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# *IN-VITRO* ANTIMICROBIAL ACTIVITY OF VARIOUS EXTRACTS OF *DYSCHORISTE LITTORALIS* NEES

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**ABSTRACT**

During an appraisal of medicinal plants, it was found that plants belongs to acanthaceae family was potential for its anti-pyretic, anti-inflammatory and analgesic activity. The leaves and the root of selected plant *Dyschoriste littoralis* nees are considered a very efficacious remedy for all sorts of coughs, being administered along with ginger. The leaves are also used for rheumatism. The leaves were dried, made into cigarettes and are smoked in asthma and their juice is used in treatment of diarrhoea and dysentery. In the present study the *in-vitro* anti-bacterial and anti-fungal activities of petroleum ether, chloroform, ethyl acetate and methanol extracts of *Dyschoriste littoralis* nees was evaluated against various strains of bacteria and fungi. The aerial part of the plant extracts were tested for the anti-bacterial activity against gram positive (*Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis*) and Gram negative (*Escherichia coli, Salmonella paratyphi, Pseudomonas aeruginosa, Klebsiella pneumoniae, Vibrio cholerae*) bacteria. The anti-fungal potency was tested against *Aspergillus fumigatus, Aspergillus niger, Monococcus purpura, Candida albicans* and *Tinea capitis.* The preliminary anti-microbial activities were done by agar well diffusion method. Petroleum ether and chloroform extracts displayed very less anti-microbial activity; whereas ethyl acetate and methanol extracts showed very good anti-microbial activity with widest zone of inhibition which was comparable to standard drug. Hence these two extracts were further tested for their MIC by micro broth dilution method. From the study it was found that ethyl acetate and methanol extracts of *Dyschoriste littoralis* nees exhibited very good anti-microbial activity against the tested micro-organism.

**Key words:** *Dyschoriste littoralis* nees, Anti-bacterial, Anti-fungal.

## INTRODUCTION

The investigation of the efficacy of plant based drugs used in the traditional medicine have been paid great attention during the course of reviewing the traditional medicinal plants in various states of India because they are cheap and have little side effects. According to WHO still about 80 % of the world population rely mainly on plant based drugs (Kumara NKVMR, 2001). It is well known that many plants showed remarkable effects on pyrexia, pain, infections, and liver ailments when used as an aqueous extract, churna, or arista.

During an appraisal of medicinal plants, it was found that the plants belongs to acanthaceae family were potential for its anti-pyretic, anti-inflammatory and analgesic activity. In 1931 Kiritikar and basu mentioned in his book about the collection of medicinal plant belongs to acanthaceae family and its traditional medicinal use, folklore of the plant and tribal medicine in various parts of the India. In Malabar coast juices of *Cardanthera uliginosa* leaves were used with salt as a blood purifier. Leaves of *Asteracantha longifolia* are sweet, sour, bitter, tasty, oleaginous, tonic, aphrodisiac, hypnotic and useful in diarrheas and dysenteries, thirst, urinary calculi, urinary discharges, inflammations, biliousness, diseases of the eye, pains, abdominal troubles, anemia, constipation and anuria. The leaves

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are good for cough; applied for gleets, and in lumbago and pains in the joints. The seeds are tasteless, fattening, aphrodisiac, tonic; improve the blood.

During the cold stage of intermittent fever *Strobilanthes auriculatus* leaves are rubbed on the body. *Blepharis edulis* leaves are acrid with a flavor; cooling, astringent to the bowels, aphrodisiac, appetizer, were useful in tridosha, fevers, urinary discharges, leucoderma, mental derangements, applied to wounds and ulcers. White flowered *Barleria prionitis* which are bitter, sweetish, heating, alexiteric and useful in toothache, leucoderma, blood complaints, bronchitis, itch; whereas yellow flowered variety are bitter, acrid, heating, appetizer; useful in diseases of the skin and the blood, itching, purities, inflammation.

A paste is made from the root which is applied to disperse boils and glandular swellings, and a medicated oil is made by boiling the leaves and stems with sweet oil until all the water has been driven off which is used for cleaning wounds. The plant *Barleria cristata* is bitter; heating; useful in inflammations, fevers, bronchitis, blood diseases, biliousness, tympanitis, pains, asthma. The roots and leaves are used to reduce swelling, and an infusion is given in cough. Decoction of the root of *Barleria courtallica* is given in rheumatism and pneumonia. The plant *Andrographis paniculata* is very useful in general debility, dysentery and certain forms of dyspepsia. The roots and the leaves are febrifuge, stomachic, tonic, alterative and anthelmintic. A saturated infusion of the whole plant in a dose of about half a pint, is administered to fever patients by the Mundas of Chota Nagpur. The plant *Justicia gendarussa* is pungent, bitter, hot, dry and useful in bronchitis, inflammations, vaginal discharges, dyspepsia, tympanitis, eye diseases, fevers.

The leaves and tender shoots are diaphoretic and they are given in chronic rheumatism in the form of decoction. The plants *Adhatoda vasica* is pungent, bitter, acrid, cooling which are useful in bronchitis, leprosy, blood impurities, heart troubles, thirst, asthma, fever, vomiting, loss of memory, leucoderma, consumption, jaundice, tumors, disease of mouth. The root facilitates the expulsion of the foetus; useful in strangury, and in leucorrhea with blood discharges (Kiritikar and Basu, 1931). The leaves and the root of this plant are considered a very efficacious remedy for all sorts of coughs, being administered along with ginger. The leaves are also used for rheumatism. The dried leaves were placed in cigarettes and are smoked in asthma and their juice is used for diarrhoea and dysentery.

From the above background, we plan to evaluate the plant used by most of traditional practitioners in Tamilnadu and ethno medical information given in various literatures named as *Dyschoriste littoralis* nees belongs to the family Acanthaceae was taken for our study. Since no detailed previous work relating to the anti-microbial studies of the plant *Dyschoriste littoralis* nees has been brought on record. Hence we decided to make a thorough and detailed study for anti-microbial activity of *Dyschoriste littoralis* nees against various strains of bacteria and fungi.

## MATERIALS AND METHODS

### Microorganism used

In the present study the following microorganism were used to determine the anti-microbial effect of *Dyschoriste littoralis* nees. The anti-bacterial effects were studied against Gram positive (*Staphylococcus aureus* NCIM 2079, *Micrococcus luteus* NCIM 2169 and *Bacillus subtilis* NCIM 2063) and Gram negative (*Escherichia coli* NCIM 2065, *Salmonella paratyphi* NCIM 2501, *Pseudomonas aeruginosa* NCIM 2200, *Klebsiella pneumoniae* NCIM 2707 and *Vibrio cholerae*) bacteria. The bacterial strains were obtained from MNR Medical College, Sangareddy. The anti-fungal potency of the plant was tested against *Aspergillus fumigatus* MTCC 1811, *Aspergillus niger* MTCC 1344, *Monococcus purpura* MTCC 1090 and *Candida albicans* MTCC 3100. The standard fungal strains were collected from MNR Medical College, Sangareddy.

### Preparation of culture media

Dehydrated media were purchased from Hi-Media Laboratories Ltd, India. All the media were prepared in sterile glass petri plates (4 mm thickness) according to the manufacturer's instructions. Chloramphenicol (10 µg/ml) and Griseofulvin (25 µg/ml) was used as standard drug for comparison of anti-bacterial and anti-fungal activity respectively. DMF was used as a solvent.

### Collection of raw material and preparation of extract

The *Dyschoriste littoralis* nees herb was collected from the moist places near Tirunelveli, Tamil Nadu, India. The plant was identified and authenticated by Mr. V. Chelladurai, Research Officer of Botany, Central Council for Ayurveda and Siddha, Government of India. The voucher specimen was preserved in our laboratory for future reference. After collection of the plant, the root was removed and the aerial part was washed thoroughly in tap water and dried in shade for about 10 days under controlled temperature (25 ± 2 ºC). Then the raw material was powdered and passed through a 40 mesh sieve and stored in a well closed container for further use. Coarsely powdered dried aerial plant (1.3 kg) was successively soxhlated using petroleum ether, chloroform, ethyl acetate and methanol for 72 h at room temperature respectively. The extracts were filtered and the solvents were evaporated to dryness under reduced pressure in an Eyela rotary evaporator at 40 to 45 ºC. The percentage yield was noted as 2.91 % for petroleum ether,

4.58 % for chloroform, 3.50 % for ethyl acetate and 8.91 % for methanol. The preliminary phyto chemical investigations of aerial plant extract of *Dyschoriste littoralis nees* were carried out by the standard methods (Harborne 1973; Kokate 2001).

**Determination of anti-microbial activities of extract**

The anti-microbial activities of petroleum ether, chloroform, ethyl acetate and methanol extracts were determined by agar well diffusion method. All bacterial and fungal strains were grown in nutrient broth (NB) and Sabouraud dextrose broth (SDB) for 4-6 hours at specified temperatures. The turbidity of the broth culture was adjusted to 0.5 McFarland units. This gives a suspension containing approximately 1-2 x $10^6$ cfu/ml (Mackie & Mac Cartney 1996).

An aliquot of microbial culture was added to agar medium at 45 °C and poured into the petri plate. After solidification of the agar, medium was punched with a sterile cork borer (5.0 mm diameter) to cut uniform wells. Different concentrations of the extracts (125, 250 and 500 µg/ml) were prepared using DMF as solvent and added to the wells. Bacterial cultures were incubated at 37 °C for 24 hours and fungal cultures at 25 °C for 48 hours. Anti-microbial activity was determined by measuring the zone of inhibition surrounding the well. The zones of inhibition were then measured, recorded and compared with positive standard controls, Chloramphenicol (10 µg/ml) and Griseofulvin (25 µg/ml) for anti-bacterial and anti-fungal activity respectively. The assays were carried out under aseptic conditions. DMF was used as a negative control.

**Determination of Minimum Inhibitory Concentration (MIC)**

The minimum inhibitory concentration (MIC) of the extracts was determined by micro broth dilution method (Andrews JM 2001). For MIC, four fold serial dilutions of the extracts were prepared (15.625, 31.25, 62.5 and 125 µg/ml) in microtitre wells. Incubation of the microtitre plates was carried out at 37 $^oC$ for 24 hours for bacteria and at 25$^oC$ for 48 hours for fungi. After incubation, microtitre wells were observed for any visible growth. The bacterial suspensions were used as positive control and extracts in broth were used as negative control. The MIC was interpreted as the lowest concentration of the extract that did not show any visible growth when compared to control tubes.

**RESULTS**

**Phytochemical screening**

The phytochemical screening of aerial part of the plant different extracts revealed the presence of phytosterols and fats and fixed oils in petroleum ether extract, slight reaction for alkaloids in chloroform extract, gave positive results for flavonoids, phenolic acids and tannins in ethyl acetate and methanol extracts.

**Anti-microbial activities**

The results of the preliminary anti-microbial activities (zone of inhibition) are presented in table 1. All test strains of bacteria were found to be sensitive to Chloramphenicol and fungal strains were sensitive to Griseofulvin. DMF was used as the negative control which did not showed any zone of inhibition against tested bacteria and fungi.

Petroleum ether and chloroform extract does not displayed any anti-microbial activity against tested micro-organism at all three tested concentration (100, 250 and 500 µg/ml).

Ethyl acetate and methanol extracts displayed anti-bacterial and anti-fungal activity against all the tested bacteria and fungi at all three tested concentrations (125, 250 and 500 µg/ml). Against all the tested micro-organism ethyl acetate extracts displayed better activity (highest zone of inhibition) than methanol extracts. Highest activity was observed at 500 µg/ml concentration for ethyl acetate and methanol extracts. Both the extracts displayed more anti-bacterial activity than anti-fungal activity.

Minimum inhibitory concentration (MIC) was tested for the ethyl acetate and methanol extracts of *Dyschoriste littoralis* nees and the results are presented in table 2. The MIC was considered as the lowest concentration of the extract that did not show any visible growth when compared to control tubes.

MIC of ethyl acetate extract was found to be 15.625µg/ml against *Bacillus subtilis*, *Salmonella paratyphi*, *Aspergillus niger* and *Candida albicans.* The MIC for the same extract against *Escherichia coli. Klebsiella pneumonia, and Monococcus purpura* was found to be 31.25µg/ml. The MIC was found to be 62.5µg/ml for ethyl acetate extract against *Staphylococcus aureus, Pseudomonas aeruginosa, Vibrio cholerae* and *Aspergillus fumigatus.* 125µg/ml was found as MIC for ethyl acetate extract against *Micrococcus luteus* and *Tinea capitis.*

MIC of methanol extract was found to be 15.625µg/ml against *Bacillus subtilis.* The MIC for the same extract against, *Salmonella paratyphi, Aspergillus niger* and *Candida albicans* was found to be 31.25µg/ml. The MIC was found to be 62.5µg/ml for ethyl acetate extract against *Staphylococcus aureus, Escherichia coli*, and *Vibrio cholerae.* 125µg/ml was found as MIC fore ethyl acetate extract against *Micrococcus luteus, Pseudomonas aeruginosa, Klebsiella pneumonia, Aspergillus fumigatus, Monococcus purpura and Tinea capitis.*

**Table 1. Anti-microbial activity (zone of inhibition in mm) of four extracts of aerial part of *Dyschoriste littoralis* nees**

| Micro organism | Extract / Drug | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Petroleum ether | | | Chloroform | | | Ethyl acetate | | | Methanol | | | Std. Drug | DMF |
| | 125 | 250 | 500 | 125 | 250 | 500 | 125 | 250 | 500 | 125 | 250 | 500 | | |
| *S. aureus* | - | - | - | - | - | - | 7 | 15 | 20 | 5 | 12 | 19 | 21 | - |
| *M. luteus* | - | - | - | - | - | - | 6 | 11 | 22 | 4 | 8 | 16 | 22 | - |
| *B. subtilis* | - | - | - | - | - | - | 10 | 14 | 23 | 9 | 16 | 20 | 24 | - |
| *E. coli* | - | - | - | - | - | - | 8 | 15 | 22 | 5 | 11 | 18 | 20 | - |
| *S. paratyphi* | - | - | - | - | - | - | 9 | 13 | 24 | 7 | 10 | 17 | 23 | - |
| *P. aeruginosa* | - | - | - | - | - | - | 7 | 11 | 22 | 4 | 9 | 18 | 21 | - |
| *K.pneumoniae* | - | - | - | - | - | - | 8 | 16 | 25 | 3 | 10 | 16 | 23 | - |
| *V. cholerae* | - | - | - | - | - | - | 7 | 10 | 23 | 5 | 10 | 19 | 24 | - |
| *A. fumigatus* | - | - | - | - | - | - | 7 | 14 | 20 | 4 | 11 | 17 | 22 | - |
| *A. niger* | - | - | - | - | - | - | 9 | 12 | 18 | 5 | 8 | 14 | 21 | - |
| *M. purpura* | - | - | - | - | - | - | 8 | 14 | 20 | 4 | 9 | 16 | 23 | - |
| *C. albicans* | - | - | - | - | - | - | 10 | 15 | 22 | 6 | 12 | 16 | 25 | - |
| *T. capitis* | - | - | - | - | - | - | 6 | 10 | 17 | 3 | 9 | 17 | 22 | - |

- : indicates no zone of inhibition; Std. Drug: Chloramphenicol (10 µg/ml) and Griseofulvin (25 µg/ml) for anti-bacterial and anti-fungal activity respectively; DMF: Dimethyl formamide.

**Table 2. MIC (in µg/ml) of ethyl acetate and methanol extracts of aerial part of *Dyschoriste littoralis* nees**

| Microorganism | Ethyl acetate extract | Methanol extract |
|---|---|---|
| *S. aureus* | 62.5 | 62.5 |
| *M. luteus* | 125 | 125 |
| *B. subtilis* | 15.625 | 15.625 |
| *E. coli* | 31.25 | 62.5 |
| *S. paratyphi* | 15.625 | 31.25 |
| *P. aeruginosa* | 62.5 | 125 |
| *K. pneumoniae* | 31.25 | 125 |
| *V. cholerae* | 62.5 | 62.5 |
| *A. fumigatus* | 62.5 | 125 |
| *A. niger* | 15.625 | 31.25 |
| *M. purpura* | 31.25 | 125 |
| *C. albicans* | 15.625 | 31.25 |
| *T. capitis* | 125 | 125 |

## DISCUSSION

The anti-microbial potentials of substances are useful tools in the control of various infections caused by micro-organisms especially fungal infections. Antibiotic resistance is a major concern and development of new agents from plants could be useful in meeting the demand for new anti-microbial agents with improved safety and efficacy (Srivastava *et al.,* 2000). Newer anti-microbial from plant extracts may be useful in many (food, dairy and pharmaceutical) industries to prevent contamination by limiting the microbial growth.

In this study, it was found that the ethyl acetate and methanol extracts of aerial parts of *Dyschoriste littoralis n*ees exhibited highest anti-microbial activity than the petroleum ether and chloroform extracts. The difference in the anti-microbial efficacy could be due to the presence of variable phytochemical compounds in different extracts.

The bacterial organisms found in this study to be susceptible include *E. coli*, *S. aureus* and *B. subtilis* which have been implicated in many systemic infections such as respiratory and genitourinary tract infections. The phyto chemical screening of aerial part of the plant different extracts revealed the presence of phytosterols and fats and fixed oils in petroleum ether extract, slight reaction for alkaloids in chloroform extract, gave positive results for flavonoids, phenolic acids and tannins in ethyl acetate and methanol extracts. The phyto chemical screening of this plant showed the presence of flavonoids in both of the ethyl acetate and methanol extracts which have been shown to possess anti-microbial properties (Hostettman *et al*., 1995; Oboh *et al*., 1998). Flavonoids are known for their anti-inflammatory, anti-arthritic and anti-microbial properties (Trease and Evans, 1989). Therefore, the anti-microbial activities of this plant may be ascribed to the presence of flavonoids. Bioassay directed fractionation of the most active extract is in progress to isolate and identify the compounds responsible for the anti-microbial activity.

## CONCLUSION

Crude extracts from *Dyschoriste littoralis n*ees have medicinal applications from olden days and very little work has been done on the biological activity and plausible medicinal applications of isolated compounds of *Dyschoriste littoralis n*ees. Hence a drug development program undertaken to investigate the anti-microbial potency of *Dyschoriste littoralis* nees. From the study it was found that ethyl acetate and methanol extracts exhibited very good anti-microbial activity against the tested micro-organism. The good activity may be attributed to the presence of flavonoids on these extracts.

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