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Chemical composition and antimicrobial effect of *Melissa officinalis* and *Angelica sylvestris* on selected microbial pathogens

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Abstract. *Saleh RH, Omran AM, Bash HS. 2023. Chemical composition and antimicrobial effect of* Melissa officinalis *and* Angelica sylvestris *on selected microbial pathogens. Biodiversitas 24: 1871-1877. Melissa officinalis* L. and *Angelica sylvestris* L. have been used for traditional herbal medicines due to their antimicrobial action against pathogenic microbes. The purpose of this research was to determine the chemical content of the aqueous and ethanol extracts of *M. officinalis* and *A. sylvestris* and their antimicrobial activity against selected bacteria and fungi. This study was done from March 2022 to July 2022. The antimicrobial activity of the extracts was carried out against three bacterial isolates (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*) and one fungal isolate (*Candida albicans*). The fungal isolate was isolated from the different patients. The concentrations of extracts were 10, 20, 30, 40 mg/mL. The antimicrobial activity was performed using agar diffusion methods and compared to standard gentamycin and fluconazole disc. It has been shown that the major phytochemical composition in *M. officinalis* and *A. sylvestris* aqueous and ethanolic extracts were: alkaloids, phenols, glycosides, and flavonoids. The extracts of *M. officinalis* and *A. sylvestris* exhibit different antimicrobial activity against the tested microorganisms, and their activity increased with increasing extract concentration. *Melissa officinalis* extracts inhibited the growth of all tested microorganisms except *C. albicans*. Aqueous and ethanol extracts of *M. officinalis* were more effective against *E. coli*. *A. sylvestris* aqueous extract was the most effective against *S. aureus*. The most potent antimicrobial activity of the ethanolic extract was detected against *K. pneumoniae*. The antimicrobial properties of *M. officinalis* and *A. sylvestris* are related to their chemical compounds, such as phenols, alkaloids, glycosides, and flavonoids. Both plants extract showed good inhibition against most microorganisms. Based on the research results, *M. officinalis* and *A. sylvestris* are potential new sources of antimicrobial agents.

Keywords: *Angelica sylvestris*, antimicrobial activity, medical plants, *Melissa officinalis*

INTRODUCTION

One of the serious problems in infectious illness is the increasing prevalence of antibiotic resistance in pathogenic microbes (Cave et al. 2021). Several antibiotics are becoming less potent due to the development of antibiotic resistance, which is caused by the overuse of antibiotics. Antibiotic resistance mechanisms are mainly due to the efflux of antibiotics by transporters, and the prevention of antibiotic interaction with targets through mutation, modification, and target protection. These mechanisms result from the inherent structural or functional resistant characteristics, the acquired resistance by mutational change or horizontal gene transfer, and the adaptive antibiotic resistance (Ogawara 2019). Hence, it is essential to obtain different options to overcome antibiotic resistance (Marasini et al. 2015). Herbal medicines have been the primary source of therapeutic compounds. World Health Organization reported that up to 80% of the world's population still rely on herbal therapies for their medical needs (Ekor 2014) because herbal medicine is safe and has few side effects compared to synthetic drugs (Saleh et al. 2017). In the last ten years, research on herbal medicines and their bioactive components has increased (Subba and Basnet 2014). Plants produce secondary metabolites with antimicrobial activity in response to pathogenic microbes or stress response. These compounds frequently act as defense mechanisms against herbivores, insects, and microbial pathogens. (Duško et al. 2006). The essential oils (EO) of medicinal plants comprise several secondary metabolites like alkaloids, tannins, and flavonoids, which may have antimicrobial properties (Klūga et al. 2017).

Melissa officinalis L. and *Angelica sylvestris* L. have been used as herbal medicines. *Melissa officinalis* is a plant species belonging to Lamiaceae, indigenous to Iran, Central Asia, and Europe. It is also called lemon balm (Okmen 2017). It is a perennial plant that smells strongly like lemon. It is an erect, bushy shrub that grows to a height of about 1 m, with heart-shaped hairy leaves and 2-8 cm long (Petrisor et al. 2022). The leaf border is scalloped or toothed, and the leaf's surface is rough and extensively veined. In the summer, white or pale pink flowers comprise tinny clusters of 4-12 blossoms (Bagdat and CosgeO 2006). *Melissa officinalis* essential oil exhibit antimicrobial, antioxidative, anti-inflammatory, and antidiabetic properties (Al-Mijalli et al. 2022). In many countries, it is a powerful medicinal herb used in conventional medicine to cure headaches, nervousness, gastritis, colic, cold, infectious disorders, fever, and cough. The lemon balm extract can be used as a lotion to speed up the recovery of herpes mouth sores (recurrent herpes labialis), prevent their spread, and reduce itching symptoms (Stefanović and

Comic 2012). It has been reported that *M. officinalis* could prevent cancer cells from producing proteins. Essential flavonoids and phenolic acids like rosmarinic and caffeic acids have been associated with these biological properties (Abdellatif et al. 2014). Phenolics are related to plant antioxidants. Generally, essential oils and their ingredients are safe and have many potential uses in several functional applications (Chouhan et al. 2017). Natural medicines benefit from their low drug resistance phenomena, even in prolonged use (Vitullo et al. 2011).

The other medicinal plant was *A. sylvestris* belongs to Apicaceae and is a wild perennial plant. It is endemic in eastern and northern Europe countries. *A. sylvestris* is herbaceous, has no odor, and has thick, long, brown tapering parts on the outside and white on the inside. Pinnated leaves consist of oval jagged, uniform pinnae, including an irregular one on the terminal. Flowers are grouped in broad compound umbels, either white or slightly pink. The main parts used for herbal medicine are the seed and root, rarely the aerial parts (Aćimović et al. 2018). It is generally used to treat digestive, nervous, and respiratory tract illnesses. It is also indicated an antipyretic, anticarcinogenic effect, analgesic, headache, and arthritis (Budniak et al. 2022). Studies on the *A. sylvestris* root revealed essential oil with nonane as the primary ingredient and glycosides. At present, essential oils are becoming more popular in industries including food, cosmetics, and pharmaceuticals. Because essential oils are complex mixes with a variety of components, they have a range of bioactivity (Ağalar et al. 2020). Furthermore, the seeds possess fatty acids, with linoleic and oleic acids predominating. They also include essential oils, especially limonene and -pinene serving as the main ingredients. The aerial parts of *A. sylvestris* only had 0.05% essential oil, with two main components being limonene (75.3%) and -pinene (9.5%) (Aćimović et al. 2017). The root extract has been demonstrated to have antibacterial activity against *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecium, Bacillus subtilis,* and *Listeria monocytogenes* (Canli et al. 2016). The current investigation aims to evaluate the antimicrobial property of aqueous and ethanolic extracts of *M. officinalis* and *A. sylvestris* against different selected bacteria.

MATERIALS AND METHODS

Preparation of extracts

Aqueous extract

10 g of air-dried powder from every plant was put in a 500 mL conical flask, then added with 250 mL distilled water. The mixture was boiled slowly for 2 h before filtered using a muslin cloth (8 layers). The filtrate was centrifuged at 5000 RPM for about 10 min, and the supernatant was recovered. The extract was concentrated in an oven (45°C) until completely dry, and the dried extracts were kept at 4°C) for further use (Parekh and Chanda 2007).

Alcoholic /ethanolic extract

200 mL of ethanol in a conical flask was added with 10 gm of the plant powder plant, sealed with cotton, and shaken in a horizontal shaker (140-220 RPM) for 24 hours. The mixture was filtered using muslin cloth consisting of 8 layers and centrifuged (5000 RPM) for approximately ten min. The supernatant was obtained and dried in an oven (4°C). Dried extracts were kept at (4°C) for further use (Parekh and Chanda 2007).

Qualitative tests of phytochemicals

Test for alkaloids

Tannic acid reagent. 2 mL of 10% tannic acid solution was added to the extract (5 mL). The presence of alkaloids is indicated by orange precipitate (Neelima et al. 2011).

Alkaloids test (Hager's test). Hager's reagent is a saturated Picric acid solution ($C_6H_3N_3O_7$). The presence of alkaloids was indicated by yellow precipitate after Hager's reagent addition (Neelima et al. 2011).

Phenols test

Lead acetate test. Dissolved extract (50 mg) in distilled water (5 mL) and then with 3 mL of lead acetate (10%). A massive white precipitate referred to phenol presence (Tamilselvi et al. 2012).

Flavonoids test

Flavonoids test based on the method by Aziz et al. 2012. First, 10 g of the extract from each sample was dissolved in 5 mL of 95% ethyl alcohol, and then the mixture was filtered. Second: a potassium hydroxide solution (10 mL) was mixed with 10 mL of 50% ethyl alcohol. Equal parts of the two solutions are combined, and the yellow color indicates the existence of flavones.

Glycosides test

Fehling's test was used to determine the presence of glycosides. Fehling's A solution: Copper sulfate ($CuSO_4$) in distilled water. Fehling's B: Potassium tartrate ($K_2C_4H_4O_6$) and sodium hydroxide (NaOH) dissolved in distilled water. Fehling's A was mixed with the amount of Fehling'S B. The extract was added with some drops of mixture reagent and heated to a boil. The presence of glycosides was indicated by red precipitate (Neelima et al. 2011).

Assessment of antibacterial activity

Microorganisms

Staphylococcus aureus, *Escherichia coli*, and *Klebsiella pneumonia* were used for the antibacterial test, and *Candida albicans* was used for the fungal activity. Bacterial isolates were activated and cultured three times and stored on a nutrient agar slant at 4ºC. The bacteria were identified by conventional biochemical assays. Their sensitivity to the reference antibiotics was checked for antimicrobial activity. Gentamycin antibiotic discs (10 mcg) (Sigma, USA) were used as a positive control for antibacterial activity, and amphotericin B (25 mcg) (Sigma, USA) was used for antifungal activity.

Inoculum preparation

The inoculum was prepared by adding colonies from nutrient agar to 5 mL of 0.85% sterile normal saline to reach 0.5 McFarland turbidity, equivalent to a suspension of bacterial cells 1.5 x 10^8 CFU/mL.

MacFarland standards preparation

0.5 McFarland standard was made by mixing 0.05 mL of concentrated sulfuric acid (H_2SO_4) (1.0%) with 9.95 mL of barium chloride (1.17%). Its turbidity is approximately equivalent to 1.5 x 10^8 bacterial cells per mL.

Antimicrobial susceptibility testing

The Bauer-Kirby method determined the sensitivity of bacterial isolates to conventional antibiotics. A bacterial suspension (0.1 mL) was inoculated to agar plates, then distributed evenly with a sterile glass spreader. The antibiotics disks were added in the middle of an agar plate. The experiment was carried out in triplicate. After finishing inoculation, plates were incubated overnight at 37°C, and the inhibition zone was measured (mm) after incubation. The results were evaluated by recommendations from the National Committee for Clinical Laboratory Standards (CLSI 2019). Antibiotics used in this study were Ceftazidime Amikacin, Gentamicin (10 µg), Trimethoprim, Ciprofloxacin, Nalidixic acid, Nitrofurantoin, and Fluconazole.

Testing the antimicrobial efficacy of plant extract

The antimicrobial efficacy of aqueous and ethanolic extracts of *M. officinalis* and *A. sylvestris* at various concentrations (10, 20, 30, and 40 mg/mL) was determined using the disk diffusion test. A loop of every bacterial isolate was cultivated into the nutrient broth and incubated for 18 hours at 37°C. Normal saline was used to dilute the bacterial suspensions. To produce a homogenous suspension with 1.5×108 CFU/mL, The turbidity of bacterial suspension was compared to a reference tube McFarland (number 0.5). A cotton swab was soaked in adjusted bacterial dilution and streaked to the Mueller-Hinton agar plates. The plates were left to dry at room temperature for one to 5-15 min. A cork borer was used to create wells (5 mm in diameter) in the inoculated agar media. 0.1 mL of extract at different concentrations was added to the wells, while the well in the center was filled with distilled water). The plates were then incubated at 37°C for 24hr. After overnight incubation, every extract was evaluated for the diameter of the inhibition zone (mm). The test was performed in triplicates. For each replicate of the test, readings were taken in three distinct fixed directions, and the average values were recorded. Gentamicin (10 mcg) was used as a positive control for antibacterial activity, while amphotericin B (25 mcg) was used as a positive control for antifungal activity (Forbes et al. 2007).

RESULTS AND DISCUSSION

Determination of chemical content

The secondary metabolites of aqueous and ethanol extracts are shown in Table 1. All extracts contained alkaloids, phenols, glycosides, and terpenes.

A previous study by Abdel-Naime et al. (2019) showed that *M. officinalis* ether extract contained flavonoids, phenolic acids, volatile oils, and triterpenes/ triterpenoids. Another study showed that different extracts of *M. officinalis* contained phenolic compounds, flavonoids, tannin, rosmarinic acid, vanillic acid, caffeic acid, and protocatechuic acid (Miraj et al. 2017). These chemical compounds are commonly found in plant parts, i.e., roots, leaves, flowers, and bark (Behbahani and Shahidi 2019). A previous study showed that the methanol extract of *A. sylvestris* contains polyphenols (Stanković et al. 2016). A study by Aćimović et al. (2018) showed that *A. sylvestris* root contained glycosides and essential oil with nonane A as the essential ingredient. The main constituents of the oil were α-pinene, δ-3-carene, limonene, and α-phellandrene. The differences in the chemical contents of *M. officinalis* and *A. sylvestris* among studies may be related to genotypic change, climatic *circumstances*, different solvents, and different solvents used in extraction.

Antibiotic susceptibility test

The antimicrobial sensitivity of testing microorganisms to various antibiotics and an antifungal are shown in Table 2. Results indicated that Trimethoprim was the most potent antibiotic against bacteria, and Fluconazole has an inhibitory zone of 11 mm against *C. albicans*. On the other hand, *Klebsiella pneumoniae* and *E. coli* are resistant to several tested antibiotics. Previous studies by Assefa et al. 2022 and Al-Hasnawy et al. 2018 showed that *E. coli* was also resistant to the standard and available antibiotics. Nitrofurantoin has remarkable antibacterial activity. Other studies like (Shakti and Veeraraghavan 2015), reported related findings. Genetic mutation may result in resistance caused by the upregulation of efflux systems and the production of various enzymes, including extended-spectrum beta-lactamases. High antibiotic resistance is attributable to multi-drug efflux pumps' concerted action with a chromosomally encoded antibiotic resistance gene and the low permeability of bacterial cellular envelopes (Zahedani et al. 2021).

Table 1. Qualitative tests of phytochemicals

Phytochemicals	*Melissa officinalis* extract		*Angelica sylvestris* extract	
	Aqueous	**Alcoholic**	**Aqueous**	**Alcoholic**
Alkaloids	+	+	+	+
Phenols	+	+	+	+
Flavonoids	+	+	+	+
Glycosides	+	+	+	+

Table 2. The antibiotic sensitivity against several microorganisms

Bacteria	Antibiotics							
	TMR	**GN**	**AK**	**CIP**	**NA**	**CAZ**	**F**	**FLU**
S. aureus	S	R	R	S	R	R	S	-
E. coli	R	R	R	R	R	R	S	-
K. pneumoniae	S	R	R	R	R	R	S	-
C. albicans	-	-	-	-	-	-	-	S

Notes: TMR: Trimethoprim, GN: Gentamicin, AK: Amikacin, CIP: Ciprofloxacin, NA: Nalidixic acid, CAZ: Ceftazidime, N: Nitrofurantoin, F: Fluconazole, S: sensitive, R: resistant

Antimicrobial activity test of plants

The antibacterial activity of aqueous and ethanolic extract *M. officinalis* and *A. sylvestris* at different concentrations were evaluated in vitro against *S. aureus, K. pneumoniae, E. coli* and *C. albican* (Table 3) and compared to standard gentamycin and fluconazole. The aqueous and alcohol extracts of *M. officinalis* at various concentrations had antimicrobial activities against tested bacteria with inhibition zone ranging from 10-2 3mm. The aqueous and ethanol extracts of *M. officinalis* had maximum inhibition activity against *E. coli* but no inhibitory activity against *C. albicans*. Figures 1 and 2 show the inhibitory activity of *M. officinalis* extracts against tested bacteria.

A study by Okmen (2017) showed that the aqueous, ethanol, and ethyl acetate *M. officinalis* extracts at the concentration of 10 mg/mL had moderate inhibitory activity against *S. aureus.* Canadanovic-Brunet et al. (2008) reported that the aqueous, chloroform, ethyl acetate, petroleum ether, and n-butanol extracts of *M. officinalis* had moderate or strong inhibitory activity against *Bacillus cereus* and *S. aureus*; however, not active against *E. coli* and *Pseudomonas aeruginosa*. In contrast to our study, (Erturk 2006) reported that *M. officinalis* extracts exhibited antifungal activity against *C. albicans* and *A. niger* with inhibitory zones ranging from 10-16 mm. A study by Mutalib (2015) showed that ethanolic extract of *M.officinalis* leaf had antibacterial activity against *Klebsiella spp.* and *S. aureus*, but it did not active against *P. aeruginosa*, *E. coli*, *Streptococcus pyogenes*, *Streptococcus pneumoniae,* and *Bacillus spp*. Rabbani et al. (2016) reported that *M. officinalis* extract possesses significantly higher antibacterial activity against Gram-positive bacteria, such as *Staphylococcus epidermidis* and *S. aureus*, than Gram-negative bacteria. The most sensitive Gram-negative bacteria against *M. officinalis* was *E. coli*. The leaf and the herb of *M. officinalis* were utilized in herbal medicines. Still, due to its therapeutic properties, western pharmacopeias prefer to use the leaf instead of other plant components (Uyanik and Gurbuz 2014). The phytochemical compounds entirely rely on regional and environmental variables, which affect their bioactivity and medicinal importance. Several compounds that contribute to antibacterial activity in the extract of *M. officinalis* could be tannin, phenolic compounds, caffeic acid, p-coumaric acid, rosmarinic acid, and vanillic acid, as well as essential oils and flavonoids (Abolfazl et al. 2016).

The aqueous and ethanolic extracts of *A. sylvestris* inhibited the growth of tested microorganisms, and increasing concentration increased the inhibition zone (Table 4). The highest inhibitory effect of aqueous extract was observed against *S. aureus*, and the ethanolic extract had maximum inhibition at 40mg/mL against *K. pneumoniae.* Figures 3 and 4 show the effect of *A. sylvestris* extract on tested microorganisms.

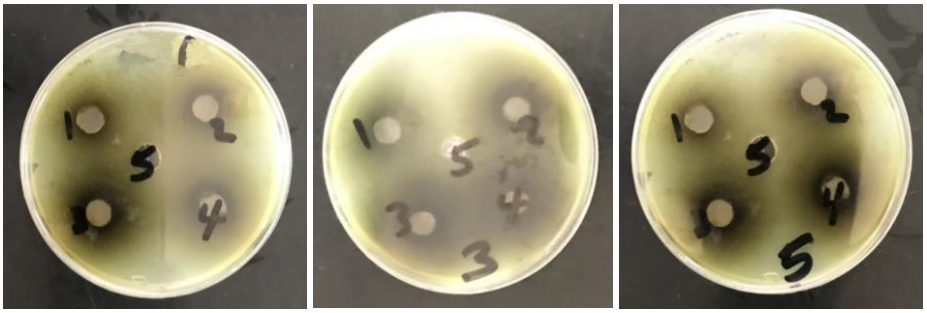
A previous study by Canli et al. (2016) showed that *A. sylvestris* roots ethanol extracts inhibit the growth of several Gram-positive and Gram-negative microbes, including *Enterococcus faecium, S. epidermidis, S. aureus, B. subtilis, C. albicans P. aeruginosa,* and *K. pneumonia*. In contrast, a study by Sarker et al. (2003) showed that *A. sylvestris* extracts possess low antimicrobial activity against *S. aureus, E. faecalis,* and *E. coli*. Most species of *Angelica* contain significant amounts of furanocoumarins, which have been shown to have antibacterial properties. The essential oil of *A. sylvestris* possesses antimicrobial activity due to the combined interactions between its minor and major compounds. The major compounds of *A. sylvestris* essential oil were limonene, α- pinene. The major compounds of *Angelica pancicii* oil were β-phellandrene, α-pinene, and α-phellandrene, were previously demonstrated as potent antimicrobial agents (Stankovć et al. 2020). The isocaryophyllene oxide, α-bisabolol, and α-pinene were the major compounds in the roots of *A. sylvestris* (Raal et al. 2022).

Table 3. The inhibitory zone of aqueous and ethanolic *Melissa officinalis* extract against various microorganisms

Microorganism	***M. officinalis* extracts concentrations (mg/mL)**									
	Aqueous				**Ethanolic**				**GN(µg)**	**FLU (µg)**
	10	**20**	**30**	**40**	**10**	**20**	**30**	**40**	**10**	**25**
S. aureus	14	15	15	16	9	12	15	17	11	-
S. aureus	16	17	20	21	12	20	22	23	17	-
K. pneumoniae	10	11	13	15	15	18	19	20	18	-
C. albicans	0	0	0	0	0	0	0	0	-	S

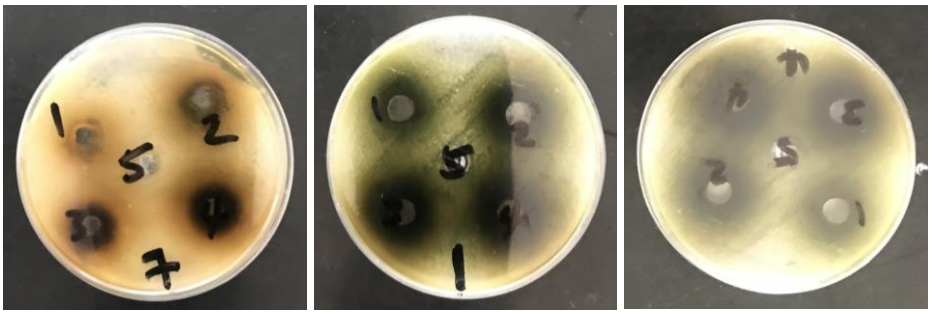
Table 4. The inhibitory zone of aqueous and ethanolic *Angelica sylvestris* against various microorganisms

Microorganism	*A. sylvestris* extracts concentrations (mg/mL)									
	Aqueous				Ethanolic				GN(µg)	FLU (µg)
	10	20	30	40	10	20	30	40	10	25
S. aureus	11	15	16	18	6	8	9	15	11	-
S. aureus	0	12	15	17	13	14	15	16	17	-
K. pneumoniae	0	0	14	16	8	14	16	20	18	-
C. albicans	0	11	14	15	0	10	11	15	-	S



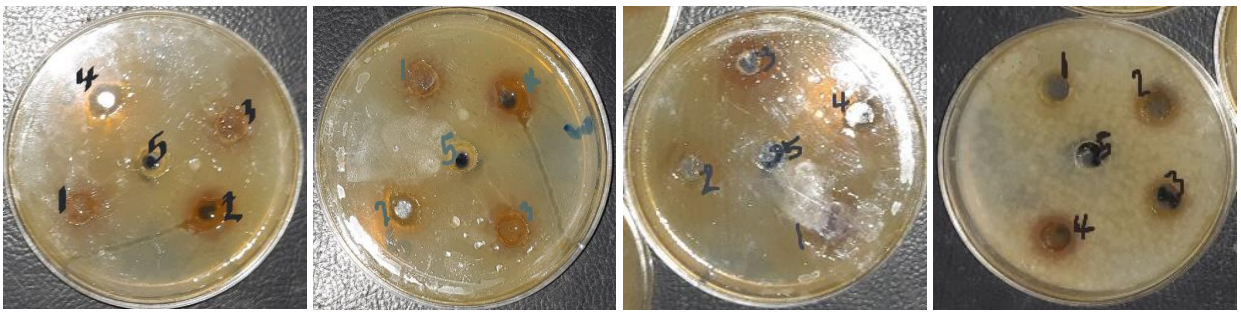
A **B** **C**

Figure 1. The growth inhibition of *M. officinalis* aqueous extract at different concentrations against: A. *S. aureus*, B. *K. pneumoniae*, C. *E. coli*, D. C. *albicans*. 1. 10 mg/mL; 2. 20 mg/mL; 3. 30 mg/mL; 4. 40 mg/mL. GN: Gentamicine, F: Fluconazole, mm: Inhibition zone



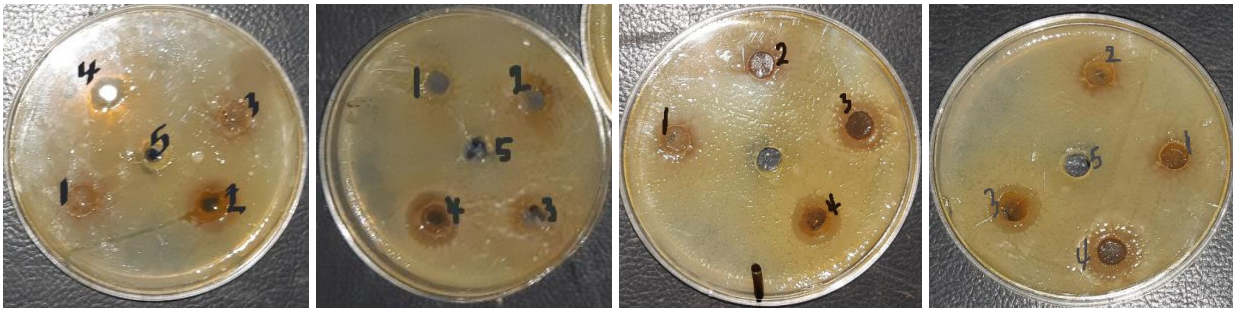
A **B** **C**

Figure 2. The growth inhibition of *M. officinalis* ethanolic extract at different concentrations against: A. *S. aureus*, B. *K. pneumoniae*, C. *E. coli*, D. C. *albicans*. 1. 10 mg/mL; 2. 20 mg/mL; 3. 30 mg/mL; 4. 40 mg/mL. Diameter of inhibition was not visible.



A **B** **C** **D**

Figure 3. The inhibitory zone of aqueous *A. sylvestris* extracts at different concentrations against: A. *S. aureus*, B. *K. pneumoniae*, C. *E. coli*, D. C. *albicans*. 1. 10 mg/mL; 2. 20 mg/mL; 3. 30 mg/mL; 4. 40 mg/mL. GN: Gentamicine, F: Fluconazole, mm: Inhibition zone



A **B** **C** **D**

Figure 4. The inhibitory zone of ethanolic *A. sylvestris* extract at different concentrations against: A. *S. aureus*, B. *K. pneumoniae*, C. *E. coli*, D. C. *albicans*. 1. 10 mg/mL; 2. 20 mg/mL; 3. 30 mg/mL; 4. 40 mg/mL

In conclusion, medicinal plants are recognized as safe and possess low side effects. The current study revealed that aqueous and ethanolic extracts of *M. officinalis* and *A. sylvestris* Showed inhibitory effects on tested bacteria, in which *M. officinalis* extracts prevented the growth of all assessed pathogens excluding *C.albicans*. *Angelica sylvestris* suppressed microbes, and the inhibition zone increased as the concentration increased. The antimicrobial properties of these plants may relate to the phytochemical constituents, including flavonoids, phenols, alkaloids, and glycosides.

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