Article

Bio-evaluation of different fractions of *Matricaria chamomile* L. plant

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Abstract
Chamomile is one of the oldest therapeutic plants on the planet. It belongs to the Asteraceae family, and two common types are German Chamomile and Roman Chamomile. Chamomile's dried flowers are high in terpenoids, phenolics, and flavonoids, all of which contribute to its therapeutic qualities. Chamomile essential oils are widely utilised in cosmetics and aromatherapy. Chamomile has spawned a slew of various concoctions. Chamomile is well-known for being utilised in a variety of applications. Many individuals recommend and utilise chamomile flower dry powder for a variety of traditional health concerns. In this paper, we discuss how chamomile has been used in traditional medicine to evaluate its curative and preventive capabilities, as well as contemporary findings for its development as a therapeutic agent for human health. It has been used to treat eye swelling, skin irritation, and infections in the form of compresses, while the oily version has been used to treat wounds and burns. The phenolics and flavonoids fractions in the Chamomile plant were evaluated and defined using a liquid chromatography – mass spectrometry (LC-MS) test. In addition, an antibacterial bioassay was conducted to determine Chamomile's antimicrobial activity in comparison to control ethanol. All the identified chemicals have been shown to have a wide range of actions in the Chamomile plant. These chemicals have been employed in the treatment of a variety of ailments as well as in traditional applications.

Keywords chamomile; *Matricaria chamomilla*; phenolics; flavonoids; LC-MS; antimicrobial activity.

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1 Introduction

Chamomile (*Matricaria chamomilla* L.) is a well-known medicinal plant species from the Asteraceae family that has been dubbed "the star of therapeutic species." It is now a popular and widely used therapeutic herb in folk and traditional medicine. Years of traditional and scientific use and research have confirmed its multitherapeutic, aesthetic, and nutritional properties. The chemical ingredients of essential oil and plant parts, as well as their pharmacological effects, are mentioned because chamomile is a rich source of natural substances. Furthermore, the plant's biochemistry, biotechnology, market demand, and trade are all given special attention. This is an attempt to collect and document information on various aspects of chamomile, as

well as to emphasise the need for further research and development (Franke, 2005). Its inflorescences (so called anthodia) contain over many beneficial health effects such as antioxidant (Hernández-Ceruelos et al., 2010), anti-allergic (Chandrashekhar et al., 2011), anti-inflammatory (Bulgari et al., 2012), anti-microbial (Silva et al., 2012) and anticancer (Matić et al., 2013).

The phenolic compounds in chamomile extract, primarily the flavonoids apigenin, quercetin, patuletin, luteolin and their glucosides, quercetin and rutin (flavonols), and naringenin (flavanone), but also the principal components of the essential oil extracted from the flowers, such as -bisabolol and its oxides, and azulenes, including camazu (Hadaruga and Hadaruga, 2009). Pino and Bayat (2002) found more than 120 chemical constituents in chamomile flower as secondary metabolites, including 28 terpenoids, 36 flavonoids, and 52 other chemicals with potential pharmacological activity (Mann and Staba, 1986). Terpenoids: chamazulene, bisabolol; flavonoids: quercetin, apigenin, luteolin; coumarins: scopoletin-7-glucoside; and other components like angelic and tiglic acid esters, anthemic acid, fatty acids and other components like angelic and tiglic acid esters, anthemic acid, fatty acids and other components like angelic and tiglic acid esters, anthemic acid,and choline. Optimum chamomile extracts contain about 50 percent alcohol. Normally standardized extracts contain 1.2% of apigenin which is one of the most effective bioactive agents. Aqueous extracts, such as in the form of tea, contain quite low concentrations of free apigenin but include high levels of apigenin-7-O-glucoside (Carnat, 2004).

Plant secondary metabolites, flavonoids, and other phenolics are found in a variety of plant species; however, the type and amount of chemical components vary by species and are influenced by environmental factors such as mineral availability and geographic origin (Lopes et al., 2018). There is a considerable increase in the number of studies concentrating on natural phenolic compounds and flavonoids that are being conducted on the potential of medicinal plant species for pharmaceutical and medical applications. Despite the fact that many flavonoids and other phenolic compounds have been studied in medicinal plant species, a substantial number of local or endemic medicinal plants have yet to be surveyed and their new components observed through profiling studies. These procedures are necessary for the advancement of medication research and development (Su et al., 2017).

Interestingly, while many flavonoids and phenolics have antibacterial properties, flavonoids from this plant have the strongest antibacterial activity against both gram-positive and gram-negative bacteria, including *E.coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Staphylococcus aureus* Rosenbach (Lee et al., 2009; Dzoyem et al., 2018). Flavonoids and other phenolics have also been found to be effective antibacterial agents against P. acnes, the most common cause of skin acne.

This study aims to identify the fractions of phenolics and flavonoids of chamomile plant using LC-MS, besides identifying the antibacterial activity of chamomile.

2 Materials and Methods

2.1 Plant materials

Matricaria chamomilla is often called "German chamomile" or "Water of Youth was purchased from “Agriculture Research Center – Giza”.

2.2 Extraction procedure

Dried and powdered chamomile aerial parts (5 g) were separately extracted by maceration with 10 ml methanol using a shaker apparatus at room temperature for 24 h. The residual was rinsed with 50 ml of solvent and the recovered fractions were filtered and evaporated completely under vacuum.

2.3 Sample preparation

The dried and powdered aerial parts of M. chamomile (0.2 g) was transferred into a screw-capped extraction

tube and extracted with 10 ml of 70% aqueous methanol at 75ºC for 60 min. After centrifuging at 4000 rpm for 5 min, 3 ml of supernatant was transferred to a 5 ml vial. The solution was filtrated through a syringe filter of 0.2 µm membrane for quantification of apigenin and apigenin 7-glucoside in plant material by LC-MS.

2.3.1 Alkaline hydrolysis

Apigenin is normally present both free and as esters with glycoside in this herb. For measurement of total apigenin 7-glucoside, the herb and extracts were separately subjected to alkaline hydrolysis according to the procedure reported in British Pharmacopoeia (British Pharmacopoeia, 2010). In this process, various acetylated derivatives of apigenin 7-glucoside are converted to apigenin 7-glucoside. The hydrolysates were filtrated through a syringe filter of 0.2 µm membrane to assay total apigenin 7-glucoside by UPLC.

2.3.2 Acid hydrolysis

A simple approach to obtain the aglycone is mild acid hydrolysis of the samples which releases the glycoside moiety without promoting decomposition of the remaining aglycone skeleton. Hydrolysis of apigenin glycosides in the extracts and herb were performed in acidic medium following the procedure described in the literature with minor modifications (Haghi and Hatami, 2010). A 100 mg of herb or 20 mg of dry extract were separately hydrolysed with 10 ml of a mixture of methanol : HCl (7:1) at 100°C in a caped tube for 2 h. The solution was cooled, centrifuged for 5 min and filtered through a 0.2 µm filter for the measurement of total apigenin by UPLC method.

2.4 Total phenolic content (TPC)

Total phenolic content was estimated using the Folin–Ciocalteu colorimetric method as described elsewhere (Wang et al., 2003) with minor modification. 0.2 ml of each extract (1 mg/ml) was transferred into a 5 ml volumetric flask and swirled with 3 ml of deionised water. 0.25 ml of Folin-Ciocalteu's reagent was added and swirled. After 3 min, 0.75 ml of 20% (w/v) sodium carbonate solution was added and mixed. This was recorded as time zero. Deionized water was added to make up the volume to 5 ml exactly. The solution was mixed thoroughly and allowed to stand at ambient temperature for 2 h until the characteristic blue color developed. Quantification was done on the basis of the standard curve of gallic acid at 760 nm by a spectrophotometer (Perkin-Elmer Lambda EZ-210 UV/VIS). All tests were conducted in triplicate and averaged. Results were expressed as gram of gallic acid equivalent (GAE) per 100 g of dry material.

2.5 Total flavonoid content (TFC)

Stock solution of quercetin (0.5 mg/ml) prepared in methanol was diluted with 80% (v/v) ethanol (ranging from 25 to 100 µg/ml) to construct the calibration curve. A 0.5 ml of standard solutions or the crude extract were separately transferred into a 5 ml volumetric flask containing 2 ml of 80% ethanol, 0.1 ml of 10% (w/v) aluminum chloride and 0.1 ml of 1 M potassium acetate, diluted with distilled water to the mark and mixed. After 30 min, the absorbance was measured at 415 nm with a Perkin-Elmer Lambda EZ-210 spectrophotometer. The TFC in the crude extracts (1 mg/ml) were determined as above method. TFC expressed as g of quercetin equivalent per 100 g of dry material (Chang et al., 2002).

2.6 Antimicrobial bioassay of ethanol extract

The ethanol extract of the three species leaves was tested against Gram- negative bacterial strains *Escherichia coli* (DH5α). This assay was applied using the agar plate diffusion method according to Kavanagh (1972). One ml of the standardized bacterial stock suspension (108 – 109 CFU/ml) was mixed with 100 ml of molten sterile nutrient agar which was maintained at 45°C. Twenty ml aliquots of the inoculated media were distributed into sterile Petri-dish plates (9 cm). The bacterial cultures were maintained on nutrient agar and 100 µl of plants ethanol extract was inoculated in wells, then incubated at 37°C overnight. The control bioassay was applied by inoculating only ethanol in wells.

2.7 Statistical analysis

In Minitab 19, the data was subjected to an analysis of variance test. Standard deviations, and mean averages were calculated. Excel 2019 was applied for histogram drawing.

3 Results

3.1 The cultivated plant

The seeds of chamomile were germinated in peatmoss soil and left for growth for about 30 days. The resulted plat was branched, erect, with acicular leaves, and has fine fragrance (Fig. 1).

Fig. 1 The Germinated plant of chamomile plant.

3.2 Determination of total phenolic and total flavonoid contents

The dry extract yield of dry material was methanol extract. The total phenolic and total flavonoid contents were calculated on the basis of gallic acid and quercetin and expressed as equivalents of gram gallic acid and quercetin per 100 g of dry material, respectively. The amounts of total phenolics and total flavonoid are shown in Fig. 2. The analysis showed that total phenolic and total flavonoid contents in methanol extract were with a mean value of 2.75 and 1.23 mg/g respectively.

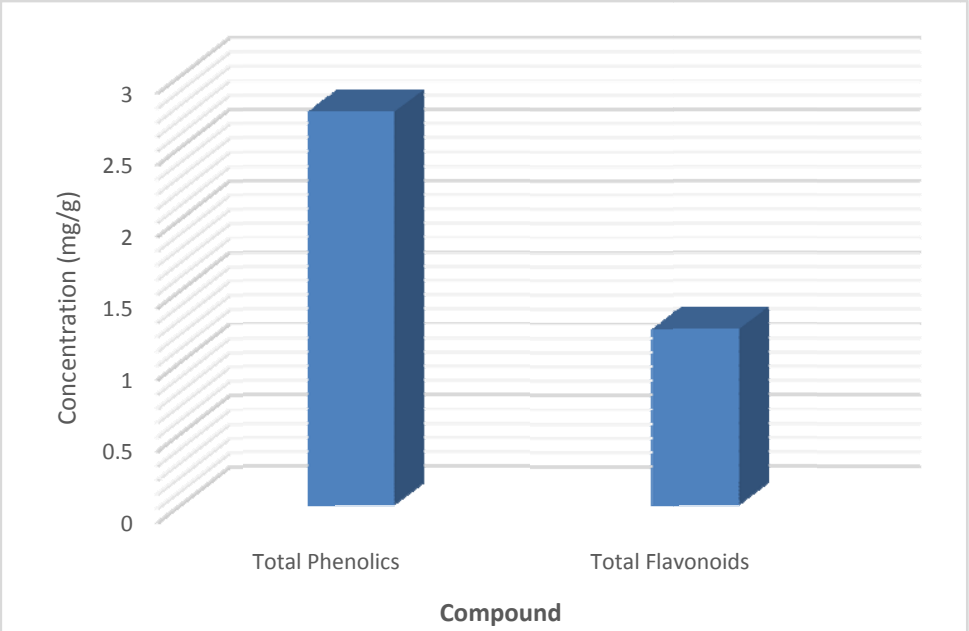


Fig. 2 The total phenolics and flavonoids of chamomile plant.

3.3 Estimation of the phenolics and Flavonoids Fractions

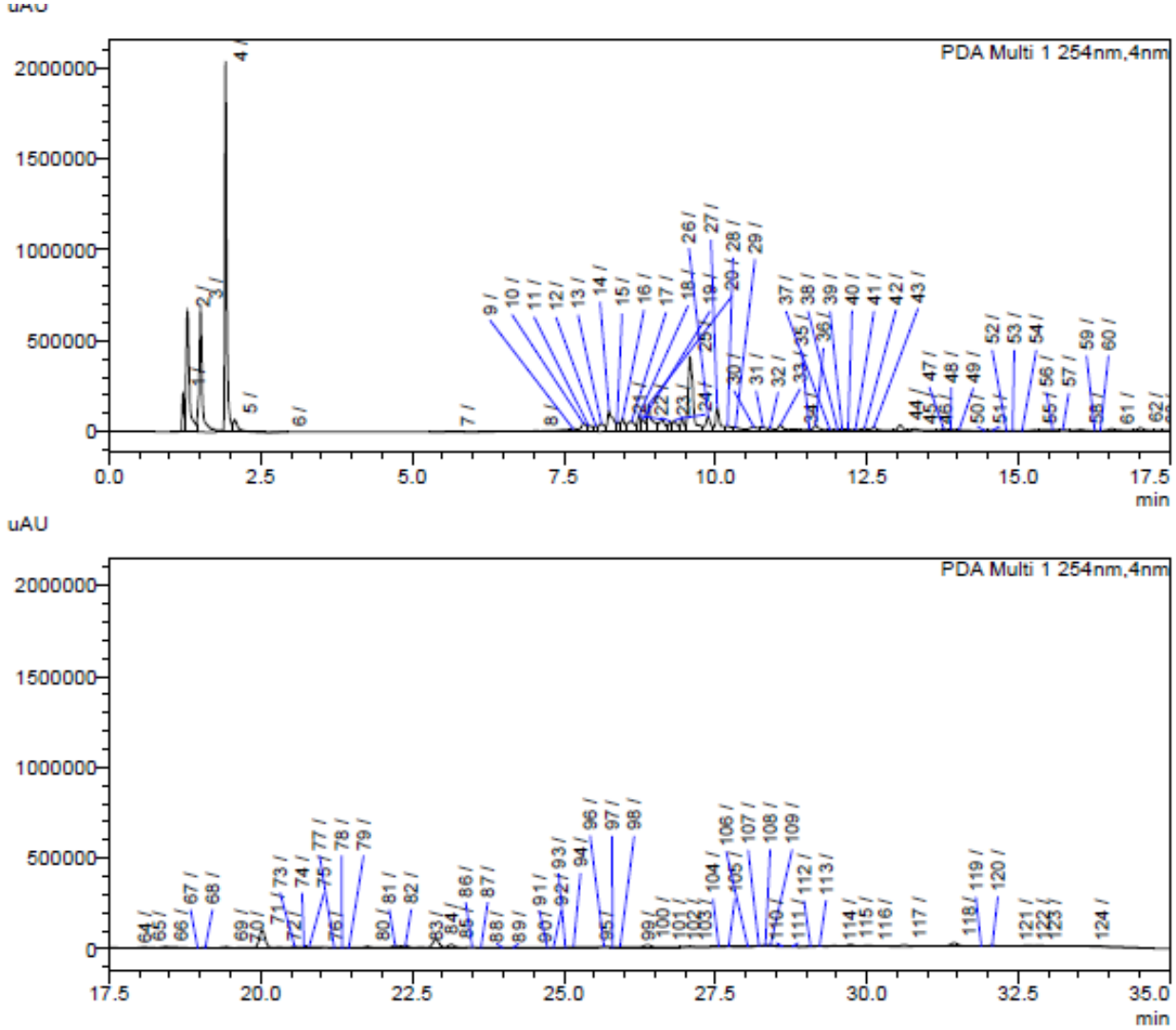
The total fractions (phenolic and flavonoid) in chamomile plant were identified using LC-MS analysis. The identified fractions were tabulated in Table 1. Some of the fractions were illustrated in Figs 3 and 4.

Table 1 The identification of the different compounds in chamomile plant.

Fraction No.	Nature	Fraction/Compound	Fragment size m/z
1	Phenolics	1-Caffeyolquinic acid	191
2		3-Caffeoylquinic acid	179
3		Chlorogenic acid	135
4		4-Caffeoylquinic acid	167
5		Caffeic acid	163
6		Caffeoyltartaric acid	179
7		5-Feruloylquinic acid	193
8		Ferulic acid glucose	193
9		1,4-Dicaffeoylquinic acid	353
10		3,4-Dicaffeoylquinic acid	353

11		1,5-Dicaffeoylquinic acid	191
12		3,5-Dicaffeoylquinic acid	179
13		3-Caffeoyl-5-feruloyquinic acid	367
14		3,4,5-Tricaffeoylquinic acid	515
15		Hexahydroxyflavone 3-O-hexoside	317
16		Pentahydroxyflavone 7-O-hexoside	303
17		Leteolin 7-O-rutinoside	287
18		Pentahydroxyflavone 7-O-hexoside	301
19		Luteolin 7-O-glucoside	285
20		Hexahydroxyflavone 3-O-dihexoside	317
21		Apigenin 7-O-glucoside	287
22		Apigenin 7-O-caffeoyllucoside	595
23		Apigenin 7-O-malonylacetylglucoside	561
24		Apigenin 7-O-malonylacetylglucoside	559
25	**Flavonoids**	Apigenin 6,8-di-C-glucoside	285
26		Dihydroxy-tetramethoxyflavone	373
27		Quercetin 3-O-glucuronide-7-O-galactoside	641
28		Quercetin 3-O-glucuronide-7-O-glucoside	639
29		Hexahydroxyflavone 3-O-dihexoside	641
30		Quercetin 3-O-rhamnosylgalactoside	611
31		Quercetin 3- O-rutinoside	609
32		Petuletin 3-O-robinobioside	465
33		Pentahydroxyflavone 7-O-hexoside	463
34		Quercetin 3-O-galactoside	461
35		Kaempferol-3-O-rutinoside	593
36		sorhamonetin-3-O-rhamnosylgalactoside	623
37		Syringenin 3-O-rhamnosylhexosideb	655
38		Kaempferol 3-O-glucoside	449
39		Petuletin 3-O-manolylrhamnosylhexoside	739

40		Isorhamnetin 7-O-glucoside	479
41		Tetrahydroxy-dimethooxyflavone 7-O-glucosideb	507
42		Pentahydroxymethoxyflavonecaffeoylglucoside	655
43		Pentahydroxymethoxyflavonecaffeoylglucoside	657
44		Quercetin 3-O-caffeoylglucoside	627
45		Quercetin 7-O-glucoside	465



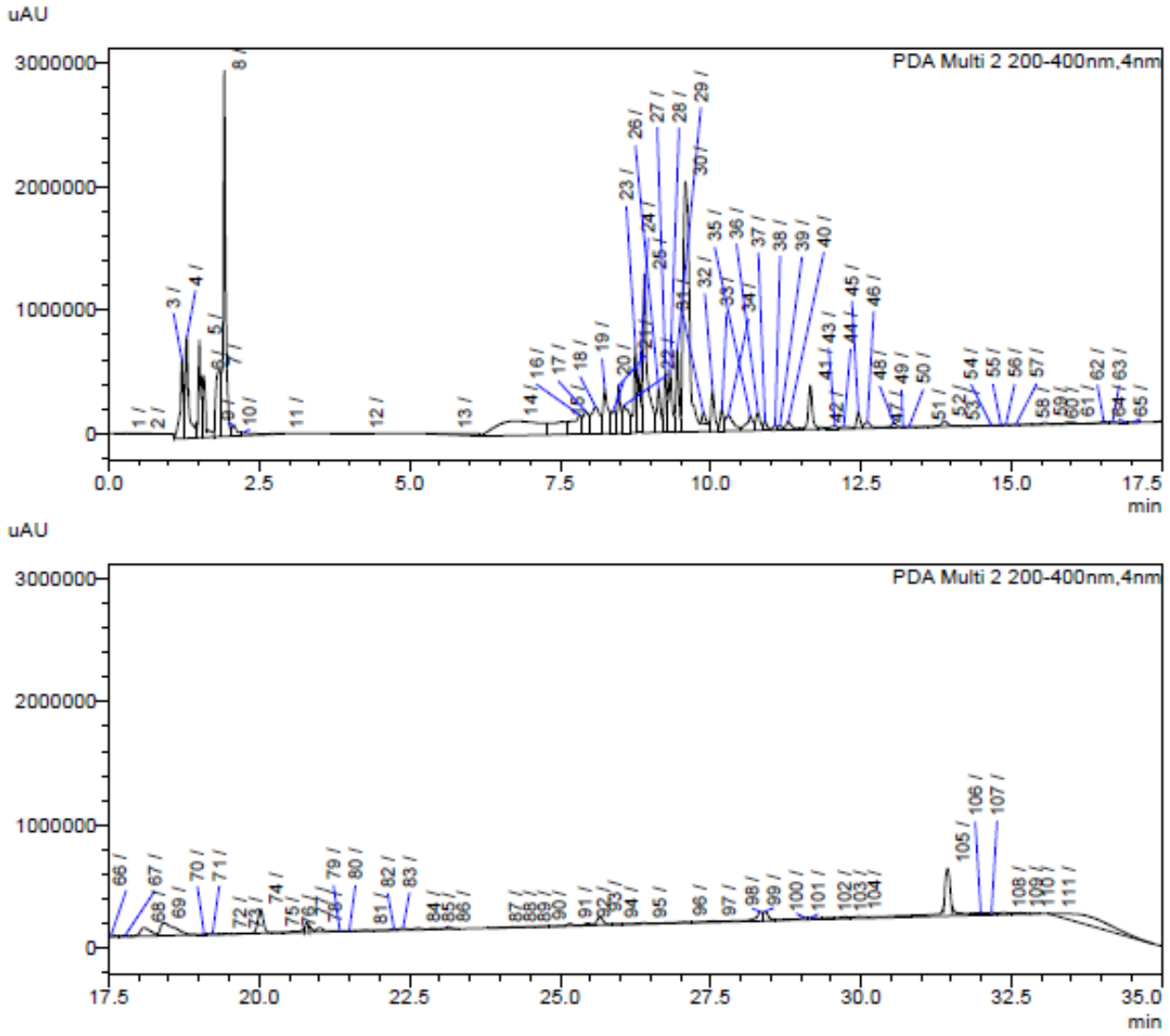


Fig. 3 The fractions of phenolics and flavonoids of chamomile plant.

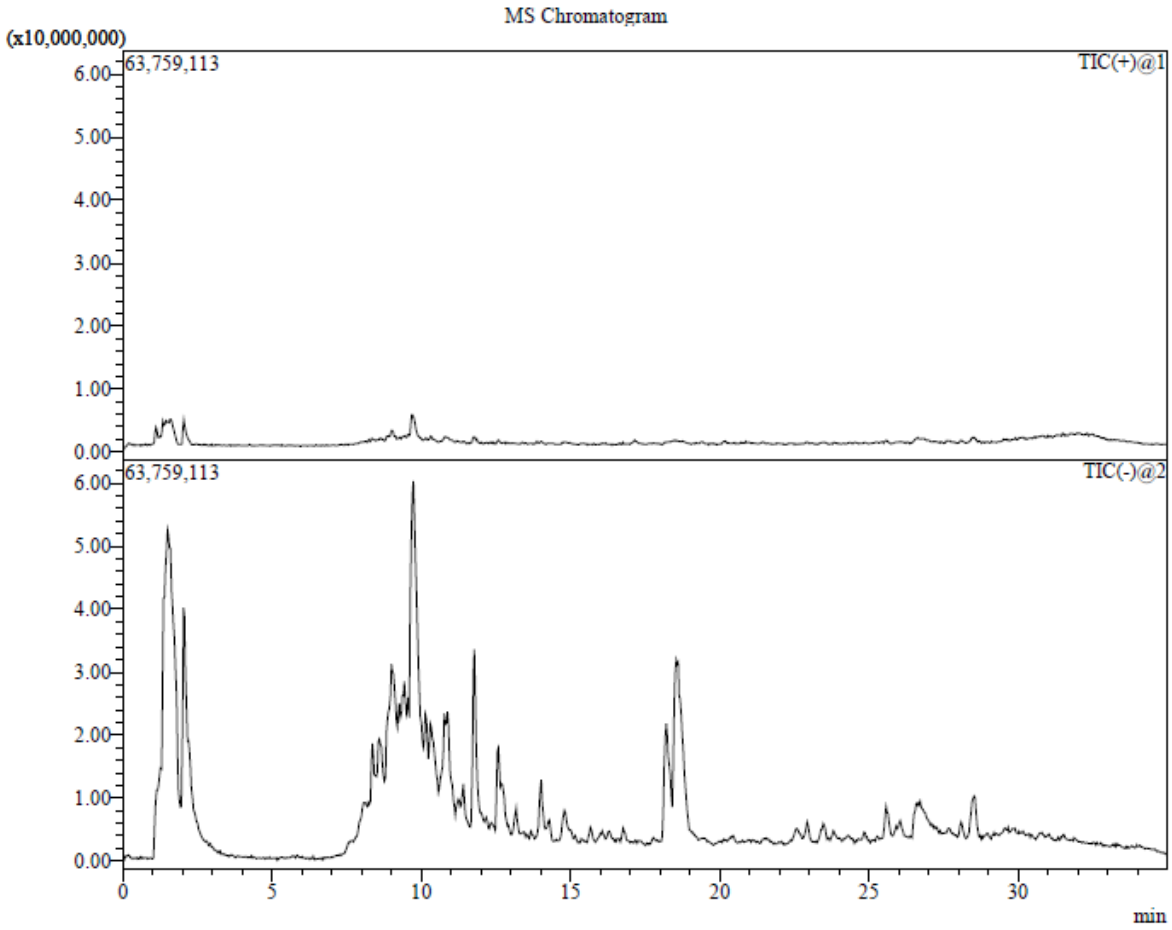


Fig. 4 The MS chromatogram of the fractions of chamomile plant.

3.4 Antimicrobial Activity

The antimicrobial activity of the chamomile plants was estimated against *E. coli*. The plants proved the antimicrobial activity via the clear zones shown in Figs 5 and 6.

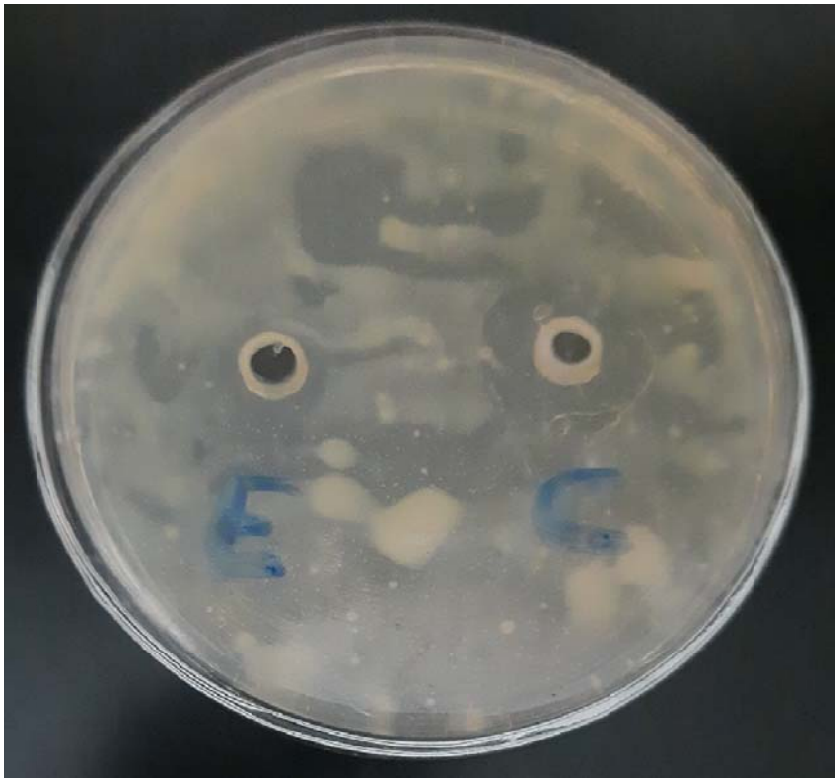


Fig. 5 LB agar plate showing the antimicrobial activity of chamomile plant extract against *E. coli* comparing to control ethanol (E: control, C: chamomile).

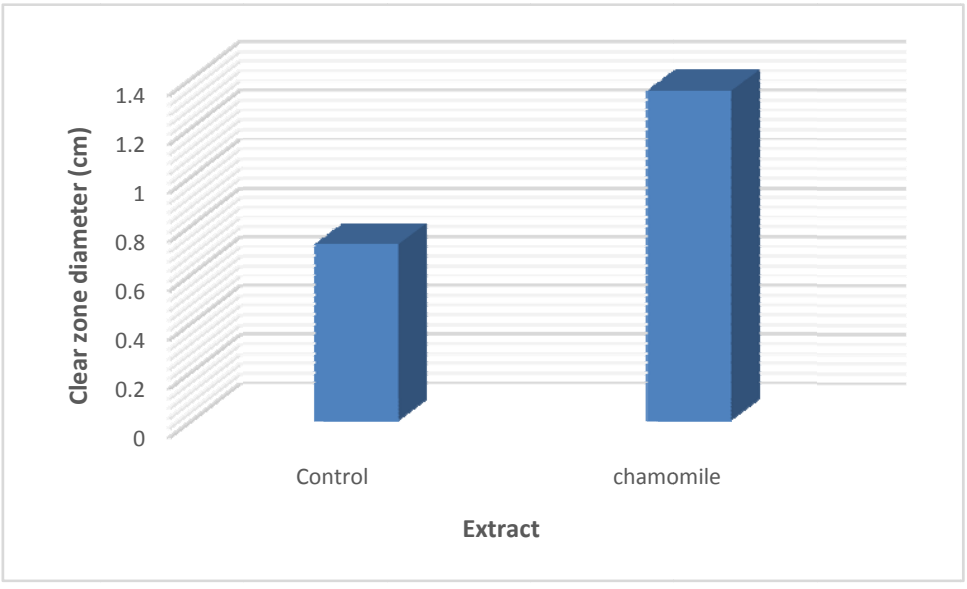


Fig. 6 Histogram showing the antimicrobial activity of the three plant species extract against *E. coli* comparing to control ethanol.

4 Discussion

Several chamomile items, including beverages, detergents, scents, hair products, infusions, lotions, and soaps, have been proven to be commercially available by scientists. The EO of this plant has been shown to have antibacterial activity, as it was effective against 25 distinct Gram-positive and Gram-negative bacteria, including *E. coli* (Abad et al., 2013).

M. chamomilla is a member of a large family of cultivated medicinal herbs. It includes a diverse range of therapeutically useful and active chemical classes. The most important ingredients of the chamomile medicine are sesquiterpenes, flavonoids, coumarins, and polyacetylenes. In *M. chamomilla*, herniarin, umbelliferone, and other minor coumarins are present (Kotov et al., 1991). The glucoside precursor of herniarin, (Z)- and (E)-2—d-glucopyranosyloxy-4-methoxycinnamic acid (GMCA), was described as a natural chemical in chamomile. Gupta et al. (2010) discovered eleven bioactive phenolic compounds in chamomile extract, including herniarin and umbelliferone (coumarin), chlorogenic acid and caffeic acid (phenylpropanoids), apigenin, apigenin-7-O-glucoside, luteolin and luteolin-7-O-glucoside (flavones), quercetin and rutin (flavonols), quercetin.

We used LC/MS to further define the presence of components in chamomile extracts. The existence of a combination of multiple apigenin glucosides and parent glycone, apigenin, in both aqueous and methanolic chamomile extracts was confirmed by negative ion spectra acquired by mass spectrometry. Aqueous chamomile extract has also been found to contain a tiny fraction (5–7% of total essential oil) of the flower's composition (Heuskin, 2009). This could be owing to essential oil's relative insolubility non aqueous medium. Chamazulene, -bisabolol, bisabolol oxides A and B, acyclic ether, and other hydrocarbons insoluble in the aqueous phase make up the essential oil content of the methanolic extract (Marczal and Petri, 1989). The methanolic chamomile extract has been shown to have a high concentration of apigenin-7-O-glucoside, as well as various polyphenolic compounds such as caffeic acid, luteolin, and luteolin-7-O-glucoside, among other common flavonoids (Fonseca et al., 2007).

5 Conclusion

Chamomile has been used as a herbal medicine since antiquity, is still popular today, and is likely to remain so in the future because it includes a variety of bioactive phytochemicals that may have therapeutic properties. Chamomile can be used as an aromatherapy treatment to help with sleeping and anxiety, as well as a topical treatment to guard against inflammation and skin burns, activate the immune system, and provide some cancer prevention. Because many herbal medications are free of side effects, easy to obtain, deemed healthful, and generate cash, there has been an upsurge in the use of natural ingredients rather than synthetic drugs. For improved quality control of the intended bioactive components, chamomile should be grown.

Chamomile is in high demand around the world due to its wide range of therapeutic effects and excellent pharmacological properties. In addition, the use of natural substances rather than synthetic chemicals has increased because many herbal treatments have few side effects, are easy to obtain, are deemed healthful, and generate revenue. It is a well-known truth that unrestricted harvesting of wild populations and the growth of urban areas are threatening chamomile plant variety. As a result, cultivating chamomile is recommended for better quality control of the target bioactive components. This method also enables the creation of consistent plant material in the needed quantities at specified intervals. There is a significant desire to screen the various chemotypes of chamomile growing in various phytogeographical areas. Similarly, biodiversity study at the morphologic, biochemical, and genetic levels will help the research community understand the extent of variability in the current chamomile germplasm, and hence aid in the plant's conservation. However, there is still a lot of need for more research into the various features of chamomile.

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