



PHYTOCHEMICAL ANALYSIS OF THE METHANOL EXTRACT FROM LEAVES OF HEMIDESMUS INDICUS

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ABSTRACT

Our aim of study to investigate the presence of various plant chemicals like salts steroids, triterpenes, alkaloids, saponins, tannins, anthraquinones and flavonoids iso flavonoid compounds or their derivatives with their biomedical applications. Plant used for study are traditionally known we carry out phytochemical study of the dried leaf of the plant, to extract and fractionate the leaf and thin layer chromatography of the different fractions to study different components. In the phytochemical investigation of the plant, the powdered leaves was gives tests positive for steroids, triterpenes, alkaloids, saponins, tannins, anthraquinones and flavonoids. In panvel region theses leaves are used on wound healing activity like tridex procubace and gulmohar tree.

KEYWORDS: flavanoids, alkaloids, materials, tests, phytochemical, chromatography, tannins, extraction, colour.

MATERIALS AND METHODS

Collection and preparation of plant material

Plant leaves are used for the study of plant material collected from panvel region of raigad district indentified by Dr. B.K. Auti of RKMM College Ahmednagar and herbarium is preserved in Rayat SK3hikshan Sansthsa Karmaveer Bhaurao Patil college Vashi as voucher no (001) The leaves were dried at room temperature for 13 days and when properly dried the leaves were powdered using clean pestle and mortar and the powdered plant was size reduced with a sieve. The fine powder was then packed in airtight container to avoid the effect of humidity and then stored at room temperature.

Climber found throughout India. Leaves are 2.5 inches long and alternatively arranged in a pair. Upper part is oval in shape and it is very soft. Stem of this plant is cylindrical with thick nodes. Woody roots are underground part and are very aromatic. Flowers of this vine is found in cluster yellow and greenish purple in color.^[1-3] Fruits of this vine are divergent long follicles 2-4 inch long. This plant is used as household remedy for various disorders. Leaves and roots have medicinal properties a great herb which has many versatile properties. It is very effective in managing gastritis and many other pitta disorders in the body.

It is also known as "Sariva" and is used in managing disorders of the reproductive system in females. It is a very good natural detoxification herb and helps in

managing health naturally.^[3-6] It is a very useful plant in the Indian system of medicine. There are two varieties of this shrub which are demarcated by the color of their flowers, one of them is called as shveta sariva (bearing white flowers) and the other one is called as Krishna sariva^[6-11] (black variety) but both of them seem to have similar medicinal value. The roots are woody and aromatic while the stems are slender, green in color and numerous tender, twining shrub which is commonly found in most parts of India. Commonly it is available in North Western parts of Himalaya.^[10-12] It is also cultivated in Punjab. It is a very useful plant in the Indian system of medicine.

Extraction of the powdered leaf of the plant

Maceration

Collected plant leaves around 100 gm are soked in 350 ml of methanol in conical flask was cork with Teflon and shake on shaker sor 30 min. and kept at room temperature for 48 years then refluxed for 2 hours, then cool and evaporate all methanol to dryness, in evaporating dish.

Fractionation of the methanol extract

The methanol extract (2 g) was placed at the top of a silica gel (28 g) wet packed in a chromatographic column and eluted with gradient of hexane and ethyl acetate at ratio of 8:2. 20 ml of the elute were collected in small bottles and labeled 1, 2, 3....19, successively. The fractions collected were spotted on thin layered

chromatography (TLC) plate and developed. The plate was allowed to dry and sprayed with 20% sulphuric acid then heated in an oven at 105°C for 15 min.

Fractionation of the methanol extract

2 gm of powdered leaves placed on column around 30 gm of silica gel in taken and eluted with solvent hexane and ethylacetate (8:2) and eluent are collected in conical flask labelled as 1-20 depend on TLC observed during column.

Phytochemical analysis of *Paullinia pinnata*

Identification of sterols and triterpenes

Three grams of the powdered leaves was placed in a test tube and 10 ml of 50% alcohol was added, the tube was then placed on a water bath and heated for 3 min. It was then allowed to cool to room temperature and filtered. The filtrate was then evaporated in an evaporating dish to dryness and 5 ml of petroleum ether was added to the dish and stirred for 5 min, the petroleum ether portion was then decanted and discarded. 10 ml of chloroform was then added and stirred for about 5 min, it was then transferred into test tube and 0.5 mg of anhydrous sodium sulphate was added and shaken gently and filtered, the filtrate was then divided into two test tubes and used for the following tests.

Lieberman-Burchard's reaction

To test tube I, equal volume of acetic anhydride was added and gently mixed. Then 1 ml of concentrated H₂SO₄ was added down the side of the tube. The appearance of a brownish-red ring at the contact zone of the two liquids and a greenish colour in the separation layer indicates the presence of sterols and triterpenes.

Salwoski's test

To test tube II, 2 to 3 drops of concentrated sulphuric acid was added to form a lower layer. Reddish-brown colour at the inter phase indicates the presence of steroidal ring.

Identification of alkaloids

The powdered leaves (2 g) were boiled in a water bath with 20 ml of 5% sulphuric acid in 50% ethanol. The mixture was cooled and filtered. A portion was reserved. Another portion of the filtrate was put in 100 ml of separating funnel and the solution was made alkaline by adding two drops of concentrated ammonia solution. Equal volume of chloroform was added and shaken gently to allow the layer to separate. The lower chloroform layer was run off into a second separating funnel. The ammoniacal layer was reserved. The chloroform layer was extracted with two quantities each of 5 ml of dilute sulphuric acid. The various extracts were then used for the following test:

Mayer's test

To the filtrate in test tube I, 1 ml of mayer's reagent was added drop by drop. Formation of a greenish coloured or

cream precipitate indicates the presence of alkaloids (Evans, 2002).

Dragendoff's test

To the filtrate in test tube II, 1 ml of dragendoff's reagent was added drop by drop. Formation of a reddish-brown precipitate indicates the presence of alkaloids (Evans, 2002).

Wagner's test

To the filtrate in tube III, 1 ml of wagner's reagent was added drop by drop. Formation of a reddish-brown precipitate indicates the presence of alkaloids (Evans, 2002).

dentification of tannins

Two grams of the leaves was extracted with 10 ml of 50% alcohol, it was then filtered and the filtrate was divided into three portions for the following tests.

Ferric chloride test

Three drops of diluted solution of FeCl₃ was added to the test tube I, production of a blue or greenish-black colour that changes to olive green as more ferric chloride is added indicates the presence of tannins (Evans, 2002).

Bromine water test

Three drops of bromine water was added to the second portion of the filtrate. A buff coloured precipitate indicates condensed tannins while hydrolysable tannins gave none (Evans, 2002).

Lead sub-acetate test

Three drops of lead sub acetate solution was added to the third portion. Occurrence of a coloured precipitate indicates the presence of tannins (Evans, 2002).

Identification of anthraquinones

Borntrager's test (for free anthracene derivatives)

The powdered leaves (0.5 g) was taken in a test tube and 5 ml of chloroform was added and shaken for 5 min. The mixture was filtered and the filtrate shaken with equal volume of 10% ammonia solution. A pink, red or violet colour in the aqueous layer after shaken indicates the presence of free anthraquinone (Evans, 2002).

Modified Borntrager's test (for combined anthracene derivatives)

One gram of the powdered leaves was boiled with 5 ml of 10% hydrochloric acid for 3 min. The hot solution was filtered in a test tube, cooled and extracted gently with 5 ml of benzene. The upper benzene layer was pipetted off and shaken gently in a test tube with half of its volume of 10% ammonium hydroxide solution. A rose pink to cherry red colour in the ammonia layer indicates the presence of anthraquinone (Evans, 2002).

Identification of saponins**Frothing test**

The powdered leaves (0.5 g) was placed in a test tube and 10 ml of distilled water was added and shaken vigorously for 30 s. It was then allowed to stand for 30 min and observed. Formation of honey comb froth indicates the presence of saponins (Safowora, 1993).

Haemolysis test

One gram of the leaves was extracted with distilled water and 2 ml of aqueous NaCl solution was placed in a test tube and 2 ml of the filtrate was added to the test tube. Then 3 drops of an animal blood was added to the tube by means of a syringe and mixed gently by inverting the tube (no shaking) and allowed to stand for 15 min. The settling down of the red blood cells denotes the presence of saponins.

Identification of flavonoids

Two gram of the powdered leaves sample was completely detanned with acetone. The residue was extracted with warm water after evaporating the acetone on a water bath. The mixture was then filtered while hot, the filtrate was allowed to cool and used for the following test:

Shinoda's test

Few magnesium chips were added to 3 ml of the aqueous solution and 2 drops of dilute hydrochloric acid was added and warmed. A pink or red colour indicates the presence of flavonoids (Evans, 2002).

Sodium hydroxide test

To test tube II, 2 mls of 10% NaOH solution was added, yellow solution indicates the presence of flavonoids which on adding dilute hydrochloric acid becomes colourless (Evans, 2002).

FeCl₃ test

To test tube III, 3 drops of FeCl₃ solution was added, production of greenish-black colour indicates the presence of phenolic nucleus (Sofowora, 1993).

Thin layer chromatography**Parameters used**

Absorbent (silica gel) Merck, Germany 120 mesh size, eluting solvent- n-hexane: ethyl acetate (8:2); technique: ascending; visualization aids: day light, methanol-sulphuric acid and heated at 105°C for 15 min.

Development of thin layer chromatography for the extract

The extract was applied onto the plate about 1.5 cm above the edge and 0.5 cm away from the margin, when the spot was dried, the plate was observed and then sprayed with methanol-sulphuric acid and then heated in oven at 105°C for 15 min. The solvent used for the mobile phase was n-hexane and ethyl acetate (8:2).

Column chromatography of the extract

The methanolic extract of the powdered leaf was added into a column pre-packed with silica gel. It was then run using n-hexane/ethyl acetate (8:2) and the separated fractions were collected separately in bottles.

TLC of the fractions

Using capillary tubes, the various fractions collected from column chromatography were spotted on a silica gel pre-coated plate 1.5 cm from the base and 0.5 cm away from the edge. Each plate was allowed to dry before putting it in a chromatographic tank containing specific solvent system. The developed plate was sprayed using methanol sulphuric acid.

RESULTS

The followings are the results of analysis of phytochemical constituents in *hemidesmus indicus* leaf.

Identification of sterols and triterpenes**Lieberman-burchard's test**

A violet ring was formed at the contact zone of the two liquids; the upper layer becomes green which indicates the presence of sterols.

Salwoski's test

A reddish brown colour was observed at the interphase which indicates the presence steroid ring.

Identification of tannins**Ferric chloride test**

A greenish precipitate was formed which indicates the presence of condensed tannins.

Lead sub-acetate test

A coloured precipitate was observed indicating the presence of tannins.

Bromine water test

A buff colour precipitate was observed which indicates the presence of tannins.

Identification of alkaloids**Mayer's test**

A cream (buff) coloured precipitate was formed which indicates the presence of alkaloids.

Dragendoff's test

A reddish-brown precipitate was formed which indicates the presence of alkaloids.

Wagner's test

A reddish-brown precipitate was formed which indicates the presence of alkaloids.

Identification of flavonoids**Ferric chloride test**

A greenish-black colour was observed which indicates the presence of flavonoids.

NaOH test

A yellow coloured solution was formed which indicate the presence of flavonoids.

Shinoda's test

A pinkish coloured solution was observed which indicates the presence of flavonoids.

Identification of saponins**Frothing test**

A honey comb froth was formed which persisted for about 10 minutes indicating the presence of saponins.

Extraction Fraction	Colour After Spray	Number of spots	Retention Factor
1	Light yellow	5	0.64
	yellow		0.20
	pink		0.19
	Green		0.54
	Purple		0.78
2	Light yellow	3	0.67
	pink		0.90
	Green		0.50
3	Pale Purple	3	0.70
	Green		0.90
	Voilet		0.45
4	Violet	1	0.56

Haemolysis test

The red blood cell settled down in the test tube which indicates the presence of saponins.

Identification of anthraquinones**Borntrager's test**

A pink colour solution was formed showing the presence of free anthracene derivative.

Modified Borntrager's test

A pinkish colour was formed in the ammonia layer which indicates the presence of anthraquinone.

Layer chromatography

Technique used: Ascending; eluting solvent: n-hexane:ethyl acetate (8:2); visualization aids: Day light, methanol-sulphuric acid and heated at 105°C for 15 min.

(a) Before spray: Number of spot = 3; Colour: Spot 1: Light yellow, Spot 2: Yellow, Spot 3: Green.

(b) After spray: Number of spot = 6; Colour: Spot 1: Light yellow, Spot 2: Yellow, Spot 3: Pink, Spot 4: Green, Spot 5: Violet, Spot 6: Purple.

Retention factor (Rf) = Distance moved by the Component / Distance moved by the solvent.

Spot 1 Rf value = $1.4/7.2 = 0.19$	Spot 2 Rf value = $4.6/7.2 = 0.64$	Spot 3 Rf value = $5.7/7.2 = 0.79$
Spot 4 Rf value = $6.1/7.2 = 0.85$	Spot 5 Rf value = $6.4/7.2 = 0.89$	Spot 6 Rf value = $6.9/7.2 = 0.96$

DISCUSSION

Phytochemical analysis of the leaves are was carried out, hexane/ethyl acetate at ratio (8:2) was found to be a good solvent system for the separation of the active constituents of the plant and using TLC, the separation of these constituents on the chromatogram was carried out. The powdered leaf was tested positive for steroids, triterpenes, alkaloids, saponins, tannins, anthraquinones and flavonoids. These results agreed with the literature review on the plant which showed these chemical constituents to be present. The TLC chromatograms of elutes collected showed different spots and colours ranging from fairly coloured to distinctively visible colours after spraying with 20% sulphuric acid indicating the presence of such chemical constituents (Plates 1 to 4 and Table 1).

CONCLUSION

The phytochemical constituents of the leaf of *hemidesmus indicus* was investigated. The leaf was found to constitute steroids, triterpenes, alkaloids, saponins, tannins, anthraquinones and flavonoids. The leaf is an African woody vine widely used in traditional medicine for the treatment of malaria and as a remedy against different forms of pains and as a natural cure (Jimoh et al., 2007). The presence of the constituents was also found to be similar to those reported for most medicinal plants. In East Africa, the leaves are used against snake bites, rabies, mental problems, blindness and eye troubles, together with the roots, against gonorrhoea, paralysis, wounds, threatened abortion, malaria, ancylostomiasis and to expel the placenta. Roots are applied against eczema, as a tonic and as a styptic

medicine (Abourashed et al., 1999). The whole plant is applied for bad skin conditions, for wounds and microbial infections. The root decoction is drunk in the case of nausea and vomiting. In Nigeria, the research work is still in the initiation stage and the work was carried out to find its phytochemical constituents in relation to the leaf elsewhere in Africa.

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