Trop J Nat Prod Res, May 2020; 4(6):223-227

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

In-vitro Antioxidant Activities of Different Stem Bark Extracts of *Irvingia gabonensis* (Irvingiaceae)

Abdurahman E. Mukhtar[1], Abdulhakim Abubakar[2]*, Onyemelukwe G. Chukwubuike[3]

[1]*Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria*
[2]*Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria*
[3]*Department of Medicine, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria*

ARTICLE INFO

Article history:
Received 16 April 2020
Revised 30 June 2020
Accepted 01 July 2020
Published online 02 July 2020

ABSTRACT

Oxidative stress is implicated in the pathogenesis of many diseases. *Irvingia gabonensis* is used in Nigeria for the management of diabetes mellitus, inflammation, liver diseases, viral infections and dementia. In order to explore the antioxidant potential of the stem bark of *I. gabonensis*, the powdered plant was subjected to soxhlet extraction with solvents of varying polarities (petroleum ether, butanol, and water) to obtain the various extracts. The extracts were subjected to phytochemical screening and evaluated for antioxidant activities, total phenolic and flavonoid contents using established protocols. Phytochemical screening revealed the presence of flavonoids, tannins, triterpenes, steroids, and saponins. Total phenolic contents (TPCs) of the petroleum ether, butanol and aqueous extracts were 109.3, 147.7 and 96.3 µg of gallic acid equivalent per mL, respectively, while the total flavonoid contents (TFCs) were 56.3, 43.3, and 20.3 µg of rutin equivalents per mL for the petroleum ether, butanol and aqueous extracts, respectively. Antioxidant activities of the three extracts using ferric ion reducing power were significant compared to ascorbic acid. In the phosphomolybdate assay, the petroleum ether extract at 500 µg/mL was not different from ascorbic acid. In the hydrogen peroxide scavenging assay, the petroleum ether and butanol extracts at 250 and 500 µg/mL were significantly increased compared to ascorbic acid. In the DPPH assay, the butanol extract at 500 µg/mL showed antioxidant activity compared to ascorbic acid. The findings of this research indicate that *I. gabonensis* possesses *in vitro* antioxidant activity which was prominent in the petroleum ether and butanol extracts.

Keywords: Antioxidant, Flavonoids, Phenolics, *Irvingia gabonensis*

Introduction

Reactive oxygen species (ROS) comprise of free radicals that have molecules of oxygen with unpaired electrons.[1] The Human body is continuously exposed to ROS which transfer their free electrons to cause oxidation of cells and a distortion in the levels of antioxidants and free radicals.[1] This distortion leads to oxidative stress [2] which is greatly involved in the pathogenesis of many ailments [3] some of which include; diabetes mellitus, cancer, inflammation, atherosclerosis, neurological, hepatic and renal disorders.[2-6] To neutralize the deleterious effects associated with oxidative stress, antioxidant compounds play a significant role in inactivating the ROS. [7, 8] Natural products from medicinal plants are invaluable and widely utilized for their pharmacological benefits.[9-11] Many medicinal plants and their bioactive components make up the basis of natural antioxidants which are being clamored to replace the synthetic ones.[12] *Irvingia gabonensis* is commonly known as 'African Bush Mango' In Nigeria where both the seeds and fruits are well consumed. The plant is locally called "*Ogbonno*" by the Igbos, "*Goronor*" by the Hausas, "*Mbukpabuyo*" by the Efiks and Ibibios, "*Aapon*" by the Yorubas, "*Ogwi*" by the Bini people and "*Apioro*" by the Deltans [personal communication]. Ethno-medicinal therapies use the leaves, bark, kernels and roots for the treatment of many diseases.[13] The stem bark extract is used for pain management,[14] the seeds are reported to improve libido and reproductive function in men.[15] The seeds are also used for hernia, yellow fever and cases of poisoning.[16] The kernels are utilized for weight reduction and for the management of type 2 diabetes.[17] The antioxidant activity of the kernel has also been reported.[18] The following compounds were isolated from the stem bark of *I. gabonensis*: 3-friedelanone, butulinic acid, oleanolic acid, 3,3,4-tri-O-methylellagic acid, 3,4-di-O-methylellagic acid and hardwickiic acid.[19] This research focused on investigating the *in vitro* antioxidant activities of the petroleum ether, butanol and aqueous extracts of the stem bark of *I. gabonensis*.

*Corresponding author. E mail: abdulhakimevuti@gmail.com
Tel: +234-(0)8036412047

Citation: Mukhtar AE, Abubakar A, Chukwubuike OG. *In-vitro* Antioxidant Activities of Different Stem Bark Extracts of *Irvingia gabonensis* (Irvingiaceae). Trop J Nat Prod Res. 2020; 4(6):223-227. doi.org/10.26538/tjnpr/v4i6.2

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Materials and Methods

Chemicals and reagents

DPPH, Dipotassium hydrogenphosphate, Potassium ferricyanide, Potassium persulfate, Trichloroacetic acid, Ferric chloride, monosodium dihydrogenphosphate, Rutin and Folin-Ciocalteu, Ammonium molybdate, Gallic acid (Sigma Aldrich, St Louis, USA), Ascorbic acid (MP Biomedicals, France), Petroleum ether, butanol (Sigma Aldrich, USA).

Plant collection and preparation of extracts

I. gabonensis plant was collected at the Forestry Research Institute of Nigeria, Ibadan, Nigeria on the 9th of August, 2019. It was identified and documented with the specimen number (103947) at the Herbarium of the Institute. The stem bark was dried under shade and powdered

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

after which 200 g was extracted with various solvents (petroleum ether, butanol and water) using a soxhlet extractor. The extracts were dried and then subjected to preliminary phytochemical screening using the methods of Evans.[20]

Total phenolic and total flavonoid contents
The total phenolic and total flavonoid contents were assessed in accordance with standard protocols.[21, 22]

Antioxidant studies
The antioxidant activity of different extracts of *I. gabonensis* was evaluated using a myriad of tests viz; Ferric ion reducing power assay [23], hydrogen peroxide scavenging activity,[24] phosphomolybdate assay [25-27] and DPPH scavenging activity.[27-30]

Statistical analysis
Results generated were presented as Mean ± Standard Deviation and the variations between means were analyzed by one way Analysis of Variance (ANOVA) followed by Dunnett post hoc test using statistical package for social sciences (SPSS, Version 20) and values of $p \leq 0.05$ were taken into account to be significant statistically.

Results and Discussion

Phytochemical constituents
The phytochemical investigation of the different extracts of *I. gabonensis* showed the presence of phyto-constituents such as flavonoids, steroids, tannins and triterpenes. However, anthraquinones and cardiac glycosides were absent (Table 1).

Total phenolic and flavonoid contents
The total phenolic contents of the petroleum ether, aqueous and butanol extracts of *I. gabonensis* were determined to be 109.3, 96.3 and 147.7 µgGAE/mL respectively (Figure 1) while the total flavonoid content of *I. gabonensis* was determined to be 56.3, 43.3 and 20.3 µg RE/mL for petroleum ether, butanol and aqueous extracts, respectively (Figures 1 and 2).

Antioxidant activities
The ferric ion reducing antioxidant activity of the aqueous, butanol and petroleum ether extracts of *I. gabonensis* at all the concentrations tested were significantly ($p < 0.001$) reduced compared to ascorbic acid (Figure 3). The H_2O_2 scavenging activity of the aqueous, butanol and petroleum ether extracts of *I. gabonensis* at 100 µg/mL was significantly ($p < 0.001$) reduced compared to ascorbic acid. However at 250 and 500 µg/mL, the butanol and petroleum ether extracts were significantly ($p < 0.001$) increased compared to ascorbic acid (Figure 4). The total antioxidant capacity of the aqueous, butanol and petroleum ether extracts of *I. gabonensis* at 100, 125 and 250 µg/mL was significantly ($p < 0.001$) reduced compared to ascorbic acid (Figure 5). The DPPH radical scavenging activity of the aqueous, butanol and petroleum ether extracts of *I. gabonensis* at 100, 125, and 250 µg/mL was significantly ($p < 0.001$) reduced compared to ascorbic acid.

Table 1: Phytochemical Constituents of Different Extracts of *Irvingia gabonensis*

Constituents	Inference		
	Aqueous	Butanol	Pet. ether
Alkaloids	–	–	–
Anthraquinones	–	–	–
Cardiac Glycosides	–	–	–
Flavonoids	+	+	+
Saponins	+	+	–
Steroids	+	+	+
Tannins	+	+	+
Triterpenes	+	+	+

Key: Absent – , Present +

However, at 500 µg/mL, the DPPH radical scavenging activity of the butanol extract was not significantly different from the ascorbic acid (Figure 6).
The search for phytochemicals having antioxidant activities has gained prominence.[12] Several plants elicit this activity [31] and have the ability to counter free radicals which makes them useful in many ailments. [32] *I. gabonensis* has been used for its nutritional and medicinal benefits in several African countries including Nigeria. It is also utilized in folk medicine for many ailments.[13-17] The stem bark of *I. gabonensis* is rich in phenolic and flavonoid constituents which play significant roles as antioxidants. The phenolic and flavonoid contents which are highly present in the petroleum ether and butanol extracts of *I. gabonensis* may be attributed to its antioxidant activity.[33, 34]
The reducing capacity and scavenging properties of antioxidants are known to hinder free radicals[35] and this can be assessed by ascertaining the capability of the extracts to convert Fe^{3+} to Fe^{2+} and to give out an electron.[36] Regarding the ferric ion reducing activity, the various extracts of *I. gabonensis* did not show marked effect compared to ascorbic acid.
Phenolic compounds are a class of phyto-constituents with powerful antioxidant activities and this relationship has long been established.[37, 38] The petroleum ether extract elicited the best total antioxidant capacity at 500 µg/mL as the activity was not significantly different from ascorbic acid. This activity means some of the antioxidant compounds of *I. gabonensis* are non-polar and are highly soluble in petroleum ether. Indeed, studies have also shown that polyphenols are readily soluble non-polar solvents.[39,40]
Hydrogen peroxide can readily cross membrane of cells and also react with Cu^{2+} and Fe^{2+} ions to result in the formation of free radicals which are implicated in toxicity. [41] The butanol and petroleum ether extracts of *I. gabonensis* produced a marked inhibitory effect on hydrogen peroxide and this is an indication that the plant possesses free radical scavenging and antioxidant activities.[35]
DPPH radical scavenging activity is also a sensitive technique used in establishing the antioxidant capacity of medicinal plants. [42] The DPPH radical scavenging activity of the butanol extract of *I. gabonensis* at 500 µg/mL was comparable to ascorbic acid. This inhibition of DPPH by *I. gabonensis* is an indication that it possesses antioxidant activity. [43, 44] In this study, there is a link between total phenolic content, total flavonoid content and antioxidant activity (Total antioxidant capacity, H_2O_2 and DPHH assays) by the petroleum ether and the butanol extracts of *I. gabonensis*. The observed activity can be connected with polyphenolic, flavonoids and other constituents of plants [45, 46] which are present in the various extracts of *I. gabonensis*.

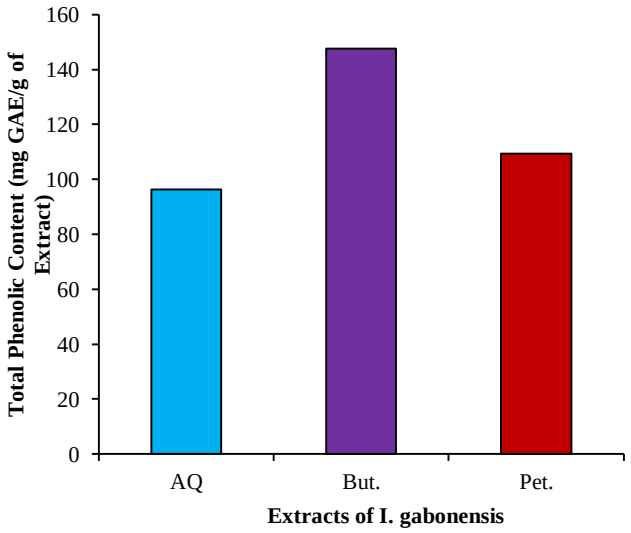

Figure 1: Total phenolic Content of *Irvingia gabonensis* extracts. Values are presented as Mean ± SD in µg GAE/mL. AQ = Aqueous, But = Butanol, Pet.=Petroleum ether, n = 3

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

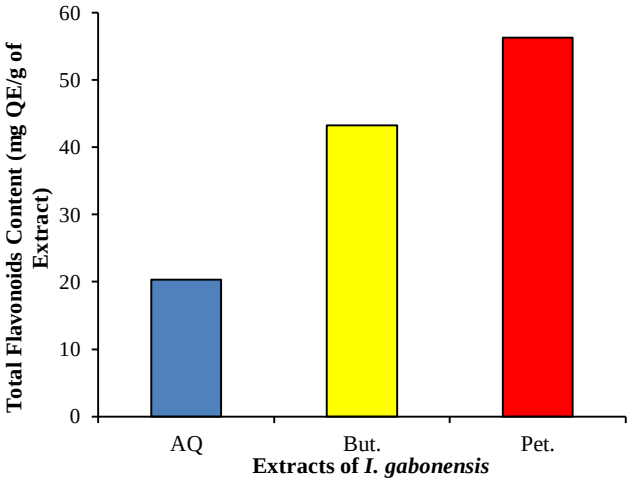

Figure 2: Total flavonoid Content of *Irvingia gabonensis* extracts. Values are presented as Mean ± SD in µg RE/mL. AQ=Aqueous, But=Butanol, Pet.=Petroleum ether, n=3

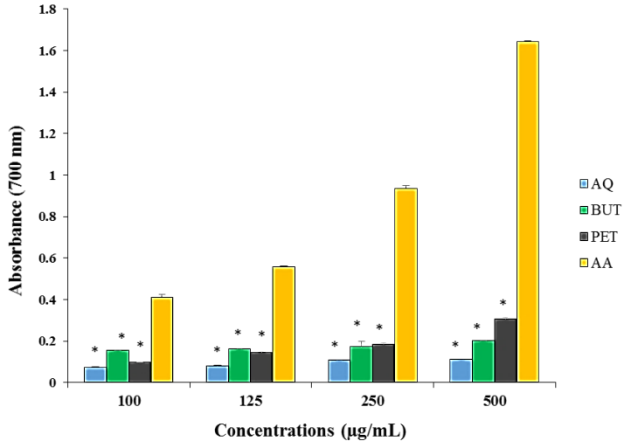

Figure 3: Ferric Ion Reducing Antioxidant Power of *Irvingia gabonensis* extracts in different solvents. Values are presented as Mean SD, *= p<0.001 compared to AA - One way ANOVA followed by Dunnett post hoc test, AQ= Aqueous, BUT =Butanol, PET= Petroleum ether and AA = Ascorbic acid, n = 3

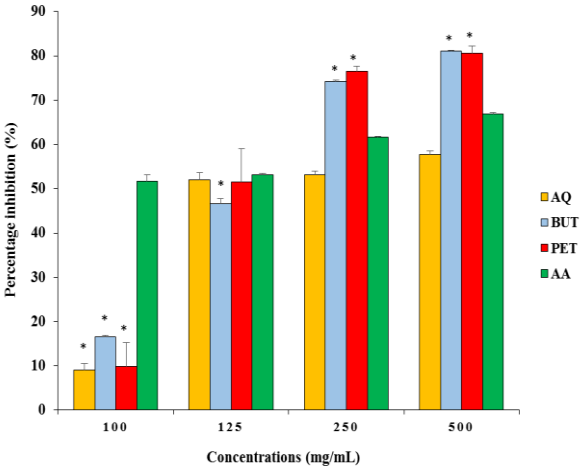

Figure 4: Hydrogen peroxide scavenging activity of *Irvingia gabonensis* extracts in different solvents. Values are presented as Mean SD, *= p<0.001 compared to AA - One way ANOVA followed by Dunnett post hoc test, AQ= Aqueous, BUT =Butanol, PET= Petroleum ether and AA = Ascorbic acid, n = 3

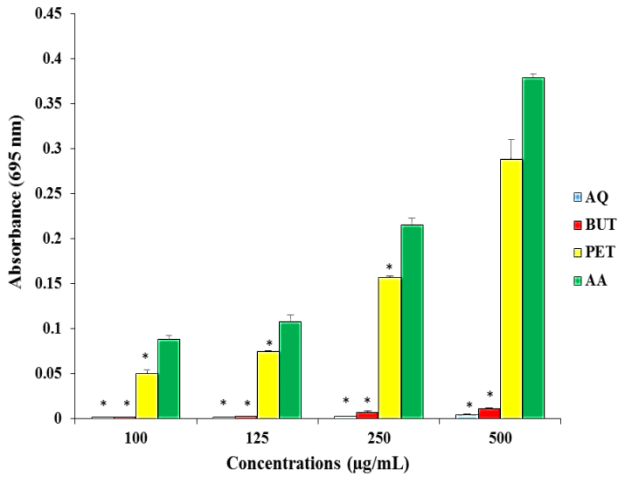

Figure 5: Total antioxidant capacity of different extracts of *Irvingia gabonensis* extracts in different solvents. Values are presented as Mean SD, *= p<0.001 compared to AA - One way ANOVA followed by Dunnett post hoc test, AQ= Aqueous, BUT =Butanol, PET= Petroleum ether and AA = Ascorbic acid, n = 3

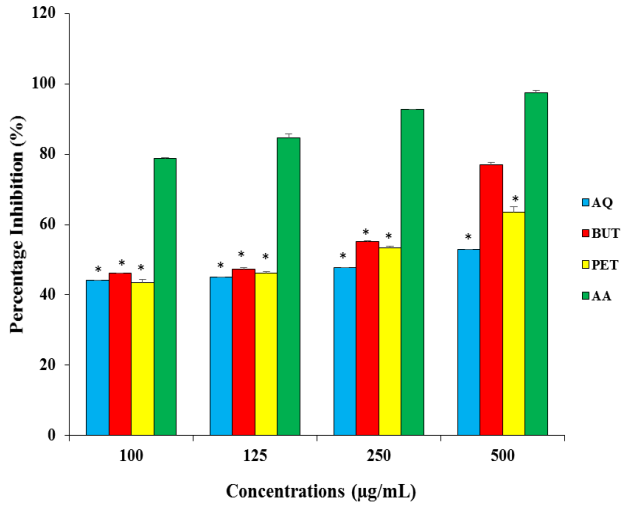

Figure 6: DPPH radical scavenging activity of *Irvingia gabonensis* extracts in different solvents. Values are presented as Mean SD, *= p<0.001 compared to AA - One way ANOVA followed by Dunnett post hoc test, AQ= Aqueous, BUT =Butanol, PET= Petroleum ether and AA = Ascorbic acid, n = 3

Conclusion

The findings of this research indicate that *I. gabonensis* possesses in vitro antioxidant activity which was prominent in the petroleum ether and butanol extracts.

Conflict of interest

The authors declare no conflicting interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors wish to acknowledge and appreciate the technical assistance of Aliyu Mansir of the Department of Biochemistry, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria.

References

1. Chen B, Lu Y, Chen Y, Cheng J. The role of Nrf2 in oxidative stress-induced endothelial injuries. J Endocrinol. 2015; 225:R83–R99.
2. Asmat U, Abad K, Ismail K. Diabetes mellitus and oxidative stress- A concise review. Saud Pharm J. 2016; 24:547-553.
3. Karuna DS, Dey P, Das S, Kundu A, Bhakta T. In vitro antioxidant activities of root extract of *Asparagus racemosus* Linn. J Trad Compl Med. 2018; 8:60-65.
4. Yildirim A, Oktay M, Bilaloglu V. The antioxidant activity of the leaves of *Cydonia vulgaris*. Turk J Med Sci. 2001; 31:23-27.
5. Govindappa M, Bharath N, Shruthi HB, Gustavo S. In vitro antioxidant activity and phytochemical screening of endophytic extracts of *Crotalaria pallida*. Free Rad Antioxid. 2011; 1:79-86.
6. Neethu P, Haseena P, ZevaluKezo, Thomas SR, Goveas SW, Abraham A. Antioxidant properties of *Coscinium fenestratum* stem extracts on Streptozotocin-induced type 1 diabetic rats. J Appl Pharm Sci. 2014; 4:29-32.
7. Gomathi D, Ravikumar G, Kalaiselvi M, Devaki K, Uma C. Efficacy of *Evolvulus alsinoides* (L.) L. on insulin and antioxidants activity in pancreas of streptozotocin-induced diabetic rats. J Diabetes Metab Disord. 2013; 12:12-39.
8. Sindhi V, Gupta V, Sharma K, Bhatnagar S, Kumari R, Dhaka N. Potential applications of antioxidants – a review. J Pharm Res. 2013; 7:828–835.
9. Newman DJ and Cragg GM. Natural products as sources of new drugs from 1981 to 2014. J Nat Prod. 2016; 79:629–661.
10. Thenmozhi K, Karthika K, Jamuna S, Paulsamy S, Manian, S, Chitravadivu C. In vitro antioxidant and radical scavenging abilities of aqueous methanolic extracts of *Cassia obtuse* L. plant parts (Caesalpiniaceae). Int J Pharm Pharm Sci. 2015; 7:340–344.
11. Falodun A and Irabor EEI. Phytochemical, Proximate, Antioxidant and Free Radical Scavenging Evaluations of *Calliandria surinamensis*. Acta Poloniae Pharm Drug Res. 2008; 65(5):571-575.
12. Djacbou DS, Pieme CA, Biapa PC, Penlap BV. Comparison of in vitro antioxidant properties of extracts from three plants used for medical purpose in Cameroon: *Acalypha racemosa, Garcinia lucida* and *Hymenocardia lyrata*. Asian Pac J Trop Biomed. 2014; 4:S625-S632.
13. Ude GN, Dimkpa CO, Anegbeh PO, Shaibu AA, Pillay M, Tenkouano A, Pillay M, Tchoundjeu Z. Analysis of genetic diversity in accessions of *Irvingia gabonensis*. Afr J Biotech. 2006; *5*:219-223.
14. Okolo CO, Johnson PB, Abdurahman EM, Abdu-Aguye I, Hussaini IM. Analgesic effect of *Irvingia gabonensis* stem bark extract. J Ethnopharmacol. 1995; 45:125-129.
15. Obianime AW and Uche FI. Effects of aqueous extracts of *Irvingia gabonensis* seeds on the hormonal parameters of male guinea pigs. Asian Pac J Trop Med; 2010:200-204.
16. Ayuk ET. Uses, management and economic potential of *I. gabonensis* in humid low lands of Cameroon. For Ecol Man. 1999; 113:1-9.
17. Kuete V, Wabo GF, Ngameni B, Mbaveng AT, Metuno R, Etoa FX, Ngadjui BT, Beng VP, Meyer JJ, Lall N. Antimicrobial activity of the methanolic extracts, fraction and compounds from the stem Bark of *Irvingia gabonensis.* J Ethnopharmacol. 2007; 114:54– 60.
18. Arogba SS and Omede A. Comparative Antioxidant Activity of Processed Mango *(Mangifera indica)* and Bush Mango *(Irvingia gabonensis)* Kernels. Nig Food J. 2012; 30:17-21.
19. Kuete V, Wabo GF, Ngameni, B, Mbaveng AT, Metuno R, Etoa F, Ngadjui BT, Beng VP, Meyer, JJM, Lall N. Antimicrobial activity of the methanolic extract, fractions and compounds from the stem bark of *Irvingia gabonensis* (Ixonanthaceae). J Ethnopharmacol. 2007; 114:54-60.
20. Evans, WC. Trease and Evans Pharmacognosy, 16th ed. London, U.K: Elsevier Health Sciences; 2009. 135-144 p.
21. Slinkard K and Singleton VL. Total phenol analysis: Automation and comparison with manual methods. Am J Enol Vitic. 1977; *28*:49-55.
22. Ahmed D, Khaizran F, Saeed R. Analysis of phenolic and flavonoid contents, and theanti-oxidative potential and lipid peroxidation inhibitory activity of methanolic extract of *Carissa opaca* roots and its fractions in different solvents. Antioxid. 2014; 3:671–683.
23. Oyaizu M. Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr. 1986; 44:307–315.
24. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinog. 1989; 10:1003-1008.
25. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem. 1999; 269:337–341.
26. Jan S, Khan MR, Rashid U, Bokhari J. Assessment of antioxidant potential, total phenolics and flavonoids of different solvent fractions of *Monotheca Buxifolia* fruit. Osong Pub Health Res Perspect. 2013; 4:246–254.
27. Mashwani Z, Khan MA, Irum S, Ahmad M. Antioxidant potential of root bark of *Berberislycium* Royle. Galliyat, western Himalaya, Pakistan. Pak J Bot. 2013; 45:231-234.
28. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol. 1995; 28:25-30.
29. Shah NA, Khan MR, Ahmad B, Noureen F, Rashid U, Khan RA. Investigation on flavonoid composition and anti-free radical potential of *Sida cordata*. BMC Compl Altern Med. 2013; 13:276.
30. Zengin G, Aktumsek A, Guler GO, Cakmak YS, Yildiztugay E. Antioxidant properties of methanolic extract and fatty acid composition of *Centaurea urvillei* DC. Subsp. Hayekiana Wagenitz. Rec Nat Prod. 2011; 5:123-132.
31. Kanatt SR, Chander R, Sharma A. Antioxidant potential of mint (*Mentha spicata* L.) in radiation-processed lamb meat. Food Chem. 2007; 100:451-458.
32. Sindhi V, Gupta V, Sharma K, Bhatnagar S, Kumari R, Dhaka N. Potential applications of antioxidants – a review. J Pharm Res. 2013; 7:828–835.
33. Govindappa M, Bharath N, Shruthi HB, Santoyo G. In vitro antioxidant activity and phytochemical screening of endophytic extracts of *Crotalaria pallida*. Free Radicals Antioxid. 2011; 1:79-86.
34. Puravankara D, Boghra, V, Sharma RS. 2000. Effect of antioxidant principles isolated from mango (*Mangifera indica* L.) seed kernels on oxidative stability of buffalo ghee (butter-fat). J Sci Food Agric. 2000; 80:522-526.
35. Amir M, Khan A, Mujeeb M, Ahmad MA, Siddiqui NA. Phytochemical Screening and *in vitro* Antioxidant Activity of Jawarish Amla. A Poly Herbal Formulation. Pharmacog J. 2011; 3:54-60.
36. Rao AS, Reddy SG, Babu PP, Reddy AR. The antioxidant and anti-proliferative activities of methanolic extracts from Njavara rice bran. BMC Compl Altern Med. 2010; 10:1-9.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

37. Newell AM, Yousef GG, Lila, MA, Ramirez-mares, DeMejia EG. Comparative *in vitro* bioactivities of tea extracts from six species of *Ardisia* and their effect on growth inhibition of HepG2 cells. J Ethnopharmacol. 2010; 130:536–544.
38. Misbah H, Aziz AA, Aminudin N. Antidiabetic and antioxidant properties of *Ficus deltoidea* fruit extracts and fractions. BMC Compl Altern Med. 2013;13:1-12.
39. Choe E and Min DB. Mechanisms of antioxidants in the oxidation of foods. Compr Rev Food Sci Food Saf. 2009; 8:345-358.
40. Haminiuk CWI, Plata-Oviedo MSV, de Mattos G, Carpes ST, Branco IG. Extraction and quantification of phenolic acids from *Eugenia pyriformis* using different solvents. J Food Sci Technol. 2014; 51:2862-2866.
41. Xiaoli L, Chun C, Mouming Z, Jinshui W, Wei L, Bao Y, Yueming J. Identification of phenolics in the fruit of emblica (*Phyllanthus emblica* L.) and their antioxidant activities, Food Chem. 2008; 109:909–915.
42. Kumar PS, Sucheta S, Deepa VS, Selvamani P, Latha S. Antioxidant activity in some selected Indian medicinal plants. Afr J Biotechnol. 2008; 7:1826-1828.
43. Rojsanga P, Gritsanapan W, Suntornsuk L. 2006. Determination of berberine content in the stem extract of *Coscinium fenestratum* by TLC densitometry. Med Princ Pract. 2006; 15:373-378.
44. Tushar KV, Satheesh G, Remashree AB, Balachandran I. *Coscinium fenestratum* (Gaertn.) Colebr.-a review on this rare, critically endangered and highly-traded medicinal species. J Plant Sci. 2008; 3:133-145.
45. Wohlmuth H, Leach DN, Smith MK, Myers SP. Gingerol content of diploid and tetraploid clones of ginger (*Zingiber officinale* Roscoe). J Agric Food Chem. 2005; 53:5772-5778.
46. Al-Mamary M, Al-Habori M, Al-Zubairi AS. The in vitro antioxidant activity of different types of palm dates (*Phoenix dactylifera*) syrups. Arab J Chem.2014; 7:964–971.