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ANTIMICROBIAL EVALUATION AND HPTLC FINGER PRINT PROFILING OF THE LEAVES AND STEM OF *RHYNCHOTECHUM PERMOLLE* (NEES)B. L. BURTT

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ABSTRACT

The family Gesneriaceae comprises a diverse group of plants that are valued not only for their ornamental importance but also for their medicinal potential **[1]**. Many species belonging to this family are extensively cultivated as ornamental plants because of their attractive foliage and brightly coloured flowers **[2]**. In addition to their aesthetic value, several members of the family have been traditionally used in different systems of medicine for the treatment of various ailments. Phytochemical studies on Gesneriaceae species have revealed the presence of numerous bioactive compounds such as glycosides, steroids, saponins, flavonoids, and terpenoids. These secondary metabolites play a vital role in plant defense mechanisms and are also responsible for the diverse pharmacological activities exhibited by these plants. Different plant parts, including leaves, stems, roots, and flowers, contain these compounds in varying concentrations. The bioactive constituents isolated from members of the Gesneriaceae family

possess a wide range of biological activities, including antioxidant, antimicrobial, anti-inflammatory, and wound-healing properties. Consequently, plant extracts and purified compounds derived from these species are widely investigated for their potential use in the treatment and management of infections, inflammatory disorders, and oxidative stress–related diseases. The rich diversity of phytochemicals present in Gesneriaceae highlights its significance as an important source of natural products for both traditional and modern medicine, emphasizing the need for further detailed research into their therapeutic applications.

Keywords: Gesneriaceae; Medicinal Plants; Phytochemicals; Bioactive Compounds; Pharmacological Activities

INTRODUCTION

The plant generally occurs in moist and shady localities. It grows up to 18–65 cm in height, with alternate leaves having serrate to denticulate margins. The corolla is white and distinctly two-lipped in the upper region. The plants are commonly found in tropical forests, particularly in moist and shady habitats along streams and riverbanks **[1-3]**. The genus is recognized for its ethnobotanical significance and is traditionally used by several tribes inhabiting Northeast India **[4]**. Moller revealed that fleshy fruits of Rhynchotechum dispersed by birds **[5]**. The inflorescence of R.permolle is not very noticeable. A few Rhynchotechum species are used as food,medicine, smolking and fibre by indigenous peoples in different geographical areas. Phytochemistry is the branch of science that deals with the study of secondary metabolites isolated from plants, including their characterization, chemical reactions, and biological activities. Plant-derived compounds are widely utilized in medicines, pesticides, fragrances, and various other useful products. Chromatographic techniques such as Gas Chromatography (GC) and High-Performance Liquid Chromatography (HPLC) play an important role in the separation and analysis of individual chemical constituents. Gas chromatography is a widely used analytical method for the qualitative analysis of compounds by comparing the retention data of analytes with those of known standards. Aroma chemicals are mainly characterized by two sensory properties: odor intensity and odor quality. Fresh plant tissues, especially leaves, stems, roots, and flowers, contain a high amount of moisture and therefore cannot be directly extracted using solvents such as petroleum ether or other organic solvents. Extraction is commonly performed using a Soxhlet extractor. However, when high-boiling solvents are employed, the extract remains exposed to elevated temperatures for an extended period during

the extraction process. Although this method ensures nearly complete extraction of soluble compounds from the plant material, it also carries the risk of degrading or losing heat-sensitive constituents.

MATERIALS AND METHODS

2.Materials and Methods

2.1Antimicrobial activity

The antibacterial activity was evaluated using the agar well diffusion method described in the European Pharmacopoeia with slight modifications **[6, 7]**. Sterile Mueller–Hinton Agar (MHA) plates were employed for the assay. Wells of 8 mm diameter were aseptically punched into the agar using a sterile cork borer. Freshly subcultured bacterial strains were inoculated into 1 mL of nutrient broth and incubated at 37°C for 2–3 hours to obtain log-phase cultures. The turbidity of the cultures was adjusted to match the 0.5 McFarland standard, corresponding to approximately 1–2 × 10^8 CFU/mL. A volume of 100 µL of each standardized bacterial suspension was uniformly spread over the surface of MHA plates using a sterile swab to ensure an even lawn of growth. The inoculated plates were allowed to dry for 5 minutes, after which different concentrations of the test samples, dimethyl sulfoxide (DMSO) as the negative control, and a standard antibiotic as the positive control were carefully introduced into the respective wells. The plates were then incubated at 37°C for 24 hours. Antibacterial activity was assessed by measuring the diameter of the zones of inhibition around each well, expressed in millimeters (mm).

2.2 Antifungal activity

The agar well diffusion method, as outlined in the European Pharmacopoeia with minor modifications, was used to evaluate antifungal activity (Berkow *et al*., 2020). Sterile Sabouraud Dextrose Agar (SDA) plates were prepared for the assay. Freshly subcultured fungal strains were inoculated into 1 mL of Sabouraud Dextrose Broth (SDB) and incubated at 37°C for 2–3 hours to obtain log-phase growth. The turbidity of the culture was adjusted to correspond to the 0.5 McFarland standard, equivalent to approximately 1–2 × 10^8 CFU/mL. Subsequently, 100 µL of the standardized fungal suspension was evenly spread onto the surface of SDA plates using a sterile swab to ensure uniform distribution. The inoculated plates were allowed to dry for about 5 minutes. Wells of 8 mm diameter were then aseptically punched into the agar using a sterile cork borer. Each well was filled with 25 µL of the test extract or antimicrobial agent at the desired concentration. The plates were incubated at 37°C for 24 hours. Following incubation, antifungal activity was determined by measuring the diameter of the clear zones of inhibition around each well, expressed in millimeter

2.3 HPTLC fingerprint profile

HPTLC analysis was carried out using aluminium-backed, pre-coated silica gel 60 F_{254} TLC plates. Sample application was performed with a CAMAG Automatic Sampler V. HPTLC fingerprint profiling was conducted using methanolic extracts of the stem of *R.notonianum*. The results are presented in Tables 3 and 4. The optimized mobile phase for the methanolic extract consisted of toluene, ethyl acetate, and methanol in the ratio of 7:3:1, whereas for the hexane extract, a solvent system of toluene and ethyl acetate in the ratio of 9:1 was found to be most suitable.

3.RESULTS AND DISCUSSION

Anti-microbial activity

Table 1: Antibacterial screening of Sample *Rhynchotechum permolle*, *Streptococcus aureus* and *Escherichia coli*

Sample mg/ml	Zone of inhibition (mm in diameter)	
	S. aureus	*E. coli*
Streptomycin (+CP)	29	21
Negative Control (-CN)	NA	NA
200mg (Stem)	14	15
400mg (Stem)	20	17
200mg (Leaf)	NA	NA
400mg (Leaf)	NA	NA

***NA indicates No Activity**

Anti-microbial activity including anti-bacterial and antifungal activity of methanol extract of stem of *R.notonianum* was determined by agar well diffusion method **[6, 7]**. In the present study the antimicrobial activity was evaluated by determining the zone of inhibition developed for each extract. Gram positive bacteria including *Staphylococcus aureus* and gram-negative bacteria like *Escherichia coli* were tested in present study. *Candida albicans* and *Aspergillus niger* were fungal strains tested for antifungal activity. 200,400 µg/1ml of methanol plant extracts were used to derive maximum activity against the microbes under investigation. The result showing zone of inhibition obtained against tested organism is given in **Table 1, 2** and corresponding plate were shown in **Figure 1**. The present study of the antibacterial activity of stem extract of *R.permolle* exhibited inhibitory against *Streptococcus aureus*. The zone of inhibition of the extract of *Streptococcus aureus* 200 mg of stem is 14 whereas 400 mg of stem is 20. There is no inhibitory effect was shown against 200 mg leaf and 400 mg leaf.

Anti-fungal activity

Table 2: Antifungal screening of Sample *Rhynchotechum permolle*, *Streptococcus aureus* and *Escherichia coli*

Sample	Zone of inhibition (mm in diameter)	
	Candida albicans	*Aspergillus niger*
Clotrimazole (+CN)	20	18
Negative control (-CN)	NA	NA
200mg (Stem)	NA	17
400mg (Stem)	NA	17
200mg (Leaf)	NA	15
400mg (Leaf)	NA	15

***NA indicates No Activity**

The present study of the antifungal activity of leaf and stem extract of *R.permolle* exhibited inhibitory against *Aspergillus niger.* The zone of inhibition of the extract of *Aspergillus niger* 200 mg of stem is 17 whereas 400 mg of stem is 17. There is no inhibitory effect was shown against 200 mg leaf and 400 mg leaf of *Candida albicans*.

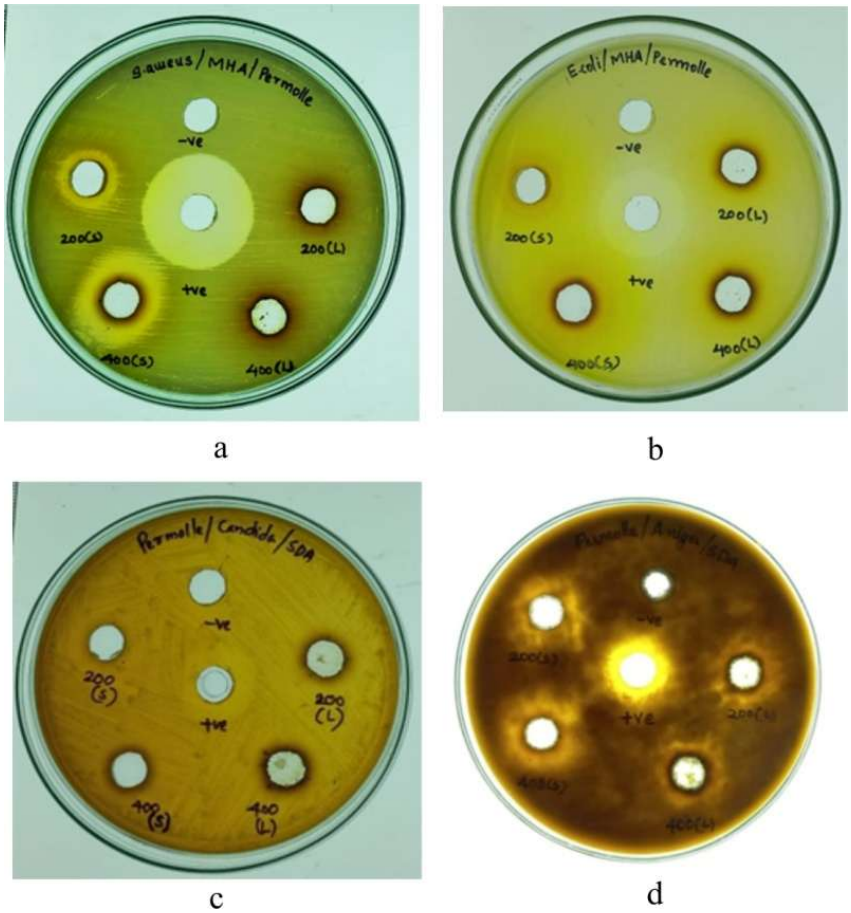

Figure 1: Antimicrobial activity of methanol stem extract of a. *S.aureus*, b. *E.coli*, c. *C.albicans*, d. *A.niger*

R.permolle leaves methanol

Table 3: HPTLC fingerprint profile of methanol extract of stem of *R.permolle at* different wave length

254 nm			366nm		
No of peaks	Rfvalue	Area %	No of peaks	Rfvalue	Area %
1	0.05	1.76	1	0.02	0.36
2	0.12	2.66	2	0.06	1.28
3	0.14	0.77	3	0.08	0.84
4	0.30	5.18	4	0.16	0.30
5	0.34	3.46	5	0.22	1.52
6	0.39	4.73	6	0.27	1.37
7	0.42	4.45	7	0.34	4.54
8	0.45	2.64	8	0.39	6.93
9	0.53	5.65	9	0.42	4.79
10	0.56	8.31	10	0.45	3.59
11	0.63	5.94	11	0.50	5.19
12	0.67	3.88	12	0.56	18.95
13	0.76	2.57	13	0.63	13.64
14	0.80	9.83	14	0.67	5.13
15	0.87	11.19	15	0.75	1.97
16	0.94	26.98	16	0.80	12.59
			17	0.86	15.07
			18	0.94	1.94

The results showing number of peaks, R_f values, and area percentage are presented in **Table 3**. In the HPTLC densitometric the finger prints profile of Permolle leaf in methanol extract under 254nm revealed 16 peaks, major peak at R_f 0.94 with area percentage of 26.98, followed by peak at 0.87 with area percentage 11.19 a minor peak at 0.05 with area percentage of 1.76. In 366nm revealed 18 peaks major peak at R_f 0.94 with area percentage 1.94 followed by peak at 0.86 with area percentage 15.07, minor peak at 0.02 with area percentage 0.36.

R.permolle leaf hexane

Table 4: HPTLC fingerprint profile of methanol extract of stem of *R.permolle* at different wave length

254 nm			366nm		
No of peaks	Rfvalue	Area %	No of peaks	Rfvalue	Area %
1	0.44	1.03	1	0.54	3.96
2	0.75	4.95	2	0.62	4.28
3	0.80	5.66	3	0.75	5.30
4	0.88	15.34	4	0.83	47.50
5	0.93	73.03	5	0.88	31.61
			6	0.95	7.36

The results showing number of peaks, R_f values, and area percentage are presented in **Table 4**. In the HPTLC densitometric the finger prints profile of Parmolle leaf in hexane extract under 254nm revealed 5 peaks, major peak at R_f 0.93 with area percentage of 73.03, followed by peak at 0.88 with area percentage 15.34 a minor peak at 0.44 with area percentage of 1.03. In 366nm revealed 6 peaks major peak at R_f 0.95 with area percentage 7.36 followed by peak at 0.88 with area percentage 31.61, minor peak at 0.54 with area percentage 3.96.

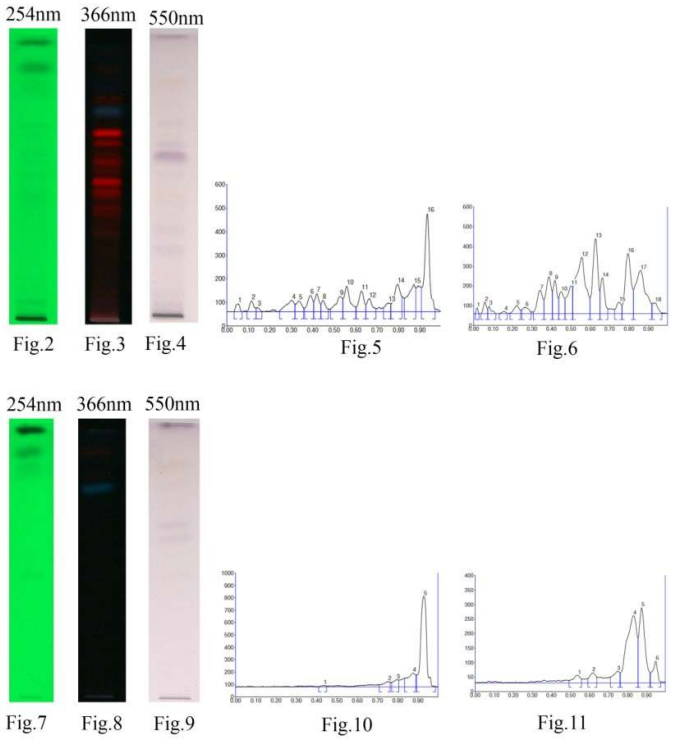

Figure 2-4: Densitogram of methanol extract of leaf of *R.permolle*, Fig.5.Chromatogram of methanol extract of *R.permolle* 254 nm, Fig.6-9 Chromatogram of hexane extract of leaf of *R.notonianum* 366 nm

R.permolle stem methanol

The results showing number of peaks, R_f values, and area percentage are presented in table. In the HPTLC densitometric the finger prints profile of Parmella leaf in methanol extract under 254nm revealed 4 peaks, major peak at R_f 0.94 with area percentage of 68.19, followed by peak at 0.89 with area percentage 20.44 a minor peak at 0.14 with area percentage of 3.09. In 366nm revealed 4 peaks major peak at R_f 0.96 with area percentage 7.76 followed by peak at 0.92 with area percentage 18.02, minor peak at 0.45 with area percentage 25.15.

R. permolle stem hexane

Table 5: HPTLC fingerprint profile of methanol extract of stem of *R.permolle* at different wave length

254 nm			366nm		
No of peaks	**Rrvalue**	**Area %**	**No of peaks**	**Rrvalue**	**Area %**
1	**0.80**	**11.00**	**1**	**0.83**	**56.29**
2	**0.93**	**89.00**	**2**	**0.89**	**47.71**

The results showing number of peaks, R_f values, and area percentage are presented in **Table 5**. In the HPTLC densitometric the finger prints profile of Parmella stem in hexane extract under 254nm revealed 2 peaks, major peak at R_f 0.93 with area percentage of 89.00, a minor peak at 0.80 with area percentage of 11.00. In 366nm revealed 2 peaks major peak at R_f 0.89 with area percentage 47.71, minor peak at 0.83 with area percentage 56.29.

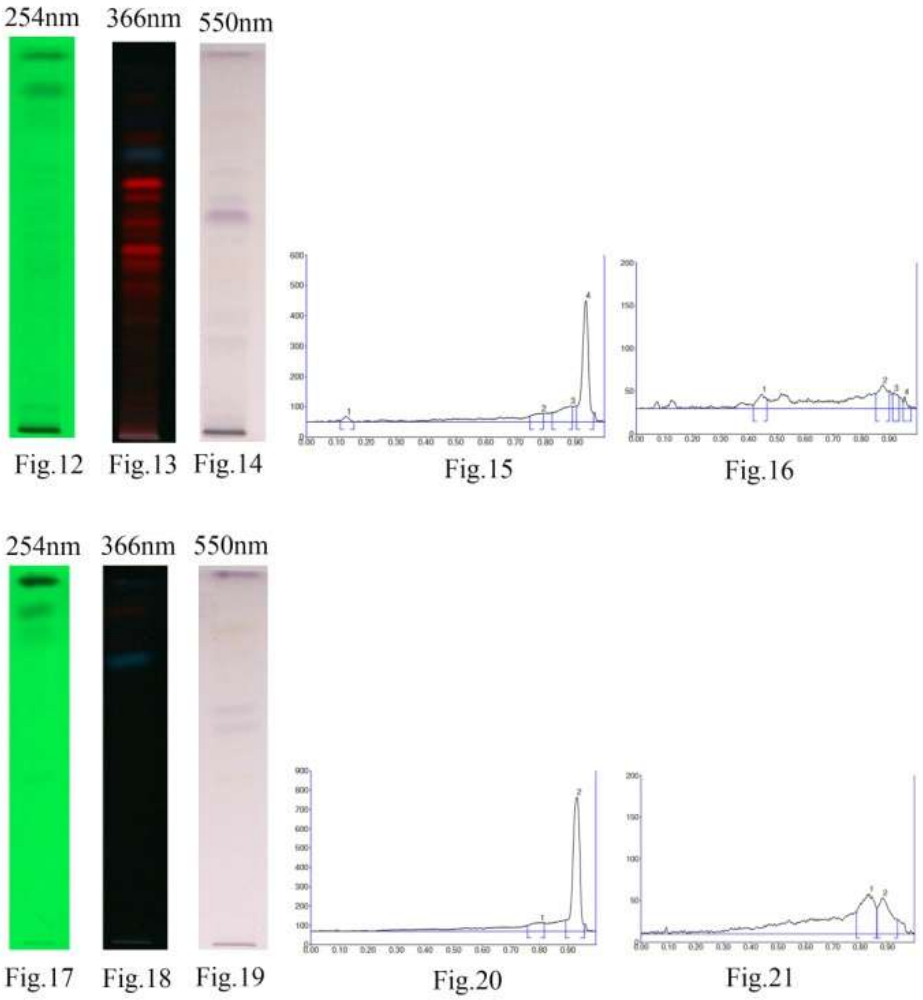

Figure12-14: Densitogram of methanol extract of stem of *R.permolle*, Fig.15-16 Chromatogram of methanol extract of stem of *R.permolle* 254 nm, Fig.17-19 densitogram of hexane extract of stem of *R.permolle* 366 nm, 20-21 chromatogram of hexane extract of stem of *R.permolle* 366nm

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