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Phytochemical Constituents, Acute Toxicity and Free Radical Scavenging Activity of Methanol Extract of *Ficus glumosa* Leaves

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ABSTRACT

Background: *Ficus glumosa* parts have been previously used as food and in folklore medicine in several African countries. It therefore necessitated this study to scientifically substantiate the active ingredients that may be responsible for the efficacy of the plant and to know whether or not the consumption poses any deleterious effect on the cells.
Objectives: This study investigated the presence of phytochemicals in *Ficus glumosa* leaves as well as its toxicity and free radical scavenging activity.
Methods: The crude methanol leaves extract of *Ficus glumosa* was dissolved in distilled water and repeatedly partitioned using *n*-hexane, ethylacetate and *n*-butanol. The fractions obtained were concentrated and subjected to phytochemical, toxicological and free radical scavenging activity analyses.
Results: Flavonoid, alkaloid, tannin and saponin were present in the crude methanol extract. The *n*-butanol fraction has higher concentrations of total phenol and flavonoid [9.76±0.63 (mg/100g) Gallic Acid Equivalent and 5.27±0.23 (mg/100g) Quercetin Equivalent respectively]. There was an increasing IC_{50} value in the order of *n*-butanol fraction (0.41±0.07 mg/ml) < aqueous residue (0.71±0.05 mg/ml) < ethylacetate fraction (0.93±0.13 mg/ml) < *n*-hexane fraction (2.77±0.49 mg/ml). Consequently, no death was recorded at 10, 100, 1000, 1600, 2900 and 5000 mg/kg body weight respectively. The LD_{50} of *n*-butanol fraction was taken to be >5000 mg/kg body weight.
Conclusion: The findings suggested that the various fractions of the *Ficus glumosa* leaf extract contain useful phytochemicals and possess free radical scavenging activity. Furthermore, it was revealed that the methanol leaf extract of the plant demonstrated no cytotoxicity on the rats.

Keywords: Phytochemical toxicity, free radicals, IC_{50}, antioxidant, *Ficus glumosa*

INTRODUCTION

Plants usually produce chemical compounds as part of their normal metabolic activities. These chemicals could be primary metabolites such as carbohydrates, proteins and fats found in all plants as well as secondary metabolites which are present in few plants and serving more specific roles such as interaction with other organisms within their environment (Meskin, 2002). Some secondary metabolites are toxins used for protection against predation while others are pheromones used to attract insects for pollination. However, other secondary metabolites and pigments most often possess therapeutic actions in humans and can serve as drugs when refined as in the case of inulin from the roots of dahlias, quinine from the cinchona, morphine and codeine from the poppy, quercetin from *Moringa oleifera* leaf and digoxin from the foxglove (Mariam *et al.*, 2015). Plant usually synthesis varieties of phytochemicals but most are derivatives of a few biochemical motifs such as alkaloids (cocain, caffeine, nicotin atropine), polyphenols (anthocyanins, isoflavonone, tanins, phytoestrogens), glycosides (cyanoglycosides) and terpenes (carotenoids) (Springbob and Karin, 2009).

Ficus glumosa is commonly called Fig Tree or African Rock Fig. In Nigeria, it is readily present in the southern part and is referred to as "Baure" in Hausa language (Umar *et al.,* 2013), "Obata" in Yoruba, "Obadan" in Edo language (Aigbokhan, 2014). The leaf of this plant is used as a vegetable in preparation of local delicacies and in traditional medicine in Nigeria to treat illness such as diabetics. In Cote d'Ivoire, the Central Africa Republic and Zimbabwe, the latex of *Ficus glumosa* is used to ameliorate pains from

sprains, treat diarrhoea and sore eyes, whereas in central Africa, Senegal, East Africa and Tanzania, the stem bark is used as mouth wash agents to alleviate toothache, to prevent conjunctivitis, treatment of jaundice, dysentery, typhoid fever and stomach disorders (Kwazo *et al.,* 2015). This plant is used in traditional medicine in East Africa, Cameroon and Senegal for the treatment of oedema, hypertension, diabetes, haemorrhoids, rheumatism, skin diseases and stomatitis (Orwa *et al.,* 2009).

This study investigated the presence of phytochemicals in methanol extract of *Ficus glumosa* leaves as well as its toxicity and free radical scavenging activity.

MATERIALS AND METHODS

Preparation and extraction of plant material

The plant leaves were rinsed in clean water and air-dried at room temperature to constant weight. The dried leaves were pulverized into powder using the Thomas-Wiley laboratory mill (model 4) before being extracted by cold maceration.

Exactly 500 g of the pulverized plant leaves was then suspended in 2.5 L of absolute methanol and the solution was left standing for 48 h in large amber bottles with intermittent shaking. At the end of the extraction, the crude methanol extract was filtered using Whatman No. 1 filter paper (1 mm mesh size) and then concentrated using water bath maintained at 45 $^{\circ}$C until dark residue was obtained.

The concentrated methanol extract was stored in an air-tight sample container in a refrigerator for further analysis.

The percentage yield was then calculated using the formula:

$$\text{Percentage yield of crude extract} = \frac{\text{weight of extract (g)}}{\text{weight of sample (g)}} \times 100$$

Fractionation of crude methanol extract of *Ficus glumosa* leaves

The crude methanol extract (20.13 g) was dissolved in 300 ml of distilled water and repeatedly partitioned in a separating funnel with 400 ml of *n*-hexane for three times with vigorous shaking. At each portioning, the mixture was allowed to stand for 30 minutes to separate into distinct layers of hexane and aqueous. The *n*-hexane fraction was collected and concentrated using a water bath.

The aqueous layer was then repeatedly partitioned with 400 ml of ethylacetate three times to obtain ethylacetate fraction. The aqueous layer from the above was then saturated with distilled water and repeatedly partitioned with 400 ml of *n*-butanol for three times after which the *n*-butanol fraction and aqueous residue were obtained. The fractions were concentrated using a water bath maintained at 45 $^{\circ}$C until the residues were obtained. The residual fractions were kept in sealed containers and refrigerated for further use.

The percentage yield was then calculated using the formula:

$$\text{Percentage yield of fraction} = \frac{\text{weight of fraction (g)}}{\text{weight of crude extract (g)}} \times 100$$

Qualitative screening of some phytochemical constituents of methanol extract of *Ficus glumosa* leaves

Test for Alkaloids

Meyers Test: Three drops of the reagent were added to 1ml of a sample of the extract in a test tube and a green precipitate formed indicates the presence alkaloids (Trease and Evans 1983).

Test for Saponins

Frothing test: Exactly 0.5g of the extract was dissolved in 10ml of distilled water. This was then shaken vigorously for 30 seconds and was allowed to stand for 30 minutes. A honeycomb formed for more than 30 minutes indicates saponin presence (Trease and Evans, 1983).

Test for Flavonoids

Sodium Hydroxide Test: Three drops of aqueous NaOH were added to 5ml of extract, a yellow colouration shows the presence of flavonoid (Trease and Evans, 1983).

Test for Tannins

Ferric chloride test: Exactly 0.5g of the extract was dissolved in 10ml of distilled water and filtered. Three drops of ferric chloride solution were added to the filtrate. Formation of a blue-black precipitate indicates hydrolysable tannins and green precipitates indicate the presence of condensed tannin (Trease and Evans, 1983).

Quantitative screening of some key phytochemical constituents in fractions of methanol extract of *Ficus glumosa* leaves:

Determination of total phenolic

Total phenolic content was estimated by Folin Ciocalteu's method as described by Bhalodia *et al.* (2011).

One ml of sample (1 mg/5 ml) and standard gallic acid (0.625, 1.25, 2.5, 5, 10 and 20 µg/ml) were positioned into the test tubes and 5 ml of distilled water and 0.5 ml of Folin Ciocalteu's reagent was added, mixed and shaken. After 5 minutes, 1.5 ml of 20 % sodium carbonate was added and volume made up to 10 ml with distilled water. The mixture was incubated for 2 h at room temperature after which intense blue colour was developed. After incubation, absorbance was measured at 750 nm. The blank was performed using reagent blank with solvent. The calibration curve was plotted using standard gallic acid.

The data for total phenolic contents of solvent fractions of crude methanol leaves extract of *Ficus glumosa* were expressed as mg of gallic acid equivalent weight (GAE)/100 g of dry mass.

Determination of total flavonoid

Total flavonoid content was measured with the aluminium chloride colorimetric assay described by Pallab *et al.* (2013).

One ml of sample (1g/5ml) and 1ml standard quercetin solution (10, 20, 30, 40, 50 and 60 µg/ml) were positioned into test tubes and 4 ml of distilled water and 0.3 ml of 5 % sodium nitrite solution was added into each tube. After 5 minutes, 0.3 ml of 10 % aluminium chloride was added. At 6^{th} minute, 2 ml of 1 M sodium hydroxide was added. Finally, the volume was made up to 10 ml with distilled water and mixed well. Orange yellowish colour was developed and the absorbance was measured at 510 nm. The blank was performed using distilled water. The calibration curve was plotted using standard quercetin. The data of total flavonoids of solvent fractions of methanol extract of *Ficus glumosa* leaves were expressed as mg of quercetin equivalents/100 g of dry mass.

Determination of ascorbic acid content

Ascorbic acid was determined using the method described by Barros *et al.,* (2007). One gram of each fraction was diluted with 10 ml of 0.5% oxalic acid and the mixture was shaken and left for 20 minutes at room temperature and was filtered through Whatman No. 4 filter paper. Precisely 1 ml of the filtrate was mixed with 9 mL of 0.1 M of 2, 6-dichlorophenolindophenol reagent. A reagent blank using distilled H_2O instead of the sample was prepared. The absorbance was read within 30 minutes at 515 nm against the prepared blank. This test was carried out in triplicates. The ascorbic acid content was calculated using the calibration curve, prepared from standard L-ascorbic acid (0.65, 1.25, 2.5, 5 and 10 mg/ml). The data obtained were expressed as mg L-ascorbic acid equivalent per gram of dry matter.

Determination of free radical scavenging activity of fractions of methanol extract of *Ficus glumosa* leaves

The antioxidant activity of fractions of methanol extract of the plant was assayed by the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method described by Karadag *et al.,* (2009).

The assay mixture contained 2ml of 1.0 mM DPPH radical solution prepared in methanol and 1 ml of standard or extract solution of different concentrations (10 – 500 µg/ml). The solution was rapidly mixed and incubated in dark at 37 ^{o}C for 20 minutes. The decrease in absorbance of each solution was measured at 517 nm using a spectrophotometer. Ascorbic acid was used as a positive control while 2 ml of 1.0 mM DPPH radical solution with 1 ml ethanol was taken as blank.

The percentage of radical scavenging (%) was calculated as:

% Free Radical Scavenging Activity = $A_c - A_s/A_c \times 100$

Where A_c = Absorbance of control at 517 nm

A_s = Absorbance of the sample at 517 nm

The concentration of sample required to scavenge 50% of DPPH free radical (IC_{50}) was determined from the curve of percentage inhibitions plotted against the respective concentrations.

Acute toxicity study of *n*-butanol fraction of methanol extract of *Ficus glumosa* leaves

The median lethal dose (LD_{50}) of *n*-butanol fraction of methanol extract of *Ficus glumosa* leaves was conducted to select suitable safe doses for the evaluation of effects of the *n*-butanol fraction. This was done using the method described by Lorke (1983). In the initial phase, rats were divided into three groups of three rats each and were treated with 10 mg, 100 mg and 1000 mg of

n-butanol fraction per kg body weight orally. They were observed for 24 h for signs of toxicity, including behavioural changes and death. In the final phase, three rats were divided into three groups of one rat each and were treated with *n*-butanol fraction based on the findings in the first phase. Based on the survival from phase one, 3 rats were separately treated with 1600, 2900 and 5000 mg/kg body weight of the *n*-butanol extract fraction in the second phase respectively, the number of death within 24 h were recorded. The LD_{50} was calculated from the results of the final phase as the square root of the product of the lowest lethal dose and the highest non-lethal dose, that is, the geometric mean of the consecutive doses with 0 and 100% survival rates were recorded.

RESULTS

Percentage yield of crude methanol extract of *Ficus glumosa* leaves and its fractions

The Percentage yield following the methanol extraction of *Ficus glumosa* leaves and the various fractions is shown in Table 1. The percentage yield (% (w/w)) of the methanol crude extract was 4.03 % (w/w) while that of aqueous, *n*-butanol, ethylacetate and *n*-hexane were 21.31, 11.73, 6.51 and 4.07 % (w/w) respectively.

Qualitative phytochemical constituents of methanol extract of *Ficus glumosa* leaves

Qualitative phytochemical constituents of methanol extract of *Ficus glumosa* leaves are presented in Table 2. There was presence of flavonoid, alkaloid, tannin, saponin but absence of cardiac glycoside in the crude methanol extract.

Quantitative phytochemical constituents of fractions of methanol extract of *Ficus glumosa* leaves

Table 3 shows the concentrations of total phenol, total flavonoid and ascorbic acid of various solvent fractions of methanol extract of *Ficus glumosa* leaves. The *n*-butanol fraction has higher concentrations of total phenol and flavonoid [9.76±0.63 (mg/100g) Gallic Acid Equivalent and 5.27±0.23 (mg/100g) Quercetin Equivalent respectively] as compared to aqueous, *n*-hexane and ethylacetate fractions. However, the aqueous fraction was found to have the highest concentration of ascorbic acid (0.387±0.08 mg/ml) as expressed in terms of L-Ascorbic Acid Equivalent.

Free radical scavenging activity of fractions of methanol extract of *Ficus glumosa* leaves

The free radical scavenging ability of the fractions on DPPH was investigated and the % inhibition values at different concentrations of *n*-hexane, ethylacetate, *n*-butanol and aqueous residue of methanol extract of *Ficus glumosa* leaves were used to determine the IC_{50} values as presented in Table 4. There was an increasing IC_{50} value in the order of *n*-butanol fraction (0.41±0.07 mg/ml) < aqueous residue (0.71±0.05 mg/ml) < ethylacetate fraction (0.93±0.13 mg/ml) < *n*-hexane fraction (2.77±0.49 mg/ml). The *n*-butanol fraction with the lowest IC_{50} value (0.41±0.07 mg/ml) was then adopted as the fraction with the most active antioxidant potential.

Acute toxicity study of *n*-butanol fraction of methanol extract of *Ficus glumosa* leaves

Table 5 shows the behavioural change of *albino* rats when performing LD_{50} of *n*-butanol fraction of methanol extract of *Ficus glumosa* leaves. It was obvious that most behaviours stated on the table remained normal at 10, 100, 1000, 1600, 2900 and 5000 mg/kg body weight, except for the activity that slightly increased at the higher doses of 2900 and 5000 mg/kg body weight respectively.

The Median Lethal Dose (LD_{50}) of *n*-butanol fraction of methanol extract of *Ficus glumosa* leaves on the tested *albino* rats is described in Table 4.6. There was no death recorded at 10, 100, 1000, 1600, 2900 and 5000 mg/kg body weight respectively. The LD_{50} of *n*-butanol fraction was taken to be >5000 mg/kg body weight.

Table 1: Percentage Yield of Crude Methanol Extract of *Ficus glumosa* Leaves and its Fractions

Extract	Percentage Yield % (w/w)
Crude Methanol	4.03
Aqueous	21.31
n-Butanol	11.73
Ethylacetate	6.51
n-Hexane	4.07

Table 2: Some Qualitative Phytochemical Constituents of Methanol Extract of *Ficus glumosa* Leaves

Phytochemical	Status
Flavonoid	+++
Alkaloid	++
Tannin	++
Saponin	+

+++ = Highly present; ++ = moderately present; += low; - = absent

Table 3: Some Qualitative Phytochemical Constituents of Solvent Fractions of Methanol Extract of *Ficus glumosa* Leaves

Solvent	Total phenol GAE (mg/100g)	Total flavonoid QCE (mg/100g)	Ascorbic acid LAA (mg/ml)
n-Butanol	9.76±0.63^{a}	5.27±0.23b^{a}	0.359±0.03^{ab}
Ethylacetate	4.73±0.20^{b}	2.19±0.42^{b}	0.202±0.07^{a}
Aqueous	2.60±0.24^{c}	1.46±0.50^{b}	0.387±0.08^{b}
n-Hexane	0.20±0.15^{d}	0.12±0.09^{c}	0.032±0.03^{c}

n=3; Results are in mean±standard deviation; values with different superscript down the columns are significantly different at P<0.05; GAE = Gallic Acid Equivalent, QCE = Quercetin Equivalent, LAA = L-Ascorbic Acid

Table 4: DPPH Scavenging Activity of Fractions of Methanol Extract of *Ficus glumosa* Leaves (IC_{50})

Methanol Extract fraction	DPPH Scavenging Activity (IC_{50}) mg/ml
n-Butanol	0.41±0.07^{a}
Aqueous	0.71±0.05^{a}
Ethylacetate	0.93±0.13^{a}
n-Hexane	2.77±0.49^{b}

n=3; Results are in mean±standard deviation; values with different superscript down the columns are significantly different at P<0.05; DPPH = 1, 1-Diphenyl-2-picrylhydrazyl; IC_{50} = 50% Inhibitory Concentration

Table 5: Behavioural Changes of Albino Rats following Administration of *n*-Butanol Fraction of Methanolic Leaf Extract of *Ficus glumosa*

Parameter	Dose (mg/kg/bw)					
	10	100	1000	1600	2900	5000
Social Interaction	+	+	+	+	+	+
Activity	+	+	+	+	++	++
Aggressiveness	+	+	+	+	+	+
Reaction to Noise	+	+	+	+	+	+
Reaction to Touch	+	+	+	+	+	+
State of Tail	+	+	+	+	+	+
State of Excrement	G	G	G	G	G	G

+ = Normal; ++ = slightly increased; G = granular

Table 6: Median Lethal Dose (LD_{50}) of *n*-Butanol Fraction of Methanol Extract of *Ficus glumosa* Leaves after 24 hour

	Dose (mg/kg/ bw)	Number of Rat	Mortality	% Lethality
Phase I	10	3	0/3	0
	100	3	0/3	0
	1000	3	0/3	0
Phase II				
	1600	1	0/1	0
	2900	1	0/1	0
	5000	1	0/1	0

LD_{50} >5000 mg/kg Body Weight

DISCUSSION

Medicinal plants have been appreciated for a very long time for their numerous pharmacological effects which are attributed to the presence of secondary metabolites like alkaloids, flavonoids, phenols, glycosides tannins and saponins (Fatma *et al.,* 2013). Some of these plants have been evaluated for antioxidant property which is essential to limit the risk and progression of certain acute and chronic diseases (Ali *et al.,* 2008).

From this study, the presence of saponins, tannins, alkaloids and flavonoids in the crude methanol extract of *Ficus glumosa* leaves correlates with the earlier report by Tanko *et al.* (2012) that showed the presence of these compounds in methanol leaves extract of this plant.

These results may indicate a possible cellular protective effect of *Ficus glumosa* against oxidative damage probably owing to the antioxidative properties of these compounds (Jayathilakan *et al*., 2007). Plant phenolic acids, flavonoids and ascorbic acid constitute major groups of phytochemicals acting as primary *in vitro* antioxidants or free radical scavengers (El-Sayed *et al*., 2012). Therefore, it was reasonable to determine their concentrations in the various fractions to utilise the fraction with the highest concentration of these *in vitro* antioxidants for further analyses. Quantitatively, flavonoids, phenols and ascorbic acid were found in varying concentrations in the *n*-butanol, ethylacetate, *n*-hexane and aqueous fractions of the crude methanol leaf extract of *Ficus*

glumosa. This variation may be, however, attributed to the difference in polarity of the solvents and molecular size of compounds present in the plant extracts (Jongkwon *et al.,* 2014; Ali *et al.*, 2011).

Free radicals scavenging potentials of *n*-butanol, aqueous, ethylacetate and *n*-hexane fractions may depend on phenolic acids, flavonoids and ascorbic acid unique structure, number and position of the hydroxyl groups as reported by Pazos *et al*. (2005). The low free radical scavenging IC_{50} value (0.42±0.04mg/ml) of *n*-butanol fraction suggests it is a better free radical scavenger compared to ethylacetate, *n*-hexane and aqueous fractions. This finding conforms with Jamuna *et al.* (2014), which showed an inverse relationship between IC_{50} and free radical scavenging activity of *Hypochaeris radicata*. This result accounts for the fact that the higher the free radical scavenging activity of the fraction the lower the inhibitory concentration that would be required to prevent oxidative damage that may arise from reactive oxygen species (free radicals).

There were no deaths of animals even at high doses of crude extract administration except for mild changes in activities immediately after administration that were normalized in short while. The high LD_{50} (>5000mg/kg) of *n*-butanol fraction of methanol extract of *Ficus glumosa* leaves may be due to the presence of useful phytochemical constituents that serve as a protective mechanism to the cells rather than poison.

CONCLUSION

The findings suggested that the various fractions of the *Ficus glumosa* leaf extract contain useful phytochemicals and possess free radical scavenging activity. Furthermore, it was revealed that the methanol leaf extract of the plant demonstrated no cytotoxicity on the rats. Further work could be done using the n-butanol fraction to treat oxidative stress-induced in animals using chemicals such a carbon tetrachloride or diethylnitroamine.

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