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Research Article
Antimicrobial Activity and Chemical Composition of Flowers of *Matricaria aurea* a Native Herb of Saudi Arabia

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Abstract

Objective: The main objective of the present study was to evaluate the phytochemical constituents and antimicrobial activity of flowers of *Matricaria aurea*, a native herb of Saudi Arabia against pathogenic bacteria and fungi. **Methodology:** Five different organic solvents with increasing polarity were used for extraction purpose. Antibacterial activity was determined by Agar Well diffusion method, while poisoned food technique was used to determine the antifungal ability of the extracts. Scanning electron microscopy was done to evaluate the extent of damage caused by extracts. Furthermore, GC-MS and FTIR studies were performed to analyze the bioactive compounds and functional groups present in most potent solvent extract. **Results:** The results show the flower extracts to possess varied but significant antimicrobial activity against test pathogens. The minimum inhibitory concentration for bacteria and fungi varied between 0.2-50 mg mL^{-1}, respectively. In addition, GC-MS and FTIR results revealed the presence of important phytochemicals and functional groups belonging to coumarins, phenols, esters and ketones. Scanning electron micrographs of treated cells show aggregation of cells, morphological changes and cell damage. **Conclusion:** Based on the results it was concluded that ethanol and methanol extracts of *Matricaria aurea* flowers possess broad spectrum antimicrobial activity. Presence of various important phytoconstituents points *M. aurea* as potential candidate of pharmacological importance and prospective source of antimicrobials.

Key words: *Matricaria aurea*, SEM, GC-MS, coumarins, FTIR

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Matricaria aurea L. is golden chamomile native to Saudi Arabia and grows as an aromatic herb. It is a member of Asteraceae family growing to a height of 20 cm with a slender ascending stem. Flower heads are golden yellow, dome shaped mostly terminal or sometimes axillary. Locally it is known as "Babunaj". Dried flower heads are used for preparing infusions or consumed as tea to treat ailments like colic pains, abdominal cramps and stomachaches by Bedouins or villagers. They are various uses of dried flowers of chamomile in folk medicine like its infusion is gargled to treat of inflamed mucous membrane of mouth and throat, to treat sore throat, as carmative, for flavoring and to induce sleep[1,2]. Recent study has shown aerial extracts of the plant to possess significant antioxidant activity, which could be due to the phenolic compounds[3]. Phytochemical analysis of essential oil from *Matricaria aurea* showed several chemicals compounds many of which are important antimicrobial and antioxidants[4].

Chamomile is one of the oldest known medicinal plant often referred to as "Star of medicinal species"[5]. It is widely represented by two known varieties the Romanian chamomile (Chamaemelum nobile) and German chamomile (*Matricaria chamomilla* L.). Drug resistance is a major global concern. Herbs and plant-derived products have always served as safer and effective alternative drugs in treating various ailments. Worldwide there is a herbal renaissance, though plants have been explored for therapeutic potential since ages yet there are many untapped sources. One such plant is *M. aurea*, which has not been explored thoroughly for its antimicrobial potential. Present study aims to explore the antimicrobial potential of *Matricaria aurea* extracts against pathogenic microbes and determine its chemical composition.

MATERIALS AND METHODS

Plant material: Flowers of *Matricaria aurea* were collected from Riyadh, Saudi Arabia and authenticated by Dr Mona Alwhibi, Department of Botany, King Saud University. Flowers were washed thoroughly and then completely dried in shade.

Preparation of extracts: Dried flowers of *Matricaria aurea* were weighed and crushed to fine powder. Preperation of crude extract was done by following the method of Kaur and Arora[6] with slight modifications. Five organic solvents (Chloroform, ethyl acetate, acetone, ethanol and methanol) with increasing polarity were used for extraction purpose. Powdered flowers were dissolved in different solvents and kept for 72 h on a shaker at 200 rpm. The extracts were then filtered and centrifuged at 10,000× g for 15 min. The supernatant was used for antimicrobial assays. The concentration of the extract used was 200 mg mL^{-1}.

Test organisms: All the bacterial strains were obtained from King Khalid Hospital, Riyadh, Saudi Arabia. Methicillin resistant *Staphylococcus aureus* (MRSA) (ATCC 12498), *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Streptococcus pyogenes* (ATCC 19615), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumonia* (ATCC 700603) were used as test organisms. The fungal isolates used were *Fusarium oxysporum*, *Fusarium solani*, *Alternaria alternata*, *Aspergillus niger*, *Aspergillus flavus* and *Colletotrichumg gleosporoides* were isolated from fruits and vegetables and identified following their macro and micro morphological characteristics.

Antibacterial screening: Antibacterial activity was determined by Agar Well diffusion method of Valgas *et al.*[7] with slight modifications. Sterile nutrient broth was used to prepare bacterial suspension for all the test organisms (0.5 MacFarland) and 100 µL of this suspension was inoculated on the surface of solidified Muller Hinton agar plates. Inoculated plates were allowed to dry after which 6 mm diameter wells were punched on the medium. Each well was filled with 100 µL of the seed extract and incubated at 37°C for 24 h. Antibacterial activity was measured as the diameter of the zone of inhibition (mm) around each well and all tests was carried out in triplicates. Antibiotic susceptibility test was carried out with different antibiotics discs as positive control and the best zone were chosen and tabulated. Sulphamethoxazole trimetoprine 25 µg, vancomycin 30 µg, optichin 10 µg, tetracycline 30 µg and impenem (Im 10 µg, Oxoid) served as positive control, whereas negative control was the extracting solvent.

Antifungal screening: Poisoned food technique[8,9] was used to determine the antifungal ability of the extracts. To a sterile petri plate (9 cm), 1 mL of extract was added followed by Potato dextrose agar (19 mL). The mixture was allowed to solidify after gentle swirling. After solidification, the medium was inoculated with a mycelial plug of 6 mm that was removed from 7-9 days old colony. Culture plates were incubated at 25±2°C and diameter of the fungal colonies was measured after 7 days. Control treatment was without the extract. Each sample was assayed in triplicates and the mean values were observed.

Percentage inhibition of mycelial growth was evaluated by measuring the relative growth of fungus in treatment and control was calculated by using the following equation:

$$I = \frac{C\text{-}T}{C} \times 100$$

where, I is the percentage inhibition, C is the mean growth rate of control and T that of the treatment.

Minimum inhibitory concentration: Tube dilution assay was used to determine the MIC of both fungi and bacteria. All tests were performed in Muller Hinton Broth (MHB) for bacteria and Potato Dextrose Broth (PDB) for fungi. A double fold dilution of the extracts was made using MHB and PDB to obtain concentrations ranging from 0.2-100 mg mL^{-1}. Both, extract and broth were added in equal volumes to sterile test tubes containing 5×10^5 CFU mL^{-1} of bacterial inoculums and for fungi 2×10^6 mL spore suspension. The tubes were incubated for 24 h at 37°C for bacteria and 28±200°C for 72 h for fungi. The highest dilution (lowest concentration) showing no visible growth was regarded as MIC. The contents from the tubes showing no visible growth were sub-cultured on Muller Hinton agar plates and incubated at 37°C for 24 h to determine if the inhibition was permanent. All experiments were performed in triplicate. Control tube contained only broth and inoculum without extract. Macrodilution method according to the CLSI standard[10,11] with minor modification was used[12].

Minimum Bactericidal Concentrations (MBCs) and Minimum Fungicidal Concentrations (MFCs) of *Matricarai aurea* against the test isolates: The minimum fungicidal and bactericidal concentration of extracts was performed as an adjunct to MIC test following the method of Espinel-Ingroff *et al.*[13] and Olorundare *et al.*[14]. Briefly samples were taken from test tubes with no visible growth in the MIC assay and were inoculated onto sterile Muller Hinton agar plates for bacteria and Potato dextrose agar plates for fungi. The plates sub cultured with bacteria were incubated at 37°C for up to 72 h, while fungal plates were incubated at 27°C for up to 96 h. The MBC or MFC was taken, as the concentration of the extract that did not show any bacterial or fungal growth on fresh agar medium. All the test pathogens were tested for MIC MBC and MFC irrespective of their poor inhibition zones on antimicrobial assays.

Scanning electron microscopy: Scanning electron microscopy was carried out for *B. subtilus*. For this, the bacterial suspension of cells with methanol extract at an MIC of 0.4 mg mL^{-1} was incubated for 24 and 36 h, while control was without extract. The bacterial suspension was taken and centrifuged at 8000×g for 10 min, the supernatant was discarded and the bacterial suspension was spread on a glass slide and fixed by immersing it in 2.5% (4°C) glutaraldehyde overnight followed by rinsing it thrice in phosphate buffer (pH 7.2) and then post fixed in 1% osmium tetroxide for 1 h. The bacterial cells were then dehydrated through a series of ethanol gradient (60, 70, 80, 90 and 100%) and dried with CO_2. Finally the dried samples were coated with gold and were examined under LTD JSM-6060LV (JEOL-Japan) scanning electron microscope.

GC-MS: Extracts were directly used for the GC-MS analysis. Analysis was carried out on Clarus 500 mass spectrometer. Two microliter of ethyl acetate and methanol extracts were employed for the GC-MS analysis[15]. Clarus 500 GC used in the analysis employed a fused silica column packed with Elite-1 (100% dimethyl poly siloxane, 30×0.25 nm ID×1 µm df) and the components were separated using helium as carrier gas at a constant flow of 1 mL min^{-1}. Two microliter sample extract injected into the instrument was detected by the Turbo gold mass detector (Perkin Elmer) with the aid of the Turbo mass 5.1 software. The oven temperature program was 2 min at 45°C, 1.5°C min^{-1} to 100°C, 2°C min to 200°C during the GC extraction process, split ratio 25:1. The injector temperature was set at 250°C (mass analyzer). The different parameters involved in the operation of the Clarus 500 MS were also standardized (Inlet line temperature 200°C, ion source temperature 230°C). Mass spectra were taken at 70 eV, solvent delay time 5 min, acquisition mode-scan 40-550 amu. The GC run time was 90 min. The identification of the phytocompounds and interpretation of mass spectrum of GC-MS of the analytes with those of authentic standards from the database of NIST libraries.

Fourier transform infrared (FTIR) fingerprint analysis: Fourier transform infrared (FTIR) spectrophotometer was used to identify the characteristic functional groups in the flowers of *M. aurea*. The extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1 filter paper by using high-pressure vacuum pump. The sample was diluted to 1:10 with the same solvent. The FTIR spectrum was obtained using Perkin Elmer 2000 spectrophotometer system with a scan range from 400-4000 cm^{-1} and analyzed using Bruker OPUS software.

Statistical analysis: All the tests were performed in triplicates and values are expressed as Mean±Standard Deviation.

RESULTS

Antibacterial activity of flower extracts: The results of the present study indicate that the extracts of *Matricaria aurea* flowers to possess substantial antibacterial activity. Bacteria screened in this study responded to various solvents extracts differently. Maximum zone of inhibition was observed with methanolic extracts. Amongst all the bacterial strains screened *Bacillus subtilis* exhibited maximum inhibition (24.83±0.57) followed by *Streptococcus pyogenes* (23.00±0.00), *Staphylococcus aureus* (21.00±1.00) and *Klebsiella pneumoniae* (21.00±0.86). The MRSA, *Escherichia coli* and *Enterococcus facecalis* were inhibited moderately in comparison to the above mentioned, while *P. aeroginosa* was resistant and was not inhibited. Ethanol extracts also exhibited good antibacterial activity after methanol extracts by inhibiting *Bacillus subtilis* with a maximum zone of inhibition of (23.00±0.50) followed by *S. aureus* (22.83±0.57) and *K. pneumoniae* (20.00±0.00). Chloroform extracts showed the least inhibitory effects on test isolates (Table 1).

Antifungal activity of flower extracts on radial growth of fungi: Flower extracts of *Matricaria aurea* showed variable antifungal activity with different solvents. Though methanol extracts showed highest mycelial inhibition but ethyl acetate extracts was the most potent solvent as it inhibited all the fungi screened. *Alternaria alternata* was completely inhibited by ethanol extracts followed methanol and chloroform (79.66±0.57 and 62.42±0.26), respectively. *Colletotrichum gleosporoides* showed significant inhibition with all the solvents and ranged between (50.00±0.00 to 66.22±0.38). Both *Fusarium oxysporum* and *F. solani* were the most resistant test isolates, as they showed poor to no response to the extracts (Table 2).

Minimium inhibitory and minimum bactericidal concentration (MIC/MBC): The minimum inhibitory concentration for bacteria varied with different solvents and ranged between 0.4-50 mg mL^{-1} (Table 3). Methanolic extracts followed by ethanolic extracts were effective at lower concentrations in comparison to other solvent extracts used. *Bacillus subtilus*, *Staphylococcus aureus* and *Klebsiella pneumoniae* was inhibited at a lowest concentration of 0.4 mg mL^{-1} with methanol extracts, while MRSA required a higher concentration of 50 mg mL^{-1}. In general *Enterococous facecalis* and *Escherichia coli* were resistant to the extracts of *Matricaria aurea*. The MBC ranged between 0.4 to >100 mg mL^{-1}, lowest was exhibited by *Bacillus subtilis* and highest by MRSA (>100 mg mL^{-1}).

Table 1: Antibacterial activity of organic extracts of *Matricaria aurea* against pathogenic bacteria

	Zone of inhibition					
	Extracts used					
Bacteria tested	Chloroform	Ethyl acetate	Acetone	Ethanol	Methanol	Control
MRSA	9.50±0.50	12.33±0.57	10.00±0.00	18.33±0.28	16.16±0.57	26.00±0.00 (V)
Staphylococcus aureus	10.50±0.86	14.83±0.76	15.50±0.00	22.83±0.57	21.00±1.00	27.67±1.16 (A)
Bacillus subtilis	11.50±0.50	15.00±1.00	13.66±1.15	23.00±0.50	24.83±0.57	27.33±0.58 (T)
Streptococcus pyogenes	9.33±0.28	16.66±0.57	8.00±0.86	18.16±0.28	23.00±0.00	22.60±0.20 (ST)
Enterococcus faecalis	NI	NI	NI	14.00±0.00	11.83±0.76	25.83±0.57 (O)
Escherichia coli	NI	9.00±0.50	18.83±0.28	13.66±1.15	16.50±0.50	36.40±0.17 (T)
Klebsiella pneumonia	13.00±0.00	15.50±0.86	14.00±1.00	20.00±0.00	21.00±0.86	29.00±0.00 (ST)
Pseudomonsa aeruginosa	NI	NI	NI	NI	NI	9.83±0.28 (IM)

NI: No inhibition, MRSA: Methicillin resistant *Staphylococus aureus*, values are Mean inhibition zone (mm)±SD of three replicates. Antibiotics used, Ampicillin (A, 10 µg), Tetracycline (T, 30 µg), Sulphamethoxazole trimetoprine (ST, 25 µg), Impenem (Im, 10 µg), Vancomycin (V, 30 µg) and Optichin (O,10 µg)

Table 2: Effect of extracts on the radial growth of fungi

	Percentage inhibition of mycelial growth					
	Organic extracts					
Fungi tested	Chloroform	Ethyl acetate	Acetone	Ethanol	Methanol	Control
Alternaria alternata	62.42±0.26	50.74±1.28	52.96±0.64	100	79.66±0.57	100.00 (M)
Aspergillus niger	34.11±1.64	53.44±0.50	33.22±0.19	36.44±0.38	43.92±0.55	72.00±0.00 (C)
Aspergillus flavus	44.14±0.25	43.11±0.19	31.48±1.69	35.55±1.38	38.96±1.00	92.00±0.00 (C)
Fusarium oxysporum	NI	35.18±1.69	NI	NI	NI	98.66±0.06 (M)
Fusarium solani	NI	40.37±0.64	41.03±0.60	NI	NI	90.16±0.28 (M)
Colletotrichum gleopsoroides	66.22±0.38	62.40±0.52	50.00±0.00	56.92±0.88	54.99±0.55	100.00±0.00 (C)

NI: No inhibition, C: Carbendazem, M: Mancozeb (0.2%), all values are expressed as Mean inhibition zones (mm)±SD of three replicates

Table 3: Minimum inhibitory concentration (mg mL^{-1}) of organic extracts of the *Matricaria aurea* flowers

	Organic extract				
Organisms	Chloroform	Ethyl acetate	Acetone	Ethanol	Methanol
Bacteria					
MRSA	50	25	50	3.12	6.25
Staphylococus aureus	50	12.5	6.25	0.8	0.4
Bacillus subtilis	12.5	12.5	25	0.4	0.4
Streptococcus pyogenes	12.5	3.12	25	1.56	1.56
Enterococcus faecalis	NI	NI	NI	6.25	12.5
Escherichia coli	NI	50	12.5	50	25
Klebsiella pneumonia	50	12.5	25	0.8	0.4
Fungi					
Alternaria alternata	0.8	0.8	1.56	0.2	0.4
Aspergillus niger	50	25	50	12.5	12.5
Aspergillus flavus	25	25	50	25	12.5
Fusarium oxysporum	NI	100	NI	NI	NI
Fusarium solani	NI	50	50	NI	NI
Colletotrichum gleosporoides	0.4	1.56	6.25	0.8	1.56

Table 4: Minimum microbicidal concentration (MBC/MFC, mg mL^{-1}) of organic extracts of *Matricaria aurea* flowers

	Organic extract				
Organisms	Chloroform	Ethyl acetate	Acetone	Ethanol	Methanol
Bacteria					
MRSA	>100	50	50	12.5	6.25
Staphylococus aureus	50	12.5	6.25	0.8	0.8
Bacillus subtilis	25	12.5	50	0.8	0.4
Streptococcus pyogenes	25	6.25	50	3.12	1.56
Enterococcus faecalis	NI	NI	NI	25	50
Escherichia coli	NI	50	25	50	25
Klebsiella pneumonia	50	50	25	1.56	0.8
Fungi					
Alternaria alternata	0.78	0.78	1.56	0.2	0.8
Aspergillus niger	>100	50	25	25	12.5
Aspergillus flavus	100	50	100	25	25
Fusarium oxysporum	NI	100	NI	NI	NI
Fusarium solani	NI	100	100	NI	NI
Colletotrichum gleosporoides	1.56	3.12	12.5	0.8	3.12

E. coli required higher concentration (25-50 mg mL^{-1}). Since *E. facecalis* was not inhibited it was not tested for bactericidal activity (Table 4).

Minimum inhibitory and minimum fungicidal concentration (MIC/MFC): Amongst all the fungal isolates screened *Alternaria alternata* and *Colletotrichum gleosporoides* were inhibited at lower concentrations (0.2-6.25 mg mL^{-1}), while *Aspergillus niger* and *A. flavus* were moderately sensitive (12.5-50 mg mL^{-1}). However *Fusarium solani* was resistant, while *F. oxysporum* was not inhibited (Table 3). The minimum fungicidal concentration ranged between (0.2-100 mg mL^{-1}) (Table 4). The fungicidal effect was quite pronounced on both *Alternaria alternata* and *Colletotrichum gleosporoides* at lower concentration with all the extracts (0.2-12.5 mg mL^{-1}), while the rest of the fungal isolated were highly resistant.

Scanning electron microscopy: Scanning electron microscopy images clearly shows the marked morphological changes in treated and control cells. The micrograph of the control cells shows actively dividing intact rods of *Bacillus subtilus* with smooth well-defined margins. Extract treated cells showed clustering and change in shape and size. Cell destruction and damage was also observed after 12 and 24 h of treatment with the extracts (Fig. 1).

GC-MS analysis: The GC-MS analysis identified 15 phytoconstituents in methanol and ethanol extracts of *M. aurea* (Fig. 2 and 3). Compounds mainly comprised of hydrocarbons, esters, alcohols, aromatic compounds phenols and ketones. Methanol extracts possessed the following compounds 1,2-Benzene carboxyacid, monoethyl ester, Benzofuran-1, 3-dione, O-xylene, phenol, 2, 3, 5, 6-tetramethyl, phenol, 3-methyl-5-(1-methyl ethyl)-, methyl carbamate, benzene acetic acid, α-hydroxy-ethyl ester,

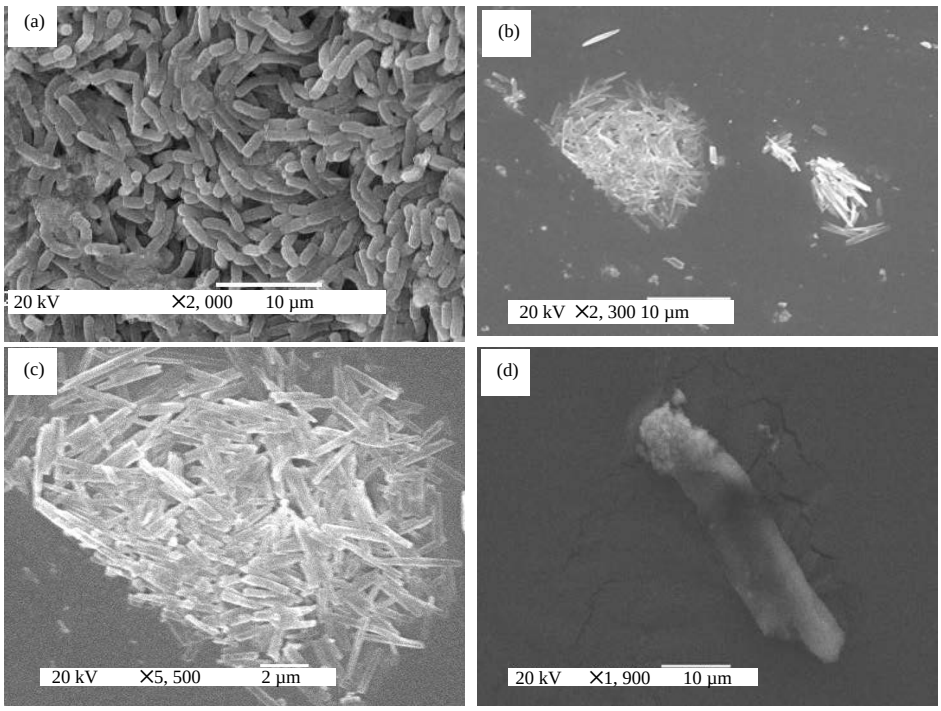

Fig. 1(a-d): Scanning electron micrograph showing the untreated *B. subtilus* cells (control), (b-d) treated with methanol extracts of *Matricaria aurea*. Micrograph (a) shows actively dividing regular and intact cells (control), (b-c) shows clustered and elongated cells with distorted shape and (d) Damaged and broken cell

benzenemethanol, alpha-(ethoxymethyl), 7-Acetoxy-4-methylcoumarin, benzene,1-methoxy-3-phenoxy. The following are the list of compounds identified from ethanol extracts phenyl cyclohexyl ketone, 7-Acetoxy -4-methylcoumarin, hymecrome, phenol, 2, 3, 5, 6-tetramethyl, 1, 2, 3, 4, 5, 6-Hexahydro-1, 1, 5, 5, tetramethyl-2, 4a-methanopnap-7(4aH)-ONE, benzene acetic acid, α-hydroxy –ethyl ester, benzene, 1-methoxy-3-phenoxy.

FTIR analysis: Methanol extract of *Matricaria aurea* was subjected to FTIR analysis, which revealed the presence of characteristics absorption spectrum (Fig. 4). The biological active functional groups were obtained from the absorption bands of the IR spectrum. The IR spectrum shows a maximum absorption peak at 3338 cm^{-1} indicating the presence of phenolic compounds with hydroxyl group. The CH and CH_2 asymmetric and symmetric stretching were observed at 2941.91 and 2831.51 cm^{-1}. The band at 1635 cm^{-1} is due to C=O vibration of bonded conjugated ketones, aldehydes, quinones and esters, while the band at 1448.23 cm^{-1} indicates C-C stretch. Peaks obtained at 1115.84 and 1021.52 cm^{-1} represents C-O stretch, while the peak at 634 cm^{-1} is due to C-H bending.

DISCUSSION

The study reveals *Matricaria aurea* flower extracts to possess significant antimicrobial activity. During this study, some isolates of both bacteria and fungi were effectively controlled, while others either responded very poorly or were not inhibited. Among the bacterial isolates screened, Gram negative bacteria were resistant to the extracts, while Gram positive isolates were sensitive and exhibited significant zones of inhibition and MIC values. The MIC for sensitive strains was in the range of 0.4-12.5 mg mL^{-1}, while others exhibited an MIC value of 25-50 mg mL^{-1}. The different level of sensitivity to the antimicrobial compounds found in the extract explains the variation in the MIC value. Similarly AlKuraishy *et al.*[16] screened alcoholic extracts of *M. chamomilla* against five bacterial strains and found the MIC and MBC to be in the range of 8-32 and 16-64 mg mL^{-1}. In another study, phenols and alkaloids were isolated from *M. chamomila* flowers and tested against *S. aureus* and *E. coli.* They reported an MIC in the range[17] of 25, 50, 75 mg mL^{-1}. Thus results are in agreement with Lo Cantore *et al.*[18]. They reported Gram negative bacteria, especially *E. coli* to be less sensitive to *M. chamomile* oil. The resistance of Gram negative bacteria is because they possess an outer membrane made of lipopolysaccharide, which is impermeable to the

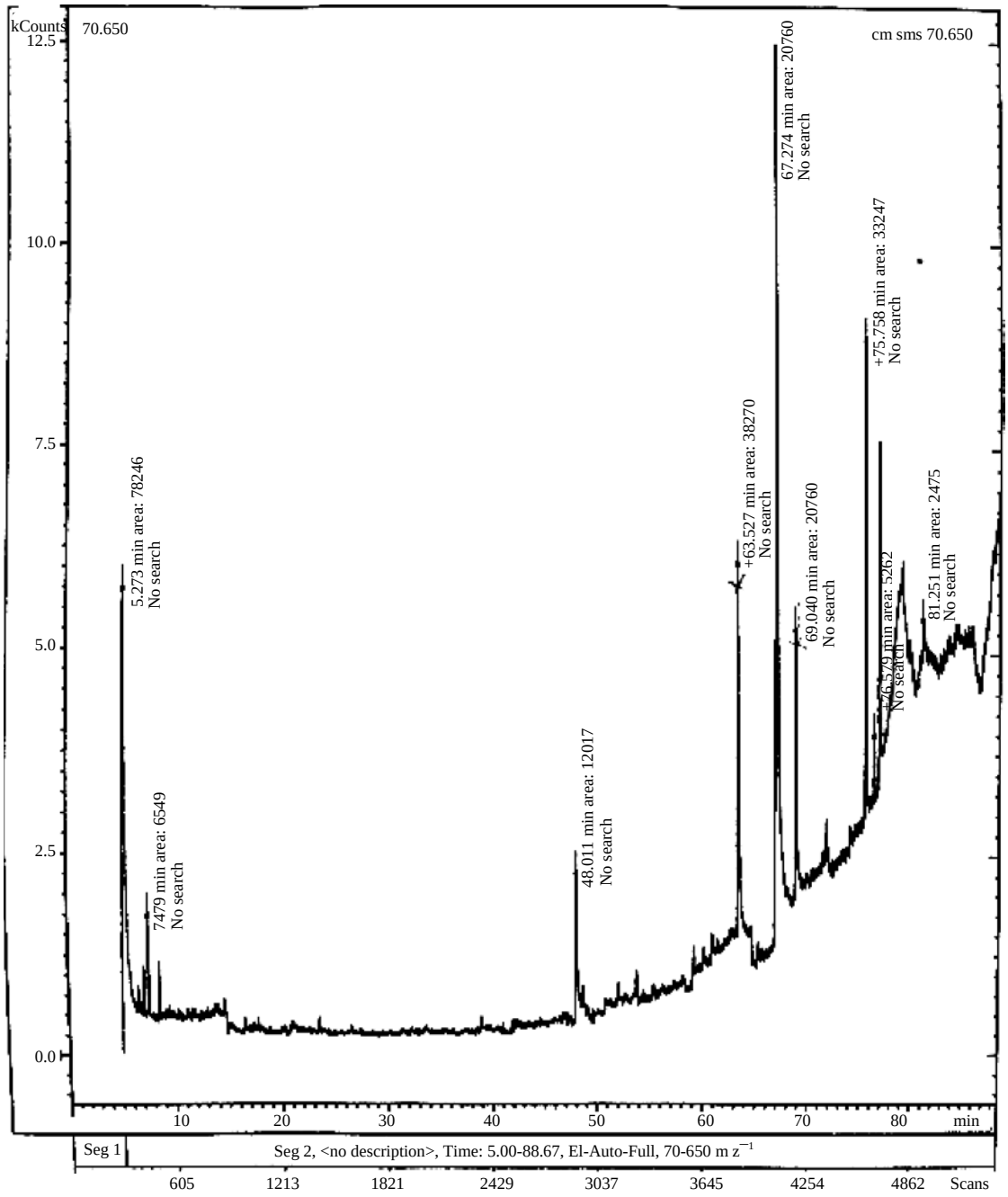

Fig. 2: GC-MS chromatogram of methanolic extracts of *Matricaria aurea*

antimicrobial compounds present in the plant extracts. Hence, it serves as an effective shield by restricting the entry of antimicrobials thereby conferring resistance to many plant derived bioactive compounds[19]. Another view states the presence of multidrug efflux pump found in Gram negative bacteria helps them to extrude the bioactive compounds[20,21].

Amongst all the solvents screened, methanol followed by ethanol extracts exhibited highest antimicrobial activity. Earlier reports suggest similar observation with *M. chamomile* methanolic extracts against a panel of bacteria and fungi, where significant inhibition was observed for *S. aureus*, *B. cereus* and *A. flavus*[22-24]. The high antimicrobial activity of methanol and ethanol extracts in this study could be due to the presence of different phenolic compounds, coumarins, flavanoids and their derivatives. The GC-MS results clearly highlight the presence of all these phytochemicals in both the extracts. It is also reported that methanol and ethanol are better solvents in extracting phenolic compounds from plant material[25]. In another study flavanoids and its derivatives were recorded from methanolic extracts of chamomile flowers as

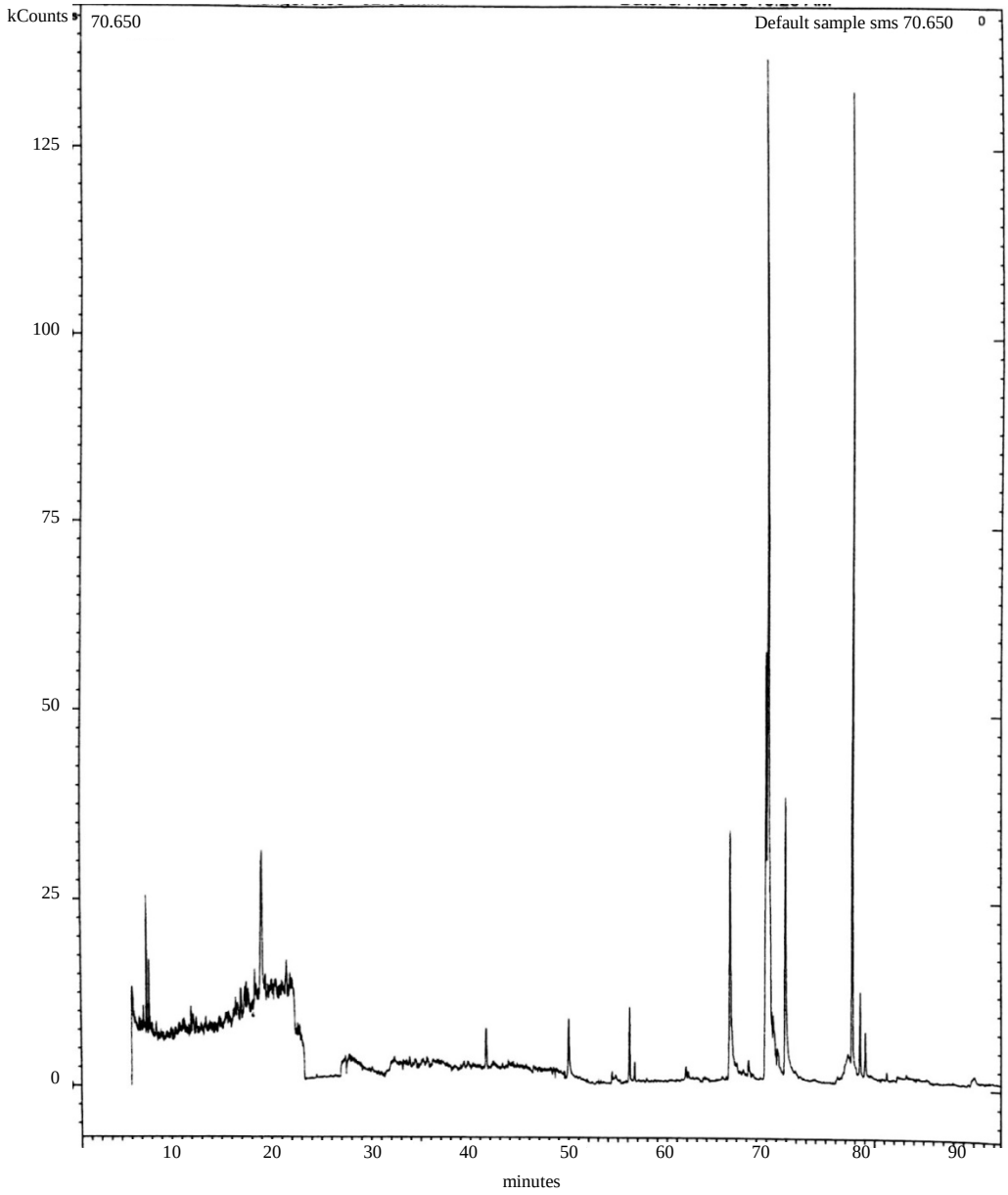

Fig. 3: GC-MS chromatogram of ethanolic extracts of *Matricaria aurea*

main constituents[22]. All the above mentioned compounds are known to suppress the growth of microorganism by inhibiting the cytoplasmic membrane function and nucleic acid metabolism[26,27]. The variation in inhibition rates is due to the sensitivity of microbes towards secondary metabolites present in different solvents extracts with varying polarities[28]. The scanning electron micrographs show the *Bacillus subtilus* cells with damaged structural integrity after being treated with methanol extracts. It is clear that the bioactive antimicrobials found in the extracts acts on the cell membrane thereby altering its permeability resulting in cell destruction[29,30]. The most competent solvent extracts (methanol and ethanol) of *M. aurea* flowers, exhibiting strong antimicrobial activity were further subjected to GC-MS analysis. Analysis revealed the presence of various chemical compounds. All the compounds identified have been reported earlier from various plants and are known to possess antimicrobial properties. In the present study flower extracts contained O-xylene, which is known for its antifungal, antibacterial and antioxidant activity. Similarly it was also isolated from leaves of *M. recutita* acetone extract earlier[31]. The coumarins and its derivatives like hymecrome and

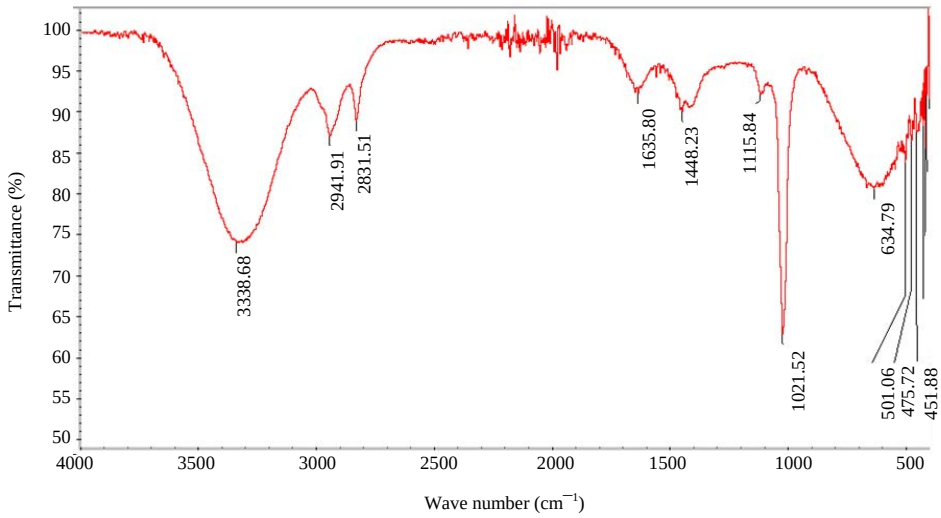

Fig. 4: FT-IR spectrum of Methanol extract of *Matricaria aurea*

7-acetoxy-4 methyl coumarin were identified in both the methanol and ethanol extracts in this study. Coumarins are phenolic substances made of fused benzene and α-pyrone rings[32]. They are known bioactive compound exhibiting antibacterial and antifungal properties and is found to be present in many aromatic plants[33]. In another study phenol, 2, 3, 5, 6-tetramethyl has been reported to possess antibacterial activity[34]. This compound was identified in the present investigation study as well. The other phytocompounds identified in this study are benzene acetic acid, benzene derivatives, phenols and esters. Interestingly similar compounds have been reported from acetone and ethanol extracts of chamomile[31,35]. Various reports suggests these compounds to exhibit antifungal, antibacterial and defensive functions[36,37].

However, this findings of the present study are not in agreement with the findings of other reports, wherein they have isolated α-bisabolol, α-bisabolol oxide and chamulazene from extracts and essential oils of German and Roman chamomile[22]. Previous studies have explained that medicinal plants grown in different ecological conditions produce different active phytochemicals including secondary metabolites[38]. Hence, this explains the variation in chemical composition of *M. aures* in this study.

It is interesting to note the presence of diverse bioactive compounds belonging to aromatic benezene and its derivates, phenols, coumarins, ketones and esters in the extracts of *Matricaria aurea.* Earlier reports suggest all of the above-mentioned constituents as potential antimicrobial agents[5,39]. Similarly other researchers have reported the polyphenols to be a major component of chamomile and stated that its antimicrobial action is related to interactions that lead to inactivation of microbial adhesion, inhibition of hydrolytic enzymes and cell envelope transport proteins[40,41].

A few animal studies on *M. chamomilla* and *M. recutita* are available but reports on *in vivo* activities of *M. aurea* on animals and humans are lacking as per my knowledge. In a study conducted earlier, highest number of mice survived lethal doses of *E. coli* when treated with chloroform extracts of *M. recutita* (10.5 mg mL^{-1})[42]. In another study on rabbits, alcoholic extracts of *M. chamomilla* flowers inhibited eye infections induced by *S. aureus* at a dose[43] of 40 mg mL^{-1}. Similarly, topical application of extracts based cream of *M. recutita* completely cured skin lesions on mice induced by injecting 10^7 CFU mL^{-1} of *S. aureus*[31].

CONCLUSION

The present study demonstrates significant *in vitro* antibacterial and antifungal activity in *Matricaria aurea* flowers growing in Saudi Arabia. Phytochemical and FTIR analysis of flower extracts showed the presence of major constituents like phenols, benzene, coumarins and their derivatives. Hence, it can be concluded that *Matricaria aurea* possesses natural bioactive compounds of widespread pharmacological and medicinal properties.

Various varieties of chamomile have been screened for their antimicrobial activities *in vitro*, however, *in vivo* studies are scanty. To the knowledge of the researcher, there are no reports on *in vivo* studies of *M. aurea* on animals

and humans. Though chamomile has been declared as safe (GRAS) by the FDA , it can be further explored for potentially relevant novel compounds and applied in clinical trials for potentially developing promising therapeutic agents. Therefore, future studies need to focus on clinical formulations, bioavailability of active ingredients, therapeutic dosages and potential toxicity assessment through *in vivo* studies.

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