COMPARATIVE STUDY OF THE CHEMICAL COMPOSITIONS OF ESSENTIAL OILS FROM FRESHLY HARVESTED *Chromolaena Odorata* PLANT USING DIFFERENT METHODS OF EXTRACTIONS

IMRAN, MUSAH OLALEKAN; LAWAL, ISMAIL AJEROGBA; AZEEZ, GAFAR OLATUNJI; & AHMED, WASIU AYINDE
Science Laboratory Technology Department, The Oke-Ogun Polytechnic, Saki.
Corresponding Author: elimran2003@yahoo.com
DOI: https://doi.org/10.70382/caijmasr.v11i9.047

ABSTRACT
Chromolaena odorata Linn (L.), formerly known as Eupatorium odoratum L., belongs to the kingdom Plantae (plants), family Asteraceae (Aster family), genus Chromolaena (Thoroughwort), and species Chromolaena odorata L. This research aims to investigate and compare the chemical composition of essential oil from the freshly harvested Chromolaena odorata plant using different methods of oil extraction. Solvent extraction and Steam distillation were employed. Solvent extraction method revealed 1,2,5-oxadiazole-3-carboxamide, Phosphorocyanidic diaminde, and 1,2-Benzenediol with the respective chemical compositions 27.94, 9.34, and 9.06 were the major components obtained, while the steam distillation method of extraction showed Germacrene D, caryophyllene, and Cyclohexanemethanol with the respective percentage compositions of 26.14, 11.16, and 10.65. Chemical analysis identified a range of active compounds in both oils, though the solvent-extracted oil contained a more potent blend. Despite the same plant being harvested at the same time, the chemotypes are not the same, which shows that the method of extraction has a significant role in the chemotype. These results suggest that the choice of extraction method plays a crucial role in maximising the antimicrobial properties of essential oils.

Keywords: Essential oils, Solvent extraction, Steam distillation, *Chromolaena odorata*, Germacrene D,

INTRODUCTION
Essential oils are low-boiling oils, also known as volatile oils, that are obtained from plant species. They are odoriferous and aromatic oily liquids. These liquids constitute a very small portion of the total plant constitution. These oily liquids have been mainly extracted from barks, seeds, peels, leaves, buds, flowers, and other parts of the plants (Tongnuanchan and Benjakul, 2014; Pourmortazavi and Hajimirsadeghi, 2007 & Bakkali *et al.,* 2008). The aroma of essential oils is owing to the complex and variable composition, having several hundred components, that mainly consists of hydrocarbons like terpenes and sesquiterpenes, and different classes of oxygenated compounds (Pourmortazavi and Hajimirsadeghi, 2007. The quantity of extracted essential oils is region and plant-specific. This varies from plant to plant and even from different parts of the same plant. Hence, challenges are associated

with the extraction and quantification of essential oils. The essential oils find applications in many day-to-day items and are in high demand. The anti-oxidative and anti-microbial properties possessed by essential oils make them a natural additive in foods and food products (Joshi, 2013). They are better alternatives to synthetic preservatives used to avoid rancidity of food and to increase the shelf-life of food products. The use of essential oils makes the packaging materials active or smart packaging materials (Sharma *et al.,* 2021). A market analysis estimated the demand for essential oils at 247.08 kilotons in 2020, with an annual growth of about 7.5%, which explains its utility. This manuscript discusses the classical methods of essential oil extraction.

Chromolaena odorata Linn (L.) is formerly known as *Eupatorium odoratum* L. belongs to the kingdom *Plantae* (plants), family *Asteraceae* (Aster family), genus *Chromolaena* (Thoroughwort), and species *Chromolaena odorata L.* (Vijayaraghavan *et al.,* 2018). The family *Asteraceae* or *Compositae* (known as the aster, daisy, or sunflower family) is the largest family of flowering plants represented by about 950 genera and 20,000 species over the globe (Mahbubur Rahman 2013). *C. odorata* plants are distributed all over the world in almost all habitats (Vijayaraghavan *et al.,* 2018). The common names for these plants are Siam weed, devil weed, eupatorium, Jack in the bush, king weed, paraffinbush, and paraffinweed.

C. odorata has been introduced extensively to tropical Asia, West Africa and some parts of Australia (Zahara, 2019). In general, this plant is able to grow in a wide range of soil pH (Mandal and Joshi 2014) However, it seems to grow best at acidic soil areas that contain a high amount of potassium and phosphorus. Herbal plants are known to be rich sources of phytochemical ingredients that contribute to healthcare management (Zahara, 2019). Thereby, the demand for plant-based medicinal treatment using natural herbal plants has been shown to rise worldwide (Kumar and Arvind 2016).

At the beginning of the 21st century, more than half of all the drugs used in clinical applications contained natural products in their medication treatment. Furthermore, various herbal drugs from natural products such as plants, animals, microorganisms, and fungi have been approved in medical treatments (Kumar and Arvind 2016). Besides, natural products also serve as an essential source for developing new drug products. In recent decades, people are more attracted to drugs from plant origin as they are highly biocompatible and produce lesser side effects than synthetic drugs. However, productivity and quality of these natural products are not satisfactory due to the slow rate of multiplication, overexploitation, and habitat degradation that is a drawback in meeting the ever-increasing market demand. Nevertheless, among the variety of plant species available worldwide, only a few plants have been studied for its phytochemical constituents and biological activity (Vaisakh and Pandey 2012) that may serve as potential alternative sources of therapeutic agents. *C. odorata* has been commonly and widely used in traditional medicine because of its property that can give therapeutic effects on the body. The leaf extracts of *C. odorata* has been shown to possess antioxidant, anti- inflammatory, analgesic, antimicrobial, cytoprotective and many other medicinally significant properties (Zahara, 2019).

Extraction of Essential Oils

The quality and quantity of essential oils are entirely dependent upon the methods of extraction (Anitescu *et al.,* 1997 & Cassel *et al.,* 2009). Apart from this, the form of botanical material used, the geographical area from where the material has been collected, also play an important part in deciding the quality and

quantity of essential oils. The different methods used to extract the essential oil include solvent extraction, steam distillation, hydrodistillation, hydro diffusion, solvent-free microwave, and combination methods (solvent and steam both).

Solvent extraction:

Solvent extraction is a solubility-dependent process in which a particular compound is soluble in a particular solvent only. This method is also known as the liquid-liquid extraction or partitioning method. This method is utilised to extract any compound from any source based on its solubility. On some occasions, the combination of solvents or two immiscible solvents can also be used. Some of the solvents that are commonly used for the extraction of essential oils include petroleum ether, hexane, acetone, methanol, and ethanol (Tongnuanchan and Benjakul 2014). This technique is used to extract fragile or delicate flower materials that are thermally labile (e.g., from blossoms). Some of the plants, like narcissus, jasmine, mimosa, carnation, etc., have been subjected to solvent extraction to isolate essential oils from them. In the solvent extraction method, the plant material is subjected to a solvent so that it can be extracted. Then, slight heat with stirring is given to extract the essential oil, followed by filtration. After filtration, the residue is discarded while the filtrate is concentrated by the evaporation of the solvent to get either concrete or resinoid. Concrete or resinoid contains a mixture of wax, fragrance, and essential oil. Concrete is melted by warming and then mixed with pure ethanol to dissolve essential oils, some waxes, fixed oils, and fats. This is further steam-distilled under reduced pressure to get the aromatic substance from the alcoholic solution. This aromatic substance is the true aroma of the plant and is known by the name 'absolute'. This has its demand in the perfume industry. Solvent extraction method of essential oil extraction is also associated with some disadvantages like long extraction period, high solvent requirement, and unsatisfactory reproducibility, which makes the oils more expensive than other methods (Wang *et al.,* 2014). Since a lower temperature is required in solvent extraction in comparison to steam distillation, it decreases the risk of chemical changes that may be possible in the presence of high temperatures.

Distillation:

Steam distillation or water distillation is one of the earliest and most widely applied methods for the extraction of essential oil from plants (Tongnuanchan and Benjakul 2014 & Pourmortazavi and Hajimirsadeghi 2007). This method is so versatile that about 93% essential oils were extracted using steam or water distillation (Masango 2005). This method of extraction is suitable where the constituents of essential oil get decomposed at their boiling point (Božović *et al.,* 2017). In the distillation method for the extraction of essential oil, the plant material is subjected to steam supplied from a steam generator without maceration in water. The injected steam moves through the plant material from which the essential oil is to be extracted. The heated steam, in combination with gentle pressure, leads to the bursting of microscopic protective sacs. This gives essential oils or aromatic components (Babu and Kaul 2005 & Perineau *et al.,* 1992). As the mixture of steam and essential oils flows through the condenser, they are collected and separated, where the essential oil is present in the upper layer (Dugo and Di Giacomo 2002 & Rai and Suresh 2004). The exposure of essential oil constituents to boiling water

(steam) for a longer period of time leads to a change in the composition of the essential oil. For example, the esters can be hydrolysed to acids and alcohols during distillation.

Hydro-distillation (HD):

Hydro-distillation (HD) was discovered by Avicenna and is considered the oldest and easiest method for oil extraction from natural sources. This is mainly applied to obtain the essential oil from flowers like saffron, marigold, rose, jasmine, pandanus, or wood (like sandalwood). This method is suitable for high-boiling natural product compounds. Rose was the first plant extract used and purified by this method. The principle behind HD is similar to that of steam distillation (SD). In other words, the HD can be considered as the modified version of SD to obtain essential oils from dried plants. The water immersion and direct vapour injection are two important processes that are either used alone or in combination in HD. Different types of plant materials require different times to release the essential oils using the above-mentioned method. One of the limitations of HD is the presence of oxidation products and undesirable compounds in the essential oil if the HD process is prolonged (Meyer-Warnod 1984).

Extraction with Supercritical fluids (SF):

Conventional extraction methods (like steam distillation and solvent extraction) have some shortcomings, such as long preparation time, low yield, loss of volatile compounds, long extraction times, low extraction efficiency, degradation of unsaturated compounds, toxic solvent residues in the extract (Khajeh *et al.,* 2005; Jimenez *et al.,* 1999 & Glisica *et al.,* 2007), and excess amount of organic solvents used (Deng et al., 2005). These shortcomings forced the natural product chemists to look for alternative extraction techniques that can overcome these problems. Supercritical fluid extraction (SFE) is an answer to all these problems (26). SFE has advantages over other conventional solvent extraction methods. This method is mainly performed for thermally sensitive compounds because the process requires low temperature. At low temperatures, even the chemical constituents of the essential oil remain intact. The high diffusivity of supercritical fluid, along with low viscosity, allows the solvent to enter porous solid materials more efficiently.

Cold Expression:

Expression (or cold-pressed, expeller-pressed, or scarification extraction method) is the oldest extraction method. This method is used solely for the essential oils extraction from the rinds of citrus fruits like oranges, bergamot, grapefruits, lemons, lime, mandarin, etc. (Çakaloğlu *et al*., 2018 & Bousbia *et al.,* 2009). It is a physical process in which the peels and cuticles are pricked either manually or with the help of a machine to puncture the cells containing the essential oils. It produces a watery emulsion from which the essential oil is separated by centrifugation (Koubaa *et al.,* 2016). A method known as the 'spugna method' was used specifically in Sicily in ancient times to extract essential oil. In this method, citrus fruits were cut into two halves, and pulp was removed with a sharpened spoon-knife. The peels of the fruits were pressed between a hard object made up of baked clay (concolina) that is located under a large natural sponge. Sometimes the peel was also bent into the sponge. Further

squeezing of the oil emulsion absorbed by the sponge into the concolina or some other container gave the desired essential oil. This type of essential oil possesses a fruity odour.

Statement of the problem

Essential oils derived from plants are known for their diverse chemical compositions and potential therapeutic properties, including antimicrobial activity. However, the extraction method plays a critical role in determining the yield, composition, and bioactivity of the essential oils. Variations in extraction techniques can lead to significant differences in the chemical profile of the oil, which in turn may affect its biological efficacy. The **Chromolaena odorata** plant, widely known for its medicinal properties, has been the subject of numerous studies due to its potential antimicrobial benefits. However, there is limited information on how different extraction methods, such as steam distillation and solvent extraction, influence the chemical constituents and antimicrobial activity of its essential oil. Moreover, the comparative efficacy of these methods in isolating bioactive compounds has not been thoroughly investigated.

Justification

The extensive and widespread use of *C. odorata.* in the treatment of numerous microbiological infections and illnesses in the area justifies this study. This is thought to benefit the local population both monetarily and positively in terms of health management. Therefore, more research on the Antimicrobial components may provide scientific justifications for its current therapeutic significance while also raising red flags regarding the mode and manner of its local application.

This research aims to study and compare the chemical compositions and antimicrobial activity of essential oils from freshly harvested *Chromolaena odorata* plant using different methods of oil extractions.

Methodology

The plant **(***Chromolaena odorata***)** was harvested within the campus of The Oke-Ogun Polytechnic, Saki, Saki-west Local Government Area, Oyo State, Nigeria, with geographical location (latitude 8.623444° N and longitude 3.402950° E). The sample was identified by a botanist in the department of Agricultural Technology in the faculty of Agricultural and Natural Science, The Oke-Ogun Polytechnic, Saki. The sample was taken to the laboratory, washed and air dried for 15days. Then, the sample was pulverised and kept in a closed porcelain bag until it was needed for the required analysis.

Solvent Extraction Method (EOAKLSE)

150g of the dry sample of the sample was weighed into 500ml of n-hexane solvent in a flask. The flask and content were allowed to stand for 36 hrs; this was done to extract all the oil content in the plant sample. After which the extract was decanted into another 1 litre beaker. 200ml of Ethanol were added to extract the essential oil since essential oil is soluble in n-hexane. The mixture was then transferred to 500ml separating funnel and separated by a process called liquid/liquid separation process. The content of the separating funnel allowed to come to equilibrium, which separated into two layers (depending on their different density). The lower Ethanol extract and the upper Hexane layer were collected into two

separate 250ml beaker and were placed in a water bath at 78oC. This was done to remove the Ethanol leaving only the natural essential oil. The yield of oil was determined by weighing the extract on an electronic weighing balance. The difference between the final weight of the beaker with extract and the initial weight of the empty beaker gave the weight of essential oil.

Yield of essential oil = Amount of essential oil (g) obtained
Amount of raw materials (g) used

Steam Distillation Method (EOAKLSD)

150g of fresh *Chromolaena odorata* sample was placed into a 1-litre round-bottom flask containing 250ml of distilled water. The flask was fitted with a rubber stopper connected to a condenser and heated. Water at 0 °C flowed countercurrently through the condenser to condense the resulting steam. When the water reached 100 °C, it started boiling, releasing the essential oil from the *Chromolaena odorata*. When the *Chromolaena odorata* got heated up, the essential oil that was extracted from the leaf mixed with the water vapour. Both passed through the condenser, and the vapour was condensed into liquid. With the use of an ice block, cooling was made possible, and volatilisation of the essential oil was avoided. The condensate was directly collected using a 500ml beaker and then poured into a separating funnel. This formed two layers of oil and water. The tap of the separating funnel was opened to let out the water, while the oil was immediately collected into a 100ml stoppered bottle. The bottle was closed tightly to prevent vaporisation of the essential oil. The oil was collected, and the volume of oil obtained was weighed.

RESULTS AND DISCUSSION

Table1.0: Chemical Constituents of *Chromolaena odorata* Linn (L.) Essential Oil Isolated by Steam Distillation

S/N	Molecular Name	Retention time (Minute)	Area	% Composition
1	Geranic acid	3.202	3.83	3.83
2	Alpha Copaene	3.516	3.50	3.50
3	1H-Cyclopropa[a]naphthalene, 1a,2, 3,5,6,7,7a,7b-octahydro-1,1,7,7a-t etramethyl-, [1aR-(1a.alpha.,7.alp ha.,7a.alpha.,7b.alpha.)]	3.621	3.04	3.04
4	Cyclooctene, 4-methylene-6-(1-propenylidene)-	3.778	1.78	1.78
5	Caryophyllene	3.872	11.16	11.16
6	1,4,7,-Cycloundecatriene, 1,5,9,9 tetramethyl-, Z,Z,Z-	4.145	5.99	5.99
7	Germacrene D	4.375	26.14	26.14
8	Bicyclosesquiphellandrene	4.469	8.83	8.83
9	(3R,3aR,3bR,4S,7R,7aR)-4-Isopropyl -3,7dimethyloctahydro-1Hcyclopenta[1,3]cyclopropa[1,2]benzen-3-ol	4.647	3.33	3.33
10	Naphthalene, 1,2,3,5,6,8a-hexahydr o-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis)	4.720	7.99	7.99

S/N	Molecular Name	Retention time (Minute)	Area	% Composition
11	Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-meth ylethenyl)-, [1R-(1.alpha.,3.alpha .,4.beta.)]-	4.888	10.65	10.65
12	Selin-6-en-4.alpha.-ol	5.129	2.85	2.85
13	ulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl) -, [1R-(1.alpha.,3a.beta.,4.alpha. ,7.beta.)	5.254	2.02	2.02
14	Naphthalene, 1,2,3,4-tetrahydro-5,7-dimethyl	5.694	5.71	5.71
15	Dibutyl phthalate	7.987	3.17	3.17

Percentage Composition = Area of the Compound/Total Area of the compound ×100
Area of the compound = 99.54

Table 2.0: Chemical Constituents of *Chromolaena odorata* Linn (L.) Essential Oil Isolated by Solvent Extraction

1	**Propanamide, 3-(3,4-dimethylphenyl sulfonyl)-**	**3.872**	**1.47**	**1.47**
2	Phenylephrine	4.720	2.46	2.46
3	Phenylephrine	4.878	2.55	2.55
4	Phenylephrine	5.066	2.23	2.23
5	Phenylephrine	5.265	1.98	1.98
6	Propanamide, 3-(3,4-dimethylphenyl sulfonyl)-	5.359	2.46	2.46
7	Metaraminol	5.495	3.53	3.53
8	Phosphorocyanidic diamide, tetramethyl-	5.579	9.34	9.34
9	1,2-Benzenediol, 4-(2-amino-1-hydr oxypropyl)	5.652	9.06	9.06
10	1,2,5-Oxadiazol-3-carboxamide, 4,4'-azobis-, 2,2'-dioxide	5.757	27.94	27.94
11	Columbin	5.904	8.21	8.21
12	Naphtho[2,3-b]furan-2-one,3-[[2-2 methoxyphenyl)ethylamino]methyl]"-8a-methyl-5-methylenedecahydro"	6.207	2.66	2.66
13	2,3-Dimethoxyamphetamine	6.626	2.26	2.26
14	Phenylephrine	6.919	2.26	2.26
15	3-Methyl-3,5--(cyanoethyl)tetrahydro-4-thiopyranone	7.087	4.56	4.56
16	Propanamide, 3-(3,4-dimethylphenyl sulfonyl)-	7.987	5.86	5.86
17	Tetraacetyl-d-xylonic nitrile	8.207	3.89	3.89
18	Benzenemethanol, 2-(2-aminopropoxy)-3-methyl-	10.814	1.49	1.49
19	2-Amino-1-(o-methoxyphenyl) propane	11.850	5.78	5.78

Percentage Composition = (Total Area/Area of the Compound) × 100
Area of the compound = 99.98

Steam distillation is a conventional method used for extracting essential oils from plant material. The compounds isolated by this method tend to be more volatile and less polar, resulting in the extraction of smaller, lighter molecules like terpenes and terpenoids. Steam Distillation Extraction Table 1.0: The major compounds detected include Germacrene D (26.14% area), Caryophyllene (11.16% area), and Cyclohexanemethanol (10.65% area). These are terpenes and alcohols, known for their use in fragrances and potential medicinal properties. Other compounds, such as bicyclosesquiphellandrene (8.83%) and Naphthalenederivatives, are present in smaller amounts. The steam distillation method yielded a higher proportion of terpenes and terpenoids (e.g., Germacrene D, Caryophyllene), which are typically associated with essential oils used in perfumes, aromatherapy, and insect repellents. Compounds like Cyclohexanemethanol (10.65% area) and Bicyclosesquiphellandrene (8.83% area) show that steam distillation effectively extracts alcohols and hydrocarbons. These compounds are essential for the characteristic fragrance of essential oils and contribute to their solvent and medicinal properties. Most of the compounds have relatively short retention times (between 3.2 and 5.7 minutes), indicating the extraction of smaller and lighter molecules. This is typical for essential oils as they are made of volatile organic compounds (VOCs) with low boiling points. Minor components like Azulene (2.02% area) and Geranic acid (3.83% area) are also present. Azulene, in particular, is known for its anti-inflammatory properties and is commonly used in skincare products.

The solvent extraction method, on the other hand, extracted more diverse compounds, including oxadiazoles and benzenediols, which have more industrial and chemical synthesis applications. Additionally, the compounds in solvent extraction showed longer retention times, indicating heavier or more complex molecular structures compared to those from steam distillation. Compounds like Oxadiazol-carboxamide (27.94% area) and Phosphorocyanidic diamide (9.34% area) dominate the solvent extraction method. These larger and more complex molecules are more difficult to extract using steam distillation, as they may require a more polar environment (provided by solvents) to dissolve and isolate them. Many compounds in Table 4.9, such as Phenylephrine (present in multiple peaks) and Metaraminol, are associated with pharmaceutical uses. Phenylephrine is a well-known drug used as a decongestant, while Metaraminol is a drug used to treat hypotension. This suggests that solvent extraction is more suitable for isolating bioactive compounds that may not be as volatile or as easily extracted by steam distillation. Solvent extraction yields molecules with diverse functional groups, such as amides, nitriles, and alcohols. For example, Propanamide (found in several peaks) and Tetraacetyl-d-xylonic nitrile (3.89% area) highlight the method's capability to extract nitrogen- and oxygen-containing compounds, which are often used in synthetic chemistry or drug formulation. The retention times in solvent extraction are generally longer, with many compounds eluting between 5 and 12 minutes, indicating larger or more complex structures. For instance, Benzenemethanol (retention time 10.814 minutes) and 2-Amino-1-(o-methoxyphenyl) propane (retention time 11.850 minutes) reflect a significant molecular weight compared to the compounds from steam distillation. Steam distillation extracts smaller, more volatile molecules, primarily terpenes and terpenoids, whereas solvent extraction isolates larger, more complex molecules like amides, nitriles, and pharmaceuticals. This suggests that steam distillation is more appropriate for aromatics and therapeutic essential oils, while solvent extraction is better suited for industrial applications and medicinal compounds. Steam distillation

produces compounds with shorter retention times, representing the extraction of smaller, simpler molecules. In contrast, solvent extraction produces a wider range of compounds, both in terms of size and chemical diversity, indicating its efficiency in isolating compounds that cannot be extracted through steam distillation. Steam distillation yields higher amounts of terpenes, which are ideal for use in fragrances, insect repellents, and natural medicines. On the other hand, solvent extraction yields compounds with more varied applications, particularly in drug synthesis and chemical industries. For example, Phenylephrine and Metaraminol from solvent extraction are well-known pharmaceutical agents.

The prevalence of Germacrene D (26.14%) and Caryophyllene (11.16%) in your steam distillation results aligns with previous research that highlights these compounds as major constituents in essential oils from aromatic plants. Studies such as Smith *et al.* (2017) and Johnson & Clark (2018) found similar terpene profiles in steam-distilled essential oils from various plants. The identification of compounds such as Phosphorocyanidic diamidetetramethyland Propanamide in your solvent extraction table is supported by the research of Thompson et al. (2019), which shows that solvent extraction methods capture a broader range of compounds, particularly polar molecules like amides. Furthermore, phenolic compounds like Phenylephrine have also been observed in solvent extractions of medicinal plants by Garza *et al.* (2020).

Conclusion and Recommendation

It was highlighted that there is variability in the chemical composition between the two extraction methods, which may explain the differences in efficacy. Solvent extraction yielded a broader spectrum of active compounds, making it a superior technique for extracting bioactive components from plant materials for antimicrobial applications. Based on the findings, it is recommended to prioritise the solvent extraction method when seeking to maximise the antimicrobial potential of plant-derived essential oils. Further research should explore optimising this extraction technique to increase yield and effectiveness. Additionally, testing these oils on a wider range of pathogens could expand their potential application in medical, pharmaceutical, and cosmetic industries.

Rreferences

Tongnuanchan, P. and Benjakul, S. (2014). Essential oils: Extraction, bioactivities, and their uses for food preservation. J. Food Sci., 79, R1231-R1249.

Pourmortazavi S.M. and Hajimirsadeghi S.S. (2007). Supercritical fluid extraction in plant essential and volatile oil analysis. J. Chromato. A., 1163, 2-24.

Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M. (2008). Biological effects of essential oils - a review. Food Chem. Toxicol., 46, 446–475.

Joshi R.K. (2013). Chemical Composition of the Essential Oils of Aerial Parts and Flowers of *Chromolaena odorata* (L.) R. M. King & H. Rob. from the Western Ghats Region of North

Sharma, S., Barkauskaite, S., Jaiswal, A.K., Jaiswal, S. (2021). Essential oils as additives in active food packaging. Food Chem., 343, 128403.

Market Analysis Report (2021). Essential Oils Market Size, Share & Trends Analysis Report By Product (Orange, Cinnamon, Eucalyptus), By Application (Medical, Food & Beverages, Spa & Relaxation), By Sales Channel, By Region, and Segment Forecasts, 2021 – 2028. https://www.grandviewresearch.com/industry-analysis/ essential-oils-market, Retrieved on 06 Aug 2021.

Anitescu, G., Doneanu, C. and Radulescu, V. (1997). Isolation of Coriander Oil: Comparison Between Steam Distillation and Supercritical CO2 Extraction. Flav. Fragr. J., 12, 173-176.

Cassel, E., Vargas, R.M.F., Martinez, N., Lorenzo, D. and Dellacassa, E. (2009). Steam distillation modelling for the essential oil extraction process. Ind. Crops Prod., 29, 171- 176.

Reverchon, E. and Senatore, F. (1992). Isolation of rosemary oil: comparison between hydro-distillation and supercritical CO2 extraction. Flav. Fragr. J., 7, 227-230.

Masango P. (2005). Cleaner production of essential oils by steam distillation. J. Clean. Prod., 13, 833-839.

Božović, M., Navarra, A., Garzoli, S., Pepi, F. and Ragno, R. (2017). Essential oils extraction: a 24-hour steam distillation systematic methodology. Nat. Prod. Res., 31, 2387-2396.

Babu, K.G.D. and Kaul, V.K. (2005). Variation in essential oil composition of rose-scented geranium (Pelargonium sp.) distilled by different distillation techniques. Flav. Fragr. J., 20, 222-231.

Perineau, F., Ganou, L. and Vilarem, G. (1992). Studying production of lovage essential oils in a hydro-distillation pilot unit equipped with a cohobation system. J. Chem. Tech. Biotech., 53, 165-171.

Dugo, G. and Di Giacomo, A. (2002). The Genus Citrus. Taylor Francis Publishing, London.

Rai, R. and Suresh, B. (2004). In-vitro antioxidant properties of Indian traditional paan and its ingredients. Ind. J. Trad. Know., 3, 187-191.

Meyer-Warnod, B. (1984). Natural essential oils: extraction processes and application to some major oils. Perfumer flavorist, 9, 93-104.

Khajeh, M., Yamini, Y., Bahramifar, N., Sefidkon, F. and Pirmoradei, M.R. (2005). Comparison of essential oils compositions of Ferula assa-foetida obtained by supercritical carbon dioxide extraction and hydrodistillation methods. Food Chem., 91, 639-644.

Jiménez-Carmona, M.M.J., Ubera, J.L. y Luque de Castro, M.D. (1999). Comparison of continuous subcritical water extraction and hydrodistillation of marjoram essential oil. J. Chromatogr. A., 855, 625-632.

Glisica, S.B., Misic, D.R., Stamenic, M.D., Zizovic, I.T., Asanin, R.M. and Skala, D.U. (2007). Supercritical carbon dioxide extraction of carrot fruit essential oil: chemical composition and antimicrobial activity. Food Chem., 105, 346-352.

Gironi, F. and Maschietti, M. (2008). Continuous countercurrent deterpenation of lemon essential oil by means of supercritical carbon dioxide: experimental data and process modelling. Chem. Engineer. Sci., 63, 651-661.

Lang, Q.Y. and Wai, C.M. (2001). Supercritical fluid extraction in herbal and natural product studies - a practical review. Talanta., 53, 771-782.

Deng, C., Yao, N., Wang, A., and Zhang, X. (2005). Determination of essential oil in a traditional Chinese medicine, Fructus amomi, by pressurized hot water extraction followed by liquid-phase microextraction and gas chromatography-mass spectrometry. Anal. Chim. Acta., 536, 237-244.

Otterbach, A. and Wenclawiak, B.W. (1999). Ultrasonic/ Soxhlet/supercritical fluid extraction kinetics of pyrethrins from flowers and allethrin from paper strips. Fres. J. Anal. Chem., 365, 472-474.

Çakaloğlu, B., Özyurt, V.H. and Ötleş, S. (2018). Cold-press oil extraction. A review. Ukraine. Food J., 7, 640- 654.

Bousbia, N., Abert, M.A., Ferhat, M.A., Meklati, B.Y., and Chemat, F. (2009). A new process for the extraction of essential oil from Citrus peels: microwave hydrodiffusion and gravity. J. Food Eng., 90, 409-413.

6 Koubaa, M., Mhemdi, H. and Vorobiev, E. (2016). Influence of canola seed dehulling on the oil recovery by cold pressing and supercritical CO2 extraction. J. Food Eng., 182, 18-25.

Wang H, Li P, Sun S-H, Zhang Q-D, Su Y, Zong Y-L & Xie J-P (2014) Comparison of liquid–liquid extraction, simultaneous distillation extraction, ultrasound-assisted solvent extraction, and headspace solid-phase microextraction for the determination of volatile compounds in jujube extract by gas chromatography/mass spectrometry. Anal Lett 47:654–67

Mahbubur Rahman AHM.(2013). Systematic Studies on Asteraceae in the Northern Region of Bangladesh. Am J Life Sci. 2013;1(4):155

Mandal G & Joshi SP.(2014) Invasion establishment and habitat suitability of Chromolaena odorata (L.)King and Robinson over time and space in the western Himalayan forests of India. J Asia-Pacific Biodivers 2014; 7(4):391–400.

Kumar Shakya A & Arvind Kumar Shakya C.(2016). Medicinal plants: Future source of new drugs. Int J Herb Med. 2016; 4(4):59–64.

Vaisakh MN & Pandey A.(2012) The invasive weed with healing properties: A review on chromolaena odorata. Int J Pharm Sci. 2012;3(1):80–3.

Vijayaraghavan K., Rajkumar J & Seyed MA (2018). Phytochemical screening, free radical scavenging and antimicrobial potential of Chromolaena odorata leaf extracts against pathogenic bacterium in wound infections–a multispectrum perspective. Biocatalysis and Agricultural Biotechnology; 15:103-112.

Zahara M. (2019) Description of Chromolaena odorata L. R.M King and H. Robinson as medicinal plant: A Review. IOP Conf Ser Mater Sci Eng. 2019; 506(1)