

Full Length Research Paper

Comparative study of the phytochemical composition of the leaves of five Nigerian medicinal plants

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The percentage phytochemical composition of the leaves of pawpaw, bitter kola, tetrapleura, neem and ginger was investigated using the methods of the Association of analytical chemists, Harbone and the Alkaline picrate method. The saponin content of pawpaw, though not significantly different from bitter kola, was the highest among other plants while ginger had the least. The alkaloid content of tetrapleura was significantly higher than other plants studied ($P < 0.05$) while pawpaw had the least. The tannin contents of all the plants indicated that they were in moderate amounts. Pawpaw and ginger whose tannin contents were statistically the same contained higher quantities of tannins than other plants while bitter kola contained the least. In addition, the tannin contents of both neem and tetrapleura were also the same. The flavonoids in ginger, pawpaw, tetrapleura and neem were observed to be statistically the same while they were higher than that of bitter kola. Neem contained the highest amounts of cyanogenic glucosides among other plants while pawpaw contained the least. Results show that the cyanogenic glucoside contents of the plants were below the toxic level for man. The presence of tannin in all the plants investigated indicated that they could be used in the treatment of burns and wounds. Finally, the high alkaloid and flavonoids contents of the plants, suggests their antioxidant potentials and justifies their therapeutic actions, which could be used in drug formulation.

Key words: Comparative Evaluation, Leaves, Therapeutic, Phytochemical

INTRODUCTION

Phytochemicals are compounds that occur naturally in plants. They contribute to the color, flavor and smell of plants. In addition, they form part of a plant's natural defense mechanism against diseases. Their therapeutic values to human health and disease prevention have been reported (Okwu, 2004).

Pawpaw (*Carica papaya*) is a plant that belongs to the family of *Caricaceae*. It is a herbaceous succulent plant with self-supporting stems (Dick, 2003). Elizabeth (1994) has reported that unripe pawpaw fruit has therapeutic potentials on patients with ulcer and impotence.

Pawpaw plants produce natural compounds (*Annonaceous acetogenins*) in leaf bark and twig tissues that possess both highly anti-tumor and pesticidal properties. It has been suggested that a potentially

lucrative industry based simply on production of plant biomass could develop for production of anti-cancer drugs, pending Food and Drug Agency approval, and natural pesticides (McLanghlin, 1992). The high level of natural self-defense compounds in the plant makes it highly resistant to insect and disease infestation (Peter, 1991). Pawpaw leaf extract has been reported as a tumor-destroying agent (Walter, 2008).

Fresh green pawpaw leaf possesses antiseptic properties, whilst the brown, dried leaf can serve as a tonic and blood purifier (Atta, 1999). Chewing the seeds of ripe pawpaw fruit also helps to clear nasal congestion (Elizabeth, 1994). The green unripe pawpaw has a therapeutic value due to its antiseptic quality. It cleans the intestines from bacteria, more so that only a healthy intestine is able to absorb vitamin and minerals, especially vitamin B₁₂. Tea, prepared with the green papaya leaf, promotes digestion and aids in the treatment of ailments such as chronic indigestion, overweight,

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obesity, arteriosclerosis, high blood pressure and weakening of the heart (Mantok, 2005). Although there is sufficient information on the phytochemical constituents of the fruits, there is scarcity of such information on the leaves.

Bitter kola (*Garcinia kola*) is also known as an African wonder nut. It comes from *Garcinia kola* trees, which belong to the family of *Clusiaceae* and it grows in the coastal rainforests in the South Western and South Eastern parts of Nigeria. Traditionally, these nuts were chewed as a masticatory substance, to stimulate the flow of saliva (Leakey, 2001) but are now widely consumed as snack in West and Central Africa. The kernels of the nuts are widely traded and eaten as a stimulant (Leakey, 2001; Omode *et al.*, 1995). Bitter kola is also rich in caffeine and threobromine and is also believed to be an aphrodisiac. Unlike other kola nuts, bitter kola is believed to clean the digestive system, without side effects such as abdominal problems, even when a lot of the nuts are eaten. In folk, *G. kola* is used in folklore remedies for the treatment of ailments such as liver disorders, hepatitis, diarrhea, laryngitis, bronchitis and gonorrhoea (Iwu, 1993). The seed is masticatory and also used to prevent and relieve colic, chest colds, cough and can as well be used to treat headache. Iwu (1993) reported the use of this plant for the treatment of jaundice, high fever and as a purgative. The plant is also found useful in the treatment of stomach ache and gastritis (Ajebesone and Aina, 2004).

The phytochemical compounds isolated from *G. kola* include oleoresin (Onayade *et al.*, 1998), tannins, saponins, alkaloids, cardiac glycosides (Ebana *et al.*, 1991). Other phytochemical compounds so far isolated from *G. kola* seeds are biflavonoids such as kola flavone and 2-hydroxybi-flavonols (Okunji and Iwu, 1991; Terashima *et al.*, 1999; Okunji *et al.*, 2002). However, there is paucity of such information on the leaves.

Tetrapleura tetraptera, one of the medicinal plants in Nigeria, is known in the South Eastern Nigeria as *uhiokiriho*. The documented biological or pharmacological activities are found to be molluscicidal, cardiovascular, neuromuscular, hypotensive, anti-conversant, anti-ulcerative, anti-inflammatory and anti-microbial. (Enwere, 1998). The pods notably have an appealing culinary use for mothers from the first day of delivery to post parturition and as a lactation aid (Enwere, 1998).

The dry fruit has a characteristic pleasant aroma which makes it a popular seasoning spice in the Southern and Eastern Nigeria (Essien *et al.*, 1994; Okwu, 2004). At the same time, most of the folkloric chains agree in the traditional use of the fruit for management of convulsion, leprosy, inflammation and rheumatoid pains.

Neem (*Azadirachta indica*) commonly called 'Indian Lilac' belongs to the family '*Meliaceae*'. In Nigeria, the plant is known by the name '*Dogonyaro*'. Neem possesses maximum useful non-wood products (leaves, bark, flowers, fruits, seed, gum, oil and neem cake) than

any other tree species. These non-wood products are known to have anti-allergenic, anti-dermatic, anti-feedent, antifungal, anti-inflammatory, anti-pyorrhoeic, antiscabic, cardiac, diuretic, insecticidal, larvicidal, nematicidal, spermicidal and other biological activities. Because of these activities, neem has found enormous applications making it a green treasure. The extracts from neem are recommended in ancient medical texts for gastrointestinal upsets, diarrhea and intestinal infections, skin ulcers and infections, and malaria (Schmutterer, 1995).

Ginger (*Zingiber officinale* Roscoe) is a widely used herb and food-flavoring agent. Its nutraceutical properties have long been of interest to the food processing and pharmaceutical industries. The roots are commonly used as spices. It is medicinally used for its antioxidant (Sekiwa *et al.*, 2000; Eleazu and Eleazu, 2012), androgenic (Kamtchouing *et al.*, 2002) and hypoglycemic (Al-Amin *et al.*, 2006) actions.

The research into the discovery of new potent drugs from plants is based on the use of plant bio principles from extracts and essential oils to treat infection or diseases. Since prevention is a more effective approach than treatment for chronic diseases, constant supply of phytochemical containing plants with desirable health benefits beyond basic nutrition is essential to furnish the defense mechanism as this can help in reducing the risk of chronic diseases in humans.

This work is thus targeted at investigating the phytochemical constituents of the leaves of these five Nigerian plants: Paw paw, Tetrapleura, Bitter kola, Neem and Ginger.

MATERIALS AND METHODS

The fresh leaves of the plants were collected and identified by a Taxonomist in Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria.

Preparation of the extracts

Ten gram each of the plant leaves was oven dried at a temperature of 50°C for 24hrs before being processed to flours using a food processor and stored in an air tight container prior to analysis.

Determination of tannin content

The percentage composition of tannin in the plants was determined using the AOAC methods (1980) with some modifications. Folin-Denis reagent and saturated sodium carbonate were prepared in accordance with the procedure to analyze the tannin content. Standard solution of tannic acid was freshly prepared by dissolving 10 mg of tannic acid in 100 ml water. A series of tannic

acid standard were prepared in the range of 0-2.5 ml aliquots in 25 ml volumetric flasks then added with 1.25 ml Folin-Denis reagent and 2.5 ml sodium carbonate solution. The mixture was made up to the volume and the color was measured after 30 min at 760 nm using a spectrophotometer (Perkin Elmer). The samples were prepared by boiling 1 g of their dried powder in 80 ml of water for 30 min. The samples were cooled, transferred into a 100 ml volumetric flask and diluted to mark. The solution was filtered to get a clear filtrate and analyzed as in the standard. Tannin content was determined by a tannic acid standard curve and expressed as milligrams of tannic acid equivalence (TAE) per 100 g of dried sample.

Determination of saponin content

Saponin determination was done using the method of AOAC (1990). Saponin extraction was done using two different solvents. The first solvent, acetone, was used to extract crude lipid from the samples while the second solvent (methanol) was used for the extraction of the saponin proper. Two gram of the sample was folded into a thimble and put in a soxhlet extractor and a reflux condenser fitted on top. Extraction was done with acetone in a 250cm³ capacity round bottomed flask for 3 hours, after which the apparatus was dismantled and another 150cm³ capacity round bottomed flask containing 100cm³ of methanol was fitted to the extractor and extraction was carried on for another 3 hours. The weight of the flask was taken before and after the second extraction in order to make the change in weight. At the end of the second extraction, the methanol was recovered by distillation and the flask was oven-dried to remove any remaining solvent in the flask. The flask was then allowed to cool and the weight of the flask taken. The saponin content of the sample was calculated thus:

$$\%Saponin = \frac{\text{Weight of Saponin} \times 100}{\text{Weight of Sample}}$$

Determination of Alkaloid Content

The gravimetric method of Harborne (1973) was used in the determination of total alkaloid contents of the leaves. Five gram of the dried samples was dispersed into 50ml of 10% acetic acid solution in ethanol. The mixture was well shaken and allowed to stand for 4hours before filtering. It was evaporated to one quarter of its original volume and drop wise concentration of ammonium hydroxide was added to precipitate the alkaloids. The precipitate was filtered off with a pre-weighed filter paper and washed with 1% ammonium hydroxide solution. The precipitate was oven dried for 30 mins at 60°C and reweighed. The alkaloid contents of the samples were determined by difference using the equation:

$$\text{Percentage alkaloid} = \frac{W_2 - W_1 \times 100}{W}$$

Where W = weight of sample; W₁ = Weight of empty filter paper; W₂ = Weight of paper + precipitate.

Determination of flavonoid content

Ten grams of the plant samples were extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper no. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed (Boham and Kocipai, 1994).

Determination of cyanogenic glucosides content

The alkaline picrate method (Onwuka, 2005) was employed for the determination of cyanogenic glycosides. A portion (5g) of each sample was grinded into paste and dissolved in 50 ml distilled water in a corked conical flask. The extraction was allowed to stay overnight (12 hrs). The sample was filtered and the filtrate was used for the cyanide determination. To 1 ml of the sample filtrate in a corked test tube, 4 ml of alkaline picrate was added and incubated in a water bath for 5 minutes and the absorbance was read at 490 nm. The absorbance of the blank which contained only 1 ml distilled water and 4 ml alkaline picrate solution was read and used to stabilize the spectrophotometer before taking the absorbance of the samples. The cyanide content was extrapolated from a cyanide standard curve. The cyanide content was calculated using the formula:

$$\text{Cyanide} \left(\frac{\text{Ug}}{\text{g}} \right) = \frac{\text{Absorbance} \times \text{Gradient factor} \times \text{Dilution factor}}{\text{Weight of Sample}}$$

RESULTS AND DISCUSSION

Tannins are dietary anti-nutrients that are responsible for the astringent taste of foods and drinks (Chikezie *et al.*, 2008). Tannins bind to both proteins and carbohydrates which has several implications for commodities containing tannins. Their presence can cause browning or other pigmentation problems in both fresh foods and processed products. The presence of tannin in the plants implies they may have astringent properties and in addition, could quicken the healing of wounds and burns (Farquar, 1996). This justifies their usage in herbal medicine.

Pawpaw and ginger leaves whose percentage tannin contents were observed to be the same, contained

Table 1. Phytochemical composition of the leaves of 5 varieties of Nigerian plants

	Bitter kola	Neem	Tetrapleura	Pawpaw	Ginger
Saponin(%)	1.92± 0.820 ^{bc}	1.00±0.028 ^{ab}	1.00±0.042 ^{ab}	2.00±0.057 ^c	0.80±0.000 ^a
Alkaloid(%)	4.00± 0.212 ^b	4.00± 0.156 ^b	9.00±0.396 ^d	3.00±0.170 ^a	6.00±0.283 ^c
Tan(mg/100g)	0.26±0.007 ^a	0.36±0.025 ^b	0.38±0.021 ^b	0.43±0.004 ^c	0.43± 0.004 ^c
Flavonoid(%)	1.10±0.849 ^b	7.20±0.566 ^a	6.80±0.000 ^a	5.70±1.556 ^a	6.70±0.424 ^a
HCN(ug/g)	0.52±0.001 ^b	0.67±0.002 ^e	0.56±0.001 ^c	0.33±0.001 ^a	0.62±0.002 ^d

Each value in the table is the average ± standard deviations of triplicate experiments. Values with the same superscripts along each row are not significantly different from each other at P > 0.05. Tan = Tannin

HCN = Cyanogenic glucosides

higher proportions of tannins among other plants studied while bitter kola had the least. The tannin contents of both neem leaves and tetrapleura were also observed to be the same.

Saponin has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties (Eleazu et al., 2010) and bitterness (Sodipo et al., 2000). The saponin contents of neem and tetrapleura were observed not to differ from each other. The percentage composition of saponin in Pawpaw, though not significantly different from that of bitter kola, was the highest among other plants studied while that of bitter kola, though not statistically different from neem and tetrapleura, was the least. The moderate amounts of saponin in all the plants investigated suggested that they may not be deleterious to the user.

Alkaloids are basic natural products occurring primarily in plants. They occur as one or more heterocyclic nitrogen atoms and are generally found in the form of salts with organic acids. Alkaloids are the most efficient therapeutically significant plant substances. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents because of their analgesic, antispasmodic and anti-bacterial properties (Stray, 1998). The alkaloid content of tetrapleura was significantly higher than other plants studied while that of pawpaw was the least. The alkaloid contents of bitter kola and neem leaves were observed to be statistically the same. The high alkaloid contents of all the plants investigated especially tetrapleura justify the therapeutic use of these plants.

The large amounts of flavonoids in all the plants investigated infer that the plants have biological functions such as protection against allergies, inflammation, free radical, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumor (Okwu, 2004).

Flavonoids are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anticancer activity and protect against the different levels of carcinogenesis (Okwu, 2004). Flavonoids in the intestine lower the risk of heart

diseases. The antioxidant potentials of plants have been linked with their flavonoids contents. In addition, the therapeutic potentials of plants have been linked with their antioxidant potentials (Akinmalodun *et al.*, 2007; Eleazu *et al.*, 2011). As antioxidants, the flavonoids from these plants may provide anti-inflammatory activity. Thus the high alkaloid and flavonoid contents of these plants explain their therapeutic use in herbal medicine especially in the treatment of wounds, burns and ulcers.

Cyanogenic glucosides are compounds that yield glucose, hydrogen cyanide and aldehyde or ketone upon hydrolysis with an acid or enzyme. Dietary exposure to cyanide occurs mainly in the consumption of food stuffs rich in endogeneous cyanide in the form of cyanogenic glucosides. This cyanide could be lethal as it intercalates with cytochrome oxidase for aerobic function (Bolhius, 1954). Only free cyanide (CN⁻) is toxic and if hydrolysis does not occur, the glycoside remains stable and the food using this product becomes safe.

Values obtained for all plant samples investigated were below the lethal dose for man (Bolhius, 1954) indicating the safety in the therapeutic usage of these plants. (Table 1)

Conclusion

All the different species of plant leaves that were assayed contained significant quantities of saponin and cyanide though in moderate levels suggesting that they may not be deleterious to the user. They were also observed to contain high quantities of alkaloids and flavonoids suggesting their antioxidant potentials and justifying their therapeutic uses which could be utilized in drug formulation. Finally, the low quantities of tannin in all the plants studied suggest that usage of these plants in herbal medicine can neither interfere with dietary iron absorption nor inhibit digestive enzymes.

REFERENCES

Ajebesone PE, Aina JO (2004). Potential African Substances for Hops in Tropical Beer Brewing. *J. Food Technol. Afr.*, 9(1):

- 13-16.
- Akinmalodun AC, Ibukun EO, Afor E, Akirinlola BL, Onibon TR, Akinboboye AO, Obuotor EM, Farombi EO (2007). Chemical constituents and antioxidant activity of *Alstonia boonei*. Afr. J. Biotechnol., 6(10): 1197-1201.
- Al- Amin, ZM, Thomson M, Al Qattan KK, Peltonen-Shalaby R, Ali M (2006). Antidiabetic and hypolipidemic properties of ginger (*Zingiber officinale*) in streptozotocin - induced diabetic rats. Br. J. Nutri., 96(4): 660-664.
- AOAC (1990). Official Methods of Analysis, 15th edn. Association of Official Analytical Chemists, Arlington, VA.
- Atta K (1999). The Power of Garlic. Cardiovascular disease prevention Association, Buea, Cameroon. p. 72.
- Boham AB, Kocipai AC (1994) Flavonoid and condensed tannins from leaves of Hawaiian vaccinium vaticulum and vicalycinium. Pacific Sci., 48: 458-463.
- Bolhuis GG (1954). The toxicity of cassava roots. Neth. J. Agric., 2: 176-185.
- Chikezie PC, Agomuo EN, Amadi BA (2008). Biochemistry, Practical/Research Method, A Fundamental Approach. Vol 2, Mega soft publishers, p. 51-53.
- Dick G (2003). Papaya: A tantalizing taste of the Tropics. Maricopa County Master Gardener Volunteer information, University of Arizona Cooperative Extension. <http://ag.arizona.edu/maricopa/garden/html/pubs/mgjournal.htm>.
- Ebana RU, Madunagu BE, Ekpe ED, Otung IN (1991). Microbiological exploitation of cardiac glycosides and alkaloids from *Garcinia kola*, *Borreria ocymoides*, *Kola nitida* and *Citrus auratifolia*. J. Appl. Bacteriol., 71(5): 398- 401.
- Eleazu CO, Eleazu KC (2012). Physico-chemical properties and antioxidant potentials of 6 new varieties of ginger (*Zingiber officinale*). Am. J. Food Technol., 7(4): 214-221.
- Eleazu CO, Okafor PN, Ahamefuna I (2010). Total Antioxidant Capacity, Nutritional Composition and Inhibitory Activity of Unripe Plantain (*Musa paradisiaca*) on Oxidative Stress in Alloxan Induced Diabetic Rabbits. Pakistan J. Nutrition, 9(11): 1052-1057.
- Eleazu CO, Okafor PN, Amajor J, Awa E, Ikpeama AI, Eleazu KC (2011). Chemical Composition, antioxidant activity, functional properties and inhibitory action of unripe plantain (*M. Paradisiaca*) flour. Afr. J. Biotechnol., 10(74): 16948-6952
- Elizabeth K (1994). Immense help from nature's workshop. 1st Ed. Elikaf Health Services Ltd. Ikeja, Lagos. pp. 207-209.
- Enwere NJ (1998). Foods of Plant Origin. Int. J. Microbiol., 9 (94): 329-334.
- Essien EU, Izunwane BC, Aremu CA, Eka CA (1994). Plant Food and Animal Nutrition. Longman London. pp. 45- 49.
- Farquar JN (1996). Plant Sterols, their biological effects in human. Handbook of Lipids in Nutrition BOCA Rotan HL CRC Press. pp. 101-105.
- Harborne JB (1973). *Phytochemical methods*. London: Chapman and Hall, Ltd. p. 113.
- Iwu MM (1993). Handbook of African medicinal Plants. Boca Raton: CRC Press Inc. pp. 223-224.
- Kamtchouing P, Mbongue-Fandio GY, Dimo T, Jatsa HB (2002). Evaluation of androgenic activity of *Zingiber officinale* extract in male rats. Asian J. Androl., 4: 299-301.
- Leakey R (2001). Potential for novel food production from agroforestry trees: A Review. <http://www.wanatca.org.au/acotanc/Papers/Leakey>.
- Mantok C (2005). Multiple Usage of Green Papaya in Healing at Tao Garden. Tao Garden Health spa & Resort.Thailand.WWW.TAO-GARDEN.COM.
- McLanghlin JL, Ratanyake S, Rupprecht JK, Potter WM (1992). Evaluation of various parts of the pawpaw tree, *Asimina triloba* (Annonaceae), as commercial source of the pesticidal annonaceous acetogenins. J. Econ. Entomol., 85: 2353-2356.
- Okunji CO, Tantalia AW, Hicks RP, Iwu MM, Skanchy DJ (2002). Capillary electrophoresis determination of biflavonones from *Garcinia kola* in three traditional African medicinal formulations. Plant Med. 68:440 - 444.
- Okunji CO, Iwu MM (1991). Molluscidal activity of *Garcinia kola* biflavonones Fitoterapia, 67: 74-76
- Okwu DE (2004). Phytochemicals and vitamin content of indigenous species of South Eastern Nigeria. J. Sustain Agric. Environ., 6: 30-34.
- Omode AA, Fatoki OS, Olagun KA (1995). Physico-chemical properties of some underexploited and unconventional oilseeds. J. Agric. Food. Chem. 43: 2850-2853.
- Onayade OA, Looman AMG, Scheffer JJC, Gbile ZO (1998). Lavender lactone and other volatile constituents of the oleoresin from seeds of *Garcinia Kola* Hechel. Flavour Frangrance J., 13(6): 409-412
- Onwuka GI (2005). Food Analysis and Instrumentation. Theory and Practice. Naphthali Prints. p. 140-146.
- Peter RN (1991). Pawpaw (*Asimina*). In: J. N. Moore and J. R. Ballington (eds). Genetic resources of temperate fruit and nut trees. Acta. Hort., 290: 567- 600.
- Schmutterer H (1995). The Neem Tree. In: Schmutterer, H., Ascher, K. R. S., Isman, M.B., eds. *The Neem Tree: Azadirachta indica A Juss and other Meliaceous Plants*. Weinheim, Germany. pp. 1-3.
- Sekiwa Y, Kubota K, Kobayashi A (2000). Isolation of novel glycosides from ginger and their antioxidative activity. J. Agric. Food Chem., 8: 373-379.
- Sodipo OA, Akiniyi JA, Ogunbamosu JU (2000). Studies on certain characteristics of extracts of bark of pansinystalia macrucas (K schemp) pierre Exbeille. Global J. Pure Appl. Sci., 6: 83-87.
- Terashima K, Kondo Y, Aqil M, Waziri M (1999). A study of biflavanones from the stem of *Garcinia kola*. Heterocycles 50: 238-290.
- Walter L (2008). Cancer remedies. www.health-science-spirit.com/cancer6-remedies