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STUDY OF THE PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *ACALYPHA INDICA*

K. Radhika[1], A. Kavitha Amirthanayagi[2] and V. Ramamurthy[3]*

[1]Assistant Professor, Department of Zoology, Govt. Arts College for Women (Autonomous), Pudukkottai, Tamil Nadu, India.
[2]Assistant Professor, Department of Zoology, Kunthavai Naacchiyaar Govt. Arts College for Women (Autonomous), Thanjavur-613 007.
[3]Assistant Professor, Department of Biochemistry, Marudupandiyar College, Vallam, Thanjavur, 613 403, Tamil Nadu, India.

***Corresponding Author: V. Ramamurthy**
Assistant Professor, Department of Biochemistry, Marudupandiyar College, Vallam, Thanjavur, 613 403, Tamil Nadu, India.

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ABSTRACT
Ethanol extracts of *Acalypha indica* were used traditionally in India for the treatment of skin diseases. The present study was investigated for *invitro* antimicrobial activity against pathogens namely *Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes, Pseudomonas auroginosa, Klebsiella pnemonia, Aspergillus níger, Trichoderma viride* and *Candida albicans* using the agar well diffusion method. The results relevant that the plant leaf extract possessed the highest inhibitory activity against the bacteria (*Klebsiella pnemonia* in 22 mm) and fungi (*Aspergillus* in 15 mm). Among the leaf extracts of *Acalypha indica* possess the highest inhibitory activity then the root extracts. In parallel study was performed to identify the distribution and the concentration of the phytocompounds in the roots and leaves of this plant. For this purpose we have prepared methanolic extracts from each part of the plant and we have studied them separately.

KEYWORDS: Antimicrobial activity, *Acalypha indica*, Phytochemical analysis.

INTRODUCTION
Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many of them based on their use in traditional medicine. Various medicinal plants have been used for daily life to treat disease all over the world. They have been used as a source of medicine. The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible has been traced to the occurrence of natural products with medicinal properties. In fact plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines. Higher plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry (Boominathan and Ramamurthy, 2009).

There has been a revival of interest in herbal medicines. This is due to increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new molecular structures as lead compounds from the plant kingdom. Plants are the basic source of knowledge in modern medicine. The basic molecular and active structures for synthetic fields are provided by rich natural sources. The worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in health care. The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is founds in “Rigveda”, which is said to have been written between 4500-1600 B.C. and is supposed to be the oldest repository of human knowledge. It is Ayurveda, the foundation of medicinal science of Hindu culture, in its eight division deals with specific properties of drugs and various aspects of science of life and the art of healing (Rastogi and Mehrotra, 2002).

Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including

hypersensitivity, immune-suppression and allergic reactions. This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants (Ramamurthy and Naveen, 2017).

Acalypha indica (English: Indian Acalypha, Indian Mercury, Indian Copperleaf, Indian Nettle, Three-seeded Mercury) is an herbaceous annual that has catkin-like inflorescences with cup-shaped involucres surrounding the minute flowers. It is mainly known for its root being attractive to domestic cats, and for its various medicinal uses. It occurs throughout the Tropics.

An erect annual herb that can be easily distinguished by the cup-shaped involucre that surrounds the small flowers in the catkin-like inflorescence. It can grow up to 1.2 m (3.9 ft) tall in favorable circumstances, but is usually smaller. The leaves are broad ovate, the leaf base is rounded to shortly attenuate. The leaf margin is basally 5-nerved and is crenate-serrate with an acute or obtuse apex. The petiole is 1.5–5.5 cm (0.59–2.17 in) long. The flower spikes are axillary, 2.5–6 cm (0.98–2.36 in) long, monoecious, with a rachis terminating in a triradiate hood. The tiny male flowers are white-green, located on the upper part of the flower spikes, and are ebracteate, minute, and clustered with vermiculiform anthers. The green female flowers are located lower on the spikes, and are subtended by 3–7 mm (0.12–0.28 in) long suborbicular-cuneiform, many-nerved, toothed bracts that are foliaceous. The ovary is hispid, 3-lobed. Styles are 3, each 2-fid. Capsules are hispid, 3-valved and concealed by a bract. The stem is striate (longitudally ribbed) and pubescent. The fruit is 1.5–2 mm (0.059–0.079 in), 3-lobed, tuberculate and pubescent.

Acalypha indica (Euphorbiaceae) is an herb distributed throughout India and other topical regions of the world. The various parts of the plant (leaves, roots, seeds and seed and seed oil) are widely used in a variety of ailments in traditional system of medicine such as Ayurveda and Siddha. The paste of plant leaves is used for the treatment of skin diseases by rural people. The aim of present research is, to determine the preliminary phytochemical constituents, antimicrobial activity of Ethanol, Methanol, Hexane and water extracts of the leaves and stems of *Acalypha indica*. Traditional medicines derived from medicinal plants are used by about 60% of the world's population. Though there are various approaches to control diseases and their secondary complications, herbal formulations are preferred due to lesser side effects and low cost. The use of and search for drugs and dietary supplements derived from plants has been increased in recent years. Botanists, Ethno pharmacologists, microbiologists, and chemists are combing the earth for phytochemicals and drugs which could be developed for treatment of highly infectious diseases in a natural way. While 30 to 50% of current pharmaceuticals are derived from plants, only a few of them are used as antimicrobials. Traditional healers have long used plants to prevent or cure infectious conditions. Plants are rich in a wide variety of secondary metabolites, such as Terpenoids, Tannins, Alkaloids, Flavonoids, saponins and Anthraquinones which have been found in vitro to have antimicrobial properties. *Acalypha indica* belonging to family Euphorbiaceae, commonly called Indian Copperleaf grows along the sides of the road which is often mistaken as a weed plant inspite of immense medicinal properties. Indian Copperleaf is a small erect herb, growing up to 60 cm or more. The ascending branches are angled and velvet-hairy. Leaves are broadly ovate, nearly triangular, rather coarsely toothed. Leaf stalks are as long as or longer than the 3-5 cm long blades. Flowers are stalkless, borne on erect axillary spikes longer than the leaves. Male flowers are minute, crowded distally. Female flowers are scattered along the inflorescence axis, each subtended by a conspicuous semicupular leaf-like toothed green bract nearly 7 mm long. Capsule is bristly, 1 mm broad (Pant et al., 2003; Prajapati et al., 2003; Qureshi et al., 2008). Some of the medicinal properties used by folk are: Juice of the root and leaves given to children as expectorant and emetic. The leaves, in decoction or powdered form, are used as a laxative. For constipation, an anal suppository of the bruised leaves helps relax the constricted sphincter ani muscle.In Philippines, decoction of leaves used for dysentery. Leaves mixed with common salt applied to scabies. In Indian pharmacopoeia, it is used as an expectorant. Also used for the prevention and reversal of atherosclerotic disease. Used for pneumonia, asthma and rheumatism. In Tamilnadu, India, the Paliyar tribes of Shenbagathope use the entire plant for bronchitis, a decoction of the herb for tooth and ear aches and paste of the leaves is applied to burns. Poultice of bruised leaves used for syphilitic ulcers, to maggot-eaten sores and as an emollient to snake bites. Decoction of leaves used as instillation for earaches and for periauricular poultice or compress. Leaves mixed with garlic used as anthelminthic Root, bruised in water, used as a cathartic. Powdered dried leaves used for bed sores. Juice of, fresh leaves, mixed with oil or lime are used for rheumatic complaints. Bruised leaves used as "suppository" in constipation, assumed to work through decrease of the sphincter anti contraction. Against this background information and appreciating the knowledge of medicinal plants an effect has been made in this study to evaluate the antimicrobial efficacy of *Acalypha indica* medicinal plants and also characterizing them by screening preliminary by phytochemical analysis. The study also pertains to inculcate the subject about the utilization of natural flora as therapeutic agents.

MATERIALS AND METHODS

Acalypha indica belongs to the family Euphorbiaceae was collected from Pudukkottai District, Tamil Nadu,

India and identified by the special key given Cambell flora. The leaves of *Acalypha indica* were washed with sterile distilled water. After, the leaves were shade dried and powdered by using pestle and mortar. Twenty five gram of powder was filled in the thimble and extracted successively with ethanol using a Soxhlet extractor for 48 h. The extracts were concentrated using rotary flash evaporator and preserved at 5^{o}C in airtight bottle until further use. All the extracts were subjected to phytochemical analysis and antimicrobial activity assay.

Phytochemical Analysis

The preliminary phytochemical evaluation of leaves was carried on extract prepared by successive extraction method in Soxhlet. The previously dried powdered leaves (50 gm) were extracted in a Soxhlet apparatus with ethanol and water successively. The resultant extracts were evaporated to dryness under vacuum. These extract were subjected to chemical test for different phytoconstituents viz. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, mucilage and resins etc.

Chemical tests were carried out on the ethanol and aqueous extracts using procedures to identify the phytochemicals as described by Sofowara (1993); Trease and Evans (1983); Harborne (1973). Alkaloids, carbohydrates, tannins and phenols, flavonoides, gums and mucilage, fixed oils and fats and saponins were qualitatively analyzed.

Antimicrobial Assay: The following organisms were employed for this study as test organisms: Bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pyogenes*, *Pseudomonas auroginosa* and *Klebsiella pnemonia.* Fungi such as *Aspergillus níger*, *Trichoderma viride* and *Candida albicans*. The test microbial pathogen cultures were obtained from the stock cultures maintained in specific agar medium.

Antibacterial and antifungal activity of above mentioned extracts were tested using the agar diffusion method described by Collins and Lyne, (1970). All the above-mentioned bacteria were inoculated into nutrient agar medium and fungi inoculated to potato dextrose agar medium. The well of 8 mm diameter was punctured in the culture medium using sterile cork borer. Different extracts were administered to fullness in each well. Culture plates were incubated at 37^{o}C for 24 h in bacteria and incubated at 37^{o}C for 4 days in fungi. Bioactivity was determined by measuring diameter of inhibition zones in mm. Solvents used for extraction served as control.

RESULTS AND DISCUSSION

Qualitative phytochemical analyses for alkaloids, carbohydrates, tannins, phenols, gums and mucilage, fixed oils and fats, saponins, proteins, volatile oils, flavonoids and steroids were screened in ethanolic extracts of the selected medicinal plants *Acalypha indica*. The screening of the extract indicated the presence of alkaloids, tannins and saponin in the ethanolic extracts of leaves (Table 1). The chemical test of hydroalcoholic extract of *Acalypha indica* revealed that it contains alkaloids tannins and saponins, this observation was accordance with the earlier phytochemical reports on this plant. Incidentally many of that alkaloids other plant sources have been identified to impair release of aetocoids in inflammation. Previous study in the naturally the ethanolic extracts of *Cassia alata* were subjected for phytochemical analysis. Phytochemical screening of the crude extract revealed the presence of alkaloids, cardiac glycosides, terpenoids, saponins, tannin, flavonoids, and steriods, but reducing sugars, carbonyl (aldehyde) and Phlobatanin show negative results (Makinde *et al*., 2007).

This plants growing under natural conditions contain the spectrum of secondary metabolites such as phenols, flavanoids, quinones, coumarins, tannins and their glycosides, alkaloids, essential oils etc., the importance of these substance as microbial agents against the pathogen has been emphasized by several workers (Sofowara, 1993). In the present study, it was clearly understood that the alcohol extracted maximum amount of the different type of metabolites present in the *Acalypha indica*. Boominathan and Ramamurthy (2009) reported that the phytochemical analysis of the *H. indicum* and *C. procumbens* extracts showed the presence of tannins, alkaloids, flavonoids and phenolic compounds. Tannins have been found to form irreversible complexes with proline-rich proteins.

Ethanolic extracts were tested against bacteria and fungi. Among the extracts, the leaf extract of *Acalypha indica* were effective against bacteria and fungi. The antibacterial activity crude extract is shown in Table 2. The extracts showed maximum activity against *Staphylococcus aureus*, *Streptococcus pyogens*, *Klebsiella pnemonia* and *Pseudomonas aurogonosa*. These data revealed that leaf extracts of *Acalypha indica* exhibited significant antimicrobial activity. In testing, inhibition zone increased with increase in drug concentrations and thus exhibiting concentration dependent activity. The plants are the vital source of innumerable number of antimicrobial compounds. Several phytoconstituents like flavanoids (Tsuchiya *et al.*, 1996), phenolics and polyphenols (Mason and Wasserman, 1987), tannins (Ya *et al.*, 1988), terpenoids (Scortichini and Pia Rossi, 1991), sesquiterpenes (Goren, 1996) etc., are effective antimicrobial substances against a wide range of microorganisms.

The extracts showed maximum activity against *E. coli*, *Enterobacter aerogenes* and *Alcaligenes faecalis*. These data revealed that extracts of *R. tetraphylla* exhibited significant antibacterial activity (Suresh *et al*., 2008). Apart from antimicrobial activity exhibited by tannins, they also react with proteins to provide the typical tanning effect. Medicinally, this is important for the

treatment of inflamed or ulcerated tissues (Mota *et al.*, 1985). Tannins have important roles such as stable and potent antioxidants (Trease and Evans, 1983). Herbs that have tannins as their main component are astringent in nature and used for treating intestinal disorders such as diarrhoea and dysentery, thus exhibiting antimicrobial activity. One of the largest groups of chemical produced by plant is the alkaloids and their amazing effect on humans has led to the development of powerful pain killer medications (Raffauf, 1996).

H. indicum and *C. procumbens* are used for the treatment of inflammation, wound healing, antitumor and antianelgesic, hence different formulations could be prepared for clinical trials (Boominathan and Ramamurthy, 2009). It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin. Studies are in progress to further evaluate the mechanisms of action *Acalypha indica* extracts on some organisms associated with human diseases. Hence, the present study suggests that pathogenic microorganisms may become resistant to existing drugs. Moreover, this study shows that some plants show much promise in the development of phytomedicines having antimicrobial properties. In this endeavour, traditional herbal medicines must perforce be granted the benefits of modern science and technology to serve further global needs. The drugs derived from herbs may have the possibility of use in medicine because of their antibacterial activity. With onset of scientific research in Ayurvedic system of medicine, it is becoming clearer that the medicinal herbs have a potential in today's synthetic era, as numbers of medicines are becoming resistant. According to one estimate only 20% of the plant flora has been studied and 60% of synthetic medicines owe their origin to plants. Ancient knowledge coupled with scientific principles can come to the forefront and provide us with powerful remedies to eradicate the diseases.

Table 1: Qualitative Phytochemical screening on extracts of *Acalypha indica*.

S. No	Name of Test	Test applied / Reagent used	Leaf extract
1	Alkaloids	A] Mayer's B] Wagner's C] Hagner's D]) Dragndorff's test	+ + + +
2	Flavanoids	HCl and magnesium turnings	+
3	Carbohydrate	Molisch's test	+
4	Tannins & Phenols	A] 10% Lead acetate B] $Fecl_3$	+ +
5	Test for Steroids	A] Salkowski's Test B] Libermann-Burchard's Test	+ +
6	Gums & Mucilages	Alcoholic Precipitation	-
7	Fixed oil & Fats	Spot test	+
8	Saponins	Foam test	-
9	Phytosterols	LB test	+
10	Volatile oils	Hydro distillation method	+
11	Protein & free amino acids.	A] Biuret test B] Ninhydrin test C] Xanthoprotein test	+ + +

-, absents; +, present;

Table 2: Antimicrobial efficacy of *Acalypha indica*.

S. No	Organism	Zone of inhibition in mm
	Bacterial species	
1	*Staphylococcus aureus*	21
2	*Bacillus subtilis*	14
3	*Streptococcus pyogens*	24
4	*Pseudomonas aurogonosa*	18
5	*Klebsiella pnemonia*	22
	Fungal species	
6	*Aspergillus niger*	15
7	*Trichoderma viride*	10
8	*Candida albicans*	12

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