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Phytochemical Screening and Antioxidant Activity of *Balanites Aegyptiaca* Root Bark Extracts: Influence of solvent

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Abstract *Balanites aegyptiaca (L) Del is a medicinal plant used in Africa's folk medicines to treat wide range of diseases. Phytochemical screening of aqueous and ethanolic extracts of Balanites aegyptiaca (L) Del root indicated the presence of flavonoids, saponins, tannins and phenols.The ethanolic extract of the plant root had in addition carbohydrate, cardiac glycoside and steroids while n-hexane extract indicated the presence of anthraquinone, carbohydrates, cardiac glycoside, terpenoids and resins. The extracts were also evaluated for their free radical scavenging potential, total phenolic and total flavonoid contents. The antioxidant activity was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, the total phenolic content was evaluated using Folin-Ciocalteau assay, while the total flavonoid content was assessed by the aluminium chloride colorimetric assay. The results obtained revealed that the ethanolic extract exhibited higher total antioxidant capacity in the range of 55.23±0.4 to 81.04±0.9% while concentrations in the aqueous extract ranged from 20.15±0.2 to 35.11±0.2% compared to 80 % of standard ascorbic acids. Estimated total phenolic content in aqueous and ethanolic extracts were 179.48±1.99 and 260.07±2.31 mg/gallic acid equivalent (GAE)/g respectively. However, the total flavonoid content for aquoues and ethanolic extracts were 69.17±0.32 and 95.52±0.41 mg/ quercetin equivalent (QE)/g respectively. Total phenol and flavonoid were absent in normal hexane extract. The results obtained from the study indicated that the choice of solvent can influence the components that can be extracted from Balanites aegyptiaca (L) Del root and that for optimum extracts to be obtained, the best solvent is ethanol.*

Key Words: *Extraction, phytochemicals, antioxidant activity*, *Balanites aegyptiaca root, effect of solvent.*

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1.0 Introduction

Most phytochemicals are more useful in extracted forms and the extraction is normally done by using suitable solvents because the required components may not involves the whole plant and in some cases, unsueful toxicant has to be eliminated (Ekop and Eddy, 2005).Phytochemicals are most useful in sevral applications aspect of life including corrosion inhibiton (Eddy *et al*., 2011, 2011, 2012), pharmaceutical applications (Thouri *et al*., 2017) and others. Studies have indicated that different solvents extract phytochemicals to different extent. Thouri *et al*. (2017) investigated the effect of water, methanol, absolute acetone and aqueous acetone as solvemts for the extraction of phytochemicals and biological activity assay of Tunisia date seed. They also conducted in vitro antioxidant activities, in vitro hyperglycemia key enzymes inhibtion and in vitro anti-inflammatory properties and concluded that the polar solvent exhibited the highest amount of bioactive compounds. Truongo *et al*. (2019) investigated impact

of different solvents on extraction yields, phytochemical and antioxidant constituents and in vitro anti-inflammatory activities of *S. buxifolia*. Their results indicated that methanol was the most effective solvent for the extraction and gave the highest extraction yield, highest content of phenolic, flavonoid, alkaloid and terpenoids. The extract obtained from methanol also exhibited high capacity of antioxidant and in vitro anti inflammatory activity. Iboki-Assanga *et al*. (2015), also investigated the effect of different solvents in the extraction and biological assays of *Bucida buceras* L. and *Phoradendron californicum* and found that hexane phase of *B. buceras* and *P. californicum* (mesquite) extracts revealed the presence of carotenes, triterpenes/steroids, and lactonic groups, the ethanol and aqueous extraction phases indicated the presence of a diverse range of compounds, including tripterpenes/steroids, lactonics groups, saponins, phenols/tannins, amines, amino acids and flavonoids/anthocyanins. Highest concentrations of total phenolics and flavonoids were obtained from methanol and aqueous extracts of the plants. The acetone extract exhibited the highest levels of DPPH radical-scavenging activity and reducing power as well as the highest superoxide radical scavenging activity. In order to investigate the suitability of diethyl ether, acetone and methanol as solvents for the extraction of phytochemicals and for testing antioxidant and antimicrobial activities of *Ecballium elaterium* seeds and its fruit peels, the total phenolic, flavonoid, flavonol, condensed tannins and carotenoids contents were estimated for each of the extract obtained from the respective solvents. Their results indicated that methanol extract gave the highest antioxidant activity with respect to IC50, DPPH, EC50 and ABTS values. Acetone and diethyl extracts were observed to showed best antibacterial activity against *Micrococcus luteus*. See *et al*. (2017) had reported that ethyl acetate performed better than methanol in extracting phenolic components of *G. benthamiana* and also displayed high total phenolic concentrations and scavenging activity against 2,2-diphenyl-1-picylhydrazyl (DPPH) free radicals. In a series of studies conducted to investigate the performance of different solvents in extracting phytochemical constituents of several medicinal plants Kannamba *et al*. (2017) concluded that phytochemical composition of leaves extracts highly varied with the extraction method and with the solvent used for extraction. Consequently, the present study is designed to investigate the effect of hexane, ethanol and water as solvents on the yield and biological assay of *Balanites aegyptiaca* root.

Balanites aegyptiaca (L) Del (Zygophyllaceae), also known as 'Desert dates' in English, belongs to the genus *balanites*, which consists of nine species and eleven intra-specific taxa (Sands, 2013). It is an arid and semi arid multibranch evergreen tree reaching up to 10 metres in height and is widely distributed in the Sudano-Sahielian region of Africa, the Middle East and South Asia (Hall, 1992). *B. aegyptiaca* has good adaptive mechanisms to grow and thrive under different soil types, salinity stresses and climatic moisture levels (Chothani and Vaghasiya, 2011, Elfeel, 2017). It is also a multipurpose tree with a wide range of products and values such as food, fodder, shade, oil and traditional medicine (Gour *et al*., 2012, Kamel *et al*., 1991, Okia *et al*., 2011). *B. aegyptiaca*, has been used traditionally in the management of different diseases such as jaundice, intestinal worm infection, wound healings, malaria, syphilis, epilepsy, dysentery, stomach aches, constipation, diarrhea, haemorrhoid and asthma (Al-Thobaiti and Abu Zeid, 2018, Chothani and Vaghasiya, 2011, Ojo *et al*., 2006, Zarroug *et al*., 1990, Bashir *et al*., 1984).

The pharmacological investigation of different parts of this tree shows that the fruit pulp, leaves, root and stem bark have several useful properties including insecticidal (Wiesman & Chapagain, 2003, Chapagain and Wiesman, 2005), antibacterial (Doughari *et al*., 2007, Parekh and Chandra, 2007, Otieno, *et al*., 2007), antifungal (Margesi *et al*., 2008, Chapagain *et al*., 2007), hepatoprotective (Ali *et al*., 2001), anticancerous and antioxidant (Speroni *et al*., 2005), antihelminthic and molluscicidal (Koko *et al*., 2005, 2007), antidiabetic (Zaahkouk et al., 2003, Kamal *et al*., 1991) and anti-inflammatory activity (Speroni et al., 2005, Gaur *et al*., 2008). Several studies have reported *B. aegyptiaca* root bark to have high antioxidant and anticancerous properties (Speroni *et al*., 2005; Doughari *et al*., 2007; Chothani and Vaghasiya, 2011). These activities were assumed to be due to the presence of active ingredients such as phenols and flavonoids (Doughari *et al*., 2007). Saponins obtained from different parts of this plant have also been reported to have wide range of industrial and pharmaceutical applications (Gnoula *et al*., 2008, Patil *et al*., 2010). Hence, there need to investigate the different roles solvent can impact on the extraction of phytochemical constituents of the useful components of this plants.

2.0 Materials and Methods

2.1 Sample collection and preparation

The root bark of *B. aegyptiaca* was collected from a mature tree found around boys hostel in the Nasarawa State University Keffi and was authenticated at the Department of Forestry and Wildlife of the same University (with a Voucher No FWF/O132) and deposited at the Departmental herbarium. The samples were washed with distilled water, and were air dried at room temperature to constant weight before been grounded with a laboratory pistle and motar. The pulverised fine particles obtained were preserved in a desicator prior to further experiments.

2.2 Extraction of the sample

The grounded and dried root bark of *B. aegyptiaca* samples were extracted using the method described by Yinusa *et al.,* (2016) with slight modification. Grounded root bark (50 g) was suspended in 200 ml of the respective extracting solvents (n-hexane, aqueous or ethanol) for three days with occasional agitations. The extracts were decanted and filtered out using Whatman No 1 filter paper. Each batch of the filtrates was re-concentrated using rotary evaporator, weighed and store in the refrigerator at 4 °C.

2.3 Phytochemical screening

The root bark extracts of *B. aegyptiaca* were screened for the presence of secondary metabolites such as tannins, saponins, alkaloids, steroids, terpenes, anthraquinone, flavonoids, cardiac glucosides, phenols, resins, carbohydrates and phlobatannins using standard procedures described by Odebiyi and Sofowora (1999).

.2.3.1 Total phenolic content assay

The total phenolic content of each extract was determined using the Folin-Ciocalteau method as described by Ghasemi *et al*. (2009). 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 gallic 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. 50 % of ethanol solution was used as the blank. 10 µl of of the samples required for analysis was introduced into the cuvette 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 calibtation curve and expressed as gallic acid equivalents (GAE).

2.3.2 Total flavonoid content assay

Total flavonoid content of the extract was estimated using the aluminum chloride ($AlCl_3$) colorimetric method reported by Ordónez *et al*. (2006), 5 mg of the extract was dissolved in 1 ml of the test solvent to obatianed 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). 100 µl of each extract or quercetin concentration was aliquoted into the cuvettes in triplicates. 100 µl of 2% aluminium 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 flavonoid content of each extract was calculated from the regression equation of the quercetin standard curve and expressed as quercetin equivalents (QE).

2.3.3 DPPH-free radical scavenging activity

Analysis of DPPH radical scavenging activity of the plant extracts was performed according to the method described by Koleva *et al.* (2002). Stock solution was prepared by dissolving 100 mg of the extract in 1 ml of methanol and five, two fold serial dilutions were 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 to each of them 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. The control solution was prepared by mixing 3.5 mL of methanol and 0.3 ml of DPPH radical solution. The percentage of scavenging activity (Z %) was calculated according to equation 1

$$Z\% = \frac{A_{Sample} - A_{Blanl}}{A_{Control}} \times \frac{100}{1} \quad (1)$$

where A_{Sample}, A_{Blank} and $A_{Control}$ are the absorbances of the sample, blank and contro; respectively.

3.0 Results and Discussion

Results obtained for phtochemical screening of extract of *B. aegytiaca* root are presented in Table 1. From the results obtained, flavonoid, saponins and tannins were identified in aqueous and ethanol extracts of the root of *B. aegytiaca* but was absent in the hexane phase. Similar results has been reported by Kannamba *et al*. (2017), in their studies, where flavonoid was identified in most of the solvents phases except in hexane solvent phase. Saponins, tannins, phenol, phlobatannins, alkaloids and steroids were also absent in the hexane phase. However, the hexane phase indicated the presence of anthaquionone, terpenoids and resins which were absent in aqueous and ethanol phases. Carbohydrate and cardiac glycosides were also identified in hexane and ethanol extracts but not in aqueous extract. Ther presence of phenol was common in aqueous and ethanol phases

Table 1: Phytochemical screening of *B. aegytiaca* root bark extracts

Test	Hexane extract	Aqueous extract	Ethanol extract
Flavonoid	-	+	+
Saponins	-	+	+
Tannins	-	+	+
Anthraquinone	+	-	-
Phenols	-	+	+
Phlobatannins	-	-	-
Carbohydrates	+	-	+
Cardiac glycosides	+	-	+
Terpeniods	+	-	-
Resins	+	-	-
Alkaloids	-	-	-
Steroids	-	-	+

+ = Present — = Absent

Ethanol also exhibited the capacity to extract steroids, which was not found in aqueous or hexane phases. Ethanol and water are solvents that have a common feature of undergoing autoionization, which can be represented according to the following equation,

$$2H_2O = H_3O^+ + OH^-$$

$$2CH_3CH_2OH = CH_3CH_2OH_2^+ + CH_3CH_2O^-$$

Autoionization is a property of aqueous solvents and they are know to have the potential of coveying solutbility for several substances. The autoionization concentant for water and ethanol at 298 K are 1×10^{-14} and 1×10^{-20} respectively. According to Ferreira and Pinho (2012), flavonoid and glycosides are very soluble in water, methanol, ethanol, acetone, and ethyl acetate, but are insoluble in ethyl ether and chloroform. This explain why flavonoid was present in the aqueous and ethanol phases and not in hexane. Solubility of flavonoid in hexane has not been established to be significant. Saponins and tannins have also been reported to be soluble in water and ethanol and not in hexane. Therefore, ethanol and water are the best solvents for extraction of saponnins in the root of the studied plant (Chua *et al*., 2019). This also explain why saponnins and tannins were identified in the ethanol and aqueous phases. As a rule, polar solvents can extract polar phytochemicals and vice versa. Phenol is aromatic and due to the presence of hydroxyl group, it can easily be extracted by ethanol and water and not by hexane, which is an organic solvent that can not undergoes autoionization. Although carbohydrate and cardiac glycosides are very soluble in water, it was not presence in the aqueous extract. These maybe due to the difficulty of recovery the compound from the solvent due to high solubility (Hameister and Kragl, 2006). In practice, hexane is the solvent of choice in recovery carbohydrate from this plant. Most terpenoids are insoluble in water and ethanol but soluble in chloroform and diethyl ether. This implies that they are not polar indicating that hexane is also a better solvent than water and ethanol (Uwah *et al*., 2013; Dougahri, 2008). The presence of terpenoid in the ethanol phase and not in the water phase is due to the fact that ethanol possess hydrophobic (due to hydrocarbon) and hydrophilic (due to hydroxyl group) properties, hence its ability to attract terpenoid for extraction more than

water. Non of the chosen solvents (water, ethanol and hexane) was able to extract phlobatannins and alkaloids out of the sample. This indicates that this phytochemical is not presence in the root of the plant or the solvent is not able to extract it. For example, Truong *et al*. (2019) reported that the best solvents for extraction of phlobatannins and alkaloids is methanol.

Babayi *et al*. (2004) reported that many traditional healers make herbal teas, infusions or decoctions using water to extract the active compounds from this plants for medicinal purposes, because water is easily available and very safe for consumption but satisfactory isolation of active compounds from plant material depends to a large extent the nature of solvent used in the extraction process as explained by Masoko et al. (2008). The presence of these metabolites have been reported to be responsible for the pharmacological actions associated with this plant (Yadav and Panghal, 2010).

The total phenolic contents of the aqueous root extract, calculated from the calibration curve (Fig.1) (R^2 = 0.9956) was 179.48±1.99 mg GAE/g while the concentration for th ethanolic root extract deduced from the same plot (R^2 = 0.9981) was 260.07±2.31 mg GAE/g. Similarly, the total flavonoid content of the aqueous and ethanolic root extracts calculated from the calibration curves (R^2= 0.9919 and 0.9984) were 69.17±0.32 and 95.52±0.41 mgQE/g (Calibra933tion curve is presented in Fig 2. Conversely, hexane extracts recorded no flavonoid and phenolic contents.

The presence results agree with the findings of Ignat *et al.,*2011) who reported that phenolic and flavonoid compounds have high solubility in alcohols (ethanol or methanol) than in water. The higher solubility can be attributed to the differences in autoionization constants and to the fact that ethanol, in addition to hydrophilic property, also has hydrophilic property. It has also been reported that alcoholic extracts of medicinal plants exhibited high phenolic and flavonoids contents as well as antioxidant activities than water, ethyl acetate, chloroform and acetone extracts (Khettaf *et al*., 2016, Mostefa *et al*., 2018).

Phenolic compounds are important secondary metabolite which is widely distributed in plant kingdom. They are mainly classified into phenolic acids and flavonoid. They are reported to be involved in growth and reproduction of the plant, and play an important role in defense against ultraviolet radiation and pathogens (Pawar and Dasgupta, 2018). Although, Folin-Ciocalteau assay is easy to perform, rapid and applicable in routine laboratory use and low-cost for total phenolic determination but studies have showed that Folin-Ciocalteau reagent react with other phytoconstituents beside phenol in an extract which could result to high amount of phenolic content (Amorati and Valgimigli, 2015, Piazzon *et al*., 2012),

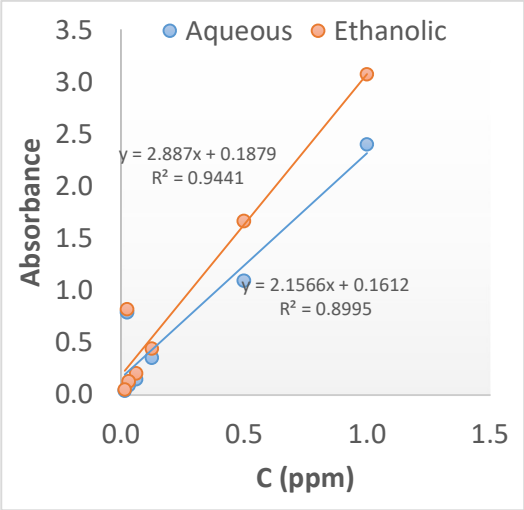

Fig. 1:Calibration curve for total phenolic contents

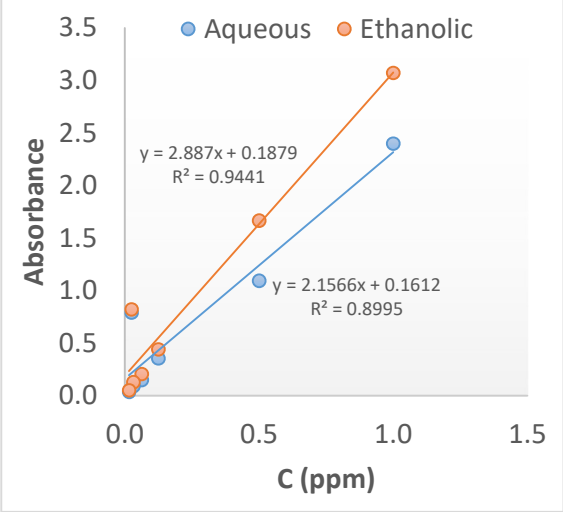

Fig. 2: Calibration curve for total flavonoids contents

Table 2 shows antioxidant activities of ethanol and aqueous extract of *B. aegytiaca* root. Ascorbic acid was used as a reference sample. The antioxidant activity is a measure of DPPH free radical scavenging ability of each of the n-hexane, aqueous and ethanolic root extract. The aqueous and ethanol extracts generally demonstrated a concen-

tration-dependent antioxidant potency with ethanol extracts exhibiting higher antioxidant activity while n-hexane extract shows no activity.The high antioxidant activity of this plant extract is due to the presence of total flavonoid and phenolic contents, and these metabolites have redox properties that allow them to act as antioxidant because their free radical scavenging ability is facilitated by the presence of free hydroxyl groups (Soobrattee *et al.,* 2005). Several studies have revealed that flavonoids suppresses reactive oxygen formation, chelate trace elements involved in free radical production and are highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various other free radicals implicated in several diseases (Agati *et al.,* 2012, Baba and Malik, 2015).

Table 2: Antioxidant activities of ethanol and aqueous extracts of *B. aegytiaca* root bark extract and ascorbic acid standard.

Concentration (mg/ml)	Ethanol extract	Aqueous extract	Ascorbic acid
2.0	81.04 ±0.9	35.11±0.2	86.92±1.1
1.5	78.12±1.2	30.20±0.4	84.27±0.9
1.0	68.15±1.9	25.85±0.2	85.14±1.2
0.5	61.13±0.7	22.76±0.3	84.33±1.4
0.25	55.23±0.4	20.15±0.2	85.12±1.5

****Values are presented as mean (SD of three replicates)**

4.0 Conclusion

The results and findings of this study, it can be concluded that the root of *Balanites aegyptiaca (L) Del* root bark contained various phytochemical compounds but not all can be extracted by a single solvent. Different components of its phytochemical has different affinities for different solvents. Therefore, for optimum extraction, the type of phytochemicals requires for extraction and the choice of solvent must be considered.

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