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PHYTOCHEMICAL COMPOSITION, TANNIN CONTENT AND ANTIBACTERIAL ACTIVITY OF LEAF AND CALLUS EXTRACTS OF *PHYLLANTHUS VIRGATUS* G. FORST

M. R. Ramachandra Kumar*[1], S. Ravi Kumar[2]* and B. Janarthanam[3]

[1]Research Scholar PG and Research Department of Botany, Presidency College, Triplicane, Chennai 600 005. Tamil Nadu, India.

[2]Assistant Professor PG and Research Department of Botany, Presidency College, Triplicane, Chennai 600 005. Tamil Nadu, India.

[3]Chief Scientist Omnigreen Organic Biopark Pvt. Ltd., Plant Biotechnology Division, Chennai- 600 087, Tamil Nadu, India.

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***Corresponding Author**
M. R. Ramachandra Kumar
Research Scholar PG and Research Department of Botany, Presidency College, Triplicane, Chennai 600 005. Tamil Nadu, India.

ABSTRACT

The present study has been conducted to examine the phytochemical composition, tannin content, and antibacterial activity of leaf and callus extracts of *Phyllanthus virgatus*. Phytochemical screening of various extracts such as aqueous, ethanol, chloroform, acetone and petroleum ether of leaf and callus extracts, revealed the presence of tannins, saponins, phenols, flavonoids, cardiac glycosides, coumarins, terpenoids, alkaloids and steroids. The leaf and callus extracts were evaluated for tannins content with tannic acid as standard. The optimum yield of tannins was found in ethanol callus extract (8.01±0.2 mg TAE/ g) of *Phyllanthus virgatus.* Different concentrations of ethanolic leaf and callus extracts were tested for the anti-bacterial activity against *Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Escherichia coli* using the agar disc diffusion technique. The ethanolic callus extracts from *Phyllanthus virgatus* had a superior level of antimicrobial activity. The powerful antibacterial effect is attributed to the greater amount of tannins compound in the ethanolic callus extracts of *Phyllanthus virgatus*.

KEYWORDS: *Phyllanthus virgatus*, phytochemical analysis, Tannins. Antibacterial activity.

INTRODUCTION

Medicinal plants are the most exclusive source of life-saving drugs for majority of the world's population. The utilization of plant cells for the production of natural or recombinant compounds of commercial interest has gained increasing attention over the past decades.[1] The secondary metabolites are known to play a major role in the adaptation of plants to their environment and also represent an important source of pharmaceuticals.[2]

Tannins are high polyphenolic compounds present in plants, foods, and beverages, soluble in water and polar organic solvents. These tannins are classified as hydrolysable and condensed tannins based on their chemical structure and biological activity.[3] Both types of tannins are capable of forming strong complexes with certain type of proteins depressing the rate of their digestion.[4] Tannins may also bind to bacterial enzymes or form indigestible complexes with cell wall carbohydrates reducing the cell wall digestibility.[5] In recent years, tannins have been investigated to possess high antioxidants,[6] antimicrobial,[7] gastro protective, and anti-ulcerogenic activities,[8] Moreover, tannins have been investigated as potent inhibitors of lipid peroxidation in heart mitochondria[9] and possess anti-fibrotic effects.[10] Due to these therapeutic properties, tannins can be used in the treatment of various diseases to improve human health.

Free radicals (superoxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide, hypochloric acid and peroxynitrite) produced during aerobic metabolism in the body, can cause oxidative damage of amino acids, lipids, proteins and DNA.[11] Oxidative stress, induced by oxygen radicals, is believed to be a primary factor in various degenerative diseases as well as in the normal process of ageing. Several biochemical reactions in our body generate Reactive Oxygen Species (ROS) and these are capable of damaging crucial bio-molecules. If they are not effectively scavenged by cellular constituents, they lead to disease conditions. Antioxidants stabilize or deactivate free radicals, often before they attack targets in biological cells.[12] Phenols and flavonoids are widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-

inflammatory, anticarcinogenic activity, etc.[13] It has been established that oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others.[14,15,16]

In the last few decades, plants belong to the genus *Phyllanthus* (Euphorbiaceae) came in focus due to their wide distribution, diversity in the genus, broad therapeutic potential and variety in their secondary metabolites. Substantial amount of the genus are used widely in traditional medicine for the treatment of flu, dropsy, diabetes, jaundice, gall bladder calculus, liver disease.[17] *Phyllanthus virgatus* is rich in polyphenols and is known traditionally for its antioxidant.[18] Antimicrobial, antiseptic, anti-inflammatory agent, anticancer activity and antidiabetic properties of various *Phyllanthus* species have been investigated in experimental models.[19] Therefore, the purpose of the present investigation was to evaluate the total tannin content and antibacterial activity of leaf and callus extracts of Phyllanthus *virgatus*.

MATERIAL AND METHODS

Collection of plant material

The healthy wild *Phyllanthus virgatus* plants (figure 1) were collected from Chengalpattu, Tamilnadu, India and were raised in pots containing soil and farm yard manure (1:1) under greenhouse conditions at PG & Research Department of Botany, Presidency College, Chennai and heathy leaves explants used for further experimental studies.

Preparation of the plant extract

Preparation of the extracts was done according to combination of the methods used by Pizzale *et al.*,[20] and Lu and Foo [21]. About 15g of dried leaf and callus fine powder of *Phyllanthus virgatus* plant materials were extracted with 150 ml acetone, ethanol (75%), chloroform, petroleum ether and aqueous extract for 1 min using an Ultra Turax mixer (13,000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman No.1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-evator at 40°C to a constant weight and then dissolved in respective solvents. The concentrated extracts were stored in airtight container in refrigerator below 10ºC.

Phytochemical Screening from leaf and callus extracts of *Phyllanthus virgatus*

The phytochemical screening of peel extracts were assessed by standard methods.[22,23] Phytochemical screening was carried out on the peel extracts using different solvents to identify the major natural chemical groups such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides, cardiac glycosides**,** coumarins and steroids. General reactions in these analyses revealed the presence or absence of these compounds in the peel extracts tested.

Estimation of Tannins content in leaf and callus extracts of *Phyllanthus virgatus*

Tannins content in leaf and callus extract of *Phyllanthus virgatus* was estimated by standard method.[24] The ethanol peel extracts (1 ml) were mixed with Folin-Ciocalteau's reagent (0.5 mL), followed by the addition of saturated sodium carbonate (Na_2CO_3) solution (1 mL) and distilled water (8 mL). The reaction mixture was allowed to stand for 30 min at room temperature. The supernatant was obtained by centrifugation and absorbance was recorded at 725 nm using UV-Visible Spectrophotometer. Different concentrations of standard tannic acid were prepared and the absorbance of various tannic acid concentrations was plotted for a standard graph. The tannin content was expressed as µg tannic acid equivalent (TAE) per gram of the sample.

Antibacterial activity from leaf and callus extracts of *Phyllanthus virgatus*

The ethanol leaf and callus extracts of *Phyllanthus virgatus* plant were used for antibacterial study.[25,26] Different concentration (10, 20 and 30 mg/ml) of the concentrated ethanol leaf and callus extracts was tested for its antimicrobial strain such as *Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Escherichia coli.* The bacterial cultures were grown in Mueller Hinton Agar and Mueller Hinton broth (Himedia).[27]

Antibacterial activity was measured using the standard method of diffusion disc plates on agar.[28] Then 0.1ml of each culture of bacteria was spread on agar plate surfaces. For antibacterial assay, all bacterial strains were grown in Mueller Hinton Broth Medium (Hi media) for 24 hours at 37°C and plated on Mueller Hinton Agar (Hi media) for agar diffusion experiments. Paper disc (6mm in diameter) were placed on the agar medium to load 20µl of different concentrations of ethanol peel extracts of *Phyllanthus virgatus* were tested. Inhibition diameters were measured after incubation for 24 - 48 hours at 37°C.

Blanks of solvent only (processed in the same way), were also tested for antibacterial activity.

RESULTS AND DISCUSSION

In the present study, the phytochemical screening of five different extracts such as ethanol, chloroform, petroleum ether, acetone and aqueous studied, showed that the ethanolic callus extract of *Phyllanthus virgatus* were rich in secondary metabolites such as tannins, saponins, flavonoids, quinones, cardiac glycosides, terpenoids, phenol, steroid, coumarins and alkaloids followed by other extracts (Table 1). The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, steroids, etc.,[29] Thus, the preliminary screening test may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development.[30] The presence of alkaloids and saponins in the leaf extract, the biological function of alkaloids and their derivatives are very important and are used in analgesic, antispasmodic and bactericidal activities.[31] Saponins have properties of precipitating and coagulating red blood cells, and they also have cholesterol binding properties, formation of foams in aqueous solutions and hemolytic activity[32] and traditionally saponins have been extensively used as detergents and molluscicides, in addition to their industrial applications as foaming and surface active agents and also have beneficial health effects.[33] Plant steroids are known important for their cardiotonic activities and also used in nutrition, herbal medicine and cosmetics.

The result of the present study recorded highest Tannins content in the leaf and callus extract of *Phyllanthus virgatus* and the tannins content was expressed as mg tannic acid equivalent (TAE) per gram of the sample. The optimum yield of tannins was found to be 8.01±0.7 mg TAE/ g dry weight from callus of *Phyllanthus virgatus* followed by leaf extract (7.34±0.45) (Table 2). The effect of ethanol on extraction of tannins from *Phyllanthus virgatus* callus extracts was found to be good. The results corroborates with the findings of Singh *et al.*,[34] who has reported the maximum yield of Tannins from ethanolic extract of *Artemisia absinthium.* Tannins are the natural polyphenolic compounds which can influence the nutritive value of different food stuffs utilized by human and other animals. Tannins also have large influence on the phytochemical and phytotherapeutic value of medicinal plants. Various methods have been used to increase

the extraction efficiency of tannins from different medicinal plants for their use in pharmaceutical field.[35] Ethanol has been found to be the most commonly used solvent for the extraction of tannins rather than other organic solvents.[36] Tannins have stringent properties, hasten the healing of wounds and inflamed mucous membranes.[37]

The data presented in Table 3, indicate that the leaf and callus extracts of *Phyllanthus virgatus* inhibit the growth of some microorganism in various concentration. The concentrations of 50 mg/ml -100 mg/ml ethanolic callus extract showed antimicrobial activity against *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli* (Fig. 3). The maximum clear zone of inhibition was found at 30mg/ml of ethanolic callus extract of Phyllanthus *virgatus***.** In leaf and callus extract, there is no zone of inhibition was found in lower concentration 10mg/ml. Similar results were obtained on ethanol peel extract of *Mangifera indica*, *Citrus sinensis* and *Citrus aurantium* which exhibited antibacterial activity.[38,39] The antimicrobial activities of ethanol extract may be due to the presence of tannins, triterpenoids and flavonoids.[40] Thus from our findings, it is concluded that the ethanolic extracts from dry powdered peel of *Phyllanthus virgatus* had superior level of antimicrobial activity. The powerful antibacterial effect is attributed to the greater amount of tannins compound in the ethanolic callus extracts of *Phyllanthus virgatus*.

Table 1: Phytochemical screening from leaf extracts of *Phyllanthus virgatus.*

Phytochemicals Tested	**Leaf extracts of *Phyllanthus virgatus***				
	Aqueous	**Ethanol**	**Acetone**	**Petroleum ether**	**chloroform**
Tannins	++	+ +	++	-	-
Saponins	+	++	-	-	-
Quinones	+	+	+ +	-	+
Terpenoids	+ +	+ +	+	+	+
Steroids	+	+ +	+ +	-	+
Flavonoids	+	+	+	-	+
Phenol	++	+ +	-	-	+
Alkaloids	+	+	-	-	-
Glycosides	-	-	-	-	-
Cardiac glycosides	+	+	+	-	+
Coumarins	+	+	-	-	+

Table 2: Phytochemical screening from callus extracts of *Phyllanthus virgatus.*

Phytochemicals Tested	Callus extracts of *Phyllanthus virgatus*				
	Aqueous	Ethanol	Acetone	Petroleum ether	chloroform
Tannins	+	+ +	+	-	-
Saponins	+	+	-	-	+
Quinones	+	++	+	-	+
Terpenoids	+	+ +	+	+	+
Steroids	+	+ +	+	+	+
Flavonoids	+	+ +	+	-	+
Phenol	++	+ +	-	-	-
Alkaloids	+	+	-	-	-
Glycosides	-	-	-	-	-
Cardiac glycosides	+	+	+	-	+
Coumarins	+	+	+	-	+

Table 3: Determination of tannin content from leaf and callus extracts of *Phyllanthus virgatus.*

Phyllanthus virgatus	Tannin Content (mg tannic acid equivalent/g dry material)
Leaf	7.34 ± 0.45
Callus	8.01 ± 0.7

Table 4: Antibacterial activity of leaf extracts of *Phyllanthus virgatus.*

Zone of inhibition (mm in diameter)*			
Micro-organisms Tested	Concentrations of extract		
Ethanol leaf extracts of *Phyllanthus virgatus*	10mg/ml	20mg/ml	30mg/ml
Bacillus subtilis• MTCC No. 10224	8	10	13
Bacillus cereus• MTCC No. 10211	9	11	12
Pseudomonas aeruginosa• MTCC No. 14676	-	8	10
Staphylococcus aureus• MTCC No. 9542	-	8	11
Escherichia coli• MTCC No. 1563	-	8	10

Table 5: Antibacterial activity of callus extracts of *Phyllanthus virgatus.*

Zone of inhibition (mm in diameter)*			
Micro-organisms Tested	Concentrations of extract		
Ethanol callus extracts of *Phyllanthus virgatus*	10mg/ml	20mg/ml	30mg/ml
Bacillus subtilis• MTCC No. 10224	10	13	15
Bacillus cereus• MTCC No. 10211	11	12	14
Pseudomonas aeruginosa• MTCC No. 14676	-	10	13
Staphylococcus aureus• MTCC No. 9542	-	12	15
Escherichia coli• MTCC No. 1563	-	10	13

• This strain was obtained from MTCC

* Includes diameter of disc (6mm); Average of three replicates

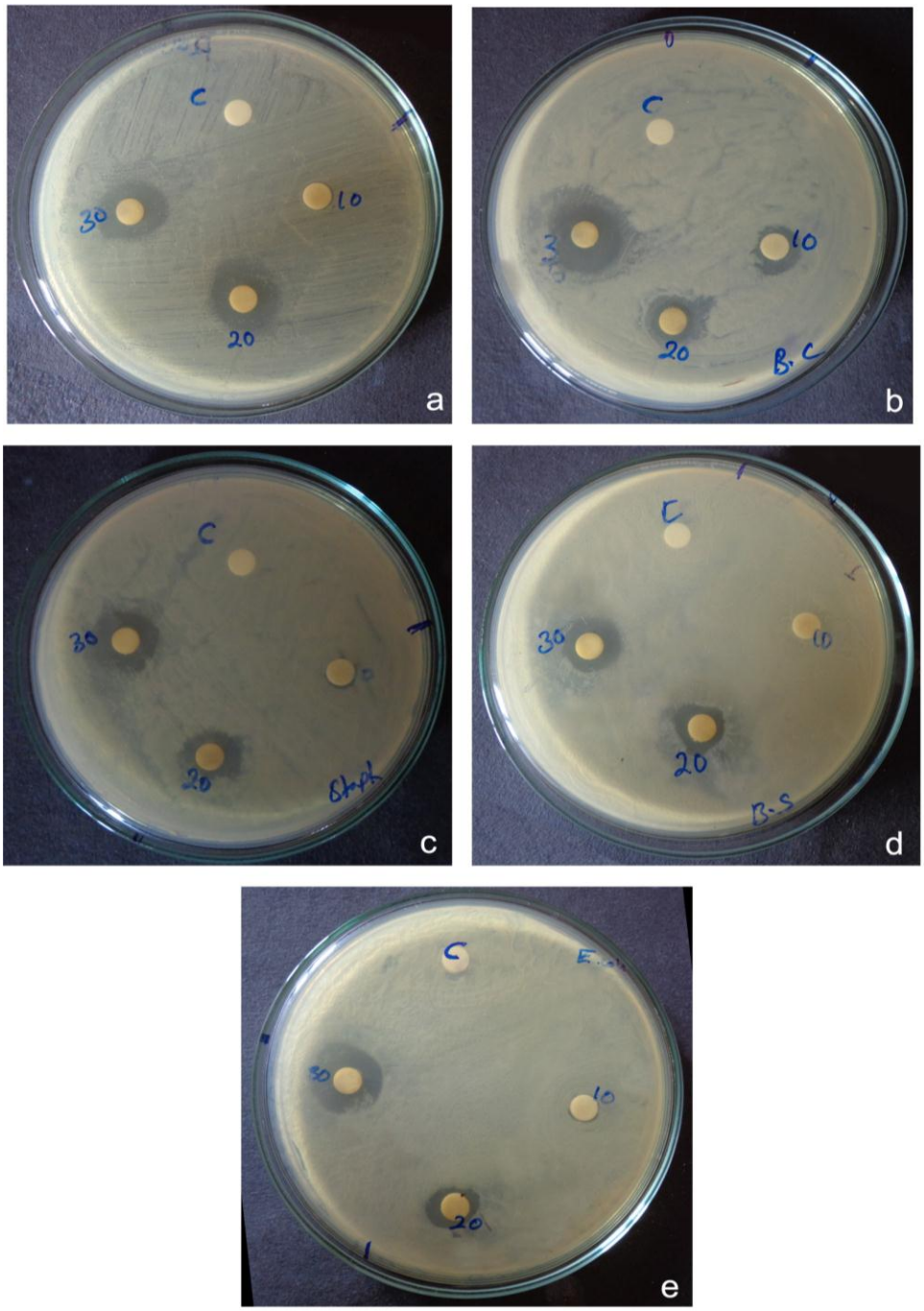

Figure 1: Antibacterial activity of leaf extracts of *Phyllanthus virgatus.*

Antibacterial activity of leaf extracts of *Phyllanthus virgatus* against (a) *Bacillus subtilis* (b) *Bacillus cereus* (c) *Pseudomonas aeruginosa* (d) *Staphylococcus aureus* (e) *Escherichia coli*.

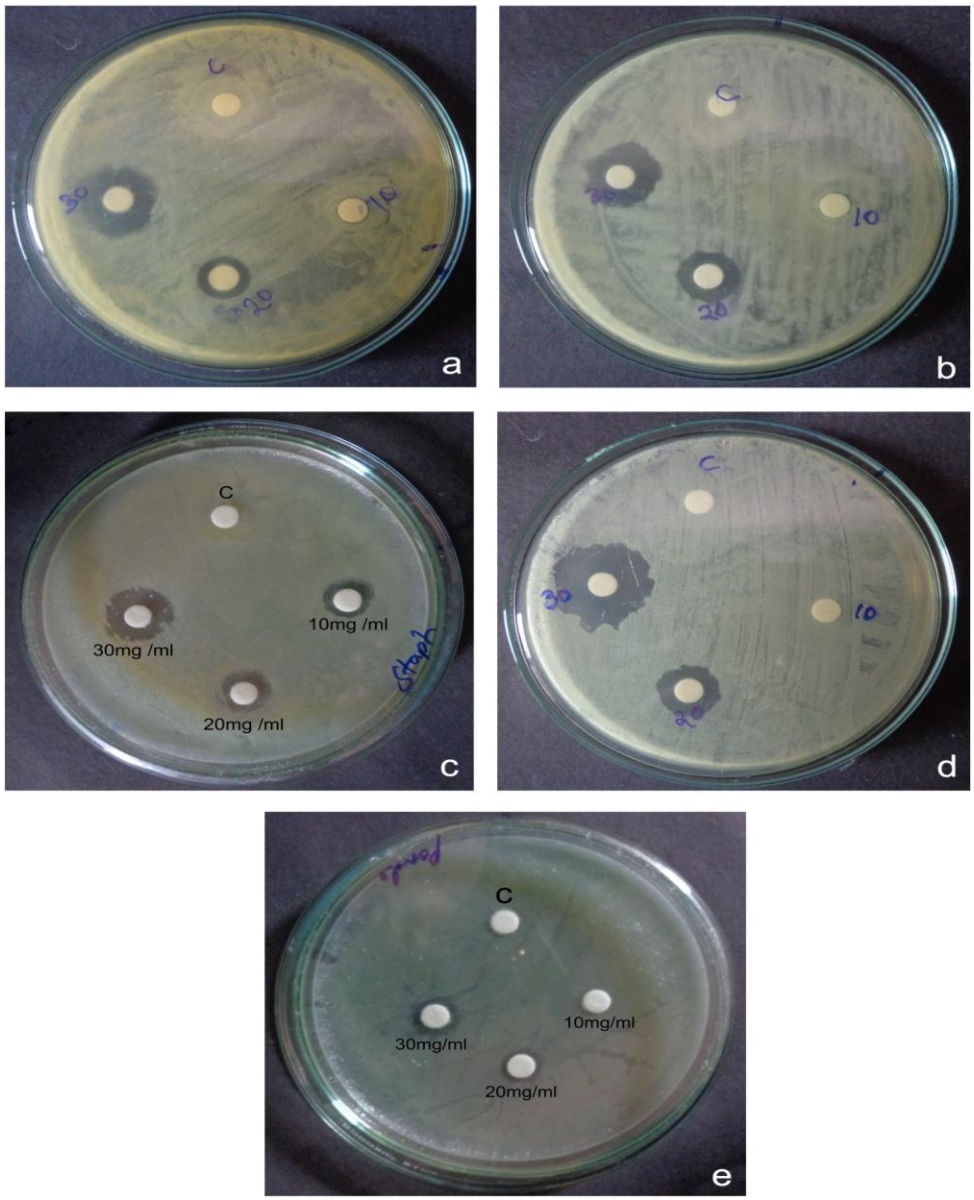

Figure 2: Antibacterial activity of callus extracts of *Phyllanthus virgatus.*

Antibacterial activity of callus extracts of *Phyllanthus virgatus* against (a) *Bacillus subtilis* (b) *Bacillus cereus* (c) *Pseudomonas aeruginosa* (d) *Staphylococcus aureus* (e) *Escherichia coli.*

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