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Modified microwave assisted extraction of essential oils from Ceylon clove (*Syzygium aromaticum*) as a cost-effective and sustainable extraction method

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Abstract
Essential oils, also known as volatile oils are liquid extracts obtained from plants that have been known to contain numerous health benefits. The main objective of this study was to extract the Sri Lankan clove (*Syzygium aromaticum*) essential oil, using a green microwave assisted extraction method with low energy use and reduced wastage without compromising the quality. Domestically modified microwave assisted extraction was used to extract the clove essential oil as a clean technology in which directly heated the in situ water and exploded to extract all the bioactive compounds. Nevertheless, the method is price competitive with a high quality of the essential oil. 50g of dried clove powder/ 400 ml of water provided the maximum yield of 16.5%. The composition profile of clove essential oil was obtained by Gas chromatography, Mass spectrometry which resulted in 37.67% of Eugenol.

Keywords: Microwave assisted extraction method, sonication, gas chromatography mass spectrometry

1. Introduction
Essential oils, also known as volatile oils are liquid extracts obtained from plants that have been known to contain numerous health benefits. The use of these medicinal plants as cosmetics, food preservatives, and for the treatment of various ailments, date back to centuries ago. With the advancements made in science, a deeper approach into the various uses of essential oils and their constituents have received great interest [1]. Such uses of essential oils can be seen in the food industry as flavorings, aromatics, and preservatives, also in the cosmetics industry as fragrances and in the pharmaceutical industry as natural alternatives for various medications [2]. The Bioactive compounds found to be present in essential oils have resulted in contributing to the various properties of essential oils such as anti-fungal, anti-oxidative and anti-bacterial effects. Essential oils are made up of a complex of bioactive compounds such as aromatic compounds, terpenes, and terpenoids (such as aldehydes, ketones, phenols and alcohols) which give rise to these properties [3-4]. Oils can be extracted from various plant parts such as roots, barks, berries, seeds, rhizomes, leaves and flowers, using various techniques such as hydrodistillation, steam distillation, solvent extraction and super critical extraction method. Microwave assisted extraction method is a recently adopted green technique that is used in obtaining essential oils from the plant materials different from the traditional methods. Electromagnetic waves disrupt the cells to release the essential oils. This technique has advantages over the traditional methods such as short extraction time, reduced solvent consumption less environmental pollution and higher extraction yield whilst preserving the quality of the oils with minimal to no thermal degradation [5].
In this paper a cost effective, green extraction method was conducted by modified Microwave Assisted Extraction method (MAE) to extract the clove essential oil from Sri Lankan clove buds (*Syzygium aromaticum*). Recent article shows that the use of microwave assisted extraction method has an advantage of obtaining the pure eugenol from cloves without traces of acetyl eugenol, which is an advantage over the existing extraction methods [6].
Clove (*Syzygium aromaticum*) is an aromatic bud cultivated mostly in the mid wet zone of Sri Lanka and now being considered to cultivate in the low country of Sri Lanka consists of high amount of phenolic compounds having various properties as antioxidant, antibacterial, antifungal activities and Insecticidal activities[7]. According to the Food and Drug Administration (FDA), clove essential oil is classified as generally recognized as Safe (GRAS) hence it can be used in the industries of medicine, food, cosmetics and sanitary products [8].

Around thirty different types of compounds have been found in clove essential oil eugenol being 50% [6]. Eugenol is a phenolpropanoid compound which could vary in colour from colorless to yellow with an intense odour [9]. According to Banerjee *et al*., 2020 eugenol was observed to have an anti-inflammatory effect and wound healing ability [10]. Furthermore Eugenol has shown anti-cancer activity against colon, breast, prostate, and skin cancers and also in melanoma and leukemia [11]. Eugenyl acetate is a phenylpropanoid derivative of Eugenol which exhibits antibacterial, anticancer, antimutagenic, antioxidant and antivirulent activity [12]. However, the current study does not have any traces of Eugenyl acetate. Humulene is another component found in clove essential oil which has the ability of anti-inflammatory, antitumor, anti proliferative activity that helps in many cancers such as lung cancer, colon cancer, prostate cancer [13]. β-caryophyllene is a sesquiterpene found in clove oil, has demonstrated antimicrobial, anti-inflammatory and anti-carcinogenic properties and has shown to be effective against many cancers such as prostate cancer, breast cancer, skin cancer, leukemia, lymphatic cancer and cervical cancer [12].

2. Materials and Methods

Clove buds were plucked from Meetiyagoda a small village in the low country of Sri Lanka as shown in figure 1. Clove buds were sun dried and the buds were grounded at 2500 rpm to a fine powder using a high power grinder (Figure 2). Distilled water was used as the solvent to extract the clove essential oil.

Fig 1: Sun dried Clove buds prior to grinding plucked from a low country village in Sri Lanka

Fig 2: Grinding process by using high power 25000 rpm grinder

2.1 Sonication

Ultrasonic sonicator 100W with a titanium horn was used to enhance the extraction; 10 g of clove powder was dissolved in 50 ml water and subjected to sonication. The sonication cavitation [14] cause implosion of bubbles. Hence the agitation results in extreme high pressure that leads to the breakdown of cell walls and in turn enhancing the release of essential oils. Sonication was carried out for 20 minutes [15].

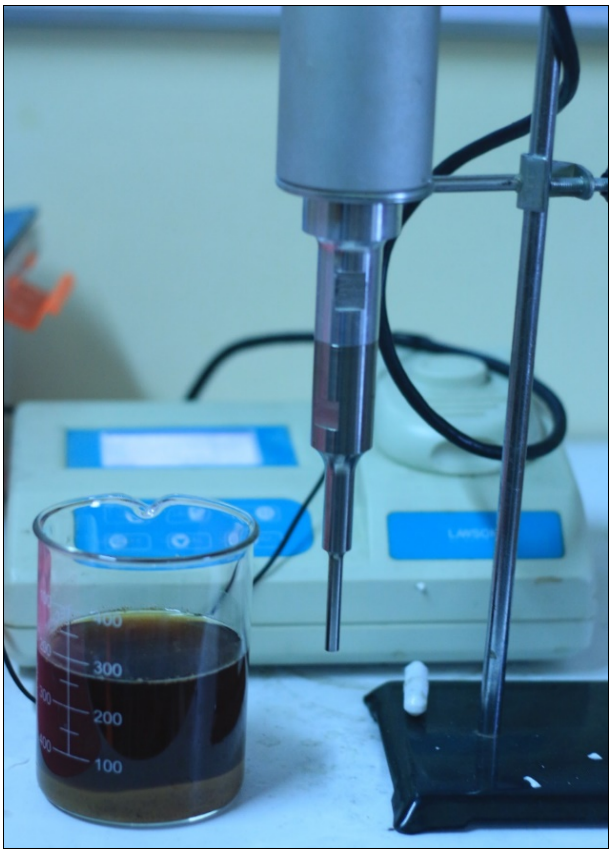

Fig 3: Sonication of the clove powder dissolved in 50 ml water using ultra sonic sonicator (100 W with a titanium horn)

2.2 Instrumentation of the modified microwave extraction plant

Microwave extraction method was carried out using a modified domestic microwave oven as shown in figure 4, which made it possible to consistently run the machine without overheating the component or burning the high power capacitor. The modification of the microwave oven was done using a microwave reactor 800W according to several previous studies [16-17]. As the microwave full power was 1000W, using it in 800W means the microwave would power periodically which allowed the partials to mixed evenly in the solution, This also reduced the bumping caused by overheating avoid any location with very high temperatures. Clevenger apparatus with a reflux condenser is connected to the hole created in the microwave oven. 2000 ml round bottom flask was used as the vessel as this allowed other possibilities with the same machine. Some of the internal components such as the high voltage capacitor were taken out and was cooled separately to avoid any disruption. External cavity was separately cooled, as such machines are meant to be used for shorter periods of time several changes had to be made to the device to make possible to consistently use. An additional stirrer was added to the top to mix the clove solution homogenously. This would also help to avoid the excessive bumping issue which can overflow the solution.

Fig 4: Modified Microwave Assisted Extraction Plant

2.3 Microwave assisted extraction of the clove essential oil

Several extraction rounds were carried out using different powder to water ratios as mentioned in Table 1. Distilled water was used as the solvent. The best yield was obtained with 50g of clove powder and 400 ml of distilled water. After half an hour of extraction at 90 ^{0}C -100 ^{0}C with a flow rate of approximately 8 ml per minute, Two phases of condensates were obtained the oil phase and aqueous phase, The 50g of clove powder to 400 ml distilled water ratio was extracted at different timings and 30 minutes of extraction gave the best yield. The clove oil is denser than water, hence most of the oil was trapped in the bottom of the Clevenger trap and some of the less dense oil was floating on the aqueous layer.

Table 1: Ratio of Clove powder (g): Water (ml)

Clove powder(g):	Water(ml)
50	350
50	500
50	400

Micropipette was used to filter out the water and the remaining oil was separated by centrifugation at 2500 rpm for 30 minutes by using a LD_{50} centrifuge

The obtained yield of the clove essential oil was calculated from the below equation Eq. (1).

2.4 Equations

$$\text{Yield} = \frac{\text{Weight of the oil} \times 100}{\text{Weight of the dry clove powder}} \quad \text{Eq. (1)}$$

2.4 Gas chromatography – mass spectrometry (GC-MS) analysis of chemical components

It is necessary to determine the quality of the clove essential oil and to determine the components of it. The analysis was performed using GC-MS (Agilent 6980N chromatography gas coupled with Agilent 5973 inert mass spectrometry). The sample was injected at a 250 °C inlet temperature with a split ratio of 1:50. Flow rate was maintained at a rate of 1ml per min inside the column. Wiley's mass spectral data library (version 7) was used to identify the chromatographic peaks.

3. Results and Discussion

There are many methods of extracting the essential oils from clove buds and are mostly being classified as the conventional methods and the novel methods. The conventional extraction methods are based on the distillation process through injecting steam or water through the plant material [18]. However with the advancement of technology, novel methods have been introduced such as super critical extraction, ultrasonic assisted extraction, sub critical fluid extraction and microwave assisted extraction [19]. In this current study clove oil was extracted by microwave assisted extraction enhanced by ultrasonic sonication, which is carried out for 30 minutes, followed by centrifugation for the separation of oil. The yield percentages are indicated in Table 2.

Table 2: The yield obtained by different ratios of clove powder and water

Ratio Clove powder(g): Water(ml)	Average Yield (%)
50g:350 ml	14.8
50g:500 ml	15.3
50g:400 ml	16.5

In this study the clove extraction was carried out with and without sonication to understand the importance of sonication on extraction on clove essential oil. When the particles were subjected to direct microwave extraction without processing them through sonication the yields were observed to be about 20% lower for the same extraction time and same ratio of clove powder (g): water (ml). This can be explained by the fact that the sonication provided the additional agitation to support the rupture of the hardened exterior walls of the clove pods.

The maximum oil was obtained by the oily fraction isolated from the clove essential oil which had a light-yellow color with a strong aroma, which accounted for 16.5% of the clove essential oil, However further studies are necessary to identify the effect of time on the yields. The yield obtained was higher than the yield obtained by using ethanol as a solvent as in the findings of Britany J *et al*., 2019. According to El Asbahani *et al*., 2015, Microwave assisted extraction method improved obtaining the organic compound by applying microwave energy which helped in disrupting the plant cell walls. Microwave assisted extraction method has low energy consumption, less heating time, no organic extract degradation and also it preserves the antibacterial activity of clove [20].

According to the GC- MS analysis, Eugenol, Carbopylene and phenol are major constituents separated based on mass to charge ratio. The detailed compounds discovered in clove oil are tabulated in Table 3. Eugenol obtained in the tested sample was 37.67% and Kennouche *et al*., 2015 reported 65-71% of eugenol which is higher than tested sample. According to Ratri *et al*., 2020, contrasting results were obtained in commercially available clove oil which had 81.35% eugenol and 12.05% acetyl eugenol. On the same study the values from steam distillation resulted in 85.01% eugenol and 13.06% acetyl eugenol. The eugenol percentage obtained in this study was low, comparatively with the

literature that eugenol carries a percentage between 70%-80% [21]. However no traces of acetyl eugenol was obtained in the tested sample in which similar results were obtained in Brittany J *et al*., 2019. Eugenol is the highest component of the clove oil and is responsible for the medicinal properties of the essential oil [22].

According to GC MS data 37.67% Eugenol, 27% Caryophylene and 21% phenol was obtained. Further 2.79% of copaene,3.48% Humulene, 0.67% bicycle, 4.97% Naptholene and 1.35% Caryophylene oxide components were present in low levels which was on similar percentages with D'Ameila *et al*., 2017 [23].

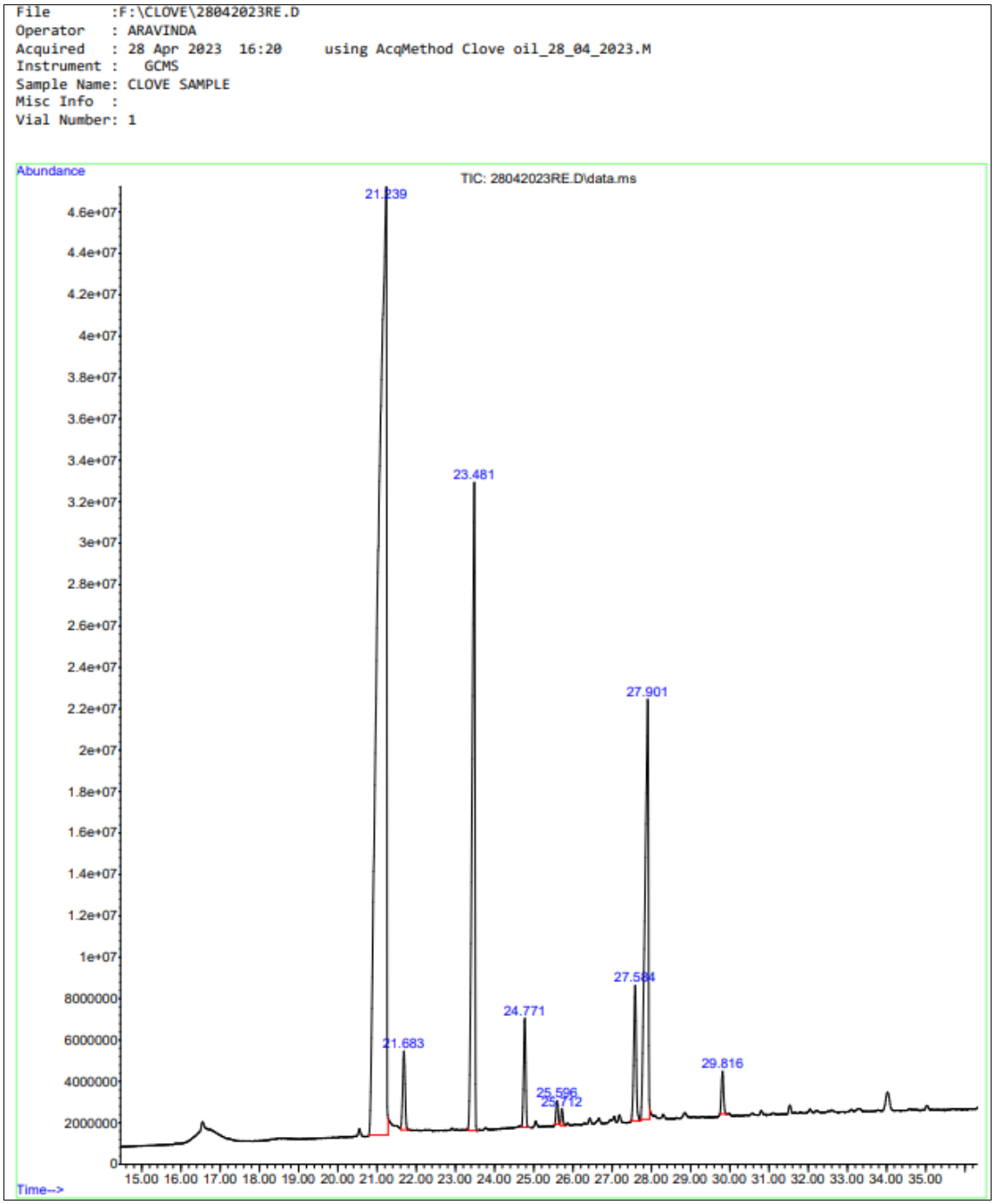

Fig 5: Chromotogram of GC-MS Analysis

Table 3: Constituents Separated on Mass to Charge Ratio

Compound	Percentage
Eugenol	37.67%
Caryophylene	27.264%
Phenol	21.304%
Copaene	2.798%
Humulene	3.48%
Bicyclo	0.672%
Napthalene	4.977%
Caryophylene oxide	1.358%

According to Kennouche *et al*., 2014 the main volatile compounds obtained from Hydrodistillation and Microwave assisted extraction method indicated similar results [20]. Eugenol being present in the highest percentage, β-caryophyllene, α Humulene and Eugenyl acetate were the other main compounds found in both the methods. Same study obtained a pale yellow color clove essential oil, which was similar to the results of the current study as shown in figure 6, while Soxhlet extraction using ethanol produces a brown extract due to impurities, waxes and organic waste [12].

Fig 6: Clove Essential oil obtained from Microwave Assisted Extraction

Golkamani *et al*., 2017 reported that the clove essential oil extraction yield from microwave assisted extraction method after 6o minutes was similar to the extraction yield from hydro distillation for 240 minutes [8]. Microwave assisted extraction method reduces the extraction time and also increased the yield by two folds as the extraction temperature reaches faster than the hydro distillation. Current study obtained 16.5% of a clove essential oil yield after 30 minutes. According to Kennouche, A *et al*., 2015 a comparison was conducted between hydro distillation and Microwave assisted extraction methods in which microwave assisted extraction gave a higher eugenol percentage than hydro distillation [20] Contrastingly Gonzalez-Rivera J et at 2015 reported a higher eugenol percentage from hydro distillation compared to the microwave assisted extraction [23].

4. Conclusions

In the present study microwave assisted extraction was the method used to isolate clove essential oil, keeping the extraction time for 30 minutes and the maximum obtained yield was 16.5%. The two major components of the essential oil were eugenol and carbophylene. Phenol was obtained in a substantial amount. Microwave Assisted Extraction is a green method which is energy efficient and cost effective for extracting essential oil from the clove buds.

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