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Phytochemical Screening and Antimicrobial Activity of Leave and Stem Bark of *Dillenia indica* Linn

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Abstract This study was aimed at determining the phytochemical profile and antimicrobial activity of leave and stem bark. The phytochemical and antimicrobial activity of ethanol extracts of leave and stem bark of *Dillenia indica* was evaluated using standard methods. The phytochemical screening of the leave and stem bark extracts revealed the presence of saponins, glycosides, phenols, alkaloids, flavonoids, tannins, carbohydrates and resins in varying quantities while anthraquinones was absent in both leave and stem bark extract. *Dillenia indica* ethanol extracts of the leave and stem bark exhibit moderately proven potential to contain antimicrobial agents of pharmacological interest. The *Dillenia indica* plant could be exploited as potential therapeutic agents in the treatment of various diseases due to the rich phytochemical profile and promising antimicrobial activity.

Keywords Dilleniaceae, Leave Extract, Stem Bark Extract, Phytochemicals, Antimicrobial

1. Introduction

Dillenia indica belongs to the family of Dilleniaceae, a shrubs, sub-shrubs, or climbers comprising of about 12 genera [1]. It is also known as elephant apple, a medium-sized tree and geographically distributed in the forests of India, Indo-Malaysian region and Tropical Australia [1-3]. The flowers are mainly bisexual or rarely unisexual with colourful petal (white, yellow, or red) and visible reproductive component. *Dillenia indica* fruits (calyx) are used by the Indian communities as a flavouring agent for curries, and preparation of jam and jelly [1]. The fruit is follicle or berrylike, sometimes edible, either dehiscent or indehiscent and enclosed with fleshy calyx [4].

Mixture of leaf, stem bark and fruits juice of *Dillenia indica* are normally consumed orally for the treatment of cancer and diarrhea [5]. The fruit juices of *Dillenia indica* fruit is usually taken as a cooling beverage to treat fever, relieve fatigue and also has laxative properties [6]. The ripped fruits are taken orally to increase appetite, overcome weakness and relieve abdominal pain. The plant is rich in secondary metabolite such as lupeol group of triterpenoids like betulinic acid and flavonoids that possess diverse pharmacological activities [6].

Therefore, increase in failure due to chemotherapeutic and antibiotic resistance leads to screening of several medicinal plants for their antimicrobial potency. However, the Nigerian species of *Dillenia indica* have been under-utilized. Hence, there has been a dearth of scientific information on their phytochemicalprofile and biological activity. The present study was undertaken to determine the phytochemical constituents and antimicrobial activity of the leave and stem bark extracts of *Dillenia indica* plant.

2. Materials and Methods

2.1. Plant Collection

The *Dillenia indica* leaves and stem bark were collected from botanical garden of the University of Ibadan, Oyo state, Nigeria, in March, 2012. The plant sample was identified and authenticated at the Forestry Research Institute of Nigeria (FRIN) Ibadan; where voucher specimens (UIL-0027) were deposited.

2.2. Sample Preparation and Extraction

The plant materials were carefully collected, washed, air-dried for three days and pulverized. The pulverized plant sample (250 g) of both the leaves and stem bark of *Dillenia indica* were soaked separately in 1.2 L of ethanol for 72 hours under frequent agitation at room temperature. The extract was filtered with what-man filter paper and concentrated using rotary evaporator at 40 ^{o}C.

2.3. Phytochemical Screening

Standard methods for phytochemical screening (alkaloids, flavonoids, saponins, tannins, carbohydrates, sterols and triterpenes) were employed. Alkaloids determination was done using Mayer's and Dragendoff's reagents following the methods of Sofowora [7]; tannins and phlobatannins [8]. The methods described by Harborne [9] and Trease and Evans [8] were used for determining flavonoids, phenol and cardiac glycosides. The persistent frothing, sodium bicarbonate and carbonate tests, as described by Trease and Evans [8] and Safowora [7] were used for saponins. Carbohydrates, sterols and triterpenes determination were done using Fehling's reagent following the method described by Harborne [9].

2.4. Evaluation of Antimicrobial Activity

Agar well diffusion method was adopted for the antimicrobial activity of the extract [10]. The crude extracts (0.1g, 0.2g, 0.3g, 0.4g) was dissolved in 5ml of the stock solution with a sterile cork-borer of 5mm. Holes of equidistant diameter was made on the surface of the seeded plates and various concentrations of each extracts were aseptically made to fill the holes such that each isolate was tested on different concentrations of the extracts. The plates were incubated at 37°C for 24 hours. Antimicrobial activity of each extracts against test organisms was evaluated by measuring their zones of inhibition in millimeters. Control experiment was carried out using commercial antibiotics and solvent (stock). These were set up alongside with extracts. Gentamicin antibiotic (80 mg) was used for bacterial isolates plates were inoculated for 24 hours and 48 hours respectively. Zones of inhibition were measured and recorded.

2.4.1. Microbial Strains

Microorganism used were standard strains of bacteria obtained from centre of drug Research institute, (CDRI) Lucknow, India. *Bacillus subtilis* (ATTC 14579), *Bacillus cereus* ATTC 33923, *Salmonella typhi* ATTC 25179, *Pseudomonas aeruginosa* ATTC 27856 and *Proteus mirabilis* ATTC 21784 were used in this study; Each strain was stored in sterile peptone water. Cell densities were estimated from the pour plate method on plate count agar.

3. Results and Discussion

The results of phytochemical profile of ethanol extract of leave and stem bark is presented in Table 1. The phytochemical screening indicated varying quantity of carbohydrate, sterol, phenol, saponins, glycoside, tannins, alkaloids and flavonoids in the leave and stem bark extracts. The phytochemical profile revealed that the ethanol leave extracts contains high amount of resin, flavonoids, saponins, alkaloids, cardiac glycosides, terpenoids, tannins, phenol and carbohydrate justifying their pharmacological usage in the treatment of diabetes mellitus, anti-inflammatory, antidiarrheal, cancer, antimicrobial and antioxidant activities [1]. Anthraquinones were not detected in any of the extracts. The presence of these metabolites in leave of *Dillenia indica* has been demonstrated previously by Bose *et al*., 2010. However, alkaloids were found to be present in the leaf and stem bark of *Dillenia indica* which is contrary of the findings of Bose *et al* [11]. This might be due to season of collection, time of

collection, climatic and environmental factors. Phytochemical constituents in the various part of the plant vary significantly. The *Dillenia indica* leaf and stem bark ethanol extract with their phytoconstiuents are reported for diabetes mellitus, anti-inflammatory, antidiarrheal, cancer, antimicrobial and antioxidant activities [1]. Phenolic compounds are also reported in the ethanol extract of *Dillenia indica* have anti-inflammatory effects. Therefore, the ethnomedicinal usage of the extracts in the treatment of anti-inflammatory diseases might be attributed to the high concentration of phenolic compounds. The presence of flavonoids and tannins in the leaf and stem bark of *Dillenia indica* ethanol extract is likely to be responsible for the free radical scavenging effects reported by Ayoola, 2008. Flavonoids and tannins are phenolic compounds and plant phenolics are major group of compound that act as primary antioxidants or free radical scavengers [12].

Table 1: Phytochemical screening of ethanol extracts of *Dillenia indica*

Test	Leaves	Stem bark
Carbohydrate	++	++
Saponins	+++	++
Alkaloid	++	+++
Flavonoids	+++	+++
Resins	++	++
Anthraquinones	─	─
Tannins	++	++
Phenol	+++	+++
Glycoside	+++	+++

+++: High, ++: Moderate, +: Trace, - : Not detected

The antimicrobial potency of the crude extracts of the plant was estimated using standard conventional methods. The study showed that the different extracts of the plant inhibited the growth of some microorganisms used in the assay, justifying the presence of antimicrobial compounds in the *Dillenia indica* ethanol extract. These antibacterial actions could be attributedto the chemical components present in the crude extracts. The antimicrobial activity of the ethanol extracts of leave and stem bark against pathogenic bacteria at various concentrations (0.05, 0.10, 0.15, 0.20 g/ml) is presented in Table 2. The results revealed that all the extracts tested indicated varying degree of antimicrobial activities against the test microbial strains. The zones of inhibition differ with the extract and the organism tested. It was observed that the some zones of inhibition for some microorganism increases with increase in concentration as improved antimicrobial activity is concentration dependent.

The moderate activity of the ethanol extracts against most bacterial strains investigated in this study correlates with its phytochemical profile (Table 1) and consistent with previous work which reveals that the extract of the plant generally show moderate antibacterial activities. Joseph *et al.* stated that the medicinal properties and biological activities of plants are usually due to their chemical profile. This suggests that the saponins, alkaloids, flavonoids, resins, tannins and phenols in the ethanol extracts of *Dillenia indica* in this study may be implicated for its moderate antimicrobial effect shown. These differences in activity may be due to thesource of the microbial strains, plant part utilized in the studies or environmental factors. The *Dillenia indica* leave and stem bark crude extracts can serve as potential sources of new antimicrobial agent. Further research such bioassay should be done on the isolated active principles which could be used in the development of new drugs.

Table 2: Zones of inhibition of *Dillenia indica* leave extract in millimeters (mm)

Organisms	0.05	0.10	0.15	0.20	Gentamicin
B. subtilis	0.7	1.0	1.1	1.2	1.8
B. cereus	1.3	-	1.0	-	2.0
S. typhi	-	1.2	1.9	2.1	3.0
P. aeruginosa	1.4	1.0	2.0	1.6	2.5
P. mirabilis	1.0	1.5	1.9	1.4	2.2

Table 3: Zones of inhibition of *Dillenia indica* stem bark extract in millimeters (mm)

Organisms	0.05	0.10	0.15	0.20	Gentamicin
B. subtilis	0.9	1.3	1.0	1.2	1.8
B. cereus	1.2	1.1	1.7	0.9	2.0
S. typhi	2.0	-	1.9	-	3.0
P. aeruginosa	1.0	-	2.0	-	2.5
P.mirabilis	1.2	1.5	1.9	1.8	2.2

4. Conclusion

The phytochemical screening revealed that *Dillenia indica* ethanol extracts contained alkaloids, glycosides, saponins, phenols, carbohydrate, tannins, resins and flavonoids in various quantities. These biologically active metabolites therefore suggest the antimicrobial potency of the ethanol extract. The inhibitory effect of the leave and stem bark extracts of *Dillenia indica* against pathogenic bacterial strains (*B. subtilis, B. cereus, S. typhi, P. aeruginosa and P. mirabilis*) lends credence to the plant as a potential candidate for bio-prospecting for antibiotic drug development for the treatment of ailments caused by these pathogens.

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