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In vitro Antioxidant Activity, Phytochemical analysis and Cytotoxicity of *Diospyros mespiliformis* (leaves)

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Abstract
Interest in natural products as a source for innovation in drug discovery and agrochemicals is still growing worldwide. Natural products, whose immense diversity has been appreciated for many years, may become in a rich source of novel chemical structures. The present study was conducted to investigate the *in-vitro* antioxidant (DPPH assay), total phenolic, total flavonoids content, phytochemical screening and cytotoxicity (MTT-assay) of methanol, ethanol and petroleum ether for *Diospyros mespiliformis* leaves extracts. The antioxidant activity of methanol, ethanol and petroleum ether extract were (81±0.10, 79±0.09 and 19±0.11 RSA %) respectively, in comparison to the control of propyl galate levels (82±0.02RSA %). The methanol extract of *D. mespiliformis* were found to contain higher amount of phenolic, and flavonoid compounds. And in addition cytotoxicity (MTT assay) with different concentration (500, 250 and 125 ppm) in comparison to triton-x100 (the reference control) which verified the safety of the examined all extracts with an IC_{50} less 100 µg/ml.

Keywords: *In vitro,* antioxidant, total phenolic, flavonoid, cytotoxicity and *Diospyros mespiliformis.*

Introduction

Recently in many African countries comprehensive research was conducted on medicinal plants for the treatment of different diseases and conditions, such as diabetes, malaria, anemia and cancer. The availability and relatively cheaper cost of medicinal plants in sub-Saharan Africa, makes them more attractive as therapeutic agents when compared to 'modern' medicines (Agbor *et al*., 2005) [1]. Medicinal plants are still invaluable source of safe, less toxic, lower price, available and reliable natural resources of drugs all over the world. People in Sudan and in other developing countries have relied on traditional herbal preparations to treat themselves. Therefore, it is useful to investigate the potential of local plants against these disabling diseases (Amaral *et al*., 2006; Koko *et al*., 2008) [2, 9].

Reactive oxygen species (ROS) have been implicated in the induction of various types of oxidative damage to biomolecules that results against cancer neurodegenerative diseases, atherosclerosis, malaria, several pathological events in living organisms and different other diseases associated with our life-style (Shahidi and Naczk, 2006; Halliwell *et al.*, 1992) [12, 7]. These molecules can induce changes in different biological tissues and cell biomolecules such as lipids, proteins, DNA or RNA. Free radicals can also affect food quality; reducing its nutritional content and promoting the development of food deterioration (Nickavar and Abolhasani, 2009) [13].

Recently in many African countries comprehensive research was conducted on medicinal plants for the treatment of different diseases and conditions, such as diabetes, malaria, anaemia and cancer. The availability and relatively cheaper cost of medicinal plants in sub- Saharan Africa, makes them more attractive as therapeutic agents when compared to 'modern' medicines (Agbor *et al.*, 2005) [1]. The medicinal properties of plants have been investigated, in the light of recent scientific developments, throughout the world due to their potent pharmacological activities and economic viability. Many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, anticarcinogenic, antibacterial or antiviral activities to a greater or lesser extent (Sala *et al*., 2002) [17]. *Diospyros mespiliformis* (Hochst) It is a member of the (family: Ebenaceae) is a plant that grows wild in tropical Africa. The plant has white fragrant flowers and soft sweet fruit-pulp, used in some parts of Sudan to make a fermented drink (Dalziel, 1937) [3]. *Diospyros mespiliformis* is reportedly one of the most important genera of Ebenaceae which species have been used over the millennia in traditional medicinal systems including Ayurveda, Chinese and African folklores (Mallavadhani *et al*., 1998) [10]. The leaves are used in sleeping sickness, malaria, headache and antihelmintic, (Kerharo, 1974; Etkin, 1997) [8, 5] and extraordinary remedy for fever and for wounds, barks and roots are used to treat malaria, pneumonia, syphilis, leprosy, dermatomycoses and diarrhea, facilitation of delivery and as psycho-pharmacological drug (Mohamed *et al.*, 2009) [11]. This paper is conducted to study the antioxidant activities, phytochemical screening and Cytotoxicity of *Diospyros mespiliformis* (leaves) in Sudan.

Materials and Methods

Collection of *D. mespiliformis*

The *D. mespiliformis* leaves were collected from central Sudan between January 2015 and February 2015. The plant was identified and authenticated by the taxonomists of Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI). The leaves were air-dried, under the

shadow with good ventilation and then ground finely in a mill until their uses for extracts preparation.

Preparation of crude extracts

Extraction was carried out for *D. mespiliformis* by using overnight maceration techniques according to the method described in Harbone (1984) [6]. About 50 g round material was macerated in 250 ml of ethanol for 3 h at room temperature. Occasional shaking for 24 h at room temperature was performed and, the supernatant was decanted. Thereafter, the supernatant was filtered under reduced pressure by rotary evaporatorion at 55 °C. Each residue was weighed and the yield percentage was calculated and then stored at 4 °C in tightly sealed glass vial ready for use. The remaining extracts which were not soluble were successively extracted using methanol and petroleum ether with the described technique. The extracts were kept in freez dryer for 48 h, (Virtis, USA) until they were completely dried. The residue was weighed and the yield percentage was calculated. The extracts were kept and stored at 4 °C until required.

Antioxidant activity *D. mespiliformis* extracts

DPPH radical scavenging assay

The DPPH radical scavenging was determined according to the method by (Shimada *et al*., 1992) [19] with some modification. In 96-wells plate, the test samples were allowed to react with 2.2Di (4-tert-octylphenyl)-1-picryl hydrazyl stable free radical (DPPH) for half an hour at 37 °C. The concentration of DPPH was kept as 300 µm. The extract was dissolved in DMSO (500µg/ml. concentration), while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517 nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group and propyl gallate (PG). All tests and analysis were run in triplicate.

Phytochemical Screening

Phytochemical screening is of great importance in providing us with information about chemicals found in the plant in term of their nature and range of occurrence. This information would enable us to correlate between the nature and range of occurrence of these chemicals and biological assays held to investigate a certain bioactivity of the mentioned plant. In this study the preliminary phytochemical screening was conducted according to (Haborne, 1984) [6].

Preparation of the Extracts

10 mg of the powdered leaves of each plant were refluxed with 100 ml of ethanol 80% for 4 hours. The cool solution was filtered and enough ethanol 80% was passed through the volume of the filtrate 100 ml. This prepared extract (PE) was used for the various tests.

Test for Unsaturated Sterols and Triterpenes

10 ml of the prepared extract (PE) was evaporated to dryness on a water bath and the cooled residue was stirred several times with petroleum ether to remove most of the coloring materials. The residue was then extracted with 20 ml of chloroform. The chloroform solution was dehydrated over sodium sulphate anhydrous. 5 ml of chloroform solution was mixed with 0.5 ml acetic anhydride followed by two drops of conc. Sulphuric acid. The gradual appearance of green, blue pink to purple color was taken an evidence of the presence of sterol (green to blue) and or triterpenes (pink to purple) in the sample (Harborne, 1984) [6].

Test for Alkaloids

7.5 ml of (PE) was evaporated to dryness on a water bath. 5 ml of HCl (2N) was added and stirred while heating on the water bath for 10 minutes, cooled filtered and divided into two test tubes. To one test tube few drops of Mayer's reagent were added. While to the other tube few drops of Valser's reagent were added. A slight turbidity or heavy precipitate in either of the two test tubes was tanked as presumptive evidence for the presence of alkaloids (Harborne, 1984) [6].

Test for Flavonoids

17.5 ml of the (PE) was evaporated to dryness on a water bath, cooled and the residue was defatted with petroleum ether and the defatted residue was dissolved in 30 ml of ethanol (80%) and filtered. The filtrate was used for the following tests: (A) To 3 ml of the filtrate in a test tube 1 ml of 1% aluminum chloride solution was in methanol was added. Formation of yellow color indicated the presence of Flavonoids, (Flavones and / or chalcone). (B) To 3 ml of the filtrate in a test tube 1 ml of 1% potassium hydroxide solution was added. A dark yellow color indicated the presence of the Flavonoids compounds (flavones or flavanones) chalcone and/or flavonol. (C) To 2 ml of the filtrate 0.5 ml of magnesium turnings were added. Producing of defiant color to pink or red was taken as presumptive evidence that flavanones were present in the plant sample (Harborne, 1984) [6].

Test for Tannins

7 ml of the (PE) was evaporated to the dryness on water bath. The residue was extracted several times with n-hexane and filtered. The insoluble residue was stirred with 10 ml of saline solution. The mixture was cooled, filtered and the volume of the filtrate was adjusted to 10 ml with more saline solution. 5 ml of this solution was treated with few drops of gelatin salt reagent. Formation of immediately precipitate was taken as an evidence for the presence of tannin in plant sample. To another portion of this solution, few drops of ferric chloride test reagent were added. The formation of blue, black or green was taken as an evidence for the presence of tannins.

Test for Saponins

1 g of the original dried powdered plant material was placed in a clean test tube. 10 ml of distilled water was added and the tube was stoppered and vigorously shaken for about 30 seconds. The tube was then allowed to stand and observed for the formation of (honeycomb). The appearance of honeycomb, which persisted for least an hour, was taken as an evidence for the presence of Saponins.

Test for Anthraquinone Glycosides

10 g of the powdered plant sample were boiled with 10 ml of 0.5N KOH containing 1 ml of 3% hydrogen peroxide solution. The mixture was extracted by shaking with10 ml of benzene. 5 ml of the benzene solution was shaken with 3 ml of 10% ammonium hydroxide solution and the two layers were allowed

to separate. The presence of Anthraquinones was indicated if the alkaline was found to have assumed pink or red color.

Test for Coumarins

3 g of the original powdered plant sample was boiled with 20 ml of distilled water in a test tube and filter paper was attached to the test tube to be saturated with the vapor after a spot of 0.5N KOH put on it. Then the filter paper was inspected under UV light, the presence of coumarins was indicated if the spot has found to be absorbed the UV light.

Determination of total flavonoid contents

The content of flavonoids in the examined plant extracts was determined according to a modified colorimetric assay with aluminium chloride (Quettier *et al.*, 2000) [14]. 1 mL of methanol solution of the extract (1 mg/mL) was added to a test-tube, followed by the addition of 0.3 mL of solution of $NaNO_2$ (0.05 g/L). After 5 min, 0.3 mL of a 0.1 g/L solution of $AlCl_3$ was added and 5 min later, 2 mL of NaOH (1 mol/L) was added to the mixture, the solution was mixed and the absorbance was measured at 415 nm against a blank. quercetin was used as the standard for the construction of a calibration curve in different concentration (0 – 100 mg/L) Figure (1).

Flavonoid content was expressed in terms of quercetin equivalents (QE) (mg of Q/g of extract).

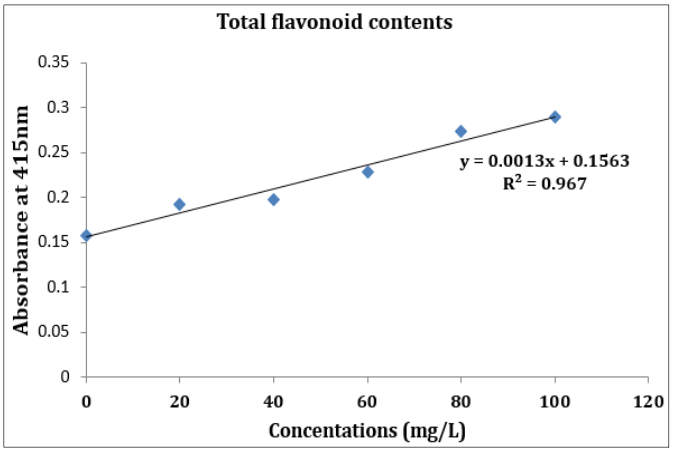

Fig 1: Total flavonoid contents of quercetin equivalents.

Determination of total phenolic contents

The concentration of phenolics in plant extracts was determined using Folin–Ciocalteu method (Singleton *et al.*, 1999) [20]. Methanolic solution of the extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of methanolic solution of extract 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in water and the contents were mixed. After 3 min, 2.5 mL 7.5% $NaHCO_3$ solution was added. The samples were incubated at 45 ^{0}C for 45 min. The absorbance was determined using spectrophotometer at 765 nm against the blank. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. A calibration curve was constructed using gallic acid standard solutions (0–100 mg/L) Figure (2).

Then the content of phenols in extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

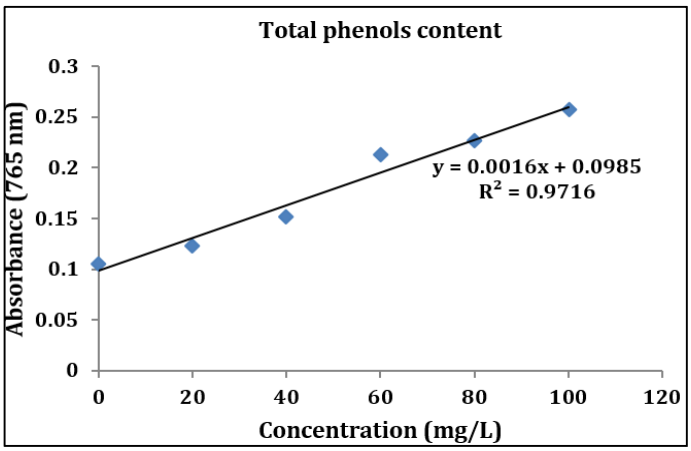

Fig 2: total phenolic contents of gallic acid.

Cytotoxicity Screening

Microculture tetrazolium MTT assay was utilized to evaluate the cytotoxicity of the *D. mespiliformis.*

Microculture Tetrazolium (MTT) Assay

Principle

This Colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3- (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, blue colored formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells (Patel, *et al*., 2009) [16].

Preparation of *D. mespiliformis* extract

Using a sensitive balance 5 mg of each extracts were weighed and put in eppendorf tubes. 50 µl of DMSO were added to the extract and the volume was completed to 1 ml with distilled water obtaining a concentration of 5 mg/ml. The mixture was vortexed and stirred by magnetic stirrer to obtain a homogenous solution.

Cell Line and Culturing Medium

Vero (Normal, African green monkey kidney) cells were cultured in a culturing flask containing a complete medium consisting of 10% fetal bovine serum and 90% minimal essential medium (MEM) and then incubated at 37 ºC. The cells were sub cultured twice a week.

Cell line used

Vero cells (Normal, African green monkey kidney).

Cell counting

Cell counts were done using the improved Neubauer chamber. The cover slip and chamber were cleaned with detergent, rinsed thoroughly with distilled water and swapped with 70% ethanol, then dried. An aliquot of cell suspension was mixed with equal volume of 0.4% trypan blue in a small tube. The chamber was charged with cell suspension. After cells had settled, the chamber was placed under light microscope. Using 40 X objective, cells in the 4 large corner squares (each containing 16 small squares) were counted. The following formula was used for calculating cells:

$$\text{(Cells/ml) } N = \frac{\text{Number of cells counted X Dilution factor X } 10^4}{4}$$

Procedure

The monolayer cell culture formed in the culturing flasks was trypsinized and the cells were put in centrifuging tube and centrifuged for 5 minutes separating the cells from the supernatant that flicked out. 1 ml complete medium was added to the cells and all the cell suspension was contained in a basin. In a 96- well microtitre plate, serial dilutions of each extracts were prepared. 3 duplicated concentrations for each extracts i.e. 6 wells for each of 8 extracts. All wells in rows A, B and C were used in addition to first 4 wells from each rows D, E and F. The first 2 wells of row G were used for the negative control and the first 2 wells of row H were used for the positive control Triton X. 20 µl complete medium pipetted in all wells in rows B, C and mentioned wells of rows E and F. Then 20 µl from each extracts were pipetted in rows A and B and first 4 wells of rows E and F. 20 µl taken from row B were pipetted and mixed well in row C from which 20 µl were taken and flicked out. The same was done from E to F. After that 80 µl complete medium were added to all used wells. Then adjusting the cell account to 3000 cell/well, 100 µl of cell suspension were added completing all wells to the volume 200 µl. Now, we have duplicated three concentrations 500, 250, 125 µg/ml for each extract. Then the plate was covered and incubated at 37 ºC for 96 hours.

On the fourth day, the supernatant was removed from each well without detaching the cells. MTT suspension stock (5 mg/ml) prepared earlier in 100 ml phosphate buffer solution (PBS) was diluted (1:3.5) in a culture medium. To each well of the 96-well plate, 50 µl of diluted MTT were added. The plate was incubated for further 4 hours at 37 ºC. MTT was removed carefully without detaching cells, and 100 µl of DMSO were added to each well. The plate was agitated at room temperature for 10 minutes then read at 540 nm using microplate reader. The percentage growth inhibition was calculated using the formula below:

$$\% \text{ Cell inhibition} = 100-\{(Ac-At)/Ac\} \times 100$$

Where,

At = Absorbance value of test compound;
Ac =Absorbance value of control.

Statistical analysis

All data were presented as means ± S.D. Statistical analysis for all the assay results were done using Microsoft Excel program (2007).

Results and Discussion

Ethanolic, methanolic and petroleum ether of *D. mespiliformis* extract were screened for antioxidant screening for their free radical scavenging properties using 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl (DPPH), while propyl galate was used as standard antioxidant, total phenolic content was determined using Folin–Ciocalteu method, while gallic acid equivalent was used as standard, total flavonoids content was determined according to a modified colorimetric assay with aluminium chloride, while quercetin equivalents was used as standard, phytochemical screening and cytotoxicity (MTT assay) with different concentration (500 ppm, 250 ppm and 125 ppm) and compare tritonx-100 (the reference control).

Antioxidant activity of *D. mespiliformis* extract

This table indicate the anti DPPH of ethanol extract of *D. mespiliformis*, propyl gallate was used as standard drug level. The tested antioxidant activity gave (79±0.09, 81±0.10, 19±0.11) RSA% sequentially in comparison to the control of propyl gallate levels gave (82 ± 0.02 RSA %). As shown in Table (1), the results of antioxidant activity *D.mespiliformis* ethanol and methanol showed high antioxidant activity against the DPPH free radical. (This result was similar to that reported by (Oulare, 2015) [15]. Using free radical scavenging activity of *D.mespiliformis* using 1, 1-diphenyl-2-picrylhydrazyl radicals (DPPH).

Table 1: Antioxidant activity *D. mespiliformis*

No	Name of samples	solvent	%RSA* ± SD (DPPH)
1	*D. mespiliformis*	Ethanol	79±0.09
		Methanol	81±0.10
		petroleum ether	19±0.11
2	*Control	PG	82±0.02

Key: RSA* = Radicals scavenging activity ***Control = P.G** = Propyl Gallate.

Phytochemical analysis of *D. mespiliformis*

The Phytochemical analysis of crude ethanolic extract of *D.mespiliformis* by the method described earlier and then and then analyzed for phytocompounds like steroids or terpenoids, alkaloids, flavonoids, coumarins, saponins, tannins and anthraquinone preliminary analyzed and present in the Table (2).

The Phytochemical analysis of crude ethanolic extract *D.mespiliformis* performed by the method described earlier and then and then analyzed for phytocompounds like steroids or terpenoids, alkaloids, flavonoids, coumarins, saponins, tannins and anthraquinone preliminary analyzed and present study. The Alkaloids is absent in both the case of plant extracts. This result was similar to that reported by (Dangoggo, 2012) [4].

Table 2: Preliminary Phytochemical Screening analysis of *D. mespiliformis* extract

No.	Tested	Diospyros mespiliformis
1	Unsaturated Sterol And/or Triterpenes	+
2	Alkaloids	+
3	Flavonoids	+
4	Tannins	+
5	Saponins	+
6	Anthraquinone glycoside	+
7	Coumarins	+

+ = Present - = Absent.

The total phenolic and flavonoid content of the methanolic and ethanol and petroleum ether extracts of *D. mespiliformis* is given in Table 3. The methanolic and ethanolic extracts of *D. mespiliformis* were found to contain significantly higher phenolic and flavonoid, the values presented as the mean ± SD of three measurements.

Table 3: The total phenolic and flavonoid content of *D. mespiliformis*

Test	Methanol	Ethanol	Petroleum ether
Total Phenolics (mg GA*/g extract)	187.48±0.37	163.98±0.55	46.43±0.99
Total Flavonoids (mg of Q**/g of extract)	1167.83±1.53	955.22±0.89	255.97±0.84

Key: * gallic acid ** quercetin

Determined the total phenolic and flavonoid content of the methanol, ethanol and petroleum ether extracts of *D. mespiliformis.*

The methanol extract was found to have higher flavonoid and phenol content than ethanol extract on the other hand the petroleum ether extract have low quantity of phenolics and flavonoid, the level of phenolic compounds in plants mainly depends on the cultivation techniques and growing conditions, type of the plants, ripening process, as well as storage conditions. Furthermore, the quantification of phenolics is done via different extraction processes and methods. Therefore, the phenolic content is also influenced by the way of extraction and also by the polarity of the solvent used for the the extraction process (Naczk, 2006) [12]. The considerable amounts of total phenolics and flavonoids might lead to the potential antioxidant activity of *D. mespiliformis.*

Cytotoxicity assay *D. mespiliformis* extract

Interestingly, the cytotoxicity assays were conducted in this study to evaluate the cytotoxicity effects of ethanolic, methanolic and petroleum ether extract of *D. mespiliformis* by using MTT-assay including (Vero cell line). Table 4 indicated the inhibition percentage (%) of Vero cell line growth *in vitro* by ethanolic, methanolic and petroleum ether extract of *D.mespiliformis* for different concentrations 125 to 500 µg/ml and showed an IC_{50} >100 (µg/ml) which is verifying the plant safety. The maximum concentration used was 500 µg/mL. When this concentration produced less than 50% inhibition, the IC_{50} cannot be calculated.

Table (4): Cytotoxicity of Diospyros mespiliformis extract on normal cell lines (Vero cell line) as measured by the MTT assay

S. NO	Name of Extracts	Concentration (µg/ml) Inhibition (%) ± SD			IC_{50} (µg/ml)	IC_{50}
		500	250	125		
1	Ethanol	61.1 ± 0.02	45.5 ± 0.05	35.1 ± 0.01	296.4	>100
2	Methanol	61.4 ± 0.04	40.4 ± 0.02	30.9 ± 0.03	329.1	100>
3	Petroleum ether	41.3 ± 0.09	30.1 ± 0.04	20.6 ± 0.01	625.9	100>
4	*Control	95.3 ± 0.00				< 30

Key: *Control=Triton-x100 was used as the control positive at 0.2 µg/mL.

Conclusion

From complete investigation about antioxidant, pharmaceutical screening and cytotoxicity studies of *D. mespiliformis* leaves it can be recommended that extracts could be used as a easily available foundation of natural antioxidants (total flavonoids content and total phenolic content), which can be used as supplement to aid the therapy of free radical mediated diseases such as cancer, diabetes, inflammation, etc., diabetes swelling. Further studies are needed on the isolation and elucidation of their chemical structures of antioxidant components, and other related pharmacological studies such as *in vivo* investigation, drug formulation and clinical trials are highly recommended.

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References

1. Agbor GA, Oben JE, Ngogang JY. Haematinic activity of Hibiscus cannabinus. African journal of Biotechnology. 2005; 4(8):833-837.
2. Amaral FMM, Ribeiro MNS, Barbosa-Filho JM, Reism AS, Nascimento FRF, Macedo RO. Plants and chemical constituents with giardicidal activity. Braz. J. Pharmacogn. 2006; 16:696-720.
3. Dalziel JM. The Useful Plants of West Tropical Africa. Crown Overseas Agents for the Colonies and Administration, London, 1937, 347-348.
4. Dangoggo SM, Hassan LG, Sadiq IS, Manga SB. Phytochemical Analysis and Antibacterial Screening of Leaves of Diospyros Mespiliformis and Ziziphus Spina-Christi. Journal of Chemical Engineering. 2012; 1(1):31-37.
5. Etkin NL. Antimalarial Plants used by Hausa in northern Nigeria. Tropical Doctor, 1997; 27(1):12-16.
6. Harbone B. Phytochemical methods. 2nd. New York, Champan Hall, 1984; 4:4-7.
7. Halliwell B, Gutteridge J, Cross E. Free radicals and human disease: Where are we now? J. Lab. Clin. Med. 1992; 119:598-620.
8. Kerharo Adam JG. Pharmacopée sénégalaise traditionnelle, plantes médicinales et toxiques. Vigot et Frères; Paris, 1974, 1011.
9. Koko WS, Mesaik MA, Yousaf S, Galal M, Choudhary MI. In vitro immunomodulating properties of selected Sudanese medicinal plants. J. Ethnopharmacol. 2008; 118:26-34.
10. Mallavadhni UV, Panda AK, Rao YR. Pharmacology and chemotaxonomy of Diospyros. Phytochemistry, 1998; 49:901-51.
11. Mohamed IE, El Nur EE, Choudhary MI, Khan SN. Bioactive natural products from two Sudanese medicinal plants Diospyros mespiliformis and Croton zambesicus. Rec. Nat. Prod. 2009; 3:198-203.
12. Naczk M, Shahidi F. Phenolics in cereals, fruits and vegetables: Occurence, extraction and analysis. Journal of Pharmaceutical and Biomedical Analysis. 2006; 41:1523-1542.
13. Nickavar B, Abolhasani FA. Screening of antioxidant properties of seven umbelliferae fruits from Iran. Pak. J. Pharm. Sci. 2009; 22:30-35.

14. Quettier DC, Gressier B, Vasseur J, Dine T, Brunet C, Luyckx MC, *et al*. Phenolic compounds and antioxidant activities of buckwheat (Fagopyrum esculentum Moench) hulls and flour. J. Ethnopharmacol. 2000; 72:35-42.
15. Oulare K, Barry MS, Bah F, Keita N, Haba NL, Balde AM. Anti-oxidative activity of fruit extracts of some medicinal plants used against chronic diseases (diabetes, hypertension) in Kankan, Guinea. Journal of Plant Sciences. 2015; 3(1-2):1-5.
16. Patel S, Gheewala N, Suthar A, Shah A. In-Vitro Cytotoxicity Activity of Solanum nigrum Extract Against Hela Cell Line And Vero Cell Line. International Journal of Pharmacy and Pharmaceutical Sciences. 2009; 1(1):38-46.
17. Sala A, Recio MD, Ginner RM, Manez S, Tournier H, Schinella G, Rios JL. Anti‐inflammatory and antioxidant properties of Helichrysum italicum. J. Pharmacy and Pharmacology. 2002; 54(3):365-371.
18. Shahidi F, Naczk M. Phenolic Compounds in Fruits and Vegetables: Food Phenolics, Sources, Chemistry. Technomic Publishing Co. Inc., Lancaster, PA., 1995, 75-107.
19. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the antioxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food. Chem. 1992; 40:945-8.
20. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 1999; 299:152-178.