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Antimicrobial Potency and Bioactive Ingredients of Different Extracts of *Prosopis africana* against Some Selected Human Pathogenic Microbes: An *In-vitro* Approach

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Authors' contributions

This research work was carried out in collaboration among all authors. Author ARA conceptualized the study, wrote the proposal writing, discussion/choice of the protocol, performed the laboratory experiments (bench work), interpreted the results and wrote the manuscript for publication. Authors ZJK and ILM did the literature researches, discussion/choice of the protocol, proposal writing proof reading and supervised the bench work. Authors SBM and YY carried out literature researches, discussion/choice of the protocol, proof read the manuscript and made the necessary corrections. All authors read and approved the final version of the manuscript.

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ABSTRACT

Background: The resistance to conventional drugs by microbes is at alarming rate and poses extra challenges in the field of medical and pharmaceutical industries. This study was aimed to determine the active ingredients and assess the antimicrobial potency of *Prosopis africana* leave extract using system of different solvents as aqueous, ethylacetate and n-hexane.

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Methodology: The plant specimen was collected, identified, prepared and cold macerated using aqueous, ethylacetate and n-hexane as solvent. The resulted crude extracts were further assayed for anti-infective potency; adopting agar well diffusion protocol against some selected human pathogenic isolates viz *Streptococcus pyogenes, Klebsiella pneumoniae, Salmonella typhi,* Methicillin-Resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* and *Candida albicans*. The Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) were ascertained using the standard protocol of microdilution assay.
Results: The antimicrobial screening of the crude extracts showcased that *P. africana* leave is susceptible against all the tested microbial stains by exhibiting significant degrees of inhibition zone of diameter (0 to 25 mm). The positive controls likewise were observed to be vulnerable to the tested isolates while *P. aeruginosa* was resistant. The MIC was observed at 50.0 to 12.5 mg/mL and MBC at 50.0 mg/mL against some bacteria strains. *P. aeruginosa* and *C. albicans* has no bactericidal and fungicidal properties. The phytochemical analysis led to the isolation of anthraquinones, carbohydrates, saponins, terpenes, steroids and cardiac glycosides as the active constituents of the plant part.
Conclusion: This finding provides a logical justification to the traditional healers for the utilization of the plant in the management of different ailments caused by the tested microbes.

Keywords: Antimicrobial potency; bioactive ingredients; pathogenic microbes; microdilution assay.

1. INTRODUCTION

It is eminent that diseases caused by pathogenic microbes are prevalent globally, which account for high-level of well-being problems, particularly in the developing nations like Nigeria. In recent years, there has been an increasing trend in the emergence of resistance to chemotherapeutic products and series of conventional antibiotics have lost their efficacy as a result of advancement in microbial strains resistant, mainly via the countenance of resistance genes [1,2]. Aside from insufficiency and exorbitancy of some conventional drugs, some of the clinically significant orthodox medicines required to alleviate and obviate these ailments are coupled with major undesirable and unacceptable confrontational drug reactions. Furthermore, [3] reported that most standard antibiotics at some points are associated with setback on the host together with suppression of healthy immune response, depletion of advantageous gut and mucosal microbes, hypersensitivity, allergic reactions and other setbacks. These have prompted to the necessity for urgent search and development of new pharmaceuticals to combat infectious ailments and curtail antimicrobial resistance.

Medicinal plants are wellsprings of critical amounts of compound substances which can start distinctive biological activities that are valuable in the management of human illness [4, 5]. Literature demonstrates that medicinal plant products have been a crucial fountain of medications on disease causing agents [5]. Currently, people should fully understand the benefits of plant materials and their products as the beginning of drug discovery. By tradition, a lot of plant species are well-known for their healing potentials and a limited number of such species have been scientifically screened with reference to their healing properties such as complementary and alternative medicine [6]. The scientific research of traditionally used plants has drawn interest in resolution of multidrug resistance to the current antimicrobial products.

Therefore, typical biological active constituents from the leave of *P. africana* shall function as conceivable and promising sources of alternative therapy against the pathogenic microbial infections; meanwhile biopharmaceuticals are derivatives of plant and animal materials which are of paramount important to human, animal and public health as well. It is therefore of interest to scrutinizing this plant organ for its natural biological active compounds and antimicrobial efficacy.

2. MATERIALS AND METHODS

2.1 Plant Specimen Collection and Identification

The plant specimen was collected in Bida, Niger State, Northern part of Nigeria. The plant material was taxonomically identified, authenticated and the voucher specimen was prepared and deposited in the herbarium unit,

Department of Medicinal Plant Research and Traditional Medicine (MPR&TM), National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria.

2.2 Plant Sample Preparation

The freshly collected leaves were properly washed under clean-running tap water to dislodge the dirt's and shade dried at room temperature for seven (7) days. Then, the dried plant specimen was ground into fine powder and sieved. The pulverized sample was weighed, stored in a container properly labeled and tightly covered until use.

2.3 Plant Sample Extraction

2.3.1 Aqueous extraction

The aqueous extraction of the leave was done by cold maceration in 500 mL of sterile distilled water. One hundred grams (100 g) of the pulverized samples was added to the distilled water, stirred using glass rod and allowed to stand for 24 h at laboratory temperature. The macerated sample was then filtered using Whatman No 1 filter paper and the filtrate was concentrated under control temperature by using water bath to evaporate the extract to dryness at 45^{o}C.

2.3.2 Ethylacetate and n-hexane extraction

One hundred grams (100 g) of the pulverized leave of *P. africana* was accurately weighed and subjected to cold maceration in 500 mL of ethylacetate and n-hexane for 72 h at laboratory temperature. The macerated extracts were filtered using Whatman No.1 filter paper. The extraction was repeated thrice for the maximum extraction of the active ingredients and to obtain reasonable yield (crude extract). The filtrates were dried using water bath at 45ºC until all the solvent evaporated out.

2.4 Phytochemical Screening of the Extracts

The plant extracts were phytochemically screened for the presence of carbohydrates, flavonoids, phenols/tannins, saponins, terpenes, steroids, alkaloids, cardiac glycosides and anthraquinones using the standard protocol of [7,8,9].

2.5 Antimicrobial Assay

The antimicrobial assay of the extracts was determined in the Microbiology Laboratory, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria.

2.6 The Test Microbes

Antimicrobial activity of aqueous, ethylacetate and n-hexane extract of leaves of *Prosopis africana* was investigated against five bacterial isolates and one fungus isolate which were obtained from Vaccine Discovery and Research Laboratory Unit, Centre for Genetic Engineering and Biotechnology, Federal University of Technology, Minna, Nigeria. The bacteria strains which were used included *Salmonella typhi, Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, Methicillin-Resistant *Staphylococcus aureus* (MRSA) and the fungus used for the study was *Candida albicans*. The tested bacteria were maintained on Nutrient agar at 37°C for 24 h and C*andida albicans* on Potato Dextrose Agar at 30°C for 48-72h.

2.7 Preparation of Standard Inoculums for Antimicrobial Assay

The stock cultures were maintained on nutrient agar (NA) slants, active cultures for the experiments were prepared by transferring a loopful of microbial cells from the stock cultures into bottles containing sterile Mueller-Hinton Broth (MHB) for bacteria and Sabouraud Dextrose Broth (SDB) for fungi in order to revive them by culturing overnight at 37^{o}C. The overnight cultures were further sub cultured with freshly prepared MHB and SDB and the turbidity was adjusted to $BaSO_4$ turbidity standard which is equivalent to 0.5 McFarland standards in order to obtain the microbial density consistent with $1.5x10^8$ CFU/mL cells for bacteria, and $1.5x10^7$ spores/mL for fungi strain.

2.8 Preparation of the Crude Extracts

The extracts were prepared by weighing 0.5 g into sterile plane tubes and dissolved in 1mL of 2% (v/v) Dimethylsulfoxide (DMSO), then vortexed in order to attain complete dissolution of the extracts. Thereafter, 9mL of sterile distilled water was added to each tube so as to get the stock solution of 50.00 mg/mL. From the stock solutions, double fold dilutions were carried out

to give extract concentrations of 25.00 mg/mL, 12.50 mg/mL and 06.25 mg/mL respectively.

2.9 *In-vitro* Antimicrobial Susceptibility Assay of the Crude Extracts

The susceptibility test of the extracts was done adopting agar-well diffusion protocol as described by [10,11]. Twenty-three millilitres (23 mL) of MHB and SDB plates were inoculated with 100 µL (0.1 mL) of MHB and SDB cultures of every aliquot microbial isolate comparable with 0.5 McFarland standard into sterile petri dish. The seeded plates were carefully rocked for even distribution of the isolates and allowed to solidify. Thereafter, five wells of 8 mm in diameter were bored on the agar plates using sterile metallic cork borer and properly labelled according to the serial diluted concentration of the prepared extracts and the wells were also seeded with a drop of MHA and SDA. Subsequently, the same volumes of different concentrations of the extract (200 µL) were carefully added in each well using mechanical micropipette. For the extracts, replicate trials were made against every isolate.

The control experiments were realized using chloramphenicol (Fidson, Nigeria) and fluconazole (Pfizer, UK) as standard drugs, sterile MHA and SDA plates were used as Media Sterility Control (MSC) and MHA and SDA plates streaked with the used organisms as Organism Viability Control (OVC). Hence, the prepared plates were left in the bio safety cabinet for 2 h for the proper diffusion of the extracts and then incubated at 37°C for 24 h for bacteria and fungi at 25°C for 48 h. The plates were closely observed for the clear zone of inhibition around the wells. The diameters of each zone were accurately measured using a spotless and translucent ruler in millimetres (mm) in vertical and horizontal manner and average values were determined.

2.10 Determination of Minimum Inhibitory Concentration (MIC)

The MIC was carried against the isolates using the standard broth microdilution protocol in 96 micro wells titre plates as described by Bauer et al. [10] with little modification. A volume of 50µL of the sterilized media (MHB and SDB) was dispensed into each well except the first row which only contained 50µL of the extract (stock solution). 50µL of the extract (50 mg/mL) was added to the second row and properly mixed, and then 50µL was aspirated from same row and transferred to the third row, the serial dilution continued down to row 7 where 50µL was discarded. Thereafter, each well in row 1-7 received 50µL of 0.5 McFarland aliquot microbial inoculums.

Control experiment were realized such that wells in row 8, 9 and 10 were made up to 100µL by 50µL of organism as OVC, 50µL of media as MSC and 50µL of drug (Chloramphenicol and Fluconazole) respectively and the plates were incubated at 37ºC for 24 h. Thereafter, 50µL of tetrazolium dye was applied into each well with 2 h incubation at 37ºC and colour change was observed. Any well with reddish-pink colour signified the microbial growth, which was recorded and document as positive (MIC). The test was carried out in duplicate and the values were express in means.

2.11 Determination of Minimum Bactericidal and Fungicidal Concentration (MBC & MFC)

The MBC and MFC were determined from the Minimum Inhibitory Concentration (MIC) results by subculturing from the wells that showed no sign of turbidity in the MIC test and streaked on the freshly prepared MHA and SDA plates and incubated at 37ºC for 24 to 48 h and the plates were observed for the presence or absence of the growth.

3. RESULTS

3.1 Colour and Percentage Yield Obtained from *P. africana* Leave Extracts Using Various Solvents

The colouration of the plant organ was put into cognizant and documented in the course of maceration. The aqueous, ethylacetate and n-hexane leave of *P. africana* revealed lemon green colouration as shown in Table 1. The same table also demonstrates the percentage yield of the crudes obtained by different solvents where aqueous, ethylacetate and n-hexane displayed 12.23%, 1.41% and 0.81% respectively.

3.2 Bioactive Screening

The three *P. africana* solvent extracts aqueous, ethylacetate and n-hexane were assessed for the active constituents. As can be seen in Table 2,

Table 1. Colour and percentage yield obtained in various solvents

Solvents	Colour of the extracts	% Yield
Aqueous	Lemon green	12.23
Ethylacetate	Lemon green	01.41
Hexane	Lemon green	00.81

Table 2. Phytochemical screening of the extracts

Test	Observation	Inference
1. Molisch's Test	Appearance of purple ring/violet at the interphase	Carbohydrates present
2. i Alkaline Test	No formation of an intense yellow colouration	Flavonoids absent
ii Shinoda Test	No appearance of pink scarlet colour	Flavonoids absent
3. $FeCl_3$ Test	No appearance of blue-green or black colouration	Phenols/Tannins absent
4. Froth Test	No formation of the stable foam	Saponins absent
5. Libermann's Test	Change in colouration from violet to blue	Terpenes present
6. Salkoski's Test	Formation of reddish brown colour	Steroids present
7. Keller-kilani Test	Formation of brown ring at the interphase	Cardiac glycosides present
8. i Dragendoff's Test	No turbidity of the precipitate	Alkaloids absent
ii Hagner's Test	No turbidity of the precipitate	Alkaloids absent
iii Wagner's Test	No turbidity of the precipitate	Alkaloids absent
9. Borntrager's Test	Formation of yellow gelatinous precipitate	Anthraquinones present

Table 3. Phytochemical constituents of leaves

Phytocemicals	Tests	ALE	HLE	ELE
Carbohydrates	Molisch	-	+	+
Flavonoids	Alkaline	-	-	-
Phenol/Tannins	Ferric Chloride	-	-	-
Saponins	Froth	+	-	-
Terpenes	Liebermann	+	+	+
Steroids	Salkowski	+	+	+
Alkaloids	Dragendoff's	-	-	-
	Hagner's	-	-	-
	Wagner's	-	-	-
Cardiac glycosides	Keller-Kilani	+	+	+
Anthraquinones	Borntrager's	+	+	-

Key: + = Present - = Absent

the screening of the biological active compounds of *P. africana* leaves extracts shows the presence of anthraquinones, carbohydrates, saponins, terpenes, steroids and cardiac glycosides, though flavonoids, phenol/tannins while alkaloids were not present.

3.3 Antimicrobial Assay

The aqueous, ethylacetate and n-hexane leaves of *P. africana* were assayed for their antimicrobial efficacy against some bacteria isolates, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Salmonella typhi*, Methicillin-Resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* and a fungus *Candida albicans*. As shown in Tables 4, 5 and 6, the extracts exhibited some level of antimicrobial properties. The inhibitory activity of aqueous extract ranged from 10 – 25 mm, ethylacetate extract 10 – 20 mm while n-hexane extract ranged from 0 – 12 mm. The standard controls

Table 4. Antimicrobial efficacy of aqueous extract

Isolates	Concentration of the crude extract (mg/mL)/zone of inhibition (mm)				
	50.00	**25.00**	**12.50**	**06.25**	**C**
S. pyogenes	25	15	10	-	31
K. pneumoniae	17	12	-	-	28
S. typhi	21	16	11	-	26
MRSA	20	15	11	-	28
P. aeruginosa	18	10	-	-	-
C. albicans	21	10	-	-	28

Key: C = Control, - = No zone

Table 5. Antimicrobial efficacy of ethylacetate extract

Isolates	Concentration of the crude extract (mg/mL)/zone of inhibition (mm)				
	50.00	**25.00**	**12.50**	**06.25**	**C**
S. pyogenes	14	10	-	-	27
K. pneumoniae	10	-	-	-	30
S. typhi	20	11	-	-	26
MRSA	12	10	-	-	28
P. aeruginosa	10	-	-	-	-
C. albicans	11	-	-	-	28

Key: C = Control, - = No zone

Table 6. Antimicrobial efficacy of n-hexane extract

Isolates	Concentration of the crude extract (mg/mL)/zone of inhibition (mm)				
	50.00	**25.00**	**12.50**	**06.25**	**C**
S. pyogenes	-	-	-	-	32
K. pneumoniae	-	-	-	-	29
S. typhi	-	-	-	-	27
MRSA	-	-	-	-	28
P. aeruginosa	12	-	-	-	-
C. albicans	-	-	-	-	27

Key: C = Control, - = No zone

Table 7. Minimum inhibitory concentrations of the extracts (mg/mL)

Test isolates	ALE	ELE	n-HLE
S. pyogenes	25.0	50.0	-
K. pneumoniae	25.0	50.0	-
S. typhi	12.5	50.0	-
MRSA	12.5	50.0	-
P. aeruginosa	50.0	-	50.0
C. albicans	50.0	50.0	-

Key: ALE = Aqueous Leaves Extract, ELE = Ethylacetate Leaves Extract, n-HLE = n-hexane Leaves Extract, - = not tested

Table 8. Minimum bactericidal and fungicidal concentrations of the extracts

Test isolates	ALE	ELE	n-HLE
S. pyogenes	50.0	-	-
K. pneumoniae	50.0	-	-
S. typhi	50.0	-	-
MRSA	50.0	-	-
P. aeruginosa	-	-	-
C. albicans	-	-	-

Key: ALE = Aqueous Leaves Extract, ELE = Ethylacetate Leaves Extract, n-HLE = n-hexane Leaves Extract, - = not tested

(Chloramphenicol and Fluconazole) likewise showed appreciable susceptibility against the tested isolates with inhibitory zones of inhibition ranging from of 26 – 32 mm.

4. DISCUSSION

In spite of the fact that plants do have a tendency to be greenish in colour owing to the presence of chlorophyll, there is a far more extensive range of colours in plants than what we may think. Therefore, the colouration of the *P. africana* leave extracts were taken into account and the result obtained (lemon green) is not far from the thoughts that plants are seen to be greenish in colour. The percentage of the crude extracts obtained in aqueous, ethylacetate and n-hexane were (12.23%), (1.41%) and (0.81%) respectively (Table 1). The differences experienced in percentage yields could be accounted for the strength and solubility of the solvents used to dissolve the plant part so as to extract the bioactive ingredients.

The phytochemical screening of *P. africana* leaves extracts in this study revealed the presence of secondary metabolites like anthraquinones, saponins, terpenes, steroids and glycosides, though flavonoids, phenol/tannins and alkaloids were not present. This finding reinforces the preceding report of [12] that phytochemicals like anthraquinones, cardiac glycosides, carbohydrates, steroids, saponins and tannins were isolated from *P. africana*. Correspondingly, our analysis is to some extent in compliance with the reports of [13] where some similar components were extracted from similar plant but alkaloids, tannins and flavonoids were not present. This variation could be due to different plant organ and the solvent used. Some studies also used water for extraction and found some of the secondary metabolites like alkaloids, saponins, flavonoids, anthraquinones and tannins from same plant organ [14].

The finding from this study shows no flavonoids and alkaloids compare to [14] in their study. This variability might be due to climatological condition, geographical location and period at which the plant was collected, sampling soil, plant age, storing situation and preparation protocol. The manifestation of phytochemical ingredients like glycosides, terpenes, alkaloids, saponins, steroids and tannins are of medically importance since they have antimicrobial potency [15,16].

The antimicrobial activity of this plant against the tested organisms; *Klebsiella pneumoniae*, *Salmonella typhi*, *Streptococcus pyogenes*, Methicillin-Resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* and *Candida albicans* proved effective against them. This may be the reason why it has been used by traditional medical practitioners in the management of different ailments in Nupe kingdom of Bida, Niger State, Nigeria. However, the aqueous and ethylacetate extracts of the plant showed appreciable antimicrobial potency against all the test isolates at different concentrations whereas, n-hexane extract only displayed activity on *P. aeruginosa* while *S. pyogenes*, *K. pneumoniae*, *S. typhi*, Methicillin-Resistant *Staphylococcus aureus* (MRSA) and *Candida albicans* resisted the extract across all the concentrations used. The inhibitory levels of the extracts were not equal. Probably, the causes could be the strength of each solvent used in the course of extraction to extract the active chemicals from the plant or the genetic makeup of the microbes Tables 3, 4 and 5.

From this study, the maximum and minimum potency of the aqueous extract was against *S. pyogenes* (25 mm) and *K. pneumoniae* (17 mm), this is to say that the aqueous extract demonstrates less vulnerability on Gram negative bacteria (*K. pneumoniae*) compared to Gram positive (*S. pyogenes*) bacteria, since it has been reported that Gram negative group of bacteria possess high permeability barrier for numerous antibiotic molecules [17,18]. The result of aqueous extract in this study is in agreement with the report of [2], where the aqueous and methanol extract of *P. africana* demonstrates antibacterial properties against fifteen bacteria, including *Klebsiella pneumoniae*, Methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa* among others at a fixed concentration of 25 mg/mL and recorded zone of inhibition ranging from 5 mm to 17 mm. However, the study by [19] showed no activity against *K. pneumoniae*, most likely, it could be ascribed to the bacteria strain or the concentration of the extract used. The study by [20] revealed the activity against *Pseudomonas aeruginosa, Staphylococcus aureus, and Bacillus subtilis* at concentrations comparable to the findings of this study.

The ethylacetate extract of *P. africana* exhibited antimicrobial efficacy ranging from 10 – 14 mm against all the tested isolates but for n-hexane, the antimicrobial response was noted only on *P.*

aeruginosa with 12 mm of inhibition zone. This inhibitory variation of these two organic solvent extracts (ethylacetate and n-hexane) could be due to the fact that the ethylacetate which is medium in polarity has high tendency of extracting more of the natural biological active metabolites from the plant than the n-hexane which is apolar in nature. As different classes of solvents exhibit different spectrum of solubility for the phytochemical constituents and the valuable medicinal properties of different plant extracts are as a result of several active compounds they possess [21,22].

Remarkably, the antimicrobial screening of *P. africana* extract expressed appreciable results across all the test isolates with lesser MIC value of 12.5 mg/mL against *S. typhi* and MRSA, 25.0 mg/mL against *S. pyogenes*, *P. aeruginosa* and *K. pneumoniae*. These MIC analyses ascertained with respect to the crude extracts are quite interesting and of course promising, because the high activity of antimicrobial agent at relatively lower concentration is very fundamental in search for new drugs as their toxicity is believed to be less. It is noteworthy that the tested plant part had antimicrobial property against all the tested isolates at reasonable MIC. This is great, because further purification of the plant extracts might enhance the antimicrobial potency against the isolates which could lead to discovery of a new lead antimicrobial product.

5. CONCLUSION

These present findings bequeath a rationale for the utilization of *P. africana* in folk medicine for the management of different infectious ailments particularly the ones associated with the tested microbes. It could also be the basis of discovery of new and efficient biomolecules, considering the plant expressed broad-spectrum antimicrobial profile which can be related to the presence of secondary metabolites. However, phytochemicals are of high clinical value in challenging the microbial infections. It is therefore recommended that the future work should centre on fractionation and characterization of the crude extracts and toxicological assessment of the plant in order to ascertain if the plant is potable for consumption as some plants are more detrimental than infectious ailments while some have long term effect(s).

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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