

Full Length Research Paper

Phytochemical constituents of some selected medicinal plants

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Accepted 24 September, 2009

Tannins, saponins, phlobatannins, flavonoids, anthraquinones, terpenoids, steroids, alkaloids, carbohydrates and glycosides distribution in four medicinal plants belonging to different families were investigated and compared. The medicinal plants investigated are *Carica papaya*, *Ocimum gratissimum*, *Adenia cissampeloides* and *Cymbopogon citratus*. All the plants were found to contain tannins, flavonoids, terpenoids, steroids and carbohydrates while anthraquinones were absent in all. Alkaloids were absent in both *O. gratissimum* and *C. citratus*. Glycosides were absent in only *C. papaya*, saponins were absent in only *O. gratissimum* while phlobatannins were absent in only *C. citratus*. The extraction of oils was carried out by solvent extraction and steam distillation methods and the percentage yield of extracts by each method determined. Solvent extraction method gave percentage yield of 7.40, 6.30, 6.75 and 5.63% for *C. papaya*, *O. gratissimum*, *A. cissampeloides* and *C. citratus* respectively. For steam distillation, *C. papaya*, *O. gratissimum*, *A. cissampeloides* and *C. citratus* gave percentage yield of 5.60, 5.80, 5.44 and 3.82% respectively. The significance of the plants in traditional medicine and the importance of the distribution of these chemical constituents were discussed with respect to the role of these plants in ethnomedicine in Nigeria.

Key words: Ethnomedicine, medicinal plants, natural products, phytochemicals.

INTRODUCTION

Medicinal plants contain some organic compounds which produce definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Edoga et al., 2005; Mann, 1978). In most cases these substances appear to be non-essential to the plant producing them. For example, penicillin produced by a few species of fungi (Family: *Penicillinae*) have great value to man as antibiotic, but appears to serve no useful purpose in the microorganisms producing it (Mann, 1978; Sofowora, 1984). Many of these natural products have vital roles as mediators of ecological interactions; that is, they have functions in ensuring a continued survival of particular organisms in often hostile environments where there is competition with other organisms (Mann, 1978). Such roles include being attractant to pollinators, allelo-

pathic agents or defence against predators and pathogens (Hill, 1985). For example, ipsdienol, a major constituent of the floral fragrance of several orchid species and azadirachtin, present in *Azadiracta indica*, have roles as attractant to bees and defence mechanism against insects respectively (Hill, 1985; Swaminathan and Kochhar, 1989).

Medicinal plants are of great importance to the health of individuals and communities (Edeoga et al., 2005). Many of these indigenous medicinal plants are used as spices and food plants. They are sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes (Okwu, 1999, 2001). Medicinal plants are generally used in traditional medicine for the treatment of many ailments (Njoku and Ezeibe, 2007; Ogukwe et al., 2004). *Carica papaya*, *Ocimum gratissimum*, *Adenia cissampeloides* and *Cymbopogon citratus* are extensively used in herbal medicine in South Eastern Nigeria. Their various species, families and uses in traditional medicine are reviewed in Table 1. Despite extensive applications of these plants in traditional medicine, little information is

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Table 1. Medicinal uses of *C. papaya*, *O. gratissimum*, *A. cissampeloides* and *C. citratus*.

Species	Family	Uses	References
<i>C. papaya</i>	Caricaceae	The flowers are used for treatment of jaundice; the leaves are used as a febrifuge and laxative and the root for kidney and bladder problems.	Watt, 1984
<i>O. gratissimum</i>	Lamiaceae	The leaves are used for treatment of intestinal worms and venereal diseases.	Dalziel, 1985
		The leaves are used in steam baths for the treatment of rheumatism while a decoction of it is used to treat venereal diseases.	Usher, 1984;
<i>A. cissampeloides</i>	Passifloraceae	The plant is used for expelling worms from the intestines.	Sofowora, 1984
		The crushed plant is used as a fish poison. The pollens and other extracts from the flowers are used by bees and other insects to manufacture honey.	Usher, 1984; Lobreu-Collen et al., 1989; Thomas et al., 1988.
<i>C. citratus</i>	Graminae	The plant is used for the treatment of sore throats, respiratory and skin problems. It could be infused as tea, used for inhalation, massage and as food flavour.	Dalziel, 1985

available on their phytochemical constituents.

In this study, the presence of phytochemical constituents in these Nigerian medicinal plants was investigated. The phytochemical compounds responsible for the reported therapeutic uses of these plants were determined. The percentage yield of extracts obtained from these plants by solvent extraction and steam distillation methods were also determined.

MATERIALS AND METHODS

Collection and preparation of plant materials

Fresh leaves of *C. papaya*, *O. gratissimum*, *A. cissampeloides* and *C. citratus* were collected from uncultivated farmlands located in the South-eastern parts of Nigeria. All the four plant samples were identified by a botanist. The voucher specimens were deposited in the Biotechnology laboratory of Imo State University, Owerri, Nigeria. The leaves were dried at 40°C in a thermostatically controlled oven until they attained a constant weight. The samples were then crushed to powder, using a manual grinding machine, so as to enhance effective contact of solvent with sites on the plant materials.

Steam distillation method

50 g of the powdered leaves were placed in round bottom flasks and 200 ml of water added to each. The resulting suspensions were heated on a heating mantle. The steam-volatile oils volatilized with the steam and were condensed and collected in conical flasks as distillates. The distillation process was carried out for a period of 2 h. The oils settled on top of water and were removed with the aid of separating funnel. The oils obtained were stored in bijou bottles in a refrigerator until they were required for use. The respective weights of the oils were recorded and used for percentage yield calculations.

Solvent extraction method

40 g of the powdered leaves were weighed, tied up in filter papers and put into the thimble. A reflux condenser and a round bottom flask were fitted above and below the thimble respectively. This assembly of apparatus, known as a Soxhlet extractor, was clamped firm into position and 250 ml of absolute ethanol poured into the round bottom flask. *C. citratus* is soluble in ethanol; therefore petroleum ether was preferably used in place of ethanol for the extraction of *C. citratus* oil. Petroleum ether is more selective towards true lipids. The Soxhlet extractor was then heated electrically on a heating mantle. Continuous extraction was carried out for a period of 8 h with about eighteen refluxes. The samples were then removed and the ethanol recovered. The oils were poured into bijou bottles and the flasks washed with some quantity of ethanol and transferred into the bottles. These were later evaporated on the heating mantle and stored in a refrigerator. The weights of the oils obtained were recorded and used for percentage yield calculations.

Partitioning of essential oils

The crude oil extracts were partitioned according to the methods of Edeoga et al. (2005). The oil was poured into a clean dry separating funnel. 10 ml of aqueous (50%) ethanol was added into the separating funnel and additional 40 ml of aqueous ethanol was later added. 50 ml of organic solvent (chloroform-ether mixture) was added.

The mixture was then shaken vigorously and allowed to stand for about 30 min to partition. The two fractions were separated and put into two conical flasks and evaporated to dryness. The organic phase, which appeared above, was labelled as organic while the phase below was labelled as aqueous.

Phytochemical Screening

Chemical tests were carried out on the aqueous extracts to identify the constituents using standard procedures as described by

Sofowora (1993), Trease and Evans (1989) and Harborne (1973).

Test for tannins

About 2 ml of the aqueous extract was stirred with 2 ml of distilled water and few drops of FeCl_3 solution were added. The formation of a green precipitate was an indication for the presence of tannins.

Test for saponins

5 ml of aqueous extract was shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication for the presence of saponins.

Test for phlobatannins

About 2 ml of aqueous extract was added to 2 ml of 1% HCl and the mixture was boiled. Deposition of a red precipitate was taken as an evidence for the presence of phlobatannins.

Test for flavonoids

To 1 ml of aqueous extract was added 1 ml of 10% lead acetate solution. The formation of a yellow precipitate was taken as a positive test for flavonoids.

Tests for anthraquinones

(a) *Borntrager's test*: 3 ml of aqueous extract was shaken with 3 ml of benzene, filtered and 5 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of a pink, red or violet colour in the ammoniacal (lower) phase indicates the presence of free anthraquinones.

(b) 3 ml of the aqueous extract was boiled with 3 ml of aqueous sulphuric acid and filtered while hot. 3 ml of benzene was added to the filtrate and shaken. The benzene layer was separated and 3 ml of 10% NH_3 added. A pink, red or violet colouration in the ammoniacal (lower) phase indicates the presence of anthraquinone derivatives.

Test for terpenoids

2 ml of the organic extract was dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and heated for about 2 min. A greyish colour indicates the presence of terpenoids.

Tests for steroids

(i) A red colour produced in the lower chloroform layer when 2 ml of organic extract was dissolved in 2 ml of chloroform and 2 ml concentrated sulphuric acid added indicates the presence of steroids.

(ii) The development of a greenish colour when 2 ml of the organic extract was dissolved in 2 ml of chloroform and treated with sulphuric and acetic acids indicates the presence of steroids.

Test for alkaloids

3 ml of aqueous extract was stirred with 3 ml of 1% HCl on a steam

bath. Mayer's and Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Tests for carbohydrates

(a) Molisch's test: 3 ml of the aqueous extract was added to 2 ml of Molisch's reagent and the resulting mixture shaken properly. 2 ml of concentrated H_2SO_4 was then poured carefully down the side of the test tube. A violet ring at the interphase indicates the presence of carbohydrate.

(b) To 3 ml of the aqueous extract was added about 1 ml of iodine solution. A purple colouration at the interphase indicates the presence of carbohydrates.

Tests for glycosides

(a) Liebermann's test: 2 ml of the organic extract was dissolved in 2 ml of chloroform and 2 ml of acetic acid was added and the solution cooled well in ice. Sulphuric acid was then added carefully. A colour change from violet to blue to green indicates the presence of a steroidal nucleus (that is, a glycone portion of glycoside).

(b) Salkowski's test: 2 ml of each extract was dissolved in 2 ml of chloroform. 2 ml of sulphuric acid was added carefully and shaken gently. A reddish brown colour indicates the presence of a steroidal ring (that is, a glycone portion of glycoside).

(c) Keller-Kiliani test: 2 ml of each extract was dissolved in 2 ml of glacial acetic acid containing one drop of FeCl_3 solution. The mixture was then poured into a test tube containing 1 ml of concentrated H_2SO_4 . A brown ring at the interphase indicates the presence of a deoxy sugar, characteristic of cardenolides.

RESULTS

The phytochemical characteristics of the four medicinal plants investigated are summarized in Table 2.

The results reveal the presence of medicinally active constituents in the four plants studied. From Table 2, tannins, flavonoids, terpenoids, steroids and carbohydrates were present in all the plants while anthraquinones were absent in all the plants. Alkaloids were absent in both *O. gratissimum* and *C. citratus*. Glycosides, were absent in only *C. papaya*, saponins were absent in only *O. gratissimum* while and phlobatannins were absent in only *C. citratus*.

Quantitative estimation of the percentage yield of the oil extracts from the four medicinal plants studied is summarized in Table 3.

O. gratissimum showed the highest percentage yield of 5.80% by steam distillation while *C. papaya* showed the highest percentage yield of 7.4% by solvent extraction method.

DISCUSSION

The results confirm the presence of constituents which are known to exhibit medicinal as well as physiological activity (Sofowora, 1993). *C. papaya* oil is acidic having phenolic groups which could be attributed to the presence of tannins. Tannins are soluble in water and hence

Table 2. Phytochemical constituents of the extracts of *C. papaya*, *O. gratissimum*, *A. cissampeloides* and *C. citratus*.

Chemical constituent	Plant			
	<i>C. papaya</i>	<i>O. gratissimum</i>	<i>A. cissampeloides</i>	<i>C. citratus</i>
Tannins	+	+	+	+
Saponins	+	-	+	+
Phlobatannins	+	+	+	-
Flavonoids	+	+	+	+
Antraquinones	-	-	-	-
Terpenoids	+	+	+	+
Steroids	+	+	+	+
Alkaloids	+	-	+	-
Carbohydrates	+	+	+	+
Glycosides	-	+	+	+

+ = Presence of constituent; - = Absence of constituent

Table 3. Percentage yield of oil extracts from *C. papaya*, *O. gratissimum*, *A. cissampeloides* and *C. citratus* as obtained by the various methods.

Method	<i>C. papaya</i> (%)	<i>O. gratissimum</i> (%)	<i>A. cissampeloides</i> (%)	<i>C. citratus</i> (%)
Steam distillation	5.60	5.80	5.44	3.82
Solvent extraction	7.40	6.30	6.75	5.63

there is partial solubility of the oil extract in water. The presence of alkaloids in *C. papaya* gives its oil a bitter taste (The New Encyclopedia Britannica, 1992). Indian investigators have isolated from the dry leaves of *C. papaya*, 0.11% carpaine ($C_{14}H_{25}ON$) and 0.01% pseudo-carpaine, an isomer of carpaine (Watt, 1984). *C. papaya* could be said to contain more alkaloids as a result of aggregation of precipitated alkaloids although this could also be attributed to a particular alkaloid-precipitating reagent (Sim, 1970). The use of *C. papaya* as a soap substitute is attributed to the presence of saponins, which are used as cleaning agents of all kinds (Sofowora, 1984). The *C. papaya* oil is slightly viscous. This indicates the presence of high molecular weight compounds present in the oil. A lower percentage of oil extract obtained on steam distillation, justifies the presence of high molecular weight compounds. Similar results were obtained for all the other plants studied. Investigators have identified caricaxanthin ($C_{40}H_{56}O$) and violaxanthin ($C_{40}H_{56}O_4$), both high molecular weight carotenoids (terpenoids) present in the leaves (Watt, 1984).

The presence of glycosides was detected in *O. gratissimum*, *A. cissampeloides* and *C. citratus*. Glycosides have been known to lower blood pressure, although some workers have attributed the cardiac action of these oils to the presence of the alkaloid, carpaine (Watt, 1984). Nyarko and Addy (1990) had earlier shown aqueous extracts from *A. cissampeloides* to have antihypertensive effect on blood pressure and serum analyses of hypertensive patients. This effect could be attributed to

the presence of steroidal nucleus and deoxy-sugar both of which are present in glycosides. The presence of compounds with phenolic groups gives oils acidic properties and could possibly be responsible for its antimicrobial activities. Thymol, for example, has a phenolic group and has been identified as making up to 75% (in some cases) of steam volatile oil (Sofowora, 1993). According to the British Pharmacopoeia (1988), thymol is an antimicrobial agent and is used in wound dressing. This is attributed to the presence of tannins, which has styptic property as well as precipitates protein which renders it resistant to attack by proteolytic enzymes (The New Encyclopedia Britannica Volume II, 1992). The oil from *A. cissampeloides* is acidic with mainly phenolic groups which are present in tannins (e.g. digallic acid) and flavonoids while the carboxylic acid groups are found in resins and carotenoids (apocarotenoid) both terpenoids as well as fats and oils.

The antimicrobial effects of the plants studied are attributed to the presence of tannins in the aqueous extract. The presence of higher terpenoids that have carboxylic acid groups could also be responsible for the activity of the organic extracts. Several workers have reported on the analgesic properties of alkaloids (Antherden, 1969; Harborne, 1973) as well as the anti-inflammatory and anti-bacterial properties of tannins (Duguid et al., 1989). These classes of compounds are known to show curative activity against several bacteria and it is not surprising that these plant extracts are used traditionally by herbalist to cure bacteria related ill-health. Tannins with its protein-

precipitating and vasoconstriction effect could be advantageous in preventing ulcer development (Aguwa and Nwankwo, 1988; Dahiru et al., 2006). The diuretic and antibacterial activity of plant extracts containing flavonoids have been documented (Enwerem et al., 2001, 2003; Monache et al., 1996; Rao et al., 1996; Sofowora, 1993). The alkaloids contained in plants are used in medicine as anaesthetic agents (Herourat et al., 1988). The presence of saponins in plants have been reported to be responsible for the tonic and stimulating activities observed in Chinese and Japanese medical herbs (Alinnor, 2008). The results obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive constituents responsible for the efficacy of the leaves of the plants studied. The presence of some of these compounds have been confirmed to have antimicrobial activity (Odebiyi and Sofowora, 1978), hence it could be inferred that the plant extracts could be a source for the industrial manufacture of drugs useful in the chemotherapy of some microbial infections (Kubmarawa et al., 2007).

The percentage oil extract yield of 5.8% from *O. gratissimum* by steam distillation is higher than 4.2% reported by Sofowora (1993). This difference could be as a result of the total number of refluxes (eighteen in this case) allowed, thus making Soxhlet extraction a more efficient method of extraction. The low oil yield from the fresh leaves of the four medicinal plants studied by steam distillation showed that water is not a good extracting solvent for these materials. Drying up the leaves would maximise the yield.

Conclusion

The results reveal the presence of medicinally active constituents in the four plants studied. The phytochemical compounds identified in this study have earlier been proved to be bioactive. The presence of some of these compounds have been confirmed by previous workers to have medicinal as well as physiological activity and therefore could be said to be responsible for the efficacy of the leaves of the plants studied in treatment of different ailments. The plant extracts could therefore be seen as a potential source for useful drug. The continued traditional medicinal use of these plants is therefore encouraged while it is suggested that further work should be carried out to isolate, purify and possibly characterize the active constituents responsible for the activity of these plants. Also additional work should be embarked upon with a view to elucidate the possible mechanism of action of these extracts.

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