and do not require substantial heating of the environment. Therefore a temperature gradient exists in both conventional and microwave heating as a result of the way heat is transferred/generated in the object as shown in Fig. II.6.

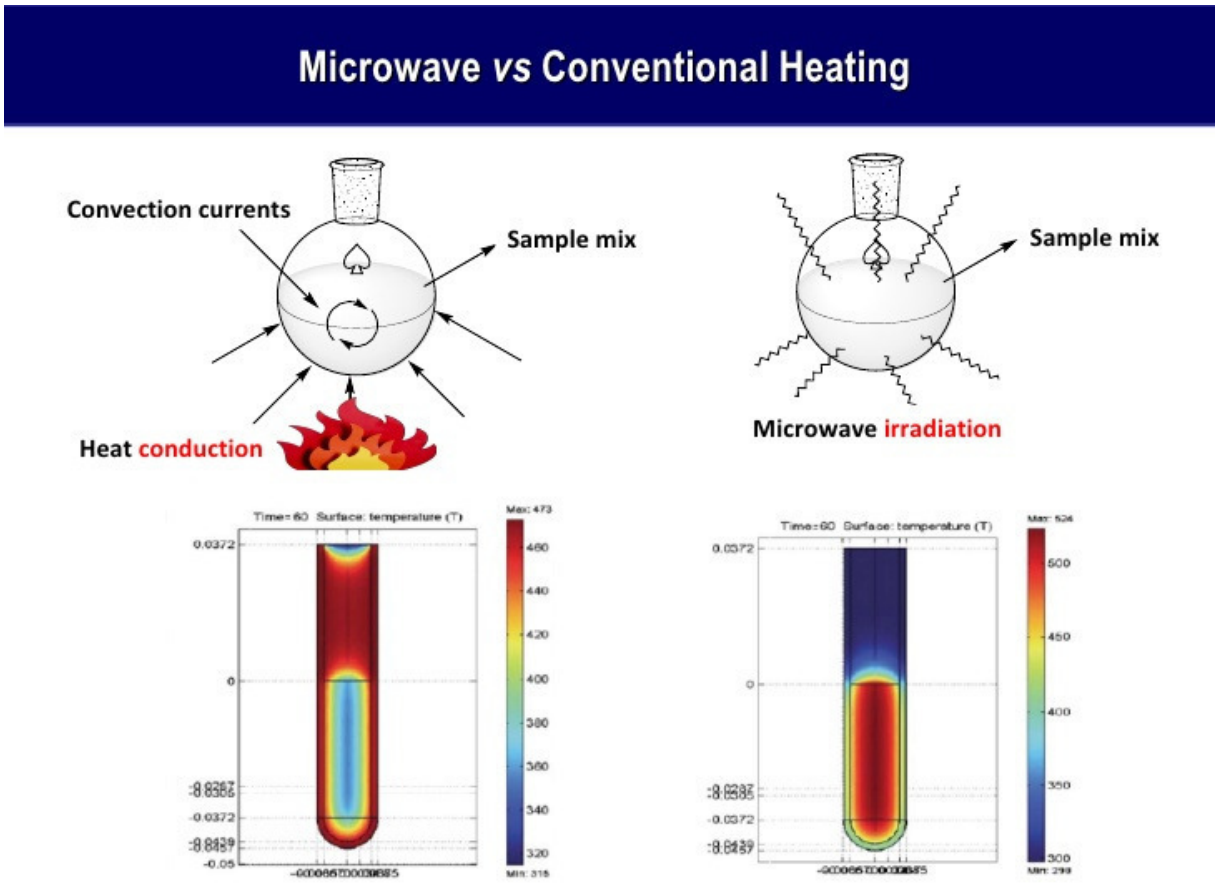

Figure. II.6: Conventional and microwave heating mechanisms(Kahrilas 2012).

1.5. Microwave–material interaction aspects

When microwaves are directed towards a material, part of the energy is reflected, part is transmitted through the surface, and of this latter quantity, and part of it is absorbed. The proportions of energy, which fall into these three categories, have been defined in terms of the dielectric properties. The dielectric properties of materials dictate, to a large extent, the behaviour of the materials when subjected to radio-frequency (RF) or microwave field for the purposes of heating, drying or processing the materials. The characterisation of dielectric properties is vital for understanding the response of a material to design of microwave thermal processes can be described in terms of them.(Venkatesh and Raghavan 2004).

1.6. Mechanism of Microwave in Green Extraction

Even though dried plant material is used for extraction in most cases, but still plant cells contain minute microscopic traces of moisture that serves as the target for microwave heating. The moisture when heated up inside the plant cell due to microwave effect, evaporates and generates tremendous pressure on the cell wall due to swelling of the plant cell (Al-Harahsheh and Kingman 2004). In MAE, the process acceleration and high extraction yield may be the result of a synergistic combination of two transport phenomena: heat and mass gradients working in the same direction. On the other hand, in conventional extractions the mass transfer occurs from inside to the outside, although the heat transfer occurs from the outside to the inside of the substrate Fig. II.7.

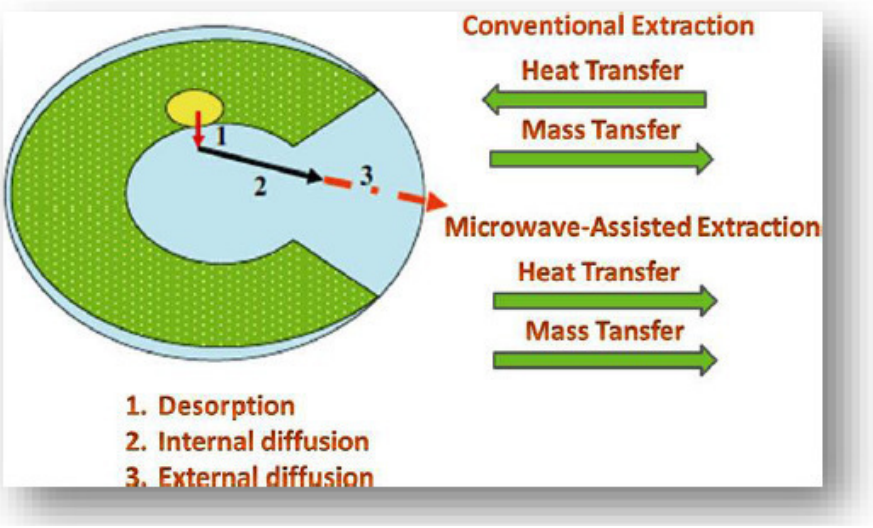

Figure. II.7: Basic heat and mass transfer mechanisms in microwave and conventional extraction of natural products (Veggi, Martinez et al. 2012).

The pressure pushes the cell wall from inside, stretching and ultimately rupturing it, which facilitates leaching out of the active constituents from the ruptures cells to the surrounding solvent thus improving the yield of phytoconstituents. This phenomenon can even be more

intensified if the plant matrix is impregnated with solvents with higher heating efficiency under microwave. Higher temperature attained by microwave radiation can hydrolyze ether linkages of cellulose, which is the main constituent of plant cell wall, and can convert into soluble fractions within 1 to 2 min. The higher temperature attained by the cell wall, during MAE, enhances the dehydration of cellulose and reduces its mechanical strength and this in turn helps solvent to access easily to compounds inside the cell. This observation suggests that microwave treatment affects the structure of the cell due to the sudden temperature rise and internal pressure increase. During the rupture process, a rapid exudation of the chemical substance within the cell into the surrounding solvents takes place. This mechanism of MAE based on exposing the analytes to the solvent through cell rupture is different from that of heat-reflux extraction that depends on a series of permeation and solubilisation processes to bring the analytes out of the matrix. Destructive changes in the plant tissue of fresh orange peel due to microwave treatment was also observed using scanning electron micrographs (Chandrasekaran, Ramanathan et al. 2013). These changes in the plant tissue due to microwave heating gave a considerable increase in the yield of extractable pectin.

1.6.1. Factors Affecting Microwave Extraction

a. Solvent nature and volume

A correct choice of solvent is fundamental for obtaining an optimal extraction process. Solvent choice for MAE is dictated by the solubility of the target analyte, by the interaction between solvent and plant matrix, and finally by the microwave absorbing properties of the solvent (Letellier, Budzinski et al. 1999). Preferably the solvent should have a high selectivity towards the analyte of interest excluding unwanted matrix components. Another important aspect is the compatibility of the extracting solvent with further chromatographic analytical steps. MAE can also be performed with the same solvent as used for the conventional extraction

methods. However, the optimal extraction of solvents for MAE cannot always be deduced from those used in conventional procedures. On the other hand use of ethanol as the extracting solvent gave significantly higher yield than Soxhlet ethanol extraction(Alfaro, Bélanger et al. 2003). This can be accounted due to the difference in dielectric properties of the solvent. Thus Dielectric properties of the solvent towards microwave heating play an important role in microwave extraction. Both the efficacy and selectivity of MAE depend significantly on the dielectric constant of the extracting solvent mixture.

b. Microwave power

Microwave power and irradiation time are two such factors, which influences each other to a great extent. A combination of low or moderate power with longer exposure may be a wise approach. Amount of ginsenosides extracted by MAE method under different microwave conditions were studied (Nemes and Orsat 2011). In general, the extraction efficiency was improved by raising microwave power from 30 to 150 W. During short extraction time (1 and 2 min), recovery was enhanced with increased microwave power. However, high microwave power might increase the product temperature overly high, and decrease the extraction yield through product damage or compound breakdown.

c. Extraction time

Generally, by increasing the extraction time, the quantity of analytes extracted is increased, although there is the risk that degradation may occur. Often 15 – 20 min is sufficient, but even 40 and 43 sec have been demonstrated to have given excellent recovery(Li, Chen et al. 2004). MAE of polyphenols from tomatoes was found to increase up to 2.04 min and later decreased with the increase of time(Li, Deng et al. 2012). Irradiation time is also influenced by the dielectric properties of the solvent. water, ethanol, and methanol may heat up tremendously

on longer exposure thus risking the future of thermolabile constituents (Mandal, Mohan et al. 2007). But some extraction reports also reveal that varying extraction time does not significantly improves recovery (Mandal, Mohan et al. 2007).

d. Temperature

Microwave power and temperature are very interrelated to each other and needs to be given special attention particularly when working with closed vessel system. In closed vessel systems, temperature may reach well above the boiling point of the solvent (Letellier and Budzinski 1999)). These elevated temperatures result in improved extraction efficiencies, since desorption of analytes from active sites in the matrix will increase. Solvents have higher capacity to solubilize analytes at higher temperatures, while surface tension and solvent viscosity decrease with temperature, which will improve sample wetting and matrix penetration, respectively. However, some studies have shown that the temperature is not a significant factor. (Eskilsson and Björklund 2000, Zhang, Yang et al. 2008).

e. Matrix characteristics

The plant particle size and the status in which it is presented for MAE can have a profound effect on the recoveries of the compounds. The particle sizes of the extracted materials are generally in the range of 100 μm – 2 mm (Wang and Weller 2006). Fine powder can enhance the extraction by providing larger surface area, which provides better contact between the plant matrix, and the solvent, also finer particles will allow improved or much deeper penetration of the microwave. One of the disadvantages associated with the use of finer particles is difficulty of separation of the matrix from the solvent after microwave irradiation. Generally, centrifugation or filtration is applied to satisfy the above purpose and use of very fine particles may pose some technical problems. In the MAE of ginseng saponins, extraction yield increased

with the decrease in particle size (Kwon, Lee et al. 2003), but it was also seen that particles less than 60 meshes are not suitable for the filtration of the extracts. The status of the plant matrix presented for MAE also needs to be evaluated during the extraction process. Sample pretreatment prior to MAE can bring about effective and selective heating of the plant matrix.

1.6. Mechanism of Microwave drying

In recent years, microwave drying was an alternative way to improve the quality of dehydrated products. Usually, drying is not induced by dielectric heating alone, but most microwave drying systems combine microwave and conventional heating. While the microwave is processing, microwaves radiate from a source in all directions. These waves carry energy and during the drying process, material absorbs this energy and converts it to heat by polar molecules. Water is the common polar molecule and a component of foods. So, during this process, water molecules convert microwave energy to heat. Then, the water molecules start to evaporate as a result of this heat, and so the material starts to dry .The heating may take place in separate operations or simultaneously. Microwave drying, like conventional drying, is caused by water vapor pressure differences between interior and surface regions which provide a driving force for moisture transfer. It is most effective at product moisture contents below 20% (Maskan 2001). Therefore, essentially for economic reasons, it has been suggested that microwave energy should be applied in the falling rate period or at a low moisture content (where conventional drying takes a long time) to finish drying. Microwave drying has been used in drying of herbs (Giese 1992), potato (Bouraoui, Richard et al. 1994)), raisins (Karathanos, Kostaropoulos et al. 1995), apple and mushroom (Funebo and Ohlsson 1998), diced apples (Feng and Tang 1998), carrots ((Prabhanjan, Ramaswamy et al. 1995) (Litvin, Mannheim et al. 1998) and banana (Maskan 2000).

However one of disadvantages of microwave drying is that excessive temperature along the corner or edges of food products results in scorching and production of off-flavors especially during final stages of drying (Zhang, Tang et al. 2006). Hence, it is necessary to combine microwave drying with a pretreatment in order to maintain product quality.

2. Ultrasound Technology - An Overview

In the last two decades, these shortcomings have led to the consideration of the use of enhanced and efficient extraction techniques amenable to automation such as ultrasound-assisted extraction. Shorter extraction times, reduced organic solvent consumption, energy and costs saved, were the main tasks pursued. Driven by these goals, advances in ultrasound assisted method have resulted in a number of innovative extraction techniques such as ultrasound-assisted Soxhlet extraction, ultrasound-assisted Clevenger distillation, continuous ultrasound-assisted extraction, and combination of ultrasound with drying techniques such as microwave drying. Ultrasound is a key-technology in achieving the objective of sustainable ‘‘green” chemistry and extraction. Ultrasound is well known to have a significant effect on the rate of various processes in the chemical and food industry. Using ultrasound, full extractions can now be completed in minutes with high reproducibility, reducing the consumption of solvent, simplifying manipulation and work-up and giving higher purity of the final product. Several classes of food components such as aromas, pigments, antioxidants, and other organic and mineral compounds have been extracted, analyzed and formulated efficiently from a variety of matrices (mainly animal tissues, microalgae, yeasts, and food and plant materials).

In addition, ultrasound has been implemented as an alternative pretreatment method for drying, and the results have shown that this pretreatment can greatly reduce the overall processing time (Duan, Zhang et al. 2008, Aversa, Van der Voort et al. 2011) (Mothibe, Zhang

et al. 2011) which can attribute to the following factors; Increase in the mass transfer rate(García-Pérez, Cárcel et al. 2009), , (Cárcel, Garcia-Perez et al. 2011),, Loss of cellular adhesion, rupture of the cell walls and formation of large channels (He, Yang et al. 2012)..

2.1. Ultrasounds waves

Ultrasound is defined as sound waves having frequency that exceeds the hearing limit of the human ear (~20 kHz). Some animals utilize ultrasound for navigation (dolphins) or hunting (bats) using the information carried by back-scattering sound waves. Ultrasound is one of the emerging technologies that were developed to minimize processing, maximize quality and ensure the safety of food products. Ultrasounds are mechanical waves that are capable of moving in an elastic medium at a frequency higher than the maximum audible limit of the human ear (16 kHz) (Fig. II.8). Based on frequency range, the applications of ultrasound in food processing, analysis and quality control can be divided into low and high. Low energy (low power, low intensity) ultrasound has frequencies higher than 100 kHz at intensities below 1 W·cm2, which can be utilized for non-invasive analysis and monitoring of various food materials during processing and storage to ensure high quality and safety. High energy (high power, high-intensity) ultrasound uses intensities higher than 1 W·cm−2 at frequencies between 20 and 500 kHz, which are disruptive and induce effects on the physical, mechanical or chemical/biochemical properties of foods. These effects are promising in food processing, preservation and safety(Awad, Moharram et al. 2012).

2.1. 1. Low power ultrasound

Low power ultrasound (LPU) along with spectroscopy and nuclear magnetic resonance (NMR) are currently the most popular, practical and widely used nondestructive analytical methods. For many years, LPU has been successfully utilized for studying the physicochemical and structural properties of fluid foods (McClements and Gunasekaran 1997).

2.1.1.2. Basic principles of LPU for food analysis

Sound propagates through food materials as mechanical waves causing alternating compressions and decompressions (Blitz 1971). These ultrasound waves have characteristic wavelength, velocity, frequency, pressure and period. The interaction of sound waves with matter alters both the velocity and attenuation of the sound waves via absorption and/or scattering mechanisms (McClements 1994). The velocity of sound is the product of frequency and wavelength, thereby high frequency sound waves have short wavelength while low frequency waves have long wavelength. Ultrasonic velocity (v) is determined by density (ρ) and elasticity (E) of the medium, according to the Newton–Laplace equation (Blitz 1971):

$$V = \sqrt{\frac{E}{P}} \qquad \text{Eq. I.1.}$$

This equation implies that the ultrasound velocity of the solid form of a material is larger than that of its liquid form (e.g., solid and molten chocolate). For the analysis of food materials, ultrasound velocity is very sensitive to molecular organization and intermolecular interactions, which make ultrasound velocity measurements (UVM) suitable for determining composition, structure, physical state and various molecular process (Buckin, O'Driscoll et al. 2003)

Other ultrasound parameters that correlate with many physicochemical properties of materials are attenuation coefficient and acoustic impedance. Attenuation is caused by the energy loss in compression and decompression in ultrasonic waves due to both absorption and scattering contributions (Buckin, Kudryashov et al. 2002). The absorption contribution of attenuation is associated with homogeneous materials whereas the scattering only exists in heterogeneous ones. Attenuation is affected by viscosity, compressibility, wall material, and

scattering and adsorption effects (Povey 1997)which give information about the physicochemical properties of food materials such as molecular relaxation, microstructure, phase composition, bulk viscosity and rheology(McClements 1994), kinetics of fast chemical reactions and droplet sizing and stability in emulsions (Buckin, Kudryashov et al. 2002). In addition, the attenuation coefficient for a given material is highly dependent on the way in which the material was manufactured (Umchid, Gopinath et al. 2009) which may be useful in quality control assurance of some products. Acoustic impedance is the product of density and sound velocity passing through the boundary of different materials, which affects the reflection coefficient. Materials with different densities will have different acoustic impedances, which results in reflections from the boundary between two materials with different acoustic impedances. Attenuation (A) and acoustic impedance (z) are expressed by the following relationships (McClements 1994):

$$A = A0e^{-ax} \qquad \text{Eq. I.2.}$$

$$R = \frac{AT}{At} = \frac{Z\,1-Z2}{Z1+Z2} \qquad \text{Eq. I.3.}$$

where: Ao is the initial (unattenuated) amplitude of the wave. x is the distance traveled R is the ratio of the amplitude of reflected wave (AT) to the incident wave (At) reflection coefficient z1 and z2 are the acoustic impedances of two materials.

2.1.2. High power ultrasound

The propagation of ultrasound through a biological material induces compressions and decompressions (rarefactions) of the medium particles, which imparts a high amount of energy. High power ultrasound with frequency higher than 20 kHz has mechanical, chemical and/or biochemical effects, which are used to modify the physicochemical properties and enhance the

quality of various food systems during processing (J Mason, Chemat et al. 2011). The mechanical effect has many applications such as extraction of flavors, degassing, destruction of foams, emulsification, enhancement of crystallization and modifying polymorphism (Higaki, Ueno et al. 2001). High power ultrasound can be applied using sonication baths or ultrasonic immersion probes with different lengths, diameters and tip geometries depending on applications. High intensity focused ultrasound using lens-shaped transducers is another technique that is used in medicine to destroy diseased or damaged tissue through ablation.

2.1.2.1. Principles of high power ultrasound

In general, energy, intensity, pressure, velocity and temperature are the main parameters affecting power ultrasound. High power ultrasound can be described by the following pattern(Patist and Bates 2008):

$$Pa = Pamax \sin(2\pi ft)\ . \qquad \text{Eq. I.4.}$$

Pa is the acoustic pressure (a sinusoidal wave), which is dependent on time (t), frequency (f) and the maximum pressure amplitude of the wave. Pa max is related to the power input or intensity (I) of the transducer:

$$I = \frac{Pamx}{2pv} \qquad \text{Eq. I.5.}$$

Where ρ is the density of the medium and v is the sound velocity in the medium. With low intensities (or high frequencies), acoustic streaming is the main mechanism(Leighton 2007). Acoustic streaming is the motion and mixing within the fluid without formation of bubbles. Higher intensities (low frequencies) induce acoustic cavitation (Mason 1998)) due to the generation, growth and collapse of large bubbles, which causes the liberation of higher energies (Alzamora, Guerrero et al. 2011).

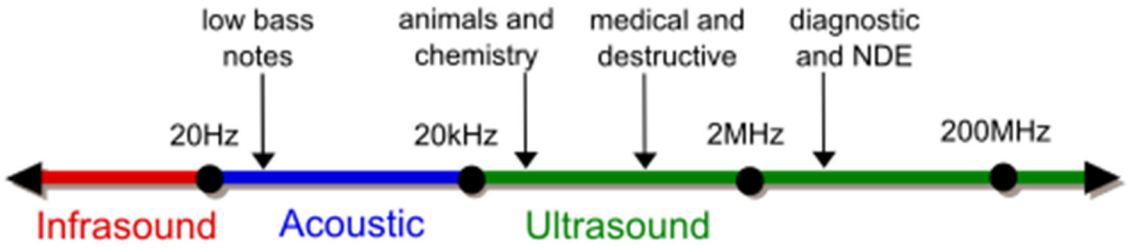

Figure. II. 8 : Diagram of ultrasounds ranges

2.3. Instrumentation

2.3.1. Electrical Generator

The electrical generator is the source of energy for the ultrasonic system, which must drive the transducer. Generally, an electrical generator produces electrical current with a specified power rating. Most generators allow the power to be set only indirectly through voltage (V) and current (I) settings. The voltage represents the potential energy stored in the electrons (measured in volts); the current represents the net charge of electrons traversing an area over some time interval (measured in amps); and the power is the product of these two represented in (Eq. I.13)[(Bermúdez-Aguirre, Mobbs et al. 2011):

$$P = I.V\ (W\ ou\ volt.amps) \qquad \text{Eq } I.6.$$

Electrical generators that are designed specifically for ultrasound mostly focus on industrial cleaning, and therapeutic, welding, disinfecting applications and extraction and operate in the lower frequency range (10–40 kHz)(Bermúdez-Aguirre, Mobbs et al. 2011).

2.3.2. Transducer

The transducer converts electrical energy (or mechanical energy, in the case of the liquid whistle) into sound energy by vibrating mechanically at ultrasonic frequencies(Povey 1989) . The transducer attached to an electrical generator will transform, for instance, 20-kHz electrical energy from the generator into ultrasounds energy of the same frequency by vibrating at 20,000 mechanical cycles per second (Povey 1989) summarize three main types of transducers: liquid-driven (mechanic), magnetostrictive, and piezoelectric (pzt). Liquid-driven transducers rely on purely mechanical energy to produce ultrasound, but magnetostrictive and piezoelectric transducers convert electrical and magnetic energy into mechanical, ultrasonic energy. While liquid whistles make excellent mixers and homogenizers, most power ultrasound equipments use piezoelectric or magnetostrictive transducers today.

2.3.3. Emitter (Baths, Horns, and Sonotrodes)

Emitters may also fulfill the role of amplifying the ultrasonic vibrations while radiating them. The two main forms of emitters are baths and horns (i.e., probes); horns often require the attachment of a horn tip known as a sonotrode (Fig. II. 9) (Povey 1989). Baths usually consist of a tank to which one or more transducers have been attached. The tank holds a sample in solution and the transducers radiate ultrasounds directly into the sample. In horn-based systems, a horn is attached to the transducer to amplify the signal and bring it to the sample. The tip of the horn, often a separate attachable device known as a sonotrode, radiates the ultrasonic wave into the sample. The shape of the horn determines the amount of amplification. Hence, the intensity of radiation can be controlled by selecting differently shaped horns. The main difference in equipment used in laboratories versus commercial processing plants is the type of emitter. More robust emitters that do not wear down after many hours of use are required in food manufacturing.

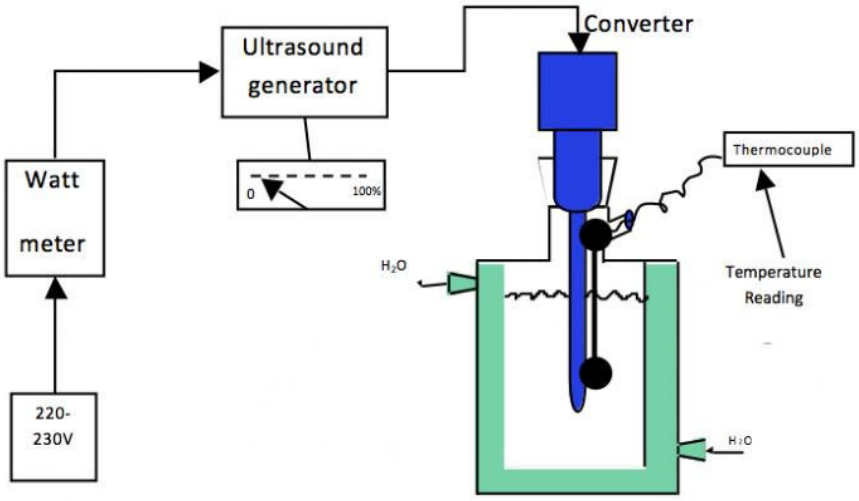

Figure. II.9: Diagram of an ultrasonic device

2.4. Phenomenology of cavitation

Each liquid medium has a critical molecular distance (critical distance d in Fig. II.10): below this distance the liquid remains intact but above this characteristic threshold the forces maintaining the cohesion of the liquid are overcome and Bubbles Compression waves propagating in the medium Molecules of an elastic medium Sound wave Rarefaction zone . Cavitation containing liquid vapor and dissolved gases appear. This phenomenon, called cavitation, has been studied theoretically and experimentally. Neppiras and Noltingk(Noltingk and Neppiras 1950) initiated the hot spot theory that implosion of a cavitation bubble generates very high local values of temperature and pressure (thousands of degrees Kelvin and thousands of kPa). This theory was then taken up by Suslick and Price(Suslick and Price 1999) . The behavior of the bubbles depends on their size and the nature of the local ultrasonic field which determines their stability or their implosion.

2.4.1. Factors Influencing Cavitation

- **Presence of dissolved gases**

The cavitation bubbles may consist of vapor of the liquid in which they are generated. Cavitation originates from the nuclei, which are constituted by the gaseous occlusions within the liquid. The presence of dissolved gases will therefore favor the phenomenon of cavitation (Achat 2013).

- **Pressure of the medium**

The cavitation bubbles can be generated when the pressure applied to the medium (PL) drops below the vapor tension of this liquid (PV). The pressure applied to the medium when subjected to ultrasound can be calculated as the sum of the hydrostatic pressure (Ph.) and the sound pressure (Eq.II.7). Consequently cavitation will be possible in the liquid if the vapor pressure of the liquid is greater than the sum of the hydrostatic pressure and the sound pressure (Eq.8). This means that the higher the pressure applied to the medium, the more difficult it will be to cause cavitation. Indeed, to provoke it, it will be necessary to increase the acoustic pressure and thus to increase the intensity of the ultrasounds (Achat 2013).

$$P_L = P_h + P_a \qquad \textbf{Eq II. 7.}$$

$$P_v > P_h + P_a \text{ or } Pa > P_h - P_v \qquad \textbf{Eq II.8.}$$

Ultrasonic intensity: there is a direct relationship between the ultrasonic intensity and the amplitude of the sound wave pressure that correlates with the sound pressure. Since acoustic pressure is a key parameter in ultrasound generation, the more the ultrasound intensity will be increased, cavitation can be achieved (Achat 2013).

- **Frequency of ultrasound**

The frequencies most commonly used are between 20 and 40 kHz. Higher frequencies make cavitation more difficult. Indeed, the cavitation bubbles require a small amount of time to be generated during the rarefaction phase. The higher the frequency, the shorter the phases of rarefaction, the less likely the bubble is to be created. This is the reason why high frequency ultrasound is said to be non-destructive; the frequency is too high to allow cavitation.

- **Temperature**

The importance of this parameter is difficult to evaluate because it modifies the viscosity of the medium and the saturating vapor pressure, which can influence the cavitation capacity of the study medium. The closer one gets to the boiling point of the liquid, the more easily the cavitation bubbles are generated, but they lose their implosion capacity, which is why the majority of the sonochemical reactions are favored by low temperatures.

- **Impurities**

Each impurity present in the liquid or on the surface of the glassware used can act as a new cavitation site. Indeed, gases can be trapped in the interstices of the impurities and the depressions generated by the rarefaction cycles will dislodge these gases from the impurity and constitute a new cavitation core (Achat 2013).

The different experimental conditions will produce different types of cavitation bubbles. Some are said to be stable, others are said to be transient. Transient bubbles exist only during a few acoustic cycles before imploding violently. Knowing that the lifetime of such bubbles is too low to observe a transfer of matter by diffusion of gas into or out of the bubble, their implosion is not damped and proceeds with great violence. According to Mason and Lorimer (Mason and Lorimer 2002), it is thus possible to reach temperatures close to 5000 K and pressures of the order of 1000 atmospheres (**Table II**). Stable cavitation bubbles are gas and

vapor bubbles that oscillate non-linearly around a pseudo-equilibrium size for several number of cycles. At each cycle, their volume increases because the balance of exchanges between the desorption and condensation (entering the bubble) and absorption and vaporization (bubble exit) phases is positive. The bubble then implodes when it reaches a critical size. This implosion is less violent because it is damped by the gases present and the temperatures reached then are around 1800 K (Flynn 1975).

Table II.2: Characteristics and effects of ultrasound according to the type of cavitation induced

Type de cavitation	Stable	Transitoire
Intensité	1 à 3 W/cm2	> 10 W/cm2
Type d'oscillations	faibles et autour de l'équilibre	Fortes
Cycle	plusieurs cycles	1 à 2 cycles
Durée de vie des bulles	Longue	Courte
Phénomène local	simple diffusion gaz/liquide maisrisque de résonance	températures et pression très élevées
Nature de la bulle	gaz résiduel ou vapeur du liquideenvironnant	vapeur du liquide seulement
Effet	à court terme : dégazage à long terme : mélange	violents : érosion, émulsification, sonochimie, sonoluminescence

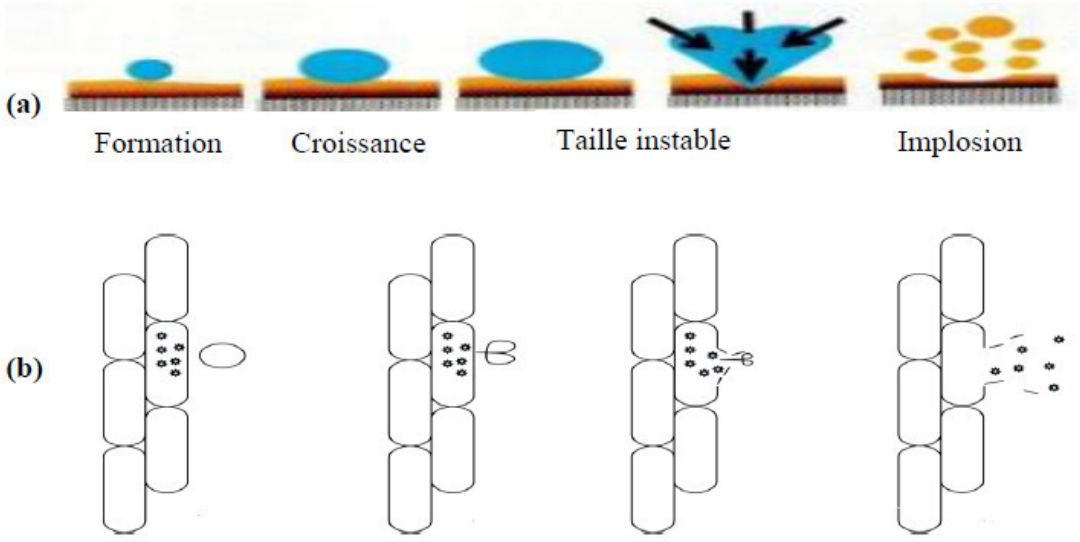

Figure. II.10: Evolution of a cavitation bubble near a solid surface (a) and a plant cell (b) (Achat 2013)

Ultrasound has mechanical and physical actions, especially during the explosion of cavitation bubbles. The presence of an adjacent obstacle causes the loss of symmetry of the system with respect to an implosion within the solution (Figure II.10). The main physical and mechanical effect of ultrasound is the production of micro-jets directed to a solid surface during the implosion of the cavitation bubbles. (Over 120 m / s) and they could have a preponderant influence on the effects induced by cavitation and on the increase of agitation at the obstacle / reaction medium interface. In addition to their mechanical and physical effects, ultrasound produces chemical effects which also result from the cavitation phenomenon. When an aqueous solution is subjected to ultrasound, free radicals can be generated and initiated chain reactions. These entities can then react with species in solution or recombine to form hydrogen, hydrogen peroxide or other radicals. If the nature of the chemical species is highly dependent on the dissolved gases, the frequency greatly influences the reaction kinetics (Veillet S., et al 2009).

2.5. Laboratory and industrial equipment

Two types of equipment are commonly used in laboratories. The first is the ultrasonic tank, which is inexpensive and finds many applications for sample preparation, homogenization, dispersion, degassing and cleaning (Fig.II.11). It generates frequencies between 25 and 50 kHz and acoustic powers from 1 to 5 W.cm-2. This type of instrumentation has the advantage of being simple to use but requires the use of a container for the mixture to be treated which will act as a dampener for the effect of ultrasound which can only act indirectly (Achat 2013) .

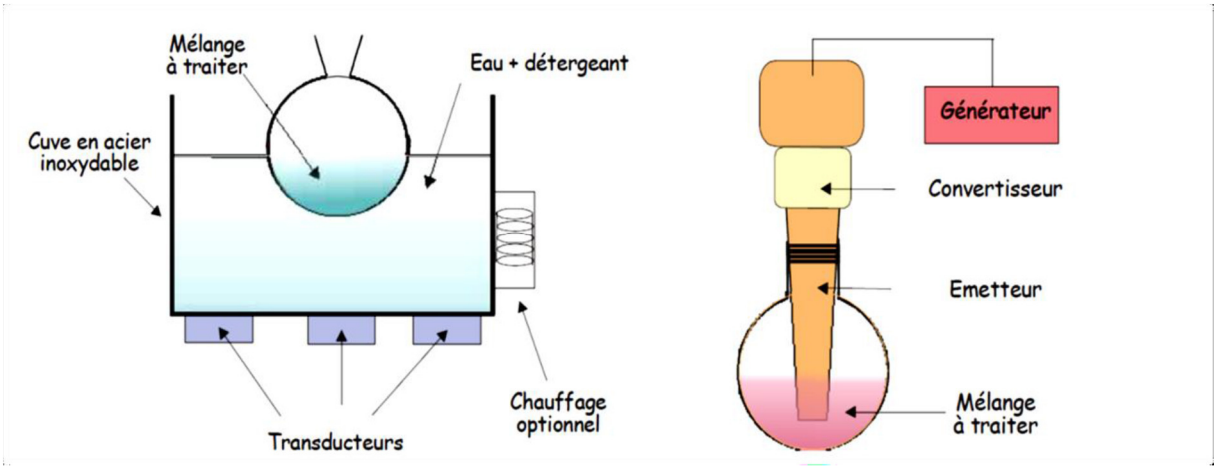

Figure II.11: Diagram of ultrasonic devices: tray and probe

The second apparatus is the ultrasonic probe. Unlike the tank, it allows direct irradiation of the medium while generating considerably higher acoustic powers (100 times higher). However, its use remains only adapted to the treatment of small volumes of liquid and particular attention must be paid to this handling because the sample can undergo a rapid increase in temperature. The Coupling of these two types of equipment to pump systems allows a Continuous mode because it promotes uninterrupted flow of "fresh" solvent through the sample subjected to ultrasound. Industrial systems are commonly used in combination with tanks

Buffer and pumps (on-line processing) or batch mode with Tanks equipped. The latter have been used for a number of years to plant compounds to accelerate maceration. A range of new devices has been developed (Fig. II.12). The Ultrasonic power of approximately 1 W / cm², the frequency of use is of 25 kHz. The other specificities of this reactor are to have a dual system Enabling to thermostat the system and the possibility of working directly In the reactor inducing the direct application of ultrasound to the reaction mixture (Chemat and Khan 2011).

(A) (B)

Figure II.12: Ultrasonically assisted extraction laboratory reactors: (A) batch and (B) continuous .

2.6. Applications of ultrasound

The diversity of ultrasonic devices and actions allows a wide range of applications. In the field of agri-food, these may be of the order of extraction of drying or of the processing, food products(Mason and Lorimer 2002).

2.6.1. Ultrasound-assisted extraction (UAE)

After interaction with subjected biological or non-biological material, ultrasound waves alter their physical and chemical properties and their cavitational effect facilitates the release of extractable compounds and enhances the mass transport by disrupting membranes, plant cells walls and other structures. The most established application is to accelerate conventional extraction methods. UAE, also reported as ultrasound assisted leaching (USAL), has been widely used to release inorganic compounds using acidic and basic solutions (Domínguez-Vega, García et al. 2010, De La Calle, Cabaleiro et al. 2011, Jiang, Zhang et al. 2012).

These procedures do not involve total destruction of the sample matrix but the breakdown of the chemical bonds between the trace elements and the constituents of the sample matrix. The principle of high-power ultrasound has been attributed to the acoustic cavitation phenomenon that appears when high-intensity acoustic waves are generated in a fluid (Jiang, Zhang et al. 2012) . The extraction mechanism involves two types of physical phenomena: diffusion through the cell walls and washing out the cell content once the walls are broken (Vinatoru 2001) . Ultrasound waves modify their physical and chemical properties after their interaction with subjected plant material, and their cavitational effects facilitate the release of extractable compounds and enhance mass transport by disrupting the plant cell walls (Li, Pordesimo et al. 2004).

2.6.2. Combination of ultrasound with innovative extraction techniques

Several extractions can be performed simultaneously, and, as no specialized laboratory equipment is required, the technique is relatively inexpensive compared to most modern extraction methods. A wide range of solvents or solutions can be used and the solvents can be collected easily. However, the extraction is still time-consuming and a large volume of organic

solvents is required. Due to its characteristics, UAE can be used as pretreatment prior to more sophisticated extraction. For example, a UAE/solid-phase extraction (SPE)/supercritical fluid extraction (SFE) method was developed for trace concentrations of is flavones in algae and cyanobacteria. During the sonication pretreatment, certain parts of the matrix (e.g., walls of the cells or organelles in plant material) .

The combination of ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) by means of simultaneous irradiation (UMAE) is one of the most promising hybrid techniques for fast, efficient extractions. Since the pioneering work of Cravotto et al. (Cravotto, Boffa et al. 2008), several applications of combined US/MW irradiation for plant extraction have appeared in the literature, the great potential of this hybrid technique has not yet been adequately exploited (Fig. II. 13). Due to the high efficiency and the dramatically short extraction time, we believe that UMAE has a great potential for academic and industrial research activity. Ultrasound can dramatically improve the extraction of a target component mainly through the phenomenon of cavitation.

The mechanical ultrasonic effect promotes the release of soluble compounds from the plant body by disrupting cell walls, enhancing mass transfer and facilitating solvent access to cell content. Meanwhile, MW heats the whole sample very quickly inducing the migration of dissolved molecules. The simultaneous irradiation increases solvent penetration into the matrix, facilitates analyte solvation and usually increases the solubility of target compounds.

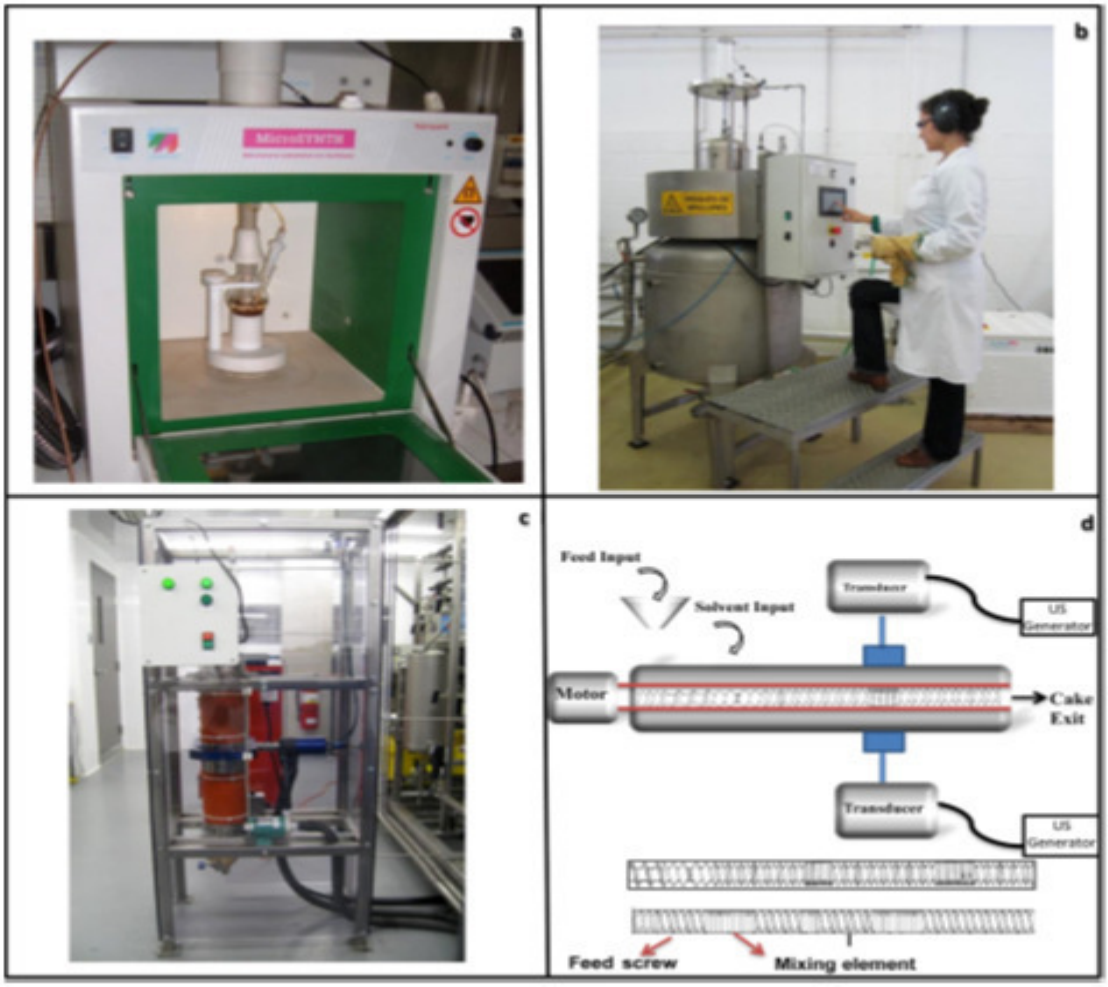

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Figure II.13. Combined innovative extraction techniques ((a) ultrasound-microwave, (b) ultrasound – DIC, (c) ultrasound-SFE, (d) ultrasound-extrusion).

2.6.3. Application in materiel drying

Drying or dehydration, the oldest method of food preservation, is based on the use of thermal energy such as sun, hot air, smoking, drum and convection drying(Cohen and Yang 1995). However, heat can deteriorate the quality of the final product causing undesirable food flavor, color, vitamin degradation and loss of essential amino acids (Mousa and Farid 2002, Min, Chunli et al. 2005);(Zhang, Tang et al. 2006). In addition seen one of disadvantages of microwave

drying is that excessive temperature along the corner or edges of food products results in scorching and production of off-flavors especially during final stages of drying (Zhang, Tang et al. 2006). ultrasound has been implemented as an alternative pretreatment method for drying, and the results have shown that this pretreatment can greatly reduce the overall processing time (Mothibe, Zhang et al. 2011)) which can attribute to the following factors; Increase in the mass transfer rate (García-Pérez, Cárcel et al. 2009), (Cárcel, Garcia-Perez et al. 2011),), Loss of cellular adhesion, rupture of the cell walls and formation of large channels (He, Yang et al. 2012).

Ultrasonic dehydration is a very promising technique since it can be utilized at low temperature, which prevents the degradation of food at high temperatures. Power ultrasound also improves heat and mass transfer phenomena in drying processes (Cárcel, Garcia-Perez et al. 2011). Acoustic dehydration relies on cavitation (Tarleton 1998)) and also on the effects of compressions and expansions induced by sound waves passing through the food medium, which generates high forces and maintains the moisture inside the capillaries of the material thus making the moisture removal easier (De la Fuente-Blanco, De Sarabia et al. 2006)). The application HPU for the dehydration of vegetables using forced-air drying assisted by air-borne ultrasound and ultrasonic dehydration have been carefully studied by the Power Ultrasonic Group of the Institute of Acoustics in Spain (De la Fuente-Blanco, De Sarabia et al. 2006) .

2.7. Ultrasound-Assisted Extraction (UAE) Versus MAE and conventional method

Several studies reported the comparison of UAE (mainly in terms of recoveries and duration) with other extraction techniques, either classical or recent Figure II.14.

2.7.1. Microwave-assisted extraction (MAE)

Among the more recently developed solvent extraction techniques, MAE showed the highest extraction yields in food processing, with short extraction times and easy operation, due to the high degree of automation. However the disadvantage of MAE extraction was the higher

temperature causing the chemical compounds degradation. In comparison, UAE offered a simpler extraction procedure, requiring amounts of solvent similar to MAE and PLE, but longer analysis times (Chandrapala, Oliver et al. 2012, Chandrapala, Oliver et al. 2013).

2.7.2. Conventional techniques

As the majority of UAE applications deal with solid matrices, the classical techniques are commonly manual shaking, Soxhlet extraction, steam distillation or reflux. Most often, UAE affords several advantages over classical techniques (e.g., lower solvent consumption, speed, and the potential to recover tightly bound residues not easily released by conventional techniques). In addition, UAE is well suited to routine analysis, and solvents already used for classical methods may be readily adaptable to UAE. The recoveries of both analytes obtained by ultrasonic extraction were better than those of than shaking [i.e., 90% and 88% versus 76% and 79%, respectively]. Furthermore, ultrasound is easy to handle and saves time. Several studies reported similar results for UAE or Soxhlet extraction in applications {e.g., PBDEs from dust samples(Król, Zabiegała et al. 2012) or chlorobenzenes from soil (Wang, Wang et al. 2012).

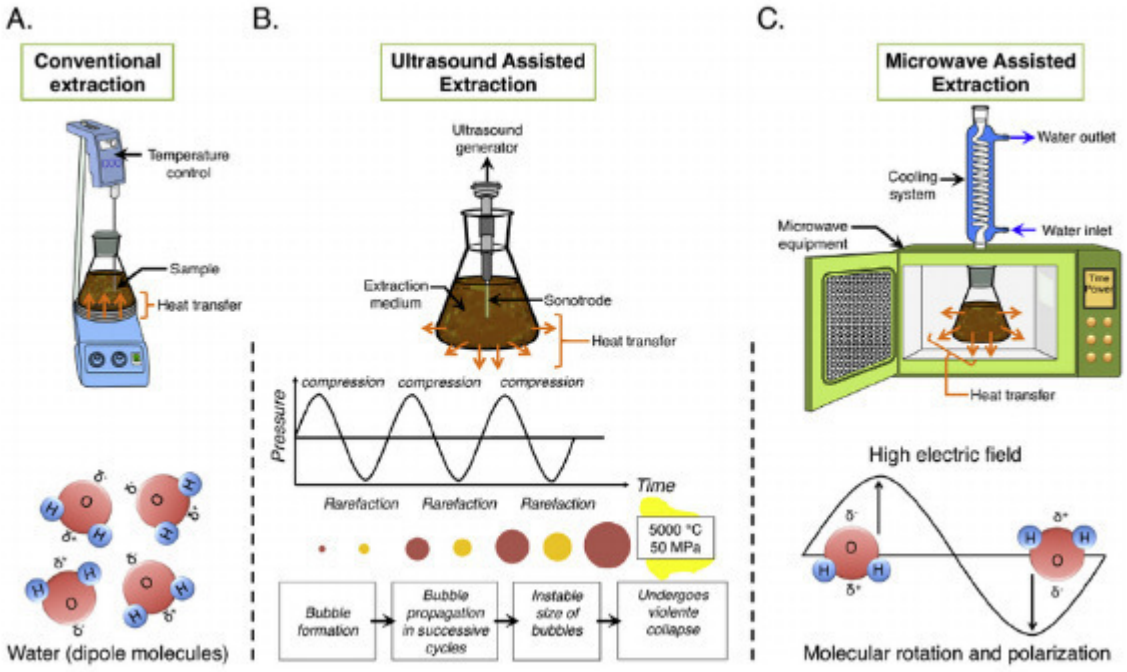

Figure. II. 14. A) Experimental set-up for conventional extraction of high-added value molecules from plant matrices used at laboratory scale. B) Ultrasound assisted extraction principle and cavitational phenomenon. C) Microwave assisted extraction equipment used at laboratory scale showing the molecular rotation mechanism.

2.8. Cost, investment and environmental impact

In general, an ultrasound-assisted extraction process has an optimum of between 15 and 30 minutes of treatment at room temperature, whereas conventional extractions (maceration, distillation, etc.) are very long and / or carried out at the boiling temperature of solvent for several hours. For a 1 liter reactor, the energy consumption of the conventional extraction is 5 kilo Watt hours, while the ultrasonic assisted extraction requires only 0.25 kWh. The tests were carried out using a Wattmeter at the inlet of the heating system for conventional extraction and 106 at the inlet of the ultrasonic generator for ultrasonic assisted extraction. This directly affects the environmental impact of the process. While the US extraction process releases only 200 g of CO2 into the atmosphere, the conventional process releases more than 4000 g of CO2. This

was calculated according to the rule that to obtain one kWh of electricity, one must burn either natural gas, oil or coal, and this results in a release of 800 g of CO2. Prices for industrial ultrasonic reactors vary between 7,000 euros (3 liters) and 200,000 euros (1000 liters). The choice in an ultrasonic reactor induces only 25% more investment compared to a conventional reactor. However, taking into account the process times which are divided by a factor ranging from 10 to 100 and an energy and pollution reduction by a factor of 10, the ultrasonic-assisted processes have a much lower cost of production to conventional methods (Chemat and Khan 2011).

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Part II

(Personal work)

Chapter III
Phytochemical analysis

of Myrtus communis plant:

Conventional versus

microwave assisted-extraction

procedures

Phytochemical analysis of *Myrtus communis* plant*:* Conventional versus microwave assisted-extraction procedures

Abstract

Microwave assisted extraction (MAE) of total phenolic compounds (TPC) from myrtle leaf, stems, pericarp, and seeds was studied and the results were compared with those of the conventional method extraction (CME) in terms of extraction time, extraction yield/efficiency, and antioxidant activity which was measured using radical scavenging assay ($DPPH^{\bullet}$) and reducing power The results show that the MAE was higher in terms of saving energy, extraction time (62s) and extraction efficiency of bioactive compound compared to CME (2h). Leaf presented the optimum content of total phenols (250 mg GAE.g^{-1} DW) and flavonoids (13.65 mg GAE.g^{-1} DW). However, the anthocyanin content was most important in pericarp extract (176.50 ± 2.17 mg Cyd-3-glu g^{-1} DW). The antioxidant activity was important in all parts, mainly in leaves. The results indicated that appropriate microwave treatment could be an efficient process to phenolic compounds recovery and thus, better the antioxidant activity of myrtle extract. Principal component analysis (PCA) applied to the experimental data shows that the distribution of the myrtle phenolic compounds depended on their plant part localization as well as the extraction method.

Keywords: *Myrtus communis*, microwave-assisted extraction, antioxidant activity, phenolic compounds.

Introduction

Myrtle (*Myrtus communis L*) belongs to the family of Myrtaceae is an evergreen shrub, which grows wild in several regions all over the world (Aydın and Özcan 2007). Different parts of this plant found various uses in the food industry, such as for savoring meat and sauces, and in the cosmetic industry (Chalchat, Garry et al. 1998). The leaf decoction was used for virginal washing enemas, and against respiratory diseases (Marchini and Maccioni 1998). A decoction or infusion of leaves and fruits of this plant were used as stomachic, hypoglycemic, cough, infectious and oral diseases. They were also used for constipation, appetizing envy, and externally as anti-hemorrhagic for wound healing (Aydın and Özcan 2007). Regarding the chemical composition, previous studies on *Myrtus communis* aerial parts have revealed the presence of several compounds. Leaves and flowers contain essential oils, tannins, phenolic acids, and flavonoids (Messaoud, Zaouali et al. 2005, Aidi Wannes and Marzouk 2013). Fruits are mostly composed of volatiles oils, tannins, anthocyanins, fatty acids, sugars, and organic acids such as citric and malic acids (Messaoud and Boussaid 2011).

Extraction represents the primary step to get a crude extract from plants; then the obtained extracts should undergo further analysis of their active components. Different techniques such as conventional method extraction (CME) methods including soaking, maceration (Aspé and Fernández 2011, Dahmoune, Boulekbache et al. 2013), water percolation, soxhlet extraction have been used (Jun, Deji et al. 2011). These techniques are based on the correct choice of solvents and the use of heat or/and agitation to increase the solubility of the desired compounds. Moreover, these techniques require longer extraction time and as a result cause thermal degradation for most of constituents (Luque de Castro and García-Ayuso 1998). Over the past decade, various novel extraction techniques have been introduced and investigated, most of which were claimed to be better in terms of efficiency, extraction time and solvent

consumption. The techniques are microwave-assisted extractions (MAE) (Dahmoune, Boulekbache et al. 2013), supercritical fluid extraction (SFE), pressurized solvent extraction (PSE), and ultrasonic extraction (UE) (Jun, Deji et al. 2011).

MAE has drawn significant research attention in various field, in particular medicinal plant research, due to its special heating mechanism, moderate capital cost and its good performance under atmospheric conditions. MAE is an innovative solvent extraction technology which offers a better alternative to several thermal applications due to its efficient volumetric heat production, and the fact that it has many advantages over CME , such methods improved efficiency, reduced extraction time, lower solvent consumption, higher selectivity toward target molecules, and higher level of automation (Aspé and Fernández 2011). In addition, a wider range of solvents can be used in MAE, as the technique which is less dependent on solvent affinity (Yang and Zhai 2010).

Several investigations have focused on the natural antioxidants compounds of Myrtle leaves (Amensour, Sendra et al. 2010, Aidi Wannes and Marzouk 2013). However, the emphasis has been given to the conventional extraction method. To our best knowledge, no literature report exists on the MAE of natural phenolic contents from different parts of myrtle (stem, pericarp, and seed) with exception of the study by Dahmoune, Nayak et al. (2015) in the case of myrtle leaf polyphenols. Since this is the most common form of using, the species and it may constitute an interesting dietary source of health protective compounds. Therefore, the aim of the present work is:(*i*) to compare the effects of MAE and CME on the extraction efficiency from different myrtle parts (in terms of TPC, flavonoids, anthocyanins and condensed tannins), and (*ii*) to estimate the recovery and the antioxidant capacity of the extracts.

2. Materials and methods

2.1 Chemicals

Sodium carbonate (Na_2CO_3), Folin–Ciocalteau's phenol reagent and disodium hydrogen phosphate (Na_2HPO_4), aluminium chloride ($AlCl_3$) were obtained from Prolabo (Loire, France), and 1,-diphenyl-2-picryl-hydrazil (DPPH) from Sigma Aldrich (Germany). Gallic acid, ferric chloride ($FeCl_3 \cdot 6H_2O$), potassium ferricyanide ($C_6N_6FeK_3$), trichloroacetic acid and sodium dihydrogen phosphate (NaH_2PO_4) were purchased from Biochem-chemopharma (Loire, France). All solvents used were of analytical grade.

2.2 Plant material and sample preparation

Myrtus communis L. samples were collected at optimal fruit maturity (November, 2012), from Addekar (Bejaia; North East of Algeria; 36° 41′ 32.54″ N, 4° 40′ 21.44″ E; altitude 1092 m). A voucher specimen has been stored in the Plant Ecology Herbarium of Es-Senia university (Oran, Algeria), under voucher number 1856. Leaves, stems and fruits were isolated manually from the aerial parts. The samples were washed with a tap water then distilled water to remove any adhering soil and dust. Myrtle parts were dried at room temperature in a ventilated darkroom to protect the active compounds from light oxidation. The drying time was about 5, 3, and 7 days for the leaves, stems, and fruits, respectively. Fruit samples were peeled manually and seeds were recovered. All parts were ground with an electrical grinder (IKA modelA11 Basic, staufen, Germany). The obtained powder was passed through standard 250 nm sieve and only the fraction with particle size < 250 nm was used. The powder was stored in airtight bags until use.

2.3 Extraction procedures of phenolic contents

2.3.1 Microwave assisted extraction MAE)

Phenolic contents were extracted using a domestic microwave oven (Samsung MW813ST, Kuala Lumpur, Malaysia) modified with the addition of a condenser, generated during extraction procedure. It operates at a frequency of 2450 MHz and a maximum output power of 1000 W with a 100 W increment. The size of the heating cavity is 37.5 cm (L) × 22.5 cm (W) × 38.6 cm (D). For the extraction, a volume of 32 mL of ethanol/water (42/58, v/v) was added to 1 g of the powders in flat bottomed flask. The mixture was irradiated at 500 W for 62 s (optimization conditions) then filtered with a sintered glass at 0.45 µm using a vacuum pump. The obtained extract was stored at 4 °C until further analysis (Dahmoune, Boulekbache et al. 2013).

2.3.2. Conventional Method extraction (CME)

Regarding the CME, one gram of each powder was placed in a conical flask, and 50 mL of ethanol/water (42/58, v/v) ethanol were added. After stirring for 2 hours, the mixture was the mixture was vacuum filtered. The obtained extract was stored at 4 °C until further analysis (Dahmoune, Boulekbache et al. 2013).

2.4. Phytochemical analysis

2.4.1. Total phenolic content (TPC)

The total phenolic content in the extracts was assessed according to the method of George, Brat et al. (2005). Briefly, 500 µL of diluted and filtered extract from the different parts were added to 2.5 mL of 10-fold diluted (v/v) Folin–Ciocalteau reagent. The solution was mixed and incubated at room temperature for 2 min. 2 mL of 7.5% (m/v) sodium carbonate were added and the solution was then incubated at 50 °C for 15 min. The absorbance of the sample was measured at 760 nm against a blank using a UV–VIS

Spectrophotometer (SpectroScan 50, Nkesia, Cyprus). The assay was performed in triplicate. For quantification, a calibration curve was generated with the standard solution of gallic acid, (R^2 = 0.998). The TPC were expressed as mg of gallic acid equivalent (GAE) per gram of dry weight (DW) basis (mg GAE g^{-1} DW).

2.4.2. Total flavonoid content

The total flavonoid content was estimated by the aluminum chloride method according to Quettier-Deleu, Gressier et al. (2000), based on the formation of a flavonoid-aluminum complex. Briefly, 1mL of different extracts was mixed with 1mL of 2% (m/v) aluminum chloride. After 15 min of incubation in the dark, the absorbance of the mixture was measured at 430 nm. Each analysis was carried out in triplicate. The total flavonoid content was calculated from a calibration curve made with rutin (R^2 = 0.997) and expressed as milligrams of rutin equivalent per gram of dry weight (DW) basis (mg REg^{-1} DW).

2.4.3. Total monomeric anthocyanin content

Total monomeric anthocyanin content was determined by the pH-differential method of Lee, Durst et al. (2005), based on the structural change of the anthocyanins chromospheres between pH 1.0 and 4.5. The absorbance was measured at 520 nm and at 700 nm in buffers at pH 1.0 and 4.5 respectively. The concentration of anthocyanins was obtained using equation (Eq.1). Results are expressed on a Cyanidin-3- glucoside basis.

$$\textit{Anthocyanin pigment (cyanidin-3-glucoside equivalents, mg/g DW)} = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times l} \quad \text{Eq. III.9}$$

$$\text{Where } A = \left(A_{520nm} - A_{700nm}\right)_{pH1.0} - \left(A_{520nm} - A_{700nm}\right)_{pH4.5}$$

MW (molecular weight): 449.2 g mol^{-1} for cyanidin-3-glucoside (cyd-3-glu); *DF*: dilution factor; *l*: path length in cm; ε: 26 900 molar extinction coefficient, in L × mol^{-1}× cm^{-1}, for cyd-3-glu; and 10^3: factor for conversion from g to mg.

2.4.4. Condensed tannin content

The condensed tannin content was determined by the HCl–Vanillin method as described by Aidi Wannes, Mhamdi et al. (2010). One mL of extracts was mixed with 5 mL of reagent (HCl + Vanillin). The mixture was kept in the dark room for 20 min. The absorbance was determined at 500nm versus a blank. All Analyses were performed in triplicate. Total tannins were expressed as mg catechin equivalents per gram of dry weight basis (mg CE·g^{-1} DW) through a calibration curve made against catechin standard (R^2 = 0.996).

2.5. Antioxidant activity

The antioxidant properties of the active compounds are very important due to the deleterious role of free radicals in foods and biological systems (Gülçin, Mshvildadze et al. 2006). The antioxidant activity of all parts of plant (leaves, steams, pericarp and seeds of fruits), was evaluated by $DPPH^{\bullet}$ radical scavenging assay and reducing power test. The higher percentage inhibition test rate is the greater the hydrogen donating ability, thus the higher antioxidant activities.

2.5.1. Radical scavenging activity assay

The free radical-scavenging activity of the extracts was determined using the 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^{\bullet}$) (Choi, Kim et al. 2002). It is a highly colored free radical that can abstract labile hydrogen atoms from phenolic antioxidants with concomitant formation of a color-less hydrazine (DPPH-H). The free radical scavenging activity (RSA) of an extract can be expressed as the percentage of DPPH reduced by a given amount of extract.

The RSA was measured, following the method of Dudonné, Vitrac et al. (2009). DPPH• radicals have an absorption maximum at 515 nm (Choi, Kim et al. 2002), which disappears with reduction by an antioxidant compound. A DPPH• solution in absolute methanol (60 M) was prepared, and 3 mL of this solution were mixed with 1 mL of the different extracts. The samples were incubated for 20 min at 37 °C in the dark, then, the decrease in absorbance at 515 nm was measured. The α-tocopherol served as a positive control. All the tests were performed in triplicate, and the inhibition rate was calculated according the equation (Eq.III.10).

$$\%\ Scavenging = \frac{\left(A_{control} - A_{extract}\right)}{A_{control}} \times 100 \qquad \text{(Eq.III.10)}$$

Where $A_{control}$ is the absorbance of DPPH• at = 0 min; $A_{extract}$ is the absorbance of DPPH•in the presence of the sample at t = 20 min.

2.5.2. Reducing power

The yellow color of the test solution changes to green depending on the reducing power of test specimen. The presence of reductants in the solution causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, Fe^{2+}can be monitored by the measurement of the absorbance at 700 nm (Zou, Lu et al. 2004). One mL of different extracts was mixed with 2.5 mL of a 0.2 M (m/v) sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% (m/v) Potassium ferricyanide ($K_3Fe(CN)_6$). The mixture was incubated in a water bath at 50 °C for 20 min. Then, 2.5 mL of 10% (m/v) trichloroacetic acid were added. Finally, 1mL of the obtained solution was added to 5 mL of distilled water and 1mL of 0.1% (m/v) ferric chloride ($FeCl_3$), the intensity of the blue green color was measured at 700 nm. Tests were carried out in triplicate.

2.6. Statistical analysis

The analysis of variance (ANOVA) was performed using XLSTAT release10 (Addinsoft, Paris, France), Tukey's multiple range test (HSD) was used to compare between TPC content and antioxidant activity means as affected by microwave assisted extraction (MAE) or conventional methods extraction (CME). Principal component analysis (PCA) was performed to detect structure in the relationships between variables, allowing its classification and the separation of each parts. All parameters analyzed were used as variables in PCA.

PCA is a multivariate ordination technique used to display patterns in multivariate data. It aims to graphically display the relative positions of data points in fewer dimensions while retaining as much information as possible, and explore relationships between dependent variables. In this study, PCA was applied to the phenolic compounds, flavonoid, anthocynins, tannin content and antioxidant activity (Table 2) of different myrtle parts for the two Tunisian cultivars and two factors were selected justifying 68.73% of total variance.

3. Results and discussion

3.1. Phytochemical analysis

3.1.1. Total phenolic content (TPC)

As one of the most important antioxidant plant components, phenolic antioxidants have been widely investigated in many medicinal plants (Djeridane, Yousfi et al. 2006). Their antioxidant activity is believed to be mainly due to their redox properties (Zheng and Wang 2001). The phenolic content of the different parts of the studied plant was presented in Fig. III. 15. The results showed a clear difference in the distribution of bioactive compounds in all parts, which confirm those reported in the literature (Aydın and Özcan 2007). The main significant differences were found in TPC contents among different parts. In fact, leaf extract presented the higher TPC (63.11 ± 0.35 mg GAE g^{-1} DW) compared to that of pericarp and stem extracts. Seed samples presented the lowest TPC (56.32 ± 11.81 mg GAE g^{-1} DW) than the other myrtle parts. These results were in agreement with the finding of Aidi Wannes and

Marzouk (2013), showing that Tunisian myrtle leaves extract possessed the highest TPC (33.67 mg GAE g^{-1} DW) as compared to that of the stem (11.11 mg GAEg^{-1} DW). In the other hand, Gardeli, Vassiliki et al. (2008) showed that Greece myrtle leaves possessed higher TPC (373 mg GAE g^{-1} DW) compared to that found in the present work. However, Aidi Wannes and Marzouk, who quantified TPC of fruits parts from *M. communis* var. L., revealed a content of 23.87 mg GAE g^{-1} DW for seeds and 2.76 mg GAE g^{-1} DW for pericarp fruits, which are low compared to those obtained in the present study. These differences could be due to the cultivar plant, environmental factors, collection period, geographical origin (Piras, Dettori et al. 2009), and the methods used for extraction (Prior and Cao 1999).

To evaluate the MAE effects on the extraction efficiency, the amounts of phenolic contents obtained by MAE were comparable to those obtained by CME. The TPC of leaves and pericarp are significantly higher ($p < 0.05$) than that obtained by CME. It increased from 128.73 ± 6.84 to 249.86 ± 9.2 mg GAE g^{-1} DW and from 76.38 ± 7.27 to 119.60 ± 8.4 mg GAE g^{-1} DW, respectively. However, stems and seeds have denoted no significant difference in their concentration (67.89 ± 1.73 mg GAE g^{-1} DW, 72.06 ± 0.81 mg GAE g^{-1} DW, respectively).

The high TPC obtained by MAE may be explained by the exposure of plant cells to microwave field. The dried plant material used for extraction contains traces of moisture and as microwave energy is absorbed and subsequently converted into heat, the moisture begins to evaporate. The vaporization of water generates pressure within the cell wall that eventually leads to cell rupture, thereby facilitating the leaching out of active constituents into the surrounding solvent and improving extraction yield (Cheung, Cheung et al. 2003). To verify this fact, scanning electron microscopy (SEM) was employed by several authors to study the mechanism of MAE (Mandal, Mohan et al. 2007). Dahmoune, Nayak et al. (2015) had treated myrtle leaves by scanning electron microscopy (SEM) after MAE, they showed that

microwave heating caused a higher cellular damage helping the rapid release of solutes into the solvents and enhancing the well-known main heating effect of microwaves. These results confirm that microwave radiations have a destructive effect on extraction sample matrix and the rapid extraction occurred when the active compounds elute and dissolve in solvent once the cell is ruptured.

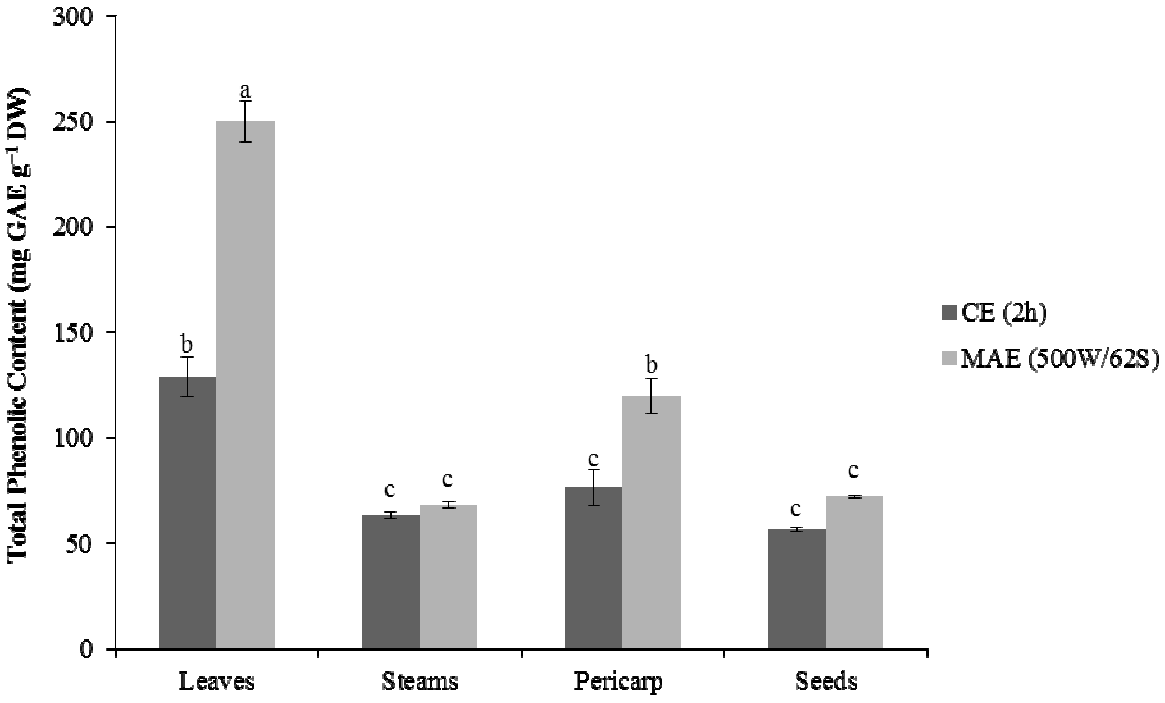

Figure. III. 15. Total phenolic content of different extracts of myrtle aerial parts extracted with microwave assisted extraction (MAE) and conventional solvent extraction (CME).

3.1.2. Total flavonoids content

As can be seen in the Fig. III. 16**,** flavonoid distribution within myrtle depends on the analyzed part. The highest values of flavonoid contents were observed in leaves and pericarp extracts, followed by seeds. However, the lowest flavonoid content was found in stem extract

(0.74 ± 0.01 RE g^{-1} DW). This finding is in contrast with the results of Aidi Wannes and Marzouk (2013) who reported also that the highest values of flavonoids are in fruit pericarp. Compared CME with MAE, the yield increase in all parts namely in leaves (from 9.14 ± 0.05 to 13.65 ± 0.09 mg RE g^{-1} DW) and pericarp (6.95 ± 0.20 to 11.50 ± 0.26 mg RE g^{-1} DW).

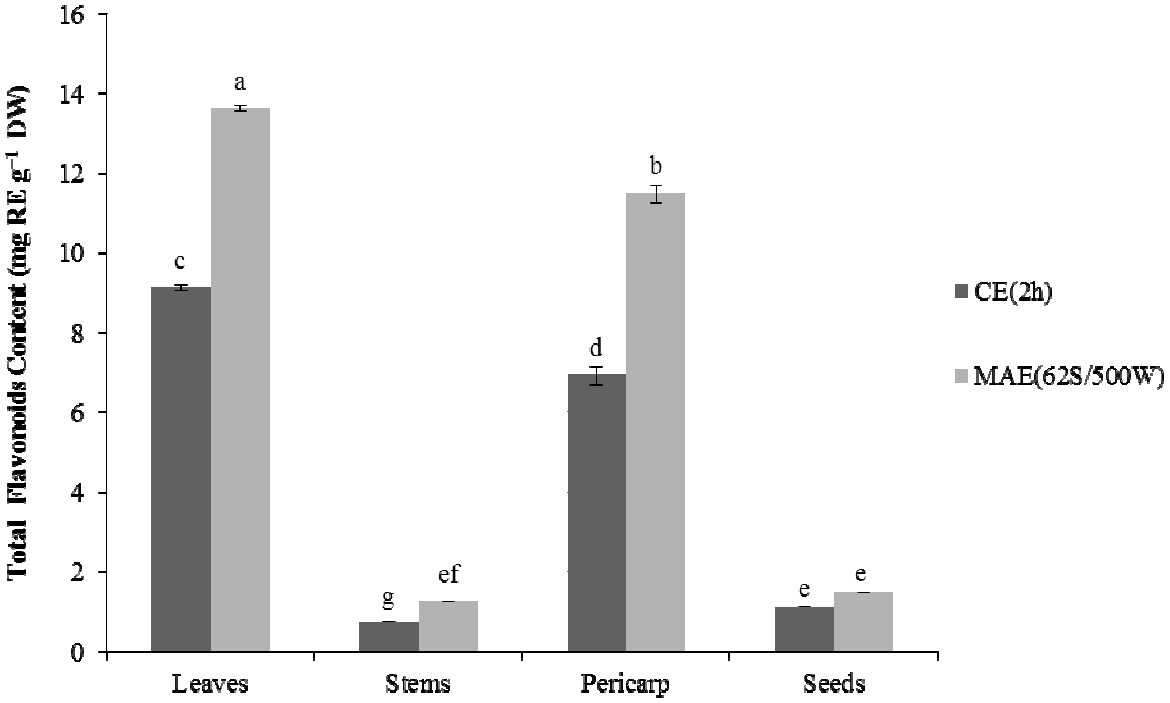

Figure. III.16. Total flavonoids content of different extracts of myrtle aerial parts extracted with microwave assisted extraction (MAE) and conventional solvent extraction (CME).

3.1.3. Total anthocyanin content

The distribution disparity of anthocyanin content was also found among different parts. Anthocyanins are the most important phytochemicals in myrtle plant and play an important role in its organoleptic properties (JIA, DONG et al. 2011). Anthocyanins were predominant in pericarp extract (135.26 ± 3.66 mg Cyd-3-glu g^{-1} DW) than in seeds (9.79 ± 1.99 mg Cyd-3-glu g^{-1} DW). The lowest contents were observed in stem and leave extracts (1.00 ± 0.13 mg Cyd-3-glu g^{-1} DW and 1.32 ± 0.16 mg Cyd-3-glu g^{-1} DW, respectively) (Fig.

III. 17)**.** These results are in agreement with those reported by (Montoro, Tuberoso et al. 2006). The high anthocyanins content in myrtle pericarp and seeds could be explained by their increase during repining that is related to the change in the color of fruits surface from yellow-white to dark-blue. The anthocyanins concentration of microwave extracts is significantly different ($p < 0.05$), they were higher than that obtained by CME, mainly in the pericarp extracts (176.50 ± 2.17 mg Cyd-3-glu g^{-1} DW) than in the seeds (0.31 ± 0.10 mg Cyd-3-glu g^{-1} DW). The farmer results agreed with those found by JIA et al., 2010; who report that microwave extraction increases the yield of anthocyanins compounds.

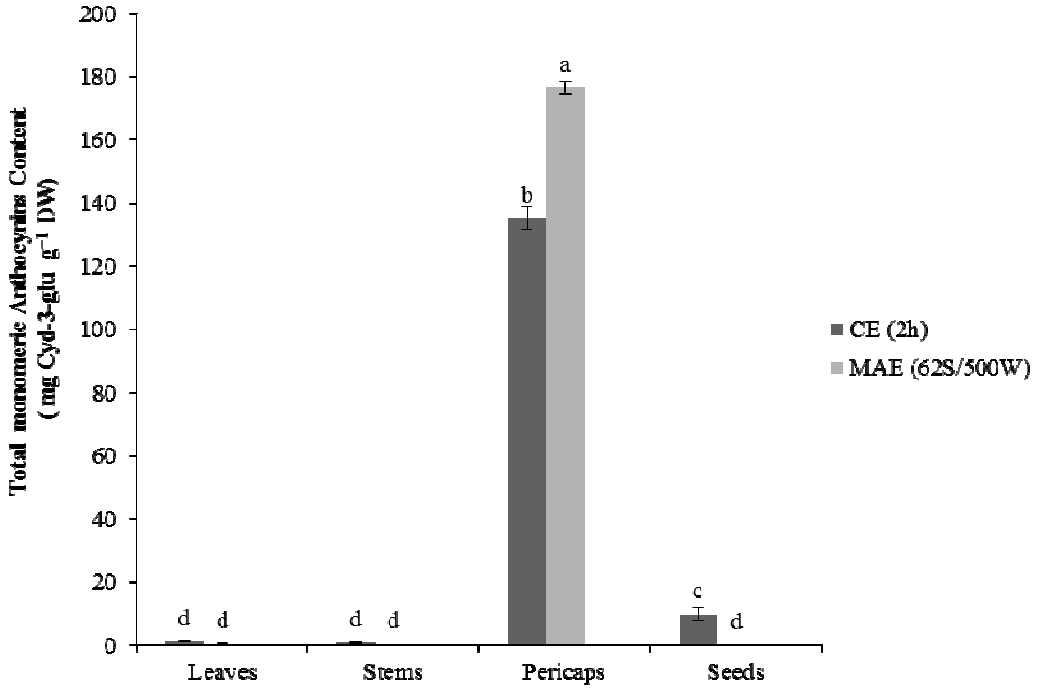

Figure. III. 17. Total monomericanthocyanin's content of different extracts of myrtle aerial parts extracted with microwave assisted extraction (MAE) and conventional solvent extraction (CME).

3.1.4. Total tannin content

The same discrepancy was also observed for condensed tannin content. In fact, tannin contents were higher in fruit pericarp, it is about 220.81 ± 1.21 mg CE g^{-1} DW, while other

parts presented a lower value, they were about 22.14 ± 0.26; 35.74 ± 0.26 and 36.01 ± 0.20 mg CE g^{-1} DW for seeds, stem, and leaves extracts, respectively (Fig. III. 18). Fruits are very astringent and are used as a condiment, a substitute for pepper, and considered as a rich source of tannins (Canhoto, Lopes et al. 1998). This result was in agreement with the work of Aidi Wannes and Marzouk (2013) who studied the methanolic extract of the fruit from Tunisian myrtle, they reported that condensed tannin content was relatively low in pericarp and seeds, while the highest concentration was found in the whole fruit extract (0.96 mg CE g^{-1} DW). Compared CME with MAE, higher tannins content was observed in the pericarp (333.77 ± 1.85mg CE g^{-1} of DW). These results were in agreement with those found by Dahmoune, Nayak et al. (2015) and Jia, Dong, Dong [32] who report that microwave extraction increases the yield of tannin compounds from myrtle leaves.

The results of MAE at 500 W/62 s shows that the amount of phenolic contents of leaves (63.15%), stems (9.04%), pericarp (43.73%), and seeds of fruits (24.53%) was higher than that obtained conventionally (1:50) for 7200 s. This results are in accordance with those reported in the literature (Pérez-Serradilla and Luque de Castro 2011). Several authors reported the advantages of MAE compared to CME, such as reduced process time, lower solvent, energy demand, and higher yield (Chen, Xie et al. 2008, Proestos and Komaitis 2008).

Conventional solvent extraction without microwave assistance is a time-consuming process that uses heat to increase the mass transfer rate of the extraction system (Proestos and Komaitis 2008). The reduction of extraction time was due to the heating mechanism of microwave. It offers a rapid transfer of energy to the extraction solvent and raw plant materials (Zhou and Liu 2006). A significant increase was obtained for the MAE as compared to that of the same sample extracted using the CME method. In addition, extraction time was

significantly reduced in microwave extraction. Thus, microwave extraction method can be recommended for leaching phenolic compounds from myrtle.

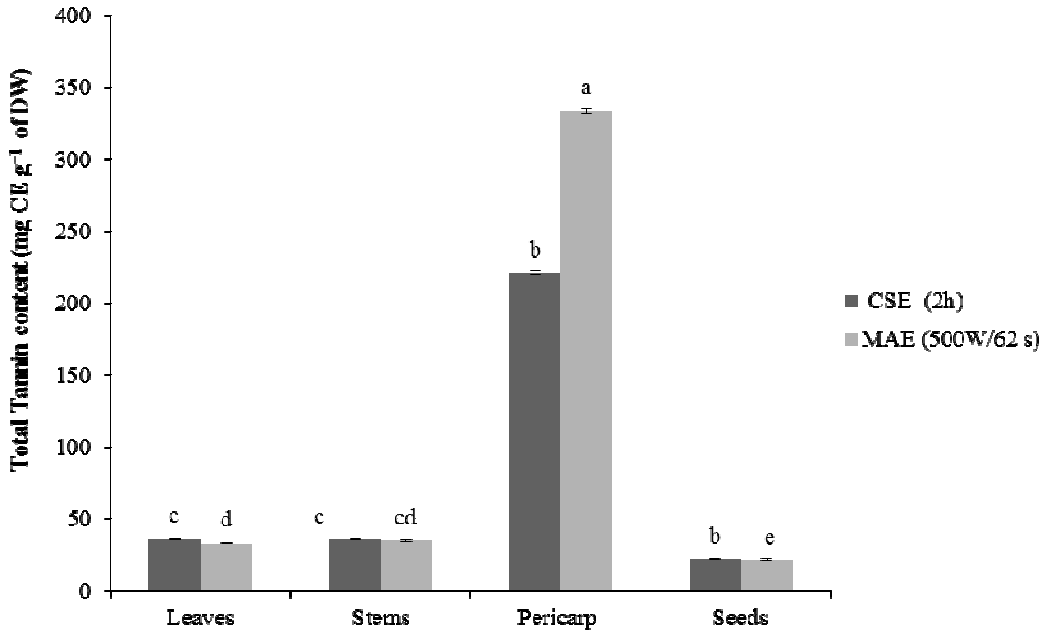

Figure III. 18. Total tannin content of different extracts of myrtle aerial parts extracted with microwave assisted extraction (MAE) and conventional solvent extraction (CME).

3.2. Antioxidant activity

The amount of such compounds in each part of the plant is usually different. Myrtle was a source of natural antioxidants because of the activity of secondary metabolites, such as phenylpropanoids and essential oils. In the present study, the antioxidant activity of ethanolic extracts obtained by CME and MAE of myrtle leaf, stem, pericarp, and seeds of fruits, was determined by measuring the free radical scavenging activity ($DPPH^{\bullet}$) and the reducing power.

The effect of antioxidant on $DPPH^{\bullet}$ scavenging was conceived to their hydrogen donating ability (Chen, Xie et al. 2008). The $DPPH^{\bullet}$ scavenging ability of the ethanolic extracts of

myrtle parts was higher than that of α-tocopherol ($p < 0.05$). The greatest antioxidant activity of the different parts of the studied plant, was obtained in leaves extract (94.78 ± 0.37%) which is similar to that obtained by Ferchichi, Le Ray et al. (2009) with a higher level in leaves of myrtle black fruit (86.54%). The inhibition effect of DDPH$^{\bullet}$ radical by antioxidant from stems, is about 88.72 ± 0.65%. The antioxidant activity of seeds was higher (88.41 ± 0.64%) than that of pericarp (88.03 ± 0.37%). Same results were reported by Aidi Wannes W and Marzouk B concerning the Tunisian myrtle fruits. Thus, it has been reported that free radical-scavenging activity is greatly influenced by the phenolic composition of the extract. The HPLC analysis of the phenolic contents of different fruit parts showed that the seed was rich in hydrolysable tannins, which were absolutely absent in the pericarp (Aidi Wannes and Marzouk 2013). Additionally, Aidi Wannes and Marzouk (2013) reported also that leaves were rich in hydrolyzable tannin. According to Yoshimura, Amakura et al. (2008), these hydrolyzable tannins exhibited a strong antiradical activity compared to other compounds such as gallic and quinic acids.

Concerning the reducing power activity, the presence of reductants (antioxidants) in the samples would result in the reduction of the Fe^{3+}ferricyanide complex to its ferrous form (Fe^{2+}) by donating an electron. Hence, the Fe^{2+} can then be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Chou, Chiang et al. 2006). Higher absorbance value indicates higher reducing power (Pan, He et al. 2010). The results in Table III.3 showed the Fe^{3+} reducing power ability of ethanolic extracts from different parts. Leaves extracts had higher reducing power than pericarp ones. However, stems and seeds exhibited moderate reducing capacity.

The results shown in table. III. 3, suggests that the content of phenolic compounds can play a major role in the antioxidant activity of all extracts. Phenolic contents are the antioxidants that contribute to the high antioxidant capacity observed in different parts of plants (Simopoulos

2004). Effectively, leaves extracts showed a higher antioxidant activity than other extracts, which correlated with its highest content of phenolic compounds. The different values of antioxidant activity obtained with each extract parts can be ascribed to their different chemical compositions (Ordoñez, Gomez et al. 2003). MAE extraction gives the higher values of DPPH• scavenging capacity in leaves followed by those of seeds, pericarp than stem. The same tendency was observed using reducing power test.

Despite the high levels of phenolic contents obtained by microwave extraction, no correlation was observed between antioxidant activity and total phenolic content. Similar results were reported in the literature (Sengul, Yildiz et al. 2009). Furthermore, the study of (Chiang, Kadouh et al. (2013)) reported that high TPC was not always correlated with high antioxidant activity. The type of extraction can explain this discrepancy, because the microwave radiations have an effect on the structure of phenolic compounds (Zhang, Yang et al. 2008, Yang and Zhai 2010). The chemical nature affects also the content of polyphenols (Prior and Cao 1999). In addition, Hayat K et al. (Hayat, Zhang et al. 2010) have reported that microwave irradiations could induce free radicals formation within the liquid medium, thus causing oxidation and degradation of the active compounds and the high phenolic content causes the association of the latter thereby preventing the DPPH• radical to access these compounds to induce antiradical activity. However, there is a correlation between DPPH• radical scavenging activity assay and reducing power test (r = 0.11).

Table III.3: Antioxidant activity of different extracts of myrtle aerial parts extracted with microwave assisted extraction (MAE) and conventional solvent extraction (CME).

Extraction Methods	Plant Parts	DPPH (%)	Reducing Power (Abs)
MAE	Leaf	88.35 ± 0.47^{a}	0.661 ± 0.002^{b}
	Stem	87.09 ± 0.28^{b}	0.301 ± 0.003^{f}
	Pericarp	87.16 ± 0.28^{b}	0.439 ± 0.006^{e}
	Seeds	88.09 ± 0.28^{ab}	0.308 ± 0.002^{f}

CME	Leaf	94.78 ± 0.37^{a}	0.865 ± 0.001^{a}
	Stem	88.72 ± 0.65^{ab}	0.406 ± 0.0001^{e}
	Pericarp	88.03 ± 0.37^{ab}	0.426 ± 0.001^{d}
	Seeds	88.41 ± 0.64^{ab}	0.442 ± 0.0003^{e}

3.3. PCA analysis

PCA is a multivariate ordination technique used to display patterns in multivariate data. It aims to graphically display the relative positions of data points in fewer dimensions while retaining as much information as possible, and explore relationships between dependent variables. In this study, PCA was applied to the phenolic compound; flavonoid, anthocyanins, tannin contents and antioxydant activity of different myrtle parts and two factors were selected justifying 75.19 % of total variance. PC1 explained 46.32 % of the total variance in the data set while PC2 explained 28.87%.

The sample score plot for PC1 vs. PC2 is shown in Fig. III. 19. Samples were located in four different plots, indicating their composition, and repartition part. The position of each variable in this loading plot describes its relationship to the other variables (Fig. III. 19. 1, 2). Fig. III. 19.b shows four distinctive groups. The first and second groups are composed by pericarp and leaves myrtle respectively, which are ported positively by PC1. The third group show the positive correlation between CME leaves and DPPH, and reducing power test. The last group seed pericarp, which are ported negatively by PC1. Using the plots in Fig. III.19, it is possible to restart all phenolic compounds in myrtle part and to select the adequate method of extraction. Phenolic compounds of pericarp are represented mainly by tannins 62.8%, which are better extracted by MAE. In the other hand, microwave extraction of the TPC and flavonoids content in leaves was selected 72.2% and 80.4%, respectively. Concerning antioxidant activity, the DPPH and reducing power test were correlating positively with phenolic compound leaves obtained by CME. The anthocyanin compound was represented

mainly in seed of fruit with a higher yield obtained by CME (56.2%) in seeds pericarp and stem. The results of this study revealed the importance of comparing and exploring the variance of phenolic compound from different myrtle parts and extraction method.

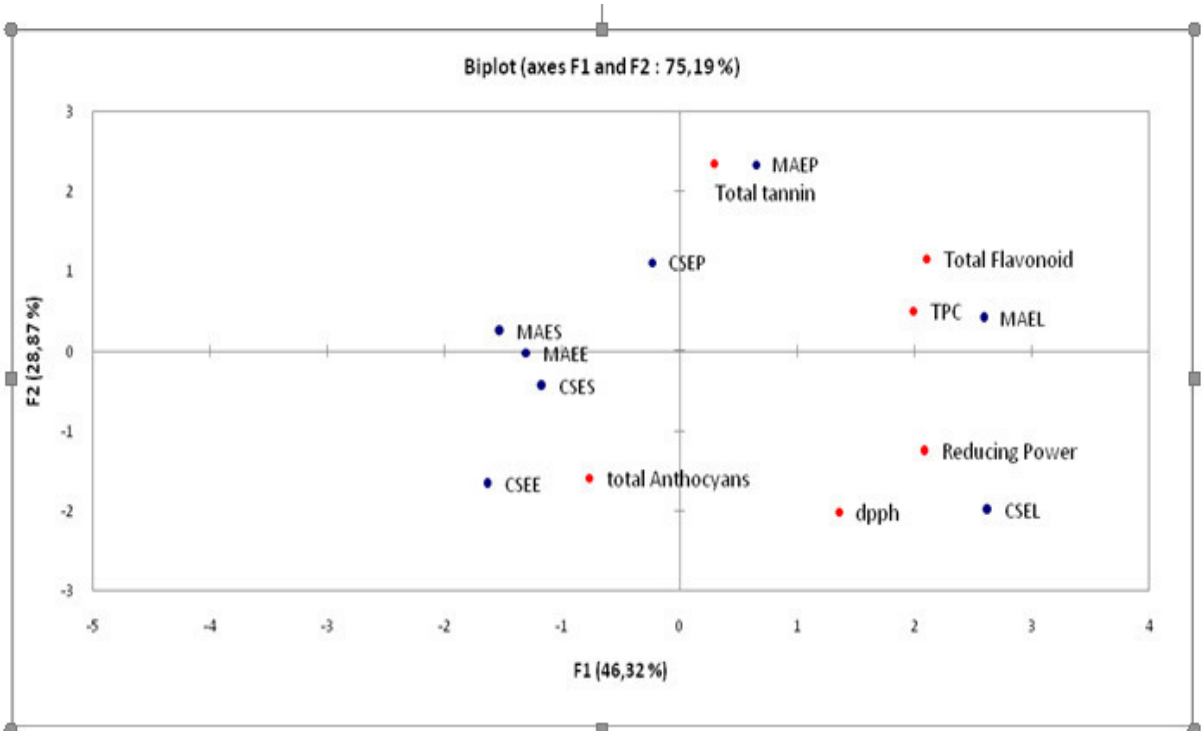

Figure. III.19. Principal Component analysis of myrtle part samples based on the main important factors (MAE, CME, Leaves, Steams, pericarp and Seeds).

4. Conclusion

Myrtle aerial parts are potential source of active natural substances such as phenolic compounds. MAE was found to be highly effective enabling a considerable reduction in extraction time (62 s against 7200 s), and the efficiency of extraction of phenolic contents from all myrtle parts was improved in comparison with the CME method with an increase of TPC but with antioxidant activity similar to those of CME. This showed great potential for industrial application in the near future.

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Chapter IV

Optimization of ultrasound-assisted extraction of phenolic compounds from Myrtus communis L. pericarp

Optimization of ultrasound-assisted extraction of phenolic compounds from Myrtus communis L. pericarp

Abstract

Pericap of Myrtus. *Communis w*as subjected to phenolic extractions. Response surface methodology (RSM) was used to optimize extraction conditions of total phenolic compounds (TPC), and antioxidant activity with ultrasound-assisted extraction (UAE). Results were compared with those obtained by microwave-assisted extraction (MAE) and concentional solvent extraction (CME) methods. The individual phenolics of the optimized extract were then identified by means of ultrahigh performance liquid chromatography coupled with diode array detection and electrospray ionization mass spectrometry (UHPLC-DAD-ESI-MS^n). The yield of TPC extraction was affected more significantly by ethanol concentration, irradiation time, liquid solvent to solid ratio ($P < 0.0001$) and amplitude ($P = 0.0421$). UAE is more efficient than MAE and CME. The predicted extraction yield of TPC was 235.52 ± 9.9 mg GAE/g DW that was consistent with the experimental yield of 241.66 ± 12.77 mg GAE/g DW confirming the validity of the predicted models. Higher antioxidant capacity was observed in UAE myrtle pericarp extract, which is mainly due to its phenolic contents. The presence of twenty three phenolics was established, which can be divided into five different groups (phenolic acids, gallotannins, flavonols, anthocyanins and non-prenylated acylphloroglucinols), with the predominance of flavonol derivatives (myricetin-3-*O*-galactoside isomer and myricetin-3-*O*-rhamnoside). UAE is an effective method for the extraction of phenolics from myrtle pericarp.

Keywords: *Myrtus communis*, phenolic compounds, antioxidant activity, UHPLC-DAD-ESI-MS^n analysis, response surface methodology.

Introduction

Myrtus genus, belonging to the Myrtaceae family, comprises about 50 species that are native of the Mediterranean basin. Among those, *Myrtus communis* is a sub-shrub (high: 1–3 m) with white flowers (blossoming time: June to July) (Barboni, Venturini et al. 2010) and dark blue ripe berries, which have a long history of application in the perfumery, cosmetic, food and pharmaceutical industries (Nuvoli and Spanu 1996). Volatile oils, tannins, anthocyanins, fatty acids, sugars, and organic acids such as citric and malic acids are important components of these fruits (Messaoud and Boussaid 2011). In general, the myrtle berries are accepted as being rich in phenolic compounds, which are thought to be responsible for their beneficial effects. Particularly in Sardinia (Italy), they have been associated with the prevention of degenerative diseases, such as cancer and cardiovascular diseases (Liu 2003).

Due to countless beneficial characteristics of phenolic compounds in human health, researches have been intensified aiming to find fruits, vegetables, plants, agricultural and agroindustrial residues as sources of these bioactive components. Obtaining of these molecules often requires many long and costly steps, such as extraction, isolation and identification (Dahmoune et al., 2013) and several times result in their thermal degradation for most constituents (Luque de Castro and García-Ayuso 1998). Development of new extraction methods such as ultrasound-assisted extraction (UAE) is one of the major challenges in technological innovation in the direction of "Green chemistry". UAE is particularly attractive for its simplicity, low cost of equipment, efficiency in extracting analytes from different matrices, low energy requirement, reduced solvent and time consumption. The enhancement of the extraction process by ultrasounds is attributed to the disruption of the cell walls, reduction

of the particle size and the increased mass transfer of the cell content to the solvent, caused by the collapse of the bubbles produced by acoustic cavitation (Chemat, Rombaut et al. 2017).

The processing parameters optimization and interpretation of experiments compared to others has been previously done through RSM (Yan, Yu et al. 2011). Indeed, response surface plots of any model is an adequate manner to study the interaction between the independent and dependent variables (Wei, Liao et al. 2009). The model allows to determine the optimum value of the independent variables (Xi), as well as those of the dependent ones (Y).

Previous studies focusing phenolic coumpounds and/or the antioxidant abilities of myrtle pericarp have been performed with extracts obtained by conventional methods (Montoro, Tuberoso et al. 2006, Amensour, Sendra et al. 2009); (Tuberoso, Rosa et al. 2010), while to our knowledge, there is no available information on the optimization of ultrasonic procedure for the extraction of phenolic compounds from this matrix. Therefore, the present study is aimed at the optimization of UAE process parameters using a RSM, including ethanol concentration, extraction time, irradiation amplitude and liquid-to-solid ratio, in order to maximize the content of extracted phenolics. The yield of phenolic compounds and antioxidant activity in the *M. communis* extract obtained under the optimum setting parameters (UAE-OPT extract) were compared with those obtained by MAE and CME methods. Then, the individual phenolic compounds present in the optimized extract were identified by UHPLC-DAD-ESI-MS^n.

2. Materials and methods

2.1. Plant material

The fruits of *M. communis* were harvested in November 2015 from spontaneous plants in ADAKAR, Bejaia, located in the North East of Algeria. The collected samples were identified by the Vegetable Ecological Laboratory of the Algiers University, Algeria. The fruit berries were washed with tap water followed by distilled water, dried in a static oven at 40 °C for one

week. Pericarps were separated manually from seeds and further grounded in an electrical grinder (IKA model A11Basic), which was then sieved in order to obtain a fine powder (< 250 µm). The resultant powder was stored in airtight bags under darkness until use.

2.2. Reagents

Sodium carbonate (Na_2CO_3), Folin–Ciocalteau's phenol reagent and disodium hydrogen phosphate (Na_2HPO_4) were obtained from Prolabo (France), and 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^{\bullet}$) from Sigma Aldrich (Germany). Gallic acid, hexahydrated ferric chloride ($FeCl_3.6H_2O$), potassium ferricyanide [$K_3Fe(CN)_6$], trichloroacetic acid and sodium dihydrogen phosphate (NaH_2PO4) were purchased from Biochem-chemopharma (Loire, France). All solvents used were of analytical grade and purchased from Prolabo (Loire, France).

2.3. Obtaining of phenolic extracts

2.3.1. UAE extraction

UAE was performed in an ultrasonic apparatus (SONICS Vibra cell, VCX 75115 PB, SERIAL No. 2012010971 MODEL CV 334) with a working frequency fixed at 20 kHz. For extraction, one gram of the pericarp powder was placed in a 250 mL amber glass bottle containing the extraction solvent. The suspension was exposed to acoustic waves under distinct setting parameters. The temperature was maintained constant by circulating external cold water and checking the temperature using a T-type thermocouple. After the extraction, the solution was filtered through a sintered glass filter of porosity 2.

To determine the effect of ethanol concentration, irradiation time, ultrasound amplitude and solvent-to-solid ratio on the extraction yield of phenolic coumpounds from myrtle pericarp, RSM was applied with a Box–Behnken Design (BBD). This design resulted in the testing of four factors in a single block of 30 sets of test conditions (Table 1). The constant values for

irradiation time, liquid-to-solid ratio and ethanol concentration in the UAE trials were 10 min, 50 mL/g and 50% (v/v), respectively. To investigate the influence of ethanol concentration and sonication time, the amplitude of ultrasound was set at 50%. On the basis of the single-factor experimental results, major influence factors were selected. Then, an RSM based on a BBD for UAE was made (Vázquez, Fernández-Agulló et al. 2012). Regression analysis of the data to fit a second-order polynomial equation (quadratic model) was carried out according to the following general equation (Eq. IV.11) which was, then, used to predict the optimum conditions of the extraction process.

$$\boldsymbol{Y = B0 + \sum_{i=0}^{k} BiXi + \sum_{i=1}^{k} Biix^2 + \sum_{i>1}^{k} BijXiXj + E} \qquad \text{(Eq. IV. 11)}$$

Where Y represents the response function (in this case the TPC yield); B_0 is a constant coefficient; B_i, Bii and Bij are the coefficients of the linear, quadratic and interactive terms, respectively, and Xi and Xj represent the coded independent variables. According to the analysis of variance, the regression coefficients of individual linear, quadratic and interaction terms were determined. In order to visualize the relationship between the response and experimental levels of each factor and to deduce the optimum conditions, the regression coefficients were used to generate 3-D surface plots from the fitted polynomial equation. The factor levels were coded as −1 (low), 0 (central point or middle) and 1 (high), respectively. The variables were coded according to the following equation (Eq. IV.12):

$$\boldsymbol{X_i = (X_i - X_0) / \Delta X \ldots} \qquad \text{(Eq. IV.12)}$$

Where x_i is the (dimensionless) coded value of the variable X_i, X_0 is the value of X at the center point and ΔX is the step change.

Analysis of variance was performed for the response variable using the full model where P-values (partitioned into linear and interaction factors) indicated whether the terms were

significant or not. To verify the adequacy of the models, additional extraction trials were carried out at the optimal conditions predicted with the RSM and the obtained experimental data were compared to the values predicted by the regression model.

2.3.2. Microwave-assisted extraction

Phenolic compounds were extracted using a domestic microwave oven (Samsung MW813ST, Kuala Lumpur, Malaysia) modified with the addition of a condenser, generated during extraction procedure. It operates at a frequency of 2450 MHz and a maximum output power of 1000 W with a 100 W increment. The size of the heating cavity was 37.5 cm (L) × 22.5 cm (W) × 38.6 cm (D).

A volume of 32 mL of 42% ethanol concentration was added to 1 g of pericarp *Myrtus* powder in a flat-bottomed flask. The mixture was irradiated at 500 W for 62 s (Dahmoune, Nayak et al. 2015) then filtered throught a sintered glass filter of porosity 2. The resultant extract was stored at 4 °C until further analysis.

2.3.3. Conventional solvent extraction

For CME, one gram of myrtle powder was placed in a conical flask, and 50 mL of 50% (v/v) ethanol were added. After stirring for 2 h, the mixture was filtered throught a sintered glass filter of porosity 2 and the extract was stored at 4 °C until further use (Dahmoune, Nayak et al. 2015).

2.4. Analytical determinations

2.4. 1. Total phenolic content (TPC)

The total phenolic content in the UAE, MAE and CME extracts was assessed according to the method of George, Brat et al. (2005). Briefly, 500 µL of extract were added to 2.5 mL of 10-fold diluted (v/v) Folin–Ciocalteau reagent. The solution was mixed and incubated at room

temperature for 2 min. After incubation, 2 mL of 7.5% (m/v) sodium carbonate were added. After incubation at 50 °C for 15 min, the absorbance of the sample was measured at 760 nm against a blank using a UV–VIS Spectrophotometer (SpectroScan 50, Nkesia, Cyprus). The TPC were expressed as mg of gallic acid equivalent (GAE) per gram of myrtle pericarp powder on dry weight (DW) basis (mg GAE g^{-1} DW).

2.4. 2. Total flavonoid content

The total flavonoid contents were estimated by the aluminum trichloride method according to Quettier-Deleu, Gressier et al. (2000), based on the formation of flavonoid-aluminum(III) complexes. Briefly, one mL of extracts was mixed with 1 mL of 2% (m/v) aluminium trichloride. After 15 min of incubation in the dark, the absorbance of the mixture was measured at 430 nm and the results were expressed as mg of rutin equivalent per g of myrtle pericarp powder on dry weight basis.

2.4. 3. Total monomeric anthocyanins contents

Total monomeric anthocyanin content was determined by the pH-differential method of Lee, Durst et al. (2005), based on the structural change of the anthocyanins chromophore between pH 1.0 and 4.5. Absorbance was measured at 520 nm and 700 nm in buffers at pH 1.0 and 4.5. Results were expressed as mg cyanidin-3-glucoside equivalents per g of myrtle pericarp powder on dry weight basis.

2.4. 4.Condensed tannin content

The condensed tannin content was determined by the HCl–vanillin method as described by Aidi Wannes, Mhamdi et al. (2010). 1 mL of the extract was mixed with 5 mL of reagent (HCl + vanillin). The mixture was kept in the dark room for 20 min. The absorbance was determined

at 500 nm versus a blank. Total tannins were expressed as mg catechin equivalents per g of myrtle pericarp powder on dry weight basis.

2.4.5. Antioxidant activity

2.4.5.1. Radical scavenging activity assay

The stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•) was used for determination of free radical scavenging activity of the extracts (Choi et al., 2002). This was measured following the method of Dudonné, Vitrac et al. (2009). Briefly, a DPPH• solution in absolute methanol (60 µM) was prepared, and 3 mL of this solution were mixed with 1 mL of the extracts. The samples were incubated for 20 min at 37 °C in the dark, then, the absorbance at 515 nm was measured. The inhibition rate of the extracts was calculated according the equation (Eq. IV. 13).

$$\%\ Scavenging = \frac{\left(A_{control} - A_{extract}\right)}{A_{control}} \times 100 \qquad \text{(Eq. IV.13)}$$

Where $A_{control}$ is the absorbance of DPPH• and distilled water A_{sample} is the absorbance of DPPH• and sample extract.

2.4.5.2. Reducing power method

1 mL of desired dilution was mixed with 2.5 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% (m/v) potassium ferricyanide [$K_3Fe(CN)_6$)]. The mixture was incubated in a water bath at 50 °C for 20 min. Then, 2.5 mL of 10% (m/v) trichloroacetic acid were added. Finally, 1 mL of the obtained solution was added to 5 mL of distilled water and 1 mL of 0.1% (m/v) ferric chloride ($FeCl_3$).The presence of reductants in the solution causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by the measurement of the absorbance at 700 nm (Zou, Lu et al. 2004).

2.5. Identification of phenolic compounds by UHPLC-DAD-ESI-MS^n

The phenolic compounds of the UAE-OPT extract were characterized by UHPLC-DAD-ESI-MS^n analysis on an Ultimate 3000 (Dionex Co., USA) apparatus equipped with an ultimate 3000 Diode Array Detector (Dionex Co., USA) and coupled to a mass spectrometer. Analysis was run on a Hypersil Gold (Thermo Scientific, USA) C18 column (100 mm length; 2.1 mm i.d.; 1.9 μm particle diameter, end-capped) and its temperature was maintained at 30 ºC. The mobile phase was composed of (A) 0.1% of formic acid (v/v) and acetonitrile (B). The solvent gradient started with 5% of solvent (B), reaching 40% at 14 min and 100% at 16 min, followed by the return to the initial conditions. The flow rate was 0.1 mL min^{-1} and UV–Vis spectral data for all peaks were accumulated in the range 200–700 nm while the chromatographic profiles were recorded at 280, 340 and 530 nm.

The mass spectrometer used was a Thermo LTQ XL (Thermo Scientific, USA) ion trap MS equipped with an ESI source. Control and data acquisition were carried out with the ThermoXcaliburQual Browser data system (Thermo Scientific, USA). Nitrogen above 99% purity was used and the gas pressure was 520 kPa (75 psi). The instrument was operated in negative-ion and positive modes with ESI needle voltage set at 5.00 kV and an ESI capillary temperature of 275 ºC. The full scan covered the mass range from *m/z* 100 to 2000. CID –MS /MS and MS^n experiments were simultaneously acquired for precursor ions using helium as the collision gas with collision energy of 25–35 arbitrary units.

2.6. Statistical analysis

Each extraction trial and all the analyses were carried out in three independent analysis performed in triplicate. Influence of each factor on the TPC yield in the single-factor experiment for UAE was statistically assessed by ANOVA and Tukey's post hoc test with 95% confidence level. Data obtained from the BBD and CCRD trials for the UAE, were statistically analyzed using ANOVA for the response variable in order to test the model significance and suitability. $P < 0.05$ and $P < 0.01$ were taken as significant and highly significant level, respectively. The JMP (Version 7.0, SAS) and Design-Expert (Trial version 8.0.7.1) software were used to construct the BBD and CCRD and to analyze all the results. PCA was applied to detect the relationships between phenolic compounds, flavonoid, anthocyanins, tannin contents, antioxidant activity and their extraction method .i.e. UAE, MAE and CME.

3. Results and discussion

3.1. Optimization of UAE conditions

Extraction of natural products using ultrasound has been proposed to improve the efficiency and/or speed of this step. The pulses emitted by the ultrasound probe can often result in higher yield of extraction since they promote the breaking of cellulose cell walls.

3.1.1. Modeling and fitting the model using response surface methodology

The experimental design and subsequent response allied to TPC are summarized in Table IV.4. The least square technique was used to calculate the regression coefficients of the intercept, linear, quadratic, and interaction terms (Zhang, Hu et al. 2013) (Table IV.5). Notably, the linear parameters, namely ethanol concentration, irradiation time and liquid solid ratio ($P < 0.0001$), followed by amplitude ($P = 0.0421$) have significantly affected the extraction content of phenolics. The quadratic terms X_2^2, X_4^2 were highly significant at the level $P < 0.001$ (Table IV.5), while the X_1^2, X_3^2 terms were insignificant ($P > 0.05$). In the UAE yield, the interaction

of ethanol concentration with amplitude of ultrasound (X_1-X_3) and with liquid to solid ratio (X_1-X_4), and that of irradiation time amplitude of ultrasound (X_2-X_3) were highly significant ($P < 0.0001$), followed by irradiation time with liquid to solid ratio ($P = 0.0054$) , amplitude of ultrasound with liquid to solid ratio ($P < 0.0094$) and ethanol concentration with irradiation time ($P = 0.0367$). Those significant terms played a dominant role in myrtle pericarp extraction by ultrasound. Based on the significant terms, the regression equation for the UAE efficiency was obtained as follows:

$$Y=205.032+10.998X_1+14.043X_2-3.994X_3+27.390X_4+5.049X_1X_2-11.460X_1X_3-10.588X_1X_4+15.196X_2X_3-7.198X_2X_4-6.580X_3X_4-1.526X_1^2-11.561X_2^2-0.888X_3^2-17.876X_4^2 \quad \text{(Eq IV.14).}$$

The significance of coefficient was tested using the *p*-value in Table IV.5. The corresponding variables become more effective as the *p*-value becomes smaller. In addition, note that the *p*-value can be employed to check the interaction strength between independent factors. From the analysis, *p*-value < 0.0001 indicated that the response surface quadratic model was significant, which mean that the model represented the data satisfactorily. The R^2adj and R^2 were 0.9553 and 0.9776 respectively, which implied that the sample variations of 97.76% for the UAE efficiency of myrtle pericarp phenols were attributed to the independent variables, and only 2.24% of the total variations could not be explained by the model, indicating a good degree of correlation between experimental and predict values of the TPC yield (Karazhiyan, Razavi et al. 2011). In addition, the low value of coefficient of variance (3.71%) clearly indicated that the model was reproducible and reliable (Karazhiyan, Razavi et al. 2011). All results indicated that the model could work well for the prediction of TPC extract from myrtle pericarps.

Table. IV.4 : Central composite design with the observed responses and predicted values for yield of total phenolic compounds of *M. communis* pericarp using the UAE method.

GAE: gallic acid equivalent.

TPC results are expressed as means ± standard deviation.

Run	X_1- Ethanol (%, v/v)	X_2- Irradiation time (min)	X_3- Amplitude (%)	X4- Solvent-to solid ratio (mL/g)	Recovery of TPC (mg GAE/g Dw)
1	50	2.5	50	25	134.93 ± 11.37
2	70	10	70	30	195.24 ± 0.99
3	30	5	30	20	105.29± 11.72
4	70	5	30	30	221.73± 3.64
5	50	7.5	50	25	200.50 ± 12.82
6	50	7.5	50	15	78.9 0 ± 10.45
7	50	7.5	50	25	200.90 ± 28.02
8	50	7.5	50	25	200.10 ± 23.11
9	50	7.5	90	25	200.02±13.23
10	70	10	30	30	214.07± 14.66
11	50	7.5	10	25	210.72± 2.70
12	50	7.5	50	25	210.21± 15.39
13	10	7.5	50	25	170.43 ±9.38
14	30	10	70	20	159.56±10.02
15	30	10	70	30	228.39± 12.96
16	50	7.5	50	25	203.34± 16.40
17	70	10	70	20	200.42±14.47
18	70	10	30	20	185.51± 13.34
19	50	7.5	50	35	195.94 ± 12.80
20	50	12.5	50	25	190.43±15.47
21	70	5	70	30	142.96±9.60
22	30	10	30	20	118.92± 12.08
23	70	5	70	20	115.45± 16.12
24	30	5	30	30	219.12± 18.72
25	30	10	30	30	180.77±9.38
26	70	5	30	20	161.68±9.42
27	30	5	70	30	179.22± 9.70
28	90	7.5	50	25	235.21±17.36
29	50	7.5	50	25	210.21± 16.60
30	30	5	70	20	111.38± 7.63

Table. IV.5: Estimated regression coefficients for the quadratic polynomial model for *M. communis* pericarp and the analysis of variance (ANOVA) for the experimental results.

Parametrs	Estimated coefficients	Standard error	DF[a]	Sun of squares	F ratio[b]	Prob> F
Model			14	46971.996	43.8356	<0.0001
Intercept	205.032	3.912523			52.40	<.0001
Linear						
X1-Ethanol	10.99875	1.736676	1	2903.340	37.9327	<0.0001
X2-Time	14.04375	1.736676	1	4733.446	61.8434	<0.0001
X3-Amplitude	-3.994583	1.736676	1	382.961	5.0035	0.0421
X4-Ratio	27.390417	1.736676	1	18005.638	235.2474	<0.0001
Quadratic						
$X1^2$	-1.526438	1.717541	1	60.454	0.7898	0.3892
$X2^2$	-11.56144	1.717541	1	3468.113	45.3116	<0.0001
$X3^2$	-0.888938	1.717541	1	20.503	0.2679	0.6128
$X4^2$	-17.87644	1.717541	1	8291.469	108.3279	<0.0001
Interaction						
X1-X2	5.049375	2.187167	1	407.939	5.3298	0.0367
X1-X3	-11.46062	2.187167	1	2101.535	27.4570	<0.0001
X1-X4	-12.58812	2.187167	1	2535.37	33.1252	<0.0001
X2-X3	15.196875	2.187167	1	3695.120	48.2775	<0.0001
X2-X4	-7.198125	2.187167	1	829.008	10.8312	0.0054
X3-X4	-6.580625	2.187167	1	692.874	9.0525	0.0094
Lack of fit			10	94.0987	4.550	0.0911
Pure error			4			
R^2					0.9776	
Adjusted R2					0.9553	
C.V. %	3.71%.					
RMSE	8.7186					
CorTotal [c]			28	48043.545		

3.1.2. Response surface analysis (RSA)

To provide a better understanding of the interaction between factors, the 3D response surface plot was constructed (Fig. IV.20) using the Eq. 5. The graphs were generated by plotting the response using the z-axis against two independent variables while keeping the other independent variable at the fixed level. Fig. IV.20. A, 20.B, 20.C show the interactions between the ethanol concentration and each of the three other factors respectively (irradiation time, amplitude and liquid to-solid ratio) on the recovery of total phenolic content. As shown, an increase of ethanol concentration from 20% to 80% (v/v), or extraction time from 5 to 10 min resulted in a rapid enhancement of TPC content with a maximum of 235.21 mg GAE/g being recovered with an irradiation time of about 8 min and ethanol concentration of 70% (v/v). Note that the solvent used in UAE was ethanol (EtOH) due to its non-toxicity and its ability to dissolve the bioactive compounds of interest. The high phenolic content obtained indicates that the mixture ethanol / water at 70% (v/v) has allowed the solubilization of phenolics of *M. communis* pericarp, thus confirming the results of the single factor experiments (Toma, Vinatoru et al. 2001) that explained the efficiency of the ultrasonic method by the fact that sonication improved the hydratation and fragmentation process and facilate hence the mass transfer of solutes to the extraction solvent.

For the extraction yield of TPC performed at fixed extraction time and liquid-to-solid ratio, with varying ethanol concentration and amplitude (Fig. IV.20.B), it was possible to conclude that maximum recovery of TPC could be achieved for 70% (v:v) of ethanol and an amplitude of the ultrasond about 35% which was about 210 ,05 mg GAE/g. This fact can be explained by the larger amplitude ultrasonic wave that promotes the liquid medium to produce more cavitation bubbles, thus resulting in a stronger pressure, which is capable of destroying the cell wall and accelerating mass transfer. Besides, the ultrasound amplitude is believed to be the driving force for the complete dispersion of liquid into solid, as previously reported by (Şahin,

Aybastıer et al. 2013)(Şahin, Aybastıer et al. 2013). These authors also showed that ultrasonication could increase the recovery of bioactive compound from *Artemisia absinthium*.

Fig.IV. 20.C shows an enhancement of TPC content that reached a peak value about 230.15 mg GAE/g for 70% (v:v) ethanol and 30 mL/g of liquid-to-solid ratio. A higher ratio corresponds to a greater concentration difference between the exterior solvent and the interior tissues of *Myrtus* pericarp. It prominently prompted the TPC compound to be rapidly dissolved, which results in an increase in extraction yield. The response surface plot for the significatif interactive effect of irradiation time and amplitude of ultrasound on the response value at a fixed ethanol concentration and liquid-to–solid ratio is shown in Fig. IV. 20. D. A higher TPC content was obtained with the irradiation time at 10 min and amplitude at 30%. The herein obtained results are similar to those of (Dahmoune, Moussi et al. 2014, Dahmoune, Spigno et al. 2014), who reported the recovery of TPC by ultrasonic energy to be a function of the interaction effect of extraction intensity and time.

Fig. IV. 20.E shows an interaction between extraction time and the liquid-to-solid ratio ($p < 0.05$). The best content (148 mg GAE /g was found with the solid-liquid ratio of 30 mL/ g and the radiation time of 10 min. The increase of the ethanol proportion required high sonication intensity to generate the cavitation bubbles. However, a higher increase in the liquid-to-solid ratio diminished the supply of ultrasonic energy density and negatively affected the extraction yield.

The yield of TPC constantly improved with the increase of both amplitude of ultrasound and liquid-to-solid ratio, reaching a maximum when X_3 and X_4 became 32% and 20% (v/v), respectively (Fig. IV.20.F). Beyond this level, the yield of TPC reduced with the increase of X_1 and X_4. Hence, the interactive effect of X_3 and X_4 was remarkable. Overall, these results

indicated that the TPC extraction yield was more significantly affected ($P < 0.0001$) by linear parameters, namely ethanol concentration, irradiation time and liquid-to-solid ratio.

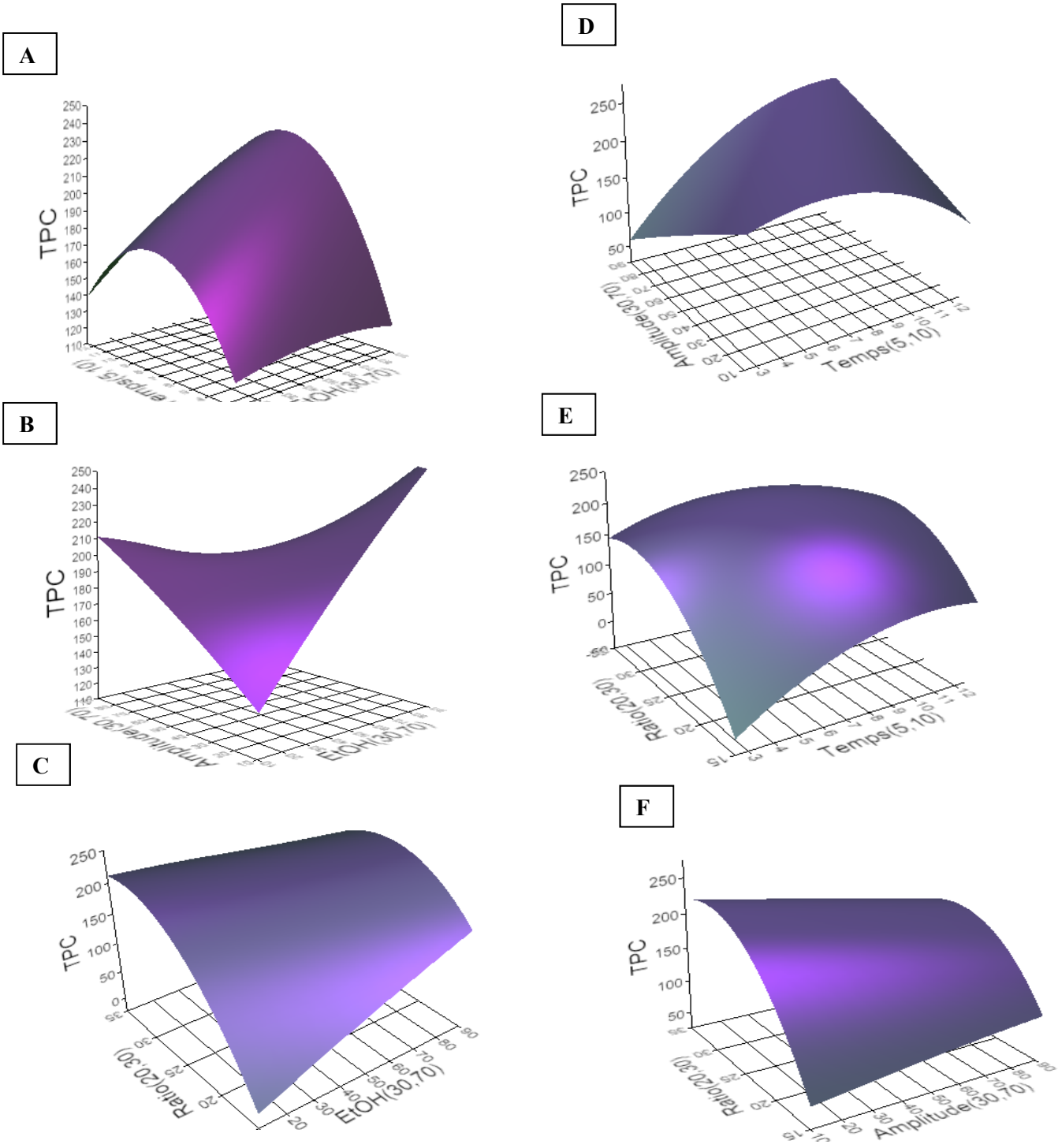

Figure 1V.20: Response surface analysis for the total phenolic yield from *Myrtus communis* pericarp with ultrasonic assisted extraction with respect to ethanol concentration and irradiation time (A); ethanol concentration and amplitude (B); ethanol concentration and solvent-to-solid ratio (C); extraction time and amplitude (D); extraction time and solvent-to-solid ratio (E); amplitude and solvent-to-solid ratio (F).

3.1.3. Validation and verification of the predictive model

The aim of this study was to maximize the extraction yield of TPC compounds from myrtle pericarp, in applied UAE, within extraction parameters range. According to the result of response surface and prediction by this built model, the optimal conditions were thus obtained for the following conditions: ethanol at 70% (v/v), 7.5 min extraction time, 30% amplitude and a liquid-to-solid ratio of 28 mL/g. To ensure that the predicted result was not biassed to the practical value, experimental rechecking was performed using this deduced optimal conditions. The predicted extraction yield of TPC was 235.52 ± 9.9 mg GAE/g that was consistent with the experimental yield of 241.66 ± 12.77 mg GAE/g DW (Table. IV.5.). The results indicated no significant difference between the experimental and the predicted values. This strong correlation between experimental and the predicted values indicates that the response of regression model is adequate to reflect the expected optimization for the extraction of antioxidants of *M. communis* pericarp (Zhang, Hu et al. 2013).

3.2. Comparison between UAE, MAE and CME methods

The yield of recovered phenolic compounds and antioxidant activities of UAE-OPT, MAE and CME extracts are summarized in Table IV.6. Remarkably, the highest TPC was obtained by UAE technique (241.60 ± 12.77 mg GAE/g). It was 4 and 3 times higher than that obtained by MAE and CME methods, respectively, thus indicating that the application of UAE has a positive effect on the extraction of TPC content. The highest levels of TPC in UAE-OPT extract was reflected by its superior amounts of flavonoids, anthocyanins and tannins (18.99 ± 1, 31 mgQE/g; 25.06 ± 0.36 mg/g, 35.56±0.36 mgCE /g respectively). These findings are consistent with those reported by (Ghafoor, Choi et al. 2009) and is mainly attributed to the fact that ultrasound radiation can facilitate mass transfer and accelerate the extracting process so

that the extraction of bioactive compounds may be improved. Hence, according to the overall data, it is possible to conclude that the herein optimized UAE process yields higher amount of bioactive compounds in a short time and requires less solvent consumption than MAE and CME methods. Note that in this study, operating temperature in the UAE-OPT was kept constant at room temperature, excluding any heating effect. This might positively or negatively influence the phenols recovery depending on the applied amplitude

The antioxidant capacity of the extracts was assessed by DPPH$^{\bullet}$ scavenging and ferric reducing antioxidant power assays. The first is believed to involve both hydrogen-transfer and electron-transfer mechanisms (Molyneux 2004), whereas reducing power assay measures the electron-donating capacity to reduce Fe^{3+} to Fe^{2+}. The results showed that UAE-OPT extract presented higher DPPH$^{\bullet}$ scavenging ability (90.71% inhibition) when compared with CME (88.03% inhibition) and MAE (87.16% inhibition) extracts. The same tendency was also observed for reducing power assay, since the absorbance at 700 nm for UAE-OPT extract was considerable higher than than those obtained for MAE and CME extracts (23.92 % and 21.6 %, respectively). This means that UAE method is more efficient for antioxidant compounds recovery than the herein tested microwave and conventional-solvent extraction methods (Ghafoor, Choi et al. 2009).

Table. IV.6: Comparison of extraction yield of polyphenols from M. *communis* pericarp extracts obtained by ultrasound-assisted (UAE), microwave-assisted (MAE) and conventional solvent (CME) methods.

Extracti-on method	Ethanol proporti-on (%)	Extrac-tion time (min)	Amplitude of ultra-sound (w/power)	Ratio of liquid-to- solid (mL/g)	Recovery of TPC (mg GAE/g)	Recovery of flavonoids (mgQE/g)	Recovery of Anthocyans (mg/g)	Recovery of tannins (mg CE/g)	DPPH (%)	Reducing power (abs 700 nm)
UAE-OPT	70	7.5	30	28	241.6±2.7^{a}	18.99±1,3^{a}	25.06±0.36 ^{a}	35.5±0.3 ^{a}	90.71±0.23^{a}	0.568±0.002^{a}
MAE	42	62	500	32	119,6±8.4^{b}	11.5±0,01^{b}	5.64±0.06^{c}	31.7 ±1.0^{b}	87.16±0.28^{b}	0.439±0.006^{b}
CME	50	7200		50	76.4 ± 7.27^{c}	6.95±0.2^{c}	6. 96-±0.72^{b}	30.7±0.17^{c}	88.03±1.04^{b}	0.429±0.01^{b}

Results of TPC, flavonoids, anthocyans, tannins coumpounds, and expressed as means ± standard deviation. Different letters in the same row indicate significant differences ($P<0.05$) according to the ANOVA test.

This information was also confirmed by PCA analysis. PCA was apllied to the extracts (UAE-OPT, MAE and CME) for the total contents of phenolic compounds (TPC, flavonoids, anthocyanins and tannins) and to the antioxidant activity, where the two choosen factors justified 100.0% of total variance. The resulting plots allowed to select the better extraction method of different compounds of myrtle pericarp, and clearly divided the samples into three groups, depending on the extraction method (Fig IV.21). For PC1, which explains 95.91% of the total variance, the firts group showed a positive correlation with PC1, thus confirming that UAE was the best extraction method for phenolic compounds and antioxidant activity. The highest correlation was found between antioxidant activity (DPPH$^{\bullet}$ and RP essay) and anthocyans, hence suggesting that these compounds might have a key influence on the antioxidant capacity of the extracts. The best correlation between the MAE and TPC, flavonoids and tannins compounds was observed in the second group. PC2 explains only better 4.09% of the experimental variability which could be essentially associated to the CME method and anthocyans content (the third group).

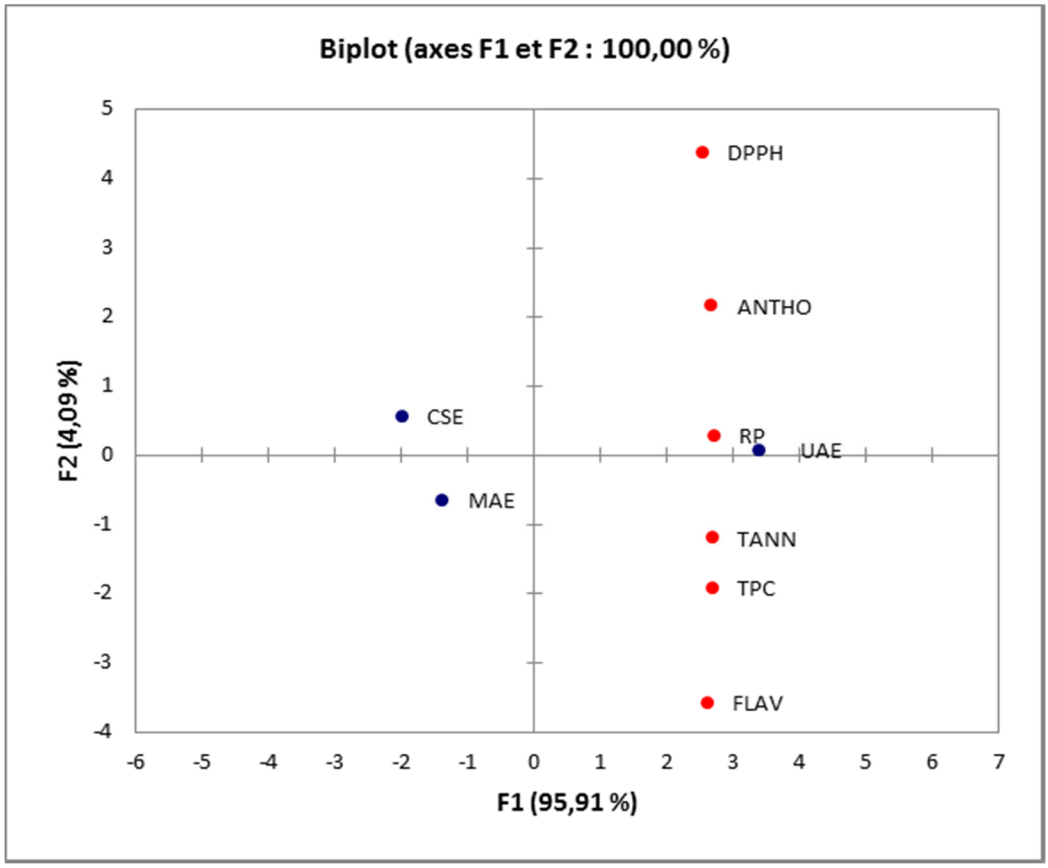

Figure IV.21. Principal component analysis of phenolic compounds for *M. communis* pericarp with UAE, MAE and CME extraction.

3.3. Identification of phenolic compounds by UHPLC-DAD-ESI-MS^n analysis

M. communis pericarp optimized extract was analysed by UHPLC-DAD-ESI-MS^n in order to further elucidate its phenolic profile. The registered chromatogram at 280 nm is shown in Figure 3 and the UV-Vis and MS^n spectral data of eluted peaks are summarized in Table IV.7. Among the distinct phenolic groups found in the extract, flavonols were the prevalent components. Overall, eleven flavonol glycosides were detected in pericarp of *M. communis*, being myricetin glycosides, in particular the myricetin-3-*O*-galactoside isomer eluted in peak 10 ([M-H]$^-$ at *m/z* 479) and myricetin-3-*O*-rhamnoside eluted in peak 14 ([M-H]$^-$ at *m/z* at 463), the major abundant ones. For each of these compounds, a low abundant isomer could also be

found in peaks 11 and 15, respectively. Note that myricetin-3-*O*-galactoside and myricetin-3-*O*-rhamnoside isomers have been previously reported for distinct organs of *M. communis* plant (Aidi Wannes and Marzouk 2013); (Pereira, Cebola et al. 2016); (Romani, Pinelli et al. 1999);(Romani, Coinu et al. 2004); (Romani, Campo et al. 2012); (Taamalli, Iswaldi et al. 2014); Yoshimura et al., 2008, Tuberoso et al., 2010; Martin et al., 1999; Messaoud et al., 2002;(Babou, Hadidi et al. 2016).

Besides the above compounds, four other myricetin glycosides could be found in the extract. The compound eluted in peak 9, showing a $[M-H]^-$ at *m/z* 631 corresponded to a myricetin-*O*-galloyl-hexoside, since the main fragments in MS^2 spectrum were formed by the loss of 152 Da (equivalent to a galloyl moiety) and 332 Da (equivalent to the simultaneous loss of galloyl and hexosyl units). This could possibly correspond to myricetin 3-(6´´-*O*-galloyl galactoside), that has been previously reported in leaves (Romani, Pinelli et al. 1999);(Romani, Coinu et al. 2004); (Taamalli, Iswaldi et al. 2014); (Pichon, Joseph et al. 1993); (Yoshimura, Amakura et al. 2008) and berries (Tuberoso, Rosa et al. 2010). In addition, the compounds with $[M-H]^-$ at *m/z* 449 (peak 13) and at *m/z* 625 (co-eluted in peak 18) were respectively assigned to myricetin-3-*O*-arabinoside and myricetin-3-*O*-rutinoside, according to their fragmentation pattern which showed the loss of a pentosyl (132 Da) and deoxyhexosyl plus hexosyl (308 Da) moieties, respectively. In turn, the compound eluted in peak 19 at 14.6 min with a pseudomolecular ion at *m/z* 569 and fragment ions at *m/z* 485 (equivalent to galloyl ester moiety) and 317 (myricetin), was tentatively assigned to a galloyl ester of myricetin, which to our knowledge has not been previously detected in myrtle.

The three remaining flavonols detected in the UAE-OPT extract were assigned to quercetin and kampferol derivatives. From those, the compound eluted in peak 12 was characterized by a $[M-H]^-$ at *m/z* 615 and fragment ions at *m/z* 463 (-152 Da, loss of galloyl group) and 301 (-162 Da, loss of an hexosyl group), and was tentatively assigned to quercetin-3-*O*-β-galactoside 6”-*O*-

gallate on the basis of data reported in the literature (Boulekbache-Makhlouf, Medouni et al. 2013); (Saldanha, Vilegas et al. 2013); (Sobeh, ElHawary et al. 2016). This compound has been already reported in Myrtaceae family, namely in *Eucalyptus* species (Cadahía, Conde et al. 1997); (Amakura, Yoshimura et al. 2009); (Okamura, Mimura et al. 1993, Boulekbache-Makhlouf, Medouni et al. 2013)) and two other species from the same family such as *Myrcia multiflora* extracts (Cascaes, Guilhon et al. 2015) and *Eugenia edulis* (Hussein, Hashem et al. 2003). To our knowledge, this is the first report of this compound in the *M. communis* species. In addition, the compound eluted in peak 17 with a deprotonated ion at *m/z* 447 and a base peak fragment ion at *m/z* 301 (-146, equivalent to the loss of a deoxyhexose unit), was identified as quercetin-3-*O*-rhamnoside according to literature data (Saldanha, Vilegas et al. 2013), (Silva, Matias et al. 2005); (Sobeh, ElHawary et al. 2016). This flavonoid was previously detected in pericarp (Aidi Wannes and Marzouk 2013), berries (Barboni, Venturini et al. 2010, Pereira, Cebola et al. 2016) and leaves (Pereira, Cebola et al. 2016); (Romani, Pinelli et al. 1999); (Taamalli, Iswaldi et al. 2014) of *M. communis*. Finally, flavonol eluted in peak 16 ($[M-H]^-$ at *m/z* 447) presented the main fragment ion at *m/z* 285 in the MS^2 spectrum, which in turn showed a fragmentation pattern coherent with kaempferol. Based on UV-Vis spectra (UV_{max} at 265, 353) and MS^n spectral data, this compound was assigned to kaempferol-*O*-hexoside which is herein described for the first time in *M. communis*.

Besides flavonols, other flavonoids in UAE-OPT extract corresponded to anthocyanins, that were eluted from 7.6 min to 9.7 min (peaks 6 – 8). Note that in general, anthocyanins are preferred detected as $[M]^+$ in ESI in the positive mode, while typically they show $[M-2H]^-$ in the negative mode (Sun, Lin et al. 2012), as represented in Table 4. Overall, according to UV-Vis and MS^n spectral data, these compounds were assigned to delphinidin, petunin and malvidin derivatives. In more detail, the compound in peak 6 exhibiting a $[M-2H]^-$ at *m/z* 463 and a base peak MS^2 fragment ion at *m/z* 301 (-162 Da) was assigned to delphinidin-*O*-hexoside by

comparison with data reported in the literature (Bochi, Godoy et al. 2015); (Dias, Bronze et al. 2010, Lopes-Lutz, Dettmann et al. 2010). In turn, petunidin-*O*-hexoside and a petunidin-*O*-hexoside derivative were eluted in peaks 7 and 8, respectively. The first showed a $[M-2H]^-$ at *m/z* 477 and a main MS^2 fragment ions at *m/z* 315/314 (Lopes-Lutz, Dettmann et al. 2010),(Bochi, Godoy et al. 2015) ,(Dias, Bronze et al. 2010) while ions corresponding to petunidin-*O*-hexoside and its hydrated form (at *m/z* 477 and *m/z* 495, respectively) were predominant in MS^2 spetrum of the latter compound. The petunidin-*O*-hexoside derivative was co-eluted with malvidin-*O*-hexoside ($[M-2H]^-$ at *m/z* 477→329). It should be remarked that, excepting for the petunidin-*O*-hexoside, the remaining anthocyanins herein detected have already been described in distinct organs from *M. communis*, including pericarp (Aidi Wannes and Marzouk 2013, Scorrano, Lazzoi et al. 2017); (Pereira, Cebola et al. 2016); (Sumbul, Ahmad et al. 2011); (Montoro, Tuberoso et al. 2006)

Several non-flavonoid compounds could also be observed in UEA-OPT extract, including caffeoyl hexoside, gallic acid and galloyl derivatives. The first ($[M-H]^-$ at *m/z* 341→179, eluted in peak 1) was the only hydroxycinnamic acid found in the extract which has not reported previously in *Myrtus communis*. In constrast, gallic acid ($[M-H]^-$ at *m/z* 169→125, eluted in peak 4), has been described, in the literature, for extracts obtained from the pericarp (Aidi Wannes and Marzouk 2013), berries (Pereira, Cebola et al. 2016); (Tuberoso, Rosa et al. 2010), and leaves (Pereira, Cebola et al. 2016); (Romani, Pinelli et al. 1999); (Romani, Pinelli et al. 2002, Yoshimura, Amakura et al. 2008, Romani, Campo et al. 2012).

Regarding galloyl derivatives (typical UV_{max} at 273-276 nm), these enclosed esters of mono- or di-galloyl groups with a hexose or quinic acid unit, or even with myrtucommulone-type groups. In detail, the compound eluted in peak 2 with a $[M-H]^-$ at *m/z* 331 and corresponding fragments at *m/z* 271, 169, 241, 211, 193 and 125, was assigned to a galloylhexoside (Abu-Reidah, Ali-Shtayeh et al. 2015); (Fröhlich, Niemetz et al. 2002), presumably galloyl-3-*O*-*β*-D-

galactoside-6-*O*-gallate, since this latter has been previously reported in *M. communis* leaves (Pichon, Joseph et al. 1993); (Sumbul, Ahmad et al. 2011); (Yoshimura, Amakura et al. 2008). Besides, two isomers of galloylquinic acid ($[M-H]^-$ at *m/z* 343→191, 169, 125) could be found in peaks 3 and 5, while a digalloylhexoside ($[M-H]^-$ at *m/z* 483→271, 331, 313, 439, 193, 169) and digalloylquinic acid ($[M-H]^-$ at *m/z* 495→343, 325, 191, 169) were detected as co-eluted compounds in peak 6. Note that all these galloyl derivatives have been previously detected only in *M. communis* leaves and thus this is their first report in the fruit organ.

Moreover, four gallomyrtucommulone-type derivatives were found in UAE-OPT extract. All these compounds showed a UV_{max} at 276 nm, and similar fragment ions in MS^n spectra, including ions at *m/z* 331, 313, 271 and 211, that are typically formed in galloylglucoside (Taamalli, Iswaldi et al. 2014). Indeed, the ion at *m/z* 331 correspond to the galloyl glucoside moiety, while ions at *m/z* 271 and *m/z* 211 result from the cross-ring fragmentation of the hexose unit in the galloyl glucoside moiety and that at *m/z* 313 can be formed due to the loss of H_2O molecule from the latter. Among these compounds, those eluted in peaks 20 and 21 ($[M-H]^-$ at *m/z* 583 and 567, respectively) were assigned to gallomyrtucommulone F and gallomyrtucommulone C, which have been previously reported in *M. communis* leaves ((Alipour, Dashti et al. 2014); (Appendino, Bianchi et al. 2002), (Asgarpanah and Ariamanesh 2015); (Taamalli, Iswaldi et al. 2014). Besides these two compounds, the extract also contained two isomeric unidentified gallomyrtucommulone-type derivatives (MW 468 Da) that presumably vary in their acyl chain regarding to those previously identified.

The UHPLC-DAD-ESI-MSn analysis to the UAE-OPT extract also detected the presence of an organic acid in peak 1. Based on its fragmentation pattern ($[M-H]^-$ at *m/z*191→173, 127, 111, 93), this compound was assigned to quinic acid, which has been previously detected in leaves of the studied plant (Taamalli, Iswaldi et al. 2014).

Table IV.7: UHPLC-DAD-ESI-MS^2 data for *M. communis* pericarp extract obtained under optimized UAE conditions

No peak	t_R (min)	λmax (nm)	Molecular ion (*m/z*)	MS^nions (*m/z*)	Probable compound
1	1.4	275	191[a]	MS^2[191]: 173, 127, 111, 93	Quinic acid
			341[a]	MS^2[341]: 179	Caffeoyl hexoside
2	1.9	276	331	MS^2[331]: 271, 169, 241, 211, 193, 125; MS^3[271]: 211, 169	Galloyl hexoside
3	2.1	273	343	MS^2[343]: 191, 169, 125	Galloylquinic acid (isomer 1)
4	2.3	271	169	MS^2[169]: 125	Gallic acid
5	6.7	274	343	MS^2[343]: 191, 169, 125	Galloylquinic acid (isomer 2)
6	7.6	276, 525	463[a,b]	MS^2[463]: 301, 300, 337,315	Delphinidin-*O*-hexoside
			495[a]	MS^2[495]: 343, 325, 191, 169	Digalloyl quinic acid
			483[a]	MS^2[483]: 271, 331, 313, 439, 193, 169 ; MS^2[271]: 211, 169	Digalloyl hexoside
7	8.8	274, 525	477[b]	MS^2[477]: 315, 314	Petunidin-*O*-hexoside
8	9.7	274, 525	647[a,b]	MS^2[647]: 495, 477	Petunidin-*O*-galloyl-hexoside derivativ
			491[a,b]	MS^2[491]: 329	Malvidin-*O*-hexoside
9	10.3	265, 356	631	MS^2[631]: 479, 299, 317	Myricetin-*O*-galloyl-hexoside
10	11.0	260, 356	479	MS^2[479]: 316, 317	Myricetin-3-*O*-galactoside (isomer 1)
11	11.2	260, 356	479	MS^2[479]: 316, 317	Myricetin-3-*O*-galactoside (isomer 2)
12	11.4	265, 356	615	MS^2[615]: 463, 301 ; MS^2[463]: 179, 151	Quercetin 3-*O*-β-gallactoside-6"-*O*-gallate
13	11.8	263, 356	449	MS^2[449]: 316, 317	Myricetin-3-*O*-arabinoside
14	12.1	261, 351	463	MS^2[463]: 316, 317	Myricetin-3-*O*-rhamnoside (isomer 1)
15	12.2	261, 351	463	MS^2[463]: 316, 317	Myricetin-3-*O*-rhamnoside (isomer 2)
16	12.9	265, 353	447	MS^2[447]: 285; MS^2[285]: 267, 257, 241	Kaempferol-3-*O*-hexoside
17	13.4	257, 350	447	MS^2[447]: 301; MS^2[301]: 179,	Quercetin-3-*O*-rhamnoside
18	14.5	265, 352	431[a]	MS^2[431]: 271; MS^3[271]: 211,	Galloylderivative
			625[a]	MS^2[625]: 479, 317	Myricetin-3-*O*-rutinoside
19	14.6	273, 350	569	MS^2[569]: 485, 317	Myricetin-3-*O*-galloyl ester
20	16.5	276	583	MS^2[583]: 271, 565, 313, 211, 331; MS^3[271]: 211, 169	Gallomyrtucommulone F
21	16.8	276	567	MS^2[567]: 271, 313, 211, 169; MS^3[271]: 211, 169	Gallomyrtucommulone C
22	17.6	276	467	MS^2[467]: 271, 313, 169, 211; MS^3[271]: 211, 169	Gallomyrtucommulone-type (isomer 1)
23	17.8	276	467	MS^2[467]: 271, 313, 169, 211; MS^3[271]: 211, 169	Gallomyrtucommulone –type (isomer 2)

Peak numbers correspond to those depicted in Figure 3; [a] Co-eluted compounds in a peak fraction; [b] the respective $[M]^+$ ions were registered in the positive mode.

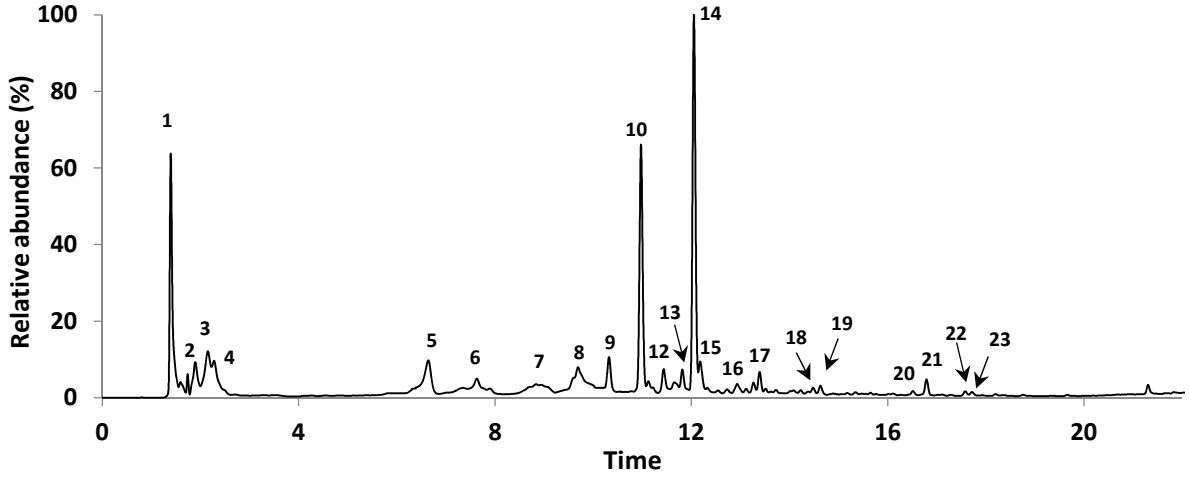

Figure IV.22. Chromatographic profile at 280 nm of *M. communis* pericarp extract obtained by UAE extraction at optimized conditions. Numbers in the figure correspond to the eluted UHPLC peaks for which UV and MS data is summarized in Table IV.7.

Conclusion

The response surface methodology was successfully employed to optimize total phenolic extraction yield from dried *M. communis* pericarp by non-conventional solvent extraction process, namely UAE. As compared to MAE and CME extractions, the proposed UAE method allowed a higher phenolic recovery yield and antioxidant activity with a short working time and a lower solvent consumption. The quantification of the amount of phenolic compounds in the three types of extract complemented with PCA analysis also allowed to conclude that the *M. communis* pericarp extract obtained under optimal UAE experimental conditions contained higher levels of flavonois, tannins and anthocyanin than the remaining extracts and particularly, the latter phenolic components could be correlated to its antioxidant activity. According to UHPLC-DAD-ESI-MS^n analysis, anthocyanins in UAE-OPT extract enclosed delphinidin, petunin and malvidin derivatives. Besides those, the extract was particularly enriched in myricetin glycosides, and also contained several galloyl derivatives, as described before for several organs of *M. communis*. Apart from that, this analysis allowed to identify distinct compounds, namely a caffeyol hexoside, a galloylester of myricetin, a kaempferol-*O*-hexoside, a petunidin-*O*-hexoside derivative and gallomyrtucommulone-type compounds were herein detected for the first time in *M. communis* plant and/or pericarp, thus contributing for an improvement of the knowledge of the phenolic profile of this botanic species.

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Chapter V

Effect of ultrasound pretreatment on the water state, phenolic compounds and antioxidant activity in Myrtus communis fruits during the microwave drying.

Effect of ultrasound pretreatment on the water state, phenolic compounds and antioxidant activity in *Myrtus communis* fruits during the microwave drying.

Introduction

Among the medicinal plants, *Myrtus communis* is one of the important aromatic and medicinal species from the Myrtaceae family (Aidi Wannes et al., 2010). It has been also used in folk medicine because of its astringent and balsamic properties .Different parts of the plant have found various uses in the food industry, such as, in the cosmetic and pharmaceutical industries (Messaoud and Boussaid, 2011)**.**The fruits are very rich in anthocyanin it was used as a condiment as a substitute for pepper**.** In addition the oils extracted by steam distillation of fruits are used both in flavor and fragrance industries (Aydın and Özcan, 2007).

Before plan preservation, drying is the most common method of food preservation and is used to reduce post-harvest loss and to produce several dried fruits, which can be directly consumed or used in processed foods tuffs. Conventional air-drying is energy intensive and consequently cost intensive because it is a simultaneous heat and mass transfer process accompanied by phase change ; which can result in serious damage to flavor, color, rehydration capacity and nutrients of the treated material as well as long low energy efficiency (Özbek and Dadali, 2007**)**. Owing to these reason, development of new methods of drying for such perishable fruits (Myrtle) is essential for food preservation, which can save time and energy and minimize quality degradation.

Since, the microwave drying has gained popularity as an alternative drying method to overcome above problems for a wide variety of food products (Bouraoui et al., 1994)**.** However one of disadvantages of microwave drying is that excessive temperature along the corner or edges of food products results in scorching and production of off-flavors especially during final stages

of drying (Zhang et al., 2006).Hence, it is necessary to combine microwave drying with an pretreatment in order to minimized product quality degradation

In recent years, ultrasound has been implemented as an alternative pretreatment method for drying, and the results have shown that this pretreatment can greatly reduce the overall processing time (Jangam, 2011) (Mothibe et al., 2011) which can attribute to the following factors; Increase in the mass transfer rate**,** Loss of cellular adhesion, rupture of the cell walls and formation of large channels (García-Pérez et al., 2011) Therefore, the aim of this study was to evaluate the effect of ultrasound pre-treatments on pericarp drying. The influence of pre-treatments on water loss, total phenol content and their antioxidant activity were analyzed. The comparison between the microwave drying assisted by ultrasound pretreatment, conventional and microwave drying was also investigated.

1. Material and methods

1.1. Preparation of samples

Myrtus communis, were collected at optimal maturity (January), from Addekar (Bejaia, North-east of Algeria). Fruits were isolated manually from the aerial parts. It has been washed with a tap and distilled water to remove any adhering soil and dust. Finally, fruits were blotted with filter paper and pre-treated by ultrasound.

1.2. Water content

To determine the water content, one tests moisture is to carry out, for the fruit of Myrtus, three samples of 10g were dried with 103± 2°C, the weight of the sample was taken each 3 hours until its stabilization. The result is average of three samples.(Bourkhiss et al., 2009)**.** The water content was given according to the following equation:

$$H\% = \left(\frac{w_i - w_f}{w_i}\right) * 100 \quad \text{Eq .15}$$

Where

H %: moisture;

W_0: represent the initial weight of the sample;

W_f: represent the dry weight of sample;

1.3. Ultrasound pre-treatment (UP)

The samples were immersed in distilled water and placed in an ultrasonic bath (Ctra.NII:585 Abrera (Barcelona) Spain, Ultrasound H-D, frequencies: 20 to 60 KHZ, Power: 80 to 600 W). The fruits were placed in a biker next to each other in the bath and covered with the metal net to void flow out of the samples. After that, the distilled water was added into the ultrasonic bath. The pre-treatment was carried out at room temperature (25C°). The ratio of raw material to water was set at 1:4, as recommended by (Fernandes et al., 2008a), b. The ultrasound frequency was 25 kHz; the ultrasound energy was applied for 10, 20, 30, 45 and 90 min. After the treatment, the plant materials was blotted with filter paper. Before and after ultrasound treatment the mass of the samples, dry matter content and water temperature were measured. The temperatures increase during the experiments maximally by 2 to 5 C°. The experiments were conducted in triplicate for each drying process.

1.4. Microwave drying assisted ultrasound pretreatment (MD-AUP)

After ultrasound pretreatment, the fruits were dried in microwave by two power (500W, 700 W).Drying treatment was performed in domestic digital microwave oven with the technical feature of 230 V, 50Hz and 2450 W. The size of the heating cavity is 37.5 cm (L) x 22.5 cm (W) x 38.6 cm (D).The microwave oven consisted of a rotating glass plate with 300 mm diameter at the base of the oven. The apparatus was equipped with a digital control system for irradiation time and microwave power (the latter linearly adjustable from (100 to 900W). The microwave oven was operated by a control terminal, which could control microwave power

level and emission time, two different microwave power (500 700W) were used. After drying Fruit samples are peeled manually and seeds are recovered. The pericarps was ground with an electrical grinder (IKA model A11 Basic, staufen, Germany), the obtained powder was passed through standard 125µm size and only the fraction with particle size < 125µm was used. The powder was stored in airtight bags until use.

1.5. Ultrasound assisted extraction (UAE)

Extraction of phenolic compounds using ultrasound has been proposed to improve the efficiency and/or speed of this step. An ultrasonic apparatus (SONICSVibra cell, VCX 75115 PB, SERIAL No. 2012010971 MODEL CV 334) was used for UAE with working frequency fixed at 20 kHz. For the extraction, one gram of the powder was placed in a 250 mL amber glass bottle containing the extraction. The suspension was exposed to acoustic waves under with a concentration of 28 ml of ethanol at 70%, irradiation time (7min30s), and amplitude at 30 %. The temperature (27±2 °C) was controlled continuously by circulating external cold water and checking the temperature using a T-type thermocouple. After the extraction, the solution was filtered through filter paper.

1.6. Analytical determinations

1.6.1. Total phenolic content (TPC)

The determination of total phenol compounds in the extracts was done according to the method of. (George et al., 2005)**.** A volume of 500 µL of diluted fruits extract with distilled water was added to 2.5 mL of 10-fold diluted Folin–Ciocalteau reagent. The solution was mixed and incubated at room temperature for 2 min. After 2 min, 2 mL of 7.5% sodium carbonate (Na_2CO_3) (v/v) were added. After incubation at 50° C for 15 min, the absorbance of the sample was measured at 760 nm against a blank (made as reported for the sample) by using a UV–VIS Spectrophotometer (SpectroScan 50, Nkesia, Cyprus) figure n°7. The assay was performed in

triplicate. For quantification, a calibration curve was generated with the standard solution of gallic acid, (R^2= 0.998). The TPC was expressed as mg of gallic acid equivalent (GAE) per gram of powder on dry weight (AW) basis (mg GAE g^{-1} DW).

1.6.2. Total flavonoid content

The total flavonoid contents were estimated according to the aluminum chloride method of (Quettier-Deleu et al., 2000) based on the formation of a complex flavonoid-aluminum (Chang et al., 2002). Briefly, 1 mL of pericarps extracts was mixed with 1 mL of 2 % AlCl3. After 15 min of incubation in the dark, the absorbance of the mixture was determined at 430 nm (figure n°8). Each analysis was carried out in triplicate. The total flavonoid content was calculated from a calibration curve made with rutin and expressed as milligrams of rutin equivalent per gram of powder an dry weight (AW) basis (mg RE g^{-1} DW).

1.6.3. Total monomeric Anthocyanin contents

Total monomeric anthocyanin content was determined by the pH-differential method (Lee et al., 2005**)**, based on the structural change of the anthocyanin chromospheres between pH1.0 and 4.5. Absorbance was measured at 520 nm and at 700 nm in buffers at pH 1.0 and 4.5. The concentration of anthocyanin was obtained using the following equation (Eq. V.16). Results are expressed on a Cyanidin-3- glycoside basis.

$$Anthocyanin\ pigment\ \left(cyanidin\text{-}3\text{-}glucoside\ equivalents,\ mg/g\ DW\right)=\frac{A\times PM\times FD\times 10^3}{\varepsilon\times l} \quad (16)$$

Where $A = \left(A_{520nm} - A_{700nm}\right)_{pH1.0} - \left(A_{520nm} - A_{700nm}\right)_{pH\,4.5}$

MW (molecular weight): 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu); DF: dilution factor; l: path length in cm; ε: 26 900 molar extinction coefficient, in L × mol^{-1}× cm^{-1}, for cyd-3-glu; and 10^3: factor for conversion from g to mg.

1.6.4. Total condensed tannin content

Total tannin content was determined by the HCL–Vanillin procedure according to (Ba et al., 2010). 1 ml of the extract was mixed with 5 ml of reagent (HCL + Vanillin).The mixture is put in the dark for 20 minutes. The absorbance versus prepared blank was read at 500 nm. All analyses were performed in triplicate. Total tannins expressed as mg Catechin equivalents per gram (mg C/g) through the calibration curve with Catechin.

1.7. Antioxidant activities

The antioxidant activity of plants is mainly contributed by the active compounds and phenolic fraction present in them such as flavonoids(Pietta et al., 1998) and anthocyanin (Montoro et al., 2006b).The antioxidant activity of pericarp was evaluated by DPPH radical scavenging assay and reducing power. The higher percentage inhibition test rate is, the greater the hydrogen donating ability, thus the higher antioxidant activities.

1.7.1. DPPH radical

The stable 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extracts (Choi et al., 2002). It highly colored free radical that can abstract labile hydrogen atoms from phenolic antioxidants with concomitant formation of a color-less hydrazine (DPPH-H). The free radical scavenging activity (RSA) of an extracts can be expressed as the percentage of DPPH reduced by a given amount of extract. The RSA was measured, following the method of (Dudonné et al., 2009). DPPH radicals have an absorption maximum at 515nm (Choi et al., 2002) which disappears with reduction by an antioxidant compound. A DPPH$^{\bullet}$ solution in absolute methanol (60 µM) was prepared, and 3 mL of this solution were mixed with 1 mL of the different diluted extracts. The samples were incubated for 20 min at 37°C in the dark, then, the decrease in absorbance at 515 nm was measured (figure n°9). The α-tocopherol served as a positive control. All the tests were

performed in triplicate, and the inhibition rate was calculated according to the following equation (Eq. V.16).

$$\%\ Scavenging = \frac{\left(A_{control} - A_{extract}\right)}{A_{control}} \times 100 \qquad \text{Eq.V.17}$$

Where $A_{control}$ is the absorbance of DPPH radical + distilled water, A_{sample} is the absorbance of DPPH radical + sample extract.

1.7.2. Iron reducing power

In this study, the yellow color of the test solution changes to green depending on the reducing power of test specimen. The presence of reductions in the solution causes the reduction of the Fe^{3+}/ ferricyanide complex to the ferrous form. 1 mL of desired dilution with distilled water of fruits extracts was mixed with 2.5 mL of a 0.2 M sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% Potassium ferricyanide ($K_3Fe(CN)_6$). The mixture was incubated in a water bath at 50°C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid was added. At the end, 1mL of the obtained solution was added to 5 mL of distilled water and 1 mL of 0.1% ferric chloride ($FeCl_3$), the intensity of the blue green color was measured at 700 nm. Tests were carried out in triplicate (Pan et al., 2010).

1.8. Statistical analysis

The analysis of variance (ANOVA) was performed using XLSTAT release 10 (Addinsoft, Paris, Framed),Tukey's multiple range test (HSD) was used to compare between TPC content and antioxidant activity as affected by microwave assisted by ultrasound (MD-AUP), microwave (MAE) and conventional drying methods (CME).

2. Results and discussion

2.1. Ultrasound pretreatment-assisted microwave drying (UP-MD)

Drying kinetics were studied until a final moisture content of 56 ± 0,005 %. Ultrasound US treatment pplied prior to microwave drying (MD), improves the mass transfer phenomena and the durattion drying time of many fruits (Fernandes et al., 2008a, 2009). In the present study, the pre-treatment with ultrasound for 10, 20 and 30 min had a positive effect on water loss of myrtle fruit tissue compared to the MD treatment alone (Fig. V. 23. 24), especially in the first 30 min of the treatment. This could be due to the microscopic channels creation during ultrasound pre-treatment, which may ease moisture removal and increase the diffusivity of the water (Fernandes et al., 2008a) (Fernandes et al., 2008b)

The results illustrate that the ultrasonic pretreatment is interesting when the quantity of water in the fruit was very high such our case. The ultrasonic waves can cause a very fast series of compression and alternative expansions, in the manner similar to a sponge when it is tightened and released on several occasions. The forces implied in this mechanical process can be much larger than those due to the surface tension, which holds moisture inside the capillaries of the fruit creating the microscopic channels, which can relieve the removal of moisture (Fernandes et al., 2008a; Fernandes et al., 2008b)**.** This result confirms the observations of (De la Fuente-Blanco et al., 2006) which report that increased water diffusivity during the microwave drying process reducing the time required for drying**.** The MD-AUP affect the time duration that show a short time duration (6mn, 5mn30s at 500w, 700w) respectively compared with microwave drying (18 mn at 500w, 11mn at 700w). In addition the higher temperature to the longest duration in the microwave can cause the phenolic compounds degradation (Yang and Zhai, 2010)**.**

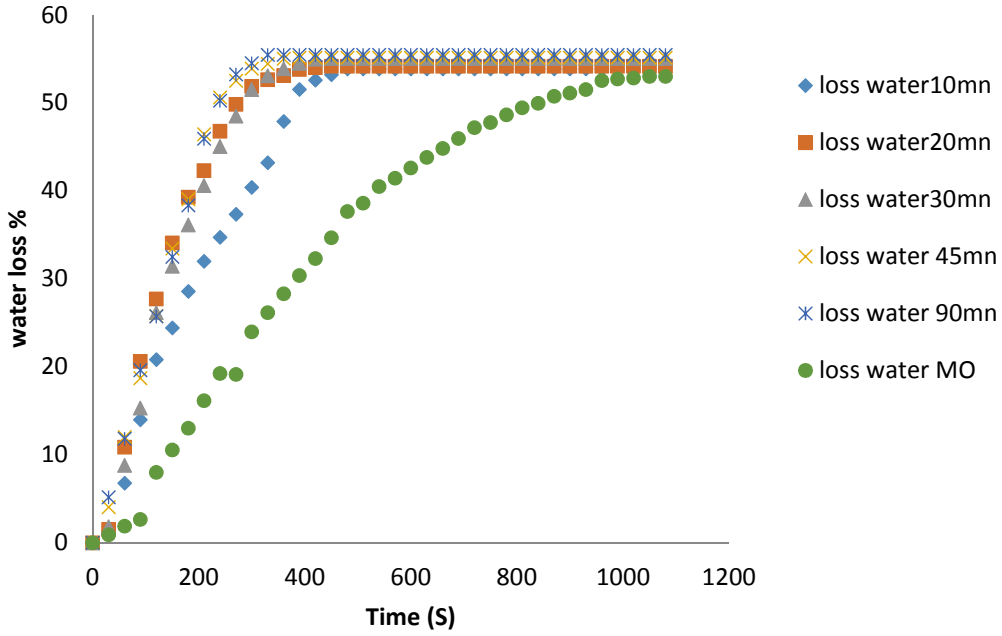

Figure V.23: Influence of ultrasonic time on the dehydration kinetic process at 500w of Myrtle pericarp

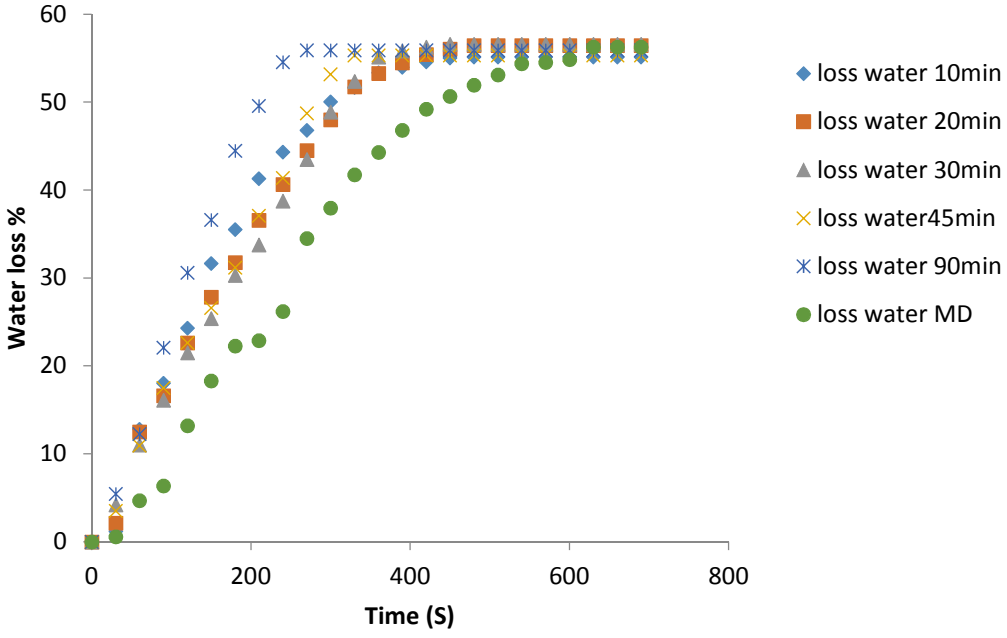

Figure V.24: Influence of ultrasonic time on the dehydration kinetic process at 700w of Myrtle pericarp

2. 2. Analytical determination

In order to evaluate the UP-MD effect on phytochemical content and antioxidant activities, different parameters were determined.

2.2.1. Total phenolic content (TPC)

As one of the most important antioxidant plant components, phenolic compounds have been widely investigated in many medicinal plants (Djeridane et al., 2006)). This antioxidant activity is believed to be mainly because of their redox properties (Zheng and Wang, 2001), which play an important role in adsorbing and neutralizing free radicals (Laranjinha et al., 1995), quenching singlet and triplet oxygen (Hatano et al., 1988). The (Table. V.8.), showed the results obtained for the TPC content of *Myrtus communis* fruits obtained by microwave drying at 500 w and 700w assisted by ultrasound pretreatment.

The main significant differences were found in TPC content among different ultrasound pretreatment time. In fact, the highest content was observed with a pretreatment time of 90 mn at 500W (219.90±0,14 mg GAE/g), and the lowest TPC content was observed with sample pre-treated for 10 min (115.78 ± 0.04 mg GAE/g) which correspond to 47 % TPC reduction compared to 90 min result. These observations are in correlation with kinetic drying result; i.e., increase ultrasound treatment reduce MD time and preserve TPC from thermos-degradation. Microwave drying at 700W showed that TPC content had the same tendency that 500 W one, but TPC yield was decreased by 7%. This lowest content could be due to the thermal degradation of the phytochemicals at higher microwave power ((Naczk and Shahidi, 2004; Shahidi and Naczk, 2004).

Total flavonoid content of myrtle using different microwave drying methods were represented in Table. V.8.The total flavonoid content represent a significant difference (<0, 05) between all samples. The highest amount was attributed to sample pre-treated for 90 min (5.75

±0.09 mg RE/g), and the lowest content was found with sample pre-treated for 10 min (3.69 ± 0.09mg RE/g MS), and this is in agreement with TPC content. According to the work of (Oliveira et al., 2011) who studied dehydration of Malay Apple using ultrasound as pre-treatment, explained that to reduce the initial moisture content of the fruit by 90%, the total processing time can be reduced by 233 min when Malay apples are subjected to ultrasound during 60 min that leading to better preservation of nutrients content.

Anthocyanins are the largest water-soluble natural pigment. They belong to a large group of flavonoids. Anthocyanins have anti-inflammatory, anticarcinogenic, prevention of cardiovascular disease (Basu et al., 2010).The results of total monomeric anthocyanin contents of *Myrtus communis* fruit using UP-MD method was represented in Table. V.8. The result showed that the highest total anthocyanin content was observed in samples pretreated by ultrasound at 90 mn assisted microwave drying at 500W (11.30 ± 0.02 mg E cy-3-glu/g), and also the lowest yield was observed by 10 min (6.46±0.06 mg E cy-3-glu/g).The results can be explained by the reduction of duration time in the ultrasonic bath, which can contribute to the preservation of nutritive compounds(Sledz et al., 2016)**.** However, the microwave drying at 700W showed an amount at 9.46 ± 0.02 mg E cy-3-glu/g; the lowest content of anthocyanin could be due to its degradation by the high temperatures and the largest duration time in microwave (Gao et al., 2007)**.**

The total condensed tannins for myrtle fruits obtained by MD-AUP were showed in Table V.8. The results show that the total condensed tannin in the samples obtained with microwave drying at 500W was the highest with significant difference ($p<0.05$) between all samples, it is of 200.50 ± 0.7 mg CE/g at 90 min, followed by sample pre-treated at 45 min, min (158.60 ± 0,7 mg CE/g). While the lowest content of condensed tannins was attributed to, the sample pre-treated for 10 min at 111 ± 0.54 mg CE/g. Furthermore, the significant difference was observed in the condensed tannins content at power of 700W (192.23±0.84mg CE/g). This

result may be due to the degradation of these compounds under the influence of microwaves irradiation (Zhang et al., 2011)**.**

Table V.8: The phenolic composition of myrtle fruits obtained with MD-AUP drying at 500 and 700 w.

	Conditions methods	**Recovery Of TPC (mg GAE/g)**	**Recovery of flavonoid (mg RE/g)**	**Recovery of anthocyans (mg /g)**	**Recovery of tannin (mg QE /g)**
500W	UAD-10mn	115,78 ± 0,26	3,69 ± 0,05	6,46 ± 0,06	111±0,54
	UAD-20mn	140,25 ± 0,77	3,81± 0,05	6,86±0,1	119,83±0,8
	UAD-30mn	160,34 ±0,22	4,10±0,01	7,99±0,40	136,38±0,28
	UAD-45mn	173,11 ±0,18	4,70±0,09	10,51±0,01	158,6±0,46
	UAD-90mn	219,90 ±0,69	5,75± 0,01	11,30±0,02	200,56±0,76
700W	UAD-10mn	108,84 ±0,43	2,49±0,01	5,79±0,06	85,61±0,72
	UAD-20mn	130,82 ± 0,03	2,80±0,04	6,46±0,47	110,06±1,6
	UAD-30mn	154,17 ± 0,36	3,17±0,03	6,98±0,17	153,15±0,9
	UAD-45mn	167,10 ± 0,24	3,85±0,005	7,95±0,02	141,86 ±0,16
	UAD-90mn	204,43 ± 0,43	4,75± 0,04	9,47±0,02	192.23±0.83

3.2. 5. Antioxidant activity

The active compounds and phenolic fraction present in them such as flavonoids and anthocyanin mainly contribute the antioxidant activity of plants (Montoro et al., 2006a). Their antioxidant properties are very important due to the deleterious role of free radicals in foods and biological systems (Gülçin et al., 2006).The antioxidant activity of, myrtle pericarp, was evaluated by DPPH radical scavenging assay and reducing power test. The higher percentage inhibition test rate is, the greater the hydrogen donating ability, thus the higher antioxidant activities.

3.2.5.1. DPPH radical scavenging assay

The effect of antioxidant on DPPH radical scavenging was conceived to their hydrogen donating ability (Chen et al., 2008**).** Concerning the extracts obtained by microwave drying at 500W assisted by ultrasound (Fig V.25) show that the inhibition effect of DPPH radical

antioxidant was most important for sample pretreated for 90 min (98.63 %) , followed by that pretreated for 45 min, and the lowest is recorded with that pretreated for 10 min (80.80 %). A positive correlation was observed between antioxidant activity and total phenolic compounds. These results were in agreement with these obtained by (Ao et al., 2008)**;**(Zainol et al., 2003), which proved that high total polyphenol contents increase the antioxidant activity. Effectively, the sample drying with microwave at 700W presented a low content of bioactive compounds since tannins by comparison with those of 500W. Our results were in agreement with those founded by (Dairi et al., 2014) and (Oliveira et al., 2012).

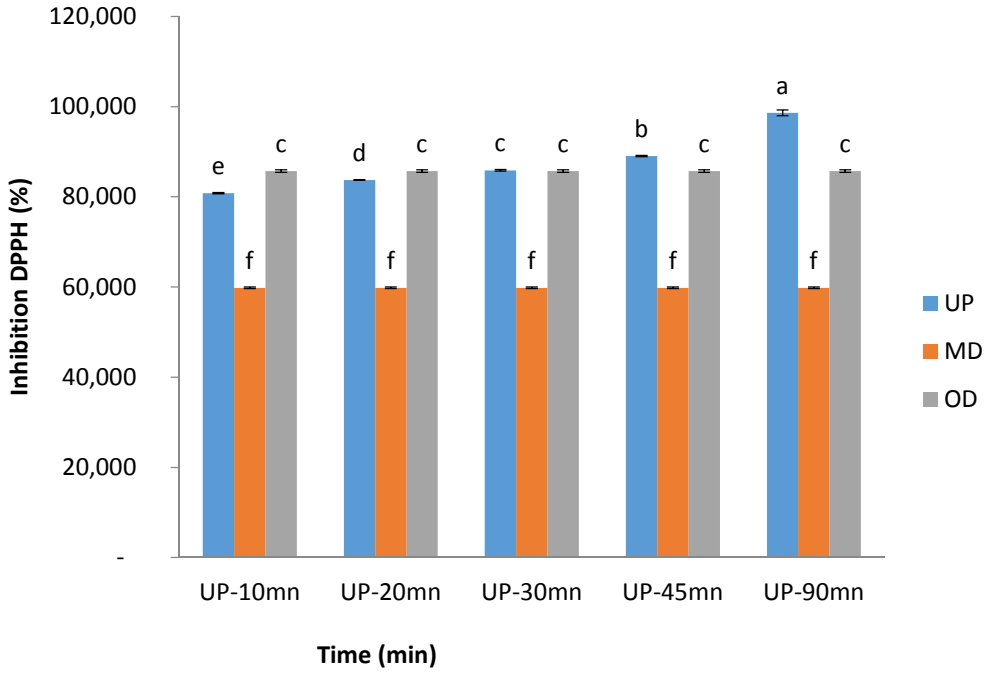

Figure V.25: Radical DPPH with deferent methods of drying

3.2.5.2. Iron reducing power

The reducing power was based on the capacity of the phenolic compounds to reduce the ferric iron ferrous Fe^{3+} on iron Fe^{2+}; the power of reduction is one of the antioxidant mechanisms (Karagözler et al., 2008**).** The reducing power of Myrtle fruit obtained by MD-AUP at (500, 700W) was showed in Fig. V.26.

The result show that the highest absorbance was observed to fruits pretreated at 90mnUS-500W (0.90 UA), and the lowest was attributed to those pretreated at 10mn (0.59UA). The same correlation between reducing power and phenolic compounds was observed. That confirmed by (Hrenovic et al., 2012).

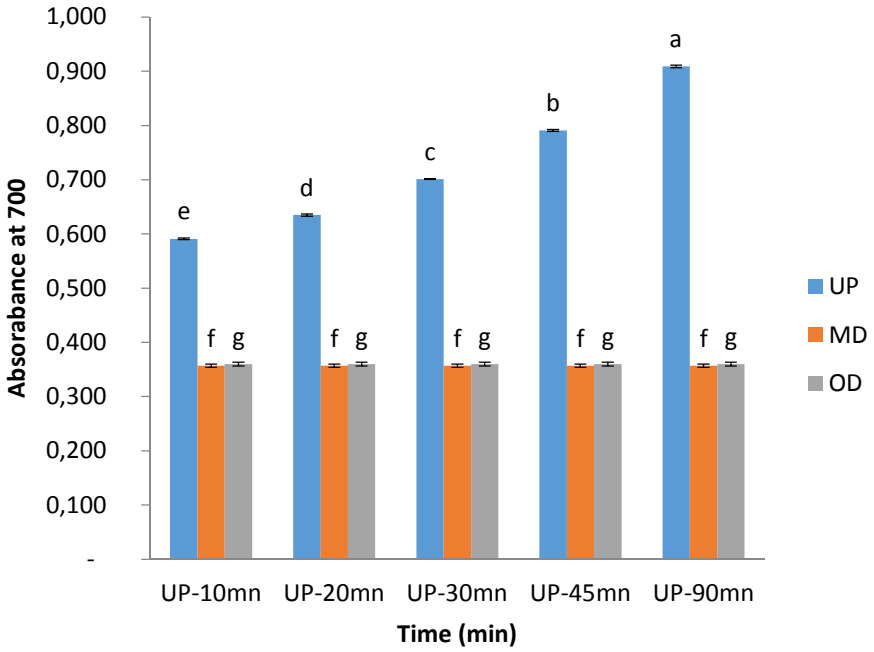

Figure V.26: Reducing power with deferent methods of drying

3.3. Comparison of MD-UAP, MD and conventional drying methods

Drying can be considered as the most common method of food preservation (Vega-Mercado et al., 2001) (Deng and Zhao, 2008). The aim of this study was to investigate the effect of ultrasound on the mass transfer, water removal and extraction yield from Myrtle pericarp. The selection of a drying method would mainly depend on the advantages and disadvantages of the processes, such as extraction yield, complexity, production cost, environmental friendliness and safety. The conditions of different techniques and their results are summarized in Table V.9:.The results indicated that the highest TPC, total flavonoids, total anthocyanins

and total tannins were obtained by MD-UAP that gave a significantly higher value ($p < 0.001$) at 500W assisted by ultrasound at 90 mn (p< 0, 05) and giving about 219.90±0,14 mg GAE/g, 5.75 ±0.09 mg RE/g ,11.30 ± 0.02 mg E cy-3-glu/g and 200.50 ± 0.7 mg EQ/g, respectively compared with that obtained by microwave and conventional drying. Concerning the antioxidant activities, a comparison of the mean total antioxidant activity of samples was presented in the figures 3.4**.** Using all the two assays (DPPH and RP essay), the antioxidant activities were higher in pericarp extracts using by MD UAP compared to MD and conventional drying ($p < 0.05$), however there is not difference between microwave and conventional drying to the reducing power at (0.35UA) and (0.36UA) respectively .The efficiency of ultrasound pretreatment was to minimize the compound degradation caused by the higher temperatures on microwave. The combination of microwave and ultrasound can be carried out at ambient temperature (Fernandes and Rodrigues, 2007). What has been proven by the works of (Simal et al., 1998) we studies the apples and the pineapples by (Fernandes et al., 2009**)** who shows that such combination gives high speeds of water removal and solid gain even at low temperatures, thus leading to better maintenance of a natural aroma, color and nutrients content.

Table V.9: The phenolic composition of myrtle fruits obtained with different drying methods

Drying methods	Recovery of TPC (mg GAE/g)	Recovery of flavonoid (mg RE/g)	Recovery of anthocyans (mg/g)	Recovery of tannin (mg CE/g)
UAD-10mn	115,78 ± 0,26	3,69 ± 0,05	6,46 ± 0,06	111±0,54
MD	193.79±0.99	4.41±0.10	0.85 ±0.01	18.22±02
CD	148.16 ± 0.95	5.03 ±0.06	7.43 ± 0.20	41.38± 05
UAD-20mn	140,25 ± 0,77	3,81± 0,05	6,86±0,1	119,83±0,8
MD	193.79±0.99	4.41±0.10	0.85 ±0.01	18.22±02
CD	148.16 ± 0.95	5.03 ±0.06	7.43 ± 0.20	41.38± 05
UAD-30mn	160,34 ±0,22	4,10±0,01	7,99±0,40	136,38±0,28
MD	193.79±0.99	4.41±0.10	0.85 ±0.01	18.22±02
CD	148.16 ± 0.95	5.03 ±0.06	7.43 ± 0.20	41.38± 05
UAD-45mn	173,11 ±0,18	4,70±0,09	10,51±0,01	158,6±0,46
MD	193.79±0.99	4.41±0.10	0.85 ±0.01	18.22±02
CD	148.16 ± 0.95	5.03 ±0.06	7.43 ± 0.20	41.38± 05
UAD-90mn	219,90 ±0,69	5,75± 0,01	11,30 ±0,02	200,56±0,76
MD	193.79±0.99	4.41±0.10	0.85 ±0.01	18.22 ±02
CD	148.16 ± 0.95	5.03 ±0.06	7.43 ± 0.20	41.38± 05

Conclusion

The use of ultrasound as pretreatment has been demonstrated to facilitate water loss during drying. The present work showed that US pretreatment performed for 90 mn than microwave drying at 500w has a significant effect on phenolic composition and their antioxidant activity.

The efficiency of ultrasound pretreatment was to minimize the compound degradation caused by the higher temperatures on microwave. The combination of microwave and ultrasound can be carried out at ambient temperature.

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General Conclusion

General conclusion

Medicinal plant research is aimed at the isolation and identification of naturally occurring substances. Chemical analysis of extracts from plant material such as *M. communis* leaves ,steam, pericarp and seeds may represent an abundant, inexpensive source of natural antioxidants will play a central role in the development and modernization of herbal medicine. The majority of extraction procedures for the determination of plant metabolites are developed in such a way that the final extract introduced into the GC and HPLC columns contains only the analytes with all interferences removed. This is one area where conventional techniques have spelled utter disaster. UAE and MAE have risen rapidly in the last decade, and for most applications they have proven to be effective in all aspects compared to traditional extraction techniques.

The present study show that quite good recoveries of total phenolic from *M. communis* leaves, stems, and seeds by a combination of relatively short times of microwave treatments and ultrasound. MAE was found to be highly effective enabling a considerable reduction in extraction time and the efficiency of extraction of phenolic contents from all myrtle parts was improved in comparison with the CME method .The highest phenolic content in the leaves and pericarp extracts from 128.73±6.84 by MAE to 249.86±9.2 mg GAE g^{-1} DW by CME and, from 76.38 ±7.27 MAE to119.60 ± 8.4 mg GAE g^{-1}DW by CME, respectively, was obtained using (62 s against 7200) extraction time. However, the mathematical model fitted to the data and the response surface plots obtained in optimization of ultrasound assisted extraction of myrtle pericarp show a higher results of phenolic compounds (235.52 ± 9.9 mg GAE g^{-1} DW) and their antioxidant activity (90.71±0.23 %) was obtained by ethanol at 70% (v/v), 7.5 min extraction time, 30% amplitude and a liquid-to-solid ratio of 28 mL/g conditions.

The discussion presented in the current work has clearly established the superiority of ultrasound-assisted extraction compared to the conventional extraction processes. Major achievement of ultrasound-assisted extraction is the reduction of processing time for the same yield in addition to other benefits such as operation at lower temperature, requirement of lower amounts of solvents and sometimes higher recoveries with enhanced purity of the final recovered products. UAE technology can potentially enhance extraction of components such as polyphenolics, anthocyanins.

In the other hand, the use of ultrasound as pretreatment during the microwave drying (MD-UAP) myrtle fruits has been demonstrated to facilitate water loss during drying caused reduction of the drying time in comparison with untreated sample. The results indicated that the highest TPC, was obtained by MD-UAP at 500W assisted by ultrasound at 90 mn that gave a significantly higher value ($p < 0.001$ about 219.90±0, 14 mg GAE).

Therefore, more identification work is needed to obtain a more complete profile of the extracts phenolic composition of *M. communis part. In addition,* the study of the metabolism of extracts obtained in vivo and its microbiological activities might be needed to use it's in the agro-food and pharmaceutical industries.

Appendix

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Phytochemical analysis of *Myrtus communis* plant: Conventional versus microwave assisted-extraction procedures

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Abstract:
Background: Myrtle (*Myrtus communis L*) may constitute an interesting dietary source of health protective compounds. Microwave-assisted extraction (MAE) of total phenolic compounds (TPC) from myrtle leaf, stems, pericarp, and seeds was studied and the results were compared with those of the conventional method extraction (CME) in terms of extraction time.
Methods: Extraction yield/efficiency and antioxidant activity were measured using radical scavenging assay (DPPH$^{\bullet}$) and reducing power.
Results: The results show that the MAE was higher in terms of saving energy, extraction time (62 s) and extraction efficiency of bioactive compound compared to CME (2 h). Leaf presented the optimum content of total phenols (250 mg GAE.g^{-1} DW) and flavonoids (13.65 mg GAE.g^{-1} DW). However, the anthocyanin content was most important in pericarp extract (176.50±2.17 mg Cyd-3-glu g^{-1} DW). The antioxidant activity was important in all parts, mainly in leaves. The results indicated that appropriate microwave treatment could be an efficient process to phenolic compounds recovery and thus, better the antioxidant activity of myrtle extract.
Conclusions: Principal component analysis (PCA) applied to the experimental data shows that the distribution of the myrtle phenolic compounds depended on their plant part localization as well as the extraction method.

Keywords: antioxidant activity, microwave-assisted extraction, *Myrtus communis*, phenolic compounds
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Introduction

Myrtle (*Myrtus communis L*) belongs to the family of Myrtaceae and is an evergreen shrub, which grows wild in several regions all over the world [1]. Different parts of this plant found various uses in the food industry, such as for savoring meat and sauces, and in the cosmetic industry [2]. The leaf decoction was used for virginal washing enemas and against respiratory diseases [3]. A decoction or infusion of leaves and fruits of this plant were used as stomachic, hypoglycemic, cough, infectious, and oral diseases. They were also used for constipation, appetizing envy, and externally as anti-hemorrhagic for wound healing [1]. Regarding the chemical composition, previous studies on *Myrtus communis* aerial parts have revealed the presence of several compounds. Leaves and flowers contain essential oils, tannins, phenolic acids, and flavonoids [4, 5]. Fruits are mostly composed of volatiles oils, tannins, anthocyanins, fatty acids, sugars, and organic acids such as citric and malic acids [6].

Extraction represents the primary step to get a crude extract from plants; then the obtained extracts should undergo further analysis of their active components. Different techniques such as conventional method extraction (CME) methods including soaking, maceration [7, 8], water percolation, soxhlet extraction have been used [9]. These techniques are based on the correct choice of solvents and the use of heat or/and agitation to increase the solubility of the desired compounds. Moreover, these techniques require longer extraction time and as a result cause thermal degradation for most of constituents [10]. Over the past decade, various novel extraction

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techniques have been introduced and investigated, most of which were claimed to be better in terms of efficiency, extraction time, and solvent consumption. The techniques are microwave-assisted extractions (MAE) [8], supercritical fluid extraction (SFE), pressurized solvent extraction (PSE), and ultrasonic extraction (UE) [9].

MAE has drawn significant research attention in various field, in particular medicinal plant research, due to its special heating mechanism, moderate capital cost, and its good performance under atmospheric conditions. MAE is an innovative solvent extraction technology which offers a better alternative to several thermal applications due to its efficient volumetric heat production, and the fact that it has many advantages over CME, such methods improved efficiency, reduced extraction time, lower solvent consumption, higher selectivity toward target molecules, and higher level of automation [7]. In addition, a wider range of solvents can be used in MAE, as the technique which is less dependent on solvent affinity [11].

Several investigations have focused on the natural antioxidants compounds of Myrtle leaves [5, 12]. However, the emphasis has been given to the conventional extraction method. To our best knowledge, no literature report exists on the MAE of natural phenolic contents from different parts of myrtle (stem, pericarp, and seed) with exception of the study by Dahmoune, Nayak [13] in the case of myrtle leaf polyphenols. Since this is the most common form of using the species and it may constitute an interesting dietary source of health protective compounds. Therefore, the aim of the present work is (i) to compare the effects of MAE and CME on the extraction efficiency from different myrtle parts (in terms of TPC, flavonoids, anthocyanins, and condensed tannins) and (ii) to estimate the recovery and the antioxidant capacity of the extracts.

Materials and methods

Chemicals

Sodium carbonate (Na_2CO_3), Folin–Ciocalteau's phenol reagent and disodium hydrogen phosphate (Na_2HPO_4), aluminum chloride ($AlCl_3$) were obtained from Prolabo (Loire, France), and 1,-diphenyl-2-picrylhydrazil (DPPH) from Sigma Aldrich (Germany). Gallic acid, ferric chloride ($FeCl_3{\cdot}6H_2O$), potassium ferricyanide ($C_6N_6FeK_3$), trichloroacetic acid and sodium dihydrogen phosphate (NaH_2PO_4) were purchased from Biochem-chemopharma (Loire, France). All solvents used were of analytical grade.

Plant material and sample preparation

Myrtus communis L. samples were collected at optimal fruit maturity (November, 2012), from Addekar (Bejaia, North East of Algeria; 36° 41′ 32.54″ N, 4° 40′ 21.44″ E; altitude 1092 m). A voucher specimen has been stored in the Plant Ecology Herbarium of Es-Senia University (Oran, Algeria), under voucher number 1856. Leaves, stems, and fruits were isolated manually from the aerial parts. The samples were washed with a tap water then distilled water to remove any adhering soil and dust. Myrtle parts were dried at room temperature in a ventilated darkroom to protect the active compounds from light oxidation. The drying time was about 5, 3, and 7 days for the leaves, stems, and fruits, respectively. Fruit samples were peeled manually and seeds were recovered. All parts were ground with an electrical grinder (IKA modelA11 Basic, Staufen, Germany). The obtained powder was passed through standard 250 nm sieve and only the fraction with particle size < 250 nm was used. The powder was stored in airtight bags until use.

Extraction procedures of phenolic contents

Microwave-assisted extraction

Phenolic contents were extracted using a domestic microwave oven (Samsung MW813ST, Kuala Lumpur, Malaysia) modified with the addition of a condenser, generated during extraction procedure. It operates at a frequency of 2450 MHz and a maximum output power of 1000 W with a 100 W increment. The size of the heating cavity is 37.5 cm (L)×22.5 cm (W)×38.6 cm (D). For the extraction, a volume of 32 mL of ethanol/water (42/58, v/v) was added to 1 g of the powders in flat-bottomed flask. The mixture was irradiated at 500 W for 62 s (optimization conditions) then filtered with a sintered glass at 0.45 μm using a vacuum pump. The obtained extract was stored at 4 °C until further analysis [8].

Conventional method extraction

Regarding the CME, 1 g of each powder was placed in a conical flask, and 50 mL of ethanol/water (42/58, v/v) was added. After stirring for 2 h, the mixture was vacuum filtered. The obtained extract was stored at 4 °C until further analysis [8].

Phytochemical analysis

Total phenolic content

The total phenolic content (TPC) in the extracts was assessed according to the method of George, Brat [14]. Briefly, 500 µL of diluted and filtered extract from the different parts was added to 2.5 mL of 10-fold diluted (v/v) Folin–Ciocalteau reagent. The solution was mixed and incubated at room temperature for 2 min. 2 mL of 7.5 % (m/v) sodium carbonate was added and the solution was then incubated at 50 °C for 15 min. The absorbance of the sample was measured at 760 nm against a blank using a UV-VIS Spectrophotometer (SpectroScan 50, Nkesia, Cyprus). The assay was performed in triplicate. For quantification, a calibration curve was generated with the standard solution of gallic acid (R^2=0.998). The TPC was expressed as mg of gallic acid equivalent (GAE) per gram of dry weight (DW) basis (mg GAE g^{-1} DW).

Total flavonoid content

The total flavonoid content was estimated by the aluminum chloride method according to Quettier-Deleu, Gressier [15], based on the formation of a flavonoid–aluminum complex. Briefly, 1 mL of different extracts was mixed with 1 mL of 2 % (m/v) aluminum chloride. After 15 min of incubation in the dark, the absorbance of the mixture was measured at 430 nm. Each analysis was carried out in triplicate. The total flavonoid content was calculated from a calibration curve made with rutin (R^2=0.997) and expressed as milligrams of rutin equivalent per gram of dry weight (DW) basis (mg RE g^{-1} DW).

Total monomeric anthocyanin content

Total monomeric anthocyanin content was determined by the pH-differential method of Lee, Durst [16], based on the structural change of the anthocyanins chromospheres between pH 1.0 and 4.5. The absorbance was measured at 520 nm and at 700 nm in buffers at pH 1.0 and 4.5 respectively. The concentration of anthocyanins was obtained using equation (eq. 1). Results are expressed on a cyanidin-3-glucoside basis.

$$\text{Anthocyanin pigment (cyanidin-3-glucoside equivalents, mg/g DW)} = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times l} \quad (1)$$

where

$$A = (A_{520nm} - A_{700nm})_{pH1.0} - (A_{520nm} - A_{700nm})_{pH4.5}$$

MW (molecular weight): 449.2 g mol^{-1} for cyanidin-3-glucoside (cyd-3-glu); DF: dilution factor; l: path length in cm; ε: 26 900 molar extinction coefficient, in L × mol^{-1}× cm^{-1}, for cyd-3-glu; and 10^3: factor for conversion from g to mg.

Condensed tannin content

The condensed tannin content was determined by the HCl-Vanillin method as described by Aidi Wannes et al. [17]. One mL of extracts was mixed with 5 mL of reagent (HCl+Vanillin). The mixture was kept in the dark room for 20 min. The absorbance was determined at 500 nm versus a blank. All analyses were performed in triplicate. Total tannins were expressed as mg catechin equivalents per gram of dry weight basis (mg CE•g^{-1} DW) through a calibration curve made against catechin standard (R^2=0.996).

Antioxidant activity

The antioxidant properties of the active compounds are very important due to the deleterious role of free radicals in foods and biological systems [18]. The antioxidant activity of all parts of plant (leaves, steams, pericarp, and seeds of fruits) was evaluated by DPPH• radical scavenging assay and reducing power test. The higher percentage inhibition test rate is the greater the hydrogen donating ability, thus the higher antioxidant activities.

Radical scavenging activity assay

The free radical scavenging activity (RSA) of the extracts was determined using the DPPH• [19]. It is a highly colored free radical that can abstract labile hydrogen atoms from phenolic antioxidants with concomitant formation of a color-less hydrazine (DPPH-H). The free RSA of an extract can be expressed as the percentage of DPPH reduced by a given amount of extract. The RSA was measured, following the method of Dudonné, Vitrac [20]. DPPH• radicals have an absorption maximum at 515 nm [19], which disappears with reduction by an antioxidant compound. A DPPH• solution in absolute methanol (60 M) was prepared, and 3 mL of this solution was mixed with 1 mL of the different extracts. The samples were incubated for 20 min at 37 °C in the dark, then, the decrease in absorbance at 515 nm was measured. The α-tocopherol served as a positive control. All the tests were performed in triplicate, and the inhibition rate was calculated according to (eq. 2).

$$\%Scavenging = \frac{(A_{control} - A_{extract})}{A_{control}} \times 100 \quad (2)$$

where $A_{control}$ is the absorbance of DPPH• at = 0 min; $A_{extract}$ is the absorbance of DPPH•in the presence of the sample at t=20 min.

Reducing power

The yellow color of the test solution changes to green depending on the reducing power of test specimen. The presence of reductants in the solution causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by the measurement of the absorbance at 700 nm [21]. One mL of different extracts was mixed with 2.5 mL of a 0.2 M (m/v) sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1 % (m/v) potassium ferricyanide ($K_3Fe(CN)_6$). The mixture was incubated in a water bath at 50 °C for 20 min. Then, 2.5 mL of 10 % (m/v) trichloroacetic acid was added. Finally, 1 mL of the obtained solution was added to 5 mL of distilled water and 1 mL of 0.1 % (m/v) ferric chloride ($FeCl_3$), the intensity of the blue green color was measured at 700 nm. Tests were carried out in triplicate.

Statistical analysis

The analysis of variance (ANOVA) was performed using XLSTAT release10 (Addinsoft, Paris, France), Tukey's multiple range test (HSD) was used to compare between TPC content and antioxidant activity means as affected by microwave-assisted extraction (MAE) or conventional methods extraction (CME). Principal component analysis (PCA) was performed to detect structure in the relationships between variables, allowing its classification and the separation of each part. All parameters analyzed were used as variables in PCA.

PCA is a multivariate ordination technique used to display patterns in multivariate data. It aims to graphically display the relative positions of data points in fewer dimensions while retaining as much information as possible, and explore relationships between dependent variables. In this study, PCA was applied to the phenolic compounds, flavonoid, anthocyanins, tannin content, and antioxidant activity of different myrtle parts for the two Tunisian cultivars and two factors were selected justifying 68.73 % of total variance

Results and discussion

Phytochemical analysis

Total phenolic content

As one of the most important antioxidant plant components, phenolic antioxidants have been widely investigated in many medicinal plants [22]. Their antioxidant activity is believed to be mainly due to their redox properties [23]. The phenolic content of the different parts of the studied plant was presented in Figure 1. The results showed a clear difference in the distribution of bioactive compounds in all parts, which confirm those reported in the literature [1]. The main significant differences were found in TPC contents among different parts. In fact, leaf extract presented the higher TPC (63.11±0.35 mg GAE g^{-1} DW) compared to that of pericarp and stem extracts. Seed samples presented the lowest TPC (56.32±11.81 mg GAE g^{-1} DW) than the other myrtle parts. These results were in agreement with the finding of Aidi Wannes and Marzouk [5] showing that Tunisian myrtle leaves extract possessed the highest TPC (33.67 mg GAE g^{-1} DW) as compared to that of the stem (11.11 mg GAE g^{-1} DW). In the other hand, Gardeli, Vassiliki [24] showed that Greece myrtle leaves possessed higher TPC (373 mg GAE g^{-1} DW) compared to that found in the present work. However, Aidi Wannes and Marzouk, who quantified TPC of fruits parts from *M. communis* var. L., revealed a content of 23.87 mg GAE g^{-1} DW for seeds and 2.76 mg GAE g^{-1} DW for pericarp fruits, which are low compared to those obtained in the present study. These differences could be due to the cultivar plant, environmental factors, collection period, geographical origin [25], and the methods used for extraction [26].

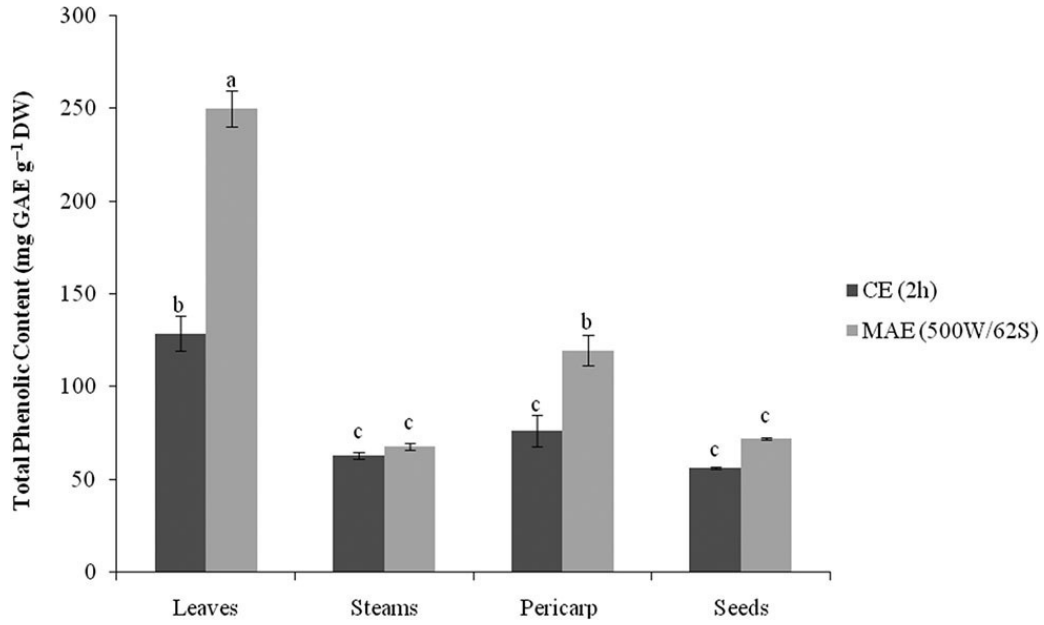

Figure 1: Total phenolic content of different extracts of myrtle aerial parts extracted with microwave-assisted extraction (MAE) and conventional solvent extraction (CME).
Values with different letters (a–c) were significantly different (Tukey, $p<0.05$) for the different aerial parts; the results are ranked in decreasing order: a > b > c.

To evaluate the MAE effects on the extraction efficiency, the amounts of phenolic contents obtained by MAE were comparable to those obtained by CME. The TPC of leaves and pericarp is significantly higher ($p<0.05$) than that obtained by CME. It increased from 128.73±6.84 to 249.86±9.2 mg GAE g^{-1} DW and from 76.38±7.27 to 119.60±8.4 mg GAE g^{-1} DW, respectively. However, stems and seeds have denoted no significant difference in their concentration (67.89±1.73 mg GAE g^{-1} DW, 72.06±0.81 mg GAE g^{-1} DW, respectively).

The high TPC obtained by MAE may be explained by the exposure of plant cells to microwave field. The dried plant material used for extraction contains traces of moisture and as microwave energy is absorbed and subsequently converted into heat, the moisture begins to evaporate. The vaporization of water generates pressure within the cell wall that eventually leads to cell rupture, thereby facilitating the leaching out of active constituents into the surrounding solvent and improving extraction yield [27]. To verify this fact, scanning electron microscopy (SEM) was employed by several authors to study the mechanism of MAE [28]. Dahmoune, Nayak [13] had treated myrtle leaves by scanning electron microscopy (SEM) after MAE, they showed that microwave heating caused a higher cellular damage helping the rapid release of solutes into the solvents and enhancing

the well-known main heating effect of microwaves. These results confirm that microwave radiations have a destructive effect on extraction sample matrix and the rapid extraction occurred when the active compounds elute and dissolve in solvent once the cell is ruptured.

Total flavonoids content

As can be seen in the Figure 2, flavonoid distribution within myrtle depends on the analyzed part. The highest values of flavonoid contents were observed in leaves and pericarp extracts, followed by seeds. However, the lowest flavonoid content was found in stem extract (0.74±0.01 RE g^{-1} DW). This finding is in contrast with the results of Aidi Wannes and Marzouk [5] who reported also that the highest values of flavonoids are in fruit pericarp. Compared CME with MAE, the yield increase in all parts namely in leaves (from 9.14±0.05 to 13.65±0.09 mg RE g^{-1} DW) and pericarp (6.95±0.20 to 11.50±0.26 mg RE g^{-1} DW).

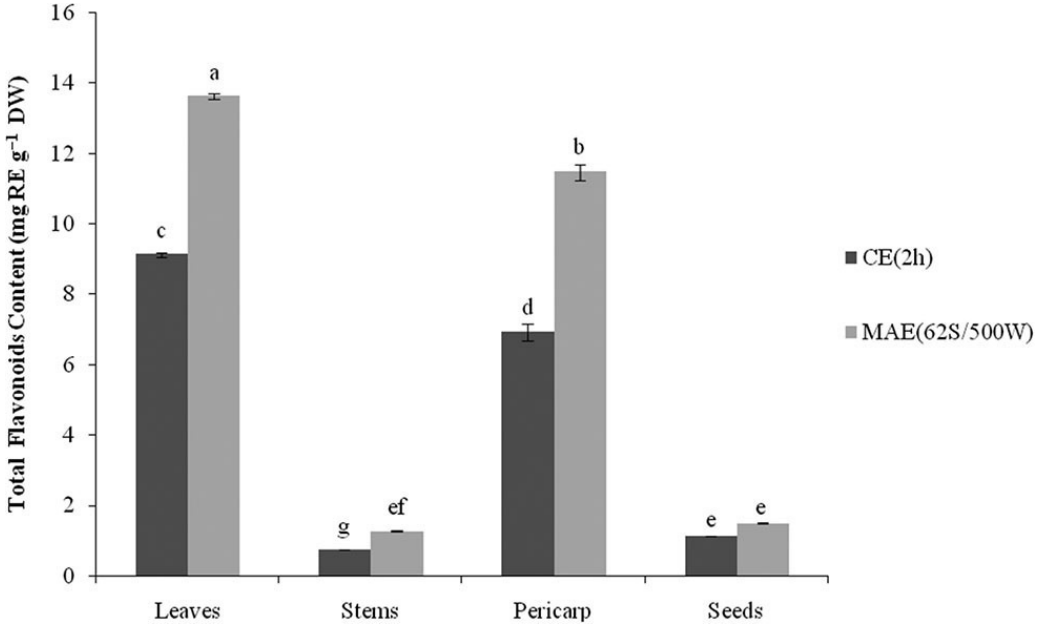

Figure 2: Total flavonoids content of different extracts of myrtle aerial parts extracted with microwave-assisted extraction (MAE) and conventional solvent extraction (CME).
Values with different letters (a–g) were significantly different (Tukey, $p<0.05$) for the different aerial parts; the results are ranked in decreasing order: a > b > c > d > e > f > g.

Total anthocyanin content

The distribution disparity of anthocyanin content was also found among different parts. Anthocyanins are the most important phytochemicals in myrtle plant and play an important role in its organoleptic properties [29]. Anthocyanins were predominant in pericarp extract (135.26±3.66 mg Cyd-3-glu g^{-1} DW) than in seeds (9.79±1.99 mg Cyd-3-glu g^{-1} DW). The lowest contents were observed in stem and leave extracts (1.00±0.13 mg Cyd-3-glu g^{-1} DW and 1.32±0.16 mg Cyd-3-glu g^{-1} DW, respectively) (Figure 3). These results are in agreement with those reported by [30]. The high anthocyanins content in myrtle pericarp and seeds could be explained by their increase during repining that is related to the change in the color of fruits surface from yellow-white to dark-blue. The anthocyanins concentration of microwave extracts is significantly different ($p<0.05$), they were higher than that obtained by CME, mainly in the pericarp extracts (176.50±2.17 mg Cyd-3-glu g^{-1} DW) than in the seeds (0.31±0.10 mg Cyd-3-glu g^{-1} DW). The farmer results agreed with those found by Jia et al. 2010, who report that microwave extraction increases the yield of anthocyanins compounds.

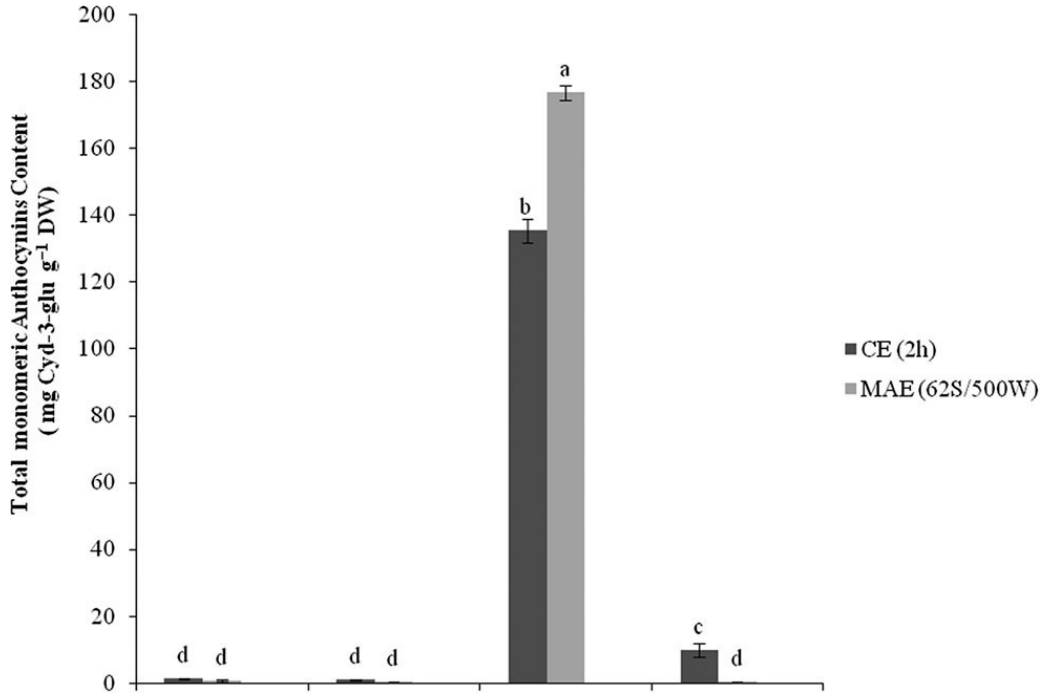

Figure 3: Total monomeric anthocyanin's content of different extracts of myrtle aerial parts extracted with microwave-assisted extraction (MAE) and conventional solvent extraction (CME).
Values with different letters (a–d) were significantly different (Tukey, $p<0.05$) for the different aerial parts; the results are ranked in decreasing order: a > b > c > d.

Total tannin content

The same discrepancy was also observed for condensed tannin content. In fact, tannin contents were higher in fruit pericarp, it is about 220.81±1.21 mg CE g^{-1} DW, while other parts presented a lower value, they were about 22.14±0.26; 35.74±0.26 and 36.01±0.20 mg CE g^{-1} DW for seeds, stem, and leaves extracts, respectively (Figure 4). Fruits are very astringent and are used as a condiment, a substitute for pepper, and considered as a rich source of tannins [31]. This result was in agreement with the work of Aidi Wannes and Marzouk [5] who studied the methanolic extract of the fruit from Tunisian myrtle, they reported that condensed tannin content was relatively low in pericarp and seeds, while the highest concentration was found in the whole fruit extract (0.96 mg CE g^{-1} DW). Compared CME with MAE, higher tannins content was observed in the pericarp (333.77±1.85 mg CE g^{-1} of DW). These results were in agreement with those found by Dahmoune, Nayak [13] and Jia, Dong, Dong [29] who report that microwave extraction increases the yield of tannin compounds from myrtle leaves.

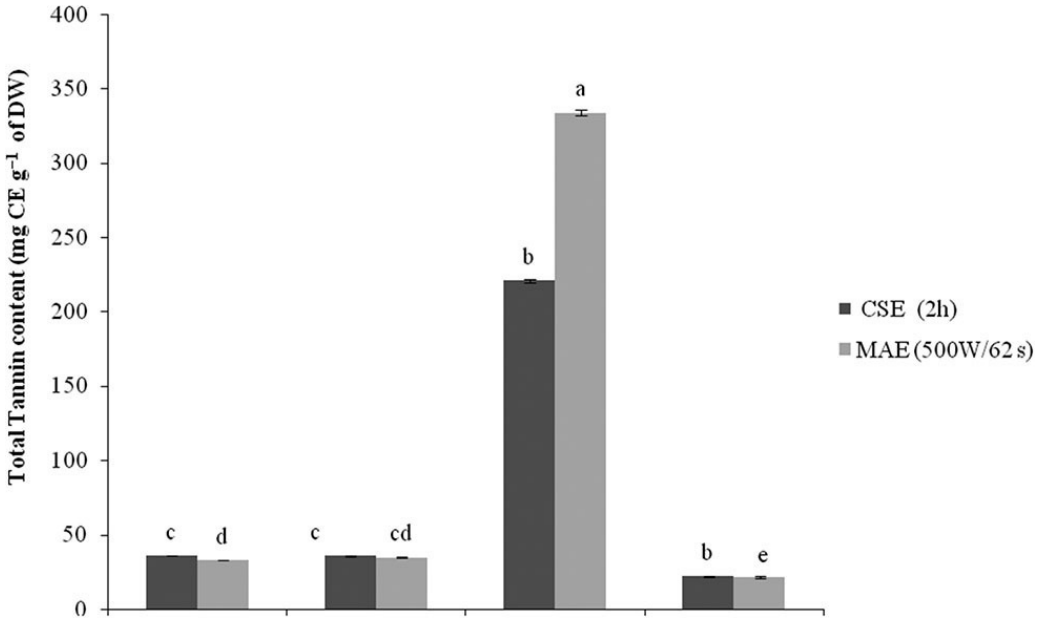

Figure 4: Total tannin content of different extracts of myrtle aerial parts extracted with microwave-assisted extraction (MAE) and conventional solvent extraction (CME).
Values with different letters (a–e) were significantly different (Tukey, $p<0.05$) for the different aerial parts; the results are ranked in decreasing order: a > b > c > d > e.

The results of MAE at 500 W/62 s show that the amount of phenolic contents of leaves (63.15 %), stems (9.04 %), pericarp (43.73 %), and seeds of fruits (24.53 %) was higher than that obtained conventionally (1:50) for 7200 s. This results are in accordance with those reported in the literature [32]. Several authors reported the advantages of MAE compared to CME, such as reduced process time, lower solvent, energy demand, and higher yield [33, 34].

Conventional solvent extraction without microwave assistance is a time-consuming process that uses heat to increase the mass transfer rate of the extraction system [33]. The reduction of extraction time was due to the heating mechanism of microwave. It offers a rapid transfer of energy to the extraction solvent and raw plant materials [35]. A significant increase was obtained for the MAE as compared to that of the same sample extracted using the CME method. In addition, extraction time was significantly reduced in microwave extraction. Thus, microwave extraction method can be recommended for leaching phenolic compounds from myrtle.

Antioxidant activity

The amount of such compounds in each part of the plant is usually different. Myrtle was a source of natural antioxidants because of the activity of secondary metabolites, such as phenylpropanoids and essential oils. In the present study, the antioxidant activity of ethanolic extracts obtained by CME and MAE of myrtle leaf, stem, pericarp, and seeds of fruits was determined by measuring the free RSA ($DPPH^{\bullet}$) and the reducing power.

The effect of antioxidant on $DPPH^{\bullet}$ scavenging was conceived to their hydrogen donating ability [34]. The $DPPH^{\bullet}$ scavenging ability of the ethanolic extracts of myrtle parts was higher than that of α-tocopherol ($p<0.05$). The greatest antioxidant activity of the different parts of the studied plant was obtained in leaves extract (94.78±0.37 %) which is similar to that obtained by Ferchichi et al. [36] with a higher level in leaves of myrtle black fruit (86.54 %). The inhibition effect of $DDPH^{\bullet}$ radical by antioxidant from stems is about 88.72±0.65 %. The antioxidant activity of seeds was higher (88.41±0.64 %) than that of pericarp (88.03±0.37 %). Same results were reported by Aidi Wannes and Marzouk concerning the Tunisian myrtle fruits. Thus, it has been reported that free RSA is greatly influenced by the phenolic composition of the extract. The HPLC analysis of the phenolic contents of different fruit parts showed that the seed was rich in hydrolysable tannins, which were absolutely absent in the pericarp [5]. Additionally, Aidi Wannes and Marzouk [5] reported also that leaves were rich in hydrolyzable tannin. According to Yoshimura et al. [37], these hydrolyzable tannins exhibited a strong antiradical activity compared to other compounds such as gallic and quinic acids.

Concerning the reducing power activity, the presence of reductants (antioxidants) in the samples would result in the reduction of the Fe^{3+} ferricyanide complex to its ferrous form (Fe^{2+}) by donating an electron. Hence, the Fe^{2+} can then be monitored by measuring the formation of Perl's Prussian blue at 700 nm [38]. Higher absorbance value indicates higher reducing power [39]. The results in Table 1 showed the Fe^{3+} reducing power

ability of ethanolic extracts from different parts. Leaves extracts had higher reducing power than pericarp ones. However, stems and seeds exhibited moderate reducing capacity.

Table 1: Antioxidant activity of different extracts of myrtle aerial parts extracted with microwave-assisted extraction (MAE) and conventional solvent extraction (CME).

Extraction Methods	Plant parts	DPPH•, %	Reducing Power, Abs
MAE	Leaf	88.35±0.47 [a]	0.661±0.002[b]
	Stem	87.09±0.28[b]	0.301±0.003[e]
	Pericarp	87.16±0.28[b]	0.439±0.006[d]
	Seeds	88.09±0.28[ab]	0.308±0.002[e]
CSE	Leaf	94.78±0.37[a]	0.865±0.001[a]
	Stem	88.72±0.65[ab]	0.406±0.0001[d]
	Pericarp	88.03±0.37[ab]	0.426±0.001[c]
	Seeds	88.41±0.64[ab]	0.442±0.0003[d]

All the values are mean±SD; SD, standard deviation. [a–e]Column wise values with same superscripts of this type indicate no significant difference ($p<0.05$).

The results shown in Table 1 suggest that the content of phenolic compounds can play a major role in the antioxidant activity of all extracts. Phenolic contents are the antioxidants that contribute to the high antioxidant capacity observed in different parts of plants [40]. Effectively, leaves extracts showed a higher antioxidant activity than other extracts, which correlated with its highest content of phenolic compounds. The different values of antioxidant activity obtained with each extract parts can be ascribed to their different chemical compositions [41]. MAE extraction gives the higher values of DPPH• scavenging capacity in leaves followed by those of seeds, pericarp than stem. The same tendency was observed using reducing power test.

Despite the high levels of phenolic contents obtained by microwave extraction, no correlation was observed between antioxidant activity and total phenolic content. Similar results were reported in the literature [42]. Furthermore, the study of Chiang et al. [43] reported that high TPC was not always correlated with high antioxidant activity. The type of extraction can explain this discrepancy, because the microwave radiations have an effect on the structure of phenolic compounds [11, 44]. The chemical nature affects also the content of polyphenols [26]. In addition, Hayat et al. [45] have reported that microwave irradiations could induce free radicals formation within the liquid medium, thus causing oxidation and degradation of the active compounds and the high phenolic content causes the association of the latter thereby preventing the DPPH• radical to access these compounds to induce antiradical activity. However, there is a correlation between DPPH• radical scavenging activity assay and reducing power test (r=0.11).

PCA analysis

PCA is a multivariate ordination technique used to display patterns in multivariate data. It aims to graphically display the relative positions of data points in fewer dimensions while retaining as much information as possible, and explore relationships between dependent variables. In this study, PCA was applied to the phenolic compound; flavonoid, anthocyanins, tannin contents, and antioxidant activity of different myrtle parts and two factors were selected justifying 75.19 % of total variance. PC1 explained 46.32 % of the total variance in the dataset while PC2 explained 28.87 %.

The sample score plot for PC1 vs. PC2 is shown in Figure 5. Samples were located in four different plots, indicating their composition, and repartition part. The position of each variable in this loading plot describes its relationship to the other variables. Figure 5 shows four distinctive groups. The first and second groups are comprised of pericarp and leaves myrtle respectively, which are ported positively by PC1. The third group shows the positive correlation between CME leaves and DPPH, and reducing power test. The last group seed pericarp are ported negatively by PC1. Using the plots in Figure 5, it is possible to restart all phenolic compounds in myrtle part and to selected the adequate method of extraction. Phenolic compounds of pericarp are represented mainly by tannins 62.8 %, which are better extracted by MAE. In the other hand, microwave extraction of the TPC and flavonoids content in leaves was selected 72.2 % and 80.4 %, respectively. Concerning antioxidant activity, the DPPH and reducing power test were correlating positively with phenolic compound leaves obtained by CME. The anthocyanin compound was represented mainly in seed of fruit with a higher yield obtained by CME (56.2 %) in seeds pericarp and stem. The results of this study revealed the importance of comparing and exploring the variance of phenolic compound from different myrtle parts and extraction method.

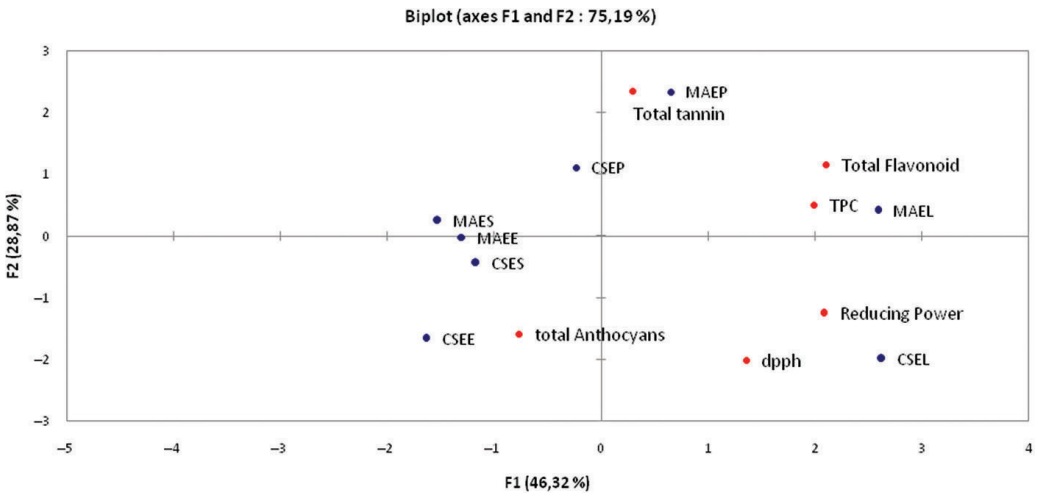

Figure 5: Principal component analysis of myrtle part samples based on the main important factors (MAE, CME, leaves, steams, pericarp, and seeds).

Conclusions

Myrtle aerial parts are potential source of active natural substances such as phenolic compounds. MAE was found to be highly effective enabling a considerable reduction in extraction time (62 s against 7,200 s), and the efficiency of extraction of phenolic contents from all myrtle parts was improved in comparison with the CME method with an increase of TPC but with antioxidant activity similar to those of CME. This showed great potential for industrial application in the near future.

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Summary:

Myrtus communis is one of the important aromatic and medicinal species from the Myrtaceae. Different parts of the plant have found various uses in the food industry, such as, in the cosmetic and pharmaceutical. The current study investigated the capability of microwave and ultrasound-assisted solvent extraction (MAE and UAE) and microwave assisted –ultrasound pretreatment (MD-AUP) in improving the extraction and n of bioactive phenolic compounds. The use of microwave, ultrasound in the pharmaceutics industry present numerous advantages, such as productivity, decrease of the costs and environmental impact, resulting in the release of better natural bioactive compounds. The first part of this study, a complete picture of current knowledge on *Myrtus communis* plant, its distribution, composition and its use in the pharmaceutical industries are described. In addition, it provides the necessary theoretical background and some details about extraction by microwaves, ultrasound, the mechanism, some applications, and environmental impacts. The second part consists of three distinct chapters. Firstly, show the comparison of the effects of MAE and CME on the extraction efficiency from different myrtle parts in terms phenolic compounds, and to estimate the recovery and the antioxidant capacity of the extracts. The second chapter consist on the optimization of UAE process parameters using a RSM to maximize the content of extracted phenolic. From the acquired knowledge, with these empirical approaches of UAE optimized methodology, it is possible to make a qualitative and quantitative comparison between these methods (microwave and ultrasound) and with one traditional extraction. In the chapter three, the evaluate the effect of ultrasound pre-treatments on pericarp drying was investigated. The influence of pre-treatments on water loss, total phenol content and their antioxidant activity were analyzed. The comparison between the microwave drying assisted by ultrasound pretreatment, conventional and microwave drying was also investigated. The purpose behind this generalization approach is, to offer ultimately , a drying process and antioxidant separation method, green simple, fast and effective (Green separation).

Résumé:

Myrtus communis est l'une des principales espèces aromatiques et médicinales des Myrtacés. Les différentes parties de la plante ont trouvé diverses utilisations dans l'industrie alimentaire, telles que, dans le domaine cosmétique et pharmaceutique. Le présent travail étudié la capacité de l'extraction au solvant assisté par micro-ondes et aux ultrasons (EAM et EAU) et le séchage par micro-ondes assisté par un prétraitement aux ultrasons (SM-APU) dans l'amélioration de l'extraction et de la nature des composés phénoliques bioactifs. L'utilisation des micro-ondes, des ultrasons dans l'industrie pharmaceutique présente de nombreux avantages tels que la productivité, la diminution des coûts et l'impact environnemental, ce qui entraîne la libération de meilleurs composés bioactifs naturels. La première partie de cette étude, montre une description complète des connaissances actuelles sur la plante de *Myrtus communis*, sa distribution, sa composition et son utilisation dans les industries pharmaceutiques. En plus des généralités nécessaires et certains détails sur l'extraction et le séchage par micro-ondes, ultrason, le mécanisme, certaines applications et les impacts environnementaux. La deuxième partie comprend trois chapitres distincts. Le premier étudié la comparaison entre EMA et conventionnelle extraction CE sur l'efficacité d'extraction des composé phénoliques de différentes parties de myrtes et leur capacité antioxydante. Le deuxième chapitre consiste à optimiser les paramètres des processus des EAU en utilisant un plan d'expérience pour maximiser le contenu du phénolique extrait. À partir des connaissances acquises, avec ces approches empiriques de la méthodologie optimisée des EAU, il est possible de faire une comparaison qualitative et quantitative entre ces méthodes (micro-ondes et ultrasons) et avec la méthode d'extraction traditionnelle. Dans le chapitre trois, on a évalué l'effet des ultrasons sur le séchage des fruits du mythe par microonde. L'influence des ultrasons sur la perte d'eau, la teneur totale en phénol et leur activité antioxydante ont été analysées. La comparaison entre le séchage par micro-ondes assisté par prétraitement aux ultrasons, séchage conventionnel et à micro-ondes a également été étudiée. Le but de cette approche de généralisation est, en définitive, un procédé de séchage et une méthode de séparation des antioxydants vert, simple, rapide et efficace (séparation verte).

الملخص

االريحان هي واحدة من الأنواع العطرية والطبية الرئيسية .الدراسة الحالية التحقيق في قدرة الميكروويف وبمساعدة الموجات فوق الصوتية الاستخلاص بالمذيبات والميكروويف ساعد المعالجة في تحسين استخراج النشطة بيولوجيا . استخدام الميكروويف والموجات فوق الصوتية في صناعة المستحضرات الصيدلانية هذا العديد من المزايا، مثل الإنتاجية وانخفاض التكاليف والآثار البيئية الناجمة عن ذلك وفي الإفراج عن أفضل المركبات النشطة بيولوجيا الطبيعية. الجزء الأول من هذه الدراسة، ا نبات، وتوزيع وتكوينها وموصوفة استخدامه في الصناعات الدوائية. وبالإضافة إلى ذلك، فإنه يوفر الخلفية النظرية اللازمة وبعض التفاصيل عن استرجاع بواسطة الميكروويف والموجات فوق الصوتية، وآلية، وبعض التطبيقات والتأثيرات البيئية؛ وبالإضافة إلى ذلك، تم تنفيذ مجموعة من التجفيف الميكروويف مع الموجات فوق الصوتية الموجات فوق الصوتية التي تظهر على أنها طريقة المعالجة البديلة للتجفيف، يتكون من ثلاثة فصول منفصلة. أولا، يقارن بين آثار على كفاءة الاستخراج المركبات مختلفة في المركبات الفينولية الفصل الثاني تثبت شروط استخراج لمركبات باستعمال الموجات فوق صوتية ا لتحقيق أقصى قدر من المحتوى من الفينولية المستخلصة. . الفصل 3، تقييم تأثير الموجات فوق الصوتية تجفيف النبات. و المقارنة بين تجفيف الميكروويف بمساعدة الموجات فوق الصوتية، مع التجفيف التقليدية والميكروويف . الهدف م هذه الدراسة هو تقديم نهاية المطاف، عملية التجفيف وطريقة فصل المضادة للأكسدة، بسيطة وسريعة وفعالة