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INVESTIGATION OF PHYTOCHEMICALS AND ASSESSMENT OF ANTI-BACTERIAL ACTIVITY OF *Acalypha indica* L. LEAVES.

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ABSTRACT

In the present study was to investigate the phytochemical screening, and antibacterial activity of leaves *Acalypha indica*. The phytochemical screening of *Acalypha indica* leaves showed that the presence of tannin, saponin, flavonoids, terpenoids, steroids, alkaloids, triterpenoids, glycosides, polyphenol and coumarins in both extract. Anthraquinone presence only aqueous extract. Quantitative analysis showed that significant amount of phytochemicals such as flavonoids, phenol and terpenoids were present in *Acalypha indica* leaves. Histochemical studies further proved the presence of phytochemicals in *Acalypha indica* leaves. *Acalypha indica* leaves shows potential antibacterial activity confirmed against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* species of bacteria strains.

Keywords: *Acalypha indica* leaves, Phytochemical, Antibacterial activity

INTRODUCTION

Owing to the global trend towards improved 'quality of life', there is considerable evidence of an increase in demand for medicinal plant (Kotnis et al., 2004). Use of plants for treating various ailments of both man and animal is as old practice as man himself. India is richly endowed with a wide variety of plants having medicinal value. These plants are widely used by all sections of the society whether directly as folk remedies or indirectly as pharmaceutical preparation of modern medicine (Bhagwati Uniyal, 2003). In recent times, focus on plant research has increased all over the world and a large body of evidence collected to show immense potential of medicinal plants used in various traditional systems (Ayurveda, Siddha and Unani) (Dahanukar et al., 2000).

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 *Acalypha indica* leaves.

MATERIALS AND METHODS

Collection of plant materials

The whole plants of *Acalypha indica* were collected from Nagachi, Thanjavur Dt, Tamil Nadu, India were washed. The plant were identified then there were washed under running tap water to remove the surface pollutants and the whole plants were air dried under shade, the dried sample was powdered and used for further studies

Preparation of plant extract

1 gram of the powder of *Acalypha indica* leaves were transferred in to different conical flask (250ml). The conical flask containing 50ml of different solution (methanol and water). The conical flask containing *Acalypha indica* 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.

Phytochemical screening

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

In the present study was carried out on the *Acalypha indica* leaves revealed the presence of medicinally active constituents. The phytochemical characters of the *Acalypha indica* leaves investigated and summarized in Table-1 and figure 2 and 3. The phytochemical screening *Acalypha indica* leaves showed that the presence of tannin, saponins, steroids, flavonoids, terpenoids, triterpenoids, alkaloids, polyphenol, glycosides and coumarins in methanol and aqueous extracts. Anthroquinones was present only aqueous extract.

Table.1: Qualitative phytochemical analysis of *Acalypha indica* leaves extract

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

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

Quantitative analysis

Quantitative analysis revealed that the *Acalypha indica* leaves has flavonoids, terpenoids and phenol. Significant amount of flavonoids (40mg/gm), terpenoids

(10.00mg/gm), and phenol (183.00mg/gm) were presented (Table 2). The above phytoconstituents were tested as per the standard methods.

Table.2: Quantitative analysis of phytochemicals in *Acalypha indica* leaves powder

Phytochemicals	Result (mg/gm)
Flavonoids	40.00 ± 1.84
Poly Phenol	183.00 ± 4.28
Terpenoid	10.00 ± 1.37

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, immunomodulating, 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 phytochemicals (Krishnan *et al.*, 2001).

In the present study, *Acalypha indica* leaves were treated with specific chemicals and reagents. The *Acalypha indica* leaves powder treated with diluted ammonia and H₂SO₄ gave yellow colour indicates flavonoids. The *Acalypha indica* leaves powder treated with few drops of H₂SO₄ gave Yellow color indicates the presence of saponins. Plant powder treated with Toluidine 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 4). These results further confirmed the presence of phytochemicals.

Table 3: Histochemical analysis of *Acalypha indica* leaves powder

Phytochemical	Result
Saponins	+
Terpenoids	++
Polyphenol	++
Flavonoids	++

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

Saponins

Terpenoids

Polyphenol

Flavonoids

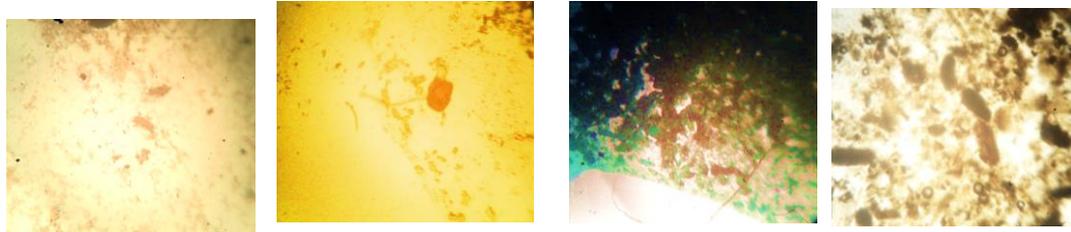


Figure 4: Histochemical analysis of *Acalypha indica* leaves powder

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

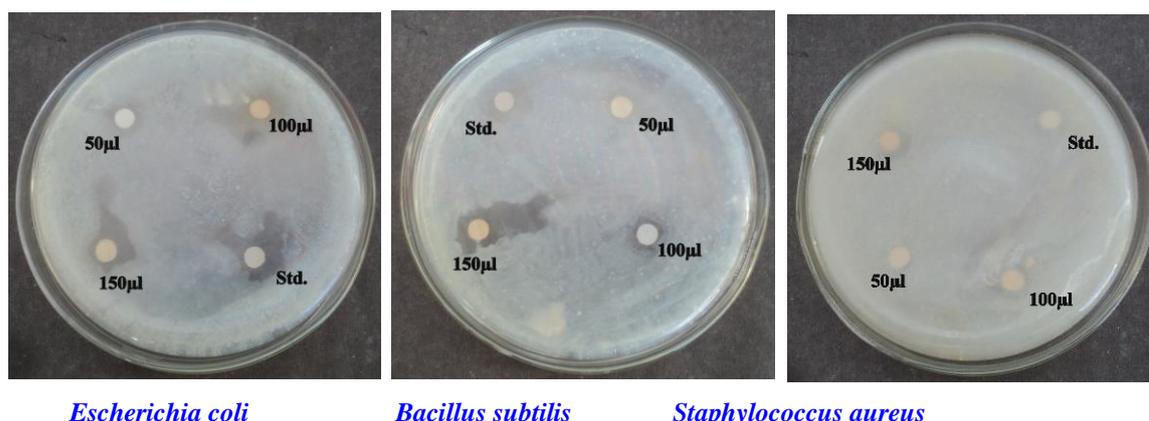
identified the antifungal properties of plant extracts.

Table 5: Anti-microbial activity of *Acalypha indica* leaves extract

Microorganisms	Concentrations (µl/ml)			Std. (30µl/ml)
	50	100	150	
<i>Escherichia coli</i> (mm)	1.50±0.10	4.50±0.31	8.25±0.57	10.50±0.73
<i>Bacillus subtilis</i> (mm)	1.00±0.07	3.25±0.22	7.50±0.52	10.00±0.70
<i>Staphylococcus aureus</i> (mm)	0.50±0.03	2.75±0.19	6.50±0.45	7.25±0.50

Values were expressed as Mean ± SD

Bacterial standard : Chloramphenicol



Escherichia coli

Bacillus subtilis

Staphylococcus aureus

Fig. 6: Antibacterial activity of *Acalypha indica* leaves extract

Conclusion

Overall, it can be concluded from the present study that *Acalypha indica* leaves extract highly against *E. coli* bacteria was caused by diabetic foot infection, contains rich source of phytochemicals and possess

antibacterial activity. This study is the first scientific report that provides convincing phytochemicals and antibacterial evidence for the relevance of *Acalypha indica* leaves thus providing scientific validity to its traditional consumption by the local populace of south India.

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