PHYTOCHEMICAL SCREENING, EVALUATION OF *IN-VITRO* ANTIOXIDANT ACTIVITIES AND ACUTE TOXICITY EFFECT OF ORGANIC EXTRACTS OF *DORSTENIA MANNII* (MORACEAE).

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ABSTRACT

Background: Many oxidative stress related diseases are as a result of accumulation of free radicals in the body. A lot of researches are going on worldwide directed towards finding natural antioxidants of plants origins. The aims of this study were to screen for phytochemical constituents, evaluate their *in vitro* antioxidant activities and acute toxicity effect of the crude organic extracts of *D. mannii*.

Methods: The phytochemical screening was done using the method of Trease and Evans. 1989, The flavonoid, proanthocyanidin, tannins and phenolic contents of the extracts were also determined using standard phytochemical reaction methods. Antioxidant potential of crude organic extract of *D. mannii* was assessed using tests involving inhibition of, DPPH, NO and metal chelating. The acute toxicity study was performed according to the OECD, 2004 protocol.

Results: Phytochemical screening revealed the presence of alkaloids, saponines, tannins, and flavonoids in all plant extracts. Glycosides and phlobatannins were also present in all plant extracts except, ethanolic Leaves (EL). Twig extracts had the highest polyphenol content (ET= 815.51 ± 0.57 mg EqCat vs HT= 727.27± 0.36 mg EqCat; P< 0.05), compared to leaves (P< 0.05), thus retaining twigs as the best plant part. The antioxidant activity measurement revealed that, both twig extracts had a comparable scavenging activities of DPPH free radical; IC_{50} (ET= 5.58 mg/ml vs HT= 6.82 mg/ml) and NO; IC_{50} (ET= 0.08 mg/ml vs HT= 0.10 mg/ml) respectively, with ET being the most active compared to HT respectively. Both extracts were also observed to significantly chelate metals best at concentrations 0,5 mg/ml and 0,75 mg/ml, for HT and ET respectively, with IC_{50} (HT= 1.03 x 10^{-10} mg/ml vs ET= 5.04 x 10^{-10} mg/ml; P< 0.05), with HT being the most active compared to ET. For the acute toxicity studies, no death of rats was neither recorded in the control nor in the treated groups indicating that the $LD_{50} > 5000$ mg/kg BW. The animals exhibited no changes in general behaviour or physiopatological activities. Although there was a change in the weights of rats in the treated groups, this was significantly reduced in the HT group and statistically not significant in the ET group though higher compare to the control (P< 0.05). Results of blood parameters indicated a significant decrease in AST and ALT levels between the negative control and the treated groups (P< 0.05), with HT lowering AST more than ET.

Conclusions: Our findings provide evidence that the crude extract of *D. mannii* is a potential source of natural antioxidants, and this justified its uses in folkloric medicines.

Key words: Phytochemical, antioxidant, acute toxicity, *Dorstenia mannii*,

INTRODUCTION

The term 'antioxidant' refers to the activity of numerous vitamins, minerals and other phytochemicals to protect against the damage caused by reactive oxygen species (ROS). By their ability to react with and damage many structures in the body, ROS are involved in various related physiological processes and diseases such as aging, cancer and atherosclerosis. (Khlifi *et al.*, 2006)

Several studies have demonstrated that plants produce potent antioxidants and represent an important source of natural antioxidants. (Es-Saffi et al, 2005) Among these, the members of the Genus *Dorstenia* contain approximately 458 species. Some of these plants have been proven to be used as anti-snakebite, anti-infection and anti-rheumatic, anti diabetic, anti malaria, anticholesterol, antioxidant (Kwete *et al.*, 2007*)* remedies in the medicinal plant

therapy of many countries in Africa, Central and South America (Abegaz *et al*., 2002)

.Scientific research work already done on this genus include, Geranylated and prenylated flavonoids from the twigs of Dorstenia mannii (Ngadjui *et al.,* 1998), antioxidant activity of prenylated flavonoids from the West African medicinal plant *Dorstenia mannii* (Dufall *et al.,* 2003), and the *in vitro* evaluation of the antibacterial properties of the crude extracts of the leaves of *Dorstenia mannii* (Moraceae) (Nelly, 2009). Although widely used for the above mentioned biological activities, no phytochemical screening, *in vitro* antioxidant activities and acute toxicity study of the crude organic extracts of *D. mannii*. have been reported. The aims of this study were to screen for phytochemical constituents, evaluate their *in vitro* antioxidant activities and acute toxicity effect of the crude organic extracts of *D. mannii*.

MATERIALS AND METHODS

Plant material: *Dorstenia mannii Hook.f. var mannii* was harvested in the South West Region of Cameroon, specifically at 18km West of Bota (West of limbe), between Batoke and Bakingili on the 19th to 20th June 2010. The plant was identified and compared with the voucher specimens conserved at the National Herbarium in Yaoundé, Centre Region under the reference number 25395/ SRF / Cam.

Preparation of crude extract: The plant material was washed with water to remove all unwanted plant materials and sand, separated into leaves and twigs. The twigs were sliced and both parts were air-dried in the shade, at room temperature and separately grind into powder. 125 g of each powder was macerated for 48 hours at room temperature with 1L ethanol (95%, v/v) to obtain ethanolic extract and 1L mixture of water-ethanol (1:1v/v) to obtain hydroethanolic extract. The solvent extract were filtered using a funnel and whatman N°.1 filter paper, and the filtrate was concentrated by evaporation through air-drying in the drying room at (31°C).

Phytochemical screening of the plant extract
A small portion of each dry extract was used for the phytochemical tests for compounds which include tannins, flavonoids, alkaloids, saponins, and steroids in accordance with the methods of Trease and Evans. (1989) with little modifications.

Polyphenols were tested by dissolving 250 mg of each extract in 4ml distilled water and heated for 15 minutes, then allowed to cool, followed by the addition of 2 drops of freshly prepared ferric cyanate solution (1 ml $FeCl_3$ 1% and 1 ml $K_3Fe(CN)_6$.) The formation of a greenish-blue

coloration indicated the presence of polyphenols. Alkaloides were detected using the test of Meyer where by 100 mg of the plant extract was heated in 2 ml of H_2SO_4 2% for 2 minutes after which the mixture was filtered. Few drops of the Meyer reagent (obtained by corrosive sublimation ($HgCl_2$), water and potassium iodide) were added in 1ml of the filtrate and the formation of a white precipitate or turbid solution was indicative of alkaloides. Saponins were tested using the foam production test whereby 250 mg of the extract was added in 5 ml of distilled water. After homogenisation the mixture was vigorously heated to boil, the appearance of foam that persisted even after 20 minutes was indicative of the presence of saponines. Exactly 5 g of extracts was macerated in 10 ml of distilled water and then filtered (using Whatman No 1. Filter paper). A blue-black or greenish-dark coloration resulted from the addition of Few drops of 3% ferric chloride reagent to the filtrate indicated the presence of tannins. Meanwhile exactly 0.5 g of the extract was macerated in 10 ml of distilled water and then filtered. 2 ml of HCl was added to 2ml of the filtrate and heated at 80°c for 3 minutes, then the solution was neutralised with 2 ml of caustic soda 1 N. Drops of Fehlings solution (A+B) were added one after another and the appearance of a brique red precipitate was indicative of the presence of reducing sugars that constitute glycosides. Flavonoids were revealed by dissolving 0.5 g of the extract in 5ml of normal caustic soda (NaoH; 1 N) and the decolorisation of the yellow colour obtained on addition of concentrated HCl 1 N confirmed the presence of flavonoides. Phlobatannins were revealed by mixing, 100 mg of extract with 2 ml distilled water. The solution obtained was filtered and 0.5 ml of the filtrate was boiled in aqueous HCl 1%. The presence of a red precipitate indicated the presence of phlobatannins.

Determination of total phenol
The amount of phenol in the ethanolic and hydroethanolic extracts of *D. mannii* was determined with Folin-Ciocalteu reagent 0.2N using the method of singleton and Rossi, 1965. Exactly 1ml of 10% Folin-Ciocalteu reagent was added to 30ul of each plant extract(1mg/ml) in triplicate. The absorbance of the samples was measured at 750 nm after 30 minutes using a spectrophotometer. Results were expressed as milligrams of catechin (0-1mg/ml) dissolved in ethanol.

Estimation of total flavonoids
Aluminum chloride colorimetric method was used in which, 1ml of sample (1 mg/ml) was mixed with 3 ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1M potassium acetate and 5.6 ml of distilled water. The mixture was allowed to stand at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at

420 nm with UV visible spectrophotometer. The content was determined from extrapolation of calibration curve (Y) which was made by preparing quercetin solution (0-1000 ug/ml) in distilled water same as catechin. The concentration of flavonoid was expressed in ug/ml of quercetin (Aiyegoro and Okoh, 2010).

Determination of Total Proanthocyanidines

Total proanthocyanidin was determined based on the procedure of Sun et al **(Aiyegoro and Okoh, 2010)**. The mixture of 3ml of vanillin-methanol (4% v/v), 1.5 ml of concentrated hydrochloric acid was added to 0.5ml (1mg/ml) of extract and vortexed. The resulting mixture was allowed to stand for 15minutes at room temperature followed by the measurement of the absorbance at 500 nm. Total proanthocyanidin content was expressed as cyanidine equivalent (ug/ml) from its standard curve equation(Y) (cyanidine standard was prepared similarly to catechin standard).

Determination of Tannins.

Tannin content was determined using the method described by Bainbridge *et al.* (1996). One milliliter of the extract was collected and mixed with 5 ml of reaction solution (50 g of vanillin + 4 ml of hydrochloric acid in 100 ml distilled water) and the mixture was incubated at 30°C for 20 minutes. The absorbance was read at 500 nm against a blank (without extract). The tannin content in the extract was calculated with the help of a standard (Gallic acid 1 mg/ml). The results were expressed in µg/ml Gallic acid.

Concentration = $\dfrac{OD\ sample}{OD\ standard}$ x concentration of standard

Antioxidant assay

The antioxidant activity of the plant extracts was determined using acts prepared by Five mg/ml stock solution of plant extracts were prepared by weighing 200 mg of each plant extract and dissolved in 40 ml of distilled water. This solution was later used to prepare 20 ml daughter stock solution of varied concentration from 0-5 mg/ml. This was used to test for the following:

Scavenging effect of extract on DPPH (1, 1-diphenyl-2-picrilhydrazyl) free radical

➢ **Principle**

The determination of the antioxidant activity based on the DPPH free radical method has a principle based on the decrease in absorbance measured at 517 nm resulting from the change of colour from brown to yellow as the free radical becomes trapped by the antioxidant through the transfer of a hydrogen atom to form a stable DPPH-H (Katalinie *et al.*, 2003).

➢ **Reagents preparation**

- **DPPH reagent (0.3 mM)**: 0.024 g of DPPH reagent was weighed and dissolved in a little volume of methanol and the volume was completed to the 200 ml mark of a volumetric flask by adding methanol. The reagent was covered and kept in the dark.

➢ **Procedure**

Non hydrolysed extracts (20 μl) were introduced in 2 ml of a methanolic solution of DPPH (0.3 mM). After 30 minutes of incubation in the dark, the absorbance was measured spectrophotometrically at 517 nm. The control consisted of DPPH reagent only. The percentage inhibition of the free radical by the specimen was calculated using the formula of Yen and Duh. (1994).

% inhibition = [(Ac (0)–As(t) / Ac(0)] x 100
Ac (0) = absorbances of control at time 0mins
Ac (t) = absorbances of sample after 30 mins of incubation.

II.2.4.2: Inhibiting effect of extracts of *D. mannii* on the NO-radical.

➢ **Principle:**

Nitric oxide is generated by sodium nitroprusside at physiological pH on interaction with oxygen to produce nitrite and nitrate. The nitrite ion is measured using Griess reagent; the more the extract is reactive, the lesser the production of nitrite ion and the weaker the absorbance (Sreejayan and Rao, 1997).

➢ **Reagent preparation**

- Sodium Nitroprusside 10 mM (in Phosphate buffer 50 mM, pH 7.4).
- 0.33% Sulfanilic acid (0.33 g of sulfanilic acid in 100 ml of 20% glacial acetic acid (10 ml acid in 80 ml distilled water).
- Naphtylethylenediamine dihydrochloride; NED (0.1%)

➢ **Procedure**

In 1 ml of extract was added to 2 ml of sodium nitroprusside 10 mM (in phosphate buffer 50 mM, pH 7.4) and homogenised and incubated at 25°C for 15 minutes. After incubation, 0.5 ml of the mixture was mixed with 1 ml of sulfanilic acid (0.33% in glacial acetic acid 20%). The mixture was allowed to stand for 15 minutes for diazotization then, 1 ml of NED (0.1%) was added, homogenised and incubated at room temperature for 30 minutes. A pink chromophore was formed in diffused light whose absorbance was read at 540nm against a blank (consisting of the reaction mixture without nitroprusside). The control is prepared in the same way except that the extract is replaced by the solvent of dissolution.

%inhibition = (Ac – As) / Ac] x 100, Ac = absorbance of control, As = absorbance of sample

II.2.4.3: Evaluation of the metal chelating activity of twig extracts. (Dinis et al., 1994)

➤ **Principle:**

This method is based on the competition between ferrozine and the bioreactive components of the plant extracts in trapping ferrous ions. This is translated by a reduction in the absorbance at 562 nm of the ferrozine (Fe $^{2+}$) complex.

➤ **Reagent preparation**

- Ferrous chloride 2 mM (0.0507 g of ferrous chloride was weighed and dissolved in a small quantity of water and the volume was made up to the 200 ml mark by adding distilled water).
- ferrozine 5 mM (0.5 g of ferrozine was weighed and dissolved in a small volume of water and the volume was made up to 200 ml by adding distilled water).

Procedure

In 0.5 ml of plant extract was added 25 μl of 2 mM FeCl$_2$ and 100 μl of ferrozine. The mixture was vigorously shaken and left to stand at room temperature for 10 minutes. A control tube was prepared similarly except that the extract was replaced with the solvent of dissolution. The absorbance was measured spectrophotometrically at 562 nm. All measurements were done in triplicate and the % inhibition was calculated as follows.

%inhibition = [(Ac − As)/Ac] x 100.

Toxicity study

Experimental animals

Female Albino Wistar rats (121g $\pm$ 2) were obtained from the animal laboratory of the Biochemistry Department of the Yaounde I University, Cameroon. All the rats were kept under room temperature and had free access to water and food. These rats were deprived of food but not water (16-18 h) prior to the administration of the extract.

Acute toxicity

The bioassay was conducted according to the protocol of the 'limit test' proposed by the **OECD, (2004)**, which the administration of a unique dose (5000 mg/kg of body weight) of the substance to experimental animals (rats) by gavage followed by an intensive observation and record of the physiological variation of the animals within 48 hours.

For the study, albino Wistar rats were divided into three groups of four rats each. The extract (1.8105 g), was weighed and dissolved in 3 ml distilled water, such that 1 ml of this solution contained a dose of 5000 mg/kg body weight of extract. This solutions were administrated in a single oral dose of 1ml of 5000 mg/kg body weight to the respective groups by intra gastric gavages using a feeding needle. The control group received an equal volume of distilled water as vehicle.

Observations of toxic symptoms were made and recorded systematically for 2 weeks after administration of the extract. The number of rats that survived were noted after 24 h and then maintained for the further 14days with daily observations. This visual observation included skin changes, mobility, and aggressiveness, sensitivity to sound and pain, as well as respiratory movements. The toxic effects of the extract were assessed on the basis of mortality, which was expressed as LD50 . During the experiment, the animals were weighed at time 0, 7, 14days. At the end of the experiment, all surviving animals fasted overnight and sacrificed by decapitation. The organs such as liver, kidney, heart, pancreas, spleen, lungs and brain were removed, washed in 0.9% NaCl and weighed. The pathological observations of these tissues were performed on gross. The blood samples were also collected freshly in EDTA tubes for preparation of plasma. This plasma was assayed for biochemical parameters.

Determination of biochemical parameters

Blood collected into EDTA tubes was centrifuged at 3400 rpm for 10 min to separate the plasma which was the supernatant. This was used to evaluate the liver enzyme function through some biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) by the kinetic UV method and creatinine by the kinetic method without proteinization (Muray *et al*., 1984) and protein by Biuret.

Statistical analysis:

Statistical analysis of results was done using the statistical package for social science (SPSS) software, version 10.0 for windows. Analysis of variances between groups were done using ANOVA (analysis of variances) at one factor. Post hoc test of least significant difference (LSD) and Tamhane were used to compare results of *in vivo* studies, and all results were expressed as mean $\pm$ SEM, with signifance difference considered at P < 0.05

RESULT

The yield was 9.09%, and 11.24% for hydroethanolic twigs and hydroethanolic leaves respectively and 4.11% and 3.12% for ethanolic twigs and ethanolic leaves respectively.

III.1.1: Phytochemical content of extracts of *D. mannii.*

Table VII below illustrates the results of the Phytochemical screening which showed the presence of alkaloids, saponines, tannins, and flavonoids in all plant extracts. Glycosides and

| Extract	EL	HL	HT	ET
Polyphenol	+	+	++	+++
Saponines	+	+	+	++
Tannins	+	+	+++	++
Glycosides	-	+	+	+
Flavonoids	+	+	+	++
Alkaloides	+	+	+	+
Phlobatanins	-	+	+	+

phlobatannins were present in all other plant extracts except in ethanolic leave extracts.

+ = Presence   **-** = Absence

III.1.2: Polyphenol content of extracts of *D. mannii*

Results of the evaluation of the polyphenol content of *D. mannii* extracts illustrated in figure 11 below revealed that, twig extracts, had the highest ± 10,20 vs 690.75± 1, 36 mgEqCat).

polyphenol content compared to leave extracts (P< 0.05), with ET extracts having the highest content compared to HT (815.51 ± 0.57 vs 727.27 ± 0.36 mg EqCat; P< 0.05). HL and EL had lower comparable polyphenol content (689.23
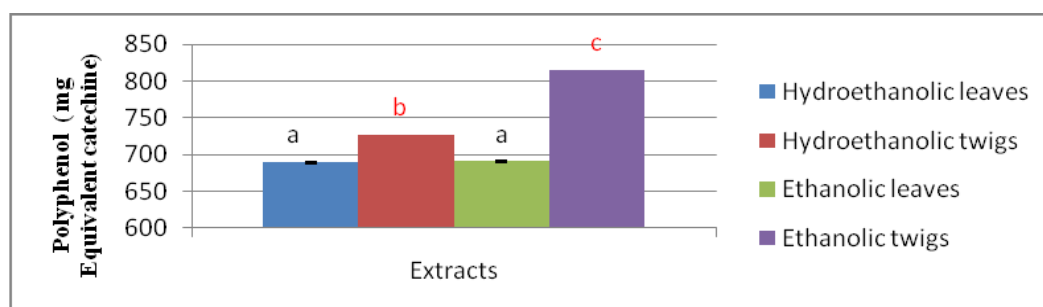
Bars with different letters are significantly different (P< 0.05)

Figure 1: Polyphenol content of extracts of *D. mannii*

From the evaluation of the phytochemical content and polyphenol content of extracts of *D. mannii*, which revealed that twigs contained all the phytochemicals screened for and had the highest polyphenol content compared to leave extracts, twigs were retained as the best plant part of *D. mannii*.

III.1.3: Proanthocyanidines, Tannins, and Flavonoids content in hydroethanolic and ethanolic twig extracts of *D. mannii*

Table VIII below illustrates the quantities of proanthocyanidines, tannins and flavonoids present in HT and ET extracts of *D. mannii*. Results showed that proanthocyanidins and flavonoids content were significantly higher in ET compared to HT(P< 0.05) meanwhile tannin content was higher in HT compared to ET (P< 0.05).

Table I: Results of proanthocyanidins, tannins and flavonoids content in *D. mannii* Hydroethanolic and ethanolic twig extracts.

Extracts	Proanthocyanidins (µg/ml Eq cyanidine)	Tannins (µg/ml Eq gallic acid)	Flavonoids (µg/ml Eq Quercetine)
HT	59.916 ± 2.347[a]	301.000 ± 10.136 [a]	4.761 ± 0.362[a]
ET	108.083 ± 1.900 [b]	185.333 ± 0.000[b]	75.761 ± 2.449 [b]

Values with different letters are significantly different (P< 0.05)

And more to that, the general polyphenol content was revealed to be higher in ET than in HT as observed in the quantitative determination of polyphenols done before. This could be explained by the higher content of proanthocyanidins and flavonoids present in ET compared to HT.

III.I.4: Antioxidant capacity of twig extracts of *D. mannii*

III.I.4.1: DPPH free radical Scavenging activity by twig extracts of **D. mannii**

From the results illustrated on figure 12 below, it was observed that, both twig extracts had a comparable DPPH free radical scavenging activity, which increased with increasing concentration, with ET extract inhibiting best with an IC_{50} value of 5.58 mg/ml and a maximum percentage inhibition of 47.45% compared to the HT extract with an IC_{50} value of 6.82 mg/ml and a maximum inhibition of 49.31%
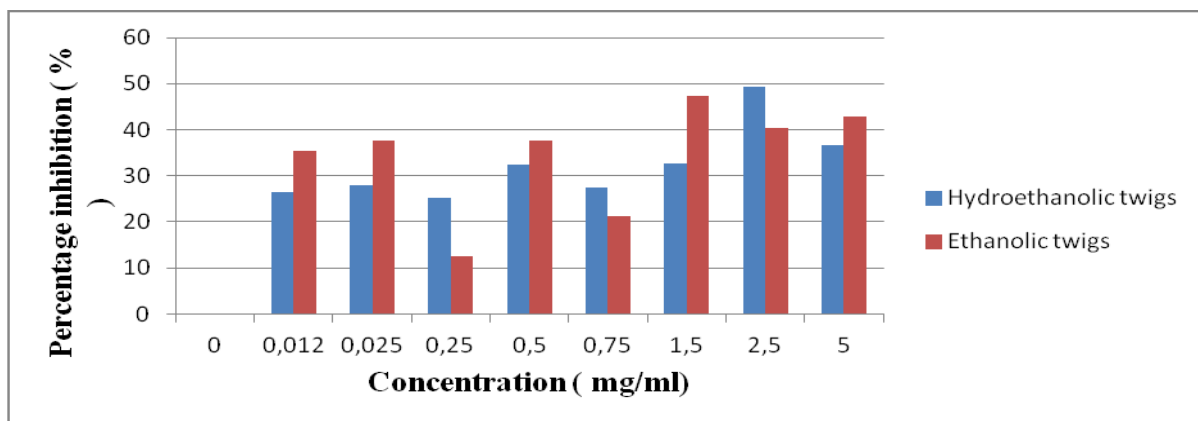

Figure 2: Scavenging effect of extracts of *D. mannii* on DPPH free radicals

III.1.4.2: Nitric Oxide radical scavenging activity by extracts of **D. mannii**

It was also observed that, NO inhibition by both extracts was comparable and decreased with increasing concentration of extracts, with ET inhibiting best with an IC_{50} of 0.08 mg/ml and a maximum inhibition of 60.35%, compared to HT with an IC_{50} of 0.10 mg/ml and a maximum inhibition of 63.00%. But at concentrations 2.5 mg/ml it was observed that, ET inhibition increased meanwhile HT inhibition was negative as shown in figure 13 below Both extracts probably have the property to counteract the formation of nitric oxide radical
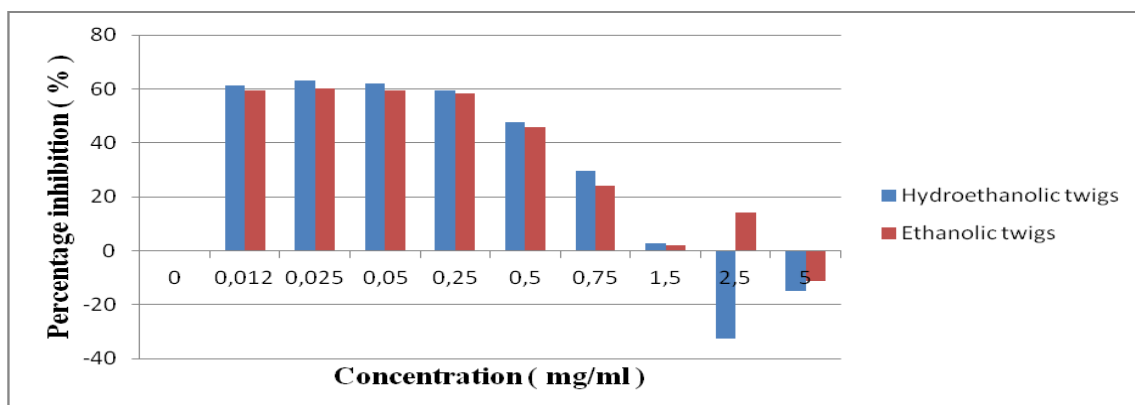

Figure 3: Scavenging activity of Nitric Oxide radical by extracts of *D. mannii*

III.1.4.3: Metal chelating activity of extracts of *D. mannii*.

Both extracts were observed to chelate metals best at concentration 0,5mg/ml and 0.75 mg/ml for HT and ET respectively with HT being the most active with IC_{50} 1.03 x10^{-10} mg/ml and a maximum inhibition of 87.342% compared to ET with an IC_{50} of 5.04 10^{-10} mg/ml and a maximum inhibition of 84.355% as indicated in figure 14 below.
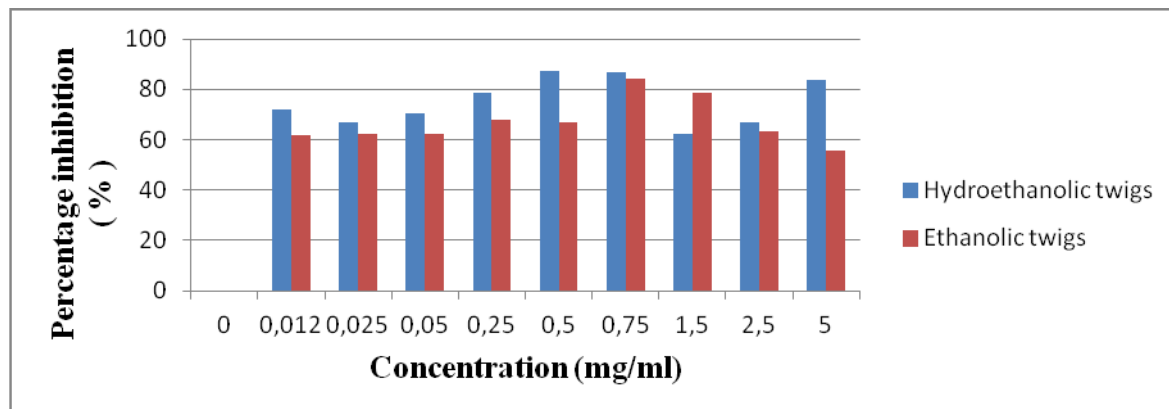

Figure 4: Metal chelating activity of *D.mannii*

From the determination of some polyphenols (proanthocyanidines, tannins and flavonoides) and the evaluation of the antioxidant activity of *D. mannii* HT and ET extracts, it was generally observed that Ethanol (100%), was the best solvent system since ET extract had most phytochemicals screened for and at higher concentrations explaining why it has a good antioxidant activity than HT extract ethanol (50%).

Acute toxicity
**III.1.5: Acute toxicity effects of twig extracts of *D. mannii***
III.1.5.1: Effect of twig extracts of *D. mannii* on the behavior of rats during treatment

Table IX illustrates results obtained from the behavioural partern observed in rats within 48hrs of acute toxicity treatment. For this study, no death of rats was recorded neither in the control nor in the treated groups indicating that the LD_{50} > 5000 mg/kg BW. The animals exhibited no changes in general behavior or in physiopathological activities.

Table II: Behavioural parten of animals during acute toxicity treatment

Groups	Negative control	HT	ET
General mobility	Normal	Normal	Normal
Sensitivity to noice	Normal	Normal	Normal
Feeding habits	Normal	Loss of apetite after 24hrs	Very little apetite after 24hrs
Aspect of feaces	Normal	Normal	Normal
Death	No death	No death	No death

For the acute toxicity studies, no death of rats was neither recorded in the control nor in the treated groups. The animals exhibited no changes in general behaviour or physiopatological activities. Although there was a change in the weights of rats in the treated groups, this was significantly reduced in the HT group and statistically not significant in the ET group though higher compare to the control (P< 0.05).
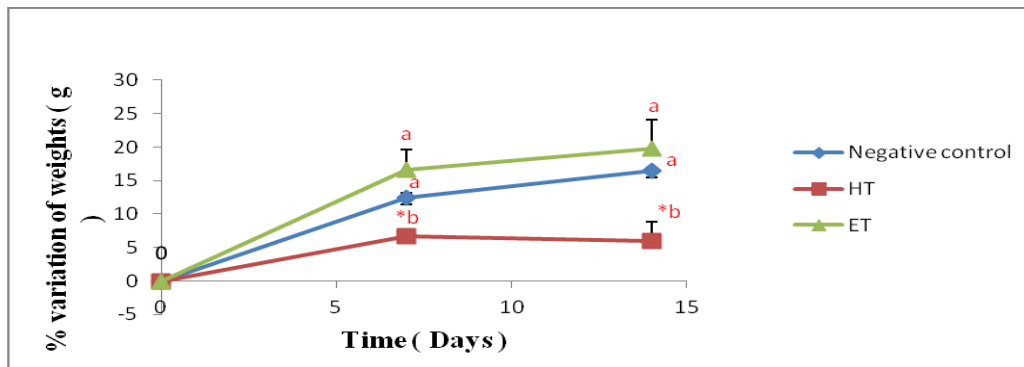
* = P<0.05 significant difference between Negative and positive control groups, Values with different letters are significantly different (P< 0.05)
Figure 5: Effect of extracts of *D. mannii* on the variation of body weights of rats after 14days of acute toxicity treatment

The pathological examinations of the tissues on a gross basis indicated no detectable abnormalities at the end of the experiment. Results of blood parameters indicated a significant decrease in AST and ALT levels between the negative control and the treated groups (P< 0.05), with HT lowering AST more than ET, meanwhile, though not significant, a slight increase in creatinine levels was also observed as indicated in Table III.

Table X : Effect of extracts of *D. mannii* on AST/ALT and Creatinine levels after 14 days of acute toxicity treatment.

GROUPS	AST (U/l)	ALT (U/l)	Creatinine (mg/dl)
Negative Control	91.956 ± 0.448^a	31.913 ± 1.316^a	0.815 ± 0.007^a
HT5000 mg/kgBW	75.918 ± 4.388^b	20.564 ± 1.359^b	0.868 ± 0.422^a
ET5000 mg/kgBW	87.429 ± 4.652^a	24.185 ± 1.272^b	$0.859 \pm 0.035a$

Values with different letters are significantly different (P< 0.05)

DISCUSSION

Results from the evaluation of the preventive effect of the extracts of *D. mannii* on hyperglycemia, hyperlipidemia and oxidative stress on rats fed with HF/HF diet results obtained revealed the following:

Phytochemical screening showed the presence of bioactive components such as, alkaloids, saponins, tannins, and flavonoids in all plant extracts. Glycosides and phlobatannins were present in all plant extracts except in Ethanolic leaves. This is in relation to the work done by Abegaz *et al*. (2002) who reported that prenylated flavonoides were from African *Dorstenia* only and more to that, *D. mannii* furnished the novel chalcone with many more prenylated flavonoids as Dorsmanins of various types. Some of these phytochemicals are believed to be responsible for the blood glucose lowering effects of these plant materials (Ojewole 2002).

Twigs extracts were observed to have the highest polyphenol content compared to leave extracts (P< 0.05), with ET extracts having the highest content compared to HT (815.51 ± 0.00 vs 727,27 ± 0.00 mg EqCat; P< 0.05). HL and EL had lower comparable polyphenol content (689.23 vs

690.75 mgEqCat). Many studies have shown that there is a strong link between the antioxidant activity of tropical plants and the amounts of polyphenols they contain (Ayoola *et al.,* 2008). This implies that plants with high levels of phenolic compounds are good sources of antioxidants used in the prevention and control of oxidative stress, associated disorders

The quantities of proanthocyanidines, tannins and flavonoids present in HT and ET extracts of *D. mannii*, were significantly higher in ET compared to HT extracts (P< 0.05) meanwhile tannin content was higher in HT compared to ET (P< 0.05). And more to that, the general polyphenol content was revealed to be higher in ET than in HT as observed in the quantitative determination of polyphenols done before. This could be explained by the higher content of proanthocyanidins and flavonoids present in ET compared to HT. This correlates with the work done by Watchueng. (2004), which explains that, the species of the genus Dorstenia were a source of a variety of flavonoids with most of its component belonging to the family of flavonoids. This result explains why the polyphenol content of ET was higher than HT

due to their significant differences in tannin, proanthocyanidines and flavonoids content.

The antioxidant activity revealed that, both twig extracts, had potent antioxidant activity. Their DPPH free radical scavenging activity increased to the HT with an IC_{50} value of 6.82mg/ml and a maximum inhibition of 49.31%. This inhibition is probably due to the presence of a hydrogen donating compound in the extracts that could reduce the stable radical DPPH to its non radical form; DPPH-H. This is in relation with the work done by Hajimahmoodi *et al.(*2008) which revealed that antioxidant activity of polyphenols is due to their redox properties, which allow them to act as reducing agents through hydrogen donation and that, the presence of flavonoids, and tannins in all plant extracts are likely to be responsible for the free radical scavenging effect observed since they are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers Ayoola et al. (2008). Implying that, this hydrogen donating compounds are polyphenols particularly flavonoids as reported by Dufall *et al.* (2003) who revealed that prenylated flavonoides were potent scavengers of the stable free radical DPPH, meaning that ET inhibits DPPH best probably due to the fact that it contained a higher concentration of flavonoids compared to HT, meaning that the degree of action of flavonoids is directly proportional to its concentration in the extract.

Likewise, both extracts had a comparable potent scavenging power against NO of which, NO inhibition decreased with increasing concentration of extracts, with ET inhibiting best with an IC_{50} value of 0.08 mg/ml and a maximum inhibition of 60.35%, compared to HT with an IC_{50} of 0.10 mg/ml and a maximum inhibition of 63.00 %. But at concentrations 2.5 mg/ml of extracts, it was observed that, ET inhibition increased while HT inhibition was negative. Hajimahmoodi *et al.(*2008) reported that flavonoids efficiency in protecting against peroxynitrite toxicity depended on its the general behavior or physiopathological activities of animals and no death of the rats was observed. According to the OECD protocol, extract of *D. mannii* could be classified as non toxic since the limited dose of an acute toxicity is generally considered to be 5000mg/kg BW *(Assam et al.,* 2010*)*. If no mortality is observed at this level, a higher dosage is generally not necessary (Wallace, 1989). However, although there was a change in the weights of rats in the treated groups, this change was significantly reduced in the HT group and statistically not significant in the ET group though higher, compared to the control (p< 0.05). This result showed that ET extract slightly stimulated the appetite of rats and probably did not irritate directly the gastrointestinal tract, meanwhile HT treated group significantly reduced the weight (P<0.05). A

with increasing concentration, with ET extracts inhibiting best with an IC_{50} value of 5.58 mg/ml and a maximum percentage inhibition of 47.45% compared

structure where by two very important pharmacophores have been identified in flavonoids (C_6-C_3-C_6 or C_{15}), namely the catechol group in ring B and the hydroxyl (OH) group at the 3-position. Whereby a flavonoid with this 2 pharmacophores and other OH- groups in minus or addition at positions other than position 5 and 7 could be potent scavengers of peroxynitrite otherwise they become toxic. This explains why the NO inhibition decreases with increasing concentration of extracts probably due to the fact that, as the concentration of flavonoids increase, with increasing extract concentration, diverse substitutions (hydroxylation, methylation, acetylation, etc) occur at the levels of rings A and B of the flavonoids structure thereby forming less potent scavengers that could even become toxic meanwhile at 2.5mg/ml the type of flavonoid formed was a potent scavenger.

And finally both extracts were also observed to chelate metals best at concentration of 0.5mg/ml and 0.75mg/ml for HT and ET respectively with HT being the most active with IC_{50} value of 1.03×10^{-10} mg/ml and a maximum inhibition of 87.34% compared to ET with an IC_{50} value of 5.04×10^{-10} mg/ml and a maximum inhibition of 84.35%. In addition to Mira *et al.*, (2002); Hajimahmoodi *et al.* (2008), explained that flavonoids also have metal chelating properties but this is influenced by their structure. This explained why extracts were observed to chelate metals best at concentration 0.5mg/ml and 0,75mg/ml for HT and ET respectively.

The acute toxicity test was investigated to establish the adverse effects of the administration of the extracts (HT and ET) of *D. mannii* on some behavioural and biochemical parameters of rats. The results indicated no change in significant decrease in AST and ALT activities between the negative control and the treated groups (P< 0.05), with HT lowering AST more than ET after treatment of rats with 5000 mg/kgBW of extract implied no injury of the liver as well as the heart or other sources of these enzymes. Although the variation of ALT and AST activities are associated with the hepato-cellular damage, only ALT is specific for the evaluation of liver damage meanwhile AST is highly concentrated in cardiac muscle, liver, skeletal muscle and kidneys. The creatinine level of treated rats increased but remained less than one-fold greater compare with the control.
This last result confirmed that kidneys were slightly affected at the dose of 5000mg/kg body weight of the extract (Assam et al., 2012)

CONCLUSIONS

Our study which was based on the hypothesis that *D. mannii* crude organic extracts had anti-hyperglycemia, anti-hyperlipidemia and antioxidant activity showed that

The twig extracts were the best plant part extract of *D. mannii,* becaused they contained bioactive components such as alkaloids, saponins, tannins, glycosides, phlobatannins and flavonoids and very high polyphenol content particularly ET with a high tannin and flavonoids content, which confer it a very good antioxidant activity.

The administration of the twig extracts at a dose of 5000 mg/kg BW was revealed to be non toxic since no death of rats was recorded.

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