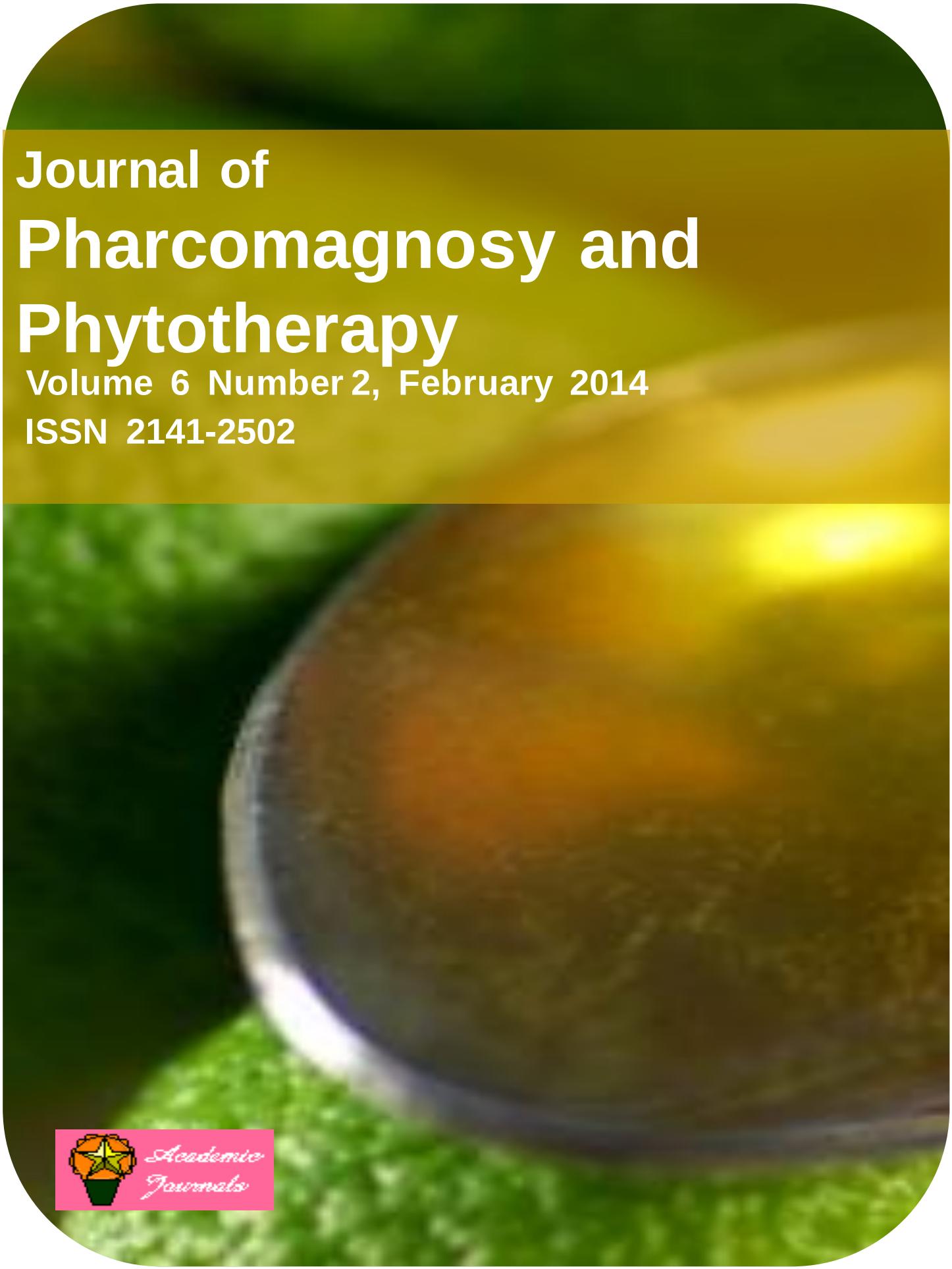

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Charnley AK (1992). Mechanisms of fungal pathogenesis in insects with particular reference to locusts. In: Lomer CJ, Prior C (eds), Pharmaceutical Controls of Locusts and Grasshoppers: Proceedings of an international workshop held at Cotonou, Benin. Oxford: CAB International. pp 181-190.

Jake OO (2002). Pharmaceutical Interactions between *Striga hermonthica* (Del.) Benth. and fluorescent rhizosphere bacteria Of *Zea mays*, L. and *Sorghum bicolor* L. Moench for Striga suicidal germination In *Vigna unguiculata*. PhD dissertation, Tehran University, Iran.

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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

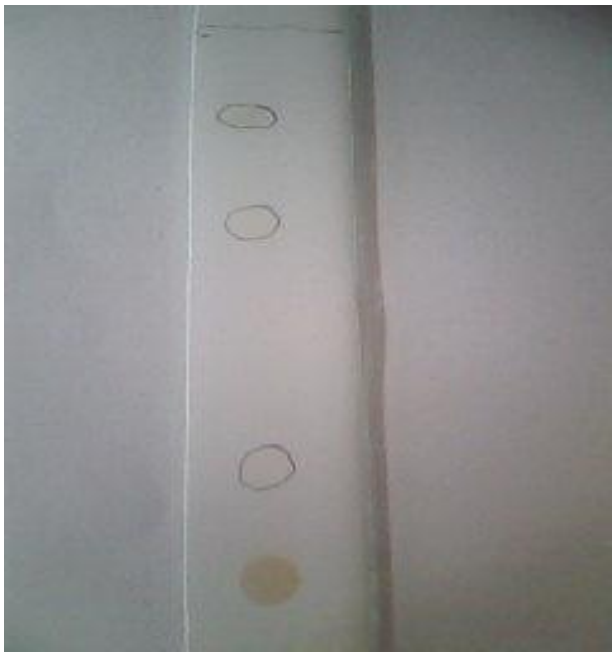

Plate 1. Chromatogram of methanol extract developed in hexane/ethyl acetate (8:2) before spray.

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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Full Length Research Paper

Phytochemical profiling of phytoconstituents of grape, *Jatropha curcas* and Neem (*Azadirachta indica*) extracts

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This study investigated the phytoconstituents in Neem (seed and flower), *Jatropha curcas* (stem and root bark) and grape (stem bark and leaves) in some selected solvents. Phytochemical screening revealed the presence of bioactive compound saponin in all the parts of the three plants in water extract only. Saponin was absent in other solvents used (ethanol, ethyl acetate, propan-2-ol, methanol, n-butanol and acetone). Ethanol, ethyl acetate and methanol were the most promising solvents to extract flavonoids in both the seed and flower of Neem plant. Acetone and ethanol were the most promising solvents to extract flavonoids in the stem bark of *J. curcas*. In the root bark of *J. curcas*, acetone and ethyl acetate were the most promising solvents to extract flavonoids. In grape (*Citrus paradisi*) leaves, ethanol, water and acetone were the most promising solvents to extract flavonoids. In the stem bark of grape, water, ethyl acetate and acetone proved promising as extraction solvents for flavonoids. All the parts of the plants studied were positive for alkaloid in ethanol and acetone extracts. The seed extract of Neem (*Azadirachta indica*) was strongly positive in ethanol only. Terpenoids were detected in ethyl acetate and n-butanol in all the parts of the three plants. Aged flower extract of Neem plant with pale pink colour could be exploited as a novel source of colourant. Volatile oil was not restricted to *J. curcas* stem and root barks, it was also present in *C. paradisi* stem and root barks. The nutritional significance, economic and toxicological implications of phytoconstituents analysed in the plants were discussed.

Key words: Phytomedicine, bioactive compounds, natural product, drug discovery, preventive medicine.

INTRODUCTION

Plant extracts or secondary metabolites have served as antioxidants in phytotherapeutic medicines to protect against various diseases for centuries (Halliwell, 1996). The grape fruit (*Citrus paradisi*) is a subtropical citrus tree known for its bitter fruit (Sean and Henry, 2003). Grape contains many bioactive constituents such as flavonoids, polyphenols, anthocyanins and stilbene derivatives of resveratrol (Cetin and Sagdic, 2009). Grape fruit mercaptan, a sulphur-containing terpene, is one of the substances which have a strong influence on the taste and odour of grape fruit (Buettner and Schieberle, 1999). Grape is used in the treatment of B and C type viral hepatitis (Block et al., 1994). Grape oil is used in aromatherapy, and its historically known for its aroma (Ann, 1991). The seeds have antioxidant (Yigit et al., 2009) and cardioprotective (Falchi et al., 2006) properties. The grape seed extract displayed reduction of platelet adhesion and aggregation and generation of superoxide radical (Olas et al., 2008).

The utility of *Jatropha curcas* oil and its esters as replace-ment for petrodiesel is well documented (Roach et al., 2012; Kywe and Oo, 2009). The seed oil of the plant is rich in phorboesters (Roach et al., 2012). The antifungal effect of the seeds is due to its phorbol esters

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(Saetea and Suntornsuk, 2010). The fruit possessed abortifacient property (Goonasekera et al., 1995). The latex and leaf extracts of the *J. curcas* showed the highest *in vitro* antioxidant activity and the extracts of different plant parts contained various levels of phenolics, flavonoids and saponins (Oskoueian et al., 2011; Sawant and Joshi, 2010). Saponin isolated from the plant is useful in managing inflammation (Just et al., 1998). The aqueous branch extract of the plant inhibited HIV-1 (Matsuse et al., 1999).

Azadirachta indica (Neem) belongs to the mahogamy family (Meliaceae) (Girish and Shankora, 2008). The leaf powder of *A. indica* is used as a biosorbent for the removal of congo red from water (Bhattacharyya and Sarma, 2003). Tignic acid is responsible for the bitter taste of the seed oil (Lale, 2002). Beta-nimbolides, flavonoids and carotenoids are the constituents of the flower of *A. indica* (Srithanaudomchai et al., 2005). The genotoxic effect of the plant is due to the most active principle (azadirachtin) (Khan and Aswathy, 2003). Neem oil is an indigenous product and a practical solution to curtail mosquito nuisance (Mishra et al., 1995).

The phytochemical screening of the aqueous, ethanolic and methanolic extracts of the *J. curcas* has been reported (Igbinosa et al., 2009), but the authors failed to categorize the bioactive compounds in the three solvents. The methanolic leaf extract of the plant revealed the presence of bioactive compounds like flavonoids, tannins, alkaloids, saponin, steroids and triterpenoids (Uche and Aprioku, 2008). Sharma et al. (2012) reported the presence of bioactive compounds such as alkaloids, saponins, tannins, terpenoids, steroids, glycosides, phenols and flavnoids in the extracts of root, stem and leaf of the plant in one solvent only (methanol), but failed to utilize several solvents. Daniel et al. (2012) reported the bioactive principles in the leaves, bark and seed extracts of the plant using methanol alone, but failed to use many solvents.

This study was designed to add more knowledge to the bioactive compounds in several solvents (seven solvents), which include water, ethanol, ethyl acetate, n-butanol, propan-2-ol, methanol and acetone.

MATERIALS AND METHODS

Collection of plant

The seeds and flowers of Neem (*A. indica*) were collected from the premises of Ladoke Akintola University of Technology, Ogbomoso, Nigeria on the 14th of February, 2010. The root and stem barks of *J. curcas* was collected from Oke-Anu area of Ogbomoso, Nigeria on the 6th March, 2010. The stem bark and leaves of grape fruit plant was collected from the premises of Soun High School, Ikuye, Ogbomoso, North Local Government, Ogbomoso, Nigeria on the 14th March, 2010.

Phytochemical analysis

The phytochemical analysis was carried according to standard methods (Parekh and Chanda, 2006; Trease and Evans, 2002; Sofowora, 1993; Harborne, 1973) with little modification. For qualitative test for alkaloid, Wagner's test was utilized. To 1 ml of extract, 1 ml of 1% HCl was added and the mixture steamed in a water bath. To the solution, 6 drops of Wagner's reagent was added. Formation of brownish precipitate is indicative of alkaloids.

For flavonoid detection, Shibata's test was used, and it involved the addition of 0.4 ml of concentrated HCl to 1 ml of extract, followed by the addition of few pieces of magnesium ribbon. Pink colour indicates the formation of flavonoid. Volatile oil was detected in the extract by the addition of 0.1 ml NaOH solution to 1 ml of extract, followed by small quantity of dilute HCl. A white precipitate indicates the presence of volatile oil. Frothing test was employed for the qualitative identification of saponin. To 2 ml extract, 2 ml of distilled water was added and the mixture shaken. Pesistent/stable foam was used to detect saponin.

Tannin in the extract was detected by adding few drops of 0.1% $FeCl_3$ to 1 ml extract. Bluish black indicates tannin. For phlobatanin detection, 1 ml of 1% HCl was added to 1 ml extract and the mixture was steamed in a water bath for 10 min. Formation of a red precipitate indicates phlobatanin. For hydrolysable tannin detection, 2 ml of 10% ammonia solution was added to 1 ml extract and formation of emulsion indicates the compound. For steroid identification, 1 ml of acetic anhydride was added to 0.5 ml extract, followed by 2 drops of concentrated H_2S0_4. Formation of a violet/brown ring at the junction indicates the presence of steroid.

For cardiac glycoside identification, legal test was used. To 1 ml of extract, 0.5 ml of glacial acetic was added, followed by 9 drops of $FeCl_3$ solution, and 0.5 ml of concentration slowly near the side of the test tube. A brown ring at the junction is positive for cardiac glycoside. For cardenolide aglycone, legal test was employed. To 1 ml of extract, 7 drops of pyridine, 7 drops of NaOH solution and 7 drops of sodium nitroprusside were added. Formation of a deep red colour that fades to brown indicates cardenolide aglycone.

RESULTS

Table 1 showed the phytochemical data for flavonoid in different parts of Neem, *J. curcas* and *C. paradisi* in solvents of interest. The water extracts of *A. indica* and *J. curcas* lacked flavonoid. The leaves and stem bark extracts of *C. paradisi* showed the presence of flavonoid. All the butanol extracts lacked flavonoids in all the parts of the three plants investigated. Acetone proved most promising in the extraction of flavonoid in all the parts of all the plants except the seed of *A. indica*.

Saponin was detected only in the water extract of all the parts of the three plants. Other solvents lacked saponin (Table 2).

Ethanol and acetone were the promising solvents for all the parts of the plant investigated (Table 3). Volatile oil was detected in all the parts of *C. paradisi*, *J. curcas* and *A. indica* with water, and butanol as separate extraction solvents. Ethyl acetate and ethanol were suitable solvents except that both solvents failed to detect volatile oil in *A. indica* flower (Table 4).

Cardenolide aglycone was absent in the water, ethanol, ethyl acetate, propan-2-ol in all the parts investigated (Table 5). It was only present in n-butanol seed extract of *A. indica*, but absent in other parts. Acetone was the most promising for the detection of the bioactive compound in all parts investigated, except the acetone seed

Table 1. Flavonoids (Shibata's test).

Parameter	*A. indica*		*J. curcas*		*C. paradisi*	
	Seed	**Flower**	**Stem bark**	**Root bark**	**Leaves**	**Stem bark**
Water	-ve	-ve	-ve	-ve	+ve	+ve
Ethanol	+ve	+ve	+ve	-ve	+ve	-ve
Ethyl acetate	+ve	+ve	-ve	+ve	-ve	+ve
Propan-2-ol	-ve	-ve	-ve	-ve	-ve	-ve
Methanol	+ve	+ve	-ve	-ve	-ve	-ve
n-butanol	-ve	-ve	-ve	-ve	-ve	-ve
Acetone	-ve	+ve	+ve	+ve	+ve	+ve

Table 2. Saponin (Frothing test).

Parameter	*A. indica*		*J. curcas*		*C. paradisi*	
	Seed	**Flower**	**Stem bark**	**Root bark**	**Leaves**	**Stem bark**
Water	+ve	+ve	+ve	+ve	+ve	+ve
Ethanol	-ve	-ve	-ve	-ve	-ve	-ve
Ethyl acetate	-ve	-ve	-ve	-ve	-ve	-ve
Propan-2-ol	-ve	-ve	-ve	-ve	-ve	-ve
Methanol	-ve	-ve	-ve	-ve	-ve	-ve
n-butanol	-ve	-ve	-ve	-ve	-ve	-ve
Acetone	-ve	-ve	-ve	- ve	-ve	-ve

Table 3. Alkaloid (Wagner's test).

Parameter	*A. indica*		*J. curcas*		*C. paradisi*	
	Seed	**Flower**	**Stem bark**	**Root bark**	**Leaves**	**Stem bark**
Water	+ve	+ve	+ve	+ve	-ve	+ve
Ethanol	Strongly +ve	+ve	+ve	+ve	+ve	+ve
Ethyl acetate	Trace	Trace	Trace	Trace	+ve	Trace
Propan-2-ol	+ve	+ve	+ve	+ve	-ve	+ve
Methanol	+ve	+ve	+ve	+ve	Trace	+ve
n-butanol	+ve	+ve	-ve	-ve	-ve	-ve
Acetone	+ve	+ve	+ve	+ve	+ve	+ve

Table 4. Volatile oil.

Parameter	*A. indica*		*J. curcas*		*C. paradisi*	
	Seed	**Flower**	**Stem bark**	**Root bark**	**Leaves**	**Stem bark**
Water	+ve	+ve	+ve	+ve	+ve	+ve
Ethanol	+ve	-ve	+ve	+ve	+ve	+ve
Ethyl acetate	+ve	-ve	+ve	+ve	+ve	+ve
Propan-2-ol	-ve	+ve	-ve	-ve	-ve	-ve
Methanol	-ve	+ve	-ve	-ve	-ve	-ve
n-butanol	+ve	+ve	+ve	+ve	+ve	+ve
Acetone	Trace	Trace	-ve	-ve	Trace	Trace

seed extract of *A. indica*.

Cardiac glycoside was absent in water, ethanol, propan-2-ol and methanol extracts in all the parts investigated (Table 6). Cardiac glycoside was detected in

Table 5. Cardenolide aglycone (Legal test).

Parameter	*A. indica*		*J. curcas*		*C. paradisi*	
	Seed	Flower	Stem bark	Root bark	Leaves	Stem bark
Water	-ve	-ve	-ve	-ve	-ve	-ve
Ethanol	-ve	-ve	-ve	-ve	-ve	-ve
Ethyl acetate	-ve	-ve	-ve	-ve	-ve	-ve
Propan-2-ol	-ve	-ve	-ve	-ve	-ve	-ve
Methanol	-ve	-ve	-ve	-ve	-ve	-ve
n-butanol	+ve	-ve	-ve	-ve	-ve	-ve
Acetone	-ve	+ve	+ve	+ve	+ve	+ve

Table 6. Cardiac glycoside.

Parameter	*A. indica*		*J. curcas*		*C. paradisi*	
	Seed	Flower	Stem bark	Root bark	Leaves	Stem bark
Water	-ve	-ve	-ve	-ve	-ve	-ve
Ethanol	-ve	-ve	-ve	-ve	-ve	-ve
Ethyl acetate	Positive	-ve	-ve	-ve	-ve	-ve
Propan-2-ol	-ve	-ve	-ve	-ve	-ve	-ve
Methanol	-ve	-ve	-ve	-ve	-ve	-ve
n-butanol	+ve	-ve	-ve	-ve	-ve	-ve
Acetone	-ve	-ve	+ve	+ve	+ve	+ve

Table 7. Tannin.

Parameter	*A. indica*		*J. curcas*		*C. paradisi*	
	Seed	Flower	Stem bark	Root bark	Leaves	Stem bark
Water	trace	Trace	-ve	Trace	Trace	Trace
Ethanol	-ve	+ve	+ve	+ve	+ve	+ve
Ethyl acetate	-ve	-ve	-ve	-ve	-ve	-ve
Propan-2-ol	-ve	-ve	-ve	-ve	-ve	-ve
Methanol	-ve	-ve	-ve	-ve	-ve	-ve
n-butanol	-ve	-ve	-ve	-ve	-ve	-ve
Acetone	-ve	-ve	-ve	-ve	+ve	-ve

the stem and root acetone extract in *J. curcas* and *C. paradisi*.

Tannin was absent in the ethyl acetate, propan-2-ol, methanol and n-butanol extracts of all the parts of the three plants (Table 7). Tannin was positive in ethanolic extracts of the parts of the plants except the ethanolic seed extract of *A. indica*.

Hydrolysable tannin was present in n-butanol and ethyl acetate extracts of the three plants. Phytochemical screening showed the absence of hydrolysable tannin in water, ethanol, propan-2-ol, methanol and acetone extracts (Table 8).

Phytochemical screening revealed that steroid was strongly positive in methanolic flower extract of *A. indica* (Table 9). The aqueous, ethyl acetate, propan-2-ol and acetone extracts in all the parts of the plants lacked steroid.

Terpenoid was positive in ethyl acetate and n-butanol extracts in all the parts of the plants. The aqueous, ethanol, propan-2-ol and methanolic extracts lacked terpenoid in all the parts of the three plants.

Phlobatanin was only positive in methanolic leaf extract of *C. pardisi* (Table 11). Meth indicates methanol, while n-butan and pro indicate n-butanol and propan-2-ol, respectively. After 20 days of soaking, saponin was present in both the Neem flower and seed extracts. Flavonoid and

Table 8. Hydrolysable tannin.

Parameter	*A. indica*		*J. curcas*		*C. paradisi*	
	Seed	**Flower**	**Stem bark**	**Root bark**	**Leaves**	**Stem bark**
Water	-ve	-ve	-ve	-ve	-ve	-ve
Ethanol	-ve	-ve	-ve	-ve	-ve	-ve
Ethyl acetate	+ve	+ve	+ve	+ve	+ve	+ve
Propan-2-ol	-ve	-ve	-ve	-ve	-ve	-ve
Methanol	-ve	-ve	-ve	-ve	-ve	-ve
n-butanol	+ve	+ve	+ve	+ve	+ve	+ve
Acetone	-ve	-ve	-ve	-ve	-ve	-ve

Table 9. Steroids.

Parameter	*A. indica*		*J. curcas*		*C. paradisi*	
	Seed	**Flower**	**Stem bark**	**Root bark**	**Leaves**	**Stem bark**
Water	-ve	-ve	-ve	-ve	-ve	-ve
Ethanol	-ve	+ve	-ve	-ve	-ve	-ve
Ethyl acetate	-ve	-ve	-ve	-ve	-ve	-ve
Propan-2-ol	-ve	-ve	-ve	-ve	-ve	-ve
Methanol	Trace	Strongly +ve	Trace	Trace	trace	Trace
n-butanol	-ve	+ve	-ve	-ve	-ve	-ve
Acetone	-ve	-ve	-ve	-ve	-ve	-ve

Table 10. Terpenoid.

Parameter	*A. indica*		*J. curcas*		*C. paradisi*	
	Seed	**Flower**	**Stem bark**	**Root bark**	**Leaves**	**Stem bark**
Water	-ve	-ve	-ve	-ve	-ve	-ve
Ethanol	-ve	+ve	-ve	-ve	-ve	-ve
Ethyl acetate	+ve	+ve	+ve	+ve	+ve	+ve
Propan-2-ol	-ve	-ve	-ve	-ve	-ve	-ve
Methanol	-ve	-ve	-ve	-ve	-ve	-ve
n-butanol	+ve	+ve	+ve	+ve	+ve	+ve
Acetone	Trace	Trace	Trace	Trace	Trace	Trace

Table 11. Phlobatatannin.

Parameter	*A. indica*		*J. curcas*		*C. paradisi*	
	Seed	**Flower**	**Stem bark**	**Root bark**	**Leaves**	**Stem bark**
Water	trace	Trace	-ve	-	Trace	Trace
Ethanol	-ve	-ve	-ve	-	-ve	-ve
Ethyl acetate	Trace	Trace	-ve	-	Trace	Trace
Propan-2-ol	-ve	-ve	trace	-	-ve	-ve
Methanol	-ve	-ve	-ve	-	+ve	-ve
n-butanol	-ve	-ve	-ve	-	-ve	-ve
Acetone	-ve	-ve	-ve.	-	-ve	-ve

Table 12. Phytochemical screening of Neem (*Azadirachta indica*) seed and flower extracts.

Parameter	Seed extract after 20 days of soaking					Flower extract after 20 days of soaking				
	Water	ethanol	Meth	n-butan	prop	water	ethanol	Methanol	n-butanol	Prop
Tannin	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Flavonol	-ve	-ve	-ve	-ve	-ve	-ve	Trace	-ve	-ve	-ve
Saponin	+ve	-ve	-ve	-ve	-ve	+Ve	-ve	-ve	-ve	-ve

tannin were absent in seed and flower extracts of Neem plant after 20 days of soaking (Table 12).

DISCUSSION

In this study, phytochemical screening revealed the presence of bioactive compound alkaloid in the stem and root of *J. curcas* in methanol, which is consistent with the finding of Sharma et al. (2012). However, this study failed to detect the presence of flavonoids, saponin and cardiac glycosides, terpenoids and tannin in the methanolic extracts of the root and stem of the plant, while Sharma et al. (2012) detected the presence of the bioactive compounds. Phytochemicals are chemical compounds formed during the plants normal metabolic processes; these chemicals are often referred to as secondary metabolites of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, polysaccharides, phenols, tannins, terpenes and terpenoids (Okwu, 2004).

In this work, flavonoid was detected in the methanolic seed extract of *A. indica*, which is in agreement with the earlier investigation (Daniel et al., 2012). The two bioactive compounds (saponin and tannin) previously identified with methanol as extraction solvent in other laboratory (Daniel et al., 2012) were not detected in the present study. In this study, alkaloid in the methanolic seed extract of *A. indica* was detected, which was not consistent with the work of Daniel et al. (2012). Methanol and ethanol have been proved as effective solvents to extract phenolic compounds (Siddhuraju et al., 2003).

Water was a promising solvent for the extraction of bioactive compounds like flavonoids, cardiac glycosides and terpenoids for the leaf of *A. indica* (Selvan et al., 2012), but the same solvent failed to detect these bioactive compounds *in A. indica* flower in this work. Moreover, saponin was detected in the water flower extract of the plant. .Saponin had earlier been reported to be present in the water extract of *A. indica* leaf (Selvan et al., 2012).

Saponins are amphipathic glycosides grouped phenomenologically by the soap-like foaming they produce when shaked in aqueous solutions (Hostettmann and Marston, 1995). Most saponins function as antioxidants, because they possess a special moiety (2,3-dihydri-2,5 –dihyroxy6-methyl-4-pyran -4-one) which act by forming hydroperoxide intermediates thus removing free radicals (Hu et al., 2002). Saponins possesss haemolytic action on human erythrocytes (Baumann et al., 2000). Saponins with acyl residues or oxide-ring moiety tend to show haemolytic activity (Oda et al., 2000).

Flavonoids are important in human diet and are present in plant extracts that have been used for centuries in oriental medicine (Di Carlo et al., 1999). Antioxidant properties, reactive oxygen species scavenging, and cell function modulation of flavonoids could account for the large part of their pharmacological activity (Limasset et al., 1993).

Raw oil of *J. curcas* has been used as a substitute for petrol-diesel both in modified and unmodified diesel engines (Jingura et al., 2010). *J. curcas* plant found in Nigeria has the potential of boosting the economy in term of biodiesel production (Belewu et al., 2010). n-Hexane may be preferable in the extraction of biodiesel oil from *J. curcas* (Belewu et al., 2010), although petroleum ether had been used with lower yield (Adebayo et al., 2011).

Some alkaloids are known to precipitate hepatocyte necrosis and cytoskeleton disorganization (Lekhehal et al., 1996). Phenolic alkaloids such as caffeic acid phenyl ester (CAPE) have been reported to possess beneficial effects such as anti-tumor property against human breast cancer line (Grunberger et al., 1988) and in the treatment of acute inflammation (Orban et al., 2000).

Cardiac glycosides are class of natural product, which are used to increase the cardiac contractile force in patients with congestive heart failure and cardiac arrhythmias (Hauptman et al., 1999).

In this work, the water flower extract of *A. indica* afforded pale colour after 20 days of soaking in water. The water flower extract of the plant could be exploited as a colourant, although with caution. The aqueous flower extract with pale colour was positive for saponin. This requires future research. This could be due to browning process. The enzymatic oxidation of polyphenols, particularly, flavonoids, occurs during storage when cell integrity is affected (Cheynier, 2005, 1994). Flavonoids, particularly, ortho-diphenols, can be oxidized to their corresponding semiquinones and quinones by oxidases such as polyphenol oxidase and peroxidases (Yoruk and Marshall, 2003; Walker and Ferrar, 1998).

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