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Recent advances in the identification and the study of composition and activities of medicinal plants

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ABSTRACT

Medicinal and aromatic plants are the foremost source of life economy drugs for the majority of the world's population. Secondary plant metabolites are essential for life as drugs, fragrances, pigments and food additives. Botanical study of plants is an inevitable step in the ethno-pharmacological approach and it is the reference system since it allows sitting scientifically research that follows from of these new concepts. So, the purpose of many scientists is to advance a new approach useful for identifying plants within a folk pharmacopoeia which deserve the increased research attention of phytochemists and pharmacologists. In the objective to study medicinal plants, usually we start by extraction of the bioactive compounds, which is an essential stage. So, new important extraction processes were elaborated to recover bioactive constituants from herbal plants by augmenting the yield at inferior fee. Recent approachs and methods used in isolation and characterization of active compounds from herbal preparations are also given according to their important roles. Besides, in the present work, an update on some biological activities properties of medicinal plants is reviewed.

1. Introduction

Traditional medicine can be defined by the sum total of the knowledge, skills and practices based on theories, beliefs and experiences indigenous to different cultures that are used to maintain and improve health, as well as to prevent, diagnose, and treat physical and mental illnesses[1]. Nowadays, there is a renewed interest in drugs of natural origin simply because they are considered as green medicine which is always supposed to be safe in addition to their ease of availability with affordable prices. Another factor which emphasizes the attention to the incidences of harmful nature of synthetic drugs which are regarded as harmful to human beings and environment[2].

It is estimated that 70%–80% of people worldwide rely mainly on traditional, largely herbal medicines to meet their primary healthcare needs. The global demand for herbal medicine is not only large, but also growing one[3]. In recent years, these medicinal herbs are much studied since their great employ in traditional medicine to treat regular ailments like gastrointestinal disorders, cold, fever and at present, other medicinal claims hold up with reliable scientific confirmations[4].

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Complementary researches are needed to study plants with healing properties. After the harvesting and the botanical identification, it is necessary to extract, identify, isolate and purify the compounds of various chemical groups found in plants and study their relative position in the treatment of different pathological conditions (Figure 1).

The botanical identification plays an essential role in many domains. Any scientific publication based on a phytochemical study requires precise identification of the studied plant. It is the same for any food or artisanal application of a plant, any recovery of its use among the population, but also for the preservation of biodiversity[3]. The people, by their experience and empirical knowledge transmitted through previous generations, recognize medicinal plants from their natural environment by different aspects: smell, flower color, leaf shape, location, season, *etc*. However, there is the risk of confusion by the population[5]. At present, it is still indispensable to form specialized botanists in flora from different regions and in all cases, accurate identification of plants is of course essential.

After the botanical identification, the study on medicinal plants is started with extraction procedures that play a critical role to the extraction outcomes (*e.g.* yield and phytochemicals content) and also to the consequent assays performed. A wide range of technologies with different methods of extraction is available nowadays[4]. This review describes the frequently employed techniques of extraction with models in latest years such as microwave-assisted extraction (MAE), ultrasound-assisted

extraction (UAE) and supercritical fluid extraction (SFE).

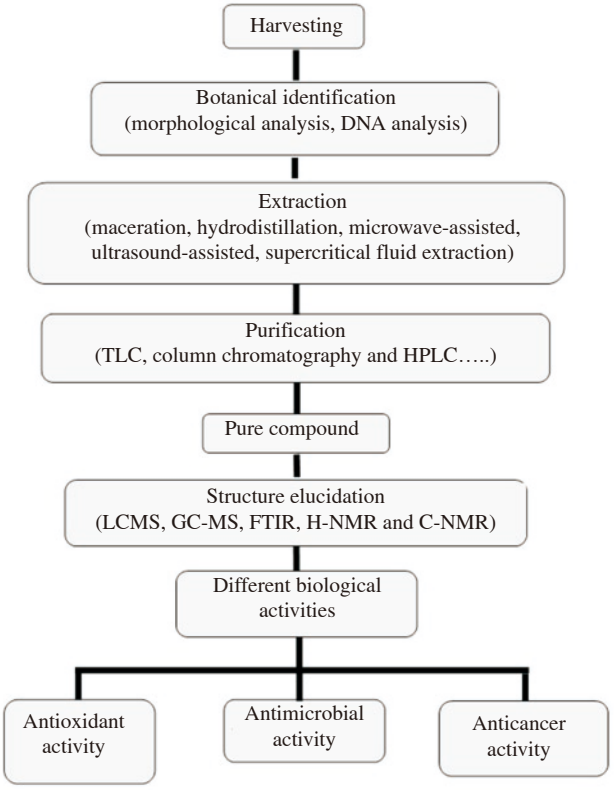

Figure 1. A brief summary of the general approaches in study of medicinal plants.

TLC: Thin layer chromatography; HPLC: High performance liquid chromatography; LC-MS: Liquid chromatography-mass spectrometry; GC-MS: Gas chromatography-mass spectrometry; FTIR: Fourier transform infrared spectroscopy; H-NMR: H-nuclear magnetic resonance; C-NMR: C-nuclear magnetic resonance.

The analysis of bioactive compounds presents in the plant extracts involving the applications of common phytochemical screening assays, chromatographic techniques such as TLC, HPLC, ultra-high performance liquid chromatography (UHPLC), hydrophilic interaction liquid chromatography (HILIC), gas chromatograph (GC), *etc*. as well as nonchromatographic techniques such as spectrophotometric techniques [infrared (IR), nuclear magnetic resonance (NMR), mass spectrometry (MS), *etc*.] and capillary electrophoresis (CE). Therefore, this review is to portray and contrast the major frequently employed techniques established on their rules to assist assessing the appropriateness and economic achievability of the techniques.

Extraction, separation and identification of bioactive constituents from herbal reserves are followed by pharmacological screening. The antioxidant, antimicrobial and anticancer activities are the most valued biological properties of plant extracts. This review provides the most recent methods used to study these activities.

2. Harvesting and identification of medicinal plants

2.1. Harvesting

Herbal medicine is used worldwide and all cultures of plants from ancient times to the present day are used as a source of medicines[6,7]. Due to the rising claim for the herbal therapies, and fragmentation of their natural habitats, many of the medicinal plants are invalidly collected and extremely endangered[8]. In addition, over 90% of the medicinal plants traded are harvested from the wild and most of them are faced with an unsustainable manner and around 1 000 species are estimated to be facing various degrees of threat throughout the world[9]. The durable accessibility to medicinal herbs is also imperative, for the practitioners and for the conservation of native healing information. Accordingly, it is important to know the general rules on the sustainable collection of medicinal plants in ways that preserve these plants and environment[8,7]. Medicinal plants should be harvested sustainably with the pay of attention for the collection time, requested plant parts, collection methods, collection utensils and conditions of conservation. It is also important to recognize the season and the instance of day. In general, the aboveground parts of plants are best to harvest in the spring and summer, before or during flowering. Roots are best to harvest early in spring or late in the fall. The ideal time of day for harvesting is after the morning dew has evaporated, and before the full strength of the sun has potentially wilted the plant in late afternoon. A careful storage of the plant materials should also be undertaken to avoid dehydration and fermentation and to collect medicinal herbs in the best likely climatic circumstances (sunny day). However, it is not suggested to collect herbal parts which are infected with fungus and insects or sprayed with pesticides, herbicides or fertilizers[6,8].

When the plants are in optimum conditions, some specific recommendations for harvesting different parts of medicinal plants (root, bark, leaf and fruit) are necessery. Concerning root harvesting, it is suggested to only collect the lateral ones, because if several plants are deracinated, they will finally exist. The leaf collection is considered not destructive but it is recommended to pluck individual leaves as a substitute of leaf striping. It is also important to collect only some leaves which cannot sever the branches and twigs. For the fruits, it is suggested to collect them only from some trees and in order to permit of the high-quality fruits to germinate and it's recommended to do not collect all good quality ones. Subsequent to collection, the suitable processing must be undertaken, counting visual examination, removal of unwanted materials and contaminants, cleaning and wounding before passing to drying and storing[6,8].

For handling and storage purposes, reducing the water content of freshly harvested medicinal plants is imperative and can avoid microbial degradation. The methods available for drying medicinal plants can be grouped into natural and artificial drying on the basis of heat source or energy utilization[6]. Then, the dried plants should be packed in bags or containers, allowing air exchange. The main aim of storing medicinal plants is also to prevent the deterioration of their quality, reduce metabolic activity and render the medicinal plants less susceptible to deterioration. This can be achieved by either reducing the moisture content to a safe level or cooling the plants or by modifying the atmospheric condition of the system where the medicinal plants are stored. This is done also through the control of moisture and air movement[6,8].

2.2. Identification of medicinal plants

Some methods can be used to rigorously identify medicinal plants and to ensure the effectiveness and lack of toxicity of an herbal remedy. In addition to the identification of plant both botanically and genetically, a series of analyzes exist for a quality control in order to aid the differentiation and identification of medicinal plants. These methods consisted of (i) a study of macromorphological and micromorphological characters of different parts of the plant (leaves, fruits, seeds, stems, barks, *etc*.), (ii) genetic profiling (DNA fingerprint, evaluation of chloroplast and nuclear DNA barcodes), and (iii) protein profiling.

After taking into account recommendations of the harvest,

herbarium can be used for easy identification of samples. Specimens for morphological studies must be dried according to standard herbarium techniques. Then, voucher samples were kept in the herbarium and photographs were taken using digital camera.

Anatomical investigations were performed on cross-sections of the root, young and old stems, leaves, fruits and seeds which were preserved in 70% alcohol containing 5% glycerol and on air-dried finely powdered samples. For the DNA isolation, freeze-dried whole young small leaves were ground in liquid nitrogen to fine powder[10,11].

2.2.1. Botanical study

In botanical study, macroscopic examination (morphological studies) and microscopic examination were used. Macroscopic examination focuses specifically on morphological characteristics and is used to observe all criteria of the plant: morphology, color, flavor, size of leaves, barks, the underground parts (roots and rhizomes), fruits and different parts of the plant. Measurements were taken on well developed parts of plants[10].

For the rods, the review focuses on the shape, color, the presence or absence of hair, the implantation of the leaves and the presence of knots. While for the leaves, it is necessary to dwell on the color, the general shape, more or less pronounced ribs, the edge of the sheet, the presence or absence of fuzz and the presence of petiole. Concerning the fruits and the seeds, the shape, size, flavor and color were examined. Finally, the flowers, the bracts and petals are the determinants. Then, the plants have been documented and the photographs were taken using a digital camera to both the general appearance and morphological details (Figure 2)[12].

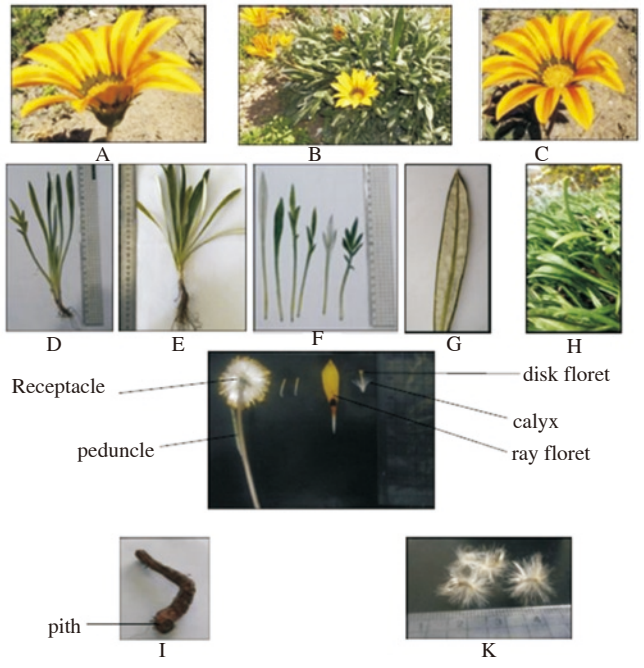

Figure 2. Photographs of different parts of *Gazania longiscapa* (Asteraceae).

A: The flower (0.4×); B: Whole plant (0.1×); C: Top view of the flower (0.4×); D and E: Photos showed the insertion of leaves (0.1×); F: Leaves showed variable lamina (0.2×); G and H: Leaves showed revolute margins (0.5×), disk and ray floret (0.5×), I: The rhizome (0.2×); K: The seed (1.5×)[12].

Recent advances on microscope technology and corresponding improvements in computing increased the accuracy and capabilities of microscopy as a mean of botanical identification. The histological characterization can be employed as a fast and cheap method to the botanical control by microscopic obeservations. Nevertheless, it needs extremely qualified persons and a standard references libraries accessible for comparison are limited. Polarizing microscope, phase contrast microscope, light microscope (LM), and scanning electron microscope (SEM) are the most important microscopes used for the pharmacognostic evaluation[13].

LM adjusted to finest resolution was used in anatomical investigations to view the cross-sections of roots, young and old stems, leaves, fruits and seeds which were preserved in 70% alcohol containing 5% glycerol and on air-dried finely powdered samples. Microphotographs were obtained using digital camera focused through the microscope eyepiece[11]. The examination of micromorphological characters by LM comprises the preparation of slide momentary mounts with slim pieces of plant parts and soaked mounts of grinded plant materials[13]. According to the study of Ismail *et al*., *Farsetia aegyptia* Turra is characterized microscopically by the presence of myrosin cells and non-glandular branched unicellular two-armed hairs in the stems, leaves and fruits. While the root showed sclereids with a wide or narrow lumen and pitted lignified walls[11].

However, the images obtained by SEM possess high resolution and are three-dimensional. So, this method was the most exploitable to examine the surface topology of plant materials, hence it has a very important function in diagnosis of whole botanicals and in fragmentized or powdered type. The classical steps are used in the preparation of the samples for analysis[13,14]. The mainly pertinent histological features by which each herbal drug may be identified, predominantly inside the morphological group to which it fit in, *i.e.* leaf, flower, stem, root and rhizome, bark, fruit and seed. Some botanical characteristics of different kinds of plant tissues and stromata (epidermal trichomes, papillae, as well as the size, shape of cells and cell contents like calcium oxalate crystals) are particularly significant for the botanical identification[13].

As exemple, micromorphological properties of *Nigella* seeds (Ranunculaceae) were investigated by using LM and SEM. Segregation was performed micromorphologically. Studied *Nigella* taxa have a diverse macro- and micro-morphological characters that are utilized to separate them from each other to assess the systematics of *Nigella*[15].

2.2.2. Genetic profiling (DNA fingerprinting)

The DNA fingerprinting was performed using random amplified polymorphic DNA technique to facilitate differentiation between plants belonging to the same genus, even between varieties[10]. Whole fresh leaves of the plant under investigation were lyophilized and ground to a fine powder under liquid nitrogen prior to extraction of DNA plant materials[11]. The collected fresh leaves can be immediately stored in ziplock bags with silica gel and stored at 80 °C to the laboratory for extraction of DNA[16].

DNA can be extracted from 10 g of leaf tissue in 1.5 mL microcentrifuge tubes using the DNA extraction method described by Williams *et al*.[17].

Three molecular markers techniques were performed (i) random amplified polymorphic DNA-PCR analysis (randomly amplified polymorphic DNA) was given as exemple, oligonucleotide primers used in the method described by El Kady *et al*.[12]; PCR amplification was described using six random deca-mer arbitrary primers synthesized by Operon Biotechnologies, Inc. Germany, with the following sequences OP-A09: 50 GGGTAA CGC C 30; OP-B09: 50 TGG GGG ACT C 30; OP-B11: 50 GTA GAC CCG T 30; OP-C04: 50 GAT GAC CGC C 30; OP-C19: 50 GTT GCC AGC C 30; OP-M01: 50 ACG GCG PCR reactions were performed in polypropylene tubes according

to Williams *et al*.[17]; (ii) inter simple sequence repeat (ISSR)-PCR analysis: according to El Kady *et al*.[12], PCR amplification was performed using six ISSR primers synthesized by Operon Biotechnologies, Inc. Germany, with the following sequences HB-08: 50 GAG AGA GAG AGA GG 30; HB-10: 50 GAG AGA GAG AGA CC 30; HB-11: 50 GTGTGT GTG TGT TGT CC 30; HB-12: 50 CAC CAC CAC GC 30; HB-15: 50 GTG GTG GTG GC 30. PCR was done according to the method described by Williams *et al*.[17], and (iii) start codon targeted-PCR amplification: PCR was performed in polypropylene tubes according to Williams *et al*.[17]. The effectiveness of start codon targeted, directly amplified minisatellite DNA and ISSR markers was quite similar in finger printing of genotypes and might be employed to identify polymorphism for genotypes of chick pea (*Cicer arietinum*)[18].

The evaluation of chloroplast and nuclear DNA barcodes was also used. Species of *Terminalia* belong to four sections and identification of species within the sections is considered to be complex due to the lack of sufficient taxonomical characters and the existence of morphotypes. Therefore, Nithaniyal and Parani tested the effectiveness of three chloroplast DNA barcodes (*rbc*L, *mat*K and *trn*H-*psb*A) and a nuclear DNA barcode (*ITS2*) for the discrimination of *Terminalia* species[19]. A DNA barcode reference library consisting of 120 DNA barcodes from ten species of *Terminalia* was created. Intra-specific divergence was not observed among the accessions for any markers. Inter-specific divergence of *trn*H-*psb*A (10.6%) was the highest, followed by *ITS2*, *mat*K and *rbc*L markers. The success of species differentiation by DNA barcodes was 100% with *trn*H-*psb*A, 80% with *mat*K and *ITS2*, and 10% with *rbc*L. In the phylogenetic trees, the *rbc*L marker did not differentiate the species in any sections. Two species from the section catappa were not differentiated by *mat*K and *ITS2* markers. Only *trn*H-*psb*A resolved all the species and ranked the best among four markers for species identification. However, regarding species relationship studies, *ITS2* was found to be better than other markers because it formed a separate clade for each section[19].

2.2.3. Protein profiling

Sample preparation of protein extraction was conducted by separately mixing 0.2 g of powdered plant tissue of each species with 1 mL sample buffer [tris borate solution (pH 8.2) (1:3 v/v)]. The slurry was centrifuged at 6 000 r/min for 10 min. The supernatant containing the protein extract was used immediately for electrophoresis. The proteins of *Gazania longiscapa* and *Gazania rigens* were analyzed using continuous polyacrylamide gel electrophoresis[12].

The protein profiles of both *Gazania* species were studied and the observed protein banding profile was shown. Protein band patterns were coded 0 or 1 depending on their absence or presence in each species. The highest number of bands (17) was recorded for *Gazania rigens*. The molecular weight observed within the studied samples ranged between 15 and 115 kDa. The similarity coefficient between both samples was 77.7%. Protein fingerprint revealed that the distance between the two studied *Gazania* species did not exceed 22.2%, which indicated little variation among their sodium dodecyl sulfate polyacrylamide gel electrophoresis profiles and further explained any resemblance on the chemotaxonomical level[12].

3. Extraction methods

The selection of the appropriate extraction technique is an important step in the qualitative and quantitative studies of bioactive constituants from herbal materials[20,21]. The priority in this selection maintains the activity of the extracted compound(s). The basic principle is to separate the medicinally active fractions of plant tissue from inactive/inert components by using selective solvents and extraction technology. During the extraction, organic solvents diffuse into the solid plant tissues and solubilize compounds of similar polarity[22]. Adjacent to traditional procedures, various innovative methods have been recognized, namely, MAE and UAE. Thus, the development of modern sample preparation techniques with significant advantages over conventional methods for extraction and analysis of these molecules is likely to play an important role in the overall effort of ensuring and providing high-quality products to consumers.

3.1. CE techniques

Conventional solvent extraction may be thought of as a transfer of solutes from one phase (*e.g.*, solid phase) into another (the solvent)[23]. Several conventional techniques can be used to extract bioactive constituents from different parts of the plants including soxhlet extraction, thermal desoption, maceration, phytonic desorption, infusion, extraction leaching, surfactant mediated extraction, accelerated solvent extraction, pressurized liquid extraction, steam distillation, percolation, membrane process, decoction, sample disruption method, counter current extraction and enfleurage, and the majority of them are based on the effectiveness of the solvents chosen and the utilization of temperature and/or combination (Figure 3A)[24,25].

Extraction efficiency of any conventional method depends mainly on the selection of extraction solvents which is critical for the plant matrices as it will determine the amount and type of bioactive compounds being extracted[26]. According to Puri *et al*., the organic solvents used for the separation of components based on their polarity are given in Table 1[27].

3.2. Modern extraction techniques

The major challenges of conventional extraction are longer extraction time, requirement of costly and high purity solvent, evaporation of the huge amount of solvent, low extraction selectivity and thermal decomposition of thermolabile compounds[28]. In order to overcome these limits, modern extraction techniques (MAE, UAE and SFE) were developed specially in studying medicinal plants[4]. These methods are considered as green techniques as they are conform to standards set by Environmental Protection Agency, USA (http://www.epa.gov/greenchemistry/pubs/about_gc.html). So, they reduce or eliminate the use or generation of hazardous substances from chemical products and processes and improve all types of

Table 1
Solvents used for the extraction of bioactive compounds from plants[27].

Polarity of solvants	Solvent used	Product
Apolar	Cyclohexane, hexane, toluene, benzene, ether, chloroform, ethyl acetate	Alkaloids, terpenoids, coumarins, fatty acids, flavonoids, terpenoids
Polar	Acetone, acetonitrile, butanol, propanol, ethanol, methane	Flavanols, lectins, alkaloids, quassinoids, flavones, polyphenols, tannins, saponins

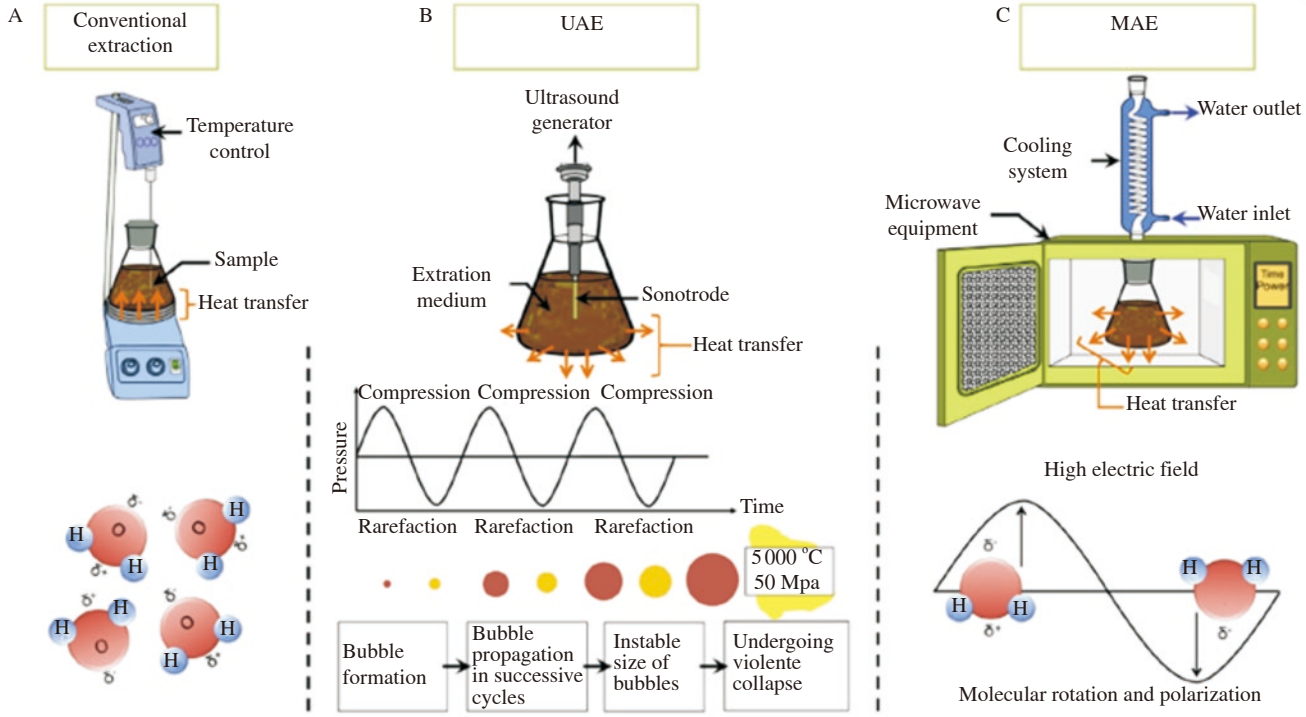

Figure 3. Several conventional techniques used to extract bioactive constituents.
A: Experimental set-up for conventional extraction of high-added value molecules from plant matrices used at laboratory scale; B: UAE principle and cavitational phenomenon; C: MAE equipment used at laboratory scale showing the molecular rotation mechanism[30].

chemical products and processes by reducing the impacts on human health and environment[25].

3.2.1. UAE

Although the use of ultrasonic energy to aid the extraction of medicinal compounds from plant materials can be found in the literature as early as the nineteen-fifties, mechanistic aspects of the usefulness of UAE are worth noting[29]. UAE involves the use of ultrasound, a sound wave beyond human hearing, ranging from 20 kHz to 2 000 kHz[24]. The technique is based on the acoustic cavitation phenomenon (Figure 3B) which allows the formation, growth, and burst of the bubbles (micro size) inside the liquid phase[23,30]. Advantages of UAE can be seen in powder of plant sample since ultrasound energy makes easy organic and inorganic compounds percolating from herbal matrix[25]. The mechanical effects of ultrasound induce a greater penetration of solvent into cellular materials and improve mass transfer. Ultrasound in extraction can also disrupt biological cell walls, facilitating the release of contents. Therefore, efficient cell disruption and effective mass transfer are cited as two major factors leading to the enhancement of extraction with ultrasonic power[31].

SEM have provided evidence of the mechanical effects of ultrasound, mainly shown by the destruction of cell walls and release of cell contents. In contrast to conventional extractions, plant extracts diffuse across cell walls due to ultrasound, causing cell rupture over a shorter period[32]. Effectiveness of UAE is governed by a variety of parameters including nature of solvent, humidity of sample, crushing degree and particle dimension, as well as temperature, pressure, frequency and time of sonication[25].

Two different types of ultrasound equipments are commonly used in laboratory. The first one is the ultrasonic cleaning bath (UB) and the second one is the ultrasonic probe or horn (UP) system. The UAE using UB is easy to handle and economically advantageous. But the delivered intensity is low and is highly attenuated by the water in the bath and the walls of the glassware used for the experiment. However, the UAE using UP is much more powerful because the ultrasonic intensity is delivered on a small surface compared to the UB. In UP, the probe is directly immersed into the reaction flask, so less attenuation can happen[33]. A special sono-extraction reactor (from 0.5 to 3 L) has been developed by R.E.U.S. (Contes, France), with a frequency of 25 kHz. The main advantage of this type of apparatus is that the natural products and the extraction solvent are mixed into a container and ultrasounds are directly applied to the mixture. To run out industrial trails or to scale-up laboratory experiments, R.E.U.S. has also developed reactors from 30 to 1000 L[34].

The industrialization of ultrasounds extraction processes has been reported in literature for the recovery of bioactive compounds from plant materials, showing great results as a relevant, rapid and sustainable alternative process to conventional procedure[35], and the main studies were summarized in Table 2.

3.2.2. MAE

MAE is another advanced method of extraction which has become popular for the separation of bioactive compounds from plant matrices[51]. Kaufmann and Christen firstly described the use of MAE for plant resources[52]. This method is based on the direct impact of microwave energy on polar compounds. Microwaves are electromagnetic fields in the frequency ranging from 300 MHz to 300 GHz and 915 MHz is considered most useful for industrial applications with its greater penetration depth, while 2.450 MHz frequency is generally used in domestic microwave ovens and for extraction applications with a wide range of commercial units designed for analytical chemistry purposes[53]. In the system, following ionic conduction and dipole rotation mechanisms, a heat was generated by transformation of electromagnetic energy (Figure 3C)[54].

MAE process is assumed to involve three sequential steps: (i) separating the solutes from the active sites of the sample matrix under increased temperature and pressure, (ii) diffusing solvent across the sample matrix and (iii) releasing the solutes from the sample matrix to the solvent[55]. Numerous parameters influence

Table 2

Recent applications of UAE, MWA and SFE in plant material.

Extraction technique	Matrix	Analytes	Extraction conditions	References
UAE	*Arachis hypogaea* (peanuts)	Antioxidants	UAE bath: dual or triple frequency combinations (25 and 40 kHz; 25 and 80 kHz; 40 and 80 kHz and 25, 40 and 80 kHz; 80% ethanol)	[36]
	Mentha spicata leaves (spearmint)	Flavour compounds	UP: 20 kHz, 200 W and 70% ethanol (v/v)	[37]
	Citrus sinensis L. peel (orange)	Flavonoids	UAE bath: 40 °C, a sonication power of 150 W and 4:1 (v/v) of ethanol: water	[38]
	Salvia miltiorrhiza root (sage)	Phenolic acid (salvianolic acid B)	UAE bath: 45 Hz; 60% ethanol; 30 °C; extraction time duration: 25 min; ratio of solvent to material: 20:1 (v/w, mL/g)	[39]
	Punica granatum L. peel (pomegranate)	Antioxidants	UAB: temperature of (25 ± 2) °C and a ratio of water: peel at 50:1 (w/w); continuous UAE: 59.2 W/cm^2 for 6 min; pulsed UAE 59.2 W/cm^2, 5 s, 5 s and 8 min (intensity level, pulse duration and interval and extraction time respectively)	[40]
	Forsythia suspense (weeping forsythia)	Lignan (phyllirin)	About 1 g plant sample with 10 mL of 20% methanol and the extraction for 60 min at 60 °C under ultrasonic irradiation.	[41]
	Olea europea leaves (olive)	Total phenolic compounds, oleuropein	UAE bath of 60 W, temperature of 16 °C and sonication duration of 45 min	[35]
	Ligustrum lucidum Ait fruit	Triterpene (oleanolic acid and ursolic acid)	About 95% ethanol, the ratio of material to liquid at 1:20 and extraction for 10 min at 40 °C under ultrasonic irradiation	[42]
	Sparganium stoloniferum Buch.-Ham. Rhizome (sparganii)	Total phenols, phenolic acids and flavonoids	Ethanol (75.3%), a time of 40 min and a solvent-to-material ratio of 19.21 mL/g; ethanol (80%), a time of 33.54 min and solvent-to-material ratio of 22.72 mL/g	[43]
	Laurus nobilis leaves (laurel)	Phenolic compounds	UAE bath at a frequency of 40 kHz, 1 g plant sample with 12 mL of 35% ethanol, for 40 min at room temperature	[44]
	Annona glabra leaves	Diterpene	About 2 g of plant sample, 25 mL of solvent, 50% amplitude, 0.5 s^{-1} for cycle and an extraction time of 15 min	[45]
	Abutilon theophrasti leaves (velvet leaf)	Flavonoids	Extraction temperature at 60 °C, extraction time of 12 min, liquid-solid ratio of 32 mL/g and a fixed ultrasonic power of 50 W	[46]
	Lonicera caerulea L. Haskap berries	Anthocyanins	UAE bath: liquid/solid ratio of 25:1 (mL/g), 80% ethanol and 0.5% of formic acid, 35 °C for 20 min	[47]
	Ascophyllum nodosun (brown seaweed)	Total phenolics, fucose and uronic acid	Extraction time of 25 min, acid concentration of 0.03 mol/L HCl and ultrasonic amplitude of 114 μm	[48]
	Allium ursinum L. (wild garlic)	Total phenolics, total flavonoid	Ultrasonic power of 20.06 W/L, temperature at 80 °C, 70% ethanol during 79.8 min	[49]
	Phyllanthus amarus aerial parts	Phenolic content	Ultrasonic intensity of 301 W/cm^2, and liquid-solid ratio of 40 mL/g for 7 min	[50]
MAE	*Silybum marianum* (milk thistle)	Phenols (flavonolignan)	Microwave power of 600 W, extraction time of 12 min, 80% ethanol, preleaching time of 20 min and 25:1 (mL/g) as the solvent to material loading ratio	[67]
	Gymnema sylvestre (Gymnema)	Triterpenoid aglycone (gymnemagenin)	40% microwave power, 6 min, 85% methanol, pre-leaching time of 15 min and 25:1 (mL/g) as the solvent-to-material ratio	[68]
	Arachis hypogaea skins (peanut)	Polyphenols	Microwave power of 855 W, 30% ethanol, irradiation time of 30 s and skins of 1.5 g	[69]
	Cuminum cyminum (cumin)	Phenolic compounds	Ethanol/water (50 %) of 20 mL/g, 200 W of 50% nominal power and a temperature of 50 °C for 18 min	[70]
	Vitis vinifera skins (grape)	Anthocyanins	2 g of sample, 500 W, 100 °C, 40% methanol in water as extraction solvent	[71]
	Quercus infectoria gall (aleppo oak)	Polyphenols (tannins)	About 15.3 g/125 mL of methanol at 67 °C for 30 min under microwave irradiation of 300 W	[72]
	Solanum lycopersicum fruit (tomato)	Phenolic compounds	Microwave power of 100 W, 1:50 (w/v), 96.5 °C, extraction time of 2.06 min and 66.2% ethanol	[73]
	Brassica oleracea (broccoli)	Antioxidants	Temperature of 71.11 °C; microwave power of 167.03 W; solvent concentration 75.95% for 16.34 min	[74]
	Vitis vinifera fruit (grape)	Total polyphenols and anthocyanins	Microwave reactor with frequency of 2.45 GHz and variable power (10 W increments, maximal value of 900 W) for 20 min	[75]
	Citrus limon residues (lemon)	Total phenolic compounds	About 48% ethanol as extraction solvent, 28:1 mL/g of solvent: solid ratio, 123 s and 400 W for irradiation time and power	[76]
	Pistacia lentiscus leaves (mastic)	Total phenols, tanins and flavonoid	About 46% ethanol, extraction time of 60 s, potency density of 17.86 W/mL and liquid/solid ratio of 28:1	[66]
	Abutilon theophrasti leaves (velvet leaf)	Flavonoids	Temperature of 60 °C, extraction time of 12 min, liquid-solid ratio of 32 mL/g and microwave power of 534 W	[46]
	Myrtus communis L. leaves (myrtle)	Total polyphenols and flavonoids	About 42% ethanol concentration, microwave power of 500 W, irradiation time of 62 s and solvent-material ratio of 32 mL/g	[77]
	Agaricus bisporus (mortobello mushroom)	Sterols (ergosterol)	Extraction time of (19.4 ± 2.9) min, (132.8 ± 12.4) °C and (1.6 ± 0.5) g/L	[78]
	Paramignya trimera root (Xao Tam Phan)	Terpene (saponin)	About 100% methanol, extraction time of 40 min and ratio of solvent to sample of 100 mL/g	[79]
SFE	*Helianthus annuus* L. leaf (sunflower)	Diterpenes	Temperature: 50 °C; pressure: 300–500 bar; CO_2 and co-solvent: 2–126 kg CO_2/kg sample; extraction yield: 0.1%–1.2 %	[82]
	Rosmarinus officinalis L. leaf (rosemary)	Triterpene	Temperature: 40–60 °C; pressure: 100–180 bar; CO_2 and co-solvent: 0–25 kg CO_2/kg sample of EtOH; extraction yield : 0%–3%	[83]
	Illicium verum seed (Chinese star anise)	Fatty acids	Temperature: 30–50 °C; pressure: 100–300 bar; ethanol: 0%–15%; extraction yield: 6.80%–23.72%	[84]
	Laurus nobilis L. leaf (laurel)	Monoterpenes, oxygenated derivates	Temperature: 40 °C; pressure: 100 bar; CO_2 and co-solvent: 17 kg CO_2/kg sample; extraction yield: 1.37%	[85]
	Olea europaea L. leaf (olive)	Oleuropein	Temperature: 50–100 °C; pressure: 100–300 bar; CO_2 and co-solvent: 72 000 L CO_2/kg sample	[86]
	Arbutus unedo L. fruit (strawberry)	Phenols	Temperature: 40–80 °C; pressure: 150–6 300 bar; CO_2 and co-solvent: 30 kg CO_2/kg sample of EtOH (0%–20%)	[87]
	Amaranthus paniculatus seed (amaranth)	Squalene	Temperature: 60–100 °C; pressure: 110–280 bar; CO_2 and co-solvent: 1 200–600 L CO_2/kg sample	[88]
	Momordica charantia L. fruit	Flavonoid	Temperature: 30–50 °C; pressure: 250–350 bar; CO_2 and co-solvent: 555–1 000 L CO_2/kg sample of EtOH; extraction yield: 1.2%–1.5 %	[89]
	Arrabidaea chica leaf (Humb. Bonpl.)	Anthocyanins	Temperature: 40 °C; pressure: 300 bar; CO_2 and co-solvent: 0–140 kg CO_2/kg sample of EtOH (0%–20%); extraction yield: 0%–3.6%	[90]
	Pinus pinaster wood (cluster pine)	Phenolics	Temperature: 30–50 °C; pressure: 100–250 bar; ethanol: 0%–20%; extraction yield: 0.3%–2.1%	[91]
	Coffea arabica and *Coffea robusta* residue (coffee)	Oil and diterpenes	Temperature: 40–70 °C; pressure: 140–190 bar; CO_2 and co-solvent: 91 kg CO_2/kg sample; extraction yield: 0%–12%	[92]
	Origanum basilicum L. (sweet basil)	Essential oils	Temperature: 40 °C; pressure: 30 MPa; CO_2 and co-solvent: CO_2 flow rate of 60 g/min	[93]
	Eruca sativa (rocket salad)	Phenolic and glucosinolate	Temperature: 75 °C; pressure: 25 MPa; CO_2 and co-solvent: flow rate of 30 000 kg/h of CO_2 Water; extraction yield: 41%	[94]

EtOH: Ethyl alcohol.

MAE including selection of the appropriate solvent, volume of the solvent, extraction time, microwave power, matrix characteristics, and temperature[56,57].

In MAE, mostly two types of equipment are single-modes and multi-modes. In the first one, a rapid extraction of the bioactive compounds was induced by a direct application of high density microwave energy on the sample matrix. Nevertheless, in the second type, the volume and the amount of sample that can be extracted are relatively limited[52,58]. Thus, microwave extraction has resulted in a number of techniques such as microwave-assisted solvent extraction[59], compressed air microwave distillation[60], vacuum microwave hydrodistillation[61], solvent free microwave extraction[62], and microwave hydro-diffusion and gravity[34].

Several advantages of MAE have been reported: quicker heating for the extraction of bioactive substances from plant materials; reducing thermal gradients; reducing equipment size and increasing extract yield[63-65]. MAE can extract bioactive compounds more rapidly and a better recovery is possible than conventional extraction processes. The observation of images under SEM showed more rupturing in microwave-assisted sample compared to untreated one[32,66]. This releases the active constituents from the cell on the outside milieu. The release of active compounds is increased by heating or soaking the plant matrix with solvents with a higher dissipation factor[57]. MAE was applied for the recovery of valuable compounds from different vegetable matrix (Table 2).

3.2.3. SFE

Other method such as SFE is also being used in the extraction of plant materials. This method is less popular due to high cost despite the efficiency of the methods[4]. SFE is currently a well established method for extraction and separation because its design and operating criteria are now fully understood. The most important cause for the attention in SFE was the ability to extract natural compounds at temperature close to ambient one, as a result the substance of interest can be protected from thermal degradation. The rapid extraction due to less viscosity and high diffusivity is linked with supercritical fluids[51,57]. All substances have three essential states which are solid, liquid and gas. Supercritical state is a characteristic state and can just be achieved if a material is subjected to temperature and pressure outside its critical point.

This point is defined as the characteristic temperature and pressure above which distinctive gas and liquid phases do not exist[25]. To carry out a successful SFE, some parameters such as the choice of supercritical fluids, plant material preparation, modifiers and extraction conditions must be concerned[32]. The most frequently employed extraction solvent in SFE is CO_2 which is not alone discriminating but its ability and selectivity of extraction can be enhanced by using a co-solvent or modifier[24].

In view of the vast diversity of molecules found in natural matrices, vegetables are typically matter of research for more than one application. Depending on the species and plant component studied, processes of SFE can be devoted to many naturally occurring compounds, due to its advantages such as clean, environmental friendly and an innovative technology[80]. SFE extracts obtained from vegetable matrices are typically mixtures of the following family of compounds: triglycerides, fatty acids, fatty alcohols, terpenoids, phytosterols, tocopherols, tocotrienols and phenolics[81]. A wide-ranging compilation of works in this field was presented in Table 2.

4. Identification of the secondary metabolites in medicinal plants

The invention in analytical technology is behind the development of natural products chemistry. The determination of the components in complex mixtures raises the use of advanced techniques, which should provide information on the structure of the constituents to be analyzed, as well as good sensitivity and selectivity. An analysis method is selected depending on the chemical nature of the analyte[95]. There are various techniques that are used for determination of active components in herbs like HPLC, GC, UHPLC and TLC[96]. Moreover, the recent approaches including hyphenated chromatography and spectroscopy like HPLC-diode array detection (DAD), GC-MS, HPLC-MS, CE-DAD and HPLC-NMR might give additional spectral information which may be helpful for the qualitative and quantitative analysis and even for the structural elucidation[97,98]. With spectral information, the hyphenated instruments show greatly improved performances in terms of the elimination of the instrumental interferences, retention time shift correction, selectivity, chromatographic separation abilities, measurement and precision[97].

4. 1. Spectrophotometric techniques

4.1.1. IR spectroscopy

In food industry, spectroscopy techniques in the IR wavelength region of the electromagnetic spectrum have been used to monitor and evaluate food composition[96]. Low molar absorptivities and scattering are characteristics of near infrared (NIR) spectroscopy. These two characteristics allow nearly effortless evaluation of pure materials. New computers allow NIR spectroscopy to have many applications in various fields including the medical, pharmaceutical and food analysis[96].

NIR spectroscopy is a rapid and non-destructive method with high resolution that is used for analysis of multi-component of almost any matrixes[96,99,100]. In recent years, NIR spectroscopy has gained a wide appreciation within the pharmaceutical industry for raw material testing, product quality control and process monitoring. The growing pharmaceutical interest in NIR spectroscopy is probably a direct consequence of its major advantages over other analytical techniques, namely, an easy sample preparation without any pretreatments, the probability of separating the sample measurement position by use of fiber optic probes, and the expectation of chemical and physical sample parameters from one single spectrum[100]. Thus, it was used for the prediction of polyphenols in tea leaves[101]. The NIR spectroscopy was also used to quantify vitamin E (α-, β- and γ-tocopherols) in leaves of alfalfa (*Medicago sativa*) and also to determinate the terpenoids responsible of flavor and pungency in black and white pepper (*Piper nigrum* L.)[96]. When NIR spectroscopy was related with multivariate data analysis, numerous fascinating insights were opened particularly in qualitative and quantitative pharmaceutical analysis[100].

4.1.2. NMR spectroscopy

Over the last few years, a variety of state-of-the-art approaches have been presented and found a widespread application of NMR in both pharmaceutical and academic research. Recently, in the aim to find out the impurity of drug products, characterize their composition, and quantify them in pharmaceutical formulations and biological solutions, NMR was used in quantitative analysis[100].

A whole series of 2D correlation experiments, such as correlation spectroscopy, heteronuclear multiple bond correlation, heteronuclear single quantum correlation are now widely employed in structure elucidation by NMR[95]. Thus, for structural elucidation of phenolic compounds, various NMR spectroscopy techniques have been used. This includes the 1H-NMR, the 13C-NMR homonuclear bidimentional, the correlated NMR spectroscopy (correlation

spectroscopy), heteronuclear correlation of the chemical shift, *etc*. [101,102]. The application of 13C-NMR spectroscopy has probably had the greatest impact on the structure of flavonoids. The development of advanced dimensional techniques still revolutionized the analysis of the structure of these compounds[103].

Capillary NMR as an innovative method has improved the sensitivity of NMR. A capillary tube and an radiofrequency coil were used, permitting an easier shimming and very low spending of deuterated solvent and hence economizing time[95].

4.1.3. MS

The identification of compounds in plants was also carried out using mass spectrometry based on the determination of *m/z* ratio after the ionization of the molecules. One of technical developments concerns the ionization of the molecules to be analyzed. Thus, different ionization modes are used such as chemical ionisation, fast atom bombardment, thermospray, electrospray ionization (ESI) and atmospheric pressure chemical ionisation[95]. For qualitative and quantitative studies on various *Panax* spp. extracts, ESI negative mode has been used. With the MS-MS experiments, 25 ginsenosides have been identified during the fragmentation ion [M - H][104]. ESI-MS was used for structural confirmation of phenols[101]. Matrix assisted laser desorption ionization-time of flight-MS is a powerful tool for the qualitative analysis of procyanidins present in the matrix without sample preparation; however, relatively high concentrations are needed[102,105].

In recent years, flow injection-MS lets the sample to be positioned in a hauler to move directly into the atmospheric pressure interface of the mass spectrometer.

Since, ambient MS provides information very rapidly using minimal sample preparation, it's considered advantageous. Ambient MS and direct analysis in RT has been used for the analysis of 7 hypoglycemic drugs[106].

4.2. Chromatographic techniques

4.2.1. TLC

This technique is employed in order to analyse rapidly samples to recognize herbal products and to distinguish between herbal species[97]. This is especially the separations of very different polarity substances which are carried out very effectively with TLC[108]. However, in TLC, it is difficult to manage some factors that influence its precision such as sample spotting, saturation of the developing chamber and the instability of color appearance. Nonetheless, as TLC is an economical technique, fast and easy to use, it is still used as an analytical technique. Recent advances and changes in technology have significantly improved resolution, sensitivity and reproducibility of the technique[97]. In last few years, TLC is developed into a high performance method [high performance thin layer chromatography (HPTLC)] with finer particle size of silica phases and narrower particle size distribution and automation of different steps which increase the reproducibility and resolution[97,109]. HPTLC is a fast separation technique and flexible enough to analyze a wide variety of samples. This technique is advantageous in many means as it is simple to handle and requires a short analysis time to analyze the complex or the crude sample. HPTLC evaluates the entire chromatogram with a variety of parameters without time limits. Moreover, there is simultaneous but independent development of multiple samples and standards on each plate, leading to an increased reliability of results[100,110].

Thus, procyanidins dimers up to heptamers have been successfully separated using HPTLC[95,102,111]. Furthermore, HPTLC-densitometric technique was developed for the quantitative determination of rosmarinic acid in Algerian mints. The densitometric quantification was found to be simple, allowing the analysis of many samples in a short time with reasonable precision[112].

The micro emulsion-TLC was an additional employed TLC and it augmented effectiveness of separation and signal improvement and consequential better sensitivity. Other accepted modifications in herbal analysis include 2D-TLC and and coupled-layer planar chomatography (graft-TLC)[97].

4.2.2. HPLC

HPLC is the most popular analytical technique which is easy to operate and a fully automatable technique with high rates of precision, resolution, selectivity and sensitivity[97,104]. Considering the variety of natural products and no single technique for effective detection, choosing a suitable detector in HPLC is essential such as ultraviolet[98,101,104], DAD[113], the chemical color sensor colorimetric, the detection technique by chemical reaction, voltammetry[101], the fluorescence detection[98,101,102], evaporative light scattering detector and chemoluminescence detectors, NMR and MS[97,98]. The findings of analytical HPLC were used in the quantitative determination of plant components, in the control of the purity of the natural products, and in the chemotaxonomic investigations[95]. HPLC-DAD method, combined with chemometrics, was demonstrated to be very helpful in searching *Ziziphus jujuba* resources and possibly useful in chemotaxonomic characterization. A total of 10 triterpenoid acids were determined simultaneously[97].

The HPLC finger print analysis has been widely used for the identification, authentication, characterization and classification of herbal medicines and it is accepted and advised as an efficient method to control the quality of herbal medicines by World Health Organization and Food and Drug Administration[98]. An important factor in finger print development is the selection of stationary phase. The performance of columns is a function of the particle size. Smaller size of particles greatly increases the pressure. As the current HPLC instrumentation can withstand up to 6000 psi, higher efficiencies and shorter separation times by decreasing particle size and increase of flow rates are limited[97]. The introduction of reverse phase columns has considerably improved the HPLC separation of different classes of phenolic compounds[101,102,114]. HPLC can be used with 2D chromatography[97].

4.2.3. UHPLC

The UHPLC is a technique near to HPLC and is based on the same principles. The UHPLC offers two interesting possibilities: either very fast separation (1 min) using short columns at high flow or ultra high resolution[115]. Thus, UHPLC makes it possible to perform higher solution separations superior to HPLC analysis by using solid phase particles of less than 2 mm in diameter to achieve superior sensitivity and resolution. Smaller particle size leads to higher separation efficiency and shorter columns size leads to reduce the time of analysis with low solvent consumption[95,97]. UHPLC analysis, in comparison to HPLC, recorded a reduced analysis time until eight by a factor with preservation of information. UHPLC technique has also the advantage of greatly improving the selectivity in comparison to conventional HPLC[97]. To control the quality of products derived from plants, UHPLC was shown recently as a feasible technique[95,97].

4.2.4. High temperature HPLC

For reducing the percentage of organic modifier and the dilution step, high temperature of HPLC was used. At higher temperatures water becomes appreciably more non-polar by itself so at

temperatures of 150–200 ˚C much less organic modifier is necessary and correspondingly less dilution is required. This demands some adaptations of the technical part and the method only works for natural products which are stable in water at the chosen temperature. The technical changes constist of a larger column oven, a preheater for the mobile phase before the column and a cooler for the mobile phase after the column. One study showed that the retention time with methanol-water [10:90 (v/v)] decreased from 24 to 2 min when the temperature was increased from 90 to 205 ˚C[115,116].

4.2.5. Hydrophilic interaction chromatography (HILIC)

HILIC has gained attention in herbal finger printing because of good separation quality of hydrophilic compounds. HILIC which shows better separation of aqueous solutions can extract many polar compounds of medicinal plants. HILIC is a technique that can replace the normal phase liquid chromatography, with polar stationary phase and water as mobile phase, HILIC can separate the polar compounds[97]. Furthermore, the mobile phase of HILIC solubilizes better polar compounds. Few papers have been published about the use of HILIC in herbal products analysis because it is relatively new. Majority papers describe a methodology exploiting the orthogonal character of the HILIC and reversed-phase liquid chromatography methods for quality control[97,117]. The offline coupling between the two columns allows the first column to separate and split, and the second to analyse the obtained fractions.The composition of *Carthamus tinctorius* was established by combining between the material of reversed phase and HILIC[117].

4.2.6. GC

GC is commonly used for the characterization, quantization and identification of volatile compounds. The GC is an important tool for analysis of essential oils due to its high efficiency separation and sensitive detection[97,117]. Currently, GC has been utilized to analyse drugs like cocaine and impurities of pharmaceuticals and to determine residual solvents in betamethasone valerate. The molecules with high mass confine the scope of this technique. Its main constraint rests in the comparative non-volatility of the drug substances therefore, derivatization is virtually compulsory[100]. Thus, it is considered as a method for analysis of procyanidins but after derivatization[102].

GC-MS analysis of essentials oils, showing faster analysis and high efficiency, made use of micro-bore capillary columns with reduced stationary phase film thickness with rapid temperature programming, fast data acquisition by flame ionization detector and high split ratio[95,97]. Thus, GC-MS is a fine technique to help identify the chemical composition of *Melissa officinalis* L. Indeed, it has identified the molecular composition of the volatile extract of the plant which is composed essentially of terpene, terpene oxide, and sesquiterpene ester[118]. In several recent studies, GC with MS detection has been used to analyse pertinent herbs of traditional Chinese medicine. Concerning separation and detection, there are slight novel descriptions compared to previous papers. Nevertheless, progression concern sample preparation where a process usually used is headspace solid-phase microextraction[117]. As HPLC and TLC, GC can also be used with 2D chromatography, but to reach a maximum capacity, a major limitation is very long time span needed[97].

4.3. CE

Another useful and effortless technique used for herbal components analysis is CE, which is fast, cheap, and needs smaller amount of the samples[95,106]. It is a relatively new analytical technique based on the separation of charged analytes through a small capillary under the impact of an electric field. In this technique solutes are perceived as peaks as they pass through the detector and the area of individual peak is proportional to their concentration, which allows quantitative estimations[100,117].

Several modes of CE have been developed and used as zone capillary electrophoresis, capillary gel electrophoresis, micellar electrokinetic chromatography, capillary isotachophoresis and capillary electrochromatography. As flavonoids are negatively charged, CE is a specifically adapted method to separate these compounds. The coupling between the capillary isotachophoresis and zone capillary electrophoresis was applied for the quantitative determination of flavonoids[95,100,106].

4.4. Coupling techniques (hyphenated technique)

In the case of a mixture, which is the case of biological matrices, or small quantities of the sample, one method does not allow to have information on the structure of analytes. To overcome this problem, the combinations were made between the different techniques. In order to expand hyphenated techniques, coupling and on-line separation techniques were used[98]. Hyphenated techniques are methods that combine two or more techniques in one tool. A notable progress was showed in these techniques where a variety of them such as LC-MS, GC-MS, LC-NMR, capillary electrophoresis coupled to inductively coupled plasma mass spectrometry and CE-MS were useful in the study of pharmaceuticals[95,98].

According to Bansal *et al*.[97], LC-MS, GC-MS, and LC-NMR have been increasingly used in complex chemical identification. This advancement in instrumentation is able to generate enormous amounts of data which record small differences between samples and this enables us to provide large implications for the discrimination of herbal plants. The HPLC-MS couple (LC-MS) is one of the most important techniques. The combination offers the opportunity to employ the chromatography as a method of separation and MS as identification tool[95]. It is an explanation procedure to provide informations on quantities and structures of compounds in herbs and other botanical products[106]. This coupling has been commonly used for structural characterization of phenolic compounds. Thus, the coupling HPLC-MS-thermospray permits detection of flavan-3-ols monomers and dimeric proanthocyanidins[101,119]. Also, combinations chromatography-diode- array detection-mass spectrometry and liquid chromatography-solid-phase extraction-nuclear magnetic resonance enable reliable identification of the active components without the need to isolate them in a classical sense[115].

When the metabolites are present only in the nanogram, NMR is impossible, but the GC-MS couple after derivatization enables the elucidation of the structure[115].

Other combinations have been cited in the literature, for example

LC-SPE combination which leads to an improvement in sensitivity. Chromatography ultra-thin layer can also be combined with the MS[115], bi-dimentionnel NMR and mass spectrometry FAB[101], LC-MS-MS and GC-MS[106], NIR-UHPLC to quantify the amount of bioactive compounds in red peony root[121] and UHPLC–MS/MS method revealed the presence of 34 compounds of different families in the methanolic extract of *Marrubium vulgare* (horehound) leaves[122].

4.5. Analysis and interpretation of multivariate data (chemometrics)

The combination of spectroscopic or/and chromatographic techniques with high resolution with new mathematical data processing techniques provide a good opportunity for mining more useful chemical information for structural elucidation[101]. These methods allow obtaining useful informations and provide various data processing methods, are known to chemometrics. It was defined by the International Society of Chemometrics as the science of measurements made on a chemical system or process by application of mathematical or statistical methods[97]. Among these methods, the method of partial least squares, principal component analysis (PCA) and hierarchical cluster analysis[97,98,101]. Thus, the difference between fruits, stems and leaves of *Tribulus terrestris* L. were visualized by effective components profiles and chemometrics (PCA and partial least square discriminant analysis), and this approach would be helpful for quality control of for example, Chinese medicine, especially those with different medical parts[120]. According to Bansal *et al*.[97], the combined data matrices of both HPLC-DAD and GC-MS were utilized for the analysis of complex traditional Chinese medicinal plant *Rhizoma curcumae* and evaluation of data by the use of PCA showed a reasonable separation of the samples for each technique.

In their study, Gao *et al*.[123] by UHPLC-tandem quadrupole time-of-flight-MS and triple quadrupole MS method, then multivariate statistical analysis (PCA, back propagation-artificial neural network model, and partial least square discriminant analysis model), were found that three compounds are in combination contributed to species characterization of varied *Lonicera* samples were found and applied to their specific tests. This strategy provides an applicable approach to select chemical markers for quality control of closely herbal medicines.

5. Biological effects of medicinal plants

Increasing attention is given to the study of natural products, which may treat and prevent multiple human diseases such as neurodegenerative diseases, ageing, rheumatoid arthritis, metabolic diseases such as atherosclerosis, diabetes, hypertension, cancer, stroke, autism and others. In this line, different medicinal plants have been re-evaluated[124].

It is increasingly being realized that many of today's diseases are due to "oxidative stress". So, the scientific world's particular interest in studying the antioxidant properties of various plant extracts. The literature has also focussed on the antimicrobial and antitumor activities.

5.1. Antioxidant activity

Oxidative stress results from an imbalance between the formation and neutralization of prooxidants. It is initiated by free radicals [reactive oxygen (ROS) and reactive nitrogen species], which seek stability through electron pairing with biological macromolecules such as proteins, lipids, and DNA in healthy human cells and cause protein and DNA damages along with lipid peroxidation[125,126].

The studies on "discovering natural antioxidants" have become a subject of great interest in many areas, such as food chemistry and natural pharmaceutical products. Potential sources of antioxidant compounds derived essentialy from plants[127]. Exogenous antioxidants and bioactive compounds (polyphenols, vitamins, tocopherols, among others) from herbal plants have been possibly used by humans to neutralize and prevent the deleterious effects induced by ROS in the cells[128].

The antioxidant activity of the different plant extracts was analyzed using a number of different chemical-based assays, such as UV-vis spectrometry, chromatography and electrochemistry[126]. The most widely used analytical methods including 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), 2,2-diphenyl-1-picryl-hydrazyl (DPPH)[129], ferric reducing antioxidant power[130], reducing power[131], oxygen radical absorbance capacity[130], inhibition of linoleic acid oxidation[112], superoxide radical scavenging activity[132]. Recently, other methods were proposed for the evaluation of the antioxidant activity like the metallic nanoparticles and electrochemical assays, a cellular-based activity was also used by several authors[128].

5.1.1. Metallic nanoparticles method

Nano-scale materials with a size range of 1–100 nm find a niche in materials science owing to their defined size, shape, optical, mechanical and biological properties[133]. Currently, a large number of methods like biological ones can be used to synthesize different types of nanoparticles[134]. In order to avoid adverse effects during synthesis and application of nanoparticles, there is a growing need to develop eco-friendly and sustainable methods for synthesizing nanomaterials that do not use toxic chemicals during the process[135]. Nowadays, green synthesis of metallic nanoparticles is a growing area of research because of their potential applications in different domains[134].

Investigation of the antioxidant activity of silver nanoparticle (AgNPs) biosynthesisted using *Clerodendrum phlomidis* leaf extract showed that these AgNPs exhibit remarkable antioxidant activity than the crude extract[136]. Thatoi *et al*.[137] biosynthesized AgNPs and zinc oxide nanoparticles using the aqueous extracts of bark of *Heritiera fomes* and leaves of *Sonneratia apetala*. Both AgNPs and zinc oxide nanoparticles have a potential radical scavenging capacity of the DPPH•compared to the catechol, which was taken as a standard. Recentely, Tułodziecka and Szydłowska-Czerniak[126], developed a novel gold nanoparticles spectrophotometric assay for the antioxidant activity determination of *Brassica* oil seeds, white flakes and flour.

5.1.2. Electrochemical measurement of antioxidant capacity

Results obtained from spectrophotometric methods are not

constantly compatible and materials used for these analyses are costly [2, 2'-azobis (2-amidinopropane, 2,20-azinobis(3-ethylbenzothiazoline-6-sulfonic acid, DPPH, *etc*.]. That is why Keyrouz *et al*.[138] thought to manage a new competent system, which can offer systematically results and can economise expensive raw materials.

Cyclic voltammetry method is frequently preferred for various types of physicochemical analysis in redox system due to their high sensitivity, rapidity, simplicity, the possibility of performing analysis in colored or turbid solutions as well as the portability of the instrumentation[127]. This assay measures the decrease of the oxidation peak current value of the O_2•− which is produced electrochemically in cyclic voltammetry from the dissolved oxygen reduction[129,139].

This test is applied to evaluate the antioxidant activity of different plant extracts: herb extracts from the Lamiaceae family[140], phenol fractions of the brown seaweed *Ascophyllum nodosum*[139], extracts of *Berberis lycium*, *Zanthoxylum armatum* and *Morus nigra*[141], *Mentha* species extracts[129], three species of *Ericaceae*[142] and *Limoniastrum feei* aqueous extract[127].

Another application of the electrochemical method is the use DNA-based biosensor. This method was employed by Barroso *et al*.[128] for the assessment of the antioxidant activity of nine plant infusions. It is useful because the principle of measurement of the biosensors is closer to the activity of antioxidant in biological systems (by mimicking the damage caused *in vivo* by ROS).

5.1.3.Cellular-based antioxidant activity (CAA)

Wolfe and Liu[143] have developed a much needed method to measure antioxidant activity in cell culture. The CAA assay addresses this need for a biologically relevant protocol. Compared to chemical assays, cell culture models provide a new approach for the determination of antioxidant activity that is cost-effective, relatively fast, and addresses some of the issues associated with uptake, distribution, and metabolism[143,144].

The CAA assay took into account cellular uptake and distribution of the bioactive compounds and therefore had some important advantages over chemical-based methods for measuring antioxidant activity[144]. This approach was adopted in 2016 by Li *et al*[144] to assess the antioxidant capacity of phenolics (proanthocyanidins) isolated from *Choerospondias axillaris* peels.

5.2. Antimicrobial activity

Recent studies have been carried out accessing the antimicrobial potential of some plant species, but essential oils are the main used extract preparations[145]. Several techniques are used to screen plant extracts for antimicrobial activity essentially by determining their minimum inhibitory concentration (MIC). However, in order to discover a novel natural antimicrobials, the development of new bioassay methods which are sensitive enough to detect small amounts of biologically active coumpounds is required[146].

5.2.1. Detection of antimicrobial agents

Flow cytofluorometric and bioluminescent methods can provide rapid results of the antimicrobial agents' effects and a better understanding of their impact on the viability and cell damage inflicted to the tested microorganism. Howerver, they are not widely used because they require specified equipment and further evaluation for reproducibility and standardization[147].

Other standard tests can be conveniently divided into diffusion and dilution methods. Common diffusion tests include agar well diffusion, agar disk diffusion and bioautography, while dilution methods include agar dilution and broth micro/macrodilution[146].

Othman *et al*.[148] found that the pour plate disc diffusion and broth-based turbidometric assays were the optimal methods for assessing the potential antibacterial activities of plant extracts.

5.2.1.1. Agar disk diffusion method

Disk diffusion method is an additional recognized technique to assess *in vitro* susceptibility of microorganisms to various drugs[149]. Nowadays, many accepted and approved standards are published by the Clinical and Laboratory Standards Institute (CLSI) for bacteria and yeasts testing.

This method have been widely used recently by several authors to test the antimicrobial activity of plant extracts. Martins *et al*.[145] studied the potential of the hydromethanolic extracts of some plants against *Candida* species using agar disc diffusion method. Brahmi *et al*.[150,151] employed this assay in evaluating the antimicrobial activity of *Mentha* species essentials oils. This method was also used to determine the antimicrobial activity of leaf and stem ethanol extracts from *Muntingia calabura* L.[152]. Furthermore, the antibacterial potential of the silver and zinc oxide nanoparticles synthetised using aqueous extracts of two mangrove plant species, *Heritiera fomes* and *Sonneratia apetala* was determined against six different pathogenic bacteria by agar disc diffusion assay[137].

5.2.1.2. Broth microdilution

The micro-titre plate (broth microdilution method)was a very used technique for determining MICs of numerous samples. The minimum bactericidal concentration (MBC) is determined also by this method[146,153].

This method was recently used by several authors to evaluate the antimicrobial activity of plant extracts. For example: the antibacterial activity of the methanol extracts of plants from Ericaceae was determined using the broth microdilution method[142]. Ngezahayo *et al*.[154] employed this assay in order to characterize the antibacterial activity of the isolated triterpenic acids from aerial tissue of *Plastotoma rotundifolium*.

5.2.1.3. Drug combinations activity

The tradition of synergy in herbal medicine is being used as a source of research ideas. A literature review of antimicrobial research and plant synergy published in a five year period was carried out by Mundy *et al*.[155]. The *in vitro* findings were that most of the research reported synergy both within plants and between plants and antibiotics. Whole plant extracts and combinations of compounds were shown to be more effective antimicrobials than isolated constituents. The methods used to assess combinations are the checkerboard and/or disk diffusion methods.

In the checkerboard technique, the activity of drug combinations was determined by calculating the MIC of each drug in the combination and the activity of the combination. A fomula which takes into account the mutual effects of each drug on the other component is used. Hence, it is possible to determine whether there is a synergistic or antagonistic effect by using the two drugs together, or no effect at all as compared to the single drug[146,156].

Combinations between antibiotics and other antimicrobial substances such as plant essential oils represent one of the most promising advances against drug-resistant microorganisms. Additional effect was noted with ciprofloxacine and *Thymus numidicus* essential oil combination tested against *Staphylococcus aureus* (*S. aureus*)[157]. Knezevic *et al*.[158] obtained *in vitro* synergistic interactions of antibiotics ciprofloxacin, gentamicin and polymyxin B with *Eucalyptus camaldulsensis* essential oils against multidrug-resistant *Acinetobacter baumannii* isolates. *S. aureus* and *Pseudomonas aeruginosa* were used to test the effect of combination of *Erica arborea* and *Erica multifloram* ethanol extracts with two antibiotics (cefotaxim and streptomycin). These plant extracts showed additive effects with cefotaxim and streptomycin against *S. aureus*[142].

Rondevaldova *et al*. determined anti-staphylococcal activity of thymoquinone in combinations with antibiotics by checkerboard method using ethylene vinyl acetate capmat™ as a vapor barrier[159]. Synergy was obtained for combination with oxacillin against three strains, with penicillin against one strain and with tetracycline against two strains tested.

5.2.3. Antitumor activity

Cancer is one of the most fatal diseases, which causes millions of death worldwide per annum and it is second to cardiovascular diseases as a leading cause of death. It is the single cause of nearly 12.5% of overall mortality rates and it is expected that by 2030, the number of cases and deaths caused by cancer will rise above 21.4 million and 13.1 million, respectively[160].

Although the mortality due to cancer is high, many advances have been made both in terms of treatment and understanding the biology of the disease at the molecular level. It is estimated that plant derived compounds in one or the other way constitute more than 50% of anticancer agents. Numerous cancer research studies have been conducted using traditional medicinal plants in an effort to discover new therapeutic agents that lack the toxic side effects associated with the present chemotherapeutic agents[125].

At present colorimetric assays using the tetrazolium salt thiazolyl blue, also termed methyl-thiazolyl-tetrazolium (MTT) are widely used to measure the *in vitro* cytotoxic effects of drugs and plant extracts on cell lines. It is based on the conversion of MTT into formazan crystals by living cells, which determines mitochondrial activity. For most cell populations the total mitochondrial activity is related to the number of viable cells[161,162]. MTT gives a yellowish aqueous solution which, on reduction by dehydrogenases and reducing agents present in metabolically active cells, yields a water insoluble violet-blue formazan[162].

Several studies have investigated the anticancer properties of diverse medicinal plants and the isolated compounds from their extracts. Shaikh *et al*.[125] showed the potent cytotoxic activity of *Helicteres isora* against some cancer cells (HeLa-B75, HL-60, HEP-3B, and PN-15). Negri[163] isolated polyacetylenes from terrestrial plants that have important cytotoxic activity. Araliadiol showed potent cytotoxic activity against human breast adenocarcinoma cell line. Moreover it has been demonstrated that this compound inhibits the cell cycle progression at the G1–S transition through downregulation of CDK4 and cyclin D3, and upregulation of p21WAF-1/Cip1 by a p53 independent mechanism. Besides, stipudiol and stipuol were extracted from the rhizomes of *Panax stipuleantus*. Both compounds markedly inhibited the proliferation of human acute promyelocytic leukemia and human colon cancer cells by inducting apoptosis. Cubebin, isolated from the seeds of *Piper cubeba*, and its five different types of derivatives (a total of 17), with varying functionalities, were tested for their *in vitro* anticancer activity against six human cancer cell lines (A549, K562, SiHa, KB, HCT116 and HT29) using MTT assay. Cubebin as well as its derivatives containing lactone and amide groups showed significant anticancer activity[160].

On the other hand, a handful recent studies have been considered on the cytotoxicity of metal nanoparticles synthesized from plant extracts. Among the these nanoparticles, AgNPs may have considerable potential for development as cytoxic agent because of their unique properties to enhance potential therapeutic efficacy[134].

Mollick *et al*.[164] recently synthesized successfully silver nanoparticles (AgNPs) using $AgNO_3$ via an eco-friendly and simple green route using *Abelmoschus esculentus* pulp extract. The synthesized AgNPs are capable of showing anti-proliferative effects in a dose dependent manner. In earlier studies, biosynthesized AgNPs using *Lonicera hypoglauca* have shown enhanced anticancerous effect against MCF-7 breast cancer cells *in-vitro*[134]. The mechanism implicated either the up-regulation of the p53 tumor suppressor gene or the increases in the expressions of pro-apoptotic Bax, caspase-3 and caspase-9. Silver NPs down-regulated also the mRNA levels of anti-apoptotic Bcl-2 and curtailed (chercher synpyme) the JAK/STAT signaling in MCF-7 cancer cells. Besides, Sriranjani *et al*.[136] synthesized AgNPs from aqueous extract of *Clerodendrum phlomidis*. It was found that these nanoparticles possess excellent anticancer activity than extract alone and it showed 91.84% and 84.91% inhibition against Ehrlich ascites carcinoma and human colorectal adenocarcinoma (HT-29) cells, respectively. Furthermore, the study carried by Rajkuberan *et al*.[133] reveals the rapid biosynthesis of AgNPs using aqueous latex extract of *Euphorbia antiquorum* L as a potential bioreductant. *In vitro* cytotoxicity assessment of bioformulated AgNPs has shown potential anticancer activity against human cervical carcinoma cells (HeLa). The nanoparticles can exhibit cytotoxicity by producing free radicals, which cause cellular degradation and eventually results in cell death[136].

6. Conclusion

Medicinal and aromatic plants are used more preferable than western drugs. However, an important point is to raise awareness of the hazards associated with no scientific basis for their uses.

Therefore, the scientific and pharmaceutical communities received recently more attention to study these plants. So, several researches and different papers have studied various aspects related to medicinal plants and to the use of their active ingredients in therapeutic and other domains. The aim is to prove the validity of the request of their biological effects.

In this review we presented the most advanced methods in the study of medicinal plants which allowed us to conclude that the research progresses in this field to facilitate the exploitation of these plants. Particular focus is on the use of the effective methods in the extraction and isolation of the active compounds. This has enabled exploitation of plants for the treatment of different pathologies and in the development of new herbal drugs. However, the word of plants is complex and further systematic and rigorous studies are very important and necessary. On the other hand, it is not easy to compare results obtained from different used methods and conclusive ones may be considered if tehniques are submitted for standardization and to be approved.

Conflict of interest statement

We declare that we have no conflict of interest.

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