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Evaluation of the Phytochemical and Antibacterial Activity of Some Plants Against Some Pathogenic Bacteria

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Abstract

The aim of this study was to evaluate the antibacterial activity of *Citrus aurantium, Sclerocarya birrea and Tamarindus indica* against the selected pathogenic bacteria. The ethanolic and aqueous leaves extracts of *Citrus aurantium, Sclerocarya birrea and Tamarindus indica* were screened for the presence of some phytochemicals and antibacterial activity against *Bacillus subtilis, Escherichia coli, Salmonella dysenteriae, Salmonella paratyphi, Staphylococcus aureus and Streptococcus faecalis.* The phytochemicals were analyzed using the standard methods of phytochemical analysis, while the antibacterial activities were analysed using agar well diffusion method. The results indicated presence of tannins, saponins, flavonoids, alkaloids and cardiac glycosides in the two extracts. The results revealed that the two extracts possess broader antibacterial activities against the tested organisms at a concentration of 100 mg/ml when compared with the standard antibiotic ciprofloxacin as positive control. However, ethanolic extract demonstrated a better activity than the aqueous extract with the most susceptible organism being *Bacillus subtilis*, while the minimum antibacterial activity was reported for *Staphylococcus aureus.* The ethanol leaves extracts of the three plantsmay therefore provide a good target for drug discovery.

Keywords

Citrus Aurantium, Phytochemicals, *Sclerocarya birrea*, *Tamarindus indica*, Antibacterial Activity and Tannins

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1. Introduction

Plants and their extracts are used for centuries in traditional African medicinal practice to treat diseases and infections. This practice was known for centuries all over the world. Traditional medicinal practitioners in Nigeria use a number of plants and herbs in ethno medicinal practice [1]. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties [2, 3].

A large number of plants with medicinal or antimicrobial activities in this country were recognised and acknowledged [4, 5]. Also, some of the active components of varied extracts of a number of these plants were isolated, tested and documented [6, 7].

The present scenario of emergence and spread of resistance to current antibiotics by human pathogenic organisms has necessitated a search for new antimicrobial substances from other sources including plants for the treatment of infections and diseases of microbial origin. Although, several plants with antimicrobial activities were identified and reported, a large number still remain to be identified.

The present work was aimed at evaluating the antibacterial activities of ethanolic and aqueous leaves extracts of *Citrus aurantium, Sclerocarya birrea* and *Tamarindus indica* against *B. subtilis, E. coli, S. dysenteriae, S. paratyphi, S. aureus* and *S. faecalis*.

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2. Materials and Methods

2.1. Plant Sample

Fresh disease-free leaves of *Citrus aurantium, Sclerocary abirrea and Tamarindus indica* were separately collected from Aliero, Kebbi State, Nigeria in July, 2011 and was identified and authenticated by a Botanist at the Biological Sciences Department, Kebbi State University of Science and Technology, Aliero, Kebbi State, Nigeria. Voucher samples were prepared and deposited in the Herbarium of the Botany Department of Kebbi State University of Science and Technology, Aliero, for reference. The samples wereair-dried, ground and keptin air-tight containers till further use.

2.2. Preparation of Plant Extracts

The ethanolic extract was prepared by soaking a sample (20g) of powdered plant material in 90% ethanol (200 ml) for 48 h. Aqueous extract was prepared by soaking a sample (20g) of powdered plant material in warm sterile water (200ml) for 48 h. At the end of the extraction, each extract was filtered using Whatman filter paper. The filtrate was concentrated in vacuum at 30°C and stored at 4°C until further use.

2.3. Text Organisms

Clinical isolates of *B. subtilis, E. coli, S. dysenteriae, S. paratyphi, S. aureus* and *S. Faecalis* were obtained from the Department of Microbiology, Faculty of Science, Kebbi State University of Science and Technology, Aliero. The bacteria were grown and maintained on Trypticase Soy agars (TSA) at 37°C and were sub-cultured once every week.

2.4. Phytochemical Screening

The two extracts were screened for the presence of major phytochemicals using standard qualitative methods as described previously [8, 9, 10]. The plant extracts were screened for the presence of saponins, tannins, alkaloids, flavonoids, terpenoids, steroids, phenols, cardiac glycosides and anthraquinones as outline below:

Test for Phenols

0.5ml of the extract, 5ml of Folin Ciocalteu reagent and 4ml of aqueous sodium carbonate were added. Appearance of blue colour indicates the presence of phenols [8, 9].

Test for Saponins

To 2ml of the extract, 2ml of distilled water was added and it was agitated in a test tube for 5minutes. The formation of foams indicates the presence of saponins [8, 10].

Test for Tannins

5 drops of 0.1% ferric chloride was added to 2ml of the extract, a brownish green or blue-black colouration indicated the presence of tannis [8].

Test for Alkaloids

To 2ml of the extract, 2ml of 10% hydrochloric acid was added. To the acidic medium, 1ml Hager's reagent (saturated picric acid solution) was added. Presence of alkaloids is confirmed by the formation of yellow colored precipitate [9].

Test for Anthraquinones

2ml of the extract was boiled with 5ml of 10% hydrochloric acid for 3 minutes. 5ml of chloroform was added. 5 drops of 10% ammonia was further added. A rose pink coloration indicates a positive result [8, 9].

Test for Glycosides

2ml of acetic acid was added to 2ml of the extract. The mixture was cooled in cold water bath. 2ml of concentrated H_2SO_4 was then added, color development from blue to bluish green indicates the presence of glycosides [8, 9].

Test for Flavonoids

2ml of 10% Sodium hydroxide was added to 2ml of the extract in a test tube. An intense yellow colour was formed which turned colourless upon addition of 2ml of dilute hydrochloric acid indicating the presence of flavonoids [8, 9].

Test for Phlobatannins

2ml of the extract were boiled with 1% aqueous hydrochloric acid. Formation of red precipitate indicates the presence of Phlobatannins [8, 9].

Test for Terpenoids

5ml of the extract was mixed in 2ml of chloroform and 3ml of concentrated sulphuric acid was carefully added to form a layer. A reddish brown colouration at the interface indicates presence of terpenoids [8, 9].

Test for Steroids

2ml of extract were dissolved in 10ml of chloroform and then 10ml of concentrated sulphuric acid was added by the side of the test tube. The upper layer turned red whereas the sulphuric acid layer turned yellow with green fluorescence. This indicates the presence of steroids [8, 9].

2.5. Antibacterial Activity Assay

Agar well diffusion method was employed to assay for the antibacterial activity [11]. The test organisms (bacterial isolates) were first grown in already prepared nutrient agar for 15 hours. The inoculum solutions were standardized and then the antibacterial activities of the two plant extracts tested at a concentration of 100 mg/ml each in the medium. The plates were later incubated at 37°C for 24 h after which zone of inhibition (diameter) formed was determined as an

indication of antibacterial activity. These effects were compared with that of the standard antibiotic ciprofloxacin at a concentration of 1 mg/ml.

3. Results

The result for the phytochemicals analysis of both the ethanolic and aqueous leaves extracts of *Citrus aurantium, Sclerocarya birrea and Tamarindus indica* is shown in table 1. While table 2 represents the results for the antibacterial activity of the leaves extracts of *Citrus aurantium, Sclerocarya birrea and Tamarindus indica* against the test bacteria.

Table 1. Phytochemical constituents present in the ethanol and aqueous leaves extracts of *Citrus aurantium, Sclerocarya birrea and Tamarindus indica.*

Plant extracts						
Phytochemical	***Citrus aurantium***		***Sclerocaryabirrea***		***Tamarindusindica***	
	Ethanol	**Aqueous**	**Ethanol**	**Aqueous**	**Ethanol**	**Aqueous**
Tannins	++	+	+	+	++	+
Saponins	+++	+	++	+	++	++
Flavonoids	+++	++	++	++	+	++
Alkaloids	++	+	+	+	++	+
Steroids	+	+	+++	-	+	+
Terpenoids	+	-	+	-	++	-
Phenols	++	+	+	+	++	-
Anthraquinones	-	++	+	+	+	+
Cardiac glycoside	+	+	+	++	++	+

Note: +: slightly present; ++: moderately present; +++: highly present; -: not detected.

Table 2. Zone of inhibition (mm) of the solvent extracts (100mg/ml) of the leaves of *Citrus aurantium, Sclerocarya birrea and Tamarindus indica* against tested bacteria.

Test bacteria	**Inhibition zone (mm)**						
	Citrus aurantium		***Sclerocaryabirrea***		***Tamarindusindica***		**Ciprox.**
	E	**W**	**E**	**W**	**E**	**W**	
B. subtilis	23±0.2	14±0.6	15±0.5	11±3.3	20±0.7	21±1.1	26±0.6
E. coli	17±1.2	11±3.6	12±2.8	0±0.0	16±1.7	10±0.3	11±2.1
S. dysenteriae	21±2.2	17±2.3	16±1.9	14±1.3	19±0.2	18±0.6	23±1.2
S. paratyphi	19±0.5	7±2.8	19±2.2	15±0.6	22±1.8	20±1.4	21±0.3
S. aureus	15±0.9	4±0.3	0±0.0	0±0.0	17±2.4	3±1.3	10±0.5
S. faecalis	19±2.1	15±2.0	10±1.8	0±0.0	23±0.4	20±0.9	22±1.2

Note: Values are presented as mean ± standard deviation of three replicates.
Key: E = ethanol extract, W = aqueous extract, Ciprox = ciprofloxacin

4. Discussion

The result for the phytochemical analysis is presented on table 1. The result revealed the presence of different phytochemicals that slightly varied with the solvent used for the extraction. The results show the presence of saponins, tannins, alkaloids, flavonoids, and cardiac glycosides in both the two extracts of leaves of *Citrus aurantium, Sclerocarya birrea and Tamarindus indica*. Anthraquinones and terpenoids were not found in the ethanolic and aqueous leaves extracts of *Citrus aurantium* respectively. Also, terpenoids and steroids were not detected in the aqueous leaves extract of *Sclerocarya birrea*. While terpenoids and phenols were not detected in the aqueous leaves extract of *Tamarindus indica.* This might be as a result of different extraction abilities of varied solvents [12]. The phytochemicals might have been better extracted with ethanol than with water as a solvent of extraction.

The antibacterial activity of the leaves extracts of *Citrus aurantium, Sclerocarya birrea and Tamarindus indica* is presented on table 2. In this study the results showed that the two leaves extracts of the three plants possess wider antimicrobial activities against the tested organisms at a concentration of 100 mg/ml (Table 2). The two leaves extractscompared favourably with the standard antibiotic ciprofloxacin control. However, the ethanolic leaves extract demonstrated a better activity than the aqueous leaves extract with the most susceptible organism being *Bacillus subtilis* and the minimum antibacterial activity was reported for

Staphylococcus aureus. The present study compares favourably with that of the antibacterial activity of the methanolic extract of *P. guajava* on *B. subtilis*, *S. aureus, E. coli* and *P. aeruginosa* [13]. The study is also in agreement with that of Antimicrobial and phytochemical studies on 45 Indianmedicinal plants against multi-drug resistant human pathogens [14].

The presence of alkaloids, saponins, terpenoids and tannins in the leaves extracts of *Citrus aurantium, Sclerocarya birrea and Tamarindus indica* has medicinal implications. These phytochemicals are known to be biologically active. The presence of tannins was found to play a role in antifungal, antibacterial, astringent and antibiotic activities [15, 16]. Tannins were also found to form irreversible complexes with proline-rich proteins [17] resulting in the inhibition of the cell protein synthesis.

In addition to antimicrobial activity exhibited by tannins, they also react and form complex with proteins to provide the typical tanning effect. This is important medicinally for the treatment of inflamed or ulcerated tissues [18]. Tannins-containing herbs as their main component are astringent in nature and are used in the treatment of intestinal disorders such as diarrhoea and dysentery, thus exhibiting antimicrobial activity. One of the largest groups of chemical produced by plants is the alkaloids and their amazing effect on humans has led to the development of powerful pain killer medications. Terpenoids also act as antibiotics to protect plants from pathogenic microorganisms [19].

5. Conclusion

The results of the present work revealed the presence of most of the phytochemicals with various biological activities. This might be responsible for the observed antibacterial activity against test organisms. The results also indicate that both the ethanolic and aqueous extracts demonstrated wider antibacterial activities against the test organisms that compared well with the standard drug ciprofloxacin. The ethanolic leaves extracts of *Citrus aurantium, Sclerocarya birrea and Tamarindus indica* may therefore provide good target for drug discovery.

Further research is needed in the identification, isolation and characterisation of the active components responsible for the antibacterial activities.

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