**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*, *Citrobacter* 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.

**Keywords**

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**Materials and Methods**

1. **Plants 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...
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, Antraguine, Cardiac Glycosyls, Flavonoids, 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, including 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 bacte-ria 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 pre- pared 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 parafins to avoid eventual evaporation of the test samples. The anti-bacterial assay plates were incubated at 37°C for 24 h. The diameter of the zones of inhibition around each of the discs was taken as measure of the antibacterial activity. Each ex- periment 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 sol-vent extracts of A. nervosa, the alkaloids, flavanoids, steroids, Triterpenoids, saponin, were present in extracts, but carbo-hydrate was absent in extract. The various secondary metabolites like flavanoids, polyphenols, quinines tenterpenoids, 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

<table>
<thead>
<tr>
<th>Secondary metabolites</th>
<th>Name of test</th>
<th>Methanol</th>
<th>Water</th>
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<tr>
<td>Steroids and Triterpenoids</td>
<td>Salkowski Test</td>
<td>++</td>
<td>+++</td>
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<tr>
<td></td>
<td>Libermann – Buchard Test</td>
<td>++</td>
<td>+++</td>
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<tr>
<td>Glycosides</td>
<td>Legal Test</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Sodium Nitroprusside Test</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Benedit’s Test</td>
<td>Ab</td>
<td>AB</td>
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<tr>
<td></td>
<td>Fehling’s Test</td>
<td>Ab</td>
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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 Bacil-lus 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 antracnis, Salmonella typhi, Salmonella paratypi, Shigella boydii, Shigella flexneri, Streptococcus β-haemolyticus and Bacillus subtilis (Batra et al., 1985; Mishra et al., 1978 ). In Ut- tarp 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, Citrobacter 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. aerugi-nosa (14.5) > C. bacter (12.75)>E.coli (10.5). Ethanolic ex-tracts of the leaves showed antibacterial activities against various bacterial strains such as E.coli, Proteus vulgaris, Bar-cillus 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. aer-uginosa (16.87 mm), C. bacter (13.75) and E.coli (11.75 mm). In both cases of methanol and water extract zones of inhibi-tion decreased with decreasing concentration of the extracts.

George and Pandalai (1949) reported that the alcoholic ex-tract 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 num-ber of fungal species such as Aspergillus flavus, Colletotri-chum capsici, 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 metha-nol and water extract showed antibacterial activity against all tested organisms and had large inhibition against S. aureus.
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.

**REFERENCE**