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In-vitro antimicrobial screening of some medicinal plants

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ABSTRACT

The extracts of Uvaria chamae, Cassia arereh, Aristolochia albida, Pseudocedrella kotchyii, Tamarindus indica and Nebouldia laevis were evaluated for secondary metabolites and antimicrobial activity. Phytochemical investigations revealed the presence of alkaloids flavonoids, steroids, glycosides, cardiac glycosides, tannins, saponins and anthraquinones. Brine shrimp lethality tests carried out indicate high activities for methanol and ethyl acetate extracts of the plants (BST LC_{50} 3.4 – 114.4 µg/cm^3) The extracts exhibited various levels of activities against Escherichia coli, Streptococcus pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, Aspergillus flavus and Aspergillus niger.

Keywords: Phytochemical screening, medicinal plants, antimicrobial activity, ethnomedicine.

INTRODUCTION

The application of poultices and imbibing infusion of hundreds or thousands of indigenous plants dated back to prehistory. There is evidence to suggest 60,000 years ago, Neanderthals living in present day Iraq used plants such as holy hock for medicinal purposes [1,2]. These plants are still widely used in ethnomedicine around the world. The use of medicinal plants as herbal remedies to prevent and cure ailment differs from community to community [3]. Medicinal uses of plants range from administration of the roots, barks, stems, leaves, and seeds to the use of extracts and decoction from the plants [4]. Recently, synthetic drugs have come into use, and in many instances these are chemically identified compounds in plants [3].

The intractable problem of antimicrobial resistance has led to the resurgence of interest in herbal products as sources of novel compounds to fight the ever increasing problems of emergence of newer diseases and those thought to be brought under control [5]. To this end, the world health organization (WHO) is actively encouraging national governments of member countries to use their traditional system of medicines with regulations suitable to their national healthcare system [5].

The goal of the medicinal plant chemist is to find compounds that have potent effect on specific diseases while producing no or minimal side effect. Most compounds are found by screening a lot of compounds randomly. Several workers have reported bioactive compounds from plants [7]. The active principles found in plants differ from plant to plant due to biodiversity and these active principles produce a definite physiological effect on the human body

[8]. The Nigerian traditional medicinal system needs evaluation to understand how Nigerian plants can be used to treat diseases. It may be possible to develop new drugs from Nigerian plants with fewer side effects [8]. As part of our zeal to search for bioactive compounds from plants, we report the investigation of the following Nigerian plants: *Uvaria chamae, Cassia arereh, Aristolochia albida, Pseudocedrella kotchyii, Tamarindus indica* and *Nebouldia leavis*.

MATERIALS AND METHODS

Sample Collection.
The stem-bark of *Uvaria chamae, Pseudocedrella kotchyii, Tamarindus indica* and *Nebouldia laevis,* the root-bark of *Cassia arereh* and the rhizome of *Aristolochia albida* were collected from Rigachikun, Giwa local government area, Kaduna State, Nigeria. The plants were identified and authenticated by U.S. Gallah of Herbarium Unit, Department of Biology, Ahamdu Bello University, Zaria.

The plant materials were air-dried, grounded and stored in clean polythene bags at ambient temperature. Clinical isolates of *Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae,* and *Pseudomonas aeruginosa,* were obtained from the Microbiology Section, Ahmadu Bello University Teaching Hospital, Zaria. Fungal isolates of *Candida albicans, Aspergillus flavus* and *Aspergillus niger* were obtained from the Department of Biology, Nigerian Defence Academy, Kaduna, Nigeria.

Extraction
A portion (50g) each of the respective parts of *U. chamae, C. arereh, P. kotchyii, A. albida, T. indica and N. laevis* was percolated in 200cm^3 each of methanol, ethyl acetate and n-hexane respectively for 2 weeks. They were separately filtered and evaporated to dryness at 40^{o}C using rotary evaporator. Each residue was then allowed to cool, weighed and stored in refrigerator until needed.

Phytochemical Screening
The extracts were screened for the presence of alkaloids, flavonoids, steroids, glycosides, cardiac glycosides, anthrquinones, tannins and saponins according to standard protocol [9,10].

Brine Shrimp Lethality Test (BST)
Fractions obtained were evaluated for lethality to brine shrimp using standard methods [11,12]. In this test a drop of DMSO was added to vials of the test and control substances to enhance the solubility of test materials.

Antimicrobial Assay
The antibacterial and antifungal activities of the plants crude extracts were determined according to the methods described by [13].

RESULTS AND DISCUSSION

The results of phytochemical analysis of the extracts (see Table 1) indicate that alkaloids were detected in the methanol extracts of the screened plants except that of *P. kotchyii*. Flavonoids were detected in ethyl acetate and n-hexane extracts of *A. albida* and *N. laevis* as well as methanol and n-hexane extracts of *T. indica* and *U. chamae* respectively. Flavonoids have been reported to possess anti-inflammatory, oestrogenic, antimicrobial, antiallergic, antioxidant, vascular and cytotoxic antitumour activities as well as enzyme inhibition [14]. Steroids were detected in methanol and ethyl acetate extracts of *C. arereh, P. kotchyii* and *T. indica*. They were also present in methanol extract of *U. chamae*. Glycosides were detected in methanol and ethyl acetate extracts of *U. chamae, C. arereh* and *T. indica*. They were also detected in methanol and ethyl acetate extracts of *N. laevis* and *P. kotchyii* respectively. However, they were absent in the extracts of *A. albida*. Cardiac glycosides were present in the extracts of *T. indica* and methanol and ethyl acetate extracts of *C. arereh, A. albida,* and *P. kotchyii*. They were also present in methanol and n-hexane fractions of *U. chamae*. Anthraquinones were detected in methanol extracts of *U. chamae* and *C. arereh* as well as ethyl acetate extracts of *P. kotchyii* and *T. indica*. Tannins were detected in methanol and ethyl acetate extracts of *U. chamae* and *P. kotchyii*. They were also present in methanol extracts of *C. arereh* and *A. albida*. However, they were absent in the extracts of *T. indica* and *N. laevis*. Saponins were detected in n-hexane extracts of *U. chamae* and *A. albida*, ethyl acetate and n-hexane extracts of *P. kotchyii* and ethyl acetate extract of *T.*

indica. Extracts containing alkaloids, tannins and glycosides were found to exhibit broad spectrum antibacterial activities [15,16]. Anthraquinones have been reported to have great antimicrobial properties and also provide a source of stable free radicals [1].

TABLE 1: Results of phytochemical screening of extracts.

Plant	Solvent of Extraction	1	2	3	4	5	6	7	8
U. chamae	methanol	+ +	_	+ + +	+ + +	+ + +	+++	_	+ +
	ethylacetate	+	_	_	+ +	_	+	_	_
	n-hexane	+	+	_	_	+ +	_	+	_
C. arereh	methanol	+ +	_	+ + +	+ + +	+++	+	_	+
	ethylacetate	+	_	+ + +	++ +	++	_	_	_
	n-hexane	_	_	_	_	_	_	_	_
A. albida	methanol	+ ++	_	_	_	+ +	+	_	_
	ethylacetate	+	+ +	_	_	+ +	_	_	_
	n-hexane	+	+ +	_	_	_	_	+	_
P. kotchyii	methanol	_	_	+ + +	_	+ + +	+ +	_	_
	ethylacetate	_	_	+ +	+ + +	+ + +	+	+	+ +
	n-hexane	_	_	_	_	_	_	+	_
T. indica	methanol	+ +	+ +	+ + +	+ + +	+ + +	_	_	_
	ethylacetate	_	_	+ +	+ +	+ +	_	+	+
	n-hexane	+	_	_	_	+ + +	_	_	_
N. laevis	methanol	+	_	_	+ + +	_	_	_	_
	ethylacetate	_	+ +	_	_	+	_	_	_
	n-hexane	_	+ + +	_	_	_	_	_	_

KEY:-1 = Alkaloids; 2= Flavonoids; 3= Steroids; 4= Glycoside; 5 = Cardiac glycoside;
6 = Tannins; 7 = Saponins; 8 = anthraquinones

+ + + *Present in large quantity*
+ + *Present in moderate quantity*
+ *Present in low quantity*
\- *Absent*

Table 2: Results of Brine shrimp lethality test (BST) of extracts

Plant	Solvent of Extraction	BST LC_{50} (µg/cm^3)*
U. chamae	methanol	3.3930 (11.6693/0.0675)
	ethyl acetate	54.5853 (80.8363/34.7275)
	n-hexane	106.9819 (166.2031/69.2411)
A. albida	methanol	40.6261 (72.6326/20.1397)
	ethyl acetate	114.441 (178.4152/74.2648)
	n-hexane	869.8740 (1857.1930/533.1820)
C. arereh	methanol	19.0934 (33.2836/9.1378)
	ethyl acetate	18.4889 (30.3426/10.1682)
	n-hexane	355.7308 (452.0542/125.4423)
P. kotchyii	methanol	42.4260 (71/9671/23.4678)
	ethyl acetate	63.0653 (103.9621/37.5103)
	n-hexane	3988.95 (187234/1329.60501)
T. indica	methanol	68.7815 (111.9992/41.7068)
	ethyl acetate	662.9206 (1153.4350/422.3471)
	n-hexane	3988.95 (187234/1329.60501)
N. laevis	methanol	2351.0350 (59224.6700 / 1029.5980)
	ethyl acetate	3988.95 (187234/1329.60501)
	n-hexane	3988.95 (187234/1329.60501)

**Upper/Lower Limit 95% Confidence Interval*

The cytotoxicity tests presented in Table 2 indicate that methanol and ethyl acetate extracts of the plants were generally active (BST LC_{50} 3.4 – 114.4 µg/cm^3) against the shrimp larvae. This is expected in view of the

phytochemicals detected in the plants. However, with the exception of *U. chamae* and *C.arereh* whose extracts recorded high and moderate activities respectively, all the n-hexane extracts were inactive.

The results of antimicrobial analysis are presented in Tables 3 – 5. The methanol extract of *U. chamae* exhibited very high activities (zones of inhibition 27mm and 34 mm) against *S. aureus* and *Candida albicans* respectively at the highest concentration. It also recorded a high acitivity (zone of inhibition 20 mm) against *S. pneumoniae*. These findings are in tandem with the high cytotoxicity (BST LC_{50} 3.4µg/cm^3) exhibited by the plant. Methanol extract of *C arereh* exhibited moderate activity against the bacterium, *S. pneumoniae* and the fungi *C. albicans*, *A. flavus* and *A. niger*. The methanol extracts of *A. albida* and *P. kotchyii* exhibited moderate activities against the bacteria *P. aeruginosa*, *S. aureus* and the fungus *C. albicans*. The methanol extracts of *T. indica* recorded moderate activities against *S. pneumoniae*, *P. aeruginosa* and *C. albicans*. Generally, the ethyl acetate extracts of the plants recorded low to moderate activities against some of the test organisms. Similarly, low to moderate activities were recorded by the methanol and ethyl acetate extracts of most of the plants against *E. coli*. With the exception of *U. chamae* and *A. albida* which recorded low activities against tested organisms, the n-hexane extracts were inactive.

CONCLUSION

The methanol extract of *U. chamae* was very active against the bacteria *S. pneumonia*, *S. aureus* and the fungus *C. albicans.* Constituents of the plant may be useful in the treatment of a variety of diseases caused by these agents. Further research is recommended to isolate the bioactive compounds responsible for this activity.

TABLE 3: Zone of inhibition diameter (mm) of bacterial and fungal growths in methanol extracts of plants.

Plant	Concentration (x10^2 µg/cm^3)	1	2	3	4	5	6	7
U. chamae	7	8	20	NI	27	34	16	12
	6	6	18	NI	22	33	11	10
	5	NI	15	NI	18	30	8	10
	4	NI	13	NI	18	27	NI	NI
C. arereh	7	12	18	NI	NI	12	NI	NI
	6	10	12	NI	NI	9	NI	NI
	5	10	11	NI	NI	NI	NI	NI
	4	NI	8	NI	NI	NI	NI	NI
A. albida	7	8	13	13	14	9	NI	NI
	6	8	9	10	12	7	NI	NI
	5	NI	NI	8	9	NI	NI	NI
	4	NI	NI	NI	NI	NI	NI	NI
P. kotchyii	7	13	NI	14	12	15	NI	NI
	6	10	NI	10	9	10	NI	NI
	5	8	NI	NI	NI	10	NI	NI
	4	NI	NI	NI	NI	8	NI	NI
T. indica	7	12	10	14	NI	10	NI	NI
	6	8	9	12	NI	8	NI	NI
	5	NI	7	NI	NI	NI	NI	NI
	4	NI	NI	NI	NI	NI	NI	NI
N. laevis	7	NI	7	9	NI	9	NI	NI
	6	NI	NI	7	NI	8	NI	NI
	5	NI	NI	NI	NI	NI	NI	NI
	4	NI	NI	NI	NI	NI	NI	NI

KEY: 1 = E. coli; 2 = S. pnemonae; 3 = P. aeruginosa; 4 = S. aureus; 5 = C. albicans; 6 = A. flavus; 7 = A. niger; NI = No inhibition

TABLE 4: Zone of inhibition diameter (mm) of bacterial and fungal growths in ethyl acetate extracts of plants.

Plant	Concentration ($x10^2$ µg/cm^3)	1	2	3	4	5	6	7
U. chamae	7	NI	10	8	10	10	13	NI
	6	NI	8	NI	9	9	11	NI
	5	NI	NI	NI	9	7	9	NI
	4	NI	NI	NI	6	6	8	NI
C. arereh	7	10	13	NI	NI	10	18	NI
	6	8	10	NI	NI	9	15	NI
	5	7	8	NI	NI	8	12	NI
	4	NI	6	NI	NI	7	8	NI
A. albida	7	10	6	6	9	NI	NI	NI
	6	7	NI	NI	NI	NI	NI	NI
	5	NI	NI	NI	NI	NI	NI	NI
	4	NI	NI	NI	NI	NI	NI	NI
P. kotchyii	7	10	8	10	12	NI	NI	NI
	6	8	NI	7	NI	NI	NI	NI
	5	NI	NI	NI	NI	NI	NI	NI
	4	NI	NI	NI	NI	NI	NI	NI
T. indica	7	8	7	10	8	NI	NI	NI
	6	NI	NI	8	NI	NI	NI	NI
	5	NI	NI	NI	NI	NI	NI	NI
	4	NI	NI	NI	NI	NI	NI	NI
N. laevis	7	NI	NI	NI	NI	NI	NI	NI
	6	NI	NI	NI	NI	NI	NI	NI
	5	NI	NI	NI	NI	NI	NI	NI
	4	NI	NI	NI	NI	NI	NI	NI

KEY: 1 = E. coli; 2 = S. pnemonae; 3 = P. aeruginosa; 4 = S. aureus; 5 = C. albicans; 6 = A. flavus; 7 = A. niger; NI = No inhibition

TABLE 5: Zone of inhibition diameter (mm) of bacterial and fungal growths in n-hexane extracts of plants.

Plant	Concentration ($x10^2$ µg/cm^3)	1	2	3	4	5	6	7
U. chamae	7	6	8	7	9	7	11	NI
	6	NI	NI	NI	7	6	9	NI
	5	NI	NI	NI	7	NI	7	NI
	4	NI	NI	NI	6	NI	6	NI
C. arereh	7	NI	NI	NI	NI	NI	NI	NI
	6	NI	NI	NI	NI	NI	NI	NI
	5	NI	NI	NI	NI	NI	NI	NI
	4	NI	NI	NI	NI	NI	NI	NI
A. albida	7	NI	NI	NI	NI	8	NI	NI
	6	NI	NI	NI	NI	7	NI	NI
	5	NI	NI	NI	NI	NI	NI	NI
	4	NI	NI	NI	NI	NI	NI	NI
P. kotchyii	7	NI	NI	NI	NI	NI	NI	NI
	6	NI	NI	NI	NI	NI	NI	NI
	5	NI	NI	NI	NI	NI	NI	NI
	4	NI	NI	NI	NI	NI	NI	NI
T. indica	7	NI	NI	NI	NI	NI	NI	NI
	6	NI	NI	NI	NI	NI	NI	NI
	5	NI	NI	NI	NI	NI	NI	NI
	4	NI	NI	NI	NI	NI	NI	NI
N. laevis	7	NI	NI	NI	NI	NI	NI	NI
	6	NI	NI	NI	NI	NI	NI	NI
	5	NI	NI	NI	NI	NI	NI	NI
	4	NI	NI	NI	NI	NI	NI	NI

KEY: 1 = E. coli; 2 = S. pnemonae; 3 = P. aeruginosa; 4 = S. aureus; 5 = C. albicans; 6 = A. flavus; 7 = A. niger; NI = No inhibition

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