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Characterization of the Phytochemicals and Antioxidant Properties of Extracts From *Wrightia Tinctoria* R.Br.

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Abstract

In the present investigation, phytochemical screening of different extracts of *Wrightia tinctoria* has been tested by using standard procedures. The findings showed the presence of alkaloids, flavonoids, phenols, steroids, terpenoids and glucosides. Among the four solvents tested (aqueous, ethanol, N-hexane and ethyl acetate) ethanol extract of leaf showed the best result. The free radical scavenging activity of both the extract was measured by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The aqueous and ethanol extracts of *Wrightia tinctoria* leaf shows slightly different antioxidant activity. The remarkable activity showed by the plant is due to the synergic effect of active compounds present in it.Characterization of phytochemicals was done by using UV-Visible spectrophotometry, FTIR analysis and Thin layer chromatography.

Keywords: Wrightia tinctoria leaf extract, phytochemical screening, antioxidant, UV, FTIR, TLC.

INTRODUCTION

Wrightia tinctoria R.Br. is a famous medicinal plant commonly called Pala indigo plant. *W. tinctoria* is distributed throughout the world and mainly in India [1]. It is a small deciduous tree belongs to apocyanaceae family. Various part of the plant has been used in traditional medicine [2]. The bark is very smooth, yellowish brown in colour and produces milky white latex. The leaves are simple, oppositely arranged and leaf stalks are very short. Flowers have 5 white petals, which turn creamish yellow as they age. Fruiting is in August, the fruit is cylindrical and blackish green in colour. The seeds are brown and flat with minute white hairs. The seed is dispersed by wind and pollination is by insects. The plant is grows in a wide range of soil types such as arid, semi-arid, rocky soils and moist regions. The flowers, leaves, fruits are edible. The leaves, flowers, roots are source of indigo-yielding glucoside, which produces a blue dye or indigo like dye. Approximately 100-200 kilos of leaves are needed to prepare 1 kilo of dye. In Ayurveda the plant is called

Shwetha kudaja and its seeds are called Indrajava. Ethan- medically, the bark of this plant is used to treat abdominal pain, skin diseases and wounds. It is also used as an antidote for snake poison. The seeds of this plant are used as an aphrodisiac [3]. The leaves are very useful to treat skin infections and also used to treat mumps and herps. In folk medicine, the dried and powdered roots of *Wrightia tinctoria* along with *Phyllanthus amarus* (Keezharnelli) and *Vitex negundo* (Nochi) are mixed with milk and orally administrated to women for improving fertility. The plant is used to treat breast cancer [4] The bark and leaves are very effective against psoriasis and non-specific dermatitis.

It has anti-inflammatory and anti-dandruff properties, so it is used in hair oil preparations. During headache, stem and leaf pastes are applied on the forehead or administrated orally[8]. The terpenoids and flavonoids of *W. tinctoria* are found to have anti-microbial property against pathogenic bacteria, fungus, virus and protozoans. Ethanol and methanol extract of *Wrightia tinctoria* were found to have strong inhibitory activity against *Staphylococcus sp*. and *Bacillus species* [5,6,7]. *W.tinctoria* is also found to contain important enzyme. Proteases are commercially important class of enzymes and the hydrolytic property of the enzyme is exploited in various biotechnological processes. A serine protease, Wrightin, has been isolated from the latex of *Wrightia tinctoria*. The stable thermodynamic properties of Wrightin make it an economical source of protease for commercial exploitation [12].

Various diseases caused by microbes are treated by available drugs. They treated the human population by the resistance of microorganisms to the available drugs. *Wrightia tinctoria* is used for skin disorders in different parts of the country [13]. The terpenoids and flavonoids of *W. tinctoria* are found to have anti-microbial property against pathogenic bacteria, fungus, virus and protozoans. *Pityrosporum ovale* is a fungi belonging to the family Basidiomycetes, which is causing major cosmetic problem of dandruff. The active constituents of *W. tinctoria* have shown significant inhibition against *P. ovale* and *C. albicans* [14].The ethanol extract of W. tinctoria leaves was found to inhibit a wide range of fungal genera viz. *Curvularia, Bortrytis*, and *Aspergillus* was found to have significant inhibition against dermatophytes with extract concentration up to 1000ppm [15].

An indole alkaloid of tryptanthrin in naturally found in *W. tinctoria* leaves had been found to be active against *Leishmania spcies* and *Plamodium falsiparum* [16.17]. The methanol extract of the W. tinctoria leaves was found to be have anti-viral activity against hepatitis C virus using Huh 5.2 cell line (a cell line with persistant viral replication) [18]. Psoriasis is a auto immune disorder of skin that is charaterised by skin redness, itching and patchy looks. Traditionally, *W. tinctoria* is used to treat psoriasis, eczema, scabies etc. and that was clinically proved[19,20]. Hydro –alcoholic extract of *Wrightia tinctoria* was found to have anti-psoriatic activity [21,22]. Methanol and aqueous extracts of *W. tinctoria* leaves showed comparable anti-helminthic activity [23].

Ethanol extract of *Wrightia tinctoria* bark is found to have anti- diarrhoeal activity by showing its effects on prostaglandin inhibition and decreasing intestinal spasmodic movement [24]. The hydro-alcoholic extracts of *Wrightia tinctoria* leaves shows very good anti –oxidant activity in DPPH,H2O2 and nitric acid scavenging assays [25]. Anti-inflammatory activities of *Wrightia tinctoria* bark was first reported by Tharkar *et al*. in 2010 [25]. Aqueous and alcohol extracts of *Wrightia tinctoria* leaves were found to be significantly and comparably active in increasing the urinary water and electrolyte (Na+, Cl-, K+) concentration when compared to standard drug furosemide, in an experiment done according to the CPCSEA guidelines by Sathianarayanan et al.[26]. The present investigation designed to screen antioxidant activity of *Wrightia tinctoria* and its phytochemical constituents.

MATERIALS AND METHODS

Collection of leaf sample: Leaves that appeared healthy was collected from different branches of *Wrightia tinctoria* from Palakkad district of Kerala and was authenticated by Dr. S.S. Hameed., Scientist 'E', Botanical survey of India, Tamilnadu(No: BSI/SRC/5/23/2023/Tech – 547).

Preparation of extract: *Wrightia tinctoria* R.Br leaf was collected from Palakkad district of Kerala. In the laboratory, the leaf samples were washed 2-3 times with running fresh water, leaf material was then air dried under shade after complete shade drying, the plant material (500g) was grinded with mechanical grinder, the powder was kept in small labeled plastic bags. The extract was prepared by using soxhlet extraction at 60-80℃. The leaves were extracted with water, ethanol, hexane and ethyl acetate for 72 hours to get crude extract and it was filtered through Whatman No.1 filter paper. The extract was concentrated under vaccum and dried at 45℃ for complete removal of the solvent [34]

Preliminary qualitative phytochemical analysis: The various extracts of leaves of *Wrightia tinctoria* were analysed for the presence of various phyto constituents like alkaloids, flavonoids, glycosides, steroids, phenols, saponins, terpenoids, sugar, proteins, quinines according to standard methods [5].

Quantitative estimation of chemical constituency:

Determination of total phenol content:

Total phenolic content of the extract was studied with the Folin-Ciocalteau reagent method with modifications. To 0.5ml of each extract 2.5ml of 0.2N Folin-Ciocalteau's reagent was added, mixed by gentle shaking and kept for 10 minutes in room temperature. To the mixture 2 ml of Na2CO3 (20%, w/v) was added and incubated at 30℃ for 20 minutes. Three replicates were maintained per each experimental procedure. The absorbance of the sample was recorded at 765nm (Zahin et al, 2017) using UV/vis spectrophotometer (Labtronics LT291, Microprocessor). The phenolic content of the extract was estimated from the standard curve of Gallic acid and the results were expressed in Gallic acid equivalent GAE/g (Gallic acid standard was prepared in methanol at the concentration of 20,40, 60,80, and 100 µg ml-1) of extract [37].

Determination of total flavonoid content:

Total flavonoid content was determined by spectrophotometrically using the method of Aluminium chloride assay. 1ml of the extract was dissolved with 0.1ml of 10% Aluminium chloride solution and 0.1ml of sodium potassium tartarate followed by 2.8ml of distilled water. After adding the reagents the tubes were incubated in room temperature for 30 minutes and the measurement was taken in the nanometer of 415 using spectrophotometer (ELICO SL 159). Blank was maintained without adding the sample and standard quercetin was used to calculate the mg/g of the flavonoid content [38].

Determination of total alkaloid content:

TAC was also quantified by spectrophotometric method. This method is based on the reaction between alkaloid and bromocresol green. The plant extract (1mg/ml) was dissolved in 2N HCl and then filtered. The pH of phosphate buffer solution was adjusted to neutral with 0.1N NaOH. 1ml of this solution was transferred to a separating funnel, and 5ml of BCG solution along with 5ml of phosphate buffer were added. The mixture was shaken and complex formed was extracted with chloroform by vigorous shaking. The extract was collected in 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in the chloroform was measured at 470nm. The whole experiments are conducted in three replicates [39].

Pigment analysis

Determination of carotenoid:

Total carotenoid was calculated using the absorption coefficient for the mixture of the carotenoids in hexane. This approach was checked versus three types of calibrations: calibration in hexane, calibration in real blank.

Antioxidant property - DPPH Free Radical Scavenging Assay:

The DPPH free radical is a stable free radical, which has been widely accepted as a tool for estimating free radical scavenging activities of antioxidants. Hydrogen or electron donation abilities of the compounds were measured from the bleaching of the purple coloured methanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH). A solution of 0.1mM DPPH in methanol was prepared and 0.5mL of this solution was mixed with different concentration of sample (250,500,750 and 1000µL) along with 50mM tris HCl solution. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30minutes. The absorbance of the mixture was measured spectrophotometrically at 517nm [35, 36].

Percentage DPPH radical scavenging activity was calculated by the following equation:

% DPPH radical scavenging activity= (A0− A1)/A0×100

Where, A0 is the absorbance of the control, and A1 is the absorbance of the extractives/standard.

Characterization of compounds

UV-Visible spectrophotometry

UV-visible study of the ethanol extract using UV-visible spectrophotometer (UV-Visible spectrophotometer, LT291) with slit width of 100nm, using a 0.5nm absorbance at room temperature. The extract was examined under visible and UV light in the wave length ranging from 200-800 nm for proximate analysis, the extract

was centrifuged at 3000 rpm for 10 minutes and filtered through Whatman No. 1 filter paper the final extract was extract taken.

Fourier Transform Infrared Spectroscopy (FTIR):
FTIR was used to identify the characteristic functional groups in the extract. A small quantity of the extract was kept in the disc and was placed in a sample cup of a diffuse reflectance accessory. The IR spectrum was obtained using Shimadzu, infrared spectrometer. The sample was scanned from 4000 to 400 cm-1. The peak values of the FTIR were recorded and analysed. It provides the information about the structure of a molecule could frequently be from obtained from its absorption spectrum.

Thin layer chromatography (TLC): TLC is also used to identify the compound in a mixture when the Rf of a compound is compared with the Rf of a known compound. The extracts were added as a spot using capillary tubes (20µl) on the one end of the thin layer plate at above 1cm. plate was allowed it for dry, then it was placed in a beaker containing solvent of Toluene: ethyl acetate: methanol and formic acid in the ratio of 1:2:1:1 .The samples were allowed to run towards the other end of the plate. The sheet was removed and allowed it to air dry and incubated iodine chamber for 5 to 10 minutes [40, 41] Calculated Rf value using the given formula;

$$\text{Rf value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

RESULTS AND DISCUSSION

Preliminary qualitative phytochemical analysis
Phytochemical evaluation was performed with aqueous, ethanol, hexane and ethyl acetate extracts of *Wrightia tinctoria.* Ethanolic extract revealed the presence of alkaloids, terpenoids, phenols, sugars, flavonoids, protein, steroids and glucosides. Aqueous extract showed the presence of phenolics, saponins, flavonoids, proteins, steroids and glucosides. Hexane extract showed the presence of alkaloids, terpenoids and proteins. Ethyl acetate showed the presence of sugar and glucosides and the result were summarized in Table 1. Phytochemicals are naturally found in plants, they are biologically active and function to plants against invasion, disease and infection. Phytochemicals produced by plants through primary and secondary metabolism, and play an important role in plant growth.

Table 1: Preliminary Phytochemical Analysis of *Wrightia tinctoria* Extracts

Chemical components	Aqueous extract	Ethanol extract	Hexane extract	Ethyl acetate extract
Alkaloids	-	+	+	-
Terpenoids	+	+	+	-
Phenols	+	+	-	-
Sugar	-	+	-	+
Saponins	+	-	-	-
Flavanoids	+	+	-	-
Quinines	-	-	-	-
Proteins	+	+	+	-
Steroids	+	+	-	-
Glucosides	+	+	-	+

(+) = Present, (-) = Absent

Quantitative estimation of phytochemical constituents
Plant bioactive compounds have played a vital role worldwide in preventing and curing numerous human ailments. It is because of their broad spectrum of chemical and biological activities. Phyto-constituents of plants help in the production of complex chemical compounds as well as screening of their biological activities. The ethanol and aqueous leaf extracts of *Wrightia tinctoria are* subjected to quantitative analysis by standard methods and analyzed for phenols, flavonoids, alkaloids respectively. The results obtained from the quantitative analysis of aqueous leaf extract of *Wrightia tinctoria* showed the presence of phytochemicals from highest to least extent. The aqueous leaf extract of Wrightia *tinctoria* shows 24µg/g of total alkaloids

and total flavonoid content of 310µg/g and shows 80µg/g of total phenols. The results obtained from the quantitative analysis of ethanol leaf extract of *Wrightia tinctoria* showed the presence of phytochemicals from highest to least extent. The ethanol extract of *Wrightia tinctoria* shows 24µg/g of total alkaloid, 101µg/g of total flavonoid and 72µg/g of total phenols. The *Wrightia tinctoria* leaf extract shows 0.618µg/g of carotenoids respectively.

Figure 1: Preparation of extracts using the leaves of *Wrightia tinctoria*

Antioxidant property – DPPH free radical scavenging assay:

The DPPH free radical is a stable free radical, which has been widely accepted for estimating free radical-scavenging activities of antioxidants. DPPH free radical scavenging activity of ethanol and aqueous extracts is represented in figure 1. Both the extracts were found to be having slightly different free radicals scavenging capacity. The antioxidant activity of aqueous leaf extract of *W. tinctoria* absorbance at 517nm with the percentage of antioxidant as 61.20% and ethanol extract showing absorbance at 517nm with the percentage of antioxidant as 39.71% with DPPH.

UV-Visible spectrophotometry:

UV-VIS analysis performed for identification of phytoconstituents in ethanol extract of *Wrightia tinctoria*. The profile showed the peaks at 365nm, 440nm, 565nm, 635nm and 690nm with the absorption 0.368, 2.479.0.529, 0.404 and 0.930 respectively. Figure 2 shows the absorption spectrum of *W. tinctoria* extract. The spectrum for *W.tinctoria* extract shows peaks at 365nm and 440nm. This confirms the presence of Tannins and Carotenoids. The appearance of peak at 635 and 690nm is the clear indication of the presence of Chlorophyll.

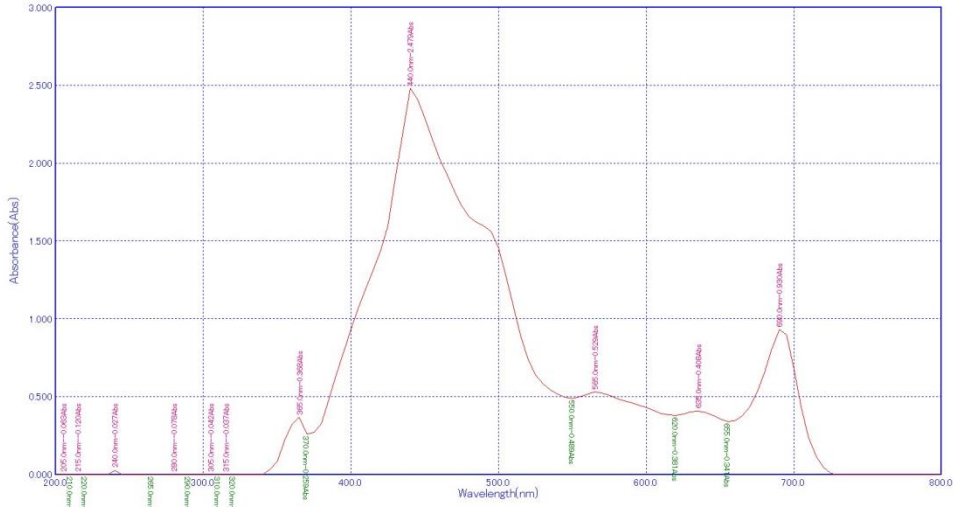

Figure 2: UV-VIS spectra of ethanolic *W. tinctoria* leaf extract

Fourier Transform Infrared Spectroscopy (FTIR Analysis):

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The FTIR spectrum of *W.tinctoria* ethanolic plant extract is shown in the figure 2. The band at 2113.4 is due to C≡C stretching. A notable band at 1345.6 is due to NO_2 stretch. The band at 1021.3 is indicate the presence of C-F group (alkyl halides) respectively.

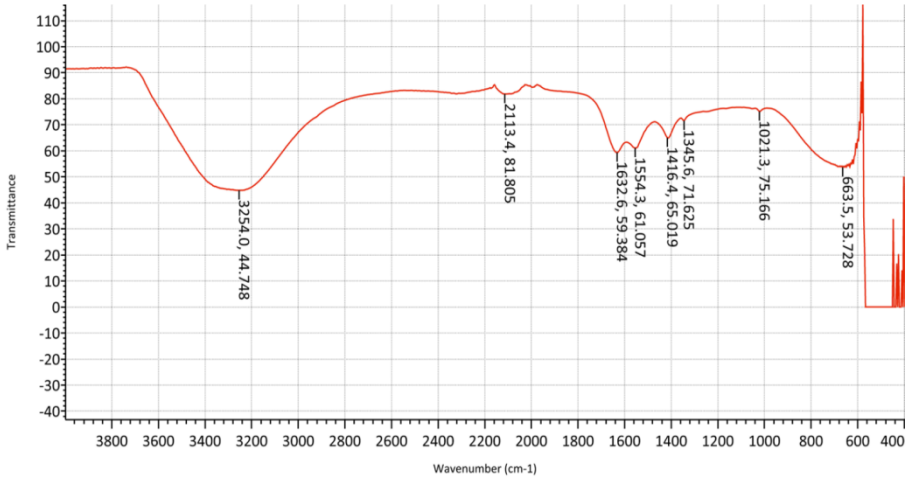

Figure 3: FTIR spectra of ethanolic *W. tinctoria* leaf extract

Thin layer chromatography (TLC):
TLC is also used to identify the compound in a mixture when the Rf of a compound is compared with the Rf of a known compound. TLC was performed with all extracts in which ethanolic extract showed the best result. The phyto constituent showed separation on system after spraying with iodine solution. UV scanning of the plates under 365 and 254nm (fig 4,5,6)showed the Rf values 0.4 to 0.6 compared with the standard quercetin. The maximum concentration of phyto constituent was found in the ethanol extract with Rf 0.67.

Fig 4: 365nm

Fig 5: 254nm

Fig 6: 365nm and 254nm

CONCLUSION

The present work concludes that *Wrightia tinctoria* is important plant with very effective medicinal property. The plant shows the presence of phytochemical constituents which are responsible for their significant medicinal and pharmacological property. The findings of this study indicate that the aqueous and ethanolic extracts of *Wrightia tinctoria* leaf showed significant antioxidant activity.

CONFLICT OF INTEREST:

We declare that we have no conflict of interest.

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