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Antibacterial Potential of *Calotropis procera*, *Jatropha tanjorensis*, and *Adansonia digitata*: A Comparative In Vitro Study

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Abstract
Plants provide important source of the world's pharmaceuticals and have become quite useful in the treatment of diseases as they exhibit antifungal, antibacterial, anticonvulsant, anti-inflammation activities. However, since a single plant may contain many substances, hence different abilities, it is important to ascertain their exact disease-curing abilities. Therefore, this research was aimed at assessing the antibacterial potential of three medicinal plants. Ten medicinal plants selected from Wudil, Kano State were screened for the presence of their phytochemicals using ethanol extraction method. Three (Sodom apple, catholic vegetable and Baobab) out of these were chosen and used to test for their anti-bacterial effects because they contain all the phytochemicals screened for. Phytochemical analysis was done using appropriate methods. The plant extracts were serially diluted; absolute (100 %), 80 %, 60 %, 40 %, 20 % and 0 % (control). Bacteria isolates from culture media were identified using macroscopic, microscopic as well as biochemical tests. The results revealed that all the plant extracts used had highly significant effects on the tested organisms. The highest effect was observed at 80 % for all the extracts. Staphyloccocus aureus was most sensitive to the three plant extracts with Sodom apple and Catholic vegetable showing the greatest effect (17.7 and 17.3 mm respectively). It can be concluded that, Sodom apple, Catholic vegetable and Baobab are highly efficacious against bacteria agents especially Staphylococcus aureus.

Keywords: Anti-bacterial, Assessment, Medicinal plants, phytochemicals

Introduction

A medicinal plant is any plant part which, in one or more of its organ, contains substances that can be used for therapeutic proposes or which are precursors for the synthesis of useful drugs (Abayomi, 2008). Medicinal plants have become so important to the health of individuals and communities that, it has created great awareness which has necessitated the need for knowledge of the food nutrients and phytochemicals present in the various parts of different plants. Many plants contain phytochemicals that are largely responsible for the definite physiological activity they exert on human body (Ighodaroro et al., 2012). Plants have become quite useful in the treatment of diseases and they provide important source of the world's pharmaceuticals. According to khobe et al. (2017), plants in all aspects of life have served as valuable starting materials for drug development. The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents.

Phytochemicals may be regarded as Primary compounds such as; Chlorophyll, proteins, amino acids and common sugars or secondary compounds which include terpenoids, alkaloids, essential oils, flavonoids, tannins, saponins, reducing sugar and phenolic compounds (Wadood et al., 2013). Phytochemicals have antifungal, antibacterial, anticonvulsant, anti-inflammation activities (Oladeji, 2016). Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for treatment of bacterial infections (Herin et al.,2012). However, since a single plant may contain many substances, the effects of taking plants as medicine can be complex. As such there is need to study the phytochemicals contained in the experimental plants to determine their efficacy as treatment of bacterial infections.

Materials and Methods

Study Area

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The study was conducted in the Biological Sciences Laboratory of Nigeria Police Academy, Wudil Kano State and in Microbiology Laboratory of the Murtala Muhammed Specialist Hospital, Kano. Kano State.

Sample Collection

Ten medicinal plants were collected locally from the Nigeria police Academy Wudil Kano State. The plant was identified by the indigenous local people for the Hausa names and then taken to the Department of Botany, Ahmadu Bello University Zaria for proper identification which was done by Dcf. Namadi Sunusi.

Ten medicinal plants selected from Wudil, Kano State were screened for the presence of their phytochemicals using ethanol extraction method. Three (Sodom apple, catholic vegetable and Baobab) out of these were chosen and used to test for their anti-bacterial effects because they contain all the phytochemicals screened for. Phytochemical analysis was done using appropriate methods (Usunobun et al., 2015). The plant extracts were serially diluted; absolute (100 %), 80 %, 60 %, 40 %, 20 % and 0 % (control). Bacteria isolates from culture media were identified using macroscopic, microscopic (Fawole and Oso, 2004) as well as biochemical tests according to the methods of Archarya (2013.) and Martina and Vladimira (2016).

Identification of bacteria

Pure cultures of bacteria were prepared using the streak method, culture were characterized and identified using various morphological and biochemical tests such as macroscopic characteristics, microscopic characteristics, catalase, urease, triple sugar test (triple sugar iron test, hydrogen sulphide (H_2S) test and Gas test), citrate, coagulate and indole test.

Table 1a: The 10 plant materials used for the phytochemical analysis research and their names

S/N	Common names	Botanical names	Hausa names	Family name
1	Camel foot	*Piliostigma thonningii*	*Kalgo*	Fabaceae
2	Moringa	*Moringa oleifera*	*Zogole*	Moringaceae
3	Baobab	*Adansonia digitate*	*Kuka*	Malvaceae
4	Sickle senna	*Senna obtusifolia*	*Tapasa*	Fabaceae
5	Acacia gum	*Acacia suberiana*	Bagaruwa	Fabaceae
6	Sodom apples	*Calotropis procera*	*Tunfifiya*	Asclepidaceae
7	Moshi medicine	*Guiera senegalensis*	Sabara	Combretaceae
8	Catholic vegetable	*Jatropha tanjorensis*	Bini da zugu	Euphorbiaceae
9	Scent leaves	*Ocinum gratissium*	Daidoya	Labiatae
10	Balsam apple	*Ctenolepis cerasiformis*	Garafuna	Cucurbitaceae

Table 1b: The selected plants used for the research

S/N	Common Name	Botanical Name	Hausa Name	FamilyNname
1	Baobab	*Adansonia digitate*	*Kuka*	Malvaceae
2	Sodom apples	*Calotropis procera*	*Tunfifiya*	Asclepidaceae
3	Catholic vegetable	*Jatropha tanjorensis*	Bini da zugu	Euphorbiaceae

Macroscopic Examination

The bacteria colonies in the petri dishes were examined using a magnifying lens. Features observed included shape, pigmentation, texture, elevation and hemolysis.

Microscopic Examination

This was done using the oil immersion objective lens (×100) to check
Gram's Reaction, Appearance, Morphology, Spore formation

Biochemical Examination

The tests done included
Catalase, Urease, Citrate, Indole test. Coagulase, Triple sugar test (triple sugar iron test), Hydrogen sulphide (H_2S) test and Gas test),

Statistical Analysis

Standard errors of mean of three replicates were calculated for the measured zone of inhibition.
Data obtained were subjected to one-way analysis of variance (ANOVA) in SPSS for windows.

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Results

Macroscopic examination of Bacteria

The result of the macroscopic examination of the bacteria is presented in Table 2. The results revealed that two types of shape were observed. Round shape was observed in both *Staphylococcus aureus* and *Klebsiellia pneumoniae* while rod shape was observed in *E. coli*. Furthermore, the results revealed different pigmentation in different colonies. *E. coli* had creamy pigmentation, *Staphylococcus aureus* was golden while *Klebsiellia pneumoniae* had pale yellow pigmentation. On blood agar all the bacteria were non-hemolytic. The bacterial colonies had different texture as *E. coli* had a non-mucoid texture; *Staphylococcus aureus* is smooth and *Klebsiellia pneumoniae* had a mucoid texture. In the elevation of the bacteria, *E. coli* and *staphylococcus aureus* appeared to be convex while *Klebsiellia pneumoniae* had concave elevation (Table 2).

Table 2: Macroscop charateristics of *E. coli*, *S. aureus* and *Klebsiella pneumoniae*

Bacteria	**Shape**	**Pigment**	**Texture**	**Elevation**	**Hemolysis**
E.coli	Rod	Creamy	Non mucoid	Convex	non-hemolytic
S.aureus	Round	Golden	Smooth	convex	non-hemolytic
K.pneumoniae	Round	pale yellow	Mucoid	concave	non-hemolytic

Microscopic examination

The results of microscopic examination showed that 66.67% of the bacterial examined were gram negative bacteria (*E. coli* and *Klebsiellia pneumonia*) and 33.33% were gram positive bacteria (*Staphylococcus aureus)*. The shapes of bacteria under the microscope showed that, *Esccherichia coli* and *Klebsiellia pneumoniae* were straight rod while *Staphylococcus aureus was* cocci. Similarly, the appearance of the bacteria differed under the microscope. *E. coli* appeared singly, *Staphylococcus aureus* appeared in grape like cluster and *Klebsiellia pneumoniae* appeared to be in chain. The result shows that *Klebsiellia pneumoniae* was the only spore forming bacteria compared to the others (Table 3)

Table 3: Microscopic characteristics of different bacterial cells

Bacteria	Gram's stain	Morphology	Spore	Appearance
E.coli	_	straight rod	_	Singly
S.aureus	+	Cocci	_	Grape like cluster
K.pneumoniae	_	straight rod	+	Chain

Key
+=positive - = negative

Biochemical Reactions

The results of the biochemical test showed that, bacteria isolated were all triple sugar irons positive and were hydrogen sulphide negative. Similarly, *Staphylococcus aureus* and *Klebsiella pneumoniae* were positive for urease and TSI tests but were negative for indole test. Escheritchia coli was observed to be positive for catalase, TSI, Indole and Gas but negative for all other tests (Table 4).

Table 4: Biochemical reactions of the different bacteria cells

Bacteria	Catalase	Urease	TSI	Citrate	Indole	Gas	H_2S	Coagulase
E.coli	+	_	+	_	+	+	_	_
S.aureus	+	+	+	+	_	_	_	+
K.pneumoniae	_	+	+	+	_	+	_	_

Key += positive -=negative TSI= Triple Sugar Irons H_2S= Hydrogen Sulphide

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Sensitivity Test

The antibacterial effects of the plant materials indicated that, there was a correlative effect on the bacterial isolates. As the treatment (concentration) increased, the effect also increased. The highest effect was observed at 80% in all the extracts on all the bacteria studied (17.67, 17.33 and 15 mm) zone of inhibition for *Calotropis procera*, *Adansonia digitata* and *Jathropha tajorensis* respectively except, in Sodom apple where 16.33 mm was recorded at 100 % concentration (Table, 5). The results also revealed that, *Staphylococcus aureus* was most vulnerable to all the plant extracts at 80 % concentration. (Table 5).

Table 5: The sensitivity of bacteria toward the plant extracts

Plant extract	Concentration (%)	*E. coli* (mm)	*S. aureus* (mm)	K. pneumonia (mm)
Sodom apple	100	12.00±0.00	12.33±0.88	16.33±0.33
	80	12.33±1.20	17.67±1.45	13.33±0.88
	60	9.33±1.76	9.67±0.33	10.67±1.20
	40	8.00±1.00	6.67±0.33	8.67±1.33
	20	7.76±1.67	6.00±0.00	7.67±1.67
	0	6.00±0.00	6.00±0.00	6.00±0.00
Baobab	100	11.33±2.91	11.00±1.52	10.00±2.00
	80	13.33±3.71	17.33±1.33	10.67±2.40
	60	10.33±0.33	8.67±0.67	7.00±0.58
	40	6.67±0.67	6.33±0.33	6.00±0.00
	20	6.67±0.67	6.00±0.00	6.00±0.00
	0	6.00±0.00	6.00±0.00	6.00±0.00
Catholic vegetable	100	10.00±1.00	9.00±1.53	10.67±0.67
	80	12.00±1.15	15.00±1.15	13.00±2.89
	60	8.00±1.15	7.00±0.58	8.00±1.00
	40	6.00±0.00	6.67±0.33	7.33±0.33
	20	6.00±0.00	6.00±0.00	7.33±1.33
	0	6.00±0.00	6.00±0.00	6.00±0.00

Keys %=percentage mm=millimeter

Discussion

The results of phytochemical analysis of the ten medicinal plants selected from Wudil, Kano State, revealed that all the selected medicinal plants have secondary metabolites which contribute significantly toward the biological activities of medicinal plants such as antimicrobial, anti-inflammatory, antioxidant, anti-malarial activities etc. One of the largest groups of chemical arsenal produced by plants are alkaloids. The study indicated that in the phytochemical analysis using methanol and aqueous extracts, alkaloid was found to be present in all the selected plants except scent leaves and acacia gum which were absent in aqueous extraction method and alkaloid was absent in moshi medicine in methanol extraction method this was not strange since Raffauf (1996) in his work reported that there are some species that do not contain any of these bitter, nitrogenous compounds. The absence of alkaloid in these plant extracts may be due to insolubility of active compounds in water or the solvent used was not effective in removing all the alkaloids from the plant tissue. This situation according to Gholamreza et al. (2017) who investigated on some plants in Aligudarz, is common among Lamiaceae (Labiatae) family. They found that, out of the twenty five species of the Lamiaceae (Labiatae) family worked on, only four had alkaloid present. Okoro et al. (2011) also reported the absence of alkaloid in acacia gum which is similar to the result obtained in this study.

Saponins which are another important secondary metabolite found in the selected medicinal plants, is known for its formation of stable soap-like foam in aqueous solution. Mayank et al. (2011) reported that majority of saponins-producing species have been found within dicotyledonous plants. This study revealed that, saponins was present in moshi medicine which was previously reported by Edward (2015) after working on Malian medicinal plants in order to identify a potential cancer drug. The study also revealed that saponins was absent in camel's foot (*Piliostigma thonningii*), *a*nd this was similarly reported by Ugwoke et al. (2017).

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Flavonoid which is known for its antioxidant properties which can protect humans from free radicals and reactive oxygen species was observed in the selected medicinal plants. The study also revealed that, flavonoid was absent in scent leaf and balsam apple in the methanol extraction method while it was absent in acacia gum in both aqueous and methanol extracts. This may be due to inability of methanol to liberate the active component in the plant extracts. Flavonoid have increased water solubility and aqueous solutions are suitable while it is difficult to dissolve in methanol, ethanol or water-alcohol mixture. Its solubility has been reported to depend on the P^H of water-containing solution (Oyvind & Kenneth, 2006). Karumi et al. (2004) who carried out their study on balsam apple also reported the absence of flavanoid in methanol extract and its presence in aqueous extracts.

Terpenoids are other groups of compound observed in this study. The study revealed that terpenoids are present in all the selected plants but was absent in scent leaf in methanol extract and absent in balsam apple in both extraction methods. Phytochemicals are better recovered from leaves by application of heat, while better recovery of tannins, flavanoids and terpenoid is by aqueous extraction method (Abah & Egwari, 2011). Selvakumar and Monica (2018) also reported the absence of terpenoid in balsam apple in both aqueous and methanol extracts. Reducing sugars are end products of many biological processes and enzymatic reactions. In this study it was observed to be present in most of the selected plants except in Moringa, sickle senna and acacia gum in both methods of extraction, maybe it is absent in the plants generally and not due to extraction method used since it was also reported by Pinal et al. (2014) and Wadood et al (2013) in their work on Moringa and Acacia gum respectively.. However, Preshant et al. (2011) reported that the best method of extraction of reducing sugar is by aqueous method.

The anti-microbial activities of the three selected plants in this study showed that all the plants had significant effect on the micro-organisms isolated. Baobab which contained all the phytochemicals in this study was used for its anti-bacterial activities. Vertuani *et al.* (2002); Besco et al. (2007); Brady (2011) who worked on baobab fruit pulp revealed that it is high in vitamin C, which acts as antioxidant which may be because of the flavonoid present. Gholamreza *et al.* (2017) reported that alkaloids which is another phytochemical found in the extract, have anti-diarrheal and antibacterial properties This anti-bacterial activity of aqueous baobab fruit pulp extract was attributed probably to the presence of flavanoid, saponins and terpenoid in the extract (Ramadan *et al.*, 1993). Yagoub (2008), reported that, the petroleum ether, methanol and aqueous extracts of baobab showed antimicrobial activity. However, Abiona et al. (2015) who investigated on antimicrobial effect of baobab and found out that the aqueous extract is significant against all the tested micro-organism; fungal and Bacteria except Klebsiella pneumoniae.

Sodom apple extract was also used for its anti-bacterial effect and was observed to be highly significant against all the tested bacteria. Mainasara et al. (2012) who investigated on phytochemical and antibacterial properties of root and leaf extracts of *Calotropis procera* showed that, the extracts was active against tested bacteria; *S. aureus*, *E. coli*, *K. pneumoniae*, *Salmonella typhi* and *Streptococcus pyogenes* which was similar to the result obtained in this studied and Asoso et al. (2016) also reported that, Sodom apple was active against human pathogens. Catholic vegetable was observed to contain all the phytochemicalso and was observed to be active on all the tested microorganisms. Previous phytochemical screening on *J. tanjorensis* leaf also revealed the presence of alkaloid, saponin, flavonoid, tannin, reducing sugar and steroid. Thus, it appears that the presence of this components makes the plant active against bacteria (MacDonald et al., 2014). Ogunnusi et al. (2016) also observed the anti-bacteria activity of *J. tanjorensis*, using both aqueous and methanol extraction methods after serial dilution. They reported that, the extract was highly active against *E. coli* and *S. aureus* while it was mildly active against *K. pneumoniae.* This is similar to the result obtained in this study using methanol extract. Akhigbe et al. (2009) and Ananti et al. (2009) who used some solvents such as chloroform, methanol, petroleum ether and water showed activities of this plant against organisms including *K. pneumoniae* and *E. coli.* This is in agreement with this result.

However, the bioactive molecule extracted depends upon the type of solvent used in the extraction procedure (Shoba et al., 2014). Variation in extraction methods usually depends upon; length of extraction period, solvent used, P^H of solvent, temperature and particle size of the plants tissue (Prashant et al., 2011).

Conclusion

This study has shown that the medicinal plants tested have broad spectrum of activities against bacteria especially *E. coli, S. aureus* and *K. pneumoniae.* In the phytochemical analysis of the medicinal plants, it was observed that the methanol extraction method is better than the aqueous method although the difference is not much. The result also showed that phytochemicals such as alkaloid, terpenoid, flavonoid, saponins and reducing sugars are present in all the selected plants. The plants extracts especially the three tested against bacteria can inhibit the growth of bacteria and is more active when diluted to 80 %. Among the three selected plants, Sodom apple was observed to be highly effective against all the tested bacteria compared to catholic vegetable and baobab.

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