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Phytochemical and Antibacterial Activity Screening of Acalypha Indica and Ocimum Tenuiflorum

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Abstract: ***The aim of this study was to assess the secondary metabolities of Ocimum tenuiflorum and Acalypha indica. The ethanol and methanol extracts were screened phytochemically to check for the presence of various bioactive compounds. Furthermore, the extracts were qualitatively analysed by high-performance liquid chromatography, which showed the presence of flavonoids (keampferol and quercetin) and tannins (tannic acid, gallic acid and resorcinol). The extracts at various concentrations were used against common pathogens isolated from street food. It was seen that as the concentration increases, the activity exhibited also increased. It also showed less activity against the natural beneficial bacteria present in the human body. Methanol extracts, of both the plants, showed more activity when compared with the ethanol extract. This can be due to the ability of the methanol solvent to extract low-molecular-weight bioactive compounds, which results in the accumulation of more bioactive compounds.***

Keywords: Methanol extract; Ethanol extract; Flavonoids; Tannins; High-performance liquid chromatography

I. INTRODUCTION

Plants are the naturally available medicines that are used as a potential material for maintaining good health and conditions. The reasons for considering herbal plant sources are as follows: (1) herbs can act in pathways similar to those of pharmaceutical medications, (2) the bioactive compounds find their way into the arsenal of the antimicrobial drug easily, and (3) the public is becoming aware of the use of chemical antibiotics, their side effects, and starts looking for alternative sources. Many potential drugs have been derived from various plants that have interdependent pathways for synthesizing effective metabolites. The higher the concentration of phytochemical in a plant, the greater therapeutic potency or medicinal importance of that plant. Research shows that plants exhibit antimalarial, antibacterial, antioxidant, antitumour, antifungal, anticancerous, and antiviral properties, which have been exploited extensively for developing new drugs against a wide range of ailments. The potential of higher plants as a source of drugs is still unexplored (Verma *et al.*, 2008).

A. Herbal Extracts as Antimicrobial Agents

Antibiotics are effective treatments for the microbial diseases. But, in recent years, bacteria under continuous stress due to various environmental factors result in developing resistance, which is an adaptation of bacteria to the changing environment (Schlundt *et al.*, 2004). Owing to the lack of new antimicrobial agents to combat them, there is a rapid emergence of resistance pathogens. The safe and effective compounds that fight against these drug resistant pathogens, especially the pathogens associated with the food borne illness, could be plant-derived antimicrobial agents (Gyawali R *et al.*, 2017). Plant extracts in combination with the antibiotics can restore the efficacy of the already existing drugs against resistant pathogenic bacteria (Abreu *et al.*, 2012).

Plant extracts, organic acids, essential oils, and bacteriocins are natural antimicrobial agents that could be used as a good alternative to ensure food safety.

Food antimicrobial agents are substances that cause microbial death or delay the microbial growth in food. The major target for such antimicrobial agents is food-poisoning microorganisms and spoilage organisms whose metabolic end products cause off-odours, off-flavours and discoloration (Davidson, 2001).

Antimicrobial agents from plants are mostly used in the form of biofilms and edible coatings in food systems. These antimicrobial films and coatings slowly diffuse inside the food package, thus extending the duration of the antimicrobial activity. The direct usage of antimicrobial agents in food system is avoided as these agents could diffuse into food bulks, and the concentration of antimicrobial agents at food surface could reduce and eventually results in microbial growth and spoilage at surface. The antimicrobial films are used in meat industry, beverage industry, and pharmaceuticals industry as per the FDA-prescribed format.

B. Acalypha indica

A. indica, commonly known as kuppaimeni in Tamil, Indian copperleaf, or three-seeded mercury, is widely used in traditional medicinal systems such as Ayurveda, unani, siddha, and so on. The whole plant has been extensively used as a medicinal plant owing to the presence of phytochemicals such as alkaloids, flavonoids, phenolic compounds, saponins, and sterols (Chitravadivu *et al.*, 2009). This plant is an emetic, expectorant, laxative and diuretic, which is used to treat bronchitis, pneumonia, asthma, and pulmonary tuberculosis. Leaves have laxative and antiparasiticide properties. Powdered dry leaves are used to expel intestinal worms. Fresh leaf juice is used to treat arthritis and scabies.

C. Ocimum Tenuiflorum

O. tenuiflorum, popularly known as holy basil, tulasi in tamil, is native to the Indian subcontinent widely used in the traditional medicinal system. O. tenuiflorum is a very important herb in the Ayurvedic tradition. It is a pungently aromatic, warming, antiseptic herb; Some of its applications are that it induces perspiration, lowers fevers, relaxes spasms, eases pain, clears bacterial infections, strengthens the immune and nervous systems, reduces inflammations, and benefits the digestive system. This herb is used externally as antiseptic to treat skin infections. The seeds are used as a tonic. The herb has been suggested to possess antifertility, anticancer, antidiabetic, antifungal, hepato protective, cardio protective, antiemetic, antispasmodic, analgesic, adaptogenic and diaphoretic actions.

II. MATERIALS AND METHODS

A. Plant Collection

O. tenuiflorum (Tulasi) was purchased from a flower shop in Chennai, and *A. indica* (Kuppaimeni) was collected from the Loyola College campus. The leaves of these plants were washed, shade-dried for 24 hours, powdered, and stored in air tight containers for further analysis.

B. Plant Extract

Extracts of *A. indica* and *O. tenuiflorum* were prepared by dissolving 20 gms of the leaf powder in 200 ml of ethanol and methanol separately. This mixture was kept in a shaker for 24 hours, filtered, and allowed to evaporate. The extract was stored at -20°C for further use.

C. Phytochemical Screening

1) *Test for Flavonoids:* To 1 ml of the extract, 2 ml of NaOH was added, and the appearance of intense yellow colour occurs, which on addition of dilute acids, appearance of pale yellow colour indicated the presence of flavonoids.
2) *Test for Saponins:* Few amount of the extract was dissolved in a test tube containing 3 ml of hot distilled water. Then, the mixture was shaken vigorously for 1 minute, and the foam was observed.
3) *Test for Terpenoids:* To 2 ml of the extract, 2 ml of chloroform and 3 ml of concentrated sulphuric acid was added, and the appearance of reddish brown colour indicates the presence of terpenoids.
4) *Test for Tannins:* To the extract, 2 ml of bromine water was added. Disappearance of yellow colour indicates the presence of tannins.
5) *Test for Glycosides:* To the extract, 2 ml of sulphuric acid was added, and the red colouration indicates the presence of glycosides.
6) *Test for Steroids:* To the extract, 2 ml of chloroform and concentrated sulphuric acid was added. The red layer appears at the lower level of chloroform.

D. High-Performance Liquid Chromatography

The specifications of HPLC for the analyses of the samples are as follows:
Equipment: HPLC, Waters make, pump-515
Column: RP, C-18; (5µm particle size)
Injection volume : 20µl
Temperature: 28°C
Mobile phase:
Solvent A, phosphate buffer (pH 5.8)
Solvent B, acetonitrile
Flow rate: 1 ml/min

Detector: UV–VIS detector (model 2487 Waters make)
Wavelength: 254 nm
The analyzed samples were compared with the standards from the literature to identify the compounds present in the analyzed samples.

E. Antibacterial Assay

The antibacterial activity for the leaf extract of *A. indica* and *O. tenuiflorum* was evaluated by agar well-diffusion method on Muller–Hinton Agar. The leaf extracts were prepared at different concentrations (100 mg to 600 mg) with dimethylsulfoxide as a solvent. The bacterial cultures isolated from the food samples (previous work by the authors) were used to examine the antibacterial activity of the leaf extract. Fresh inoculum was prepared by inoculating the bacterial culture in nutrient broth and incubated for 2 hours. The Muller–Hinton agar plates were prepared, and wells were punched. The cultures were spread evenly by the swabbing method. The wells were filled with 25µl of the extract with streptomycin antibiotic discs as a positive control. The plates were incubated at 37°C for 24 hours. Using the standard scale (mm in measurement), the zone of clearance was measured.

III.RESULTS

A. Phytochemical Screening

Phytochemical analyses revealed that *A.indica* and *O.tenuiflorum* contained alkaloids, tannins, saponins, terpenoids, flavonoids, and phenolic compounds in the extracts which are shown in the Table 1.

TABLE 1: PHYTOCHEMCIAL SCREENING

Phytochemical test	Acalypha indica		Ocimum tenuiflorum	
	Methanol extract	Ethanol extract	Methanol extract	Ethanol extract
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Tannins	+	-	+	+
Saponins	+	+	+	-
Carbohydrates	+	-	-	-
Terpenoids	+	-	-	+
Glycosides	-	-	-	-

Note(s): +, Presence; -, Absence.

B. High-Performance Liquid Chromatography

High-performance liquid chromatography was performed for the methanol extract of both the plants. This is a qualitative analysis carried out to determine the presence of various bioactive compounds. The chromatogram shows various peaks at different retention period of time. The chromatographs are shown in Fig 1 and 2. The bioactive compounds that were identified are given in the tables 2 & 3. The compounds kaempherol and quercetin belongs to the group of flavonoids, whereas gallic acid, tannic acid, and resorcinol belong to the group of tannins.

TABLE 2: HPLC ANALYSIS OF METHANOL EXTRACT OF *A. INDICA*

Peak	Retention time (min)	Area (mAU*s)	Amount/area	Compound
1	5.899	1819.96753	5.4946	Kaempherol
2	29.298	144.19958	6.9434	Quercetin

TABLE 3: HPLC ANALYSIS OF METHANOL EXTRACT OF O.*TENUIFLORUM*

Peak	Retention time (min)	Width (min)	Area (mAU*s)	Height (mAU)	Area %	Compound
1	1.579	0.2128	3633.00146	219.80565	17.3301	Gallic acid
2	3.451	0.2999	1446.57849	67.52967	6.9005	Tannic acid
3	7.502	0.2862	336.06171	16.7901	1.6031	Resorcinol
4	29.194	0.7351	998.53998	17.51589	4.7632	Quercetin

C. Antibacterial Activity of the Extracts

A total of 7 isolates were used against the prepared extracts. These isolates were isolated from a common street food, pani puri water, and were biochemically characterized. As per the test results, possible genus of the isolates were Bacillus, Escherichia, Streptococcus, Pseudomonas, and Klebsiella. When these organisms were tested against the extracts of the plant, zone of clearance was observed. The efficiency of the plant extracts on the bacterial cultures is shown in Table 4 and 5.

TABLE 4: EFFICIENCY OF A.INDICA EXTRACT AGAINST BACTERIAL CULTURES

Acalypha indica												
Isolates	Ethanol extract concentration (mg)						Methanol extract concentration (mg)					
	100	200	300	400	500	600	100	200	300	400	500	600
1	-	-	10	10	11	11	-	-	10	10	11	11
2	-	-	-	-	10	11	-	10	11	11	12	12
3	-	8	10	11	11	12	-	-	10	12	14	15
4	-	8	10	10	12	12	-	10	10	10	11	11
5	-	6	11	11	12	12	-	12	12	13	14	14
6	-	9	10	11	11	12	-	10	10	11	12	13
7	-	6	10	10	11	12	-	10	11	11	12	12

TABLE 5: EFFICIENCY OF O.TENUIFLORUM EXTRACT AGAINST BACTERIAL CULTURES

Ocimum tenuiflorum												
Isolates	Ethanol extract concentration (mg)						Methanol extract concentration (mg)					
	100	200	300	400	500	600	100	200	300	400	500	600
1	-	9	10	11	12	12	-	10	11	12	12	13
2	-	9	10	11	11	12	-	10	10	11	11	12
3	-	8	10	10	11	11	-	-	10	11	11	12
4	-	8	10	11	11	12	-	9	10	10	11	11
5	-	-	10	11	11	12	-	10	11	12	13	13
6	-	6	10	11	12	12	-	10	11	11	12	12
7	-	8	10	11	12	12	-	10	11	12	13	13

IV. DISCUSSION

A. Phytochemical Screening

Phytochemical screening showed the presence of various bioactive compounds such as alkaloids, flavonoids, tannins, and saponins. These compounds are non-nutritive chemicals, which have enormous significance due to their beneficial effects on human health. HPLC analyses showed the presence of bioactive compounds: flavonoids and tannins. Flavonoids, hydroxylated phenolic substances, are synthesized by the plants in response to the microbial infections. They exhibit antifungal and antibacterial activity against some human pathogenic fungi and bacteria (Owoyale JA *et al.*, 2005).

Their antibacterial activity is due to the formation of complex with extracellular and soluble proteins thus inactivating the proteins. This leads to the disruption of microbial membranes (Tsuchiya *et al.*, 1996). Kaempferol is an important flavonoid found abundantly in tea, broccoli, apples, strawberries, and beans (Somerset & Johannot, 2008). It is a strong antioxidant and helps to prevent oxidative damage of cells, lipids and DNA. Kaempferol seems to prevent arteriosclerosis by inhibiting the oxidation of low density lipoprotein and the formation of platelets in the blood (Kowalski *et al.*, 2005). Studies have also confirmed that kaempferol acts as a chemopreventive agent as they exhibit less toxicity to normal cells in comparison to chemotherapy drugs (Zhang *et al.*, 2008). Fig. 1 shows the molecular structure of kaempferol.

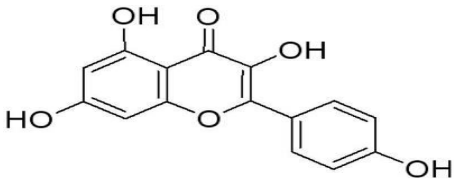

Fig. 1: Structure of kaempferol

Quercetin is categorized as a pigmented flavonol, a subclass of flavonoid compounds that are abundant in variety of foods including apples, berries, grapes, onions, shallots, and tomatoes. It possesses anti-inflammatory potential that can be expressed on different types of cells, both in human and animal models (Chirumbolo S, 2010). They also possess antiproliferation and gene expression–changing capacities in vitro (Boots *et al.*, 2008). Fig. 2 shows the structure of quercetin

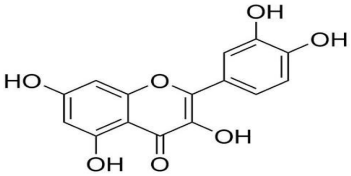

Fig. 2: Structure of quercetin

Tannins are polyphenols present in medicinal plants. They have astringent properties which hasten the healing of wounds and inflamed mucous membrane due to their physiological activities such as antioxidant, antimicrobial, and anti-inflammatory properties (Sule *et al.*, 2010). Tannic acid (Fig. 3), gallic acid (Fig. 4), and resorcinol (Fig. 5) were the tannins present in the analysed samples, which provide the antibacterial property to the plant. The antimicrobial mechanisms of tannins can be summarized as follows. (i) The astringent property of the tannin may induce complexation with enzymes or substrates. Many microbial enzymes in raw culture filtrates or in purified forms are inhibited when mixed with tannins. (ii) A tannin's toxicity may be related to its action on the membranes of the microorganisms. (iii) Complexation of metal ions by tannins may account for tannin toxicity (Chung *et al.*, 1998).

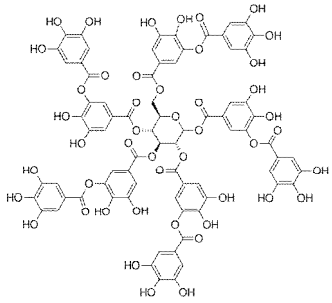

Fig. 3: Structure of tannic acid

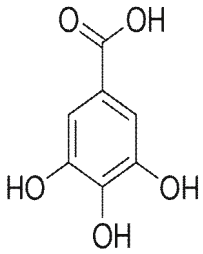

Fig. 4: Structure of gallic acid

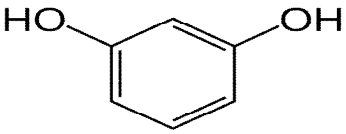

Fig. 5: Structure of resorcinol

B. Antibacterial Assay

The antibacterial assay by disc diffusion method showed no activity against the pathogens. This negative result could be due to the difference in the phytochemicals present or the composition of the extracts and the methodology of the antimicrobial tests (Pochapski *et al.*, 2011). The plant extracts were inoculated directly into the wells of the culture swabbed agar to diffuse. After incubation, the extracts showed antibacterial activity against the inoculated organism. The methanol extracts of both the plants *A. indica* and *O. tenuiflorum* showed higher effect when compared with ethanol extracts. Ethanol and methanol are polar solvents used frequently to recover polyphenols from the plants. Ethanol has been commonly used for polyphenol extraction and is safe for human consumption. Methanol was found to be more efficient in the extraction of lower molecular weight polyphenols (Quy Diem Do *et al.*, 2014). Hence, the methanol extract may contain maximum amount of alkaloids and flavonoids compared with ethanol extract which could be reason for higher antibacterial activity.

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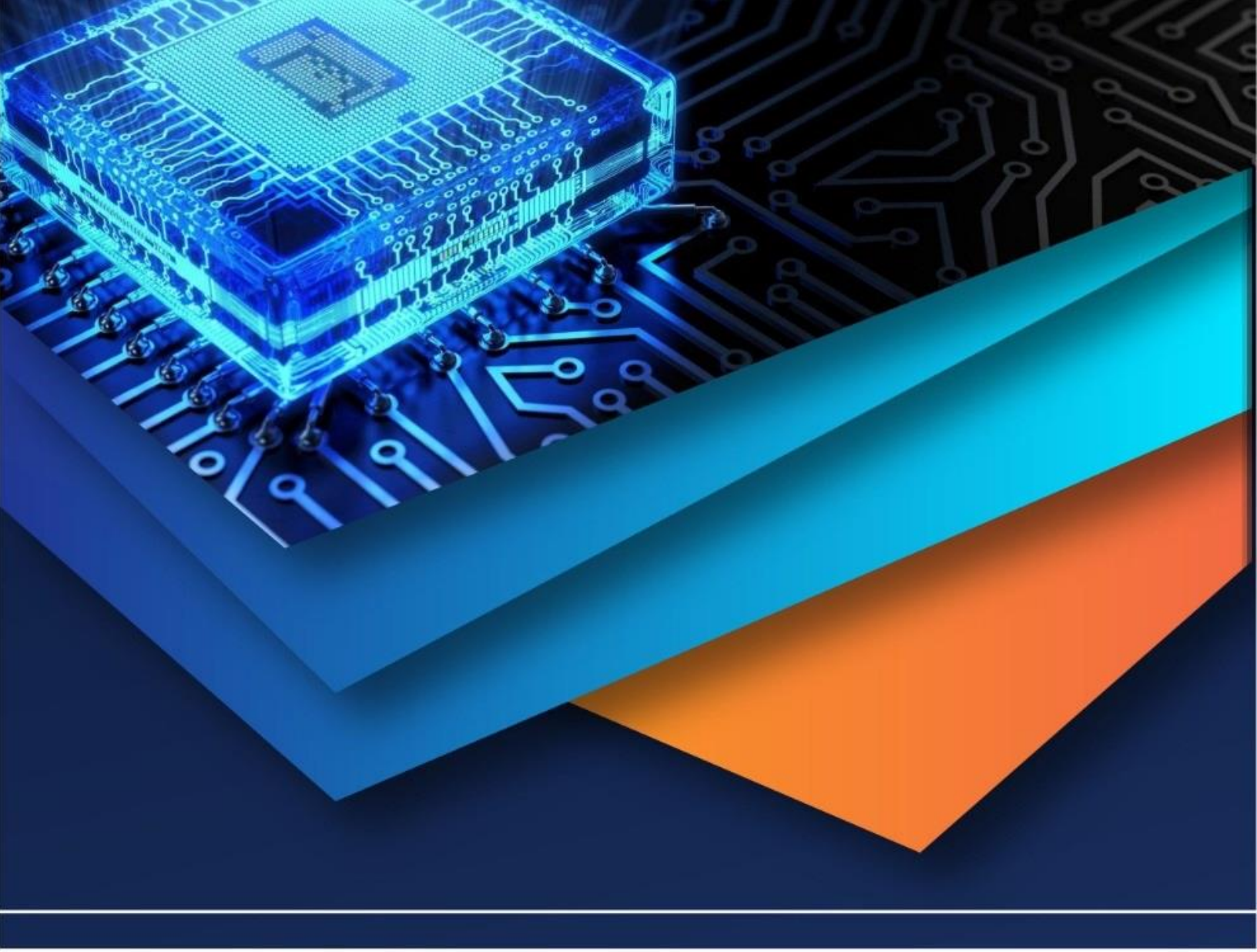

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