ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 5, Issue, 03, pp. 3852-3859, March, 2015

International Journal of
DEVELOPMENT RESEARCH

Full Length Research Article

ANTIMICROBIAL AND ANTIFUNGAL ACTIVITIES OF *ZEHNERIA SCABRA* (L.F.) SOND AGAINST HUMAN PATHOGENS

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ARTICLE INFO

Article History:
Received 27th December, 2014
Received in revised form
24th January, 2015
Accepted 04th February, 2015
Published online 31st March, 2015

Key words:
Zehneia scabra,
Antibacterial,
Antifungal

ABSTRACT

Solvent extracts of Ethanol obtained from the tubers of *Zehneria scabra* were screened for antibacterial and antifungal activities. The extracts displayed wider spectrum of antimicrobial activity.

INTRODUCTION

Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides, etc., which have been found in vitro to have antimicrobial properties (Dahanukar *et al.,* 2000; Cowan, 1999). Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action (Runyoro *et al.,* 2006; Shahidi, 2004). In last five decades, these plants have been extensively studied by advanced scientific techniques and reported for various medicinal properties viz, anticancer activity, antibacterial activity, antifungal activity, antidiabetic activity, antioxidant activity, hepatoprotective activity, haemolytic activity, larvicidal activity and anti-inflammatory activity (Shoeb, 2006; Riaz *et al.,* 2008). Since not much work has been carried out on the antimicrobial and antifungal activity of the tubers of *Z. scabra*, hence the current study is undertaken. *Z. scabra* (L.F.) Sond (Cucurbitaceae) is a vine having ovate leaf, tendrils simple, glabrescent, base truncate, margin denticulate, apex acuminate, flowers dioecious, umbellate racemes, corolla greenish white, petals ovate, fruit ovoid, glabrous and apically beaked. *Z. scabra* tubers are valued article of medicinal importance. Being a climbing or trailing herb, it can go up to 10 m in length. Stems become woody with corky-ridged bark as they grow old (Matthew, 1982).

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Medicinal Properties

Z.scabra has enormous ethnobotanical value, as used by tribes for various treatments such as stomach pain, fever and skin diseases etc. It acts as an important medicine for livestock in various ailments. Fruits are reported to cure stomachache. Tribal people used the root of *Z. scabra* to hang in front of their house believing that it will prevent the entry of disease causing pathogens. Root of the plant is used with milk in fever and diarrhoea (Kirtikar *et al.,* 1975; Anand *et al.,* 2011). In Gingee hills, the tubers of *Z. scabra* are ground into powder form with bark of *Syzygium cuminii*, leaf of *Gymnema sylvestre* and leaf of *Nilgirianthu sciliatus* and powdered and is orally taken with honey to cure snake bite and also to cure diabetes (Thamacin and John Britto, 2014). *Z. scabra* is grown around the house to keep away the snakes. Traditionally in Ethiopia the flowers of the plant have reportedly been used for topical treatment of alopecia, wound and eczema along with other herbals mixed together (Messele *et al.,* 2004). Additionally, the leaves (Gedif *et al.,* 2003), fruit, and flower have been used for the treatment of abdominal colic in decoction of water and taken orally (Woldegerima *et al.,* 2004).

Preliminary Phytochemical tests in the Aqueous, Ethanolic and Chloroform Extracts

Plant	Part used	Phytoconstituents	Aqueous	Ethanolic	Chloroform
Zehneria scabra	tuber	Phenol	+	+	+
		Steroids	+	+	-
		Tannins	-	-	+
		Flavanoids	-	-	+
		Alkaloids	-	-	-
		Saponins	-	-	
		Glycosides	+	+	-
		Proteins	+	+	+
		Amino acids	+	+	+

(+) = Present, **(-)** = Absent

Disc Diffusion Method for Ethanoland Aqueous extracts of tubers in *Zehneriascabra*

S.No.	Bacterial Organisms	Concentration of Ethanol solvent				Concentration of Aqueous solvent		
		Control*	6.25 mg/disc	7.5 mg/disc	8.75 mg/disc	6.25 mg/disc	7.5 g/disc	8.75 mg/disc
		Zone of Inhibition (mm)				Zone of Inhibition (mm)		
1	*E. coli*	18	-	7	-	7	7	8
2	*V. cholerae*	18	-	-	-	-	-	-
3	*E. aerogenes*	16	-	8	-	-	-	-
4	*K. pneumoniae*	15	-	-	18	-	-	-
5	*S. marcescens*	18	-	-	9	-	-	-
6	*S. paratyphi*	19	-	7	-	-	-	-
7	*P. aeruginosa*	20	7	-	-	-	7	7
8	*S. aureus*	14	-	-	-	-	-	-
9	*P. mirabilis*	16	7		12	-	8	8
10	*P.vulgaris*	15	7	7	-	-	-	-
11	*B. cereus*	14	-	-	-	7	-	-
12	*B. subtilis*	16	-	15	15	-	-	8
13	*S. pneumonie*	15	-	10	-	-	7	7

* Streptomycin 30 µg

Disc Diffusion Method for Ethanol extracts of tubers of *Zehneria scabra*

S.No.	Fungal Organisms	Concentration of Ethanol Solvent						
		Control*	6.25 mg/disc	7.5 mg/disc	8.75 mg/disc	10 mg/disc	11.25 mg/disc	12.5 mg/disc
		Zone of Inhibition (mm)						
1	*A. niger*	19	9	8	9	8	8	8
2	*A. flavus*	18	9	8	10	8	9	8
3	*A. fumigatus*	18	9	10	10	9	8	8
4	*M. indicus*	17	9	9	10	8	9	9
5	*C. albicans*	19	8	6	7	7	7	7

* Nystatin 50 µg

The traditional use of the leaves of this plant for the treatment of diarrhoea is also reported in rural central Ethiopia and Burundi besides its use for skin reaction (Woldegerima *et al.,* 2004). Scientific works to testify to any of the claims are almost non-existent except a few studies that conducted antimicrobial activity test for the various extracts of the dried, powdered leaves of *Z. scabra*.

MATERIALS AND METHODS

Plant Material

The tuber of *Zehneria scabra* was collected from Gingee hills, Villupuram, Tamilnadu during January, 2014. Taxonomic identification of these plants was carried out by John Britto and the voucher specimens were deposited at Rapinat Herbarium (RHT) St. Joseph's College of Tiruchirappalli (RHT65356). Dried ground leaves of 50grams were extracted in Soxhlet apparatus in 300 ml of ethanol and water. The process was run for 48 hrs after which the sample was concentrated using rotary evaporator and freeze dried to powdered form. The freeze dried extracts were weighed and kept in labeled sterile specimen bottles.

Test Organisms

The test pathogens used for screening the efficacy of plant extracts were *E. coli* (MTCC # 119), *Vibrio cholera* (ATCC # 14104), *Enterobacter aerogenes* (MTCC # 2990), *Klebsiella pneumoniae* (MTCC # 3040), *Serratia marcescens* (MTCC # 2645), *Salmonella paratyphi* (MTCC # 734), *Pseudomonas aeruginosa* (MTCC # 2474), *Staphylococcus aureus* (MTCC # 3163), *Proteus mirabilis* (MTCC # 1429), *Proteus vulgaris* (MTCC # 1771), *Bacillus subtilis* (MTCC # 441), *Bacillus cereus* (ATCC # 4342), *Streptococous pneumoniae* (ATCC # 7066); *Aspergillus niger* (MTCC # 2612), *A. flavus* (MTCC # 2813), *A. fumigatus* (MTCC # 2584), *Mucor indicus* (MTCC # 3318), *Candida albicans* (MTCC # 1637) (Table 1, 2).

Disc Diffusion Assay

The freeze dried extract was reconstituted with DMSO to obtain a stock solution of 100 mg/ml, 50 mg/ml, 25 mg/ml,

and 12.5 mg/ml were prepared. Overnight broth culture of the respective bacterial strains was adjusted to turbidity equivalent to 0.5 McFarland standards. (0.2 ml culture of the organisms was dispensed into 20 ml sterile nutrient broth and incubated for 24 hrs and standardized at 1.5×10^6 CFU/ml by adjusting the optical density to 0.1at 600nm PERKIN-ELMER UV-spectrophotometer). For fungus the 24 hr overnight culture in Potato Dextrose Broth was standardized 5.0×10^6 spores/ml by adjusting the optical density to 1.0 at 530nm PERKIN-ELMER UV- spectrophotometer). For MIC both bacterial and fungal inocula were agitated for 15 s with a Vortex mixer and were diluted 1:100 using sterile saline (0.9%) to get a concentration of 1.5×10^8 CFU/ml and 1.0×10^8 spores/ml respectively. Nutrient agar (Hi Media Laboratories Pvt. Ltd. Mumbai) plates were swabbed using sterile cotton swabs with the adjusted broth culture of the respective bacterial strains. Discs of 6 mm were punched from Whatmann No.1 filter paper. Up to 10µl of each concentration of the extract were respectively introduced in the discs using sterile automatic pipettes. The discs were allowed to dry at room temperature for 2 hrs and were placed at equidistance in each of the plates using a sterile forceps. The plates were incubated to 37◦C for 24 h. The control antibiotic Streptomycin (30µg) (Hi Media Laboratories Pvt. Ltd. Mumbai) was used. Diameters of the inhibition zones were measured. The antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the plant extract.

RESULTS AND DISCUSSION

Preliminary Phytochemical Study

The preliminary triphytochemical screening (aqueous, ethanolic and chloroform) of *Zehneria scabra* (tuber) showed the presence of relatively very less amount of the secondary metabolites in the screening of the extracts. Phenol, proteins and amino acids were present in all the extracts and while steroids and glycosides were observed in only aqueous and ethanolic extracts. Only chloroform extracts showed the presence of tannins and flavanoids.

Antimicrobial Activity

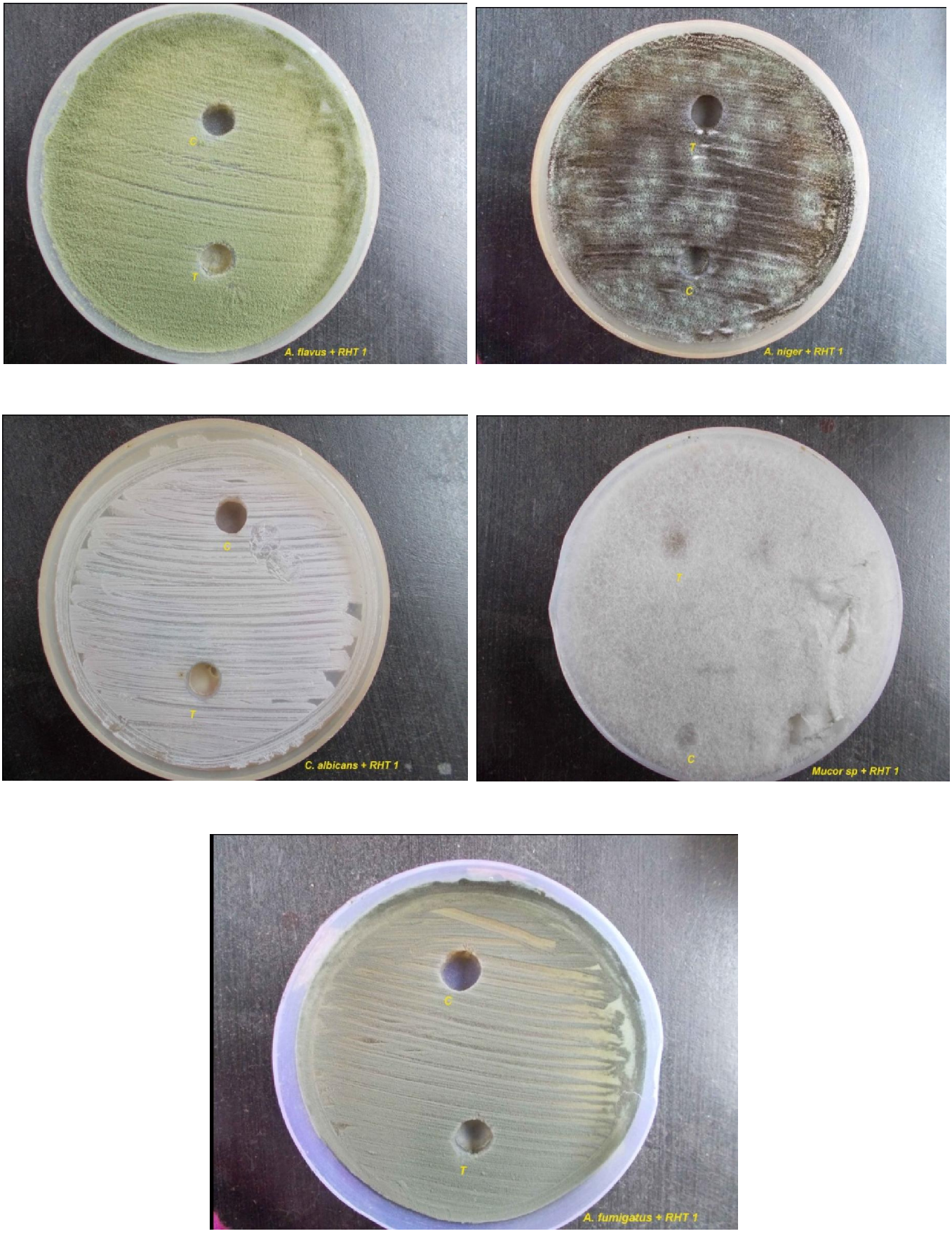
The ethanolic extract of *Zehneria scabra* (tuber) showed antimicrobial activity against 11 microorganisms. *Z. scabra* (tuber) showed strong inhibition against *K. pneumoniae* (18 mm), *S. paratyphi* (18 mm), *B. subtilis* (15 mm), and *P. mirabilis* (12 mm) and while minimum inhibition zones were against *S. pneumoniae* (10 mm), *S. marcescens* (9 mm), *S. aureus* (8 mm), *E. aerogenes* (8 mm), *E. coli* (7 mm), *P. aeruginosa* (7 mm) and *P. vulgaris* (7 mm). Among them, *B. subtilis, S. aureus* and *S. pneumoniae* were G^{+ve} bacteria and *K. pneumoniae, P. mirabilis, S. paratyphi, S. marcescens, E. aerogenes, E. coli, P. aeruginosa* and *P. vulgaris* were G^{-ve} bacteria. G^{-ve} bacteria has shown more number of inhibition zones than G^{+ve} bacteria in this plant. The aqueous extract of *Z. scabra* (tuber) showed antimicrobial activity against only two microorganisms. Both the microorganisms have shown inhibition zones to minimum level *E. coli* (7 mm) and *B. cereus* (7 mm). The former is a G^{-ve} bacteria and the latter is a G^{+ve} bacteria. Other bacteria tested for the antimicrobial activity has shown zero inhibition zones.

Bruck (2004) found that the root of *Z. scabra* exhibited antimicrobial activities against one of the most common bacterial pathogens, namely *S. aureus*. A similar result was also observed by Gelana (2011) that the root extract of *Z. scabra* in ethyl acetate showed the highest inhibition diameter against *S. aureus* (22.6±0.33mm) and in acetone the inhibition diameter of 19.3±0.33 against *S. aureus.* He found that chloroform showed no inhibition zones against the test microorganisms. Apart from these findings, no other works on antimicrobial activities has been done on the root/tuber extracts until now. The findings of the others and the present findings of ethanolic extract of *S. aureus* (8 mm) on the root/tuber extract of *Z. scabra* prove that the study plant was most susceptible against *S. aureus*. Moreover, the present findings have shown more number of inhibition zones against microorganisms both in ethanolic and aqueous extracts. Sood *et al.,* (2012) studied the most commonly available and readily consumed plants of Cucurbitaceae in India and screened for antimicrobial activity.

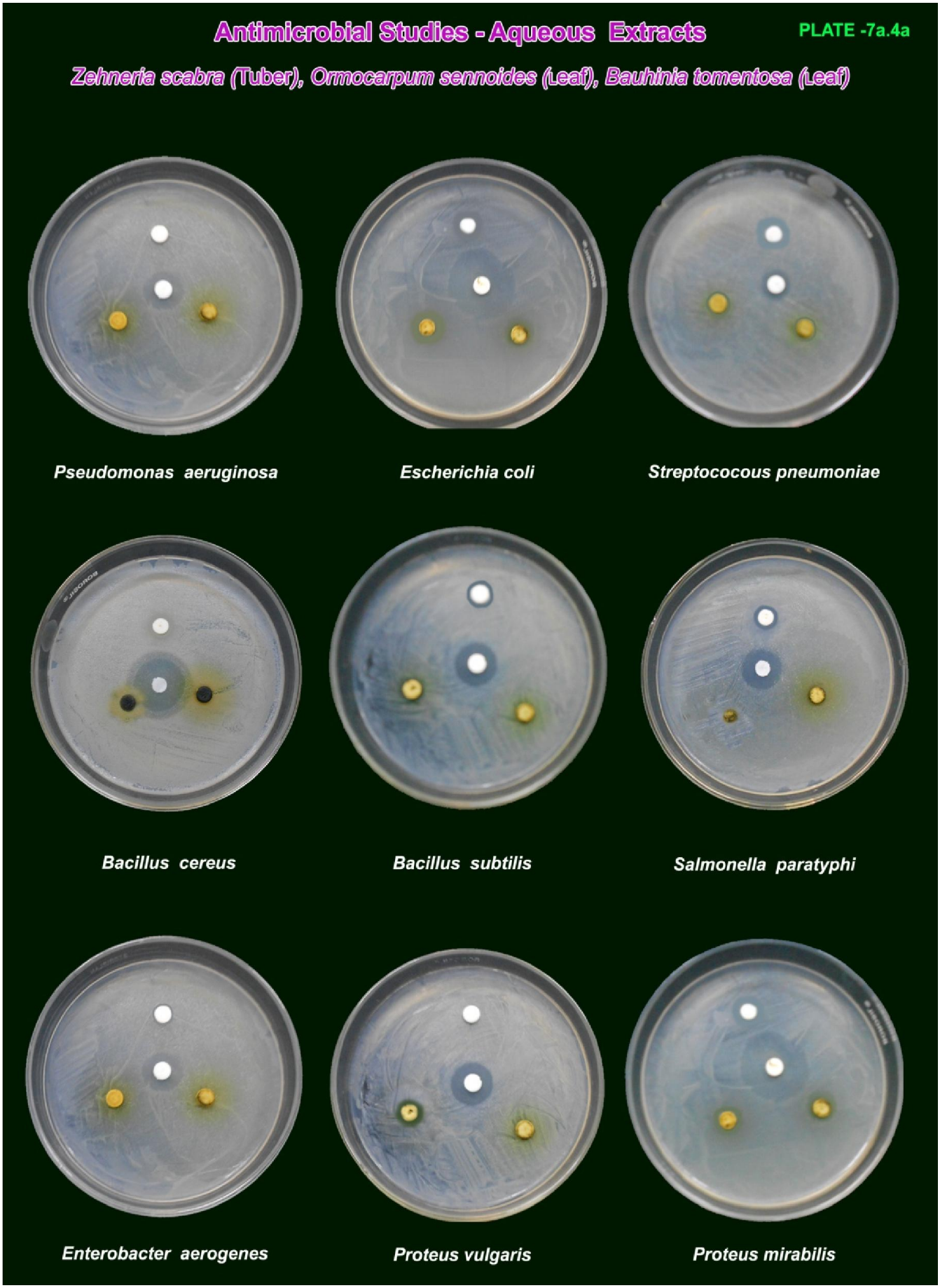
The seeds extract of *Momordica charantia, Cucumis sativa, Praecitrullus fistulosus, Cucurbita pepo* and *Lagenaria siceraria*. Results of antimicrobial activity revealed that all the seeds extracts were very effective against *S. marcescens, E. coli* and *S. thermophilous*. Extracts of various plant parts of *M. charantia*, including leaf, fruit and seeds have been investigated and found to be pharmacologically active against microbes. A leaf, in addition to whole plant extracts have been shown to have anti-HIV activity (Sharma *et al.,* 2012). *B. subtilis, E. coli, P. aeruginosa, S. aureus* are inhibited by the extracts of chloroform and ethanol (95%) of dried fruit (Sharma *et al.,* 2012). Leaf and stem extracts of *Bryonopsis laciniosa* exhibited antimicrobial activity against different Gram positive and Gram negative bacteria (Bonyadi *et al.,* 2009). Antimicrobial activities of *Trichosanthes cucumerina* in petroleum ether, chloroform, ethyl acetate and methanol extract of the leaves gives activity against various pathogenic bacteria such as *B. cereus, E. faecalis, S. paratyphi, S. aureus* and *E. coli* by agar well diffusion method. The antimicrobial potency of this plant extract is due to the presence of phenolic compounds flavonoids and carotenoids (Reddy *et al.,* 2010). The antimicrobial activities of root extracts from *Coccinia grandis* were examined by Hasanuzzaman *et al.,* (2013). The results suggested that very strong inhibition zones were observed in crude ethanolic extract, n-hexane, carbon tetrachloride, dichloromethane and aqueous extracts. Gram positive bacteria such as *S. aureus, B. cereus* and *Sarcina lutea* showed inhibition zones in almost all the extracts.

Antifungal Activity

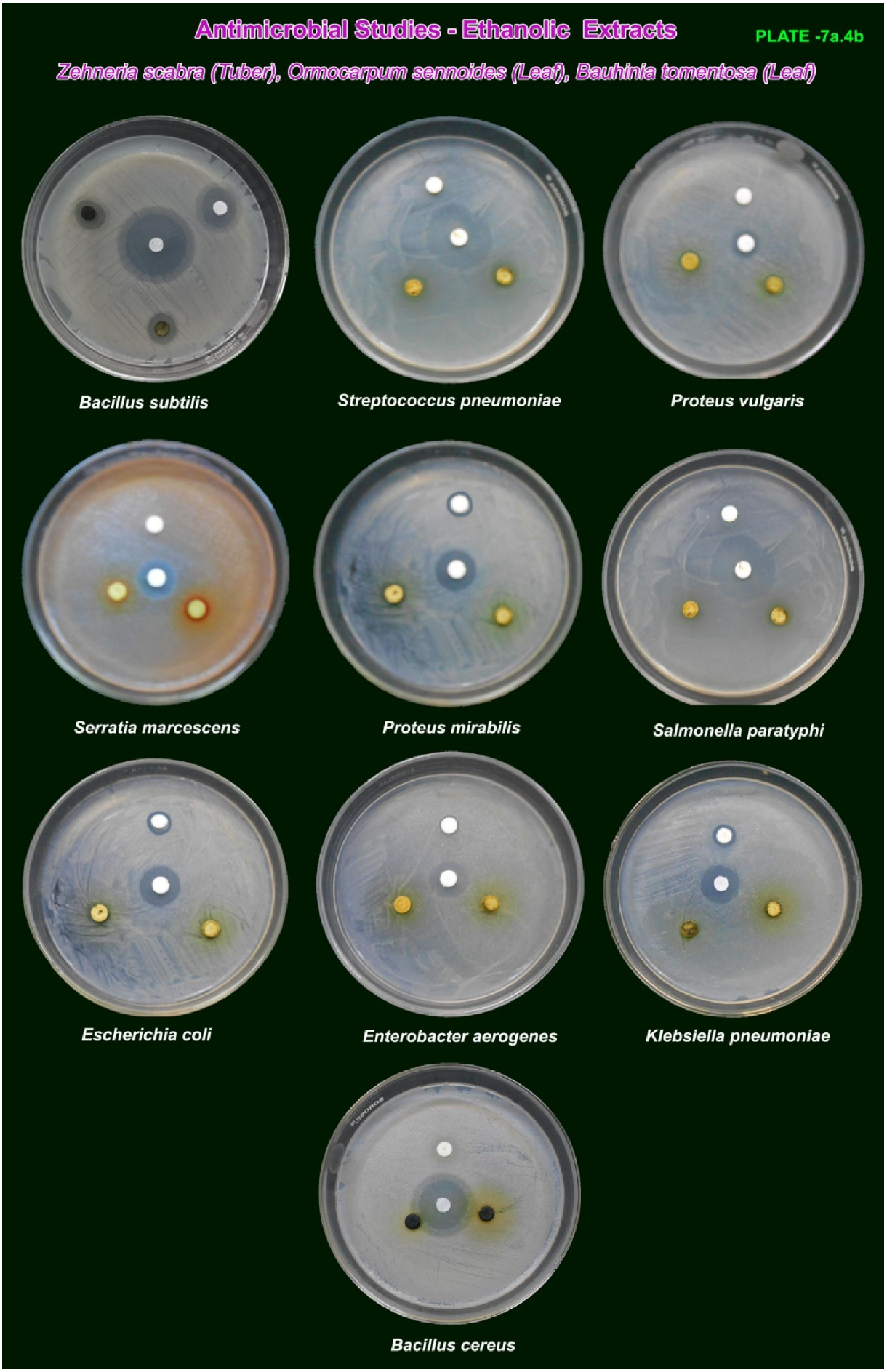
The ethanolic extract of *Zehneria scabra* (tuber) showed a moderate antifungal activity against all the test fungi. *Z. scabra* (tuber) showed inhibition zones in six different concentrations against *Aspergillus flavus* (9, 8, 10, 8, 9, 8 mm), *Aspergillus fumigatus* (9, 10, 10, 9, 8,8 mm), *Aspergillus niger* (9, 8, 9, 8, 8, 8 mm), *Candida albicans* (8, 6, 7, 7, 7, 7 mm)and *Mucor indicus* (9, 9, 10, 8, 9, 9 mm) respectively (Table 7b.4; Plate 7b.4). Among the clinically relevant fungi, the non-dermatophytic fungi showed good inhibition zones followed by mould species and yeast species.



Antifungal Activities in *Zehneria scabra* (Ethanolic Tuber Extract)



(Top White Disc is *Zehneria scabra* Tuber)



(Top White Disc is *Zehneria scabra* Tuber)

as confirmed by preliminary phytochemical screening suggest that the study plant *Z. scabra* might play an important role for cytotoxicity and antifungal effects. The chloroform, ethyl acetate, acetone, methanol, ethanol extracts of the leaves of *Z. scabra* did not show inhibitory effect against the two test fungal organisms, *Botrytis* and *Fusarium* (Gelana, 2011). Since the fungal studies on *Z. scabra* were less done by the researchers, further findings in the members of Cucurbitaceae family were sought. The ethanolic and chloroform extracts of *Cucumis sativus* showed moderate antifungal activities against all tested fungal organisms with zones of inhibition ranging from 4.40 ± 0.18 to 1.67 ± 0.08 mm and 3.45 ±0.04 to 1.50 ± 0.12 mm, respectively. Ethanolic extracts of *C. sativus* showed more potent cytotoxicity and *Aspergillus niger* was the most susceptible fungal (Das *et al*., 2012).

At various concentrations of ethanolic fruit extract of *Luffacyl indica* has exhibited inhibition against *A. fumigatus, A. niger* and *C. albicans* fungi (Devi *et al.,* 2009). In the leaves of *Lagenaria siceraria* two fungal strains *C. albicans* and *A. niger* were used for antifungal activity using griseofulvin as a standard. The extracts showed a moderate antifungal activity against the test microorganisms (Badmanaban and Patel, 2010). The methanol callus tissue extract of *Cucumis anguria* has shown good antifungal properties for all the fungal strains tested in particular *A. flavus, A. fumigatus* and *A. niger*. A Similar result was also reported in methanol extract of *C. anguria* fruits at 500 µg/ml against *Aspergillus, Penicillium, Microsporum* and *Trichophyton* (Senthil Kumar and Kamaraj, 2011, Anusharaj *et al.,* 2013, Abubacker *et al.,* 2008). Moreover, leaf and fruit extracts of the same plant in methanol had antifungal activities against *C. albicans, Fusarium oxysporium* and *Cryptococcus neoformans* (Jigna *et al.,* 2007, Philip *et al.,* 2009, Jigna *et al.,* 2008).

Conclusion

Zehneria scabra is a less researched medicinal plant. The reason could be either it is medicinally less known or less common in tropical climate. Two extracts, namely ethanolic and aqueous has been used to study the antimicrobial activity in *Zehneria scabra* (tuber). The present research suggests that the ethanol is a very efficient solvent for the antimicrobial studies in *Z. scabra*. Findings in Cucurbitaceae family show that almost all parts of the plants are of medicinal importance and all parts have shown antimicrobial activities. Members of this family also show strong inhibitions against many microorganisms. Particularly, microorganisms such as *S. aureus* and *E. coli* were the most susceptible and have shown strong inhibitions. The presence of phenolic compounds, saponins and proteins in the plant indicate that this plant may be used as an antimicrobial agent. From the present findings and findings of others, fungi like *A. fumigatus, A. niger, A. flavus* and *C. albicans* are commonly used as test organisms for the members of the Cucurbitaceae family. In the present study of the ethanolic tuber extract of *Z. scabra*, all the five test fungi showed moderate inhibitions indicating the potent antifungal properties. *Zehneria scabra* is a valuable medicinal plant but very fewer studies of antimicrobial and antifungal activities have been carried out. Although the plant has been less studied, it could enthuse pharmacologists in further productive drug development research. Moreover these test organisms were also found to be most susceptible in determining the antifungal properties. Family Cucurbitaceae contains more bioactive compounds such as cucurbitacin, triterpenes, sterols and alkaloids (Yuan *et al*., 2006; Das *et al.,* 2012) and also many early studies had reported that the members of Cucurbitaceae showed more pronounced antifungal activity (Bola y*et al.,* 2010; Sangeetha *et al.,* 2010; Wayne, 2000). Several workers have reported that water extracts do not have much activity against fungi in Cucurbitaceae family (Martin, 1995; Paz *et al.,* 1995; Vlietinck *et al.,* 1995). The overall results of the study revealed that the ethanolic crude extracts of the selected study plant contains phytoconstituents like alkaloids, flavonoids and phenolic compounds which are necessary for the effectiveness of antifungal activity. The present work also suggests the same. *Z. scabra* is unquestionably the most sought medicinal plant in Gingee hills.

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