Full Length Research Paper

Phytochemical constituents of some selected medicinal plants

V.O. Njoku^{1*}, C. Obi² and O.M. Onyema²

¹Department of Chemistry, Imo State University, P.M.B. 2000 Owerri, Nigeria. ²Department of Pure and Industrial Chemistry, University of Port Harcourt, P.M.B. 5323 Port Harcourt, Nigeria.

Accepted 19 August, 2011

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 were *Carica papaya*, *Ocimum gratissimum*, *Adenia cissampeloides* and *Cymbopogan citratus*. All the plants were found to contain tannins, flavonoids, terpenoids, steroids and carbohydrates while anthraquinones were absent in all. Alkaloids were absent only in *O. gratissimum* and *C. citratus*. Glycosides were absent only in *C. papaya*, saponins were absent only in *O. gratissimum* while phlobatannins were absent only in *C. citratus*. Extraction of oils was carried out by solvent extraction and steam distillation methods and the percentage yield of extracts by each method was 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: Penicillinaceae) 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, allelopathic agents or defence against predators and pathogens (Hill, 1985). For example, ipsdienol, a major constituent of the floral fragrance of several orchid species, and azadichtin, 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 Cymbopogan 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

^{*}Corresponding author. E-mail address: viconjoku@yahoo.com. Tel: +234 8033430656.

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

Species	Family	Use	Reference
С. рарауа	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. The leaves are used for treatment of intestinal worms and	Wattand and Breyer- Brandwyk, 1984
		venereal diseases.	Dalziel, 1985
O. gratissimum	Lamiaeceae	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
		The plant is used for expelling worms from the intestines.	Sofowora, 1984
A. cissampeloides	Passifloraceae	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.
C. citratus	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

extensive applications of these plants in traditional medicine, little information is 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

50 g of the powdered leaves were placed in round bottom flasks and 200 ml of water was added to each. The resulting suspensions were heated on a heating mantle. The steam-volatile oils volatilized with the steam 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 the water and were removed with the

aid of separatory 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

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 18 refluxes. The samples were then removed and the ethanol recovered. The oils were poured into bijou bottles and the flasks washed with 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 separatory funnel. 10 ml of aqueous (50%) ethanol was added into the separatory 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 before, was labelled as organic while the later phase was labelled as aqueous.

Ohamiaal aanatituant	Plant					
Chemical constituent —	C. papaya	O. gratissimum	A. cissampeloides	C. citratus		
Tannins	+	+	+	+		
Saponins	+	_	+	+		
Phlobatannins	+	+	+	_		
Flavonoids	+	+	+	+		
Anthraquinones	_	_	_	_		

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

Phytochemical screening

Terpenoids Steroids Alkaloids Carbohydrates Glycosides

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) with some modifications.

RESULTS AND DISCUSSION

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 the table, 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.

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 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 Britanica, 1992). Indian investigators isolated from the dry leaves of *C. papaya* 0.11% carpaine (C₁₄H₂₅ON) and 0.01% pseudocarpaine, an isomer of carpaine (Watt and Breyer-Brandwyk, 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 ($C_{40}H_{56}O_4$); both are high molecular weight cartenoids (terpenoids) present in the leaves (Watt and Brever-Brandwyk, 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, and carpaine (Watt and Breyer-Brandwyk, 1984). Nyarko and Addy (1990) earlier shown aqueous extracts from cissampeloides to have antihypertensive effect on blood pressure and serum analytes of hypertensive patients. This effect could be attributed to the presence of steroidal nucleus and deoxy-sugar both of which are present in alycosides. 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

^{+ =} 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	C. papaya (%)	O. gratissimum (%)	A. cissampeloides (%)	C. citratus (%)
Steam distillation	5.60	5.80	5.44	3.82
Solvent extraction	7.40	6.30	6.75	5.63

mainly phenolic groups which are present in tannins (for example, digallic acid) and flavonoids while the carboxylic acid groups are found in resins and carotenoids (apocarotenoid) and in 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 antiinflammatory 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 herbalists to cure bacteria related illhealth. 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; Enwerem et al., 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.

Conclusions

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.

REFERENCES

Aguwa CN, Nwankwo SO (1988). Preliminary studies on the root extract of *Naulea latifolia* S. for antiulcer properties. Nig. J. Pharmaceutical Sci. 4(1): 16-23.

Alinnor J (2008). Preliminary phytochemical and antibacterial activity screening of leaves of *Varnonia amygdalina*, J. Chem. Soc. Nigeria, 33(1): 172-177.

Antherden LM (1969). Textbook of Pharmaceutical Chemistry, 8th edn. Oxford University Press, London, pp. 813-814.

British Pharmacopoeia (1988). Volume 1. Her Majesty's Stationery Office, London, p. 154.

Dahiru D, Onubiyi JA, Umaru HA (2006). Phytochemical screening and antiulcerogenic effect of *Moringa oleifera* aqueous leaf extract. Afr. J. Trad. Comp. Alt. Med. 3(3): 70-75.

Dalziel JM (1985). The Useful Plants of West Tropical Africa. Royal Botanic Gardens, Kew, 1: 302.

Duguid JP (1989). A guide to the laboratory diagnosis and control of infection. In Collee et al. (eds) MacKie and McCartney Medical Microbiology, 13th edn., Churchill Livingstone, London, 1: p. 163.

Edeoga HO, Okwu DE, Mbaebie BO (2005). Phytochemical constituents of some Nigerian medicinal plants, Afr. J. Biotechnol. 4(7): 685-688.

Enwerem NM, Wambebe CO, Okogun JI, Akah PA, Gamaniel KS (2001). Anthelmintic screening of the stem bark of *Berlina grandiflora*. J. Natural Remedies, 1: 17-23.

Enwerem NM, Okogun JI, Wambebe CO, Ajoku GA, Okorie DA (2003). Antibacterial principle from the stem bark of *Berlina grandiflora*. J. Chem. Soc. Nig., 28(1): 52-54.

Harborne JB (1973). Phytochemical Methods. Chapman and Hall Ltd., London, pp. 49-188.

Herourat D, Sangwin RS, Finiaux MA, Sangwan-Norrell BS (1988). Variations in the leaf alkaloid content of androgenic diploid plants of *Daturu innoxia*, Planta medical. J. Med. Plant Res. 54: 14-20.

- Hill RA (1985). Terpenoids. In Thomson RH (ed) Chemistry of Natural Products, Blackie Academic and Professional, London, pp. 106-134.
- Kubmarawa D, Wase GA, Ayinla OG (2007). Preliminary studies on phytochemical analysis and antimicrobial evaluation of extracts of Commiphora kerstingii, J. Chem. Soc. Nig., 32(1): 38-40.
- Lobreu-Collen D, Darchen R, Le-Thomas A, Thomas A (1989). The Plants Visited by Apis Mellifered Adansonu in Gabon and Ivory Coast. Int. Bee Res. Association, London, pp. 234-235.
- Mann J (1978). Secondary Metabolism. Oxford University Press, London, p. 154.
- Monache GD, Botta B, Vinciguerra V, de Mello J, Chiappeta A (1996).

 Antimicrobial isoflavanones from Desmodium canum.

 Phytochemistry, 41(2): 537-544.
- Njoku PC, Ezeibe AU (2007). Phytochemical and elemental analyses of *Helianthus annus* and its use as blood clotting agent. J. Chem. Soc. Nigeria, 32(2): 128-132.
- Nyarko AA, Addy ME (1990). Effects of aqueous extract of *adenia cissampeloides* on blood pressure and serum analyte of hypertensive patients. Phytother. Res. 4(1): 25-28.
- Odebiyi A, Sofowora AE (1978). Phytochemical screening of Nigerian medicinal plants. Lloydia, 41(3): 234-246.
- Ogukwe CE, Oguzie EE, Unaegbu C, Okolue BN (2004). Phytochemical screening on the leaves of *Sansevieria trifasciata*, J. Chem. Soc. Nig., 29(1): 8-10.
- Okwu DE (1999). Flavouring properties of spices on cassava Fufu. Afr. J. Roots Tuber Crops, 3(2): 19-21.

- Okwu DE (2001). Evaluation of the chemical composition of indigenous spices and flavouring agents. Global J. Pure Appl. Sci. 7(3): 455-459.
- Rao CP, Prashant A, Krupadanam GLD (1996). Two prenylated isoflavans from *Milletia racemosa*, Phytochemistry, 41(4): 1223-1224.
- Sim K (1970). Medicinal Plant Alkaloids 2nd edn. University of Toronto Press, Toronto, pp. 60-61.
- Sofowora A (1984). African Medicinal Plants, University of Ife Press, Ile-Ife, Nigeria, p. 104.
- Sofowora A (1993). Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria, pp. 191-289.
- Swaminathan MS, Kochhar SL (1989). Plants and Society, Macmillan Publishers Ltd., London, pp. 25-28
- The New Encyclopedia Britannica Volume II (Micropaedia) (1992). University of Chicago, USA. p. 514.
- Thomas A, Lobreu-Collen D, Darchen B, Darchen R (1988). Comparative analysis of the pollen resources and flower: visiting strategies of three species of trigonids in Ivory Coast, Institute Francais de Pondichery, 25: 83-88..
- Trease GE, Evans WC (1989). Pharmacognosy, 11th edn., Bailliere Tindall, London, pp. 45-50.
- Usher G (1984). A Dictionary of Plants Used by Man. C.B.S Publishers and Distributors, Delhi-110032, India, p. 220.
- Watt JM, Breyer-Brandwyk MG (1984). Medicinal and Poisonous Plants of Southern and Eastern Africa. E. & S. Livingstone Ltd., London, pp. 105-106.