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Phytochemical, antioxidant and antimicrobial studies of *Terminalia gella* Dalz- A potential medicinal plant

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Abstract
The present paper deals with phytochemical, antioxidant and antimicrobial studies on *Terminalia gella*, a potential medicinal plant from Simhachalam hills (Vishakhapatnam), Andhra Pradesh, India. The stem bark, leaf and fruit samples were screened for phytochemical composition, antioxidant content and antimicrobial evaluation. The phytochemical studies revealed that the presence of phenolic compounds especially alkaloids, coumarins, terpenoids etc., while antioxidant studies revealed that high content of antioxidants present in methanolic extracts when compare to ethyl acetate and petroleum ether extracts. The antimicrobial studies revealed that the most susceptible microorganisms were found to be *Bacillus cereus, Candida albicans, Escherichia coli, Micrococcus luteus, salmonella typhimurium, Pseudomonas aeruginosa* and *Staphylococcus aureus.* Phytochemical analysis of various test extracts revealed that the phenolic compounds especially alkaloids and coumarins are responsible for antimicrobial activity. *Terminalia gella* could be exploited in the infectious management of various diseases.

Keywords: *Terminalia gella*, potential medicinal plant, phytochemical, antioxidant, antimicrobial studies.

1. Introduction
The medicinal value of plants lies in some chemical constituents that produce a definitive physiological action on human body. According to WHO report above 80% of the world population are taking interest in indigenous medicinal plant remedies [1]. Therefore it is essential to investigate traditional medicine with a view to identity and exploit safe and effective remedies for ailments of both microbial and non-microbial origin. The use of phytochemicals, antioxidants with established antimicrobial properties, could be of great significance in therapeutic approaches. It is estimated that about 75% of the biologically active plant derived compounds, presently in use worldwide, have been derived through follow up researchers to verify the authenticity of data from folk and ethnomedicinal uses. So there is a great scope for new drug discoveries based on traditional plant uses [2]. The ethnobotanical information obtained from the traditional herbal practitioners may serve as an initial lead for isolation and characterization of bioactive compounds. Phytoconstituents are the natural bioactive compounds, exhibit potential therapeutic properties, work with nutrients and fibres to form an integrated part of defensive system in which alkaloids, flavonoids, saponins, terpenoids, phenolics, tannins etc., considered as major constituents in crude drugs. Antioxidants are the common compounds in low concentration which can prevent biomolecules (proteins, nucleic acids, polyunsaturated lipids, sugars) from undergoing oxidative damage through free radical mediated reactions [3]. The methanolic extracts of stem bark, leaf and fruit exhibiting high content of antioxidants. These plant derived compounds are considered to be active against human pathogenic microorganisms. In the present study *Terminalia gella*, a potential medicinal plant from Simhachalam hills of Vishakhapatnam district, Andhra Pradesh, was selected and screened against multidrug resistant bacteria and fungal strain.

2. Material & Methods
2.1 Plant material
The plant material was collected from Simhachalam hills of Vishakhapatnam district and identified with the help of regional floras [4, 5]. The ethnomedicinal properties of the species were recorded based on interviews conducted with elder people from tribal communities, inhabited in and around the forests. The identification was confirmed by comparing with

authentic specimens in Sri Krishnadevaraya University Herbarium (SKU), Ananthapuramu, Madras herbarium (MH), Coimbatore and Central National Herbarium (CNH) Kolkata and the voucher specimens was deposited in Sri Krishnadevaraya University Herbarium (SKU), Ananthapuramu.

2.2 Phytochemistry

The plant material (Stem bark, leaf and fruit) was shade dried, powdered (100 g) and successively extracted with ethyl acetate, methanol and water using Soxhlet apparatus for 6 hours [6]. The extracts were filtered, concentrated under reduced pressure to dryness and subjected for phytochemical screening using standard procedures [7 and 8]. The positive reaction was observed for 30 different groups of phytochemical compounds. Alkaloids, phenols, anthocyanins, anthracene glycosides, saponins and steroids were recorded as most predominant chemical derivatives followed by flavones, catecholic compounds, proteins, gallic tannins, etc. (Table-1).

2.3 Preparation of Plant extracts for quantitative analysis of antioxidants

The plant parts (stem bark, leaf and fruit) were separated and washed with distilled water and shade dried at room temperature. Fifty grams of each part pounded and successively extracted with Petroleum ether, Ethyl acetate and Methanol using soxhlet apparatus for 6-8 hours. The extracts were filtered and concentrated under reduced pressure to dryness and the extracts used for the assays. One gram of sample after the successive extraction was taken and soaked in distilled water for 24 hours and filtered. The filtrate served as water extract and used in the present experiments. The decoction was prepared by boiling the 1 g of plant material in distilled water for 3-4 hours and the filtrate was used as the direct decoction (DC).

2.4 Total phenolic content (TPC)

The extraction of total phenolics was performed using the Folin–Ciocalteu assay [9]. In total, 100 µl of each extract (1 mg/ml) was added to a test tube containing 50 µl of the phenol reagent (1 M). A further 1.85 ml of distilled deionized water was added to the solution and allowed to stand for 3 min after vortexing; then 300 µl Na_2CO_3 (20% in water, v/v) was added and vortexed and the final volume (4 ml) was obtained by adding 1.7 ml of distilled deionized water. A reagent blank was prepared using distilled deionized water. The final mixture was vortexed, and then incubated for 1 h in the dark at room temperature. The absorbance was measured at 725 nm using spectrophotometer. A standard curve was prepared using 0, 65.5, 125, and 250 mg/l gallic acid in methanol: water (50:50, v/v). Total phenolic values were indicated in terms of gallic acid equivalents (GAE) in milligrams per gram plant extract. All determinations were performed in triplicate.

2.5 Total flavonoid content (TFC)

A 0.5 ml sample (1 mg/ml) was mixed with 0.1 ml of 10% aluminium nitrate and 0.1 ml of potassium acetate (1 M) and 4.3 ml of 80% ethanol was added to make a total volume of 5 ml. The mixture was vortexed and the solution was allowed to stand for 40 min for reaction at room temperature. The absorbance was measured spectrophotometrically at 415 nm. All determinations were performed in triplicate. Total flavonoid values are expressed in terms of quercetin equivalents (QE) per gram of plant extract [10].

2.6 Total antioxidant activity

For total antioxidant activity assay [11], 0.1 ml of the extract (10 mg/ml) dissolved in DMSO was combined in a eppendorf tube with 1 ml of reagent solution (0.6 M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95 °C for 90 min. After cooling to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. Ascorbic acid was used as the standard and the total antioxidant activity expressed as ascorbic acid equivalents (AAE) per gram of plant extract.

2.7 Antimicrobial assay

The antimicrobial activity was performed by employing the pour plate as well as disc diffusion methods [12, 13 and 14]. The crude extracts of each sample dissolved in dimethyl sulfoxide (DMSO) and the concentrations of 10 to 100 mg/ ml were prepared. 25-30 µl of each sample was applied to sterile Whatmann filter paper discs.

All the bacterial and fungal strains were grown in respective media for overnight at 37^0 C. The suspension of microorganisms adjusted to 10^5 to 10^7CFU/ml in broth media. In this method (Pour-plate method) 100 µl of the suspension of bacterial/ Candidal suspension microorganisms were prepared in the nutrient broth were inoculated in the nutrient agar in petri dishes at room temperature in sterile condition and mixed thoroughly to ensure uniform growth. This was allowed to stand for 15 minutes so that the medium got solidified. The sterile filter paper discs of 5 mm diameter containing different concentrations of crude extracts were aseptically placed on the agar plates. All the seeded petri dishes were incubated at 27+/- 2 ^{0}C for twenty-four hours in case of bacteria and 48- 72 hours for fungal species. The ethyl acetate and methanol used as control for the crude extracts of ethyl acetate and methanol respectively. The assessment of antimicrobial activity was based on measurement of inhibition zones formed around the discs. The appearance of bacterial free zone around the disc is known as inhibitory zone, is considered as positive response. The diameter of the zones of inhibition around each disc measured and recorded at the end of the incubation period. The diameters of the inhibition zones were measured and expressed in millimetres. Each test was performed in three replicates and repeated twice. Model values were selected.

3. Results and Discussion

The present study was designed to evaluate the phytochemical, antioxidant and antimicrobial activity of different parts (stem bark, leaf and fruit) of *Terminalia gella*. Phytochemical studies [Table-1] revealed that among the three solvents the methanol extracts exhibited positive reaction for maximum no of secondary metabolites followed by ethyl acetate and water extracts. Alkaloids, anthocyanins, anthracene glycosides, coumarins phenols and saponins were present in all solvents. Maximum (13) compounds were present in stem bark followed by fruit (10) and leaf (5).

Table 1: Distribution of Phytochemical constituents in *Terminalia paniculata*

S. No	Compound	Stem bark			Leaf			Fruit		
		EA	M	AQ	EA	M	AQ	EA	M	AQ
1	Alkaloids	+	+	+		+			+	+
2	Anthocyanins		+	+	+	+			+	+
3	Anthocyanidins			+						
4	Anthracene glycosides		+						+	
5	Anthroquinones							+		
6	Acubins									
7	Carbohydrates				+				+	
8	Carotenoids			+			+			
9	Catecholic compounds			+						
10	Coumarins		+		+				+	+
11	Dihydrochalcones									
12	Emodins									
13	Flavonoids		+			+				
14	Flavonols								+	
15	Flavonones				+					
16	Flavones									
17	Fatty acids									
18	Gallic tannins		+							
19	Glycosides									
20	Iridoids								+	
21	Lignans				+					
22	Phenols	+	+	+		+			+	+
23	Proteins									
24	Polyoses									
25	Reducing compounds		+			+				
26	Saponins		+						+	+
27	Steroids									
28	Tannins									
29	Triterpenoids	+								
30	Volatile oils	+							+	

EA-Ethyl acetate; M-Methanol; AQ-Aqueous

Phenols and flavonoids are well established to show antioxidant activity [Table-2] and contribute to maintain human health. Methanolic extracts showing high content of antioxidants when compare to ethyl acetate and petroleum ether extracts. High content of phenols present in methanol extracts of stem bark (23.5 mg/g), whereas low content was found in petroleum ether extracts of leaf (0.7 mg/g). Total flavonoid content was high in methanol extracts of stem bark (29.8 mg/g), while low content was found in petroleum ether of fruit (1.25 mg/g) extract. Total antioxidant content was high in methanol extract of stem bark (26.75 mg/g), low content was found in petroleum ether extracts of fruit (1.72 mg/g).

Table 2: It shows the Total amount of plant phenols, flavonoids and antioxidants of *Terminalia gella*

Name of the plant	Part	Total phenolic compounds GAE mg/g DW			Total flavonoids QE mg/g DW			Total antioxidants AAE mg/g DW		
		Pet. ether	Ethyl acetate	Methanol	Pet. ether	Ethyl acetate	Methanol	Pet. ether	Ethyl acetate	Methanol
Terminalia gella	Sb	1.92	10.2	23.5	1.92	16.52	39.8	2.5	12.24	26.75
	Lf	0.7	0.9	19.5	1.42	18.42	22.6	1.89	12.45	24.65
	Fr	1.8	10.5	19.45	1.25	15.62	26.75	1.72	14.25	22.50

GAE-Gallic acid equivalents; QE-Quercetin equivalents; AAE-Ascarbic acid equivalents; DW-Dry weight; Pet. ether- Petrolium ether; Sb-Stem bark; Lf-Leaf; Fr-Fruit

In vitro antimicrobial studies of *Terminalia gella* [Table-3] revealed that all crude drug extracts had significant antimicrobial activity against the test pathogens. The methanol extracts of stem bark was more effective on test pathogens when compare to leaf and fruit. The methanol extract of stem bark exhibited maximum inhibition of zone (18 mm) against *Klebsiella pneumoniae* (MTCC-7028) followed by *Salmonella typhimurium* (16mm; MTCC-98), gram negative bacteria. The leaf extracts of ethyl acetate shows minimum inhibition activity (6 mm) against *Bacillus cereus* (MTCC-4079), a gram positive bacterium.

Table 3: It shows the Antimicrobial activity of Ethyl acetate and Methanol extracts of *Terminalia paniculata*.

Organism											
	Part	**mg/ml**	**Bc**	**Bs**	**Ca**	**E. coli**	**Kb**	**MI**	**Pa**	**Sa**	**St**
Ethyl acetate: Inhibition Zone (mm^{-1})	Leaf	C (EA)	6	8	7	6	8	-	8	7	-
		10	6	8	-	-	10	10	-	10	-
		25	-	-	-	-	15	14	-	12	-
		50	-	8	-	-	15	15	12	-	-
		MIC	-	312	-	-	156	625	156	312	-
	Stem bark	C (EA)	8	-	-	7	8	8	-	-	7
		10	-	-	-	-	6	-	10	6	-
		25	-	-	8	-	7	-	12	10	-
		50	-	-	10	-	12	-	15	12	-
		MIC	-	-	156	-	156	-	312	156	-
	Fruit	C (EA)	7	10	8	-	-	10	-	6	7
		10	-	-	-	8	8	-	-	-	-
		25	9	10	-	10	10	-	-	-	-
		50	-	-	-	12	15	-	-	-	10
		MIC	312	312	-	312	156	-	-	-	312
Methanol: Inhibition Zone (mm^{-1})	Leaf	C (M)	7	-	-	8	10	8	-	-	-
		10	8	-	10	-	-	-	-	8	6
		25	10	-	12	12	12	-	-	10	10
		50	12	10	-	15	15	-	-	14	15
		MIC	156	156	312	156	156	-	-	625	625
	Stem bark	C (M)	8	6	-	-	10	8	-	-	-
		10	6	10	-	-	-	-	6	6	9
		25	10	12	-	-	-	-	6	7	10
		50	12	14	-	-	18	16	7	8	12
		MIC	312	156	-	-	-	-	312	312	312
	Fruit	C (M)	8	6	10	7	7	-	-	-	6
		10	-	10	8	-	-	-	10	6	-
		25	-	11	7	-	-	-	12	7	-
		50	-	12	8	-	-	-	14	16	-
		MIC	-	312	312	-	-	-	156	312	-

Bc-Bacillus cereus; Bs-B. subtilis; Ca-Candida albicans; E. coli-Escherichia coli; Kb-Klebsiella pneumoniae; Ml-Micrococcus luteus; Pa-Pseudomonas aeruginosa; Sa-Salmonella typhimurium; St-Staphylococcus aureus; C (EA)-Ethyl Acetate control; C (M)-Methanol control; MIC-Minimum inhibitory concentration.

* Each test was performed in three replicates and repeated twice.

4. Conclusion

The present work revealed that *Terminalia gella* has rich and diversified phytochemical compounds like alkaloids, coumarins, gallic-tannins and other compounds. The plant parts were also rich in phenols and flavonoids, major group of antioxidants. These antimicrobial agents with its significant inhibition activity against various clinical isolates suggest to conduct further studies for isolation and characterization of active principles. The molecular studies have been conducted in laboratory for elucidation of bioactive compounds.

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