



Qualitative and Quantities Estimation Phytochemical of *Saccopetalum Tomentosum* Leaf Extracts

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KEYWORDS

Saccopetalum tomentosum, antidiabetic, phytochemical etc.

ABSTRACT:

Natural products from plants continue to serve as promising sources of biologically active compounds for drug development. *Saccopetalum tomentosum* (Annonaceae), a deciduous tree widely found in India and Southeast Asia, is traditionally used for its antibacterial, anticancer, anthelmintic, and antiparasitic properties. This study aimed to evaluate the pharmacognostical, phytochemical, and in vitro antioxidant activities of *S. tomentosum* leaves collected from the Bhopal region of Madhya Pradesh. Standard methods were used for pharmacognostical and phytochemical analysis. Quantitative estimation of total phenolics and flavonoids was performed using Folin-Ciocalteu and aluminum chloride methods, respectively. Ethanolic extracts showed significant phenolic (323.667 mg/100 mg) and flavonoid (448.333 mg/100 mg) contents. Antioxidant activity of chloroform, ethyl acetate, and ethanolic extracts was assessed using DPPH, SOS, and reducing power assays, revealing concentration-dependent activity. The presence of diverse phytochemicals such as alkaloids, flavonoids, glycosides, and triterpenoids supports the plant's potential as a therapeutic agent in modern and traditional medicine. The high TPC and TFC values in *Saccopetalum tomentosum* leaf extracts suggest a strong presence of bioactive compounds. These phytochemicals are known for their health benefits, including antioxidant and antidiabetic effects, supporting the plant's traditional use. The results validate its potential for further pharmacological and therapeutic investigations.

Introduction: Phytochemicals are naturally occurring, non-nutritive bioactive compounds derived from plants, playing essential roles in plant defense against pathogens, herbivores, and environmental stress. Although not vital for basic human nutrition, these secondary metabolites, including flavonoids, alkaloids, tannins, and phenolic acids, are increasingly recognized for their health-promoting properties. Epidemiological studies have linked phytochemical-rich diets to a reduced risk of chronic diseases such as diabetes, cardiovascular disorders, and cancer. In particular, flavonoids and polyphenols have demonstrated antioxidant, anti-inflammatory, and antidiabetic effects by modulating glucose metabolism, enhancing insulin sensitivity, and inhibiting carbohydrate-digesting enzymes. *Saccopetalum tomentosum* (Annonaceae), a deciduous tree traditionally used for its antibacterial, anticancer, and antiparasitic activities, is a promising source of such compounds. This study investigates the pharmacognostical, phytochemical, and in vitro

antioxidant properties of *S. tomentosum* leaf extracts, focusing on total phenolic and flavonoid content, to validate its therapeutic potential and support its traditional use in managing oxidative stress and diabetes.

Material and method: Plant material The Pinnacle Biomedical Research Institute (PBRI), near the Bharat Scout and Guides Campus, Shanti Marg, Shyamla Hills Road, Depot Chouraha, Bhopal, Madhya Pradesh 462003, India, collected the leaves of *S. tomentosum*. Botanist Dr. Saba Naaz from the Saifia College of Science in Bhopal's Department of Botany carried out the plant's identification and authentication. For future use, a voucher specimen with the number 287/Saif./Sci./Clg/Bpl for *S. tomentosum* was conserved in the department of botany at Saifia College of Science, Bhopal.

Chemical reagents The Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd.



(Mumbai, India), and SRL Pvt. Ltd. (Mumbai, India) provided all the chemicals used in this work. The investigation only employed analytical-grade compounds.

Detailed phytochemical testing was performed to identify presence or absence of different phytoconstituents in *S. tomentosum* leaves extracts by using standard procedures¹³⁻¹⁴. The extracts prepared in Petroleum ether, Ethyl acetate, Chloroform and Ethanol were subjected to following tests:

Tests for carbohydrates:

Molisch test: To 1ml of extract, 2-3 drops of alcoholic α -naphthol solution was added. Conc. sulphuric acid was added along the side of the test tube. The appearance of purple ring at the junction of two liquids was observed, which confirms the presence of carbohydrates in the test samples.

Fehling's test: To 1 ml of extract, similar quantity of Fehling's solution A and B was added and heated on a water bath for few minutes. The development of brick red precipitate was observed. **Benedict's test:** Equal volume of Benedict's reagent and extract were mixed in a test tube and heated in the water bath for 5-10 minutes. Solution appears green, yellow or red depending on the

amount of reducing sugar present in the test solution which indicated the presence of reducing sugar.

Barfoed's test: 1 ml of extract and Barfoed's reagent were mixed in a test tube and heated on water bath for 2 minutes. Red colour due to formation of cupric oxide indicates the presence of monosaccharide.

Quantitative analysis of phytochemicals by spectrophotometer: The activity of any kind of phytomedicine principally depends upon the presence of phytochemicals. Phytochemicals present in plant act as the source for the treatment of different health problem. Different phytochemical have different therapeutic value. In this study, we have quantified the presence of total phenolic and total flavonoid content. Total phenolic content (TPC) was measures by using Folin-ciocalteau's reagent method and total flavonoid content (TFC) of *S. tomentosum* was measured by Aluminum chloride method. The TPC and TFC of the extracts were expressed as milligram of gallic acid equivalent per gram of extracts i.e. mg GAE/g extract and milligram of rutin equivalent per gram of extract i.e. mg RE/g extract respectively. The TPC and TFC in ethyl acetate and ethanolic extract were found to be 123.333, 323.667mg/gm and 216.333, 448.333mg/gm respectively

Table 1.1 Standard table for Gallic acid

| S. No. | Concentration ($\mu\text{g/ml}$) | Absorbance* |
|--------|------------------------------------|-------------------|
| 1. | 20 | 0.120 \pm 0.002 |
| 2. | 40 | 0.191 \pm 0.003 |
| 3. | 60 | 0.241 \pm 0.002 |
| 4. | 80 | 0.280 \pm 0.001 |
| 5. | 100 | 0.333 \pm 0.004 |

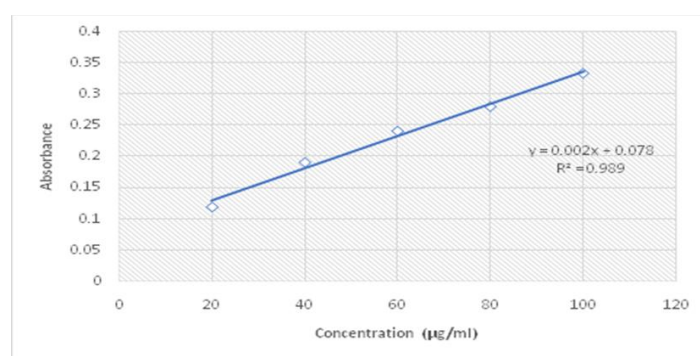


Figure 1.1: Graph represent standard curve of Gallic acid



Table 1.2: Standard table for Rutin

| S. No. | Concentration ($\mu\text{g/ml}$) | Absorbance* |
|--------|------------------------------------|-------------------|
| 1. | 20 | 0.144 \pm 0.003 |
| 2. | 40 | 0.160 \pm 0.002 |
| 3. | 60 | 0.178 \pm 0.004 |
| 4. | 80 | 0.204 \pm 0.003 |
| 5. | 100 | 0.255 \pm 0.001 |

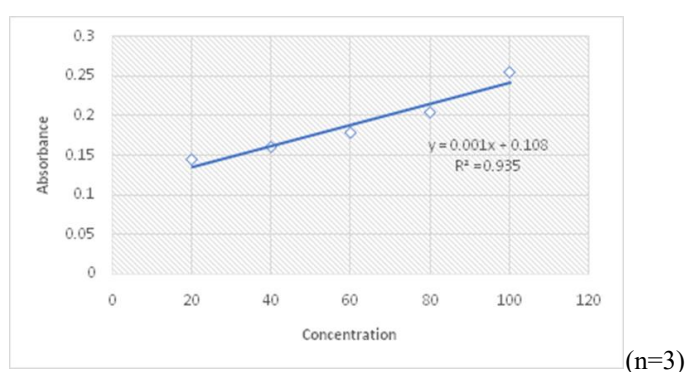


Figure 1.2.: Graph represent standard curve of Rutin

Total phenolic content (TPC) estimation:

Table 1.3: Total Phenolic content (TPC) of extract of *S. tomentosum* leaves

| Extracts | Total phenolic content (mg/gm equivalent to Gallic acid) | | |
|-----------------------------|---|-------------------|-------------------|
| | Chloroform | Ethyl acetate | Ethanol |
| Absorbance Mean \pm SD | 0.172 \pm 0.003 | 0.144 \pm 0.008 | 1.325 \pm 0.024 |
| TPC | 47.167 | 123.333 | 323.667 |

(n=3)

Total flavonoid content (TFC) estimation:

Table 1.4: Total Flavonoid content (TFC) of extract of *S. tomentosum* leaves

| Extracts | Total flavonoid content (mg/gm equivalent to rutin) | | |
|-----------------------------|---|-------------------|-------------------|
| | Chloroform | Ethyl acetate | Ethanol |
| Absorbance Mean \pm SD | 0.236 \pm 0.004 | 0.324 \pm 0.003 | 0.456 \pm 0.005 |
| TFC | 128.333 | 216.333 | 448.333 |

(n=3)



Isolation and purification: In vitro antioxidant assays was followed by Thin Layer Chromatography (TLC) of the crude extracts. This is the initial step for isolation of bioactive components from plants.

Thin Layer Chromatography of crude extracts:

| Solvent system number | Solvent System | Solvent Ratios | Rf values | | | |
|-----------------------|--|----------------|--------------------|--|---|-------------------|
| | | | Pet. ether extract | Ethyl acetate extract | Chloroform extract | Ethanolic extract |
| 1 | Toluene: Ethyl formate: Formic acid | 50:40:10 | 0.97 | 0.24 0.19 0.58 0.73 0.09 0.04 | 0.08 0.12 0.37 0.94 0.37 | 0.26 |
| 2 | Benzene: Acetone | 90:10 | 0.99 | 0.09 0.15 0.22 0.45 0.56 0.73 0.35 0.78, 0.81 | 0.95 0.09 | Nil |
| 3 | Toluene: Chloroform: Acetone | 40: 25: 35 | 0.99 | 0.44 0.50 0.55 0.11 0.15 0.64 0.74 0.82 0.88 0.96 | 0.13 0.87 0.96 0.98 0.05 0.13 | 0.11 |



| | | | | | | |
|---|-----------------------|---------|------|------|------|-----------|
| | | | | | | 0.05 |
| | | | | | | 0.14 0.24 |
| | | | | 0.07 | 0.88 | 0.34 0.72 |
| | Butanol: Acetic acid: | 40: 10: | 0.99 | 0.87 | 0.99 | 0.81 0.88 |
| 4 | Water | 50 | 0.96 | 0.96 | 1.00 | 1.00 |
| | | | | | | 0.74 |
| | | | | | | 0.91 |

TLC of isolated compound: The compound extracted & isolated as flavonoid. TLC of FR-1 developed in mobile phase, Ethanol + Acetic Acid + Water (40:10:50) ratio, showing bright spot at blue background at RF

value of 0.87. The fraction after isolation by column chromatography was subjected for spectral analysis by UV, IR NMR & Mass Spectroscopy.

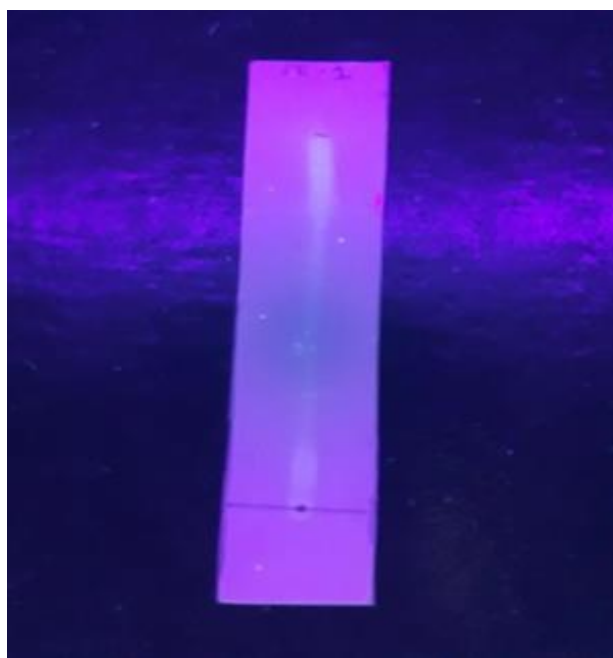


Figure 1.3: TLC of isolated compound FR-1

Interpretation UV Spectrum: When UV Spectrum of flavonoids recorded mostly three peaks from 250 to 350 nm were observed. The compound showed peaks at

λ_{max} 259, 293 & 302nm indicating the compound is a flavonoid.

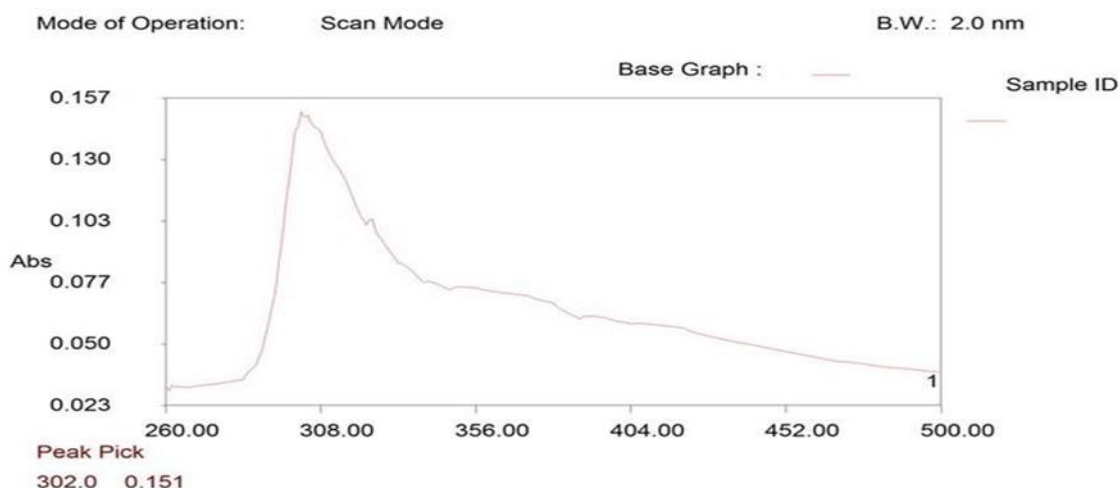
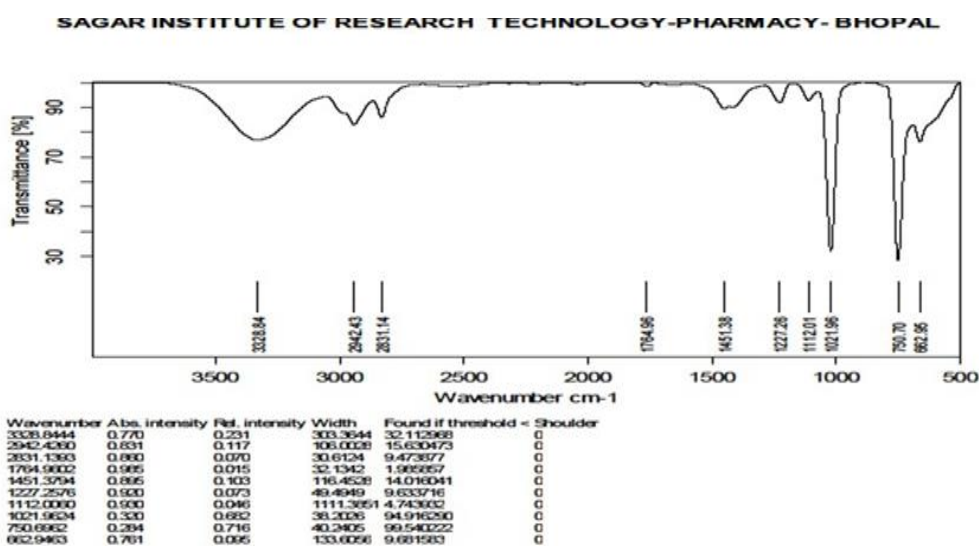


Figure 5.4: UV spectrum of isolated fraction FR-1

Interpretation of IR Spectra of FR-1: Infra red Spectroscopy mainly provides information regarding organic functional groups in the isolated phytoconstituents which supports to prove the structure

of the compound. The recorded peaks for the compound at various analytically useful frequencies are The IR spectrum data supports presence of Hydroxyl, alkene, carbonyl, aromatic groups in the compound.





Interpretation of 1H NMR of FR-1: The PMR data shows major peaks their positions & splitting as follows:

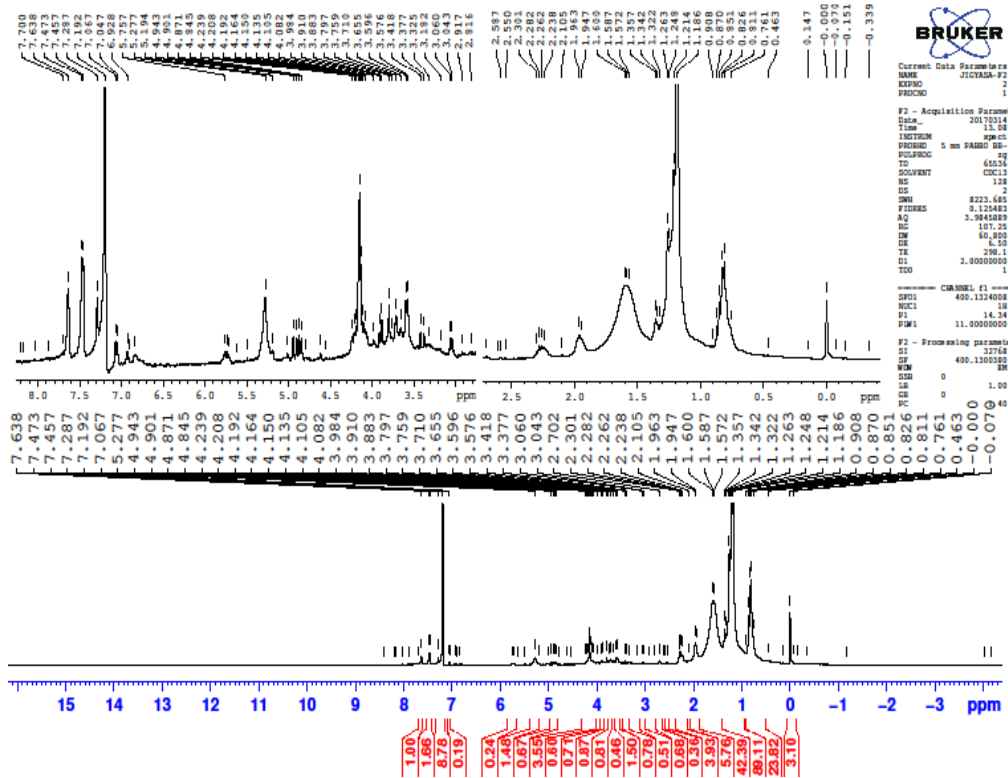
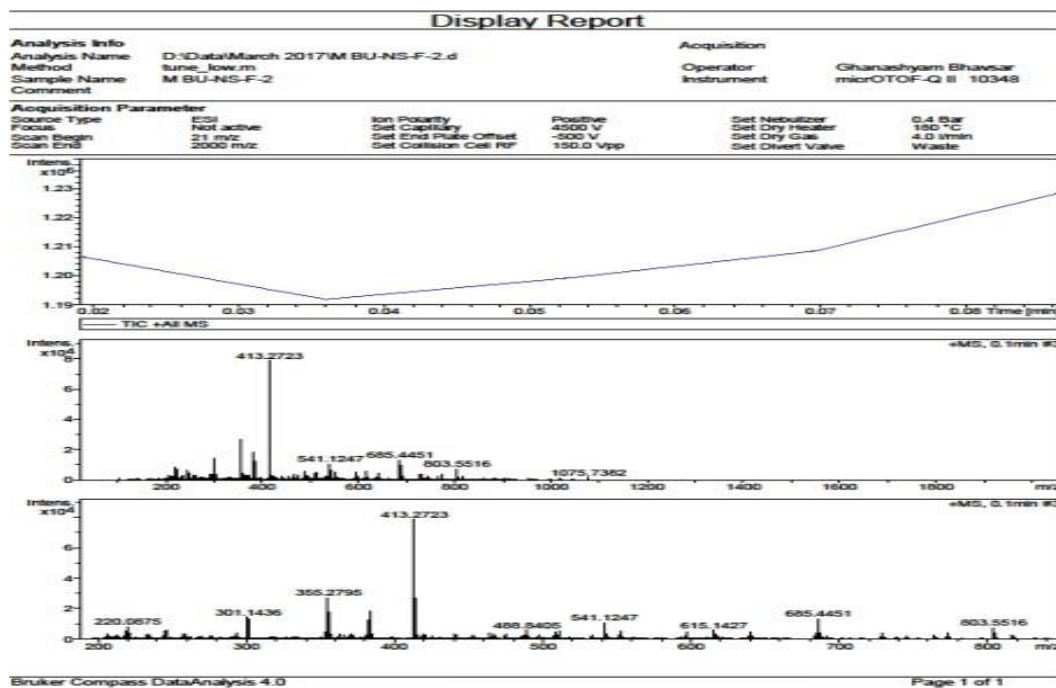


Figure 5.6: 1H NMR spectra of isolated compound FR-1

Interpretation of Mass Spectrum of FR-1:





Discussion: The high TPC and TFC values in *Saccopetalum tomentosum* leaf extracts suggest a strong presence of bioactive compounds. These phytochemicals are known for their health benefits, including antioxidant and antidiabetic effects, supporting the plant's traditional use. The results validate its potential for further pharmacological and therapeutic investigations.

Conclusion: The qualitative and quantitative phytochemical analysis of *Saccopetalum tomentosum* leaf extracts confirmed significant levels of phenolics and flavonoids. TPC and TFC values, expressed in gallic acid and rutin/quercetin equivalents, indicate a rich phytochemical profile, supporting the plant's potential therapeutic, antioxidant, and antidiabetic applications.

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