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Determination of Phytochemical and Antimicrobial Activity of Ethanolic Extract of the Leaves of *Cochorus Capsularis* (Lalo) on some Selected Bacterial Spcies.

[1]Mohammed, A.,[2]Kocifa A M. and [3]Abubakar I.,
[1,2,3]Department of Biomedical and pharmaceutical technology, Federal Polytechnic, Mubi Adamawa State Nigeria
Corresponding author Email: aliyumohammed1234@gmail.com

ABSTRACT
The studies on the Phytochemial and Antibacterial activity of the ethanolic extract of the leaves of Cochorus capsularis was carried out the leaves of the plant collected between the Months of August and October 2019. Results obtained showed the presence of flavonoids, steroids, terpenoids and phlabotannins, while alkaloids,saponins, tannins and anthroquinones were absent. Antibacterial activity of various (40, 20, 10, 5, 2.5, 1.25 and0.625) concentrations of the extract showed no zone of inhibition from 40mg/ml to 10mg/ml concentrations on the test bacteria. Klebsiella and Staphylococcus species had zones of inhibition of 1.12 and 1.90 respectively at 5mg/ml concentrations of the extract. Minimum inhibitory concentration had Bacillus, Pseudomonas species and Escherichia coli at 2.5mg/ml, while, Staphylococcus and Klebsiella species at 5mg/ml concentration of the Cochorus capsularis. The antibacterial activity may be attributed to the presence of phytochemical components
Keywords: Antimicrobial, Sensitivity, Phytochemical, Bacteria, Jute

INTRODUCTION

Corcorus capsularis (lalo in Hausa), Ewedu inYoruba and white long jute in English, it is one of the main cash crops grown in Bangladash for its fibre content. The genius cochorus was first described by Limneaus in his work species plantarum in 1753 [1,2]. It is derived from the ancient greek word (Korkhoros or Korkoros) which referred to wild plant of uncertain identity, possibly Jutes or wild as paragus [3,4,5,6]. In the recent years, research on medicinal plants has attracted a lot of attention globally [7,8]. Large body of evidence has accumulated to demonstrate the promising potential of medicinal plants used in various traditional, complementary and alternate system of treatment of human diseases [9]. Medicinal plants are those that contain one or more of its phytochemical constituents that can be used for the synthesis of useful therapeutic agent [10].Medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi synthesis. Such a plant will have its parts including leaves, roots, rhizomes, stems, barks containing chemical components that are medically active [11]. These non-nutrient plant chemical compounds or bioactive components are often referred to as phytochemicals („phyto-„from Greek - *phyto* meaning „plant") or phytoconstituents.The wide range of medicinal plants like flowers, leaves, barks, stems, fruits and roots extract have been processed for variety of pharmacological activities [12] It was observed that *C. capsularis* is rich in beta-carotene for good eye sight, iron for healthy RBCs, calcium for strong bones and teeth and vitamin C, for smooth and clear skin, strong immune cells and fast wound healing. Antioxidants from Jute leaves have been implicated with protection from chronic diseases such as diabetes, heart diseases and hypertension. In Africa, today, up to 80% of the population uses traditional medicine in primary health care [13]. The beneficial medicinal effects of plant material typically result from the

secondary metabolites such as alkaloids, flavonoids, steroids, tannins, phenol, etc. which is capable for producing definite physiological action on the body [13]. Current problems associated with the use of antibiotics, increased prevalence of multiple-drug resistant (MDR) strains of a number of pathogenic bacteria such as methicillin resistant *Staphylococcus aureus, Helicobacter pylori,* and MDR *Klebsiela pneumonia* has revived the interest in plants with antimicrobial properties. In addition, the increase in cases of opportunistic infections and the advent of Corona virus (Covid-19),Acquired Immune Deficiency Syndrome (AIDS) patients and individuals on immunosuppressive chemotherapy, toxicity of many antifungal and antiviral drugs has imposed pressure on the scientific community and pharmaceutical companies to search alternative and novel drug sources.

Materials and Methods

Sample Collection

Plant samples were collected at a school farm in Federal Polytechnic Mubi in the month of July, 2020 and taken to the Botany Department of Adamawa State University Mubi for taxonomical identification. Freshly collected*Corhcorus capsulains*(Lalo)leaves were dried at room temperature, grinded to powder using well sterilized pestle and motar. The dried powder was stored in an air tight sterilized container for further analysis. Microbial test samples including*Pseudomonas* spp*Escherichia coli,Staphylococcus* spp*, Klebsiella* sppand *Bacillus* spp were collected at the General Hospital Mubi Diagnostic Laboratory. Biochemical tests such as sugar fermentation, methyl red, voges-prokuer test, indole , catalase, oxidase tests and grams staining reactions were carried out to further confirm the identity of the bacterial isolates as described by Cheesbrough, 2006 and also Owuama, 2015.

Metabolic Extraction

This was based as described the Association of Official Analytical Chemists [1,4] in which Ethanol was selected as the solvent for extraction of leaves of *C.capsularis*.The powdered leaf of *C. capsularis*,was soaked in methanol for 4 days and filtered by using whattman numer 1 filter paper It was stirred with a clean glass rod to ensure the maximum amounts of constituents present in the blended leaves become soluble into ethanol. To prevent the evaporation of the solvent and volatile compounds from the medium, the beaker was covered by aluminium foil. The extract was then concentrated with a rotary evaporator and phytochemical constituents were determinedusing standard procedure (terpenoids, tannins, flavonoids, saponins, phlobatannins, staroids,anthraquinones and alkaloids).

Phytochemical Screening

This was based on the standard methods described [1]. Five (5) ml of ethanolic extract was mixed with 2ml of Chloroform ($CHCl_3$) in a test tube. 3mls of concentrated H_2SO_4 was carefully added to the mixture to form a layer. A reddish brown coloration of the interface shows positive test for the presence of terpenoids. Zero point five (0.5mls) of the ethanolic extractr in a test tube and 0.1% of $FeCl_3$ was added and observed for brownish green or a blue black coloration, which shows the presence of tannins. A 1% NH_3 solution was added to 0.2mls of ethanolic extract be supplementary to the aqueous extract in a test tube. A yellow color indicates the presence of flavonoids.About 2mls of the extract was treated in a test tube with 10ml of 1%HCl for 30 min in a water bath. The suspension was filtered into a test tube using cotton and divided. Five drops of Mayer's reagent was added. Appearance of whitish opalescence indicates the presence of alkaloids. To 0.5mls of chloroform in a test tube, and 2mls of the extract, 1mls of concentrated sulphuric acid was added to form a lower layer. A reddish brown interface indicates the presence of steroids. Phlbatanins and

anthraquinines were also detected using methods described by AOAC, 1990.

Method for antibacterial activity

The agar well diffusion method was employed to assay the plant extracts for antimicrobial activity. Petri dishes were plated, Muller Hinton agar and prepared according to the manufacturer's instructions and were allowed for 45 minutes to solidify. The test organisms were then spread on the surface of the media using a sterile wire loop. Cork borer (3mm in diameter) was used to bore wells in the media. . The different concentration of the plant extracts were dispensed into the wells using a sterile pipette. These were then allowed a diffusion time of 1 hour after which it was incubated at 37^0C for 24 hours. Zones of inhibition were measured using a Vernier calipper and the mean recorded in millimeter as described by Owuama, 2015.

Minimum Inhibitory Concentration (MIC)

The ethanolic extracts which showed significant zones of inhibition were chosen to assay for MIC. MIC was determined by the standard method described by [6]. Nutrient broth was prepared and sterilized using autoclave. One 1 ml of the prepared broth was dispensed in to the test tubes numbered 1-9 using sterile pipette. A stock solution containing 30mg/ml of the extract was prepared. Then 1 ml of the solution was dispensed into the tubes numbered 1. Subsequently, from tube 1, serial dilution was carried out and 1 ml from tube 1 was transferred up to tube number 7 and 1 ml from the tube 7 was discarded. Tube 8 was used as control for sterility of the medium and tube 9 for viability of the organisms. An overnight culture (inoculums) of each of the test isolates were prepared in sterile nutrient broth. 1 ml of the inoculum was transferred into each tube from tube 1 to tube 9 with exception of tube 8, to which another sterile nutrient broth was added. The final concentration of the plant extract in each of the test tubes numbered 1-7 after dilution 30, 15, 7.5, 3.75, 1.875, 0.938 and 0.469mg/ml, were incubated at 37°C for 24 h and were examined for growth. The last tube in which growth failed to occur was recorded as the MIC tube [8].

Results and Discussions

Phytochemical screening of the ethanolic extract of the leaves of *Cochorus capsularis*showed the presence of flavonoids, steroids, terpenoids and phlabotannins, while alkaloids,saponins, tannins and anthroquinones were absent as can be seen on table one(1).

Table 1 Phytochemical sSreening of Ethanolic Extracts of C. capsularis Leaves.

Phytochemical	Ethanolic extracts
Flavonoids	+
Alkaloids	-
Saponins	-
Steroids	+
Terpenoids	+
Tannins	-
Phlobatanins	+
Anthraquinones	-

Antibacterial activity of various concentrations of the extract showed no zone of inhibition frpm 40mg/ml to 10mg/ml concentrations on the test bacteria. *Klebsiella* and *Staphylococcus* species had zones of inhibition of 1.12 and 1.90 respectively at 5mg/ml concentrations of the extract (table ii. Results of minimum inhibitory concentration in (table iii) had *Bacillus*, *Pseudomonas* species and *Escherichia coli* at 2.5mg/ml, while, *Staphylococcus* and

Klebsiella species at 5mg/ml concentration of the *Cochorus capsulari*. The antibacterial activity may be attributed to the presence of phytochemical components

Table 2. Antimicrobial Activity of the Ethanolic Extract of *Cochorus capsularis* on each test bacterial isolate measured in millimeters (mm).

Leaves concentration (mg/ml)	*Pseudomonas aeruginosa*	*Klebsiella* sp*p*	*Bacillus* spp	*E. coli*	*Staph. Spp*
40.00	*0.00*	0.00	0.00	0.00	0.00
20.00	0.00	0.00	0.00	0.00	0.00
10.00	0.00	0.00	0.00	0.00	0.00
5.00	0.00	1.12	0.00	0.00	1.90
2.50	1.80	4.30	2.14	2.30	2.80
1.250	3.20	7.20	2.80	5.80	6.70
0.625	5.70	9.0	12.30	7.40	11.40

Table 3.Minimum Inhibitory Concentration (MIC) of Ethanolic Extract of *Cochorus capsularis* Leaves on Test Bacterial Isolates

Name of Bacteria	MIC (mg/ml)
Pseudomonas aeruginosa	2.50
Klebsiella sp*p*	5.00
Bacillus spp	2.50
conE. Coli	2.50
Staph. Spp	5.00

CONCLUSION AND RECOMMENDATIONS

Cochorus capsularis leaves contain some phytochemical constituents such as, flavonoids, steroids, terpenoids and phlabotannins, while alkaloids,saponins, tannins and anthroquinones were found to be absent. Antibacterial activity of various concentrations of the extract showed no zone of inhibition from 40mg/ml to 10mg/ml concentrations on the test bacteria. *Klebsiella* and *Staphylococcus* species had zones of inhibition of 1.12 and 1.90 respectively at 5mg/ml concentrations of the extract. Minimum inhibitory concentration, had*Bacillus*, *Pseudomonas* species and *Escherichia coli* at 2.5mg/ml, while, *Staphylococcu*s and *Klebsiella* species at 5mg/ml concentration of the *Cochorus capsulari*. Recommendations were made on the need to encourage the use of this beneficial plant.

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