Preliminary Phytochemical and Antimicrobial Investigations on Melia Dubia Bark

Department of Pharmaceutical Chemistry, J.S.S. College of Pharmacy, Rocklands, P.B. No.20, Ootacamund – 643 001, Tamilnadu

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Abstract : The various parts of Melia dubia (Meliaceae) plant was observed to be used by the local tribes of Nilgiris for various infections. There was no report on antimicrobial activity of Melia dubia. Therefore, a preliminary phytochemical analysis and antimicrobial investigations were carried out on different extracts of Melia dubia bark. Ethanolic and aqueous extracts of the bark were found to posses significant antibacterial activity against Staphylococcus aureus.

INTRODUCTION

The use of plant-based medical therapeutics by primitive man is as old as the history of man himself. As civilization advances, these practices tend to disappear from our sight. Therefore, a great deal of awareness is generated among the scholars to focus immediate attention on plant based crude drugs and validate their folk claims for their phytochemical and pharmacological properties. Since the Nilgiri district is a treasure-house for medicinal plants in South India harbouring both indigenous and exotic medicinal flora of good therapeutic value, we felt it is worthwhile to investigate certain select medicinal plants of Nilgiris. It was during the course of the ethno pharmacological survey, we have noticed that the local tribal groups of Nilgiris district are using various parts of Melia dubia (Meliaceae), commonly referred to as Hill Neem, Malai Vembu, Munnattikaraka, etc., for wide array of skin infections of microbial origin and other ailments related to gastro-intestinal tract.

Literature reveals that fruits of Melia dubia is considered to be important in colic and skin diseases and also as anthelmintic. Leaves and seeds of this plant were reported to possess two tetranotriterpenoids, compositin and compositolide. Fruits gave the bitter principle, Salannin and heartwood yielded a triterpenoid. There have been no previous reports of screening for any biological and pharmacological properties of this plant in spite of its curative potential as observed from the literature. We therefore report the preliminary phytochemical and antimicrobial evaluation studies on the bark part of this plant in this communication.

MATERIALS AND METHODS

COLLECTION AND EXTRACTION

The trunk bark of Melia dubia was collected from the forest near Burliar of Niligiri district, Tamilnadu. The plant was identified by the locals at Burliar and authenticated at the Botanical Survey of India, Coimbatore. A voucher specimen has been deposited at the Department of Pharmacognosy of our institution. It was dried in shade, coarsely powdered in a disintegrator and the dried powder (400 gms) obtained was successively extracted with petroleum ether (60-80°), ethyl acetate, ethanol 95% and water. The estimation of percentage extractive values, behavior of the bark powder with different chemical reagents, physio-chemical and
phytochemical nature of different extracts of bark were conducted by standard methods\textsuperscript{5a,b,6} and the results are presented in Tables 1-5, respectively.

The crude extracts thus obtained were concentrated under vacuum at 30\(^{0}\)C. The final products which weighed around 9 gms. in each case were stored at 4\(^{0}\)C prior to testing.

**ANTIMICROBIAL EVALUATION**

The crude extracts were reconstituted in dimethyl sulphoxide and tested for antimicrobial activity. Antibacterial activity of the extracts was determined by Minimum Inhibitory Concentration method\textsuperscript{7}, Minimum Cidal Concentration method\textsuperscript{7} and Cup plate method\textsuperscript{8} against two gram positive bacteria, \textit{Bacillus subtilis} and \textit{Staphylococcus aureus} and two gram negative bacteria, \textit{Escherichia coil} and \textit{Pseudomonas aeruginosa}. In case of cup-plate method, the test samples were employed at the concentrations of 5mg/ml and 10 mg/ml using Chloramphenicol (100µg/ml) as reference standard. Antifungal activity was also determined in similar way against two fungi, \textit{Candida albicans} and \textit{Aspergillus flavus} using Clotrimazole (100µg/ml) as the reference standard and the results are presented in Tables 6 and 7.

**RESULTS AND DISCUSSION**

Results on preliminary phytochemical studies indicate that all the present extracts were found to be acidic in nature. Ethanolic extract was found to contain alkaloids while aqueous extract gave tests for the presence of carbohydrates, glycosides, phenolic compounds tannins, gums and mucilages.

Antibacterial evaluation studies revealed that the extracts under investigation were found to be completely ineffective against all the bacteria employed, except the gram positive bacteria, \textit{Staphylococcus aureus}, up to the dose as high as 10,000 µg/ml. The minimum inhibitory effect and minimum cidal effect of different extracts against different tests organisms have been performed and the results indicate all the extracts found to possess significant inhibitory effect and at two fold concentration significant cidal effect against the gram positive bacteria, \textit{Staphylococcus aureus} only, while they are completely ineffective against the rest of the organisms employed. It could be noted that the results obtained in all the three methods were in agreement with one another. However, the antibacterial activity elicited by the extracts against S.aureus was not comparable with that of the standard, chloramphenicol which may be because of the crude nature of the samples and the antibacterial action of the extracts was found to be dose dependent.

Of all the extracts studied, aqueous extract and ethanol extract were found to be superior in their antibacterial action which may be attributed to the presence of phenolic compounds and alkaloids respectively followed by ethyl acetate extract while the petroleum ether extract being the least effective one against S.aureus.

Surprisingly, all the extracts were completely devoid of any action against the two fungi employed.

**CONCLUSIONS**

1. Preliminary phytochemical evaluation of the extracts of bark revealed the presence of alkaloids in ethanolic extract and the presence of carbohydrates, glycosides, phenolic compounds,
tannins, gums and mucilages in aqueous extract.

2. All the extracts were effective against *Staphylococcus aureus* only while they did not show any activity against rest of the organisms employed.

3. Aqueous and ethanolic extracts showed better antibacterial profile, however it was not comparable with that of the standard, chloramphenicol.

4. None of the extracts inhibited the growth of *Candida albicans* and *Aspergillus flavus*.

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REFERENCES

1. *Dictionary of Indian Medicinal Plants*, Central Institute of Medicinal Aromatic Plants, Lucknow, 292, (1992).

2. Purushothaman, K. K., Duraiswamy, K. and Conolly, J. D., Phytochemistry, 23 (1), 135 – 137, (1984).

3. De Silva, L.B., Stocklin, W. Geissman, T.A., Phytochemistry, 8, 1817 – 1819, (1969).

4. Madhusudana Rao, M., Murali Krishna, E., Gupta, P.S. and Singh, P.P., Indian J.Chem., 16B, 825 – 826, (1978).

5. Pharmacopoeia of India, Controller of Publications, New Delhi, 3rd edition, Vol.II (1985), a) A.73-76; b) 63.

6. Harborne, J.B., Phytochemical Methods, Jackmann Hall, London, 1-34, (1973).

7. Irobi, O.N., Moo-Young, M. and Anderson, W.A., Int. J. Pharmacog., 34(2), 87- 90, (1996).

8. British Pharmacopoeia, Pharmaceutical Press, London, 796, (1953).

**TABLE – 1**

| Extractive Value | Chloroform – water | Alcohol (Ethanol) |
|------------------|--------------------|------------------|
|                  | 10.027 % w/w       | 14.526 % w/w     |
### TABLE – 2
**Ash values of bark of Melia dubia**

| Ash values          | % w/w |
|---------------------|-------|
| Total ash           | 5.2   |
| Acid insoluble ash  | 0.123 |
| Water soluble ash   | 1.621 |
| Sulphated ash       | 4.572 |

### TABLE – 3
**Behaviour of powder with different chemical reagents**

| S. No. | Treatment                        | Observation        |
|--------|----------------------------------|---------------------|
| 1      | Powder (neat)                    | Reddish brown      |
| 2      | 1N NaOH                          | Dark brown         |
| 3      | Picric acid                      | Yellowish brown    |
| 4      | Acetic acid                      | No change          |
| 5      | 1N HCl                           | No change          |
| 6      | 1N HNO3                          | No change          |
| 7      | 5% Iodine                        | Yellowish brown    |
| 8      | 40% NaOH + few drops of lead acetate | Pinkish brown |
| 9      | HNO3 + Ammonia solution          | Dark brown         |

### TABLE – 4
**Successive solvent extraction of air-dried plant material**

| S. No. | Extracts    | Colour          | Consistency | pH    |
|--------|-------------|-----------------|-------------|-------|
| 1      | Petroleum ether | Dark brown     | Semisolid   | Acidic |
| 2      | Ethyl acetate     | Yellowish brown| Solid       | Acidic |
| 3      | Ethanol          | Reddish brown  | Semisolid   | Acidic |
| 4      | Water            | Black          | Solid       | Acidic |

### TABLE – 5
**Qualitative chemical examination of various extracts**

| Plant constituent | Extracts |
|-------------------|----------|
| Test / Reagent used | Pet. Ether (60º -80º) | Ethyl acetate | Ethanol | Water |
| 1. Alkaloids       |          |               |         |
| a. Mayer’s reagent | -        | -             | +       | -     |
| b. Dragendorf’s reagent | -    | -             | +       | -     |
| c. Hager’s reagent | -        | -             | +       | -     |
2. Carbohydrates & glycosides
   a. Molisch’s reagent
   b. Fehling’s solution
   c. Barfoed’s test
   d. Benedict’s reagent
   e. Brontrager’s test

3. Phenolic compounds & tannins
   a. Ferric chloride solution
   b. Gelatin solution
   c. Lead acetate solution
   d. Aq.bromine solution

4. Gums & Mucilages
   a. Alcoholic precipitation
   b. Molisch’s test

### TABLE – 6
Antibacterial activity of MIC method & MCC method against *S.aureus*

| Extracts         | Mic (µg/ml) | MCC (µg/ml) |
|------------------|-------------|-------------|
| Petroleum ether  | 5,000       | 10,000      |
| Ethyl acetate    | 5,000       | 10,000      |
| Ethanol          | 2,500       | 5,000       |
| Water            | 2,500       | 5,000       |

### TABLE – 7
Antibacterial activity by cup plate method against *S.aureus*

| Extract          | Concentration used (µg/ml) | Zone of inhibition (mm) | Average |
|------------------|----------------------------|-------------------------|---------|
| Petroleum ether  | 5,000                     | 18 18 18 18            | 18.33   |
| Ethyl acetate    | 5,000                     | 18 17 17 17            | 17.33   |
| Ethanol          | 5,000                     | 23 24 24 24            | 23.33   |
| Water            | 5,000                     | 16 21 22 22            | 19.67   |
| Chloramphenicol  | 10                        | 32 33 33 33            | 32.33   |