Estimation of Phytochemical, Total Phenolic and Total Flavonoid Contents of Methanol Extract of *Voacanga africana* Root Bark and its Fractions

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Abstract

Phenolic and flavonoid contents in plants play a great role in scavenging free radicals in the body and act as antioxidants; thus making their determination very vital. Total phenolic and flavonoid contents of the methanol extract of Voacanga africana root bark and its fractions (n-hexane, ethyl acetate and n-butanol) were carried out in this study. The total phenolic content was determined by using Folin-Ciocalteau assay while the the total flavonoid content was determined by the aluminium chloride colorimetric assay method. The result obtained showed that n-hexane fraction of the plant exhibited the highest (116.607±95.13 mgGAE/g) total phenolic content (TPC) at all concentrations followed by ethyl acetate fraction of the plants. The highest total flavonoid content across concentrations (300, 250, 200, 100, 50 and 25 µg/ml) was exhibited by n-hexane fraction (467.143±44.22 mg QE/g). From the results, it was concluded that n-hexane fraction of V. Africana root bark exhibited the highest total phenolic and total flavonoid contents at all concentrations (ppm), followed by ethyl acetate. It could be deduced that V. africana root bark possesses phenolic and flavonoid contents depending on the type of extract or fraction; thus justifying its folkloric use in the treatment of diseases.

Key words: Phytochemicals, Phenols, Flavonoids, *Voacanga africana*, Root bark, Fraction.

Introduction

Plants are a major source of phenolic compounds, which are synthesized as secondary metabolites during normal development in response to stress conditions, such as wounding and UV radiation among other. Plants may contain simple phenolics, phenolic acids, coumarins, flavonoids, stilbenes, hydrolysable and condensed tannins, lignins and lignans. Distribution of phenolics in plants at the tissue, cellular and subcellular levels is not uniform. Insoluble phenolics are found in cell walls, while soluble phenolics are present within the plant cell vacuoles.

Voacanga africana is a deciduous, mesophytic plant of the Apocynaceae family found in the tropical rainforest of Nigeria and the Guinea Savanna. A mature V*oacanga africana* grows about 6 m high, not more than 10 m with low widely spreading crown distributed mainly in West Africa. It is known locally as kokiyar in Hausa, pete-peteinigbo, kirongasi in swahili, and ako-dodo in yoruba. Flowers are white borne in axillary or terminal loosely branched inflorescent, spherical mottled green fruits occur mainly in pairs with seeds wrapped in yellow pulp. The plant is used to treat leprosy, generalized oedema and as infant tonic (Iwu, 1993).

A decoction of the stem bark and root is used to treat mental disorder and the latex is applied to carious teeth .the decoction of the bark is considered analgesic, and is added to embrocating mixtures used as paste during fracture repairs. Root and bark decoctions are also used to treat cardiac spasms. The fruit decoction is used as a disinfectant and the leaf decoction to treat asthma in children (Neuwing, 2000). In the South Eastern part of Nigeria, the plant is featured in many healing rituals preparation of the extract (Iwu, 1993) including some induced hallucinations and trance in religious rituals. In Congo, traditional medicine preparation of extracts containing *Voacanga africana* are used as anti-amoebial against intestinal amoebiasis, which is one of the current diseases in tropical regions causing diarrhea. It has been reported that *V. africana* has activity against *Entamoeba hystolitica in vitro* (Crowwell and Otvos, 2004).

V. africana is also used to treat painful hernia. Analysis of root and bark extract of the plant

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showed the presence of alkaloids including voacamine, voacangine and vobasine (Oliver-Bever, 1986). The leaves and stem decoction of the plant have been implicated in folk medicine for the treatment of malaria, diarrhea, infant convulsion, mental disorder and heart aches (Burkill, 1995). Other compounds found in the plant include voascritine, voacamidine, voacamine, voaphylline, vobustine and voalpolidine which occur in the leaves and tabers is a constituent of the seeds (Iwu, 1993). The alkaloid ibogaine is a powerful hallucinogen also found in Voacangin supporting its use in the treatment of withdrawal symptoms and craving in drug addicts (Correar and Calixto, 1993).

Phenolic acids contain carboxylic acid in the chemical composition, and each of the hydroxycinnamic and hydroxybenzoic is a main pillar of phenolic acids according to figure 2. Moreover, scientists have noted that p-coumaric, caffeic, ferulic, and sinapic acids are the main component part of the hydroxycinnamic.

The molecular weight for flavonoids is low (Coultate, 1990). Flavane is the main part of flavonoids which contains two benzene rings (A and B) within its chemical composition. As these two rings connected to each other through pyrane ring (C), so all of flavones, isoflavones, flavonoids, flavonols, flavanones, anthocyanins, and pro anthocyanidins are part of flavonoids as classified according to a new nomenclature and classification.

Aim of the Study: The aim of this project work is to determine the total phenolic and total flavonoid contents *Voacanga africana* root bark

Materials and Methods

Materials

Chemicals and Reagents: All chemicals and reagents used in this study were of analytical grade. Sodium carbonate (M & BD England), aluminium chloride (Fluka Garantie Germany), sodium nitrite (Qualikems, India), quercetin (BDG, England), methanol (Sigma- Aldrich, Germany), Sulfuric acid, (BDG, England), Sodium phosphate (JHD, China), ammonium molybdate (Kiran Light Lab, India) and ascorbic acid (BDG, England). Distilled water was obtained from National Centre for Equipment Maintenance and Development (NCEMD), University of Nigeria, Nsukka.

Major Apparatus: Balances (Adventurer OHAUS Corp, China), UV-visible spectrophotometer (UV-1800, Schimadzu Corperation, Japan), rotary evaporator (Biichi Rotavapour, Germany), volumetric flask, measuring cylinder, reagent bottle, Eppendorff vials, Syringes (1, 2 and 10 ml), water-bath and test tubes.

Plant Material: The root bark of *Voacanga africana* plant was collected and used for extraction studies.

Methods

Plant Collection and Identification: The root barks of *Voacanga africana* were collected from Idah Local Government Area, Kogi State, and identified by Mr. Alfred Ozioko of Bio-resources Diversity and Conservative Programme (BDCP), Nsukka, Enugu State of Nigeria.

Preparation of Plant Extract: The root barks were chopped into pieces, air-dried for two weeks and then pulverized to coarse powder form using a mechanical grinder. A known weight, 200 g of the powdered *Voacanga africana* root was soaked in 80% methanol and extracted by cold maceration for 48 hrs at room temperature. The extract was then concentrated *in vacuo* using a rotary evaporator (Büchi, Rota vapor R-200).

Solvent-Solvent Partitioning of the Extract: Ten grams (10 g) of the methanol (MeOH) extract was dissolved in 400 ml of 20% MeOH and the aqueous solution partitioned using *n*-hexane, ethyl acetate and *n*-butanol to obtain three solvent fractions *viz n*-hexane, (HF), ethyl acetate (EF) and *n*-butanol (BF), respectively.

Qualitative Phytochemical Analysis of the Extract and Fractions: Phytochemical analysis of the plant extracts was done to determine the presence of some bioactive compounds such as flavonoids, tannins and phenolics according to standard methods described by Harborne and Baxter (1993).

Total Phenolic Content (TPC) Determination of Methanol Extract of *V.africana* and its Fractions: Folin-Ciocalteau method was used for the determination of the total phenolic content of the extract and fractions of the plant using gallic acid as an internal standard. Exactly, 1 ml each of the extract and fractions (*n*-hexane, ethyl acetate and *n*-butanol) was mixed with 9 ml of distilled water in a 25 ml volumetric flask. A quantity, 2.5 ml of a 10-fold dilute Folin Ciocalteau phenol

reagent (FCPR, 1:10) was added. After 5 minutes, 10 ml of 7.5% Na_2CO_3 solution was added to the mixture and made up to desired volume with distilled water. The mixture was incubated in the dark at room temperature for 90 minutes. A set of standard solution of gallic acid (20, 40, 60, 80,100 µg/ml) were prepared in the same manner as described for the extract and its fractions. The absorbance of the extract and fractions and standard solutions were read against the reagent blank at 760 nm with a UV/Visible spectrophotometer (UV-1800, Shimadzu, Japan). The total phenolic content was determined from the calibration curve and expressed as milligram of gallic acid equivalent (GAE) per gram of the extract and its fractions. The determination of the total phenolic content was carried out in triplicate.

Determination of Total Flavonoid Content (TFC) of Methanol Extract of *V. africana* and its Fractions: Aluminium-chloride colourimetric assay was used to determine the total flavonoid content in the extract and fractions in the plant (Kostic *et al.,* 2013). A quantity, 1 ml of the extract (1 mg/ml) was mixed with 4 ml of distilled water in a 10 ml volumetric flask. A known volume, 0.30 Ml of 5% sodium nitrite was added to the flask. After 5 min, 0.30 ml of 10% $AlCl_3.6H_2O$ solution was added to the mixture, followed by addition of 2 ml of 1.0 M NaOH after another 5 min and diluted to the mark with distilled water. A set of standard solutions of quercetin (20, 40, 60, 80, 100 µg/ml) was prepared in the same manner as described for the extract and fractions. The absorbance of the extract, fractions and standard solutions were measured against the reagent blank at 510 nm with a UV/Visible spectrophotometer. The total flavonoid content was determined from the calibration curve as shown in the Appendix and expressed as milligram of quercetin equivalent (QE) per gram of extracts. The determinations of total flavonoid in the extracts and standards were carried out in triplicates.

Results and Discussion

Qualitative Phytochemical Composition of Extract of *V. africana* Root Bark and Its Fractions

The phytochemical constituents in the extract and fractions investigated are shown in Table 1. From the analysis for qualitative phytochemical constituents in the plant extract and fractions, ethyl acetate fraction exhibited the highest presence of flavonoids and tannins with moderate presence of phenol in ethylacetate as shown in Table 1. On the other hand, a relatively low concentration of phenol was observed in the extract and all fractions except ethylacetate fraction. In the same vein, phenol and tannin contents were found to be relatively low in n-butanol fraction. All other fractions showed a moderate presence of phytochemicals (tannins, phenols and flavonoids).

Table 3: Qualitative phytochemical constituents of the extract and fractions of *Voacanga africana* root bark

Phytochemicals	Extract and Fractions of *V. africana*			
	Extract	*n*-hexane	Ethyl Acetate	*n*-butanol
Flavonoids	++	++	+++	++
Phenols	+	+	++	+
Tannins	++	++	+++	+

Key:
+ = Present in low amount; ++ = Present in moderate amount
+++ = Present in high amount; ND = Not detected

Total Phenolic Content in Extract of *V. africana* Root Bark and Its Fractions

Across extract and fractions of *V. aficana* root bark, the total phenolic content in methanol extract exhibited a significantly ($p < 0.05$) lower level than the level observed for fractions of the plant (*n*-hexane, ethyl acetate and *n*-butanol). The *n*-hexane fraction exhibited the highest level of total phenolic content, followed by *n*-butanol and ethyl acetate fraction, i.e. *n*-hexane > *n*-butanol > ethyl acetate. At 50 to 200 ppm concentration, methanol extract exhibited a significantly ($p < 0.05$) lower level of total phenolic content when compared to all the fractions of the plant while *n*-hexane fraction showed the highest total phenolic content (TPC) followed by ethyl acetate and then *n*-butanol fraction at 50 ppm. At 250 and 300 ppm, there was non- significant ($p > 0.05$) difference of total phenolic content between *n*-hexane fraction and ethyl acetate fraction as well as between methanol fraction and *n*-butanol as shown in Table 2.

Total phenolic content of extract and fractions of *V. africana* compared at different concentrations (25, 50, 100, 200, 250, 300 ppm) exhibited significantly ($p < 0.05$) higher levels as concentrations increased progressively. At 25 ppm, showed a significantly ($p < 0.05$) lower level of total phenolic content than the levels observed at higher concentrations whereas the highest total phenolic content was observed at 300 ppm; however, at 25 ppm, total phenolic content compared with the content at 50 ppm was of non-significant ($p > 0.05$) effect as shown in Table 2.

Table 2: Total phenolic content of *V. africana* root bark

Concentration (ppm)	Methanol Extract	*n*-hexane Fraction	Ethyl acetate Fraction	*n*-butanol Fraction
25	5.633 ± 0.32^{a}	21.757 ± 1.05^{be}	7.213 ± 1.05^{ce}	17.517 ± 0.53^{de}
50	6.817 ± 1.74	29.640 ± 1.82^{af}	27.820 ± 4.82^{abf}	23.573 ± 1.39^{bf}
100	16.303 ± 0.53^{g}	55.393 ± 3.79^{ag}	58.730 ± 2.41^{ag}	28.730 ± 1.82^{cg}
200	43.273 ± 3.28^{ah}	105.393 ± 2.78^{bh}	103.880 ± 2.28^{bh}	38.427 ± 0.53^{ch}
250	52.667 ± 2.29^{a}	132.063 ± 3.67^{bi}	138.427 ± 18.86^{bi}	42.060 ± 0.52^{ai}
300	61.453 ± 1.57^{aj}	161.457 ± 1.82^{bj}	158.120 ± 6.45^{bj}	54.483 ± 2.29^{cj}

n=3. Results are expressed in mean ± SD. Mean values with different letters as superscripts across rows and columns are considered significant ($p < 0.05$) while mean values with the same letters as superscripts across rows and columns are considered non significant ($p > 0.05$).

Total Flavonoid Content of Extract of *V. africana* Root Bark and Its Fractions

Across the extract and fractions of *V. aficana* root bark, for 25 and 50 ppm, there was no significant ($p > 0.05$) difference of total flavonoid content between methanol extract and that of the fractions (*n*-hexane, ethyl acetate and *n*-butanol) whereas at 100 ppm, the total flavonoid content of methanol extract showed a significantly ($p < 0.05$) lower level when compared to that of the fractions of the plant (*n*-hexane, ethyl acetate and *n*-butanol). Significantly ($p < 0.05$) lower content of total flavonoid was observed in the ethyl acetate fraction than in *n*-hexane fraction and significantly ($p < 0.05$) lower content of total flavonoid of *n*-butanol fraction than that of *n*- hexane fraction. Ethyl acetate fraction showed significantly ($p < 0.05$) higher total flavonoid content when compared to that of *n*-butanol fraction. Ethyl acetate fraction had the highest total flavonoid content followed by *n*-hexane and *n*-butanol fractions. The least total flavonoid content was exhibited by methanol extract as shown in Table 3.

The total flavonoid contents of all extract and fractions of *V. africana* compared at different concentrations (25, 50, 100, 200, 250, 300 ppm) exhibited significantly ($p < 0.05$) higher levels as the concentrations increased progressively, i.e. concentration at 25 ppm exhibited a significantly ($p < 0.05$) lower level of total flavonoid content when compared to that of all fractions of the plant and the highest flavonoid content was observed at 300 ppm; however, the flavonoid content at 25 ppm showed non-significant ($p > 0.05$) difference compared to that obtained at 50 ppm as shown in Table 3.

Table 3: Total flavonoid content of *V. africana* root bark

Conc (ppm)	Methanol Extract	*n*-hexane Fraction	Ethyl acetate Fraction	*n*-butanol Fraction
25	$1.33.333 \pm 5.78^{ac}$	120.000 ± 20.00^{ae}	130.000 ± 20.00^{ae}	100.000 ± 20.000^{ae}
50	126.66 ± 11.55^{f}	170.000 ± 0.00^{af}	153.333 ± 15.28^{abe}	150.000 ± 10.00^{abf}
100	133.333 ± 5.78^{af}	256.667 ± 20.82^{bg}	210.000 ± 0.00^{cf}	173.333 ± 15.28^{dg}
200	156.667 ± 5.78^{ag}	313.333 ± 5.77^{bh}	456.667 ± 40.41^{cg}	176.667 ± 20.82^{afg}
250	160.000 ± 0.00^{ag}	436.667 ± 15.28^{bi}	523.333 ± 11.55^{ch}	240.000 ± 10.00^{dh}
300	180.000 ± 0.00^{ah}	473.333 ± 5.77^{bj}	573.333 ± 5.77^{ci}	276.667 ± 15.28^{di}

n=3. Results are expressed in mean ± SD. Mean values with different letters as superscripts across rows and columns are considered significant ($p < 0.05$) while mean values with the same letters as superscripts across rows and columns are considered non significant ($p > 0.05$).

According to World Health Organization (WHO) over 80% of the world's population depends on medicinal plants for their health care needs (Upadhyay *et al.,* 2011). Plant-derived medicines are relatively cheaper than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment (Abubakar, 2009). The medicinal value of the plants depends on their chemical constituents that provide a definite physiological action on the human body (Kumar *et al.,* 2009). These bioactive constituents include tannin, flavonoids, alkaloids, phenol compounds, saponin, e.t.c. (Adegoke *et al.,* 2010). The phenolic and flavonoid contents are widely distributed secondary metabolites in plants having anti-oxidatiants activity and wide range of biological activities such as anti-apoptosis, anti-aging, anti-carcinogenic, anti-inflammatory, anti-atherosclerotic, cardiovascular protection and improvement of endothelial function as well as inhibition of angiogenesis and cell proliferation activities (Rice-Evans *et al.,* 1996). Recent studies have shown that many dietary polyphenolic constituents derived from plants are more effective antioxidants *in-vitro* than vitamin E or C, and thus might contribute significantly to the protective effects *in vivo* (Rice-Evans *et al.,* 1996). Many of these plants are readily available in rural areas, thus making traditional system of medicine relatively cheaper than orthodox medicine therefore this study estimated the total phenolics, total flavonoids and *in vitro* antioxidant activities of methanol extract of *Voacanga africana* root bark and its fractions.

The extract and various fractions (methanol extract-ME, *n*-hexane fractions- HF, ethyl acetate fractions- EF, and *n*-butanol fraction-BF) of *V. Africana* root bark were tested for different phytoconstituents like taninins, phenolic compounds and flavonoids. Ethyl acetate fraction showed the highest content for tannins, phenols and flavonoids. This could be as a result of the polarity of ethyl acetate and its ability to dissolve these phytochemicals as earlier reported in the works of Kumara *et al*. (2011) in which the highest content of phtoconstituents were observd in ethyl acetate fraction.

The total phenolic and flavonoid content of methanol extract – (ME), *n*-hexane fraction – (HF), ethyl acetate fraction – (EF), and *n*-butanol fraction – (BF) of *V. africana* root bark were estimated using standard gallic acid equivalent of phenols and quercetin standard for flavonoids. The *n*-hexane and ethyl acetate fraction were found to have maximum phenolic and flavonoid components simultaneously as a non-significant ($p > 0.05$) difference of total phenolic and total flavonoid content was observed between *n*-hexane fraction and ethyl acetate fraction of the plant. . This may be one of the reasons for their possession of maximum antioxidant activities than other two extracts. This is in agreement with the report of Sim *et al.* (2010) in which high potential of phenolic and flavonoid activity was observed in ethyl acetate and *n*-hexane fraction.

Conclusion

The results of this study showed that *n*-hexane fraction of *V. Africana* root bark exhibited the highest total phenolic and total flavonoid contents at all concentrations (ppm), followed by ethyl acetate. It could be deduced that *V. africana* root bark possesses phenolic and flavonoid contents depending on the type of extract or fraction, thus justifying its use in the treatment of diseases such as cardiovascular diseases, cancer, inflammation and allergy and other oxidative stress related diseases.

Conflict of Interest

There is no conflict of interest regarding the manuscript.

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