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Phytochemical Examination and Antimicrobial Activity of Various Solvent Extracts and the Selected Isolated Compounds from Roots of *Tragia involucrata* Linn.

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ABSTRACT
A large number of medicinal plants have been used for years in daily life to combat diseases, world over. Presently herbs, embodied with due importance in healthcare system, create a herbal renaissance, spread with a greater speed throughout the world. The herbal products today symbolize safety in contrast to the synthetic drugs that are regarded as unsafe to human environment. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases, have led to increase emphasis on the use of plant materials as source of medicines for a wide variety of human ailments. It is therefore, essential to search for the efficacious plants of medicinal value for better manifestations. In the present study, the fresh roots of young matured plants of *Tragia involucrata* Linn. were selected for phytochemical and antimicrobial screening to justify the folklore uses of the plant parts in various microbial infections.

Keywords: *Tragia involucrata* Linn., solvent extracts, phytochemical examination, isolated compounds, antimicrobial activity.

INTRODUCTION

Nature is the paradise of medicinal principles offers to the humanity through plants which act as richest source of phytochemicals since time immemorial. An impressive number of modern drugs have been isolated from the floristic resources; many being tapped basing on their use in the treatises of traditional medicines. Various medicinal plants have been used for years in daily life to combat diseases, world over. The widespread use of herbal remedies in healthcare preparations, such as those described in ancient texts described as 'Ethno-medicines', has been traced as the natural products with medicinal properties. In fact, plants (otherwise termed as botanicals) produce a diverse range of bioactive molecules, making them a rich source of various types of medicaments. Presently herbs, embodied with due importance in healthcare system, create a herbal renaissance, spread with a greater speed throughout the world. The herbal products today symbolize safety in contrast to the synthetic drugs that are regarded as unsafe to human environment. The traditional systems of medicine continue to be widely practised on many accounts even at present. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases, have led to increase emphasis on the use of plant materials as source of medicines for a wide variety of human ailments. India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely used as raw material for manufacture of drugs and allied products. Ayurveda is gaining momentum and prominence as the natural system of health care all over the world.

Presently, more emphasis is laid on use of herbal drugs because of their easy availability and cost effectiveness comparing to the synthetic drugs which are not always affordable by the common people and also the synthetic drugs are having unwanted side effects. It is therefore, essential to search for the efficacious plants of medicinal value for better manifestations. On personal interaction and enquiry by the author with the local people of Mahuda village it was selected to work out on the probable antimicrobial properties of various extracts as well as isolated compounds of root of *Tragia involucrata* Linn. collected from the hilly areas of Mohuda village, Ganjam District, Odisha.

MATERIALS AND METHOD

Collection of Plant material

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Fresh roots of young matured plants of *Tragia involucrata* Linn. were collected from the local scrub hilly areas of Mohuda village. After authentication, the roots were collected in bulk, washed under running tap water to remove adhering dirt followed by rinsing with distilled water. The roots were then fully shade dried and pulverized in a mechanical grinder followed by sieving (sieve no. 40) to obtain coarse powder.

Preparation of extracts

The dried powdered roots (500 g) were separately extracted successively with various solvents *viz*. petroleum ether (40-60°C), ethyl acetate and methanol in increasing order of polarity using a Soxhlet extractor. The period of extraction was fixed at 48 h for every solvent at every stage of the extraction process. The solvents were purified by distillation prior to extraction. [1]

Phytochemical examination of selected extract

The ethyl acetate root extract of *T. involucrata* Linn. was subjected to column chromatography using silica gel (60-120 mesh size), and eluted with various solvent ratios of n-Hexane: Ethyl acetate. Five different fractions were collected from the column. The elutes collected were monitored by thin layer chromatography for homogeneity and the similar fractions were pooled together. Five compounds (TIR-01, TIR -02, TIR -03, TIR -04 and TIR -05) were isolated from these fractions.

Spectroscopy of isolated compounds

The IR spectrum of **TIR-01** showed absorption bands at 3416.6 (O-H, free hydroxyl group), 2925.0 and 2853.8 (Ali-C-H, str), 1734.7 (C=O, ester), 1616.8 (C=C stretch), 1529.2 and 1447.3 (C-C ring stretch),1286.5 (C-C stretching), 1071.1 (C-O-C), 779.9 and 622.8 (monosubstituted in aromatic ring) and 518.4 (out of plane ring C=C, bend) cm $^{-1}$. The ^{1}H-NMR spectrum of the compound displayed the characteristic signals at δ_H 6.570 (Ar-H), 5.338 (2H, s, H-2 and H-3), 4.867 (1H), 3.650 (OCH_3-), 3.300 (H-6), 1.436 (s, 3H), 1.280 (H, CH), 0.890 (d, CH_3). The mass data showed m/z = 402 (100) [M^+], an indicative of $C_{27}H_{47}O_2$ with other fragments 307[402-C_6H_7O]$^+$, 165 [307-$C_{10}H_{22}$]$^+$, 134 [307-$C_{11}H_9O_2$]$^+$, 96 [134-CH_4O_2]$^+$. On the basis of above spectral data and related literatures [2-3], the compound TIR-01 was identified as 10, 13-dimethoxy-17-(6-methylheptan-2-yl)-2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthrene.

The IR spectrum of the compound **TIR-02** showed absorption bands at 3778.6 (Hydrogen bonding), 3691.8 to 3376.1 (O-H, free hydroxyl group), 3022.6 (Cyclic C-H, str), 2927.2 (Ali- C-H, str), 2401.1, 2337.4 and 2269.1 (Alkyne group), 1812.9 (C=O, ester), 1661.7 (C=C stretch), 1588.7, and 1434.1 (C-C ring stretch), 1218.1 (C-C stretching), 929.2 (O-H, out of plane bend), 720.9 and 673.6 (monosubstituted in aromatic ring), and 487.8 (out of plane ring C=C, bend).The ^{1}H-NMR spectrum of the compound displayed the characteristic signals at δ_H 3.68 (1H, s), 6.50 (1H, m), 2.06 (3H, s), 1.90 (3H, s), 1.67 (3H, s), 3.79 (1H, m), 3.76 (1H, m), 1.23 (3H, s), 1.21 (3H, s), 1.18 (3H, s). The FTIR and ^{1}H-NMR spectral results of this compound were compared with the reported literatures [4-5] and the mmp reading as well as Co-TLC data by comparison with authentic sample could characterize the compound to be Stigmasterol.

The IR spectrum of compound **TIR-03** showed absorption bands at 3388.4 (O-H, free hydroxyl group), 2932.3 (Ali- C-H, str), 1630.3 (C=C stretch), 1387.2 (C-C ring stretch), 1074.6 (C-O-C), 933.1 (O-H, out of plane bend), 779.1 (monosubstituted in aromatic ring), 620.9 (out of plane ring C=C, bend).The ^{1}H-NMR spectrum of compound displayed the characteristic signals at δ_H8.12 (1H, H-6'), 7.96 (1H, H-2'), 7.31 (1H, H-5'), 7.29 (1H, H-8), 7.20 (1H, H-6), 4.52 (1H, m, H-3), 3.36(1H, m, H-3), 2.54(1H, H-8), 1.24 (1H, H-6) ppm. The FTIR and ^{1}H-NMR values of this compound were compared with the reported literatures [6-7] and the mmp reading as well as Co-PC data by comparison with authentic sample gave inference that the compound could be characterized as Quercetin.

The IR spectrum of the compound **TIR-04** showed absorption bands at 3366.5 to 3286.9 (O-H, free hydroxyl group), 3063.0 (Cyclic C-H, str), 1705.7 (C=O, ester), 1619.2 (C=C stretch), 1541.0, 1448.2.3 and 1339.1 (C-C ring stretch), 1245.0 (C-C stretching), 1024.3 (C-O-C), 867.1 (O-H, out of plane bend), 764.8 and 701.1 (monosubstituted in aromatic ring), 570.3 (out of plane ring C=C, bend).The ^{1}H-NMR spectrum of compound displayed the characteristic signals at δ_H8.04 (1H, *d*, H-2), 6.96 (1H, *d*, H-5), 6.53 (1H, *d*, H-6), 7.28 (1H, H-6), 6.66 (1H, H-8), 7.12 (1H, *s*, OH-3), 6.94 (1H, *s*, OH-4), 9.21 (1H, *s*, OH-5) of aglycone; 4.40 (1H, H-1), 3.49 (1H, H-2), 3.35 (1H, *m*, H-3), 3.21 (1H, *m*, H-4), 3.26 (1H, *m*, H-5), 1.20 (3H, H-6) of rhamnose of rutinose; 5.04 (1H, H-1), 3.28 (1H, *m*, H-2), 3.49 (1H, H-3), 3.43 (1H, H-4., 3.30), (1H, *m*, H-5., 3.37 (1H, *m*, H-6), 3.51 (1H, H-6) of glucose of rutinose; 5.03 (1H, *d*, H-1), 3.54 (1H, H-2), 3.59 (1H, *m*, H-3), 3.34 (1H, *m*, H-4), 3.94 (1H, H-5), 3.93 (1H, H-6), 4.33 (1H, H-6) of glucose of 6-*O*-benzoyl glucose; 6.94 (2H, H-2, H-6), 6.66 (2H, H-3, H-5), 6.59 (1H, H-4) of benzoyl moiety. The spectral results of the compound were well comparable with that of the related literatures [8-9] and simultaneously, the mmp and Co-PC data were compared with the authentic sample which gave inference that this compound could be characterized as rutin.

The IR spectrum of **TIR-05** showed absorption bands at 3415.9 (O-H, free hydroxyl group), 2923.6 and 2852.1 (Ali-C-H, str), 1637 and 1617.2 (C=C stretch), 1516.5, and 1456.0 (C-C ring stretch), 1288.0 (C-C stretching), 1075.9 (C-O-C), 779.3 and 620.7 (monosubstituted in aromatic ring) and 518.4 (out of plane ring C=C, bend), 481.4 and 417.9 (out of plane ring C=C, bend) cm $^{-1}$.The ^{1}H-NMR spectrum of the compound TIR-05 displayed the characteristic signals at δ_H8.023 (d, H2), 7.612 (1H, s, H-4), 6.752 (Ar-H), 5.387 (2H, s, H-2 and H-3), 3.772 (m, H-2), 3.632 (OCH_3-), 3.389 (m, 1H), 2.909 (OPh), 1.278 (H, CH), 0.867 (d, CH_3). The mass data showed m/z = 330 (100) [M^+], an indicative of $C_{19}H_{22}O_5$ with other fragments 284[330-$C_2H_6O_2$]$^+$, 233 [284-C_4H_4]$^+$, 120 [233-$C_7H_{13}O$]$^+$.On the basis of above spectral data and related literatures [10-13], the compound TIR-05 was identified as "3-(2,4-dimethoxyphenyl)-6,7-dimethoxy-2,3-dihydrochro-men-4-one".

Antimicrobial activity study of root extracts and isolated compounds (first time reported)

Standard drugs used as reference for activity studies

Chloramphenicol and Ketoconazole, two pure polymers, procured from M/s Science World, Bhopal (MP), were used as standard drugs in the present work for antibacterial and antifungal activity studies respectively.

Microorganisms used

For the present study, the bacteria and fungi used, include the gram +ve bacteria such as Staphylococcus *aureus* (MTCC

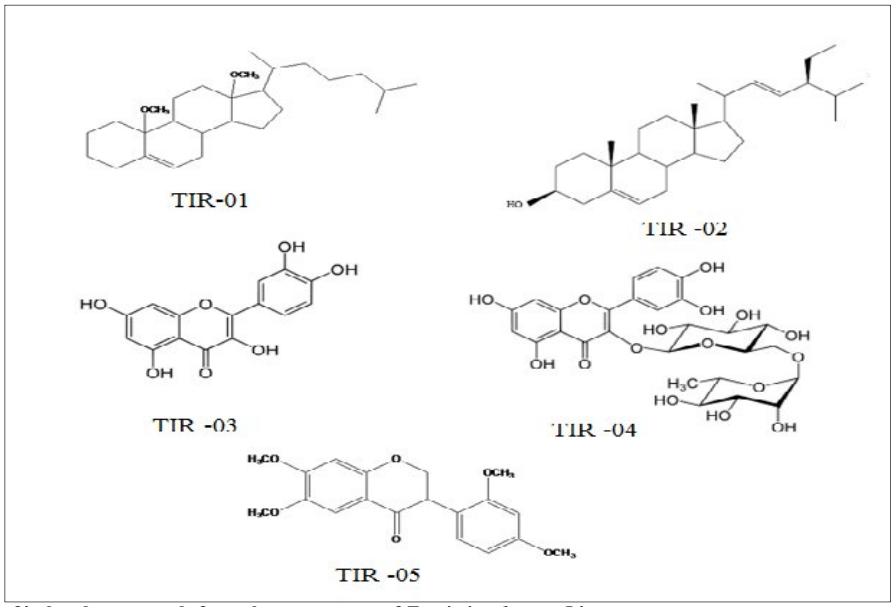

Fig. 1: Chemical structures of isolated compounds from the root extract of *Tragia involucrata* Linn.

Table 1: Data showing zone of inhibition (mm) of *T. involucrata* root extracts against selected gram stains of bacteria and fungi

Various Conc. of Extracts	GM. + VE BACTERIA				GM. – VE BACTERIA				FUNGI	
	Sta. epi.	*Sta. aur.*	*Baci. Sub.*	*Baci. Brev.*	*Esch. coli*	*Vib. chol.*	*Shi. dys.*	*Pseu. aeru.*	*Mala. fur.*	*Tric. Rub.*
Petroleum ether Extract										
50 mg/ml	-	-	-	-					-	-
100 mg/ml	-	0.6±0.02	-	-	-	-	-	-	-	-
150 mg/ml	-	1.5±0.05	0.4±0.02	-	0.8± 0.03	-	-	-	-	-
200 mg/ml	-	2.1±0.12	1.2±0.04	-	1.4± 0.08	-	-	-	-	-
250 mg/ml	0.6±0.03	2.8±0.15	1.6±0.07	-	1.5± 0.27	-	-	0.5± 0.01	0.5± 0.02	-
Ethyl acetate Extract										
50 mg/ml	1.4±0.03	5.6±0.44	1.2±0.02	-	1.7± 0.26	0.5± 0.02	-	0.6± 0.01	-	-
100 mg/ml	3.2±0.17	9.2±0.83	4.5±0.08	0.8±0.04	3.5± 0.27	-	-	-	-	-
150 mg/ml	7.1±0.26	16.4±1.15	7.3±0.16	1.4±0.05	8.2± 0.24	-	0.4± 0.02	-	2.3± 0.06	-
200 mg/ml	12.2±0.87	19.3±1.29	11.5±0.62	3.9±0.13	10.3±0.92	-	1.3± 0.04	3.2± 0.08	7.1± 0.26	-
250 mg/ml	18.3±1.14	22.4±1.87	14.1±0.92	5.7±0.23	13.2±1.26	1.5± 0.05	2.5± 0.38	5.1± 0.56	13.5± 0.86	3.7± 0.04
Methanol Extract										
50 mg/ml	-	2.4±0.22	-	-	17.1±1.69	4.7± 0.21	3.7± 0.34	9.4± 0.83	-	-
100 mg/ml	-	3.8±0.26	1.9±0.06	-	1.4± 0.12	-	-	-	-	-
150 mg/ml	0.6±0.02	8.7±0.48	2.5±0.14	0.4±0.01	3.4± 0.18	-	-	-	-	-
200 mg/ml	1.5±0.05	10.8±0.76	4.3±0.18	0.8±0.03	4.3± 0.25	-	0.6± 0.02	1.2± 0.06	1.8± 0.14	-
250 mg/ml	2.8±0.13	11.6±1.06	6.7±0.32	1.6±0.11	5.8± 0.56	-	1.2± 0.03	2.2± 0.21	2.6± 0.21	0.4± 0.02
	Chloramphenicol								**Ketoconazole**	
5 µg/ml	5.4±0.52	17.2±1.23	18.1±1.26	6.4±0.72	10.3±0.96	5.7± 0.66	8.9± 0.13	18.1±1.26	8.9± 0.04	5.4± 0.06
25µg/ml	12.1±0.97	21.5±1.85	23.6±1.93	13.2±1.21	16.5±1.26	8.3± 0.82	14.6±1.62	23.6±1.93	14.2± 1.04	11.7± 0.07
50µg/ml	18.3± 1.47	27.3±2.02	28.1±2.36	21.4±1.43	23.7±1.82	12.6±1.20	22.9±2.68	28.1±2.36	19.8± 1.47	14.3± 0.85
100µg/ml	21.2± 1.64	30.2±2.57	31.8±2.15	25.6±1.62	29.3±2.32	18.2±1.21	28.6±2.32	31.8±2.15	31.1± 1.88	21.5± 1.32
200µg/ml	26.3± 2.68	36.1±2.41	39.5±2.59	32.2±1.81	34.7±1.94	25.6±1.59	32.7±3.04	39.5±2.59	37.5± 1.94	27.1± 1.67

(*Sta.epi.- Staphylococcus epidermidis, Sta.aur.- Staphylococcus aureus, Baci.Sub.- Bacillus subtilis, Baci. Brev.- Bacillus brevis, Esch. coli- Escherichia coli, Vib. chol.- Vibrio cholera, Shi. dys.- Shigella dysenteirae, Pseu. aeru.- Pseudomonas aeruginosa, Mala. fur.- Malassezia furfur* and *Tric. Rub. - Trichophyton rubrum*. Mean ± SEM, n = 3. The results were the mean values of tests repeated three times after every 24 h and 72 h of inhibitions for bacteria and fungi respectively, at 37° C. ' – ' : No inhibition)

7443), *Bacillus subtilis* (MTCC 619), *Bacillus brevis* (MTCC 4832) and *Staphylococcus epidermidis* (MTCC 2639); gram –ve bacteria such as *Escherichia coli* (MTCC 1687), *Shigella dysenteriae* (Lab. isolate from stool), *Pseudomonas aeruginosa* (MTCC 1688) and *Vibrio cholera* (MTCC 3904) and fungi such as *Trichophyton rubrum*(MTCC 296) and *Malassezia furfur* (MTCC 1765). MTCC strains of the above micro-organisms were procured through M/s Growtips Biotech Research Lab., Bhopal (MP) and rest other strains were procured from the 'Region of Diagnostic Centre', Capital Hospital, Bhubaneswar (Odisha).

Preparation of stock solutions [14-15]

Stock dilutions of the standard drug solutions of concentrations 1000 and 100µg/L were prepared as per requirement from original stock solution (10,000 mg/L). Then two rows of 12 sterile (7.5×1.3cm) & capped tubes were arranged in the rack. 8ml of broth containing required concentration of the standard drug solution (required for first tube of each row) was prepared from the existing stock solution and kept in a sterile 30ml (Universal) screw capped bottle. The contents of the universal bottle were properly mixed and 2ml from it was transferred to the first tube in each row.

Table 2: Data showing zone of inhibition (mm) of isolated compounds (first time reported) of *T. involucrata* roots against selected gram stains of bacteria and fungi

Various Conc. of Isolates	GM. + VE BACTERIA				GM. – VE BACTERIA				FUNGI	
	Sta. epi.	*Sta. aur.*	*Baci. Sub.*	*Baci. Brev.*	*Esch. coli*	*Vib. chol.*	*Shi. dys.*	*Pseu. aeru.*	*Mala. fur.*	*Tric. Rub.*
Compound : TIR – 01										
5µg/ml	0.8±0.06	3.8±0.37	0.6±0.04	-	0.8±0.07	-	-	-	-	-
25µg/ml	2.4±0.23	7.9±0.44	2.8±0.32	-	3.7±0.24	-	-	-	-	-
50µg/ml	5.9±0.38	14.3±1.02	6.2±0.57	0.4±0.02	6.9± 0.39	-	-	1.2±0.11	1.8± 0.32	-
100µg/ml	10.2±0.52	16.4±1.32	9.8±0.39	2.7±0.29	9.4±0.67	-	0.9±0.02	3.6±0.38	6.4± 0.63	-
200µg/ml	14.8±1.52	20.1±1.87	12.9±1.16	4.9±0.38	13.2±0.83	1.9±0.28	1.7±0.13	7.8±0.59	12.6± 0.83	3.1± 0.42
Compound : TIR – 05										
5µg/ml	-	2.1±0.17	-	-	2.7±0.27	-	-	-	-	-
25µg/ml	1.8±0.23	5.4±0.23	-	-	7.4±0.58	-	-	-	-	-
50µg/ml	2.7±0.31	8.9±0.53	-	-	9.6±0.64	-	0.4±0.02	2.3±0.26	1.3± 0.23	-
100µg/ml	6.9±0.39	12.5±0.84	-	-	12.3±0.89	0.5±0.02	1.8±0.13	4.3±0.31	5.7± 0.34	-
200µg/ml	11.5±0.76	16.7±1.72	0.9±0.07	-	16.2±1.23	3.9±0.38	3.2±0.31	8.7±0.69	11.9± 0.78	1.8± 0.23
	Chloramphenicol								**Ketoconazole**	
5µg/ml	5.4±0.52	17.2±1.23	18.1±1.26	6.4±0.72	10.3±0.96	5.7± 0.66	8.9± 0.13	18.1±1.26	8.9± 0.04	5.4± 0.06
25µg/ml	12.1±0.97	21.5±1.85	23.6±1.93	13.2±1.21	16.5±1.26	8.3± 0.82	14.6±1.62	23.6±1.93	14.2± 1.04	11.7± 0.07
50µg/ml	18.3±1.47	27.3±2.02	28.1±2.36	21.4±1.43	23.7±1.82	12.6±1.20	22.9±2.68	28.1±2.36	19.8± 1.47	14.3± 0.85
100µg/ml	21.2±1.64	30.2±2.57	31.8±2.15	25.6±1.62	29.3±2.32	18.2±1.21	28.6±2.32	31.8±2.15	31.1± 1.88	21.5± 1.32
200µg/ml	26.3±2.68	36.1±2.41	39.5±2.59	32.2±1.81	34.7±1.94	25.6±1.59	32.7±3.04	39.5±2.59	37.5± 1.94	27.1± 1.67

(*Sta.epi.- Staphylococcus epidermis, Sta.aur.- Staphylococcus aureus, Baci.Sub.- Bacillus subtilis, Baci. Brev.- Bacillus brevis, Esch. coli- Escherichia coli, Vib. chol.- Vibrio cholera, Shi. dys.- Shigella dysenteirae, Pseu. aeru.- Pseudomonas aeruginosa, Mala. fur.- Malassezia furfur* and *Tric. Rub. - Trichophyton rubrum*. Mean ± SEM, n = 3. The results were the mean values of tests repeated three times after every 24 h and 72 h of inhibitions for bacteria and fungi respectively, at 37° C. ' – ' : No inhibition)

Table 3: Data showing minimum inhibitory concentration (MIC) of *T. involucrata* root extracts against selected gram stains of bacteria and fungi

	Bacteria and Fungi used in the study	P.E. Ext.	E.A. Ext.	M. Ext.
Gram positive bacteria	*Staphylococcus epidermis*	62.5	0.78	37.5
	Staphylococcus aureus	50	6.25	3.12
	Bacillus subtilis	37.5	12.5	12.5
	Bacillus brevis	-	25	75
Gram negative bacteria	*Escherichia coli*	50	6.25	12.5
	Vibrio cholera	25	6.25	31.25
	Shigella dysenteriae	-	50	75
	Pseudomonas aeruginosa	50	37.5	37.5
Fungi	*Malassezia furfur*	62.5	9.37	25
	Trichophyton rubrum	-	15.62	62.5

(P.E. Ext. – Petroleum ether extract, E.A. Ext. – Ethyl acetate extract and M. Ext. – Methanol extract)

Table 4: Data showing minimum inhibitory concentration (MIC) of isolated compounds (first time reported) of *T. involucrata* roots against selected gram stains of bacteria and fungi

	Bacteria and Fungi used in the study	TIR-01	TIR-05
Gram positive bacteria	*Staphylococcus epidermis*	2.5	3.12
	Staphylococcus aureus	0.62	1.25
	Bacillus subtilis	2.5	3.14
	Bacillus brevis	3.12	12.5
Gram negative bacteria	*Escherichia coli*	1.25	1.25
	Vibrio cholera	1.25	12.5
	Shigella dysenteriae	12.5	12.5
	Pseudomonas aeruginosa	6.25	3.12
Fungi	*Malassezia furfur*	6.25	3.12
	Trichophyton rubrum	12.5	12.5

TIR-01 & TIR-05 : Isolated compounds (first time reported) of *T. involucrata* roots

Then, 4ml of the broth was added to the remaining 4ml content in the universal bottle by the help of a fresh pipette and and mixed well. Again, 2ml from it was transferred to the second tube in each row. In this way, dilutions were prepared. 2ml of antibiotic free broth was placed in the last tube in each row. One row was inoculated with one drop of an overnight broth culture of the test organism diluted approximately to 1 in 1000 in a suitable broth and the second row with the control organism of known sensitivity (similarly diluted). The result of the test was significantly affected by the size of the inoculums as the test mixture was containing 10^6organisms / ml. Finally the tubes were incubated for 18 hours at 37°C. A tube containing 2ml broth with the organism was inoculated and kept in a refrigerator at 4°C overnight which was to be used as standard for the determination of complete inhibition.

Determination of zone of inhibition by disc diffusion method

Antimicrobial activity of *T. involucrata* root extracts as well as isolated compounds (first time reported) was determined by "disc diffusion" method in reference to the reported literatures. [16-19] Petri-plates containing 20 ml of agar medium were seeded with a 24 h culture of the microbial strains. The sterilized filter paper discs (Whatman no.1) of 6mm in diameter were impregnated individually with plant extracts as well as isolated compounds at various concentrations ranging from 50 to 250 mg/ml and 5 to 200µg/ml respectively and placed on the inoculated agar. The inoculum size was adjusted so as to deliver a final inoculum of approximately 108 colony-forming units (CFU)/ml. Incubation was performed for both bacteria and fungus at 37ºC for 24 h and 37ºC for 72 h respectively. The assessment of antimicrobial activity was based on measurement of the diameter of the inhibition zone formed around the disc. A standard drug, chloramphenicol was used as a positive control for comparison of antibacterial activity and ketoconazole for antifungal activity respectively. All assays tests were carried out in triplicate for each and every concentration of root extracts as well as isolated compounds (first time reported) and the results were depicted in Tables 1 and 2.

Determination of Minimum inhibitory concentration (MIC)

Dilution susceptibility testing method was used for MIC determination in reference to the cited literatures [20-21] wherein, 75µl of sterile nutrient broth media was decanted into each well of a sterile 96-well micro plate. Highest concentration of the plant extract/isolate was added at 75µl to the first well. After mixing of the above, 75µl of the same was transferred to the second well and in this way, the dilution procedure was continued for the subsequent wells to

attain a series of dilutions of 1/2, 1/4, 1/8, 1/32, 1/64, 1/128, 1/256, 1/512 and 1/1024 respectively. Inoculum solution at 1.5µl was added to every well. Being incubated for 24 h at 37ºC, the tubes were monitored for turbidity growth and non-turbidity as no growth. The MIC values were interpreted as the highest dilution (lowest concentration) of the sample, which showed clear fluid with no development of turbidity. Solvent blanks and positive controls were also included. All the tests were carried out in triplicate. The MIC values of all the root extracts as well as isolated compounds (first time reported) of *T. involucrata* were calculated for all the used microbes (bacteria & fungi) and the results were recorded in the Tables 3 and 4.

RESULTS AND DISCUSSION

The ethyl acetate extract of *T. involucrata* was selected to phytochemical examination based on results on preliminary screening of five compounds, namely TIR-01, TIR-02, TIR-03, TIR-04 and TIR-05 were isolated and identified by comparing their spectral results with that of the related compounds (reported) in case of first time reported compounds and the mixed melting points and Co-TLC/Co-PC data of the isolated compounds (known) with that of the authentic samples (Fig. 1).

The antimicrobial activities noticed among the various solvent extracts of roots of *T. involucrata* were found to be maximum in case of ethyl acetate extracts in the concentration of 250mg/ml (Table 1). Maximum zone of inhibition (ZOI) against gram positive strains of bacteria was found to be 22.4 mm against *Staphylococcus aureus* (gm. +ve bacteria) which was comparable with that of the standard drug, Chloramphenicol (21.5mm inhibition) in a concentration of 25 µg/ml, 13.2 mm against *Escherichia coli* (gm. –ve bacteria) which was comparable with that of 16.5 mm inhibition of Chloramphenicol in a concentration of 25 µg/ml and 13.5 mm against *Malassezia furfur* (fungi) which was comparable with that of 14.2 mm inhibition of the standard drug, Ketoconazole in a concentration of 25 µg/ml. The lowest MIC (minimum inhibitory concentration) against almost all selected gram positive and gram negative bacteria and fungi were observed in ethyl acetate extract of *T. involucrata* (Table 3).

In a similar manner, the antimicrobial activities noticed in the selected isolated compounds in a concentration of 200µg/ml were well comparable with that of the standard drugs in the concentrations of 25µg/ml against some selective strains of bacteria and fungi (Table 2). Maximum ZOI of 20.1 mm was found in case of TIR-01 against the gm. +ve bacteria, *Staphylococcus aureus*; 16.2 mm in case of TIR-05 against the gm. –ve bacteria, *Escherichia coli* and 12.6 mm in case of TIR-01 against the fungi, *Malassezia furfur*. While comparing the MIC values of the two selected isolated compounds (TIR-01 and TIR-05), the lower MIC was observed in TIR-01 against almost all selected bacteria (Gm. + ve and – ve) and fungi with a few exceptions against *P. aeruginosa* (Gm. – ve bacteria) and *M. furfur* (fungi) and these were found to have low MIC in TIR-05 (Table 4).

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