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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *ALLIUM SATIVUM, CALOTROPIS PROCERA, ACACIA NILOTICA,* AND *MITRACARPUS SCABER* MIXED HEXANE EXTRACTS

I. Sani*, F. Bello, D. Abdul-Kadir

Department of Biochemistry, Kebbi State University of Science and Technology, Aliero, P.M.B. 1144, Birnin Kebbi, Kebbi State, Nigeria.

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***Correspondence for Author**
Ibrahim Sani
Department of Biochemistry, Kebbi State University of Science and Technology, Aliero, P.M.B. 1144, Birnin Kebbi, Kebbi State, Nigeria.

ABSTRACT

Plants remain the main sources of natural products for new therapies particularly in poor countries, because most of them are less cost, and can affect a wide range of infectious microorganisms, and another interesting reason is that, the herbal medicines have fewer adverse effects compared to the conventional ones. Therefore, plants are important sources of potentially useful substances for the development of new therapeutic agents. This work is aimed at evaluating the possibility of applying mixed hexane extracts of *Allium sativum* bulbs, *Calotropis procera* leaves, *Acacia nilotica* pods, *and Mitracarpus scaber* whole parts as antibacterial on some selected bacterial strains (*Staphylococcus aureus, Klebsilla pneumoniae* and *Streptococcus pneumoniae*). The efficacies of the mixed extracts were tested by using Agar well diffusion assay method and zones of growth inhibition were measured in millimetre (mm). Interestingly, the combination of the Four plants (synergetic effect) showed highest antimicrobial activity against the tested organisms (*Staphylococcus aureus* 6.5 ± 0.707*; Klebsilla pneumoniae* 12.5 ± 0.707; and *Streptococcus pneumoniae* 8.0 ± 1.414). Therefore, this study ascertained the value of these medicinal plants to be used as alternatives in the treatment of infections cause by the tested bacterial strains.

KEYWORDS: *Acacia nilotica* pods, *Allium sativum* bulbs, *Calotropis procera* Leaves, *Mitracarpus scaber*, Mixed hexane extracts.

INTRODUCTION

A medicinal plant is any plant in which one or more of its organ contains substances that can be used for therapeutic purposes and which are precursors for the synthesis of useful drugs. [1] In other words, Medicinal plants can be defined as the group of plants that possess some special properties or virtues that qualify them as articles of drugs and therapeutic agents and are used for medicinal purpose. [2] Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. These Chemical compounds in plants can exert antimicrobial effects on the human body through processes similar to those chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines. [3] Medicinal plants have been identified and used throughout human history. They are used locally in the treatment of various infections cause by fungi, bacteria, virus and other parasites. [4] A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compound. [5] Medicinal plant extracts offer considerable potential for the development of new agents effective against infections currently difficult to treat. [6] The plants (*Allium sativum, Calotropis procera, Acacia nilotica, and Mitracarpus scaber*) were selected due to their Ethno-medical applications in the traditional treatment of various diseases in Hausa land (north-western part of Nigeria).

MATERIALS AND METHODS

Plant Sampling and Authentication

The healthy plants, *Allium sativum, Calotropis procera, Acacia nilotica, and Mitracarpus scaber* were obtained within Birnin Kebbi Local Government Area, Kebbi State. The plants samples were identified and authenticated in the Botany Unit, Department of Biological Sciences, Kebbi State University of Science and Technology, Aliero, Kebbi State, Nigeria.

Preparation of the Plant Sample

The required parts of the plants to be used were cleaned and air-dried in a room. The dried samples were pulverized using a mortar and pestle. The ground samples were used for the preparation of the hexane extracts of the plant samples.

Clinically Isolated Bacterial Strains

The bacterial strains (*Staphylococcus aureus, Klebsilla pneumoniae* and *Streptococcus pneumoniae*) were obtained from Sir Yahaya Memorial Hospital Birnin Kebbi, Kebbi State,

Nigeria. They were identified on the basis of cultural and morphological characteristics.

Culture and Maintenance of Microorganisms

The isolates were maintained by sub-culturing them into a new prepared nutrient agar slant and stored in an incubator at 37^{o}C.

Inoculums Preparation

The Bacterial inoculums were prepared by sub-culturing of the test organisms from nutrient agar slants on another prepared nutrient agar plates and incubated at 37^{o}C for 24 hours. The pure cultures on the nutrient agar plates were used as the inoculums.

Preparation of Extracts

The hexane extracts of the plants were obtained according to a described method,[7] with slight modifications. 100g of each dried powder of each plant samples was mixed with 400 ml of n-hexane. The mixture was gently stirred, tightly covered with cotton wools and foiled, then allowed to stand for 3 days at room temperature. Each extract was decanted and filtered through muslin cloth. The filtrates obtained were placed in a water bath and allowed to stand to evaporate the solvent. The residues obtained were used for the phytochemical screening and their mixtures were used for testing the antibacterial activities.

Qualitative Phytochemical Screening: Phytochemical analyses were carried out using standard methods. [8, 9]

Test for Saponins

To 2 ml of extract, 2 ml of distilled water was added to the test tube and shaken vigorously for about 5 minutes. Formation of foam which persisted for 10 minutes indicates the presence of saponins.

Test for Flavonoids

2 ml of extract was treated with few drops of 10% sodium hydroxide solution. The formation of intense yellow colour, which become colourless upon addition of dilute hydrochloric acid indicates the presence of flavonoids.

Test for Alkaloids

To 2 ml of extract, few drops of Dragendroff's reagent (solution of Potassium Bismuth Iodide) were added. The formation of orange brown precipitate indicates the presence of alkaloids.

Test for Tannins

To 2 ml of extract, 3 drops of 0.1% ferric chloride ware added. The formation of bluish black colour indicates the presence of tannins.

Test for Phenols

2 ml of extract was treated with 3 drops of ferric chloride solution. The formation of brownish solution indicates the presence of phenols.

Test for Terpenoids

5 ml of extract was mixed with 2 ml of chloroform and then, 3 ml of concentrated sulphuric acid was carefully added to form a layer. A reddish brown coloration at the interface indicates the presence of the terpenoids.

Tests for Antibacterial Efficacy

The Agar well diffusion method. [10] Was used to determine the antibacterial activity of the mixed plant extracts. The Mueller-Hinton agar media were prepared according to a reported method. [11] The synergetic actions of the mixed extracts were tested against the three bacterial strains using a described method. [12] The three combinations were *Cp/As* (*C. procera* and *A. sativum)*, *Ms/An* (*M. scaber* and *A. nilotica*), and final combination was the mixture of all the four plant extracts (*Cp/As/Ms/An*). The concentration of each combination was 100 mg/ml. Gentamycin (50 mg/ml) was used as positive control. The cultures were incubated at 37^{O}C for 24 hours. The zones of inhibition around the wells were measured in millimetres (mm) as an indication of the antibacterial activity.

RESULTS

The results of the phytochemical screening of each plant are presented in Table 1 while those of the antibacterial activities are presented in Table 2.

Table 1: Phytochemical Screening of each Plant Extract.

Phytochemicals	Observations/Results			
	A. nilotica	***M. scaber***	***A. sativum***	***C. procera***
Terpenoids	++	+	+	++
Flavonoids	+	+	+	+
Tanins	++	+	-	-
Saponins	+	+	+	-
Alkaloids	+	-	+	-
Phenols	+	+	-	+

+ = present, ++ = moderately present, +++ = highly present, − = not detected.

Table 2: Synergetic (Combined) Antibacterial effect of the Plant Extracts.

Bacterial Strain	Zone of Inhibition (mm)			
	As/Cp	***Ms/An***	***As/Cp/Ms/An***	**GEN (50mg/ml)**
K. pneumoniae	6.0 ± 1.414	12.0 ± 1.414	12.5 ± 0.707	11.0 ± 1.414
S. aureus	2.5 ± 0.707	4.0 ± 2.828	6.5 ± 0.707	21.0 ± 0.707
S. pneumoniae	1.5 ± 0.121	7.5 ± 2.121	8.0 ± 1.414	4.5 ± 0.364

Values are presented as mean ± standard deviation of triplicates. GEN (Gentamycin) was use as a positive control. ***Ms*** = *Mitracarpus scaber*, ***An*** = *Accacia nilotica*, ***As*** = *Allium sativum*, ***Cp*** = *Calotropis procera*. The concentration of each combined mixture was 100mg/ml.

DISCUSSION

Bacterial infectious diseases represent an important cause of morbidity and mortality worldwide, and the problem of microbial resistance is growing and the outlook for the use of synthetic commercial antibacterial drugs in the future is still uncertain. The search for antibacterials from natural sources has received much attention and efforts have been put in to identifying compounds that can act as suitable to replace the synthetic ones.

Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of microorganism. These compounds have significant therapeutic applications against human pathogens including bacteria, fungi or virus. [13] Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activities as well as for the discovery of new antimicrobial compounds. So, a return to natural substances is an absolute need of our time.

The first step towards this goal is the *in vitro* antibacterial activity assay. Many reports are available on the antibacterial properties of these plants. Some of these observations would help in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings. However, not many reports are available on the plants for developing commercial drug for applications to overcome antibiotic resistant in the resistant strains. Hence, in this investigation, the hexane extracts of *A. nilotica*, *Allium sativum*, *M. scaber* and *C. procera* were evaluated for exploration of their antimicrobial activity against certain Gram negative (*Klebsilla*) and Gram positive bacteria (*S. aureus* and *Streptococcus*), which were regarded as human pathogenic bacteria that are resistant to many antibiotic drugs. The phytochemical screening was conducted to test the presence of some terpenoids, flavonoids, tannins, saponins, alkaloids and phenols. These metabolites are reported to have antibacterial activities in their pure form and are responsible for the most

antibacterial activities of many plants extracts. [13, 14] The Phytochemical screening of the hexane extracts of the four plant samples, revealed the presence of terpenoids, and flavonoids in all the plant extracts as presented in Table 1. All the tested metabolites; terpenoids, flavonoids, tanins, saponins, alkaloids and phenols were detected in the hexane extract of *A. nilotica*. This result is in line with an earlier report. [15] Also in the *M. scaber*, all the tested metabolites were detected, except alkaloid, which corresponds with another reported result. [16] In *A. sativum*, tannins and phenols were absent among the tested phytochemicals. This result is in line with another reported research. [17] The hexane extract of *C. procera* showed only the presence of terpenoids, flavonoids and phenols among the tested phytochemicals. This result is in line with that reported by [18] who detected the presence of these three phytochemicals in methanolic extract of the plant.

In comparing, the contrariety of this phytochemical screening results with those reported in different literatures, it may be due to the differences in environmental conditions and geographical locations of the places where the plant materials were obtained and/or the use of different solvent (aqueous or organic solvent) and/or procedure in the extraction or method adapted for detection of the metabolites. The active principles identified in this study exhibited antimicrobial activity against all the test organisms. Several plants, which are rich in alkaloids, tannins and flavonoids, etc., have been shown to possess antimicrobial activity against a number of microorganisms. Therefore, the antibacterial activities of the plants extract were attributed to the presence of these active metabolites.

The combined actions (synergetic effect) of the tested plant extracts against the three (3) bacterial species are presented in Table 2. The mixture of the four plant extracts, (*As/Cp/Ms/An*) gave the highest antibacterial activity than the other mixtures. The highest zone of inhibition was on *K. pneumoniae* (12.5 ± 0.707 mm) which was exhibited by the mixture of the four (4) plants (*As/Cp/Ms/An*). Then, the least was on *S. pneumoniae* with zone of inhibition of (1.5 ± 0.121 mm)exhibited by the combination of the two plant extracts (*As/Cp*). The synergic action obtained between the plant extracts when tested in combinations, may be due to an increase in the concentration of the most bioactive metabolites in the mixture or due to the combined effect of two (2) or more different bioactive metabolites. This result was supported by earlier reported results. [12]

CONCLUSION

This research has shown that, the n-hexane solvent extraction was suitable to verify the antibacterial properties of these medicinal plants. Phytochemical analysis showed that, the antibacterial activities of the plant extracts ware due to the presence of the phytochemical compounds such as alkaloids, tannins, saponins, flavonoid, phenolic compounds etc. These plants have very promising antibacterial activities and thus can be used traditionally to cure various infectious diseases cause by the tested bacterial species, and could serve as useful source of new antibacterial agents.

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