

Investigation of Ethanol Soluble Bioactive Compounds in Tender Fruits of *Thespesia Populnea*

Mitter C. S.

Department of Zoology, Sathaye College, Vile-Parle (E), Mumbai, 400 057, Maharashtra, India

Abstract: Hot and cold extracts of tender fruits of *Thespesia populnea* were made in ethanol and evaluated against ten Gram positive and Gram negative human bacteria by Agar cup method. Both the extracts were effective against *Bacillus subtilis*, *Streptococcus pyogenes*, *Corynebacterium diphtheriae* and *Klebsiella pneumonia* whereas *Pseudomonas aeruginosa* was susceptible to only cold extract. Minimum Inhibitory Concentrations (MIC) of cold and hot extracts, Activity guided fractionations showed comparable antimicrobial activity with antibiotics, Gentamicin (G-10mcg) and Ampiclox (ACX-20mcg).

Keywords: *Thespesia populnea*, Activity guided fractionations, MIC, antimicrobial activity, human bacteria.

1. Introduction

Plants have a great potential for producing new drugs for human benefit. The use of medicinal plants as source of drugs is as old as mankind. The demand for more and more drugs from plant sources is continuously increasing. More than 50% of drugs used in western pharmacopeia are isolated from plants (Ahmad *et al.*, 1989). The plant extracts provide natural antimicrobial substances (Del Campo *et al.*, 2000). Ghose *et al.*, (2007) studied antimicrobial properties of *Terminalia bellirica*, *T. chebula*, *Emblica officinalis*, *Punica granatum*, and *Lawsonia inermis*. Plant *Vitex negundo* is with antimicrobial properties (Panda *et al.*, 2009, P. Sumathi and Parvathi., 2010).

2. Literature Survey

Thespesia populnea is an evergreen middle sized tree, indigenous to India. It is commonly known as Portia tree, belonging to family Malvaceae. It is used as folklore medicine in the treatment of several diseases. It is an effective remedy for scabies, psoriasis, skin diseases, dysentery, piles, diabetes (Joshi, 2000). A flavonoid of *Thespesia populnea* is isolated from fruits, gossypol, from flowers. (Khare, 2007) Biochemical analysis also shows presence of populneol, populin, herbacetin and calycopterin (Rastogi and Malhotra, 1995). Fruits of this plant are helpful in wound healing (Nagappa and Cheriyan, 2001). Phytochemical screening of methanol extracts of flower of this species reveals the presence of alkaloids, flavanoids, tannins, gums and mucilage (Saravankumar *et al.*, 2009). It is therefore essential to evaluate higher plants for medicinal value systematically for various human infections caused by bacteria and determining whether the use in folklore medicine is justified. In view of this present study is an attempt to investigate the bioactive principles from tender fruits of *Thespesia populnea*.

3. Materials and Methods

Tender fruits of *Thespesia populnea* were collected from Sanjay Gandhi National park Mumbai, Maharashtra (India) in the month of April. It was taxonomically identified by an expert taxonomist. Fruits were washed thoroughly under the

running tap water in order to remove dirt and other contaminants. Some fresh fruits were kept for cold extract while remaining air dried till crisp at 40°C - 45°C. The dried material was then ground to fine powder, sieved through 100 μ mesh sieve and store in clean glass jars for further use.

3.1 Test Microorganisms and Media:

The clinical isolates used for antimicrobial testing were obtained from Haffkine's Institute, Parel, Mumbai. The microorganisms selected were four gram positive bacteria i.e. *Bacillus subtilis*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Corynebacterium diphtheria* and six gram negative bacteria i.e. *Klebsiella pneumoniae*, *Salmonella typhi*, *Salmonella paratyphii-A*, *S. paratyphii-B*, *Pseudomonas aeruginosa* and *Escherichia coli*. They were maintained on nutrient agar at 4°C and sub-cultured at regular time period. These microorganisms were inoculated in liquid medium to obtain 24hrs growth culture for bioassay.

3.2 Extraction Preparation (50 % w/v)

Cold extract was made by homogenizing 10 gm of tender fruit in mortar and pestle using ethanol and was kept aside for 45 minutes for extraction. It was then filtered and made up to 20ml. Hot extract (50% w/v) were prepared from dry powder of tender fruits by soxhlet apparatus (Vogal, 1958). All extracts were stored in air tight glass bottle at 4°C in refrigerator for further use.

3.3 Bio-Assay

Agar cup method was employed for primary screening (Spooner and Sykes, 1972). 0.4ml of suspension was mixed with 20-25ml of nutrient agar and poured in a sterile petriplate. The plate was kept on flat surface and allowed to solidify. When the nutrient agar had set, wells were made with a sterile 8 mm cork borer as per requirement. In each well 80 μ of sample was loaded using micropipette. One of the well contained equal quantity of the pure solvent in which extract served as negative control. All the tests were carried out in triplicate and the average values for zones of Inhibition in millimeters (mm) were taken after 24 hrs and 48 hrs of incubation at 37°C or room temperature depending upon the culture used. Based on primary screening results

the extracts giving more than 16mm zone of inhibition were selected for MIC.

Hot and cold extracts of ethanol evaporated to dry residues and using fresh solvents, different concentrations viz. 0.5 mg/ml, 1 mg/ml, 5 mg/ml and 10 mg/ml were made respectively. The bioassay of all the concentrations was carried out by Agar cup method as mentioned above. The results were observed after 24 and 48 hrs as zones of inhibition in mm.

3.4 Activity Guided Fractionation

The Eluotropic series as suggested by Trappe (Eloff, 2004., Kirchner, 1978) was employed for fractionation using polar to non-polar solvents. Hot and cold ethanol extracts were evaporated in Rota evaporator to dryness and residues collected were serially dissolved in different solvents in the order of petroleum ether, chloroform, benzene, ethyl acetate, acetone, ethanol, methanol and water centrifuged at 3000 rpm for 30 min, collected the filtrates and designated as fraction "a" to "g" respectively. Bioassay screening was applied to the entire different fractions and compared with antibiotics, Gentamicin (G-10mcg) and Ampiclox (ACX-20 mcg).

4. Results

Cold extract exhibited better zone of inhibition (ranged between 14-22 mm) than hot (ranged between 13-18 mm) (Table: I). Maximum zone of inhibition was noted against *C. diphthiae* (22 mm), *S.aureus* (21mm) followed by *P. aeruginosa* (20 mm) and *B. subtilis* (18 mm) by cold extract and *C. diphthiae* (18 mm), *B. subtilis* (17 mm) and *S. aureus* (17 mm) by hot extract. *S. typhi*, *S. paratyphii-A*, *S. paratyphii-B*, and *E. coli* were resistible to hot and cold extracts. MIC of cold and hot extracts were displayed at 1 mg/ml against susceptible pathogen (Table- II).

Among Activity Guided Fractionations various polar and non-polar fractions viz. acetone, ethanol, methanol, petroleum ether and benzene showed inhibitory activity with different potency against susceptible organisms. These findings support active principles are soluble in polar as well as in non polar solvents. Chloroform, ethyl acetate, and water fractions did not show inhibitory activity against susceptible clinical isolates. Isolated fractions are comparable with antibiotics, Gentamicin and Ampiclox (Table- III).

5. Discussion

Bioassay results indicated that tender fruits of *T. populnea* contain wide range of antimicrobial principles. These findings are in agreement with Saxena (2010), Sarvankumar (2009) and Jhurani and Jadhav (2010). A few reports noted similar results in activity guided fractionation of other mangrove plants occurring along Mumbai coast (Pagare, 2003, Shah 2005), which supports the present finding. Both cold and hot extracts recorded antimicrobial activity against susceptible microorganisms, indicating bioactive principles are thermolabile and thermostable.

6. Conclusion

It is suggested that ethanol is the best solvent for the extraction of antimicrobial principles from mangrove plants. The present scientific investigation confirms the bioactive compounds present in this plant are comparable with antibiotics, Gentamicin and Ampiclox. Therefore it is suggested that the isolated fractions from *T. populnea* may serve as useful antimicrobial in infectious diseases. However, further studies are required to isolate and determine actual bioactive principles in this plant part.

References

- [1] Ahmad F. B. and Salleh K. M. (1988). "Penyaringan fitokimia tumbuhan Sabah" proceedings. Seminar on Malaysia Traditional Medicine, Soepadmo, E. p 147-158.
- [2] Del Campo J, Amiot MJ, Nguyen C. (2000). Antimicrobial effect of Rosemary extract. J. Food Product. 63: 1359 -1368.
- [3] Eloff J. N. (2004). Quantification of the bioactivity of plant extracts during screening and bioassay guided fractionation. *Phytomedicine: International Journal of phytotherapy and phytopharmacology*, **60**: 1-8.
- [4] Jhurani B. S. A. and Jadhav B. L. (2010). Evaluation of Antimicrobial Properties and Activity Guided Fractionation of Mangrove species *Rhizophora Apiculata*. *Asian Jr. of Microbial Biotech. Env. Sc.*, 12(4): 1-4.
- [5] Joshi S. G. (2000). Medicinal plants, Oxford and IBS Publishing Co. Pvt. Ltd, New Delhi, Culcutta. Pp. 205.
- [6] Khare C.P. (2007) Indian medicinal plants: An illustrated dictionary. **Pub:** Springe, 659
- [7] Kirchner J. G. (1978). Developing the Chromatogram, **In:** Thin Layer Chromatography. (2nd Ed.). Techniques of Chemistry. **Pub:** John Wiley and Sons. **XIV**, 250-253.
- [8] Nagappa and Cheriyan (2001). Wound healing activity of the aqueous extracts of *Thespesia populnea* fruit. *Fitoterapia*, **72(5)**: 503-506.
- [9] Pagare B.G. (2003). Studies on the bioactive constituents of *Exocoecaria agallocha*. **Ph.D.** Thesis (Life Sciences), University of Mumbai, Mumbai-400098.
- [10] Pande S.K, Thatol H.N, and Dutta S.K. (2009). Antibacterial activity and phytochemical screening of leaf and bark extracts of *Vitex negundo* from Simlipal biosphere reserve, Orissa. *Journal of Medicinal Plants Research* **Vol.** 3(4), pp. 294-300.
- [11] Rastogi R.P, Malhotra B.N. (1995). Compendium of Indian Medicinal Plants. Vol-4 New Delhi: National Institute of Science Communication.
- [12] Saravanakumar, Venkateshwaram A. K., Vanita J., Ganesh M., Vasudevan M. and Sivakumar (2009). Evaluation of Antibacterial Activity. Phenol and Flavonoid contents of *Thespesia populnea* flower extracts. *Pakistan Journal of Pharmacology Science*, **22(3)**: 282-286.
- [13] Saxena P.K, Mishra S.B, Das T and Maurya A. (2010). Phytochemical investigation and antimicrobial activity of stem bark extracts of *Thespesia populnea*.

International Journal of Pharmacy Research. Vol 1(1), pp. 24-29.

- [14] Shah N.J. (2005). Pharmacognosy studies on methanol extracts of Mangrove species *Aegicerus corniculatum*. Ph.D. Thesis (Life Sciences). University of Mumbai, Mumbai- 400 098.
- [15] Sumanthi P. and Parvathi A. (2010). Antimicrobial activity of some traditional medicinal plants. *Journal of Medicinal Plants Research* Vol. 4(4), pp. 316-321.
- [16] Sykes G. and Spooner D.F. (1972). Laboratory assessment of antimicrobial activity. **In:** Methods of Microbiology. Norris J.R., and Ribbons D.W. (Ed.) **Pub.:** Academic press, London. **7B:** 217-224.
- [17] Vogel A. I. (1958). Experimental Technique. **In:** Practical organic chemistry (3rd Ed.). **Pub.:** English Language Book Society, 152-154.

Table 1: Primary screening of ethanol extracts of tender fruits on microorganism.

| Cold Extract | | | | | | | | | |
|--------------|--------------|------------|------------|------------|------------|------------|-------------|-------------|---------------|
| <i>B.sub</i> | <i>S.pyo</i> | <i>S.a</i> | <i>C.d</i> | <i>K.p</i> | <i>E.e</i> | <i>S.t</i> | <i>St.A</i> | <i>St.B</i> | <i>Ps.aur</i> |
| 18 | 14 | 21 | 22 | 17 | - | - | - | - | 20 |
| Hot Extract | | | | | | | | | |
| 17 | 15 | 17 | 18 | 13 | - | - | - | - | - |

Keywords: - no zone inhibition.

Table 2: MIC of ethanol extracts of tender fruits

| Extracts | Organisms | Dilution of extract mg/ml | | | |
|---------------|-----------------------|---------------------------|----|----|-----|
| | | 10 | 5 | 1 | 0.5 |
| Cold extracts | <i>B. subtilis</i> | 20 | 16 | 14 | - |
| | <i>S.aureus</i> | 20 | 15 | 12 | - |
| | <i>Kl. Pneumonia</i> | 14 | 12 | 11 | - |
| | <i>C. diphtheriae</i> | 19 | 14 | 11 | - |
| | <i>Ps. aeruginosa</i> | 17 | 15 | 11 | - |
| Hot Extracts | <i>B. subtilis</i> | 19 | 16 | 12 | - |
| | <i>S.aureus</i> | 16 | 14 | 11 | - |
| | <i>C.diphtheriae</i> | 17 | 14 | 11 | - |

Keywords: - no zone inhibition.

Table 3: Effects of bioactivity guided fractions of tender fruits of *T. populnea* ethanol extracts on microorganisms (Inhibition zones in mm)

| Organisms and extracts | Fr. a Pet ether | Fr. b Benzene | Fr. e Acetone | Fr. f Ethanol | Fr. g Methanol | Gentamicin | Ampiclox |
|--------------------------|-----------------|---------------|---------------|---------------|----------------|------------|----------|
| <i>B. subtilis</i> (H) | 12 | - | 14 | 15 | 17 | 17 | 20 |
| <i>S.aureus</i> (H) | 12 | 11 | 14 | 16 | 17 | 16 | 19 |
| <i>C.diphtheriae</i> | 11 | 12 | 13 | 12 | 14 | 15 | 19 |
| <i>Kl. Pneumonia</i> (C) | - | - | 12 | 15 | 16 | NT | NT |
| <i>C.diphtheriae</i> (C) | 12 | 11 | 14 | 15 | 19 | 16 | 17 |
| <i>B. subtilis</i> (C) | 13 | 12 | 14 | 16 | 16 | 17 | 19 |
| <i>Ps. aure</i> (C) | - | - | 13 | 15 | 15 | NT | NT |
| <i>S.aureus</i> (C) | 11 | 12 | 12 | 13 | 13 | 17 | 20 |

Author Profile



Prof. (Dr.) Chhaya S. Mitter is Head of Zoology Department, Sathaye College, Vile Parle (E), University of Mumbai, Mumbai-400057, Maharashtra, India. Associate Professor for 26 years. Successfully

completed university research projects. Published various research papers. Worked on various bodies in the college and university. Active in social fields. Worked as associate editor for Bio-chemical Science.