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Full Length Research Paper

Phytochemical analysis of the methanol leaves extract of *Paullinia pinnata* linn

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The aim of this study seeks to investigate the presence of steroids, triterpenes, alkaloids, saponins, tannins, anthraquinones and flavonoids compounds as the possible agent responsible for the medicinal activities of the leaf of the plant *Paullinia pinnata* and also to carry out phytochemical screening of the dried leaf of the plant, to extract and fractionate the leaf and finally to carry out thin layer chromatography of the different fractions. In the phytochemical investigation of the plant, the powdered leaves was tested positive for steroids, triterpenes, alkaloids, saponins, tannins, anthraquinones and flavonoids. 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 placenta. Roots are applied against eczema, as a tonic and as a styptic medicine.

Key words: *Paullinia pinnata*, phytochemical, chromatography, flavanoids, alkaloids, materials, tests, tannins, extraction, colour, spray, fractionate.

INTRODUCTION

There are a large number of medicinal plants whose scientific importance has not been explored. All over the world, plants have served as the richest source of raw materials for traditional as well as modern medicine, particularly in Africa and Asia (Tsakala et al., 2006). Knowledge acquired by ancient people was transmitted from generation to generation and new knowledge added to it by the next generation. Gradually, a group of people in each generation started specializing in collecting and processing medicinal plants and using them against various diseases even though many of them had not been identified scientifically. Diseases are best controlled through pharmacotherapy. The study of the many chemical constituents contained in plant is very essential because most drugs used as medicines were later synthesized after a careful study of their constituents and structures (Ghani, 1990). The problem however remains that some drugs are scarce, costly and unavailable to the common man, hence a study of the medicinal importance of plants, scientifically and a confirmation of the use of

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these plants towards curing diseases is a possible solution to development of less costly and effective drugs from our local raw materials (Zamble, 2006).

The medicinal and pharmaceutical properties of plants are due to the type of chemical substance they produce and store. These include compounds that are utilized as food by man and other animals and also other compounds that exert physiological effects on them. This second group of chemical substances often referred to as secondary metabolites, give plants their therapeutic properties. The usual term used to refer to these various chemical substances present in plant is “constituents”. The constituent which possess pharmacological properties are called ‘active constituents’. Phytochemistry is concerned with the chemical study of these plant constituents (Evans, 2002).

The test used in phytochemical screening should be simple, standard and one should be aware of false positive result and hence the need for carrying out confirmatory tests. The chemical constituents that are of medicinal importance are mainly the secondary metabolites, and the examination of the chemical constituents of the plant can only reveal those compounds that have accumulated to some extent at a specific organ of a given plant. The presence or absence of such compounds depends largely on the extent of accumulation, the amount of plant material used and the analytical method employed (Harborne, 1973). There are a large number of medicinal plants whose scientific importance has not been explored. All over the world, plants have served as the richest source of raw materials for traditional as well as modern medicine, the study of the medicinal importance of plants, scientifically and a confirmation of the use of these plants towards curing diseases is a possible solution to development of less costly and effective drugs from our local raw materials.The plants are distributed in Africa including Nigeria (Brako and Zarucchi, 1993).

Paullinia pinnata is a climbing shrub, the leaves are compound with winged rachis, inflorescences stand axillary on long stalks, and bearing paired collected tendrils with white flowers. *P. pinnata* grows in evergreen and mixed forests up to an altitude of 1200 m. The plant is a perennial climber with a height of 2.5 to 8.0 m. The fruit is up to 2.5 cm long (Watson and Dalwitz, 2007). It is a stout and strong woody vine that forms mats in forest openings and ascends rocks and trees by means of forked tendrils to access full sunlight. It may reach 10 m of extension and 12 cm or more of stem diameter. The young, green stems are angularly striated; the plant is easily recognized from the leaves that have five serrated leaflets with prominent veins and a winged rachis and petiole. The fruits are red or dark pink when ripe (Watson and Dallwitz, 2007).

The major aim of this study seeks to investigate the presence of compounds as the possible agent responsible for the medicinal activities of the leaf of the plant *P. pinnata* by carrying out the phytochemical screening of the dried leaf of the plant, extract and fractionate the leaf and to carry out thin layer chromatography of the different fractions.

MATERIALS AND METHODS

Collection and preparation of plant material

In this research, the plant part utilized were the leaves. The plant was collected from Durumi market in Zaria Local Government, Kaduna state. The leaves were identified at the Herbarium Section of the Department Of Biological Sciences, Ahmadu Bello University, Zaria, with voucher number (9000185). 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.

Extraction of the powdered leaf of the plant

Maceration

The powdered leaves (100 g) was weighed and soaked in 350 ml of methanol in a conical flask. The flask containing the leaves was shaken, corked and left to stand for 48 h at room temperature. After 48 h, the mixture was filtered and the extract was collected and concentrated by evaporation to dryness in evaporating dish (Trease and Evans, 1997).

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.

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 H_2S0_4 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).

Identification 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 $FeCl_3$ 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).

$FeCl_3$ test: To test tube III, 3 drops of $FeCl_3$ 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 chromatoghraphy 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 phyto-chemical constituents in *P. pinnata* 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.

Table 1. Summary of the chromatographic analysis results for the combined fractions obtained after column chromatography.

Extract fraction	Colour after spray	Number of spots	Retention factor (R_f)
1	Light yellow	5	0.19
	yellow		0.64
	Pink		0.72
	Green		0.85
	Purple		0.96
2	Light yellow	3	0.19
	pink		0.72
	Green		0.85
3	pale purple	3	0.96
	Green		0.85
	Violet		0.89
4	Violet	1	0.89

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 ($R_{f)}$= Distance moved by the component/Distance moved by the solvent.

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

DISCUSSION

Phytochemical analysis of the leaves of *P. pinnata* was successfully 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 (Tsakala et al., 2006). 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 phytochemial constituents of the leaf of *P. pinnata* was investigated. The leaf was found to constitute

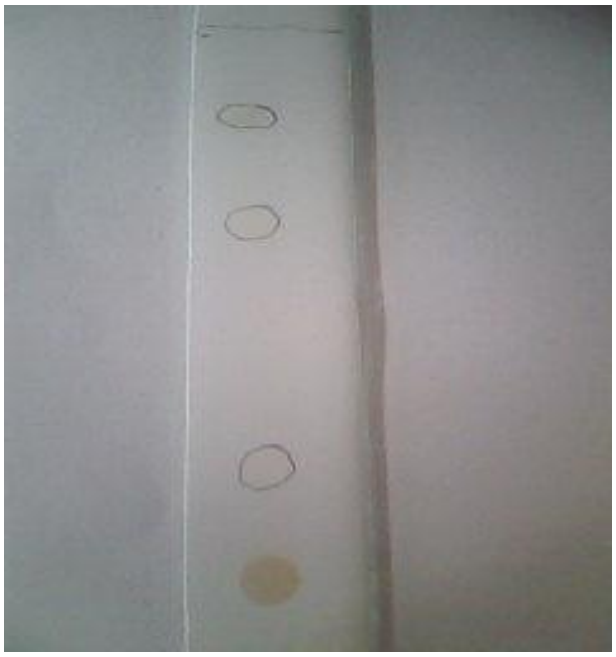

Plate 1. Chromatogram of methanol extract developed in hexane/ethyl acetate (8:2) before spray.

Plate 3. Chromatogram of column fractions in hexane/ethyl acetate before spray.

Plate 2. Chromatogram of methanol extract developed in hexane/ethyl acetate (8:2) after sprayed with 20% sulphuric acid and heated at 105°C for 15 min.

Plate 4. Chromatogram of column fractions in hexane/ethyl acetate (8:2) after spray with 20% sulphuric acid and heated at 105°C for 15 min.

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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