

A comparative phytochemical analysis of *Wrightia* species of family apocynaceae by spot tests

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Abstract

The phytochemical analysis of four species of medicinal plants from district Saharanpur was done. The plants were *Wrightia coccinea*, *Wrightia tinctoria*, *Wrightia mollissima* and *Wrightia tomentosa*, members of the genus *Wrightia* (Family: Apocynaceae). Qualitative phytochemical analysis of these plants confirms the presence of various phytochemicals like alkaloids, flavonoids and terpenoid. The presence of these phytochemicals can be correlated with medicinal potential of these plants.

Keywords: Medicinal plants, phytochemical analysis, spot test, alkaloids and flavonoids.

1. Introduction

Medicinal plants are nature's unique gift to human beings. The acceptance of traditional medicine as an alternative form of health care. Apocynaceae is A family of 180 genera and 1,500 species distributed mainly in the warmer parts of the world but few are temperate. In India the family is represented by 29 genera and 60 species occurring chiefly in eastern Himalayas and southern peninsular India [1].

A phytochemical investigation of bioactive compounds from plants has lead to the discovery of new medicinal drug which have efficient protection and treatment of various diseases including cancer [2]. Many groups of chemical compounds like aromatic oils, steroids, gums, tannins, catechins, alkaloids, anthraquinones and flavonoids etc., are called secondary constituents, as these are not directly involved in metabolism; but, these are very much important medicinally and taxonomically. The rapid development of biochemical system in the last three decades owes much to the study of these chemical

compounds. Genus *Wrightia* has recently gained attention for its excellent use in curing Psoriasis and other ailments for which medicines are not available.

Members of the genus *Wrightia* (Family: Apocynaceae) are widely distributed in Asia, Africa, and Australia [3]. Extracts of *Wrightia tinctoria* R. Br. seeds are used in traditional medicines (Ayurveda and Siddha), in India, as carminative, astringent, aphrodisiac and tonic. It is also recommended for the treatment of infections of chest (in asthma), colic and as a diuretic [3].

2. Materials and methods

2.1 Collection of plant materials

Fresh leaves of selected species of *Wrightia-tinctoria*, *Wrightia coccinea*, *Wrightia tomentosa*, and *Wrightia mollissima* were identified and collected from Saharanpur district with the help of Dr. S.K. Updadhya, formerly Ex H.O.D and Reader Department of Botany, M.S. College, Saharanpur. The flowering season of plant is basically varies March to May, peaking from April to June.

2.2 Preparation of extracts

100 g shade dried leaves were grounded in the form of coarse powder. Each powdered plant material was extracted successively with methanol in a Soxhlet apparatus.

2.4 Preparation of aqueous extract

Dried coarsely powdered leaves sample (2.0 g) were taken with 100 ml water, in conical flask and then put on water bath for few minutes and allow standing for overnight. The sample filtered and then collected the filtrate after drying on Petri dish at 105°C. Therefore no need of defatting

2.5 Analysis

According to Gibbs (1974) [4], Harborne (1973) [5], Ljkrans (1956) [6], Peach and Tracey (1955) [7], Feigl (1974) [8] and Paris (1963) [9], spot test are helpful in the testing for the presence of various secondary metabolites and phytochemicals. The extracts of different species of *Wrightia* qualitatively tested by the help of solvent extraction and got the basic idea for the correlation in between different species of *Wrightia*.

(1) Test for Lucoanthocyanin:

Lucoanthocyanin Test: Dried plant material about 0.5 g was macerated in 10 ml 2N HCl on water bath, after 5 minutes, 5 ml of iso-amyl alcohol was added and shaken, two layers formed, the upper iso-amyl layer if coloured, indicates the presence of lucoanthocyanin.

(2) Test for Indole:

Ehrlich Test 'A': Dry leaf material crushed and extracted with 50 ml Aq. ethanol (2g leaf powder/100 ml each) on water bath the extract concentrated and a spot of the extract was developed on the filter paper with the help of a glass rod. On this spot Ehrlich reagent (prepared by dissolving 1 g P-dimethyl aminobenzal-dehyde in 5ml conc. HCl in 200 ml or 95% Methanol) was added. Appearance of bright blue color spot was considered as positive test.

Ehrlich Test 'B': Acid alcohol was added on the spot, extracted by Ehrlich Test 'A'. Appearance of red color was the indication of positive test.

Ehrlich Test 'C': NH₄OH was added on the spot and heated, extracted by Ehrlich Test 'A'. Appearance of yellow color was considered to be the positive test.

(3) Saponin Test:

Dried leaves (Powdered) with 10 ml of water taken in a test tube and kept in a water bath for one minute, cooled, shaken vigorously and set for 10 minutes. Appearance of foam of about 2 cm. or more in depth indicates the presence of saponins and little or no foam indicated their absence.

(4) Tannin Test:

A small strip of filter paper was dipped in a freshly prepared 2.5% aq. ferric ammonium citrate solution, blotted gently and folded around the leaf. Then filter paper and leaf were

squeezed with rib nosed pliers. Development of a purple blue colour on the filter paper was considered as a positive test for the presence of tannins.

(5) Test for Quinones:

Juglone Test 'A': Dried leaves powder about 1 g were taken in a test tube and 5 ml CHCl₃ was added to it. The material was allowed to macerate for 24 hours at room temperature. Then the extract was transferred to another test tube, evaporate on water bath, 2 ml ether and equal volume of dilute ammonia (1:9 part water) was added and the mixture was shaken gently. An immediate appearance of purple colour in the ammonia layer indicated the presence of quinones.

Juglone Test 'B': Juglone Test 'B' was to be positive reaction if juglone test 'A' is negative and any other colour develops in the ammonia layer.

Juglone Test 'C': If there is fluorescence present in ammonia layer in daylight or in long wave ultra violet lamp then juglone test 'C' was considered to be positive.

(6) Test of oxalic acid:

Cigarette Test: A lighted cigarette was pressed gently against the back of a fresh mature leaf for about 3 seconds. Development of almost at once of a brown or black ring around the heated area was supposed to be a strongly positive reaction. Appearance of yellow color was indication of oxalic acid reaction.

(7) Test for Anthocyanins:

Betacyanin and Betanidin Test: Fresh leaves were crushed against a filter paper to obtain two separate spots and a drop of 1 M NaOH aq. solution on the second spot. Anthocyanins became deep and stable with HCl in shades of blue-green with NaOH. Betanidins became yellow with NaOH and completely lost their colour with HCl.

(8) Test for antraquinones :

Borntrager Test: About 0.25 g of powdered leaf material was extracted with ethyl alcohol on water bath. The extract was taken to another test tube and evaporated to dryness. Residues were washed with petroleum ether to remove chlorophylls, other pigments, and fatty materials. Defatted residues were dissolved in benzene (about 3 ml) and dilute ammonia solution was added to it. Immediate development of pink to red colour indicated the presence of antraquinones

Modifid Borntrager's Test: About 0.25 g powdered leaves were heated with 10 ml of 0.5 N KOH and 1 ml of H₂O₂. The extract was filtered and the filtrate was acidified with a few drops of glacial acetic acid and shaken with 10ml benzene. The benzene layer was decanting in another test tube and 2.5 ml NH₄OH solution was added to the decanting. Appearance of pink red colour in benzene layer indicated the presence of antraquinones.

(9) Test for auronones, calcones, catchins, and leucoanthocyanins:

Shinoda Test 'A': Dried leaves (About 0.25 g) were extracted in ethyl alcohol on water bath. Extract was filtered and evaporated by rotating test tube on a steam current. Residues present along the walls of the test tube were washed first by petroleum ether and then by benzene to remove fatty substances and photosynthetic pigments. Then ethyl alcohol was added to the test tube and subsequently by adding 2-3 drops of conc. HCl the colour was observed. Development of red, pink or purple colour was the indication of the presence of auronones, calcones, catchins, and leucoanthocyanins.

Shinoda Test 'B': Mg dust was added to test tube if Shinoda test 'A' was negative. Appearance of red or orange colour indicated the presence of flavonoids. The contents were divided into two parts; one part was kept reference and in the other Mg dust was added. In the presence of flavonoids along with leucoanthocyanines the intensity of the colour comparison the reference part was greater.

(10) Tests for Alkaloids: The plant extract was mixed with a few drops of acetic acid followed by dragendroff's

reagent and mixed well. A orange red precipitate is formed indicated the presence of alkaloid.

(11) Formic acid Test:

Dried leaves were extracted in ethyl alcohol in 250 ml round bottom flask, using a reflex condenser. The extract was evaporated over a water bath and washed successively by petroleum ether and benzene. The residue left behind was dissolved in water. 2 ml of this stock solution was taken in a test tube and 1 ml of 2 N HCl and Mg dust was added to it until no gas evolved. Then 3 ml of 72% H₂SO₄ and a little chromatoropic acid was added and the tube was heated at 60⁰c for 10 minutes. Appearance of a violet pink colour indicated the presence of formic acid.

(12) Test for cardiac glycosides:

Keller-Killiani Test: Dried leaves extracted by alcohol were washed with petroleum ether and dissolved in 1 g glacial acetic containing traces of ferric chloride. Then 1 ml conc. H₂SO₄ was added to it carefully by the side of test tube. Blue colour in acetic acid layer and red at the junction indicated the presence of cardiac glycosides.

Table 1: Qualitative chemical analysis of *Wrightia* species by spot tests

S. No.	Spot Tests	<i>W.tinc.</i>	<i>W. coc.</i>	<i>W. tom.</i>	<i>W. moll.</i>
1	Lucoanthocyanin Test	+	+	+	-
2	Indole Test				
i)	Ehrlich Test A	-	-	+	+
ii)	Ehrilch Test B	+	+	+	-
iii)	Ehlich Test C	+	+	+	-
3	Saponin Test	-	-	-	-
4	Tannin Test	-	-	-	-
5	Quinones Test				
i)	Juglone Test A	+	+	+	+
ii)	Juglone Test B	-	-	-	-
iii)	Juglone Test C	-	-	-	-
6	Oxalic acid Test				
i)	Cigaratte Test	+	+	+	-
7	Anthocyanins Test				
i)	Betacyanins	+	+	+	+
ii)	Betanidins Test	+	+	+	+
8	Antraquinone Test				
i)	Borntranger Test	-	-	-	-
ii)	Modified Borntranger Test	-	-	-	-
9	Auronones, Calcones, Catchins, Test				
i)	Shinoda Tast A	+	+	+	+
ii)	Shinoda Test B	+	+	+	+
10	Alkaloids Test	-	-	-	-
11	Formic Acid Test	-	-	-	+
12	Cardiac Glycoside Test				
i)	Keller-KillianiTest	+	+	+	+

Legend: *W.tinc.*=*Wrightia tinctoria*, *W.tom.* = *Wrightia tomentosa*, *W. coc.*=*Wrightia coccinea*, *W.moll.*= *Wrightia mollissima*

3. Results and discussion

Results of the qualitative analysis were reported in table no. 1, where + means positive and – means negative test. The phytochemical screening of the plants studied showed the presence of: presence of Lucoanthocyanin in *Wrightia tinctoria*, *Wrightia tomentosa*, *Wrightia coccinea* and absence in *Wrightia mollissima*. Indole is present in all species of *Wrightia tomentosa* through Ehrlich Test A, B, C. Found absent in *Wrightia tinctoria* and *Wrightia coccinea* through Ehrlich Test A. *Wrightia mollissima* also gives negative test through Ehrlich Test B and C. Saponin found absent in all species of *Wrightia*. Tannin found absent in all species of *Wrightia*. Quinone is present in all species of *Wrightia* through Juglone Test A whereas found absent through Juglone Test B and C. Oxalic acid is present in *Wrightia tinctoria*, *Wrightia tomentosa*, *Wrightia coccinea* and absent in *Wrightia mollissima*.

Anthocyanins are present in all species of *Wrightia*. Antraquinone found absent in all species of *Wrightia*. Aurones, Calcones and Catchins are present in all species of *Wrightia*. Alkaloids found absent in all species of *Wrightia*. Formic Acid is present in *Wrightia mollissima*. Cardiac Glycoside is present in all species of *Wrightia*.

It has been found in present analysis that *Wrightia* species of family Apocynaceae are rich in phytochemicals.

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