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(RESEARCH ARTICLE)

Phytochemical characterization and antimicrobial studies on four folklore medicinal plants in Semi-Arid Region of Borno State, Nigeria

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Abstract

Crude leave extracts of four folklore medicinal plants were subjected to phytochemical screening and antimicrobial assays against microbial pathogens using well diffusion method. The preliminary phytochemical investigation of the crude leave extracts of four folklore consists of Neem, Moringa, Jatropha and Balanites revealed that there is present of bioactive phytocomponents with potential antimicrobial ingredients when Soxhlet extraction was performed using different solvents (Hexane, Chloroform, Methanol, acetone and Ethyl acetate). The crude extracts showed significant antimicrobial activities against all microbial pathogens screened with highest activity in methanol and chloroform extracts of alkaloids as phytocomponents. While highest activity was recorded in methanol and chloroform extracts, faintly in ethyl acetate extracts using phenolics Phytochemical. Whereas, microbial activities was moderately present in chloroform, acetone and ethyl acetate extracts using steroids and reducing sugars phytocomponents respectively. Whereas, the antimicrobial activities against pathogens revealed remarkable sensitivity with prominent zone of inhibitions with ranging from 14mm to 26mm against Pseudomonas aeruginosa and 10mm to 24mm in streptococcus species using extracts from chloroform, ethyl acetate, Hexane and methanol extracts. Likewise, moderate zone of inhibition ranging from 14mm to 17mm was recorded in Staphalococcus aureus, 10mm to 17mm was recorded in P. pyogene and 10mm to 16mm in Escherichia coli respectively. Whereas weak zone of inhibition ranging from 10 mm to 12 mm was obtained against streptococcus mutans. The bioactive ingredients of those with antimicrobial activities are screened and recommended that more research work be conducted to explore their bioactive components for formulation into appropriate dosage as potential antibiotics for the treatment of infectious diseases in the study area.

Keywords: Folklore, Phytochemical, Characterization, Antimicrobial studies, Semi-arid, Borno state, Nigeria

1. Introduction

Traditional medicine as a source of bioactive compound for pharmaceutical and microbiological studies has been handed down from generation to generation for thousands of years [1,2]. In recent years medicinal plants and their phytocomponents are used in traditional treatments of various diseases in the developing countries like Nigeria because of the high cost of modern synthetic drugs [3]. Furthermore, the bioactive components of herbal plants appear to be rewarding as it might lead to the development of phytomedicine which are safe, effective and inexpensive [4]. According

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to [2] traditional herbal medicine is an important component of primary health care system in developing countries. Several benefits are derived from plant secondary metabolite, pharmaceuticals and antimicrobial efficiency which would play a prominent role against microbes. Hence, phytochemical survey and screening of medicinal plants used by different ethnic group and agroclimatological zone has become inevitable to explore the potential source for characterization of bioactive compounds [5]. Recent studies revealed that numerous plants and herb species has been a source of medical agents with potential antimicrobial and antiviral properties [6,7]. Several surveys have been reported in the developing countries that there has been an increasing incidence of multiple antibiotics resistances in pathogenic microorganisms [8,9]. The indiscriminate use of synthetic antimicrobial drugs commonly employed in the treatment of ailment lead to antibiotic resistance. This necessitate scientists to explore for new antimicrobial substance from various sources such as herbs and medicinal plants [10,11]. According to [12,13] some traditional healing system have already produced bioactive compounds from phytocomponents that are efficient and effective against antibiotic-resistant strains of bacteria. Earlier studies on medicinal plants have showed that almost every component of the seeds, leaves, roots, bark, trunk and branches with potential medicinal properties are used in the semi-arid regions since time immemorial [14]. Thus, the phytocomponents of plants enhances ethnobotanical and pharmacological studies leading to production of a more potent drug with high antimicrobial agent with less side effect [15,16]. In the present research work four folklore medicinal plants were evaluated for phytochemical and antimicrobial properties.

2. Material and methods

2.1. Sample collection

The fresh leaves of four folklore medicinal plant parts of Neem, *Moringa, Jatropha* and *Balanite* were collected randomly from the semi-arid region of Borno State. The plants were identified according to various literature and other pertinent taxonomic. The leaves were washed under running tap water to eliminate dust and other foreign particles. The collected leaves were chopped into pieces and coarsely powdered with suitable homogenizer.

2.2. Preparation of extracts

The powdered sample obtained after homogenization were subjected to phytoextraction with organic solvents using methanol, ethyl acetate, chloroform and Hexane by using Soxhlet extraction. The extracts were collected and distilled off on a water bath at atmospheric pressure. The residues were used with the appropriate solvents for the phytocomponents screening and antimicrobial studies. Phytochemical characterization of medicinal plant (Neem, *moringa*, *balanite* and *jatropha*) leaves extracts were conducted by extraction, isolation and identification of phytocomponents. Bioactive ingredients like alkaloids, flavonoids, phenolics, reducing sugar and fatty acids were determined using Soxhlet extraction procedure as described by [18].

2.3. Antimicrobial assay

Antimicrobial activity of the different medicinal plants extract was determined for various phytocomponents and bioactive compound responsible for antimicrobial sensitivity by adapting [19]. The microorganisms were cultured on Muller Hinton agar medium and incubated at 37^0C for 24 hours, the diameter of the Zone of inhibition was recorded in MM and susceptibility is determined by comparing with standard values [20].

2.4. Phytochemical screening

Phytochemical screening of crude leaves extract of the four folklore medicinal plants as described by Harborne, [21].

2.4.1. Detection of Alkaloids

Alkaline Reagent Test: Each plant extracts were treated with few drops of Sodium hydroxide solution. Formation of intense yellow colour after addition of dilute HCL acid indicates the presence of flavonoids as described by [22].

2.4.2. Detection of phytosterols

Liebermann Burchard's test: the plant extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphoric acid were added, formation of brown ring at the junction indicates the presence of phytosterols [23].

2.4.3. Detection of reducing sugars

The plant extracts were dissolved individually in 5ml distilled water and filtered; the filtrates were used to test for the presence of carbohydrates.

Fehling's Test: filtrates were hydrolyzed with dil. HCL, neutralized with alkali and heated with Fehling's A&B solutions. Formation of red precipitate indicates the presence of reducing sugars.

2.4.4. Detection of Tannins

Lead acetate test: the plant extracts were dissolved separately in distilled water and to each 10% lead acetate solution were added. The appearance of yellow precipitate confirmed the tannins [24].

2.4.5. Detection of Saponins

Froth Test: the plant extracts were diluted individually with distilled water up to 20ml and shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of "Honey comb" froth indicates the presence of Saponins as described by [22].

3. Results and discussion

The presence of antimicrobial substances in the plants and herbs for the treatment of illness begins since times immemorial [14]. The extracts are potential sources of novel antimicrobial and phytomedicine compounds due to the presence of bioactive phytocomponents that produce a definite physiological action on human body [25]. In this studies, the bioactive phytocomponents of four folklore medicinal plants used for the treatment of illness in semi-arid region of Borno state as shown in (Table 1).

Table 1 List of the four folklore medicinal plants used in the experiment with their phytochemical assays and therapeutic properties.

Scientific Name	Common Name/ vernacular	Plant part used	Family	Phytochemical Assay	Therapeutic properties
Azadirachta Indica	Neem/Dogon yaro	Leaves	Meliaceae	Phenols, unsaturated sterols, polysaccharides [27]. Flavoids, triterpenes and saponine, diterpenoids, limonoids [26,18] and [28].	Possess anti-inflammatory and immunomodulatory activities. Others include anticoagulation and fibrinolytic activities. Useful in earache boils, used as a gargle in stomatitis and gums.
Jatropha curcas	Physic nut/Gongolon	Seed extract	Euphorbiacae	Saponine, phenols, fatty acids [29; 30). Steroids, tannin mucilages&gum. Diterpenes, Coumarins, lignanes, triterpenes, phytosterols (31).	Possess anatural antioxidant with broad spectrum. Local inflammation, anti-spasmodic, rheumatism. Some inhibitory activities on aspergillus species.
Moringa oleifera	Horse radish/ Zogole/Allam	Leaves	Moringaceae	Benzyl-isothiocyanate, presence of alkanoids, essential oils, lipophilic flavoids[32,33].	Possess basic medicinal agents for analgesic and antibacterial [34]. Also use for treatment of stomach pain.
Balanitesae gyptiaccus	Desrt date/Aduwa/Bet to	Leaves	Balanitaceae	Xylopranosyl derivatives of soponin [35] and	It contains vermicidal ingredient and show significant anthelminthic activity

The medicinal actions of plants against specific diseases unique to a particular plant species or group, consistent with phytocomponent, bioactive and secondary metabolites in plant are toxonomical distinct (18, 26]. The results of the preliminary phytochemical screening (table 2) shows the presence of ingredients such as alkanoids, flavonoids, phenolic compounds, terpenoides, chloroform ethyl acetate and water extracts using method described by [17]. The

medicinal plants such as Neem, *Moringa, Jatropha* and *balanite* are being used traditionally for the treatment of several illness such as carminative, cough, inflammation, wound healing, rheumation and immune-modulatory activities [6,14]. Chloroform and methanol extracts revealed the presence of alkanoids, phenolics in higher concentration, while steroids and reducing sugar in moderate concentration in acetone ethyl acetate and chloroform. While Tanins and phenol faintly present Ethyl acetate extract showed the faintly presence of alkanoids, flavonoids, phenolics, steroids. While fatty acids were completely absent, the result of the antimicrobial property of various extracts against different pathogenic microorganisms were shown in table 3. This investigation revealed that the leaf extract of *Azadirachta indica* and *jatropha carcus* possess highly appreciable antimicrobial activity against *pseudomonas aerogenisa,* with faintly antimicrobial activity in *Moringa oleifera* and *balanite aegyptiaca* leaf extracts. While *staphylococcus aureus* possesses moderate antimicrobial activity in *Azadirachta indica* and faintly in *jatropha carcus*. And no antimicrobial activity in both *moringa oleifera* and *balanite aegyptiaca* respectively. Whereas, higher antimicrobial activity against all microbes examined were recorded. While, in *salmonella* species and faintly antimicrobial activity in all plant extracts against *streptococcus mutans, streptococcus pyogene* and *Escherichia coli* respectively.

Table 2 Preliminary studies of phytochemical analysis using different solvents

Phytocomponents	Hexane extract	Chloroform extract	Methanol extract	Acetone extract	Ethyl acetate extract	Water extract
Fatty acids	-	-	-	-	-	-
Alkanoids	-	+++	+++	-	-	+
Flavonoids	-	-	-	-	+	+
Phenolics	-	+++	+++	-	+	+
Tannins	-	-	-	-	+	-
Steroids	-	++	-	++	++	+
Reducing sugars	-	++	-	++	++	-

Key: - Absent, +=Faintly present, ++ = Moderately present, +++ = Highly present.

Table 3 Antimicrobial property of various extracts against different pathogenic micro

Test organism	Name of the plants			
	Azadirachta indica	*Moringa oleifera*	*Jatropha curcas*	*Balanites aegyptiaca*
P. aeruginasa	+++	+	+++	+
S. aureus	++	-	+	-
E. coli	+	+	+	+
S. pyrogene	+	+	+	+
S. mutans	+	+	+	+
S. species	+++	+++	+++	+++

Key: - Absent, +=Faintly present, ++ = Moderately present, +++ = Highly present.

Table 4 Antimicrobial activity (zone of inhibition) of different phytochemical components of leave extracts

Name of the organism	Chloroform 50 mg/ml	Ethyl acetate 50 mg/ml	Hexane 50 mg/ml	Methanol 50 mg/ml
P. aeruginasa	14	16	18	26
S. aureus	17	14	14	16
E. coli	16	12	10	10
S. pyrogene	14	10	10	17
S. mutans	12	12	12	10
S. species	24	10	10	22

Key: 20 mm-27 mm (higher zone of inhibition), 14mm-17mm (moderate zone of inhibition), 10mm-12mm (weak zone of inhibition).

4. Conclusion

The four folklore plant extracts are potential sources of novel antimicrobial and phytomedicine compounds due to the presence of bioactive phytocomponents that produce a definite physiological action. The bioactive ingredients of those with antimicrobial activities are screened and recommended that more research work be conducted to explore their bioactive components for formulation into appropriate dosage as potential antibiotics for the treatment of infectious diseases in the study area.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest

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