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Antibacterial Activity of the Leaf Extract of *Alchornea Cordifolia* (Christman Bush) Against Selected Bacteria Isolates

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ABSTRACT

This research work shows the antibacterial activity of the leaf extract of Alchornea cordifolia by determining the phytochemical constituents, antibacterial activity and minimum inhibitory concentration of the leaf extract, cool method extraction was used for the preparation of aqueous extract and ethanol extract. The phytochemical constituents of Alchornea cordifolia contains Flavonoid, tannin, Alkaloid and saponin in the both extract while anthraquinone was absent in the both extract. The bacterial isolates used were Staphylococus aureus, Klebsiella pneumoniae and Escherichia coli. Agar well diffusion methods were employed to determine the zone of inhibition, and S. aureus shows the highest Zone of inhibition in the Aqueous extract which is 12.5mm, while E. coli has the highest zone of inhibition in the ethanol extract which is 11.0mm. The both extract has antibacterial activity against the test organism used. The minimum inhibitory concentration of the two extract shows zone of inhibition, the aqueous extract had larger zones of inhibition ranging from 8.4mm to 10.1mm and the lowest M.I.C values is 100% concentration. This study has justified the traditional use of Alchornea cordifolia leaf extracts in the treatment of disease caused by bacteria.

1. Introduction

Medicinal plants are plants that have been used in human disease treatment for ages because they contain compounds that possess therapeutic values. Recently, due to pathogens resistance against the available antibiotics and recognition of the traditional medicine as an alternative form of health care has reopen the research domain for the biological activities of medicinal plants (Agbor *et al.,* 2004). The increase material worth of medical treatment and their strong physiological or chemical effect, contribute to the reason why individuals make use of herbal therapy, (Sofowora 1992). In developing countries, it has been observed that the use of herbal remedy is a common practice to maintain good health (Sofowora 1992). In addition, the use of traditional medicine in developed societies have been recognized as the basic for the chemical analysis and developing of different forms of drugs, and even those used for chemotherapy from medicinal plants that are traditional used as herbal remedies Neuwinger HD (2000).

Respiratory tract infections continue to be the most frequent and important cause of short term illnesses that compel an individual to seek medical attention not only in the developing world, but also in the developed world Zafar *et al*., (2008). It is typically the first infection to occur after birth. Respiratory tract infections are caused by a handful of bacteria, fungi and viruses and account for more than 40% of disability days, secondary to acute illnesses; pneumonia and influenza accounting to 80 - 90% of death in the elderly Hugonnet *et al*., (2000). Respiratory tract infection can be divided into two major types; the Upper Respiratory Tract Infection (URTI) and Lower Respiratory Tract Infection (LRTI). Upper respiratory tract infections a nonspecific term used to describe acute infections involving the nose, paranasal sinuses, pharynx, larynx, trachea and bronchi Mossad, (2008). URTIs such as sore throat, ear ache, laryngitis, common cold, otitis media and sinusitis are the most frequently reported infections of all human diseases Hueston *et al*., (1999). Adults develop an average of two to four colds

cycles annually Mossad, (2008). It has been reported that the majority of URTIs are of viral origin with rhinovirus, parainfluenza virus, coronavirus, adenovirus, respiratory syncytial virus and influenza virus accounting for most cases Clark *et al*., (2004) Lykova *et al.*, (2003). Apart from viruses, bacteria pathogens have been reported to cause RTI; the organisms identified include *Haemophilus influenzae*, *Staphylococcus aureus, Streptococcus pneumonia* and *Klebsiella pneumonia,* (Isenberg and D- Amato, (1985); Ndip *et al*., (2003). Lower Respiratory Tract Infections (LRTI 's) may be defined as those infections presenting with symptoms including cough, expectoration, dyspnoea, wheeze and or chest pain/discomfort usually for a period ranging from 1-3 weeks. Acute manifestations of LRTIs which may or may not involve lungs include acute bronchitis, bronchitis, influenza, community–acquired pneumonia either with or without radiological evidence, acute exacerbation of Chronic obstructive pulmonary disease (COPD) and acute exacerbation of bronchiectasis Woodhead *et al*., (2005). It is also reported that herbs have been used as sources of food and medicinal purposes for centuries and this knowledge has been passed from one generation to another Adedapo *et a*l., (2005). Medicinal plants also represent a rich source from which antimicrobial agents can be obtained Kubmarawa *et al*., (2007).

The ethanol extract of the root significantly delayed the effect of histamine-induced broncho-constriction characterized by shortness of breath in guinea pig Boampong (1992). The cytotoxicity of the crude extract as reported by Banzouzi *et al*., (2002) and Ayisi *et al*., (2003), was very low. Alcohol extracts from root-bark, stem-bark, leaves, fruits and seeds disrupted mitotic cell division in onion *Allium cepa*L. Ayisi *et al*., (2003). Adeshina *et al*., (2010) reported that ethyl acetate extract of *Alchornea cordifolia* leaves possesses antimicrobial activity against the clinical and typed isolates of *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Candida albicans*.

2. Methods

2.1 Collection, Identification and Preparation of Plant leaf

Alchornea cordifolia leaves were collected from a farm settlement at kundum durum area along Kano road Bauchi, Bauchi state. The leaves were air-dried at room temperature for ten days and the complete dry leaves was reduced to powder using mortar and pestle which was carried out in microbiology laboratory Federal Polytechnic Bauchi.

2.2 Collection of Clinical Specimen.

The Isolates were transported from Trust care laboratory opposite old JUTH Jos, plateau state to the department of science laboratory technology federal polytechnic Bauchi for further research.

2.3 Test Organisms Used

Bacteria isolate that were used for this research are; *E. coli, S. aureus, and K. pneumonia.*

2.4 Ethanol extract

A modification of ethanol to methanol extraction procedure of Boakye *et al*, (1977) was adopted.100g of powdered leave materials of the sample was soaked in 300ml of ethanol for 5days, the solution was filtered using whatman no 1 filter paper. The filtrate was poured into a beaker and labeled appropriately. The ethanol filtrate was evaporated using water bath to remove the ethanol while the residue was dried in a hot air oven at 140^0C for1hr.

2.5 Aqueous extract

100g of powdered leave materials of the sample was soaked in 300ml of distilled water for 5days, the solution was filtered using whatman no 1 filter paper. The filtrate was poured into a beaker and labeled appropriately. The residue was dried in a hot air oven at 140^0C for 1hr.

2.6 Identification of test organisms.

The isolates were sub cultured using MacConkey agar and nutrient agar and was incubated at 37^0C for 24hrs and biochemical test was carried out for confirmation of the isolate.

2.7 Determination of the photochemical content of the plant sample

Qualitative tests were carried out on the sample to determine the presence of the phytochemical constituent in the sample.

2.8 Qualitative analysis of the plant sample

Test for presence of alkaloids

The presence of alkaloids in the sample was investigated using the method described by Harborne (2002). The ethanol extract was used, 5ml of the extract was poured into a test tube and 3 drops of pirovic acid was mixed with it. The formation of light green colouration indicates presence of alkaloid.

Test for the presence of flavonoid

The determination of presence of flavonoid in the sample was carried out using the acid alkaline test described by Harborne (2002).2ml of the aqueous extract was added into a test –tube and a few drops of Bench Concentrated ammonia (NH4) was also added. The formation of a yellow colouration shows presence of flavonoid. Confirmatory test was carried out by adding few drops of concentrated hydrochloric (HCL) into the yellow solution which turned colourless.

Test for the presence of saponin

The presence of saponins in the sample was determined using Harborne J. (2004) method. Two tests were involved in the investigation, the froth test and emulsion test. In the froth test, 2 ml of the aqueous extract was mixed with 5 ml of distilled water in a test tube. The mixture was shaken vigorously. A stable froth on standing indicates the presence of saponins.

Test for the presence of Anthrequinone

The aqueous extract was shaken with 10ml of benzene, 5ml of 10 percent ammonia solution was added and was shake, the presence of pink, red or violet colour in the ammonia layer of the preparation indicate the presence of anthrequinone.

Test for the presence of tannin

The presence of tannins in the samples was determined using the method described by Harborne (2004) .2ml of the aqueous extract and 3 ml distilled water was put into a test tube. A few drops of 0.1% ferric chloride was added to the mixture. The formation of a very dark precipitate indicated presence of tannin.

Determination of antimicrobial activity

Media preparation.

Nutrient agar was use for this study and was prepared according to the manufacturer instruction

Antibacterial susceptibility test

The sensitivity of the test organism to the ethanolic and aqueous extracts of the leaves of *A. cordifolia* was carried out using the agar well diffusion method. 20ml of the nutrient agar was seeded with 0.2ml of broth culture of the test organisms in sterile Petri dishes. The Petri dishes was rotated slowly to ensure a uniform distribution of the organisms. It was left to solidify and a 5 wells of 18mm diameter was made in the agar using a sterile test tube, 1 well is the control which contain distilled water. Different concentration of the ethanol and aqueous extract of 100mg/ml, 75mg/ml, 50mg/ml and 25mg/ml was poured into the wells and the Petri-dishes was allowed to stand for about 30 minutes at room temperature to allow for the proper diffusion of the extracts to take place. The plates were then incubated at 37^{o}C for 24 hours. The zones of inhibition in millimeters was measured and recorded.

Determination of Minimum Inhibitory Concentration (MIC)

The agar diffusion method described by Baron *et al (2000)* was used to determine the minimum inhibitory concentration. Six grams of nutrient agar were dissolved in 250ml of distilled water in a conical flask. After sterilization, the nutrient agar was poured into sterilized Petri dishes to solidify. The microorganisms were introduced into the wells using wire loop. Extracts of 5mg/ml, 15mg/ml, 20mg/ml and 25mg/ml was made from the original test samples and distilled water was used as control. The petri-dishes were then placed in the incubator at 37^{o}C for 24 hours. The inhibition zones in millimeters was measured and recorded.

3. Results and findings

TABLE 1 PHYTOCHEMICAL TEST Phytochemical properties of *A.cordifolia*.

	A. cordifolia: Aqueious extract.	A. cordifolia Ethanol extract
Flavonoid	+	+
Saponin	+	+
Tanin	+	+
Alkanoid	+	+
Antraquinone	-	-

TABLE 2 ANTIBACTERIAL/SUSEPTABILITY TEST

Antibacterial Test for Aqueous Extract of A.*cordifolia*.

Bacterial Isolate	Concentration of Extract (µg/ml) diameter of zone of Inhibition (mm)				
	100	75	50	25	Control
E. coli	0	3.5	0	0	0
S. aureus	0	10	0	12.5	0
K. pnuemonia	9.5	0	7.5	10	0

TABLE 3 Antibacterial Test For Ethanol Extract of A.*cordifolia*.

Bacterial Isolate	Concentration of Extract (µg/ml) diameter of zone of Inhibition (mm)				
	100	75	50	25	Control
E. coli	11	3.5	0	0	0
S. aureus	7.5	0	6.5	6.0	0
K. pneumonia	6.0	0	0	5.5	0

TABLE 4 Minimum Inhibitory Concentration (MIC) of the Bacterial Isolate of the Extract

	Aqueous extract.	Ethanol extract
S. aureus	10.1	5.7
E. coli	3.0	4.2
K. pnuemonia	6.8	5.3

4. Discussion

The antimicrobial activity of the properties of phytochemical analysis of the plant extract are given in table 1. extract of *A. cordifolia* has all the bioactive agents present which are tannin, alkaloid, flavonoid and saponin in the two extract while Anthraquinone was absent in the two extract. The result of the antimicrobial activity of the leaf extract against *E. coli, S.aureus* and *K. pneumonia* is shown in table 2 & 3. Both aqueous and ethanol extract of *A. cordifolia* show activity against the bacterial isolate. The minimum inhibitory concentration (MIC) of the extract is shown in table 4. Both the two extract of *A. cordifolia* was found to be bacteriostatic at the concentration of 5mg/ml and 15mg/ml respectively. A. cordifolia was found to contain all the bioactive compound which are tannin, alkaloid saponin, Flavonoid while anthraquinone is absent in the both extract. there have been several report on the natural occurring plants chemicals found in these plants. These include steroids, sap phenols, flavonols, tannins. Xanthones and alkaloid Ogunlana and Ramstad (2002). The phenolic acids

are gallic acid, ellagic acid, protocaechoc acid, Lamikanra *et al.,* (1990) while the Flavonoid include queretin, hyperin and guaijaverin Ogungbamila and Samulesson (2000). This current study is in agreement with previous work.The antimicrobial activity of the Aqueous and ethanol extract was active against all bacteria isolate and *S. aureus* has the highest zone of inhibition on aqueous extract. Igbenegu *et al* (2007) reported that it was active against multi resistance S. aureus. Earlier Okeke *et al* (2000) had shown that it was very active against seventy-four bacteria strains studied in vitro. Other workers Ogunlana and Rainstad (1975) had made similar observations. This current finding is in line with earlier report the MIC against 15mg/ml -20mg/ml in our study. Based on our findings the aqueous and ethanolic extract of leave of A. cordifolia can be useful in treatment of respiratory tract infection cause by *S. aureus, E coli* and *K pneumonia*.

5. Conclusion

This study shows that the aqueous and ethanol leaf extracts of *Alchornea cordifolia* obtained from Kundum durum Bauchi state, Nigeria was found to contain phytochemical content and have antibacterial activity against *S. aureus*, *E. coli*, and *K. pneumoniae*. This study has justified the use of *Alchornea cordifolia* in the treatment of some bacterial diseases in herbal medicine.

6. Recommendations

1. Isolating the secondary metabolites will be important to explore the full potential of *Alchornea cordifolia* plant.
2. Test for safety is required with further purification for economical purposes.
3. Mechanism of action of the *Alchornea cordifolia* should be determined.
4. The findings in this study have shown the need for further investigation to establish the economic viability of exploiting *Alchornea cordifolia* plant to address health problems.

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