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Preliminary phytochemical screening of *Wrightia tinctoria* R.Br.

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Abstract
The plants are said to have medicinal properties in traditional systems and are also used by tribes around the world. It is now believed that nature cures all diseases in one way or another. In Ayurveda, plants provide relief from various diseases. Therefore, today's scientists refer to the evaluation and properties of various herbs and plants against various diseases according to the claims of herbs in the Indian traditional medicine system. Extraction of bioactive plants has always been a difficult task for scientists. This study aims to provide an overview of some extract ants and extraction processes of *Wrightia tinctoria* and to focus on the evaluation of various phytochemical components of butanol, acetone and chloroform extracts of *Wrightia tinctoria*. Our research results show that *Wrightia tinctoria* medicinal plant contains secondary metabolites such as flavonoids, alkaloids, tannins, cardiac glycosides and steroids.

Keywords: *Wrightia tinctoria*, butyl alcohol, acetone, phytomedicine, alkaloids, flavonoids

Introduction
All over the world, medicinal plants represent the most important field of traditional medicine. The study of medicinal plants is important to support the proper use of medicinal plants to determine their potential in new medicines. Medicinal plants have been used to treat diseases since recorded history. The relationship between food and medicine is "using food as medicine and medicine as food" (Gajalakshmi *et al.* 2012) [1]. The plant kingdom has been shown to be the most effective in treating diseases, and they provide a significant portion of all medicines in the world. The most important bioactive components of this plant are steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins and glycosides. Plants are important materials for drug production in all areas of life. Phytochemicals are used as examples for optimization to produce effective drugs (Chandrashekar and Rao, 2013) [2].
Microbial diseases are an important public health problem in developing countries. Antibiotics are used in the treatment of these diseases. Due to the illegal use of antibiotics, the incidence of various antibiotic resistance in human diseases is increasing. This forces scientists to search for new antibiotics from different sources such as medicinal plants. Medicinal plants as an important source of new drugs and nutraceuticals use of traditional medicine in India (Deshpande, 2013) [3].
Understanding the chemical composition of plants is necessary not only for the discovery of medicinal products, but also because this information will be useful in the development of products; the use of new financial resources such as tannins, oils, gums, precursors for the synthesis of complex drugs, etc. element. Additionally, knowledge of phytochemical components is important for discovering the benefits of folk remedies (Mojab, *et al.* 2003) [4]. Chemical ingredients may or may not work. Those that are active are called active substances, and those that are not active are called inactive substances (Iyengar, 1995) [5].

Materials and Methods
Collection of samples: The medicinal plant used for the experiment were leaves of *Wrightia tinctoria*. Collected from local Ayurvedic shop, and the plant material were identified and authenticated by botanist BSI, Allahabad (U.P.) India.

Preparation of extracts: For sample extraction, 500 grams of *Wrightia tinctoria* leaves were placed in a round-bottomed flask. Extraction was performed with 1000 ml solvent mixture for 72 hours. Once removed, all weights were placed in the reducer and the raw materials were stored in the refrigerator for later use.

Phytochemicals analysis: The extracts prepared were analyzed for the presence of alkaloids, saponins, tannins, steroids, flavonoids, anthraquinones, cardiac glycosides and reducing sugars based on the protocols available in the literature (Segelman, *et al.* 1969; Sofowora, 1982; Trease and Evans, 1989; Salehi-Surmaghi, *et al*. 1992; Siddiqui and Ali, 1997 and Adetuyi and Popoola, 2001) [6-11].

Test for alkaloids: Each weight of dry powder was evaporated to dryness in a boiling water bath. The residue was dissolved in 2N hydrochloric acid. Strain the mixture and divide the filtrate into three equal parts. A few drops of Mayer's reagent were applied to one part, an equal amount of Dragondroff's reagent was applied to one part, and an equal amount of Wagner's reagent was applied to the third part. The presence of sweet, orange and brown precipitates indicates the presence of alkaloids.

Test for saponins: About 2 ml of plant leaf extract was vigorously shaken with water in a test tube and then heated to boil. Frothing was observed which was taken as a preliminary evidence for the presence of the saponins.

Test for tannins: About 2 ml of plant leaf extract was added was in 10 ml of water in a test tube and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black coloration.

Test for steroids: 2 ml of acetic anhydride was added to 2 ml of plant extract of each sample along with 2 ml sulphuric acid. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for flavonoids: 2 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution few drops of conc. Hydrochloric acid was added and the red colour was observed for flavonoids and orange colour for flavones.

Test for anthraquinones: Take approximately 2 ml of extraction solution into a dry test tube, add 5 ml of chloroform and shake for 5 minutes. Filter the extract, add an equal amount of 10% ammonia solution to the filter and shake well. Red-red or scarlet color in the ammonia layer indicates the presence of anthraquinone.

Test for cardiac glycosides: 2 ml of extract was dissolved in 1 ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was then under layered with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a deoxysugar characteristic of cardioids.

Test for Proteins: To 2ml of extract and 1 ml of protein solution 1ml of 40% NaOH solution and 1 to 2 drops of 1% $CuSO_4$ solution was added. A violet colour indicated the presence of peptide linkage of the molecule.

Test for Amino Acids: To 2 ml of sample was added to 2 ml of Ninhydrin reagent and kept in water bath for 20 minutes. Appearance of purple colour indicated the presence of amino acids in the sample.

Test for Tri-Terpenoids: 5ml of each extract was added to 2ml of chloroform and 3ml of con. H_2SO_4 to form a monolayer of reddish brown coloration of the interface was showed to form positive result for the tri-terpenoids.

Test for Reducing Sugar: To 2 ml of extract 2 drops of Molisch's reagent was added and shaken well. 2ml of conc. H_2SO_4 was added on the sides of the test tube. A reddish violet ring appeared at the junction of two layers immediately indicated the presence of carbohydrates.

Results and Discussion

In the last decade, plant extracts and phytochemicals have become more important for their ability to prevent different diseases. Ethnobotanical plants are more useful than selected plants.

Table 1: Preliminary phytochemical constituents of acetone, butyl alcohol and chloroformic extracts of *Wrightia tinctoria*.

S. No.	Phytochemical	Acetone extract	Butyl Alcohol	Chloroform extract
1.	Flavonoid	++	++	++
2.	Alkaloids	--	++	++
3.	Saponins	++	--	--
4.	Tanins	++	++	--
5.	Amino acid	--	--	--
6.	Protein	--	--	--
7.	Terpenoids	--	++	--
8.	Reducing sugar	--	--	++
9.	Cardiac glycosides	++	++	++
10.	Anthroquinones	++	--	++
11.	Steroids	++	--	++

"++" - Positive."--" - Negative.

Table 1 shows the phytochemical composition of acetone, butyl alcohol and chloroform extracts of *Wrightia tinctoria*. Phytochemical analysis of the crude extract revealed the presence of flavonoids, alkaloids, saponins, sugars, tannins, steroids and anthraquinones. While saponins are present in acetone extracts, butanol extracts show negative results. All extracts showed positive effects of flavonoids. The butanol extract showed the absence of protein, while the acetone extract showed negative results. Terpenoids are present in the butanol extract. Reducing sugars were present in the chloroform extract and negative in the butanol extract. Cardiac glycosides are present in all extracts and amino acids are not present in all extracts. Acetone extracts are effective for steroids and anthraquinones. For alkaloids, butanol extracts showed positive results, while acetone extracts showed negative results. Amino acid results for all extracts were negative. Phytochemical analysis of *Wrightia tinctoria* revealed a number of secondary metabolites that contribute to its phytochemical pharmacological activity. This study shows that the presence of phytochemicals in *Wrightia tinctoria* will provide significant contributions to many future studies on various activities of plants.

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