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Phytochemical Screening, Antibacterial Effect, and Essential Oil Extract from the Leaf of *Artemisia afra* against on Selected Pathogens

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Abstract

Background: *Artemisia afra* is an indigenous plant used in Africa. It is used in traditional medicine to treat a variety of diseases caused by bacterial infections. As a result, developing innovative therapeutic methods using natural ingredients to combat pathogenic germs is critical. **Methodology:** *Artemisia afra* leaves were extracted using maceration extraction utilizing three solvents (petroleum ether, ethanol, and dichloromethane). Fresh leaves were extracted using hydrodistillation. The agar well diffusion method was used to test the crude extract and essential oil leaves of *Artemisia afra* against different human pathogenic bacteria strains (*E. coli*, *S. aureus*, *E. faecalis*, and *K. pneumonia*) at different concentrations in the presence of a positive (ciprofloxacin 5.0 μg) and negative (DMSO) control. **Results:** *Artemisia afra* revealed the presence of alkaloids, flavonoids, tannins, saponins, anthraquinones, terpenoids, coumarins, phenolic quinones, cardiac glycosides, and steroids. It showed that the highest antibacterial activity given by the ethanol extract had highly inhibition zones against gram-positive and gram-negative bacteria. The essential oil extract was effective against all tested bacteria. **Conclusion:** Plant crude extracts and essential oils may have antibacterial properties due to the synergistic activity of two or more active secondary metabolites.

Keywords

Artemisia afra, Antibacterial Activity, Essential Oil, Hydrodistillation, Maceration, Agar Well Diffusion

1. Introduction

Since ancient times, plants have played an important role in disease prevention

and treatment. Traditional knowledge and medicinal plants are used by the majority of the world's population to address some of their primary health care needs [1] [2] [3]. Traditional plant use in Africa has a long and rich history, and indigenous plants are still the primary source of medicine [3]. The majority of Ethiopian communities rely on local traditional medicinal herbs to address a variety of ailments [4]. Due to its significant geographical diversity, Ethiopia is endowed with a varied range of biological resources [2] [3] [4].

The country's diverse socioeconomic and cultural origins contributed significantly to the presence of rich indigenous knowledge, including the management and use of medicinal plants to treat human ailments [1] [2] [3]. In Ethiopia, the majority of traditional medical treatments are made from plants.

Artemisia afra commonly called African wormwood [5]-[14] is a member of the Compositae (Asteraceae) family [5] [6] [9] [10] [13]-[18]. It is one of the most widely used in African medicinal plants with essential oil components [5] [7] [19]. It is locally known as 'Chigugn' (Amharic) [15] [20]. It is an evergreen perennial herb or deciduous subshrub with grey or green foliage leaves containing yellow florets. It is aromatic and it exudes a pungent, sweet smell when any part of the plant is bruised [10] [13]. It grows up to a height of about 1.5 meters [10], at an altitude range of 3070 and 3600 meters [21].

Artemisia afra is the common traditional used medicinal plant use it for variety of ailments they are pneumonia, poor appetite, wound [5], flu, cardiovascular diseases, cancer, respiratory diseases influenza [6], blocked nose [7], heartburn, stomach disorders, sprains, rheumatic swellings [8], bronchitis [7] [8], dry dyspepsia, purgative [10], chills [7] [10], infant growth/weight gain, dandruff, stop bleeding [11], Smallpox, stomach ache [9] [10] [11], dental care, gout, intestinal worms [12], sore throat [5] [6] [7] [12], asthma [6] [8] [12], colic, intestinal parasitic diseases [13], fever [7] [13], headache [8] [12] [13], bladder, kidney disorders [16], diabetes [5] [6] [8] [12] [16], Cough, colds, and malaria [5] [6] [7] [8] [10] [11] [12] [13] [16].

The leaves and stems are used to prepare teas, decoctions, and tinctures from fresh or dried leaves and stems [6] [7]. Headaches, congestion, asthma, hay fever, and sinusitis can all be relieved by inhaling the steam and vapors [6]. Also, In the Bale region of Ethiopia, the juice of chopped *A. afra* leaves combined with water is traditionally consumed orally for the treatment of roundworms and stomach discomfort [13]. Fresh/dry *A. afra* leaves combined with butter are taken orally with coffee for three days [15].

Artemisia afra plant extract and essential oil have shown antifungal [6] [14], antimicrobial [7] [8] [22], antioxidant [6] [7] [10], anti-cancer [23], antituberculotic [6], antimalarial [6] [14], antiviral [8], anti-convulsant [14], based on different concentration that explained in different literature.

The objectives of this study are as follows:

1) To test and evaluate the antibacterial activity of Petroleum ether, Dichloromethane, Ethanol, and Essential Oil extracts from *A. afra* leave.

2) To determine the phytochemical content of the leaves of *A. afra* using Petroleum ether, Dichloromethane, Ethanol, and Essential Oil extract.

2. Materials and Methods

2.1. Collection of Plant Material

The fresh leaves of *A. afra* was collected from Tepi town, South Nation Nationalities, and people regional state which is 611 km southwest of Addis Ababa. It is located at a latitude and longitude of 7012'N350 27'E with a mean elevation of 1097 meters above sea level.

2.2. Experimental Site

The extraction and phytochemical screening were conducted at Mizan-Tepi, University Chemistry department laboratory room. The bacterial activity tests were performed at Amhara regional health research laboratory center, Dessie.

2.3. Extraction

The powdered leaves of *A. afra* (200.0 g × 3) were successively extracted with petroleum ether, dichloromethane, and ethanol prepared by the maceration. It was soaked in a clean flask containing petroleum ether, dichloromethane, and ethanol in 1000.0 mL for three days with frequent manual shaking. The resultant extract was filtered using filter paper (Whatman filter paper No 1), and the supernatant was concentrated using a rotary evaporator under decreased pressure at 40˚C. The dried extract was placed in vials and stored in the refrigerator at 4˚C until needed [4] [24].

2.4. Essential Oil Extraction

Fresh leaves of *A. afra* (100.0 g) were measured and soaked in 500.0 mL water in the round bottom flask. The hydrodistillation apparatus setup was adjusted. The mixture was heated at the boiling temperature of water for 6 h. Water (a total of 1500.0 mL) was added continuously to the flask via funnel for a convenient hydro distillation process. Essential oil-water mixture (1250.0 mL) was received using volumetric flasks. This mixture was allowed to stand in a separatory funnel for 8 h and then the essential oil was separated. The essential oil samples were stored at 4˚C in the refrigerator [25].

2.5. Phytochemical Screening

The leaves crude and essential oil extract were subjected to the following preliminary phytochemical studies.

Test for phenolic compounds

The extract (1.0 mL) was diluted in distilled water to 3.0 mL and then filtered. Four drops of ferric chloride solution (5%) were added to this. The presence of phenolics was identified by the formation of the dark green color [4].

Test for alkaloids

Hager's reagent (saturated picric acid solution) was used to treat the extract (1.0 mL). The presence of alkaloids was identified by the formation of the creamy white precipitate color [26].

Test for flavonoids

The extract (1.0 mL) was transferred into a test tube and treated with four drops of sodium hydroxide solution. The formation of a yellow color indicated the presence of flavonoids [4].

Test for tannins

The extract (5.0 mL) was transferred to a test tube, and 2.0 mL of $FeCl_3$ solution (5%) was added. The presence of tannins was revealed by the formation of a greenish-black precipitate color [26].

Test for terpenoids

The extract (0.5 mL) with 2.0 mL of chloroform was added to the test tube. Then 3.0 mL conc. H_2SO_4 was added carefully to form a layer. The formation of reddish-brown color indicated the presence of terpenoids [27].

Test for quinones

The extract (1.0 mL) was placed in a test tube, followed by 1.0 mL of pure sulphuric acid. The presence of quinones was detected by the formation of a reddish red color [4].

Test for saponins

A test tube was filled with the extract (2.0 mL). A solution of sodium bicarbonates was added in four drops. The test tube was violently shaken for 3 minutes. The presence of saponins was indicated by the formation of honeycomb-like foam [28].

Test for anthraquinones

The extract (1.0 mL) was shaken well with 10.0 mL benzene and filtered. Then 0.5 mL of ammonia solution was added to the filtrate and stirred. The formation of a Violet color indicated the presence of anthraquinones [4] [29].

Test for cardiac glycosides

The extract (2.0 mL) was dissolved in glacial acetic acid with four drops of ferric chloride in 2.0 mL. Then, under the layers, 2.0 mL of concentrated sulphuric acid was added. At interphase, a brown ring developed, indicating the presence of deoxy sugar, a characteristic of cardiac glycoside [29].

Test for anthocyanins

The extract (2.0 mL) was treated with 2.0 mL 2N HCl before being added to 2.0 mL ammonia. The presence of anthocyanins was revealed by the formation of blue-violet [30].

Test for coumarins

The extract (1.0 mL) was transferred into a test tube. 1.0 mL of Conc. sulphuric acid was added. The formation of red color indicated the presence of quinines [4].

Tests for steroids

The extract (1.0 mL) was shaken with chloroform, and to the chloroform layer sulphuric acid was added slowly by the sides of the test tube. The formation of

red color indicated the presence of Steroids [4] [28].

2.6. *In Vitro* Antibacterial Studies

2.6.1. Bacterial Test Organisms and Standard Antibacterial Disc

The standard American Type Cell Culture (ATCC) bacterial species of *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Klebsiella pneumonia* were obtained from Amhara national regional state health bureau Dessie regional health research laboratory. The standard antibacterial disc used for the study was ciprofloxacin 5.0 μg (positive control).

2.6.2. Antibacterial Activity Assay

The plant extracts and essential oil effect on several bacterial species were assessed by agar well diffusion methods [31]. The agar well diffusion method was used in measuring and determining the zone of inhibition of plant extract and essential oil against test organisms.

2.6.3. Media Preparation

Muller-Hinton agar powder (38.0 g) was suspended in 1.0 L of distilled water in a flat-bottomed conical flask. To completely dissolve the media, the mixture was heated with frequent agitation and boiled for one minute. The mixture was then heated with regular agitation until a clear solution was observed. Cotton wool was used to close the flask, which was then coated with aluminum foil. After autoclaving for 15 minutes at 121˚C, the mixture was allowed to cool to ambient temperature. The media was poured in a laminar flow into the Petri dishes to achieve a consistent depth of 4 millimeters. Before use, the Petri plates containing the media were placed in sterile plastic bags and stored at 6˚C [4] [32] [33].

2.6.4. Determination of Inhibition Zone

Fresh culture bacteria were suspended into 5.0 mL of sterile normal saline water and then the turbidity of suspension was adjusted equivalent to 0.5 McFarland standard by reading on the McFarland Densitometer instrument. A sterile cotton swab was dipped into adjusted bacterial suspension, rotated gently, and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculums from the swab. The swab was streaked to the entire surface of the MHA plate three times by rotating approximately 60˚ each time to ensure even distribution of the inoculums. Petri plates were left for three minutes at room temperature. Then, equal distance holes with 6 mm diameter were punched aseptically using flame sterilized cork borer tip. Prepared extract concentrations (50 μL) and essential oils (10 - 50 μL) were introduced into the labeled wells using a micropipette. The negative control (DMSO, 50 μL) and positive control (Ciprofloxacin, 5.0 μg) were placed into the labeled agar wells. The plates were placed undisturbed at room temperature for 2 h and then incubated at 37˚C for 24 h [4] [31] [33].

All tests were performed in triplicate for each bacterial species. Finally, the diameter of the inhibition zone around the wells was measured in millimeter

using a ruler. The mean zone of inhibition and standard error of the mean (Mean ± SEM) were calculated for the crude extracts and essential oil as well as for standard positive control.

2.7. Data Analysis

Phytochemical screening test results were reported as present (+) or absent (−). For the antibacterial efficacy test, the mean zone of inhibition is expressed as the mean and standard error of the mean (Mean ± SEM) for each triplicate determination.

3. Results and Discussions

3.1. Phytochemical Screening

Evaluation of the preliminary phytochemical screening of the ethanol, dichloromethane, and petroleum ether extract of the leaves of the *A. afra* plant revealed the presence of alkaloids, flavonoids, tannins, saponins, anthraquinones, terpenoids, coumarins, phenolic quinones, cardiac glycosides, and steroids. While anthocyanins were absent in all crude extracts (Table 1). The result is in line with the findings of Liu, NQ *et al.* [34], who reported terpenoids and flavonoids were present.

Moreover, in ethanol and dichloromethane crude extract phenolic compounds, alkaloids and tannins were detected. Dichloromethane and petroleum ether showed the presence of cardiac glycosides was detected. Flavonoids and saponins were only found in ethanol but not in another crude extract. Amongst

Table 1. Result of Phytochemical Screening tests of essential oil and crude extract.

Phytoconstituent	Oil extract	Crude Extract		
		Ethanol	Dichloromethane	petroleum ether
Phenolic compounds	+	+	+	−
Alkaloids	+	+	+	−
Flavonoids	+	+	−	−
Tannins	+	+	+	−
Terpenoids	+	+	−	−
Quinones	−	−	−	+
Saponins	+	+	−	−
Anthraquinones	+	−	−	−
Cardiac glycosides	−	−	+	+
Anthocyanins	−	−	−	−
Coumarins	+	+	−	−
Steroids	+	+	−	+

Observed + = Present − = Absent.

all crude extracts, the ethanol extract appeared to be relatively rich in secondary metabolites as shown from shown in (Table 1).

Phytochemical screening of the oil extract of the leaves of the *A. afra* plant revealed the presence of alkaloids, flavonoids, tannins, saponins, anthraquinones, terpenoids, coumarins, and steroids. In contrast, quinones, cardiac glycosides, and anthocyanins were not detected (Table 1). The result is in agreement with the findings of Gayathri VP [6], who reported, that phenolic compounds, coumarins, and flavonoids [5] [6] [16], and terpenoids [5] [6] [16] [31] were present. On the hand, the present finding differs from that of Gayathri *et al.* [6], who reported, that cardiac glycosides were absent. Differences in results might be related to the composition of essential oils from the leaves of *A. afra* varied from one geographical zone to another with consideration to the time of harvest [5].

3.2. Antibacterial Susceptibility Assay

3.2.1. Antibacterial Activity Leaves of *A. afra*

In this investigation, the antibacterial activities of leaves extract ethanol, dichloromethane, and petroleum ether extract were evaluated using the agar well diffusion method at a concentration of 25, 50, and 500 µg/mL as shown in Table 2 and Table 3. Among the test of the organism, the maximum average zone of inhibitions at 500 µg/mL concentration in gram-positive bacterial species was determined to be 15.00 ± 0.29 and 14.00 ± 0.58 mm for *S. aureus* and *E. faecalis* respectively. On the other hand, the maximum average inhibitions, at a similar concentration in gram-negative bacteria species were 14.67 ± 0.47 and 15.83 ± 0.60 mm for *K. pneumonia* and *E. coil* respectively. On the contrary, no zones of inhibition were observed in 25 and 50 µg/mL of petroleum ether extract against *K. pneumonia*. Dichloromethane extracted had no zones of inhibition at 25 µg/mL against *E. coli*.

The crude ethanol extract showed greater antibacterial activity against gram-positive bacterial test organisms (*S. aureus* and *E. faecalis*). The dichloromethane

Table 2. Antibacterial activities of crude extract of the leaves of *A. afra* against gram-positive bacteria.

		Concentration extracts Zone of inhibition in diameter (mm)				
Selected Bacteria	**Plant extracts**	**25 µg/L**	**50 µg/L**	**500 µg/L**	**(+) control**	**(−) control**
S. aureus	Ethanol	10.33 ± 0.17	11.33 ± 0.33	15.00 ± 0.29	24.50 ± 0.20	NA
	Dichloromethane	7.03 ± 0.16	9.33 ± 0.17	11.7 ± 0.33		
	petroleum ether	9.00 ± 0.00	10.50 ± 0.58	13.67 ± 0.47		
E. faecalis	Ethanol	9.47 ± 0.17	11.83 ± 0.17	14.00 ± 0.58	21.00 ± 0.00	NA
	Dichloromethane	8.33 ± 0.33	10.17 ± 0.17	11.33 ± 0.17		
	petroleum ether	7.35 ± 0.15	8.33 ± 0.88	10.17 ± 0.17		

Values are expressed as mean ± SD (n = 3). NA= no activity (+) control (Ciprofloxacin) and (−) control = negative control (DMSO).

and ethanol extract showed greater antibacterial activity against gram-negative bacterial test organisms (*E. coil* and *K. pneumonia*) respectively, when compared with other solvent fractions. This might be due to the higher concentration of bioactive secondary metabolites in these extracts (Table 1).

3.2.2. Antibacterial Activity of *A. afra* Essential Oil

The results indicated that essential oils applied with the same concentration have a variable antibacterial effect against *S. aureus, E. faecalis, E. coli*, and *K. pneumonia in vitro*. The essential oil was effective at all concentrations against all the test organisms with showed different inhibition zone. Among the tested bacteria, *S. aureus* (17.67 ± 0.44 mm) was the maximum average inhibitions compared to the other tested bacteria within the concentration of 50 µL of plant extract of essential oil. As depicted in Table 4, the moderate susceptible bacterium at 50 µL was *E. faecalis, Escherichia coli*, and *K. pneumonia* with of zone of inhibition (15.83 ± 0.44, 12.33 ± 0.17, and 10.17 ± 0.17 mm) respectively.

The essential oil extract showed greater antibacterial activity against gram- positive bacterial test organisms (*S. aureus* and *E. faecalis*). The result is in agreement

Table 3. Antibacterial activities of crude extract of the leaves of *A. afra* against gram-negative bacteria.

Selected Bacteria	Plant extracts	Concentration extracts Zone of inhibition in diameter (mm)				
		25 µg/L	50 µg/L	500 µg/L	(+) control	(−) control
K. pneumonia	Ethanol	9.67 ± 0.47	10.17 ± 0.17	14.67 ± 0.47	22.00 ± 0.00	NA
	Dichloromethane	NA	8.33 ± 0.33	10.33 ± 0.17		
	petroleum ether	NA	NA	7.67 ± 0.17		
E. coli	Ethanol	8.17 ± 0.17	11.33 ± 0.33	14.00 ± 0.00	28.25 ± 0.32	NA
	Dichloromethane	8.33 ± 0.88	11.83 ± 0.17	15.83 ± 0.60		
	petroleum ether	6.00 ± 0.00	8.00 ± 0.29	9.67 ± 0.17		

Values are expressed as mean ± SD (n = 3). NA = no activity (+) control (Ciprofloxacin) and (−) control = negative control (DMSO).

Table 4. *In vitro*, ınhibition results in antibacterial efficacy of *A. afra* essential oil on bacteria.

Selected Bacteria	Volume of essential oil poured on the wells in µL			(+) Control	(−) Control
	10	25	50		
S. aureus	15.83 ± 0.33	16.67 ± 0.73	17.67 ± 0.44	25.00 ± 0.00	NA
E. faecalis	12.00 ± 0.58	13.50 ± 0.29	15.83 ± 0.44	22.50 ± 0.50	NA
E. coli	9.33 ± 0.33	10.17 ± 0.17	12.33 ± 0.17	22.50 ± 0.50	NA
K. pneumonia	7.67 ± 0.17	8.50 ± 0.29	10.17 ± 0.17	22.25 ± 0.25	NA

Values are expressed as mean ± SD (n = 3). NA= no activity (+) control (Ciprofloxacin) and (−) control = negative control (DMSO).

with the findings of Suliman S *et al.* [7] and Liu, NQ *et al.* [34], who reported *E. faecalis* and *S. aureus* were inhibited. In addition, the result is in agreement with the Gayathri VP [6], Suliman S *et al.* [7], and Liu, NQ *et al.* [34] who reported *K. pneumonia* and *E. coil* were detected respectively.

At equal concentrations, the crude and oil extracts demonstrated stronger zone inhibition in gram-positive bacteria than gram-negative bacteria. This could be related to the crude and oil extract increased activity against gram-positive bacteria, as most plant crude and oil extracts were more active against gram-positive bacteria. This discrepancy could be explained by the difference in cell wall construction between gram-positive bacteria, which has a single layer, and gram-negative bacteria, which have a multi-layered and complicated structure.

In the majority of test bacteria, the essential oil and crude ethanol extract showed better activity. The number of bioactive metabolites and their synergetic effects may be linked to the increased activity of these extracts. On the other hand, the low activity of the dichloromethane and petroleum ether fractions could be attributed to the existence of fewer metabolites. As a result, plant extracts' overall antibacterial action could be attributed to the presence of concentrated bioactive components or the synergistic activity of two or more active metabolites [7].

4. Conclusion

Based on the findings of this study, it can be concluded that *A. afra* leaves and essential oil extracts have good antibacterial action against pathogen bacteria. This finding shows that *A. afra* leaves and essential oil extracts can effectively combat bacterial infections.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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