AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Phytochemical Investigation of the Bark of Parkinsonia aculeata and Rotula aquatica: A Comparative Study

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ABSTRACT

The present study investigates the phytochemical profile of the bark of Parkinsonia aculeata and Rotula aquatica, two medicinal plants known for their traditional therapeutic applications. The bark samples were subjected to extraction using alcohol and water, followed by qualitative phytochemical screening to detect the presence of secondary metabolites. The extracts were evaluated for constituents such as alkaloids, flavonoids, steroids, tannins, carbohydrates, and saponins. The results confirmed the rich presence of flavonoids and phenolic compounds in both plants, supporting their ethnomedicinal relevance. These findings contribute to the standardization and quality control of these plants in herbal drug formulations.

Keywords: Parkinsonia aculeata, Rotula aquatica, phytochemical screening, bark, flavonoids, herbal drugs

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Received 19 August 2025, Accepted 08 September 2025

Please cite this article as: Jeelani A *et al*., Phytochemical Investigation of the Bark of Parkinsonia aculeata and Rotula aquatica: A Comparative Study. American Journal of PharmTech Research 2025.

INTRODUCTION

Medicinal plants continue to be a valuable source of bioactive compounds used in modern and traditional medicine. Parkinsonia aculeata (Fabaceae) and Rotula aquatica (Boraginaceae) have long been utilized in Indian folk medicine for treating inflammatory disorders, fever, and infections. However, scientific data validating the phytochemical constituents of their bark remains limited. This study aims to evaluate the phytochemical profile of their bark to provide scientific insight into their traditional use and to aid in the quality control of plant-based pharmaceuticals. Herbal preparations are effectively and extensively used for their medicinal properties, and have become increasingly popular worldwide. Herbal medicines generally have fewer side effects than synthetic compounds, and their effectiveness can be improved by modern pharmacological.

In botany the understanding of herb is a plant with a fleshy rather than a woody stem, which after the plant has bloomed and set seed, dies down to the ground. However, the word "herb" has other meanings that expand the concept. The word is derived from the old Sanskrit *bharb*, meaning, "to eat". Generally, an herb is a plant or plant part valued for its medicinal, savory or aromatic properties. In all of these cases, an herb is a fresh or dried plant or its useful part.

The term drug has different meanings in different times and contexts. Legal definitions and common understandings vary. Most people consider drugs as medicines or substances of abuse, as nonfood items that affect function and sometimes behavior. Herbs and their products are caught in the middle of this web of nomenclature.

The word 'drug' was derived from the Dutch work *droog*, meaning, "dried," and from the Anglo-Saxon *drigan*, indicating, "to dry." As recently as 100 years ago in the pharmaceutical profession, drugs were understood as the dried herbs from which medicinal extracts were produced.

MATERIALS AND METHOD

Collection and Authentication of Plant Material

Bark of P. aculeata and R. aquatica was collected from Rajasthan and authenticated by respective botanists. The plant specimens were shade-dried, coarsely powdered, and stored in airtight containers for further use.

Extraction Procedure

The powdered bark was extracted using Soxhlet extraction with ethanol and cold maceration with distilled water. The extracts were concentrated and stored at 4°C until further analysis.

Preliminary Phytochemical Screening

Alcoholic and aqueous extracts of the bark of *P. aculeata* and *R. aquatica* were prepared using Soxhlet extraction and cold maceration. Preliminary phytochemical screening was carried out for

alkaloids, flavonoids, steroids, carbohydrates, and tannins. The bark of *P. aculeata* and *R. aquatica* were subjected to following phytochemical investigations:

A. Extraction

- **Extraction with 95 % alcohol.**
- **Cold maceration.**

B. Qualitative chemical identification tests.

C. HPTLC fingerprint profile

A. Extraction of plant material:

Extraction with 95% alcohol:

The bark of *P. aculeata* and *R. aquatica* were shade dried at room temperature, pulverized, and coarse powder was extracted exhaustively with 95% ethanol at temperature 40-60^{0}C, in a Soxhlet extractor. The extract was concentrated in a rotary flash evaporator and residue was dried in a desiccator over sodium sulfite.

Method: Cold maceration.

The bark of *P. aculeata* and *R. aquatica* were shade dried at room temperature, pulverized, and coarse powder was macerate exhaustively with water then being kept for 5 days in tightly sealed vessels at room temperature, protected from sunlight and shaken several times daily and adds preservative. Concentrate extract by distilling off the solvent and then evaporating to dryness on water –bath.

B. Preliminary phytochemical screening

Preliminary phytochemical screening was carried out for bark of *P. aculeata* and *R. aquatica* to know the nature of constituents present in them and their distribution in total alcoholic and aqueous extracts and which extract shows maximum activity then go for further phytochemical and isolation of phytoconstituents.

Tests for Carbohydrates

❖ **Molisch's test:** Treat the extract solution with few drops of alcoholic α-napthol. Add 0.2 ml of concentrated H_2SO_4 slowly through the sides of the test tube, purple to violet colored ring appears at the junction.

❖ **Benedict's test:** Treat the extract solution with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and upon boiling on water bath, reddish brown precipitate forms if reducing sugars are present.

❖ **Barfoed's test:** General test for monosaccharides**:** Heat the test tube containing 1ml reagent and 1 ml of extract solution in a beaker of boiling water; if red cuprous oxide is formed within two

minutes, a monosaccharide is present. Disaccharides on prolonged heating (about 10min) may also cause reduction, owing to partial hydrolysis to monosaccharides.

❖ **Selwinoff's test:** Hydrochloric acid reacts with ketose sugar to form derivative of furfuraldehyde, which gives red colored compound when linked with resorcinol. Add extract solution to about 5 ml of reagent and boil. Fructose gives red color within half minute. The test is sensitive to 5.5 mmol/lt. if glucose is absent. If glucose is present it is less sensitive and on addition of large amount of glucose it gives similar color.

❖ **Fehling's test:** Equal volume of Fehling's A (Copper sulphate in distilled water) and Fehling's B (Potassium tartarate and Sodium hydroxide in distilled water) reagents are mixed along with few drops of extract solution, boiled, a brick red precipitate of cuprous oxide forms, if reducing sugars are present.

❖ **Caramelisation:** Carbohydrates when treated with strong sulfuric acid, they undergo charring with the dehydration along with burning sugar smell.

❖ **Tollen's test:** To 100mg of extract add 2ml of Tollen's reagent, a silver mirror is obtained inside the wall of the test tube, indicates the presence of aldose sugar.

❖ **Bromine water test:** It gets decolorized by aldose but not by the ketose, because bromine water oxidizes selectively the aldehyde group to carboxylic group, giving raise to general class of compounds called aldonic acid.

Tests for Proteins & Amino acids

❖ **Millon's Test:** Extract solution + 2 ml of Millon's reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid) white precipitate appears, which turns red upon gentle heating.

❖ **Ninhydrin Test:** Amino acids and proteins when boiled with 0.2% solution of Ninhydrin (Indane 1, 2, 3 trione hydrate), produces violet color.

Tests for Sterols and Triterpenoids

❖ **Libermann-Burchard test:** Extract treated with few drops of acetic anhydride, boil and cool, concentrated sulphuric acid is added from the side of the test tube, A brown ring at the junction of two layers and the upper layer turns green indicates the presence of sterols and formation of deep red color indicates the presence of triterpenoids.

❖ **Salkowski's test:** Treat extract in chloroform with few drops of concentrated Sulfuric acid, shake well and allow to stand for some time, red color appears in the lower layer indicates the presence of sterols and formation **of yellow colored** lower layer indicating the presence of triterpenoids.

Tests for Glycosides

Test I: Extract 200 mg of the drug by warming in a test tube with 5 ml of dilute (10%) sulphuric acid on a water bath at 100ºC for two minutes, centrifuge or filter, pipette out supernatant or filtrate. Neutralize the acid extract with 5% solution of Sodium hydroxide (noting the volume of NaOH added). Add 0.1 ml of Fehling's solution A and B until alkaline (test with pH paper) and heat on a water bath for 2 minutes. Note the quantity of red precipitate formed and compare with that formed in Test II.

Test II: Extract 200 mg of the drug using 5 ml of and boil on water bath. After boiling add equal volume of water to the volume of NaoH used in the above test. Add 0.1 ml of Fehling's A and B until alkaline (red litmus changes to blue) and heat on water bath for two minutes. Note the quantity of the red precipitate formed.

Compare the precipitates of Test II with Test I. If the precipitate in Test-II is greater than in Test-I, then Glycoside may be present. Since Test I represent the amount of free reducing sugar already present in the crude drug, whereas Test-II represents the Glycoside after acid hydrolysis.

Tests for Alkaloids

- **Mayer's test:** (Potassium mercuric iodide solution)**:** To the extract/sample solution, add few drops of Mayer's reagent , creamy white precipitate is produced.
- **Dragendroff's Test:** (Potassium bismuth iodide solution). To the extract/sample solution, add few drops of Dragendroff's reagent, reddish brown precipitate is produced.
- **Wagner's test:** (Solution of Iodine in Potassium Iodide)**:** To the extract/sample solution, add few drops of Wagner's reagent, reddish brown precipitate is produced.
- **Hager's Test:** (Saturated solution of Picric acid) To the extract/sample solution, add few drops of Hager's reagent, yellow precipitate is produced.

Tests for Phenolic Compounds

- **Ferric chloride test:** Extract solution **gives blue-green** color with few drops of $Fecl_3$.
- **Shinoda Test: (Magnesium Hydrochloride reduction test)** To the extract solution, add few fragments of magnesium ribbon and concentrated Hydrochloric acid drop wise, yellowish, yellow-orange occasionally orange color appears after few minutes.
- **Zinc- Hydrochloride reduction test:** To the extract solution, add a mixture of Zinc dust and concentrated Hydrochloric acid. It gives yellowish, yellow- orange occasionally orange color appears after few minutes

Tests for Flavonoids

❖ **Shinoda test: (Magnesium Hydrochloride reduction test)** To the extract solution add few fragments of magnesium ribbon and concentrated Hydrochloric acid drop wise, pink scarlet, crimson red or occasionally green to blue color appears after few minutes.

❖ **Zinc- Hydrochloride reduction test:** To the extract solution, add a mixture of Zinc dust and con. Hydrochloric acid. It gives red color after few minutes.

❖ **Alkaline reagent test:** To the extract solution, add few drops of Sodium hydroxide solution, formation of an intense yellow color that turns to colorless on addition of few drops of dilute acetic acid indicates the presence of flavonoids.

Tests for Tannins

❖ **Gelatin test:** Extract solution with 1% gelatin solution containing 10% sodium chloride gives white precipitate.

❖ **Ferric chloride test:** Extract solution gives **blue-green** color precipitate with $FeCl_3$.

❖ **Vanillin Hydrochloride test:** Extract solution when treated with few drops of Vanillin Hydrochloride reagent gives purple red color.

❖ **Alkaline reagent test:** Extract solution with sodium hydroxide solution gives **yellow** to **red** precipitate within short time.

Test for Steroidal Glycosides

❖ **Kedde's test:** Extract the bark powder with chloroform, evaporate to dryness, and add one drop of 90% of alcohol and 2 drops of 2% 3,5-dinitro benzoic acid (3,5, dinitrobenzene carboxylic acid - Kedde's reagent) in 90% alcohol. Make alkaline with 20% sodium hydroxide solution. A purple color is produced. The color reaction with dinitrobenzoic acid depends upon the presence of an α,β unsaturated – γ lactone in the aglycone.

C. HPTLC fingerprint profile:

The high performance thin layer chromatography (HPTLC) finger print profile of the aqueous alcoholic extracts of all the selected plants were carried out using pre-coated Silica Gel plates as the stationary phase. 100 mg of each dried extract was reconstituted separately in 10 ml of methanol, filtered and clear filterate was used for the HPTLC fingerprint analysis. It was spotted as a band in different concentrations using a Camag Linomat IV applicator.

The plates were eluted with two different solvent systems separately. Plates were then densitometerically scanned with CAMAG TLC scanner IV using the Wincats software at multi wavelengths either under UV or visible light using Deuterium lamp or Tungsten lamp. Photo documentation was carried out using a Linomat Reprostar unit under UV light at 254 and 366 nm.

RESULTS AND DISCUSSION

Results of preliminary phytochemical screening was carried out using total alcoholic soxhlation and cold maceration of bark of *P. aculeata* and bark of *R. aqutica*. Because it was reported that almost all the constituents are expected to come into the particular solvent. Qualitative chemical examinations of bark of *P. aculeata* extracts of total alcoholic and aqueous revealed the presence of alkaloids, steroids, carbohydrates, flavonoids, Tannins, bark of *P. aculeata* extracts of total alcoholic and aqueous revealed the presence of alkaloids, steroids, carbohydrates, flavonoids, Tannins and bark of *R. aqutica* extracts of total alcoholic and aqueous revealed the presence of alkaloids, steroids, carbohydrates, flavonoids, Tannins. The overall results obtained suggested that all the selected plants were found to contain flavonoids as tested in the preliminary phytochemical screening. Flavonoids have been linked with analgesic, anti-inflammatory, antipyretic activity and antioxidant activity. The alcoholic extracts and aqueous extracts of bark and bark of *P. aculeata* and bark of *R. aquatica* might provide some justification for the folklore use in the treatment of inflammation, fever, pain and scavengers of free radicals.

Both alcoholic and aqueous extracts of the bark of Parkinsonia aculeata and Rotula aquatica tested positive for alkaloids, flavonoids, steroids, carbohydrates, and tannins. Saponins were present only in alcoholic extracts. The alcoholic extracts exhibited a higher intensity of phytochemical reactions

DISCUSSION

Extraction was carried out using total alcoholic Soxhletion and cold maceration of bark of *P. aculeata* and *R. aqutica*. Because it was reported that almost all the constituents are expected to come into the particular solvent.

Qualitative chemical examinations of bark of *P. aculeata* extracts of total alcoholic and aqueous revealed the presence of alkaloids, steroids, carbohydrates, flavonoids, Tannins

Qualitative chemical examinations of bark of *R. aqutica* extracts of total alcoholic and aqueous revealed the presence of alkaloids, steroids, carbohydrates, flavonoids, Tannins.

The presence of flavonoids and tannins in both bark extracts correlates with their known pharmacological effects, including anti-inflammatory and antioxidant activity. Alcoholic extracts yielded a broader spectrum of constituents, suggesting their suitability for extracting active components. The study confirms that the bark of P. aculeata and R. aquatica is a rich source of secondary metabolites, validating their traditional use.

CONCLUSION

Extraction was carried out using total alcoholic Soxhletion and cold maceration of bark of *P. aculeata* and bark of *R. aquatica*. Because it was reported that almost all the constituents are

expected to come into the particular solvent.

Qualitative chemical examinations of bark of *P. aculeate and R. aqutica* extracts of total alcoholic and aqueous revealed the presence of alkaloids, steroids, carbohydrates, flavonoids, tannins. By preliminary phytochemical screening it was found that all the selected herbs contain flavonoids and/phenolics. Many phenolics, such as flavonoids, functions as analgesic antipyretic, anti-inflammatory and scavengers of free radicals by rapid donation of a hydrogen atom(s) to radicals[117,118] and many plants containing flavonoids /phenolics were used in the treatment of analgesic, anti pyretic, anti-inflammatory and scavengers of free radicals. Flavonoids have been linked with analgesic, anti-inflammatory and antipyretic activity[119]. This supported the folklore use of selected plants in the treatment of pain, fever, inflammation, scavengers of free radicals. Besides flavonoids, *P. aculeata* contain various flavonoids, flavonols and flavones according to the literature (orientin, iso-orientin, vitexin, iso-vitexin, lucenin-II, vicenin-II, diosmetin 6-C-B-glucoside, apigenin, luteolin, 7-glycosyl kaempferol, chrysoeriol, epi-orientin, Parkinsonin-A, Parkinsonin-B and Parkintin. The results of aqueous extract of *R. aquatica* revealed that the extract possesses appreciable anti-oxidant activity

The phytochemical investigation of the bark of Parkinsonia aculeata and Rotula aquatica confirms the presence of therapeutically significant compounds. These results can serve as a reference for further pharmacological studies and development of herbal formulations using these plant materials.

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