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A critical analysis of publication trends from 2005–2015 in microwave assisted extraction of botanicals: How far we have come and the road ahead

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ABSTRACT

This review has been written with the objective to strategically screen the published research papers (articles) through Scopus database related to microwave assisted extraction (MAE) of bioactive(s) from plant matrix and present a critical analysis report. Scopus is world's largest abstract and indexing database being used by researchers worldwide. This review discusses the publication trends of articles from 2005–2015 related to the above mentioned theme which shall give the readers a 10 year performance report to what extent MAE has been explored for extraction of botanicals compared to other areas of research in natural products. Based on the article screening some of the widely used domains related to MAE of botanicals and the top three participating countries have been identified. Core concept of strategizing and prioritizing research on MAE of botanicals has been presented in a simplified manner.

Contents

1. Introduction

Today natural product research serves as one of the major source of inspiration for researchers involved in drug discovery programmes [1]. Secondary metabolites present in plants forms the basis of such inspiration and these complex organic molecules have always fascinated scientists owing to their remarkable medicinal properties [1]. One of the major objectives of natural product research has always been the isolation and characterization of bioactive compounds which needs to be extracted from complex plant matrix [2,3]. Thus a wise selection of extraction strategy is vital to drug discovery. Extraction of botanicals is the primary step in obtaining the crude extract which signifies the start of natural product research. The extract thus obtained then undergoes either bioactivity testing or isolation of active constituents or phyto-analysis for detection of trace components [4]. The success of the forthcoming steps largely depends upon the fact how well the extract has been prepared. Hence selection of a judicious extraction process which shall ensure exhaustive extraction and at the same time thermal safety to the constituents will be the key to success [2]. Moreover, issues related to standardization and quality control of herbal material are also suffering due to lack of a simple, reliable and powerfull analytical techniques for the chemical analysis of botanicals [5]. A wide array of problems associated with conventional extraction techniques

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pertaining to botanicals have been repeatedly mentioned by many authors who have worked on design and development of newer extraction techniques for plant based bioactive(s) [6,7]. Among the newer techniques, microwave assisted extraction (MAE), pressurized solvent extraction (PSE/ASE), ultrasound assisted extraction (UAE) has been the major focus centers which has drawn considerable research interest in the last decade [8,9]. Such newer extraction operation not only accelerates the process of drug discovery but also adds to the accuracy and precision when it comes to sample preparation compatabile with any chromatographic operation for judging the quality of the finished product [5]. A plenty of review articles have been published pertaining to different extraction methods related to medicinal plants and environmental samples [3–5,8,10]. Scopus database reveals a total of 139 review articles published with the word "microwave assisted extraction" either in their title or abstract body or keywords from 2005–2015. Scopus search using the same above search phrase revealed 15 review articles on MAE in "Trends in Analytical Chemistry" alone, out of which 7 of them are related to MAE of environmental samples and 6 of them are related to food and plants. The primary focus of these review articles has been on the recent strategic developments associated with MAE in the concerned field, factors affecting MAE, coupling techniques with different chromatographic methods, post extraction cleaning-up followed by sample enrichment and instrumentation designing. Mandal and co-workers themselves have published two review articles on MAE of botanicals [3,11]. The former contains the descriptions of different extraction protocols and factors affecting MAE of botanicals, whereas the latter dictates the different chemometric options available for optimization of MAE. In parallel to this, in 2011 another two review articles were published by Zhang et al and Chan et al dedicated purely to MAE of active ingredients from plants [4,12]. These review articles highlighted the principle and mechanisms, different technological modifications possible to enrich MAE further, instrumentation details, also gave a concise list of phytoconstituents that has been extracted using MAE, comparison of MAE performance against other conventional techniques and also even presented brief guidelines for selecting MAE techniques. In light of this situation it can be stated that probably no corner of MAE research has been left untouched for writing a review article. All these review articles are extremely valuable as they serve as building blocks for starting a research on a particular area. The fact that no area has been left out in MAE and an excellent summarization of the MAE concept providing a detailed understanding of the subject is already contained in these reviews, provided a driving force in making this particular review which is based on statistical numbers to generate a short performance report on MAE dedicated to botanicals. Writing a review again on different operational and strategic aspects in MAE of botanicals would have been a mere repetition of what already exists in the scientific domain. This review has been written with the objective to strategically screen the published research papers (articles) in Scopus database related to microwave assisted extraction of medicinally active compounds from plant matrix and present a critical analysis report. Scopus is world's largest abstract and citation database with more than 6 million records (as claimed in their official website) and most widely used by researchers worldwide. Till date no such comprehensive review exist which critically analyzes the publication trends on MAE of botanicals. This review discusses the publication trends of articles from 2005–2015 related to the above mentioned theme which shall give the readers a 10 year performance report to what extent MAE has been explored for extraction of botanicals and also it shall serve as a starting manual for any researcher planning a venture with MAE of botanicals. The analysis report that has been presented shall be a handy comprehensive report for any beginners on this area to plan their work more meticulously. Based on the publication trends as noticed we have logically opined on various situations that have come out after studying the publication records. Such reviews on critical analysis (opinions) of publication records by using bibliometrics and related data-mining methods have been of high value to researchers as it plays a critical role to study the footprints of a particular research which in turn becomes very helpful in planning further research ventures. This is evident from some literature analysis based reviews related to drug discovery published in reputed journals such as Nature Reviews Drug Discovery [13,14].

Table 1
Details of the search parameter and operational settings used to perform the Scopus search

Search parameters	Operational settings
Search term text	<microwave assisted extraction>
Search field type	Article title, abstract, keywords
Data range	2005–2015
Document type	<article>
Subject areas	Life sciences, health sciences and physical sciences
Operator used between two search terms	<and>, <or> depending upon the search requirement

2. Search on MAE of botanicals

Research on MAE of medicinal plants has been majorly on the design and development of optimum conditions for the maximum yield of target compound (bioactive) followed by comparison with conventional techniques [2,3]. In order to study the publication trends only those research papers have been screened which are associated with extraction of bioactives / pharmaceutical aids from terrestrial plants. Marine plants, extraction of pesticide residues/contents from plants or food material have been excluded. The other search parameters which were used have been depicted in Table 1.

Search results from Scopus database for a 10 year period (2005–2015) indicated a total of 2260 published articles [15]. These papers had the word "microwave assisted extraction" either in the article title or abstract body or in the keywords. Generally when a research paper based on MAE is published the occurrence of the word "microwave assisted extraction" is almost certain in any of the above mentioned three areas. Henceforth, article search using the above search field type becomes rationale. All the 2260 articles were screened by their abstract and only those articles year wise were sorted out which was according to the search parameters determined. Briefly, only those articles were sorted which dealt with extraction of bioactive from terrestrial plants. The search results are depicted in Fig. 1.

3. Critical analysis

3.1. Critical analysis: scenario of MAE of botanicals

Search results depicted in Fig. 1 clearly showed a trend that each year after 2010, minimum 50% of the publications that appeared on microwave assisted extraction where based on extraction of bioactive(s) from terrestrial plants. Pearson coefficient value of 0.982 clearly indicates a linear trend (2005–2015) between the total numbers of articles published in Scopus indexed journals on MAE and the number of articles actually related to extraction of bioactive(s) from botanicals. This is quite understandable from the fact that application of microwaves, pressurized liquids or ionic liquids are relatively new technologies which is being applied for the extraction of plant based bioactive(s). Huge technological advancements related to natural product research has taken place but innovation pertaining to extraction of botanicals has been on the slower side. This is evident from the fact that when it comes to drug

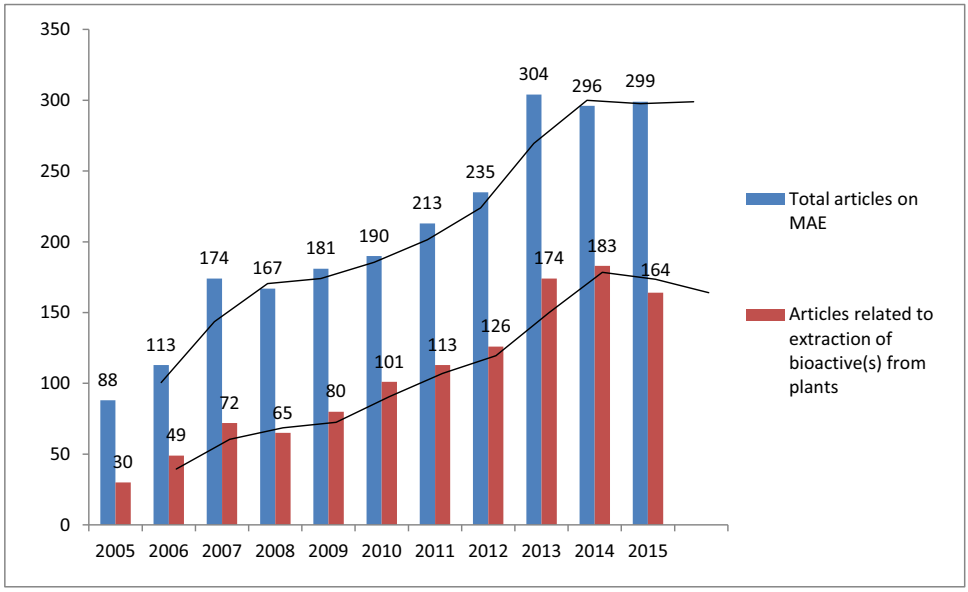

Fig. 1. Search results depicting year wise the total number of publication that appeared on MAE and the number of publication which were related to MAE of bioactive(s) from plants.
Database: Scopus, time range: 2005–2015

discovery from natural products for the management of different diseases, publication rate of research papers has always been high. Publications recorded in Scopus database on MAE of botanicals was compared with the intensity of research on ethnopharmacology of medicinal plants where basically crude extracts are prepared and evaluated for their possible activity on different disease models (*invivo* and *invitro*) and the results are depicted in Table 2. The picture as predicted earlier was found to be very dull when it comes to research on designing newer methods of extraction for botanicals as publication trends clearly showed that ethnopharmacology research clearly enjoys an upper hand. This is quite understandable because drug discovery from natural product entity can only take place through ethnopharmacology research using a holistic approach and MAE chips in later which then can be used for large scale production of such bioactives or extracts. In recent times researchers have felt the drawbacks associated with conventional extraction techniques (like Soxhlet, maceration, percolation, reflux, distillation) are really hurting their research objectives. Today with so much advancement in chromatographic system where we can achieve high resolution of complex mixtures of almost every matrix and with detection limits upto few nanograms or even below, the whole analytical process for the detection of bioactive(s) can really fall apart if a suitable extraction method is not applied for the sample preparation prior to reaching the chromatographic system [2]. Moreover, the fact that huge amount of organic solvent and time consumption associated with conventional methods also really is a matter of concern. In many cases these conventional techniques are not exhaustive enough to pull out traces of target analyte from plant matrix thus producing misleading results and also they are not automated and hence the level of precision is quite low [4]. All these understandings even though came late in the minds of the natural product researchers but now probably after 2010 as can be seen from the publication trend, sufficient interest towards these newer techniques have been raised as far as extraction of botanicals is concerned.

Table 2
Comparison of publication records between MAE of botanicals and ethnophamacology research of medicinal plants

Area of research on medicinal plants	Publications recorded in Scopus (2005–2015)
MAE of botanicals	1157
Antidiabetic	4731
Anticancer	4616
Hepatoprotective	2854
Anti-inflammatory	9626
Antibacterial	12022

The disease area mentioned has been investigated on crude extract of medicinal plants. The word "extract" was included in the keywords to locate only those articles which are concerned with plants.

3.2. Critical analysis: different domains of MAE of botanicals

The different domains in extraction of botanicals where research has been prioritized using MAE was identified and their contribution in terms of appearance as research papers in Scopus indexed journals in the 10 year stipulated tenure is depicted in Fig. 2. Trend clearly shows that extraction of antioxidant, phenolic and flavonoid principles occupies a lion's share contributing 38.89% of the publication share. This is quite understandable from the fact that research on phenolic and antioxidant principles is on the rise due to their positive involvement in the management of different chronic diseases [16]. These principles provide synergistic support to the bioactive compound and are also responsible for maintaining a balance oxidative status of the body and have profound use as dietary supplements [17]. To understand the intensity of research on phenolics and antioxidants a general scan from Scopus database was carried out. A 10 year scan revealed an enormous number of 2, 04,393 articles having either "flavonoids" or "phenolics" or "antioxidants" words in the title or keywords or abstract of the article. Whereas, on the other hand 6816 articles showed up where all these three words have been used together in the above mentioned search field type. These data's are in support of the growing research interest in the field of phenolics and antioxidant principles but also at the same time outnumbers the publications that are associated with MAE and phenolic/antioxidant principles. Most of the research based on MAE and phenolic/antioxidant principles had been on prioritizing the extraction of these principles from plant source.

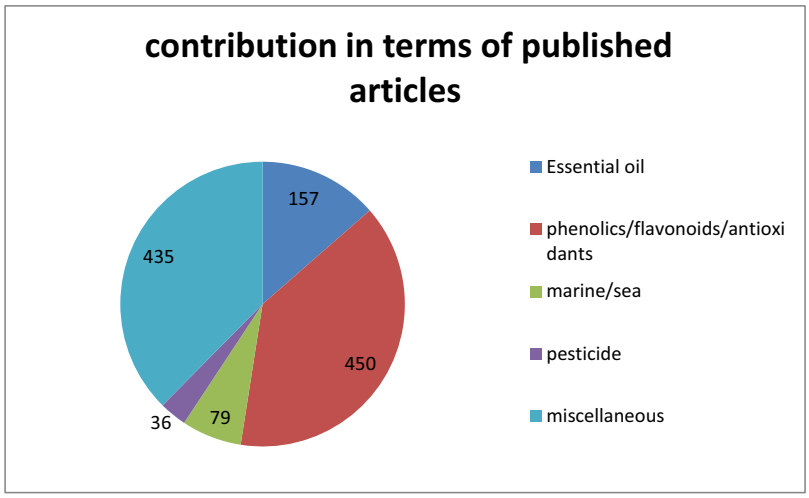

Fig. 2. Different domains in extraction of botanicals where research has been prioritized using MAE and their contribution in terms of appearance as research papers in Scopus indexed Journals.

Different optimization strategies were used for zeroing into the optimum conditions required for the maximum yield of these principles. Performance of MAE process were evaluated from the yield of total phenolic content (using Gallic acid equivalent or others) or total flavonoid content (using rutin/quercitin equivalent or others) or total antioxidant capacity (using trolox or DPPH model). Authors have also identified the individual phenolic antioxidants principles present through HPLC/HPTLC/LC-MS studies [7,18].

The publication share for miscellaneous category counts to 37.5%. In this category the extraction of mostly well established plant bioactive(s) which has immense therapeutic and commercial significance have been dealt with. Researchers have basically used MAE to develop optimized conditions for the large scale extraction of such bioactive(s) with a view that such type of research can form the basis for developing future large scale extraction techniques by industries. Such type of research revolves around the concept of irradiating the plant material immersed in a solvent with microwaves followed by quantifying the target analyte (bioactive analyte) through HPLC/GC/HPTLC using external standards [19,20]. The entire focus of the research lies on developing optimized operating conditions for obtaining the maximum yield. The performance of the procedure is evaluated on the basis of yield of target analyte. Authors in most cases have studied the extraction mechanism through analyzing scanning electron micrographs of marc left over after extraction [21,22a]. The SEM images of the marc (obtained after different extraction process) depicting destruction of surface morphology and disruption of cellular channels have been compared to understand the mechanism of acceleration behind MAE. However, Scopus search (2005–2015) revealed only 37 articles related to MAE of botanicals where the word "SEM" appears in either title or keywords or abstract body. However, even if SEM images had been reported and if the word "SEM" has not been mentioned either in the abstract or keywords then it shall not be included in our search as the search is limited to the words used only in the title, abstract or keywords. This fact also highly signifies the importance of abstract and keywords which affects the search results and also article citation. A similar case also was experienced when it was attempted to find out which technique was commonly used for optimizing several operational parameters involved in MAE. Scopus search revealed 205 articles which had used response surface methodology (RSM) for optimizing a MAE protocol in any field of research. But it may be believed that there exist more papers in Scopus database which has used RSM for optimizing an MAE method but again due to poor keywording or abstracting those articles could not be detected in the search results. However, a review article published by Mandal and coworkers gives a vivid description of different cases of MAE related to botanicals where RSM or factorial designs have been used for optimization [11].

MAE of essential oil accounted for only 13.5% of publication share which can be one of the driving forces for researchers to plan something innovative pertaining to extraction of essential oil. Such ready to use literature analysis reviews definitely helps to channelize the interest of researchers and research agendas in one particular direction. A Scopus search revealed 30,507 articles having the word "essential oil" or "volatile oil" either in their abstract body or title or keywords. This is enormous number signifying the intensity of research associated with essential oils. However, from 30,507 articles only 37 articles were found which dealt with MAE of essential oil. In case of MAE of volatile oil some of the strategies that have been applied are (a) solvent free extraction technique where fresh leaves are loaded in the microwave extraction cavity of an inverted (upside down) microwave extractor. The oil is collected in a desired vessel by the flow of gravity from the ruptured oil glands due to internal heating resulting from microwave absorption, (b) by using a classical Clevenger apparatus coupled with microwave extractor. In a normal microwave extractor operated under atmospheric pressure a reflux condenser is attached to the top of the extractor which is replaced with a clevenger apparatus in case of volatile oil extraction. An excellent review article has been published by Chemat *etal* which dictates the concepts and principles of solvent free extraction which can be effectively utilized for the extraction of essential oil [22b]. The performance of microwave extraction is determined by quantifying the volatile principles using GC-MS/GC analysis.

Extraction of bioactive principles from marine/sea plants and extraction of pesticide residue from plants presents a huge scope of research as far as developing new extraction techniques using microwaves is concerned. Pesticides are generally present in very trace amount and conventional techniques like Soxhlet extraction often do not yield promising results in this regard [23,24]. Henceforth, microwave assisted extraction can definitely be a promising venture for extraction of pesticide residue from plant matrix [24].

After analyzing all the domains where MAE has been prioritized a basic check list has been provided for beginners to further intensify their research on MAE of botanicals and shall also give a scientific merit to this article.

a) Does the solvent have enough dielectric properties to get heated up by absorbing microwaves? Too much absorption of microwaves by the extracting solvent may not allow sufficient microwaves to reach the plant matrix after crossing the solvent layer. This may not allow simultaneous heating of the plant matrix which is mainly responsible for fracture of cell wall and leaching out of target analytes [19]. Hence an optimum sample-solvent ratio must be decided upon. On the other hand if the solvent heats up under microwave to a lesser extent due to lack of dielectric properties, it can then be used for the extraction of thermolabile constituents which after leaching out of the plant matrix will find a cooler zone of bulk solvent which may protect the analytes from thermal degradation [3,19].
b) Pretreatment of the plant matrix with microwave absorbing solvent may be considered for better matrix heating [19,25]
c) A strong optimization strategy (preferably using chemometric tools) which also takes into account the interaction between different factors [11]
d) SEM images may be obtained to ascertain the effects of microwave on plant matrix with emphasis to formation of pores on the cell wall due to internal thermal stress [21].
e) Kinetics study and mathematical modeling for heat and mass transfer phenomenon [21].
f) Reproducibility studies of the proposed method and comparison with conventional techniques to ascertain the supremacy of the proposed technique. Comparison of chromatograms of microwave extracted sample with that of samples obtained from conventional methods may be done to detect any degradation products.
g) Intensification of the MAE process may be considered by application of vacuum or by providing an inert reaction environment by applying inert gasses [12].
h) The bioactivity of the extract/analyte extracted through MAE may be reconfirmed (through *invitro* analysis) to abolish any fear that electromagnetic radiation may alter its inherent biological property [21].
i) Green/energy audit may be done for the whole process of MAE by performing a life cycle analysis which shall ascertain its eco-friendly edge over conventional methods [21,26].

3.3. Critical analysis: different plant parts used for MAE

A critical analysis of different plant parts used in drug discovery and among them which plant part has been used most for MAE has also been scrutinized. The search term used was <name of the plant part > and "extract". The other search parameters remained same as used earlier. This produced results of articles where extracts of different plant parts have been used for research and logical understanding indicates that area of work related with extracts of plant parts will have some medical relevance [5,27]. A detailed graphical analysis report has been shown in Fig. 3a and 3b. Critical analysis of publication records revealed that leaves have been explored more when it comes to using crude drugs for drug discovery and so was the trend seen in case of MAE as there too leaves were used most. Search term "microwave assisted extraction" and <name of the plant part > was used to identify those articles which dealt with MAE using different plant parts. Leaves have been used more in both cases indicates that probably accumulation of bioactive(s) might be more in the leaves. Also collection of leaves doesn't endanger the plant species or cause threat to the local biodiversity as leaves can be grown back by the plants again. Moreover, leaves can be collected in large quantities which is a critical issue in natural product research because the bioactive components are present in very small quantities and only a large amount of crude extract can ensure isolation of few milligrams necessary for structural elucidation and determination of biological activity. Whereas, collection of roots, barks and other parts can considerably endanger the existence of the plant species and their collection in large quantities can further worsen the biodiversity issues [28]. This fact has been very well depicted by Atanasov *etal* where it has been explained how over collection of plant parts for drug discovery can severely affect the local biodiversity and the authors have also stated that over collection leads to crisis in availability of bioresources which is now becoming a leading cause for decline of research interest in drug discovery from natural products [28].

3.4. Critical analysis: MAE of botanicals, the global picture

Based on the search term "microwave assisted extraction" top three countries with their standing is depicted in Fig. 4. However, it is not claimed that all the articles were related to plants. The standings of different countries (top three) on different domains in MAE of botanicals are also depicted in Fig. 5a, 5b, 5c and 5d. China showed their strong involvement in all domains of MAE related to botanicals. Standings of China and India at position 1 and 2 as far as extraction of antioxdiants/phenolic principles are concerned is understandable as both the countries share a rich history of traditional medicinal knowledge through Traditional Chinese Medicine and Ayurveda respectively. As a result both the countries have ample documented knowledge on medicinal plants which can be scientifically explored.

3.5. Critical analysis: can MAE revolutionize botanical extraction?

The trends discussed in this review article give a clear indication that research interest on MAE of botanicals has been greatly uplifted after 2010. Recently concluded Paris Convention for Climate Change definitely puts more accountability towards burning carbon for our economic development. Natural product researcher not even in their wildest dreams can even think how their activities related to extraction may increase the carbon load. Conventional extraction techniques like Soxhlet involves long heating hours which in turn burns excess energy and that's where we should make way for greener technology which makes use of lesser amount of energy resources and thus protects the environment. Many articles have also accounted for the amount of carbon dioxide release by a conventional heating process (Soxhlet) and MAE [21,29]. Calculations have been based on measuring the total consumption of energy resources by the process and then using standard conversion data, the amount of CO_2 can be calculated which is supposed to be liberated by the burning of coal to generate the required power to run the said extraction process in terms of electricity. In the near future probably nothing other than greener technology can survive as environmental protection shall then be the rate limiting step for the industrial growth. In this context it becomes the need of the hour that we adapt to and accommodate new green ways for botanical extraction.

When it comes to new drug discovery from an unexplored plant probably conventional methods are more preferred because optimization of extraction is not an issue in such cases. This statement is based on the general observation that articles which explores the ethnopharmacology of traditionally used medicinal plants generally employs classical extraction methods like maceration and Soxhlet. While working with MAE, optimization becomes critical because even a few seconds of microwave irradiation in excess may be enough to char the bioactive(s) present. This is evident from the published articles of our research group (Mandal and co-workers) where the extraction efficiency with respect to time by keeping other operational parameters at optimum level has been studied [19,21]. Results have clearly indicated that an extra microwave exposure of

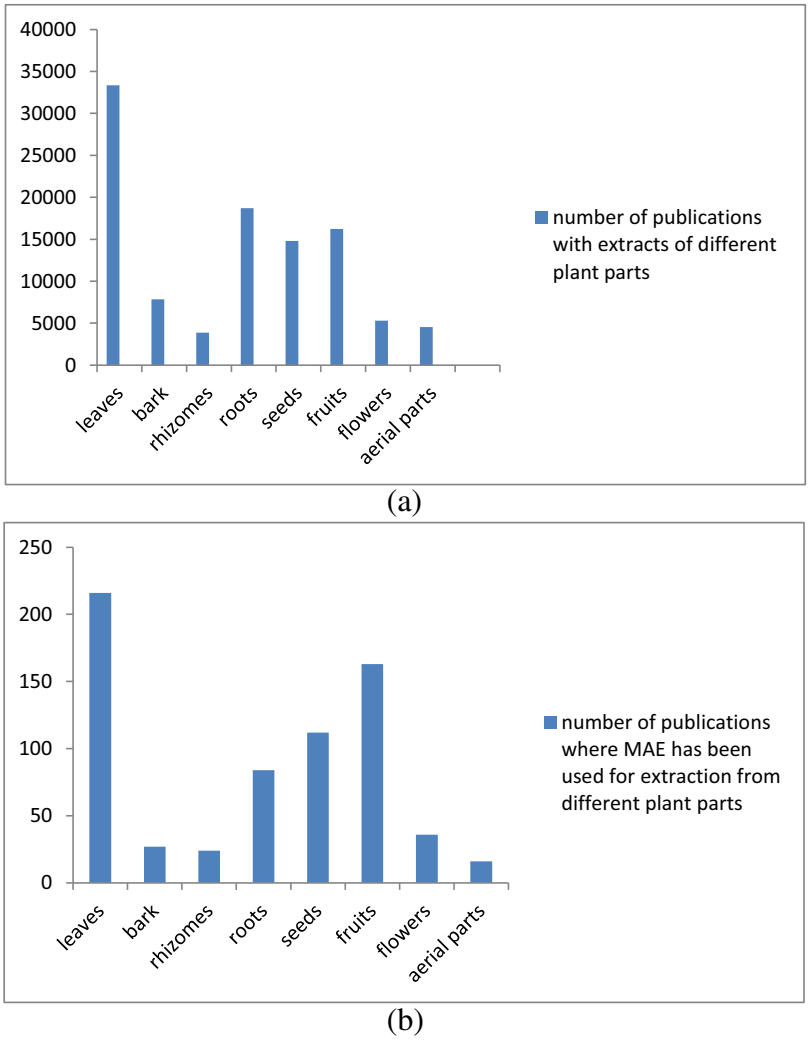

Fig. 3. (a) Graphical representation of number of publications that has appeared on different plant parts whose extracts have been used in drug discovery. (b) Graphical representation of number of publications that has appeared on different plant parts used for MAE.

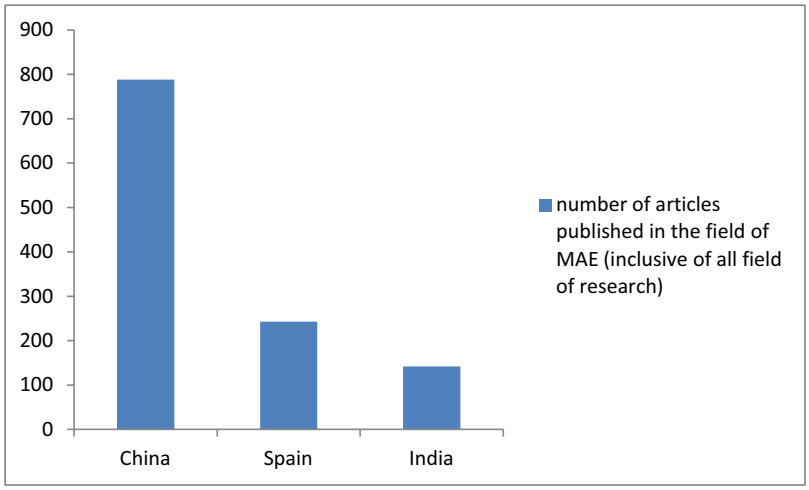

Fig. 4. Ranking of top three countries based on the number of publications on MAE (inclusive of all field) that appeared in Scopus indexed journals from 2005–2015.

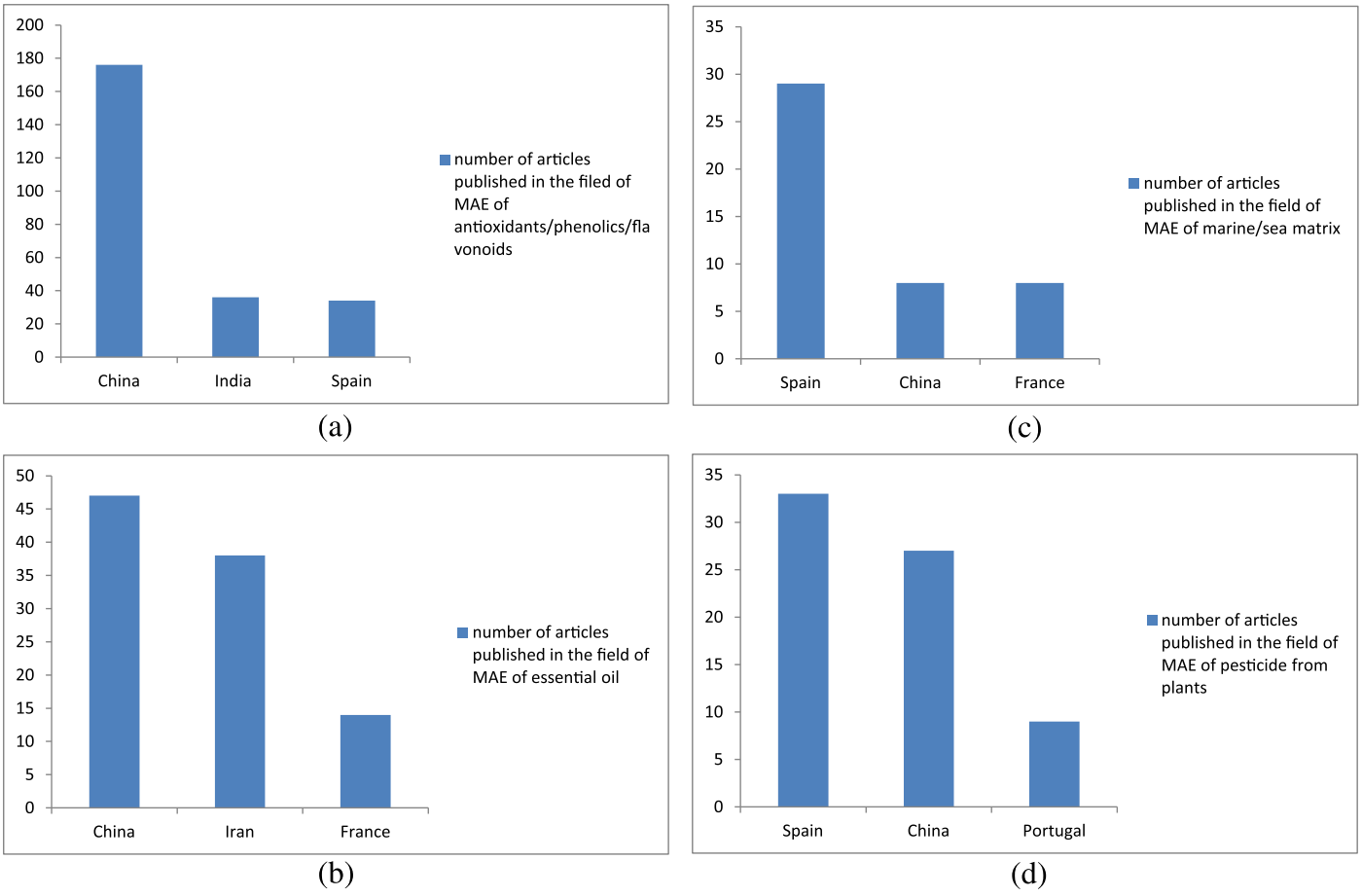

Fig. 5. (a) Ranking of top three countries based on the number of publications on MAE of **antioxidants/phenolic/flavonoids** that appeared in Scopus indexed journals from 2005–2015. (b) Ranking of top three countries based on the number of publications on MAE of **essential oil/volatile oil** that appeared in Scopus indexed journals from 2005–2015. (c) Ranking of top three countries based on the number of publications on MAE of **marine** sample that appeared in Scopus indexed journals from 2005–2015. (d) Ranking of top three countries based on the number of publications on MAE of **pesticide** residue from plants that appeared in Scopus indexed journals from 2005–2015.

even more than 1–2 minutes beyond the optimum exposure is sufficient to jeopardize the entire objective of the research by charring the target analytes. While screening the individual articles related with MAE of botanicals a trend was noticed that when exploring a new plant for its ethnopharmacology and whose chemical profile is yet to be revealed, MAE probably is not among the preferred extraction choice for natural product researchers, because in such a situation a researcher cannot decide on the settings of the operational parameters particularly the time of exposure. All articles related with MAE of botanicals had a specific target analyte to be extracted such as, phenolics, flavonoids, antioxidant principles, pesticide, organic pollutants or a specific phytoconstituent and the yield of these target analytes served as the performance indicator in optimization process of MAE [21,30,31]. The above fact can also be double checked through a carefull observation of articles dealing with ethnopharmacology of medicinal plants which shows a trend that researchers do not start their extraction with MAE for the preparation of crude extract whose activity is to be investigated but rather prefer classical methods [32,33]. Since MAE deals with sample ranging from 0.5–5 gms, detection of anatlytes even if present in trace amount may be easily done but when it comes to isolation of new bioactive(s), sufficient quantity of isolate is necessary for structural elucidation and clinical trials. Since lab scale Microwave extractor offer low sample loading so MAE may not be a preferred customer again for new drug discovery. These could have been the major reasons which might have resulted in slow growth of research interest in adapting MAE as far as extraction of botanicals are concerned.

A comparison of 10 years of publication records in application of microwaves, ultrasound, pressurized liquids and supercritical fluids in all forms of extraction has been depicted in Fig. 6. Results clearly show the supremacy of MAE over other techniques.

4. Conclusion

As already highlighted that plenty of review articles have been written on methodologies associated with MAE, this review takes a different turn to present the research foot prints so produced from MAE of botanicals. Some of the key thrust areas of research on MAE have been highlighted which clearly shows that MAE of phenolics/flavonoids/antioxidant principles has been the most dominant. Henceforth, researchers can definitely take up this area for developing industrial scalable MAE protocols for large scale production of dietary supplements. The vital leads obtained through analysis of publication trends clearly show the importance of green technologies which shall be the key to survival of industries in the near future. Nevertheless, one eventually has to move out of the box and adapt to the recent technologies. MAE along with other newer techniques such as UAE, SCFE and PLE/ASE has immense potential to bring about a green revolution in herbal drug industry. Undoubtedly the major share of research on natural products is on ethnopharmacology driven drug discovery. But sophisticated

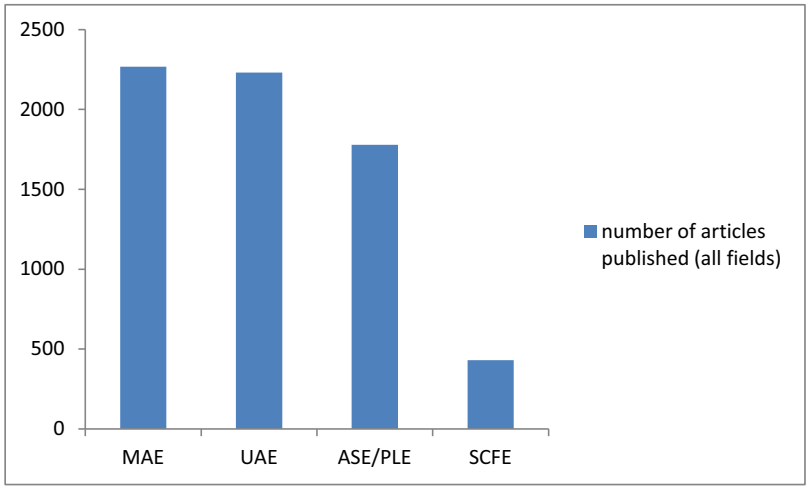

Fig. 6. Comparison of 10 years of publication records in application of microwaves, ultrasound, pressurized liquids and supercritical fluids in all forms of extraction. UAE: ultrasound assisted extraction, ASE/PLE: accelerated solvent extraction/pressurized liquid extraction, SCFE: supercritical fluid extraction.

techniques like MAE can definitely strengthen the entire drug discovery process by providing a sustainable green, economical and rapid large scale industrial source of extraction of such bioactive extracts/fractions/phytoconstituents which then can be made available to common mass. Such critical analysis report based on periodical analysis of the published articles can be very informative regarding the research agenda, the group of participants involved in research, and their priorities & interest in the type of research field. Moreover, such literature analysis can also identify innovation drivers in the field of botanical extraction.

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