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Phytochemical and ethno-microbiological survey of crude extracts of some medicinal plants growing in Nigeria

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Abstract
Phytochemicals are naturally occurring compounds present in all plant parts which together with nutrients and fibres provide protection to plants and humans against diseases. The types and the quantity of phytochemicals vary from one plant to another. This study is aimed at determining the phytochemicals and antimicrobial activities of five medicinal plants growing in Nigeria. The phytochemical analysis revealed the presence of flavonoids, saponins, tannins carbohydrate, terpenoids, glycosides and anthraquinones in the plant extracts. The antimicrobial screening was carried out using the following organisms; *B. subtilis, C. species, S. aureus, S. pyogenes, E. coli, P. aeruginosa, S. typhi, S. dysenteriae* and *C. albicans.* ***Chromolaena odorata*** leaf extract was found to contain all thesecondary metabolites under investigation with exception of anthraquinones and showed activity against all the tested organisms at 100 mg/ml. The evidence of these phytocompounds with inhibiting effect against the tested organisms could be the reasons for their uses in treatment of the various claimed diseases traditionally.

Keywords: extracts, antimicrobial, medicinal plants, organisms, phytochemistry

1. Introduction
In recorded history, medicinal plants have been in use for the treatment of man and animal diseases [1, 2]. A plant becomes a medicinal plant only when its biological activity has been ethno-botanically reported or scientifically established [3]. Medicinal plants are cheaper, more accessible to most of the population in the world, thus there is need to encourage the use of medicinal plant as potential sources of new drugs [4, 5]. This has been an upsurge in the interest in herbal remedies in several plants of the world with many of the herbal remedial been incorporated into orthodox medical practice. Industrial interest in exploiting plant for medical purposes is exclusively found in China and Japan. Some African countries have also made advances in the area of the use of plant for the production of new drugs these countries are Egypt, Burkina Faso, Ghana, Nigeria, Zimbabwe, Zambia and South Africa [6].

The indigenous knowledge on medicinal plants plays a vital role in the discovery of new herbal drugs and new sources of pharmaceuticals. The use of medicinal plants has been in practice throughout human history, whose knowledge gathered through experience of many generations. With this in context, it is important to point out that plants have contributed to the abstention of many drugs; substances with interesting activities are often present in plants. Over years, medicinal plants have been used in the treatment of snakebites, particularly in rural areas. However, the mode of action and the active components of these plants are almost unknown [7, 8] reported that the exploration of ethno medicinal data may serve as a useful source of information to the chemist and pharmacologists so that it can provide ways of identifying the bioactive components of the plant extract for the purpose of drug development.

2. Materials and Methods
2.1 Sample collection and preparation
The plants of *Ceiba pentandra (*Leaf), *Chromolaena odorata* (Leaf), *Sarcocephalus latifolius* (Leaf), *Spondia mombin* (Leaf) and *Parkia biglobosa (*Bark) were collected separately from Abbaganaram ward Maiduguri Borno State, Ire-Ekiti-Oye L.G.A of Ekiti State, Ushongu L.G.A of Benue State, Mbaukuihitte Ezinihitte L.G.A of Imo State and Yobe State respectively. The plant samples were devoid of foreign materials by beating, cut into smaller pieces and air-dried under shade. The dried plant materials were pulverized into fine texture using wooden mortar and pestle.

2.2 Extraction of the samples
Two hundred and fifty grams (250 g) of each sample was weighed using electric weighing balance, the weighed samples were transferred to flasks for maceration, and then 2 litres of 90% ethanol was poured unto the samples in the flasks. The mixtures were macerated for 72 hours, at every 24 hours the extracts were decanted and filtered and fresh 90% ethanol was added to each until the third day, the filtrates were allowed to dry under hot air oven at 50 ^{o}C, which is referred to as the crude extracts. The crude extracts were transferred into clean dried containers for further analysis.

2.3 Phytochemical analysis
Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants under study were

carried out in the extracts using the standard procedures as described by [9, 10].

2.4 Antimicrobial assay

Test organisms: The Gram positive or organisms were *Staphylococcus aureus*, *Bacillus subtilis*, *Corynaebacterium specie*, Gram negative organisms used in their study were *Salmonella typhi*, *Shigella dysenteriae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes;* and *Candida albicans* as fungal isolate. These organisms were clinical isolated obtained from the department of veterinary medicine, University of Maiduguri, Borno State, Nigeria. Anti-microbial susceptibility studies was carried out using the hole-in-plate disc diffusion technique as described by [11, 12].

The extract were made in different stock concentrations prepared by dissolving 1.0 g, 0.5 g and 0.25 g into 10 ml each in 62% of ethanol, the microorganisms were maintained on agar plants until the insular was then prepared by subjecting the test organisms in nutrient an incubated for 24 hours at 37 ^{o}C after incubation, the both cultures were diluted to 1:100 for the Gram positive bacterial and 1:5000 for the Gram negative bacteria. 1 ml of the dilute culture was inoculated into 71.75 g/500 ml sterile molten nutrient agar (48 ^{o}C) and Saba round dextrose agar prepared according to manufacture specification was poured into Petri dishes. These were gentle swelled and allowed to solidity. Inoculated nutrient agar plates using sterilized number cark borer, all the holes were filled with equal volume of 1ml of each portioned. The extraction was allowed to diffuse into the agar for an hour. Thereafter, plates were then incubated overnight at 35 ^{o}C and 37 ^{o}C for fungal and bacterial strain respectively. At the end of the incubation period, inhibition zone were recorded in millimeters as the diameter of growth free zone around the bored holes using a transparent meter rule.

3. Results & Discussion

The results of phytochemical screening of the crude extracts of five medicinal plants and their antimicrobial susceptibility assay are summarized in Tables 1 and 2 below.

Table 1: Phytochemical contents of the crude extracts of some parts of five medicinal plants

S/N	Constituents/Test	Plant Species/Parts				
		Ceiba pentandra	***Chromolaena odorata***	***Parkia biglobosa***	***Sarcocephalus latifolius***	***Spondias mombin***
1	Anthraquinones					
	Free	+	-	ND	-	-
	Combined	-	-	ND	-	-
2	Tests for Carbohydrates					
	Molisch's test	+	+	+	-	+
	Free reducing sugars	-	+	+	+	+
	Combined red. Sugars	-	+	+	+	+
3	Test for Tannins	+	+	+	ND	+
4	Test for Glycosides					
	Salkwoski's	-	+	+	+	+
	Liebermann-Burchard's	-	+	+	+	+
5	Test for Terpenoids	+	+	-	ND	+
6	Test for Saponins	+	+	+	ND	+
7	Tests for Flavonoids					
	Shinoda's	+	-	+	+	-
	Ferric chloride	+	+	+	+	+
	Lead ethanoate	-	-	+	-	-
	Sodium hydroxide	-	-		-	-
8	Test for Alkaloid					
	Preliminary test	ND	+	-	-	-

Key: + = present; - = absent; ND = not done

Table 2: Susceptibility patterns of crude leaf extracts of five medicinal plants various concentrations

Extracts	Organisms/Diameters of inhibition zone (mm) Mean±SEM at 100 mg/ml								
	B. subtilis	***C. species***	***S. aureus***	***S. pyogenes***	***E. coli***	***P. aeruginosa***	***S. typhi***	***S. dysenteriae***	***C. albicans***
Ceiba pentandra	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	12.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Chromolaena odorata	13.0±0.00	7.00±0.00	8.33±0.58	9.00±0.00	13.00±0.00	8.00±0.00	9.00±1.00	8.00±0.00	9.33±0.58
Parkia biglobosa	9.67±0.71	14.67±0.58	0.00±0.00	0.00±0.00	8.00±0.00	14.67±0.58	10.00±0.00	11.67±0.58	9.00±0.00
Sarcocephalus latifolius	8.00±0.00	8.00±0.00	8.33±0.58	9.67±0.58	0.00±0.00	9.00±0.00	8.67±0.58	7.00±0.00	10.33±0.58
Spondias mombin	0.00±0.00	12.00±1.00	9.00± 0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	8.00±0.00	0.00±0.00
Extracts	Organisms/Diameters of inhibition zone (mm) Mean±SEM at 50 mg/ml								
	B. subtilis	*C. species*	*S. aureus*	*S. pyogenes*	*E. coli*	*P. aeruginosa*	*S. typhi*	*S. dysenteriae*	*C. albicans*
Ceiba pentandra	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	9.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Chromolaena odorata	8.00±0.00	0.00±0.00	0.00±0.00	7.00±0.00	8.33±0.58	0.00±0.00	7.00±0.00	0.00±0.00	7.00±0.00
Parkia biglobosa	7.33±0.58	10.67±0.58	0.00±0.00	0.00±0.00	0.00±0.00	10.67±0.58	7.00±0.00	9.00±0.00	9.00±0.00
Sarcocephalus latifolius	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	7.00±0.00	0.00±0.00	0.00±0.00	7.67±0.58
Spondias mombin	0.00±0.00	7.69 ± 0.58	4.67± 4.04	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Extracts	Organisms/Diameters of inhibition zone (mm) Mean±SEM at 25 mg/ml								
	B. subtilis	*C. species*	*S. aureus*	*S. pyogenes*	*E. coli*	*P. aeruginosa*	*S. typhi*	*S. dysenteriae*	*C. albicans*
Ceiba pentandra	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Chromolaena odorata	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Parkia biglobosa	0.00±0.00	7.50±0.71	0.00±0.00	0.00±0.00	0.00±0.00	7.33±0.58	0.00±0.00	7.00±0.00	0.00±0.00
Sarcocephalus latifolius	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Spondias mombin	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

The preliminary phytochemical screening of five medicinal plants extracts namely: *Ceiba pentandra* (leaf) *Chromolaena odorata* (leaf), *Parkia biglobosa* (stem bark), *Sarcocephalus latifolius* (leaf) and *Spondias mombin* (leaf) extracts revealed the presence of secondary metabolites of therapeutic importance. The phytochemicals found variably from across the extracts were alkaloids, flavonoids, saponins, tannins, terpenoids, carbohydrate and glycosides. However, all the different plant extracts tested showed the presence of flavonoids and carbohydrates. Saponins and tannins were detected in all the extracts with exception of *Sarcocephalus latifolius*. Anthraquinones was only evident in *Ceiba pentandra* extract while alkaloids were only present in *Chromolaena odorata* leaf extract. Glycosides were present in all the plant extracts tested except *Ceiba pentandra*. Terpenoids were present in *Ceiba pentandra*, *Chromolaena odorata* and *Spondia mombin* leaf extracts. *Chromolaena odorata* leaf extract yielded the maximum phytochemicals among the five plant extracts as presented in Table 1.

The presence of these pharmacologically useful classes of compounds which are known to have curative activities against several pathogens and therefore could explain their uses traditionally for the treatment of wide array of illness [13, 14]. Tannins were reported to possess physiological astringent properties which hasten wound healing and ameliorate inflamed mucus membrane [15]. Saponins has expectorant action which is useful in the management of upper respiratory inflammation, saponins present in the plants is cardiotonic in nature [10, 15].

The results obtained for antimicrobial test performed on the five plant extracts are presented in Table 2. The results showed that *Chromolaena odorata* leaf extract is the only plant extract that shows activity against all the tested organisms at 100mg/ml, in comparison to the other four plants extracts. The activity of these plant extract decreases with decrease in concentration as shown in Table 2. And no activity was observed at 25mg/ml for *Chromolaena odorata* leaf extract. *Parkia biglobosa* (stem bark) extract is the only plant extract that shows activity at 25mg/ml against *C. species, P. aeruginosa* and *S. dysenteriae*, for the other four plant extracts no activity was observed against any organism at the same concentration. However, these differences that were observed among their antibacterial activities could be due to the difference in the chemical composition of these plant extracts as revealed by phytochemical analysis. The results of the present study support the traditional uses of these plants on the treatment of bacterial infections, particularly, the leaves of *Chromolaena odorata* which has been reported that is used for healing wound, pile treatment and malaria in the Northern part of Ekiti State [16, 17]. Other traditional medicinal uses include anti-diarrheal, astringent, antispasmodic, antihypertensive, anti-inflammatory also serve as heart tonic [18-20]. These claims have been validated by this paper.

4. Conclusion

The preliminary qualitative phytochemical screening reported in this paper showed that *Ceiba pentandra* Linn. (Leaf), *Chromolaena odorata* Linn. (Leaf) *Parkia biglobosa* Jacq. (Stem bark) *Sarcocephalus latifolius* (Leaf) and *Spondias mombin* Linn. (Leaf) extracts were found to contain secondary metabolites of therapeutic importance. *Chromolaena odorata* leaf extract contains the maximum phytochemicals and possesses antimicrobial activity against all the tested organisms at a particular concentration. The research correlates with previous reports on the antimicrobial activity of the plant leaves. Further study in detail may help in establishing the medicinal activity of the preparation, so it can serve as an alternate antimicrobial agent in case of antibiotic resistant.

5. References

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