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# Phytochemical screening of Petroleum ether and Methanol extract of *Bridelia retusa* (L.) A. Juss. leaves

**Ansari Vaseem Ahamad*[1], Manju Prajapati[2], Sweety Tiwari[3] and Akhlesh Kumar Singhai[4]**

[1]School of Pharmacy, LNCT University, Kolar Road, Bhopal - 462022 (India)
[2]School of Pharmacy, LNCT University, Kolar Road, Bhopal - 462022 (India)
[3]School of Pharmacy, LNCT University, Kolar Road, Bhopal - 462022 (India)
[4]School of Pharmacy, LNCT University, Kolar Road, Bhopal - 462022 (India)
Corresponding Author: Ansari Vaseem Ahamad
E-mail: waseemansari41485@gmail.com

**Abstract**

Medicinal plants are excellent treatment options having no or minimum side effects used for numerous diseases and have been used since time immemorial. The present study aims to screen the various phytochemicals from the petroleum ether and methanol extract of *bridelia retusa* leaves. The extracts were subjected to quantitative and qualitative estimation, total phenolic content (TPC), and Total flavonoid content (TFC) estimation. The extracts were found to contain various phytochemicals like alkaloids, phenolics, flavonoids, saponins, glycosides, and saponins.

**Key words :** Phytochemical screening, *Bridelia retusa*, Phytochemicals.

Medicinal plants are harvested, prepared for use in experiments, or consumed directly as herbal or traditional medicine. The idea of preparing medicinal herbs for experimentation entails timely and appropriate plant gathering, expert authentication, sufficient drying, and grinding[6]. In the ruler part of India medicinal plants are easily available and local tribal people have great information. In India due to the diversity in climate and soil hence availability of a large number of plants is easy. These herbal drugs are safe, have more potency, and low in cost, and have no side effects. *Bridelia* genus worldwide 50 species distributed in Tropical Africa, Asia ranging from India and China, Australia, and 10 species in Thailand hotter part of the world[1]. A variety of phytochemicals are reported in the leaves of the plant *Bridelia retusa* which are biologically active and belong to different classes like steroids, triterpenoids, tannins, phenols, fats, and minerals. Minerals like Calcium, Copper, Iron, Manganese, Magnesium, Phosphorus, and zinc are reported in plants. Triterpene Ketone [4 -desmethyl

eupha 7, 24 -diene -3 -one], stigmasterol, and dehydrositosterol were isolated and reported[4].

*Reagents and chemicals used :*

Glacial Acetic Acid, Nitroprusside, Sodium Hydroxide, and Ammonia, from Merck company. 95% Alcohol, Conc. HCl, Chloroform, from Clorofiltind company. Petroleum ether from Researchlab company. Conc. $H_2SO_4$ from Fizmerck company. Magnesium from Himedia company. 1% Copper Sulphate Solution from Rankem.

*Glassware used :*

Beakers, Glass rod, Volumetric flask, graduated pipette, and Test tubes all from the Borosilicate company.

*Plant collection :*

The medicinal plant *Bridelia retusa* (L.) Jhss. (300 gm) was collected. After cleaning, plant part (leaves) were dried under shade at room temperature for 3 days and then in oven dried at 45°C till complete dryness. The dried plant part (leaves) was stored in air-tight glass containers in a dry and cool place to avoid contamination and deterioration. Authentication of selected traditional plant - The medicinal plant *Bridelia retusa* was authenticated by a plant taxonomist to confirm its identity and purity.

*Extraction :*

In the present study, plant material was extracted by continuous hot percolation method using the Soxhlet apparatus. The powdered material *of Bridelia retusa* was placed in the thimble of the apparatus. Soxhlation was performed at 60°C using petroleum ether as a non-polar solvent. Exhausted plant material (marc) was dried and afterward re-extracted with ethanol solvent. For each solvent, soxhlation was continued till no visual change was observed in the siphon tube and the completion of extraction was confirmed by the absence of any residual solvent when evaporated. Obtained extracts were evaporated using a rotary vacuum evaporator (Buchitype) at 40°C. The dried extract was weighed and the percentage yield for each extract was determined using

Formula**:** % Yield = (Weight of extract / Weight of Plant Material used) * 100

The prepared extracts were labeled and stored in an airtight container until further usage after being examined for organoleptic characteristics (percentage yield, color, and odor)[2].

Figure Continuous Hot Extraction

*Phytochemical investigation :*

The experiment was performed to identify the presence or absence of different phytoconstituents by detailed qualitative

phytochemical analysis. The colour intensity or the precipitate formation was used as a medical response to tests. Following standard procedures were used[3].

*Test for Carbohydrates :*

- **Molisch's Test:** The aqueous solution of the *Bridelia retusa* extract to 1 ml was mixed with a few drops of Molish reagent (naphthol) and conc. $H_2SO_4$ (sulphuric acid) was added dropwise along the wall of the test tube. When two liquids mix up, the formation of a purple color ring at

*Tests for Alkaloids :*

- **Dragendorff's Test:** 1 ml of *Bridelia retusa* extract was taken. The alcohol was mixed and shaken well with little drops of acetic acid and Dragendroff's reagent. The presence of

*Test for Saponins :*

- **Froth Test:** 1ml of *Bridelia retusa* extract was added to distilled water and shaken well. The presence of saponin was indicated by stable froth formation.

*Test for Triterpenoids and Steroids :*

- **Liebermann-Burchard Test:** The *Bridelia retusa* extract was dissolved in chloroform. To it, 1 mL of acetic acid and 1 mL of acetic anhydride were added, then heated in a water bath and subsequently cooled. Then add a few drops of concentrated sulphuric acid along the sides of the test tube. The presence of steroids is indicated by the appearance of a bluish-green colour.

*Test for Tannin and Phenolic Compounds:*

- **Ferric Chloride Test:** Amount of *Bridelia retusa* extract dissolved in the distilled water. Add to it a few drops of dilute solution of ferric chloride. The formation of dark blue colour indicated the presence of tannins.

*Test for Flavonoids :*

- **Shinoda's Test:** A few magnesium turnings and little drops of concentrated hydrochloric acid to 1 ml of *Bridelia retusa* extract in alcohol were added. It was heated in a water bath. When the formation of red to pink colour occurred, indicated the presence of flavonoids.

*Test for Glycosides :*

- **Bontrager's Test:** Dilute sulphuric acid was added to 3 ml of test solution dilute sulfuric acid was added. It was boiled for 5 minutes and then filtrate was obtained. To the cold filtrate, an equal amount of benzene or chloroform was added and shaken well. Separation of the organic solvent layer was obtained and then ammonia was added to it. The presence of anthraquinone glycosides indicated the formation of pink to red colour in the ammonical layer.

*Test for proteins and amino acids :*

- **Biuret test:** The Biuret test was performed on a solution of *Bridelia retusa* extract dissolved in water or a suitable solvent. A few drops of biuret reagent were added to the solution, followed by gentle mixing. The appearance of a violet or purple color indicated the presence of proteins in the extract. To confirm the result, the color

change was compared to a blank solution containing only the solvent and biuret reagent.

*Test for fats and oils :*

- *Solubility test :*
- 2-3 ml of alcoholic solution of *Bridelia retusa* extract, add a few ml. of chloroform, and solubility was observed.
- 2-3 ml of alcoholic solution of *Bridelia retusa* extract. Add a few ml. of 90% ethanol and solubility was observed.

*Quantitative phytochemical estimation:*

*TPC :*

The total phenolic content of *Bridelia retusa* extract was determined using the Folin-Ciocalteu Assay. The *Bridelia retusa* extracts (0.2 mL from stock solution) were mixed with 2.5 mL of Folin-Ciocalteu Reagent and 2 mL of 7.5% sodium carbonate. This mixture was diluted up to 7 mL with distilled water. Then the resulting solutions were allowed to stand at room temperature for 2 hrs. prior to spectrophotometrically measuring the absorbance at 760 nm. Calibration curves were composed using standard solutions of Gallic Acid Equivalent (GAE) mg/gm. Gallic aid was produced at concentrations of 20, 40, 60, 80, and 100 µg/mL. Polyphenols are among the substances that the Folin-Ciocalteu reagent is sensitive to becoming reduced. They produce a blue colour upon reaction. This blue colour was measured spectrophotometrically[7].

*TFC :*

The aluminum chloride technique was used to calculate the flavonoid content. 0.5 ml of *Bridelia retusa* extract solution was mixed with 2 ml of distilled water. Then, 0.15 ml of sodium nitrite (5%) was added and mixed properly. After that, wait for 6 minutes before adding 0.15 ml Aluminium chloride (10 %) and allow to stand for 6 minutes. Then, 2 ml of 4 % sodium hydroxide was added. The mixture was shaken and mixed thoroughly. The absorbance of the mixture was estimated at 510 nm using a UV spectrophotometer. Calibration curves were composed using standard solutions of Rutin Equivalent (RE) mg/gm. Rutin was produced at concentrations of 20, 40, 60, 80, and 100 µg/mL.. Total flavonoid content was determined from the calibration curve and results were indicated as mg Rutin equivalent per gram dry extract weight[5].

*Percentage yield :*

In phytochemical extraction, the percentage yield is very crucial to determine the standard efficiency of extraction for a specific plant, various sections of the same plant, or different solvents used. The yield of extracts received from the *Bridelia retusa* is shown in

Table-1. Percentage Yield of crude extracts of *Bridelia retusa* extract

| S. No | Plant name | Solvent | Theoretical weight | Yield (gm) | % yield |
|---|---|---|---|---|---|
| 1 | *Bridelia retusa* | Pet ether | 289 | 1.71 | 0.59% |
| 2 | | Methanol | 300 | 6.11 | 2.03% |

Table-2. Phytochemical testing of extract

| S. No. | Experiment | Presence or absence of phytochemical test | |
|---|---|---|---|
| | | Pet. Ether extract | Methanolic extract |
| 1. | **Alkaloids** | | |
| 1.1 | Dragendroff's test | Present | Present |
| 2. | **Glycoside** | | |
| 2.1 | Borntrager test | Absent | Present |
| 3. | **Carbohydrates** | | |
| 3.1 | Molish's test | Absent | Absent |
| 4 | **Proteins and Amino Acids** | | |
| 4.1 | Biuret test | Absent | Absent |
| 5 | **Flavonoids** | | |
| 5.1 | **Shinoda's Test** | Present | Present |
| 6 | **Tannin and Phenolic Compounds** | | |
| 6.1 | **Ferric Chloride Test** | Absent | Absent |
| 7 | **Saponin** | | |
| 7.1 | **Foam test** | Present | Present |
| 8 | **Test for Triterpenoids and Steroids** | | |
| 6.1 | **Libbermann-Burchard's test** | Absent | Absent |

*Quantitative Analysis :*

Preliminary phytochemical testing of crude extracts confirmed the presence of phenolics and flavonoids in plant material. To estimate their amount total phenolic (TPC) and total flavonoid content (TFC) assays were performed.

*Total Phenolic Content (TPC) Estimation:*

Table-3. Standard table for Gallic acid

| S. No. | Concentration (µg/ml) | Absorbance |
|---|---|---|
| 1. | 20 | 0.144 |
| 2. | 40 | 0.176 |
| 3. | 60 | 0.194 |
| 4. | 80 | 0.235 |
| 5. | 100 | 0.272 |

*Total Phenolic Content extract :*

Table-4. Total phenolic content

| S. No | Absor-bance | TPC in mg/gm equiva-lent of Gallic Acid |
|---|---|---|
| 1 | 0.137 | 59.33 mg/gm |
| 2 | 0.178 | |
| 3 | 0.190 | |

Table-5. Total Phenolic Content of extract *Bridelia retusa*

| Extracts | Total Phenolic content (mg/ gm equivalent of Gallic acid) |
|---|---|
| Methanol | 59.33 |

*Total flavonoid content (TFC) estimation :*

Table-6. Standard table for Rutin

| S.No. | Concentration (µg/ml) | Absorbance |
|---|---|---|
| 1. | 20 | 0.177 |
| 2. | 40 | 0.203 |
| 3. | 60 | 0.277 |
| 4. | 80 | 0.313 |
| 5. | 100 | 0.331 |

*Total Flavonoid Content in extract :*

Table-7. Total Flavonoid Content

| S. No. | Absorbance | TFC in mg/gm equivalent of Rutin |
|---|---|---|
| 1 | 0.149 | 17.33 mg/gm |
| 2 | 0.163 | |
| 3 | 0.194 | |

Table-8. Total Flavonoid Content of extract *Bridelia retusa*

| Extracts | Total Flavonoid content (mg/ gm equivalent of rutin) |
|---|---|
| Methanol | 17.33 |

The phytochemical estimation of the crude extract of *Bridelia retusa* obtained using petroleum ether as a solvent had a yield of 0.59%. Methanol extract had a yield of 2.03% and contained phytochemicals such as alkaloids, flavonoids, and saponins. The extract also showed a total phenolic content of 59.33 mg/gm equivalent to Gallic acid and a total flavonoid content of 17.33 mg/gm equivalent to rutin. suggests that they can provide pharmaceuticals for contemporary medicine by serving as a source of bioactive components. Further studies are therefore required to validate their antiulcer, antioxidant, anti-microbial, hepatoprotective, activities. In addition, isolation purification and characterization of the active principles are necessary to make the plant have novel interesting studies.

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**Conflict of Interest**

The authors declare that they have no conflicts of interest related to this research project. All sources of funding and support are acknowledged appropriately.

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