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Antimicrobial And Phytochemcial Activities On (Tectona Grandis) Leave Extract On Staphylococcus Aureus, Escherichia Coli, Bacillus Subtilis And Proteus Vulgaris

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Abstract: ***Nowadays, microorganisms develop resistant against synthetic antibiotics. This is because of the emergence of resistance pathogens due to the indiscriminate use, incessant and misuse of antibiotics. One of the methods to reduce the resistance to antibiotics is by using antibiotic resistance inhibitors from plants. In these studies, Phytochemical and Antimicrobial Screening of the leaves of Tectona grandis against Staphylococcus aureus, Escherichia coil and Proteus vulgaris were determined using standard methods. The result of the phytochemical analysis on Tectona grandis was the presence of flavonoids, tannins and saponin. The result of the finding shows that Staphylococcus aureus was susceptible to the inhibitory of both ethanolic and aqueous extracts of Tectona grandis leaves. Among the test extracts, ethanolic extract is more effective than aqueous extract (15mm and 10mm of zone inhibition respectively). The control ciprofloxacin was the most effective with the zone of inhibition of 18mm, therefore Tectona grandis extract contain phytochemicals that can inhibit the growth of Staphylococcus aureus.***

Keywords: Phytochemical, Antimicrobial Screening, Tectona grandis Leaves, Bacteria

I. INTRODUCTION

Tectona grandis Linn., commonly known as teak tree or sagwan (Hindi) is known in the world for its dimensional stability, extreme durability and hardness in timber production. Keiding et al; (2006). It is classified in the division Magnoliophyta, class Magnoliopsida, order, Famiales, and the family Verbenaceae/Lamiaceae. *Tectona grandis* is one of three species in the genus *Tectona.* The other two species, *Tectona hamiltoniana* and *Tectona philippinensis*, are relatively found in particular areas with relatively small native distributions in Myanmar and the Philippines, respectively Tewari, (2012).

Tectona grandis is an excellent timber for bridge building and many other constructions while some other species in the family are notable ornamentals, such as Clerodendrum, Callicarpa, Vitex, Lantana, and Verbena. Watson et al; (2002). *Tectona grandis* wood is found useful in the treatment of headache, constipation, biliousness, burning sensation and pain, liver-related troubles, worms, cough, microbial, fungal, piles, leucoderma and dysentery infections. The oil of the nuts and flowers promotes hair growth and also useful in the treatment of scabies while the roots are useful in the treatment of urinary system-related troubles. Pankaj,(2004). Compounds such as Lapachol and its derivatives, methyl quinizarin and squalene isolated from the heart wood were found to have cytotoxic, antiulcer, wound healing and anaemia activities in experimental animals. Pathak et al; (2008).

II. BENEFITS OF COMMON TEAK

The tree is now planted in tropical to sub-tropical areas throughout the globe, not only for ornamental reasons but also in plantations for commercial timber production. Timber from this tree is an important tropical wood. Teak wood has a

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leather-like smell when it is freshly milled and is particularly valued for its durability and water resistance. The wood is used for boat building, exterior construction, veneer, furniture, carving, turnings, and other small wood projects. Teak wood is hard, durable, fine-grained, and resistant to decay if exposed to moisture and of attractive deep brown color. Teak wood is used in a variety of ways, including (a) for outdoor purposes, it is used in the manufacture of outdoor furniture, boat building, doors and window frames, and (b) for indoor purposes, and it is used for flooring, fine furniture and veneer. Teak is the national tree of Indonesia. Kaosa-ard, (2009).

III. MATERIALS AND METHOD

COLLECTION OF AND IDENTIFICATION OF SAMPLE

The plant *Tectona grandis* leaves used for this research work was collected from Iyere village, Ile-oluji, Okeigbo L.G.A., Ondo State and it was duly authenticated by Prince Olanipekun, N.O. (Botanist) of the Department of Science Laboratory Technology, Federal Polytechnic Offa, Kwara State. These were then collected in a sterile polythene bag, rinsed, air dried and made into a powdery form before use.

TEST ORGANISM

The test organism used for the antimicrobial assay of this medicinal plant include: *Pseudomonas aeroginosa, staphylococcus aureus* and *Escherichia coli.* The tests organisms mention above were collected from University of Ilorin Teaching Hospital, Ilorin, Kwara State, Nigeria.

CONFIRMATION AND IDENTIFICATION OF ORGANISM

The identity of organisms collected above were further confirmed by sub culturing each of the isolate in different selective and differential medium such as, Eosin methyl blue aga and MacConkey agar. And was further characterize with the uses of different biochemical test which include: Gram stain, Catalase, Coagulase, Oxidase, Indole, Motility e.tc. and the characteristic with a standard taxa (Cheesbrouogh, 2006).

SCREENING OF EXTRACT FOR ANTIMICROBIAL ACTIVITY (USING WELL METHOD)

To test for antimicrobial activity of plant, agar well diffusion method was employed i.e. 1g of aqueous was reconstituted in 5ml of sterile distilled water to make 20% concentration and was vortexed for homogeneity. The broth culture of the test organism was compared to the turbidity to ***05%*** McFarland standard. 3 drops of standardized culture was transferred into a sterile petri dish. Freshly prepared cooled tempered sterile molten Muller hilton agar was added to Petri dish that contained standard organism, it was rocked gently and allowed to set at room temperature. A sterile cork borer was used to make 2 well of 6mm diameter on the solidified agar, a drop of (0.1ml) each extract of both aqueous and ethanolic was introduced into the well, and was labeled respectively, it was incubated at 37°C for 24hours. Control agar plate were made in parallel and included (OVC) organism viability control, (MSC) medium sterility control and (ESC) Extract sterility control, ethanolic extract was equally treated.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (USING PAPER DISK METHOD)

To determine the minimum inhibitory concentration (MIC) of the extract paper disk method was employed 0.9g, 0.8g, 0.7g, 0.6g, 0.5g, 0.4g, 0.3 g, o.2g and O.1g of plant leaves of both aqueous and ethanolic extract were accurately weighed on a weighed balance (VIC-1561) and each was dissolved 5ml of sterile distilled water to make a concentration of 180mg/ml, 160mg/ml, I40mg/ml, 120mg/ml, 100mg/ml, 80mg/ml. 60mg/ml, 40mg/ml and 20mg/ml respectively. The sterile paper disk was soaked in each extract and allowed to air dried. Molten Muller hilton agar was poured into a sterile Petri dish, and allowed to solidify at room temperature after which they are labeled with the appropriate test organisms, the organisms were standard as described for antimicrobial assay. Each plate was then streaked with a loopful of standardized sensitive test organism (organism that were sensitive to the plant extract during determination of antimicrobial activity) each paper disk of the extract of both aqueous and ethanolic extract at different concentration was placed on a nutrient agar that contained the standardize organism. Control agar plate were made in parallel include organism viability control (OVC), medium sterility control (MSC) and extract sterility control (ESC). The plates were then incubated at 37°C for 24hours. Ethanolic extract was equally treated.

SCREENING OF EXTRACT FOR PHYTOCHEMICAL COMPONENT

Phytochemical screening was carried out on the aqueous and ethanolic extract for the qualitative determination of phytochemical constituent as described by (Trease and Evans, 2007).

IV. RESULTS

The result of antimicrobial activity of aqueous of *Tectona grandis* leaves were shown in Table 1. The result revealed that the aqueous extract of *Tectona grandis* leaves inhibited the growth of *staphylococcus aureus*, at the highest concentration of 200mg/ml with the zone diameter of 10mm while *E. coli, Bacillus subtilis, and Proteus Vulgaris* displayed no sensitivity and absence of zone of inhibition at 200mg/ml concentrations.

Test organism	Activities	Zone of inhibition (mm)
E. coli	-	-
Proteus vulgaris	-	-
Staphylococcus aureus	+	10mm
Bacillus subtilis	-	-
Control		
Ciprofloxacin	+	15mm

Key
(+) Activities

(-) No activities

Table 1: Antimicrobial Activities of Aqueous Extract of Tectona grandis leaves at concentration of 500mg/ml

The result of antimicrobial activity of ethanolic extract of *Tectona grandis* leave were shown in Table 2. The result revealed that the ethanolic extract of *Tectona grandis* leaves inhibited the growth *staphylococcus aureus*, at the highest concentration of 200mg/ml with the zone diameter of 15mm while *proteus vulgaris, E. coli and Bacillus subtilis* displayed no sensitivity and absence of zone inhibition at 200mg/ml concentration.

Test organism	Activities	Zone of inhibition (mm)
E. coli	-	-
Proteus vulgaris	-	-
Staphylococcus aureus	+	15mm
Bacillus subtilis	-	-
Control		
Ciprofloxacin	+	18mm

Key
(+) Activities
(-) No activities

Table 2: Antimicrobial Activities of Ethanolic Extract of Tectona grandis leaves at concentration of 500mg/ml

The result of minimum inhibitory concentration of Aqueous and ethanolic extract of *Tectona grandis* during determination of antimicrobial sensitivity is shown in table 3. The result reveal that aqueous extract displayed sensitivity on staphylococcus aureus at minimum inhibitory concentration of 160mg/ml while ethanolic extract displayed sensitivity at Minimum Inhibitory Concentration of 120mg/ml and ciprofloxacin at 100mg/ml.

Zone of inhibition (mm)

Extract/ Conc. (mg/ml)	180	160	140	120	100	80	60	40	20
Aqueous extract	8mm	4mm*	-	-	-	-	-	-	-
Ethanolic extract	13mm	10mm	5 mm	2 mm*	-	-	-	-	-
Control									
Ciprofloxacin	15mm	12mm	9mm	8mm	5mm*	-	-	-	-

Key
+ means activities
- means no activities
** Minimum Inhibitory Concentration*

Table 3: Determination of Minimum Inhibitory Concentration of Aqueous Extract prepared at various concentrations in mg/ml

The result of phytochemical screening of aqueous extract of *Tectona grandis* leaves was shown in Table 4. The result shows that the flavonoids, tannins, saponin, alkanoid and glycoside were found present, only anthraquinone was absent.

Phytochemical Compounds	Aqueous Extract
flavonoids	+
Tannins	+
Saponin	+
Alkanoids	+
Glycosides	-
Anthraquinone	-

Key
(+) indicate presence
(-) indicate absence

Table 4: Result of Phytochemical analyses of Aqueous Extract from the leaves of Tectona grandis

The result of phytochemical screening of ethanolic extract of *Tectona grandis* leaves was shown in Table 5. The result shows that the flavonoids, tannis and saponin were found present, only glycoside, alkanoid and anthraquinone were absent.

Phytochemical Compounds	Aqueous Extract
flavonoids	+
Tannins	+
Saponin	+
Alkanoids	-
Glycosides	-
Anthraquinone	-

Key
(+) indicate presence
(-) indicate absence

Table 5: Result of Phytochemical analyses of Ethanolic Extract from the leaves of Tectona grandis

V. DISCUSSION

It has been established that the ethanolic extracts of *Tectona grandis* leaves contains some secondary metabolic such as alkaloids, tannins, flavonoids and reducing sugar established by (Cowan, 2014), the presence of flavonoids and tannins in the leaf of this plant is indicative of its anti-inflammatory and analgesic effects. The flavonoids and tannin are compounds suggestive of its primary antioxidants as documented by Ayoola et al, 2018).

Also (Lai, 2010) established tannins and saponins as effective antioxidants and antimicrobial agents while flavonoids are known to target protaglandis which are implicated in the late phase of acute. Inflammation and pain sensitivity as affirmed by (Gupta et al; 2005).

However, anthraquinon were absent in both aqueous and ethanolic extract of *Tectona grandis* leaf studies when compared to its presence in the fruit according to the findings of (Adedapo, 2013). And the extracts were found to inhibit some clinical pathogens such as *staphylococcus aureus*. It inhibit the growth at highest concentration of 120mg/ml using the ethanolic extract and at minimum inhibitory concentration of 160mg/ml of aqueous extract, while (*Proteus Vulgaris, E. coli* and *Bacillus Subtilis*) displayed no sensitivity and absence of zone of inhibition at 200mg/ml concentration.

VI. CONCLUSION

The result of the study confirmed that *Tectona grandis* leaves has the following bioactive constitutes, flavonoids, tannins, saponin, alkanoids, which make the leave extract to have a valuable antimicrobial activities.

The presence of flavonoids and tannins in the leaf of the plant indicate anti-inflammatory and analgesic effects.

This finding justifies the traditional uses of this plant for therapeutic purpose.

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