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TECOMELLA UNDULATA (SM.) SEEM.- GC-MS ANALYSIS OF FLAVONOIDS AND INHIBITORY ACTIVITY AGAINST PATHOGENIC MICROBES

Richa Bhardwaj [*1] and R. A. Sharma [2]

Department of Botany [1], IIS University, Jaipur - 302020, Rajasthan, India.
Department of Botany [2], University of Rajasthan, Jaipur - 302007, Rajasthan, India.

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Correspondence to Author:
Dr. Richa Bhardwaj

Assistant Professor,
Department of Botany,
IIS University, Jaipur - 302020,
Rajasthan, India.

E-mail: richa.bhardwaj@iisuniv.ac.in

ABSTRACT: *Tecomella undulata* (Sm.) seem is a monotypic genus belonging to family Bignoniaceae. The plant holds the tremendous potential of medicinal value and has been traditionally used in various ailments like syphilis, leukoderma, blood disorders, to name a few. The plant has gained prominence due to the presence of some prominent secondary metabolites. The present study focuses on the GC-MS analysis of extracts of all the plant parts of *T. undulata*, which revealed the presence of certain bioactive compounds like Quericitin, Kaempferol, stigmasterol, sitosterol, thiazoline, phytol, pthalic acid, methyl alpha ketopalmitate and so forth. A total of about 20 bioactive compounds were identified. The antimicrobial activity of the extracts was assayed against pathogenic bacteria and fungi. The flavonoids from leaf extracts showed highest antimicrobial activity against *C. albicans*, *S. aureus*, *E. coli*, and *B. subtilis*. This also correlates with the highest amount of Flavonoids present in leaves followed by the amount present in roots. The study thus infers that the presence of bioactive components may be the principle behind the antimicrobial property of different plant parts, and therefore *Tecomella* forms a potential plant for herbal drug formulation.

INTRODUCTION: Awareness of medicinal plant usage is a result of the many years of struggles against illnesses due to which man learned to pursue drugs in barks, seeds, fruit bodies, and other parts of the plants. A natural product is a chemical compound or substance produced by a living organism like microorganisms, marine organisms, animal sources, plant sources. The definition of natural products is usually restricted to mean purified organic compounds isolated from natural sources that are produced by the pathways of primary or secondary metabolism.

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The importance of medicinal plants in traditional healthcare practices has provided clues to new areas of research and in biodiversity conservation is now well recognized [1, 2]. Many conventional drugs originate from plant sources, the emergence of new herbal genomics research, medicinal plant genomics consortium, together with advances in other omics information may help for the speedy discovery of previously unknown metabolic pathways and enzymes [3]. Because of the absence of an efficient excretory system plant produced secondary metabolites, secondary metabolites include volatile oils, flavonoids, alkaloids, glycosides, tannins, resins *etc.* that have been successfully exploited for vital sources for food additives, flavors, and industrially important pharmaceuticals [4].

The genus *Tecomella undulata* is a tree species that produce quality timber. In Rajasthan, it is mainly

found in western parts distributed in Barmer, Jaisalmer, Jodhpur, Pali, Ajmer, Nagaur, Bikaner, Churu, and Sikar districts. *Tecomella undulata* belongs to family Bignoniaceae (Jacaranda family). The Bignoniaceae family comprising of about 110 genera and 650 species is a family of flowering plants, commonly known as the Trumpet Creeper family, Jacaranda family, Bignonia family, or the Catalpa family. Roheda is mainly used as a source of timber. Its wood is strong, tough, and durable, excellent for firewood and charcoal. Roheda plays an important role in ecology. It acts as a soil-binding tree by spreading a network of lateral roots.

The species has been identified as an important factor for environmental conservation in arid zones as a stabilizer of shifting sand dunes, providing shelter for wildlife. It is also a very useful species for afforestation of the drier tracts due to its drought and fire-resistant properties [5, 6]. Flavonoids are phenolic substances isolated from a wide range of vascular plants, with over 8000 individual compounds known. A variety of *in-vitro* and *in-vivo* experiments have shown that selected flavonoids possess antiallergic, anti-inflammatory, antiviral and antioxidant activities, significant anticancer activity including anticarcinogenic properties, certain flavonoids possess potent inh-ibitory activity against a wide array of enzymes [7, 8].

According to the IUPAC nomenclature, they can be classified into flavonoids or bioflavonoids., iso-flavonoids, derived from 3-phenylchromen-4-one (3-phenyl-1,4-benzopyrone) structure, neoflavo-noids, derived from 4-phenylcoumarine (4-phenyl-1,2-benzopyrone) structure. Three flavonoid classes above are all ketone-containing compounds, and as such, are anthoxanthins (flavones and flavonols). This class was the first to be termed bioflavonoids [9, 10].

MATERIALS AND METHODS:

Plant Material: The different plant parts (roots, stems, leaves, and bark) of *Tecomella undulata* were collected in the month of October-December from the University of Rajasthan campus. It was washed with tap water, dried at room temperature and ground to a fine powder. The species specimen was submitted to the herbarium, Department of Botany, University of Rajasthan. Jaipur, Rajasthan, India, and got the voucher specimen no. RUBL211300.

Chemicals: All the chemicals used were of analytical grade and purchased from Hi-Media from Hi-media Laboratory Pvt. Ltd. Mumbai.

Tests for Flavonoids:

Shinoda's Test: To 2 ml of the test solution, a fragment of magnesium metal (mg++) ribbon was added into the test tube, followed by the dropwise addition of concentrated conc. HCl. The resulting pink/ scarlet/ crimson of occasionally green/ blue colors indicated the presence of flavonoids [11].

NaOH Tests: To 2-3 ml. of extract, few drops of sodium hydroxide solution were added into a test tube. Formation of intense yellow color that became colorless on the addition of a few drops of dilute HCl indicates the presence of flavonoids [12].

GC-MS Analysis of Flavonoids:

GC-MS Conditions: GCMS-QP 2010 Plus was used for identification and quantification of phytoconstituents, using MS libraries previously compiled from purchased standards. For the acquisition of an electron ionization mass spectrum, an ion source temperature of 250 °C was used. The GC was equipped with a SE-30 capillary column, a split injection piece (270 °C), and direct GC-MS coupling (280 °C). Helium (1.2 mL/min) was used as the carrier gas with a split ratio of 1:10. The oven temperature program for analyzing the extracts utilized an initial oven temperature of 100 °C, maintained for 2 min, followed by a steady climb to 200 °C at a rate of 7 °C/min allowed to increase to 190 °C at a rate of 30 °C/min. This oven temperature was again maintained at 190 °C for 5 min and then allowed to increase to 300 °C at a rate of 7 °C/min.

This oven temperature was maintained for 2 min and finally ramped to 300 °C at a rate of 10 °C/min and maintained for a further 22 min. Injection temperature was 270 °C and volume 250 °C and 1 µL, respectively. The total GC running time was about 43.28 min. The MS operating conditions were as follows, Interference temperature of 260 °C, Ion source temperature of 250 °C, mass scan (m/z)-40-450, solvent cut time 7 min, scan speed 2000 amu/s total MS running time-50.28 min and Threshold -1000.

Identification: GC-MS is a valuable aid for identifying unknown peaks as well as for

confirming the identification of identified phytoconstituents. In some cases, when no identical spectra were found, the structural type of the corresponding component was suggested only on the basis of its mass spectral fragmentation and retention data. Identification of components was based on directs comparison of the retention times and mass spectral data with those for standard compounds and computer matching with the library (Wiley library, NIST data bank, database NIST 98) as well as by comparison of the retention time.

Sources of Test Organisms:

Fungi: The fungal strains *Aspergillus niger* (NCIM 0616), *Fusarium oxysporium* (NCIM 1228), *Trichoderma reesei* (NCIM 0992), *Penicillium funiculosum* (NCIM 1075), *Candida albicans* (NCIM- 3501), *Trichoderma viride* are procured from the National Institute for Complementary Medicine.

Bacteria: The bacterial strains *Escherichia coli* (MTCC 1652), *Staphylococcus aureus* (MTCC 0087) (Gram+ve), Pseudomonas aeruginosa (MTCC 4646) (Gram+ve), *Bacillus subtilis* (MTCC 0121), *Klebsiella pneumoniae* (MTCC-0109) (Gram–ve) and *Streptomyces albudencus* (MTCC 1764), *Enterococcus faecalis* (ATCC- 29212) (gram+ve) were procured from the microbial type culture collection (Institute of Microbial Technology, Chandigarh, India).

Culture of Test Microbes: For the cultivation of bacteria, Nutrient Broth Medium (NB) was prepared using 8% Nutrient Broth (Difco) in distilled water and agar-agar and sterilized at 15 lbs psi for 25-30 min. Agar test plates were prepared by pouring ~15 ml of NBM into the petri dishes (10 mm) under aseptic conditions. A peptone saline solution was prepared (by mixing 3.56 g KH_2PO_4 + 7.23 g NaH_2PO_4 + 4.30 g, NaCl + 1 g peptone in 1000 ml of distilled water, followed by autoclaving) and the bacterial cultures were maintained on this medium by regular sub-culturing and incubation at 37 °C for 24 h. However, for the cultivation of fungi, Potato Dextrose Agar (PDA) medium was prepared by mixing 100 ml potato infusion + 20 g agar + 20 g glucose, followed by autoclaving) and the test fungi were incubated at 27 °C for 48 h and the cultures were maintained on the same medium by regular sub-culturing.

Fungicidal and Bactericidal Assay: For both fungicidal and bactericidal assays agar well diffusion method was adopted [13, 14], because of reproducibility and precision. The different test organism was proceeded separately using a sterile swab over previously sterilized culture medium plates, and the zone of inhibition were measured around wells in solidified medium (5 mm in diameter), which were containing 2mg/ml and 4mg/ml of the test extracts, control solvent or steptomycin (1mg/ml) or ketokenazol (1mg/ml) as reference separately. These plates were initially placed at low temperature for 1 hr, so as to allow the maximum diffusion of the compounds from the wells into the plate and later, incubated at 37 °C for 24 h in case of bacteria and 48 h at 27 °C for fungi, after which the zones of inhibition could be easily observed. Three replicates of each test extract were examined, and the mean values were then referred.

RESULTS AND DISCUSSION: Flavonoids have been reported to be an important constituent of medicinal plants, flavonoids have been assayed for their antioxidant activity [15, 16]. Various techniques have been employed for the quantification of flavonoids which include advanced techniques like HPLC RP-HPLC, microwave-assisted extraction [17, 18].

TABLE 1: CHROMATOGRAPHIC DATA AND COLOUR REACTION OF THE FLAVONOIDS ISOLATED FROM *TECOMELLA UNDULATA*

Flavonoids (aglycones)	Rf(×100) in						Colors by chromatogenic sprays color			
	BeAW$^+$	BAW*	TBA^{++}	Day-light	UV* ammonia	I_2 vapours	$FeCl_3$		$AlCl_3$	
							Visible	UV	Visible	UV
Luteolin	56	84	77	GN-YW	YW	YW-BN	BN	BK	DL-YW	YW-GN
Quercetin	79	64	41	GN-YW	YW	YW-BN	BT-GY	BK	DL-YW	YW-GN
Kaempferol	86	83	55	GN-YW	YW-BN	YW-BN	BN	BK	YW	YW-GN

Abbreviations :BeAW$^+$ = Benzene : Acetic acid : Water (125 : 72 : 3); BAW* = n-Butanol : Acetic acid : Water : (4: 1:5); TBA^{++} = t-Butanol : Acetic acid : Water (3:1:1) BK = Black; BN = Brown; BT = bright, BL = blue, GY = Grey; DL = dull; GN = green; YW = yellow

Isolated flavonoid content in *T. undulata* is recorded as, amongst the free form of flavonoids extracted, in the plant parts kaempferol was obtained in maximum mount while luteolin is observed in minimum amount. (in roots; kaempferol; 0.12 mg/gdw > quercetin; 0.09 mg/gdw > luteolin; 0.06 mg/gdw), (in stems; kaempferol; 0.09 mg/gdw > quercetin; 0.08 mg/gdw > luteolin; 0.07 mg/gdw), (in Bark; kaempferol; 0.11 mg/gdw > quercetin; 0.07 mg/gdw > luteolin; 0.06 mg/gdw) (in leaves; kaempferol; 0.23 mg/gdw > quercetin; 0.16 mg/gdw > luteolin; 0.13 mg/gdw) maximum amount of total free flavonoids was observed in leaves (leaves; 0.52 mg/gdw > roots; 0.27 mg/gdw stems; 0.24 mg/gdw = Bark; 0.24 mg/gdw).

Amongst the bound form of flavonoids in roots kaempferol was reported in maximum amount while quercetin was observed in minimum amount (kaempferol; 0.08 mg/gdw > quercetin; 0.06 mg/gdw > Luteolin; 0.05 mg/gdw) while in stems and leaves kaempferol was observed in higher amount while Quercetin was observed in lower amount (kaempferol; 0.07 mg/gdw > Luteolin; 0.06 mg/gdw > quercetin; 0.05 mg/gdw) in Bark kaempferol is observed in higher amount while luteolin in lower amount (kaempferol; 0.08 mg/gdw > quercetin; 0.06 mg/gdw > Luteolin; 0.04 mg/gdw) in leaves Quercetin is obserbved to be maximum while luteolin is minimum. (Quercetin; 0.12 mg/gdw > kaempferol; 0.10 mg/gdw > Luteolin; 0.09 mg/gdw). Maximum amount of total bound form of flavonoids was observed in leaves (leaves; 0.31 mg/gdw > root; 0.19 mg/gdw > stems; 0.18 mg/gdw = bark; 0.18 mg/gdw).

The total flavonoid content (F+B) was observed maximum in leaves and minimum in stems (leaves; 0.83 mg/gdw > root; 0.46 mg/gdw > stems; 0.42 mg/gdw = Bark; 0.42 mg/gdw). Other flavonoids have also been reported. Content higher than presently investigated have been reported in various plant parts of *Tecomella* in earlier studies; similarly, total flavonoid content has also been reported. Leaves and flowers of *Tecomella undulata* have been shown to have significant antioxidant activity due to the higher content of flavonoids present, the maximum amount of flavonoids are reported in leaves of *Tecomella undulata*. Accumulation of flavonoids is also affected due to seasonal variations.

TABLE 2: ISOLATED FLAVONOID CONTENT (mg/gdw*) IN *TECOMELLA UNDULATA*

Plant species	Free (F)			
	Quercetin	Kaempferol	Luteolin	Total
	T. undulata			
Root	0.09	0.12	0.06	0.27
Stem	0.08	0.09	0.07	0.24
Bark	0.07	0.11	0.06	0.24
Leaves	0.16	0.23	0.13	0.52
Plant species	**Bound (B)**			
	Quercetin	**Kaempferol**	**Luteolin**	**Total**
	T. undulata			
Root	0.06	0.08	0.05	0.19
Stem	0.05	0.07	0.06	0.18
Bark	0.06	0.08	0.04	0.18
Leaves	0.12	0.10	0.09	0.31
Plant species	**F+B**			
	Quercetin	**Kaempferol**	**Luteolin**	**Total**
	T. undulata			
Root	0.15	0.20	0.11	0.46
Stem	0.13	0.16	0.13	0.42
Bark	0.13	0.19	0.10	0.42
Leaves	0.28	0.33	0.22	0.83

The eluted compounds from TLC were pooled together according to their TLC behavior and isolate them with the solvents and evaporated yielding three flavonoids kaempferol, quercetin, and luteolin. The spectral analyses of the active constituent, (a) Luteolin (b) quercetin and (c) kaempferol from the different plant parts of *Tecomella undulata* are shown below: -

A. Luteolin: yellow needles on crystallization (mp 280° - 320°C).

UV light absorption MeOH: 242 sh, 253 sh, 267 sh, 291 sh, 349 sh.

IR: νcm^{-1}/ max KBr: 3400, 3423, 3100 (O–H), 1070, 1150, 1010(C=O), 1656, 1620, 1612 (C=C), 1514(aromatic), 1103, 1862, 1839, 1562.

^{1}HNMR (300MHz, $CDCl_3$): 3.42, (H_1), 3.49 (H_2), 3.56 (H_3), 6.30 (H_4), 3.68 (H_5), 3.85 (H_6), 5.10 (H_7), 6.63 (H_8), 6.83 (H_9), 6.95(H_{10}), 7.41(H_{11}), 7.43(H_{12}).

^{13}C NMR (300MHz, $CDCl_3$): 122.6 (C_1), 113.8 (C_2), 76.8 (C_3), 70.3 (C_4), 77.4 (C_5), 100.5 (C_6), 163.9 (C_7), 95.8 (C_8), 158.0(C_9), 106.3 (C_{10}), 165.8 (C_{11}), 146.3(C_{12}), 150.4 (C_{13}), 121.1 (C_{14}), 119.0 (C_{15}).

B. Quercetin: yellowish needles on crystallization (mp 312°-313°C).

UV light absorption MeOH: 255 sh, 301 sh, 374 sh, 440 sh.

IR: *v*cm^{-1}/ max KBr: 3420, 3380(O–H), 2800 (C-H), 2100 (C=C), 1680 (C=O), 1610 (C≡C), 1560, 1510, 1450, 1400 (aromatic), 1385, 1310, 1270, 1180, 1010.

^{1}HNMR (300MHz, $CDCl_3$): 2.45, (H_1), 2.55 (H_2), 6.79 (H_3), 6.98 (H_4), 6.49 (H_5), 2.33 (H_6), 6.38 (H_7), 2.36 (H_8), 5.37 (H_9), 1.4 (H_{10}).

^{13}C NMR (300MHz, $CDCl_3$): 137.3 (C_1), 137.9 (C_2), 14.2 (C_3), 127.0 (C_4), 126.1 (C_5), 133.8 (C_6), 142.4 (C_7), 158.2 (C_8), 114.6(C_9), 134.5 (C_{10}), 123.0 (C_{11}), 138.0 (C_{12}), 121.1 (C_{13}), 149.4 (C_{14}), 108.9 (C_{15}), 127.8.

C. Kaempferol: Brownish needles on crystallization (mp 312°-313°C). UV light absorption MeOH: 253 sh, 269 sh, 305 sh, 374 sh, 424 sh.

IR: *v*cm^{-1}/ max KBr: 3420 (O–H), 2830 (C-H), 2240 (C=C), 1700 (C=O), 1600, 1610 (C≡C), 1560, 1510, 1450, 1400 (aromatic), 1385, 1310, 1270, 1180, 1010, 815.

^{1}HNMR (300MHz, $CDCl_3$): 2.35(H_1), 7.01(H_2), 7.18 (H_3), 6.29 (H_4), 6.37 (H_5), 2.35 (H_6), 5.39 (H_7), 5.36 (H_8), 7.18 (H_9), 7.01 (H_{10}).

^{13}C NMR (300MHz, $CDCl_3$): 1.36 (C_1), 129.8 (C_2), 126.8 (C_3), 131.9 (C_4), 147.4 (C_5), 154.2 (C_6), 114.6 (C_7), 137.5 (C_8), 124.0 (C_9), 136.0 (C_{10}), 121.1 (C_{11}), 149.4 (C_{12}), 106.9 (C_{13}), 131.9 (C_{14}), 126.1 (C_{15}).

TABLE 3: SPECTRAL STUDIES OF ISOLATED FLAVONOIDS FROM *TECOMELLA UNDULATA*

Name of Compound	UV light absorption MeOH	IR: *v*cm^{-1}/ max KBr	^{1}HNMR	^{13}C NMR
Luteolin	242 sh, 253 sh, 267 sh, 291 sh, 349 sh	3400, 3423, 3100 (O–H), 1070, 1150, 1010(C=O), 1656, 1620, 1612 (C=C), 1514 (aromatic), 1103, 1862, 1839, 1562	3.42, (H_1), 3.49 (H_2), 3.56 (H_3), 6.30 (H_4), 3.68 (H_5), 3.85 (H_6), 5.10 (H_7), 6.63 (H_8), 6.83 (H_9), 6.95(H_{10}), 7.41(H_{11}), 7.43(H_{12})	13C-NMR (100 MHz, Acetone-*d*6): d 182.4 (C4), 164.5 (C7), 164.2 (C2), 162.7 (C5), 158.1 (C9), 149.4 (C4'), 145.8 (C3'), 123.1 (C1), 119.5 (C6), 116.0 (C5), 113.5 (C2), 104.7 (C10), 103.6 (C3), 99.0 (C6), 94.0 (C8).
Quercetin	255 sh, 301 sh, 374 sh, 440 sh	3420, 3380(O–H), 2800 (C-H), 1680 (C=O), 1610, 1610, 1560, 1510, 1450, 1400 (aromatic), 1385, 1310, 1270, 1180, 1010	2.35, (H_1), 2.35 (H_2), 6.89 (H_3), 6.99 (H_4), 6.29 (H_5), 2.35 (H_6), 6.37 (H_7), 2.35 (H_8), 5.39 (H_9), 5.36 (H_{10})	138.3 (C_1), 137.6 (C_2), 14.4 (C_3), 129.0 (C_4), 123.1 (C_5), 131.8 (C_6), 147.4 (C_7), 154.2 (C_8), 114.6(C_9),137.5 (C_{10}), 124.0 (C_{11}), 136.0 (C_{12}), 121.1 (C_{13}), 149.4 (C_{14}), 106.9 (C_{15}), 126.8
Kaempferol	253 sh, 269 sh, 305 sh, 374 sh, 424 sh	3420 (O–H), 2830 (C-H), 1700 (C=O), 1600, 1610, 1560, 1510, 1450, 1400 (aromatic), 1385, 1310, 1270, 1180, 1010, 815	2.35(H_1), 7.01(H_2), 7.18 (H_3), 6.29 (H_4), 6.37 (H_5), 2.35 (H_6), 5.39 (H_7), 5.36 (H_8), 7.18 (H_9), 7.01 (H_{10})	1.36 (C_1), 129.8 (C_2), 126.8 (C_3), 131.9 (C_4), 147.4 (C_5), 154.2 (C_6), 114.6 (C_7), 137.5 (C_8), 124.0 (C_9), 136.0 (C_{10}), 121.1 (C_{11}), 149.4 (C_{12}), 106.9 (C_{13}), 131.9 (C_{14}), 126.1 (C_{15})

GC-MS analysis of the extracted flavonoids from various plant parts of *Tecomella undulata* namely root, stem, bark, and leaf, was carried out. Various constituents obtained are reported, GC-MS spectra of flavonoids from roots, stem, bark, and leaves are shown in **Tables 4, 5, 6,** and **7**.

TABLE 4: RETENTION TIME, MOLECULAR WEIGHT AND % AREA BY SETTING THE TOTAL PEAK AREA TO 100% OF FLAVONOIDS IDENTIFIED BY GC-MS IN LEAVES OF *TECOMELLA UNDULATA*

Peak#	R. Time	Area%	Name	Mol. Formula	Mol. Wt
1	17.646	0.34	2-Tridecene, 2-chloro-1,1,1-trifluoro-, (z)-	$C_{13}H_{22}ClF_3$	270
2	19.303	6.78	2,6,10-Trimethyl,14-ethylene-14-pentadecne	$C_{20}H_{38}$	278
3	19.392	3.35	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}$	280
4	19.671	4.48	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{400}$	296
5	20.59	2.68	Cyclohexane, (1,1-dimethylpropyl)-	$C_{11}H_{22}$	154
6	21.167	0.24	3-Isobutylhexahydropyrrolo[1,2-a]pyrazine-	$C_{11}H_{18}N_2O_2$	210
7	21.262	0.24	1,2-Benzenedicarboxylic acid, dibutyl ester	$C_{16}H_{22}O_4$	278
8	21.524	2.92	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284
9	22.912	0.84	11,14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	322

10	22.985	3.04	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296
11	23.177	1.92	Phytol	$C_{20}H_{40}O$	296
12	23.299	0.6	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
13	23.77	1.25	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280
14	23.835	4.4	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310
15	24.148	1.13	Heptadecanoic acid, ethyl ester	$C_{19}H_{38}O_2$	298
16	25.357	0.76	n-Heptadecanol-1	$C_{17}H_{36}O$	256
17	25.467	0.59	Hexadecanoic acid, 1-[[[(2-aminoethoxy)hydroxyphosphinyl]ox	$C_{37}H_{74}NO_8P$	316
18	25.796	0.53	Eicosanoic acid, methyl ester	$C_{25}H_{50}O_2$	382
19	26.217	0.24	n-Decylmethylphosphonofluoridate	$C_{11}H_{24}FO_2P$	238
20	27.178	0.45	2,2,3,3,4,4 Hexadeuterooctadecanal	$C_{18}H_{30}DO$	274
22	27.696	1.06	4-Ethylthiane	$C_7H_{14}S$	130
23	27.895	0.73	1-Decanol, 2-hexyl-	$C_{16}H_{34}O$	242
24	28.166	0.68	1-Hydroxymethyl-2-methyl-1-cyclohexene	$C_8H_{14}O$	126
25	28.403	0.5	Heneicosanoic acid, methyl ester	$C_{22}H_{44}O_2$	340
26	28.829	0.8	1,2-Benzenedicarboxylic acid, diisooctylest	$C_{24}H_{38}O_4$	390
27	29.459	2.99	Docosanoic acid, ethyl ester	$C_{24}H_{48}O_2$	368
28	32.22	0.44	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	382
29	34.416	0.38	2-Piperidinone, N-[4-bromo-n-butyl]-	$C_9H_{16}BrNO$	233
30	34.654	1.11	Pseduosarsasapogenin-5,20-dien	$C_{27}H_{42}O_3$	414
31	35.667	0.15	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate	$C_{33}H_{54}O_3$	498
32	36.093	0.45	Diazoprogesterone	$C_{21}H_{30}N_4$	338
33	36.205	0.45	Ergosta-5,7-dien-3-ol, (3.beta.)-	$C_{28}H_{46}O$	398
34	36.328	0.22	12,15-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	290
35	36.375	0.25	Stigmastan-6,22-dien, 3,5-dedihydro-	$C_{29}H_{46}$	394
36	36.426	0.23	.gamma.-Tocopherol	$C_{28}H_{48}O_2$	416
37	36.554	0.42	3-Bromocholest-5-ene #	$C_{27}H_{45}Br$	448
38	36.605	0.24	Tetracosane	$C_{24}H_{50}$	338
39	36.673	2.55	Cholesta-4,6-dien-3-ol, benzoate, (3.beta.)-	$C_{34}H_{48}O_2$	488
40	37.03	0.96	2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecy	$C_{29}H_{50}O_2$	430
41	37.301	1.59	Tetratriacontane	$C_{34}H_{70}$	478
42	37.626	0.18	cis-p-Mentha-2,8-dien-1-ol	$C_{10}H_{16}O$	152
43	38.001	2.33	Hexatriacontane	$C_{36}H_{74}$	506
44	38.077	1.78	9,19-Cyclolanostan-3-ol, acetate, (3.beta.)-	$C_{32}H_{54}O_2$	470
45	38.301	0.17	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326
46	38.353	0.81	9,19-Cyclolanost-23-ene-3,25-diol, 3-acetate, (3.beta.,23E)-	$C_{32}H_{52}O_3$	484
47	38.455	0.15	Docosane, 1,22-dibromo-	$C_{22}H_{44}Br_2$	354
48	38.586	16.35	.beta.-Sitosterol	$C_{29}H_{50}O$	414
49	38.749	8.68	Tetratetracontane	$C_{44}H_{90}$	618
50	38.956	0.51	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3.b	$C_{32}H_{52}O_2$	468
51	39.066	0.44	7,8-Epoxy-.alpha.-ionone	$C_{13}H_{20}O_2$	245
52	39.278	0.51	4,22-Stigmastadiene-3-one	$C_{29}H_{46}O$	410
53	39.828	3.61	Cholest-4-en-3-one	$C_{27}H_{44}O$	384
54	40.145	0.64	Iron iodide complex I	$C_{26}H_{26}FeIN_4O_4$	641
55	42.434	0.48	Hexadecanoic acid, tetradecyl ester	$C_{30}H_{60}O_2$	452
56	44.221	0.6	Sulfurous acid, pentadecyl 2-propyl ester	$C_{18}H_{38}O_3S$	334

TABLE 5: RETENTION TIME, MOLECULAR WEIGHT AND % AREA BY SETTING THE TOTAL PEAK AREA TO 100% OF FLAVONOIDS IDENTIFIED BY GC-MS IN BARK OF *TECOMELLA UNDULATA*

Peak#	R. Time	Area%	Name	Mol. Formula	Mol. wt
1	19.234	1.08	1-Decene, 8-methyl-	$C_{11}H_{22}$	154
2	19.323	6.3	2,6,10-Trimethyl,14-ethylene-14-pentadecne	$C_{20}H_{38}$	278
3	19.408	2.16	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}$	280
4	19.957	2.63	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
5	20.606	5.88	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
6	21.283	0.83	Phthalic acid, 4-bromophenyl heptyl ester	$C_{21}H_{23}BrO_4$	418
7	21.534	7.40	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284
8	22.921	1.86	11,14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	322
9	22.987	6.71	7-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	268
10	23.305	1.44	Heptadecanoic acid, methyl ester	$C_{18}H_{36}O_2$	270
11	23.775	1.69	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280
12	23.834	5.61	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310
13	25.369	0.9	1-Octanol, dimethyl-	$C_{10}H_{22}O$	158

14	25.797	0.84	Hexadecanoic acid, 15-methyl-, methyl ester	$C_{18}H_{36}O_2$	284
15	27.194	0.6	Nonanoyl chloride	$C_9H_{17}ClO$	176
16	27.565	2.85	Benzene, (4-bromobutyl)-	$C_{10}H_{13}Br$	212
17	28.408	0.67	Heneicosanoic acid, methyl ester	$C_{22}H_{44}O_2$	382
18	28.842	1.33	1,2-Benzenedicarboxylic acid, dinonyl ester	$C_{26}H_{42}O_4$	418
19	29.041	1.53	2-[(Benzyloxy)carbonyl]-3-((benzyloxy)carbo	$C_{37}H_{36}N_2O_5$	588
20	29.26	2.81	(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-	$C_{22}H_{20}OS$	332
21	29.463	3.20	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312
22	32.228	1.04	Heneicosanoic acid, methyl ester	$C_{22}H_{44}O_2$	340
23	33.496	1.79	Docosanoic acid, ethyl ester	$C_{24}H_{48}O_2$	368
24	35.211	0.42	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326
25	36.559	1.15	Methyl 10,12-pentacosadiynoate	$C_{26}H_{44}O_2$	368
26	36.677	2.52	Cholesta-4,6-dien-3-ol, benzoate, (3.beta.)-	$C_{34}H_{48}O_2$	488
27	37.025	0.88	Vitamin E	$C_{29}H_{50}O_2$	430
28	38.079	1.04	2-Butanone, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-	$C_{13}H_{22}O$	194
29	38.3	1.52	Triacontanoic acid, methyl ester	$C_{31}H_{62}O_2$	438
30	38.589	23.71	.beta.-Sitosterol	$C_{29}H_{50}O$	414
31	39.846	1.84	Cholest-4-en-3-one	$C_{27}H_{40}O$	384
32	41.093	0.07	Curlone	$C_{15}H_{22}O$	218

TABLE 6: RETENTION TIME, MOLECULAR WEIGHT AND % AREA BY SETTING THE TOTAL PEAK AREA TO 100% OF FLAVONOIDS IDENTIFIED BY GC-MS IN ROOTS OF *TECOMELLA UNDULATA*

Peak#	R. Time	Area%	Name	Mol. Formula	Mol. wt
1	17.663	0.35	1-Hexanol, 3-methyl-	$C_7H_{16}O$	116
2	19.226	0.95	1-Decene, 8-methyl-	$C_{11}H_{22}$	154
3	19.316	5.74	2,6,10-Trimethyl,14-ethylene-14-pentadecne	$C_{20}H_{38}$	278
4	19.403	1.82	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}$	280
5	19.682	3.13	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
6	20.598	5.26	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
7	21.267	0.18	1,2-Benzenedicarboxylic acid, dibutyl ester	$C_{16}H_{22}O_4$	278
8	21.529	6.99	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284
9	22.916	1.39	11,14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	322
10	22.985	5	7-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	268
11	23.833	6.16	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310
12	25.79	0.7	Hexadecanoic acid, 15-methyl-, methyl ester	$C_{18}H_{36}O_2$	284
13	26.569	0.58	Eicosanoic acid, ethyl ester	$C_{22}H_{44}O_2$	340
14	27.183	0.7	Docosanoic acid	$C_{22}H_{44}O_2$	340
15	27.55	8.55	1,2-Propanediol, 3-benzyloxy-1,2-diacetyl-	$C_{14}H_{18}O_5$	266
16	28.402	0.91	Heneicosanoic acid, methyl ester	$C_{22}H_{44}O_2$	340
17	28.838	0.93	1,2-Benzenedicarboxylic acid, dinonyl ester	$C_{26}H_{42}O_4$	418
18	29.025	16.38	(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-	$C_{22}H_{20}OS$	332
19	29.462	2.33	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312
20	30.102	0.47	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	256
21	32.239	0.71	Heptadecanoic acid, methyl ester	$C_{18}H_{36}O_2$	284
22	36.376	0.46	Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)-	$C_{31}H_{50}O_2$	454
23	36.555	0.65	Kauren-19-yl-acetate	$C_{22}H_{34}O_2$	330
24	36.675	1.97	Cholesta-4,6-dien-3-ol, benzoate, (3.beta.)-	$C_{34}H_{48}O_2$	488
25	37.311	1.02	Docosanoic acid, ethyl ester	$C_{24}H_{48}O_2$	368
26	37.875	0.5	Diazoprogesterone	$C_{21}H_{30}N_4$	338
27	38.077	0.83	2-Butanone, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-	$C_{13}H_{22}O$	194
28	38.292	0.3	Triacontanoic acid, methyl ester	$C_{31}H_{62}O_2$	466
29	38.354	0.29	3-Hydroxy-6-oxo-drimenol	$C_{15}H_{24}O_3$	252
30	38.589	16.86	.beta.-Sitosterol	$C_{29}H_{50}O$	414
31	38.773	0.78	Docosanoic acid, ethyl ester	$C_{24}H_{48}O_2$	368
32	39.482	0.34	Cholesterol 3-O-[[2-acetoxy]ethyl]-	$C_{31}H_{52}O_3$	472
33	39.841	1.61	Cholest-4-en-3-one	$C_{27}H_{44}O$	384

TABLE 7: RETENTION TIME, MOLECULAR WEIGHT AND % AREA BY SETTING THE TOTAL PEAK AREA TO 100% OF FLAVONOIDS IDENTIFIED BY GC-MS IN STEMS OF *TECOMELLA UNDULATA*

Peak#	R. Time	Area%	Name	Mol. Formula	Mol. wt
1	8.213	0.44	1,1-Dioctyloxyoctane	$C_{24}H_{50}O_2$	370
2	11.931	0.24	1-Pentadecanol	$C_{15}H_{32}O$	228
3	17.616	0.4	Cyclopropane, 1-methyl-2-(3-methylpentyl)-	$C_{10}H_{20}$	140

4	19.188	0.6	1-Undecene, 9-methyl-	$C_{12}H_{24}$	168
5	19.272	5.59	2,6,10-Trimethyl,14-ethylene-14-pentadecne	$C_{20}H_{40}O$	296
6	19.365	0.83	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}$	280
7	19.638	4.07	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
8	20.551	1.11	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
9	21.528	7.49	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284
10	22.981	4.88	Cyclohexanepropanoic acid, methyl ester	$C_{10}H_{18}O_2$	170
11	23.773	1.77	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280
12	23.835	6.51	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310
13	25.366	0.36	Phosphonic acid, dioctadecyl ester	$C_{36}H_{75}O_3P$	586
14	25.469	0.32	Hexadecanoic acid, 2,3-dihydroxypropyl ester	$C_{19}H_{38}O_4$	330
15	27.189	1.06	Nonylchloroformate	$C_{10}H_{19}ClO_2$	206
16	27.698	1.05	Cyclohexaneacetic acid, .alpha.-methyl-.alpha.-propyl-, methyl	$C_{13}H_{24}O_2$	212
17	27.9	0.51	Octadecane, 1-chloro-	$C_{18}H_{37}Cl$	288
18	28.833	1.7	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	390
19	29.46	1.56	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312
20	32.218	1.72	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	382
21	33.491	3.2	Docosanoic acid, ethyl ester	$C_{24}H_{48}O_2$	368
22	34.009	0.72	14-Pentadecynoic acid, methyl ester	$C_{16}H_{28}O_2$	252
23	35.358	0.4	5-Ethyl-2-(furfurylidenehydrazino)-2-thiazolin-4-one	$C_{10}H_{11}N_3O_2S$	237
24	36.325	0.22	9,19-Cyclolanostan-3-ol, acetate, (3.beta.)-	$C_{32}H_{54}O_2$	470
25	36.373	0.28	Cholesta-6,22,24-triene, 4,4-dimethyl-	$C_{29}H_{46}$	394
26	36.432	0.3	.gamma.-Tocopherol	$C_{28}H_{48}O_2$	416
27	36.553	0.66	Kauren-19-yl-acetate	$C_{22}H_{34}O_2$	330
28	36.674	2.71	Cholesta-4,6-dien-3-ol, benzoate, (3.beta.)-	$C_{34}H_{48}O_2$	488
29	36.832	8.32	Cholest-5-en-3-ol (3.beta.)-, propanoate	$C_{30}H_{50}O_2$	442
30	37.029	0.65	dl-.alpha.-Tocopherol	$C_{29}H_{50}O_2$	430
31	37.308	0.88	Docosanoic acid, ethyl ester		
32	37.992	0.04	Cholesta-1,4-dien-3-one	$C_{27}H_{42}O$	382
33	38.076	1.93	3-Hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5	$C_{17}H_{26}O_3$	278
34	38.3	0.26	Heneicosanoic acid, methyl ester	$C_{22}H_{44}O_2$	340
35	38.352	0.88	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3.b	$C_{32}H_{52}O_2$	468
36	38.589	11.84	.beta.-Sitosterol	$C_{29}H_{50}O$	414
37	39.468	1.2	Stigmasta-3,5-dien-7-one	$C_{29}H_{46}O$	410
38	39.834	3.16	Cholest-4-en-3-one	$C_{27}H_{44}O$	384

The active principles in the plants may not be major compound(s) in it but the activity of a compound may be masked by some other components in an extract [20, 21]. To evaluate the biological or pharmacological importance [22, 23] various activities such as antibacterial, antifungal, antiviral, antitumor, anti-inflammatory, antipyretic and analgesic were tested by a number of workers, with an aim to identify the active principle(s) and their bioefficacy [24].

Table 8 shows detailed investigation of antimicrobial assay of crude extracts of flavonoids of *Tecomella undulata.*

Antifungal activity of the flavonoids extracted from different plant parts when tested against Fusarium showed that root showed maximum inhibition while stem showed minimum inhibition (Root; IZ=14.66 ± 2.51 mm > bark; IZ= 13.00 ± 2.00 mm > Leaf; IZ=12.66 ± 2.08 mm > Stem; IZ=11.00 ± 1.00 mm). When tested against *Penicilium funiculosum*, bark showed maximum inhibition while leaf showed mini (Bark; IZ =16.33 ± 1.00 mm > root; IZ = 14.66 ± 3.05 mm > stem; IZ = 9.66 ± 0.57 mm > leaf; IZ = 0.00 mm). Against *Candida albicans* leaf extract showed maximum inhibition while bark showed minimum (Leaf; IZ = 26.66 ± 2.08 mm > stem; IZ = 14.00 ± 1.00 mm > root; IZ = 13.33 ± 1.52 mm > bark; IZ = 12.67 ± 1.053 mm). Against *T. virdae*, bark showed maximum inhibition while stem showed minimum inhibition. (Bark; IZ = 26.00 ± 2.00 mm > leaf; IZ = 15.66 ± 2.08 mm > root; IZ = 14.33 ± 0.57 mm > Stem; IZ = 12.33 ± 2.51 mm).

Antibacterial activity of flavonoids against *S. aureus* was shown maximum by leaf whereas minimum by root (Leaf; IZ = 27.33 ± 2.08 mm > stem; IZ = 16.33 ± 1.52 mm > bark; IZ = 16.00 ± 1.00 mm > root; 0.00 mm). While against *E. coli* leaf showed maximum activity and root showed minimum inhibition (Leaf; IZ = 21.00 ± 1.00 mm > bark; IZ = 14.66 ± 1.52 mm > stem; IZ = 14.33 ± 2.08 mm > root; IZ = 14.00 ± 1.00 mm). Against

enterococcus, none of the extracts showed any activity (Root, stem, bark, leaf; IZ = 0.00 mm). Against *Bacillus subtilis*, leaf showed maximum activity and stem showed minimum activity (Leaf; IZ = 20.66 ± 1.15 mm > bark; IZ = 17.00 ± 2.64 mm > root; IZ = 16.66 ± 1.52 mm > stem; IZ = 15.00 ± 1.00 mm). Against *Klebsiella pneumonia*, bark showed maximum whereas root and stem showed minimum inhibitory activity (Bark; IZ = 13.00 ± 1.73 mm > leaf; IZ = 11.66 ± 2.88 mm > root, stem; IZ = 0.00 mm).

TABLE 8: BACTERICIDAL AND FUNGICIDAL EFFICACY OF FLAVONOIDS CRUDE EXTRACTS OF *TECOMELLA UNDULATA*

Microorganisms		Root	Stem	Bark	Leaves
Fungi		**Flavonoids**	**Flavonoids**	**Flavonoids**	**Flavonoids**
F. oxysporium	IZ	14.66 +2.51	11.00 +1	13.00+2	12.66 +2.08
NCIM 1228	AI	0.64	0.46	0.62	0.53
P. fumiculosum	IZ	14.66 +3.05	9.66 +0.57	16.33+1	0.00
NCIM 1075	AI	0.7	0.46	0.78	0.00
C. albicans	IZ	13.33 +1.52	14.00 +1	12.67+1.53	26.67 +2.08
NCIM 3501	AI	0.78	0.82	0.75	1.56
T. viridie	IZ	14.33 +0.57	12.33 +2.51	26.00+2	15.66 +2.08
NCIM	AI	0.53	0.46	0.96	0.51
Bacteria					
S. aureus	IZ	0.00	16.33±1.52	16.00±1	27.33±2.08
MTCC 0087	AI	0.00	0.54	0.54	0.91
E. coli	IZ	14.00±1	14.33±2.08	14.66±1.52	21.00±1
MTCC 1652	AI	0.78	0.79	0.82	1.16
E. faecalis	IZ	0.00	0.00	0.00	0.00
ATCC 29212	AI	0.00	0.00	0.00	0.00
B. subtilis	IZ	16.66±1.52	15.00±1	17.00±2.64	20.66±1.15
MTCC 0121	AI	0.83	0.75	0.85	1.03
K. pneumonia	IZ	0.00	0.00	13.00±1.73	11.66±2.88
MTCC 0109	AI	0.00	0.77	0.00	0.00

CONCLUSION: The current investigation reveals the high amount of flavonoid in leaves which also show high inhibitory activity against *C. albicans* and *S. aureus*. A variety of Compounds with high Pharmacological Value were identified to be present in different plant parts of *Tecomella undulata*. Roheda is therefore a pack of bioactive components which can be further investigated for curing of ailments, which so far have not found a cure and put to Pharmacological use.

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