Research Article

CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITIES OF SOME SELECTED TRADITIONAL MEDICINAL PLANTS USED IN THE TREATMENT OF GASTROINTESTINAL INFECTIONS IN NIGERIA

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ABSTRACT

Chemical composition and antibacterial effects of six Nigerian medicinal plants (*Cymbopogon citratus, Bambusa vulgaris, Aerva lanata, Cajanus cajan, Sorghum bicolor and Nauclea latifolus*) used in the treatment of gastroenteritis were investigated against three medicinally important gram negative bacteria using standard methods. The plant parts used for the assays were the leaves. The ash content ranged from 5.5±5.4 to 20.1± 3.4g/100g, the moisture was low: 0.92±1.1 to 3.2±1.2, protein had a range of 9.9±0.0 to 15.4±3.2g/100g, fat of 20.4±5.3 to 25.2±7.9g/100g qualified the samples as oil plants, fibre was high while available carbohydrate was moderate in all the samples. The plant samples would serve as good sources of Na, K, Ca, Mg, Zn and P but moderate sources of Fe and Mn while both Cu and Ni were not detected. The Ca/P was good in 66.7% and poor in 33.3% of the samples. The Na/K values were all greater than 0.60 thereby making them susceptible to the promotion of high blood pressure. The values of Phy: Zn, Ca: Phy and Ca x Phy: Zn showed that only *C.citratus* and *B.vulgaris* will promote the highest bioavailability of Zn among the samples. *S. sonnei* and *E.coli* were the most susceptible pathogens to the extracts of the medicinal plants while aqueous extracts performed better than the methanolic extracts.

Keywords: Chemical composition, antibacterial effects, six Nigerian medicinal plants.

INTRODUCTION

Antibacterial resistance among bacterial pathogens in recent time is a critical area of public health concern[1]. The usual causative agents of infectious diseases (especially bacteria) are becoming increasingly resistant to some or most antibiotics[2].

The cost of drugs in use today is too expensive for the majority of the population in the third world countries and therefore the search for some cheap sources of antimicrobial substances in nature become inevitable.

The use of plants as therapeutic agents in addition to being used as food is age long[3]. In developing countries, thousands of rural communities still depend mainly on folklore medicine to cure diseases[4]. Medicinal plants are cheap and handy to most of the populations on the globe [5]. As a result of proximity, reliability and age long practice, people still depend largely on traditional medicine for their health care delivery [6].

Traditional tropical herbs contain many useful compounds which are used for the treatment of diseases. Many reports confirmed the potentials of medicinal herbs in the prevention of some infectious diseases [7]. Though the therapeutic uses of plants by the primitive people lack scientific explanations[8], there is a great awareness in the use and significance of these medicinal floras by the World Health Organization in several resource- poor nations[9]. This has led to intensified efforts on the documentation of medicinal plants[10].

It is inevitable from the scientific point of view, to establish a balanced correlation between chemical, biological and therapeutic activities of medicinal flora which are precursors for most conventional drugs [11].

In this report, the chemical analyses and the antibacterial activities of selected medicinal recipes that had claimed success in folklore medicine in the south western Nigeria were investigated.

MATERIALS AND METHODS

Collection of plant materials and extraction of active ingredients:

All the medicinal plants were obtained from Ado-Ekiti, Nigeria. The plants were examined, identified and authenticated at the Herbarium of the Department of Plant Science and Forestry, University of Ado- Ekiti, Nigeria. The plants were air-dried and pulverized into powder. About 5g of the powdered sample of each medicinal plant were weighed into100ml of distilled water and 75g of the powdered sample of each medicinal plant were weighed into 175ml of methanol in a separate conical flask and was allowed to soak for five days. The extract was decanted and filtered with Whatman No.1 filter paper. The filtrate (extract) was evaporated to dryness at 40^{o}C in a rotary evaporator. The concentrated extract of each plant was stored at 4 ^{o}C until when required for use.

Source of inoculate:

The microorganisms used for this work were collected from the stock culture collection of the Department of Microbiology, University of Ado-Ekiti, Nigeria. The bacteria used were *Escherichia coli*, *Samonella* specie and

Shigella sonnei. They were grown (in separate tubes) at 37 ^{o} C in Mueller-Hilton (Oxoid) broth for 18 h with shaking and diluted to an optical density of 0.1(0.5 McFarland standard) at optical activity of 625 nm with Mueller-Hilton (Oxoid) broth and stored at 4^{o}C to arrest further bacterial multiplication.

Proximate and mineral analyses of the medicinal plants: The standard methods of AOAC [12] were used to determine moisture content, crude fibre, ether extract (crude lipid). The method of Pearson[13] was used to determine the protein content of the samples. Carbohydrate was determined by difference. Minerals were analysed using the method of AOAC[12] and standardized by the method of Techtron[14]. Phosphorus was determined by the phosphovanado- molybdate method[13]. All the proximate values were reported in g/100 g while the minerals were reported in mg/100 g.

Determination of antibacterial activity:

The antibacterial activities of the extracts were determined using the paper discs method. About 15 ml of molten nutrient agar was poured into the sterile petri dishes and allowed to set. About 0.2 ml of a 24 h old culture of each test organism was inoculated into the nutrient agar plate by sterile pipette. Sterile perforated filter paper disc was put into test tubes containing the extracts after 5 mins and allowed to dry before being placed on the nutrient agar plates seeded with the test organisms. The plates were then incubated at 37^{o} C for 24 h and the zone of inhibitions were measured (to the nearest mm) with the aid of venial caliper.

Determination of Phytate:

Phytate (Phy) was quantified using the method described by Harland and Oberleas [15]. The colorimeter used was Spectronic 20 (Gallenkamp.UK). The amount of phytate in the samples was calculated as hexaphosphate equivalents using the formula:

Phytate, mg/g sample = "mean K" x Ax 20/(0.282 x 1000) where A =absorbance; "mean K"= stdP(µg)/A/n(stds); phytate =28.2 % P. The phytate values were reported in mg/100 g.

Calculations of Ca/P and Na/K:

The calcium/phosphorus (Ca/P) and sodium/potassium (Na/K) ratios were calculated for all the samples[16]. Calculations of Phy: Zn, Ca: Phy and CaxPhy:Zn values were calculated according to the method of Wyatt and Triana-Tejas[17]. Mean standard deviations and coefficients of variations were also calculated.

RESULTS AND DISCUSSION

A number of medicinal plants have been screened and quite a lot of them have been confirmed to contain antibacterial activity[18]. *[Table 1]* shows the list of medicinal plants, local names and the parts used in this study. The proximate compositions of the six medicinal plants are shown in *[Table 2]*. The results showed that these medicinal plants contain ingredients such as fat, ash, protein, fibre, moisture and carbohydrate. Ash content which is an index of mineral content in the biota was relatively high in the *C.citratus* than other medicinal plants. The least value was recorded in *S. bicolor* with 5.5±5.4 g/100 g. The variation might be as a result of differences in species and the condition of growth [19]. The mean value of ash content of the medicinal plants was found to be 12.2±5.5 g/100 g with coefficient of variation (CV %) of 43.6. *C. citratus* had the least moisture content, 0.92 g/100 g which is an indication of stability and keeping quality [20] while the highest was recorded for *N. latifolus* (3.2±1.2 g/100 g).

The mineral composition of all the medicinal plants is depicted in *[Table 3]*. Both Ni and Cu were not detected in the plant samples. Sodium content in the samples ranges from 54.8 down to 12.7mg/100 g. Magnesium is an important mineral element in connection with circulatory diseases such as Ischemic heart diseases and with calcium metabolism in bone [21]. The Mg values in the plant materials ranged between 70 and 29.0 mg/100 g. The highest value was recorded in *C. citratus*.

Iron content of the plant samples varied considerably. Other than *B. vulgaris* and *C. cajan* (3.03 and 8.95 mg/100g respectively) the medicinal plants were not good sources of Fe. Iron is an essential trace element for haemoglobin formation, normal functioning of the central nervous system (CNS) and the oxidation of carbohydrate, protein and fat [20].

The concentration of Ca ranged from 18.4-69.1 mg/100 g. The highest level of phosphorus was detected in *C. citratus* (89.3 mg/100 g). The plants, apart from their medicinal values also serve as good sources of minerals particularly Zn (24.0-121 mg/100 g), Zn is present in about 52 enzymes [22] which play important roles in physiological activities. Ca and P are associated with each other for growth and maintenance of bones, teeth and muscles [23].

The Ca/P in *C. cajan* and *N. latifolus* was 2.10 and 2.12 respectively. Ca/P ratio must be close to unity to facilitate Ca/P absorption according to Gutenmann *et al*. [24], however Nieman *et al.* [16] reported that Ca/P greater than 2 would always be welcomed and concluded that Ca/P greater than 1.0 is good but values less than 0.50 is described as being poor. The Na/K *[Table 4]* ranged between 0.714 and 0.920. The values were less than 0.75 in *A. lanata* (0.727) and *S. bicolor* (0.714). Decreased Na/K ratio might be important for protection of hypertension and arteriosclerosis since K depresses and Na enhances blood pressure [25]; on this score, Nieman *et al.* [16] had set the standard of Na/K as 0.60 to avoid the two diseases mentioned above. The ratio of 3:4 (0.75) is considered most adequate for normal retention of protein during growth stage [26].

The levels of phytate (Phy), Phy/Zn, Ca/Phy and [Ca][Phy]/[Zn] of the samples are shown in *[Table 5]*. All the Phy values in this report were higher than those reported for *Capsicum annum, Piper nigrum, Hibiscus*

esculentus, Lycopersicon lycopersicum, Allium cepa and Irvingia gabonensis [27], the same observation also goes for 13 spices in which most of the samples were lower in Phy values than present report [28].

Oberleas and Harland [15] showed that foods with a molar ratio of Phy: Zn less than 10 showed adequate availability of Zn and problems were encountered when the value was greater than 15. From Table 5 only *C.citratus* and *B. vulgaris* had Phy: Zn less than 10. Frans *et al.* [29] demonstrated a lower availability of Zn in rats when fed with foods of high molar ratios of Phy: Zn. Wise [30] had linked the availability of Zn bound in a mineral complex in the intestines to depend on the levels of calcium. At Ca: Phy molar ratios lower than 6:1, Phy precipitation is incomplete, so that some of the dietary Zn remains in solution. The proportion remaining in solution increases with decreasing Ca: Phy molar ratios [30]. All our samples were having Ca: Phy less than 6:1. Ferguson *et al* [31] showed that the molar ratio varies with different foods and recommended that this value be used in conjunction with other data to explain the availability of Zn using the Ca: Phy ratio.

Our results for [Ca][Phy]/[Zn] that is (Ca x Phy: Zn) are shown in *[Table 5]*. Ellis *et al.* [32] and Davies and Warrington [33] indicated that the ratio of Ca x Phy: Zn is a better predictor of Zn availability and said that, if the value were greater than 0.50 mol/kg, there would be interferences with the availability of Zn. In our results, Ca x Phy: Zn values were less than 0.50mol/kg in *C.citratus, B. vulgaris, A. lanata and C. cajan,* such samples would promote Zn bioavailability among the samples.

The difference in the antibacterial effect of various medicinal plants screened might be due to the quantitative and qualitative differences in them [34, 35, 11, 36], extraction methods employed and the level of concentration of such an extract [37]. The antibacterial activities of aqueous and methanolic extracts of the screened medicinal plants are shown in *[Tables 6 and 7]* respectively.

Shigella sonnei and *E. coli* were the most susceptible pathogens. This report supports the earlier observations of Akujobi *et al.* [38] and Ibekwe *et al.* [39]. The result presented in this study showed that the tested extracts contained antibacterial properties. Though there was variation in their degree of antibacterial activity, extracts were very potent against some test bacteria. Reports of Ogueke *et al.* [40] and Oluma *et al.* [5] highlighted that methanol and water effectively extracts polar and non polar constituents of medicinal plants respectively. They act as best solvents that extract large quantity and quite a number of phytochemical compounds [41]. *S. sonnei* was highly susceptible to the water extract of *C.citratus* while it is resistant to aqueous extract of other plants. At very low concentrations the test organisms were susceptible to water extract of *C.citratus*. At concentration ≤ 33 mg/ml water extract of *A. lanata, C. cajan* and *Nauclea latifolus* showed no antibacterial effect on the test organisms, *B. vulgaris* was next to *C.citratus* in activity. Methanolic extract of *B. vulgaris* was very effective against *E.coli*. At 100 mg/ml of methanolic extract *C. cajan* was effective against all the test organisms. Aqueous extract of the medicinal plants was better in antibacterial activity than their corresponding methanolic extract.

Table 1: The scientific and common names of the medicinal plants evaluated in this study

S. No	Scientific Names	Common names	Part used
1	*Cymbopogon citratus*	Lemon grass	Leaves
2	*Bambusa vulgaris*	Common bamboo	Leaves
3	*Aerva lanata*	Native amarantha	Leaves
4	*Cajanus cajan*	Pigeon pea	Leaves
5	*Sorghum bicolor*	Guinea corn	Leaves
6	*Nauclea latifolus*	African guinea pea	Leaves

Table 3: Mineral composition of the medicinal plants under study (dry weight, mg/100g)

Plant	Na	K	Ca	Mg	Fe	Mn	Zn	P	Cu	Ni
C.citratus	54.8	59.5	39.5	70	0.024	0.952	121	89.3	ND	ND
B. vulgaris	31.8	39.9	27.3	57.6	3.03	0.455	89.4	18.9	ND	ND
A. lanata	12.7	17.5	18.4	29.0	1.11	0.952	53.2	19.8	ND	ND
C. cajan	44.7	56.6	69.1	56.6	8.95	4.08	71.1	32.9	ND	ND
S. bicolor	17.1	24.0	29.6	39.1	1.99	0.959	24.0	17.1	ND	ND
N. latifolus	20.3	26.6	13.6	40.6	2.15	0.696	36.7	15.8	ND	ND
Mean	30.2	37.3	26.2	48.7	2.91	1.35	66.0	32.3	ND	ND
SD	16.7	17.6	17.5	15.2	3.11	1.35	35.9	28.8	ND	ND
CV%	55.2	47.4	48.4	31.2	107	100	54.4	88.4	ND	ND

SD = standard deviation; CV%= coefficient of variation; ND = not detected.

Table 2: The proximate composition of the medicinal plants (dry weight, g/100g)

Plant	Ash	Moisture	Protein	Fat	Fibre	CHO
C.citratus	20.1±3.4	0.92±1.1	12.0±7.4	20.4±5.3	31.4±1.3	15.3±2.6
B. vulgaris	15.4±4.4	1.4±1.1	15.4±3.2	22.0±3.9	21.6±9.1	24.3±2.1
A. lanata	13.1±5.4	2.2±1.8	11.3±5.2	25.2±7.9	26.2±3.3	22.0±2.6
C. cajan	11.1±7.7	2.4±1.4	15.2±7.4	22.7±8.1	27.5±8.1	21.1±1.3
S. bicolor	5.5±5.4	2.8±0.5	2.8±1.0	21.8±6.7	29.8±7.7	31.7±2.5
N. latifolus	7.7±2.1	3.2±1.2	9.9±0.0	23.1±0.0	17.1±5.1	39.0±2.0

CHO= available carbohydrate.

Table 4: The calculated values of Ca/P, Na/K, mg of phytate (Phy)/MW of Phy, mg of Ca/MW of Ca and mg of Zn/MW of Zn

Plant	Ca/P	Na/K	Phy/MW	Ca/MW	Zn/MW
C.citratus	0.44	0.920	18.0	0.986	1.86
B. vulgaris	1.44	0.797	9.65	0.681	1.37
A. lanata	0.928	0.727	10.3	0.459	0.813
C. cajan	2.10	0.790	11.8	1.72	1.087
S. bicolor	1.73	0.714	41.6	0.738	0.367
N. latifolus	2.12	0.762	47.4	0.839	0.562
Mean	1.46	0.79	23.1	0.905	1.01
SD	0.67	0.07	16.9	0.438	5.55
CV%	45.9	8.86	73.2	48.4	54.4

Table 5: Concentration of Zn, Ca, Phytate and calculated Phy: Zn, Ca: Phy and Ca x Phy: Zn of the medicinal plants

Plant	Phy[a]	Ca[a]	Zn[a]	Phy/Zn[b]	Ca/Phy[c]	Ca x Phy/Zn[d]
C.citratus	11860	39.5	121	9.68	0.055	0.095
B. vulgaris	6370	27.3	89.4	7.06	0.071	0.048
A. lanata	6780	18.4	53.2	12.6	0.045	0.058
C. cajan	7800	69.1	71.1	10.9	0.146	0.187
S. bicolor	27460	29.6	24.0	113	0.018	0.947
N. latifolus	1300	33.6	36.7	84.4	0.018	0.708
Mean	15262	36.2	66.0	39.7	0.059	0.322
SD	11174	17.5	35.9	46.8	0.048	0.355
CV%	73.2	48.4	54.4	118	81.4	110

a = values in mg/100g; b = mg of Phy/ mol.wt of Phy;mg of Zn/mol.wt of Zn.
c = mg of Ca/mol.wt of Ca;mg of Phy/mol.wt of Phy; d = mol/kg Ca x mol/kg Phy/mol/kg Zn.

Table 6: Antibacterial activities of water extracts of the medicinal plants on test bacteria using paper disc method (zone of inhibition in mm)

Plant extract	Test organisms																	
	E.coli						*Salmonella* sp						*Shigella sonneri*					
	C	100	50	33	25	20	C	100	50	33	25	20	C	100	50	33	25	20
C.citratus	0.0	9.0	7.0	6.0	4.0	3.0	0.0	6.0	5.0	4.0	3.0	2.0	0.0	8.0	6.0	5.0	3.0	2.0
B. vulgaris	0.0	4.0	3.0	2.0	0.0	2.0	0.0	5.0	3.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A. lanata	0.0	5.0	2.0	0.0	0.0	0.0	0.0	3.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. cajan	0.0	4.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. bicolor	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	2.0	0.0	0.0	0.0
N. latifolus	0.0	3.0	2.0	0.0	0.0	0.0	0.0	5.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 7: Antibacterial activities of methanol extracts of the medicinal plants on the test bacteria using paper disc method (zone of inhibition in mm)

Plant extract[a]	Test organisms																	
	E.coli						*Salmonella* sp						*Shigella sonneri*					
	C	100	50	33	25	20	C	100	50	33	25	20	C	100	50	33	25	20
C.citratus	0.0	5.0	4.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.0	3.0	2.0	0.0	0.0
B. vulgaris	0.0	8.0	6.0	4.0	3.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A. lanata	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. cajan	0.0	2.0	0.0	2.0	0.0	0.0	0.0	4.0	2.0	0.0	0.0	0.0	0.0	3.0	2.0	0.0	0.0	0.0
S. bicolor	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
N. latifolus	0.0	5.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	2.0	0.0	0.0	0.0

[a] Concentration of plant extract(mg/ml).

CONCLUSION

The knowledge of chemical, biological and therapeutic activities of medicinal plants used as folklore medicine is necessary. The minerals and phytochemical components of the medicinal plants may have been responsible for the antimicrobial activities of the plants[34,42,43]. The quantification and determination of the active ingredients of the studied medicinal plants on the test organisms should be the object of further investigation with the mechanism(s) of action of their phytochemotherapy.

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