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Original Research Article

Antibacterial and antifungal activities of *Elephantopus scaber* Linn.

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Abstract

The present study was carried out with an objective to investigate the antimicrobial potentials of petroleum ether, diethyl ether, methanol and water extracts of the root and aerial part of *Elephantopus scaber* Linn. by determining the zone of inhibition and minimum inhibitory concentration (MIC) against various bacterial and fungal stains. Disc diffusion technique was used to determine in-vitro antibacterial and antifungal activities. In addition, minimum inhibitory concentration (MIC) was determined by broth dilution method. The different solvent extracts showed concentration dependent antibacterial activities against four gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus* and *Bacillus cereus*) and four gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Salmonella typhi*) bacteria. The MIC values against the tested Gram-positive bacteria ranged from 50 to 500 µg/ml and against Gram-negative bacteria from 100 to 500 µg/ml. The antifungal activities were found strong against three different fungi (*Candida albicans*, *Aspergillus niger* and *Aspergillus clavatus*). The MIC values of the extract against tested fungal stain were found in the range of 200-1000 µg/ml.

Keywords: antimicrobial, *Elephantopus scaber*, deoxyelephantopin.

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 and authentication of plant material

The whole plant of *E. scaber* was collected in the month of October-November 2012, from the

forest of Achanakmar, Chhattisgarh, India. The collected plant was authenticated by the Dr. G. P. Sinha, Scientist D, Ministry of environment and forests, Botanical survey of India, Allahabad, Uttar Pradesh. BSI/CRC/TECH/2014-15/ voucher specimen has been preserved in our laboratory for future reference.

2.2 Preparation of plant extract

The whole plant material was washed well with water to separate the adhering soil material. The collected entire plant containing roots and aerial parts, was dried in the shade. The total loss on drying was found to be 29.6%. After complete drying the roots and aerial parts were separated from each other. Dried roots consisted of thin, long roots attached to rhizomes while the aerial parts contained leaves, stems and flowers. 1 kg of the dried roots and rhizomes of *E. scaber* were comminuted to form coarse powder. The coarsely powdered crude drug material was first defatted with petroleum ether (40-60^0) in Soxhlet extractor. Defatted dried marc of the crude drug was further subjected to sequential extraction with diethyl ether and with methanol. 500 gm of coarse powder of root and rhizomes of *E. scaber* was macerated with water for 24 hrs (24x3). The same extraction procedure was followed for the extraction of aerial parts to afford petroleum ether, diethyl ether, methanol and water extracts. All the extracts obtained were concentrated and dried under reduced pressure using rotary evaporator.

2.3 Antimicrobial Activity

2.3.1 Test Microorganisms and Growth Media

Escherichia coli (MTCC 443), *Pseudomonas aeruginosa* (MTCC 1688), *Proteus vulgaris* (MTCC 8427), *Salmonella typhi* (MTCC 98), *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Micrococcus luteus* (MTCC 106), *Bacillus cereus* (MTCC 7278), and fungal strains *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 282), *Aspergillus clavatus* (MTCC 1323), were chosen based on their clinical and pharmacological importance. The bacterial and fungal cultures were incubated for 24 hours at 37°C on nutrient agar and potato dextrose agar (PDA) medium respectively, following refrigeration storage at 4°C. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37°C (bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C), whereas the yeasts and molds were grown in Sabouraud dextrose agar and PDA media, respectively, at 28°C. The stock cultures were maintained at 4°C.

2.3.2 Sample preparation

Antimicrobial activity of the extracts was tested at various concentrations ranging from 5.00-250.00 µg/ml. The roots and aerial part extracts of *E. scaber* from four selected solvents were weighed and dissolved in DMSO to prepare stock solution of 250.00 µg/ml concentrations. The same stock solution has been utilized to get desired concentrations of 5.00 µg/ml, 25.00 µg/ml, 50.00 µg/ml, 100.00 µg/ml and 250.00 µg/ml by the serial dilutions method.

2.3.3 Determination of zone of inhibition (ZOI)

The petroleum ether, diethyl ether, methanol and aqueous extracts of *E. scaber* roots and aerial parts were screened for antimicrobial activity by using the disc diffusion method [15,16]. In the assay each inoculums suspension (10^8 CFU/mL) was spread evenly over the entire nutrient agar surface by sterile collection swab. Then, discs having of diameter 6 mm were sterilized at 121^{0}C for 15 min and loaded with prepared positive control (ampicillin, 20 µg/ml) and extract solutions of *E. scaber* at various concentrations. The impregnated discs were dried for 3–5 min and dispensed onto the surface of the inoculated plates with flamed forceps. Each disc was pressed down firmly to ensure complete contact with nutrient agar surface. The discs were placed suitably apart and not relocated once contacted with the agar surface. The plates were then labeled and incubated at 37^{0}C for 24 hours for both bacteria and fungus. The results were measured and expressed in terms of zone of inhibition (ZI) of bacterial and fungal growth around each disc in millimeters.

2.3.4 Determination of Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration is determined by broth dilution method with some modification [17,18]. Serial dilutions were prepared in primary and secondary screening. In primary screening 1000 µg/ml, 500 µg/ml, and 250 µg/ml concentrations of the extracts were taken. The active extracts found in this primary screening were further tested in a second set of dilution against all microorganisms. The extract found active in primary screening were similarly diluted to obtain 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.250 µg/ml concentrations. The control tube containing no antibiotic is immediately sub cultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 ^{0}C overnight. The tubes are then incubated overnight. The MIC of the control organism is read to check the accuracy of the drug concentrations. The lowest concentration inhibiting growth of the organism is recorded as the MIC. The amount of growth from the control tube before incubation (which represents the original inoculums) is compared.

3. Result and discussion

3.1 Extractive values and preliminary phytochemical screening

The result of extractive values of petroleum ether, diethyl ether, methanol and aqueous extract of root and aerial part of *E. scaber* has been depicted in **table 1**.

Table 1: Extractive Values of different parts of *E. scaber* in different solvents

S. N.	Plant parts used	Solvent	Extract code	Extractive value (%)
1.	Underground part (Root)	Petroleum ether	RPE	4.73
		Diethyl ether	RDE	2.74
		Methanol	RME	1.84
		Water	RAQ	2.30
2	Aboveground part (Aerial part)	Petroleum ether	APL	1.70
		Diethyl ether	ADE	1.82
		Methanol	AME	3.91
		Water	AAQ	2.50

The various extracts obtained were subjected to preliminary phytochemical screening. The root extract of *E. scaber* shown the presence of various phytochemical constituent such as glycoside, steroid and terpenoid found in petroleum ether extract; alkaloid, steroid, flavanoid, terpenoid and saponin found in diethyl ether extract; carbohydrate, glycoside, alkaloid, steroid terpenoid and saponin found in methanolic extract; carbohydrate, alkaloid, flavonoid, terpenoid and saponin was found in aqueous extract. However the aerial part extracts of *E. scaber* shown the presence of glycoside and steroid in petroleum ether extract; steroid, flavonoid, terpenoid and saponin in diethyl ether extract; carbohydrate, flavonoid, tannin, terpenoid and saponin in methanolic extract and carbohydrate, alkaloid, tannin, terpenoid and saponin in aqueous extract.

Thin layer chromatographic study of the root extracts of *E. scaber* has shown three distinct spot in solvent system pet. ether: dichloromethane (6:4) by petroleum ether extract; three spot in benzene: pet. ether (9:1) by diethyl ether extract and six spot in hexane: ethyl acetate (8:2) by methanol extract. The aerial part extract of *E. scaber* has shown three spot in pet ether: chloroform (7:3) by pet ether extract; four spot in pet ether: ethyl acetate (6:4) by diethyl ether extract and six spot in hexane: ethyl acetate (8:2) by methanolic extract. Vanillin-H2SO4, phosphomolybdic acid, FeCl3-ethanol and iodine were used as the chromogenic reagents.

3.2 Antibacterial Activity

3.2.1 Zone of inhibition

The result representing antibacterial activity of petroleum ether, diethyl ether, methanol and aqueous extract of root of *E. scaber* against gram negative bacteria is presented in **Table 2**. The highest activity of plant extract has been shown in methanolic extract and was found to be 25.00 mm diameter of zone of inhibition against *E. coli* at the concentration of 250 µg/disc followed by 24.00 mm diameter of zone of inhibition against *P. aeruginosa* at concentration of 250 µg/disc. In comparison to ampicillin, chloramphenicol, ciprofloxacin and norfloxacin at 250 µg/disc as shown in **Table-2**, methanolic extract of *E. scaber* root possess significant antibacterial activity at 250 µg/disc. The antibacterial activity of *E. scaber* aerial part extracts against Gram negative organism. Petroleum ether extract of aerial part of *E. scaber* possess highest zone of inhibition that is 26.00 mm diameter of zone of inhibition against *P. vulgaris* at 250 µg/disc concentration.

Table 2: Zone of inhibition of *E. scaber* extracts and standard antibiotics against Gram negative organism

SN	Extr. code	Zone of Inhibition (mm)																			
		E. coli (MTCC 443)					*P. aeruginosa* (MTCC 1688)					*P. vulgaris* (MTCC 8427)					*S. typhi* (MTCC 98)				
		5	25	50	100	250	5	25	50	100	250	5	25	50	100	250	5	25	50	100	250
	Various extracts of *E. scaber*																				
1	RPE	-	10	12	17	19	-	8	12	15	20	-	11	14	18	22	-	10	12	16	19
2	RDE	-	12	13	15	18	-	9	13	17	19	-	9	12	16	18	-	11	13	17	21
3	RME	-	14	17	20	25	-	12	16	21	24	-	8	16	19	22	-	8	12	17	18
4	RAQ	-	14	15	17	19	-	14	15	17	19	-	12	11	14	15	-	14	17	18	21
5	APL	-	12	14	15	19	-	12	14	15	19	-	13	15	18	26	-	12	15	20	22
6	ADE	-	15	16	18	20	-	15	16	18	20	-	15	13	17	17	-	11	12	12	14
7	AME	-	14	15	17	20	-	14	15	17	20	-	12	13	15	18	-	10	11	12	15
8	AAQ	-	13	14	17	19	-	13	14	17	19	-	11	15	16	17	-	12	14	17	19
	Standard Antibiotics																				
9	AMP	14	15	16	19	23	14	15	15	18	24	15	19	20	24	28	12	15	19	24	27
10	CMP	14	17	23	23	23	14	17	18	19	23	13	16	22	25	29	10	13	18	21	25
11	CPF	20	23	28	28	28	20	23	24	26	27	17	20	23	28	31	16	19	22	25	29
12	NRP	22	25	26	27	29	18	19	21	23	26	13	19	24	27	29	15	17	21	24	28

Root extracts of *E. scaber* - RPE: Pet. ether extract; RDE: Diethyl ether extract; RME: Methanolic extract; RAQ: Aqueous extract

Aerial part extracts of *E. scaber* - APL: Pet ether extract; ADE: Diethyl ether extract; AME: Methanolic extract; AQU: Aqueous extract

Standard antibiotics-AMP: Ampicillin; CMP: Chloramphenicol; CPF: Ciprofloxacin; NRP: Norfloxacin. (-) represents the no zone of inhibition.

In comparison to standard drugs, Methanolic extract of root possess significant antibacterial properties against the Gram negative *E.coli* and *P.aeruginosa* at concentration of 250 µg/disc. Whereas the petroleum ether, methanolic and aqueous extract of *E. scaber* root represents significant antibacterial activity against Gram positive *B. cereus* and *M. Lutues* respectively.

Aqueous extract of root possess significant antifungal effect against *C. albicans* and *A. clavatus* at concentration of 250 µg/ml.

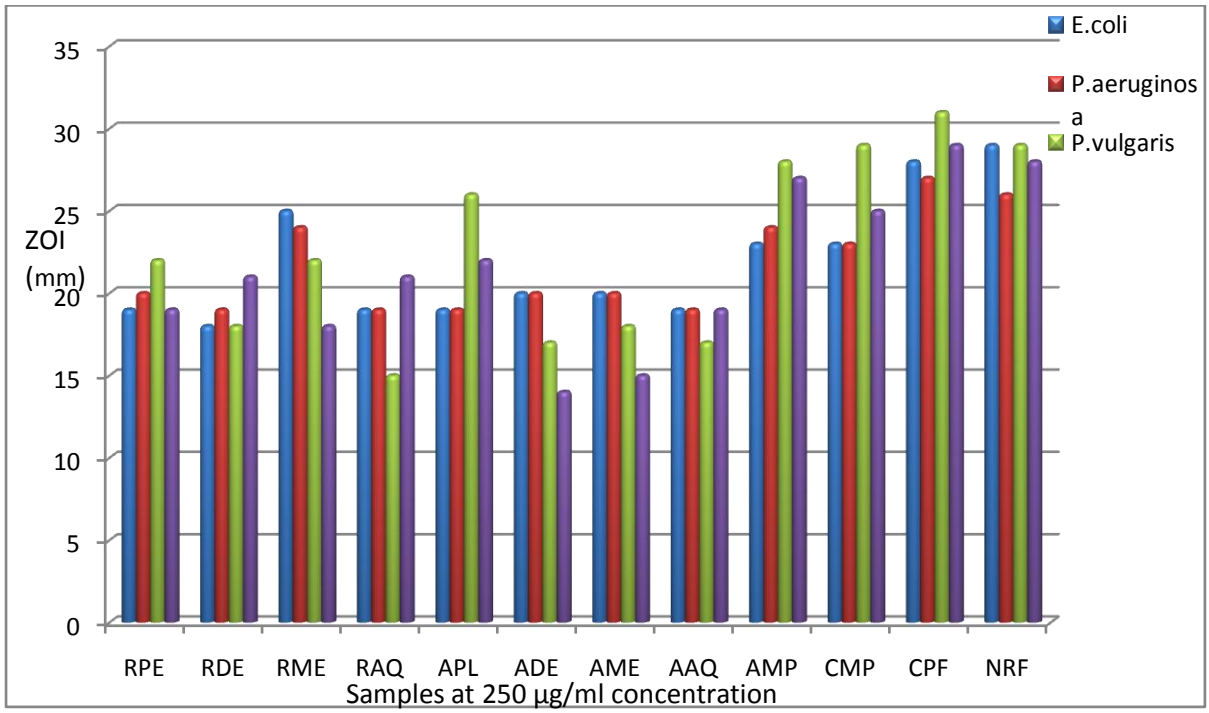

Figure 1: Graphical representation of zone of inhibition of *E. scaber* root and aerial part extract against Gram negative organism at 250 µg/ml concentration.

Against Gram positive organism, the methanolic and aqueous extract of root possess 21.00 mm and 20.00 mm diameter of zone of inhibition respectively against *M. Lutues* at concentration of 250 µg/disc. However petroleum ether extract of root possess 20.00 mm diameter of zone of inhibition against *B. cereus* at concentration of 250 µg/disc. In comparison to standard drugs, petroleum ether, methanolic and aqueous extract of *E. scaber* root represents significant antibacterial activity. Whereas the petroleum ether and methanolic extract of aerial part possess same zone of inhibition that is 21.00 mm of diameter against *S. aureus.* The diethyl ether extract of aerial part possess 20.00 mm diameter of zone of inhibition against *B. cereus* (**Table-3**).

Table 3: Zone of inhibition of *E. scaber* extracts and standard antibiotics against Gram positive organism

SN	Extr. Code	Zone of Inhibition (mm)																			
		B. subtillis MTCC 441					*S. aureus* MTCC 96					*M. lutues* MTCC 106					*B. cereus* MTCC 7278				
		5	25	50	100	250	5	25	50	100	250	5	25	50	100	250	5	25	50	100	250
Various extracts of *E. scaber*																					
1	RPE	-	10	14	17	18	-	12	14	16	18	-	14	16	17	18	-	10	13	15	20
2	RDE	-	12	13	16	19	-	13	15	17	19	-	11	13	15	17	-	11	12	15	18
3	RME	-	12	14	15	17	-	11	14	16	19	-	14	16	18	21	-	12	13	15	17
4	RAQ	-	12	13	17	18	-	11	14	15	18	-	12	15	17	20	-	11	12	16	17
5	APL	-	10	13	14	17	-	12	14	18	21	-	10	12	14	15	-	12	14	15	19
6	ADE	-	11	13	15	16	-	11	14	16	19	-	15	16	17	18	-	12	15	16	20
7	AME	-	11	14	16	19	-	12	14	16	21	-	12	14	16	18	-	12	14	15	19
8	AAQ	-	10	12	14	17	-	12	14	15	18	-	12	13	15	17	-	10	15	17	18
Standard Antibiotics																					
9	AMP	11	14	16	18	24	10	13	14	16	24	12	15	16	19	24	13	15	17	20	28
10	CMP	10	13	19	20	24	12	14	19	20	25	11	17	18	20	26	12	16	16	19	25
11	CPF	16	19	21	21	25	17	19	21	22	25	14	15	16	18	25	14	15	18	21	27
12	NRP	18	19	20	21	24	19	22	25	26	28	13	15	19	20	25	14	18	21	24	28

Petroleum ether extract of aerial part of *E. scaber* possess significant antibacterial effect against gram negative *P. vulgaris* and Gram positive *S. aureus* at 250 µg/disc concentration. Whereas the petroleum ether and methanolic extract of aerial part possess significant antibacterial activity against *S. aureus.* While the diethyl ether extract of aerial part possess antibacterial activity against *B. cereus.*

Methanolic extract and petroleum ether extract of aerial part represent significant antifungal activity against *C. albicans* and *A. clavatus* at concentration of 250 µg/ml.

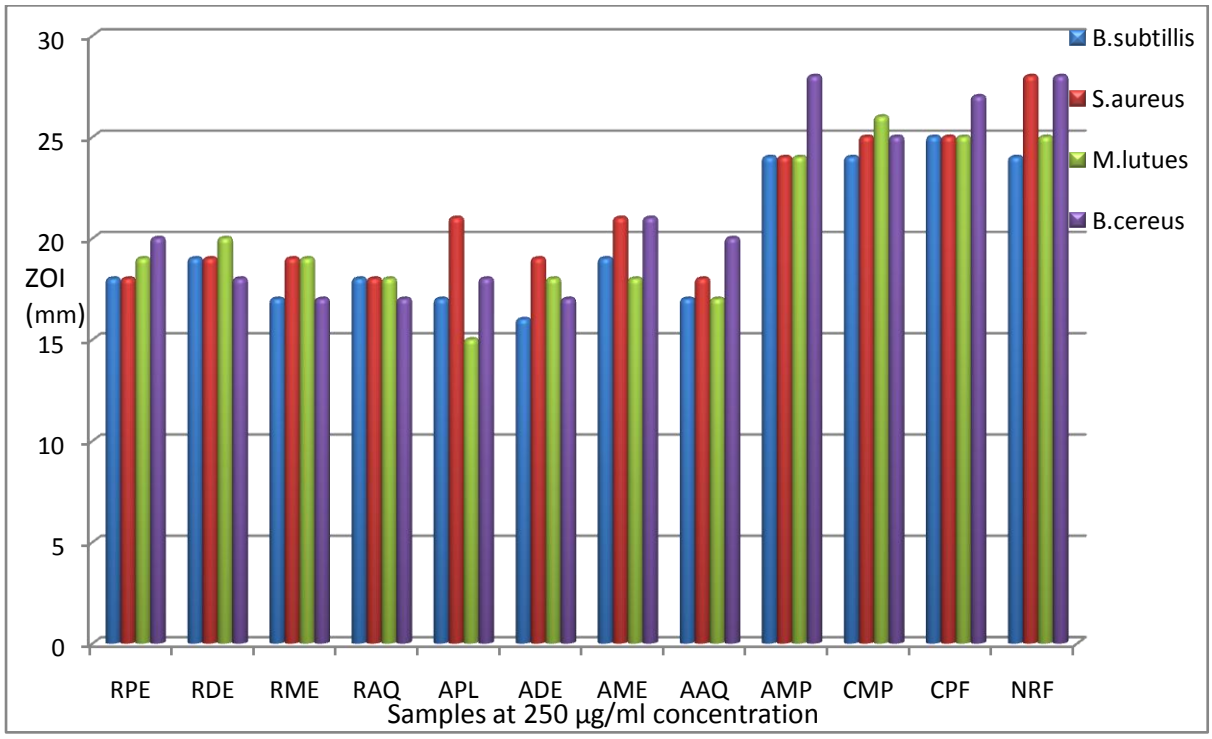

Figure 2: Graphical representation of zone of inhibition of *E. scaber* root and aerial part extract against Gram positive organism at 250 µg/ml concentration

3.2.2 Minimum Inhibitory Concentration (MIC) Measurement

The Minimum inhibitory concentration (MIC) values of the extract against tested both gram positive and gram negative bacteria were shown in **Table-4**. The MIC values were found in the range of 50-500 µg/ml against the tested organisms. The MIC values against the tested gram-positive bacteria ranged from 50 to 500 µg/ml and against gram-negative bacteria from 100 to 500 µg/ml. Antibacterial potency of plant extract against these bacteria expressed in MIC indicated the plant extract is more effective against gram-positive at lower concentration than that against gram-negative bacteria.

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

		Minimal inhibition concentration (µg/ml)							
SN	**Extract Code**	***E. coli* MTCC 443**	***P. aeruginosa* MTCC 1688**	***P. vulgaris* MTCC 8427**	***S. typhi* MTCC 98**	***B. subtilis* MTCC 441**	***S. aureus* MTCC 96**	***M. luteus* MTCC 106**	***B. cereus* MTCC 7278**
Various extracts of *E. scaber*									
1	RPE	200	250	500	250	100	100	250	200
2	RDE	250	250	250	250	500	500	500	250
3	RME	100	200	500	250	100	200	250	200
4	RAQ	200	200	200	200	200	250	500	500
5	APL	250	250	200	500	250	250	100	200
6	ADE	100	100	500	500	50	100	200	100
7	AME	100	200	500	200	100	100	500	500
8	AAQ	200	250	250	250	100	100	200	100
Standard Antibiotics									
9	AMP	100	100	100	100	200	250	100	100
10	CMP	50	50	100	50	50	50	100	50
11	CPF	25	25	25	25	25	50	25	25
12	NRF	10	10	25	10	25	10	10	10

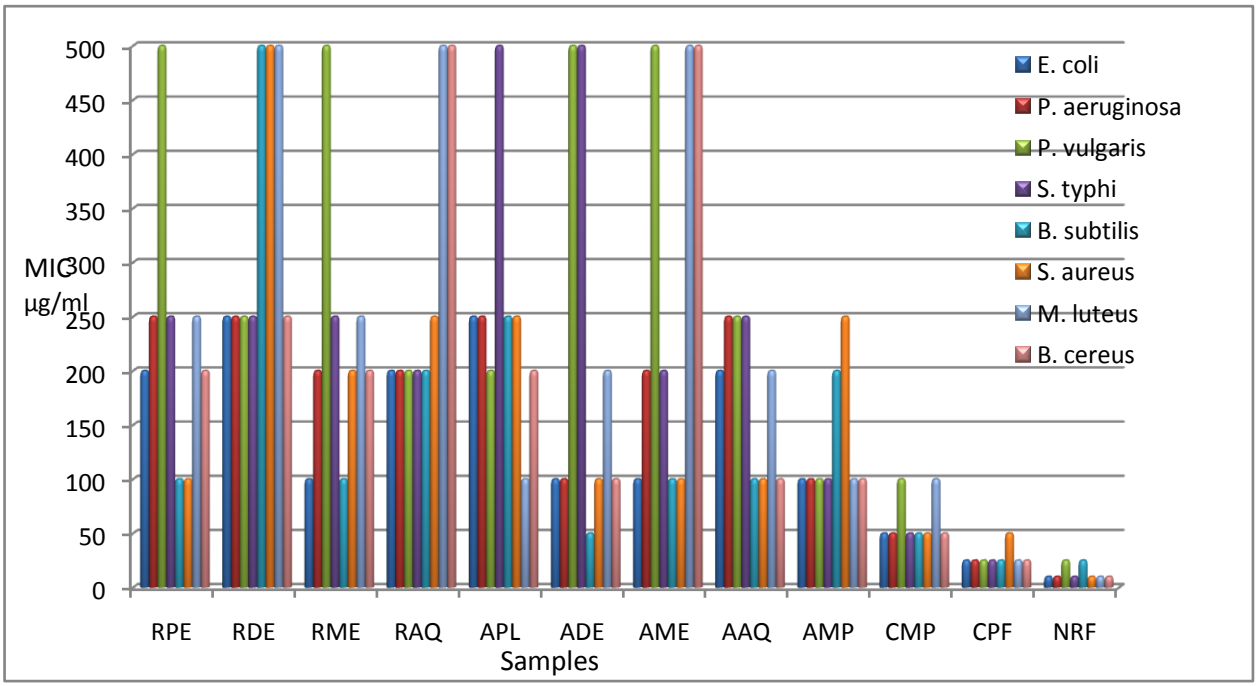

Figure 3: Graphical representation of MIC of *E. scaber* root and aerial part extract against Gram positive and Gram negative organism.

3.3 Antifungal Activity

The antifungal activity of different extract of *E. scaber* against fungal strains *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 282), *Aspergillus clavatus* (MTCC 1323) is reported in **Table-5**. Aqueous extract of root and methanolic extract of aerial part possess 27.00 mm diameter of zone of inhibition against *C. albicans* and *A. clavatus* at concentration of 250 µg/ml respectively. Petroleum ether extract of aerial part possess 26.00 mm diameter of zone of inhibition against *C. albicans* fallowed by 25.00 mm diameter of zone of inhibition against *A. clavatus.* However aqueous extract of aerial part possess 25.00 mm diameter of zone of inhibition against all three tested fungal stain.

Table-5: Antifungal activity of different extract of *E. scaber*

SN	CODE NO.	Zone of Inhibition (mm)														
		C. albicans MTCC227					*A. niger* MTCC 282					*A. clavatus* MTCC 1323				
		5	25	50	100	250	5	25	50	100	250	5	25	50	100	250
1	RPE	-	16	20	22	24	-	18	18	21	22	-	21	21	23	24
2	RDE	-	17	18	22	23	-	22	22	24	24	-	18	18	19	22
3	RME	-	19	21	22	23	-	18	20	21	22	-	18	19	21	22
4	RAQ	-	20	22	25	27	-	21	21	23	24	-	18	20	22	22
5	APL	-	22	23	25	26	-	18	18	19	22	-	21	22	23	25
6	ADE	-	18	20	22	23	-	18	19	21	22	-	19	21	22	23
7	AME	-	18	19	22	24	-	18	20	22	22	-	21	23	25	27
8	AAQ	-	21	22	23	25	-	21	22	23	25	-	23	23	25	25
Standard Antibiotics																
9	GSF	17	20	22	26	28	19	23	25	25	28	18	21	22	23	27
10	NYT	19	20	21	25	27	18	19	24	29	29	18	21	24	25	26

Root extracts of *E. scaber* - RPE: Pet. ether extract; RDE: Diethyl ether extract; RME: Methanolic extract; RAQ: Aqueous extract
Aerial part extracts of *E. scaber* - APL: Pet ether extract; ADE: Diethyl ether extract; AME: Methanolic extract; AQU: Aqueous extract
Standard antibiotics-GSF: Griseofulvin; NYT: Nystatin. (-) represents the no zone of inhibition.

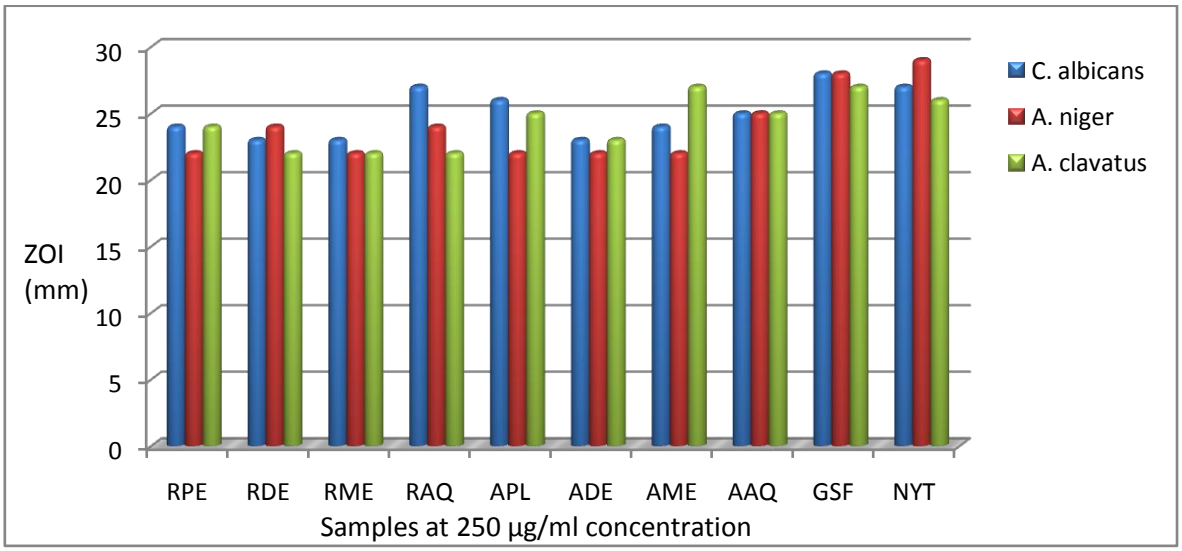

Figure 4: Graphical representation of zone of inhibition of *E. scaber* and standard drugs against fungal stains.

The Minimum inhibitory concentration (MIC) values of the extract against tested fungal stain were shown in **Table-6**. The MIC values were found in the range of 200-1000 µg/ml against the tested organisms.

Table-6: Minimal inhibition concentration of *E. scaber* extracts and standard antibiotics against fungal stains.

Minimal inhibition concentration (µg/ml)				
SN	**Extract Code**	***C. albicans***	***A. niger***	***A. clavatus***
		MTCC 227	**MTCC 282**	**MTCC 1323**
Various extracts of *E. scaber*				
1	RPE	500	1000	500
2	RDE	200	1000	250
3	RME	1000	500	1000
4	RAQ	200	500	500
5	APL	200	>1000	>1000
6	ADE	500	500	500
7	AME	1000	>1000	200
8	AAQ	200	250	250
Standard Antibiotics				
9	NYT	100	100	100
10	GSF	500	100	100

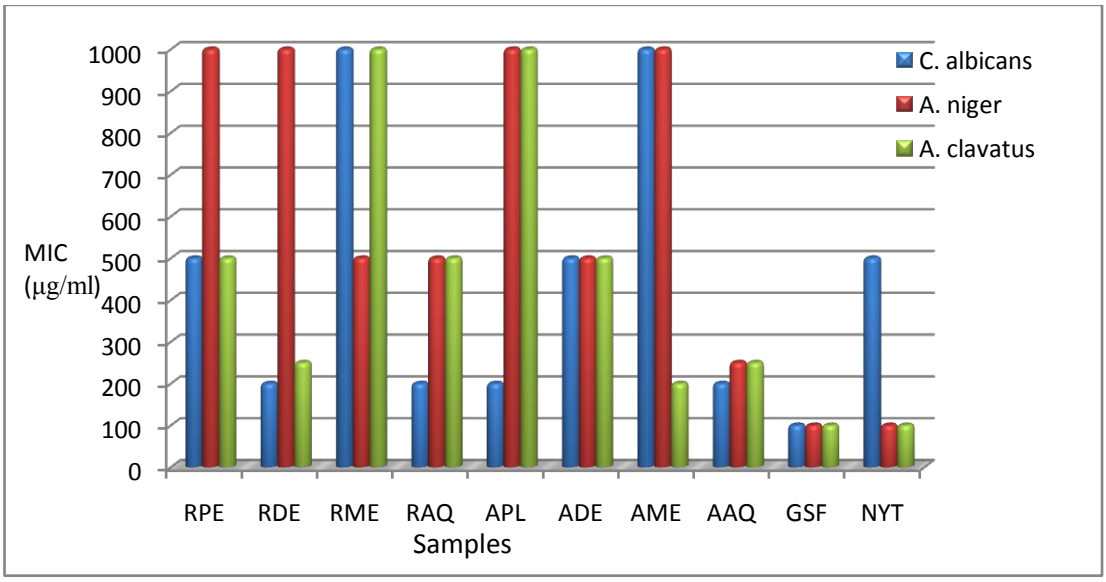

Figure 5: Graphical representation of zone of inhibition of *E. scaber* and standard drugs against fungal stains.

4. Conclusion

Various extracts of the roots and aerial parts of *E. scaber* has shown significant antibacterial and antifungal activity against the gram positive, gram negative and fungal strains in concentration dependent manner. In cases of gram negative and fungal strains the activity has been found to more significant than that of standards. The results of the antimicrobial and antifungal studies validate the ethnomedical utility of *E. scaber* in various countries for the treatment of infectious diseases.

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