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Antioxidant activity of *Guizotia abyssinica* (L. f.) Cass (Niger or Ramtil)

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Abstract: *Guizotia abyssinica (L. f.) Cass. commonly known as Niger or Ramtil belongs to family Asteraceae (Compositae). It is an oilseed crop cultivated in Ethiopia and India. It constitutes about 50% of Ethiopian and 3% of Indian oilseed production. In Ethiopia, it is cultivated on waterlogged soils where most crops and all other oilseeds fail to grow and contributes a great deal to soil conservation and land rehabilitation. It is a moderately to well branched herb which grows up to 2 m tall. The seed contains about 40% oil with fatty acid composition of 75-80% linoleic acid, 7-8% palmitic and stearic acids, and 5-8% oleic acid. The Indian Niger contain 25% oleic and 55% linoleic acids.*

In the present investigation the antioxidant activity of the methanol and n-hexane extracts of leaves and seeds of Guizotia abyssinica in vitro has been assayed. The results revealed that methanol and hexane extracts of leaves and seeds of G. abyssinica caused bleaching of the purple colour of DPPH radical that caused the development of pale spots over a purple background. This indicated that the extracts contained some active phytochemicals exhibiting antioxidant activities. The DPPH scavenging activity of the methanol and n-hexane extracts (0.5 -2.5 mg/ml) exhibited concentration-dependent free radical scavenging activity. The extracts (0.5-2.5 mg/ml) and the standard antioxidant n-propyl gallate (3.0 mg/ml) caused a concentration- dependent reduction of Fe3+ to Fe2+. The extracts (0.5 – 2.5 mg/ml) and n-propyl gallate (3.0 mg/ml) caused a concentration- dependent inhibition of linoleic acid auto-oxidation.

On the basis of the present results it can be concluded that the methanol and n-hexane extracts of leaves and seeds of Guizotia abyssinica possess significant antioxidant activity.

Key Words: *Guizotia abyssinica, Antioxidant, DPPH, Linoleic acid, Leaves, Seeds*

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I. Introduction

Botanical profile: *Guizotia abyssinica* (L. f.) cass. commonly known as Niger or Ramtil belongs to family Asteraceae (Compositae). It is an oilseed crop cultivated in Ethiopia and India. It constitutes about 50% of Ethiopian and 3% of Indian oilseed production. In Ethiopia, it is cultivated on waterlogged soils where most crops and all other oilseeds fail to grow and contributes a great deal to soil conservation and land rehabilitation.

It is a moderately to well branched herb which grows up to 2 m tall. The seed contains about 40% oil with fatty acid composition of 75-80% linoleic acid, 7-8% palmitic and stearic acids, and 5-8% oleic acid (Getinet and Teklewold, 1995) [1]. The Indian types contain 25% oleic and 55% linoleic acids (Nasirullah *et al*., 1982) [2]. The meal remaining after the oil extraction is free from any toxic substance but contains more crude fiber than most oilseed meals.

Germination is epigeal and seedlings have pale green to brownish hypocotyls and cotyledons (Seegeler, 1983) [3]. The cotyledons remain on the plant for a long time. The first leaf is paired and small and successive leaves are larger. The leaves are arranged on opposite sides of the stem; at the top of the stem leaves are arranged in an alternate fashion. Leaves are 10-20 cm long and 3-5 cm wide. The leaf margin morphology varies from pointed to smooth and leaf colour varies from light green to dark green, the leaf surface is smooth. The stem of Niger is smooth to slightly rough and the plant is usually moderately to well branched. Niger stems are hollow and break easily. The number of branches per plant varies from five to twelve and in very dense plant stands fewer branches are formed. The colour of the stem varies from dark purple to light green and the stem is about 1.5 cm in diameter at the base. The plant height of Niger is an average of 1.4 m, but can vary considerably as a result of environmental influences and heights of up to 2 m have been reported from the Birr valley of Ethiopia. The Niger flower is yellow and, rarely, slightly green. The heads are 15-50 mm in diameter with 5-20 mm long ray florets. Two to three capitulae (heads) grow together, each having ray and disk florets. The

receptacle has a semi-spherical shape and is 1-2 cm in diameter and 0.5-0.8 cm high. The receptacle is surrounded by two rows of involucral bracts. The capitulum consists of six to eight fertile female ray florets with narrowly elliptic, obovate ovules. The stigma has two curled branches about 2 mm long. The hermaphrodite disk florets, usually 40-60 per capitulum, are arranged in three whorls. The disk florets are yellow to orange with yellow anthers, and a densely hairy stigma. The achene is club-shaped, obovoid and narrowly long (Seegeler, 1983) [3]. The head produces about 40 fruits. The achenes are black with white to yellow scars on the top and base and have a hard testa. The embryo is white. Niger is usually grown on light poor soils with coarse texture (Chavan, 1961) [4]. It is either grown as a sole crop or intercropped with other crops. When intercropped it receives the land preparation and cultivation of the main crop. In Ethiopia it is mainly cultivated as a sole crop on clay soils and survives on stored moisture. A more detailed description on the agronomy of Niger is presented under Agronomy.

In Africa, *G. abyssinica* is largely found in the Ethiopian highlands, particularly west of the Rift Valley. Niger is also found in some areas in Sudan, Uganda, Zaire, Tanzania, Malawi and Zimbabwe, West Indies, Nepal, Bangladesh, Bhutan and India (Weiss, 1983) [5].

India is the largest producer and exporter of Niger (Chavan, 1961) [4]. It is cultivated in Andra Pradesh, Madhya Pradesh, Orissa, Maharashtra, Bihar, Karnataka, Nagar Haveli and West Bengal states of India of which Madhya Pradesh is the largest. During 1938 to 1948 India exported up to 6968 tons of Niger annually to Western Europe, Eastern Europe and North America.

Systematic Position: Class: Dycotyledons; Sub class: Gamopetalae; Series: Inferae; Order: Asterales; Family: Compositae (Syn. Asteraceae); Genus: *Guizotia*; Species: *G. abyssinica*

Oil Content of Niger: The oil content of Niger seed varied from 30 to 50% (Seegeler, 1983) [3]. Niger meal remaining after the extraction of oil contains approximately 30% protein and 23% crude fiber. In general the Ethiopian niger meal contains less protein and more crude fiber than the Niger meal grown in India (Chavan 1961; Seegeler 1983) [4, 3]. The oil, protein and crude fiber contents of Niger are affected by the hull thickness and thick-hulled seeds tend to have less oil and protein and more crude fiber. Niger oil has a fatty acid composition typical for seed oils of the plants of Compositae family (e.g. safflower and sunflower) with linoleic acid being the dominant fatty acid. The linoleic acid content of Niger oil was approximately 55% in seeds grown in India (Nasirullah *et al.*, 1982) [2] and 75% in seeds grown in Ethiopia (Seegeler, 1983; Getinet and Teklewold, 1995) [3, 1].

Ranges of Fatty acid composition (%) of Indian and Ethiopian Niger oil as determined by Nasirullah et al., 1982; Nagaraj, 1990; Getinet and Teklewold, 1995 are as follow:

Fatty acid	India[1]	India[2]	Ethiopia[3]
Palmitic acid	8.2-8.7	6.0-9.4	7.6-8.7
Stearic acid	7.1-8.7	5.0-7.5	5.6-7.5
Oleic acid	25.1-28.9	13.4-39.3	4.8-8.3
Linoleic acid	51.6-58.4	45.4-65.8	74.8-79.1
Linolenic acid	-	-	0.0-0.9
Arachidic acid	0.4-0.6	0.2-1.0	0.4-0.8
Behenic acid	-	-	0.4-1.5

1. Nasirullah *et al*., 1982 [2]. Nagaraj, 1990 [6]. Getinet and Teklewold, 1995 [1].

Dutta *et al.*, (1994) [7] studied the lipid composition of three released and three local cultivars of Ethiopian niger. Most of the total lipid was triacylglycerides and polar lipids accounted for 0.7-0.8% of the total lipid content. The amount of total tocopherol was 720-935 µg/g oil of which approximately 90% was a-tocopherol, 3-5% was g-tocopherol and approximately 1% was ß-tocopherol. As a-tocopherol is an anti-oxidant, high levels of a-tocopherol could improve stability of niger oil. The total sterol consists of ß-sitosterol (38-43%), campesterol (~14%), stigmasterol (~14%), D5 avenasterol (5-7%) and D7 avenasterol (~4%). The amino acid composition of niger protein was deficient in tryptophan. The protein quality of Ethiopian niger was evaluated using chemical score and essential amino acid requirement score (Haile, 1972) [8]. Using chemical score and whole egg protein as a standard, methionine, lysine, cystine, isoleucine and leucine were considered as limiting amino acids. When essential amino acids were used as a reference, lysine was the limiting amino acid. A lipoprotein concentrate was isolated from niger seed using hot water/ethanol sodium chloride solution extraction (Eklund 1971a, 1971b) [9, 10]. The lipoprotein contained 4% moisture, 12% ash, 46% protein, 20% fat, 7% crude fibre and 11% soluble carbohydrate. From the amino acid composition Eklund (1971a, 1971b) [9, 10] calculated nitrogen to protein conversion ratio of 5:9. The energy content of the niger lipoprotein concentrate was 400 kcal/100 g.

Amino acid composition of whole niger flour, Niger seed lipid concentrate, high temperature soluble (HTS) fraction concentrate, Indian Niger cake, and Ethiopian Niger meal as determined by Eklund (1974) [11], Mohan *et al*., (1983) [12] and Haile (1972) [8] are as follows:

Amino acid	Whole niger seed flour[1]	Niger seed lipid-protein concentrate	HTS Fraction[1]	Niger cake[2]	Niger meal (% of protein)3
Isoleucine	**307**	**341**	**201**	**349**	**4.66**
Leucine	**388**	**505**	**308**	**589**	**6.99**
Lysine	**294**	**279**	**199**	**335**	**4.74**
Methionine	**109**	**125**	**216**	**148**	**2.06**
Cytisine	**177**	**97**	**537**	**138**	**1.40**
Phenylelanine	**327**	**385**	**130**	**378**	**4.80**
Tyrosine	**185**	**225**	**138**	**197**	-
Threonine	**237**	**263**	**112**	**278**	**3.73**
Tryptophan	**54**	**85**	**65**	-	-
Valine	**362**	**397**	**273**	**428**	**5.76**
Arginine	**621**	**627**	**734**	**889**	**9.36**
Histidine	**162**	**192**	**97**	**190**	-
Alanine	**281**	**290**	**132**	**335**	**4.06**
Aspartic acid	**619**	**673**	**427**	**823**	**9.49**
Glysine	**375**	**357**	**295**	**502**	**5.53**
Proline	**262**	**370**	**222**	**354**	**3.86**
Serine	**347**	**390**	**390**	**456**	**6.19**

1. **Eklund (1974), Samples from Ethipia (mg/g N.);**
2. **Mohan *et al*., (1983). Based on samples from India (mg/g N)**
3. **Haile (1972) based on samples from Ethiopia (% of protein).**

The Niger plant is consumed by sheep but not by cattle, to which only Niger silage can be fed (Chavan, 1961) [4]. Niger is also used as a green manure for increasing soil organic matter. Niger seed is used as a human food. The seed is warmed in a kettle over an open fire, crushed with a pestle in a mortar and then mixed with crushed pulse seeds to prepare 'wot' in Ethiopia (Seegeler, 1983) [3]. 'Chibto' and 'litlit' are prepared from crushed Niger seed mixed with roasted cereals, and is the preferred food for young boys. In Ethiopia, Niger is mainly cultivated for its edible oil. The pale yellow oil of Niger seed has a nutty taste and a pleasant odour. The traditional method for extraction of oil from Niger in Ethiopia is through a combination of warming, grinding and mixing with hot water followed by centrifugation in an 'ensera' (a container made of clay). After an hour of centrifugation by hand on a smooth soft surface the pale yellow oil settles over the meal. Niger is also crushed in small cottage expellers and large oil mills. The small, electrically powered cottage expellers are manufactured as different brands with varying capacities in Addis Abeba and Nazreth in Ethiopia. The meal remaining after extraction of the oil using Ethiopian expellers contains 6-12% oil depending on the expeller. Many expellers are found in the provinces of Arsi, Bale, Gojam, Gonder, Shoa and Wellega of Ethiopia.

Pharmacological properties:

In India the oil is extracted by bullock-powered local 'ghanis' and rotary mills (cottage expellers) or in mechanized expellers and hydraulic presses in large industrial areas. The Niger oil is used for cooking, lighting, anointing, painting and cleaning of machinery (Chavan 1961; Patil and Joshi 1978; Patil and Patil 1981) [4, 13, 14]. Niger oil also is a substitute for sesame oil for pharmaceutical purposes and can be used for soap-making. The meal remaining after the oil extraction contains about 24% protein and 24% crude fiber (Seegeler, 1983) [3]. Niger meal from India contains higher protein (30%) and lower crude fiber (17%) levels than meal from Ethiopia. Niger cake replacing linseed cake at levels of 0, 50 and 100% was fed as a nitrogen supplement for growing calves (Singh *et al*., 1983) [15]. No significant differences in growth rate, feed efficiency and dry matter digestibility were noticed between Niger and linseed cake and it was concluded that Niger cake can replace linseed cake in calf rations (Singh *et al.*, 1983) [15]. Similarly, four levels of Niger cake (0, 50, 75 and 100%) replacing groundnut cake were fed to large White Yorkshire pigs for 9 weeks (Roychoudhury and Mandal 1984) [16]. There was no significant difference in weight gain between rations containing either Niger or groundnut cake. Niger lipoprotein concentrate was fed to growing rats as a sole protein source for 90 days and no negative effects on growth rate were observed (Eklund, 1971b) [10]. A Niger-based agar medium can be used to distinguish *Cryptococcus neoformans* (Sant) Vaill, a fungus that causes a serious brain ailment, from other fungi (Paliwal and Randhawa 1978) [17]. There are reports that Niger oil is used for birth control and for the treatment of syphilis. Niger sprouts mixed with garlic and 'tej' are used to treat coughs.

Bioactive Compounds of Niger: The GC-MS analysis by Chambhare *et al*., (2017) [18] showed the presence of potential bioactive compounds; most of these are fatty acids which are known to have antimicrobial properties. These constituents include, β-ocimene, 1-Dodecene, n-hexadecanoic acid, stigmasterol, 24S 5-stigmast-7-en-3-ol; 9,12-octadecadienoic acid (Z,Z)-methyl ester; trans-13-octadecanoic acid;tetradecanoic acid, hexadecanoic acid, octadecanoic acid.

Metabolic processes in the body generate highly reactive species, known as free radicals, which injure cellular molecules. Free radicals are highly reactive atomic or molecular species that contain an unpaired electron (Halliwell and gutteridge, 1992) [19] which contributes to their high reactivity. Free radicals react quickly with the nearest stable molecule to capture the electron they need to gain stability. The "injured" molecule loses its electron, becoming a free radical itself. They can damage vital cellular components like nucleic acids, cell membranes and mitochondria, resulting in subsequent cell death. As all aerobic organisms utilize oxygen during cellular respiration and normal metabolism, the generation of free radicals by biochemical cellular reactions and from the mitochondrial electron transport chain is inevitable (Buonocore and Groenendaal, 2007) (20]. The free radicals include reactive oxygen and nitrogen species such as superoxide ($O2.^-$), hydroxyl (OH.)-, peroxyl (ROO-), peroxinitrite ($\cdot ONOO^-$) and nitric oxide (NO•) radicals. All these are produced through oxidative processes within the mammalian body (Abdel-Hameed, 2009) [21]. They may also be generated through environmental pollutants such as cigarette smoke, automobile exhaust fumes, radiation, air pollution and pesticides (Aquil *et al*., 2006; Tivwari, 2001) [22, 23]. To protect the cells and organ systems of the body against reactive oxygen and nitrogen species, humans have evolved a highly sophisticated and complex antioxidant protection system, that functions interactively and synergistically to neutralize free radicals. These antioxidants are capable of stabilizing or deactivating, free radicals before they attack cells. Antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase destroy toxic peroxides. In addition to antioxidant enzymes, non-enzymatic molecules play important roles in antioxidant defence systems. These non- enzymatic molecules are of an exogenous nature and are obtained from foods. They include *α*-tocopherol, *β*-carotene, and ascorbic acid, and such micronutrient elements as zinc and selenium (Aqil *et al*., 2006) [22]. Normally, there is a balance between free radical generation and scavenging (Beris, 1991) [24]. Oxidative stress results from an imbalance between excessive generation of oxidant compounds and insufficient anti-oxidant defence mechanisms (Sies, 1997) [25]. When the natural antioxidant mammalian mechanism becomes inadequate, the excess of free radicals can damage both the structure and function of cell membranes in a chain reaction leading to degenerative diseases and conditions such as Alzheimer"s disease, cataracts, acute liver toxicity, arteriosclerosis, nephritis, diabetes mellitus, rheumatism and DNA damage which can lead to carcinogenesis (Abdel-Hameed, 2009) [21].

All cells in eukaryotic organisms contain powerful antioxidant enzymes. Endogenous antioxidants made in the body are believed to be more potent in preventing free radical damage than exogenous antioxidants. The major classes of endogenous antioxidant enzymes are the superoxide dismutases, catalases and glutathione peroxidases (Sies, 1997) [25], *α*-lipoic acid and coenzyme Q10. In addition, there are numerous specialized antioxidant enzymes reacting with and, in general, detoxifying oxidant compounds.

Superoxide dismutases are present in almost all aerobic cells and in extracellular fluids (Johnson and Giulivi, 2005) [26]. Superoxide dismutase enzymes contain metal ion cofactors that, depending on the isozyme, can be copper, zinc, manganese or iron. They catalyse the breakdown of the superoxide anion into oxygen and hydrogen peroxide (Zelko *et al*., 2002) [27]. Catalases, on the other hand, are enzymes that catalyse the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor (Chelikani *et al*., 2004) [28].

In the present investigation *in vitro* antioxidant activity of methanol and n-hexane extracts of leaves and seeds of *Guizotia abyssinica* was assayed.

II. Materials and Methods

Preparation of methanol and hexane extract: The leaves and seeds of *Guizotia abyssinica* were collected from Niger growing fields of Arap, Patna. These were washed with water and air-dried at room temperature for 7 days, then oven-dried at 40ºC to remove the residual moisture. The leaves and seeds were pulverized and stored in air-tight container for future use. Methanol (polar solvent) and n-hexane (non polar solvent) were used as solvent for *in vitro* antioxidant assay. Equivalent amount of powdered samples of leaves and seeds were extracted with methanol and n-hexane at room temperature for 3 days. Extraction was done in water bath at 60ºC. About 1 gm of the dried sample was added into the test tubes containing 5 ml of solvent (methanol and n-hexane) separately and was extracted at room temperature. The sample was homogenized with extraction buffer. The supernatant was collected after three rounds of extraction. The solvent was evaporated under reduced pressure in a rotary evaporator at 40^0C. To this thick paste colloidal silicon dioxide was added and dried in

vacuum tube dryer. The methanol and n-hexane extracts of leaves and seeds thus obtained were then stored separately in deep freezer at -20^{0}C until further test.

Antioxidant assay:

***In vitro* qualitative DPPH test:** The qualitative test for antioxidant activity was performed using the rapid DPPH radical scavenging assay (Cuendet *et al.,* 1997) [29]. 10 µl of the leaf and seed extracts were applied on silica gel plates 60 F254 (Merck, 0.25 mm thick) and allowed to dry completely. The plate was then sprayed with a solution of 2% DPPH in methanol. A pale yellow to white spot over a purple background indicated a radical scavenging activity of the particular extract.

Quantitative antioxidant assays of extracts

Reducing power assay: Reducing activity of the methane and n-hexane extracts was assayed as follows. Different concentrations (0.5 – 2.5 mg/ml) of both the extracts as well as the standard drug *n*-propyl gallate (3.0 µg/ml) were prepared in aqueous methanol (50% v/v) and n-hexane (50%v/v) separately and 1 ml each was taken into test tubes in triplicates. To the test tubes, 2.5 ml of sodium phosphate buffer (pH 7.0) and 2.5 ml of 1% potassium ferric cyanide solution was added. The contents were mixed well and incubated at 50^{0}C for 20 minutes. After incubation, 2.5 ml of 10% trichloroacetic acid was added and the mixture centrifuged at 3000 revolutions per minute for 10 minutes. After centrifugation 2.5 ml of supernatant was added to 2.5 ml of distilled water. To this about 1 ml of 0.1% ferric chloride was added. The absorbance was then recorded at 700 nm. A graph of absorbance was then plotted against the concentration of the extracts. Increase in absorbance was indicative of higher reducing power of the extract.

Scavenging of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical assay: The free radical scavenging activity of methanol and n-hexane extract was determined as follows. 1 ml each of the extracts (0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml in methanol and n-hexane) was added to 3 ml methanolic solution of DPPH solution (20 mg/l) in a test tube. The reaction mixture was kept at 25°C for 30 mins. The absorbance of the residual DPPH was determined at 517 nm in a spectrophotometer (Cecil CE 7200 spectrophotometer, Cecil instrument limited, Milton Technical Centre, England). One milliliter (1 ml) methanol (50%) and n-hexane (50%) was added separately to 3 ml DPPH solution, incubated at 25 °C for 30 minutes and used as control. *n*-propyll gallate (3.75-30 µg/l) was used as a standard free radical scavenger. The absorbance decreases with increasing free radical scavenging ability. Results were expressed as percentages of blank (100%). The concentration required to cause a 50% decrease in the absorbance was calculated (EC50). Each test was carried out using three replicates. The % DPPH scavenging effect (% of control) of the antioxidant was calculated as follows:

$$\% \text{ DPPH scavenging effects} = (Ac - At)/Ac \times 100$$

Where

Ac = Absorbance of the control; At = Absorbance of the test drug/ extracts

Total antioxidant capacity assay: The assay is based on the reduction of molybdenum, Mo +6 to Mo +5, by the extracts and subsequent formation of a green phosphate-molybdate (Mo +5) complex at acidic pH [26]. Test tubes containing l ml each of the extracts in five different concentrations (0.5-2.5 mg/ml) and 3 ml of the reagent solution (0.6 M sulphuric acid, 28 mM disodium phosphate and 4 mM ammonium molybdate) were incubated at 95^{0}C for 90 minutes. After the mixture had cooled to room temperature, the absorbance of the solution was measured at 695 nm. Four concentrations of ascorbic acid (0.025, 0.05, 0.1 and 0.2 mg/ml) was used to construct a calibration curve. A blank solution was prepared by adding every other solution but without extract or standard drug. The antioxidant capacity was expressed as mg of ascorbic acid equivalent (AAE) per g of extract. This procedure was used for both methanol and n-hexane extracts.

Determination of total phenolic content: The presence of phenol in the methanol and n-hexane extracts of leaves and seeds of *Guizotia abyssinica* was determined qualitatively using ferric chloride test. An intense positive colouration indicating the presence of phenols led to further quantification of total soluble phenols in the extract. The total phenol in the extract was determined by spectrophotometric assay using the Folin-Ciocalteu‟s reagent as described by Singleton *et al.,* (1999) [30] using tannic acid as standard.

1ml of the extracts (0.25-2 mg/ml) in distilled water was added to 1 ml Folin-Ciocalteu's reagent in a test tube. The content of the test tube was mixed and allowed to stand for five minutes at 25^0 C in an incubator. 1 ml of sodium bicarbonate solution (2%) was added to the mixture. The reaction mixture was allowed to stand for 2 hours with shaking at 25^{0}C in an incubator. The mixture was then centrifuged at 3000 rpm for 10 minutes and absorbance of the supernatant determined at 760 nm. Three replicates were prepared for each concentration of tannic acid and extracts. 1 ml distilled water was added to 1 ml Folin-Ciocalteu's reagent processed in the same

way as the test drugs and used as blank. Tannic acid was used as reference. Four concentrations of tannic acid (0.025, 0.05, 0.1, 0.2 mg/ml) were used to construct a calibration curve and the total phenols expressed as mg of tannic acid equivalents (TAE)/g of extract.

This method depends on the reduction of Folin-Ciocalteau reagent by phenols to a mixture of blue oxides which have a maximal absorption in the region of 760 nm. The reaction equation is as follows:

Folin: Mo^{+6} (yellow) + è (from antioxidant) → Mo^{+5} (blue)

Where the oxidizing reagent is a molybdophosphotungstic heteropolyacid comprised of $3H_2O{\cdot}P_2O_5{\cdot}13WO_3{\cdot}5MoO_3{\cdot}10H_2O$, in which the hypothesized active centre is Mo^{+6}.

Linoleic acid auto-oxidation assay: The extracts (0.5-2.5 mg/ml) in absolute alcohol were compared with *n*-propyl gallate (3.0 µgm/l) in absolute alcohol as a reference antioxidant. 2 ml of the extract, 2 ml of 2.5% linoleic acid in absolute ethanol, 4 ml of 0.05 M phosphate buffer (pH =7) and 1.9 ml of distilled water were put into test tubes with a screw cap and placed in an oven at 40^0C in the dark for 7 days. After the seven day period, 2 ml each of the extracts and standard drug was added to 20 % aqueous trichloroactetic acid solution and 1 ml of 0.6 % aqueous thiobarbituric acid solution. This mixture was placed in boiling water bath for 10 minutes and after cooling, was centrifuged at 3000 rpm for 10 minutes. The absorbance of the supernatant was measured 535 nm. Each test was carried out in three replicates. Percentage inhibition of lipid peroxidation by the test drugs was assessed by comparing the absorbance of the drug test with that of the control (linoleic acid mixture without any drug). Data was presented as percentage inhibition of lipid peroxidation against concentration. The % inhibition of linoleic acid autoxidation was calculated as follows:

$$\% \text{ inhibition} = 1 - \left(\frac{D - D_O}{C_O - C} \right)$$

Where

Co= (Full reaction mixture) is the degree of lipid peroxidation in the absence of antioxidant

C= is the underlying lipid peroxidation before the initiation of lipid peroxidation

D= is any absorbance produced by the extract/ linoleic acid mixture

Do= is the absorbance produced by the extract alone

Statistical analysis: All the experiments were conducted in replicates of three and data was recorded as mean value ± SE. The statistical analysis was performed by one way analysis of variance (ANOVA) and means were compared by least significance difference test ($P< 0.05$) using the SPSS statistical software package (SPSS, ver. 10.0; Chicago. IL, USA).

III. Results

Qualitative DPPH (2, 2-diphenyl-1-picrylhydrazyl) test for Antioxidant Activity: The methanol and n-hexane extracts of leaves and seeds of *Guizotia abyssinica* caused bleaching of the purple colour of DPPH radical that caused the development of pale spots over a purple background. This indicated that the extracts contained some active phytochemicals exhibiting antioxidant activities.

Quantitative antioxidant assay of methanol and n-hexane extracts: The methods used to determine quantitative antioxidant activity of the methanol and n-hexane extracts of leaves and seeds of *Guizotia abyssinica* included total phenolic content, total anti-oxidant capacity, reducing power, DPPH radical scavenging activity and linoleic acid autoxidation assays.

1. The total phenolic content of the methanol and n-hexane extracts was determined using the Folin- Ciocalteu's reagent and tannic acid was used as standard. The total phenolic content of the extracts was expressed as mg of tannic acid equivalents (TAE) per g of extract. The four different concentrations of each of the methanol and n-hexane extracts of leaves and seeds were used for quantitative assay. The total phenolic content in methanol and n-hexane extracts of leaves and seeds of Niger has been presented in Table-1; Fig-1 and 2.

Table-1: Total Phenolic content in extracts of leaves and seeds of *Guizotia abyssinica*

Concentration of methanol and n-hexane extract (mg/ml)	Leaf		Seed	
	Methanol extract	n-Hexane extract	Methanol extract	*n-Hexane extract*
	Mean mgTAE/g ±SE	Mean mgTAE/g ±SE	Mean mgTAE/g ±SE	Mean mgTAE/g ±SE
1.0	25.24 ±0.41	23.55 ±0.52	47.25±0.51	48.25±0.34
1.5	30.28 ±0.42	28.45 ±0.42	51.25±0.42	50.28±0.45
2.0	37.67±0.41	36.47±0.37	58.45±0.35	55.45±0.35
2.5	40.75±0.31	37.75±0.36	68.27±0.35	65.25±0.35

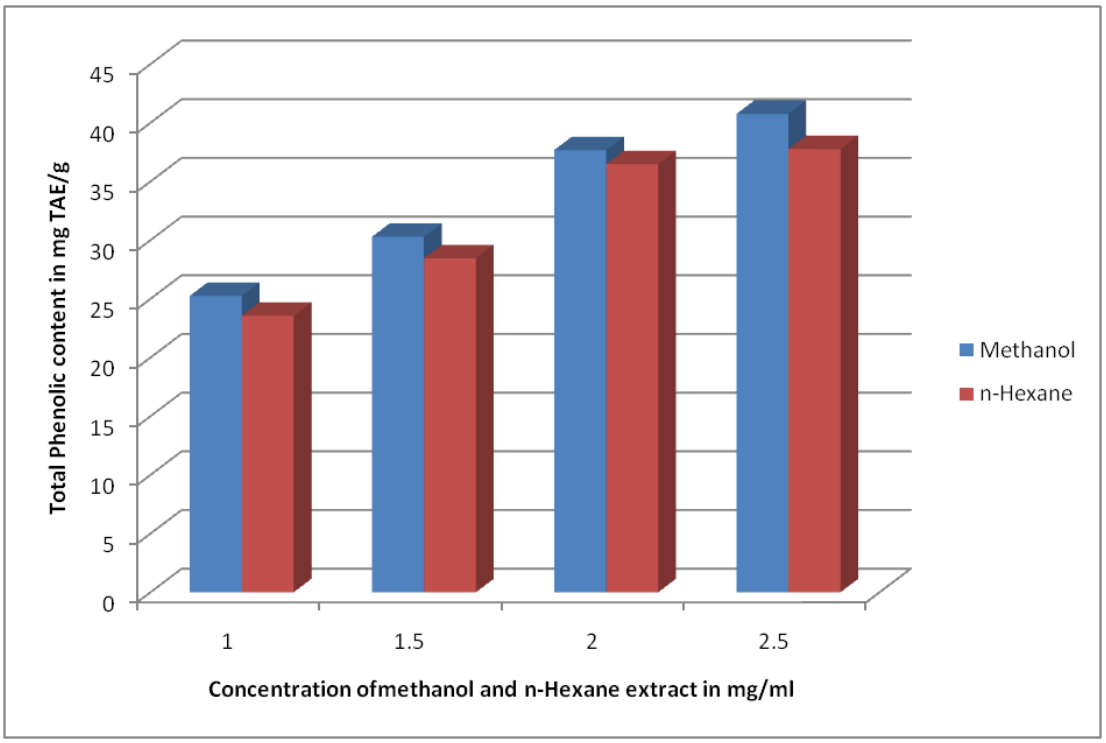

Figure-1: Phenolic content in extracts of leaves of *Guizotia abyssinica*

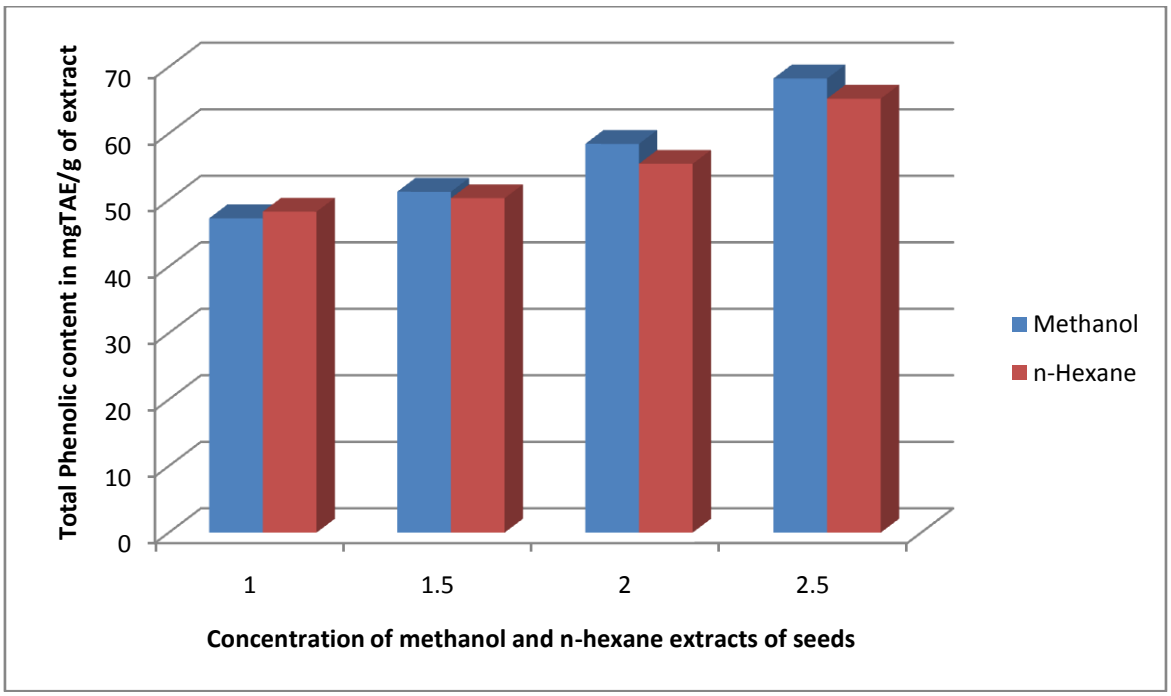

Figure-2: Phenolic content in extracts of seeds of *Guizotia abyssinica*

The results revealed that the leaves and seeds of *Guizotia abyssinica* contained appropriate phenolics. The methanol extracts contained relatively high concentration of phenolics in comparison to n-hexane extracts. The total phenolic content in 1.0 mg/ml of methanol extract of leaves of *Guizotia abyssinica* was 25.24 ± 0.41 TAE/g. Their concentration increased on increasing the concentration of methanol leaf extracts. At 2.5 mg/ml of methanol leaf extract the concentration of total phenolics was 40.75 ± 0.31 TAE/g. In n-hexane extract of leaves of *Guizotia abyssinica* 1.0 mg/ml, 1.5 mg/ml, 2.0 mg/ml and 2.5 mg/ml of the extracts contained 23.55±0.52 TAE/g, 28.45 ± 0.42 TAE/g, 36.47 ± 0.37 TAE/g and 37.75 ± 0.36TAE/g phenolics respectively (Table-1; Fig-1). The seed extracts of *Guizotia abyssinica* contained more phenolics. In sample of methanol seed extracts 1.0 mg/ml, 1.5 mg/ml, 2.0 mg/ml and 2.5 mg/ml of extract contained 44.25 ± 0.51 TAE/g, 51.25 ± 0.42 TAE/g, 58.45 ± 0.35 TAE/g and 68.27 ± 0.35 TAE/g respectively, whereas the same concentration of n-hexane extracts contained 48.25 ± 0.34 TAE/g, 50.28 ± 0.45 TAE/g, 55.45 ± 0.35 TAE/g and 65.25 ± 0.33 TAE/g of phenolics respectively (Table-1; Fig-2). The results clearly indicated that the seeds of *Guizotia abyssinica* contained more phenolic compounds in comparison to leaves.

2. Free Radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) Scavenging Activity: The results of the free radical scavenging potential of methanol and n-hexane extracts of leaves and seeds of *Guizotia abyssinica* using DPPH free radical scavenging method are depicted in Table-2 and Fig-3 and 4. The DPPH scavenging activity of the methanol and n-hexane extracts (0.5 -2.5 mg/ml) exhibited concentration-dependent free radical scavenging activity (Table- 2; Fig-3 and 4). Low free radical DPPH scavenging activity was observed at 0.5 mg/ml of methanol and n-hexane extracts of both leaves and seeds (IC_{50} 85.4 ± 0.021 µg/ml in methanol leaf extract, IC_{50}79.4 ± 0.021 µg/ml in n-hexane leaf extract, IC_{50} 81.4 ± 0.025 µg/ml in methanol seed extract IC_{50} 75.5 ± 0.026 µg/ml in n-hexane seed extract). The highest free radical scavenging activity was observed at concentration 2.5 mg/ml of both methanol and n-hexane extract (IC_{50} 935.5 ± 0.062 µg/ml in methanol leaf extract, IC_{50} 913.5 ± 0.084 µg/ml in n-hexane leaf extract, IC_{50} 925.7 ± 0.064 µg/ml in seed methanol extract and IC_{50} 918.5 ± 0.055µg/ml in seed n-hexane extract) (Table-2; Fig-3 and 4).

Table-2: DPPH scavenging activity of methanol and n-hexane extracts of leaves and seeds of *Guizotia abyssinica*

Dose of extract in mg/ml	Methanol leaf extract	n-Hexane leaf extract	Methanol seed extract	n-Hexane seed extract
	IC50 (µg/ml) ±SEM		IC50 (µg/ml) ±SEM	
0.5	85.4 ±0.021	79.4 ±0.021	81.4 ±0.025	75.5 ±0.026
1.0	235.5 ±0.082	227.5 ±0.073	235.5 ±0.065	228.5 ±0.065
1.5	565.7 ±0.084	564.5 ±0.071	566.5 ±0.064	554.5 ±0.075
2.0	736.5 ±0.082	723.5 ±0.062	727.5 ±0.083	723.5 ±0.065
2.5	935.5 ±0.062	913.5 ±0.084	925.7 ±0.064	918.5 ±0.055

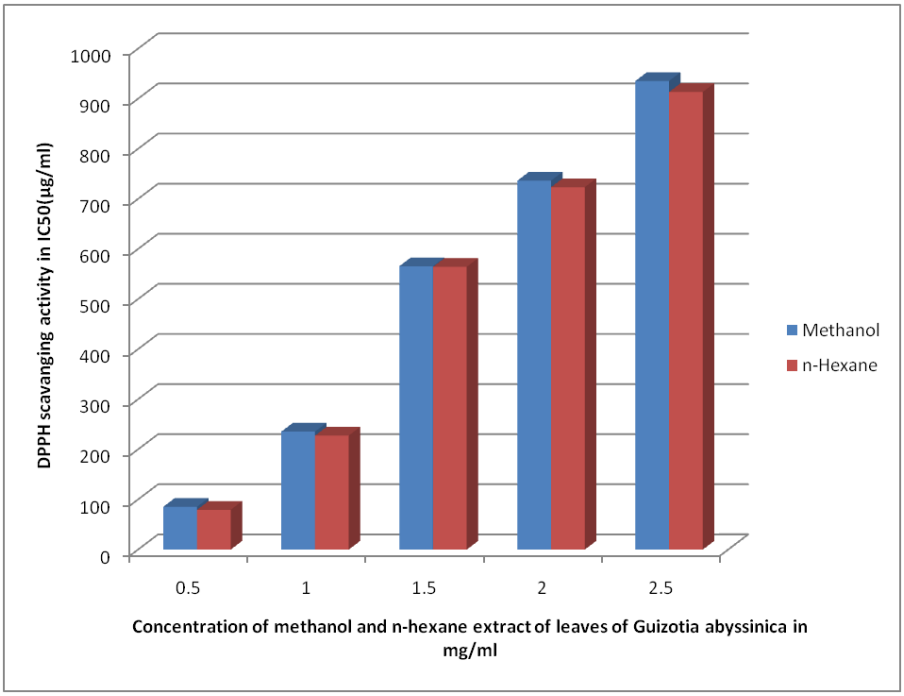

Fig-3: DPPH scavenging activity of methanol and n-hexane extracts of leaves of *Guizotia abyssinica*

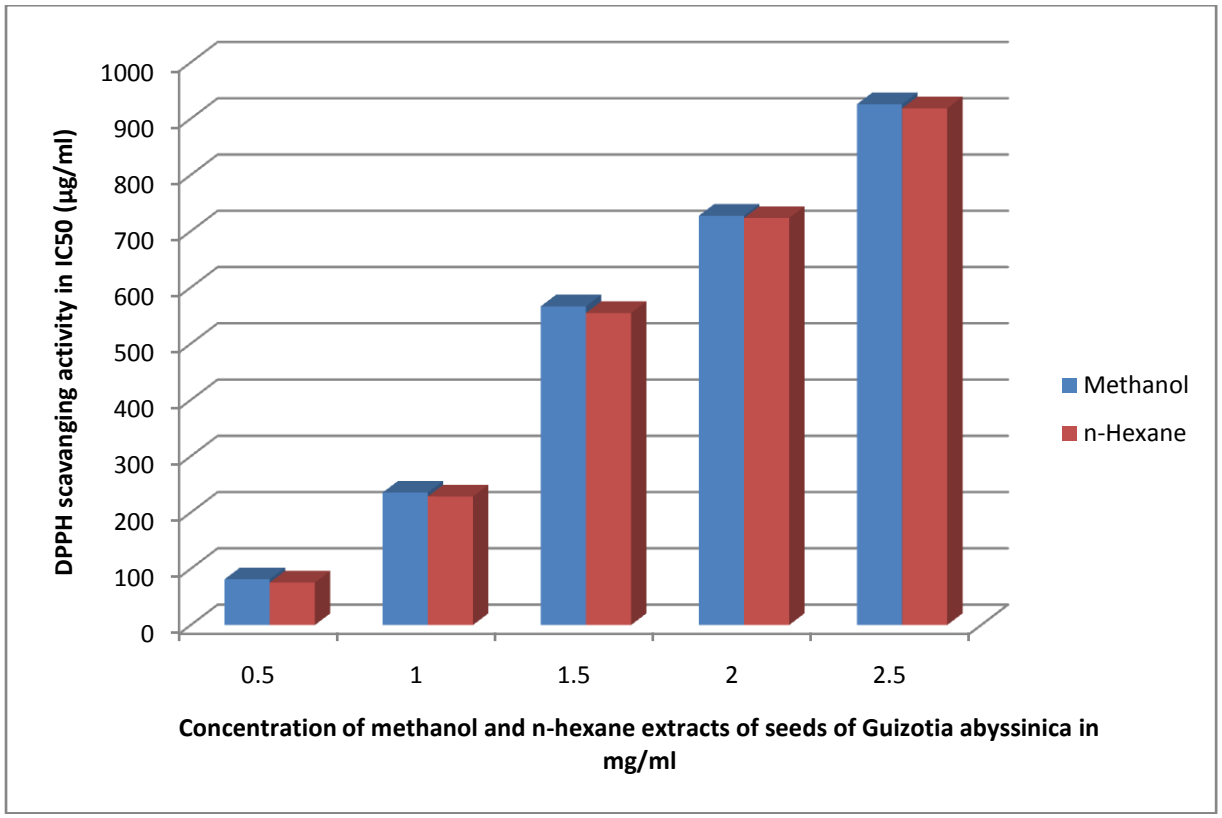

Fig-4: DPPH scavenging activity of methanol and n-hexane extracts of seeds of *Guizotia abyssinica*

3. Reducing power: The extracts (0.5-2.5 mg/ml) and the standard antioxidant *n*-propyl gallate (3.0 mg/ml) caused a concentration – dependent reduction of Fe3+ to Fe2+. From the IC50 values (Table-3; Fig-5 and 6), the methanol and n-hexane extracts of leaves and seeds of *Guizotia abyssinica* showed the highest reducing power. The reducing power activity of leaf extracts was relatively less in comparison to seed extracts. At concentrations of 0.5 mg/ml of methanol and n-hexane extracts of leaves the reducing power activity was IC_{50} 187.15 ± 0.014 µg/ml and IC_{50} 183.25 ± 0.013µg/ml respectively. In the same concentrations methanol and n-hexane extracts of seeds caused slightly higher reducing power activity (IC_{50}190.15 ± 0.013 µg/ml for methanol extract and IC_{50} 188.16 ± 0.023 µg/ml for n-hexane extract). Maximum reducing power activity was noticed for both methanol and n-hexane extract from leaves and seeds of *Guizotia abyssinica* at concentration of 2.5 mg/ml. At this concentration of extract the reducing power activity for methanol leaf extract, n-hexane leaf extract, methanol seed extract and n-hexane seed extract was IC_{50} 968.45 ± 0.035 µg/ml, IC_{50} 925.26 ± 0.015 µg/ml, IC_{50} 976.45 ± 0.031 µg/ml and IC_{50} 967.36 ± 0.027 µg/ml respectively (Table-3; Fig-5 and 6).

Table-3: Reducing power of methanol and n-hexane extracts of leaves and seeds of *Guizotia abyssinica*

Dose of extract in mg/ml	*Methanol leaf extract*	*n-Hexane leaf extract*	*Methanol seed extract*	*n-Hexane seed extract*
	IC_{50} (µg/ml) ±SEM	IC_{50} (µg/ml) ±SEM	IC_{50} (µg/ml) ±SEM	IC_{50} (µg/ml) ±SEM
0.5	**187.15 ±0.014**	**183.25 ±0.013**	**190.15 ±0.013**	**188.16 ±0.023**
1.0	**228.35 ±0.016**	**213.17 ±0.016**	**236.17 ±0.012**	**233.12 ±0.016**
1.5	**563.25 ±0.026**	**562.45 ±0.011**	**573.25 ±0.015**	**564.15 ±0.017**
2.0	**727.15 ±0.029**	**721.25 ±0.016**	**757.15 ±0.017**	**745.25 ±0.025**
2.5	**968.45 ±0.035**	**925.26 ±0.015**	**976.45 ±0.031**	**967.35 ±0.027**
3.0 n-PG	**70.25 ±0.003**	**70.25 ±0.003**	**70.25 ±0.003**	**70.25 ±0.003**

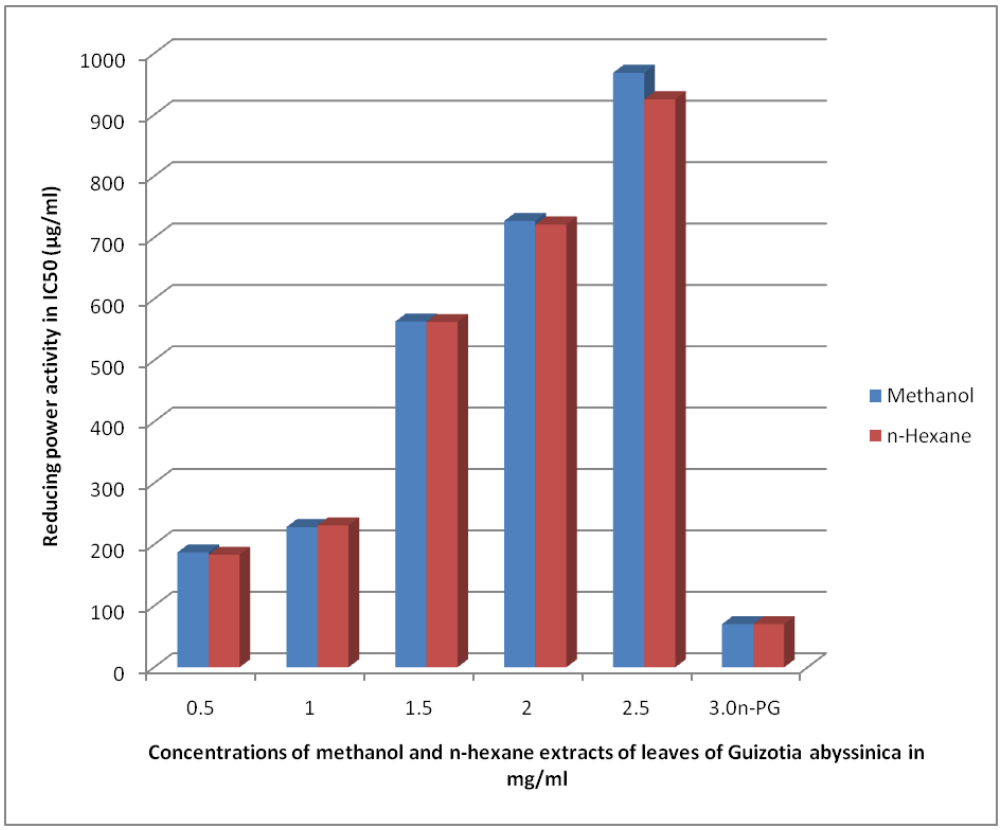

Fig-5: Reducing power activity of methanol and n-hexane extracts of leaves of *Guizotia abyssinica* in IC50 (µg/ml)

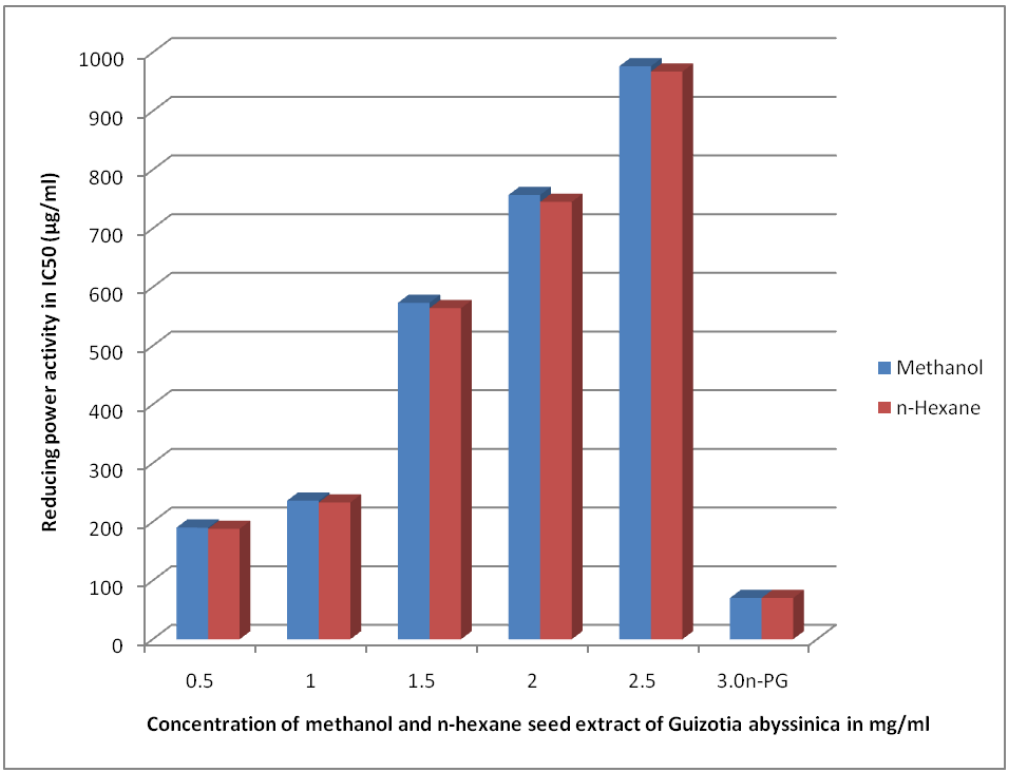

Fig-6: Reducing power activity of methanol and n-hexane extracts of seeds of *Guizotia abyssinica* in IC50 (µg/ml)

4. Lipid peroxidation: The ability of the methanol and n-hexane extracts and test drug to inhibit linoleic acid auto-oxidation was investigated. The extracts (0.5 – 2.5 mg/ml) and *n*-propyl gallate (3.0 mg/ml) caused a concentration- dependent inhibition of linoleic acid autoxidation (Table-4; Fig-7 and 8). The per cent inhibition of lipid peroxidation by methanol and n-hexane extracts of *guizotia abyssinica* increased with increasing the concentration of methanol and n-hexane extracts of both leaves and seeds. At concentration of 0.5 mg/ml of leaf and seed extracts caused minimum inhibition of lipid peroxidation (30.5 ± 0.15% by methanol leaf extract; 31.5 ± 0.13% by n-hexane leaf extract; 35.5 ±0.14% by methanol seed extract and 32.5 ±0.12% by n-hexane seed extract). Percent inhibition of lipid peroxidation increased on increasing the concentration of leaf and seed extracts. At concentration of 2.5 mg/ml all extracts caused maximum inhibition of lipid peroxidation. At this concentration methanol leaf extract, n-hexane leaf extract, methanol seed extract and n-hexane seed extract caused 95.5 ± 0.21%, 91.7 ± 0.14%, 98.5 ± 0.20% and 93.5 ± 0.21% inhibition of lipid peroxidation respectively (Table-4; Fig-7 and 8).

Table-4: % inhibition of lipid peroxidation by methanol and n-hexane extracts of leaves and seeds of *Guizotia abyssinica*

Dose of extract in mg/ml	*Methanol leaf extract*	*n-Hexane leaf extract*	*Methanol seed extract*	*n-Hexane seed extract*
	% inhibition of lipid peroxidation ±SEM	% inhibition of lipid peroxidation ±SEM	% inhibition of lipid peroxidation ±SEM	% inhibition of lipid peroxidation ±SEM
0.5	30.5±0.15	31.5±0.13	35.5±0.14	32.5±0.12
1.0	55.6 ±0.13	55.5 ±0.12	62.5 ±0.16	61.6 ±0.17
1.5	82.7±0.21	82.5±0.11	88.4±0.15	85.5±0.16
2.0	92.2 ±0.21	89.2 ±0.15	95.5 ±0.21	91.4 ±0.15
2.5	95.5 ±0.21	91.7 ±0.14	98.5 ±0.20	93.5 ±0.21
3.0 n-PG	65.5 ±0.12	65.5 ±0.12	65.5 ±0.12	65.5 ±0.12

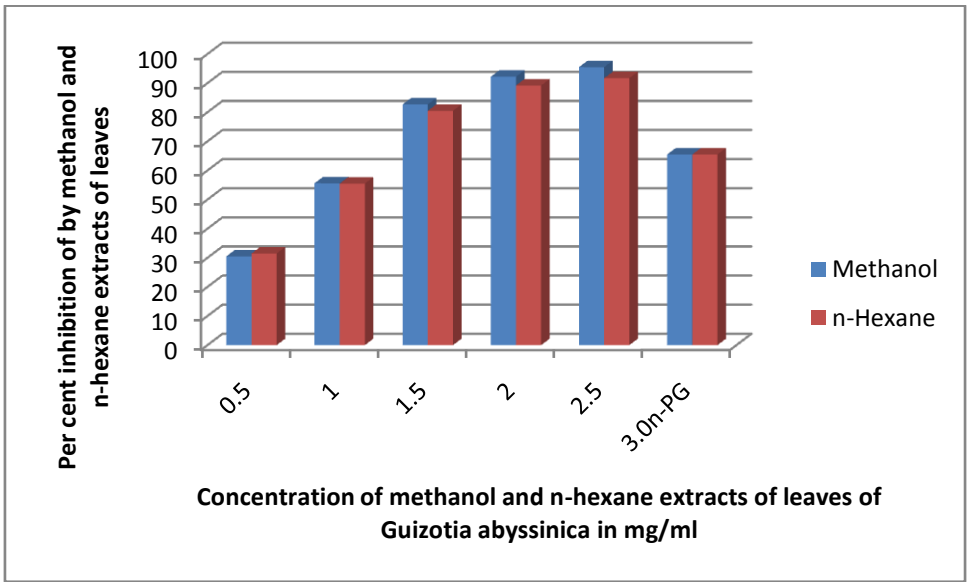

Table-7: % inhibition of lipid peroxidation by methanol and n-hexane extracts of leaves of *Guizotia abyssinica*

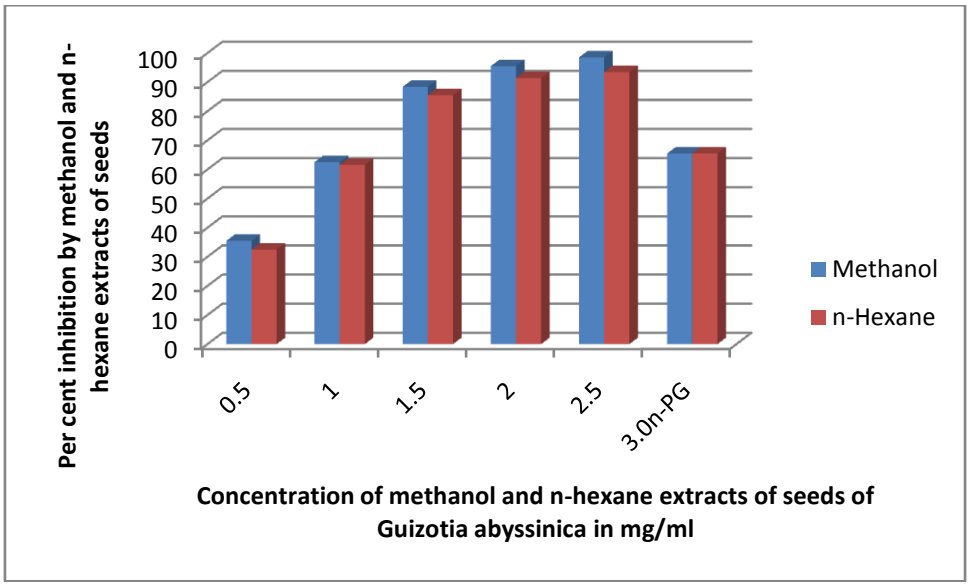

Table-8: % inhibition of lipid peroxidation by methanol and n-hexane extracts of seeds of *Guizotia abyssinica*

IV. Discussion

In the present investigation, antioxidant activity of the methanol extracts of leaves and seeds of *Guizotia abyssinica* were assayed in their methanol and n-hexane extracts by total antioxidant capacity, total phenolic content, DPPH scavenging activity, reducing power and lipid peroxidation activity. In all these assays, the antioxidant activity increased with increasing concentration of the extracts of *Guizotia abyssinica* (Table-1-4; Fig-1-8). The total methanol and n-hexane extracts showed higher reducing power and percent inhibition of linoleic acid lipid peroxidation considerably than the standard antioxidant *n*-propyl gallate. Antioxidant activity of plant extracts is not limited to phenolic compounds. The antioxidant activity may also be due to the presence of other antioxidant secondary metabolites, such as volatile oils, carotenoids and vitamins. Thus the present study has shown that the leaves and seeds of *Guizotia abyssinica* possess significant antioxidant properties and may contribute to the retardation of the inflammatory process. This is because inflammatory tissue injuries are mediated by reactive oxygen metabolites from phagocytic leukocytes (e.g neutrophils, monocytes, macrophages and eosinophils) that invade the tissues and cause injury to essential cellular components (Parfenov, E. A. and Zaikov, G. E. (2000) [31]. Compounds that have scavenging activities toward these radicals have been found to be beneficial in inflammatory diseases (Auddy., et al 2003; Koo et al., 2006) [32, 33]. The ability of the leaf and seed extracts to inhibit the peroxidation of linoleic acid supports the use of *Guizotia abyssinica* in the preservation of palm oil in indigenous societies (Umerie *et al*., 2004) [34]. The antioxidant activity of the extract may also support its traditional use for wound healing. This is because in acute and chronic wounds, oxidants cause cell damage and thus inhibits wound healing (Thang *et al*., 2001) [35]. The administration of antioxidants or free radical scavengers is reportedly helpful, notably to limit the delayed sequale of thermal trauma and to enhance the healing process (Thang *et al*., 2001) [35].

Various scientific studies show that aberrance in redox balance with elevated level of oxygen-free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS) plays an important role in the origin and progression of most human diseases including cancer (Halliwell, 2012; Valko *et al*., 2007; Halliwell , 1991; Cross, *et al*., 1987; Bhattacharyya et al., 2014 (36, 37, 38, 39, 40]. Reactive oxygen species (ROS) act as secondary messenger in intracellular signalling cascades and elevated level of ROS associated with carcinogenesis by promoting initiation, progression, and metastasis of cancer cells. It also induced DNA damage leading to genetic lesions that initiate tumorigenicity and subsequent tumor progression (Volko, 2004; Wang and Yi, 2008; Storz, 2005; Khanna *et al*., 2014 [41, 42, 43]. However, many studies also suggested that free radicals are essential mediators of apoptotic pathway for triggering cell death and therefore function as anticancer agents.Thus, free radicals production approach is used in nonsurgical therapeutic methods for cancer therapy, including chemotherapy, radiotherapy, and photodynamic therapy (Wang and Yi, 2008; Salganik, 2001; Seifried *et al*., 2003) [42, 44, 45]. Free radicals produced in cancer therapy are associated with serious side effects. Furthermore, elevated level of ROS in cancer cell leads to intercellular transfer of hydrogen peroxide (H_2O_2) to neighbouring cells, and stimulates them to acquire uncontrolled ROS production (Storz, 2005) [43]. Free radical scavenger activity plays a protective role in normal healthy cells. They prevent the ROS from spreading and ultimately protect the adjacent cells from oxidative DNA damage and check the cancer progression. Many clinical trials have also suggested that intake of exogenous antioxidants can protect the

healthy cells from oxidative stress as well as ameliorate toxic side effects of cancer therapy without affecting therapeutic efficacy (Salganik, 2001) [44]. Extracts of medicinal plants have been used for the treatment of various diseases, including cancer all over the globe, as they are easily prepared, standardized, and stored. Herbal extracts are also cost effective which increase their accessibility to the patients of all economic status (Eder and Mehnert, 1998; Vickers, 2002 [45, 46]. Global health policies promote the therapeutic use of herbal extract.World Health Organization (WHO) also encourages the use of medicinal plants in the treatment of disease (Debas *et al*., (2006); Winslow and Kroll, 1998; Pal and Shukla, 2003) [47, 48, 49].

Medicinal plants used as therapeutic agents are considered nontoxic for human consumption, while many studies reported the various side effects of medicinal plant (Chan, 2003; Ergil *et al*., 2002) [50, 51]. Medicinal plants uses for health benefit are not taken under the appropriate instruction and consultant of physician. Although people are using medicinal plants from ancient time, safety evaluation of these medicinal plants is required (Hwang *et al*., 2013) [52].

V. Conclusion

Plants are sources of new natural products used in pharmaceutical, cosmetic and food production. An *in vitro* antioxidant assay provides scientific evidence to prove the traditional claims to the *Guizotia abyssinica* (Niger). On the basis of the present results it can be concluded that the methanole and n-hexane leaf and seed extracts of this plant possess significant antioxidant activity. Presence of adequate amount of phenolics and flavonoids account for this fact. So the present investigation suggests that Niger plant is a potential source of natural antioxidant. The active phytochemicals responsible for antioxidant activity and their mechanism of action *in vivo* as well as *in vitro* require further investigation at scientific level.

Conflict of interest: Authors declare no conflict of interest directly or indirectly.

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