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ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF VARIOUS WHOLE PLANT EXTRACTS OF AERVA TOMENTOSA FORSK. (AMARANTHACEAE)

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ABSTRACT

The antimicrobial activity of ethanol, petroleum ether, dichloromethane, ethyl acetate, methanol and aqueous whole plant extracts of *Aerva tomentosa* (*Amaranthaceae)* were tested against nine human pathogens such as *Bacillus subtilis, Enterobactor aerogenes, Escherichia coli* and *Pseudomonas aeruginosa* bacterias and *Aspergillus flavus, Aspergillus niger, Candida albicans, Trichophyton rubrum* and *Penicillium crysogenum* fungus by using agar well diffusion method. Among the six extracts the aqueous cold extract of *Aerva tomentosa* showed the highest range of activity against all tested human pathogens. *E. aerogenes, E. coli*, *A. niger, C. albicans, T. rubrum* and *P. crysogenum* were found to be susceptible forming highest zone of inhibition diameter of 12-20 mm at concentration of 4 mg/ml, suggesting that *Aerva tomentosa* was strongly inhibitory towards these organisms. Appreciable activity against *A. niger* was exhibited by both cold extracts of aqueous and ethyl acetate (IZ 20.00±0.00 mm and17.30±0.33 mm, respectively). These results indicate that *Aerva tomentosa* (*Amaranthaceae)* possessed potential Antibacterial and Antifungal activities.

KEYWORDS

Aerva tomentosa, Antibacterial, Antifungal, Humanpathogens, Agar well diffusion method.

INTRODUCTION

Aerva tomentosa Forsk. (syn. *A. javanica* Juss. ex Schult): The plant belongs to the family *Amaranthaceae* and the genus *Aerva* comprises of sixty one species, distributed in the warm and desert parts of Asia and Africa. *Aerva tomentosa* is commonly known as desert cotton. English Name**:** kapok bush; Hindi Name**:** Safed Shamli; Sanskrit Name**:** Kutashamli; Guj Name: Bur; Tel Name: Magavira; Tam Name: Perumpoolia; Kan Name: Doddahindi gidda; Del Name: Dholimundi; Raj Name: Buida. *Aerva tomentosa* is a plant common to Central America, Asia, Africa, Pakistan, Sri Lanka and Myanmar. In India *Aerva tomentosa* is a deciduous dwarf shrub (up to 50-100 cm tall) widely distributed in Punjab, Rajasthan, Gujarat, Madhya Pradesh, Karnataka and Tamil Nadu. Leaves are small, alternate and of entire length of plant. Flowers are white and born on long spikes. Flowering period for this shrub is January, February, March, April, May, June and December. The shrub is suitable for pots, shrubbery and herbaceous border, propagated from seeds or cuttings. Flowers and seeds are used against swelling, headache and rheumatism[1,2,3]. Roots and flowers are reported to possess medicinal properties against rheumatism and kidney problems. Plant is reported as anthelmintic, diuretic and demulcent[4]. The decoction of the plant is administered to remove swellings[5], applied to acne like conditions of the face[6]. Medicinal uses of this plant (leaves, seeds and the roots) in Ayurveda are used for treatment of kidney stones, and as astringent. Antimicrobial and hepatoprotective activities have also been demonstrated from its perianth lobes[7]. Chemical compounds obtained: α - Amyrin and chrysin are obtained from this plant. Phytochemically, ecdysteroids (20-hydroxyecdysone and 5, 20 dihydroxyecdysone) and alkaloids from the whole plant and aervanone, chrysin-7-O-galactoside, β-sitosterol, α-amyrin and fatty acids from the roots have been reported[8,9].

Medicinal plants are an important therapeutic aid for various ailments. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century[10]. In India, from ancient times, different parts of medicinal plants have been used to cures pecific ailments. Today, there is widespread interest in drugs derived from plants. This interest primarily stems from the belief that green medicine is safe and dependable, compared with costly synthetic drugs that have adverse effects. Natural antimicrobials can be derived from plants, animal tissues, or microorganisms[11]. The short comings of the drugs available today, propel the discovery of new pharmacotherapeutic agents in medicinal plants[12]. To determine the potential and promote the use of herbal medicine, it is essential to intensify the study of medicinal plants that find place in folklore[13,14,15]. Therefore, the aim of the present study is to evaluate the antimicrobial activity of *Aerva tomentosa* and also the literature survey reveals that no reports were found on the antimicrobial activity of the whole plant extracts of *Aerva tomentosa*.

MATERIALS AND METHODS

Collection of Plant Material

The plant material of *Aerva tomentosa* Forsk. was collected from the local fields during the month of June, 2008. The botanical identity was confirmed by Herbarium, Department of Botany, University of Rajasthan, Jaipur. Voucher specimen of the plant has

been deposited at the Herbarium and Laboratory for further reference.

Preparation of the Extracts

The collected materials (whole plant) were chopped into small pieces separately, shade-dried, and coarsely powdered using a pulverizor. The coarse powders were subjected to successive extraction with organic solvents such as ethanol, petroleum ether, dichloromethane, ethyl acetate, methanol and aqueous in increasing order of polarity by Soxhlet method. The extracts were collected and distilled off on a water bath at atmospheric pressure and the last trace of the solvents was removed *in vacuo*. The different extracts were concentrated and dried using vacuum evaporator to give solid residue and were stored at 4^0 C until use for antimicrobial studies.

Bacterial and fungal used

For antimicrobial screening, Gram +ve bacteria (*Bacillus subtilis* MTCC 441) and Gram -ve bacteria (*Enterobacter aerogenes* MTCC 111; *Escherichia coli* MTCC 443 and *Pseudomonas aeruginosa* MTCC 741), obtained from IMTECH, Chandigarh, were used. The bacterial strains were maintained on nutrient agar medium. Further, fungi namely *A. flavus* (ATCC 16870), *Aspergillus niger* (ATCC 322), *Candida albicans* (ATCC 4718), *Trichophyton rubrum* (ATCC 2327) and *Penicillium crysogenum* (ATCC 5476) obtained from IARI, New Delhi, were used. These fungal strains were maintained on Sabouraud dextrose agar (SDA) medium.

Preparation of Inoculum

Stock cultures were maintained at 4^0 C on slopes nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of nutrient broth for bacteria and fungi that were incubated without agitation for 24 hours at 37^0 C and 25^0 C respectively.

Antimicrobial activity

Antimicrobial screening was performed by agar well diffusion method[16] using nutrient agar medium for antibacterial and SDA medium for antifungal activity. In the culture plates, wells were prepared with the help of sterile cork borer (6 mm in diameter) and 0.15 ml of the various plant extracts (ethanol, petroleum ether, dichloromethane, ethyl acetate, methanol and aqueous) with 4 mg concentration dispensed into each well. The extracts were allowed to diffuse into the medium for 1 hour at room temperature and then plates were incubated at 37^0 C for 24 hours for bacterial strains and 27^0 C in case of fungi for 72 hours under aerobic conditions. The diameter of the inhibition zone (IZ) around each hole was measured by inhibition zone recorder (HiMedia) in triplicate and statistically analyzed. The control was set up in a same manner except that the extract replaced with sterile distill water and ciprofloxacin, gentamycin sulphate and nystatin were used as positive standard. The experiments were conducted in triplicate.

Antibiotic assay

The selected antibiotics were obtained from a chemist. These drugs, in their high concentration, were diluted with sterile water reducing them in to a lower concentration. Wells were bored on the prepared agar with a cork borer and with the use of sterile needle and syringe; the antibiotics were poured in to the well .The zone of inhibition was observed after 24 hours and recorded.

Table 1

Zone of inhibition recorded (mm in Diameter) for extracts Antibacterial activity of various whole plant extracts of Aerva tomentosa Forsk.

Bacteria	Ethanol		Pet ether		DCM	EA		Methanol		Aqueous
	Cold	Hot	Cold	Hot	Cold	Cold	Hot	Cold	Hot	Cold
Yield in %	0.53	2.44	0.35	0.33	0.65	0.50	0.27	2.3	1.03	2.09
Bacillus subtilis	12 ± 0.816	6.5 ± 0.11	–	–	–	–	–	–	–	–
Enterobactor aerogenes	–	–	–	–	–	11.66 ± 0.34	10 ± 0	11.66 ± 0.34	–	13.33 ± 0.66
Escherichia coli	–	–	12.33 ± 0.85	–	12.33 ± 0.85	–	–	9.6 ± 0.34	–	11± 0
Pseudomonas aeruginosa	12.33 ± 0.33	9.6 ± 0.16	–	–	–	–	–	–	–	–

Values are the average of triplicate experiments and represented as mean ± standard deviation.

Table 2

Zone of inhibition recorded (mm in Diameter) for extracts Antifungal activity of various whole plant extracts of Aerva tomentosa Forsk.

Fungus	Ethanol		Pet ether		DCM	EA		Methanol		Aqueous
	Cold	Hot	Cold	Hot	Cold	Cold	Hot	Cold	Hot	Cold
Aspergillus flavus	–	–	–	–	–	–	–	–	–	–
Aspergillus niger	–	–	12.66 ± 0.33	10.6 ± 0.34	10.6 ± 0.34	17.30 ± 0.33	16 ± 0.88	12.3 ± 0.334	11.3 ± 0.667	20 ± 0..00
Candida albicans	12 ± 0.557	11 ± 0	–	–	7 ± 0	10.3 ± 0.33	10 ± 0	10.3 ± 0.33	10.3 ± 0.334	10.6 ± 0.34
Trichophyton rubrum	10.6 ± 0.336	13.30 ± 0.33	–	–	–	8.6 ± 0.668	10 ± 0	10 ± 0	14.66 ± 0.00	13 ± 0
Penicillium crysogenum	11 ± 0	10.5 ± 0.121	6.3 ± 0.33	–	6.3 ± 0.33	10.6 ± 0.34	10 ± 0	14.00 ± 0.00	11 ± 0	13.30 ± 0.33

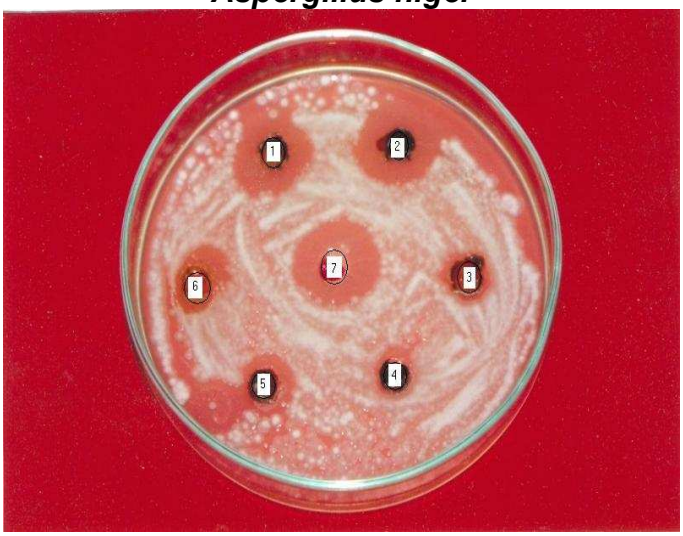
Values are the average of triplicate experiments and represented as mean ± standard deviation.

***Abbreviations**:*

PE = Pet-ether, DCM = Dichloromethane, EA = Ethyl-acetate, MeOH = Methanol, NT= not tested, "–" = not active

Picture 1

Antifungal activity of various whole plant extracts of Aerva tomentosa Forsk. against Aspergillus niger



Abbreviations:
1= Pet-ether cold extract
2 = Pet-ether hot extract
3 = Dichloromethane cold extract
4 = Ethyl-acetate cold extract
5 = Ethyl-acetate hot extract
6 = Methanol cold extract
7 = aqueous cold extract

Fig- 1

Graphical representation of comparison of Zone of inhibition recorded for Antibacterial activity of various whole plant extracts of Aerva tomentosa Forsk.

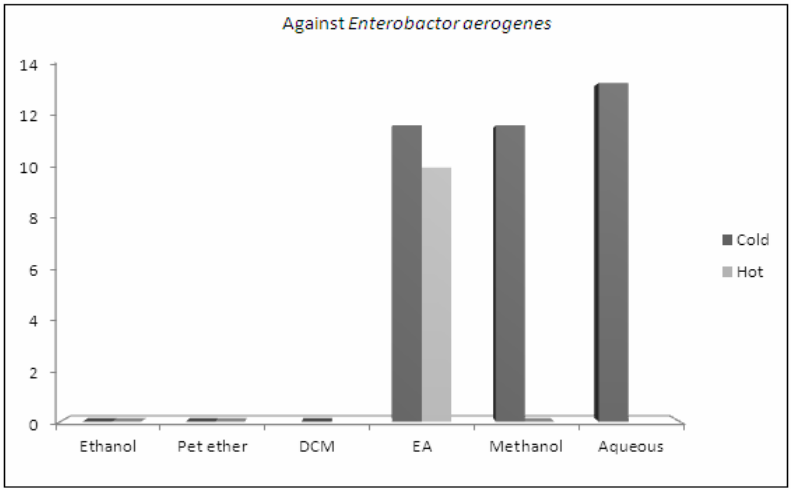
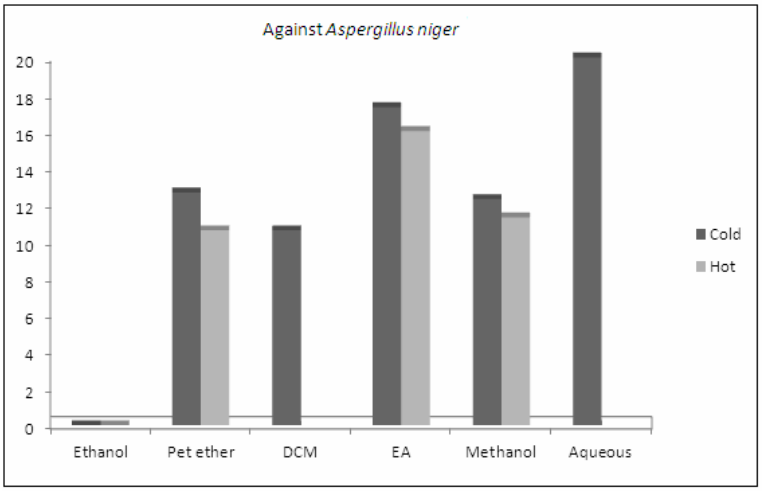


Fig- 2

Graphical representation of comparison of Zone of inhibition recorded for Antifungal activity of various whole plant extracts of Aerva tomentosa Forsk.



RESULT AND DISCUSSION

The six different extracts from whole plant of *Aerva tomentosa* (*Amaranthaceae),* like ethanol, petroleum ether, ethyl acetate and methanol as cold and hot extracts where as dichloromethane and aqueous as a cold extracts were tested against both gram positive and gram negative bacterias like *Bacillus subtilis, E. aerogenes, E.coli* and *P. aeruginosa* bacterias and also tested against fungus like *Aspergillus flavus, Aspergillus niger, C. albicans, T. rubrum* and *P. crysogenum*. Gram negative bacteria like *E.coli, P. aeruoginosa,* may cause the urinary tract infections, GIT infection, skin infection etc. The gram positive organisms like *B. subtilis* may cause the infections such as nosocomial infections, food poisoning, pyrarthirtis, endocardilis, suppurations, abscess formation, osteomyetilis and toxic shock syndrome etc[17,18,19,20]. *Aspergillus niger* is food borne pathogens if inhaled with large amounts of spores, causes a serious lung disease, aspergillosis, otomycosis pain. *Aspergillus flavus* are widely distributed in nature and causes hearing loss and in severe cases, damage to the ear canal causing considerable mortality and morbidity in the tympanic membrane[21]. As most of the *Aspergillus* infections are caused by *A. fumigatus*, the majority of studies have focused on this species and our understanding of other *Aspergillus* species is far from satisfactory[22,23]. *C. albicans* which resides as commensal in the mucocutaneous cavities of skin, vagina and intestine of humans[24], can cause infection under altered physiological and pathological conditions such as infancy, pregnancy, diabetes, prolonged broad spectrum antibiotic administration, steroidal chemotherapy as well as AIDS[25-32].

In case Gram positive bacteria, maximum activity was exhibited by cold ethanolic extract against *B. subtilis* (IZ 12.00±0.81 mm).

Among of Gram negative bacteria, aqueous cold extract showed maximum activity against *E. aerogenes* (IZ 13.33±0.66 mm); cold ethanol extract against *P. aeruginosa* (IZ 12.33±0.33 mm); Petroleum ether cold extract and dichloromethane cold extracts exhibited maximum inhibition against *E. coli* (IZ 12.33±0.85 mm in both cases); cold ethyl acetate extract demonstrated maximum inhibition against *E. aerogens* (IZ 11.66±0.34

mm).

Likewise maximum antifungal activity against *P. crysogenum* was demonstrated by cold methanol and cold aqueous extract (IZ 14.00±0.00 mm, 13.30±0.33 mm respectively). Petroleum ether extract of cold showed significant inhibitory effect against *A. niger* (IZ 12.66±0.33 mm). Appreciable activity against *A. niger* was exhibited by both cold aqueous and ethyl acetate extracts, showed in picture and Fig. 2 (IZ 20.00±0.00 mm and17.30±0.33 mm, respectively). Antifungal activity against *T. rubrum* was demonstrated by both hot methanol and ethanol extracts (IZ 14.66±0.00

The aqueous extract whole plant of *Aerva tomentosa* showed the high range of activity against all tested organisms when compared to other extracts .The diameter of zone inhibition of aqueous extract denoted in table 1 & 2. Fig.1 shows the antibacterial activity of various whole plant extracts of *Aerva tomentosa* Forsk. against *Enterobacter aerogens* whereas Fig 2 shows the antifungal activity of various whole plant extracts of *Aerva tomentosa* Forsk. against *Aspergillus niger*. The results of preliminary phytochemical analysis indicated the presence of steroids, alkaloids, flavonoids, coumarins and tannins. Flavonoids are naturally occurring phenols which possess numerous biological activities including anti-inflammatory, antiallegic, antithrombotic and vasoprotective effects[33,34]. Presence of these active constituents may responsible for the antimicrobial activity.

mm, 13.30±0.33 mm respectively).

The aqueous extract of *Aerva tomentosa* presence of these active constituents responsible for the antimicrobial activity and inhibits the growth of microorganism such as *A. niger, T. rubrum, P. crysogenum* and *E. Aerogenes* and it is also produced the highest zone of inhibitions diameter of 13 mm to 20 mm at concentration of 4mg/ml it was indicated in table 1 & 2. The dichloromethane extract not produced the activity against bacteria like *B. subtillis, E. aerogenes* and *P. aerogens*. It is showed the highest zone of inhibition against *E. coli.*

CONCLUSION

The whole plant extracts of *Aerva tomentosa* (*Amaranthaceae)* in this study showed a broad-spectrum of activity against both gram-positive and gram-negative bacteria and fungi. Bioactive substances from this plant can therefore be employed in the formulation of antimicrobial agents for the treatment of various bacterial and fungal infections including gonorrhea, pneumonia, eye infections and mycotic infections. Isolation, identification and purification of these phytoconstituents and determination of their respective antimicrobial potencies and toxicological evaluation with the view to formulating novel chemotherapeutic agents should be the future direction for investigation.

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