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PHYTOCHEMICAL AND ANTIMICROBIAL EVALUATION OF THE LEAVES OF ANTIARIS TOXICARIA, (LESCH)

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Abstract:

Antiaris toxicaria Lesch. commonly known as bark cloth tree. Its medicinal value is yet unexplored, hence this study forms a basis for the active components present in it and further isolation of the compound. The preliminary phytochemical screening in different solvent extracts of *Antiaris toxicaria* leaves, collected in the area Anaikatti, Coimbatore District exhibited variations in the presence of phytocompounds depends upon the polarity of solvents, the methanolic extract showed the presence of all phytoconstituents except alkaloids. In the present study, the methanol extract showed good antibacterial activity than chloroform and water extracts. It revealed that the methanol was the most effective solvent for the antibacterial activity. In antifungal activity, the aqueous and methanol extract showed good activity against *Cuvularia lunata* and *Aspergillus flavus* in which the zone of inhibition was higher against *Aspergillus flavus.*

Key Words: *Antiaris Toxicaria*, Anaikatti, Phytochemical, Active Components, Antibacterial & Antifungal

Introduction:

India is considered as one of the Mega Diversity Centre in the world having wide variety of plants and rich in ethnic diversity. In India, around 20,000 medicinal plant species have been recorded recently, while more than 500 traditional communities use about 800 plant species for curing different diseases (Mishra *et al*., 2011). Currently, 80% of the world population depends on plant-derived medicine for the first line of primary health care for human alleviation because it has negligible side effects (Caniago *et al*., 1998); hence it is important to have knowledge about this method of holistic healing. Identification of natural products from plants that may serve as valuable sources of bioactive agents for medicinal and agricultural uses largely depends on bioactivity directed isolation. Among the important medicinal plants there was a fair number of documentation of *Antiaris* toxicaria that would confirm some of the traditional uses (Alireza, 2011). *Antiaris toxicaria* is a tree in the mulberry and fig family, Moraceae (Plate No. I). It is the only species currently recognized in the genus *Antiaris*. *Antiaris toxicaria* Lesch. is commonly called as 'Bark cloth tree'. Traditional healers reported that decoctions of leaves and stem bark were non-toxic when taken orally for treatment of malaria. Various reports indicate that *A. toxicaria* is a potential source of phytochemicals (Hano *et al*., 1990; 1991; Kopp *et al*., 1992 ; Carter *et al*., 1997a; 1997b; Dai *et al*., 2009; Shi *et al*., 2010 ; Liu *et al*., 2013; Shi *et al*., 2014). Therefore, the present study was carried out to evaluate the qualitative phytochemicals and also the antimicrobial potential of the leaves of *Antiaris toxicaria* in various solvents.

Plate Number I: *Antiaris Toxicaria* Habit

Materials and Methods:

Plant Material:

The leaves of *Antiaris toxicaria* (Lesch.) was collected in Anaikatti area, Coimbatore district, Tamil Nadu. The plant was identified by BSI, Coimbatore,Tamil Nadu, and the voucher specimen was preserved in the Department of Botany, PSGR Krishnammal College for Women, Coimbatore District, Tamil Nadu.

Preparation of Dry Powder Samples:

Fresh leaves of *A. toxicaria* were washed to remove the dust and dried separately for about two weeks at room temperature (30 ºC±2 ºC) to get a constant weight. The dried leaf materials were ground to powder separately by mechanical device, stored and used in this work throughout the study period.

Preparation of Solvent Extract for Preliminary Phytochemical Screening:

The dry leaf powder of *A. toxicaria* was extracted separately with different solvents (petroleum ether, benzene, chloroform, acetone, methanol and water) at 20% (w/v) level in a Soxhlet apparatus. These extracts were concentrated and used for qualitative preliminary phytochemical analysis.

Qualitative Phytochemical Analysis:

The powdered leaf of *A. toxicaria* was subjected to qualitative phytochemical analysis.

Preliminary Phytochemical Screening:

The different solvent extracts of *A. toxicaria* dry leaf materials was subjected to preliminary phytochemical qualitative screening following the standard protocols (Brindha *et al*,., 1981 ; Edeoga *et al.*, 2005) to record the presence or absence of various primary and secondary metabolites such as alkaloids, glycosides, anthraquinone, flavonoids, phenol, protein and amino acids, saponins, tannin and phytosterols.

Antimicrobial Activity:

The Chloroform, methanol and water solvent extracts of *Antiaris toxicaria* were tested for their antimicrobial properties.

Source and Maintenance of Microbes:

Pure cultures of bacterial pathogens were collected from Bioline Laboratory, Sitra, Coimbatore and fungi were isolated from soil, identified in Marina Labs, Chennai and maintained in the Botany department of PSGR Krishnammal College for Women, Coimbatore.

Microorganisms Studied:

Bacteria:

- ✓ Escherichia coli
- ✓ Staphylococcus aureus
- ✓ Pseudomonas sp.

Fungi:

- ✓ Curvularia lunata
- ✓ Aspergillus flavus

Sterilization:

All glass wares were autoclaved at 121 lbs for 20 minutes. Instruments like forceps, inoculation needle were sterilized over flame after dipping in alcohol. Media were sterilized in pressure cooker for 20 minutes.

Nutrient Agar Medium

Sodium chloride	-	3 gm
Peptone	-	5 gm
Beef extract	-	3 gm
Agar	-	15 gm
Distilled water	-	1000 ml
pH	-	6.8

Potato Dextrose Agar Medium (PDA)

Potato	-	250 gm
Dextrose	-	15 gm
Agar	-	20 gm
Distilled water	-	1000 ml
pH	-	6.5

Method of Inoculation:

Sterilized petriplates containing sterile medium were inoculated with the selected microorganisms in such a way so as to get a through coverage of uniform thick lawn of growth of the inoculum.

Incubation:

The cultures were maintained at room temperature in laboratory for a period of 24 hours in the case of bacteria and 72 hours in the case of fungi**.**

Method:

The antimicrobial activity of the chloroform, methanol and water extracts of leaf powder was determined with the agar well diffusion method. Wells were bored in to the agar using a sterile 6mm diameter cork borer. Two different concentrations of 50 µl/ml and 100 µl/ml extracts were poured into the wells and allowed to stand at room temperature for about 2 hrs and incubated at 37^0C for 2 days. Chloramphenicol and Flucanazole (20 µl/ml) were used as controls for bacteria and fungi respectively and they were also set up in parallel. The plates were observed for zone of inhibition after 24 hours and 72 hours for bacteria and fungi respectively and the results are average of triplicates.

Results and Discussion:

Qualitative Preliminary Phytochemical Screening:

The results of the preliminary phytochemical screening of various solvent (petroleum ether, benzene, chloroform, acetone, methanol and water) extracts of *the* leaves of *A. toxicaria* were recorded and presented in table 1: The results revealed that the phenols and alkaloids are present in all the extracts whereas all the

phytoconstituents were present in methanol extract except alkaloids. Protein and aminoacids are present only in petroleum ether, methanol and water extracts, glycosides are present in benzene, acetone, methanol and water extracts, anthroquinone is present in acetone, methanol and water extracts, tannin is present in all extracts except water extract, flavonoids and saponins were present only in methanol and water extracts.

The presence of saponin, tannin, phlobtannin, flavonoid and terpenoids were reported earlier in the methanol leaf extract of *Antiaris toxicaria* (Omotayo *et al*., 2016). Similarly, Gopalakrishnan *et al*. (2015) reported the presence of flavonoids, alkaloids, glycosides, phenols, reducing sugars and saponins in the aqueous leaf extract of *A.toxicaria.*

Table 1 - Qualitative Phytochemical Studies on *Antiaris Toxicaria*

S.No	Solvents	Glyco sides	Anthro quinone	Phen ols	Tann ins	Protein & amino acids	Alka loids	Flav onoi ds	Phyto sterols	Saponins
1	Petroleum Ether	–	–	+	+	+	+	–	–	–
2	Benzene	+	–	+	+	–	+	–	–	–
3	Chloroform	–	–	++	+	–	+	–	++	–
4	Acetone	+	+	+	+	–	+	–	+	–
5	Methanol	+	+++	+++	++	+	-	+++	+++	++
6	Water	+	+	++	-	+	+	+	-	+++

Keys: +++ = Highly present, ++ = moderate, + = Trace, - = absent

Antimicrobial Activity of Leaves of *A.toxicaria:*

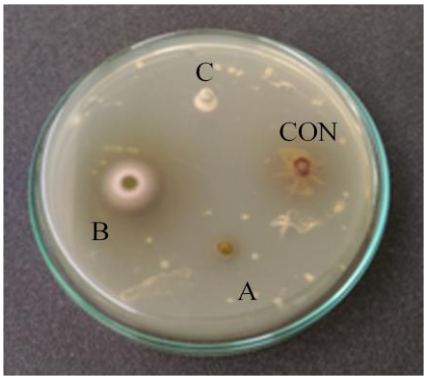
The antimicrobial activity of chloroform, methanol and aqueous leaf extracts of *A. toxicaria* was tested against bacteria such as *Escherichia coli*, *Staphylococcus aureus* and Pseudomonas sp. and fungi such as *Cuvularia lunata* and *Aspergillus flavus*.

Methanol extract showed good antibacterial activity against all the organisms tested than chloroform and water extracts (Plate No. II). The zone of inhibition was higher against *Pseudomonas* sp. The inhibitory effect increased with the increase in concentration of the extract. The control Chloramphenicol showed inhibitory effect against the organisms *Staphylococcus aureus* and *Pseudomonas* sp. and did not show any inhibitory effect against *Escherichia coli*. (Table 2). In earlier report, Hala and Al-Fadhil (2015) reported that the leaf extract of *Azadirachta indica* at the concentration of 100 mg/ml showed the highest antibacterial activity against *Pseudomonas aeruginosa* (15.8 mm inhibition zone) than *Escherichia coli*. In the present study, the methanol extract showed good antibacterial activity than chloroform and water extracts. It revealed that the methanol was the most effective solvent for the antibacterial activity.

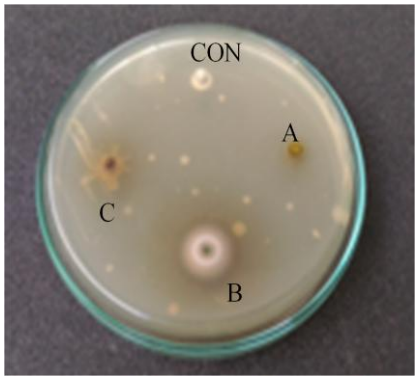
Water and methanol extract showed good antifungal activity against *Cuvularia lunata* and *Aspergillus flavus* respectively than chloroform extract (Plate No. III). The zone of inhibition was higher against *Cuvularia lunata.* The inhibitory effect increased with the increase in concentration of the extract. The allopathic antifungal drug Fluconazole at 20µg did not show any inhibitory effect (Table 3). In earlier report, it was stated that the aqueous and methanolic leaf extract of *Calotropis procera* at 100 µl/ml concentration exhibited the zone of inhibition 8 mm and 11 mm respectively against *Curvularia lunata.* (Manoorkar *et al.*, 2015). Similarly in the present investigation, the aqueous and methanol extract showed good antifungal activity against *Cuvularia lunata* and *Aspergillus flavus* in which the zone of inhibition was higher against *Aspergillus flavus.*

Plate Number Ii: Antibacterial Activity of *Antiaris Toxicaria:*

Escherichia Coli:

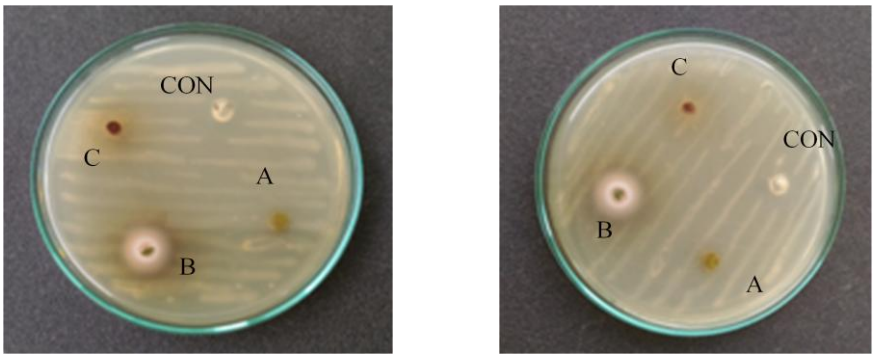


50 µl



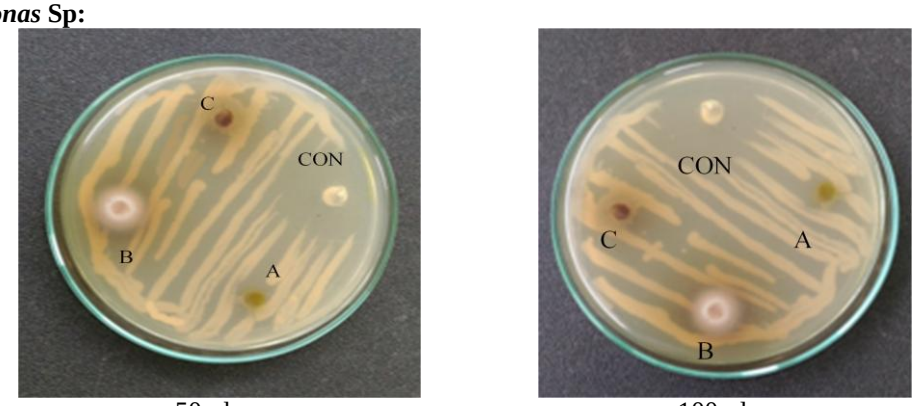
100 µl

Staphylococcus Aureus:



50 µl 100 µl

***Pseudomonas* Sp:**

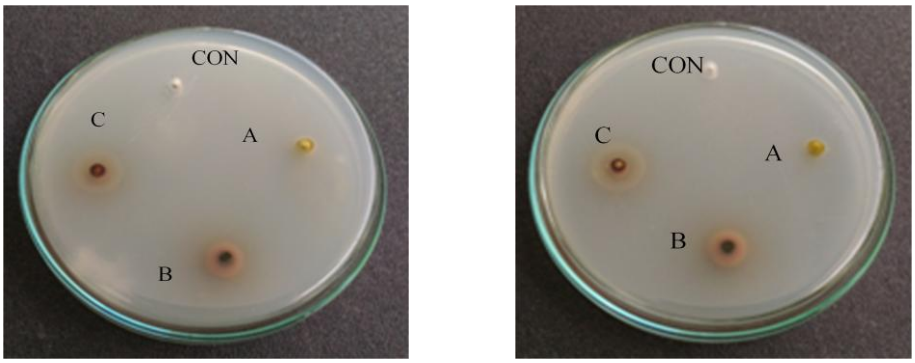


50 µl 100 µl

Control – Chloramphenicol, A – Chloroform extract, B – Methanol extract, C – Aqueous extract.

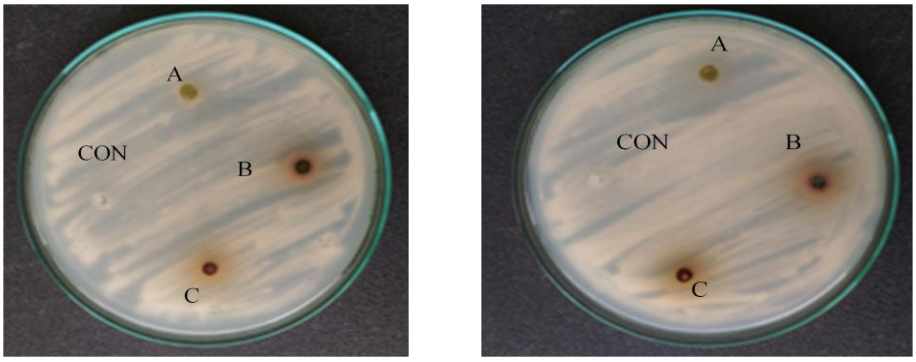
Plate Number III: Antifungal Activity of *Antiaris Toxicaria:*

Curvularia Lunata:



50 µl 100 µl

Aspergillus Flavus:



50 µl

100 µl

Control – Flucanazole, A – Chloroform extract, B – Methanol extract, C – Aqueous extract.

Table 2 - Antibacterial Activity of *Antiaris Toxicaria*

S.No	Organisms	Zone of inhibition (mm)						
		Chloroform extract (µl/ml)		Methanol extract (µl/ml)		Aqueous extract (µl/ml)		Chloramphenicol (20 µl/ml) - Control
		50	100	50	100	50	100	
1	***Escherichia coli***	-	-	5	6	-	-	-
2	***Staphylococcus aureus***	-	-	2	3	-	-	2
3	***Pseudomonas* sp.**	-	-	5	7	-	-	7

Table 3 - Antifungal Activity of *Antiaris Toxicaria*

S.No	Organisms	Zone of inhibition (mm)						
		Chloroform extract (µl/ml)		Methanol extract (µl/ml)		Aqueous extract (µl/ml)		Flucanazole (20 µl/ml) - control
		50	100	50	100	50	100	
1	***Curvularia Lunata***	-	-	3	4	4	6	-
2	***Aspergillus Flavus***	-	-	3	4	2	3	-

Conclusion:

Ethnobotany is an interdisciplinary field, combining the aspects of botany and ethnology as well as many others. The ancient man through his own observation and experience developed knowledge about the usage of plants for edible, medicinal and other purposes. In the present study a rare ethnomedicinal plant *Antiaris toxicaria* was screened to detect the presence or absence of several bioactive compounds. The preliminary studies like qualitative phytochemical analysis and antimicrobial activity were studied in the leaves of *Antiaris toxicaria*. Qualitative phytochemical studies showed the presence of alkaloids, phenols, glycosides, anthraquinone, protein, amino acids, flavonoids, phytosterols and saponins which are reported to cure different ailments.

The antibacterial studies were carried out against pathogens such as *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas* sp. Methanol extract shows good antibacterial activity against all the three organisms than water and chloroform extracts. The zone of inhibition was higher against *Pseudomonas* sp. The antifungal studies were carried out against fungi such as *Cuvularia lunata* and *Aspergillus flavus*. Aqueous extract shows good antifungal activity against the two organisms tested compared to methanol and chloroform extracts. The zone of inhibition was higher against *Cuvularia lunata*. It revealed that the methanol and water were the most effective solvents for the antibacterial and antifungal activity respectively.

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