PHYTOCHEMICAL INVESTIGATION, ANTICANCER AND ANTIMICROBIAL POTENTIAL OF *WRIGHTIA TINCTORIA* (ROXB.) R. BR.

UGC MAJOR RESEARCH PROJECT
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SUMMARY OF THE FINDINGS OF THE MAJOR RESEARCH PROJECT

Title: Phytochemical Investigation, Anticancer and Antimicrobial potential of

Wrightia tinctoria (Roxb.) R. Br.

The study aimed at the isolation of various secondary metabolites from the leaf and bark of Wrightia tinctoria (Pala Indigo) by column chromatography and characterization of the isolated compounds by spectral analysis ($^1$H NMR, $^{13}$C NMR, IR, Mass Spectrum). The study also meant to evaluate the antibacterial and antifungal activities of the plant extracts against various pathogenic bacteria and fungi. The evaluation of the antioxidant activity such as DPPH and hydroxyl free radical scavenging activity, metal chelating activity, reducing power assay, phosphomolybdenum activity and hydrogen peroxide radical scavenging activity and examining the viability of these plant extracts to incorporate in the drug formulations by comparing with the standard antibiotic drugs and thereby finding out their applications in medicine are also the objectives of the project. The study also focused on the evaluation of the anticancer potential of Wrightia tinctoria.

The phytochemical investigation of the leaves and bark of Wrightia tinctoria (Apocynaceae) resulted in the identification of five compounds which include long chain ester, alkyl derivative of Indirubin, long chain hydroxy ester and pinkish oily liquid from leaves and a waxy solid from the bark of the plant. Pinkish oily liquid on GC-MS analysis gave 10 compounds, of which 1-methyl-4-(1-methylethenyl)-cyclohexanol acetate (26.5%), Limonene (22.4%), α,α-4-trimethyl-1-methanol-3-Cyclohexene (12.5%) and 1-methyl-4-(1-methylethyl)-1,4-Cyclohexadiene(11.5%) were the major constituents. The waxy solid from the bark petroleum ether extract revealed the presence of five compounds n-Butyl laurate (27.8%), Lauric acid n-amyl ester (7.26%), Dodecanoic acid (6.13%), Ethyl citrate (4.57%) and Dodecanoic acid propyl ester (3.88%). Based on this work an article entitled “Phytochemical studies on the leaves and bark of Wrightia tinctoria R.Br. from South India” has been published in World Journal of Pharmaceutical Research Vol. 3(3); 2014: 4838-4848.
The antibacterial and antifungal activities of the bark and leaf extracts of *Wrightia tinctoria* have been studied against various pathogenic bacteria such as *Bacillus cereus*, *Enterobacter faecalis*, *Salmonella paratyphi*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Serratia marcescens* and antifungal activity against two fungi namely *Aspergillus niger* and *Penicillium chrysogenum* by ‘agar well diffusion’ method. The activity of these plant products against various microorganisms were compared with the standard antibiotics examined under similar experimental conditions. Based on this work an article entitled “Evaluation of antibacterial and antifungal activities of the leaf and bark extracts of *Wrightia tinctoria R.Br.*” has been published in *World Journal of Pharmaceutical Research* Vol. 3(3); 2014: 4849-4859.

Statistical analysis of the antibacterial and antifungal activities of leaf coconut oil extracts, bark and leaf methanol, ethyl acetate, chloroform and petroleum ether extracts of *Wrightia tinctoria* was carried out in order to evaluate the medicinal potential of these plant extracts. *Wrightia tinctoria* leaf and bark methanol extracts were found to be potent extracts and their activity is quite comparable with the standard antibiotics. Based on this work an article entitled “Statistical analysis of the antimicrobial activity of *Wrightia tinctoria* leaf and bark extracts” has been published in *International Journal of Pharmacy and Pharmaceutical Sciences* Vol. 6(7); 2014: 293-295.

The essential oil from *Wrightia tinctoria* leaf was extracted by steam distillation and it was analysed by GC-MS. Thirty seven known compounds have been identified and quantified from the leaf essential oil of *Wrightia tinctoria* by GC-MS analysis. The major compounds present in the leaf essential oil are urs-12-en-24-oic acid-3-oxo-methyl ester (34.28%), hydroquinone (13.24%), 1, 6-cyclodecadiene,1-methyl-5-methylene- 8- (1-methylethyl) (9.70%), 3-methyl-2-(2-pentenyl)- 2-cyclopentene-1-one (6.76%) and 9, 12, 15-octadecatrienoic acid (4.52%). This is the first report of extraction of essential oil from the leaves of *Wrightia tinctoria*. Based on this work an article entitled “Analysis of the essential oil from the leaves of *Wrightia tinctoria R.Br. from South India*” has
Evaluation of DPPH radical scavenging activity of the leaf, bark and seed extracts of *Wrightia tinctoria* was carried out. DPPH radical could be scavenged most effectively by *Wrightia tinctoria* bark ethyl acetate extract with IC$_{50}$ value 67.30±2.4µg/ml and was quite comparable with the standard antioxidant L-ascorbic acid (IC$_{50}$ value 62.5±2.5µg/ml). The antioxidant activity of *W. tinctoria* leaf methanol extract (IC$_{50}$:72.9±3.5µg/ml) was not significantly different from that of L-ascorbic acid (IC$_{50}$: 62.5 ± 2.5µg/ml). The remarkable antioxidant activity exhibited by the plant extracts can be attributed to the synergic effect of the active compounds present in it. The results obtained showed that the bark ethyl acetate and leaf methanol extracts of *Wrightia tinctoria* can be considered as good sources of natural antioxidants and can be incorporated into the drug formulations. Based on this work an article entitled “Evaluation of the DPPH free radical scavenging activity of *Wrightia tinctoria* R.Br. leaf, bark and seed extracts” has been published in *World Journal of Pharmaceutical Research Vol. 3(3); 2014: 5041-5048.*

Studies on the antioxidant potential of the hydro-alcoholic leaf and bark extracts of *Wrightia tinctoria* showed pronounced antioxidant activity and the percentage activity increased in a dose dependent manner. The hydro-alcoholic bark and leaf extracts of *Wrightia tinctoria* are high in phenolic and flavonoid content. Antioxidant activities of the extracts from medicinal plants are mainly attributed to the synergic effect of the active compounds such as flavonoids, polyphenols and tannins present in them. The results of scavenging activities observed against hydroxyl and hydrogen peroxide radicals, reducing power, metal chelating and phosphomolybednum activities showed that *Wrightia tinctoria* as promising natural sources of antioxidants suitable for preventing free radical-mediated diseases. Further studies are needed to explore the potential phenolics, flavonoid compounds from *Wrightia tinctoria* for application in drug delivery, nutritional or pharmaceutical fields.
The anticancer activity of the leaf and bark extracts of *Wrightia tinctoria* was studied and the ethyl acetate bark extract (EAB) and leaf oil of the plant exhibited significant cytotoxic effect to DLA (Dalton’s Lymphoma Ascites) tumour cell lines in tryphan blue exclusion method. It was found that oral administration of EAB significantly increased the life span of DLA induced ascites tumour bearing mice. The study also revealed that EAB decreased DLA induced solid tumour volume in Swiss Albino mice. Anti tumor properties of *Wrightia tinctoria* could be linked with the presence of antioxidants and cytotoxic activity. These outcomes indicate the possible potential use of *Wrightia tinctoria* as anti tumor agent.