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PHYTOCHEMICAL SCREENING AND ANALYSIS OF ANTIMICROBIAL ACTIVITY OF *Tectona grandis* LEAVES.

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ABSTRACT

In the present study to investigated the phytochemical and antimicrobial activity of Tectona grandis leaves extract. *Tectona grandis* leaves showed that the presence of tannin, saponin, flavonoids, terpenoids, triterpeniods, antroquinone, steroids, polyphenol, glycosides and coumarins in in both extract. Alkaloids presence only methanol extract. Quantitative analysis showed that significant amount of phytochemicals such as flavonoids, phenol and terpenoids were present in *Tectona grandis* leaves. Histochemical studies further proved the presence of phytochemicals in *Tectona grandis* leaves. *Tectona grandis* leaves was potential antibacterial activity confirmed against Escherichia coli and Pseudomonas aeruginosa species of bacteria strains. *Tectona grandis* leaves was promising antifungal activity evidenced against *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus* species of fungi strains.. .

Keywords: *Tectona grandis* leaves, Phytochemical, antimicrobial activity

INTRODUCTION

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. There are more than thousand known phytochemicals. It is well known that plant produce these chemicals to protect itself but recent research demonstrate that they can protect humans against diseases (Harbone, 1967). Plants synthesize a wide array of compounds that play key roles in protecting plants against herbivores and microbial infection and as attractants for pollinators and seed-dispersing animals, allopathic agents, UV protectants, and signal molecules in the formation of nitrogen-fixing root nodules in legumes. Although they have long been ignored from a nutritional perspective, the function of these compounds and their relative importance to human health are gaining significant interest (Day and Willignoson, 2001).

The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. However, the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from natural sources including plants. Keeping in view, the present study to investigate the phytochemical and antimicrobial activity of *Tectona grandis* leaves.

MATERIALS AND METHODS

Collection of plant materials

The *Tectona grandis* leaves were collected in January 2021 from Kamarasavalli, Ariyalur district, Tamil Nadu, India. The collected Tectona grandis leaves were washed several times with distilled water to remove the traces of impurities from the leaves. Then examined carefully, old infected and fungus damaged portion of the leaves were removed. Healthy leaves was dried in room temperature and grind using grinder mixture. The powder was stored for further analysis.

Preparation of plant extract

1 gram of the powder of *Tectona grandis* leaves were transferred in to different conical flask (250ml). The conical flask containing 50ml of different solution (methanol and water). The conical flask containing Tectona grandis leaves were shaken well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using Whatman filter paper No.1 and filtrate is used for further analysis.

Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973 and 1984). Total phenols estimated by the method of Edeoga *et al*., (2005). Flavonoid determine by the method of Bohm and Kocipai-Abyazan (1994). Total terpenoid content in the leaf extracts were assessed by standard method (Ferguson, 1956). Histochemical tests (John Peter Paul, 2014; Gersbach *et al*., 2001). The antibacterial activity was performed by disc diffusion method (NCCLS, 1993; Awoyinka *et al.*, 2007).

RESULTS AND DISCUSSION

Qualitative and quantitative analysis

A number of phytochemicals isolated from plant material are used in the pharmaceutical drug industry today. The phytochemicals under investigation include secondary metabolites, many which are synthesized for plant defense and adaption to environmental stress. The phytochemicals can range from medicinally useful agents to treat varieties of diseases such as diabetes, malaria, anaemia (Fola., 1993).

In the present study was carried out on the *Tectona grandis* leaves revealed the presence of medicinally active constituents. The phytochemical characters of the *Tectona grandis* leaves investigated and summarized in Table-1 and figure 2 and 3. The phytochemical screening *Tectona grandis* leaves showed that the presence of tannin, saponin, flavonoids, terpenoids, triterpeniods, antroquinone, steroids, polyphenol, glycosides and coumarins in in both extract. Alkaloids presence only methanol extract.

Table.1: Qualitative phytochemical analysis of *Tectona grandis* leaves extract

S. No	Phytochemicals	Methanol extract	Aqueous extract
1	Tannin	++	+
2	Saponin	++	++
3	Flavonoids	++	++
4	Steroids	++	++
5	Terpenoids	+	++
6	Triterpeniods	+	++
7	Alkaloids	+	-
8	Antroquinone	++	+
9	Polyphenol	++	++
10	Glycosides	++	+
11	Coumarins	++	++

(+) Presence, (++) High concentrations and (–) Absences

Quantitative analysis

Quantitative analysis revealed that the *Tectona grandis* leaves has flavonoids, terpenoids and phenol. Significant amount of flavonoids (20.00mg/gm), terpenoids (10.00mg/gm), and phenol (142.00mg/gm) were presented (Table 2). The above phytoconstituents were tested as per the standard methods.

Table.2: Quantitaive analysis of phytochemicals in *Tectona grandis* leaves powder

Phytochemicals	Result (mg/gm)
Phenol	142.00 ± 1.89
Flavonoids	20.00 ± 1.53
Terpenoid	10.00 ± 1.08

Value were expressed as Mean ± SD for triplicate

Phenol and flavonoids have become an intense focus of research interest because of their perceived beneficial effects for health, including antidiabetic, anticarcinogenic, antiatherogenic, antiulcer, anti-thrombotic, anti-inflammatory, immunemodulating, antimicrobial, varodialatory, and analgesic effects (Dewick, 2001). In the present study, *Leucaena leucocphala* leaves contain phenol and flavonoids which may possess potential antidiabetic activity.

Similarly Kumar *et al*., (2013) investigated the preliminary phytochemical screening of the leaves of the plant *Lasia spinosa* (Lour) Thwaites. The phytochemical screening showed that the methanol extract contained flavonoids, phenol, terpenoids, tannin, saponin, glycosides and alkaloid which are responsible for the biological activities.

Histochemical analysis

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues; it is a powerful tool for localization of trace quantities of substances present in biological tissues. Histochemical techniques have been employed to characterize structure and development, and to study time course of deposition and distribution of major phytocompounds (Krishnan et al., 2001).

In the present study, *Tectona grandis* leaves were treated with specific chemicals and reagents. The Tectona grandis leaves powder treated with diluted ammonia and H2SO4 gave yellow colour indicates flavonoids. The *Tectona grandis* leaves powder treated with few drops of Fecl3 gave black color indicates the presence of tannin. Plant powder treated with Toludine blue gave Blue green/Red colour indicates the presence of polyphenol. Plant powder treated with Dinitrophenol hydrazine (few drops) gave Orange colour indicates the presence of terpenoids. (Table 3 and Figure 5). These results further confirmed the presence of phytochemicals.

Table 3: Histochemical analysis of *Tectona grandis* leaves powder

Phytochemical	Result
Tannin	+
Flavonoids	+
Terpenoids	+
Polyphenol	+

Single plus (+) represents presence and double plus (++) represents high concentrations

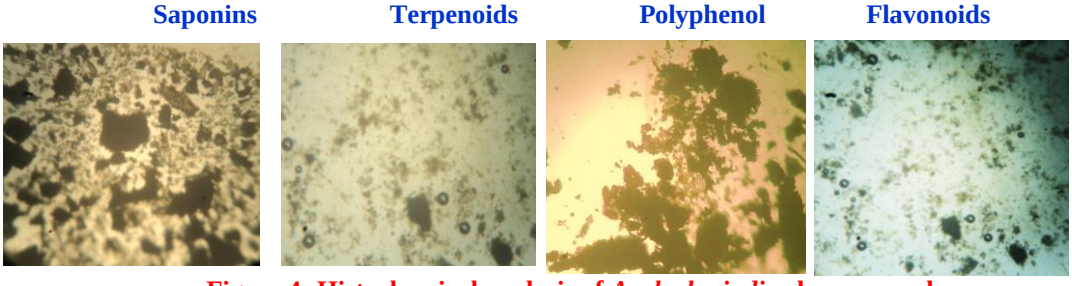


Figure 4: Histochemical analysis of *Acalypha indica* leaves powder

Antimicrobial activity of *Acalypha indica* leaves

Emergence of pathogenic microorganisms that are resistant/multi-resistant to major class of antibiotics has increased in recent years due to indiscriminate use of synthetic antimicrobial drugs. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. In addition, high cost and adverse side effects are commonly associated with popular synthetic antibiotics, such as hypersensitivity, allergic reactions,

and immunosuppressant and are major burning global issues in treating infectious diseases (Karaman *et al*., 2003). This situation forced scientists to search for new antimicrobial substances with plant origin.

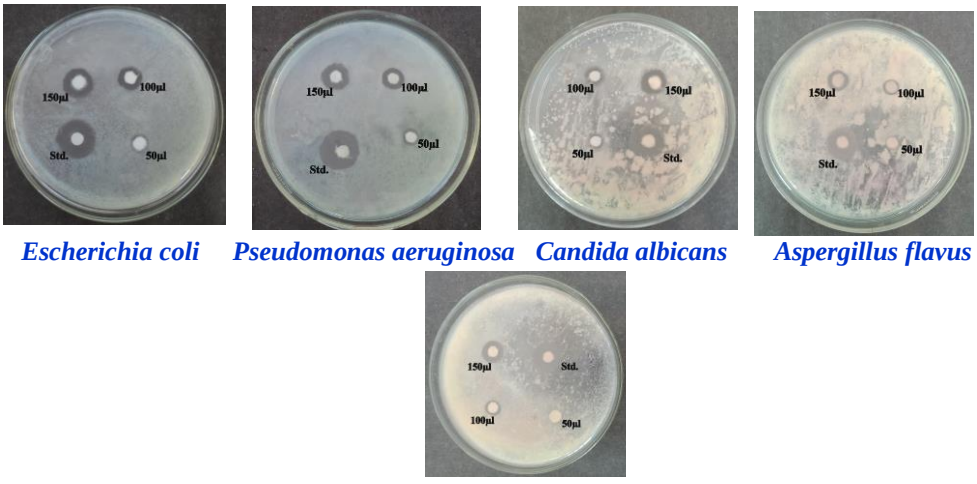
Leaves extract of *Acalypha indica* was screened against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* species of bacteria by the standard agar disc diffusion method. The in vitro antibacterial activity shows the presence of inhibition zones represented in the photograph Fig. 6 and Table 5. The inhibitory activity was reported in Table 5 were comparable with standard antibiotic viz. chloramphenicol. Among test organism the leaves of *Acalypha indica* has maximum inhibitory activity against Escherichia coli when compared to Bacillus subtilis and *Staphylococcus aureus*.There are 4 major types of Microbes: bacteria, fungi, protists and viruses. Recently, many investigators (Punnagai *et al*. 2016) have identified the antifungal properties of plant extracts.

Table. 4: Antimicrobial activity of *Tectona grandis* leaves

Microbial Strains	Concentration (µl)			Std. (30µl)
	50µl	100µl	150µl	
Bacteria				
***Escherichia coli* (mm)**	1.50±0.10	3.75±0.26	6.00±0.42	8.75±0.61
***Pseudomonas aeruginosa* (mm)**	1.00±0.07	3.20±0.22	5.25±0.36	8.50±0.59
Fungal				
***Candida albicans* (mm)**	0.90±0.06	3.00±0.21	4.90±0.34	8.40±0.58
***Aspergillus flavus* (mm)**	0.75±0.05	2.25±0.15	4.50±0.31	7.50±0.52
***Aspergillus niger* (mm)**	0.50±0.03	2.00±0.14	4.20±0.29	7.00±0.49

Values expressed as Mean ± SD for triplicates

Bacterial standard: Chloramphenicol; Fungal standard: Fluconazole



Escherichia coli *Pseudomonas aeruginosa* *Candida albicans* *Aspergillus flavus*

Aspergillus niger

Fig. 6: Antimicrobial activity of *Tectona grandis* leaves

Conclusion

The *Tectona grandis* leaves has rich source of phytochemicals and possess potential antimicrobial activity. The results of the study concluded that Tectona grandis leaves may be used for the treatment of microbial infections.

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