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RESEARCH ARTICLE

Evaluation of Phytochemical and *In Vitro* Antioxidant Activities of Methanol Stem Bark Extract of *Detarium microcarpum*

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Abstract

This study was designed to evaluate the phytochemical properties and antioxidant properties of the methanol stem bark extract of *Detarium microcarpum* and its fractions. In the quantitative estimation of phytoconstituents total phenols, alkaloids, tannin, saponin and flavonoids were estimated from the samples. The antioxidant properties of methanol stem bark extract and its fractions were evaluated by ferric reducing antioxidant power (FRAP), 2,2-diphenyl-l-picryl hydrazyl (DPPH) radical scavenging and thiobarbituric acid reactive substances (TBARS) assays. The qualitative phytochemical analysis shows that alkaloids, terpenoids, reducing sugar, saponins, tannins, flavonoids, phlobatanins, anthraquinones, phenols and cardiac glycoside were found to be present in the samples while the quantitative phytochemical investigation indicated that the level of phenols, flavonoids, tannins and alkaloids in fraction II was higher when compared with methanol extract and fraction I while the level of saponins in methanol extract was high when compared with fraction I and fraction II. FRAP, DPPH and TBARS assay indicate that all the samples (extract and its fractions) inhibited significantly higher antioxidant activity when compared to L-ascorbic acid and the inhibition activity was dose dependent, thus indicating their antioxidant activity. The highest activity was observed in fraction II.

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Introduction:-

Detarium microcarpum is an African tree belonging to the Fabaceae family (legumes) (Abdalbasit *et al*., 2009) and is a locally common plant often left when a farm land is cleared and left to fallow. It is a small tree or shrub growing up to 15 m tall but can reach 25m in moist areas. In terms of growth rate, the shoots of the trunk can reach a height of 1.5m to 2m in 1 to 2 years and are much more vigorous than seedlings which on average grow to 0.6 m after 3 years and may reach 1.5 m in 4 years (Kouyaté and Van Damme, 2006). It flowers during the rainy season (July to September/November), but the main flowering period only lasts up to 8 days. It bears fruit from September–January/May and in November; the tree sheds its leaves and produces new leaves in March (Vautier *et al.*, 2007).

D. microcarpum is widely distributed in semi-arid sub-saharan Africa which includes Benin, Burkina Faso, Cameroon, Central African Republic, Ghana, Guinea, Guinea Bissau, Niger, Nigeria, Senegal and Togo (Oibiokpa *et al.*, 2014). Unlike the other species of its family, *D. microcarpum* grows in dry savannah, in humid forest (Kouyaté and Van Damme 2006). It is most common in wooded savannahs or semi-cleared dry forest areas and fallows, growing in sandy or hard soils with high iron content as reported by Yakubu, (2015). It is commonly known in English as sweet dattock, trees. *D. microcarpum* is known locally in Ghana as Takyikyiriwa, Twutwiriwa; in

Senegal as Kpagra, Kpayhga; in Nigeria Taura, (Hausa): Ofo, (Igbo): Ogbogbo, (Yoruba): Gungorochi, (Nupe): Ejiji (Igala) Konkehi, (Fulfulde): Galapo, (Kanuri): Agashidam, Tiv as reported by Yakubu, (2015).

D. microcarpum is classified as a major African medicinal plant. Many scholars observed the following medicinal uses of *D microcarpum*: Leaves, bark and roots as infusions or decoctions are use for treatment of rheumatism, venereal disease, urogenital infections, haemorrhoids, caries, biliousness, stomache-ache, intestinal worms, diarrhoea, dysentry, malaria, leprosy and impotence. The powdered bark decoction is used for treatment of headache, sore throat, back pain, painful menstruation, measles, nocturia, hypertension, itch, and tiredness. Decoction of leaves or roots is also used for treatment of paralysis, meningitis, tiredness, cramps, difficult delivery, fainting and convulsion (Abreu et al., 1998, 1999; Abreu and Relva, 2002; Kouyaté and Van Damme, 2006; Akah *et al*., 2012)

In view of this, we have evaluated the phytochemical properties and Antioxidant properties of the methanol stem bark extract of *D. microcarpum* and its fractions.

Materials and Methods:-

Equipments

Spectrophotometer (SS1-1104, England) Weighing balance (BL5002, Water Bath (KW-1000DB, Moment West Germany), Robert Riele photometer (5010+Germany), funance, dry air oven (Gallenhamp oven 300 series plus).

Chemicals

Alcoholic potassium hydroxide solution, phenolphthalein indicator solution, (BDH Indusries Ltd, India); Ferric Reducing Antioxidant Power (FRAP) Assay Kit, 2,4-dinitrophenylhadrazine, DNPH colour reagent, (AGAPE IVD Switzerland). All other chemicals used were of analytical grade.

Plant Materials

The plant material of *D. microcarpum* was collected in the month of October/November, at Girei local Government area of Adamawa State which lies on geographical location 9° 21’53.19’’North and 12° 33’28.33’’East Google earth (2014). It was authenticated by a botanist in the Department of Biological Sciences, Modibbo Adama University of Technology, Yola, Adamawa State.

Preparation of Plant Extract

The collected plant materials were washed sliced and completely shade dry. The dried materials was ground into fine powder and used for extraction. The powdered of plant material *D. microcarpum* (200 g) was extracted with methanol 1 litre in an air tight clean flat bottomed container for 48 hours at room temperature with occasional stirring and shaking (Trease and Evans, 2002). The methanol extract was filtered first through a fresh cotton plug and then through a whatman filters. The filtrate was evaporated to dryness in vacuo by a rotary evaporator at 40-50°C and the extract was kept in a well tight sterile bottle/container under refrigerated conditions until use. The fractionation (Solvent-solvent partitioning) was used as designed and described by Kupchan and Tsou, (1973) as modified by Wagenen *et al.*, (1993).

Qualitative Phytochemical Analysis

The methanol stem bark extracts of *D. microcarpum* and its fractions were analyzed for the presence of alkaloids, terpenoids, reducing sugars, Saponins, tannins, Flavonoids, Phlobatannis, steroids, anthraquinone and phenols using the methods of Harborne, (1973); Sofowora, (1993) and Trease and Evans, (2002).

Quantitative Phytochemical Analysis

Determination of Total Flavonoids

The total flavonoids content was determined using the method of Chang *et al.*, (2002)

Determination of Total Tannins

The total tannin content was determined using the method of Schanderl, (1970).

Determination of Total Phenols

The total phenolic content was determined using the method of McDonald *et al.*, (2001).

Determination of Total Alkaloids

The total alkaloids content was determined using the method of Shamsa *et al.*, (2008).

Determination of Total Saponins Content

The total saponins content was determined using the method described by Edeoga *et al.*, (2005).

Determination of in vitro Antioxidant Activities

The quantitative antioxidant activity of methanol stem bark extract/fractions were determined using FRAP assay, DPPH assay and TBARS assay.

FRAP Assay

The antioxidants activities of the methanol stem bark extract/fractions were estimated using FRAP assay method as described by Sutharsingh *et al.*, (2011).

DPPH Assay

The antioxidant activity of methanol stem bark extract/fractions was estimated using the DPPH radical scavenging assay as described by Sutharsingh *et al.*, (2011).

TBARS Assay

The quantitative antioxidant activities (TBARS Assay) of methanol stem bark extract/fractions were determined using the method of Sutharsingh *et al.*, (2011).

Statistical Methods:-

The results of replicate experiment were represented as Mean value ± standard error of mean (SEM). Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 24.0 (SPSS, Incorporation Chicago Illinois, USA). Differences between and within the group means were analyzed using One-way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (MRT) for the post-hoc treatment. The results were considered statistically significant at $p<0.05$.

Results:-

Table 1:-Qualitative Phytochemical Screening of Methanol Extract and Fractions of *D. microcarpum.*

Phytochemicals	Methanol Extract	Fraction I	Fraction II
Alkaloids	+	+	+
Terpenoids	+	+	+
Reducing sugars	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Flavonoids	+	+	+
Phlobatannis	+	+	+
Steroids	-	-	-
Anthraquinone	+	+	+
Phenols	+	+	+
Cardiac Glycosides	+	-	+

Key:
+ = Present
- = Absent

Table 2:-Quantitative Phytochemical Contents of Methanol Extract and Fractions of *D. microcarpum.*

Phytochemicals	Methanol Extract	Fraction I	Fraction II
Alkaloids (mg/100g)	302.67 ± 0.58	188.00 ± 1.00^{b}	366.00 ± 1.00^{a}
Saponins (mg/100g)	0.89 ± 0.01^{a}	0.86 ± 0.01	0.81 ± 0.00^{b}
Tannins (mg/100g)	4.35 ± 0.00	3.95 ± 0.01^{b}	4.50 ± 0.10^{a}
Flavonoids (mg/100g)	2.60 ± 0.10^{b}	2.81 ± 0.10	2.90 ± 0.10^{a}
Phenols (mg/100g)	1372.00 ± 1.00	1314.00 ± 1.00^{b}	1430 ± 2.00^{a}

Values are Mean ± SEM for three determinations

[a] Significantly ($p<0.05$) higher compared to the values in each row
[b] Significantly ($p<0.05$) lower compared to the values in each row

Table 3:-Percentage (%) inhibition of Ferric Reducing Antioxidant Power (FRAP) of Methanol Extract and Fractions of *D. microcarpum* Stem Bark.

Conc. (mg/ml)	ME	F I	F II	ASCO. ACID
20	27.95 ± 0.01[b]	56.01 ± 0.15[a]	36.45 ± 0.38	88.79 ± 0.13
40	37.25 ± 0.13[b]	58.68 ± 013	61.85 ± 0.26[a]	89.87 ± 0.11
60	56.59 ± 0.06[b]	59.22 ± 0.24	79.15 ± 0.00[a]	91.69 ± 0.17
80	70.80 ± 0.08[b]	73.14 ± 0.17	83.95 ± 0.19[a]	92.89 ± 0.95
100	79.80 ± 0.25	77.64 ± 0.06[b]	89.46 ± 0.17[a]	93.20 ± 1.46
IC_{50}	**63.25**	**59.69**	**57.03**	**40.18**

Values are Mean ± SEM for three determinations
1. Significantly ($p<0.05$) higher compared to the test samples at the same concentration
2. Significantly lower to other samples at same concentration

Key: Asco. Acid= L-ascorbic acid, ME = Methanol Extract, F I = Fraction I and F II = Fraction II

Table 4:-DPPH Radical Scavenging Activity (% Inhibition) of Methanol Extract and Fractions of *D. microcarpum* Stem Bark.

Conc. (mg/ml)	ME	F I	F II	ASCO. ACID
20	20.13 ± 0.01[c]	29.58 ± 0.02	48.91 ± 0.01[a]	52.40 ± 0.01
40	30.81 ± 0.01[c]	58.23 ± 0.02[b]	75.61 ± 0.01[ab]	55.93 ± 0.01
60	54.75 ± 0.03[c]	70.69 ± 0.01[b]	83.87 ± 0.01[ab]	58.10 ± 0.01
80	68.19 ± 0.01[b]	77.88 ± 0.01[b]	86.77 ± 0.02[ab]	61.11 ± 0.01[c]
100	77.59 ± 0.02[b]	82.15 ± 0.01[b]	90.12 ± 0.02[ab]	64.76 ± 0.06[c]
IC_{50}	**60.66**	**50.91**	**44.01**	**60.64**

Values are Mean ± SEM for three determinations
1. Significantly ($p<0.05$) higher compared to the test samples at the same concentration
2. Significantly ($p<0.05$) higher compared to L-Ascorbic acid
3. Significantly lower to other samples at same concentration

Key: Asco. Acid= L-ascorbic acid, ME = Methanol Extract, F I = Fraction I and F II = Fraction II

Table 5:-Percentage (%) Inhibitions of Thiobarbituric Acid Reactive Substances (TBARS) of Methanol Extract and Fractions of *D. microcarpum* Stem Bark.

Conc. (mg/ml)	ME	F I	F II	ASCO. ACID
20	61.70 ± 0.02[b]	63.14 ± 0.04	74.76 ± 0.03[a]	84.17 ± 0.02
40	71.53 ± 0.03	64.46 ± 0.06[b]	80.18 ± 0.06[a]	87.76 ± 0.06
60	72.44 ± 0.02	67.83 ± 0.03[b]	83.27 ± 0.03[a]	88.79 ± 0.03
80	73.14 ± 0.04	72.26 ± 0.03[b]	85.31 ± 0.03[a]	89.80 ± 0.05
100	75.63 ± 0.03	72.49 ± 0.01[b]	85.45 ± 0.05[a]	90.47 ± 0.03
IC_{50}	**50.31**	**52.53**	**43.88**	**42.63**

Values are Mean ± SEM for three determinations
1. Significantly ($p<0.05$) higher compared to the test samples at the same concentration
2. Significantly lower to other samples at same concentration

Key: Asco. Acid= L-ascorbic acid, ME = Methanol Extract, F I = Fraction I and F II = Fraction II

Discussion:-

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack (Nyamai *et al*., 2016). These compounds are known as secondary plant metabolites and provide health benefits to humans. They are thought to act as synergistic agents, allowing nutrients to be used more efficiently by the body. Some of the beneficial roles of phytochemicals are low toxicity, low cost, easy availability and their biological properties such as antioxidant activities, antimicrobial effects, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and antineoplastic

properties (Andre *et al.*, 2010). Phytochemicals are not essential nutrients and are not required by the human body for sustaining life, but have important properties to prevent or to fight some common diseases (Holst and Williamson, 2008).

Phytochemical screening of methanol extract, fraction I and fraction II of *D. microcarpum* were carried out and the result revealed the presence of alkaloids, terpenoids, reducing sugar, saponins, tannins, flavonoids, phlobatanins, anthraquinones, phenols and cardiac glycoside. These results are in harmony with the previous reports by Yakubu, (2015), Reuben *et al*., (2016) all on stem bark of D. microcarpum.

The quantity or content of alkaloids, saponins, tannins, flavonoids and phenols were determined in methanol extract, fraction I and fraction II as indicated in table 2. The differences in the structure of phytochemical compounds also determine their solubility in solvents of different polarity as observed in this study. Therefore the type of extraction solvent as well as the isolation procedures may have a significant impact on the yield of extraction from the plant materials. It is well known that solvent polarity do play a key role in increasing phytochemicals solubility (Michiels *et al.*, 2012).

Antioxidants are chemicals that interact with and neutralize free radicals, thus preventing them from causing damage. Antioxidants are also known as “free radical scavengers.” The body makes some of the antioxidants it uses to neutralize free radicals. These antioxidants are called endogenous antioxidants. However, the body relies on external (exogenous) sources, primarily the diet, to obtain the rest of the antioxidants it needs. These exogenous antioxidants are commonly called dietary antioxidants. Fruits, vegetables, and grains are rich sources of dietary antioxidants. Some dietary antioxidants are also available as dietary supplements (Bouayed and Bohn, 2010).

Antioxidant compounds like phenolic acids, polyphenols, tannins, saponins, alkaloids and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Ravi *et al*., 2018). Studies on the antioxidant activities of methanol extract of D. microcarpum stem bark and its fractions (fraction I and fraction II) showed that the plant exhibited high antioxidant activity when compared to L-ascorbic acid being a standard antioxidant.

The antioxidant activity of methanol extract, fraction I and fraction II of *D. microcarpum* stem bark using Ferric Reducing Antioxidant Power (FRAP) is as shown in Table 3. The half maximal inhibitory concentration (IC_{50}) of methanol extract, fraction I, fraction II and L-ascorbic acid were found to be 63.25 mg/ml, 59.69 mg/ml, 57.03 mg/ml and 40.18mg/ml respectively. Fraction II exhibited high antioxidant activity than the methanol extract and fraction I. Fraction II has the most potent reducing power and thus, almost equivalent to ascorbic acid.

The antioxidant activity of methanol extract, fraction I and fraction II of *D. microcarpum* stem bark using DPPH radical scavenging activity is as shown in Table 4. The half maximal inhibitory concentration (IC_{50}) of methanol extract, fraction I, fraction II and L-ascorbic acid were found to be 60.66 mg/ml, 50.91 mg/ml, 44.01 mg/ml and 60.64 mg/ml, respectively. Fraction II exhibited high antioxidant activity than methanol extract and fraction I. Fraction II shows profound reducing activity against stable free radicals and even more than the standard antioxidant used (L-ascorbic acid).

The antioxidant activity of methanol extract, fraction I and fraction II of *D. microcarpum* stem bark using Thiobarbituric Acid Reactive Substances (TBARS) is as shown in Table 5. The half maximal inhibitory concentration (IC_{50}) of methanol extract, fraction I, fraction II and L-ascorbic acid were found to be 50.31 mg/ml, 52.53 mg/ml, 43.88 mg/ml and 42.63 mg/ml respectively. Fraction II exhibited high antioxidant activity than methanol extract and fraction I. It has potent reducing power and almost equivalent to L-ascorbic acid. The antioxidant activity has been attributed to various mechanisms such as prevention of chain initiation, the binding of transition metal ion catalysts, decomposition of peroxides, the prevention of continued hydrogen abstraction, the reductive capacity and radical scavenging and the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity as reported by Sutharsingh *et al*., (2011).

Conclusion:-

It can be concluded that the extract and its fractions are good sources of antioxidant phytochemicals.

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