



ISSN: 0976-3376

Available Online at <http://www.journalajst.com>

ASIAN JOURNAL OF
SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology
Vol. 08, Issue, 11, pp.6541-6544, November, 2017

RESEARCH ARTICLE

PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *WRIGHTIA TINCTORIA* R.BR

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ARTICLE INFO

Article History:

Received 26th August, 2017
Received in revised form
19th September, 2017
Accepted 08th October, 2017
Published online 30th November, 2017

Key words:

Phytochemicals,
Wrightia tinctoria R.Br.,
Antibacterial activity and Pathogens.

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ABSTRACT

The screening and study of selected Indian medicinal plant *Wrightia tinctoria* R.Br., were selected for phytochemical screening and antibacterial studies. The solvents used for the extraction of plant roots were ethanol, benzene, chloroform, acetone, petroleum ether and distilled water. The Gram-Positive and Gram-negative bacteria *Yeast candida*, *Aspergillus niger*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, *Pseudomonas fluorescense*, *Klebsiella pneumonia* and *Streptococcus pyogenes*. were tested. The results obtained in the present study suggest that preliminary phytochemical analysis detected the presence of Alkaloids, Flavonoids, Terpenoids, Steroids, Coumarins, Carbohydrates and Tanins. The *Wrightia tinctoria* R. Br. could be used in treating diseases caused by the test organisms.

INTRODUCTION

Medicinal plants have a long-standing history in many indigenous communities and continue to provide useful tools for treating various diseases. A large number of the country's rural population depends on medicinal plants for treating various illnesses. These plants played a significant role in various ancient traditional system of medication in India. Phytochemical, Antibacterial Screening and Spectroscopic Analysis of the Crude Samples of Stem Bark Extract of *Lonchocarpus cyanescens* (Nwokonkwo *et al.*, 2017). Preliminary phytochemical and Fourier Transform Infrared Spectral analysis and Antimicrobial Studies of solvents extracts of *Urginea indica* (Roxb.) Kunth (Liliaceae) and *Cyclea peltata* Arn. ex Wight (Menispermaceae), results were clearly revealed that the plant contained different bioactive compounds such as of Alkaloids, Anthoquinones, Coumarins, Steroids and Flavonoids compounds were rich in the extracts of *Urginea indica* (Liliaceae) and *Cyclea peltata* (Menispermaceae) are connected with defense mechanism against many microorganisms (Patil *et al.*, 2015). Plants are a source of large amount of drugs comprising to different groups

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such as antispasmodics, emetics, anti-cancer, antimicrobials etc (Tiwari *et al.*, 2011). Preliminary Phytochemical Screening and Evaluation of Anti-Inflammatory Activity of Methanolic Extract of *Barleria cristata* Linn. Roots in Experimental Animals (Banu *et al.*, 2011). Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. Kirby-Bauer method was followed for disc diffusion assay (Shihabudeen *et al.*, 2010). Preliminary studies on phytochemicals and antimicrobial activity of solvent Extracts of *Eichhornia crassipes* (Mart.) Solms. They had study the fresh plant contain alkaloids, flavonoids, phenols, sterols, terpenoids, anthoquinones and protein (Thamaraiselvi *et al.*, 2012). Studies on the phytochemistry, spectroscopic characterization and antibacterial efficacy of *Salicornia brachiata* (Krishnan *et al.*, 2014). Preliminary phytochemical screening of different solvent extracts of stem Bark and roots of *Dennetia tripetala* G. Baker (Solomon *et al.*, 2013). Seed ethanolic extract showed high content of phytochemicals, highest antimicrobial and antioxidant activity and results supported the usage of *Vernonia anthelmintica* in folk and traditional medicine (Santosh *et al.*, 2013). Phytochemical screening and antimicrobial activity of medicinal plant *Pergularia daemia* from Chandrapur Forest Region (Jogi *et al.*, 2012). Phytochemical screening, functional groups and element analysis of *Tylophora pauciflora* Wight and Arn. They had concluded that traditional use of *tylophora pauciflora* for human ailments and partly explained its use in herbal medicine

as rich source of phytochemicals with the presence of tannins, phenol, saponins, steroids, flavonoids and terpenoid (Sarlin *et al.*, 2012). Preliminary phytochemical screening of different solvent extracts of stem Bark and roots of *Denneria petala* G. Baker (Ugochukwu *et al.*, 2013). The most essential of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Many of the indigenous medicinal plants are used as spices and food (Amin *et al.*, 2013). Medicinal plants are moving from fringe to main stream use with a greater number of people seeking remedies and health approach (Saha *et al.*, 2010).

MATERIALS AND METHODS

Plant collection: The following medicinal plants were selected and collected for the study from the local area of Uttam sagar forest of Betul district. The Medicinal Plants *Wrightia tinctoria* R.Br. was collected from fallow land in and around Uttam sagar forest brought into the laboratory for further processes. The collected samples were carefully stored in sterile polythene bags and used for the further study.

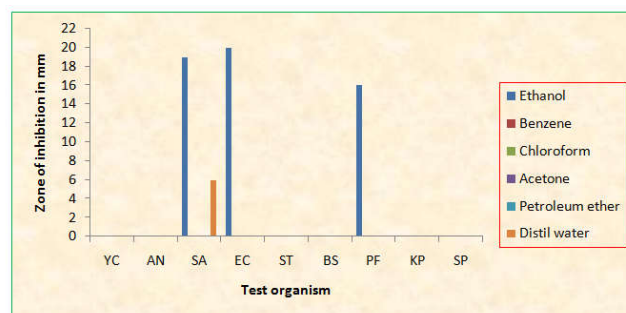


Fig. 1. Analysis of antimicrobial sensitivity of root extracts of *Wrightia tinctoria* R.Br.

Then, surface sterilized with 0.1% mercuric chloride and alcohol from few seconds. Again the materials were washed thoroughly with distilled water.

Preparation of Plant Extracts: The organic solvent extract was prepared by adding 5 gm powder of ethno veterinary medicinal plants in 250 ml of organic solvent (Absolute Alcohol, Acetone, Petroleum Ether, Benzene, chloroform and

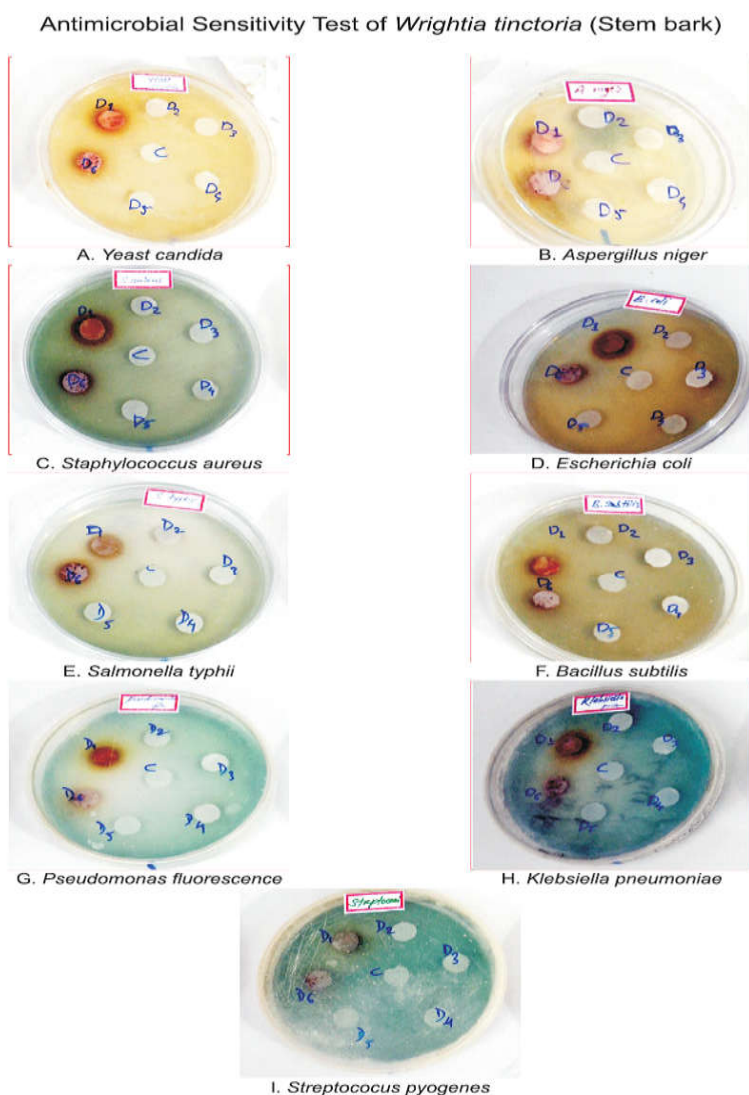


Fig. 2. Antimicrobial activity of root extracts of *Wrightia tinctoria* R.Br.

Sterilization of Plant Materials: The disease free roots were selected for this investigation. About 2gm dried roots were taken.

Distil Water) for 6 hrs. By Soxhlet method and filtrate was evaporated in controlled conditions of temperature of active constituents of preparations. Dried extracts were stored in

labelled sterile wide mouthed screw capped bottle at 40c and used for further study.

Preliminary Phytochemical screening

Phytochemical screening were performed to assess the qualitative chemical composition of different crude extracts

Alkaloids

It was found that concentration of alkaloids have been extracted in Ethanol, Acetone, and Distil water. This is evident from the positive test with Hager's reagent.

Table 1. Phytochemical activity of root extracts of *Wrightia tinctoria* R.Br.

Plant parts	Test / Reagents Used	Ethanol extract E	Benzene extract B	Chloroform Extract C	Acetone extract A	Petroleum Ether P	Distil Water extract W
Stem	Alkaloids	+	-	-	+	-	+
Bark	(Hager's Test)						
	Glycosides	-	-	-	-	-	-
	(Liebermann's Test)						
	Phenols	-	-	-	-	-	-
	Saponins	-	-	-	-	-	-
	(Foam Test)						
	Tannins	-	-	-	-	-	-
	(Braymer's Test)						
	Flavonoids	+	+	+	+	+	+
	Terpenoids	-	-	-	-	-	-
	Steroids	+	+	+	+	+	+
	(Salkowski Test)						
	Phobatanins	-	-	-	-	-	-
	(Precipitate Test)						
	Coumarins	-	-	-	-	-	-
	Proteins	+	+	+	+	+	+
	(Xanthoproteic Test)						
	Emodins	-	-	-	-	-	-
	Carbohydrates	+	+	+	+	+	+
	(Molisch Test)						

Present -- +ve Absent -- -ve

Table 2. Antimicrobial activity of root extracts of *Wrightia tinctoria* R.Br. by Disc Diffusion Method (Zone of Inhibition in mm at 100 µg/disc)

S. No	Microorganism	Ethanol	Benzene	Chloroform	Acetone	Petroleum ether	Distil water
1	YC	0	0	0	0	0	0
2	AN	0	0	0	0	0	0
3	SA	19	0	0	0	0	6
4	EC	20	0	0	0	0	0
5	ST	0	0	0	0	0	0
6	BS	0	0	0	0	0	0
7	PF	16	0	0	0	0	0
8	KP	0	0	0	0	0	0
9	SP	0	0	0	0	0	0

*Data represented in mean of three replicates.

YC = *Yeast candida*, AN = *Aspergillusniger*, SA = *Staphylococcus aureus*, EC = *Escherichia coli*, ST = *Salmonella typhi*, BS = *Bacillus subtilis*, PF = *Pseudomonas fluorescense*, KP = *Klebsiellapneumoniae*, SP = *Streptococcuspyogenes*

using commonly employed precipitation and coloration reactions, the methods of Harbone¹⁵, Trease and Evans¹⁶ were used to identify the major secondary metabolites like Alkaloids, Flavonoids, Saponins, Carbohydrate, Protein, Phenols, Steroids, Tannins, Glycosides, Terpenoids, Phlobatanins, Coumarins, Emodins, Anthoquinones, Anthocyanins, Leucoanthocyanins in the extracts.

Antimicrobial screening: All solvent extracts were screened *in vitro* growth inhibitory activity against different microbes *E. coli*, *Pseudomonas fluroscene*, *Salmonella typhi* *Bacillus subtilis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus* *Yeast candida*, *Aspergillus niger* using disc-diffusion method. The bacteria rejuvenated in Nutrient broth (Hi-media – laboratories, Mumbai, India) at 37⁰c for 18 hrs. and then stored at 40⁰c on Nutrient agar subcultures were prepared from the stock for bioassay.

Phytochemical screening: From the below table no. 1 it is clear that,

Benzene, Chloroform and Petroleum ether have shown negative test for Alkaloids.

Glycosides: All extracts have shown negative test for Glycosides with Liebermann's reagent.

Phenols: All extracts have shown negative test for phenols.

Saponins: All extracts have shown negative test for Saponins.

Tannins: All extracts have shown negative test for Tannins with Braymer's reagent.

Flavonoids: All extracts have shown positive test for Flavonoids.

Terpenoids: All extracts have shown negative test for Terpenoids.

Steroids: All extracts have shown positive test for Steroids with Salkowski reagent.

Phlobatannins: All extracts have shown negative test for Phlobatannins with precipitate test.

Coumarins: All extracts have shown negative test for Coumarins.

Proteins: All extracts have shown positive test for Proteins with Xanthoproteic reagent.

Emodins: All extracts have shown negative test for Emodins.

Carbohydrates

All extracts have shown positive test for Carbohydrates with Molisch reagent.

Antimicrobial activity

The ethanol extracts showed sound microbial zone of inhibition against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas fluorescens*. The maximum zone of inhibition of 20 mm was observed in ethanol extracts against *Escherichia coli*. Ethanol extracts were found non-reactive to other test organisms (Fig.2). The aqueous extracts were also sensitive to *Staphylococcus aureus*. The maximum zone of inhibition of 6 mm was noted against *Staphylococcus aureus* (Fig.2). The aqueous extracts were non-sensitive to other test organisms. (Fig.2) Benzene, chloroform, acetone and Petroleum ether extracts showed no any zone of inhibition to the all test organisms and reactions were nullified (Fig.2).

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