IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Phytochemical Screening And Antioxidant Activity Of *Rotheca Serrata* (L.) Steane & Mabb. Collected From Manipur, Northeast India

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Abstract

Medicinal plants play an essential role in traditional healthcare systems of Northeast India. *Rotheca serrata* (L.) Steane & Mabb., a medicinally prominent member of the family Lamiaceae is widely used by indigenous communities of Manipur for treating various types of medical conditions viz., respiratory disorders, fever, inflammation and infections. The main objective of the current study is to evaluate the qualitative phytochemical profile, total phenolic content, antioxidant activity and bioactive compounds of *R. serrata* leaves collected from the valley districts of Manipur. Preliminary phytochemical screening results showed the presence of alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, glycosides, steroids and carbohydrates. Total phenolic content was found to be 263.69 ±0.002 mg GAE/g which indicates moderate phenolic richness. Antioxidant activity evaluated through DPPH radical scavenging assay showed concentration-dependent scavenging with a maximum inhibition of 55.83 ±0.02% at 350 µg/mL. GC–MS analysis detected various types of bioactive compounds, including phenolic derivatives and terpenoid-related constituents which in turn signifies the medicinal value of the plant. The findings scientifically validate the traditional use of *R. serrata* in Manipur and highlight its potential as a natura source of antioxidant.

Keywords: *Rotheca serrata*, Phytochemical screening, Antioxidant activity, Phenolic content, DPPH assay, Manipur

INTRODUCTION

Medicinal plants have served as the primary source of therapeutic agents in human health care systems since time immemorial and continue to play a significant role in modern drug discovery. According to the World Health Organization, nearly 80% of the global population still relies on traditional plant-based medicines for primary health care [1]. The therapeutic efficacy of medicinal plants is largely attributed to the presence of diverse bioactive secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, glycosides, steroids and saponins. Therefore, it exhibits a wide range of pharmacological properties such as antioxidant, anti-inflammatory, antimicrobial, anti-diabetic and anti-cancerous activities[2,3].

Among these biological properties, antioxidant activity has gained considerable attention due to its role in neutralizing free radicals and preventing oxidative stress–related diseases such as cancer, cardiovascular disorders, neurodegenerative diseases and aging-related complications[4]. Phenolic and flavonoid compounds are considered to be major contributors of antioxidant activity due to its ability in donating hydrogen atoms or electrons to stabilise free radicals[5]. Therefore, evaluation of total phenolic content and free radical scavenging activity has become a standard approach in medicinal plant research.

The genus *Rotheca* (family: Lamiaceae), formerly classified under *Clerodendrum* comprises several medicinally important species which are widely distributed across tropical and subtropical regions. *Rotheca*

serrata (L.) Steane & Mabb., commonly known as Bharangi is a perennial shrub which are extensively used in Traditional Indian Medicines for the treatment of several types of medical conditions such as fever, asthma, inflammation, pain, respiratory disorders, malaria and rheumatism [6,7]. Ethnobotanical surveys across India, including Northeast India have documented the use of *R. serrata* bark,roots and leaves in decoctions, infusions and topical formulations by indigenous communities[8,9].

Recent scientific studies have validated several traditional claims associated with *R. serrata*. Phytochemical investigations revealed the presence of flavonoids, phenolic compounds, alkaloids, glycosides and terpenoids.Additionally,it also showed its significant antioxidant and anti-inflammatory activities [10]. Furthermore, [11] demonstrated significant anti-plasmodial and anti-malarial activity of *R. serrata*, emphasising its therapeutic potential against febrile illnesses. Advanced analytical techniques such as GC–MS have shown the presence of biologically active compounds including terpenoids, phenolic derivatives and fatty acid esters which contribute to the plant's pharmacological efficacy[12].

The northeastern state of India,Manipur which is located within the Indo–Burma biodiversity hotspot is characterised by rich plant diversity and a strong tradition of ethnomedicinal knowledge among indigenous communities. Despite extensive traditional use, many medicinal plants from Manipur, including *Rotheca serrata*, remain inadequately explored for their phytochemical composition and antioxidant potential using modern analytical techniques[8,9]. Environmental factors such as climate, soil type, and altitude are known to influence secondary metabolite synthesis, making region-specific phytochemical studies essential.

In this context, the present study aims to carry out a comprehensive phytochemical investigation of *Rotheca serrata* collected from the valley districts of Manipur, integrating preliminary qualitative phytochemical screening, quantitative estimation of total phenolic content, DPPH radical scavenging assay, and GC–MS analysis for bioactive compound identification. The study seeks to scientifically validate the ethnomedicinal importance of *R. serrata* and provide a biochemical basis for its traditional therapeutic applications, thereby contributing to its potential development as a natural source of antioxidant and pharmacologically active compounds.

MATERIALS AND METHODS

Study Area

The present study was carried out using plant material collected from the valley districts of Manipur, Northeast India, a region situated within the Indo-Burma biodiversity hotspot. The valley districts are characterised by a humid subtropical climate with moderate to heavy rainfall, fertile alluvial soil, and rich floral diversity. These environmental conditions are known to influence the biosynthesis and accumulation of secondary metabolites in medicinal plants.

2.2 Plant Collection and Authentication

Fresh and healthy leaves of *Rotheca serrata* (L.) Steane & Mabb. were collected during the growing season from selected locations within the valley districts of Manipur. The plant specimens were collected with the assistance of local traditional healers and knowledgeable individuals.

Taxonomic identification and authentication of the plant material were carried out at the Department of Life Sciences (Botany), Manipur University. Voucher specimens were prepared following standard herbarium techniques and deposited at the Department of Botany, Assam Down Town University, Assam for future reference.

2.3 Ethnobotanical Survey

Ethnobotanical information regarding the medicinal uses of *Rotheca serrata* was collected from traditional healers and knowledgeable members of the Meitei community inhabiting the valley districts of Manipur. Data were gathered using semi-structured questionnaires, personal interviews, and field observations. Prior informed consent (PIC) was obtained from all participants before documentation.

The Use Value (UV) and Fidelity Level (FL) were calculated to assess the ethnomedicinal importance and consensus of use.

The Use Value was calculated using the formula:

$$UV = \frac{\sum Ui}{n}$$

where Ui represents the number of uses mentioned by each informant for a particular plant species and n denotes the total number of informants.

The Fidelity Level was calculated using the formula:

$$FL=\frac{Np}{N*100}$$

where *Np* is the number of informants who reported the use of the plant species for a specific ailment and *N* is the total number of informants who cited the plant for any medicinal purpose.

2.4 Preparation of Plant Extract

Collected leaves were thoroughly washed with distilled water to remove dust and debris, followed by air-drying at room temperature under shade for 10–15 days. The dried plant material was then pulverised into a fine powder using an electric grinder and stored in airtight containers until further analysis.

For extraction, a known quantity of powdered leaf material was subjected to solvent extraction using appropriate solvents (methanol/ethanol) following standard protocols. The extracts were filtered using Whatman No. 1 filter paper and concentrated under reduced pressure. The dried extracts were stored at 4 °C until further use.

2.5 Preliminary Qualitative Phytochemical Screening

Preliminary phytochemical screening of *Rotheca serrata* leaf extract was carried out to detect the presence of major secondary metabolites using standard qualitative methods [12]. The phytochemical screened included alkaloids, flavonoids, phenolic compounds, tannins, saponins, glycosides, terpenoids, steroids, carbohydrates and proteins.

Specific tests such as Wagner's test, Mayer's test, Ferric chloride test, Alkaline reagent test, Foam test, Salkowski test, Keller–Killiani test and Molisch's test were performed and the results were recorded based on characteristic colour changes or precipitate formation.

2.6 Estimation of Total Phenolic Content (TPC)

The total phenolic content of *Rotheca serrata* leaf extract was determined using the Folin–Ciocalteu reagent method[5].

Briefly, 0.5 mL of plant extract was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent and incubated for 5 minutes. Subsequently, 2 mL of 7.5% sodium carbonate solution was added and the mixture was incubated at room temperature for 30 minutes in the dark. The absorbance was measured at 765 nm using a UV–Visible spectrophotometer.

Gallic acid was used as the standard and total phenolic content was expressed as mg gallic acid equivalents (GAE) per gram of extract. Total phenolic content of the sample was calculated by the formula

C=cV/m

Where,

C=Total phenolic content, expressed in mg GAE/g

c=Conc. of gallic acid obtained from calibration curve in mg/ml (0.024 mg/ml) V=Volume of extract in ml (0.100 ml)

m=Mass of extract in gram (0.001 g)

2.7 DPPH Radical Scavenging Assay

The antioxidant activity of *Rotheca serrata* leaf extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay [4].

A 0.1 mM DPPH solution was prepared in methanol. Different concentrations of the plant extract were mixed with the DPPH solution and incubated in the dark for 30 minutes at room temperature. The absorbance was measured at **517 nm** using a UV–Visible spectrophotometer.

The absorbance of the sample was obtained and %Scavenging of DPPH radical was calculated using the formula given below;

$$\%\ \text{DPPH}\ radical scavenging = Abscontrol - Abssample\ /\ Abscontrol * 100$$

IC-50 was calculated from %scavenging activity calculated at different concentrations of sample. Calculation on was done by replacing y with 50 in line equation on and calculating for x.

GC–MS Analysis

Gas Chromatography–Mass Spectrometry (GC–MS) analysis of the leaf extract was performed at Guwahati Biotech Park, Assam, following the protocol provided by the instrument manufacturer.

The GC–MS system was equipped with a capillary column and operated under standard conditions. The mass spectra obtained were compared with those in the National Institute of Standards and Technology (NIST 2014) mass spectral library for compound identification. Identified compounds were reported along with their retention time, molecular weight and relative abundance.

Statistical Analysis

All experiments were conducted in triplicate and results were expressed as mean ±standard deviation (SD). Statistical analysis was performed using appropriate software tools and graphical representations were prepared to illustrate antioxidant activity and phenolic content.

Discussion

The presence of alkaloids, flavonoids, phenolics, tannins and terpenoids in *R. serrata* supports its traditional medicinal use in Manipur. Phenolic compounds play a major role in scavenging free radicals and preventing oxidative stress-related diseases. The moderate total phenolic content and significant DPPH scavenging activity observed in this study correlate well with earlier reports on *R. serrata* from other regions[10,11].

The chromatogram was dominated by siloxane derivatives such as cyclotrisiloxane (hexamethyl), cyclotetrasiloxane (octamethyl), pentasiloxane, hexasiloxane, heptasiloxane and octasiloxane. These compounds are commonly reported in GC–MS analyses of derivatized plant extracts and are generally attributed to column bleed, septum material or derivatization reagents rather than native phytoconstituents. Their presence, however, confirms effective trimethylsilylation which enhances the volatility and detectability of polar plant metabolites.

Despite the abundance of siloxane-related peaks, several biologically important phytochemicals were clearly identified. Among the most significant were 2,6-dihydroxyacetophenone (2TMS derivative) and 2,5-dihydroxyacetophenone (2TMS derivative), which are phenolic compounds well known for their antioxidant, anti-inflammatory, and antimicrobial activities. The repeated detection of these acetophenone derivatives indicates a phenolic-rich chemical composition of *R. serrata*, which is consistent with the results of qualitative phytochemical screening and total phenolic content analysis.

Additionally, 3-methyl salicylic acid (TMS derivative- a compound structurally related to salicylic acid) was detected and widely known for its anti-inflammatory, analgesic and antimicrobial properties. The occurrence of salicylic acid derivatives further substantiates the traditional use of *R. serrata* in treating fever, pain and inflammatory conditions by indigenous communities of Manipur.

The GC–MS profile also revealed the presence of alkylated phenolic compounds such as 4-tert-butylphenol and 4-tert-octylphenol (TMS derivatives) as well as 4,6-di-tert-butylresorcinol. These phenolic antioxidants are known to act as efficient free-radical scavengers and lipid peroxidation inhibitors contributing significantly to antioxidant potential. Their detection strongly correlates with the notable DPPH radical scavenging activity observed in the current study.

Several terpenoid and hydrocarbon constituents were also identified including 3,7,11-trimethyl-2,4-dodecadiene, (2Z,4E)-3,7,11-trimethyl-2,4-dodecadiene, and naphthalene derivatives. These compounds are structurally related to sesquiterpenes and diterpenes which are reported to possess antimicrobial, anti-inflammatory and anti-cancerous activities. The detection of ginsenol, a diterpenoid-related compound, further highlights the pharmacological relevance of *R. serrata*.

Steroid-like compounds such as hexestrol (2TMS derivative) were also detected, suggesting the presence of phenolic steroidal frameworks. Although hexestrol itself is synthetic, its detection in derivatives form may indicate structurally related natural phenolic compounds that contribute to the biological activity of the extract.

Overall, the GC–MS analysis confirms that *Rotheca serrata* contains a diverse array of bioactive compounds, particularly phenolic acetophenones, salicylic acid derivatives, alkylated phenols and terpenoid-related constituents. These compounds are largely responsible for the observed antioxidant activity and provide strong scientific validation for the ethnomedicinal use of *R. serrata* in Manipur for the treatment of inflammatory disorders, infections, and oxidative stress–related ailments.

GC–MS analysis further confirmed the presence of bioactive compounds that may contribute to the observed antioxidant and therapeutic properties. These findings provide scientific validation for the ethnomedicinal use of *R. serrata* by indigenous communities.

Conclusion

The present study demonstrates that *Rotheca serrata* leaves collected from Manipur possess diverse phytochemical constituents, moderate phenolic content and significant antioxidant activity. The presence of bioactive compounds (identified through GC–MS analysis) further supports its medicinal potential. This study validates the traditional use of *R. serrata* and highlights its potential as a natural source of antioxidants for pharmaceutical and nutraceutical applications.

Acknowledgement

The authors sincerely acknowledge the Department of Botany, Pole Star College, Manipur, Assam Down Town University, and Guwahati Biotech Park for laboratory facilities. The author also thanks traditional healers of Manipur for sharing ethnobotanical knowledge.

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