Research Article

Phytochemical Screening and Antimicrobial Activity of leaves extract of *Acalypha fruticosa* Forssk (Euphorbiaceae Family)

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Abstract

The *Acalypha* genus of plants has been historically utilized in the treatment and management of various health issues, including diabetes, jaundice, hypertension, fever, liver inflammation, schistosomiasis, dysentery, and respiratory disorders such as bronchitis, asthma, and pneumonia. Additionally, these plants are employed in addressing skin conditions like scabies, eczema, and dermatitis. In the present study was aim to phytochemical screening and antimicrobial activity of ethanolic extract of *Acalypha fruticosa* Forssk leaves. **Methods**: The plant materials of *Acalypha fruticosa* Forssk leaves were extracted with ethanol for 3h and collect the extract and screening for identification of phytochemicals and their extract was assessed by antimicrobial activity using the disc diffusion method. The evaluation of antibacterial and antifungal activities involved measuring the diameter (mm) of the inhibition zone surrounding the disc and the assay was conducted three times. The antimicrobial activity was reported as the mean diameters of the leaf extract's inhibition zones of tested bacteria and fungi. **Results:** The results of the present study were observed that leaf extracts of *Acalypha fruticosa* Forssk leaves exhibited a maximum concentration of 400mg/ml extract active against maximum inhibited the growth of both tested bacteria and fungi like *Salmonella typhi, S. aureus, Candida albicans* and *Aspergillus niger*. The phytochemical constituents of phenolic compounds present in the *Acalypha fruticosa* leaves possess antibacterial and antifungal properties.

Keywords: Antibacterial activity; Antifungal activity; *Acalypha fruticosa* Forssk; leaves;phytochemicals

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1. Introduction

Antimicrobial resistance to existing commercial medications has become a major global issue; nevertheless, the prospect of creating new drugs from medicinal plants continues to show promise. Medicinal plants' use in treating human and animal diseases has long been practiced before the advent of antibiotics (Lopez, 2005; Sumner, 2008). Several studies reported that medicinal plants used in various localities possess antimicrobial activity and hence justify their use by the communities (Cheruiyot et al., 2009, Anago et al., 2011, Njume et al., 2011).

Acalypha is the fourth largest genus of the Euphorbiaceae family with approximately 450-570 species. Several *Acalypha* species are used as medicinal plants in Africa and the Mascarene Islands. Almost every part of the plant including the leaves, stem and roots is traditionally used in the treatment and/or management of diverse ailments such as diabetes, jaundice, hypertension, fever, liver inflammation, schistosomiasis, dysentery, respiratory problems including bronchitis, asthma and pheumonia as well as skin conditions such as scabies, eczema and mycoses (Seebaluck et al.,2015). The leaves are laxative, diuretic and used in the treatment of leprosy and gonorrhea. Different part of the plant is also used in infectious diarrhoea, pulmonary problems, and as an expectorant in asthma (Seebaluck et al.,2015; Siraj et al.,2016).

Acalypha hispida Burm. f. (1768) is an evergreen shrub native to New Guinea and the Bismarck Archipelago. Currently, it is widely cultivated as an ornamental and medicinal plant in tropical and subtropical areas worldwide (Dong et al., 2023). In India, *Acalypha hispida* Burm.f. also known as Indian Nettle or Indian Mercury, is a plant in the Euphorbiaceae family that is known for its medicinal uses and the attraction of its root to domestic cats. The plant is frequently used as a decorative element in outdoor and indoor settings. Historically, the leaves have been recognized for their laxative and diuretic properties and employed in the therapeutic management of leprosy and gonorrhea. Various plant components are additionally employed in treating infectious diarrhea respiratory ailments and as an expectorant for asthma (Jodh et al.,2024). The utilization of a plant species that possesses quercetagetin-7-arabinosylgalactoside, a flavonoid compound, has been extensively employed in the management of many infectious ailments (Alfarisi et al.,2022). Previous studies, anti-inflammatory activity (Sumintarti et al.,2020; Adesina et al.,2000). The plant contains ellagitannins namely, acalyphidins M_1, M_2, and D_1, anthocyanins namely, cyanidin 3-*O*-(2″-galloyl-*β*-galactopyranoside), cyanidin 3-*O*-(2″-galloyl-β-galactopyranoside), and cyanidin 3-*O*-β-galactopyranoside (Amakura et al.,1999; Reiersen et al.,2003). The present study aimed to screen for preliminary phytochemical constituents of *A. hispida* and their extract was investigated for antibacterial and antifungal properties.

2. Materials and Methods

2.1 Plant Materials

The plant materials of *A. hispida* were identified and their leaves were collected and thoroughly washed, shade-dried, ground into a powder, and subsequently extracted using ethanol (95.2%) for Soxhlet apparatus in 3h.

The extract was concentrated with the aid of a rotatory evaporator. The concentrated extract was weighed and transferred into a sterile container and stored in the refrigerator at 4°C to be used later for phytochemical analysis.

The percentage yield of the extract was as follows:

$$\text{Percentage of yields (\%)} = \frac{\text{Weight of the Extracts (g)}}{\text{Weight of the leaves powders(g)}} \text{ x100}$$

2.2. Qualitative phytochemical analyses

Qualitative phytochemical analysis of *A. hispida* was carried out using the developed method of Harborne (1983) while the quantitative phytochemical analysis was carried out using the methods of Harborne (1983); Obadoni and Ochuka (2001); Bohm and Kocipai (1994); Okeke and Elekwa (2003).

2.3 Bacterial and Fungal Strains

Five clinical isolates bacterial strains like *Klebsiella pneumonia, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli* fungal strains like *Candida albicans* and *Aspergillus niger* were used for the study.

2.4 Assay for antibacterial effect

The initial susceptibility of the tested organisms to the plant extracts was assessed using the agar well plate diffusion method (Alade and Irobi,1986). Tests were carried out in triplicate with stock solutions of leaf extracts (100mg/ml) and also at diluted concentrations of 100mg/ml, 200mg/ml and 400mg/ml. Tetracycline was used as a control. A Pasteur pipette was used to deliver the extracts or vehicle control into the two wells on the surface of each agar plate which had been previously streaked with each of the bacteria respectively and then incubated at 37 °C for 24h. The presence of a zone of inhibition around each of the wells after the period of incubation was regarded as the presence of antibacterial action while the absence would be a lack of measurable antibacterial action.

2.5 Antifungal activity

2. 5.1 Preparation of the fungi cultures

The fungal cultures of *Candida albicans* and *Aspergillus flavus* were isolates, with *Aspergillus* being cultured on malt extract agar, while *Candida albicans* were cultured on Sabouraud's dextrose agar. The isolated organisms were preserved in slant test tubes containing Sabouraud's dextrose, and agar plates were retrieved from the refrigerator and allowed to acclimatize at room temperature for two days prior to testing.

2.6 Assay for anti-fungal effect

The extracts of *A. hispida* were incorporated into the appropriate growth medium at 70 °C to achieve the concentration of 10mg/ml and then allowed to set. *Aspergilus* culturing was done on malt extract agar poured into petri dishes while for the Trichophyton species and *C. albicans* culturing was done on Sabouraud's Dextrose Agar (SDA). Incubation was at 28 ◦C growth diameter was measured after 6 days for the *Aspergilus niger* and *C. albicans* and 15 days. The fungal isolates allowed to grow on their various growth medium without the test extract were used as control. The diameter of growth on the control plates was used to compare the zones of inhibition of the extracts in the experiments. The plates were prepared in triplicate for each concentration.

The antifungal efficacy of the extracts against reference strains of *Candida albicans* and *Aspergillus niger* was tested at concentrations of 200, 100, and 50 mg/mL utilizing the agar-well diffusion

method (Bauer et al.,1996). Ketoconazole (15 µg) served as a positive control, while 5% Dimethyl sulfoxide and/or 5% Tween 80 were employed as negative controls. All experiments were performed in triplicate.

3. Results and Discussion

3.1 Preliminary phytochemical screening

In this research ethanolic extraction of *A. hispida* leaves were identified in the secondary metabolites like alkaloids, flavonoids, phenols, and anthocyanins (Table-1). Medicinal plants of *Acalypha* species contain many phytochemicals, viz. alkaloids, terpenoids, flavonoids, phenolics, saponins, coumarins, anthocyanins, tannins, and anthraquinones. In previous studies, Madziga et al., (2010) reported that phytochemical screening of *Acalypha wilkesiana* was revealed the presence of carbohydrates, tannins and flavonoid, phlobatannins, and saponins.

Table- 1: Phytochemical screening and presence of different chemical of plant A. hispida

Sl. No	Active compound	Results
		Present (+) /absent (-)
1	Alkaloids	++
2	Flavonoids	++
3	Anthocyanin	+++
4	Terpenoids/Essential Oils	++
5	Tannins	+
6	Saponin	+

3.2 Antibacterial activity

The results showed that at the concentration of 400mg/ml extract of *A. hispida* was maximum inhibited the growth of both tested bacteria *Salmonella typhi* and *S. aureus* (Table-2). Earlier studies, the antibacterial activity of acetone extract of *Acalypha indica* were more effective against *Staphylococcus aureus* and *Escherichia coli*, whereas chloroform extract was more effective against *Escherichia coli* and *Klebsiella pneumonia* (Sudhakar Chekuri et al.,2018) and extracts of the leaves of *A. ornata* were more active against *K. pneumonia* (Emeka et al.,2012). In the present study was observed that good antibacterial properties of *A. hispida* leaves.

3.2 Antifungal activity

In the present study was observed that ethanolic extract of *A. hispida* was more effective against *Candida albicans* and *Aspergillus niger* (Table-2). Previous studies, the ethanolic extract of *Acalypha indica* has shown prominent antifungal activity against *Candida albicans* and *Aspergillus niger* (Sudhakar Chekuri et al.,2018). The aqueous extract of *Acalypha wilkesiana* has shown significant antifungal properties *in vitro* (Sherifat *et al*.,2021).

The study findings suggest that the ethanolic extract derived from *A. hispida* exhibits antifungal properties against both fungi *Candida albicans* and *Aspergillus niger*. All the four extracts of *A. ornata* showed anti-fungal activity (Sudhakar Chekuri et sl.,2018). The conclusion of the present study is that the ethanolic extract of *A. hispida* leaves in the bioactive compound may have good antibacterial and antifungal properties (table-1 and 2).

Table-2: Screening of antimicrobial activity of *A. hispida*

Extract Concentrations(mg/ml)	Tested strains of Inhibition of Zone (mm)						
	Escherichia coli	*S. aureus*	*K. pneumonia*	*Pseudomonas ruginosa*	*Salmonella typhi*	*Candida albicans*	*Aspergillus niger*
50	12.33±0.9	11.00±1.2	9.33±0.9	10.00±0.6	13.00±0.6	12.33±0.9	12.33±0.3
100	15±0.6	12±0.33	12±0.9	13±0.8	15±0.33	14±0.9	13±0.9
200	16±0.8	15±0.9	14±0.8	14±0.10	18±0.9	16±0.9	15±0.8
400	17±1.1	18*±0.9	15±0.3	17±0.51	19*±0.8	19*±0.3	16±0.3
Ketoconazole (15µg)	17±0.12	19.33±0.9	18±0.3	19±0.3	20±0.3	17±0.3	24±0.3

Conclusion

The ethanolic extract of *A. hispida* leaves were the most effective plant extracts and showed good antimicrobial activities against the highly susceptible strains of *Salmonella typhi* and *Candida albicans* and *Aspergillus niger*.

Ethics approval and consent to participate
Ethics approval and consent to participate are not relevant in this case, as our research did not involve animals or human subjects.

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Conflicts of interest
There are no existing conflicts of interest.

4. References

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