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Studies on Phytochemical Constituents and Antibacterial Potentials of Extracts of *Balanites aegyptiaca* (Del.) Parts on Antibiotic Resistant Bacterial Isolates

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Authors' contributions

This research was carried out in full support of the authors. Author MYT designed the entire study and protocols with interpretations of the results, statistical analyses and prepared the first draft of the manuscript. Author FOI proof read and corrected the draft, authors TBD and GAO helped in the collection and preparations of plant materials. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To determine the phytochemicals and antibacterial potentials of parts of *Balanite aegyptiaca* on clinically important antibiotic resistant bacteria isolates
Study Design: Phytochemicals and in vitro assay of antibacterial
Place and Duration of Study: Department of Biological Science Technology, Federal Polytechnic Mubi, Adamawa State, between April, 2013 and January, 2014
Methodology: Collection of bacterial isolates; antibiogram of the bacterial isolates; preparation of plant extracts; phytochemical analyses of the plant parts on aqueous extracts; *In vitro* susceptibility test (agar well diffusion assay)
Results: The antibiogram showed that all the isolates used in this study are multidrug resistant. The results of the phytochemical analyses on aqueous extracts showed that the leaves of *B. aegyptiaca* possessed all the phytochemical components tested except anthroquinones and alkaloids, while root bark lack anthroquinones, cardiac glycosides and phlobatannins and stem bark possessed only flavonoids and polyphenols. The presence of phytochemical components in the stem bark is significantly less than those in

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the leaf and root bark ($p<0.05$). The presence of these phytochemicals has provided some biochemical basis for ethno pharmacological uses of this plant parts in the treatment and prevention of various diseases and disorders. Using agar well diffusion method, the *B. aegyptiaca* parts were screened for antibacterial activities against antibiotic resistant *Staphylococcus aureus*, *Proteus vulgaris*, *Salmonella* sp., *Shigella* sp. and *Citrobacter* sp. at 100mg/ml concentration. The results of the antibacterial activity showed that the aqueous and ethanolic extracts of all the parts of *B. aegyptiaca* has varying antibacterial activity against the tested isolates. The hot aqueous and cold aqueous extracts of leaves of *B. aegyptiaca* have no activity against *Citrobacter* spp. and *Staphylococcus aureus* respectively. The hot aqueous extract of stem bark has significant antibacterial activity against all the tested isolates except *Salmonella* spp, while the cold water extract of the same part has no activity against *Salmonella* spp., *Shigella* spp. and *Citrobacter* spp. The ethanolic, hot and cold aqueous extracts of root bark of *B. aegyptiaca* have no activity against *Salmonella* spp. Although the presence of phytochemical components in the stem bark is significantly less than those in the leaf and root bark ($p<0.05$), their antibacterial activities however, showed no significant difference ($P=0.10$) to all the isolates. The results further showed that the antibacterial activity of cold aqueous extracts of *Balanite aegyptiaca* parts is significantly lower than those of ethanolic extracts and hot aqueous extracts ($p<0.05$). However, there is no significant difference between the antibacterial activity of ethanolic extract and hot aqueous extract of all the parts on the isolates ($P=0.06$).

Conclusion: This study investigates and reports the phytochemicals antibacterial potentials of *Balanites aegyptiaca* on resistant bacteria isolates. This therefore justify the use of this plant in traditional medicine practices for the diseases caused by the microorganisms.

Keywords: Phytochemicals; antibacterial potentials; antibiotic; resistant bacteria; Balanite aegyptiaca.

1. INTRODUCTION

Despite the wide availability of clinically useful antibiotics and semi-synthetic analogues, humans have frequently used plants to treat common infectious diseases. This is because plants are invaluable sources of pharmaceutical products [1]. Most of the antimicrobial agents have considerable drawback in terms of limited antimicrobial spectrum, or serious side effect. Thus, a continuing search for new antimicrobials remains indispensable [2]. There is an urgent need to discover new antimicrobial agents for both humans and veterinary therapeutics uses, as resistance to current drugs increases in severity and extent [3]. Antibiotic resistance among bacteria is becoming more and more serious problem throughout the world. Antibiotic resistance is also a serious health concern in Nigeria, being a developing country, it has conditions and practices that promote the development and spread of pathogens that are resistant to antimicrobials [4]. Hence, the identification of new medicinal plant with impressive antimicrobial activity, hopefully new mode of action, is one of the ways of tackling this problem.

A considerable number of natural products and medicinal plants contain some active phytochemical components such as phenolics, flavonoids, terpenes, coumarins, etc, which induce different biological activities in animals including antioxidant, anti-inflammatory and anti-cholinesterase effects [5]. It is also known that the curative effect of any medicinal plant is correlated to its active phytochemical constituents [6,7].

Balanite aegyptiaca is a species of tree classified either as a member of the zygophyllaceae or the Balanitaceae. It is known as Desert date in English and 'hingoli' in Hindi [8], and 'addua' in Hausa. It is also known as soapberry tree, thorn tree in English. This tree is native to much of Africa and parts of the Middle East. It is an evergreen savanna tree, 4.5 to 6m high, woody and with small spine scents [9]. The tree grows in the Sahel-savanna regions of Nigeria, Ghana and Ivory Coast. It can be found in many kinds of habitat, tolerating a wide variety of soil types, from sand to heavy clay and climatic moisture levels [10]. Almost all part of the plant is used in traditional medicine. It has been used in the treatment of skin disease and remedy for stomach ache and jaundice [11], treatment of cough [12], treatment of diarrhea and syphilis [13] and typhoid fever [14]. The root was also reported to be used in the treatment of inflammation [15], antidote for snake bite [16], malaria, herpes zoster and veneral disease [8].

Thus, this study was undertaken to determine the phytochemicals and antibacterial potentials of parts of *Balanite aegyptiaca* on clinically important antibiotic resistant bacteria isolates

2. MATERIALS AND METHOD

2.1 Collection of Plant Materials

The leaves and stems of *Balanite aegyptiaca* were collected around Fali villages in Mubi area, while the root bark was collected in the outskirt of Yola town, Adamawa State, Nigeria. The plant parts were identified by T. B. Danchal of the Botany Unit, Department of Biological Sciences Technology, Federal Polytechnic Mubi and also by comparing their morphological and anatomical characteristics with the standard description.

2.2 Extraction Procedure

The *B. aegyptiaca* (leaves, stem bark and root bark) were air-dried at room temperature and ground to fine powder. 50g of each of the powdered plant part was soaked in 200ml distilled water and was allowed to stand for 48h at room temperature after thorough vortexing. Each mixture was filtered using whatman no.1 filter paper. The filterate were concentrated in vacuo using rotary evaporator. Similar procedure was followed to obtain hot aqueous and ethanolic extracts of the plant parts using ethanol and hot water as extracting solvents. All the aqueous (Hot and Cold) and ethanolic dried extracts of the plant parts were stored in sample bottles at 4℃ prior to use.

2.3 Phytochemical Screening

Qualitative phytochemical tests were carried out on the aqueous extract and on the powdered specimen using standard procedure to identify the constituents [17,18]. The phytochemical components screened includes; Anthraquinones, Cardiac glycosides, Phlobatannins, Polyphenols, Saponins, Alkaloidsa, Steroids and Flavonoids,

2.4 Test Organisms

The test isolates were collected from the Microbiology Laboratory of the Department of Biological Science Technology, Federal Polytechnic Mubi, Adamawa State. The antibiotic susceptibility of the bacterial species was performed on nutrient agar plates by disk diffusion

method as described by the National Committee for Clinical Laboratory Standards [19]. These isolates include; *Staphylococcus aureus*, *Salmonella* spp., *Shigella* spp., *Proteus* vulgaris and *Citrobacter* spp.

2.5 Preparation of Inoculum

The overnight cultures of the test organisms were inoculated onto peptone water and vortex thoroughly. The turbidity of the bacterial suspensions were then adjusted and compared with 0.5McFarland standards. The 0.5McFarland standards was prepared by adding 0.5 ml of 1.2% (wt/vol) barium chloride dihydrate (Bacl2.2H2O) solution to 99.5ml of 1% sulphuric acid. The turbidity standard was then aliquot into test tubes identical to those used to prepare the inoculums suspension [20].

2.6 Determination of Antibacterial Activity

The dried aqueous (cold and hot) and ethanolic extracts of *B. aegyptiaca* parts were reconstituted in glycerol to obtain a final concentration of 100mg/ml for this test. The susceptibility test was done using agar well diffusion method. 0.1ml aliquot of each test organism suspension was transferred onto dried agar plates in duplicate and was spread evenly with a sterile bent glass rod. After drying, three (3) wells were bored (using 6mm diameter cork borer) into the dried nutrient agar plates and 0.5ml each of the extracts (leaves stem bark and root bark) was aseptically introduced into two wells. Glycerol was introduced into the third well as control. The plates were then incubated at 37℃ for 24h after which zones of inhibition were measured in centimetres and recorded appropriately.

2.7 Statistical Analysis

Anova and Student T-test was used to test for significance difference in all the data obtained. All statistical analyses were carried out using the SPSS 17.0 window based program. Significance difference and non- significance difference was defined when $p \leq 0.05$ and $p \geq 0.05$ respectively.

3. RESULTS AND DISCUSSION

3.1 RESULT

Table 1 showed the antibiotic resistance profile of the isolates used for this study. All the isolates exhibit multi-drug resistance traits.

The results from Table 2 showed that leaves of *B. aegyptiaca* possessed all the phytochemical components tested except anthroquinones and alkaloids, while root bark lack anthroquinones, cardiac glycosides and phlobatannins. Stem bark possessed only flavonoids and polyphenols.

The results of the antibacterial activity showed that the ethanolic, hot and cold aqueous extracts of all the parts of *B. aegyptiaca* has varying antibacterial activity against the tested isolates Table 3. The hot and cold aqueous extracts of leaves of *B. aegyptiaca* have no activity against *Citrobacter* spp. and *Staphylococcus aureus* respectively. The hot aqueous extract of stem bark has significant antibacterial activity against all the tested isolates except *Salmonella* spp, while the cold aqueous extract of the same part has no activity against

Salmonella spp., *Shigella* spp. and *Citrobacter* spp. The ethanolic, hot and cold aqueous extract of root bark of *B. aegyptiaca* has no activity against *Salmonella* spp.

Although the presence of phytochemical components in the stem bark is significantly less than those in the leaf and root bark ($p<0.05$), their antibacterial activities however, showed no significant difference ($p>0.05$) to all the isolates. The results further showed that the antibacterial activity of cold aqueous extracts of *Balanite aegyptiaca* parts is significantly lower than those of ethanolic extracts and hot aqueous extracts ($p<0.05$). However, there is no significant difference between the antibacterial activity of ethanolic extract and hot aqueous extract of all the parts on the isolates ($p>0.05$).

3.2 Discussion

The antibiogram of the Bacterial isolates used in this study showed that the bacterial isolates were multi-drug resistant. Avasthi and Purkayastha, [21] in their study on medicinal plants revealed that their isolates were multi-drug resistant. Other studies in which plants extracts have been used on multi-drug resistance bacterial isolates include those of Camarda et al. [22], Mothana and Lindequist, [23]. The increasing incidence of antibiotic resistance among bacterial pathogens necessitates medicinal plants as an alternative approach for development of novel antimicrobial agents [24].

The Phytochemical screening in this study was carried out only on aqueous extracts of all the plant parts. The presence of phytochemicals in the roots of *Balanite aegyptiaca* observed in this study confirmed the presence of polyphenols, saponins, alkaloids, steroids and flavonoids. This was slightly similar to the observations of Kubmawara et al. [15] and [6] who reported the presence of same in the root ethanolic extract. In this study however, anthraquinones was not detected; while steroids and alkaloids were not detected in their study. In agreement with this study, the root of *B. aegyptiaca* was reported to possessed steroids and saponins [25].

Phytochemical investigation on the leaves of *B. aegyptiaca* showed the presence of cardiac glycosides, phlobatanins, polyphenols, saponins, steroids, and flavonoids. Saponins, tannins, phenols and anthraquinones were also reported in methanolic leaves extract of *B. aegyptiaca* [14]. Also, the presence of saponins and flavonoids in the leaves of *B. aegyptiaca* as demonstrated in this study confirmed the work of Samuelsson et al. [26].

In this study also, the preliminary phytochemical analysis revealed the presence of flavonoid and polyphenols on the stem bark of *B. aegyptiaca*. Contrary to the findings of this study, alkaloid was reported in the stem bark of *B. aegyptiaca* [27].

To a large extent, the chronological age of the plant, percentage humidity of the harvested material, situation and time of harvest, and the method of extraction were possible source of variation for the chemical composition, toxicity and bioactivity of the extracts [28]. In addition, variation in soil types on which the plant is growing, location or climatic conditions, extracting solvents might also be responsible for variation in the presence of the bioactive ingredients.

Phytochemicals can have complementary and overlapping mechanisms of action in the body including antioxidant effects, modulation of detoxification enzymes, stimulation of the immune system and modulation of hormone metabolism. The presence of flavonoid and polyphenols in *B. egyptiaca* parts (leaves, stem and root bark) suggest that the plant parts may be useful in preventing damage cause by free radicals by neutralizing them. This can

be explained due to the fact that flavonoids and polyphenols act as antioxidant. Antioxidants neutralize highly unstable and extremely reactive molecules, called free radicals which attack the cells of the body every day [29]. Free radical damage is believed to contribute to a variety of health related problems, including cancer, heart disease and aging [25]. In addition, the presence of flavonoids in the plant parts may suggest the usefulness of the plant parts in treating diarrhoea. This is because flavonoids can inhibit the development of fluids that result in diarrhoea by targeting the intestinal cystic fibrosis transmembrane conductance regulator Cl–transport inhibiting cAMP-stimulated Cl–secretion in the intestine [30]. To justify this, Boulos [13] reported that *B.* a*egyptiaca* is used in treating diarrhoea.

Cardiac glycosides were found to be present in the leaves of *B. aegyptiaca.* Cardiac glycoside is a compound that has been shown to aid in the treatment of congestive heart failure and cardiac arrhythma. Cardiac glycosides work by inhibiting the Na+/K+ pump. This causes an increase in the level of sodium ions in the myocytes, which then leads to a rise in the level of calcium ions. This inhibition increases the amount of Ca^{2+} ions available for contraction of the heart muscle, improves cardiac output and reduces distension of the heart [31].

Alkaloids are the most efficient therapeutically significant plant substance [32]. Pure isolated alkaloids and the synthetic derivatives are used as the basic medicinal agent because of their analgesic, anti-plamodic and bacterial properties where they showed marked physiological effects when administered to animals [32]. This may justify the use of *B. aegyptiaca* root extract in the treatment of pain, malaria, and enteric fever in folk medicine. This also justify the report of Gaur et al. [8] who revealed that *B. aegyptiaca* is used in treating malaria. In addition, the presence of alkaloids in the root bark of *B. aegyptiaca* suggests that the plant part may be used for the treatment of hypertension [33,34].

B. aegyptiaca leaves and root bark may be used to reduce blood pressure and cholesterol level in blood. This is due to the presence of saponins in the plant parts. Saponins bind to cholesterol to form insoluble complexes and excreted them via bile. This prevents cholesterol re-absorption and results in a reduction of serum cholesterol. Saponins have been found to be potentially useful for the treatment of hypercholesterolemia and thus reduce the risk of cardiovascular diseases such as hypertension [33,34]. This suggests that saponins might be acting by interfering with intestinal absorption of cholesterol [35]. Also, the findings of this study showed that the leaves and root bark of *B. aegyptiaca* can be used for anti-inflammatory treatment. This can be explained due to the presence of saponins which has anti-inflammatory properties. This is consistent with the report of Kubmarawa et al. [15] who showed that *B. aegyptiaca* is used in the treatment of inflammation.

The presence of phlobatannins in the leaves of *B. aegyptiaca* may suggest the diuretic on the plant part [36].

Furthermore, the presence of steroids on the leaves and root bark of *B. aegyptiaca* suggest that the plant part may be used as anti-inflammatory and analgesic agents [37].

In addition to all the potentials of the phytochemicals possessed by this plant parts as outlined above, the presence of these constituents has also been reported to account for the exertion of antimicrobial activity by plants [38]. Earlier studies reported that anti-dysentric and anti-diarrheal properties of medicinal plants have been due to the alkaloids, flavonoids, saponins, reducing sugars [39,40].

In this study, the ethanolic and hot aqueous extracts of all the plant parts showed significant and impressive antibacterial activity against the tested organisms with zones of inhibition ranging from 10mm to 30mm in diameter. Both the ethanolic and aqueous root bark extracts were unable to inhibit *Salmonella* spp. This was in agreement with the work of Henna et al. [6] on aqueous extract, but was in contrast with the result of Doughari et al. [14] who reported activity of aqueous root extract of *B. aegyptiaca* on *S. typhi* even though the organic solvents extract performed significantly better. These disparities may be attributed to the change in environmental conditions which may affect the production of secondary metabolites in the plant [41].

The cold and hot aqueous stem bark extracts were unable to inhibit *Salmonella* spp. Likewise *Shigella* spp and *Citrobacter* spp were not inhibited by cold aqueous extract of the same plant part. In this study, there was no significant correlation between the presence/absence of phytochemical components with antibacterial activity with regard to stem bark extracts. This is because the stem bark extract showed the presence of only polyphenols and flavonoids out of the eight phytochemical components tested. If the presence of phytochemicals is synonymous with antibacterial activity, then polyphenols and flavonoids may have been responsible for the antibacterial activity exhibited by stem bark extracts in this study. Otherwise, the presence of other phytochemical components or factors present in the stem bark extract which we are unable to unveiled or captured in the course of this study may have been responsible for the antibacterial effect. Furthermore, the impressive antibacterial activity of ethanolic stem bark extract confirmed the potentials and the efficacy of organic solvents in extracting phytochemicals and other factors responsible for antibacterial activity which ordinarily water would not be able to extract. Earlier report showed that organic solvents such as acetone have selective property of extracting phytochemicals such as tannins [42]. The results of this study also revealed that there is no significant difference between the antibacterial activity of ethanolic extract and hot aqueous extract of all the plant parts on the isolates ($p>0.05$). This suggests that subjecting the plant parts to elevated temperature in water has the potential of extracting bioactive ingredients which was replicated in its antibacterial activity. This is consistent with previous reports [43,44] which showed that high temperature increases the molecular movement that helps in extraction of phytochemicals. It further confirmed and supports the usefulness of boiling medicinal herbs as prescribed by most traditional method. In their study, Gazuwa et al. [45] revealed that although heat did not affect the bioavailability of phytochemical components, but subjecting herbs or vegetables to higher temperatures might destroy some active medicinal principles present in them.

The ethanolic and aqueous leaves extracts of *B. aegyptiaca* showed significant antibacterial activity against the tested isolates. Cold and hot aqueous extracts were not able to inhibit *S. aureus* and *Citrobacter* spp respectively. It has been reported that both aqueous and organic leaves extracts of *B. aegyptiaca* have antibacterial effect against *S. typhi* with ethanolic extracts demonstrating the highest activity [14]. In this study however, the aqueous leaves extracts demonstrates the highest antibacterial activity when compared with the ethanolic extract.

The result of the antibacterial activity showed that there was no significant difference ($p>0.05$) in the zone of inhibition between the ethanolic extract and hot aqueous extract of all the plant parts. This is in agreement with the observations of Henna et al. [6] who reported that there is no significant difference ($p>0.05$) in the zone of inhibitions between methanolic and aqueous extracts of *B. aegyptiaca* root.

Other medicinal plants with similar phytochemical constituents and antibacterial activity includes *Vernonia amygdalina* [20], *Boswellia serrata* [21], *Azadirachta indica* [42], *Sida acuta* [46]

Table 1. Antibiotic resistance pattern of bacterial isolates to commercial available Antibiotics

S/N	Bacterial isolates	Resistance pattern
1	*Staphylococcus aureus*	ck,pg,ax,ac,ct,cp,cf,cr,er,te,az
2	*Proteus vulgaris*	at,cx,fr,fu,cr,ak,of,cz,fx,cn
3	*Salmonella* spp.	at,cx,fr,fu,cr,cl,of,cz,fx,cn
4	*Shigella* spp.	at,cx,fr,na,fu,cr,cl,of,cz,fx,cn
5	*Citrobacter* spp.	at,cx,fr,fu,cr,cz,fx,cn

Key: ck= chloramphenicol, pg= penicillin-G, ax= amoxicillin, ac= amoxicillin-clavulanic acid, ct= cotrimoxazole, cp=Cephalexin, cf=Cefazolin, cr=cefuroxime, er=erythromycin, te=tetracycline, az=Azithromycin (for S.aureus only); at=Aztreonam, cx=cefotaxime, fr= ceftriaxone, fu=nitrofurantoin, cr=cefuroxime, ak=Amikacin, of=ofloxacin, cz=ceftazidime, fx=Cefixime, cl=ciprofloxacin, cn=Cefdinir (for gram negative organisms only)

Table 2. Phytochemical Screening of *Balanite aegyptiaca* parts

S/N	Phytochemical components	Leaves	Stem bark	Root bark
1	Anthroquinones	-	-	-
2	Cardiac glycosides	+	-	-
3	Phlobatannins	+	-	-
4	Polyphenols	+	+	+
5	Saponins	+	-	+
6	Alkaloids	-	-	+
7	Steroids	+	-	+
8	Flavonoids	+	+	+

KEY: + = Presence, - = Absence

Table 3. Diameter of zone of inhibitions of *Balanite aegyptiaca* parts

S/N	Organisms	Zones of inhibitions (Centimetres)								
		Leaves			Stem bark			Root bark		
		E.E	H.E	C.E	E.E	H.E	C.E	E.E	H.E	C.E
1	*Staphylococcus aureus*	1.0	1.3	-	2.2	1.3	1.4	2.3	2.5	1.0
2	*Proteus vulgaris*	3.0	1.5	1.5	3.0	2.5	2.0	2.0	-	-
3	*Salmonella* spp.	1.5	1.5	1.5	1.5	-	-	-	-	-
4	*Shigella* spp.	1.3	1.7	1.6	1.0	1.5	-	3.5	2.3	-
5	*Citrobacter* spp.	2.3	-	1.5	1.0	1.0	-	3.0	2.1	1.0

KEY: E.E= Ethanolic extract. H.E= Hot aqueous extract. C.E= Cold aqueous extract.

4. CONCLUSION

This study therefore has provided some biochemical basis for ethno pharmacological uses of this plant parts in the treatment and prevention of various diseases and disorders. The ability of *B. aegyptiaca* parts showing significant and impressive antibacterial activity against antibiotic resistant Gram positive and Gram negative bacteria shows its application as a broad spectrum antimicrobial agent and as a remedy for infections involving antibiotic

resistant organisms demonstrated in this study. This therefore justify the use of this plant in traditional medicine practices. We therefore recommend further research on this plant and its parts to optimally extract and purify the bioactive principles responsible for inhibiting the growth of the said bacterial species and formulating them into appropriate dosage for the treatment of infectious diseases involving such organisms.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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