

Comparative studies on phytochemical and proximate composition of four morphologically distinct segments of the conophor seedling (*Tetracarpidium conophorum* Hutch. & Dalziel)

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Abstract. This work attempts to determine the bioactive agents' groups probably responsible for the diverse growth characteristics and medicinal uses of *Tetracarpidium conophorum* Hutch. & Dalziel and to explore the global propagation of the climber plant. Consequently, four segments of the seedling namely: the dried epicotyls and foliage, the upper regenerative hypocotyl, the poorly regenerative swollen hypocotyl and the roots of the conophor seedlings were screened for ten bioactive compounds as well as their food values. Results showed that alkaloids and saponin concentrations were highest in the foliage. Terpene concentration was highest in the regenerative hypocotyl segment. Tannins, phenolic compounds and phlobatannin concentration were highest in the non-regenerative hypocotyl segment. Anthraquinone, cardiac glycoside and flavonoids concentrations were highest in the roots. Cardenolide concentration is generally low in all the segments of the conophor seedling studied. It can be inferred that the foliage can be used as an anti-venom agent based on its relatively high content of cardiac glycosides, alkaloids, saponins and phenols which are active substances responsible for detoxification of venoms in human system. There were also obvious variations in the amount of both bioactive agents and food content of the various segments of the conophor seedling. High proteins and terpene content of the regenerative hypocotyl segment are probably responsible for adventitious bud regeneration since terpenes are precursors of plant hormones.

Keywords: Seedling, Bioactive agents, Hypocotyl, Adventitious buds, *Tetracarpidium conophorum*, African walnut.

Introduction

There are two types of walnuts. The temperate walnut, known as *Juglans regia* L., and the tropical African walnut, known as *Tetracarpidium conophorum* Hutch. & Dalziel or *Plukenetia conophora* Miill.-Arg. The *J. regia* is planted in rows. The characteristic furrowed kernels are the cotyledons of the seeds with no

endosperms. Their kernels are eaten raw; and the young fruits are a rich source of ascorbic acid. The leaves are astringent, tonic and antihelminthic, and they yield an essential oil (Pandey, 2007). However, *T. conophorum* is a tropical, woody, perennial, climbing plant with open branches. It twines around any support, especially trees in its vicinity. It is an alley-farmed crop, grown alongside cocoa and

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kola nut and uses these trees as support for its growth. The economic importance of the species lies in the edibility of its oil-rich endospermous seed, which is consumed by diverse populations in Nigeria, Sierra Leone and the Lower Congo region. It grows along the African Coastline and is thought to originate in South-Western Nigeria (Janick and Paul, 2008). It is usually cultivated by subsistence farmers in the hot and humid zones of tropical Africa in compound gardens and backyards, just for the family and local market consumption.

Both types of walnuts are also referred to as the conophor tree (Hutchson and Dalziel, 1958; Janick and Paul, 2008). Tropical walnut belongs to the Euphorbiaceae Family (spurge). Locally in Nigeria, it has various names such as asala or awusa (Yoruba), ukpa (Ibo), oke okpokirinya (Imo) and okhue or okwe (Edo). In Sierra Leone, it is called musyabassa (Hutchson and Dalziel, 1958; Burkill, 1985).

T. conophorum is a woody climber about 6-18 m long on attainment of reproductive phase with a stem (Hutchson and Dalziel, 1958; Janick and Paul, 2008). As a successful, long-stemmed woody climbing plant that grows from ground level to the canopy of other trees. It is well adapted to the tropical forests. It has stems which could be as long as 70 m or more. It climbs up and over the tops of other very tall trees in order to benefit from full sunlight at maturity. Sometimes it may bind the trees together such that if one of the trees dies, it is held in position until it decays (Bailey, 2006). The stem can be up to 16 cm in girth and dark grey when old, but green and glabrous when young. The root is mainly fasciculated. The leaves (10 cm long and 5 cm broad) are simple, crenate and ovate with a serrated margin; they are also alternate, abruptly acuminate and rounded at the base. The leaves are three-nerved at the base with the petiole up to 5 cm long.

Conophor plant is monoecious with separate male and female flowers on the same plant. The male flowers are in narrow raceme-like panicles about as long as the leaves, with one or two female flowers near the base. The flowers are arranged

alternately on the axis of the raceme inflorescence. The style is stout and quadrangular with four spreading stigmas. The stamens are many, about 40 in number (Hutchson and Dalziel, 1958; Janick and Paul, 2008). They are forest scrambler, seed contained in compartmentalized fruits. They are found in both primary and secondary forests (Okujagu et al., 2005). The fruit is a four-winged, ribbed capsule, containing subglobose seeds with a thin, brown shell and yellowish kernel. Each seed loculus ends in a wing thereby creating ridges in between the wings. The seed is about 2.5 cm in diameter. The fruit (7 cm across) are light green to brown when ripe, while the seeds are round dark brown at maturity (covered with a hard testa). Sometimes not all the seeds develop in which cases the fruit has fewer wings (Isawunmi, 1993).

The vine is found in the moist forest zones of tropical Africa between 4° 15' N and 8° N of the Equator (Okafor, 1983). This exotic wild fruit is grown in the traditional farming system of the lowland humid regions. It can tolerate any type of soil, provided it is well drained with moderate moisture-retention capabilities (Janick and Paul, 2008). A woody liane to over 30 m long, of the bushy Savanna in Sierra Leone and from Dahomey to West Cameroons and Fernando Po and extending to Zaire. Though well-recorded from Sierra Leone and cultivated there, it is apparently indigenous. It is not recorded from Liberia, Ghana and Togo. Its presence in Sierra Leone may be due to returning slaves for it is known to the Krio by its Yoruba (Nigerian) name. Cultivation trials have been carried out in Nigeria. It is also present in Central African Republic, Congo, Gabon, and Niger (Burkill, 1985).

In Nigeria, the conophor plant flowers between November and early January and fruits between February and September with peak production in July. The immature fruits are usually green in color, but turn dark brown as they mature (Oluwole and Okusanya, 1993). They are plants having swollen, fleshy, sparsely branched stems and sometimes candlebroid in appearance (Janick and Paul, 2008). The fruit is a capsule 6-10 cm long by 3-11 cm wide containing sub-globular seeds 1-

2.5 cm long with a thin brown shell resembling the temperate walnut, hence the English name (Burkill, 1985). The shell, bark and leaves of the conophor plants are anti-parasitic, antifungal and anti-dysentric and the bark is used by local people as a mild laxative (Janick and Paul, 2008).

The medicinal uses of the bark, leaves and roots are an ancient prehistoric practice surrounded by many superstitious beliefs. The seed kernel, when eaten raw has a bitter taste like kola nut and is considered to be a tonic and aphrodisiac. Conophor plant is cultivated principally for the nuts, which are cooked and consumed as snacks along with boiled corn (Enujiugha, 2009). The leaves and young shoots occasionally eaten with cooked rice in some parts of West Africa. Food is stored in the endosperm which is white and edible after cooking. Significant concentrations of oxalates, phytates and tannins have been reported for raw, uncooked conophor nuts. These factors are effectively removed via hydrothermal treatment which the seeds are usually subjected to before consumption (Enujiugha, 2003; Enujiugha & Ayodele-Oni, 2003; Enujiugha, 2009). Conophor nuts contain 48%-50% dry weight of oil, which is liquid and golden yellow in color, with taste and odor resembling those of linseed oil. The residue after oil expression contains over 50% protein (Enujiugha, 2003; Enujiugha, 2009). Composition of the conophor oil is linolenic acid 64%, palmitic and stearic acids 15%, oleic acid 11% and linoleic acid 10%. The conophor has great potentials in the paint and varnish industry and trade awusa or n'gart (Burkill, 1985). The linseed oil has 3% myristic acid, 6% palmitic acid, 74% linoleic acid and 17% linolenic acid (Schery, 1972).

The aim of this research was to establish a relationship, if any, between the laboratory analysis for phytochemicals and proximate determination and the production of adventitious buds/shoots and to evolve methods for clonal propagation of the African walnut which has not been explored or reported in literatures.

Materials and methods

African walnut seeds (*T. conophorum*) used for this work were purchased from local markets in Oyo (Oje Farm Produce Market in Ibadan) and Ogun (Mamu and Ilishan Markets) States. The seeds were sown for germination the following day on the surface of light moist soil (at about 3cm below the soil surface) under partial shade. Germination of the endospermous seeds was visibly observed to have commenced after two weeks by the emergence of the upper part of the hypocotyl called hypocotyls hook. Germination in the African walnut is mildly epigeal. As the seedling grows and before the epicotyl emerges from the twin endosperm tissue that presses on the thin membranous cotyledon, two distinct parts of the hypocotyl are manifested. A swollen, light green to pale white lower part, which is next to the root region, becomes conspicuously differentiated. This is from the relatively thin, dark green, upper part, next to the first node that separates the hypocotyls from epicotyl. At the onset of germination, several fairly swollen roots emerged; the tap root rarely becomes identifiable at the root region of the seedling from the fasciculate roots. Any damage caused to the rapidly growing vine of the epicotyls triggered more adventitious budding on the upper hypocotyls part (regenerative hypocotyl) and auxiliary bud sprout from the vines. Complete removal of this upper part of the regenerative hypocotyls did not induce more adventitious budding or sprouting of the buds in the lower swollen hypocotyl part of the seedling which is now technically tagged as the non-regenerative part of the hypocotyls.

The sampling exercise started from the third month of germination. Fresh epicotyl foliage, regenerative hypocotyls, non-regenerative hypocotyls and roots of the *T. conophorum* seedlings were air dried and then ground into fine powder, preparatory for various laboratory analysis and determination.

Proximate analysis: Dried and ground foliage, regenerative hypocotyl, non-regenerative hypocotyl and root samples of *T. conophorum* were analyzed chemically according to the official methods of analysis described by the Association of Official Analytical Chemist (AOAC, 1990).

Moisture content evaluation: Traces of water and volatile components were removed by oven drying the samples.

Crude protein determination: The crude protein and total nitrogen were determined using the Kjeldahl method (Kjeldahl, 1883).

Crude fibre determination: Crude fiber is determined gravimetrically after chemical digestion and solubilization of other materials present. The starch and the protein are dissolved by boiling the sample with acid i then with sodium hydroxide. The residue of cellulose and lignin is washed, dried and weighed.

Crude fat determination: This was done using soxhlet method (Kjeldahl, 1883).

Carbohydrate evaluation: This component is not determined analytically but it is calculated by difference in the overall proximate analysis:

$$\text{Carbohydrates (\%)} = 100 - (\% \text{ Fibre} + \% \text{ Moisture} + \% \text{ Protein} + \% \text{ Fat} + \% \text{ Ash})$$

Phytochemical tests: Qualitative and quantitative phytochemical analysis of methanol extracts was conducted following the standard procedures (Brinda et al., 1981; Trease et al., 1989; Dhirya and Manimegalai, 2013).

Results and discussion

The results in Table 1 indicate that the moisture content was highest in the regenerative hypocotyl and decreased from the root, non-regenerative hypocotyl to the foliage in that descending order. Crude protein was highest in the regenerative hypocotyl, followed by the foliage, non-regenerative hypocotyl and lowest in the root. Ash content of the non-regenerative hypocotyl was the highest and decreased from the regenerative hypocotyl, root to the foliage. Crude fiber was highest in the non-

regenerative hypocotyl and decreased from the root, regenerative hypocotyl to the foliage. Crude fat was highest in the foliage and decreased progressively from the root, non-regenerative hypocotyl to the regenerative hypocotyl. Crude carbohydrate of the conophor seedling was generally high in all segments. The Carbohydrate content of the root was the highest, decreased from the foliage, non-regenerative hypocotyl to the regenerative hypocotyl.

In Table 2, the alkaloid complex present in the foliage was highest and decreased step-wisely the regenerative hypocotyl, non-regenerative hypocotyl and to the root of the conophor seedling. Tannin was highest in the non-regenerative hypocotyl and decreased from the regenerative hypocotyl to the foliage. The tannin present in the root was higher than in the regenerative hypocotyl but lower than the non-regenerative hypocotyl of the conophor seedling. Anthraquinone content of the root was highest and decreased from the regenerative hypocotyl to non-regenerative hypocotyl and lowest in the foliage of the conophor seedling. Cardiac glycoside was highest in the root, decreased from the foliage step-wisely to the regenerative hypocotyl and lowest in the non-regenerative hypocotyl of the conophor seedling. Flavonoid was lowest in the foliage and increased progressively from the regenerative hypocotyl, non-regenerative hypocotyl to the root of the conophor seedling. Saponin was lowest in the root and increased from the regenerative hypocotyl, non-regenerative hypocotyl to the foliage of the conophor seedling. Cardenolide concentration was generally low in the conophor seedling. Phenol and phenolic compounds were highest in the non-regenerative hypocotyl and decreased in the regenerative hypocotyl and to the foliage. The phenol and phenolic compounds present in the root was higher than in the regenerative hypocotyl but lower in the non-regenerative hypocotyl of the conophor seedling. Phlobatannins concentration was highest in the non-regenerative hypocotyl, followed by the regenerative hypocotyl, foliage and lowest in the root of the conophor seedling. Terpene was high in the regenerative

Table 1. Nutritional compositions of various segments of *T. conophorum*.

	A	B	C	D
Moisture Content	9.75	11.75	10.68	11.68
Crude Protein (%)	7.60	9.48	6.80	4.75
Ash Content (%)	3.40	6.40	6.94	4.24
Crude Fiber (%)	7.60	9.48	10.68	9.68
Crude Fat (%)	1.48	0.10	0.13	0.18
Crude Carbohydrate (%)	65.37	63.52	64.77	69.50

A = Stem epicotyl foliage; B = Regenerative upper hypocotyl; C = Non-regenerative lower hypocotyl; D = Root.

Table 2. Phytochemical constituents of various segments of *T. conophorum*.

	A	B	C	D
Alkanoids	6.468	4.564	3.547	2.478
Tannins	0.014	0.184	0.320	0.240
Anthraquinone	0.142	1.240	0.400	3.240
Cardiac Glycoside	1.403	0.648	0.375	2.380
Flavonoids	0.248	0.320	0.470	2.110
Saponin	4.470	3.204	3.640	1.680
Cardenolide	0.018	0.030	0.045	0.012
Phenolic Compounds	0.010	0.137	0.158	0.124
Phlobatannins	1.240	1.375	1.520	0.320
Terpenes	2.345	2.480	1.375	1.470

A = Stem epicotyl foliage; B = Regenerative upper hypocotyl; C = Non-regenerative lower hypocotyl. D = Root.

hypocotyl and foliage and low in the root and non-regenerative hypocotyl.

T. conophorum is a Euphorbiaceous legume (Burkill, 1985; Pandey, 2009). It is grown as a co-crop specifically for its seeds; nevertheless it has not being put into large scale, sole-crop cultivation. Consequently it is still regarded as an undomesticated crop. It is a climber that takes up to seven years to attain fruiting stage across the West African sub- region. It has in recent times been put into several uses. In this investigation, the seedling was cut into segments, and the segments designated as "A", "B", "C" and "D" with the main aim of exploring its total potential for more human uses. Segment "A" represents the stem (vine) foliage which contains the leaves, vine and stem. The foliage is dark green. Segment "B" represents the regenerative hypocotyl which is also green in color but less green relative to the stem (vine) foliage. Segment "C" represents the non-regenerative hypocotyl which is a swollen, whitish green part and segment "D" represents the root which is white or cream in color. Part of the objective of this

investigation was to find more uses for all parts of the vegetative seedling plant. The proximate analysis revealed that the moisture content of the foliage is the lowest thus probably has a longer shelf life because the higher the moisture content, the lower the shelf life of the food substance (Giuseppe and Baratta, 2000). The foliage contains 7.60% protein which makes it a potential good source of protein when consumed as an herbage vegetable. The regenerative hypocotyl contains the highest concentration of protein which probably means most of the enzymatic reactions take place there because most enzymes are proteins (Nelson and Cox, 2000) and thus this could be responsible for its adventitious bud regeneration.

The ash content of the non-regenerative hypocotyl is highest in the conophor seedling this implies that the total inorganic residue which is an indicator of mineral content (Onyeike and Osuji, 2003; Nielson, 2002) is highest in the non-regenerative hypocotyl. Crude fibre adds bulk to the food and prevent excessive intake of starchy food, thus, may guard

against metabolic conditions like hypercholesterolemia and diabetes mellitus (Mensah et al., 2008). The crude fibre content of the non-regenerative hypocotyl was the highest compared to the other parts of the conophor seedling. The fat content is highest in the foliage of the conophor seedling, dietary fat contribute to the calorific value of the food and is responsible for the odor and flavor which contributes to the palatability of food (Giuseppe and Baratta, 2000) thus probably making the foliage palatable. The carbohydrate content of the conophor seedling foliage is 65.37%. This is considered a relatively high value.

The carbohydrate content of the root is the highest compared to the other part of the conophor seedling. Carbohydrates and their derivatives play a major role in the functioning of the immune system, fertilization, pathogenesis and development (Merill and Watt, 1973) and the conophor seedling is a good source of carbohydrate.

The phytochemical screening of the various parts of the conophor seedling indicated the presence of some bioactive compounds such as alkaloids, tannins, anthraquinone, cardiac glycoside, flavonoids, saponin, and trace amount of cardenolides, phenols, phlobatannins and terpenes. Alkaloids enhance human respiration and act primarily on the central nervous system (Britannica, 2002). The foliage of the conophor seedling contains the highest concentration of alkaloid thus can probably be used as a better stimulant of human respiration than other parts of the conophor seedling. The leaves of the conophor seedling are considered a cure for headache (Burkill, 1985) alkaloids complex. For example morphine is an alkaloid and has being established to be a painkiller (Encarta, 2009). Alkaloids present in plants serve as poisons, feeding deterrents, antimicrobial defenses and germination inhibitors to reduce competition from other plants- allelopathic agents (Bowsher et al., 2008). The alkaloid is highest in the foliage and it decreases step-wisely from the regenerative hypocotyl, non-regenerative hypocotyl and lowest in the root. Tannins can be used to treat diarrhoea (in the absence of fever or

inflammation), can also be used as antimicrobial, antioxidant and antidepressants agents (Duke, 1985; Polterait, 1997) and thus the non-regenerative hypocotyl of the conophor seedling could be used compared to other parts of the seedling because it contains the highest concentration of tannin. Tannins may provide protection against microbiological degradation of dietary proteins in the semen (Abulude et al., 2010) thus the non-regenerative hypocotyl can probably be used. Tannins serve as feeding deterrents towards many insects, mammals, and birds. They also inhibit fungal growth in trees (Bowsher et al., 2008). Tannin is highest in the non-regenerative hypocotyl and decrease to the regenerative hypocotyl and lowest in the foliage, the tannin present in the root is higher than in the regenerative hypocotyl but lower than the non-regenerative hypocotyl. Anthraquinone have a laxative effect on the body (Siegers et al., 1993).

The bark of the adult conophor tree is considered a mild laxative (Jannick and Paul, 2008), probably because of the presence of anthraquinone, but the root can be used as a better laxative because it contains the highest concentration of anthraquinone in the conophor seedling. Cardiac glycoside increases the force of contraction of the heart muscle and in proper dose can treat heart failure (Leverin and McMahon, 1999) and the root contains the highest concentration of cardiac glycoside and thus can probably be used to treat heart failure better than the other parts of the seedling.

Flavonoids act as potent antioxidants, influence the risk of cancer, effective anticancer promoters and cancer chemo preventive agents (Polterait, 1997; Grotewold, 2006) they have anti-inflammatory effect (Ayoola et al., 2008). They can be used to treat hemorrhage, hypertension, radiation injuries and they are known to decrease capillary fragility (Encarta, 2009). Flavonoid concentration is highest in the root thus the root can probably be used as an anti-inflammatory agent, for cancer management and as antioxidant than the other parts of the conophor seedling. Flavonoids provide a means of signaling between plant hosts and

bacterial symbionts. They also protect the plant against the potential damaging effects of ultraviolet radiations because of its ultraviolet-absorbing properties. They are responsible for the different colors found in plants (Bowsher et al., 2008). Flavonoid is lowest in the foliage and increases gradually from the regenerative hypocotyl, non-regenerative hypocotyl and highest in the root. Saponin stimulates the immune system (Ayoola et al., 2008), can control human cardiovascular disease and reduce cholesterol (Abulude et al., 2010), can act as effective contraceptives for women (Bowsher et al., 2008).

The concentration of saponin is highest in the foliage thus the foliage of the conophor seedling can probably be used effectively to control human cardiovascular disease, as human immune system stimulator and as contraceptives for women (Bowsher et al., 2008). Phenolic compounds may reduce the risk of cardiovascular disease and certain types of cancer (Nelson and Cox, 2000; Marinova et al., 2005). There are only traces in the foliage and medium amount in the non-regenerative hypocotyl thus the non-regenerative hypocotyl can probably be used to reduce the risk of cardiovascular disease and cancer than the other parts of the conophor seedling. Plant phenolics are used for scents and pigments, poisons and feeding deterrents, allelopathic compounds, signaling molecules, structural components, and antifungal and antimicrobial agents (Bowsher et al., 2008). Phenol is highest in the non-regenerative hypocotyl and decrease to the regenerative hypocotyl and lowest in the foliage, the phenol present in the root is higher than in the regenerative hypocotyl but lower than the non-regenerative hypocotyl.

Phlobatannins are mild astringent (Marinova et al., 2005). Their concentration is highest in the non-regenerative hypocotyl and lowest in the root. Terpenes and essential oils act as effective contraceptives for women (Bowsher et al., 2008) they have antibacterial (Barre et al., 1997; Amaral et al., 1998), antifungi, antiviruses (Fujioka and Kashiwada, 1994) and antiprotozoal properties. The regenerative hypocotyl has the highest concentration of terpene thus can probably be used as contraceptives for

women, as antibacterial, antifungal, antiviruses and antiprotozoal agent better than the other parts of the conophor seedling. Terpenes precursors are used to derive plant growth regulators, abscisic acid and cytokinins, gibberellins and brassinosteroids. They also serve as phytoalexins-chemicals that have antibacterial and/or antifungal properties. They also serve as poisons, feeding deterrents (Bowsher et al., 2008). The terpene is highest in the regenerative hypocotyl and lowest in the non-regenerative hypocotyl. The terpene present in the foliage is higher than in the root. The terpene concentration of the regenerative hypocotyl is probably responsible for the adventitious bud regeneration on this segment of the conophor seedling because terpene precursors are used to derive plant growth regulators.

The conophor plant is considered as anti-venom probably because of the presence of cardiac glycoside, alkaloids, saponin and phenol which are the active substances responsible for detoxification of the venoms in the human system. Immediately a snake bite occurs, the first target of the venom is the heart but the presence of the cardiac glycoside will improve cardiac output and reduce heart dissension (Dalziel, 1937). The foliage has the highest concentration of cardiac glycoside, alkaloids, saponin and traces of phenol that is probably why it can be effectively used as anti-venom than the other parts of the conophor seedling. The foliage is consumed in some communities as food adjunct and as food probably because of its protein, fat and fibre content (Enujiugha, 2003). The foliage contains some bioactive compounds which makes it a medicinal plant thus when consumed it is nutritious and can be used for the treatment of some ailments. The bark and leaves of the conophor tree are considered as anti-parasite, antifungal and anti-dysentric (Jannick and Paul, 2008) probably because of the presence of terpenes, flavonoids, phenols, tannins and other bioactive substances. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavenger (Ayoola et al., 2008). The

presence of flavonoids and tannin in the conophor plant will probably make the conophor seedling a good antioxidant.

Conclusion

The conophor seedlings have medicinal uses because of the presence of bioactive agents. The conophor seedling is consumed as food adjunct because of its nutritional value, that is, its protein, fat and fibre content. The regenerative hypocotyl of the conophor seedlings appear to be rich in protein and terpenes relative to all other segments. These are probably responsible for the adventitious regeneration of buds on the upper part of the hypocotyl and a potential boost for its clonal proliferation and propagation. Various segments of the conophor seedling have different compositions because of either their plant growth regulator and other precursors or the type of reaction taking place in each segment.

Conflict of interest statement

Authors declare that they have no conflict of interests.

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