PHYTOCHEMICAL SCREENING, BIOACTIVE COMPOUND IDENTIFICATION AND IN VITRO ANTIOXIDANT POTENTIAL OF *WRIGHTIA TINCTORIA* R.BR

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Abstract: The present study was intended to screen the phytochemical compounds, antibacterial, larvicidal, antihelmintic and antioxidant activities of the ethyl acetate, acetone, hexane and chloroform leaf extracts of *Wrightia tinctoria*and also the GC-MS analysis of ethyl acetate leaf extract. In preliminary phytochemical screening of the extracts revealed the presence of various bioactive compounds.Ethyl acetate leaf extract of *Wrightia tinctoria* showed highest antibacterial activity in pathogenic bacterial strains and chloroform leaf extract showed the significant *in vitro* antioxidant potential. In larvicidal and antihelmintic activity chloroform and acetone extract of *Wrightia tinctoria* leaves showed potent activity respectively. In GC-MS analysis eight bioactive compounds were identified. Thus the results revealed the potential of the *Wrightia tinctoria* as a source for natural antibacterial and antioxidant agent.

Keywords-*Wrightia tinctoria*, phytochemical screening, antibacterial,larvicidal, antihelmintic activity,antioxidant analysis, thin layer chromatography and GC-MS analysis.

I. INTRODUCTION

Medicinal plants play an important role in the discovery of novel drugs due to its secondary metabolites. *Wrightia tinctoria*(Family: Apocynaceae) commonly called “Indrajau”is a small deciduous tree distributed in all districts of deciduous forest of India, which is traditionallyused in Indian System of Medicine (Khyade and Vaikos, 2011).

Fresh leaves of *Wrightia tinctoria* are pungent and are chewed for relief from toothache. Bark and seeds are considered for anti-dysenteric, carminative, astringent, aphrodisiac (Siddiqui and Hussain, 1990) and diuretic. It is also used in flatulence (Joshi, 2000), stomach pain, bilious affections and skin diseases as antidiarrheal and anti-hemorrhagic (Ghosh *et al*., 2010; Reddy *et al*., 1989). The oil emulsion of *Wrightia tinctoria* pods is used to treat psoriasis. It has anti-inflammatory and antidandruff properties. Hence is used in hair oil preparations (Chopra *et al*., 1956; Agarwal, 1986). Traditional healers of Chhattisgarh use “Indrajau” both internally and externally in the treatment of about 16 diseases. Thusthe present study mainly focus on screening the photochemical,antimicrobial, larvicidal, antihelmintic and antioxidant activity of *Wrightia tinctoria* leaf extracts and also the identification of bioactive compounds using thin layer chromatography and GC-MS analysis.

II.MATERIALS AND METHODS

2.1 Sample collection and processing

Fresh leaves of *Wrightia tinctoria* was collected from Thiruvananthapuram District, Kerala. The leaves of *Wrightia tinctoria*were washed under running tap water, air dried at room temperature and then reduced to coarse powder using an electric blender. The powders obtained were stored in airtight containers prior to extraction. The dried powder of leaves was successively extracted using Soxhlet apparatus involving different solvents such as ethylacetate, acetone, hexane, and chloroformseparately for 12-24 hours.After 5 cycles the extracts obtained were evaporated completely for dryness. 2g of dried sample were mixed with 10ml of DMSO and stored for further studies (Sukhdev Swami Handa*et al*., 2008).

2.2 Phytochemical screening

Phytochemical screening was carried out to assess the qualitative chemical composition of crude extracts with ethylacetate, acetone, hexane, and chloroform using standard procedures (Harborne, 1973; Trease and Evans, 1989; Sofowara, 1993; Okwu, 2001; Edeoga*et al*., 2005).

2.3 Biochemical analysis

2.3.1 Estimation of carbohydrate (Anthrone Method)

100mg of sample was hydrolyzed by keeping it in boiling water bath for three hours with 5ml of 2.5N HCl and then cooled to room temperature. It was neutralized with solid sodium carbonate until the effervescence ceases. Supernatant was collected after centrifugation and it was made up to 100ml. This acts as test solution (Santhi and Sengottuvel, 2016). From this 0.2 ml of sample was pipetted out and made up the volume to 1 ml with distilled water. Then 4ml of anthrone reagent was added followed by heating for 8 minutes in a boiling water bath. The tubes were cooled rapidly and the green color developed was read at 630nm (Hansen and Moller, 1975).

2.3.2 Estimation of protein (Lowry's method)

500mg sample was weighed and ground well with a mortar and pestle in 5 to 10ml of phosphate buffer. It was centrifuged and supernatant was collected and it was made up to 100 ml with phosphate buffer (AfsheenMushtaque Shah *et al*., 2010). From this 0.2 ml of sample was pipetted out and made up the volume to 1 ml with distilled water. Then 5ml of alkaline copper sulphate solution was added. Mixed well and allowed to stand for 10 minutes. Then 0.5ml of Folin- Ciocalteau reagent was added and incubated at room temperature in the dark for 30 minutes. The blue color developed was read at 660nm (Lowry *et al*., 1957).

2.3.3 Estimation of aminoacid(Ninhydrin method)

500mg sample was weighed and ground well with a mortar and pestle with the same quantity of acid washed sand (sand washed with dilute HCl). To this homogenate, 5 to 10ml of 80% acetone was added. Centrifuged and collected the supernatant. Repeated the extraction twice with the residue and pooled all the supernatants. Reduced these by evaporation and used the extract for quantitative estimation of total free amino acids. From this 0.2 ml of sample was pipetted out and made up the volume to 1 ml with distilled water. Then 2ml of buffered ninhydrin was added to all the test tubes and heated for 15 minutes in a boiling water bath and cooled to room temperature. 3ml of 50% ethanol was added to all the test tubes, and read the intensity of the purple color against a reagent blank in a calorimeter at 570nm (Magne and Larher, 1992).

2.3.4 Estimation of chlorophyll

1 g of leaf tissue was ground to a fine pulp with 20 ml of 80% acetone. The mixture was centrifuged at 5000 rpm for 5 minutes and the supernatant was collected to a 100 ml volumetric flask, the pellet was again ground with 20 ml of 80% acetone and centrifuged. The supernatant was again transferred to the same volumetric flask. The procedure was repeated until the residue become colourless. The mortar and pestle were also washed thoroughly with 80% acetone and the clear washing was collected in volumetric flask. It

was made up to 100 ml with 80% acetone. The absorbance was read at 645 and 663 nm against 80% acetone as blank (Sudhakar*et al*., 2016).

2.4 Antibacterial assay

The antibacterial assay was determinedusing Kirby-Bauer disc diffusion method. Pathogenic bacterial strains were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh. The bacterial strains *Bacillus megaterium, Bacillus subtilis, Enterobacteraerogenes, Klebsiellapneumonia, Mycobacterium tuberculosis, Pseudomonas aeroginosa, Streptococcus pyogenes*and *Salmonella typhi*are spread over the medium. Filter paper disc of uniform size (5 mm) are impregnated with specified concentrations of plant extract and then placed on the surface of Muller Hinton agar plates that has been seeded with organism to be tested. Bacterial colonies were allowed to grow overnight at 37˚C, then the inhibition zone around the disc was measured (Baur*et al*., 1966).

2.5 Larvicidal activity

Larvae of *Culex* species were exposed to test samples of ethyl acetate, acetone, hexane and chloroform of *Wrightia tinctoria.* Test solution was prepared by adding 1 ml extract dissolved in 9 ml distilled water. A control was maintained by adding 1 ml of respective solvents to 9 ml of distilled water. 5 to 10 larvae per concentration were used for all experiments and the time taken for the death of larvae was recorded (Tennyson *et al*., 2012).

2.6 Antihelmintic activity

The antihelmintic assay was performed on adult Indian earthworm *Pheretimapostuma* due to its anatomical and physiological resemblance with the intestinal round worm parasite of human beings. 1ml methanol and 9ml tap water was taken in labeled petridishes. Worms of nearly equal size were introduced in to the plates. Observations were made from the time taken for paralysis and death of individual worm, paralysis was said to occur when the worms were not able to move even in normal saline. Death was concluded when the worms lost their motility followed by their fading away of their body color. The mortality of parasite was assumed to have occurred when all signs of movement had ceased (Chatterjee, 1967; Thompson and Geary, 1995; Pavan Kumar and Subrahmanyam, 2013).

2.7 Antioxidant activity

2.7.1*In-vitro* evaluation of antioxidant activity by DPPH method

Different concentrations of leaf extracts and ascorbic acid (standard) namely 25, 50, 100, 200, 400 mg/ml were prepared in methanol. DPPH (0.002%) in methanol was used as free radical. Equal amount of different concentrations of solvent extracts and DPPH were mixed in a test tube. The tubes were then incubated at room temperature in dark for 30 minutes. The optical density was measured at 517 nm using UV-visible spectrophotometer. The degree of stable DPPH decolourization to DPPHH (reduced form of DPPH) yellow indicated the scavenging efficiency of the extract.The scavenging activity of the extract against the stable DPPH was calculated using the following equation (Sharma and Bhat, 2009).

Scavenging activity (%) = (A-B)/A x 100

Where A is the absorbance of DPPH and B is the absorbance of DPPH and extract in combination.

2.7.2*In-vitro* evaluation of antioxidant activity by reducing power method

Extracts of leaves of different concentrations in 1ml of distilled water were prepared with phosphate buffer (2.5ml, 2M, pH 6.6) and potassium ferric cyanide (2.5ml, 1%). The mixture was incubated at 50˚C for 20 minutes. A portion (2.5ml) of trichloro acetic acid (10% TCA) was added to the mixture which was then centrifuged at 1500g for 10minutes. The upper layer solution (2.5ml) was mixed with distilled water and $FeCl_3$ (0.5ml, 0.1%) and the absorbance was measured at 700nm. Increased absorbance of reaction mixture indicated increased reducing power (Jayaprakash*et al*., 2001; AbinashSahoo and ThankamaniMarar, 2018).

2.8 Thin layer chromatography

Thin layer preparation was made by using silica gel slurry (prepared by mixing 25g of silica gel in methanol and chloroform in the ratio 2:1 in 50 ml). The plate was air dried and kept in hot air oven at 80˚C for 1 to 2 minutes.Sample was loaded in plate using capillary tube. The spots should be generally placed above 1.5cm from the bottom edge of the plate. The solvent which is the mobile phase (93ml Toluene + 7ml ethyl acetate) was taken in a glass tank. It was closed with a glass plate. The TLC plate was placed vertically on the tank. It should be noted that the sample spot should not touch the solvent. The tank was again covered and the set up was kept for another 4 to 5 hours until the mobile phase travels more than ¾ of the plate. Then the plate was taken out and allowed to dry. The solvent front was immediately marked.

After drying, the plate was kept in chamber containing iodine till a brown color develops (for the detection of saponins). Then the distance moved by sample from the bottom edge of the plate was measured. From this R_f value was measured by the formula (Andrade-Neto*et al*., 2003; Basco*et al*., 1995),

$$R_f = \frac{\text{DISTANCE MOVED BY THE SAMPLE (cm)}}{\text{DISTANCE MOVED BY THE SOLVENT (cm)}}$$

2.9 GC-MS analysis

GC/MS analysis of plant extract was performed using THERMO GC-TRACE ULTRA VER: 5.0, THERMO MS DSQ II and gas chromatograph interfaced to a mass spectrometer equipped with DB 35-MS capillary standard non-polar column (30Mts, ID: 0.25mm, FILM: 0.25µm). Helium gas (flow- 1.0 ML/min) was used as the carrier gas and the injection volume of 1µl was employed. Oven temperature 80˚C was raised to 250˚C at 5˚C/min. Total GC/MS running time was 35.94 minutes. The spectrums of the components were compared with the database of spectrum of known components stored in the NIST 11 and WILEY 8 Libraries. Measurement of peak areas and data processing were carried out by GC-MS Solutions software.

III.RESULTS AND DISCUSSION

3.1 Phytochemical screening

Preliminary phytochemical screening of the leaf extracts of *Wrightiatinctoria* revealed the presence of various phytochemical compounds. The results are summarized in table 1.

Table 1: Phytochemical screening of *Wrightia tinctoria.*

Sl.no	Test conducted	Ethyl acetate	Acetone	Hexane	Chloroform
1	Tannins	+	+	-	+
2	Flavonoids	+	+	-	+
3	Saponins	-	+	+	+
4	Terpenoids	-	+	+	-
5	Alkaloids	+	+	+	+
6	Cardiac Glycosides	-	-	-	-
7	Amino acid	-	-	-	-
8	Steroids	+	+	+	+
9	Phenol	-	+	-	+
10	Glycosides	+	+	-	+

‘+’ indicates positive and ‘-‘indicates negative.

Through the phytochemical study, found that the *Wrightia tinctoria* contain alkaloids,terpenoids, tannins, phenolic compound, saponins (Khyade and Vaikos, 2011), sterols, glycosides and flavonoids and also observed the absence of phytochemical constituents such as cardiac glycosides and aminoacid. The leaf of *Wrightia tinctoria* has been reported to contain four sterols such as desmosterol, clerosterol, 24-methylene-25 methyl cholesterol and 24-dehydropollinastanol (Toshihiro Akihisa *et al*.,1988).

3.2 Biochemical analysis

Biochemical analysis revealed that the *Wrightia tinctoria*contain high amount of protein followed byamino acid, chlorophyll and carbohydrate (Table 2).

Table 2:Biochemical analysis of leaf extract of *Wrightia tinctoria*

Sl. No.	Constituents	Amount in mg/g
1	Carbohydrate	5.6
2	Chlorophyll	7.2
3	Protein	15
4	Amino acid	9

3.3 Antibacterial activity

Antibacterial activity of *Wrightia tinctoria* were studied against eight pathogenic bacterial strains. Among the four extracts, ethylacetate extract showed highest antibacterial activity against *Streptococcus pyogenes* with a zone of inhibition of 1.8 cm. The results are tabulated in table 3.

Table 3:Antibacterial activity of *Wrightia tinctoria* leaves

Sl. No.	Name of test organism	Zone of inhibition(cm)			
		Ethylacetate	Acetone	Hexane	Chloroform
1	*Bacillus subtilis*	1.5	0.4	1	1.5
2	*Pseudomonas aeruginosa*	1	1	1	1
3	*Mycobacterium tuberculosis*	1.4	0.1	1.2	0.7
4	*Klebsiellapneumoniae*	1	0.1	0.7	1
5	*Streptococcus pyogenes*	1.8	1	1	1
6	*Bacillus megaterium*	1	0.5	0.5	0.5
7	*Salmonella typhi*	0.6	0.5	0.5	0.5
8	*Enterobacteraerogenes*	0.9	0.5	0.8	0.7

In antibacterial activity greater effectivity was observed against gram positive bacterial pathogens than the gram negative strains (Rajani Srivastava, 2014).

3.4 Larvicidalactivity

In larvicidal activity, ethyl acetate, acetone, hexane and chloroform leaf extracts of *Wrightia tinctoria*showed potent larvicidal activity. The chloroform leaf extract of *Wrightia tinctoria* showed highlarvicidal activity than other extracts. The observations are tabulated in table 4.

Table 4:Larvicidal activity of *Wrightia tinctoria* leaf extracts against mosquito larvae

Extract	Time (in minutes)	Percentage of mortality
Control	15	100
Ethyl acetate	2	100
Acetone	5	100
Hexane	5	100
Chloroform	1	100

Plant may be alternative source of mosquito control agents. Kamaraj*et al*., reported the potent larvicidal activities of hexane, chloroform, ethyl acetate, acetone and methanol of dried leaf and bark extracts of *Wrightia tinctoria*, against the fourth instar larvae of malaria vector (Kamaraj*et al.*, 2011). It was analyzed in the present study that, chloroform extract of *Wrightia tinctoria* showed high level of larvicidal activity against mosquito larvae.

3.5 Antihelmintic activity

In antihelmintic activity, the acetone leaf extract of *Wrightia tinctoria*showed high level of antihelmintic activity compared to other solvents such as ethylacetate, hexane and chloroform. The activity was tabulated in Table 5.

Table 5:Antihelmintic activity of *Wrightia tinctoria* leaf extracts

Treatment	Time in minutes	
	Paralysis	Death
Control	1	2
Ethyl acetate	15	20
Acetone	30	40
Hexane	8	10
Chloroform	2	4

In the present study it was noted that chloroform extract of plant leavesshowed maximum antihelmintic activity against adult Indian earth worm and cause paralysis and death of worms in less time.

3.6 Antioxidant activity

3.6.1*Invitro* evaluation of antioxidant activity by DPPH method

DPPH(1, 1-Diphenyl-2-picrylhydrazyl) is a stable free radical with brown red colour (absorbed at 517nm).If free radicals have been scavenged,DPPH will generated it's colour to yellow. The

extracts of *Wrightia tinctoria* highly scavenge free radical when compared to standard antioxidant such as ascorbic acid. The antioxidant activity of *Wrightiatinctoria* is represented in figure 1.

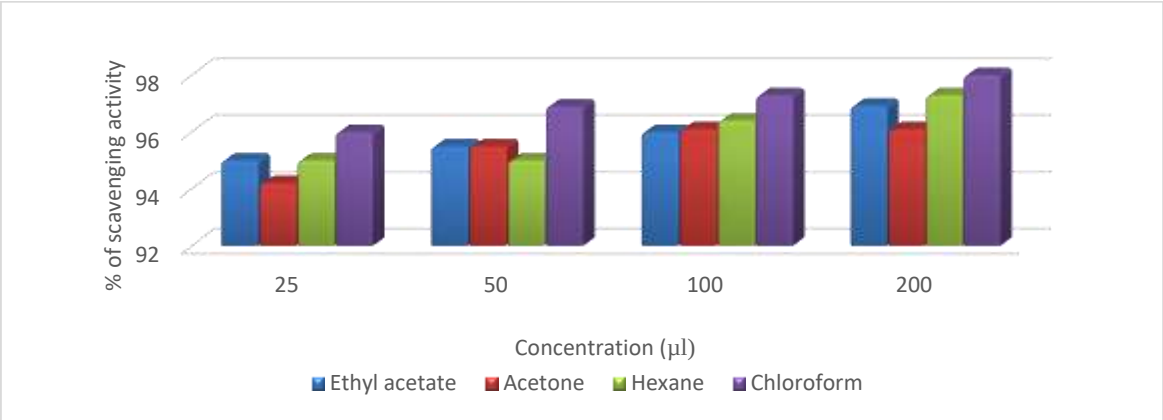

Fig.1: DPPH free radical scavenging activity of *Wrightia tinctoria*

In the present study, the chloroform extract of *Wrightia tinctoria* have high scavenging activity. There was a direct positive relationship between antioxidant activity and increasing concentration of the extract (Jose and Jesy, 2014).

3.6.2 *In vitro* evaluation of antioxidant activity by reducing power assay

In vitro evaluation of antioxidant activity of *Wrightia tinctoria* by reducing power assay results were showed in figure 2. In this assay chloroform leaf extract showed the high absorbance in increasing concentration and the absorbance was found to increase with the concentration of extract and the higher absorbance of the reaction mixture indicates higher reductive potential.

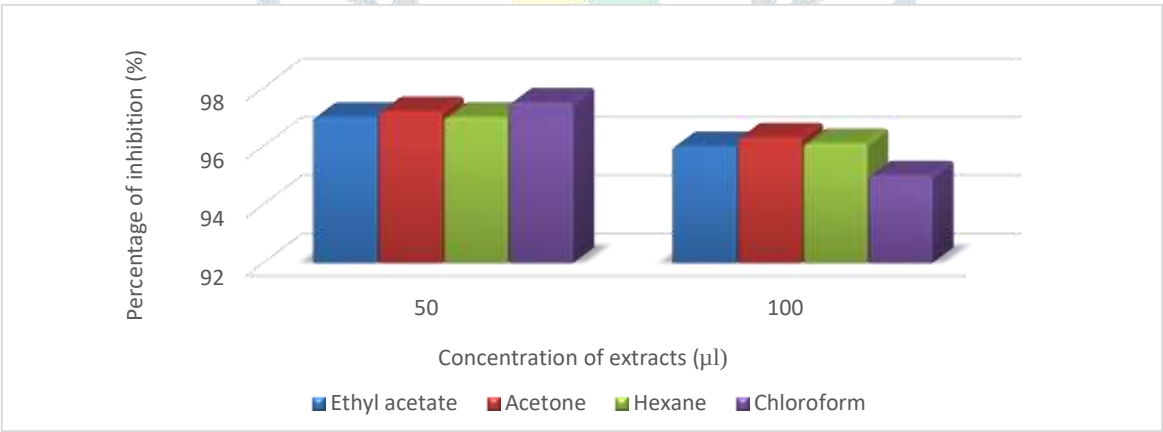

Fig.2: Reducing power assay of leaf extracts of *Wrightia tinctoria*

3.7 Thin Layer Chromatography

3.7.1 Thin layer chromatography of saponins

After drying and spraying the plate with iodine solution. Four spots of different color was obtained.The spots obtained were measured and expressed in table 6 and plates 1, 2 and 3.

Table 6: TLC of saponins

Sl.no.	Extracts	Colour of spot	R_f value
1	Chloroform	Yellow	0.9
		Light green	0.5
		Pink	0.36
		Light brown	0.16
2	Acetone	Yellow	0.90
		Light green	0.45
		Pink	0.36
		Yellowish green	0.21
3	Hexane	Yellow	0.91
		Light green	0.26
		Pink	0.33
		Greenish yellow	0.22

Plate 1: TLC of saponin in chloroform extract

Plate 2: TLC of saponin in acetone extract

Plate 3: TLC of saponin in hexane extract

Wrightia tinctoria extract revealed the presence of various secondary metabolites such as alkaloids, phenolic compounds, tannins, flavonoids, terpenoids, and saponins was detected by thin layer chromatography, which is a standard technique for separating organic compounds (Shaliniand PremaSampath Kumar, 2012). In the present study, the presence of saponins are confirmed by TLC analysis by spraying particular sprayers.

3.8 GC-MS analysis

The study was extended by analyzing the potent bioactive compound in the ethyl acetate extract of *Wrightia tinctoria* using GC-MS. Eight bioactive compounds were separated from ethyl acetate extract of *Wrightia tinctoria* leaves.The results are showed in table 9and fig.3.

Table 9: Bioactive compounds identified by GC-MS analysis

Sl. No.	Compounds	Retention time
1	Spiro[2.4]hepta-4,6-diene	1.759
2	Phosphonic acid	2.933
3	alpha.-Cubebene	9.420
4	Hexadecane, 7-methyl-	11.817
5	Phenol, 2,4-bis	13.394
6	Tetradecanoic acid	23.124
7	1,2-Benzenedicarboxylic	24.909
8	1,2-Benzenedicarboxylic	27.288

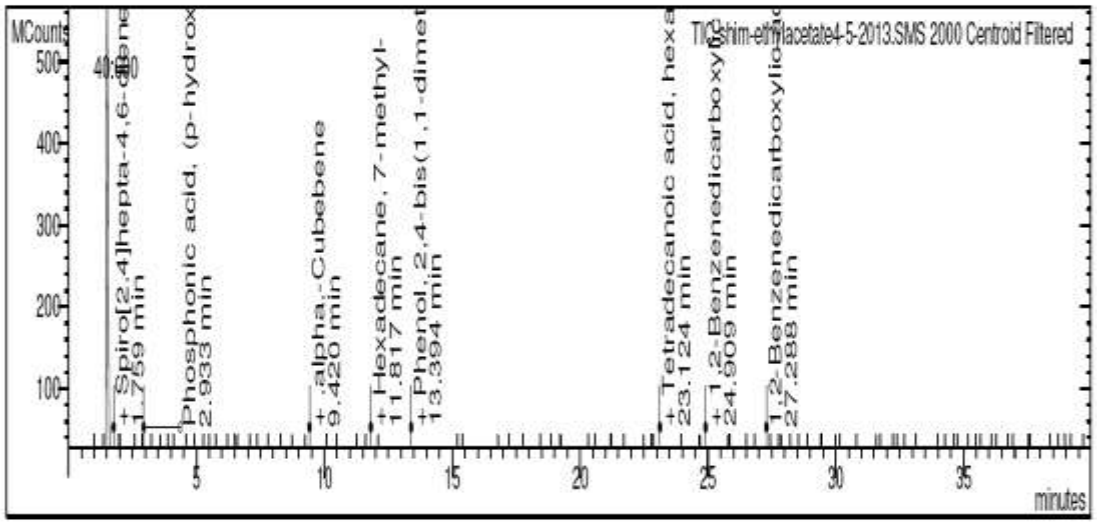

Fig.3: Chromatogram of ethyl acetate leaf extract of *Wrightia tinctoria*

The study was extended by analyzing the potent bioactive compound in the ethyl acetate extract of *Wrightia tinctoria* using GC-MS.

IV.CONCLUSION

This study revealed the presence of various phytochemical constituents in *Wrightia tinctoria*leaves.Ethyl acetateleaf extract of *Wrightia tinctoria*showed highest antibacterial activity in pathogenic bacterial strains and chloroform leaf extract showed the significant *in vitro* antioxidant potential. Thus antibacterial activity of the extracts of *Wrightia tinctoria*opens the possibility of finding clinically effective antibacterial compounds and developing new drugs. In GC-MS analysis eight compounds were separated. But more studies would be required to investigate the therapeutic potential of *Wrightia tinctoria* leaves.

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REFERENCES

1. AbinashSahooandThankamaniMarar. 2018. Phytochemical analysis, antioxidant assay and antimicrobial activity in leaf extracts of CerberaodollamGaertn.*Pharmacogn J*., 10(2): 285-292.
2. AfsheenMushtaque Shah, Mohammad Saleh Memon, Allah Nawaz Memon, Abdul Wahab Ansari andBasir Ahmed Arain. 2010. Analysis of Protein by Spectrophotometric and Computer Colour Based Intensity Method from Stem of Pea (*Pisumsativum*) at Different Stages.*Pak. J. Anal. Environ. Chem*, 11(2): 63-71.
3. Agarwal CK. 1986. Economic Plants of India.KailashPrakashan, Culcutta: 406.
4. Andrade-Neto VF, Brandao MGL, Stehmann JA, Oliveira LA andKrettli AU. 2003. Antimalarial Activity of Cinchona-like species plants used to treat fever and malaria in Brazil. *J. Ethnopharmacol.*, 87(2-3): 253-256.
5. Basco LK, Ramiliarisoa O and Le Bras J. 1995. *In vitro* activity of atovaquone against the African Isolates and clones of *Plasmodium falciparum*. *Am. J. Trop. Med.* 53(4): 388-391.
6. Bauer AN, Kirby WM, Sherries JC and Trucck M. 1966. Antibiotic susceptibility testing by standardized single disk method. *Am J ClinPathol*, 45: 493- 496.
7. Chatterjee KD. 1967. Parasitology, Protozoology and Helminthology, 6th ed, In Guha Ray SreeSaraswathy Press Ltd, Calcutta.
8. Chopra RN, Nayar SI and Chopra IC. 1956. Glossary of Indian Medicinal Plants, CSIR, New Delhi, India: 259.
9. Edeoga HO, Okwu DE andMbabie BO. 2005. Phytochemical constituents of some Nigerian medicinal plants. *Afri J Biotech*, 4: 685-688.
10. Ghosh A, Sarkar A, Mitra P, Banerji A, Banerji J, Mandal S, *et al*. 2010. Crystal structure and DFT calculations of 3, 4-seco-lup-20 (29)-en-3-oic acid isolated from *Wrightia tinctoria*: Stacking of supramolecular dimmers in the crystal lattice. *J MolStruct.* 980:7–12.
11. Hansen J and Moller IB. 1975. Percolation of starch and soluble carbohydrates from plant tissue for quantitative determination with anthrone. *Analytical biochemistry*. 68(1): 87-94.
12. Harborne JB. 1973. Phytochemical methods. A guide to modern technique of plant Analysis. Chapman Hall, London: 100-101. 33-185.
13. Jayaprakash GK, Singh RP andSakariah KK. 2001. Antioxidant activity of grape seed extracts on peroxidation models *in-vitro*. *J.Agric Food Chem*. 55: 1018-1022.
14. Jose B and Jesy EJ. 2014. Phytochemical studies on the leaves and bark of *Wrightia tinctoria* Br. from South India. *World journal of pharmaceutical research.* 3: 4838-4848.
15. Joshi SG. 2000. Medicinal plants, Oxford and IBH publishing Co. Pvt. Ltd., New Delhi: 51.
16. Kamaraj C, Bagavan A, […], and Abdul Rahuman A. 2011.Larvicidal activity of medicinal plant extracts against *Anopheles subbpictus* and *Culextritaeniorhynchus*, *Indian J Med Res*., 134(1): 101-106.

17. Khade MS and Vaikos NP. 2011. Comparative phytochemical and antibacterial studies on the bark of *Wrightia tinctoria* and *Wrightia arborea*.*IJPBS*. 2(1).
18. Lowry OH, Roseobrough NJ, Farr AL and Randall RJ. 1957. Protein measurement with folin phenols reagent.*Journal of Biological Chemistry*. 193: 265-275.
19. Magne C and Larher E. 1992. Higher sugar content of extract interfere with colorimetric determination of aminoacid and free praline.*Anal. Biochem*. 200(1): 115-118.
20. Okwu DE. 2001. Evaluation of the chemical composition of indigenous species and flavoring agents. *Global J Pure ApplSci*, 7(3): 455-459.
21. Pavan Kumar GV and Naga Subrahmanyam S. 2013. Phytochemical analysis, *in-vitro* screening for antimicrobial and antihelmintic activity of combined hydroalcoholic seed extracts of four selected folklore Indian medicinal plants. *Der Pharmacia Lettre*, 5(1): 168-176.
22. Rajani Srivastava. 2014. A review on phytochemical, pharmacological, and pharmacognostical profile of *Wrightia tinctoria*: Adulterant of kurchi.*Pharmacogn Rev*., 8(15): 36-44.
23. Reddy MB, Reddy KR and Reddy MN. 1989. A survey of plant crude drugs of Ananthapur District, Andhra Pradesh, India. *Int J Crude Drug Res*. 27:145–55.
24. Santhi, K and Sengottuvel R. 2016. Qualitative and Quantitative Phytochemical analysis of *Moringaconcanensis*Nimmo. *Int.J.Curr.Microbiol.App.Sci.*,5(1): 633-640.
25. Shalini S and PremaSampath Kumar. 2012. Phytochemical screening and antimicrobial activity of plant extracts for disease management. *INT J CURR SCI*.209-218.
26. Sharma OP and Bhat TK. 2009. DPPH antioxidant assay revisited.*Food chemistry*. 113(4): 1202-1205.
27. Siddiqui MB andHussain W. 1990. Traditional antidotes of snake poison. Fitoterapia. 61:41–4.
28. Sofowara A. 1993. Medicinal plants and Traditional medicine in Africa, Spectrum Book LTD, Ibadan, Nigeria, 289.
29. Sudhakar P, Latha P and Reddy PV. 2016. Phenotyping crop plants for physiological and biochemical traits. Elsevier, ISBN: 978-0-12-804073-7.
30. Sukhdev Swami Handa, SumanPreet Singh Khanuja, Gennaro Longo andDevDuttRakesh.2008. Extraction technologies for medicinal and aromatic plants. International centre for science and high technology, Trieste. 21-25.
31. Tennyson S, Ravindran KJ andArivoli S. 2012. Screening of twenty five plant extracts for larvicidal activity against *Culexquinquefasciatus*Say (Diptera: Culicidae). *Asian Pacific Journal of Tropical Biomedicine*. 2(2): 1130-1134.
32. Thompson DP and Geary TG. 1995. The structure and function of helminth surfaces, in: J.J. Marr. Biochemistry and Molecular Biology of Parasites. 1st ed. Academic Press, New York. 203-232.
33. Toshihiro Akihisa,Ishtiaque Ahmad and Sukhprit Singh.1988. 14α- methylzymosterol and other sterols from *Wrightia tinctoria* seeds. 27: 3231-3234.
34. Trease GE and Evans WC.1989.Pharmacognsy. 11th edition. BrailliarTiridel Can. Macmillian publishers.