Determination Antimicrobial Activity Of Leaves Extracted By Various Solvents From *(Elephantopus Scaber* L.)

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Determination Antimicrobial Activity Of Leaves Extracted By Various Solvents From (*Elephantopus Scaber* L.)

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Abstract. Medicinal plants are used traditionally in the treatment of various kinds of diseases since time immemorial. The present study was carried out to evaluate the antimicrobial and antioxidant activities of leaves from *Elephantopus scaber* by using different solvents (ethyl alcohol, acetone and aqueous). The ethyl alcohol, acetone and aqueous extracts of leaves were subjected to phytochemical analyses, which revealed the presence of alkaloids, phenols, flavonoids, saponins, steroids, tannins, coumarins, quinones and glycosides in most of the selected of leaves useing different solvents. Ethyl alcohol ,acetone and aqueous extracts of leaves were investigated for in vitro antimicrobial activity against *Escherichia coli, Salmonella typhis, Klebsiella pneumonia, Streptococcus pyogenes, Staphylococcus aureus,* and *Candida Albican* by well diffusion method by using a different concentrations (20, 40, 60, 80, 100)mg/ml. Ethyl alcohol and acetone extract of leaves showed promising antimicrobial activity against all test organisms. Ethyl alcohol of leaves has the best activity against all test organisms. Found a high effective for ethyl alcohol extract of leaves compared with the other parameters, also it was recorded a high increase for ethyl alcohol of leaves compared with the acetone extract of leaves for a concentrations of (100, 20)mg/ml. The aqueous extract of the leaves has a weak activity against all test organisms and did not inhibit the growth of *Klebsiella pneumonia* for a concentrations of (100)mg/ml but at a concentrations of (20)mg/ml did not inhibit the growth of all test organisms. The Minimum inhibitory concentration (MIC) of leaves extracts by using different solvent were also determined against different test organisms. The MIC value of leaves extract ranged from 100 mg/ml to 500 mg/ml. The ethyl alcohol, acetone and aqueous extract of leaves were selected for possible antimicrobial activity. The antimicrobial activity was studied against six microorganisms namely *Escherichia coli, Salmonella typhis, Klebsiella pneumonia, Streptococcus pyogenes, Staphylococcus aureus,* and *Candida Albican*

1.Introduction

In many developing countries, traditional medicine is one of the primary healthcare systems [1,2]. Traditional African, Ayurvedic and Chinese systems of medicine are amongst the oldest known, and have undoubtedly influenced modern drug development and the isolation of novel compounds with therapeutic value [3]. Ayurvedic medicine is originated in India more than 3,000 years ago and remains one of the country's traditional health care systems. In recent decades, research has shown that plants produce a

diverse range of bioactive molecules for industrial interest, making them a rich source of different types of medicines and have shown a promising effect in therapeutics [4]. Aromatic and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment and inhibit bacterial or fungal growth [5]. Thus medicinal plants have been representing a rich source of antimicrobial agent [6].

The lectotype species of *Elephantopus* genus, family Asteraceae, consists of 32 species of centered in the Neotropics, Europe, Asia, Australia and Africa [7-13]. *Elephantopus scaber* Linn. is one of the medicinally important species of this genus. Hiradeve and Rangari (2014) [14] has recently reviewed the ethnomedical history of *E. scaber.* The whole plant, its various parts and the extracts of *E. scaber* have been used for the treatment of a number of diseases and also as antibiotic in many countries. Literature survey indicated the antimicrobial activity of the whole plant extracts of *E. scaber* in certain microorganisms. However the roots and the aerial parts individual antimicrobial and antifungal potential has not been explored till date. Hence the present study has been undertaken to explore the antimicrobial and antifungal activity of various extracts of the root and aerial part of *E. scaber* against gram positive, gram negative bacterial and fungal stains.

2. Materials and methods

2.1 Collection the plant

The selected *Elephantopus scaber L.* was washed thoroughly and separated leaves from plants then dried under shade. The dried plants material was grounded into fine powder). The powdered material was extracted by Soxhlet apparatus for 24 hours using different solvents (ethyl alcohol, acetone and aqueous). The solvent was then evaporated using Rotary evaporator at 4^0C for further use[8]

2.2 Qualitative tests: phytochemical screening

To detect the various phytochemicals present, preliminary phytochemical screening was carried out for leaves extracts. Qualitative phytochemical tests were performed for ethyl alcohol, acetone and aqueous extracts, to get the general idea regarding the nature of phytochemicals present. Plant extracts were subjected to standard phytochemical analysis to find the presence of different phytoconstituents such as alkaloids, flavanoids, steroids, saponins, glycos ides, phenolics and tannins [14][16].

*2.2.***1** *Test for Alkaloids (Mayer's test)*

To 0.5 gm of plant material, 5 ml of 1% aqueous Hydrochoric acid was added and stirred on a water bath and the water content was filtered. To 1 ml of filtrate Mayer's reagent was added. Appearance of buff coloured precipitate indicates presence of alkaloid [17].

2.2.**2** Test for Phenolic

To 1 mL of the plant e xtract, one drop of 5 % FeCl3 (w/v) will be added. Fo rmation of greenish precipit ate indicate the presence of phenols [16].

2.2.3 Test for Flavonoids (sodium hydroxide)

0.5 gm of powdered material is dissolved in water and then filtered. To the 2 ml of filtrate 10% aqueous sodium hydroxide is added to produce yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid is an indication for presence of flavonoids [17].

2.2.4 Test for Saponins (froth test)

1 gram of plant extract was boiled in 5 ml distilled water, and the contents were filtered. To the filtrate, about 3 ml of distilled water was added and after shaking it vigorously for 5 min frothing develops. Frothing which persists on warming is an indication for presence of saponins [17].

2.2.5Test for Steroids
2 ml of acetic acid was added in 0.2 grams of plant extract. After allowing the solution to cool in ice concentrated H_2SO_4 was added carefully. Colour development from violet to blue or bluish-green indicate the presence of a steroidal ring [17].

2.2.6Test for Tannins (Ferric chloride test)
One half gram (0.5 g) of the crude extract will be diss olved and add to a tube containing 20 mL of boiling dist illed water and then boiled for an ho ur. A few drops of ferric chloride will be added and allow to stand for prop er colour development. A blue-black colouration indic ate the presence of tannins [17].

2.2.7 Test for Coumarins
In a test tube, 1 g of each of the extracts were placed and covered with filter paper moistened with dilute sodium hydroxide (NaOH), then heated on water bath for a few minutes. The filter paper was examined under UV light, yellow fluorescence indi-cated the presence of coumarins [18].

2.2.8 Test for Quinones
About 0.5 g of plant extract was taken and added 1 ml of extract and 1 ml of con. H2SO4was added formation of red colour shows the presence of quinones [15].

2.2.9 Test for Glycosides (Bromine water test)
Plant extracts were treated with bromine water. Formation of yellow precipitate indicates the presence of glycosides [15].

2.3 Prepare the Antimicrobial Samples
Prior to antimicrobial assay stock concentration of 200mg/ml extract was prepared in 0.25% DMSO (Dimethyl sulfoxide), and was filtered through 0.45 micron cellulose acetate membrane filter (Sartorious). With the filtrate, further dilutions were made to get the concentrations of (100, 80, 60, 40 and 20) mg/ml, which were then used for antimicrobial assay.

2.4 Screening of antimicrobial activity of root against test organisms (well diffusion method)
The antimicrobial assay was performed by agar well diffusion method: Method established by National Committee for Clinical Laboratory Standard [8].
About 20 ml of nutrient agar medium was poured into the Petri plates and was left to solidify. To the solidified medium 100 µl of bacterial suspension was added and was spread uniformly with the glass spreader. Four wells were prepared in the plates with the help of a cup-borer (0.8 cm). Into the tow wells, 100 µl of the plant extract (test compound) was introduced and in one well 100 µl of 0.25% DMSO (negative control) was introduced. The plates were then incubated overnight at 37^{o}C. Antimicrobial activity was determined by measuring the diameter of the zone of inhibition. For each bacterial strain, a negative control is maintained where pure solvents are used instead of the extract [9]. The experiment was performed four times and the mean values were presented.
To evaluate the synergistic antimicrobial activity of root and leave in combination of tow, well diffusion method was followed. Four wells were prepared in the plates. Into tow opposite well each extract (50 µl +50 µl) was added and in another opposite wells individual extract was added. After incubation at 37^{o}C for 24 hours the observation were made. The zone of inhibitions formed by combination of extract was compared with the zone formed by individual extract.

2.5 Determination of MIC (Minimum Inhibitory Concentration) of plant extract by Microdilution Method

The lowest concentration or highest dilution of the plant extract that inhibits the visible growth of test microorganism is known as Minimum inhibitory concentration [10]. The standardization of the bacterial cell number or preparation of bacterial suspension is of critical importance for susceptibility testing and obtaining accurate minimum inhibitory concentration. The minimum inhibitory concentration assay was performed by using agar dilution method [10].

2.5.1 Agar dilution method

Different concentration of plant extract were prepared. Around 20ml of nutrient agar was prepared in each test tube and after autoclaving at 121^{o}C, 15 lb for 15 minutes, medium was allowed to cool at 45-50^{o}C, then different concentration of plant extract were added in the respective test tubes to make the final concentration of 1-5 mg/ml, which was then poured into the Petri plates after mixing properly. The plates were left to solidify. Standard inoculums of test organisms were prepared. Test microorganisms were spotted in the agar plates with the help of micropipette set at 1µl to deliver the spots [10]. Control experiment were also done to see the effect of antibiotic and solvent alone (without plant extract) on the growth of all the test organisms [11]. The Plates were incubated at 37^{o}C for 24hrs. The lowest concentration of the plant extracts that inhibits the growth of test microorganism was considered as the MIC of the extract [10].

3. Result and discussion

3.1 Phytochemical analysis

The analysis of phytochemical of leaves extract for (ethyl alcohol, acetone and aqueous) revealed the presence of various phytoconstituents. The results are presented in table (1).

Table 1: phytochemical analysis of leaves extracts for (ethyl alcohol, acetone and aqueous) solvents

Bioactive compounds	E. scaber leaves		
	W	A	E
Alkaloids	-	-	-
Phenols	+	+	+
Flavonoids	-	-	+
Saponins	-	-	+
Steroids	+	+	+
Tannins	+	+	-
Coumarins	-	+	+
Quinones	-	-	-
Glycosides	-	-	+

The analysis of phytochemical of leaves for ethyl alcohol revealed the presence of (Alkaloids, Phenols, Saponins, Steroids, glycosides and Coumarins). Bioactive compounds constituent (Phenols, Steroids, Tannins and Coumarins) were detected in the acetone. Ethyl alcohol and acetone contains many bioactive compounds compared with aqueous because of the ability possessed by alcohols dissolve bioactive compounds compared to other organic and non organic solvents [11].

3.2 Antimicrobial activity

The antimicrobial activity of leaves from *Elephantopus scaber L*. for (ethyl alcohol, acetone and aqueous) solvent was evaluated according to their zone of inhibition against different test organisms.

Table 2: Zone of inhibition of *E. scaber* extracts and standard antibiotics against four microorganisms

SN	Extr. code	Zone of Inhibition (mm)																			
		Escherichia coli					*S.aureus*					*S. pyogenes*					*S. tphis*				
		20	40	60	80	100	20	40	60	80	100	20	40	60	80	100	20	40	60	80	100
1	ET	4.3	7.4	12	15	17	4	9	16	18	20	5	10	12	14	16	4	9	14	16	18
2	AC	4	6	8	10	13	2.3	7	9	13	17	3	6	9	12	14	2.5	3	6	8	10
3	AQ	-	4	6	8	9	-	3.2	6.4	10	14	-	5	6.3	8	11	-	3	6	8	10
Positive and negative control																					
4	GE	28.25					27.5					28.5					27.3				
5	DM	0					0					0					0				

ET. Ethyl alcohol extract; AC: Acetone r extract; AQ: *A*queous extract;
GE: Gentamicin 100 mg/ml; DM: DMSO (0.25%);

The result representing antibacterial activity of ethyl alcohol, acetone, and aqueous extract of leaves of *E. scaber* against microorganisms is presented in Table 2. The highest activity of plant extract has been shown in ethyl alcohol extract and was found to be 20 mm diameter of zone of inhibition against *S.aureus* at the concentration of 100 mg/ml followed by 18 mm diameter of zone of inhibition against *S. tphis* then 17mm against *Escherichia coli* at concentration of 100 mg/ml then followed by 16 mm diameter of zone of inhibition against *S. pyogenes* concentration of 100 mg/ml. In comparison to Gentamicin at 100 mg/ml as shown in Table-2, ethyl alcohol extract of E. scaber leaves possess significant antibacterial activity at concentration of 100 mg/ml. Acetone extract of leaves of *E. scaber* possess highest zone of inhibition that is 17.00 mm diameter of zone of inhibition against *S.aureus* at 100 mg/ml concentration. followed by 14 mm diameter of zone of inhibition against *S. pyogenes* at concentration of 100 mg/ml, then showed zone of inhibition 13mm of *Escherichia coli* then 10mm against *S. tphis*. Aqueous extract showed a low activity against all microorganisms where gave 14 mm diameter of zone of inhibition against *S.aureus* at 100

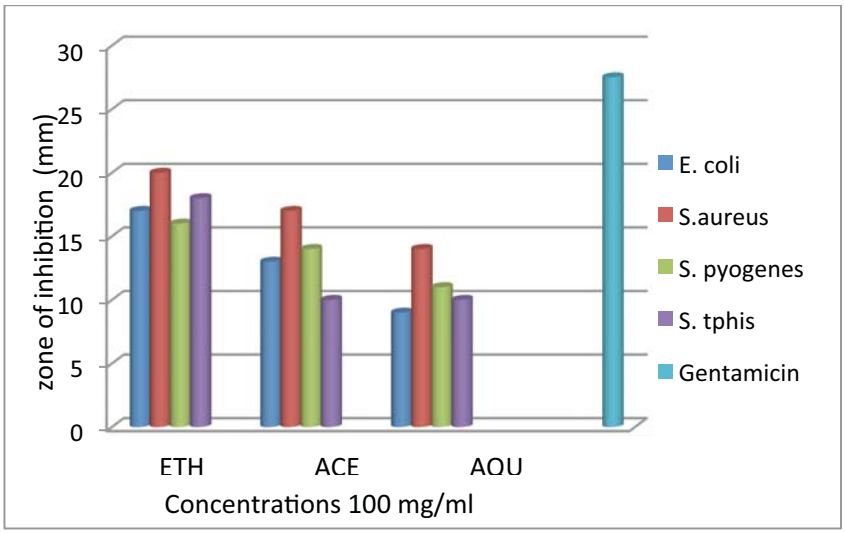
mg/ml concentration, followed by 11 mm diameter of zone of inhibition against *S. pyogenes* and 10mm against *S. tphis* then inhibition zone by 9mm against Escherichia coli at 100 mg/ml concentration.

Table 3: Zone of inhibition of *E. scaber* extracts and standard antibiotics against tow microorganisms

SN	Extr. code	Zone of Inhibition (mm)									
		K. pneumonia					*Candida Albican*				
		20	40	60	80	100	20	40	60	80	100
1	ET	-	-	-	-	3	4.5	7	10	12	14
2	AC	-	-	-	-	2.4	2	4	6	8	10
3	AQ	-	-	-	-	-	-	2	4	6.5	8
Positive and negative control											
4	GE	20					GF	22			
5	DM	0					0				

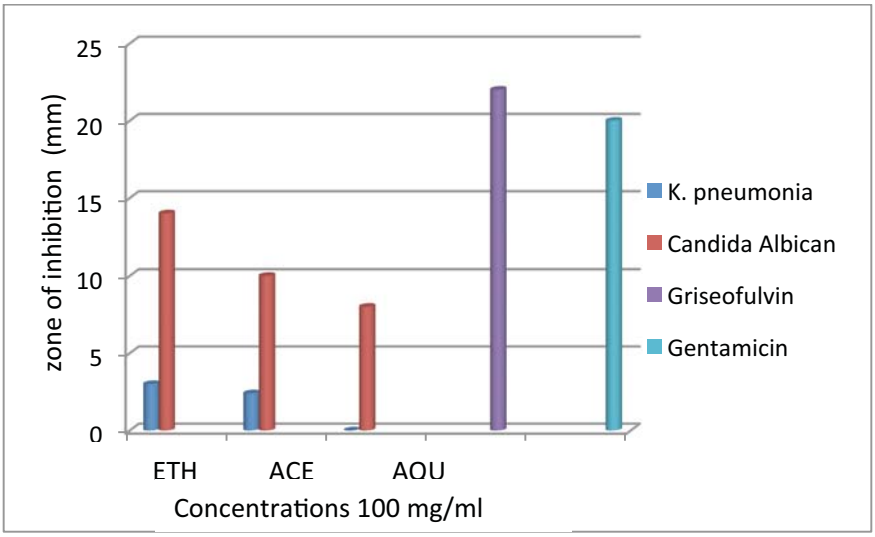
ET. Ethyl alcohol extract; AC: Acetone r extract; AQ: *A*queous extract;
GE: Gentamicin 100 mg/ml; DM: DMSO (0.25%); GF: Griseofulvin (100 µg/ml)

Finding indicated in Table-3 the Elephantopus scaber for roots extracts showed weak effective of *Klebsiella pneumoniae*. Where the leaves extract showed at the concentration of (100)mg/ml zone of inhibition (3)mm for ethyl alcohol and (2.4)mm for acetone but aqueous extract at all concentrations did not inhibit the growth of test for *Klebsiella pneumoniae.* Table (3) showed for leaves extracts the zone of inhibition against *Candida Albican* at concentration of (100)mg/ml the largest zone inhibition (14)mm was observed for ethyl alcohol extract followed acetone extract for the roots (10)mm, then aqueous extract for the roots (8)mm. All the result compared with Gentamicin 100 mg/ml as standard Figure (1, 2).



ETH. Ethyl alcohol extract; ACE: Acetone r extract; AQU: *A*queous extract

Figure 1: Antimicrobial activity of leaves extracts used (ethyl alcohol, acetone and aqueous) solvent against microorganisms compared with Gentamicin 100 mg/ml



ETH. Ethyl alcohol extract; ACE: Acetone r extract; AQU: *A*queous extract

Figure 2: Antimicrobial activity of leaves extracts used (ethyl alcohol, acetone and aqueous) solvent against microorganisms compared with Gentamicin and Griseofulvin 100 mg/ml

3.3 Minimum inhibitory concentration of E. scaber's leaves and standard antibiotics against Gram positive and Gram negative and Fungus organism.

Table (4) shows the Minimum inhibitory concentration of ethyl alcohol, acetone and aqueous extracts of *Elephanthopus scaber* for leaves. The MIC value of ethyl alcohol extract was found to be 100 mg/ml for *Escherichia coli , Salmonella typhimurium Streptococcus pyogenes, Staphylococcus aureus* and *Condida Albican*, and extracts exhibited an MIC of 300mg/ml against *Klebsiella pneumoniae*. In case of *Escherichia coli*, *Staphylococcus aureus* and *Condida Albican* the MIC value was 200 mg/ml, acetone extract inhibited the growth of *Streptococcus pyogenes* and *Salmonella typhimurium* at a concentration of 250 mg/ml, and inhibited the growth of *Klebsiella pneumoniae* at 500 mg/ml. The aqueous inhibited the growth of *Escherichia coli* and *Condida Albican* at a concentration of 250 mg/ml, the MIC value of aqueous extract of Elephanthopus scaber L. roots was found to be 300mg/ml for *Streptococcus pyogenes*, *Salmonella typhimurium*, *Staphylococcus aureus*, the aqueous extracts didn't had activity against *Klebsiella pneumoniae*.

Table 4: Minimum inhibitory concentration of roots from *E. scaber* and standard antibiotics against Gram positive and Gram negative and Fungus organism.

Minimal inhibition concentration (µg/ml)					
The microbial	**The extract**			**Standard Antibiotics**	
	ET	**AC**	**AQ**	**GE**	**CI**
Escherichia coli	100	200	250	25	20
Streptococcus pyogenes	100	250	300	50	25
Salmonella typhimurium	100	250	300	25	20
Staphylococcus aureus	100	200	300	50	25
Klebsiella pneumoniae	300	500		100	50
Fungus Condida Albican	100	200	250	**GR**	**NY**
				100	50

ET. Ethyl alcohol extract; AC: Acetone extract; AQ: *A*queous extract; GE: Gentamicin; GR: Griseofulvin; NY: Nystatin; CI: Ciprofloxacin

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