Research Article

Phytochemical screening and free radical scavenging activity of *Dialium guineense* (Jacq) fruit pulp

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Abstract

Objective: *Dialium guineese* is used in Nigerian traditional medicine for treatment of cancer, headache, pains and other diseases. This study was designed to evaluate the physicohemical parameters and antioxidant free radical scavenging activity of the Fruit Pulp. **Materials and methods**: The seed pulp was evaluated for moisture content, ash value, acid insoluble ash, water soluble and alcohol soluble extractive values using standard procedures. The fruit pulp was extracted successively by maceration using n-hexane, ethyl acetate and methanol. The phytochemical screening for the presence of saponins, phenolic compounds, steroids/triterpenoids, cardiac glycosides, vitamin C and carbohydrates were evaluated using standard methods. The free radical scavenging activity was determined using DPPH. **Results and conclusion:** The results of for moisture content, ash value, acid insoluble ash, water soluble and alcohol soluble extractive values were found to be 4.0 %, 3.0 %, 1.5 %, 10.5 % and 6.0 % respectively. The results are within the acceptable limit of most crude drugs stated in various Pharmacopoeias. The methanol gave the highest percentage yield (12.94%), followed by ethyl acetate (0.22%) and then n-hexane with the least percentage yield of 0.15%. This observed trend may due to differences in polarity of the extracting solvent. The phytochemical screening revealed the presence of saponins, phenolic compounds, steroids/triterpenoids, cardiac glycosides and vitamin C. Alkaloid was found to absent in all the extracts. The TLC profiling of the various extracts showed several spots of compounds with different R_f values. The qualitative screening of the presence of free radical scavenging compounds by TLC using DPPH showed that the n-hexane and the methanol extracts were highly active while the ethyl acetate extract was moderately active. The *in vitro* determination of free radical scavenging activity of the methanol extract at concentrations of 10 mg/mL, 5 mg/mL and 2.5 mg/mL, showed a decrease in percentage inhibition from 19.82%, 5.61% and 1.92%. The activity demonstrated by the methanol extract was concentration dependent. On the other hand, the activity demonstrated by the ascorbic acid (standard) was far greater when compared with the methanol extract. At concentration of 5 mg/mL, 2.5 mg/mL and 1.25 mg/mL, the percentage inhibition of the ascorbic acid decreases from 70.35%, 60.10% and 58.94%. The free radical scavenging activity demonstrated by the extracts has revealed the potential of *Dialium guineese* as a good source of antioxidant.

Keywords: *Dialium guineense*, Phytochemical, Antioxidant, Phenolic Content and Toxicity

Introduction

Dialium guineese (Velvet tamarind) is called *Tsamiyan Biri* in (Hausa Language of Northern Nigeria). In Igbo language (South Eastern Nigeria) it is called Nchichi. The plant belongs to the family of Fabaceae Various parts of the plant have been used in traditional medicine for treatment of different diseases: the bark in cancer, headache and pains. The usefulness of the bark for oral hygiene and stomach ache among the Esan tribe of Edo state (Nigeria) have also been reported by Idu et al. (2009); the leaves are used as a remedy in fever, prenatal pains and edema; the fruits in treatment of diarrhea and cancer (Arbonnier et al., 2004).

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The fruits of the plant are chewed among some women in south-east Nigeria to improve lactation and check genital infection (Nwosu, 2000). The leaves and stem bark are used as remedies for the treatment of infections such as diarrhea, severe cough, bronchitis, wound, stomach ache, malaria fever, jaundice, ulcer and hemorrhoids (Bero et al., 2009). The leaves can also be squeezed and applied on wounds as practiced by Wolof of Senegal (Devendra, 1988). The extracts of leaves and seed coat have been reported to be very rich in vitamin C and other micronutrient (Maduaka, 1988; Okegbile et al. 1990). *D. guineense* is used as chewing stick among the Nigerian populace (Akinpelu et al., 2011).

Antioxidants play an important role as health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Antioxidants significantly delay or prevent oxidation of oxidizable substrates when present at lower concentrations than the substrate (Halliwell, 2007). It is commonly accepted that in a situation of oxidative stress, reactive oxygen species such as superoxide (O) hydroxide (OH) and Peroxide (OOH, ROO) radicals are generated. The reactive oxygen species play an important role in degenerative or pathological processes of various diseases, such as aging (Baur et al., 1997) cataract, anterosclerosis and inflammatory disease (Aruoma, 1998).

Free radicals are formed when oxygen is metabolized in the body. These free radicals are chemical species which possess an unpaired electron in the outer (valence) shell molecules. This is why the free radical can react with protein, lipids, Carbohydrates and even DNA. These free radicals attack the nearest stable molecules, taking up its electron while the attacked molecule loses its electron. It also becomes a free radical itself beginning a chain reaction which finally results in the destruction of healthy cell in the boby (Prior et al., 1995). Free radicals may be either oxygen derived (ROS, reactive oxygen species) or nitrogen derived (RNS, reactive nitrogen species).

The main sources of naturally occurring antioxidants are whole grains, fruits and vegetables (Indian raw material dictionary, 1988). Plant sourced antioxidants like vitamin C, vitamin E, β-carotenes and garlic acid have been recognized as having the potential to reduce disease risk (Indian Herbal Pharmacopoeia, 2002). Plants have long been a source of exogenous (i.e., dietary) antioxidants. It is believed that two-thirds of the world's plant species have medicinal importance and almost all of these have excellent antioxidant potential (Krishnaiah, 2011).

Thin-layer chromatography still remains an important tool in the analysis of plant extracts and herbal preparations. Recently, the concept of using TLC for investigating biological activity of constituents, present in complex natural samples, has gained much attention (Marstron et al., 2002). It can be attributed to the commonly known advantages of planar chromatography, namely its flexibility, high sample throughput and the speed of method development. It is particularly well suited for the direct biological detection, since the separation result is immobilized prior to the detection and moreover, the open solid bed layer allows direct access to the sample (Poole, 2003). Thin layer chromatography is a simple and inexpensive analytical tool and has a wide range of application. It can be used for discovering new antioxidants in higher plants (Cuendet et al., 1997). Antioxidants can be detected on TLC plate by spraying with 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. Antioxidants reduce the free radical, producing yellow or white spots against purple background (Soler-Rivas et al., 2000; Motlhanka et al., 2008). Alternatively, the bleaching of crocin (which normally gives a yellow colour on the plate) can be used to distinguish components of plant extracts with potential antioxidant or radical scavenging properties (Hostettmann et al*.,* 1999).

Previous studies on *D. guineense* have shown that the plant contains saponins which are presumed to add to the cleaning effect of teeth and at the same time prevent carries and plaque (Okwu and Okeke, 2003). Lawal et al. (2010) reported in their findings that *D. guineense* is used as antiulcer and as vitamins supplements among some tribes in the southern part of Nigeria.

The stem bark and the leaf extracts contains cardiac glycosides, tannins, saponins, terpenoids, resins, steroids, triterpenes, alkaloids, flavonoids and carbohydrates (Gideon and Raphael, 2013; David et al., 2011; Ogu and Amiebenemo, 2012). The medicinal value of *D. guineense* lies in these chemical substances that produce a definite physiological action in human body. Therefore, this research is designed to determine the phytochemical constituents and free radical scavenging activity of *Dialium guineense* fruit pulp.

Materials and Methods

Collection and preparation of fruits for extraction

The fruits of *Dalium guineense* were bought from Gwange ward of Maiduguri, Borno State-Nigeria in November, 2016. The fruit was identified and a voucher specimen was deposited at the Herbarium of the Department of Pharmacognosy, University of Maiduguri for reference purpose. The fruits were transferred into mortar by means of hands and with gentle strikes using pestle, the dried epicarp were separated from the dried mesocarp and the seed. These were handpicked and transferred again into

mortar and with gentle striking using pestle; the mesocarp was separated from the seeds. Using sieve, the seeds were perfectly separated from the fine powdered mesocarp.

Physicochemical studies

The evaluation of the physicochemical parameters was carried out according to standard methods (WHO, 2000; WHO, 1999; WHO, 1998; African Pharmacopoeia, 1986).

Serial extraction

The extraction was carried out by maceration using n-hexane (non polar solvent), Ethyl acetate (Moderately Polar solvent) and methanol (Polar solvent). The plant sample (100 g) was macerated with 300 mL of n-hexane for 24 hours. The extract was first filtered using cotton wool and clarified using filter paper to obtain a clear n-hexane extract. The marc (residue) obtained above was dried and then macerated for 24 hours using 300 mL of Ethyl acetate. The extract obtained was filtered and clarified as described above. The process was repeated using 400 mL of methanol. The final marc was discarded. The liquid extracts obtained were concentrated by drying under the fan in the laboratory to produce solid extracts of n-hexane, ethyl acetate and methanol. The percentage yields of each extract were calculated.

Phytochemical analysis

The phytochemical analysis for the presence of carbohydrates, steroid/triterpenoids, alkaloids, tannins, flavonoids, saponins and cardiac glycosides was carried out using standard procedures (Evans, 2005; Sofowora, 2008; Trease and Evans, 2002; Mahrain et al, 1980; Brain and Turner, 1975).

Qualitative test for vitamin C (Ascorbic acid)

The methanol extract (0.5 g) was dissolved in 2 mL of distilled water. 0.1 g of sodium bicarbonate ($NaHCO_3$) was added to the solution and then followed by the addition of 2 mL of 10 % $FeSO_4$. The formation of violet coloration which turned colourless on addition of 3 drops of 1M H_2SO_4 indicates the presence of vitamin C.

Thin layer chromatography

The TLC separation profile of the hexane, ethyl acetate and methanol extracts were determine using silica gel pre-coated TLC plates (F_{254}). The n-hexane extract was resolved using solvent system; Hexane and Ethyl acetate (8:2). The Ethyl acetate extract was resolved using solvent system; Hexane and Ethyl acetate (2:8). The methanol extract was resolved using ethyl acetate, Methanol and water (8: 1: 1); Ethyl acetate, Methanol and water (7:2:1). The developed plates were view first under day light, then followed by UV light at 254 nm and then stained with 10 % H_2SO_4. The stained plates were heated at 105 °C and the R_f value of each spot was calculated.

Determination of antioxidant activity (Free radical scavenging activity)

Preparation of DPPH solution for qualitative screening of free radical scavenging compounds

The DPPH (0.01g) was dissolved in 20 mL of methanol where a deep purple coloured solution was formed.

Qualitative TLC screening of free radical scavengers

The developed TLC plates of the n-hexane, ethyl acetate and methanol extracts were stained by deeping the plates separately in the purple DPPH solution. The formation of yellow spot against the purple background indicated the presence of free radical scavenging compound.

Acute toxicity of methanol extract

Acute toxicity study was conducted in mice by using OECD 425 guidelines. Five nulliparous mice of weight (15-20g) were administered a single dose of methanol extract of *Dialium guineense* fruit pulp 5,000 mg mg/kg orally and then observed individually for the first four hours, then over a period of 24 hours and at least once daily for 14 days. General behavior, adverse effects and mortality were observed throughout the experimental period. Prior to the experiment the mice were fasted over night by withdrawal of food and water.

Results

Physicochemical evaluation

The results of the physicochemical evaluation on the powdered pulp are presented in table 1.

Table 1. Physicochemical evaluation

Parameter	% Yield
Moisture Content	4.0
Total Ash	3.0
Acid Insoluble Ash	1.5
Water Soluble extractive value	10.2
Alcohol Soluble extractive value	6.0

Extraction

The results for percentage yields of the successive extraction using n-hexane, ethyl acetate and methanol were found as 0.15, 0.22 and 12.94, respectively.

Phytochemical analysis

The results of the phytochemical analysis of the n-hexane, ethyl acetate and methanol extracts are presented in table 2.

Table 2. Phytochemical analysis of the extracts

Metabolites/Test	Hexane Extract	EtOAc Extract	Methanol Extract
Carbohydrate	-	+	+
Molisch's Test			
Saponnins	-	-	+
Frothing Test			
Phenolics	-	+	-
Ferric Chloride Test			
Steroids/Triterpenoids	+	+	+
Salkowaski's Test			
Liebermann-Burchad's Test			
Alkaloids	-	-	-
Mayer's Test			
Hager's Test			
Cardiac glycoside	-	+	+
Keller-Kiliani's Test			
Vitamin C	-	-	+

Key: + = Present; - = Absent

Thin Layer Chromatography

The thin layer chromatography profiles of the extracts are presented in tables 3 and Plates 1, 2 and 3.

Table 3. R_f value of hexane extract

S.No.	Spot	R_f Value		
		n-hexane Extract	Ethyl acetate Extract	Methanol Extract
1.	A	0.96	0.97	0.94
2.	B	0.84	0.93	0.86
3.	C	0.82	0.75	0.63
4.	D	0.77	0.42	0.52
5.	E	0.69	0.18	0.47
6	F	0.62	0.14	0.34
7	G	0.48	0.07	0.19
8	H	0.40	-	-
9	I	0.30	-	-
10	J	0.14	-	-

Qualitative DPPH , TLC screening of free radical scavengers

The results of the qualitative free radical scavenging of the n-hexane, ethyl acetate and methanol are presented in table 4 and figure 1.

Table 4. TLC Screening of free radical scavengers

S.No.	Extracts	Inference
1.	Hexane	+++
2.	Ethyl acetate	++
3.	Methanol	+++

Key: ++ = moderately Active; Highly antioxidant active

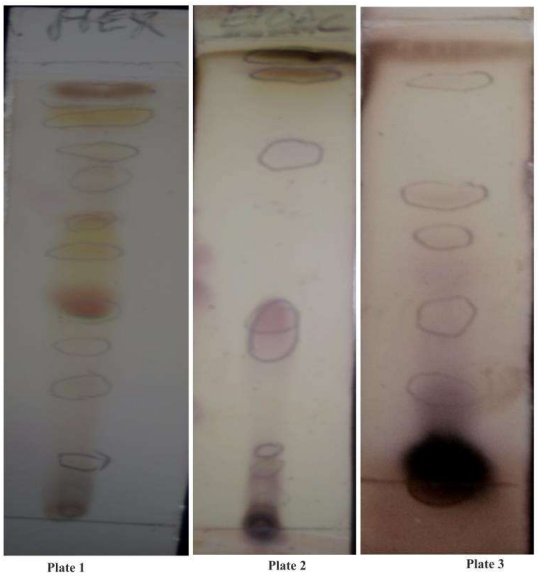

Figure 1. Thin layer chromatography of different extracts: **Plate 1**: Chromatogram of Hexane Extract, Solvent system: Hexane/EtOAc (8:2); **Plate 2**: Chromatogram of EtOAc Extract, Solvent system: Hexane/EtOAc (2:8); **Plate 3**: Chromatogram of Methanol Extract, Solvent system: EtOAc/Methanol/ Water (8:1: 1)

In vitro determination of free radical scavenging activity using DPPH

The results of the *in vitro* studies of the free radical scavenging activity are presented in table 5.

Table 5. Percentage inhibition of the extracts for antioxidant activity

Conc. mg/mL Methanol Extract	% inhibition	Conc. mg/mL Ascorbic Acid (Std)	% inhibition
10	19.82	5	70.35
5	5.61	2.5	60.10
2.5	1.92	1.25	58.94

Acute Toxicity

The rats did not show any sign toxicity or mortality after the 14 days period of observation.

Discussion

The result of the physicochemical analysis (Table 1) showed that the fruit pulp of *Dialium guineese* had moisture content, ash value, acid insoluble ash, water soluble and alcohol soluble extractive values of 4.0 %, 3.0 %, 1.5 %, 10.5 % and 6.0 %respectively. These results were found to be within the acceptable limit of most crude drugs (African Pharmacopoeia, 1986; Halilu et al., 2016a). The result of the serial extraction by maceration using n-hexane, ethyl acetate and methanol, showed methanol with the highest percentage yield (12.94 %), followed by ethyl acetate (0.22 %) and n-hexane with the least (0.15 %). This observation

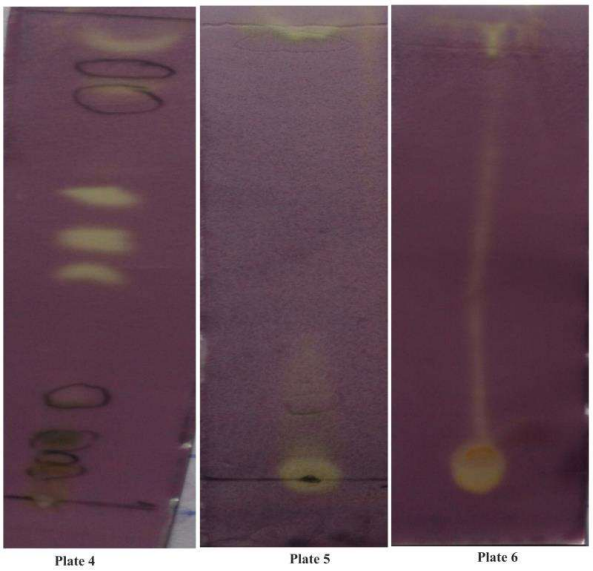

Figure 2. Thin layer chromatography of different extracts: **Plate 4**: DPPH Free Radical Activity of Hexane Extract, Solvent system: Hexane/ EtOAc (8:2); **Plate 5**: DPPH Free Radical Activity of EtOAc Extract, Solvent system: Hexane/ EtOAc (2:8); **Plate 6**: DPPH Free Radical Activity of Methanol Extract, Solvent system: EtOAc /MeOH/Water (7:2:1)

may be due to differences in polarity of the extracting solvent. Methanol being highly polar solvent has the capacity to extract more of the polar constituents. This observation is in agreement with (Halilu et al., 2016b).

The phytochemical screening (Table 2) showed the presence of saponins, phenolics, steroids/triterpenoids, cardiac glycosides, vitamin C and carbohydrates. The TLC profiling of the various extracts (Tables 3 and Plates 2, 3 and 4) showed the presence of spots with different R_f values. The n-hexane extract had 10 spots with R_f values ranging between 0.14 and 096. The ethyl acetate extract showed 7 spots with with R_f values ranging between 0.07 and 0.97. The methanol extract had 7 spots with R_f values ranging between 0.19 and 0.94. The R_f values are useful for identification of unknown compound in a mixture. This is done by comparing with standard reference compound with known R_f value. The spots also indicated that the extracts are mixtures containing many compounds. The qualitative screening of the presence of antioxidant compound by TLC using DPPH showed that n-hexane and the methanol extracts were (Plates 5 and 6) highly active while the ethyl acetate extract was moderately active (Plate 7). This observation may be due to the presence of the phytochemicals in the various extracts (Halilu et al., 2014). The *in vitro* determination of free radical scavenging activity of the methanol extract at concentrations of 10 mg/mL, 5 mg/mL and 2.5 mg/mL, showed a decrease in the percentage inhibition from 19.82%, 5.61% and 1.92%. The activity demonstrated by the methanol extract was concentration dependent. On the other hand, the activity demonstrated by the ascorbic acid (standard) was far greater when compared with the methanol extract. At concentration of 5 mg/mL, 2.5 mg/mL and 1.25 mg/mL, the percentage inhibition of the ascorbic acid decreases from 70.35%, 60.10% and 58.94%. The free radical scavenging activity demonstrated by the extracts has revealed the potential of *Dialium guineese* as a good source of antioxidant.

Conclusion

The fruit pulp of the plant contains carbohydrates, saponins, steroids, triterpenoids, flavonoids, tannins and vitamin C. The extracts all show free radical scavenging activity with the highest activity observed in the hexane. Also, the free radical scavenging activity demonstrated by the methanol was concentration dependent.

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Conflicts of interest: None

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