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EVALUATION OF PHYTOCHEMICAL COMPOSITION OF ETHANOLIC EXTRACTS OF *TECOMA STANS, GLYPHAEA BREVIS, GARCINIA KOLA, ZANTHOXYLUM MACROPHYLLA* AND *GONGRONEMA LATIFOLIUM* PLANT ROOT BARK.

Chinaka N. C.*[1], Monago-Ighorodje C. C.[1] and Chuku L. C.[1]

[1]Department of Biochemistry, Faculty of Science, University of Port Harcourt, P.M.B. 5323, Choba, Port Harcourt, Rivers State, Nigeria.

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***Corresponding Author**
Chinaka N. C.
Department of Biochemistry, Faculty of Science, University of Port Harcourt, P.M.B. 5323, Choba, Port Harcourt, Rivers State, Nigeria.

ABSTRACT

The plants *Tecoma stans*, *Glyphaea brevis*, *Garcinia kola*, *Zanthoxylum macrophylla* and *Gongronema latifolium* are acclaimed to possess numerous medicinal benefit, ranging from the treatment of burns, rheumatism, headache, stomach ache, toothache, cough and catarrh. This study aimed at investigating the phytochemical (qualitative and quantitative) potentials of these plant root barks in ethanolic extraction. The extracts were analysed for bioactive secondary metabolites using standard qualitative and quantitative spectrophotometric method. Results of qualitative analysis carried out on each plant root extract, showed the presence of important phytochemical constituents, with phenolics (tannins and flavonoids), terpenoids and alkaloids present at relatively high amount in the plant samples. The results also revealed that the total phenolic content of *Tecoma stans*, *Glyphaea brevis*, *Garcinia kola*, *Zanthoxylum macrophylla* and *Gongronema latifolium* were 0.498±0.00mg, 0.10±0.03mg, 0.47±0.02mg, 0.139±0.027mg and 0.10±0.02mg gallic acid equivalent (GAE)/mg dry weight of extract respectively. The total flavonoid content were 3.747±1.16mg, 1.65±0.20mg, 48.30±23.83mg, 3.641±0.573mg and 0.47±0.37mg rutin equivalent (RE)/mg dry weight extract respectively. Total anthocyanin content of the plants were 1985.16±18.36mg, 619.86±42.32mg, 277.86±11.35mg, 368.71±10.96mg and 323.29±22.29mg quercetin equivalent (QE)/mg dry weight extract respectively. The results therefore suggest that the extracts possess a very good phytochemical potentials and health benefit as acclaimed.

KEYWORDS: Phytochemicals, qualitative, quantitative, equivalents, extracts.

INTRODUCTION

Medicinal plants have well over the years been used as remedies for managing, treating, healing and as well for curing human diseases as a result of their rich phytochemical constituents (Nostro *et al*., 2000). Phytochemicals are known to be bioactive chemical compounds naturally occurring in plants (Hasler *et al*., 1999). They are referred to as primary and secondary compounds with chlorophyll, proteins and common sugars constituting the primary compounds while terpenoid, alkaloids and phenolic compounds constitute the secondary (Krishnaiah *et al*., 2007). According to Mahato *et al*., (1997), terpenoids are reported to exhibit various important pharmacological activities i.e., anti-inflammatory, anti-cancer, anti-malaria, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities. Alkaloids are also claimed to be used as anaesthetic agents which are present in medicinal plants.

MATERIALS AND METHODS

Chemicals

Chemicals used in the course of this study were of analytical grade. Solvents such as hexane, ethanol and ethyl acetate were purchased from EMD Biosciences (Gibbstown, NJ, USA). Sulphuric acid, sodium nitrite, potassium hydroxide (KOH), potassium dihydrogen phosphate (KH_2PO_4), acetic acid, hydrogen peroxide (H_2O_2), ferrous sulphate ($FeSO_4.7H_2O$), ferric chloride ($FeCl^{3+}$), sodium carbonate (Na_2CO_3), perchloric acid ($HClO_4$), polyvinylpolypyrrolidone, thiobarbituric acid (TBA), trichloroacetic acid (TCA) and Folin-Ciocalteu's reagent (FCR) were all purchased from Sigma Chemical Co. (St. Louis, MO).

Plants root barks were obtained from Nsukka, Enugu State and Isuofia Anambra State, both located in Eastern Nigeria.

Equipments

UV-Spectrophotometer (Jenway 6305), weighing balance (Ohaus), water bath (Genlab Ltd) and Refrigerator (Hawsley, England).

Methods

Crude extract preparation of plant root barks

Root barks of the respective plants were air-dried at room temperature and milled to fine powder. The resultant fine powder was subjected to extraction with absolute ethanol. The ethanol extract was concentrated using a rotary evaporator and stored for use at 4℃ (Gülçin, 2005; Elmastas *et al*., 2006).

Qualitative phytochemical analysis

Test for tannins, saponins, alkaloids, flavonoids, carbohydrates, resins, glycosides and terpenoids were carried out according to standard methods described by Harborne, (1973); Trease and Evans, (1989).

Quantitative phytochemical analysis

Determination of total phenolic content

Total phenolic content were determined using Folin-Ciocalteu method as described by Velioglu *et al*., (1998), with few modifications. FCR a yellowish acidic solution contains complex polymeric ions produced from phosphomolybdic and phosphotungstic heteropoly acids. Dissociation of a phenolic proton in a basic medium leads to a phenolate anion, which reduces the formation of a blue colored molybdenum oxide by FCR whose color intensity is directly proportional to the phenolic content. The standard used for plotting the calibration curve was gallic acid. Hence the total phenolic content was expressed im mg gallic acid equivalents (GAE) per mg dry weight (DW).

Determination of tannin content

The tannin content of the plant root barks were determined by using insoluble polyvinylpolypirrolidone (PVPP) which binds tannins as described by Markkar *et al*., (1993). The tannin content is obtained via calculations of the difference between total and non bound-tannin phenolic content.

Determination of flavonoids and flavonols

Determination of the flavonoid content of the plant root barks were carried out according to the method described by Kumaran and Karunakaran, (2006), with few modifications. The bases for this method is the formation of flavonoid-aluminium complex, which absorbs maximally at 415nm. Quercetin was used as a standard for calculating the amount of

flavonoids present. The values for flavonoid content were expressed in mg quercetin equivalent (QE) per mg DW. The formula for calculation is:

$$\text{Flavonoid content} = \frac{A \times m_o}{A_o \times m}$$

where A is the absorption of plant extract solution, A_o is the absorption of standard quercetin solution, m is the weight of plant extract, mg and m_o is the weight of quercetin in the solution (mg).

Determination of total anthocyanin content

Determination of the monomeric anthocyanin content of the plant extracts were ascertained using spectrophotomeric pH differential protocol as described by Giusti and Wrolstad (2001) and Wolfe *et al*., (2003), with a few modifications. The monomeric anthocyanin undergo a reversible structural transformation which brings about a change in pH that manifests different absorbance spectra. The anthocyanin content was calculated as thus:

$$\text{Total monomeric anthocyanin (mg/100g of DW)} = \frac{(A \times MW \times DF \times 1000)}{(\varepsilon \times c)}$$

where A is absorbance = $(A_{515} - A_{700})$ pH 1.0 – $(A_{515} - A_{700})$ pH 4.5; MW is the molecular weight for cyaniding 3-glucoside = 449.2; ε is the molar absorptivity of cyaniding 3-glucoside = 26900; and C is the concentration of the buffer in mg/ml. The value obtained is expressed as mg of cyaniding 3-glucoside equivalents per 100 g of dried plant extracts.

RESULTS

Qualitative analysis on the Phytochemical constituents

Qualitative analysis carried out on each plant root extract as presented in Table 1, showed the presence of important phytochemical constituents. Phenolic (tannins and flavonoids), terpenoids and alkaloids were the major phytochemical constituents present at relatively high amount in the plant samples.

Table 1: Qualitative Phytochemical constituents in the plants

Phytochemical constituent	Relative amount				
	T. stans	***G. brevis***	***G. kola***	***Z. macrophylla***	***G. latifolium***
Flavonoids	+	+	+	++	+
Tannins	+++	+	+++	-	-
Alkaloids	++	++	+++	+++	++
Steroids	+	+	+	+	+
Glycosides	-	-	+	+	+++

Saponins	++	+	+++	+	+
Resins	+	+	+	-	-
Proteins	+	+	++	+	+++
Carbohydrates	+	+	+	+	++

+ = Present in trace amount

++ = Present in moderate amount

+++ = Present in high amount

- = Absent

Quantitative analysis on Phytochemical constituents (Spectrophotometric method)

Phenolic compounds were the major class of bioactive constituents in the extracts. The amount of total phenolics was 0.498 ± 0.00, 0.10 ± 0.03, 0.47 ± 0.02, 0.139 ± 0.027 and 0.10 ± 0.02 mg gallic acid equivalent (GAE)/mg of dry plant extract for *Tecoma stans*, *Glyphaea brevis*, *Garcinia kola*, *Zanthoxylum macrophylla*, and *Gongronema latifolium* respectively (Table 2). *G. kola* had the highest flavonoid and anthocyanin contents (48.30 ± 23.83 and 1985.16 ± 18.36 mg quercetin equivalents / mg dry weight plant extract respectively) as presented in Table 2.

Table 2: Quantitative analysis on Phytochemical constituents (Spectrophotometric method)

	Concentrations				
	T. stans	***G. brevis***	***G. kola***	***Z. macrophylls***	***G. latifolium***
Total Phenol*	0.498±0.00	0.10±0.03	0.47±0.02	0.139±0.027	0.10±0.02
Total Flavonol‡	25.19±4.76	47.06±2.22	22.29±0.24	94.34±9.323	25.55±1.01
Total Flavonoid‡	3.747±1.16	1.65±0.20	48.30±23.83	3.641±0.573	0.47±0.37
Total Anthocyanins#	1985.16± 18.36	619.86± 42.32	277.86± 11.35	368.71± 10.96	323.29± 22.29

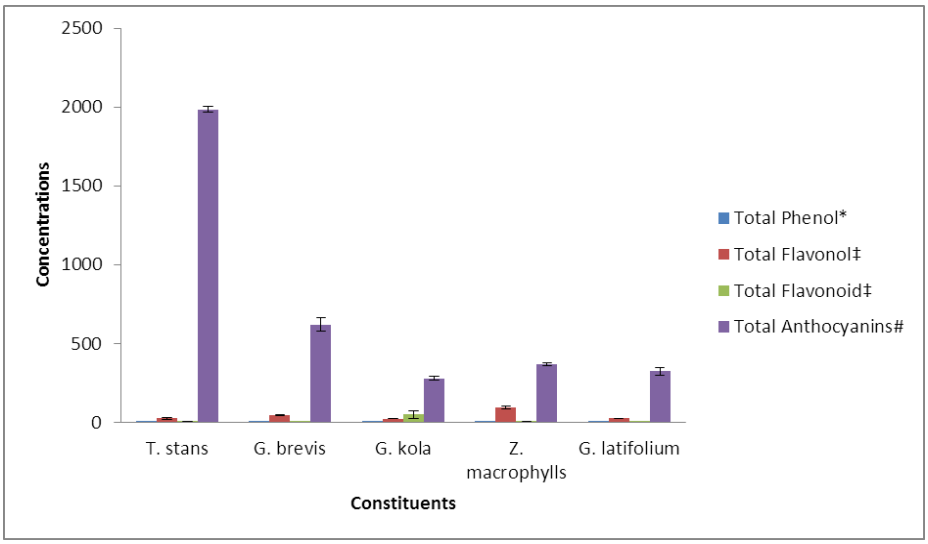
Data represented as Mean ± SEM (n=3)

* Expressed as mg gallic equivalents (GAE)/mg dry weight plant extract

‡ Expressed as mg quarcetin equivalents (QE)/mg dry weight plant extract

Expressed as mg of cyaniding 3-glucoside equivalents per 100g of dried plant extract

APPENDIX



* Expressed as mg gallic equivalents (GAE)/mg dry weight plant extract

‡ Expressed as mg quarcetin equivalents (QE)/mg dry weight plant extract

Expressed as mg of cyaniding 3-glucoside equivalents per 100g of dried plant extract.

DISCUSSION

Phytochemicals are naturally occurring and biologically active plant compounds that have potential disease inhibiting capabilities. Phytochemicals are chemicals derived from plants, these plant produce secondary metabolites in order to prevent themselves from insect attack and plant disease which in addition provides protective role for humans when consumed as it occurs in cases of oxidative stress caused by free radicals.

Qualitative analysis carried out on the plants revealed the presence of important phytochemical constituents as summarized in table 1. Phenolics (tannins and flavonoids), terpenoids and alkaloids were the major phytochemical constituents present in all the plants at significantly ($p<0.05$) high amount. The presence of alkaloids showed that the plants could be used anti-malaria drug because of quinine presence in alkaloid. The phytochemical have a lot of pharmacological properties as proved by earlier studies and the presence of tannins (saponins) could attribute the use of some plants in cosmetics industries for detergent production (Sies, 2007).

Flavonoids and flavonols are the major class of bioactive components present in the extracts. The concentration of total phenolics varied from 0.498±0.00 to 0.10±0.02 mg gallic acid equivalent (GAE)/mg of dry plant extract of the plants (table 2). *Garcinia kola* had the highest flavonoid content (48.30±23.83mg) quercetin equivalents/mg dry weight plant extract

when compared with other plant extracts studied in this work (table 2). These secondary metabolites in the plants have reported antiviral, anti-tumor, anti-inflammatory, anti-allergic, pile, dysentery and antioxidant activities (Awah *et al*., 2012).

CONCLUSION

The results however suggest that the presence of bioactive secondary metabolites like polyphenolics and flavonoids in significant amount could make them serve as good proton donors in inhibiting free radical generation during the pathogenesis of diabetes and other oxidative stress linked diseases.

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