Preliminary Phytochemical Screening and Antibacterial activity of Medicinal Plant: COCHLOSPERMUM TINCTORIUM A. RICH

A.O. ABDULSALAAM¹, DR. M.M. IDRIS² AND DR. O.W. SALAWU³.
¹&²DEPARTMENT OF CHEMISTRY, KOGI STATE UNIVERSITY, P.M.B. 1008, ANYIGBA - KOGI STATE OF NIGERIA.
&
²DEPARTMENT OF PURE AND INDUSTRIAL CHEMISTRY, BAYERO UNIVERSITY, KANO - NIGERIA.
E-mail: Absal24@yahoo.com

ABSTRACT

The crude extracts from leaf of Cochlospermum tinctorium in different solvents were subjected to phytochemical screening and antimicrobial activity against Escherichia coli, Pseudomonas, Proteus, Klebsiella and Staphylococci. The ethanol extract was used for phytochemical screening. Hexane, chloroform, ethylacetate and ethanol extract were used for antimicrobial activity. Phytochemical studies indicate the presence of carbohydrate, simple sugar, glycoside, flavanoid, steroid, saponin, Tannin and Resin. Ethanol extract exhibited significant to moderate activity at concentrations of 500µg/ml and 1000µg/ml respectively against all the tested microorganisms. Chloroform and ethyl acetate extracts showed moderate activities against E.coli, P.auregenosa, K.pneumonae and Proteus at 1000µg/ml.

Key words:Cochlospermum tinctorium, Phytochemicals, Antimicrobial

INTRODUCTION

Traditional medical option still holds a strong part in the public health delivery system of many communities in the world today[1]. For reasons of safety, effectiveness, stability, standardized dosage forms, isolation, characterization and identification of drugs of plant origin, phytochemical investigation becomes an urgent priority. Plants extracts from plants such as Actotis actotoides have been shown to inhibit the growth of bacteria and fungi[2]. Andrographis paniculata contained anti cancer and immune stimulatory compounds[3]. Artemisinin isolated from Artemisia annua L. have been used to cure resistant malaria parasite. The success recorded in the use of isolates from plant in curing resistant parasite, virus and bacteria infections has further focused attention on plants as source of drugs[4,5].

Plant Morphology

Cochlospermum tinctorium A. Rich (Cochlospermaceae) is a shrub that can grow up to 10m high. The slash is iodine-like in colour. Leaves are alternate, palmately lobed with stipules. Inflorescence consists of brightly coloured yellow flowers that are regular and borne in racemes or panicles. Fruits are elongated, 3-5 valve, capsules containing seeds that are embedded in cotton foam. The seeds are bean-shaped with brown to black colour. It contains oily endosperm with broad cotyledon. It is a savannah plant commonly found on fallow farm lands [6,7]

Ethnomedical Uses

The bark, root, seeds are used in the treatment of various ailments in different areas around the world. In Nigeria, a decoction of the root is used for treating gonorrhoea. It is used in the treatment of diabetes by the Igede people of Benue State [8,9].
The leaves are used in the treatment of malaria fever in some parts of Kogi State. In Mali, the plant is variously used against jaundice, abdominal pains, haemorrhoids, intestinal worms, helminth, bilharzia and hepatitis. It was also reported to have been used against gastrointestinal diseases like ulcer, stomach ache, flatulence and constipation [10]. The leaf pulp is used in wet dressing of wound to mature abscesses and the rhizome is used as antifungals and antibacterials [11,12].

In Ivory Coast, the use of the plant for oedematous conditions, orchites, schistosomiasis, epilepsy, pneumonia, intercostal pains, brochial infections, indigestion and in eye instillations for conjunctivitis were reported [13].

Other Uses
The bark is used as rope and as jute. Edible oil is obtained from the seed and cotton from its fruits and the yellow root used as a dye and in cooking [14].

General procedure
The solvent procured from local suppliers were redistilled prior to use. Phytochemical screening was carried out on washed and dried test tubes. The plants extracts were suspended in DMSO in test tube/vials. In vitro nutrients Agar antimicrobial assay was carried out on culture plates containing the organisms.

Plant collection
The leaves of Cochlospermum tinctorium A.Rich were collected from Ajaokuta L.G.A., Kogi State, where the natives used it to cure various ailments. The sample was identified by Baba Ali Garko and authenticated by Dr.B.S. Aliyu of the Department of Biological Sciences, Bayero University, Kano-Nigeria. The sample was air-dried in the laboratory for three weeks, crushed and ground to powder.

Extraction of plant material
In a screening program, the air-dried powdered sample (500g) was extracted using four(4) different solvents, sequentially arranged in order of their increasing polarity: Hexane, Chloroform, Ethylacetate and ethanol[15].

Each solvent was allowed to extract for a period of two weeks. At subsequent stages, the residue is air-dried before extracting with the next solvent. The extracted material were concentrated under reduced pressure using Rotar vapour(R110) at 40°c. Concentrated crude extracts were transferred into a clean weighed beaker and allowed for complete evaporation under room conditions. Weights of the crude extracts were measured and each of the solvent extracts coded as F_{hex}01, F_{chl}01, F_{ethyl}01, F_{ethyl}01 respectively.

PHYTOCHEMICAL SCREENING.

The crude ethanol extract was used for the phytochemical analysis, the extract was screened for the presence of the following components; carbohydrates, reducing sugars, flavonoids, alkaloids, steroids, glycosides, saponins, tannins and resins using standard methods[16,17].

COLLECTION OF MICROORGANISMS

In this antimicrobial screening only some selected bacteria were used. These bacteria are stock culture collection of microbiology unit of the department of biological sciences, Bayero University, Kano. They are; Escherichia coli, Pseudomonas, Proteus, Klebsiella and Staphylococci. Stock cultures were maintained on Nutrient Agar (Oxoid) at 4°c in refrigerator in accordance with standard specification[18,19]. They were identified to specie level through biochemical test, serology and growing them in differential and selective media[20].
PREPARATION OF EXTRACTS

Each of the (0.005g) was dissolved in sterile dimethylsulphoxide (DMSO)(1 ml). Thus, a concentration of 5000 µg/ml was obtained as stock for each of the extracts. Subsequent concentrations of 100µg/ml, 500µg/ml and 1000µg/ml were prepared from the stock, using dilution formula;

\[ C_1 V_1 = C_2 V_2 \]

Where \( C_1 = \) Concentration of stock solution, \( C_2 = \) Concentration of desired solution
\( V_1 = \) Unknown volume to be drawn from stock solution,
\( V_2 = \) Volume of desired concentration (\( C_2 \)) which is taken to be 1ml

a. 100µg/ml = 0.2ml of stock solution in 0.8ml of DMSO
b. 500µg/ml = 0.2ml of stock solution in 0.98ml of DMSO
These concentrations were labelled and stored in refrigerator at 4°C for further use.

PREPARATION OF SENSITIVITY DISCS

The discs were prepared in the laboratory using What man No.2 filter papers. This was achieved by punching the filter paper using a standard punching machine of diameter 6.25±0.1mm. The filter discs were then sterilized using autoclaving machine at a pressure of 20lb and temperature of 127°C for 30 minutes. The discs were then impregnated with the different concentrations of the prepared extracts, followed by drying in an incubator at 37°C for 60 minutes.

SENSITIVITY TEST

The sensitivity test was carried out using Nutrient Agar for the growth of the entire microorganisms. The test organisms were directly inoculated on the nutrient agar by direct streaking method, using a sterile wire loop. Discs of various concentrations of the extracts prepared were then placed on labelled plates containing the inoculums with the aid of sterile forceps. Control discs impregnated with DMSO only were placed at the centre of each of the petri-dishes. A pre dilution time of about 3-5 minutes was allowed for the test extracts to diffuse into each medium. The Petri-dishes were then incubated at 35°C for 24 hours. The degree of sensitivity was determined by measuring the visible zones of inhibition of the bacteria growth produced by the diffusion of extracts from the discs into the surrounding medium. The organism is then reported as ‘Resistant’, Intermediate/moderately sensitive and 'Sensitive'(susceptible) [21].

RESULTS AND DISCUSSIONS

TABLE 1: Solvent used and weight of extracts of the leaves of Cochlospermum tinctorium

<table>
<thead>
<tr>
<th>S/NO.</th>
<th>SOLVENT USED</th>
<th>WEIGHT OF EXTRACTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Hexane(FHex01)</td>
<td>2.7g</td>
</tr>
<tr>
<td>2.</td>
<td>Chloroform(FChl01)</td>
<td>1.8g</td>
</tr>
<tr>
<td>3.</td>
<td>Ethylacetate(FEthyl01)</td>
<td>5.0g</td>
</tr>
<tr>
<td>4.</td>
<td>Ethanol(FEth01)</td>
<td>10.8g</td>
</tr>
</tbody>
</table>
TABLE 2: Result of Phytochemical screening of crude F_Eth01 extract from the leaves of Cochlospermum tinctorium

<table>
<thead>
<tr>
<th>S/NO.</th>
<th>CONSTITUENTS TESTED</th>
<th>INFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Simple Sugar</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Glycoside</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Flavanoid</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Alkaloid</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Saponin</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>Tannin</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Resin</td>
<td>+</td>
</tr>
</tbody>
</table>

Key:
+ Indicates the presence of the tested constituent.
- Indicates the absence of the tested constituent.

Phytochemical Screening
Phytochemical screening on the F_Eth01 was carried out to detect the following constituents: Carbohydrates, Simple Sugar, Glycoside, Flavanoid, Alkanoid, Steroid, Saponin, Tannin and Resin. The result indicated the presence of all the tested natural products except Alkaloid. (Table 2).

Table 3: Result of antimicrobial bioassay of crude solvent extracts from the leaves of Cochlospermum tinctorium

<table>
<thead>
<tr>
<th>S/NO</th>
<th>Extract</th>
<th>Concentration (µg/ml)</th>
<th>Staph.</th>
<th>E. Coli</th>
<th>P. aureg.</th>
<th>K. Pneu.</th>
<th>Proteus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Hexane</td>
<td>1000</td>
<td>07</td>
<td>07</td>
<td>10</td>
<td>7.5</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>00</td>
<td>07</td>
<td>00</td>
<td>00</td>
<td>8.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>7.1</td>
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<tr>
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<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>2.</td>
<td>Chloroform</td>
<td>1000</td>
<td>07</td>
<td>08</td>
<td>9.2</td>
<td>08</td>
<td>08</td>
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<td></td>
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<tr>
<td>3.</td>
<td>Ethylacetate</td>
<td>1000</td>
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<td>00</td>
<td>8.4</td>
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<td></td>
<td>100</td>
<td>07</td>
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<td></td>
<td>Control</td>
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<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>4.</td>
<td>Ethanol</td>
<td>1000</td>
<td>11</td>
<td>10</td>
<td>10</td>
<td>12.5</td>
<td>11.7</td>
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<tr>
<td></td>
<td></td>
<td>500</td>
<td>09</td>
<td>09</td>
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<td>00</td>
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Key:
0-7mm zone of inhibition means resistance
8-9mm zone of inhibition means moderate
≥10mm zone of inhibition means sensitive

Antimicrobial Bioassay
Antimicrobial bioassay was carried out on the crude solvent extracts of the leaves of Cochlospermum tinctorium (Table 3).
The results showed that Ethanol extracts had sensitive activity against all the isolates at all concentration except for concentration of 500 µg/ml and 100 µg/ml against K. Pneumonia. The Ethanol extract can be used to cure the infections caused by:

i. K. Pneumonia (urinary and respiratory tract infections, necrosis, inflammations, wound infections, pneumonia, thrombophlebitis, cholecystitis, diarrhoea, osteomyelitis, meningitis, endocarditis, endophthalmitis) at concentration of 1000 µg/ml.

ii. Proteus (urinary tract infections, skin infections, diarrhoea, gastrointestinal infections) at concentration of 1000 µg/ml and 500 µg/ml.

iii. Staphylococcus aureus (skin and soft tissue infection, pneumonia, urinary tract infections and mastitis) at concentration of 1000 µg/ml and 500 µg/ml.

iv. P. aureginosa (respiratory infection, meningitis and brain abscess, external otitis, endocarditis, keratitis, sclera abscess, endophthalmitis, ophthalmia, orbital cellulites, neonatorium in children, diarrhoea, bone and joint infection, panophthalmitis, suppurative thrombophlebitis, abdominal pain, eczema gangrenosum and pastular lesion) at concentration of 1000 µg/ml and 500 µg/ml.

v. E. Coli (abdominal ceamp, diarrhoea, haemolytic uremic syndrome (HUS) at concentration of 1000 µg/ml and 500 µg/ml.

Hexane extract also exhibited significant inhibition against P. Aureginosa and Proteus at concentration of 1000 µg/ml.

The chloroform extract exhibited moderate activity against P. Aureginosa, K. Pneumonia and Proteus at concentration of 1000 µg/ml. These extracts can be used to cure infections caused by the microorganisms.

Conclusion:

This study has been a contribution to the assessment of possible antimicrobial compounds from the leaves of Cochlospermum tinctorium. The crude Ethanol extract has sensitive activity against infectious microorganisms. Generally, the plant extracts showed broad spectrum of antimicrobial activities. These provide a support for the use of the plant in treatment of various antimicrobial infections in different places around Africa and Nigeria in particular.

Recommendation:

On the basis of this study, it is recommended that further and detailed pharmacognostic studies be intensified on the extracts from these plants, so that its potential chemotherapeutic, insecticidal and other economic values could be harnessed for human benefit.

REFERENCES


