Intl. J. Bioinformatics and Biological Sci., 4(2): 77-83, December 2016

DOI: 10.5958/2321-7111.2016.00010.X

Study of Phytochemical Analysis, Antimicrobial Properties of *Flacourtia indica* (Burm.f.) Merr. and its *In vitro* Shoot Culture

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ABSTRACT

Herbal medicinal plant played an important role since ancient times in treating various chronic diseases. Frequent use of pharmaceutical drugs and its side effects have led to more research based study on traditionally available plants and folk remedies. *Flacourtia indica* (Burm.f.) Merr. is one such medicinally important plant which is commonly distributed Bangladesh, few parts of India and is becoming threatened day by day. It has immense potentiality as a medicinal plant and possess beneficial effects such as Antiasthmetic, antimalarial, antioxidant, hepatoprotective, antidiabetic, antianxiety, diuretic etc. These activities can be attributed mainly to the presence of various bioactive compounds or phytochemicals. The aim of this paper is to find out the phytochemical constituent of this plant, antimicrobial properties and standardize the *invitro* shoot culture techniques which will help to preserve the plant species and summarize the importance of this plant containing potent medicinal value and the research work being carried out till now.

Keywords: Medicinal plant, chronic diseases, folk remedies, bioactive compounds, phytochemicals, *invitro* shoot culture.

The use of medicinal plant to treat various deadly diseases is a time tested practice. Plants have the capacity to synthesize a wide variety of bioactive compounds that are used to defend from various disease causing organism. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total.[1][2] These phytochemical compound work on the human body in the same way what a conventional medicine do but decrease the chance of side effects.

Flacourtia indica (Burm.f.) Merr. commonly known as "Boichi" or "Katai" or "Indian plum" is an indigenous medicinal plant commonly distributed in Bangladesh, few parts of India and west Bengal.[3] This is a bushy shrub with a spiny trunk and branches belonging to the family Salicaceae and it becoming threatened day by day. The fruit itself is a pome, purplish in color and sweetish with an acidic tang in taste.

The plant has been used as an effected remedy of various diseases by various tribal communities from ancient age. Fruits are used as appetizer, diuretic, digestive, barks are used for the treatment of fever, roots are used in nephritic colic, gum is used in the treatment of cholera.

The modern pharmacological studies demonstrated that *F.indica* possess wide range of medicinal properties viz. Antiasthmetic, antimalarial, antioxidant, hepatoprotective, antidiabetic, antianxiety, diuretic [4],[5],[6],[7],[8],[9],[10] etc. and it may possess lots of other potentialities that haven't revealed yet.

On this current scenario the plant can be considered as the rich and valuable source of unique phyto compounds which can help to develop medicine against various diseases. To meet the purpose *invitro* shoot multiplication, phytochemical analysis, antimicrobial properties was studied.

MATERIALS AND METHODS

Phytochemical Analysis: Water extraction of leaf, stem, root of healthy plant subjected for phytochemical analysis by using standard procedures [11,12]. Explants were collected from a healthy plant. Washed thoroughly by tap water for 20 min and weighed 5 gm for each and crushed and ground by using mechanical grinder and filtered by filter paper. Each crude extract was then subjected for various phytochemical tests as followed:

- **Test for Tannins:** 0.5 g of the dried powdered samples was boiled in 20 ml of water, in a text-tube and then filtered. A few drops of 0.1 % Ferric chloride was added and observed. Brownish-green or blue-black color indicates the presence of tannins.
- **Test for Saponins:** 2g of the powdered samples were boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with three drops of olive oil and shaken vigorously and formation of emulsion was observed for the presence of saponins.
- **Test for Flavonoids:** 5 ml of dilute ammonia were added to a portion of the aqueous filtrate of each of plant extract followed by addition of 2ml concentrated H_2SO_4. A yellow coloration observed in each extract indicated the presence of flavonoids.
- **Test for Cardiac Glycosides:** 5ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was added with 1ml of concentrated sulphuric acid. A brown ring of the interface indicated a deoxysugar characteristic of cardenolides.
- **Test for Alkaloids (Harbone's test):** The method of Harbone (1973) was used in this test. 5g of the sample was weighed into a 250 ml beaker and 200ml of 10 % acetic acid in ethanol was added and covered, and allowed to stand for 4 hrs. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue, which was the alkaloids, was dried and weighed.
- **Test for Steroids:** 2ml of acetic anhydride was added to 0.5 g of ethanol extract of root, stem and leaf with 2ml H_2SO_4. The color changed from violet to blue or green in some samples indicated the presence of steroids.
- **Test for Terpenoids:** 5ml of each extract was mixed in 2ml of chloroform and concentrated H_2SO_4 and after which 3ml was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive result for the presence of terpenoids.
- **Test for phlabatannins:** Plant powder sample was mixed with distilled water in a test tube, shake it well, and filtered to take plant extract. Then 1% aqueous hydrochloric acid was added and each plant sample was then boiled with the help of Hot plate stirrer. Formation of red colored precipitate confirmed a positive result.
- **Test for anthraquinone (Borntrager's test):** A few drops of Magnesium acetate solution were added to the extract and formation of pink color shows the presence of anthraquinone.
- **Test for phenol:** Few drops of ferric chloride solution were added into 2 ml of test sample. Bluish green or red color indicates the presence of phenol.

- **Test for fixed oil (Spot test):** A small quantity of extract was passed between the filter papers. Formation of grease spot indicates the presence of fixed oils.

Antimicrobial activity

Extraction of leaf, stem, root of *invivo* grown plant was prepared by using various solvent viz. sterilized water, methanol, ethyl acetate, acetone. Those plant part was washed by running tap water and then shed dried for 48 h and ground into fine powder my using mechanical grinder. 5 gm of each powder sample was then mixed with 10 ml of various solvent. NA agar (nutrient agar) plate and PDA agar (potato dextrose agar) plate was prepared, inoculated by microorganism and studied for antimicrobial activity against different microorganism like *E.coli, Bacillus sp., Aspergillus niger, Curvularia lunata* and observed after24-48 hrs.

Comparative study of antibacterial activity

Fresh leaf, stem, root were collected from the *invivo* grown plant of *F.indica* .Those plant parts were then washed by running tap water for 20 min. Then those was subjected for distilled water washing. There after explants were allowed to shade dry for 48-72 h.10gm of each individual was weighed and ground into fine powder by using mechanical grinder and 10 ml of ethyl acetate was added to each powdered sample of *F.indica* leaf, stem, root.

MHA (Muller Hinton Agar) plates were prepared. Agar (1% Hi-media, Mumbai) was added and then P^H of MH media was adjusted to 5.8 prior to autoclaving. *E.Coli* suspension was prepared by using sterilized distilled water and it was inoculated in MH plate by "spread plate technique". 100µl of each Ethyl acetate extract of leaf, stem, root were given seperately in each palte (agar cup method) along with same volume of Amoxicillin (Mox 500), Ciprofloxacin (Cifran 500), Savlon in different wells.

In vitro shoot culture

F.indica (Burm.f.)Merr. plant was obtained from the Golapbag BU campus West Bengal India. Shoot apex and nodal segment explants of 1-1.2 cm were collected from a young plants grown in green house condition (temp28±5). They were watered twice a day. Explants were washed with running tap water for 20 min, immersed in 70% alcohol for 30secs, washed by doubled distilled water, sterilized by 0.01% tween 20 solution for 6 mins, washed twice with sterilized distilled water, immersed in 0.1%(w/v) $HgCl_2$ for 2-5 min and then washed by sterile distilled water. *F.indica* explants were then inoculated on MS medium (Murashige and Skoog, 1962), supplemented with different combination and concentration of BAP/6-Benzyl aminopurine (0.5 mg/l, 1 mg/l, 2mg/l, 3mg/l, 4mg/l, 5mg/l, 6mg/l, 7mg/l) and fixed concentration of NAA/Nicotinic acid (0.5 mg/l). Agar (0.8% Hi-media, Mumbai) was added and then p^H of MS media was adjusted to 5.8 prior to autoclaving. Those cultures were maintained at a temperature of 25± 2^0 C and 16 hrs photoperiod. The shoot and nodal explants after 30-35 days showed caulogenesis(shoot induction) as well as morphogenesis. The proliferated shoots were separated and re inoculated on MS medium. The whole procedure was repeated thrice.

Callus induction

Contamination free plant twig was obtained from the *invitro* grown shoot of *F.indica* (Burm.f.)Merr. Internodal segment explants of 0.5-1 cm were collected from the shoot twig. *F.indica* explants were then inoculated on MS medium (Murashige and Skoog, 1962), supplemented with different combination and concentration of 2,4-D/2,4 dichlorophenoxy acetic acid(0.5 mg/l, 1 mg/l, 2mg/l, 3mg/l, 4mg/l, 5mg/l,6mg/l).

Agar (0.8% Hi-media, Mumbai) was added and then p^H of MS media was adjusted to 5.8 prior to autoclaving. Those cultures were maintained at a temperature of 25± 2° C and 16 hrs photoperiod. The explants after 25-30 days showed greenish callus tissue. The callus was subjected for sub culturing on MS media again with same PGR concentration as said before.

RESULTS AND DISCUSSION

Table 1: Phytochemical analysis of *F.indica*

Test	Leaf	Stem	Root
Tannins	++	+++	+
Saponins	-	-	-
Flavonoids	+++	++	-
Cardiac Glycosides	+++	++	++
Alkaloids	++	+	+
Steroids	+++	++	-
Terpenoids	-	-	-
Phlabatannins	-	-	-
Anthraquinone	-	-	-
Phenol	-	-	-
Fixed oil	-	-	-

+ = indicates presence of phytochemicals; ++ = shows moderate concentration; +++ = shows high concentration; - = indicates absence of phytochemicals.

The qalitative analysis and visual observation of aqueous leaf extract of *F.indica* shows the presence of high concentration of flavonoids, cardiac glycosides, steroids; moderate amount of alkaloids and tannins.

The aqueous stem extract of *F.indica* shows the presence of high concentration of tannins, moderate amount of flavonoids, cardiac glycosides, steroids and low concentration of alkaloids.

The aqueous root extract of *F.indica* shows the presence of moderate concentration of cardiac glycosides and low concentration of tannins, alkaloids.

Ethyl acetate extract of leaf(a),stem(b),root(c) and aqueous leaf extract showing a clear zone of inhibition against *E.coli* with 3.1mm, 3.0mm, 3.3mm, 2.9mm respectively along with its *E.coli* control plate(ethyl acetate) containing 2 mm zone of inhibition. Aqeous extract of leaf also show 0.8mm zone of inhibition.

Methanolic extract of *F.indica* 's leaf,stem and root are showing antifungal activity upto a certain limit against *Curvularia lunata* even after 48 hrs incubation at 30^0 C along with its control plate while those methanolic extracts show no effect on *Aspergillus niger*.

The comparative study of 100µl of each standard antibiotics (amoxicillin &ciprofloxacin) ,antiseptic (savlon), ethyl acetate extract of root ,stem ,leaf of *F.indica* was also done. Ethyl acetate extract of root ,stem,leaf of *F.indica* showed very good response against *E.coli.* Ethyl acetate stem extract of *F.indica* showed a larger zone of inhibition (1.5 cm) than antiseptic savlon (1.3 cm) and the ethyl acetate leaf extract of *F.indica* showed equal size(1.5 cm) of zone of inhibition comparatively with the standard antibiotic amoxicillin.

Total 35 shoot tips were transferred into MS medium containing constant volume of 0.1mg/ltr NAA (1-napthalene acetic acid) and various concentration of BAP (Benzyl aminopurine) viz 1mg/l, 2mg/l, 3mg/l, 4mg/l, 5mg/l, 6mg/l, 7mg/l. The best result was observed at 6mg/l BAP concentration. Further study of micropropagation techniques may help to preserve the elite plant.

Table 2: Comparative Study of Ethyl Acetate Extract of *F.indica* with the Standard Antibiotic (Amoxicillin and Ciprofloxacin), Savlon

Zone of inhibition for root extract (cm)	Zone of inhibition for stem extract (cm)	Zone of inhibition for leaf extract (cm)	Zone of inhibition for salvon (cm)
1.4	1.5	1.3	1.3
Zone of inhibition for root extract (cm)	Zone of inhibition for stem extract (cm)	Zone of inhibition for leaf extract (cm)	Zone of inhibition for amoxicillin (cm)
1.3	1.4	1.5	1.5
Zone of inhibition for root extract (cm)	Zone of inhibition for stem extract (cm)	Zone of inhibition for leaf extract (cm)	Zone of inhibition for Ciprofloxacin (cm)
1.2	1.0	1.4	1.6

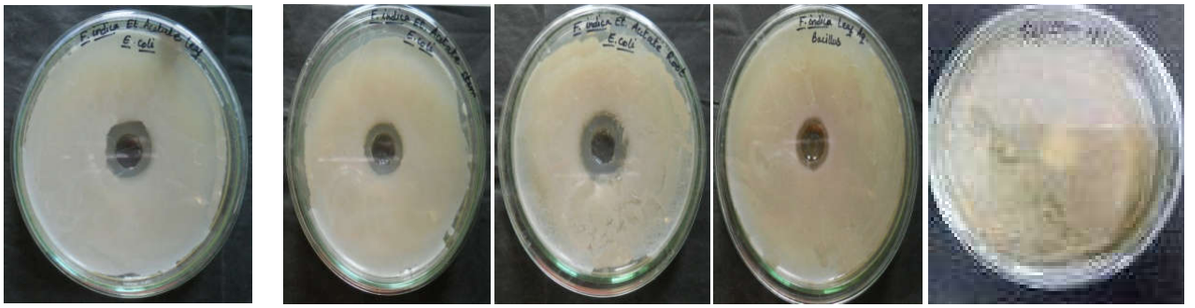

Fig. 1: Antimicrobial activity of *F. indica*: antibacterial activity of Ethyl acetate extract of leaf(a), stem(b), root(c) and aqueous leaf extract showing a clear zone of inhibition against *E.coli* with 3.1mm, 3.0mm, 3.3mm, 2.9mm respectively along with its *E.coli* control plate (ethyl acetate) containing 2 mm zone of inhibition. Aqeous extract of leaf also show 0.8mm zone of inhibition against *Bacillus* sp along with its control plate at right end

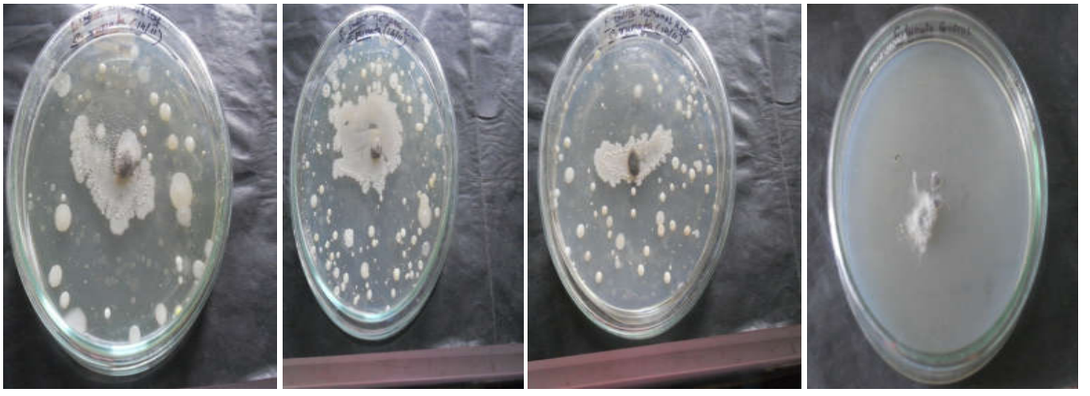

Fig. 2: Antifungal activity of *F.indica*: Methanolic extract of *F.indica*'s leaf, stem and root are showing antifungal activity upto a certain limit against *Curvularia lunata* even after 48 hrs incubation at *-.30° C along with its control plate

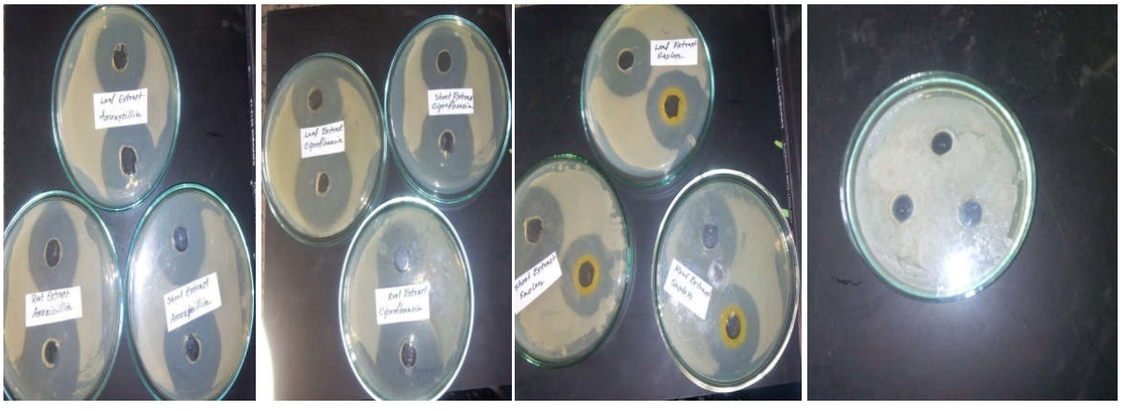

Fig. 3: Comparative study: Study of antibacterial activity of amoxicillin, ciprofloxacin, savlon along with the root, stem, leaf extract of *F.indica* with the control plate of *E.coli* at the right end respectively

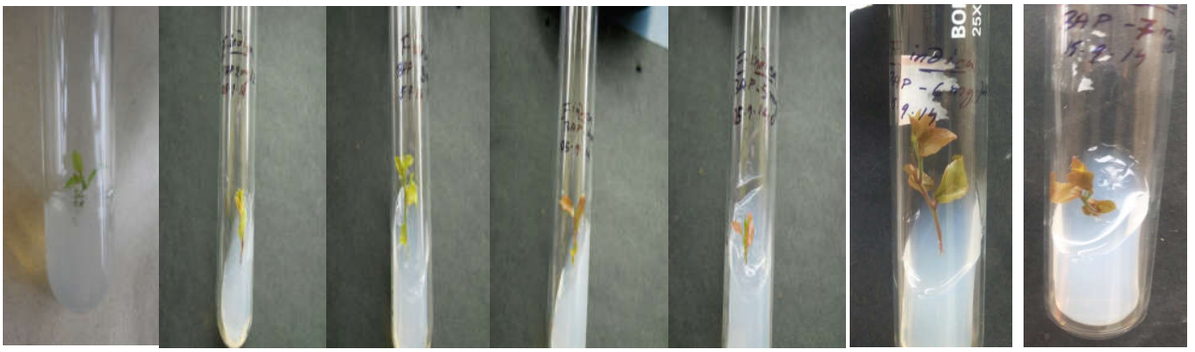

Fig. 4: Shoot multiplication at various concentration of PGR (1mg/ml, 2mg/ml, 3mg/ml, 4mg/ml, 5mg/ml, 6mg/ml, 7mg/ml BAP with fixed concentration of NAA respectively from left side)

Fig. 5: Callus induction after one month of observation in MS media with varying concentration of 2,4-D(1mg/ml, 2mg/ml, 3mg/ml, 4mg/ml, 5mg/ml respectively from left side)

The best result of callus induction was studied at 5 mg/l 2,4D concentration.

FUTURE PROSPECTS

The antimicrobial activity of *F.indica* may enhance the research interest in future and the immense medicinal properties of *F.indica* may open an alternative way to pharma industry.

F.indica stem extract can be used as the herbal antiseptic liquid in coming future.

The leaf extract of *F.indica* may be hope for the herbal antibiotic manufacturer. Further research is deserved to isolate the bioactive compounds responsible for the observed biological activity.

ACKNOWLEDGEMENTS

First of all I would like to thank almighty God for his blessings and my parents and family for their unconditional support.

I am highly thankful to The University of Burdwan and to the department of Biotechnology (DBT-sponsored) for their support in my lifelong cherishing experience.

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