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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL POTENTIAL OF *Acalypha fruticosa* FORSSK., LEAVES

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Abstract

Acalypha fruticosa Forssk. [Family Euphorbiaceae] commonly known as 'Chinnichedi' and 'Birch-leaved. *A. fruticosa* is a strong smelling bushy shrub traditionally used to treat dyspepsia, stomachache, skin diseases, wounds and poisonous bites. In the present study, the phytochemicals of the ethanol and aqueous extracts of the leaves of *Acalypha fruticosa* were analysed both qualitatively and quantitatively. The phytochemicals of the ethanol and aqueous leaf extract of *Acalypha fruticosa* revealed that the presence of triterpenoids, steroids, saponins, tannins, phenols, flavonoids, alkaloids, glycoside and coumarins respectively. Quantitative estimation of phytoconstituents in the powdered samples of *Acalypha fruticosa* showed that flavonoids were present in high amount when compared to alkaloids, tannins, phenols and saponins. To evaluate the antimicrobial activity of ethanol leaves extract of *Acalypha fruticosa* using the standard disc diffusion method against three pathogens. Among the three organisms, *Staphylococcus aureus* showed maximum zone of inhibition followed by *E. coli* and *Candida albicans* respectively.

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Introduction:-

Medicinal plant is an important element of Ayurveda medical systems in all over the world. The ethno botany provides a rich resource for natural drug research and development (Arashet al., 2010). The use of plants as medicine goes back to early man. Pollen analysis indicated that the numerous plants buried with the corpse were all of medicinal value. By the time of the ancient civilization, a great wealth of information already existed on medicinal plants. Among the many remedies prescribed were human stomachaches, dyspepsia, rheumatism, dermatitis. Ancient China is also a source of information about the early medicinal uses of plants (Farnsworth, 1990).

The use of plant extracts and phytochemicals both with known antimicrobial properties is of great significance, in the past few years several investigations have been conducted worldwide to prove antioxidant and antimicrobial activities from medicinal plants (Alonso-Paz et al., 1995). For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies (Sukanya et al., 2009). The medicinal plants are rich in secondary metabolites which are potential sources of drugs (Nadeem et al., 2010). It has been reported the free radical scavenging and antimicrobial activity of many medicinal plants are responsible.

From the beginning of human life on earth, humans have relied on medicinal plants to cure various diseases. However, in the 20th century, as the human civilization got more developed and along with the isolation of medicinally important compounds, they attempted to synthesize medicinally important compounds and their derivatives in laboratories. Although many active pharmaceutical ingredients have been prepared by the synthetic route, the importance of plants still cannot be ignored and that is reason that plants are constantly explored for novel compounds of biological importance (Memariani et al., 2021)

For a long period of time, plants have been a valuable source of natural products for maintaining human health. According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Ellof, 1998).

Acalypha fruticosa Forssk. [Family Euphorbiaceae] commonly known as 'Chinnichedi' and 'Birch-leaved Acalypha' is a strong smelling and bushy shrub. *Acalypha fruticosa* is used to treat dyspepsia, stomachache, skin diseases, wounds and poisonous bites. Hence the present study was undertaken to analyze the Phytochemicals and Antimicrobial activity of *A. fruticosa* plant ethanol extract

Material and Methods:-

Plant Material

Acalypha fruticosa is a species of flowering plant in the botanical family Euphorbiaceae it occurs widely in East and Southern Africa where it is eaten as a vegetable. It is also an important browse plant for sheep in East and southern Africa it is used as a medicinal plant in Northern Kenya arrow shafts and beehive a tea is made in Ethiopia. *Acalypha fruticosa* occurs in East and parts of southern Africa, except humid central Africa it also occurs in tropical Arabia, Southern India, Sri Lanka and Myanmar. *A. fruticosa* plant leaves were collected in Kabisthalam at Thanjavur.

Preparation of plant powder [Krishnamurthy 1993]

Acalypha fruticosa (leaves) were first cleaned using tap water in order to remove any dirt or debris and later using sterile distilled water. They were dried in laminar flow biological safety cabinet. They were crushed in a sterile mortar pestle until a fine paste was obtained. Similarly, the plant all the species cleaned and a fine powder was made. Required concentration of the plant extracts was made using ethanol

Qualitative Phytochemical Analysis

The qualitative phytochemical tests for Alkaloids, flavonoids, terpenoids, steroids, phenols compounds, tannins, saponins and coumarins were carried out on the concentrated extracts using the standard procedures to identify the constituents as described by the methodology of Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

Quantitative Phytochemical Analysis

Quantitative estimation of phytoconstituents Quantitative estimation of phytoconstituents like alkaloids [Harborne 1973], flavonoids [Bohm and Kocipai-Abyazan 1994], tannins [Van-Burden and Robinson 1981] and phenols [Edeoga et al., 2006], saponin [Obadoni and Ochuko 2001] of *A. fruticosa*

Antimicrobial activity

Antimicrobial activity of the extracts and fractions were tested using the agar diffusion method described by Collin et al., (1970). Varying concentrations of the extracts and fractions were prepared and tested against test pathogen. The plates were incubated at 37°C for 24 hours and the zone of inhibition measured.

Test Microorganisms:

Three microbial species were employed as test organism. These include, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* which were isolated from skin infected person.

For the preparation of extracts from leaves of *Acalypha fruticosa*, they were shade dried at room temperature and powdered by electric blender. 50g of each of the dried and powdered materials were macerated separately with 200 ml of ethanol for 48h. Extracts were concentrated under reduced pressure. The condensed products were weighed and kept at 4°C prior to test. The unidentified fractions were thus separated and tested for antibacterial and antifungal activities.

Preparation of Media**Nutrient Agar (NA-Himedia) Media for Bacteria****Composition of Media**

Animal's tissue	:	5.00 g	
Sodium chloride	:	5.00g	
Beef extract	:	1.50g	
Yeast extract	:	1.50g	
Agar	:	15.0g	

Preparation of medium:

Suspend 28.0 grams in 1000 ml distilled water. Heat to boiling and dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petriplates.

Potato Dextrose Agar (PDA-Himedia) Media for Fungi**Composition of Media.**

Potatoes infusion from	:	200.00g	
Dextrose	:	20.00g	
Agar	:	15.00g	

Preparation of medium:

Suspend 39.0 grams in 1000 ml distilled water. Heat to boiling and dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well before dispensing in specific work, when pH 3.5 is required; acidify the medium with sterile 10 % tartaric acid. The amount of acid required for 100 ml of sterile cooled medium is approximately 1 ml. do not heat the medium after addition of acid.

Results and Discussion:-

Phytochemical screening of the aqueous and ethanolic extracts of *A. fruticosa* leaves showed the presence of various medically active constituents (Table-1). The phytochemical constituents commonly present in both the leaf extracts include alkaloids, flavonoids, terpenoids, steroids, phenols, tannins, saponins, glycosides and coumarins. The ethanol solvent included large amounts of flavonoids, phenols, tannins, and saponins, whereas the aqueous solvent also contained high concentrations of these alkaloids. The ethanol solvent included moderate amounts of terpenoids, steroids, glycosides and coumarins, whereas the aqueous solvent contained moderate amounts of alkaloids, terpenoids, steroids, tannins, glycosides and coumarins. Ethanol is the most effective of these three solvents. As a result, we made the decision to conduct more studies using ethanol as a solvent. The presence of these powerful phytochemicals clearly demonstrated the considerable anti-microbial, anti-inflammatory, anti-diabetic, and antioxidant properties of *A. fruticosa* leaf extract.

The qualitative analysis of the phytochemicals of the various extracts of the aerial parts of *Acalypha fruticosa* indicated the presence of triterpenoids, steroids, saponins, tannins, phenols, alkaloids, flavonoids, anthraquinones and sugars in *Acalypha fruticosa* Mishra et al. 2009

Table 1:- Phytochemical screening of *Acalypha fruticosa* leaf.

S.no	Phytochemical compounds	Aqueous	Ethanol
1	Alkaloids	+	++

2	Flavonoids	++	++
3	Terpenoids	+	+
4	Steroids	+	+
5	Phenols	++	++
6	Tannins	+	++
7	Saponins	++	++
8	Glycosides	+	+
9	Coumarins	+	+

(++)Strongly present,(+) moderate present,(-)absent

Fig 1:- Phytochemical screening of *Acalypha fruticosa* in ethanol solvent.

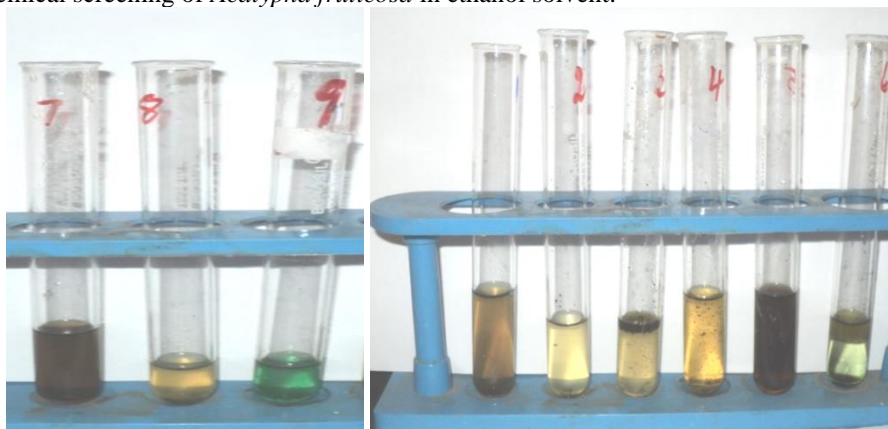
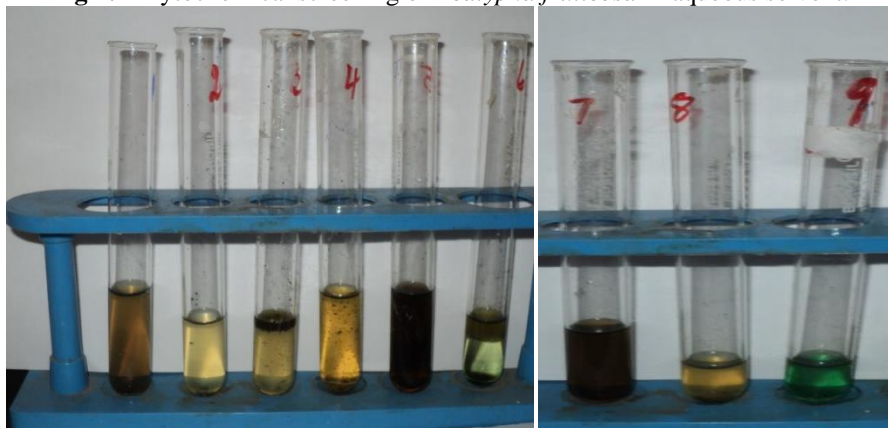


Fig 2:- Phytochemical screening of *Acalypha fruticosa* in aqueous solvent.



1. Tannin, 2. Glycoside, 3. Saponin, 4. Flavonoids, 5. Steroids, 6. Terpenoids, 7. phenols, 8. Alkaloids, 9. coumarins.

Quantitative analysis

The *Acalypha fruticosa* leaf contains a considerable amount of phenols, flavonoids, alkaloids, saponin, and tannin, according to quantitative analysis. Significant amount of total phenol (118mg/gm), tannin (45mg/gm), alkaloids (70mg/gm), saponin (80mg/gm) and flavonoids (80mg/gm) was presented (Table 2).

Quantitative estimation of phytoconstituents present in the powdered samples of *Acalypha fruticosa* showed that flavonoids were present in high amount when compared to alkaloids, tannins, phenols and steroids. These phytoconstituents are known to show medicinal activity, Mohanta et al., 2007 reported the presence of alkaloids, tannins, saponins, and cardenolides in *A. fimbriata*, *A. hispida*, *A. ornata*, *A. racemosa* and *A. wilkesiana*.

Table 2:- Quantitative analysis of *Acalypha fruticosa* leaf extract.

S.No	Test	Result (mg/gm)
1.	Phenol	80mg
2.	Flavonoids	118.8 mg
3.	Alkaloids	70 mg
4.	Saponin	80 mg
5.	Tannin	45 mg

Antimicrobial activity

The antimicrobial activity of plant extracts was detected by the indication of zone around the disc. The in vitro antimicrobial activity of the *Acalypha fruticosa* leaves extract against these bacteria and fungi were qualitatively assessed by the presence of inhibition zones represented in the photographic Fig 3. The inhibitory activities in culture media of the *Acalypha fruticosa* reported in Table 3 were comparable with standard antimicrobials viz. chloramphenicol and fluconazole.

Staphylococcus aureus is a Gram-positive extracellular bacterium that is the most common cause of skin and soft tissue infections, such as cellulitis, impetigo, and folliculitis (Todar, 2007).

Escherichia coli can cause gastroenteritis, urinary tract infections, and neonatal meningitis. In some cases, virulent strains are also responsible for haemolyticuremic syndrome, peritonitis, mastitis, septicaemia and pneumonia (McCaig, 2006).

Candida albicans is commensal and a constituent of the normal gut flora comprising microorganisms that live in the human mouth and gastrointestinal tract. Overgrowth of the fungus results in candidiasis. Candidiasis is often observed in immune compromised individuals, including HIV-infected patients. A common form of candidiasis restricted to the mucosal membranes in mouth or vagina is thrush, which is usually easily cured in people who are not immune compromised. (Ryan and Ray, 2004).

Table 3:- Antimicrobial activity of *Acalypha fruticosa* leaf Ethanolic extract.

Microbial Organism	50µl	100 µl	150 µl	Std.	Control
Escherichia coli(mm)	0.92±0.06	1.37±0.09	2.72±0.19	4.95±0.34	0
Staphylococcus aureus (mm)	1.73±0.12	3.08±0.21	5.11±0.35	7.62±0.53	0
Candida albicans(mm)	1.01±0.07	1.92±0.13	3.46±0.24	6.82±0.47	0

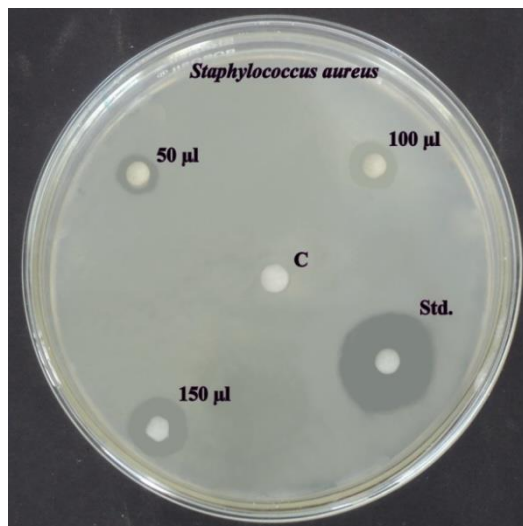
Values were expressed as Mean ± SD.

Bacterial standard - Chloramphenicol

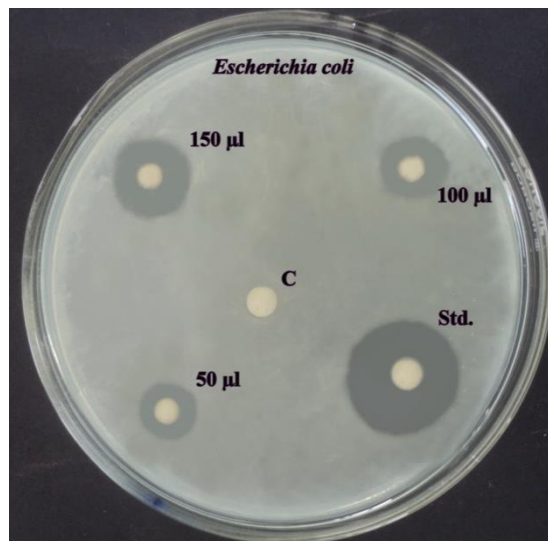
Fungal standard - Fluconazole

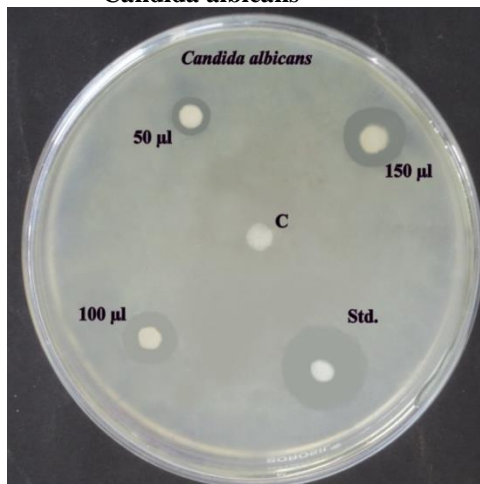
Fig 3:- Antimicrobial activity of *Acalypha fruticosa* leaf.

Staphylococcus aureus



Escherichia coli



Candida albicans**Conclusion:-**

The plants and its derivatives may be considered as good sources of natural phytochemicals for medicinal uses such as against epilepsy, stomachache, dyspepsia, venom antidote, rheumatism diseases, aging and other diseases related to radical mechanisms. Plant derived phytochemical therapy may be helpful for various free radical mediated diseases. Determination of the natural phytochemicals and antimicrobial compounds will help to develop new drug candidates for antimicrobial therapy.

The leaves of *Acalypha fruticosa* are a newly discovered potential source of natural antimicrobial compounds. The synergistic effect of plant extract against resistant bacteria and fungi leads to new choices for the treatment of infectious diseases. However, further studies are needed to understand the origin of this activity. Particularly, major constituents of the leaves need to be tested for their antimicrobial activities. Overall, the *Acalypha fruticosa* are a source of antimicrobial activity that can be important in disease prevention and health preservation.

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Conflicts of Interest

All authors declare that there are no conflicts of interest.

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