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Research Article

Phytochemical Investigation on *Wrightia tinctoria* Fruit

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ABSTRACT

Plant extracts appears to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides. The present study focuses on the phytochemical analysis of fruit of *Wrightia tinctoria.* The phytochemical screening of both Ethanolic and aqueous fruit extracts revealed the presence of various secondary metabolites such as carbohydrates, alkaloids, phytosterols, phenolic compounds, tannins, flavonoids, cumarine, amino acids, terpenoids and saponins. Present work also calculates total chlorophyll content of fruit containing peel and cellulose which shows strong chlorophyll content in peel.

The FTIR analysis of the crude extract of peel gives information about the distribution of functional groups and provides a basis for comparison of compositional differences between isolates and among samples.

keywords :- *Wrightia tinctoria*, Phytochemical, Chlorophyll Content, FT-IR.

INTRODUCTION

Wrightia tinctoria is an important medicinal plant used in the Indian system of medicine for the treatment of variety of diseases.[1] It may be used as an effective medicine with minimal side effect and can be brought to the market as a very potential chemotherapeutic drug against skin cancer in particular. Its effectiveness against cervical and lung cancer lines further demonstrates a possible use as a chemotherapeutic drug. The juice from fresh unripe fruits is used for coagulating milk. The seeds are said to be aphrodisiac and anthelminthic. The leaves are used to relieve toothache when chewed with salt. In Nepal, the milky juice is used to stop bleeding. Also the leaves and roots are pounded in water for treatment of fever. The seeds yield deep red, semi-drying oil, which has medicinal value. In Indian traditional medicine, the bark and leaves are used to treat psoriasis, stomach pains, toothache, and dysentery.[2,3]

The present study was designed to screen the phytochemical analysis of ethanolic and aqueous extracts of peel and cellulose of *Wrightia tinctoria* fruit.

MATERIALS AND METHODS

Collection of Sample**:** Fresh Fruits of *Wrightia tinctoria* were collected from Melghat Forest Near Ambegaon Village, Tal- Chikhaldara , Dist- Amravati (Central region of India) in the month of September – 2012, were authenticated by a taxonomist from Department of Botany ACS College Amravati.

Fresh Fruits of *Wrightia tinctoria* were washed well using tap water and twice using distilled water and it was dried in shade for a period of 20-25 days, at an ambient temperature of 30˚C. After drying the fruits of *Wrightia tinctoria* the peel (Green color) and cellulose (Creamiest White color) were separated by cutting them into small pieces. The dried samples were grinded properly using a mortar and pestle and later using a grinder, to obtain the powdered and fibrous form.

Preparation of Extracts: Aqueous extract: 25 gm of both samples was suspended in 200 ml of distilled water. Extraction was done at 70˚C for 30 minutes, followed by filtering of the extracts using Whatman filter paper No.1. Extracts were then evaporated at 45˚C for 72 hours to form a paste, and further transferred into sterile bottles and refrigerated until use.[4]

Ethanolic extract: 95% ethanol was added to 25 gm of sample. Extraction was allowed to stand for 72 hours at 27˚C, after which they were filtered using Whatman filter paper No.1. Extracts were then evaporated at 45˚C for 72 hours to form a paste, and further transferred into sterile bottles and refrigerated until use.[5]

Phytochemical Analysis (Qualitative Analysis): Test for Carbohydrates: Molisch's reagent was added to 2 ml of both extract. A little amount of concentrated sulphuric acid was added to it and allowed to form a layer. The mixture was shaken well, and allowed to stand for few more minutes, which was then diluted by adding 5 ml of distilled water. Purple precipitate ring showed the presence of carbohydrates.[5]

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Table 1: Phytochemical analysis of *Wrightia Tinctoria* peel and cellulose

Sr. No.	Phytochemical	Tests performed	Aqueous Extract		Ethanolic Extract	
			Peel	Cellulose	Peel	Cellulose
1	Carbohydrates	Molisch's Test	+	+	-	++
2	Sugar	Benedict Test	-	-	-	-
3	Protein	Xanthoproteic Test	+	+	+	+
4	Tannins	Gelatin Test	+	+	-	+
5	Phenolic comp	Lead acetate test	+	+	++	+
6	Phytosterols	Libermann-Burchard Test	-	-	++	++
7	Steroids	Ring test	-	+	++	++
8	Amino acids	Ninhydrin Test	++	++	+	++
9	Flavonoids	Ethyl acetate test	+	+	-	-
10	Terpenoids	Salkowski test	+	+	-	+
11	Alkaloids	Dragendorff's test	-	-	++	-
12	Anthraquinone	Borntrager's test	-	-	++	++
13	Cumarine	Fluorescence test	++	++	+	++
14	Phlobatinins	Spot test	-	-	++	-
15	Chalcones	Spot test	-	-	-	-
16	Saponins	Foam test	-	-	+	+
17	Fixed oils and lipids	Spot test	+	-	+	-

++ indicates: strong presence, + indicates: weak presence, - indicates: strong absence

Test for reducing sugars: *Fehling's test:* One ml of filtrate is boiled on water bath with 1ml each of Fehling solutions A and B. A red precipitate indicates the presence of sugar.

Benedict's test : To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 min. A characteristic coloured precipitate indicates the presence of sugar.

Test for proteins: *Xanthoproteic Test*: The extract were treated with few drop of conc. Nitric acid formation of yellow colour indicates the presences of protein.

Test for amino acids: *Ninhydrin Test* : To the sample extract, few drops of Ninhydrin reagent was added. After mixing it well, the solution was boiled in water for 2-3 minutes. A bluish-blackish color indicates the presence of amino acids.[6]

Test for Phenolic compound: *Ferric chloride test* : The extract (50 mg) is dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution is added. A dark green colour indicates the presence of phenolic compounds.

Lead acetate test : The extract (50 mg) is dissolved in distilled water and to this, 3 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

Test for phytosterols : *Libermann-Burchard Test* : A little quantity of the extract was dissolved in 5 ml of chloroform separately. This chloroform solution was treated with a few drops of concentrated sulphuric acid. To this a few drops of dilute acetic acid and 3ml of acetyl chloride was added. A bluish green color at bace & Brown ring at junction indicated the presence of phytosterols.[7,13]

Test for tannins: *Gelatin test* : To the extract 1% gelatin solution containing sodium chloride was added. Formation of white precipitation indicates the presence of tannins.

Test for steroids: 0.5 ml of the each extract was dissolved in 3 ml of chloroform and was filtered. To the filtrate, concentrated sulphuric acid was added by the sides of the test tube, which formed a lower layer. A reddish brown colour ring with a slight greenish fluorescence was taken as the indication for the presence of steroids.[9,14]

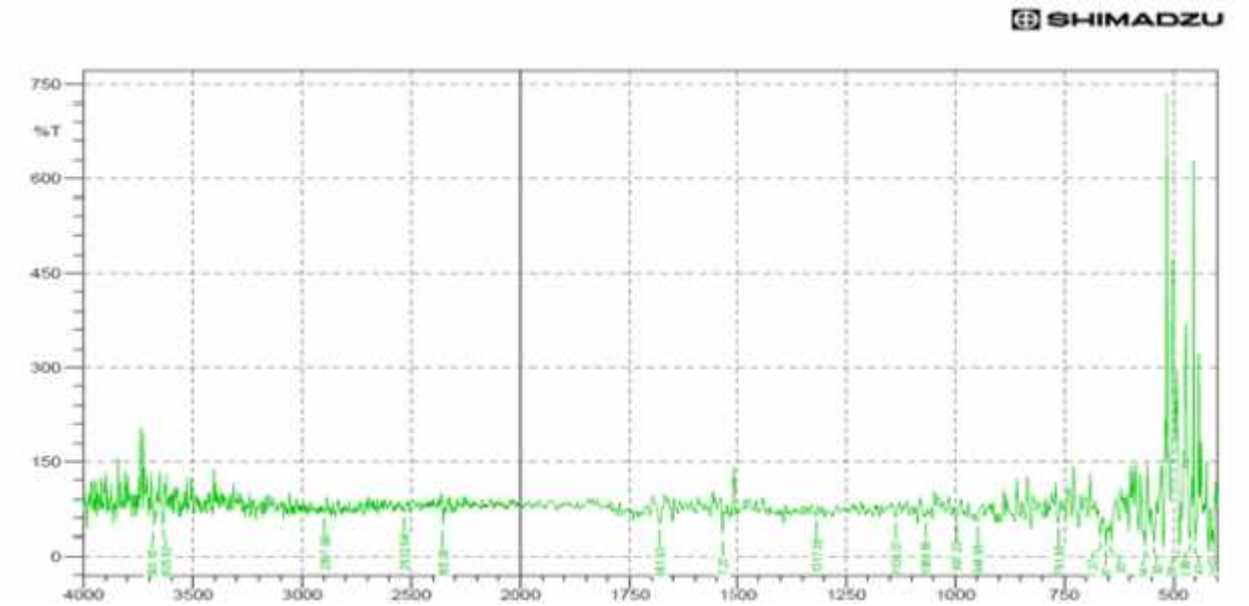
Determination of flavonoids: Two methods were used to determine the presence of flavonoids in the plant sample (Sofowara, 1993; Harbrone,1973).

1. 50 mg of each plant extract dissolved in 100 ml distilled water to form aqueous solution. 5 ml of dilute ammonia solution were added to 10 ml of aqueous filtrate of each plant extract followed by few drops addition of concentrated H_2SO_4. A yellow coloration observed in each fraction indicated the presence of flavonoids.

2. Ethyl acetate test : 50 mg portion of each plant sample was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration was observed indicating the presence of flavonoids.[5,8]

Test for terpenoids (Salkowski test): 5 ml (1 mg/ml) of each extract was mixed in 2 ml of chloroform, and then 3 ml concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the inter face was formed which showed positive results for the presence of terpenoids.

Fig:- 1. FT-IR for *Wrightia tinctoria* fruit peel



Test for Alkaloids: 0.4 g extract of each plant was mixed with 8 ml of 1% HCl, warmed and filtered. 2 ml of each filtrate was titrated separately with (a) Mayer's reagent and (b) Dragendroff's reagent Yellow precipitation for Mayer's reagent & Red precipitation for Dragendroff's reagent was observed to indicate the presence of alkaloids.[10]

Anthraquinone detection: Each extract was boiled with 6 ml of 1% HCl and filtered. The filtrate was shaken with 5 ml of benzene. The layer was removed and then 10% NH_4OH was added. Formation of pink, violet or red colour in the alkaline phase was observed for the presence of anthraquinone.

Cumarine identification : *Fluorescence test :* 0.3 g of each plant extract was taken in a small test tube and covered it with filter paper moistened with 1 N NaOH. The test tube was placed, for few minutes, in boiling water bath. Then the filter paper was removed and examined in UV light for yellow florescence to indicate the presence of Cumarine.

Identification of phlobatinins: For of Identification of phlobatinins 80 mg of each plant extract was boiled in 1% aqueous hydrochloric acid, the deposition of a red precipitate indicates the presence of phlobatinins.

Test for Chalcones: 2 ml of Ammonium hydroxide was added to 0.5 g each extract of each sample. Appearance of reddish colour showed the presence of chalcones.[8]

Test for Saponins: *Foam Test* : 0.5 gm of extract was shaken with 2 ml distilled water if foam produce persist for ten minute it , indicated the presence of saponins.

Froth test : 1gm of the each sample was weighed into a conical flask in which 10 ml of sterile distilled water was added and boiled for 5 min. The mixture was filtered and 2.5ml of the filtrate was added to 10 ml of sterile distilled water in a test tube. The test tube was stopped and shaken vigorously for about 30 second. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins[12].

Test for fixed oils and lipids: Small quantity of each extracts were separately pressed between two filter papers, and allowed to dry. Appearance of an oil stain or a grease spot on the filter paper when observed under direct sunlight, indicated the presence of fixed oils.[11]

Total Chlorophyll content of fruit : Chlorophyll content was determined by the method of Aron (1968) : Crude preparation of each extract of peel and cellulose of *W.Tinctoria* 1ml was mixed with 4ml of 80% (w/v) acetone and allowed to stand in dark at room temperature. It was centrifuged at 2000 rpm for 5 min to clear the suspension. Supernatant was used for chlorophyll determination. Absorbance of solution was read at 645 nm (chlorophyll a) and at 663nm (chlorophyll b) on spectrophotometer against 80% (v/v) acetone blank.

Total chlorophyll of each extract was calculated by = (20.2 x A645) + (8.02 x B663) mg/l

FT-IR Analysis of crude powder peel of Wrightia tinctoria : FT-IR is perhaps the most powerful tool for identifying types of chemical bonds (functional groups). The wavelength of light absorbed is characteristic of the chemical bond as can be seen in this annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Dried powder of fruit peel of *Wrightia tictoria* plant materials was considered for instrumental analysis. The powdered sample plant specimens were treated for FTIR spectroscopy

(Shimadzu, IR Affinity 1, Japan). Scan range: from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

RESULT AND DISCUSION

The *Wrightia tinctoria* fruit peel and cellulose extracts was rich in phytochemical activity, as shown in Table 1.

Total chlorophyll content of *Wrightia tinctoria* fruit peel and cellulose of both extract of aqueous and Ethanolic were found to be 4.49 & 44.852 mg/l , 12.865 &1.449 mg/l , respectively.

In *Wrightia tinctoria* fruit, FTIR- Spectrum shows strong absorption peaks at 2897.08 cm^{-1} which shows strong & wide absorbency for NH_3^+& Ar C-H, it indicates aromatic amines, peaks at 2355.08 cm^{-1} represents identical absorbency of stretching frequency for OH and NH_3^+ Group, peaks at 1317.38 cm^{-1} shows symmetrical & asymmetrical frequency for NO_2 & carboxylic acids, peaks at 1136.07 & 1068.56 cm^{-1} weak absorbency for aliphatic amines, peaks at blended frequency 761.88 cm^{-1} for C-H group.[12]

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