TOTAL ANTIOXIDANT CAPACITY, PHENOLIC AND FLAVONOIDS CONTENTS OF PARTIALLY PURIFIED AQUEOUS EXTRACT OF *Vitex doniana* LEAVES

Supported by tetfund TERTIARY EDUCATION TRUST FUND

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Abstract: In this study, the total antioxidant capacity (TAC), total polyphenolic content (TPC) and total flavonoids content (TFC) of aqueous extract fractions of *Vitexdoniana* leaves were determined. The results obtained from the study revealed that fraction 1 possessed the highest 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (282 µg/ml TE). The decrease observed in the TAC of fractions is in the order; 1>3>2>4>5 and 6. Fractions 5 and 6 have the same TAC (76 µg/ml TE) being the fractions with the lowest TAC. Fraction 2 has the highest concentration of total flavonoids content (TFC) followed by fraction 1 and the lowest TFC was observed in fraction 5. Correlation between total antioxidant capacity and total flavonoids content of different fractions obtained from aqueous extract of *V. doniana* leaves showed moderate positive correlation (R^2 = 0.6549). similarly, total antioxidant capacity and total phenolic content of fractions showed positive correlation (R^2 = 0.6423) and correlation between total phenolic and total flavonoids contents of different fractions obtained from ethanol extract of *V. doniana* leaves showed the strong positive correlation (R^2 = 0.981). From the results, it can be deduced that the antioxidant activity of the fractions to a larger extent depends on the phenolic content, especially the flavonoids.

Keywords: Total antioxidant, polyphenols, flavonoids, *Vitexdoniana*.

Introduction

Natural antioxidants from plant sources are potent and safe due to their harmless nature. A free radical in each molecule is determined as an unpaired electron that occupies an atomic or molecular orbital on its own. This reactive molecule is to another electron to pair, this in step an uncontrolled chain reaction that can damage the natural function of the living cell, resulting in different diseases (Zhishen *et al.*, 1999). Many fruits and vegetables, herbs, cereals, seeds that contain natural antioxidants can abstract the lone electron from free-radical molecules and help humans to keep control on these harmful species.

Most of these antioxidants in plants are highly colouredanthocyanines, proanthocyaninidins, flavans, flavonoids, and their glycosides, carotenoids, like β-carotene and lycopene (Matkowski *et al*., 2009). Isolation of anti- oxidants from plants depends on the polarity of these compounds. First distribution of antioxidants between a polar (aqueous, hydro ethanol) and a semi-polar solvent (n-butanol, ethyl acetate) can be used to determine the distribution factor of the compounds between phases (Matkowski *et al*., 2009). *Vitexdoniana* sweet, (family Verbanaceae) is a peren- nial shrub widely distributed in tropical West Africa, and some East African countries including Uganda, Kenya and Tanzania and high rainfall areas. It is found in the middle belt of Nigeria particularly Kogi, Benue, and parts of the savannah regions of Kaduna, Sokoto and Kano States (Etta, 1984). It is variously called vitex (English), dinya (Hausa), dinchi (Gbagyi), uchakoro (Igbo), oriri (Yoruba) ejiji (Igala) and olih (Etsako) (Burkill, 2000). V. doniana is employed in the treatment of a variety of diseases. Hot aqueous extracts of the leaves are used in the treatment of stomach and rheumatic pains, inflammatory disorders, diarrhoea dysentery and diabetes (Irvine, 1961; Etta, 1984). Yakubu *et al.* (2012; 2013) reported the antidiabetic properties of the leaves. The roots and leaves are used for nausea, colic and epilepsy (Bouquet *et al.*, 1971; Iwu, 1993). In North-Central and eastern parts of Nigeria, the young leaves are used as vegetables or sauces and porridge for meals, especially for diabetic patients.

Materials and Methods

Collection and preparation of plant materials

Fresh leaves of *V. doniana* were collected from its natural habitat in Ankpa, Kogi State. It was identified and authenticated by the Ethno- botanist in the Department of Medicinal Plant Research and Traditional Medicine of the National Institute for Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria. A voucher specimen number NIPRD/H/6415 was deposited at the herbarium of the department. The plant material was dried in the laboratory at room temperature and pulverized using laboratory mortar and pestle.

Aqueous extraction

About 400 g of the pulverized sample was soaked in 2 L of distilled water (1:5 W/V) and was allowed to stand for 24 h at room temperature according to the study of Iwueke and Nwodo (2008). The extract was filtered and the filtrate was concentrated using rotary evaporator under reduced pressure. It was allowed to dry at room temperature and stored in refrigerator at 4^{o}C prior to usage.

Fractionation

The ethanol extract was subjected to column chromatograph to separate the extract into its component fractions. Silica gel was used in packing the column while varying solvent combinations of increasing polarity were used as the mobile phase.

Packing of column

This was done according to the method of Yakubu *et al.* (2014). The lower part of the glass column was stocked with glass wool with the aid of glass rod. 75 g of silica gel (G60-200 mesh size) was dissolved in 180 ml of absolute chloroform to make the slurry. The chromatographic column (30 mm diameter by 40 mm height) was packed with silica gel and was allowed free flow of the solvent into a conical flask. The set up was seen to be in order

when the solvent drained freely without carrying either the silica gel or glass wool into the tap. At the end of the packing process, the tap was locked and the column was allowed 24 h to stabilize after which, the clear solvent at the top of the silica gel was allowed to drain down the silica gel meniscus

Elution

The method of Yakubu*et al.*, (2014) was adopted for the elution. The extract (2 g) was dissolved in 2 ml absolute methanol and the solution was applied unto a chromatographic column (30 mm diameter by 400 mm height). Elution of the extract was done with solvent system of gradually increasing polarity, beginning from chloroform, ethyl acetate, methanol and finally water. The following ratios of solvent combinations were sequentially used in the elution process: Chloroform:ethyl acetate 100:0, 50:50; ethyl acetate:methanol 100:00, 50:50; methanol:water 100:00 and 50:50. A measured volume (400 ml) of each solvent combination was poured into the column each time using separator funnel. The eluted fractions were collected in aliquots of 400 ml in fraction collection bottles.

Total antioxidant capacity

The scavenging action of the plant extracts and the resulting fractions from ethanol extract on 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined colorimetrically at 517 nm using Trolox as standard according to the method described by Singleton *et al.* (2002). About 1.0 ml trisHCl buffer was added to test tube containing 1.0 ml absolute ethanol, 2.0 ml DPPH (0.1 mM) solution was added and the solution was thoroughly mixed. The absorbance was measured within 30 s after addition of sample at 517 nm. The absorbance was measured in triplicate for each extract/fraction. Total antioxidant capacity (TAC) was calculated as mg/ml of trolox equivalent (TE) using the regression equation from calibration curve.

Total phenolic content

Total polyphenol content (TPC) was estimated colorimetrically at 765 nm as described by Lachman *et al.* (2000), using Follin-Ciocalteu reagent and expressed as gallic acid equivalent (GAE). Exactly 0.25 ml sample was added to test tube containing 2.50 ml Follin reagent. Sodium carbonate solution (2.0 ml) was added and was allowed to stand for 15-20 min at room temperature. The reactions were conducted in triplicates and absorbance of the sample was measured against the reagent blank.

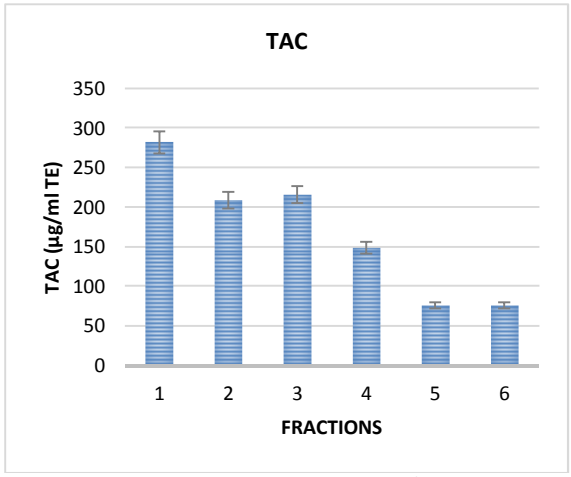
Total flavonoids content

Flavonoids were determined using the aluminum chloride colorimetric method of Chang *et al.* (2002). Quercetin standard was used for derivation of the calibration curve. Exactly 0.5 ml of the diluted sample was added into test tube containing 1.5 ml methanol. 0.1 ml of 10% aluminum chloride ($AlCl_3$) solution and 0.1 ml potassium acetate (CH_3COOK) were added. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm and the concentration of flavonoids in the sample was estimated from the calibration curve. The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. Total flavonoids were expressed as mg/ml quercetin equivalent (QE).

Results and Discussion

Total antioxidant capacity (TAC)

Fig. 1 shows total antioxidant capacity (TAC) of fractions obtained from aqueous extract of *Vitex doniana* leaves. Fraction 1 has the highest antioxidant capacity (282 µg/ml TE). The decrease observed in the TAC of fractions is in the order; 1>3>2>4>5 and 6. Fractions 5 and 6 have the same TAC (76 µg/ml TE) being the fractions with the lowest TAC.

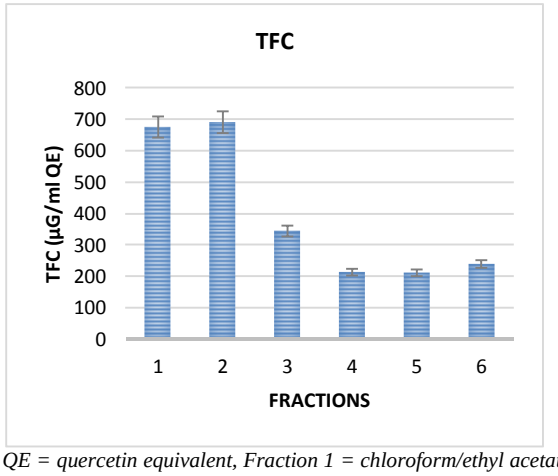


TE = Trolox equivalent, Fraction 1 = chloroform/ethyl acetate (100:0), 2 = chloroform/ethyl acetate (50:50), 3 = ethyl acetate/methanol (100:0), ethyl acetate/methanol (50:50), 4 = methanol/water (100:0), methanol/water (50:50).

Fig. 1: Total antioxidant capacity (TAC) of fractions obtained from aqueous extract of *Vitex doniana* leaves

Total flavonoids content (TFC)

Unlike the TAC in figure1, fraction 2 has the highest concentration of total flavonoids content (TFC) followed by fraction 1. The lowest TFC was observed in fraction 5 (Fig. 2).

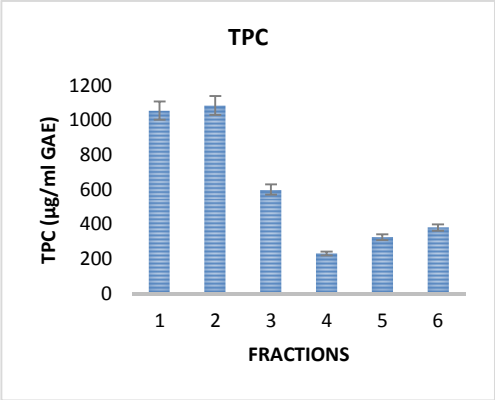


QE = quercetin equivalent, Fraction 1 = chloroform/ethyl acetate (100:0), 2 = chloroform/ethyl acetate (50:50), 3 = ethyl acetate/methanol (100:0), ethyl acetate/methanol (50:50), 4 = methanol/water (100:0), methanol/water (50:50).

Fig. 2: Total flavonoids content (TFC) of fractions obtained from aqueous extract of *Vitex doniana* leaves

Total phenolic content (TPC)

Like TFC, similar pattern of was observed in TPC of fractions obtained from aqueous extract of *Vitex doniana* leaves (Fig. 3).

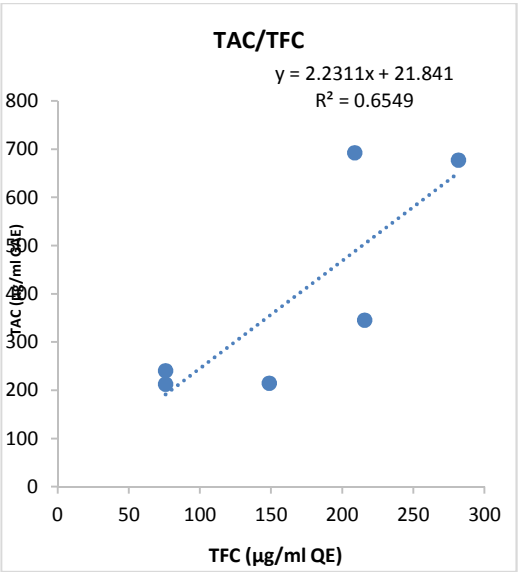


GAE = gallic acid equivalent, Fraction 1 = chloroform/ethyl acetate (100:0), 2 = chloroform/ethyl acetate (50:50), 3 = ethyl acetate/methanol (100:0), ethyl acetate/methanol (50:50), 4 = methanol/water (100:0), methanol/water (50:50).

Fig. 3: Total phenolic content (TPC) of fractions obtained from aqueous extract of *Vitex doniana* leaves

Correlation between total antioxidant capacity and total flavonoids content

Correlation between total antioxidant capacity and total flavonoids content of different fractions obtained from aqueous extract of *V. doniana* leaves showed moderate positive correlation (R^2 = 0.6549) (Fig. 4).

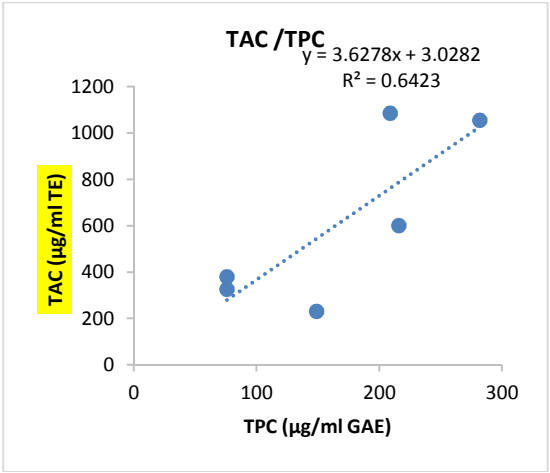


TAC = Total Antioxidant Capacity, TFC = Total Flavonoids Content, GAE = Gallic acid equivalent, QE = Quarcetin equivalent

Fig. 4: Linear correlation between total antioxidant capacity and total flavonoids content of fractions obtained from aqueous extract of *Vitexdoniana* leaves

Linear correlation between total antioxidant capacity and total phenolic content

Linear correlation between total antioxidant capacity and total phenolic content of fractions obtained from aqueous extract of *V. doniana* leaves showed positive correlation (R2 = 0.6423) (Fig. 5).

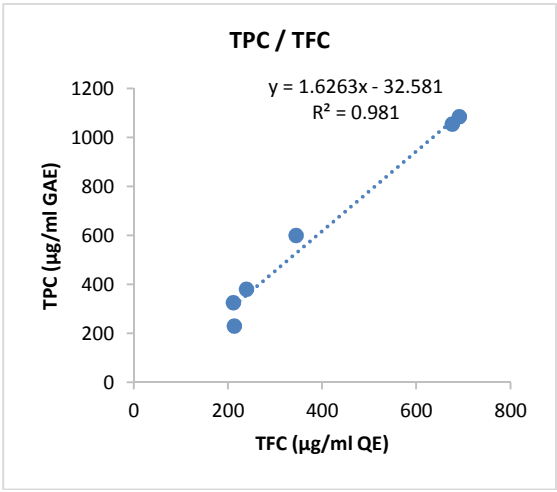


TAC = Total Antioxidant Capacity, TPC = Total Phenolic Content, GAE = Gallic acid equivalent, , TE = Trolox equivalent,

Fig. 5: Linear correlation between total antioxidant capacity and total phenolic content of fractions obtained from aqueous extract of *Vitexdoniana* leaves

Correlation between total phenolic and total flavonoids contents

Correlation between total phenolic and total flavonoids contents of different fractions obtained from ethanol extract of *V. doniana* leaves showed the strongest positive correlation (R^2 = 0.981) among all the correlated data (Fig. 6).



TFC = Total Flavonoids Content, TPC = Total Phenolic Content, GAE = Gallic acid equivalent, QE = Quarcetin equivalent,

Fig. 6: Linear correlation between total phenolic content and total flavonoids content of fractions obtained from aqueous extract of *Vitex doniana* leaves

The results from the study revealed that fractions 1, 2 and 3 possessed higher antioxidant capacity, which is in proportion to the concentration of phenolics present, especially flavonoids. In our study, flavonoids content were correlated with antioxidant activity in the DPPH. It is known that flavonoids have the strongest radical-scavenging power among all natural phenolic compounds (Wojdyło *et al.*, 2007). Moreover, it is a potent antioxidant against lipid peroxidation in mitochondrion and microsome (Wang *et al.*, 2010). Moore and Adler (2001)

reported that apolar solvents are among the most employed solvents for removing polyphenols from water. Several studies have reported on the relationships between phenolic content and antioxidant (Moure *et al*., 2001; Anjaneyulu and Chopra, 2004).

In our study, there was moderate positive relationship (R^2 = 0.6549) between antioxidant activity and total flavonoid contents and (R^2 = 0.6423) for total phenolic content of the fractions. It could be deduced however that the antioxidant capacity of the fractions is majorly dependent on its flavonoids content although there is a wide grade of variation between different phenolic compounds in their effectiveness as antioxidants (Robards *et al.*, 1999; Bjelakovic *et al.*, 2007). Furthermore, correlation between the phenolic content and that of flavonoids was stronger, indicating that flavonoid constitutes about 80% of the phenolic composition of the fractions.

Hence, concentration and pH can also play role in the antioxidant activity of phenolics (Bouayed *et al*., 2011). In addition, the chemical structure of phenolics play a role in the free radical scavenging activity, mainly depending on the number and position of hydrogen donating hydroxyl groups on the aromatic rings of the phenolic molecules (Bouayed *et al.*, 2011). The temperature during drying and extraction, affects the compound stability due to chemical and enzymatic degradation, casualties by volatilization or thermal analysis, these latter have been suggested to be the main mechanism causing the reduction in polyphenol content (Moure *et al.*, 2001). Also, for synthetic antioxidants, evaporation and analysis were the main mechanisms for the loss of activity.

In conclusion, the results of the present study showed that *V. doniana* leaves are rich in flavonoids and phenolic constituents of which flavonoids are the dominant phenolics and are largely responsible for the good antioxidant activity of the fractions and fractions 1 and 2 are potential fractions for further studies.

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