A Protocol of Phyto-Chemical & Anti Microbial Analysis of SapindusTrifoliatus L.

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Abstract*: Plant extracts are potential sources of novel anti-microbial compounds especially against bacterial pathogens because the inhibition produced by plant extracts against particular organism depends upon various extrinsic and intrinsic parameters. Anti-microbials of plant origins has enormous therapeutic potential. They are effective in the treatment of infectious diseases, while simultaneously mitigating many of the side effects. This study was carried out to assess the Phyto-Chemical & Anti Microbial analysis of Sapindus Trifoliatus L. Ethanol leaves extracts were used for carrying out this study. The preliminary phyto-chemical screening of all the taken parameters was carried out respectively of obtained ethanol leaves extract residue to determine the active phyto-constituents present in them. The antimicrobial activity of ethanolic leaf extracts of S. trifoliatus was assessed by using agar disc diffusion assay (Bauer et al., 1966). The findings of the study support the view that the ethanol leaves extract of Sapindus Trifoliatus L. plants possesses potent natural anti-biotic property. Bacillus subtilis bacteria among the other three micro-organism was found to be most active in Sapindus Trifoliatus L.*

Key words: *Anti-microbial, Phyto-Chemical, leave extract, Saponin.*

INTRODUCTION*:*

Sapindus Trifoliatus L. of family Sapindaceae commonly known as soap nut or soap berry is one of the oldest cultivated medicinal plants. It is a medium sized deciduous tree growing widely in South India for thousands of years. All parts of this plant contain saponins, the most active secondary metabolites extracted from this plant (Kasai *et al,* 1988). Saponin from soap nut is widely used in the native medicine(K.M.Nandakarni et al, 1995), Pharmaceutical Industries (Robber *et al,* 1996; Edeoga*et al*, 2006) and as a detergent (P.R.Cheeke et al, 1999). It is a good substitute for washing soap and traditionally used for washing hairs and woolen clothes. The fruit is of considerable importance for its medicinal value and commonly used in Indian Ayurvedic healing system.

Plants have been rich source of medicine because they produce a host of bioactive molecules most of which probably evolved as chemical defense against predation or infection (Cox and Balick, 1994).The powdered seeds are used for the treatment of Arthritis common cold, contraceptives, Nausea and Dental caries (Dhar et al, 1989; Akbar Syed et al, 2011). Leaves of Sapindus Trifoliatus can be used as Expectorant, Eczema, Aphrodisiac, Abortifacient, Migraine, Psoriasis, Freckles and Inflammation (Arulmozhi et al, 2000). Use of plants as a source of medicine has been an ancient practice and an important component of the health care system in India. Interest of general public, academician and government organizations in traditional medicines is growing rapidly, due to the increase in side effects of advance drug reactions and the cost factor of the modern system of medicines.

Infectious diseases account for a high proportion of health problems in the developing countries like India. Micro-organisms have developed resistance to many antibiotics and this has created immense clinical problem in treatment of infectious diseases. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world.

In view of the above and considering the importance of *Sapindus Trifoliatus*, the author has tried to assess the Phyto-Chemical & anti microbial properties of SapindusTrifoliatus L.

MATERIAL AND METHODS:

Microbial test straining of *Sapindus Trifoliatus:*

All tested bacterial strains were collected from MTCC (Microbial type culture collection), IMTECH, Chandigarh identified and provided to us by University Department of Botany, B.R.A.Bihar University. These micro-organisms were maintained on Nutrient Agarmedia slants (NAM) as a stock, at 4^0 C in refrigerator. The test micro-organisms selected were as follows –

1. Bacillus subtilis (MTCC 441)
2. Escherichia coli (MTCC 78)
3. Kelebsillapneumonae (MTCC 3384)
4. Pseudomonas aeruginosa (MTCC 3189)

The preliminary phyto-chemical screening of all the taken parameters was carried out respectively of obtained ethanol leaves extract residue to determine the active phyto-constituents present in them, as the method suggested by Sofowara (1993), Trease and Evans (1989) and Harbone (1998).

Preparation of Nutrient Agar Media:

Nutrient agar was used for the culture of micro-organisms. The components of nutrient agar culture medium (Table- 1) were measured with the help of electronic balance and dissolved separately into a conical flask using double distilled water. Then the volume of the medium was made up to 1000 ml and 2 gm molten agar was added into the prepared medium.

The pH of the medium was adjusted to 7.2 ± 0.2. The pH was measured by the pH meter and the chemicals used to adjust the pH were Hydrochloric Acid (HCl) and Sodium Hydroxide (NaOH).The medium was then sterilized by autoclaving at 121^o C for 15 minutes at 15 lb pressure and was used for analysis.

Preparation of the Inoculums:

For the preparation of inoculums, pure isolates of each micro-organism was sub cultured on the Nutrient agar medium. In this method, 20 ml of molten nutrient agar medium were poured into 9 cm Petri plates and allowed cooling. A stock plate of each micro-organism was taken and were touched with a sterile loop and transferred to the Petri plates by streaking. The inoculated Petri plates were allow to grow in an incubator at 37^0 C for 24 hrs.

From these sub-cultured colonies, inoculums were prepared into normal saline (0.85%) by transferring the micro-organisms, (minimum of four colonies)into the saline water with the help of sterile loop. The colonies of micro-organisms were mixed well by vortex mixer and the density of each microbial suspension was adjusted equal to that of 10^6colony forming unit(cfu) / ml (standardized by 0.5 McFarland standards) and used as an inoculums for antimicrobial assay.

Determination of Minimum Inhibitory Concentration (MIC):

Minimum inhibitory concentration (MIC) is defined as the lowest concentration of a compound/ extract/drug that completely inhibits the growth of micro-organism in 24 hrs. *(Thongsonet al., 2004*). In the present investigation MIC of *S. trifoliatus*ethanolic leaves extracts were determined by following the modified agar well diffusion method (*Rajasekaranet al., 2008*).

Assessment of *In Vitro* Anti-Microbial activity:

The antimicrobial activity of ethanolic leaf extracts of *S. trifoliatus* was assessed by using agar disc diffusion assay*(Bauer et al., 1966*).

Phyto-chemical analysis of ethanol leaves extracts :

Reagents used:

1. Dragendroff's reagents
2. Fehling's solution
3. Alkaline reagent
4. Ferric chloride solution
5. Starch reagent

Qualitative Phytochemical Screening:

The ethanol leaf extracts were weighed and dissolved in 20% Dimethylesulphoxide (DMSO). The volume of Dimethylesulphoxide (DMSO) was maintained at minimum concentration to avoid DMSO induced events (Tale-2). The prepared solution was used for detection of different phyto-constituents**.**

1. Test for Alkaloids
2. Test for reducing sugar
3. Test for flavanoids
4. Test for Saponins
5. Test for steroids
6. Test for tannins
7. Test for starch

RESULT AND DISCUSSION:

The Minimum Inhibitory Concentration (MIC) of the ethanol leaves extract of *S. trifoliatus* ranges from 0.008 mg/ml to 0.01 mg/ml. In the present study, according to the Table –3, ethanol leaves extract of this plant *S. trifoliatus* supposed to be strong inhibitor.

The antimicrobial activity of the ethanol leaves extract was assessed with the help of agar disc diffusion method. The *in vitro* anti-microbial activity using different bacterial strains, shows that ethanolic leaves extract of *S. trifoliatus* possess prominent activity. According to the anti-microbial assay, done in this study against certain gram negative and gram positive bacteria, the gram positive bacteria viz., *Bacillus subtilis* was the most susceptible bacteria to the ethanol leaves extract because it produces largest inhibition zone of 21 mm and 17 mm at both maximum and minimum concentrations of leaf extract, whereas the gram negative micro-organisms viz., *Escherichia coli, Pseudomonas aeruginosa* and *Kelebsiella pneumonae* were resistance variety. They form smaller zone of inhibition i.e., 19 mm, 20 mm & 20 mm at all the three concentrations under taken for investigation, in comparison to *Bacillus subtilis*. These observations are likely to be the result of the difference in cell wall structure, between gram negative and gram positive bacteria. With regard to gram negative bacteria the outer membrane acting as a barrier to many environmental substances including anti-biotic (Burt, 2004).

The findings of the study support the view that the ethanol leaves extract of *Sapindus Trifoliatus* L. plants possesses potent natural anti-biotic property. Further, Bacillus Subtilis bacteria among the other three micro-organism was found to be most active in Sapindus Trifoliatus L.

TABLES & PHOTOGRAPHS:

(a) Tables:

Table- 1 : Nutrient Agar media (for bacterial activity)

Sl. No	Ingredient	Quantity (gm)
i.	Leaf Extract	10
ii.	Peptone	10
iii.	Sodium	5
iv.	Agar	12
v.	Water	Up to 1000ml

Table – 2 : Qualitative phyto-chemical analysis of ethanol leaves extracts of *Sapindus Trifoliatus* Linn.

Sl. No.	Phytochemicals	Ethanol leaves extracts
i.	Alkaloids	+
ii.	Reducing sugar	+
iii.	Flavonoids	+
iv.	Saponins	+
v.	Steroids	+
vi.	Tannins	+
vii.	Starch	+

Key – absent; (-), present; (+)

The color of the ethanol leaves extract was brown and they showed positive (+) results for all the phyto-chemical parameters under taken for investigation.

Table – 3 : Zone of inhibition of the tested bacteria and MIC against ethanol leaves extracts of *Sapindus Trifoliatus* Linn

Sl . No.	Test organisms		Extract Concentrations (mg/ml)	Zone of inhibition(mm)	MIC value (mg/ml)
i.	Bacillus subtilis	a.	0.08	21	0.08
		b.	00.6	19	
		c.	0.05	17	
ii.	Escherichia coli	a.	0.08	19	0.02
		b.	0.06	18	
		c.	0.05	16	
iii.	Kelebsilla penumonae	a.	0.08	17	0.01
		b.	0.05	24	
		c.	0.08	20	
iv.	Pseudomonas aeruginosa	a.	0.08	20	0.01
		b.	0.06	17	
		c.	0.05	24	

(b)Photographs:

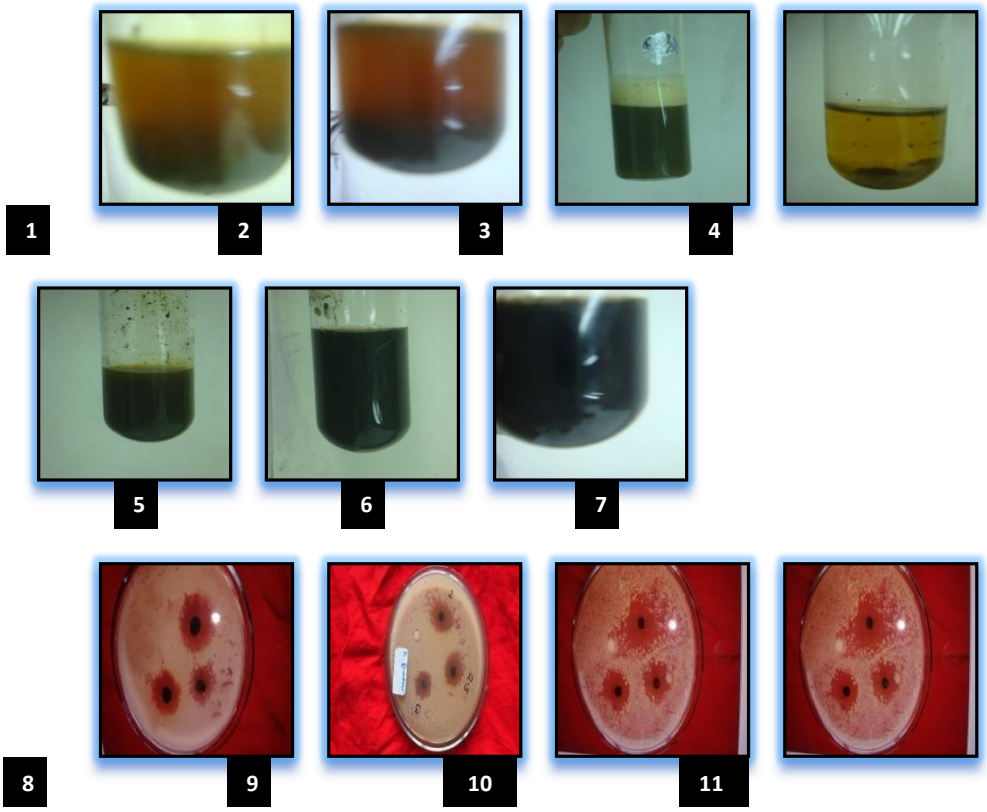

Figure 1 to 7- Phyto-chemical analysis of ethanol leaves extracts :

1:Dragendorff's reagent (1 ml) when added to 5 ml of acidic test extract formation of orange precipitate indicated the Presence of ***alkaloids***. **2:** Fehling's solution (1 ml) when added to 2ml of test extract and boiled, formation of brick red colour precipitate indicated the presence of ***reducing sugar***. **3:** Alkaline reagent (few ml) when added to 1 ml of test extract, formation of intense yellow colour indicated the presence of ***flavonoids***. **4:** 2 ml of test extract when shaken vigorously with water and left, formation of constant foam indicated the presence of ***saponin***. **5:** 1 ml of test extract when subjected to Salkowski reaction formation of yellow green fluorescent colour indicated the presence of ***steroids***. **6:** When few drops of ferric chloride solution added to 2 ml of test extract, formation of green colour indicated the presence of ***tannins***. **7:** When iodine solution (few drops) was added to 3 ml of test extract, formation of blue colour indicated the presence of ***starch***.

Figure 8 to 11- Antimicrobial activity of ethanol leaves extracts:

8: Anti-bacterial activity of ***Bacillussubtilis*** forming largest zone of inhibition (21 mm) on ethanol leaves extracts. **9:** Anti-bacterial activity ***Escherichia coli***, forming zone of inhibition (19 mm) on ethanol leaves extracts. **10:** Anti-bacterial,Activity of ***Kelebsillapneumonae***, forming zone of Inhibition (20 mm) on ethanol leaves extracts. **11:** Anti-bacterial activity of ***Pseudomonasaeruginosa***, forming zone of inhibition (20 mm) on ethanol leaves extracts.

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