Nigerian Research Journal of Chemical Sciences (ISSN: 2682-6054)
Volume 12, Issue 2, 2024

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF A TRADITIONAL MEDICINAL PLANT FROM NORTHERN NIGERIA

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Accepted: July 8, 2024. Published Online: July 17, 2024

ABSTRACT

Phytochemical screening and evaluation of antioxidant activity of medicinal plant extract are promising step of discovery of therapeutic compounds. The present study was aimed to investigate the class of phytochemical and antioxidant activity of *Balanites aegyptica* leave crude extracts. The leaves were collected, washed, dried, pulverized and subjected to sequential extraction using different solvents namely, methanol, ethanol and hexane, according to standard methods. The qualitative analysis indicated the presence of secondary metabolites including alkaloids, flavonoids, tannins, phenols, saponins and terpernoids in the extracts. Quantitative analysis of total phenolic and flavonoids contents revealed flavonoids (1.580 mg/g) and phenols (1.432 mg/g). The antioxidant activity of the crude extracts was evaluated using 2,2-di-phenyl-1-picrylhydrazyl (DPPH) assay. The results demonstrated significant antioxidant potential for *Balanites aegyptica* leaf extracts, with scavenging activity against free radicals. The findings highlight the potential of this plant as source for natural antioxidant which can be utilized in pharmaceutical and food industries.

Keywords: *Balanites aegyptica*, antioxidant, medicinal plant, phytochemical

INTRODUCTION

Traditionally medicinal plants have been used as a source of medicine since the beginning of human kind. Plant remains the natural source of organic compounds with curative properties which are still useful in healing of various diseases in many parts of the globe including Africa, Asia and South America [1]. World Health Organization (WHO) has reported that over 80% of the global population is using medicinal plant extracts for their common health problems [2]. Antioxidant derived from natural plant sources fight against many pathological health problems such as cancer, diabetes, aging, cardiovascular, and other free radical induced diseases [3].

Several researchers have studied chemical composition and biological activity of various traditional medicinal plants extracts including *Balanite aegyptica* extracts. Bhimraj reported the presence of carbohydrate, protein, amino acids, glycoside, tannins, saponins, flavonoids and phenolic compounds in leaves aqua-ethanol extract of *Banalites aegyptiaca* and shown antioxidant activity using DPPH assay [13]. Sham *et al* also reported ethanol of B. aegyptiaca Its fruits extract contains triterpenes, tannins, coumarins, saponins and flavonoids and petroleum ether of B. aegyptiaca Fruits extracts show that the plant contains coumarins, and sterols. flavonoids, and illustrated that B. aegyptiaca have potential antioxidant that could be used as therapeutic agent in healing health problems related to free radical conditions [14].

This study was aimed to investigate the phytochemicals and antioxidant activity of different solvent extracts of leaves of *Balanites aegyptiaca* so as to contribute to the existing information on promising curative agent of B. aegyptica and provide roadmap for further study into its benefit as a natural antioxidant.

MATERIALS AND METHODS

Sample collection and preparation of extracts

The leaves of *Balanites aegyptiaca* were collected from the mature tree in Wukari, Taraba State Nigeria. It was authenticated by Dr. Ademu Lawrence at the Department of Forestry and Wildlife, Federal University, Wukari, Nigeria. The ground and dried leaves of *Balanites aegyptiaca* were extracted using the method described by Natividad et al [13] with slight modification. The maceration method was followed for the extraction. Exactly 100 g powder of dried leaves were added into 400 mL of 80% ethanol, Hexane and methanol in three (6) different flasks (1000 mL capacity), two for each solvent and the resulting mixtures was vortexed well. The flasks were subjected to magnetic stirring (750 rpm) for 4 h and left to stand at room temperature for 24 h. The mixtures were filtered through 0.45 µm Whatman No1 filter paper. The operation was repeated three times. After 72 h, the filtrate was evaporated under vacuum using a rotary evaporator until the dried extract was obtained. Then the extract obtained was recovered in a tube and stored at 4 °C until use.

Phytochemical screening

Phytochemical screening the leaves of *Balanites aegyptiaca* for the presence of secondary metabolites such as tannins, saponins, alkaloids, steroids, terpenes, anthraquinone, flavonoids, cardiac glycosides, phenols, resins, carbohydrates and phlobatannins were carried out using standard procedures described by Natividad et al [4].

Total phenolic content assay

The total phenolic content of each extract was determined using the Folin-Ciocalteau method as described by Natividad et al [4]. Exactly 5 mg of the extract was dissolved in 1 ml of the extraction solvent. Calibration curves were obtained using stock solution of gallic acid. This was prepared by dissolving 5 mg of garlic acid in 100 µl of absolute ethanol and varying concentrations of the compounds were obtained through serial dilutions to i.e., 5, 2.5, 1.25, 0.625, 0.3125, 0.15625 and 0.078125 mg/ml. Exactly 50 % of ethanol solution was used as the blank. 10 µl of of the samples required for analysis was introduced into the curette in triplicates, 790 µl of distil H_2O and 50 µl of Folin-Ciocalteau reagent were added to each of the sample, mixed and incubated at room temperature for 8 minutes. This was succeeded by addition of 150 µl of Na_2CO_3 (20% w/v) and further incubated at room temperature for 2 hours. After the incubation, absorbance was read at a wavelength of 750 nm using the AT1 UNICAM UV/VIS spectrometer (UV4 coupled to Vision V3.40 computer software). From the measured absorbance, the total phenolic content of each extract was calculated through extrapolation of the calibration curve and expressed as Gallic acid equivalents (GAE). [4]. The total phenolic content of each extract illustrated in Table 3.

Total flavonoids content assay

Total flavonoids content of the extract was estimated using the aluminum chloride ($AlCl_3$) colorimetric method reported by Natividad et al [4]. Exactly 5 mg of the extract was dissolved in 1 ml of the test solvent to obtain the analyte. Calibration curve was prepared using, 1 mg of quercetin dissolved in 1 ml of absolute methanol. Seven different concentrations of the resulting quercetin solution were prepared serially in 2-folds (0.015625 - 1 mg/ml). About 100 µl of each

extract or quercetin concentration was aliquot into the corvettes in triplicates. 100 µl of 2% aluminum chloride ($AlCl_3$) was added to each well. The plate was thoroughly shaken and incubated at room temperature for 20 minutes. After the incubation, absorbance was read at a wavelength of 415 nm using AT1 UNICAM UV/VIS spectrometer (UV4 coupled to Vision V3.40 computer software). From the absorbance readings, the total flavonoids content of each extract was calculated from the regression equation of the quercetin standard curve and expressed as quercetin equivalents (QE) [4].The total flavonoids content of each extract shown in Figure1 and Table 3.

Antioxidant Activity: DPPH-Free Radical scavenging activity

The analysis of the DPPH radical scavenging activity of the plant extracts was performed according to the method described by Natividad et al [4]. Stock solution was prepared by dissolving 100 mg of extract in 1 ml of methanol and five, two fold serial dilutions was made. 0.5 ml of each of the concentrations was measured into separate test tubes and 0.3 ml of 0.5 mM DPPH was added. The reaction mixtures were vigorously shaken for 30 s in a vortex apparatus and allowed to stand in the dark at room temperature for 30 minutes. Ascorbic acid was used as a standard for the investigation of the antiradical activity and was prepared in a similar manner. The absorbance was read using spectrophotometer at 517 nm against the blank. The blank was prepared by mixing 0.5 ml of the extract or ascorbic acid with 3.3 ml of methanol. Similarly, the control solution was prepared by mixing 3.5 mL of methanol and 0.3 ml of DPPH radical solution [4].The percentage of scavenging activity (X%) was calculated according to the formula:

$$X\% = \frac{AS - AB}{AC} \times 100$$

X% = Percentage of scavenging activity

AS = Absorbance of sample

AB = Absorbance of blank

AC = absorbance of control

RESULT AND DISCUSSION

Table 1: Percentage yield of *Balanites aegyptica* leaf extract

Solvent	Extract colour	Extracts yield (mg)	Percentage recovery
n-Hexane	Green	4.223	24.491
Ethanol	Green	3.362	11.573
Methanol	Green	1.008	12.609

Table 2: Phytochemical screening of *Balanite aegyptica* leaf extract

Phytochemical	Methanol extract	n-Hexane extract	Ethanol extract
Saponins	+	-	+
froth test			
Phenol	+	+	+
Terpernoids	+	+	+
Steroid	+	+	+
Tannins	+	-	+
Phlobatannins	-	-	-
Glycosides			
Borntrager's test	+	+	+
Anthraquinone	-	-	-
Flavonoids			
Alkaline test	+	+	+
Lead acetate	+	-	+
Alkaloid			
Mayer test	+	+	+
Wagner test	+	+	+

+ = presence − = absence

Table 3: Quantitative phytochemical analysis of flavonoids and phenols contents of *Balanites aegyptiaca* leaf crude extract

Phytochemical	Concentration (mg/g)
Flavonoids	1.580
Phenols	1.432

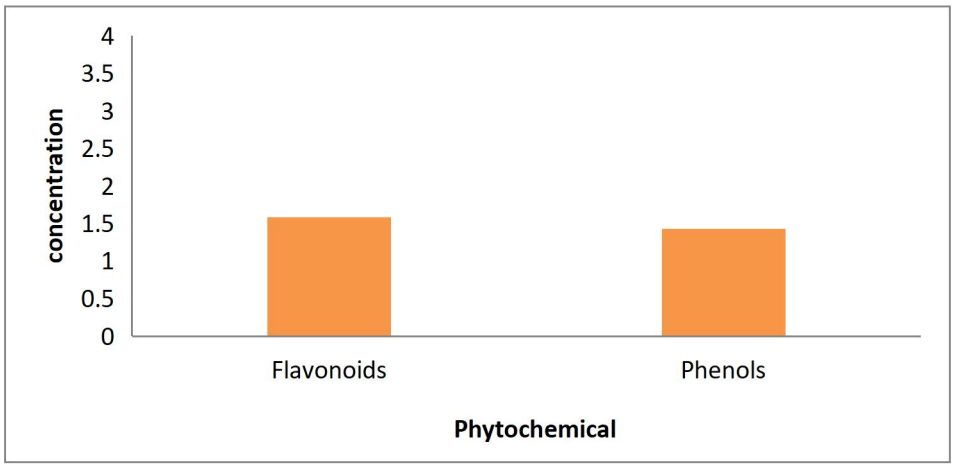

Figure 1: Graphical representation of quantitative phytochemical analysis of flavonoids and phenols content of *Balanites aegyptica* leaf extract

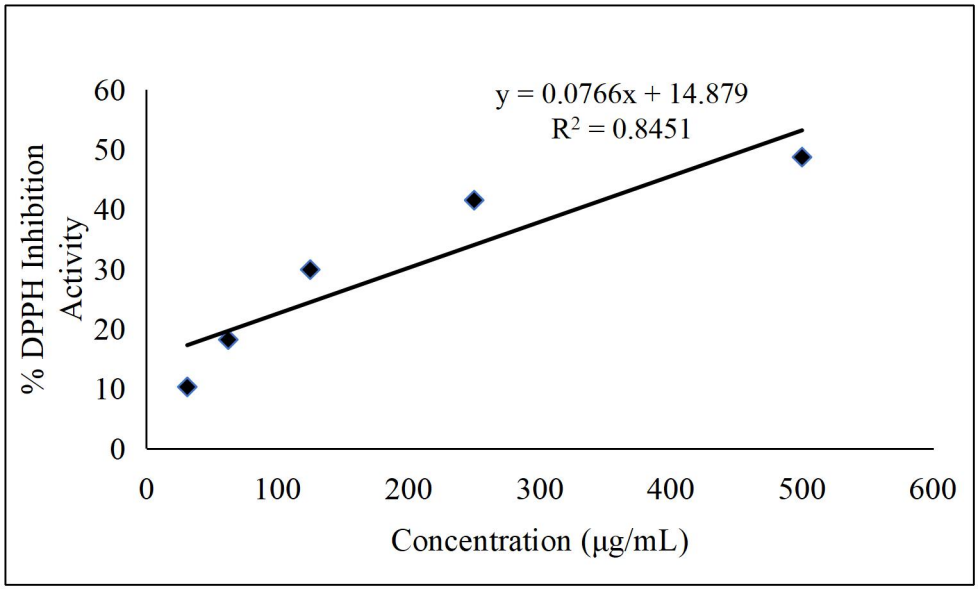

Figure 2: %DPPH Inhibition activity plot for methanol of *Balanites aegyptica*

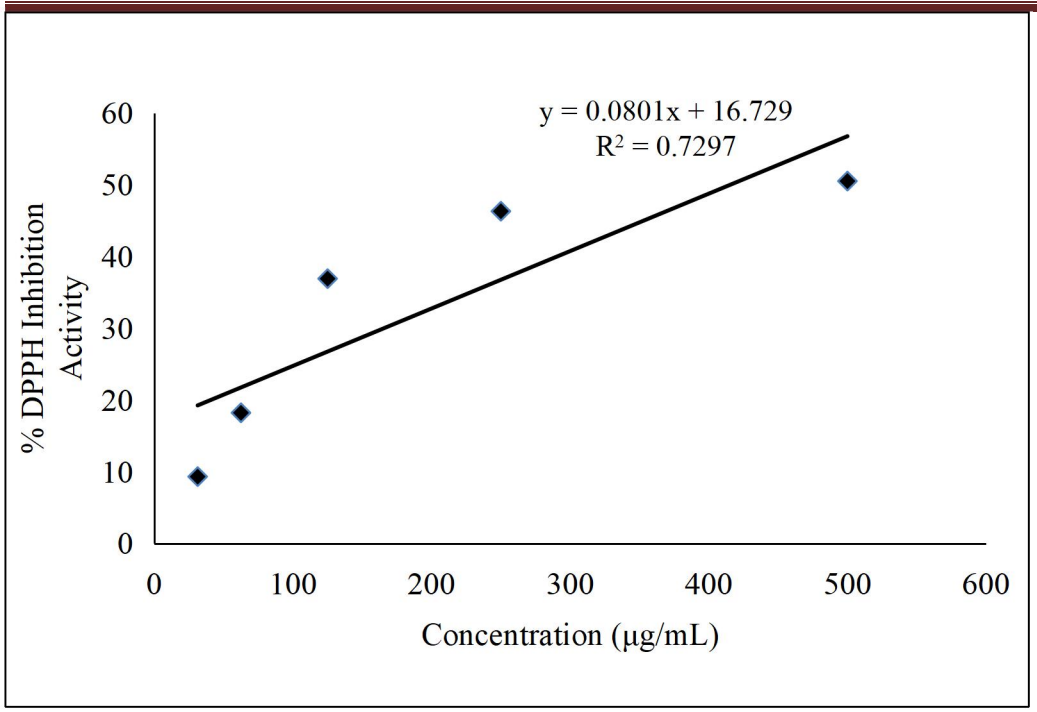

Figure 3: %DPPH inhibition activity plot for ethanol extract of *Balanite aegyptica*

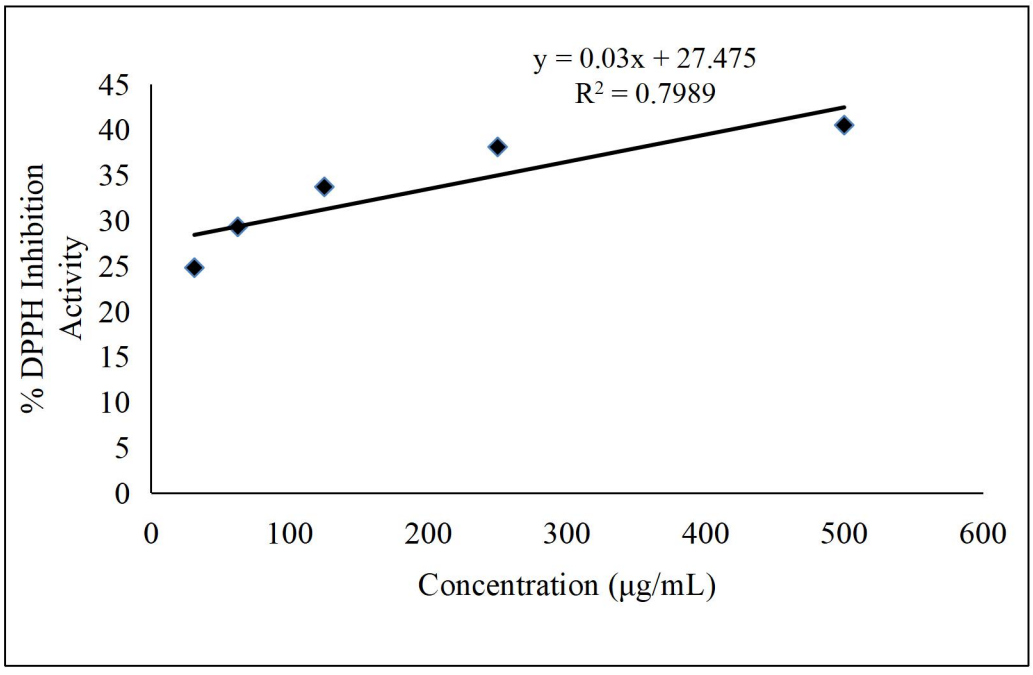

Figure 4: %DPPH inhibition activity plot for n-hexane extract of *Balanites aegyptiaca*

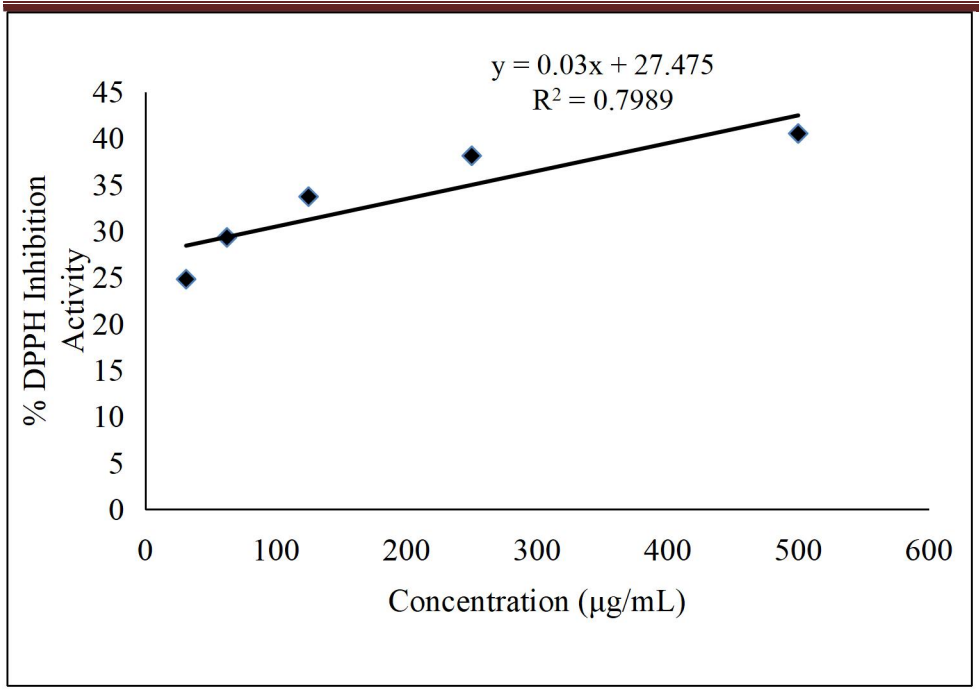

Figure 5: %DPPH inhibition activity plot for n-hexane extract of *Balanites aegyptiaca*

Percentage yield of *Balanite aegyptiaca* leaf extract

The percentage yields of these extracts were as shown in Table 1 from the result, n-hexane extract has the highest value of 4.223mg with 24.49% recovery while methanol has the least value.

Phytochemical analysis *of Balanites aegyptiaca* leaf extract

The phytochemical screening of the ethanol, methanol and n-hexane extracts of the leave of *B. aegyptiaca* revealed the presence of flavonoids, saponins, tannins, phenols, glycoside and steroids. In the n-hexane extract, only phenols, steroid, flavonoids and alkaloid were detected as presented in Table 2. Phytochemicals like phlobatannins and anthraquinone were not detected in all the sample extracts. The result shows that the presence of phytochemicasl in different extracts is dependent on the polarity of the solvents used for the extraction [5]. Studies have shown that the presence of secondary metabolites such as saponins, tannins and anthraquinones in the stem bark of *B. aegyptiaca* responsible for the antibacterial activity of this plant part [6]. Similarly, the presence of triterpenes, saponins and steroidal saponins were reported to be responsible for the

antifungal activity [7-8]. Other phytochemical like saponins, terpernoids, flavonoids, tannins and steroids have been reported to have anti-inflammatory effects[9] while saponins was reported to be responsible for the insecticidal and hepatoprotective activities of the plant [10].

Quantitative phytochemical screening of *Balanites aegyptiaca*

The Quantitative Phytochemical Screening of *Balanite Aegytica has* been illustrated in Table 3 above, Flavonoids has the concentration of 1.580mg/g while phenol has the concentration of 1.432 mg/g

%DPPH inhibition analysis of *Balanites aegyptiaca*

The ethanolic, methanolic and n-hexane extracts o of *B. aegyptiaca* were subjected to 2, 2-diphenyl-1 picryl hydrazyl (DPPH) radical scavenging assay. The ethanol extract showed a substantial free radical scavenging activity which is concentration dependent as presented in Figures 1-4. The antioxidant activity of this plant leave extract is reported to be due to the presence of flavonoids and phenolic, and these metabolites have redox properties that allow them to act as hydrogen donors, single oxygen quenchers and reducing agents. The presence of flavonoids and phenolic in food prevents lipid oxidation and thereby inhibiting many diseases as cancer and atherosclerosis [11].

CONCLUSION

This research on *Balanites aegyptiaca* leaf extracts revealed valuable insights into the phytochemical composition and antioxidant properties of the plant extracts. *Balanites aegyptiaca* extracts exhibited significant antioxidant activity, indicating their potential health benefits. The presence of phytochemicals such as flavonoids and phenols in these extracts contributes to the antioxidant properties and therapeutic potential. .The findings support the traditional medicinal uses of these plants and provide scientific evidence for their antioxidant and therapeutic properties, paving the way for further research and potential applications in the field of phyto-medicine.

REFERENCES

[1].Ghulam, M., Rawaba, A., Asia, A., Sumaira, S. & Amer, J. (2017). Bioactive Compounds from Medicinal Plants and Their Importance in Drug Discovery in Pakistan *Matrix Science Pharma (MSP)* 1(1), 17-26.

[2] Jadhao, A.B. (2022). Review on methods used in isolations of phytochemicals from medicinal plants, *Heterocyclic Letters* 12(4), *845-853.*

[3].Manyou Yu, M., Gouvinhas,i., Rocha, J. & Ana Barros A.I.R.N.A.(2021). Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources, *Scientific Reports* | 11,10041 https://doi.org/10.1038/s41598-021-89437-4

[4]. Natividad, C., Antonio S., & Juan Carlos, A. (2020) Quantification of the Antioxidant Activity of Plant Extracts: Analysis of Sensitivity and Hierarchization Based on the Method Used. *MPDI Antioxidants*, 9, 76; doi:10.3390/antiox9010076.

[5].Usman, A., Mohammed,Y., Muhammed,H.O., Usman N.L. & A. H. Zakari, A.H. (2020). Phytochemical Screening and Antioxidant Activity of Balanites Aegyptiaca Root Bark Extracts: Influence of solvent. *Communication in Physical Sciences* , 5(2):156-164.

[6].Njoku, V., Obi, C. & Onyema, O.M. (2011), Phytochemical Constituents of Plants. *African J ournal of Biotechnology*. 10(66), 15020-15024.

[7]. Parekh, J. & Chanda, S. (2007).*In vitro* screening of antibacterial activity of aqueous and alcoholic extracts of various Indian plant species against selected pathogens from Enterobacteriaceae.. *African Journal of Microbiological Research.1, 92-9*

[8].Maregesi, S.M., Pieters, L., Ngassapa, O.D., Apers, S. Vingerhoets, R., Cos, P., *et al*. (2008). Screening of some Tanzanian medicinal plants from Bunda district for antibacterial, antifungal and antiviral activities. *Journal of Ethnopharmacol*. 119, 58-66.

[9].Chapagain, B.P., Wiesman, Z. & Tsror, L. (2007).*In vitro* study of the antifungal activity of saponin-rich extracts against prevalent phytopathogenic fungi. *African Journal of Traditional Complement Alternative Medicine.* 4, 1-6.

[10].Akindele, A.J. & Adeyemi, O.O. (2007). Antiinflammatory activity of the aqueous leaf extract of *Brysocarpuscoccineus*, *Fitoterapia,* 78, 25-28.

[11]. Ojo, O.O., Nadro, M.S. & Tella, I.O. (2006), Protection of Rats by Extracts of Some Common Nigerian Trees against Acetaminophen Induced Hepatotoxicity. *African Journal of Biotechnology,* 5,755-760.

[12]. Meda, N.T., Lamien-Meda, A., Kiendrebeogo, M., Lamien, C.E., Coulibaly, A.Y., Millogo-Rasolodimby, J. & Nacoulma, O.G. (2010). *In vitro* antioxidant, xanthine oxidase and acetylcholinesterase inhibitory activities of Balanites aegyptiaca (L.) Del. (Balanitaceae). *Pakistan Journal of Biological Sci*ence, 13, 362-368.

[13]. Bhimraj, G. (2023). PhyLeaves aqua-ethanol extract, tochemical screening and antioxidant activity of Balanites aegyptiaca linn. *International Journal of Chemical Research and Development,* 5(2), 31-36.

[14]. Shams, E.K., Talib, M. A., Fatima, E., Eman, M. Z. A. & Ahmed, S.K. (2017). Antioxidant activity and Phytochemical screening of Balanites aegyptiaca fruit, *World Journal of Pharmaceutical and Medical Research,*3(10), 47-50.