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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 pyogens, Corynebacterium diphtheriae and Klebseilla 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 Cheriyen, 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 40ºC - 45ºC. The dried material was then ground to fine powder, sieved through 100µ 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 pyogens, Staphylococcus aureus, Corynebacterium diphtheria* and six gram negative bacteria i.e. *Klebseilla pneumoniae, Salmonella typhi, Salmonella paratyphii-A, S. paratyphii-B, Pseudomonas aeruginosa* and *Escherichia coli.* They were maintained on nutrient agar at 4ºC 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 4ºC 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 80µ 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 37ºC 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. diphthriae* (22 mm), *S.aureus* (21mm) followed by *P. aeruginosa* (20 mm) and *B. subtilis* (18 mm) by cold extract and *C. diphthriae* (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 Fractionation**s** 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.

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

Cold Extract									
B.sub	***S.pyo***	***S.a***	***C.d***	***K.p***	***E.c***	***S.t***	***St.A***	***St.B***	***Ps.aur***
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	*B. subtilis*	20	16	14	_
	S.aureus	20	15	12	_
	Kl. Pneumonia	14	12	11	_
	C. diphtheriae	19	14	11	_
	Ps. aeruginosa	17	15	11	_
Hot Extracts	*B. subtilis*	19	16	12	_
	S.aureus	16	14	11	_
	C.diphtheriae	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*
B. subtilis(H)	12	_	14	15	17	17	20
S.aureus (H)	12	11	14	16	17	16	19
C.diphtheriae	11	12	13	12	14	15	19
Kl. Pneumonia (C)	_	_	12	15	16	NT	NT
C.diphtheriae(C)	12	11	14	15	19	16	17
B. subtilis(C)	13	12	14	16	16	17	19
Ps. aure (C)	_	_	13	15	15	NT	NT
S.aureus (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.