ASSESSMENT OF ANTIBACTERIAL ACTIVITIES AND PHYTOCHEMICAL SCREENING OF FLAMBOYANT TREE LEAF EXTRACTS AGAINST SELECTED BACTERIA

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ABSTRACT

Delonix regia plant is one of the useful plants that have antimicrobial activities against pathogenic microorganisms. The different parts of this plant, the stem, back, pod, seed, flower and to a lesser extent leaf have been investigated for antimicrobial activities. The aim of this study is to determine the antibacterial activity and phytochemical constituents of the leaf of D. regia. The powdered leaf of D. regia was subjected to extraction with ethanol and petroleum ether using soxhlet extractor and the extracts were concentrated in rotary evaporator. The crude extracts of both ethanol and petroleum ether were assayed for antibacterial activity against Escherichia coli, Bacillus subtilis, Micrococcus luteus and Pseudomonas aeruginosa by agar well diffusion method. The MIC and MBC of the crude extracts to test organisms were determined by broth micro dilution method. The quantitative (percentage yield) as well as the phytochemical constituents of the extracts were determined. The maximum zones of inhibition observed from ethanol extract against Escherichia coli (26.33mm) and petroleum ether extract against Bacillus subtilis (21.66m). The extracts were found to contain eight phytochemicals namely: anthraquinones, tannin, cardenolides, phlobatannin, terpenes, phenol, steroid, and alkaloids. Alkaloids was the predominant phytochemical while terpene was the least from both the ethanol and petroleum ether extracts. The yield of the extract from ethanol was higher (15.47%) than that from the petroleum ether (14.40%). The leaf extracts were found to be effective against all the test organisms used in this study.

Key words: *Delonix regia*, Petroleum Ether, Bacterial Suspension, Phytochemical

INTRODUCTION

Delonix regia plant is one of the useful plants that have antimicrobial activities against pathogenic microorganism. *Delonix regia* plant which can also be called the flamboyant tree or flame tree is a flowering plant in the bean family Fabaceae, sub family Caesalpinioidea. It is 10 – 15 m high with many branches and umbrella shaped crown (Saloni and Arora, 2015). Flamboyant tree is a native of Madagascar, but now distributed in many countries of the tropical region. It is often used to prepare extract with antimicrobial effects against bacteria and fungi.
The flowers of the flame tree are orange in colour. The fruits are elongated pod like about 12 inches or more in length (Samy and Ignacimuthu 2000).

The tree is noted for its fern-like leaves and flamboyant display of flowers. In many tropical plants of the world, it is grown as an ornamental tree and in English it is given the name Royal Poinciana (Don Burke *et al*.., 2005). *D. regia* tree is widely regarded as one of the most beautiful trees in the world (Saad, *et al.*, 2012). The fern-like leaves are composed of small individual leaflets which fold up at the onset of dusk. This tree produces brown woody seed pods that reach lengths of up to 60cm which turn reddish-brown to almost dark when ripe (Saad, *et al.*, 2012). The beautiful flowers of the flame tree are pollinated by birds and produced in spring and summer.

Flamboyant tree can grow in a wide range of habitats including disturbed sites. It grows in full sun and can tolerate sandy, loamy, clay, acidic and alkaline soils.

The flame tree is most commonly propagated by seeds. The seeds are collected, soaked in warm water for at least 24 hours and planted in warm, moist soil in a semi- shaded sheltered position.

In lieu of soaking, the seeds can also be nicked or pinched (with a small scissors or nail clipper) and planted immediately. The two methods allow moisture to penetrate the tough outer casing, stimulating germination. The seedlings grow rapidly and can reach 30 cm in a few weeks under ideal conditions.

Less common, but also effective is propagation by semi-hardwood cuttings. Branches consisting of the current or last season’s growth can be cut into 30cm sections and planted in a moist potting mixture.

This method is slower than seed propagation (cutting take a few months to root) but it is the preferred method for ensuring new trees are true to form. As such cuttings are particularly common methods of propagation for the rarer yellow flowering variety of the tree.

The flowers are large with four spreading scarlet or orange red petals up to 8cm long, and a fifth upright petal called the standard which is slightly larger and spotted with yellow and white. They appear in corymbs along and at the end of the branches.

The leaves of the tree have a feathery appearance and are a characteristic light, bright green. Each leaf is 30cm -50 cm long with 20-40 pairs of primary leaflets aor pinnae, each divided into 10-20 pairs of secondary leaflets or pinnules.

Natural products from plants perform various functions in which many of them have interesting and useful antimicrobial activities (Dhanalakshmi and Manimegalai, 2013).

AIM

The aim of this research was to determine the antibacterial and phytochemical screenings of the petroleum ether and ethanol extracts of the leaf of flamboyant tree (*Delonix regia*) against selected bacteria.

MATERIALS AND METHODS

Collection and Authentication of the Plant

The leaves of *Delonix regia* were obtained within the premises of the mini campus of the Federal Polytechnic Offa. These leaves were authenticated at the Biological Garden of Science Technology Department, Federal Polytechnic, Offa.

Preparation of leaf Powder

Fresh *Delonix regia* leaves were collected, washed with clean water and air dried at room temperature for two weeks. The dried leaves were then pulverized into powdery form using an electric pulverizer. The fine powder was obtained by sieving.

Extracts Preparation

Ten (10) gram of the powdered leaf was weighted using an electric balance (Denver xs - 210). The weighed sample was made into packet using Watmann filter paper. The sample was then be subjected to extraction using soxhlet apparatus (Dhanalakshimi and manimegalai, 2013). The extraction was carried out with petroleum ether followed by ethanol extraction to enable the powders undergo extraction with solvents of increasing polarity.

Test Organisms

The test organisms used for the antibacterial activity screening were obtained from the Department of Medical Microbiology and Parasitology of the University of Ilorin Teaching Hospital, Ilorin Kwara State. The clinical isolates used were *Bacillus subtilis, Escherichia coli, Micrococcus luteus* and *Pseudomonas aeruginosa.*

Standardization of Test Organisms

The test organisms used were standardized using McFarland standard method. The McFarland Standard was used as a reference to adjust the turbidity of bacterial suspension (Test organisms) so that the number of bacteria will be within a given range to standardize microbial testing. 0.5 McFarland Standard used during this study was prepared by mixing 0.05ml of 1.175% barium chloride dehydrate with 9.95ml of 1% Tetraoxosulphate vi acid as reported by Cockerill *et al*., (2012).

Reconstitution of Extracts

The extracts obtained were reconstituted with their respective extractant to give a concentration of 200mg/L and this served as standard solution. Lower concentrations such as 100mg/L, 50mg/L,. 25.5mg/L were made (Banso and Ayodele, 2001).

Screening of Plant for Antibacterial Activity

Agar well diffusion method was used as described by Abbas *et al.,* (2016). 20ml of Mueller – Hilton agar was poured on petri plates of the same size and allowed to solidify. The agar surface of each plate was streaked by separate sterile cotton swab with the test organisms. The agar plate was then punched with a sterile cork borer of 4mm size. The molten agar was introduced in the hole to seal its base before 100 micro litre of each sample was introduced with micropipette. The plates were allowed to stand undisturbed for 30 minutes after which they were incubated at 37^0C for 48 hours.

Antibiotic Susceptibility Test

This test was carried out as described by the National Committee for Clinical Laboratory Standard (NCCLS, 2002) using agar disc diffusion method. Sterile Mueller – Hilton agar plate was swabbed with 0.5ml broth culture of the test organism. Commercially purchased antimicrobial (chloramphenicol) disc was pressed down on the agar to ensure complete contact with the agar surface. The plate was then incubated for 18 – 24 hours. The zone of inhibition was noted and measured with a ruler at right angle held at the back of the inverted plate.

Determination of Minimum Inhibitory Concentration of the Extracts

One milliliter each of the diluted extracts of different concentrations viz: 10.0, 12.5, 15.0, 17.5 and 20.0mg/L was mixed with 9ml of Mueller – Hilton broth in the test tube. The tubes were then inoculated differently with each of the test organisms and incubated for 48 hours at 37^0C. The least concentration of the extracts that was not show the visible growth of the test organisms was regarded as the minimum inhibitory concentration in each case (Abbas *et al*., 2016).

Determination of Minimum Bactericidal Concentration (MBC)

The minimum Bactericidal Concentrations were determined by first selecting the tubes that showed no growth during MIC determination. A loopful from each tube was sub cultured onto extract free Mueller Hilton agar plates, incubated for further 24 hours at 37^0c. The least concentration at which no growth was observed was taken as the Minimum Bactericidal Concentration (MBC).

Phytochemical Tests.

The two extracts were tested for the presence of chemical constituents using standard method as described by Ajayi *et al.,* (2010).

Alkaloid

0.5g each of the dried ethanol and petroleum either extract were weighed and re-extracted with 5ml of 5% hydrochloric acid. The hydrochloric acid extracts were filtered. Few drops of Drangen druff reagent were added to 2.5ml of the filtrate. A reddish - brown colour and turbidity with the reagent indicate the presence of alkaloid (Ajayi *et al.,* 2010).

Tannins

0.5g of the dried and powered sample of the leaf was stirred with 10ml of distilled water, filtered and ferric chloride was added to the filtrate. Appearance of blue or blue–black, green or blue-green coloration showed the presence of tannin. Quantitative determination was also carried out (Ajayi *et al.,* 2010).

Saponins

The leaf sample was ground and 20g was weighed and put into a conical flask and 100 cm^3 of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4 h with continuous stirring at about 55^0C. The mixture was filtered and the residue re-extracted with another 200 mL 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90^0C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethy1 ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. Sixty millimeter of n-butanol was added. The n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight; which represents the saponin content of the leaf. (Falana *et al.*, 2016).

Flavanoid

Total flavonoid content was determined by Aluminum chloride method used as standard as was reported by Falana *et al.,* 2016. One gram of test sample(leaf) and 4mL of water were added to a volumetric flask (10ml volume). 0.3m of 5% Sodium nitrite was added to the content inside the flask. 5 minutes after, 0.3mL of 10% Alumilium chloride was added. After 6 minutes incubation at room temperature, 2mL of 1M Sodium hydroxide was added to the reaction mixture. Immediately, the final volume was made up to 10mL with distilled water. The absorbance of the reaction mixture was measured at 510nm against a blank spectrophotometrically (Shimadzu UV-1609, Japan).

RESULTS

All the bacterial pathogens used in this study showed susceptibility to both the ethanol and petroleum ether extract of *Delonix regia* leaf.

The results of varying concentrations of the ethanol and petroleum ether extracts of the leaf and the chloramphenicol antibiotic disc used as control were shown in Tables 1, 2 and 3.

The leaf extract of *D. regia* from ethanol extraction against *Escherichia coli* possessed maximum zone of inhibition where as it demonstrated minimum zone of inhibition against *Pseudomonas aeruginosa*.

The petroleum ether extract of the leaf was mostly effective against *Bacillus subtilis* while it showed least effective against *Micrococcus luteus.* The antibiotic disc containing

chloramphenicol used as control was observed to have maximum and minimum zones of inhibition against *Micrococcus luteus* and *Bacillus subtilis* respectively.

Table 1: Antibacterial Activity of *D. regia* leaf extract of 100mg/L concentration against test organisms

	Diameter of Zone of Inhibition in mm		
Microorganisms	**Antibiotic disc (Chloramphenicol)**	**Ethanol**	**Petroleum ether**
E. coli.	23.22	26.33	20.36
Ballicus subtilis	21.76	23.26	21.66
M. luteus	35.45	21.33	11.33
P. aeruginosa	31.36	16.66	12.22

Table 2: Antibacterial Activity of *D. regia* leaf extract of 50mg/L concentration against test organisms

	Diameter of Zone of Inhibition in mm		
Microorganisms	**Antibiotic disc (Chloramphenicol)**	**Ethanol**	**Petroleum ether**
E. coli.	12.33	15.11	10.33
Ballicus subtilis	11.66	13.33	10.66
M. luteus	17.33	10.66	06.66
P. aeruginosa	15.22	09.36	08.33

Table 3: Antibacterial Activity of *D. regia* leaf extract of 25mg/L concentration against test organisms

	Diameter of Zone of Inhibition in mm		
Microorganisms	**Antibiotic disc (Chloramphenicol)**	**Ethanol**	**Petroleum ether**
E. coli.	07.11	07.88	05.22
Ballicus subtilis	06.33	06.22	06.88
M. luteus	09.66	04.66	03.66
P. aeruginosa	07.88	03.22	04.33

Table 4 shows Minimum Inhibitory Concentration of both the ethanol and petroleum ether extracts of the leaf of *Delonix regia*

Table 4: Minimum Inhibitory Concentrations of ethanol and petroleum ether extracts of *D. regia* leaf.

Extracts	**Concentrations (mg/L)**	**Test organisms**
Ethanol	10.0	*Escherichia coli*
	12.5	*Bacillus subtilis*
	15.0	*Micrococcus luteus*
	17.5	*Pseudomonas aeruginosa*
Petroleum ether	10.0	*Micrococcus luteus*
	12.5	*Pseudomonas aeruginosa*
	15.0	*Escherichia coli*
	17.5	*Bacillus subtilis*

Table 5: Results of the percentage yield after extraction of the dry powder part of the leaf in gram.

The extraction yield from the ethanol extraction of the leaf sample was higher than of the petroleum either.

Table 5: Percentage yield after extraction of dry powder part (g)

Sample	**Extraction solvent**	**Dry powder part (g)**	**Leaf extract yield from dry powder(g)**	**Yield (%)**
Leaf	Ethanol	150	23.20	15.46
	Petroleum ether	150	20.10	3.40

Table 6 shows the constituents of the phytochemical of the leaf extracts from ethanol and petroleum ether.

Table 6: Preliminary Phytochemical constituents of ethanol and petroleum ether extracts of the leaf of *D. regia*

Phytochemical constituents	Ethanol	Petroleum ether
Tannin	+ve	+ve
Alkaloid	+ve	-ve
Saponin	+ve	-ve
Flavonoid	+ve	+ve

Phytochemical Assay

Qualitative phytochemical analysis of the ethanol extract revealed the presence of all the active ingredients (Tannins, Saponins, Flavonoids and Alkaloid) while the extract from petroleum either did not revealed the saponin and Alkaloid. As shown in the Table 6. The active ingredient were found present in varying proportion (Figure 1). The active ingredients were found to be higher in the ethanol extract than the petroleum ether extract.

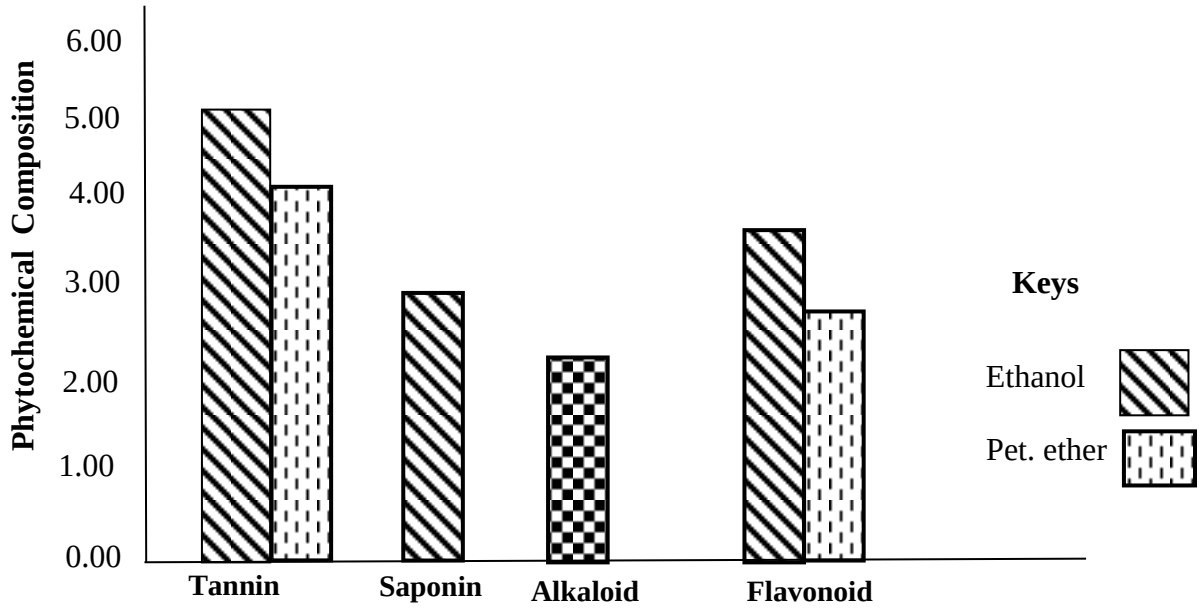

Figure 1: Quantity of Phytochemical in *D. regia* leaf extracts (mg)

Discussion

The ethanol extract of the leaf of *D. regia* showed the maximum zone of inhibition was against *E. coli (26.33mm)*. The minimum zone of inhibition observed with of ethanol extract of the leaf against *P. aeruginosa* (16.66mm). Similar observations were made by Dhanalakshmi and Manimegalai (2013) in which ethanol extract leaf of the aid seed of *D regia* showed highest activity against *E. coli.* This study revealed that the petroleum either extraction of the leaf of *D-regia* has highest effectiveness against *Bacillus subtilis* (21.6mm) zone of inhibition while its lowest activity was against *Micrococcus leteus* of 11.33mm zone of inhibition. This corroborate with the study of Ajayi *et al.*, 2010 which showed that petroleum ether extract was effective against *B. Subtilisi* from this study, the ethanol extract of the leaf of *D. regia* was observed to have phytochemical constituents such as Tanmin, Alkaloid, Flavonoid and Saponin which is similar with the results of the phytochemical constituents obtained by Singh and Sonia, 2018.

This study revealed that the petroleum ether extract of the leaf of *D. regia* has no Saponin and Alkaloid as part of the phytochemical constituents which corroborate with the work of Singh and Sonna, 2018.

The ethanol extract of the leaf of *D. regia* was showed to be effective against, *E coli, B. Subtilis, P. aeruginosa* in this study. This is similar to be work carried out by Vivek et al., 2013 that shows the effectiveness of leaf extract of *D. regia* against microorganisms

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CONCLUSION AND RECOMMENDATION

The use of plant for the treatment of infectious diseases is of utmost importance because of the emergence of antibiotic resistance pathogenic organisms.

More research on the use of herbal remedy in the treatment of infectious diseases should be embarked upon in order to overcome the predicament of antibiotics resistant pathogens.

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