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Chemical profiling, antioxidant, and antimicrobial activities of the essential oil of *Matricaria recutita* (Chamomile)

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Abstract

Matricaria recutita, commonly known as chamomile, is extensively utilized in the pharmaceutical, cosmetic, and food industries for its medicinal and essential oil properties. Local people from the Karnali Province of Nepal use this plant against skin and vaginal infection by bacteria and fungi. This research focuses on extracting essential oil through hydro-distillation, followed by Gas Chromatography-Mass Spectroscopy (GC-MS) to identify its chemical constituents and antioxidant and antimicrobial activities against bacteria and fungi responsible for common skin infections. The GC-MS analysis of the essential oil from M. recutita identified eleven chemical components. The major constituents, with respective area percentages at retention times 37.16, 11.79, and 7.88 in the GC chromatogram, were -farnesene (46.56%), bisabolol oxide-A (11.79%), and menthol (7.88%). Limonene, methyl salicylate, and -bisabolol oxide-B also constituted the lowest area percentages at 1.36%, 1.59%, and 1.62%, respectively. The essential oil exhibited significant antioxidant activity with $IC_{50} = 0.1924 \ \mu L/mL$. Additionally, the essential oil exhibited notable antibacterial activity against Staphylococcus aureus ATCC6538P and Candida albicans, displaying zones of inhibition measuring 10.64 mm and 14.44 mm, respectively. The broth method also revealed that the MIC is above the 250 mg/mLrange and has more potential for inhibition.

Keywords

 $Matricaria\ recutita,\ essential\ oil,\ GC-MS\ analysis,\ antibacterial,\ antifungal,\ antioxidant,\ MIC\ and\ MBC$.

Article information

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1 Introduction

Nature has shown to be a promising source of therapeutic compounds, with many modern medications derived from medicinal plants found in nature [1]. Herbal medicine has recently received much attention from scientists as a supplemental or replacement therapy [2]. The World Health Organization (WHO) reports that herbal medicines are the primary source of medication for 70 - 80% of the people in developing countries [3]. Among the broad assortment of plant products, essential oils (EO) have gotten much attention [4]. Essential oils are made up of highly volatile chemicals that are separated by a physical process. Essential oil combines oily fragrant volatile chemicals such as monoterpenes, sesquiterpenes, aromatic compounds, and their derivatives employed as antimicrobials, anti-inflammatory, sedatives, expectorants, and diaphoretics, among other things [5]. German chamomile (Matricaria chamomilla) is a plant find in Western Europe, West Asia, India, and North America is a source of chamomile essential oil, which has been used for its relaxing and restorative properties for a very long time [5]. It was historically used for anxiety and digestive problems in traditional medicine in Europe and the Middle East [6]. Because of its anti-inflammatory, antioxidant, and antibacterial qualities, a recent study emphasizes its potential in contemporary medicine and cosmetics [7]. Some of its constituents, such as chamazulene and bisabolol, are responsible for its medicinal properties [8]. Investigating its chemical makeup and its uses in medicine, cosmetics, and holistic well-being are the goals of this work [9–11].

It is the common name for various daisy-like plants in the Asteraceae family with yellow centers (about 1 - 1.5 cm in diameter) and white petals (between 12 - 20 in number) [12]. The essential oils are extracted from the flowering tops. Chamomile has been widely used to treat wounds, skin irritation, burns, chickenpox, ear and eye infections, and nasal inflammation [9, 12, 13]; [14]. According to scientific analysis, extracted essential oils of chamomile were discovered to include - bisabolol oxide A and B and other flavonoids with anti-inflammatory and antiphlogistic activities [8]. These plants are most recognized for their calming properties when eaten as tea, frequently served with honey or lemon [6]. Local people from the Karnali Province of Nepal use this plant against skin and vaginal infection [15]. Much chemical and biological work has been reported in this plant but chemical and biological activities of plants from Karnali Province of Nepal have not been reported so far [?, 12, 13, 16].

This research fulfills the above gap, and we have reported chemicals from the essential oil of chamomile, antibacterial and antifungal activity of microorganisms responsible for skin infection, and antioxidant activity.

2 Materials and Methods

2.1 Materials

Plant Material: The German chamomile (*Matricaria recutita*) plants were collected from the Surkhet district and the plant was identified in the National Plant Herbarium, Godawari, Lalitpur, and the collection number assigned to the herbarium

(Collection No: 79).

Chemicals: Anhydrous Na_2SO_4 and media for antibacterial activity test were bought from the local suppliers of Himedia, India, in Kathmandu. The DPPH and other chemicals used in GC were purchased from Merck.

2.2 Methods

2.2.1 Extraction of essential oil from chamomile

Fresh flowers were collected and chopped into pieces smaller than 10×10 cm, then boiled in a Clevenger apparatus unit with 200 L of distilled water. After 5-6 hours, the oil distillation stopped and a calibrated trap was used to determine the volume of essential oils [17, 18]. The distillate's essential oils were dried over anhydrous Na₂SO₄ and stored in the freezer.

2.3 Gas Chromatography-Mass spectrometry (GC-MS) analysis

The GC-MS technique was used to identify chemical compounds present in the essential oil. The GC-MS was performed on a Shimadzu GCMS-QP 2010 plus equipped with a Rtx-5MS capillary column with a column dimension of 60 m \times 0.32 mm \times 0.25 um. A capillary column that has a nonpolar stationary phase (DB-5 or HP-5, f) was used for GC. The EO sample was diluted with hexane and 1 L of the essential oil sample was injected in split mode (split ratio 10:1), the injector temperature was set at 250°C [10]. Helium gas was used as a carrier gas at a constant 4 mL/min flow rate. At first, the temperature was set at 50°C for two minutes, then ramped up to 200°C at a pace of 10°C per minute, and then to 250°C at a rate of 5°C per minute, total run time was 70 minutes. To begin scanning, the detector's temperature was set at 280° C and mass range to m/z 40–400 [19]. The ionization mode of the mass was 70 eV electron impact (EI).

2.3.1 Antibacterial activities

The Mueller-Hinton agar was used to prepare agar plates. Chamomile EO was tested against seven bacterial strains, utilizing the well-diffusion method. Overnight cultures of the respective bacterial strains, obtained from the American Type Culture Collection (ATCC), were lawn cultured on Mueller Hinton Agar (MHA). Before swabbing, the indicator strains were standardized to a 0.5 McFarland solution. Using a cork borer, wells of 5 mm diameter were created on the agar plates, into which 50 µL of chamomile essential oil (prepared in 50% DMSO) was introduced, alongside positive (antibiotic) and negative (DMSO) controls. The MHA plates were then incubated overnight at 37°C, and the subsequent zones of inhibition were measured the following day [20]

2.3.2 Antioxidant activity

The DPPH assay was conducted following a version of a previously established protocol [5]. Specifically, 100L of chamomile essential oil at concentrations of (0.01, 0.005, 0.001, 0.0005, 0.0001, and 0.00005 g/mL) was mixed with 100L of DPPH solution (0.1mM) in methanol. The reaction mixture was thoroughly shaken and incubated in darkness for 30 minutes. Following incubation, the absorbance of triplicate readings was measured at 517 nm using a microplate reader (Epoch TM 2 Microplate Spectrophotometer, Bio Tek Instruments, USA).

$$S.activity \% = \frac{(A \ sample - A \ control)}{A \ control} \times 100$$

where S. activity is Scavenging activity, A control is the absorbance of the control (DPPH solution without essential oil), and A sample is the absorbance of the sample (essential oil with DPPH). The IC₅₀ value, representing the concentration at which the essential oil scavenged 50% of DPPH radicals, was determined using Graph Pad Prism 8 software (Graph Pad Prism software, California, USA).

3 Results and Discussion

3.1 Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The essential oil was discovered as a light blue liquid with a pleasant, herbaceous aroma that smelt like straw. The components in the oil were examined using instrumental conditions set on the GC-MS and identified using direct mass spectral comparison. Figure 1 shows chromatograms of chamomile extract with varied retention times.



Figure 1: *GC- chromatogram of essential oil* extracted from chamomile (X-axis: time, Y-axis: abundance).

Table 1: List of the compounds found in the essential oils extracted from Matricaria recutita.

S. N.	Name of Compounds	Molecular	Molecular	Retention	Area
		formula	weight	time	%
1.	Tris(tert-	C18H45AsO3Si3	468.7	8.72	5.36
	butyldimethylsilyloxy)arsane				
2.	Ethylphosphonic acid,bis(tert-	C14H35O3PSi2	338.57	15.81	5.19
	butyldimethylsilyl) ester				
3.	Limonene	C10H16	136.23	17.57	1.36
4.	Decamethylcyclopentasiloxane	[(CH ₃) ₂ SiO] ₅	370.77	23.33	7.08
5.	Menthol	C10H20O	156.26	24.55	7.88
6	Methyl Salicylate	C ₈ H ₈ O ₃	152.15	25.66	1.59
7.	Phosphonoacetic Acid, 3TMS	C11H29O5PSi3	356.57	31.34	5.70
	derivative				
8.	(E)- β- Farnesene	C15H24	204.36	37.17	46.56
9.	(E, E) - α – Farnesene	C15H24	204.36	39.29	5.88
10.	α-Bisabolol oxide- B	$C_{15}H_{26}O_2$	238.37	45.36	1.62
11.	α-Bisabolol oxide- A	C15H26O2	238.37	48.77	11.79

The chromatogram abundance v/s retention time plot revealed 11 peaks, indicating the presence of 11 phytoconstituents. Table 1 shows the chemicals discovered based on retention duration and area (%). The retention time was compared with the compounds present in the NIST library to identify the chemicals present in the essential oil. The Mass spectra of major compounds were compared with the mass spectra of compounds available in the NIST library.

The principal components were discovered to include -farnesene (46.56 %), -bisabolol oxide-A (11.79 %), and menthol (7.88 %), as well as other minor components. The results revealed that essential oils isolated from *Matricaria recutita* include -Farnesene, -Bisabolol oxide-A, menthol, and nine others [20,21].

The following is the molecular structure of the critical components found in *Matricaria recutita* essential oils:



Figure 2: Structure of major compounds of EO of *Matricaria recutita*

Various compounds found in essential oil extracts of *Matricaria recutita* from GC-MS analysis

exhibit diverse pharmacological activities. -Farnesene, for instance, functions as an anticarcinogenic, antibacterial, and anti-fungal agent while stimulating gastrointestinal tract receptors [21, 22]. -Bisabolol Oxide-A showcases antiinflammatory, anti-irritant, antibacterial, and nonallergenic properties [22, 23]. Menthol, commonly used in ointments, cough drops, and nasal inhalers, is medicinal and acts as an anti-fungal agent [24]. Bisabolol is known for its anti-inflammatory and soothing properties, along with antimicrobial and antioxidant activities [22]. Chamazulene, another compound, exhibits anti-inflammatory and antioxidant properties, useful in reducing inflammation and oxidative stress [17, 22]. (-)--bisabolol also known for its antioxidant, anti-inflammatory, and anticancer properties, may also promote relaxation and reduce anxiety [25]. Luteolin, with strong antioxidant properties, is studied for neuroprotection and reducing oxidative stress in the brain [26]. Lastly, Farnesene, a sesquiterpene hydrocarbon, contributes to antimicrobial activity [27]. The bioactive compounds present in *Matricaria recutita* support its medicinal values and protect against various diseases. Thus, *Matricaria recutita* plants are considered valuable medicinal plants [20, 28].

3.2 Antioxidant activity

Chamomile essential oil exhibited promising antioxidant activity, as evidenced by its low IC₅₀ value of $0.1924 \ \mu$ l/mL compared to the standard ascorbic acid, which had an IC₅₀ of 0.0187 mg/mL. The inhibitory percentage against the concentration graph is shown in Fig. 3.



Figure 3: Antioxidant Activity of Chamomile (Matricaria recutita) Essential Oil

3.2.1 Antibacterial and antifungal activities

The antibacterial efficacy of the isolated Chamomile (*Matricaria recutita*) essential oil was assessed against *Staphylococcus aureus* ATCC6538P, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Enterococcos faecalis*, *Protecus vulgaris*, *Shigella dysenteries*, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus epidermidis*, and *Salmonella typhi* and exhibited noteworthy antibacterial activity for *Staphylococcus aureus* with a zone of inhibition measuring 14.44 mm, as compared to the positive control 27.00 mm, no significant activity was tested for *C. albicans* and showed the zone of inhibition 10.64 mm, as compared to the positive control 21.11 mm (Table 2).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC): The broth dilution method is a common approach to ascertain the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) of antibiotics or other antimicrobial agents against specific bacterial strains. In this method, a range of concentrations of the antimicrobial agent is tested against the bacteria to find the lowest concentration that inhibits visible growth (MIC) and the lowest concentration that kills the bacteria (MBC).

In this particular study, the antibacterial activity of a 100% essential oil was evaluated against Candida albicans and Staphylococcus aureus using the broth dilution method. Despite demonstrating antibacterial properties, the exact MIC of the essential oil could not be determined. The essential oil exhibited significant inhibition at a concentration of 500 mg/mL against both Candida albicans and Staphylococcus aureus. However, when the concentration was reduced to 250 mg/mL, no significant inhibitory effect was observed. This suggests that the MIC for the essential oil is somewhere above 250 mg/mL, indicating that concentrations below this level are not effective for antibacterial activity. These findings are visually represented in Figure 4, which shows the difference in bacterial growth inhibition at various concentrations of the essential oil.



Figure 4: A) Minimum Inhibitory Concentration (MIC) and B) Minimum Bactericidal Concentration (MBC) for *Staphylococcus aureus* and C) Minimum Bactericidal Concentration (MBC) *Candida albicans*.

Bacterial Strains	(M. recutita, EO)	(+) Control	(-) Control
1. Shigella dysenteriae	0	35.68	0
2. Staphylococcus aureus ATCC6538P	14.44	27.00	0
3. Bacillus subtilis ATCC 6051	0	41.30	0
4. Pseudomonas aeruginosa ATCC 9027	0	35.6	0
5. Escherichia coli ATCC 8739	0	33.68	0
6. Enterococcus faecalis ATCC 29212	0	27	0
7. Klebsiella pneumonia ATCC 70063	0	27.10	0
8. Salmonella typhi (Clinical sample)	0	38.20	0
9. Proteus vulgaris	0	33	0
10. Candida ablicans	10.64	21.14	0
11. Staphylococcus epidermidis	0	25.20	0
12. S. ceravolie	0	18.24	0

Table. 2: The zones of inhibition (ZOI) for *Matricaria recutita* were measured in this research N. C. (50% DMSO).

Chamomile essential oil's composition varies according to species, location, extraction techniques, collection time etc. In this research, we identified the major components of chamomile essential oil, which include -Farnesene (46.56%), -Bisabolol oxide-A (11.79%), and menthol (7.88%). Compared to other research papers, our findings revealed a higher percentage of -Farnesene [29]. The obtained results from Stanojevic et al. (2016) showed the presence of 52 components, with the highest content of -farnesene (29.8%), -farmesene (9.3%), -bisabolol and its oxide (15.7%), chamazulene (6.4%), germacrene D (6.2%), and spiroether (5.6%) [30]. Additionally, the study by Kazemi (2015) reported the major compounds and their percentages in chamomile essential oil as follows: -bisabolol oxide (38%), camphene (9.11%), sabinene (4.87%), limonene (6%), 1,8-cineole (7.12%), camphor (6.54%), and -pinene (6%) [20]. Amiri et al. identified the major components in chamomile essential oil as -Bisabolol oxide A (17.14%), chamazulene (15.12%), En-indicycloether (6.22%), -Bisabolone oxide (6.15%), n-Octanal (6.00%), -Bisabolol oxide B (5.17%), 1,8-Cineole (3.86%), -Terpineol (3.11%), and Germacrene D (3.02%) [21]. -Farnesene has shown antimicrobial properties, which could lead to its use in developing new antimicrobial drugs or as an ingredient in topical antimicrobial formulations [30].

The essential oil of chamomile from the Republic of Srpska's northwest exhibits significant antioxidant and antibacterial properties when compared to previous studies. In a DPPH assay, the oil showed strong antioxidant activity after 90 minutes of incubation, with an IC₅₀ value of 2.07 mg/ml [30]. Our studies show much better antioxidant activity $IC_{50} = 0.1924 \ \mu l/mL$. The bacteria *Staphylococcus aureus* is responsible for skin infection and the fungi *Candida ablicans* for skin and vagina infection, so inhibition of this bacteria and fungi by EO of chamomile validated the ethnomedicinal uses of this plant by local people.

4 Conclusion

The essential oil of Matricaria recutita was successfully extracted using the steam distillation method. The Gas Chromatography-Mass Spectroscopic (GC-MS) technique revealed the 11 different chemical constituents including -farmesene (46.56 %), -bisabolol oxide-A (11.79 %), and menthol (7.88 %) as major compounds. The EO of *M. recu*tita showed significant antioxidant potential, with an IC₅₀ value of 0.1924 µl/mL. Significant antimicrobial activities against C. albicans and Staphylococcus aureus ATCC6538P were with inhibition zones measuring 10.64 mm and 14.44 mm, respectively. It was able to efficiently inhibit Candida albicans and Staphylococcus aureus at 500 mg/mL. The concentration below 250 mg/mL showed no discernible inhibition, indicating a MIC greater than this amount. This suggests that EO can inhibit microbial growth above the concentration of 250 mg/mL. This work scientifically validates the use of this plant by local people for skin and vaginal infections.

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Conflict of Interest

The authors declare no conflicts of interest.

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