

Phytochemical Studies On *Rungia Repens* (L.) Nees– An Ethnomedicinal Plant

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ABSTRACT

Rungia repens Nees. (Acanthaceae), commonly called as Ghatipitpapa. Several ethno medicinal aspects are present in folk literature and new scientific documentation. It's reported that, *Rungia repens* Nees., the plants is used as in curing cough and fever activities, antimicrobial activity, anti-inflammatory activities etc..Alkaloids are found in medicinal plants used as anesthetic agents. Ethnobotanical and phytochemical studies were done for probing its chemically active ingredient with various standard protocols of referenced scientific methods. The plant material collected from Toranmal forest of Nandurbar District. After complete processing on crude plant material and performed their proximate analysis, phytochemical test for detection of various metabolites and their presence, *UV-VisSpectral analysis* and *Fourier Transform Infrared Spectrophotometer (FTIR)* were taken with different solvent system. As a result it is found that difference in value of leaves, stem and root parts extracts for proximate analysis for both fresh and dry material has been recorded. It is also found that in different solvent system reveals the presence of various phytoconstituents. The study followed with the help of UV visible spectral studies for the leaf extract from Methanol and Chloroform and FTIR spectral peak values and functional groups for the leaf extract from Chloroform and Petroleum ether obtained has been recorded. The complete experimental results signifies that ethnomedicinal information from folk and ancient literature reveals the therapeutic efficiency of *Rungia* which need to further elaboration in future works to investigate active ingredient for disease specific.

KEY WORDS: *Rungia*, Phytochemistry, Fourier Transform Infrared Spectrophotometer (FTIR), Secondary Metabolites.

1. INTRODUCTION

Rungia repens Nees. is a spreading decumbent herb belongs to Acanthaceae which is a large cosmopolitan family distributed mostly in the tropical and subtropical areas of the world.

The herb possesses cylindrical less fragile stems, often shows rooting near base, found throughout warmer parts of India in moist places and cultivated fields during winter (Gamble JS, 1921), (Saxena HO, Brahman M, 1995). Also found in moist places sides of water channels, bunds of paddy fields, and also under the shadow area of tree canopy. Leaves shortly petiolate, elliptic-lanceolate, acute or obtuse. Flowers blue or pale purple, in erect, terminal, 4-gonous spikes. Capsules ovoid oblong, acute, compressed, pubescent, brown seeds suborbicular, compressed, concentrically tuberculate, pale brown, flowers and fruits from August to February (Flora of Dhule and Nandurbar District).

The plant is significantly valued in traditional medicine in the treatment of fever, cough, and worms, it also credited with vermifugal and diuretic properties (Kiritikar KR, Basu BD., 1987). The fresh leaves are bruised, mixed with castor oil, and applied to the scalp in cases of *tinea capitis*, a scaly fungoid infection (Nadkarni KM, 1954). In literature the plant is recorded to possess anti-inflammatory, diuretic and antimicrobial activities (Swain SR et al, 2008). In Gujarat and Maharashtra it is used as Parjpataka (Yoganarasimhan, 2000), Ghatipitpapa (Flora of Maharashtra, Vol. 2). Whole plant dried and pulverized is given in fevers and cough by the local tribes. Leaf paste also used to heal fungal skin diseases (Vedavathy, 1992). The herb works in the treatment of cough and fever and is also attributed with vermin-fungal and diuretic properties (Trease GE 2002). Presence of alkaloids in medicinal plants acts as anesthetic agents (Mahato SB, Sen, 1997 and Tanveer et al, 2020). Flavonoids and phenolics are among the major compounds present in the *Rungia repens* plant (Karuna Modi et. al 2017 and Khairnar et al. 2018) and acts with powerful antioxidant property. Plants contain various pharmacological activities such as anti-bacterial, anti-cancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-inflammatory activities due to presence of several important phytoconstituent like terpenoids and other secondary metabolites. Hence, phytochemical investigation to explore the cause of being such medicinally active plant is needed which serves for further studies to reach around a novel drug.

2. MATERIALS AND METHODS

Field Work: *Rungia repens* was collected during the end of rainy season from forest of Toranmal region of Shahada Tehsil of Nandurbar District (21.840213° N, 74.456583° E). Collected ethno-botanically importance of the plant from local tribal peoples and authenticated with existing literature of ethno-medicine. Plant sample were collected for lab work and also recorded geographical location for revisit for the collection of plant as per need in future.

Laboratory work: Identification of plant sample was performed using taxonomic tool like Flora of Presidency of Bombay, Flora of Dhule and Nandurbar District and recorded significant taxonomical characters. Collected plant material was washed 2-3 times with distilled water and separate the plant parts i.e. leaves, stem, root and dried and placed under shade condition with due care in laboratory. These dried plant parts materials were grind and

formed a coarse powder and extraction were done by using hot percolation method- soxhlet extraction using various solvent system.

Proximate Analysis: It was taken to estimate moisture, Dry matter and Ash content.

Moisture content: The moisture of the sample was lost by volatilization caused by heat. The method of AOAC (Association of Official Analytical Chemists) 1990 was followed to determine the moisture content within the plant material. Petri plates were washed with detergents and dried at 105 °C in oven for overnight and removed from oven and then kept in desiccator for cooling and weights. The procedure for estimation accordingly were carried out in triplicate and mean values of weight of fresh sample and dry sample, both were recorded to calculate the moisture content using the relationship shown.

5 gm fresh sample of different plant part was taken separately in petri plates and placed in oven at 105 °C overnight. The moisture content in plant parts were recorded for calculated by using the following formulae:-

$$\text{Moisture content (\%)} = \frac{(\text{Weight of fresh sample} - \text{Weight of dry sample})}{\text{Weight of fresh sample}} \times 100$$

Dry matter Content: The dry matter of the sample is the amount of material remained after the complete removal of moisture from it. The method of AOAC (Association of Official Analytical Chemists) 1990 was followed to determine the dry matter content within the plant material. Petri plates were washed with detergents and then were dried at 105 °C in oven for overnight. Then plates were removed from oven and then kept in desiccator for cooling. The estimation accordingly was carried out in triplicate and the mean values of both were recorded to calculate the dry matter content in plant material.

5 gm fresh sample were taken in dishes and placed in oven at 105 °C overnight in Oven. The dry matter was calculated by using following formulae:-

$$\text{Dry matter (\%)} = \frac{(\text{Weight of dish} + \text{Weight of dried sample}) - \text{Weight of dish}}{\text{Weight of sample before drying}} \times 100$$

Ash Content: Value for ash content was determined by following the method of AOAC (1990). For this crucible were kept in muffle furnace on 600 °C for 1h. Then crucible were transferred from furnace to desiccator and then cooled at room temperature. Sample then weighed immediately to prevent absorption of external moisture in air. 5 gram of dry sample of plant part was taken in tarred silica crucible and placed in muffle furnace on 600 °C for 6h. Then crucible was transferred to desiccator and allowed for cooling at room temperature, crucible was transferred immediately to avoid moisture absorption after processing. The percentage of present ash content value was calculated by using the following formula.

$$\text{Ash (\%)} = \frac{\text{Weight of Ash}}{\text{Weight of sample}} \times 100$$

Phytochemical Studies:

Detection of Alkaloids

Dragendorff's/ Kraut's test: Few mL filtrate X + 1-2 mL Dragendorff's reagents A reddish-brown precipitate (Silva GO et al, 2017; Singh, 2017, M. B. Patil and P. A. Khan 2017a and M. B. Patil and P. A. Khan 2017b).

Mayer's/ Bertrand's/ Valser's test: Few mL filtrate X + 1-2 drops of Mayer's reagent (Along the sides of test tube) A creamy white/yellow precipitate (Silva GO et al, 2017; Singh V, Kumar R., 2017; Auwal MS et al, 2014).

Detection of Carbohydrates

Barfoed's test: 1mL filtrate Y + 1mL Barfoed's reagent+ Heated for 2 min. A red precipitate (Raaman, 2006; Sadasivam, 2005).

Detection of Glycosides

Concentrate H₂SO₄ test: 5ml plant extract + 2mL glacial acetic acid + a drop of 5% FeCl₃ + conc. H₂SO₄ A brown ring (Sheel, 2014).

Detection of Cardiac Glycosides

Keller-Killani test: 1mL filtrate + 1.5mL glacial acetic acid + 1 drop of 5% ferric chloride + conc. H₂SO₄ (along the side of test tube) A blue coloured solution in acetic acid layer (Singh V, Kumar, 2018, Nanna, 2013).

Detection of Proteins and Amino acids

Millon's test: 2mL filtrate + few drops of Millon's reagentA white precipitate (Silva GO et al, 2017; Raaman, 2006).

Detection of Flavonoids

Conc. H₂SO₄ test: Plant extract + conc. H₂SO₄ An orange colour (Tyagi, 2017).

Detection of Phenols

Ferric chloride test Extract aqueous solution + few drops 5% ferric chloride sol. Dark green/bluish black colour (Raaman, 2006; Tiwari, 2011).

Detection of tannins

10% NaOH test 0.4mL plant extract + 4mL 10% NaOH + shaken well Formation of emulsion (Singh, 2017).

Detection of Phytosterols

Salkowski's test: Filtrate Z + few drops of conc. H₂SO₄ (Shaken well and allowed to stand) Red colour in lower layer (Singh, 2017; Tiwari, 2011).

Detection of Saponins

Foam Test: 0.5gm plant extract + 2mL water (vigorously shaken) Persistent foam for 10 min (Tiwari, 2011).

Detection of Quinones

Conc. HCl test: Plant extract + conc. HCl A green colour (Basumatary, 2016).

Detection of Anthraquinones

Borntrager's test: 10mL 10% ammonia sol. + few ml filtrate g (shaken vigorously for 30 sec.) A pink, violet, or red coloured solution (Njoku, 2009; Gul, 2017; Uma, 2017).

Detection of Coumarins

NaOH test: Plant extract + 10% NaOH + Chloroform A yellow colour (Kumar, 2018).

X= 50gm solvent free extract is mixed with few ml dil. HCl and then filtered.

Y= 100mg solvent free extract is dissolved in 5ml of distilled water and filtered

Z= Equal quantity of chloroform is treated with plant extract and filtered

UV Visible Spectral Analysis:

5 gm of dry leaves powder extraction was carried out for 24 cycle of Soxhlet extraction apparatus. Two solvent were used for extraction. viz. Methanol and Chloroform. Different concentrations and dilutions were prepared for UV analysis in 200 nm to 700nm range for all three samples in replicate in table given below.

Table.1. Concentration of Solvent extract for spectral analysis

Concentration	Extract from Methanol	Extract From Chloroform
20 %	200 µl/ml	200 µl/ml
40 %	400 µl/ml	400 µl/ml
60 %	600 µl/ml	600 µl/ml
80 %	800 µl/ml	800 µl/ml
100 %	Pure extract	Pure extract

Fourier Transform Infrared Spectrophotometer (FTIR): FTIR is the most fit and applicable tool for identification of chemical bonds (functional groups), and their types present in sample. The wavelength at which the light is absorbed shows variation is their characteristic and conveys significant information for the identification of chemical bond. Graphical result shows absorbance on wavelength in the form of annotated spectrum of

FITR. Infrared absorption spectrum interprets the chemical bonds present in molecules and determination of presence of the functional group. (Yang, 2002; Martín, 2005; Duraes, 2008, M. B. Patil and P. A. Khan 2017a). Dried powder of plant sample for different solvents extracts viz. Chloroform and Petroleum Ether were used in FTIR analysis with 100 mg KBr pellet as encapsulate in sample discs. The powdered plant part sample was loaded in FTIR spectroscope (Shimadzu 8400S), with the Scanning range from 400 to 4000 cm⁻¹ with a resolution of 4 cm⁻¹.

3. RESULT AND DISCUSSIONS

Ethnobotanical Information: Information gathered from tribals, local Vaidus and doctors found to be the whole plant dried and pulverized is given in fevers and cough among the local tribes of Satpuda. Some maids use leaf paste made in castor oil for seven days, used for skin itching diseases rarely but effective.

Therapeutic uses: The plant is the valuable traditional medicine in the treatment of fever, cough, and worms, it also credited with vermifugal and diuretic properties (Kiritikar KR, Basu BD., 1987). In literature the plant possess to have anti-inflammatory, diuretic and antimicrobial activities (Swain SR et al, 2008).

Proximate Analysis:

Table.2. Result for Proximate test

Plant Part used	Moisture content	Dry matter content	Ash content
Root(w/w)	52.20 %	47.80 %	12.50 %
Stem(w/w))	68.60 %	31.40 %	10.00 %
Leaves(w/w)	88.20 %	11.80 %	7.50 %

Preliminary Phytochemistry:

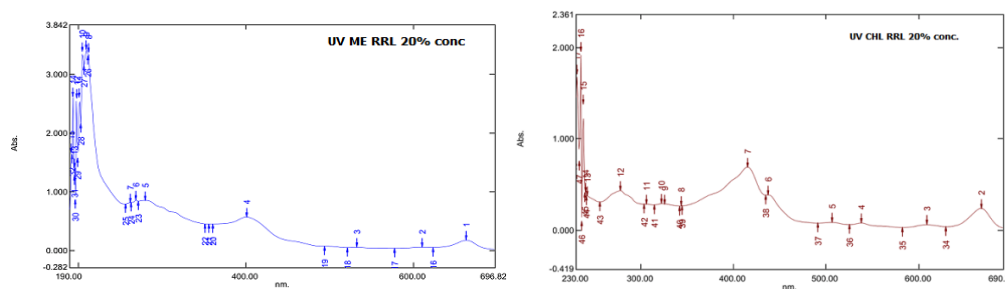
Table.3. Result for Preliminary Phytochemistry

Sr . no .	Phytoconstitu ents	<i>Rungia repens</i> (L.) Nees											
		Root				Stem				Leaf			
Solvent Systems	A q	M e	C h	Pt	A q	M e	C h	Pt	A q	M e	C h	Pt	
1.	Alkaloids	+	-	-	-	+	-	+	-	+	+	+	-
2.	Carbohydrat es and Glycosides	-	-	-	-	-	-	-	-	+	-	-	-
3.	Cardiac glycosides	+	+	-	-	+	+	+	-	+	-	+	+
4.	Proteins and	-	-	-	-	-	-	-	-	+	-	-	-

	Amino acids												
5.	Flavonoids	+	+	+	-	+	-	+	-	+	-	-	-
6.	Phenols	+	-	-	-	+	-	-	-	-	+	-	-
7.	Saponins	+	-	-	-	+	-	+	--	-	-	+	-
8.	Phytosterols	+	-	+	+	+	-	+	-	+	-	-	-
9.	Quinones	-	-	-	-	-	-	-	-	-	-	+	-
10	Anthraquinones	+	-	-	-	+	-	+	-	+	-	-	-
11	Coumarins	-	-	-	-	-	-	-	-	-	-	-	-
12	Tannins	-	+	+	+	+	+	+	+	-	+	+	+

Aq= Aqueous, Me= Methanol, Ch= Chloroform, Pt= Petroleum Ether

UV Visible Spectral Analysis:



Graph.1. UV spectra 20% Conc. for Methanol and Chloroform respectively

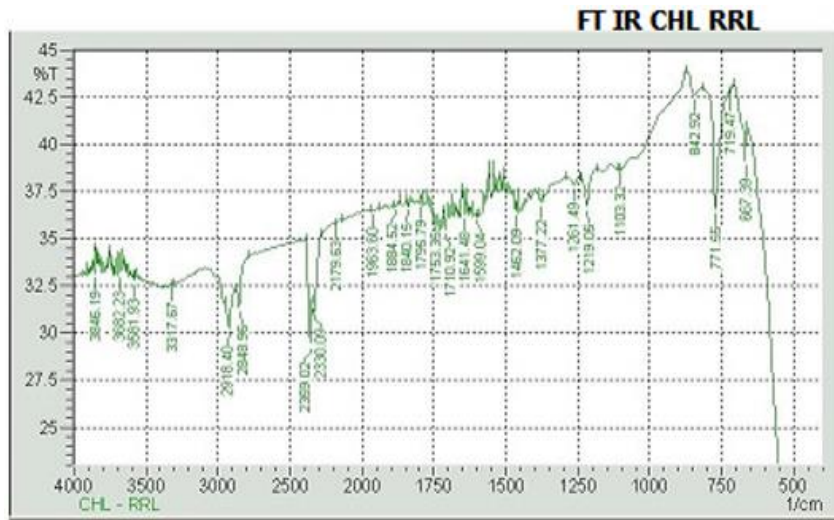
FTIR Spectral Analysis:The FTIR spectrum interpretations of leaf in different solvent.

Chloroform (CH) extract: For *Rungia repens* leaves characteristic absorption band were exhibited at 3581.93 cm⁻¹ for (-OH) group, 3317.67 cm⁻¹ for (-NH) group, 2918.40 cm⁻¹ (for -C-H- stretching), 2179.6 cm⁻¹ for (-C≡C- bonding), 1753.35 cm⁻¹for carbonyl group (-C=O-), 1710.92 cm⁻¹ for carboxyl group (-C=O-), 1641.48 and 1599.04 for -C=C- group, 1103.32 cm⁻¹ for (-C-O-)oxygen group and 771.55 cm⁻¹for a substituted benzene ring.

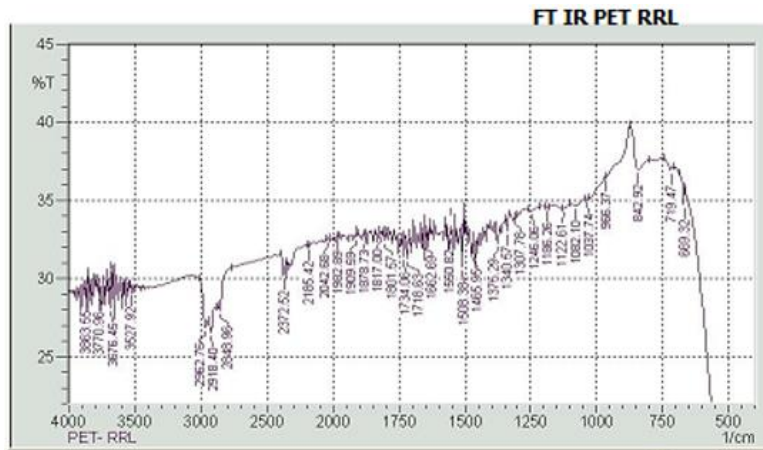
Petroleum Ether (PT) extract:The PT extract of *Rungia repens*leaves showed the characteristic absorption bands were observed at 2962.76 cm⁻¹, 2918.40 cm⁻¹ for (-C-H- stretching), 2185.42 cm⁻¹ for (-C≡C- bonding), 1718.63 cm⁻¹for (-C=O-) carboxylic group, 1162.79 cm⁻¹for (-C-O- bonding), 1186.26 cm⁻¹for (-C-O-) carbonyl group (Ester), 1082.10 cm⁻¹ for (-C-O-) oxygen group, 966.37 cm⁻¹, 842.92 cm⁻¹and 719.47 cm⁻¹for substituted benzene ring.

Table.4. FTIR Spectral Peak Values And Functional Groups Obtained For The Leaf Extract

Extracts prepared in	Peak values (cm ⁻¹)	Functional groups
Chloroform (Ch)	771.55 1103.32 1599.04 1641.48 1710.92 1753.35 2179.63 2918.40 3317.67 3581.93	Substituted benzene ring -C-O- group -C=C- group -C=C- group -C=O- carboxyl group -C=O- carbonyl group -C≡C- bonding -C-H- stretching -NH group -OH group
Petroleum Ether (Pt)	719.47 842.92 966.37 1082.10 1186.26 1162.79 1718.63 2185.42 2918.40 2962.76	Substituted benzene ring Substituted benzene ring Substituted benzene ring -C-O-group -C-O- carbonyl group (Ester) -C-O- bonding -C=O- carboxylic group -C≡C- bonding -C-H-stretching -C-H- stretching



Graph.2. FTIR of *R. repens* Leaves in Chloroform



Graph.3. FTIR of *R. repens* Leaves in Petroleum Ether

4. CONCLUSION

The current work from the survey and field observation its find that there is very significance medicinal important of *Rungia repens* among tribes. It has been found the whole plant dried and pulverized is given in fevers and cough among the local tribes of Satpuda and also the use leaf paste made in castor oil for seven days, used for skin itching diseases rarely but effective to heal. The plant has valuable traditional medicinal property in the treatment of fever, cough, and worms, it also credited with vermifugal and diuretic properties (Kiritikar KR, Basu BD., 1987). In literature the plant possess to have anti-inflammatory, diuretic and antimicrobial activities (Swain SR et al, 2008). Flavonoids and phenolics are among the major compounds present in the *Rungia repens* plant (Karuna Modi et.al 2017).

Lab and experimental reveals that Moisture content of Roots (w/w), Stem (w/w) and leaves (w/w) are 52.20 %, 68.60 % and 88.20 % respectively. Dry matter content of Roots (w/w),

Stem (w/w) and Leaves (w/w) are 47.80%, 31.40 % and 11.80 % respectively. Ash content of Roots (w/w), Stem (w/w) and Leaves (w/w) are 12.50 %, 10.00%, 7.50 % (Table.2). For another Parameter in phytochemical test for different primary and secondary metabolites it find that different solvent are effected the positive of result. Preliminary Phytochemical analysis revealed that the presence of alkaloids, carbohydrates and glycosides, proteins, cardiac glycosides, phytosterols, quinines, anthraquinones, saponins, tannins, flavonoids and phenolic compounds. For Root, Stem and Leaf aqueous samples showed most of the phytoconstituents, it was found that coumarins were not detected among all parameters in four different solvent (Table.3).

UV Visible analysis graphical representation recorded between 200:700 nm shows sharp peak in Leaf sample in 200 µl/ml of different extract (Graph 1).

FTIR spectral peak values and functional groups obtained in Chloroform (CH) are which showing nine functional group present in leaf sample such as three -C-O- group, -C=O- carboxyl group, -C=O- carbonyl group, -C≡C- bonding, -C-H- stretching, -NH group, -OH group. In Petroleum Ether (PT) ten functional group showing their presence. They are three substituted benzene ring, -C-O- group, -C-O- carbonyl group (Ester), -C-O- bonding, -C=O- carboxylic group, -C≡C- bonding, -C-H- stretching, -C-H- stretching in PT leaf sample (Table.4).

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