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Anti-microbial and Free Radical Scavenging Activity of *Chamomile* Flower Essential Oil

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ABSTRACT

Matricaria chamomilla is in use for last many years as herbal remedy in various countries it is a well known herbal plant in ancient Egypt, Greece, and Rome**.** The chamomile drug is included in the Pharmacopoeia of 26 countries. The present study was focused on the characterization of the main constituents of *Chamomile* essential oil, its antioxidant property and antimicrobial activity. The essential oil and plant extracts was extracted by Clevenger apparatus and by Soxhlet apparatus respectively with various solvents. The compound analysis of *Chamomile* essential oil was analysed by Gas chromatographic technique. After component analysis the antioxidant activity of methanolic extract was performed using DPPH assay. The antibacterial assays were carried out by the disc-diffusion in order to determine the antibacterial activity of oil against the strains *Bacillus subtilis* (ATCC 10707), *Escherichia coli* (ATCC 0157:H7), *Proteus vulgaris* (MTCC 426), and *Pseudomonas aeruginosa* (ATCC 27853). This oil was found as a good source of α-bisabolol oxides A and chamazulene which are primarily responsible for antioxidant activity and antimicrobial activity of this essential oil.

INTRODUCTION

The plant *Chamomile* belongs to the family Asteraceae and is considered as one of the oldest and precious medicinal species. The chamomile flowers and leaves contain an essential oil having the main component pro camazulene, under high temperatures it gets transformed in to camazulene compounds. The freshly obtained essential oil is blue in colour with a bitter taste and characteristic smell. Antioxidants are substances which oppose oxidation or inhibit the reactions initiated by oxygen or peroxides, many of these substances being used in preserving different products (fats, foods and soaps). Usually natural products can serve as a source of natural antioxidant. Phenolic compounds, like vitamin E and flavonoids are typical antioxidants [1].

It has been studied for its anti-inflammatory, vulnerary, deodorant, bacteriostatic, antimicrobial, anticatarrhal, carminative, sedative, antiseptic and spasmolytic properties [2]. The essential oil of *Matricaria chamomilla* has been studies for its bacteriostatic and bactericidal activity [3] Compounds in the essential oil of chamomile were effective against *Staphylococcus* and *Candida* [4]. α-bisabolol oxides A is one of the components of chamomile essential oil had the strongest activity against Gram-positive and Gram negative bacteria. The essential oil of chamomile is light blue in colour due to terpenoid chamazulene which has anti-inflammatory, anti-allergic, and antispasmodic property [5]. Chamazulene has been reported as a strong antimicrobial agent by the researchers [6] and antioxidant activity which affects free radical processes and inhibits lipid peroxidation.

MATERIALS AND METHODS

The dried flowers of *Matricaria chamomilla L.* were brought from Fragrance & Flavour Devlopment Center (FFDC), Minstry Of MSME, Govt. of India, Kannauj, U.P. 500 g of dried flowers of *Matricaria chamomilla L.* were weighed & hydro-distilled in Clevenger apparatus in 1000mL round bottomed flask with 6litres of tap water for 4h in two batches, temperature was maintained at 80°C. The distillate was collected , stored in a dark glass bottle and kept at 4°C for further analysis.

30g of dried *Matricaria chamomilla* L. was packed tightly in soxhlet extractor filled with 400mL methanol. Extraction was continued for 4 hrs at an average temperature of 40°C and the recovered extract was kept at 4°C for further analysis . Similarly extracts of chamomile with three other solvents viz: ethanol, propanol and n-hexane were also prepared.

GC analysis was carried out by HP-5 Agilent gas chromatograph Model 19091J-413, equipped with a FID detector and HP-15% phenyl methyl siloxane capillary column (30 m× 0.53 mm × 0.25 µm Hp1). The following chromatographic conditions were those of the GC validation method as follows.

Initial temperature at 50°C, held for 10.0 min, ramp 1 at 4.5 ◦C/min to 250°C held for 5 min Injection temperature: 50°C. Injection volume: 1µl. Carrier gas: N_2, at constant flow rate of 2 ml/min. Split ratio = 1:39. Detection: the GC unit has a high-frequency fast flame ionization detection (FID) system (300 Hz), at 300°C. H2 flow: 35 ml/min; air flow: 350ml/min; makeup gas flow (N2): 30 ml/min.

The antioxidant activity or radical scavenging activity of the *Chamomile* essential oils against the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical was determined spectrophotometrically at 517nm. The antioxidant activities of the essential oils were measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. The DPPH method of antioxidant activity was done by the modified method [7] .The radical scavenging activity (RSA) value was calculated by the formula

$$\%RSA = ((AC - As - Ab)/AC) \times 100$$

AC - Absorbance of control, As – Absorbance of sample &Ab – Absorbance of blank

The antibacterial assays were carried out by the disc-diffusion method[8]. In order to determine the antibacterial activity of oil against the bacteria viz: *Bacillus subtilis* (ATCC 10707), *Escherichia coli* (ATCC 0157:H7), Proteus *vulgaris* (MTCC 426), and *Pseudomonas aeruginosa* (ATCC 27853).

RESULTS AND DISCUSSION

During the present investigation it was observed that the Soxhlet method of extraction yields a higher percentage of extract than of hydrodistillation method.The extracted oil was sticky,blue in color with characteristic odour (Table No.1). GC analysis of the essential oil identified seven important components viz: Germacrene D, Bicyclogermacrene, β-Farnesene, α –Bisabolol oxide B, α- Bisabolol, Chamazulene, α -Bisabolol oxide A, Guaiazulene, Cis-z-α-Bisabolene epoxide, Cis-ene-yne-Dicycloether and Trans-ene-yne-Dicycloether (Table No.2 , Fig-2). The extracted oil was tested for its antimicrobial activity against *Proteus vulgaris,Psudomonas aureginosa,Bacillus subtilis and Escherichia coli* .The oil had shown its inhibitory activity against all the four bacterial strains(Table No.3). Antioxidant activity of methanol extract of *Chamomile* oil is shown in Fig 1.

The study was focused on the characterization of the main constituents of chamomile essential oil, its antioxidant property & its antimicrobial activity. *Matricaria chamomilla L* produces a volatile oil which is a valuable source of α-bisabolol oxides A and chamazulene which are the candidate molecules for antioxidant activity and antimicrobial activity of the essential oil. According to a study [9] the *Streptococcal* strains are more susceptible by the *Chamomile* oil, with chemical components such as (E)−β-farnesens (20.1%),guaiazulene (25.6%), α-bisabolol oxide B(7.3%), chamazulene (12.4%),α_bisabolol (7.3%) & hexadecanol (5.6%). Similar constituents are reported in the extract and has shown activity against *Proteus vulgaris,Psudomonas aureginosa,Bacillus subtilis and Escherichia coli* . Antioxidant activity results show that EC50 of the *Matricaria chamomilla* is 0.33 mg/ml as compared with EC50 of Ascorbic acid (0.0038 mg/ml) [10] The antioxidant activity of *Matricaria chamomilla* essential oil is mainly due to

Table No.2: GC analysis of extracted essential oil.

Retention time	Percentage	Compound name
18.7	2.03	Germacrene D
21.4	1.11	Bicyclogermacrene
22.4	5.9	β-Farnesene
28.7	1.0	α –Bisabolol oxide B
30.3	8.9	α- Bisabolol
35.5	16.3	Chamazulene
36.2	17.2	α -Bisabolol oxide A
37.2	4.9	Guaiazulene
37.6	7.2	Cis-z-α-Bisabolene epoxide
40.61	4.2	Cis-ene-yne-Dicycloether
42.5	3.07	Trans-ene-yne-Dicycloether

Table No.1: Percent yield of extract by different methods

Method	Extractions	Yield %	Characteristics
Clevenger method	Chamomille essential oil	0.2	Blue, sticky
Soxhlet method	Hexane extract	4.1	Green, sticky
	Methanol extract	18.0	Green, sticky
	Ethanol extract	2.4	Green, sticky
	Propanol extract	28.8	Yellow, sticky

Table No.3: Antimicrobial activity of *Chamomile* oil

Sample	Zone diameter (cm)			
	Proteus vulgaris	*Pseudomonas aeroginosa.*	*Bacillus subtilis*	*Eschereshia coli*
Matricaria chamomilla	0.7	0.7	0.5	0.5
Tetracycline disc	0.9	1.0	1.4	1.5

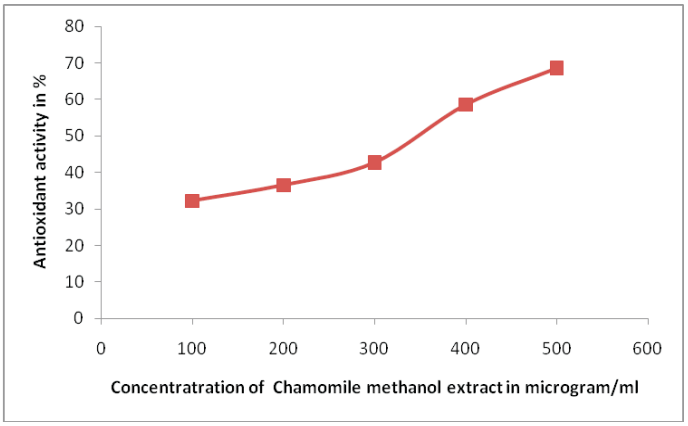

Fig No.1: Antioxidant activity of Chamomile methanol extract

chamazulene and guaiazulene [11] .EC50 value of essential oil during present investigation observed 0.33mg/ml which lower than the reported study 5.52 mg/ml [8].

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