In-vitro Antimicrobial Screening of Dendrophthoe falcata (L.F.) Ettingsh

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**A B S T R A C T**

Bioassay (petroleum ether, chloroform, ethyl acetate and methanol) of various leaf and stem extracts of Dendrophthoe falcata (L.F.) Ettingsh were investigated for an in vitro antimicrobial activity against five bacteria: Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa and Streptococcus pneumoniae and two fungi Candida albicans and Aspergillus niger. Among all the extracts of leaf and stem, methanolic and ethyl acetate shows good sensitivity against all the tested organisms. Furthermore, among fungi studied, methanolic extract of leaf showed higher antifungal activity against A. niger while in stem ethyl acetate and methanol showed moderate antifungal activity against A. niger. In C. albicans ethyl acetate extract of stem found to most active. The present investigation showed the effectiveness of crude extract of this plant against tested microorganisms.

**Keywords**

Antimicrobial screening, Phytochemical, Dendrophthoe falcata, Aravalli hills, Rajasthan.

**Article Info**

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**Introduction**

*Dendrophthoe falcata* (L.F.) Ettingsh belongs to family Loranthaceae, is an evergreen hemiparasitic plant grown on different host trees like *Boswellia serrata*, *Mangifera indica*, *Ficus religiosa*, *Madhuca latifolia*, *M. indica* and *F. rumphii* etc. (Singh and Gupta, 2013). It is also known as ‘*Vanda*’ in the Indian Ayurvedic system of medicine and ‘*Vrksadani*’ and ‘*Vrksaruha*’ in ‘Sanskrit’. It is indigenous to tropical regions especially in India, Srilanka, Thailand, China, Australia, Bangladesh, Malaysia and Myanmar (Manthri et al., 2011).

Whole plant and part/s like bark, Leaves, flower, stem and fruits possesses medicinal potential and indigenous communities use it for treatment of various human and animal ailments like rheumatic complaints (Md. Shahidullah, 2009; Shanavaskhan, et al., 2012), leucorrhoea (Shanavaskhan et al., 2012; Rothe, 2003), as contraceptive (Mairh et al., 2010), skin diseases (Kunwar et al., 2005; Ganasen et al., 2009), bone fracture (Kunwar et al., 2005; Partha & Hossain, 2007), asthma (Reddy et al., 2006; Kumar et al., 2012), wound healing (Vijigiri and Sharma, 2010; Kunwar et al., 2011), for abortion (Kunwar et al., 2005; Ganasen et al., 2006; Kaur and Mehta, 2014) and schizophrenia (Mali and Bhadane, 2011). Ethnoveterinary use of this plant is also reported by Katewa & Jain (2006), extract of...
whole plant is applied locally on uterus of cattle in volvo-vaginal-uterine prolapse. Its pharmacological activities like anti-oxidant, anti-hyperlipidaemic, anti-diabetic, diuretic and antilithiatic activity have also been studied (Tenpe et al., 2008; Pattanayak and Sunita, 2008; Aleykutty et al., 1993). The plant contains phytosterols, flavonoids, quercetin, phenolic compounds, tannin and terpins (Pattanayak and Sunita, 2008; Dashora et al., 2010) etc.

Antibacterial and antifungal screening of aerial parts of *Dendrophthoe falcata* was studied by Pattanayak & Sunita (2008) while Patil et al., (2012) studied antibacterial sensitivity of different solvent of leaves of *D. falcata* growing on *M. indica*. Perusal of literature indicates that *D. falcata*, growing hemiparasitically on *B. serrata*, yet not studied for their antimicrobial potential. Thus, the present studies undertake to check antimicrobial potential of various extracts of leaves and stem of the *D. falcata* growing on *B. serrata*.

**Materials and Methods**

**Collection of plant material**

The leaves and stem of *D. falcata* growing on *B. serrata* were collected from the southern Aravalli hills of Rajasthan. The plant was identified from its morphological features as mentioned in different standard text and flora (Hooker, 1872-1897). The voucher specimen has been deposited at VBRI, Udaipur for further reference.

**Preparation of extracts**

Stem and leaves washed, shade dried and powdered by using a pulveriser. Coarse powders (100g of stem and 90gm of leaves) then subjected to successive extraction with organic solvents such as petroleum ether, chloroform, ethyl acetate and methanol by Soxhlet method for 12 hrs. The extract were filtered and filtrate was concentrated to dryness under reduced pressure in rotary vacuum evaporator and stored at 4°C. Percent extractive yield was calculated by the following formula and are listed in table-1.

\[
\text{Percent extractive} = \frac{\text{weight of dried extract}}{\text{Weight of dried plant material}} \times 100
\]

To make stock solution of 100mg/ml of each extract (crude drug) the appropriate amount is weighed and dissolved in DMSO. The stock solution was passed through 0.2µm pyrogensic filter to sterilize the solution and further concentrations of 50 mg/ml, 25 mg/ml and 12.5 mg/ml was made by diluting with Di Methyl Sulfoxide (DMSO).

**Test microorganism**

The pathological strains of test organism *i.e.* *Escherichia coli* (MTCC 118), *Staphylococcus aureus* (MTCC 96), *Klebsiella pneumoniae* (MTCC 39), *Pseudomonas aeruginosa* (MTCC 424) and *Streptococcus pneumoniae* (MTCC *655*), *Aspergillus niger* (MTCC 281) and *Candida albicans* (MTCC 183) were obtained from MTCC, Chandigarh, India and again identified by standard methods of identification (Collee et al., 1996).

**Antimicrobial Susceptibility Testing**

**Well Diffusion Method**

The *in vitro* antimicrobial activity was determined by the agar well diffusion method (Guven et al., 2006). Cell suspensions containing $10^6$ CFU/ml cells for bacteria and yeasts and $10^5$ spore/ml of fungi were prepared and 100µl was evenly spread on the surface of the nutrient agar for bacteria and sabouraud dextrose agar medium for yeasts and fungi using glass
spreader. The wells of 6 mm diameter were made at equidistant. 100µl volumes of crude extract of each concentration were dispensed into wells, the plate were incubated at 37° C for 24 hrs for bacterial strains, 48 hrs for yeasts and 72 hrs for fungi at 28° C. The diameter of zone of inhibition was measured. As reference antibiotic Meropanum (5µg/ml) was used against all the tested bacteria and Amphotericin-B (30 µg/ml) for yeast and fungi.

**Minimum inhibitory concentration (MIC)**

The estimation of MIC of the crude extracts was carried out using the method of agar well diffusion (Mohana et al., 2008; Bais et al., 2013) with some modification. Approximate amount of extract was taken from the solution of the crude drug sample (12.5mg/ml) with DMSO and diluted it serially (1:1) with DMSO to the concentration of 0.012mg/ml. As a result, a series of the sample solution in decreasing concentration was obtained by a ratio of 0.5 (final concentration: from 6.25mg/ml to 0.012mg/ml). In this method the least concentration of each extract showing a clear zone of inhibition was taken as the MIC. The MIC value was defined as the lowest concentration to inhibit visible growth of microbes.

**Preliminary phytochemical screening**

All the extracts of leaves and stem of *D. falcata* were screened for various secondary metabolites such as tannins, alkaloids, phenols, steroids, flavonoids, and saponins using standard methodology (Panday & Tripathi, 2014).

**Results and Discussion**

*In-vitro* antimicrobial screening of various extracts of leaves and stem of *D. falcata*, was shown in Table-2 and 3. The antimicrobial activity was dose-dependent because activity at 100 mg/ml more than other concentrations against the entire tested microorganism. As shown in Table-2, the extract form the *D. falcata* leaves and stem displayed antimicrobial activity against the tested bacterial strains, with the diameter of zone of inhibition ranging between 10mm to 20mm. Among the all extract of leaf, both methanol and ethyl acetate extract produce 20 ± 0.0 mm zone of inhibition against *S. pneumoniae* and methanol extract of leaves also produce 17 ± 0.0 mm zone of inhibition against *K. pneumoniae* while in stem, ethyl acetate extract showed higher antibacterial activity against *P. aeruginosa* (20 ± 0.0) and *S. pneumoniae* (19 ± 0.0).

Furthermore, among fungi studied (Table-3), methanolic extract of leaf showed higher antifungal activity against *A. niger* with a zone of inhibition 22 ± 0.0 mm while in stem ethyl acetate and methanol showed moderate antifungal activity against *A. niger* with a zone of inhibition 22 ± 0.0 and 21 ± 0.0 mm respectively. In *C. albicans* ethyl acetate extract of stem found to most active with a zone of inhibition 11.33 ± 0.57.

The MIC of ethyl acetate extract against the entire tested microorganism was observed to be a range of 0.781 to 6.25 mg/ml (Table-4). Table-1 shows percentage extractive values of *D. falcata* leaf and stem extracts obtained with various solvents. Ethyl acetate extract gave maximum percent extractive value.

A preliminary screening was done to check the presence of various phytoconstituents in the extracts (Table-5). It was found that chloroform extract of *D. falcata* leaf shows presence of phenols. Ethyl acetate extracts shows presence of flavonoids, phenols, steroids and saponins while methanol extracts shows presence of flavonoids, phenols, saponins and tannins.
Table 1: Yield of extracts of leaves and stem of *Dendrophthoe falcata* extracted in different solvents by soxhlet apparatus.

| Solvents          | Leaves | stem  |
|-------------------|--------|-------|
| Petroleum ether   | 1.63 % | 7.17 %|
| Chloroform        | 2.33 % | 2.12 %|
| Ethyl acetate     | 21.32 %| 17.39 %|
| Methanol          | 1.44 % | 1.66 %|

Table 2: Showing zone of inhibition of different extracts of *D. falcata* against bacteria.

| Name of Organisms | Cons. Mg/ml | Leaf extracts          | Stem extracts          |
|-------------------|-------------|------------------------|------------------------|
|                   |             | PE         | Chlo     | E A         | Meoh     | PE         | Chlo     | E A         | Meoh     |
| *Escherichia coli* (MTCC 118) | 100         | 11 ± 0.0  | 12 ± 0.0 | 13 ± 0.0    | 13 ± 0.0 | -          | 12 ± 0.0 | 13.33 ± 0.57 | 12 ± 0.0 |
|                   | 50          | 10 ± 0.0  | 11 ± 0.0 | 12.66 ± 0.57 | 11.33 ± 0.57 | -          | 11 ± 0.0 | 13 ± 0.0    | 11.33 ± 0.57 |
|                   | 25          | -         | 10 ± 0.0 | 12.33 ± 0.57 | 10.33 ± 0.57 | -          | 10.33 ± 0.57 | 12.66 ± 0.57 | 11 ± 0.0  |
|                   | 12.5        | -         | -        | 12 ± 0.0    | 10 ± 0.0   | -          | 10 ± 0.0 | 12 ± 0.0    | 10 ± 0.0  |
|                   | 6.25        |           |          | 11.33 ± 0.57 |          |           |          | 11.33 ± 0.57 |          |
|                   | 3.125       |           |          | 11 ± 0.0    |          |           |          | 10.66 ± 0.57 |          |
|                   | 0.781       |           |          | 10 ± 0.0    |          |           |          | 10.33 ± 0.57 |          |
|                   | 0.390       |           |          |            |          |           |          |           |          |

| *Klebsiella pneumoniae* (MTCC 39) | Cons. Mg/ml | Leaf extracts          | Stem extracts          |
|----------------------------------|-------------|------------------------|------------------------|
|                                  |             | PE         | Chlo     | E A         | Meoh     | PE         | Chlo     | E A         | Meoh     |
|                                  | 100         | -          | 12.33 ± 0.57 | 16.33 ± 0.57 | 17 ± 0.0 | -          | -        | 17 ± 0.0    | 15 ± 0.0 |
|                                  | 50          | -          | 12 ± 0.0    | 15 ± 0.0    | 15.66 ± 0.57 | -        | -        | 16 ± 0.0    | 14.33 ± 0.57 |
|                                  | 25          | -          | 11 ± 0.0    | 13 ± 0.0    | 13 ± 0.0    | -        | -        | 15 ± 0.0    | 12.66 ± 0.57 |
|                                  | 12.5        | -          | 10 ± 0.0    | 12 ± 0.0    | 12 ± 0.0    | -        | -        | 13 ± 0.0    | 11 ± 0.0  |
|                                  | 6.25        |           | 11 ± 0.0    |          | 12 ± 0.0    |           |          | 12 ± 0.0    |          |
|                                  | 3.125       |           | 10 ± 0.0    |          |           |           |          | 11 ± 0.0    |          |
|                | 1.562  | 0.781  | 0.390  | 10.33 ± 0.57 | 10 ± 0.0 | 16.33 ± 0.57 |
|----------------|--------|--------|--------|--------------|--------|--------------|
| **Staphylococcus aureus** (MTCC 96) |        |        |        |              |        |              |
| 100            | -      | 12 ± 0.0 | 15 ± 0.0 | 15 ± 0.0    | -      | 17 ± 0.0     |
| 50             | -      | 11 ± 0.0 | 13.66 ± 0.57 | 13.33 ± 0.57 | -      | 16 ± 0.0     |
| 25             | -      | 10 ± 0.0 | 12 ± 0.0 | 12 ± 0.0    | -      | 14.66 ± 0.57 |
| 12.5           | -      | 11.66 ± 0.57 | 11.33 ± 0.57 | -      | 14 ± 0.0 | 13 ± 0.0     |
| 6.25           | -      | 11 ± 0.0 |          |            |        |              |
| 3.125          | -      | 10 ± 0.0 |          |            |        |              |
| 1.562          | -      |          |          |            |        |              |
| 0.781          | -      |          |          |            |        |              |
| 0.390          | -      |          |          |            |        |              |
| **Pseudomonas aeruginosa** (MTCC 424) |        |        |        |              |        |              |
| 100            | -      | -      | 14.66 ± 0.57 | 15.33 ± 0.57 | -      | 20 ± 0.0     |
| 50             | -      | -      | 13 ± 0.0 | 14 ± 0.0    | -      | 19 ± 0.0     |
| 25             | -      | -      | 12 ± 0.0 | 13.66 ± 0.57 | -      | 18 ± 0.0     |
| 12.5           | -      | -      | 11 ± 0.0 | 12 ± 0.0    | -      | 16.66 ± 0.57 |
| 6.25           | -      | 10 ± 0.0 |          |            |        |              |
| 3.125          | -      |          |          |            |        |              |
| 1.562          | -      |          |          |            |        |              |
| 0.781          | -      |          |          |            |        |              |
| 0.390          | -      |          |          |            |        |              |
| **Streptococcus pneumoniae** (MTCC *655) |        |        |        |              |        |              |
| 100            | -      | 12 ± 0.0 | 20 ± 0.0 | 20 ± 0.0    | -      | 12 ± 0.0     |
| 50             | -      | 11 ± 0.0 | 18 ± 0.0 | 17.66 ± 0.57 | -      | 11 ± 0.0     |
| 25             | -      | 10 ± 0.0 | 16.66 ± 0.57 | 16 ± 0.0 | -      | 10.33 ± 0.57 |
| 12.5           | -      | 14 ± 0.0 | 14 ± 0.0 | 14 ± 0.0    | -      | 10 ± 0.0     |
| 6.25           | -      | 12 ± 0.0 |          |            |        |              |
| 3.125          | -      | 10 ± 0.0 |          |            |        |              |
| 1.562          | -      |          |          |            |        |              |
| 0.781          | -      |          |          |            |        |              |
| 0.390          | -      |          |          |            |        |              |

Abbreviations: PE- Petroleum ether, Chlo- Chloroform, EA- Ethyl acetate, MeOH- Methanol
### Table 3. Showing zone of inhibition of different extracts of *D. falcata* against fungi

| Name of Organisms           | Cons. Mg/ml | Leaf extract | D. falcate | Stem extract |
|-----------------------------|-------------|--------------|------------|--------------|
|                             |             | PE           | Chlo       | EA           | Meoh         | PE           | Chlo       | EA           | Meoh         |
| *Aspergillus niger* (MTCC 281) | 100         | 10 ± 0.0     | 16.33 ± 0.57 | 19 ± 0.0     | 22 ± 0.0     | 12 ± 0.0     | 13 ± 0.0   | 22 ± 0.0     | 21 ± 0.0     |
|                             | 50          | -            | 16 ± 0.0     | 17.33 ± 0.57 | 19 ± 0.0     | 10 ± 0.0     | 12 ± 0.0   | 20 ± 0.0     | 19 ± 0.0     |
|                             | 25          | -            | 13 ± 0.0     | 17 ± 0.0     | 16 ± 0.0     | -            | 10 ± 0.0   | 18.66±0.57   | 18 ± 0.0     |
|                             | 12.5        | -            | 11.66 ± 0.57 | 16 ± 0.0     | 16 ± 0.0     | -            | -          | 17 ± 0.0     | 16.66±0.57   |
|                             | 6.25        | -            | 15 ± 0.0     | -            | -            | 16 ± 0.0     | -          | -            | -            |
|                             | 3.125       | -            | 13 ± 0.0     | -            | -            | 15 ± 0.0     | -          | -            | -            |
|                             | 1.562       | -            | 11 ± 0.0     | -            | -            | 12 ± 0.0     | -          | -            | -            |
|                             | 0.781       | -            | -            | -            | -            | 11 ± 0.0     | -          | -            | -            |
|                             | 0.390       | -            | -            | -            | -            | -            | -          | -            | -            |
| *Candida albicans* (MTCC 183) | 100         | -            | -            | -            | -            | 10.33 ± 0.57 | -          | -            | -            |
|                             | 50          | -            | -            | -            | -            | 10 ± 0.0     | -          | -            | -            |
|                             | 25          | -            | -            | -            | -            | -            | -          | -            | -            |
|                             | 12.5        | -            | -            | -            | -            | -            | -          | -            | -            |
|                             | 6.25        | -            | -            | -            | -            | -            | -          | -            | -            |
|                             | 3.125       | -            | -            | -            | -            | -            | -          | -            | -            |
|                             | 1.562       | -            | -            | -            | -            | -            | -          | -            | -            |
|                             | 0.781       | -            | -            | -            | -            | -            | -          | -            | -            |
|                             | 0.390       | -            | -            | -            | -            | -            | -          | -            | -            |

### Table 4. Antimicrobial activity of ethyl acetate extract of different plant part of *D. falcata* in term of MIC.

| Name of organisms          | E A extract *D. falcata* |
|----------------------------|--------------------------|
|                            | Leaves | Stem |
|                            | MIC mg/ml | MIC mg/ml |
| *Escherichia coli* (MTCC 118) | 0.781 | 0.781 |
| *Klebsiella pneumoniae* (MTCC 39) | 3.125 | 0.781 |
| *Staphylococcus aureus* (MTCC 96) | 3.125 | 0.781 |
| *Pseudomonas aeruginosa* (MTCC 424) | 6.25 | 1.562 |
| *Streptococcus pneumoniae* (MTCC *655) | 3.125 | 3.125 |
| *Candida albicans* (MTCC 183) | - | - |
| *Aspergillus niger* (MTCC 281) | 1.562 | 0.781 |
Table 5 Qualitative examination of secondary metabolites of extracts of *Dendrophthoe falcata* leaves and stem

| S. No. | Phytochemicals | Petroleum ether extract | Chloroform extract | Ethyl acetate extract | Methanol extract |
|--------|----------------|-------------------------|--------------------|----------------------|-----------------|
|        |                | Leaves | Stem | Leaves | Stem | Leaves | Stem | Leaves | Stem | Leaves | Stem |
| 1.     | Alkaloids      | -      | -    | -      | -    | -      | -    | -      | -    | -      | -    |
| 2.     | Flavonoids     | -      | -    | -      | -    | ++     | -    | -      | +    | -      | -    |
| 3.     | Steroids       | -      | -    | -      | -    | +      | -    | -      | -    | -      | -    |
| 4.     | Phenols        | -      | -    | +      | -    | ++     | ++   | ++     | ++   | ++     | +    |
| 5.     | Tannins        | -      | -    | -      | -    | -      | +    | -      | -    | ++     | +    |
| 6.     | Saponins       | -      | -    | -      | -    | ++     | ++   | +      | +    | +      | +    |
Patil et al., (2012) studied antimicrobial sensitivity of different solvent extracts of leaves of D. falcata growing on M. indica at the concentration of 200 mg/ml. Different extracts of leaves displayed antimicrobial activity against the tested bacterial (S. aureus, E. coli and P. aeruginosa) and fungal (A. niger and C. albicans) strains with the diameter of zone of inhibition ranging between 10 mm to 14 mm while the different extract of leaves of D. falcata growing on B. serrata found to be most active against same tested bacterial and fungal strains with zone of inhibition ranging between 10mm to 22mm at the concentration of 100 mg/ml. The present finding demonstrate that D. falcata that growing on B. serrata shows better antimicrobial activity than growing on M. indica.

The present study demonstrate that, plant extracts in ethyl acetate and methanol provided a good zone of inhibition while other two extracts were found to be less active against the tested organisms (Table 2 and 3). The present investigation clearly establishes the antimicrobial potential of the plant and suggests the need to further exploit in the management of microbial diseases caused by these bacteria in humans. From the result obtained it supports the folkloric usages of D. falcata as a therapeutic agent. Further phytochemical investigation suggests that all the extract contain certain constituents with antimicrobial properties that can be used as antimicrobial agents in new drug for the therapy of infectious diseases caused by pathogen.

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