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Antibacterial Activity of *Argyreia Nervosa* Burn.f. Against Different Strains of Bacteria

KEYWORDS

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ABSTRACT *Alcoholic and aqueous extracts of Argyreia nervosa were studied for their antibacterial activity against five bacterial strains viz., Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Citrobactor and Klebsiella pneumoniae using agar disc diffusion method. Solvents used to determine antibacterial activity were methanol and water. Both methanolic and aqueous extract showed zone of inhibition against all tested strains of bacteria. From methanolic and aqueous extract of Argyreia nervosa, methanolic extract had the most inhibitory effect on the growth of all bacterial strains tested as compared to aqueous extract. Among all the bacterial strains Escherichia coli was the most sensitive to Argyreia nervosa extracts of methanol and water. The MIC value for different strains and varieties ranged from 3.0 to 20.6 mm in diameter.*

Introduction
Medicinal plants are important source for the verification of pharmacological effects and can be natural composite sources that act as new anti-infectious agents (Ushimaru et al., 2007). Different plant parts are used for medicinal purposes i.e., bulb, gel, leaves, roots, barks, peels etc. The use of plants to treat illness is found throughout human culture (Anne-Catherine, 2007). The continuous evolution of bacterial resistance to currently available antibiotics has necessitated the search for novel and effective antimicrobial compounds. Globally, plant extracts are employed for their antibacterial, antifungal and antiviral activities. It is known that more than 400,000 species of tropical flowering plants have medicinal properties and this has made traditional medicine cheaper than modern medicine (Odugbemi, 2006). Development of bacterial resistance to the available antibiotics and increasing popularity of traditional medicine has led researchers to investigate the antibacterial compounds in plants.

Argyreia nervosa (Burm.f.), syn.A. speciosa (Sweet Hawaiian, Baby Woodrose) belongs to family Convolvulaceae is a perennial climbing shrub with woody tomentose stem. It is commonly known as Bryddhotareko in Oriya, Samudra-sok in Hindi and Elephant Creeper, Wooly morning glory in English. It is native to Indian sub-continent (upto altitude of 300m) and introduced to numerous areas worldwide, including Hawaii, Africa, Deccan and the Caribbean. Throughout India except dry western region up to 900 ft. elevation often cultivated (The Wealth of India, 1956; Prinyaka 2013). The plant of A. nervosa is of great medicinal value. In traditionally Indian medicine the whole plant is prescribed for treating stomach complaints, sores on foot, small pox, syphilis, dysentery and diarrhoeal, antifertility, anti-rheumatic, antifungal (Malahotra, 1996; The Useful Plants of India, 2000 and Guhabaksh et al., 1999.). The leaves are used externally to treat ringworm, eczema, itching and other skin diseases (The wealth of India, 1956; Malahotra, 1996; Joshi, 2000). The roots are widely used as appetitiser, anemia, aphrodisiac, anti-inflammatory, brain-tonic, cardiotonic, cerebral disorders and other diseases of nervous system (Krishaveni and Santh, 2009; Das, 2003).

Keeping in view the important role of Argyreia nervosa in inhibition of different cultures of bacteria and its role as antioxidant and antibacterial, the present study was conducted to determine the antibacterial activity of the methanol and aqueous extracts of Argyreia nervosa on some bacteria. This study also supports the use of Argyreia nervosa in traditional medicines for the treatment of bacterial infections.

Materials and Methods
1. Plant material:
Argyreia nervosa was obtained from botanical garden of Department of Botany, School of life sciences, Dr. B.R. Ambedkar University, Agra.

2. Extraction procedure:
Soxhlet apparatus was used for the extraction of Argyreia nervosa.

2.1 Soxhlet extraction:
The plant material was cut into small pieces and placed in the extraction thimble. Its weighed amount was placed in an extraction chamber which was suspended above the flask containing the solvent methanol and below a condenser. The flask was heated and the methanol evaporated and moved into the condenser where it was converted into a liquid that trickled into the extraction chamber containing the plant material. The extraction chamber was designed so that when the solvent surrounding the sample exceeded a certain level it overflowed and trickled back down into the boiling flask. At the end of the extraction process, the flask containing the methanol extract was removed and methanol was evaporated by using rotary evaporator. The weight of the extract

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was measured and percentage yield of the plant material was calculated.

3. Primary Phytochemical Screening
Phytochemical screening of different extracts of A.nervosa was carried out qualitatively for the presence of Alkaloids, Antraguinone, Cardic Glycolysis, Flavanoids, Phenols, Phlobatannin, Reducing sugars, Saponins, Steroids, Tannins, Terpenoids, Volatile Oils, Pyrolizidine Alkaloids, Pseudotannins ,Resins, Proteins, Carbohydrates, Starch.

4. Microorganisms:
Five different strains were used for testing antibacterial activity included E.coli (MTCC No. 1652), Pseudomonas aeruginosa (MTCC No. 424), Citrobacter (MTCC No. 2395), Klebsiellap neumonie (MTCC No. 821) and Staphylococcus aureus (MTCC No. 721).The test organisms used in this study were obtained from IMTECH, Chandigarh, India. The bacteria were cultured on nutrient agar slants. The cultures were maintained by subculturing periodically and preserved at 4°C prior to use.

5. Screening for antibacterial activity:
Antibacterial activity was tested by agar disc diffusion method (Mukherjee et al., 1995). Different concentrations of the Argyreia nervosa methanol and aqueous extract were prepared using serial dilution method. The culture was adjusted to approximately 10^5 CFU/ml with sterile saline solution. Five hundred micro liters of the suspension were spread over the plates containing Muller-Hinton agar using a sterile cotton swap in order to get a uniform microbial growth on test plates. Under aseptic conditions, empty sterilized discs (HI-MEDIA) were impregnated with different concentrations, (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml) of plant fraction and placed on the agar surface. All petridishes were sealed with sterile laboratory parafilms to avoid eventual evaporation of the test samples. The antibacterial assay plates were incubated at 37°C for 24 h. The diameter of the zones of inhibition around each of the disc was taken as measure of the antibacterial activity. Each experiment was carried out in triplicate and mean diameter of the inhibition zone was recorded.

Results and Discussion
Yield of extracts: Percentage yield of different extracts of Argyreia nervosa was; methanol (2.68%) and water (3.42%) respectively.

Phytochemical Analysis
The phytochemical screening showed that the different solvent extracts of A. nervosa, the alkaloids, flavanoids, steroids, Triterpenoids , saponin, were present in extracts, but carbohydrate was absent in extract. The various secondary metabolites like flavanoids, polyphenols, quinines teriterpenoids, alkaloids etc give potential opportunity for the expansion of modern chemotherapies against wide variety of organisms (Singh S et al., 2010).

Table 1: Secondary metabolites present in roots extract of Argyreia nervosa

Secondary metabolites	Name of test	Methanol	water
	Mayer's Test	++	+++
	Hager's Test	++	+++
Tannins and phenolic compounds	Ferric Chloride Test	+	+
	Vanillin Hydrochloride Test	+	+
Flavonoids	Shinoda Test (Magnesium hydrochloride reduction test)	+++	++++
	Alkaline Reagent Test	++	+++
Steroids and Triterpenoids	Salkowski Test	++	+++
	Libermann – Buchard Test	++	+++
Glycosides	Legal Test	+	++
	Sodium Nitroprusside Test	+	++
Cabohydrates	Benedit's Test	Ab	AB
	Fehling's Test	ab	AB

Antibacterial activity of different extracts of Argyreia nervosa against selected microorganisms: Antibacterial activity was studied with methanol and water extracts. Agar disc diffusion method was used to determine the zone of inhibition of bacterial growth at particular concentration of both methanol and water. Extract of A.nervosa was effective against Bacillus pumilis strain which was rather unaffected towards broad spectrum antibiotic Zentamycin. The seed oil was found to possess in vitro antibacterial activity against Klebsiella species, Escherichia coli, Pseudomonas aeruginosa, Bacillus anthracis, Salmonella typhi, Salmonella paratyphi, Shigella boydii, Shigella flexneri, Streptococcus β-haemolyticus and Bacillus subtilis (Batra et al., 1985; Mishra et al.,1978). In Uttar Pradesh folklore practice the young leaves are used for healing the wounds (Anonymous, 1985). Both the extracts showed significant inhibitory activities. Inhibition was observed against all tested bacterial strains.

The antibacterial activity test of methanol extract showed that Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Citrobactor and Klebsiella pneumoniae were inhibited at all concentrations. Methanol extract showed higher inhibitory activity against S. aureus and E.coli as compared to all other tested organisms and its inhibition zone ranged from 7 to 18.5 mm (Fig. 1a). The order of inhibition was S. aureus (20.33) and K. pneumonia (16.16) > P. aeruginosa (14.5) > C. bacter (12.75)>E.coli (10.5). Ethanolic extracts of the leaves showed antibacterial activities against various bacterial strains such as E.coli, Proteus vulgaris, Bacillus subtilis,Staphylococcus aureus and antifungal activity against the fungal strains such as Aspergillus niger, Aspergillus flavus and Candida albicans (Ashish et al., 2010) .

Water extract showed antibacterial activity against all tested microorganisms and it had large MIC than methanol extract. Water extract gave higher zone of inhibition against S. aureus (21 mm) and followed by K. pneumoniae (18.75 mm), P. aeruginosa (16.87 mm), C. bacter (13.75) and E.coli (11.75 mm). In both cases of methanol and water extract zones of inhibition decreased with decreasing concentration of the extracts.

George and Pandalai (1949) reported that the alcoholic extract of the leaves of A.nervosa showed antibacterial activity against Staphylococcus aureus but was inactive against Escherichia coli where as aqueous extract was inactive against these two organisms (George and Pandalai, 1949). The seed oil of Argyreia was found to have antibacterial activity against both gram positive and gram negative bacteria. (Batra and Mehta, 1985; Mishra and Chaturvedi, 1978) but the oil was inactive against S.aureus (Mishra and Chaturvedi, 1978). The seed oil was found to have antifungal activity against a number of fungal species such as Aspergillus flavus, Colletotrichumcapsici, Cryptococcus neoformans, Alternaria solani, Helminthosporium sp., Colletotrichum dematium, Aspergillus niger, A. sydowi and Fusarium oxysporum. Penicillium sp. was found to be resistant to the oil (Mishra and Chaturvedi, 1978).

Conclusion
Based on these results, we may conclude that both methanol and water extract showed antibacterial activity against all tested organisms and had large inhibition against S. aureus.

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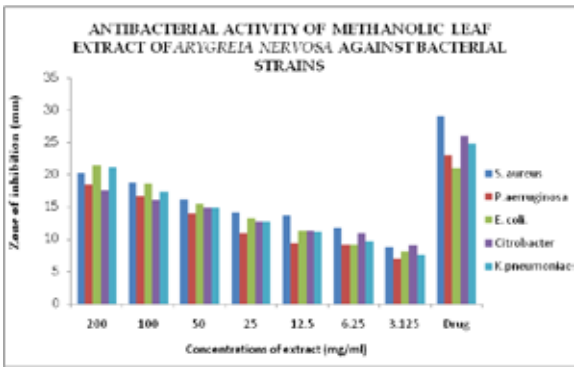
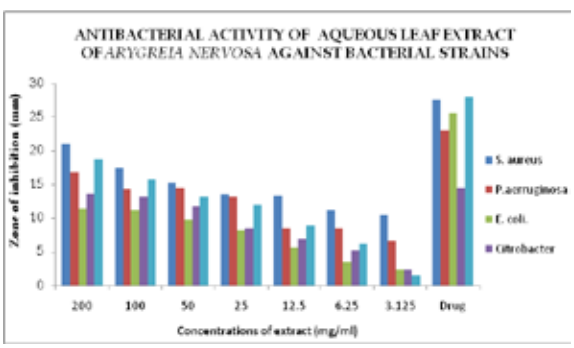
The varying degrees of sensitivity of the bacterial test organisms may be due to the intrinsic tolerance of microorganisms. The disc diffusion study revealed that aqueous extract showed more antibacterial activity against all test pathogens as compared with methanol extract.





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