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Estimation of Total Phenolic and Total Flavonoid Contents, *In-Vitro* Antioxidant Activity and Median Inhibitory Concentration of Methanol Root Extract of *Ximenia americana*, L. *Olacaceae*

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Authors' contributions

This research was carried out in collaboration among all authors. Author BGD conceived and designed the study, wrote the protocol, performed the analysis and, drafted the first manuscript. Authors SSG and MON authorized the research work read-proved the manuscript. Authors BGD, SO, WMI and CJJ supervised the bench work, managed the literature search and read the final draft of the manuscript.

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ABSTRACT

Background: This research was carried out to estimate the total phenolic and flavonoid contents, assayed the in-vitro antioxidant potential, and estimate the median inhibitory concentration (IC_{50}) of the methanol root extract of *Ximenia americana*, Linn.
Study Design: It was an experimental study design.
Methods: Total phenolic and flavonoid contents of the extract were determined by spectrophotometry using Folin-Ciocalteu reagent and Aluminum chloride colorimetric assay, readings were taken at 750 nm, and 415 nm wavelength respectively. The results were expressed in terms of Gallic acid and Rutin standards equivalent. The in-vitro antioxidant activity and the IC_{50} of

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the plant material were investigated by its ability to scavenge free radicals, using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. Similarly, the total percentage yield of the extract was estimated from the crude.
Results: The crude root powder of *Ximenia americana* showed a total extract yield of 20.06%w/w. Total phenolic and flavonoid contents were determined to be 3.501 ± 0.774 mg GAE/g, and 10.644 ± 0.20 mg RE/g extract. The extract displayed concentration-dependent DPPH free radical scavenging activity, with the highest concentration (200 µg/mL) inhibiting 60.93% of DPPH free radical, and the IC_{50} of the extract was determined to be 69.01 µg/mL.
Conclusions: *Ximenia americana* L methanol root extract has a yield of 20.06%w/w, and a total phenolic content of 3.501± 0.774 mg GAE/g, total flavonoids content of 10.644 ± 0.20 mg RE/g, and IC_{50} of 69.01 µg/mL, also, the extract possesses a potent antioxidant activity by its ability to inhibit DPPH activity.

Keywords: Antioxidant; DPPH; total phenolics; total flavonoids; IC_{50}; Ximenia americana.

1. INTRODUCTION

Ximenia americana is a tropical plant, one of the eight species of the *Olacaceae* family. This plant is very useful in traditional medicine and is used differently in different countries predominantly in African to manage varieties of ailments [1-3]. We have demonstrated the in vivo antioxidant activity of this plant extract using the *Drosophila melanogaster* model [4].

Polyphenolic compounds such as phenolics and flavonoids are secondary metabolites and a group of phytochemicals that are synthesized by plants to provide immunity for them against adversity [5]. Also, these classes of compounds have been demonstrated to possess excellent antioxidant activity that helps to scavenge or neutralize reactive oxygen and nitrogen (RON) species or free radicals that are known to cause oxidative stress in humans [6-10,1]. These secondary metabolites are useful to humans medicinally to manage, cure or prevent certain genetic, neurodegeneration, and or chronic diseases such as diabetes, cancer, cardiovascular, and for immunoregulation [10-13,2].

Beside possession of antioxidant activity, phenolics, and flavonoids, and other polyphenols have been reported to have antibacterial, anti-inflammation, skin protecting effects among other benefits [10].

The total phenolic content of the leaf extract of *Ximenia americana* has been determined [14]. Similarly, work has been done on the anti-nociceptive and anti-inflammatory properties of the root polyphenol fraction of this plant using animal model [15]. This present work was done in other to estimate the total phenolic and flavonoid contents, and to assay the in-vitro antioxidant activity of the methanol root extract of the plant. Hence, establishing the median inhibitory concentration of the methanol root extract of *X. americana*. Family *Olacaceae*.

2. MATERIALS AND METHODS

2.1 Plant Material Collection and Extraction

The collection of the plant material, identification/authentication, and the method used for the extraction were previously reported by our team [16]. The roots of the plant *Ximenia americana* were collected in Makabun Village in Kaura Local Government Area, Kaduna State, Nigeria. The plant was identified and authenticated by a plant taxonomist Mr. J. J. Azila of Federal College of Forestry Jos, Jos, Plateau State Nigeria, voucher number FHJ 243 was deposited at the herbarium of the college. The plant roots were washed with clean water, the outer scale carefully removed and air-dried at room temperature, then pulverized manually using wooden mortar and pestle. The powdered sample was stored at 4^{o}c in airtight container and properly labeled for further work.

2.2 Chemical

The chemicals used in this study include Rutin, 2,2- diphenyl-1-picrylhydrazyl (DPPH), Sodium Acetate, Sodium Carbonate, Ethanol, Methanol, Ascorbic acid (JHD® CAS No: 50-81-7, Lot No: 20141104), and Aluminum Chloride (D®, CAS No: 7784-13-6, Lot No: 20180604). All the reagents used were of analytical grades.

2.3 Determination of Percentage (%w/w) Yield

The percentage yield of the methanol root extract of *Ximenia americana* was determined from the dry weight of the extracted powder(a) and the soaked plant powder sample (b). The formula started below was used:

$$Percentage\ (\%w/w)\ yield = weight\ of\ dried\ powder\ extract\ (a)/weight\ of\ soaked\ plant\ sample\ (b) \times 100$$

2.4 Total Phenolic Content (TPC) Determination

Total phenolic content (TPC) was determined spectrophotometrically using Folin-Ciocalteu reagent (FCR). The method initially described by [17] and subsequently modified by [8] was used. First, a gallic acid standard curve was plotted from a stock solution with a concentration of 100 mg/mL to make five gallic acid standard solutions of different concentrations (0.02, 0.04, 0.06, 0.08, and 0.10 mg/mL). Next step, 1 mL of each sample as well as diluted plant extract was added to 25 mL volumetric flasks containing 9 mL of distilled H_2O. After that, 1 mL of Folin-Ciocalteu (FC) reagent was added to the flask and mixed thoroughly. Furthermore, the samples were incubated at room temperature for 5 minutes then 10 mL of 7%w/v sodium bicarbonate (Na_2CO_3) solution was added to the mixture in each flask. Subsequently, the flasks were incubated for 90 minutes at ambient room temperature (23˚c). Next, the absorbance of the sample extract, which was orange-yellow in hue, was measured at a wavelength of 750 nm using a UV spectrophotometer (Jenway UV-7513). Lastly, the TPC results were expressed as mg/g gallic acid equivalents (GAE/g) of plant methanol root extract using a standard curve. The sample was analyzed in triplicate [18]. The assay was performed in triplicates and the values were expressed in mean ± SD.

2.5 Total Flavonoid Content (TFC) Determination

The total flavonoid content of methanol root extract of *Ximenia americana* was determined using the aluminum chloride colorimetric assay procedure previously described by [19,20] and slightly modified by [8]. Briefly, standard solution of Rutin of various concentration (6.25,12.5,18.75,25,50,70 µg/mL) were prepared in 96%v/v ethanol. 50 µL of extracts (1 mg/mL) or standard solution was added to 10 µL of 10%w/v aluminum chloride solution, this was followed by 150 µL of 96%v/v ethanol. Thereafter, 10 µL of 1 M sodium acetate was added to the mixture in the test tubes. 96%v/v ethanol was used as a reagent blank. All reagents were mixed and incubated inside a dark cupboard (to protect each from light) for 30 min at room temperature. The absorbance was measured at wavelength of 415 nm using a UV- spectrophotometer (Jenway 7513). Rutin was used to make the standard calibration curve.

The total flavonoid content (TFC) was calculated from the calibration curve of Rutin plotted, and the result was expressed in mg Rutin Equivalents (RE) per g of plant extract (mg RE/g). The assay was performed in triplicates and the values were expressed in mean ± SD.

2.6 2,2-Diphenol-1-Picrylhydrazyl (DPPH) Anti-oxidant Assay

The 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity was performed using a protocol previously described by [21], and reported by [20,19,8] with little modification. Briefly, 20 µL of plant extracts (1 mg/mL) or standard solution of ascorbic acid (67 µg/mL) in absolute methanol was added to 180 µL of DPPH reagent in test tubes. Absolute methanol was used for the reagent blank. All reagents were mixed and incubated in a dark cupboard for 30 minutes at room temperature, protected from light. The absorbance was measured at 517 nm wavelength using a UV-Spectrophotometer. The experimental setup was done in triplicates. The percentages of the DPPH free radical scavenging activity were calculated as follows:

$$Percentage\ (\%w/w) DPPH\ Scavenging\ activity = \frac{(Absorbance\ of\ control\ - Absorbance\ of\ sample\ )}{(\ Absorbance\ of\ control)} \times 100$$

The percentages of the DPPH free radical scavenging activity were determined by comparing with free radical scavenging activity of ascorbic acid and expressed as mg vitamin C Equivalent Antioxidant Capacity (VCEAC) per g of dry plant extract. From the result obtained, the median inhibitory concentration required to scavenge 50% (IC_{50})) of the DPPH was estimated.

The results from this research work were analyzed using data analysis from Microsoft excel 16, and GraphPad Prism version 8.0.2 (263) for the IC_{50} analysis.

3. RESULTS AND DISCUSSION

The percentage yield of the methanol root extract of the plant *Ximenia americana* was found to be 20.06%w/w. This by implication, means that in 100 gm of crude dried root plant powder, after extraction, 20.06 gm of dried powder extract was obtained.

3.1 Total Phenolic Content

The total phenolic content of the plant extract was assayed to figure out the quantity of phenolics contain in it, this usually will correlate to the antioxidant capacity of the methanol root extract of the plant, *Ximenia americana*.

Fig. 2 showed the equation of the gallic acid calibration curve. From the regression equation of the calibration curve (Y= 0.17x + 0.0944, and a correlation coefficient, R^2 = 0.9746), the total phenolic content was estimated to be 3.501 ± 0.774 mg GAE/g (Table 1). With the correlation coefficient (R^2) 0.9746, it can be deduced that the extract has some antioxidant potential.

3.2 Total Flavonoid Content

Flavonoid is the largest class of polyphenolic compounds that are well distributed in plants [9,22]. This class of polyphenolics is made up of about six sub-classes including; anthocyanidins, flavonols, flavanones, flavones, flavonols, and isoflavones [5,23,24].

Fig. 3 showed the equation of the Rutin calibration curve, and from the regression equation of the calibration curve (Y= 0.0177x + 0.0536, and a correlation coefficient, R^2 = 0.998), The total flavonoids content in the methanol root extract of *X. americana* were calculated to be 10.644 ± 0.20 mg RE/g (Table 1).

3.3 *In vitro* Antioxidant (DPPH) ASSAY

The antioxidant activity of the methanol root extract of *X. americana* was determined via DPPH free radical scavenging assay. The ability of the tested extract to scavenge DPPH free radical is shown in Fig. 4. Our result demonstrated a dose-dependent increase in antioxidant activity of the test material. The highest concentration of the extract (200 µg/mL) scavenged DPPH radical by 60.93% as compared to the standard ascorbic acid (67 µg/mL) that showed 100% DPPH scavenging. Similarly, the median inhibitory concentration (IC_{50}), of the plant extract was 69.01 µg/mL, with a correlation coefficient (R^2) of 0.9551 (Fig. 5). These results showed that the plant material has a potent antioxidant activity and a good safety margin which correlated well with the IC_{50}.

Table 1. Total phenolic and flavonoid contents of the *X. americana* extract

Methanol extract	Total content
Phenolic (mg GAE /g)	3.501 ± 0.774
Flavonoid (mg RE/g)	10.644 ± 0.20

GAE = Gallic Acid Equivalent
RE = Rutin Equivalent

Fig. 1. Cleaned root of *Ximenia americana*

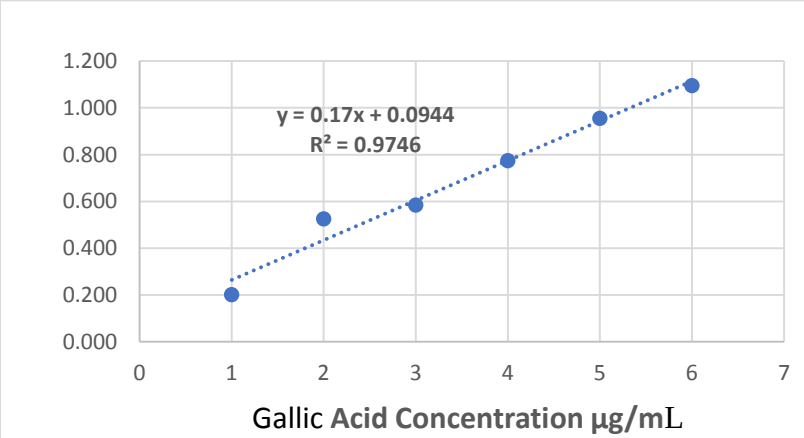

Fig. 2. Gallic acid calibration curve

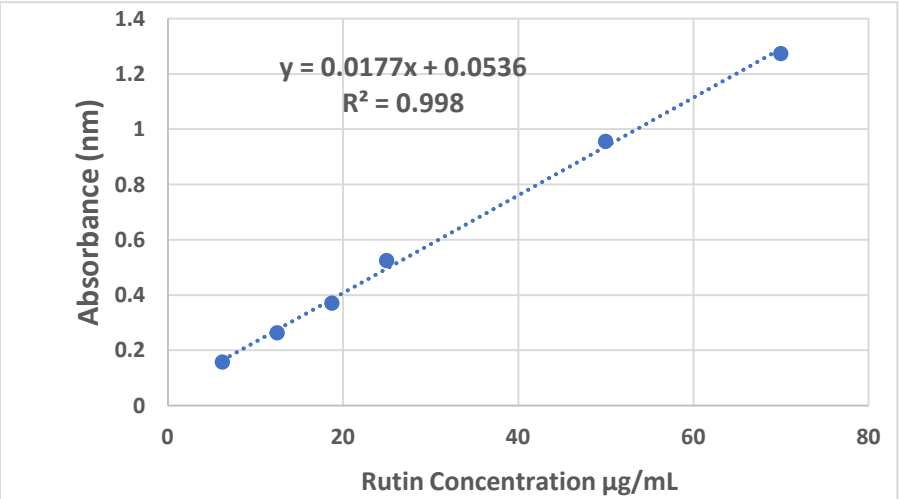

Fig. 3. Rutin calibration curve

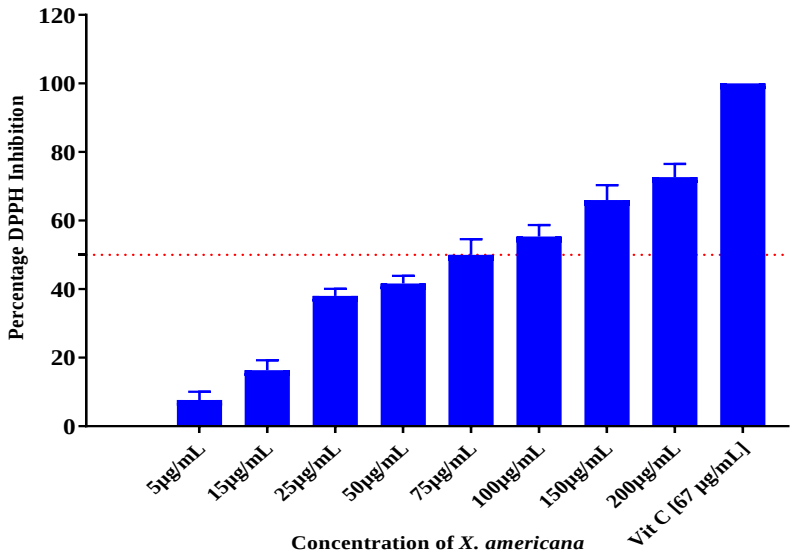

Fig. 4. Percentage DPPH activity of *X. americana*

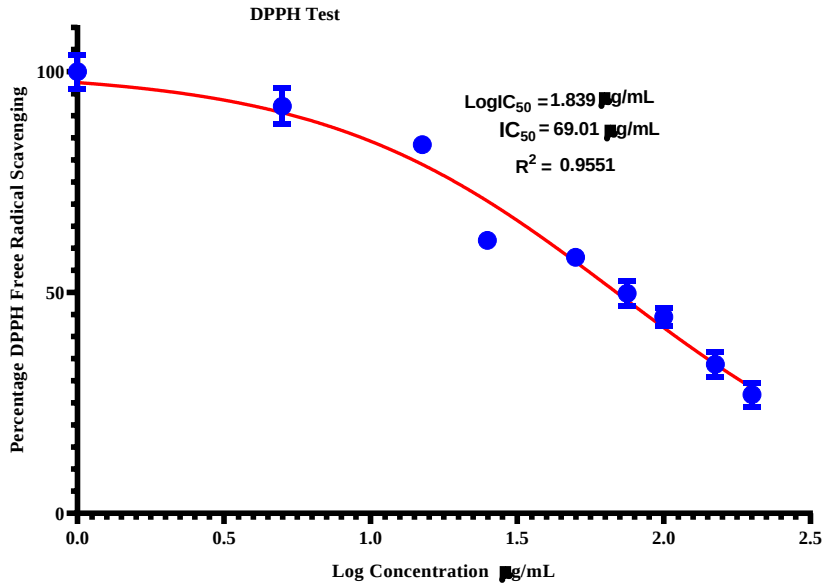

Fig. 5. DPPH median inhibitory concentration (IC_{50}) of *X. americana* root extract

4. CONCLUSION

This study has unraveled the presence and quantities of total phenolics and flavonoids that are contained in the methanol root extract of *Ximenia americana*, elucidated by in-vitro antioxidant potentials, and estimated the concentration of the extract that can inhibit 50% DPPH activity. The antioxidant potential of the plant may be due to the rich phenolics and flavonoids content, hence, its ability to scavenge DPPH free radicals. The presence of these phytochemicals in the methanol root extract of the plant could be a reason why it is used in folk medicine for the management of many disease conditions, especially those that are associated with oxidative stress. Further work needs to be done to isolate and characterize the bioactive compounds that are responsible for the observed antioxidant activity of the plant part.

ETHICAL APPROVAL

Ethical considerations were not necessary for the collection of the plant material. The plant material was collected in the wild not protected by any law, and this work did not endanger the plant species.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCE

1. Haidara M, et al. Medicinal plants used in Mali for the treatment of malaria and liver diseases. Nat. Prod. Commun. 2016;11(3): 339–352.
DOI: 10.1177/1934578x1601100309.
2. Olanrewaju TO, Odumosu PO, Eyong KO. Anti-trypanosomal evaluation of Ximenia americana root bark and chromatographic-mass spectrometric profile GSC Biological and Pharmaceutical Sciences Anti-trypanosomal evaluation of Ximenia americana root bark and chromatographic -mass spectrometric profi. GSC Biol. Pharm. Sci. 2019;07(02):108–117.
DOI: 10.30574/gscbps.2019.7.2.0051.
3. Shettar AK, Kotresha K, Kaliwal BB, Vedamurthy AB. Evaluation of in vitro antioxidant and anti-inflammatory activities of Ximenia americana extracts. Asian Pacific J. Trop. Dis. 2015;5(11): 918–923.
DOI: 10.1016/S2222-1808(15)60957-4.

4. Bagu GD, Omale S, Iorjiim WM, Uguru MO, Gyang SS. Determination of LD_{50}, Fecundity and Locomotor Effects of Methanol Root Extract of Ximenia americana Linn, in Drosophila melanogaster," Asian J. Biochem. Genet. Mol. Biol. 2020;5(2):1–9.
DOI: 10.9734/AJBGMB/2020/v5i230123.
5. Embola J, Rock C, Wang L, Reiboldt W, Ahmed S, Aliabadi S. An Assessment of Total Polyphenolic Content and Antioxidant Potential of Mauby Bark Extracts (*Colubrina arborescens*) Brewed for Different Lengths of Time. J. Food Res. 2019;8(3):26–34.
DOI: 10.5539/jfr.v8n3p26.
6. Chandra S, et al. Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: A comparative study. Evidence-based Complement. Altern. Med. 2014;1–9.
DOI: 10.1155/2014/253875.
7. Kumar S, Pandey AK. Chemistry and Biological Activities of Flavonoids: An Overview. Sci. World J. 2013;1–16.
DOI: 10.2307/j.ctt1w0ddx8.35.
8. Elin Novia Sembiring, Berna Elya, and Rani Sauriasari. Total Flavonoid Content Total Phenolic Content. Pharmacogn J. 2018;10(1):123–127. [Online].
Available:http://dx.doi.org/10.1016/j.hermed.2014.01.002.
9. Bag GC, Grihanjali Devi P, Bhaigyabati T. Total Flavonoid Content and Antioxidant Activity of Aqueous Rhizome Extract of Three Hedychium Species of Manipur Valley. Res. J. Pharm. Biol. Chem. Sci. 2014;5(5):970–976.
10. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. Medicines. 2018;5 (93):1–16.
DOI: 10.3390/medicines5030093.
11. Tristantini D, Amalia R. Quercetin concentration and total flavonoid content of anti-atherosclerotic herbs using aluminum chloride colorimetric assay. In AIP Conference Proceedings. 2019;2193:1–7.
DOI: 10.1063/1.5139349.
12. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer prevention: A Review, Journal of the American Dietetic Association. 1996;96(10):1027–1039.
DOI: 10.1016/S0002-8223(96)00273-8.
13. Akbay P, Basaran AA, Undeger U, Basaran N. In vitro immunomodulatory activity of flavonoid glycosides from Urtica dioica L. Phyther. Res. 2003;17(1):34–37.
DOI: 10.1002/ptr.1068.
14. Mariko M, Sarr SO, Diop A, Modi IA, Dackouo B, Diop YM. Antioxidant activity study and total phenolic determination of leaf extracts of *Ximenia americana L*. (Olacaceae) an anti-tumor plant used traditionally in Mali. J. Appl. Biosci. 2016;106:10258–10265.
DOI: 10.4314/jab.v106i1.5.
15. Kiessoun K, et al. Anti-Nociceptive and Anti-Inflammatory Properties of Polyphenol-Rich Fractions of Roots from *Ximenia americana L.,* (*Olacaceae*), in Experimental Mice. Int. J. Pharm. Pharm. Res. 2018;12(1):281–297.
16. Bagu GD, et al. *In Viv*o Antioxidant and Toxicity Properties of Methanol Root Extract *of Ximenia Americana, l. (Olacaceae*) in Drosophila Melanogaster. Int. J. Eng. Appl. Sci. Technol. 2020;4(12):59–66.
17. Marinova D, Ribarova F, Atanassova M. Total Phenolics and Total Flavonoids in Bulgarian Fruits and Vegetables. J. Univ. Chem. Technol. Metall. 2005;40(3):255–260.
18. Marinova D, Ribarova F, Atanassova M. Total phenolics and flavonoids in Bulgarian fruits and vegetables. J. Univ. Chem. Technol. Metall. 2005;40(3):255–260.
19. Ghosh S, et al. Phytochemical Analysis and Free Radical Scavenging Activity of Medicinal Plants Gnidia glauca and Dioscorea bulbifera. PLoS One. 2013;8(12):1–18.
DOI: 10.1371/journal.pone.0082529.
20. Chatatikun M, Chiabchalard A. Phytochemical screening and free radical scavenging activities of orange baby carrot and carrot (*Daucus carota Linn.)* root crude extracts. J. Chem. Pharm. Res. 2013;5(4):97–102.
21. Yamasaki K, Hashimoto A, Kokusenya Y, Miyamoto T, Sato T. Electrochemical Method for Estimating the Antioxidant effects of Methanol Extracts of Crude Drugs," Chem. Pharm. Bull. 1994;42(8):1663–1665..
22. Phuyal N, Jha PK, Raturi PP, Rajbhandary S. Total Phenolic, Flavonoid Contents, and Antioxidant Activities of Fruit, Seed, and

Bark Extracts of Zanthoxylum armatum DC," Sci. World J. 2020;1–7. DOI: 10.1155/2020/2803063.
23. Scalbert A, Williamson G. Dietary intake and bioavailability of polyphenols," J. Nutr. 2000;130: 2073S-2085S. DOI: 10.1093/jn/130.8.2073s.
24. Dai J, Mumper RJ. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010;15(10):7313–7352. DOI:10.3390/molecules15107313.

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