

Phytochemical Profiling and Antioxidant Potential of Methanol Extracts of *Rungia Pectinata*: Total Phenolic and Flavonoid Content Analysis

Priya Gautam¹, Pallavi^{2*}, Vinay Bhushan Kumar³

^{1&2} Department of Botany, J.N.L College Khagaul, Patliputra University, Patna

³ Department of Botany, T P S College, Patliputra University, Patna

*Corresponding author: pallavi.mp08@gmail.com

ABSTRACT

Thousands of plant species have been documented in India to be utilized as primary treatment for various ailments. Many of them, meanwhile, have not yet been investigated for their scientific validity. This study's primary goal is to analyse the extracts of *Rungia pectinata* quantitatively for flavonoids and phenolic content, as well as qualitatively for phytochemicals and antioxidants. The Soxhlet device was used to make the methanol extracts of plant materials. The results of the experiment showed that the methanol extract of *Rungia pectinata* contained phytoconstituents such as flavonoids, terpenoids, alkaloids, tannins, and phenolic compounds, but not saponins. The TFCs of *Rungia pectinata* methanol extracts were 45.78 ± 5.38 mg quercetin equivalents/g extract, and the phenolic contents were 98.22 ± 1.47 mg gallic acid equivalents/g extract. The antioxidant effectiveness of *Rungia pectinata* methanol extracts was examined using the DPPH radical scavenging assay, which produced IC₅₀ values of $95.32 \pm 1.21 \mu\text{ml}$. These medicinal plants, as far as we know, are the least exposed to their biological activities, and the findings support the traditional uses of these plants to treat illnesses. To identify the active drug candidates for the upcoming drug development process, a thorough investigation of these plants can be conducted to separate the active chemical components and assess in vivo pharmacological activities.

Keywords: *Rungia pectinata*, Qualitative phytochemical profiling, Total polyphenolic, Total flavonoid contents, Antioxidant activity.

Introduction

Many of the pharmacologically active chemicals found in medicinal plants have sparked the creation of innovative treatments (Cragg GM & Newman DJ., 2013). Their medicinal potential is highlighted by their long history of use in treating human illnesses. Because they are thought to be safer and have less side effects than synthetic medications, medicinal plants have become more and more popular in recent years (Song JX, et al, 2016). Interestingly, natural products and their derivatives make up a sizable amount of today's medications, and traditional medicine continues to be the major source of healthcare for more than 85% of the world's population (WHO, 2005).

The identification, synthesis, and development of novel lead compounds for immunomodulation, chemotherapy, drug development, and biosensor design have all benefited greatly from the utilization of natural products (Pinto DCGA & Silva AMS, 2017). These processes have also been made easier by developments in a number of scientific domains. 6. *Rungia pectinata* L., also called *Justicia pectinata*, *Rungia parviflora* var. *pectinata*, or *Rungia parviflora* var. *muralis*, is a tiny, soft annual plant with beautiful blue blooms and lance-shaped membranous leaves that is used for ethnomedical purposes.

It grows in neighbouring countries including Myanmar, China, Sri Lanka, Java, Malaysia, and the Philippines in addition to Assam, Andhra Pradesh, Bihar, Karnataka, Kerala, Maharashtra, Odisha, and Tamil Nadu in India. Key phytochemicals extracted from this plant include phytosterols, terpenes, tannins, glycosides, flavonoids, phenolic compounds, amino acids, and fixed oils. Nadkarni et al. (2007) state in "Indian Materia Medica" that the plant's leaf juice is used as a cooling agent and to treat newborn smallpox. Fresh leaf paste combined with castor oil is used to treat tinea capitis, and bruised leaves are applied topically to ease excruciating swellings and inflammations.

Tribal communities in India use the roots as vermifuges and febrifuges. According to experimental research and traditional usage, *R. pectinata* also has antipyretic, anti-inflammatory, diuretic, analgesic, antifungal, and antibacterial properties (Nadkarni AK. & Dr. K. M., 2007). The weedy *Rungia pectinata* plant has tall stems and numerous branches. This plant's leaf juice is used to treat smallpox in youngsters and acts as a cooling agent. Applying bruised leaves externally reduces uncomfortable swellings and inflammations. According to Nadkarni

(2002), plant juice can be used to treat cuts and wounds, while a decoction can be used to treat gastrointestinal issues and measles.

The secondary metabolites that are derived from plant components are important in protecting the human body from oxidative stress, diabetes, cancer, and several infectious diseases. Atherosclerosis, aging, cancer, diabetes mellitus, and inflammation are just a few of the diseases that can result from the oxidative damage that free radicals in the human body cause to biomolecules. Numerous compounds found in plants, including carotenoids, anthocyanins, flavonoids, and vitamins, have the ability to scavenge free radicals (Zhu Y. Z. et al, 2004). Antioxidants play a crucial role in protecting biological systems from oxidative stress, which has been connected to the emergence of numerous chronic diseases and conditions. By boosting the body's defences, antioxidant supplements might help lessen the harmful consequences of excessive ROS (reactive oxygen species).

Preventing oxidative stress, which has been connected to the emergence of chronic diseases and neurological disorders, is made possible by synthetic antioxidants. However, there are significant negative side effects associated with synthetic antioxidants such propyl gallate, butylated hydroxyanisole, and butylated hydroxytoluene. The usage of natural antioxidants in food has been more and more common in recent years (Mohana D. et al, 2013). The present study's primary objectives were to assess the phytochemical analysis, calculate the total phenolic and flavonoid content, and assess the medicinal plant *Rungia pectinata's* capacity to scavenge free radicals. To the best of our knowledge, the biological activities of these plants as well as the measurement of their total phenolic and flavonoid content have not yet been thoroughly investigated.

Materials and Methods

Plant Collection , Extraction and Phytochemical Analysis

In 2024, *Rungia pectinata* plant specimens were collected from Patna, Bihar. The plant components that were gathered were thoroughly cleaned, allowed to dry in the shade, and then ground into a fine powder. For the hot extraction, the 100 g of plant powder was placed in the Soxhlet apparatus's thimble. The rotatory evaporator was then used to filter and concentrate the extracts. The following formula was used to determine the extracts' % yield (Table 2) (Dirar A. I. et al, 2019). Using various phytochemical tests, the plant extracts were first analyzed to see whether certain secondary metabolites of the plants were present or absent (Bhatnagar S. et al, 2012).

Yield(%) = Weight of dried extract/Weight of dried plant material $\times$ 100

Determination of Total Phenolic Content (TPC)

With a small modification, Folin–Ciocalteu's phenol technique, as described by Hazra B. et al. (2008), was used to assess the total phenolic content in the plant extracts. To put it briefly, 5mL of distilled water was mixed with 1mL of plant extract (1 mg/mL) and 1mL of Folin–Ciocalteu's phenol. After adding 1 mL of a 10% (w/v) Na₂CO₃ solution, it was left for one hour. A UV-visible spectrophotometer was then used to measure the absorbance at 725 nm. TPC was computed in mg GAE/g of the extract, and the standard calibration curve for gallic acid was created (Bakar M. F. A., et al, 2015). Every experiment was run in triplicate (n = 3).

$$C = cV/M$$

where C = Total phenolic content in mg.GAE/g, c = concentration of gallic acid established from the calibration curve in mg/mL, V = volume of extract in mL and M = weight of plant extract.

Determination of Total Flavonoid Content (TFC)

With a small modification, Chang et al. (2002)'s aluminium chloride colorimetric method was used to quantify the total flavonoid concentration in the plant's extract. This method involves adding 0.3 mL of a 5% sodium nitrite solution after adding 1 mL of extract (1 mg/mL) to 4 mL of distilled water. Then, 0.3 mL of 20% aluminium chloride is added, and it is left for six minutes. Lastly, 2 millilitres of sodium hydroxide (1M) was added. A UV-visible spectrophotometer was used to measure the absorbance of the resultant mixed solution at 510 nm. The TFC, which was expressed as mg QE/g of the extract, was determined using a quercetin calibration curve (Bakar M. F. A., et al, 2015). Every experiment was carried out three times. The following is the formula used to determine the TFC:

$$C = cV/M$$

where C = Total flavonoid content compounds in mg QE/g, c = concentration of gallic acid established from the calibration curve in mg/mL, V = volume of extract in mL and M = weight of plant extract.

Evaluation of Antioxidant Activity

The free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was used in a quick, easy, and affordable way to assess the antioxidant activity of plant extracts. A 96-well plate reader was used to measure the extracts' antioxidant activity, slightly altering the colorimetric technique outlined by Sabudak T. et al. (2013). One milligram of plant extracts was dissolved in one milliliter of dimethyl sulfoxide (DMSO) to create the stock solution. The stock solution was serially diluted in methanol to produce the final concentrations of 10, 25, 50, 75, and 100 µg/mL. Methanol served as the negative control and ascorbic acid as the positive control. In triplicate, the plant extract, negative control, and positive control were placed into 100 L 96-well plate readers. Following that, each well received 100 L of DPPH solution. After that, it was incubated in the dark for half an hour. After half an hour, a UV-visible spectrophotometer was used to detect absorbance at 517 nm. Each sample's scavenging activity against the DPPH free radical was determined using the scavenging activity equation (Bakar M. F. A. et al, 2015). By graphing the plant extract concentration against the percentage of scavenging, the IC₅₀ was determined. Every experiment was carried out three times.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Statistical Analysis.

Graph Pad Prism version 9.0.0 was used to determine the IC₅₀ values, and all experimental data were measured in triplicate ($n = 3$). The results were displayed as mean $\pm$ SD. Microsoft Excel 2007 was utilized in the computation of the TPC and TFC.

Results and Discussion

Phytochemical Analysis

Rungia pectinata has been shown to contain secondary metabolites, including amino acids, glycosides, polyphenols, terpenes, phytosterols, tannins, flavonoids, and carbohydrates (Swain S. R. et al, 2008).

Table 1 :- Qualitative analysis of *Rungia pectinata*

S.No.	Test	<i>Rungia pectinata</i> (Methanolic extracts)
1	Alkaloids	+
2	Terpenoids	+
3	Flavonoids	+
4	Tannin	+
5	Saponin	-
6	Phenol	+
7	Steroids	+

Presence (+) Absence (-)

Total Phenolic, Flavonoid Content and Antioxidant Activity

Though their biological activities have not been well investigated, this work was carried out to assess the antioxidant activity, assessment of the total phenolic, and flavonoid content in the *Rungia pectinata*. The study's findings demonstrated that the plants are abundant in secondary metabolites, high in flavonoids and total phenolic content, and strong natural antioxidants. Widely found in plants, phenolic and flavonoid chemicals are part of the human diet and have a number of therapeutic uses, including antidiabetic and antioxidant effects. According to reports, flavonoids, the biggest family of naturally occurring chemicals, have a variety of biological functions (John B. et al., 2014).

According to the study's findings, plant extracts have secondary metabolites that might scavenge possible harm by giving hydrogen to the free radicals produced in our bodies. Oxidative stress is caused by free radicals like superoxide (Alves C. Q. et al., 2010), hydroxyl radicals, and singlet oxygen species (Meyer A. S. and Isaksen A., 1995).

The ability of phenolic and flavonoid compounds to scavenge free radicals is correlated in this study, indicating their potential as antioxidants. According to reports, the natural phenolic and flavonoid compounds found in plants are believed to benefit human health. Numerous antibacterial, antiviral, anti-inflammatory, anticancer, and antiallergic properties have been demonstrated by these phenolic and flavonoid derivatives (Montoro P. et al., 2005). The majority of oxidizing molecules, such as free oxygen and other free radicals, produced in the human body are often scavenged by flavonoid-rich plant extracts (Bravo L., 2009).

The total phenolic content (TPC) of the plant extract was estimated using the relationship ($y = 0.0035x + 0.0989$, $R^2 = 0.9732$). As illustrated in Figures 1 and 2, the relationship $y = 0.0032x - 0.0382$, $R^2 = 0.9226$ was also used to compute the total flavonoid content (TFC). The amounts of flavonoids and total phenolic content were reported in milligrams GAE/g and QE/g, respectively. Table 2 displays the TPC and TFC of plant extracts. The antioxidant activity results are displayed in Table 2, where the plant extracts' DPPH radical scavenging capacity is given as IC₅₀ (μg/mL).

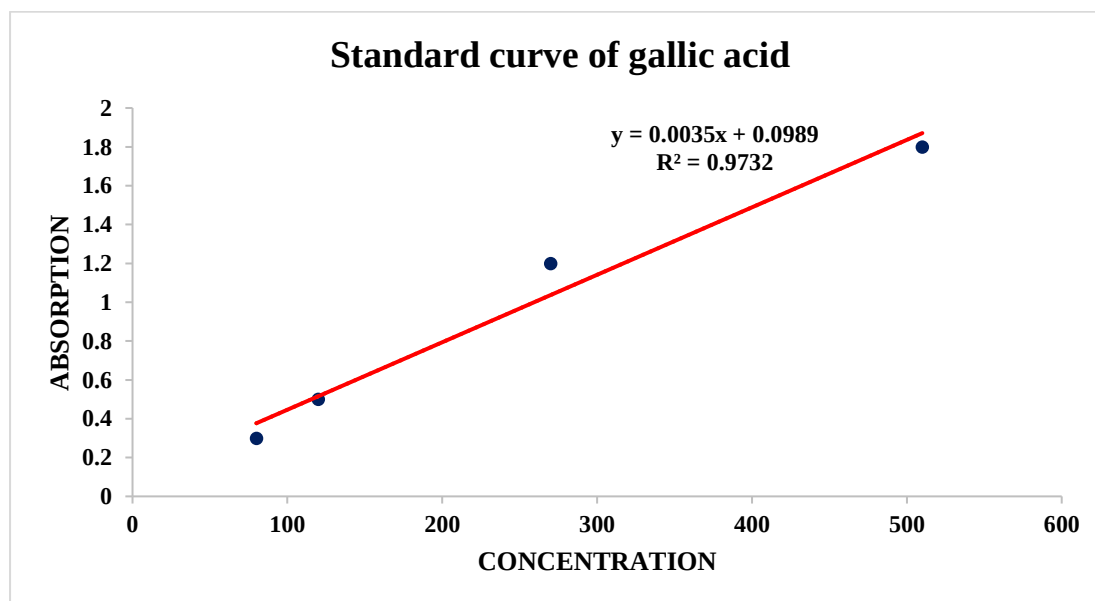


Fig. 1: Standard curve of gallic acid in μg / ml for total phenol content estimation

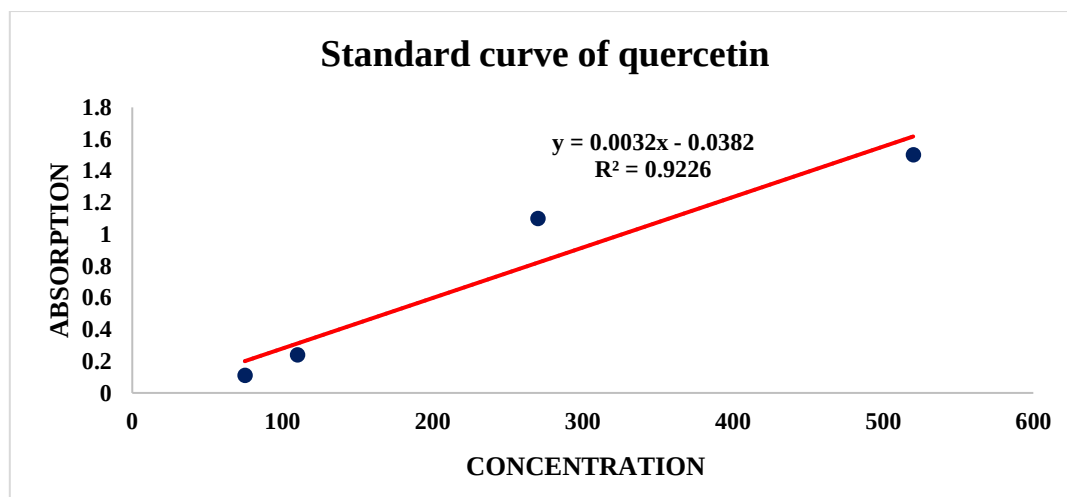


Fig. 2: Standard curve of quercetin in µg / ml for total flavonoid estimation

Table 2: Yield and assays of total phenol, total flavonoid, and DPPH radical scavenging activity of *Rungia pectinata* extract

Yield of the methanol extract (%)	Colour of extract	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)	DPPH radical scavenging activity IC ₅₀ (µg/mL)
5.54	Dark green	98.22 ± 1.47	45.78 ± 5.38	95.32 ± 1.21

DPPH: 1,1-diphenyl-2-picrylhydrazyl

Conclusions

In summary, this thorough investigation shows that *Rungia pectinata* plants have a high flavonoid and total phenolic content, a large number of secondary metabolites, and potent natural antioxidant qualities. Even though this study offers important new information about *Rungia pectinata*'s pharmacological characteristics, more experimental verification is still necessary. All things considered; this study adds significant knowledge to the field of medicinal plant research by highlighting *Rungia pectinata*'s potential as a natural treatment various diseases. The biological characteristics of *Rungia pectinata* and other medicinal plants are not well understood; therefore, more research should concentrate on *in vivo* bioactivity assessments and chemical compound isolation led by bioassay.

ACKNOWLEDGMENT

The authors are thankful to the J.N.L College Department of Botany, Patliputra University, India, for infrastructural support.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

References

Alves C. Q. , David J. M. , David J. P. , Bahia M. V. , and Aguiar R. M. , "Methods for determination of *in vitro* antioxidant activity for extracts and organic compounds," *Qu'ímica Nova*, vol. 33, no. 10, pp. 2202–2210, 2010.

- Meyer A. S. and Isaksen A. , “Application of enzymes as food antioxidants,” *Trends in Food Science & Technology*, vol. 6, no. 9, pp. 300–304, 1995.
- Montoro P. , Braca A. , Pizza C. , and Tommasi N. De , “Structure-antioxidant activity relationships of flavonoids isolated from different plant species,” *Food Chemistry*, vol. 92, no. 2, pp. 349–355, 2005.
- Bravo L. , “Polyphenols: chemistry, dietary sources, metabolism and nutritional significance,” *Nutrition Reviews*, vol. 56, no. 11, pp. 317–333, 2009.
- John B. , Sulaiman C. T. , George S. , and Reddy V. R. K. , “Total phenolic and flavonoids in selected medicinal plants from Kerala,” *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 6, pp. 406–408, 2014.
- Swain S. R. , Sinha B. N. , and Murthy P. N. , “Subchronic toxicity studies of the hydroalcoholic extract of *Rungia pectinata* leaves,” *Pharmacologyonline*, vol. 2, pp. 461–466, 2008.
- Chang C. , Yang M. , Wen H. , and Chern J. , “Estimation of total flavonoid content in propolis by two complementary colorimetric methods,” *Journal of Food and Drug Analysis*, vol. 10, no. 3, pp. 178–182, 2002.
- Biswas S. , and Mandal N. , “Antioxidant and free radical scavenging activity of *Spondias pinnata*,” *BMC Complementary and Alternative Medicine*, vol. 8, no. 1, 2008.
- Bakar M. F. A. , Karim , F. A. , Suleiman M. , Isha A. , and Rahmat A. , “Phytochemical constituents, antioxidant and antiproliferative properties of a liverwort, *Lepidozia borneensis* stephani from mount Kinabalu, Sabah, Malaysia,” *Evidence-Based Complementary and Alternative Medicine*, vol. 2015, Article ID 936215, 9 pages, 2015.
- Bhatnagar S. , Sahoo S. , Mohapatra A. K. , and D. R. Behera, “Phytochemical analysis, antioxidant and cytotoxic activity of medicinal plant *Combretum roxburghii* (family:combretaceae),” *International Journal of Drug Development & Research*, vol. 4, pp. 193–202, 2012.
- Dirar A. I. , Alsaadi D. H. M. , Wada M. , Mohamed M. A. , T. Watanabe, and H. P. Devkota, “Effects of extraction solvents on total phenolic and flavonoid contents and biological activities of extracts from Sudanese medicinal plants,” *South African Journal of Botany*, vol. 120, pp. 261–267, 2019.
- Nadkarni K. M. , *Indian Materia Medica*, Popular Prakashan, Mumbai, India, 2002.
- Zhu Y. Z. , Huang S. H. , Tan B. K. H. , J. Sun, M. Whiteman, and Y. C. Zhu, “Antioxidants in Chinese herbal medicines: a biochemical perspective,” *Natural Product Reports*, vol. 21, no. 4, pp. 478–489, 2004.
- Mohana D. , Tippleswamy S. , Manjunath K. , and R. Abhishek, “Antioxidant properties of some selected Indian medicinal plants: their correlation with total phenolic contents,” *International Journal of Green Pharmacy*, vol. 7, no. 2, pp. 117–121, 2013.
- Cragg GM, Newman DJ. Natural products: a continuing source of novel drug leads. *Biochim Biophys Acta Gen Subj*. 2013;1830(6):3670- 3695. doi:10.1016/j.bbagen.2013.02.008
- Song JX, Sun YR, Peluso I, et al. A novel curcumin analog binds to and activates TFEB in vitro and in vivo independent of MTOR inhibition. *Autophagy*. 2016;12(8):1372-1389. doi:10.1080/15548627.2016.1179404
- WHO. Traditional medicine strategy 2002-2005. Accessed July 3, 2024 <https://www.who.int/publications/item/WHO-EDM-TRM-2002.1>
- Pinto DCGA, Silva AMS. Anticancer natural Coumarins as Lead compounds for the discovery of new drugs. *Curr Top Med Chem*. 2017;17(29):3190-3198. doi:10.2174/1568026618666171215095750
- Shukla S, Bajpai VK, Kim M. Plants as potential sources of natural immunomodulators. *Rev Environ Sci Biotechnol*. 2014;13(1):17-33. doi:10.1007/s11157-012-9303-x
- Nadkarni AK. *Dr. K. M. Nadkarni's Indian Materia Medica: With Ayurvedic, Unani-Tibbi, Siddha, Allopathic, Homeopathic, Naturopathic & Home Remedies, Appendices & Indexes*. Popular Prakashan. 2007.
- Sabudak T. , Demirkiran O. , Ozturk M. , and Topcu G. , “Phenolic compounds from *Trifolium echinatum* Bieb. And investigation of their tyrosinase inhibitory and antioxidant activities,” *Phytochemistry*, vol. 96, pp. 305–311, 2013.