

Research Article

Extraction of *Paris polyphylla* Rhizome Using Different Solvents and Its Phytochemical Studies

Rajsekhar P B*, Arvind Bharani R S, Jini Angel K, Maya Ramachandran, Sharadha Priya Vardhini Rajsekhar

Rajkeerth Research Team, Rajkeerth Aromatics and Biotech Pvt. Ltd., Chennai, Tamil Nadu, India.

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ABSTRACT

Plants have been used from ancient days by humans for the treatment of various diseases. Secondary metabolites produced by plants are mainly responsible for these pharmacological activities. *Paris polyphylla* is a medicinal herb of Asia which is traditionally used for treating diseases like fever, headache, trauma, dysentery, etc. The rhizome of *Paris polyphylla* (Rhizoma Paridis) is rich in phytochemical compounds and it can be used as a potential source for the isolation of active drug compounds. In the present study, the rhizome of *Paris polyphylla* was extracted using water, methanol, ethanol, ethyl acetate and chloroform. The presence of phytoconstituents was screened by phytochemical analysis and it revealed the presence of alkaloids, flavonoids, saponins, carbohydrates, glycosides, cardiac glycosides, terpenoids, sterols, quinones, phenols and tannins. The result obtained in the present investigation proved that the Rhizoma Paridis was rich in medicinally important phytochemical compounds.

Keywords: *Paris polyphylla*; Rhizoma Paridis; Phytochemicals

INTRODUCTION

Medicinal plants have been used from ancient days for treating diverse varieties of ailments. Plants produce number of chemical compounds for biological functions and defence mechanisms. In modern medicine, these active phytochemical compounds were used rather than using the whole plant. The most important compounds are alkaloids, flavonoids, steroids, tannins, terpenoids and saponins¹. They are the promising sources for drug discovery, since their therapeutic effects were proved in clinical studies. The plant-derived drugs are safe, easily available and less expensive. The quality and quantity of the phytochemical compounds varies from plant to plant depending on various physical and chemical factors.

The genus *Paris* comprises of nearly 24 species of flowering plants which grows well in temperate forests. Among these, *Paris polyphylla*, belonging to Melanthiaceae family is a traditional medicinal herb. The term *Paris* is derived from 'pars' which means symmetry and *polyphylla* means many leaves. It originates from Asia and mainly found in China, Indian subcontinent and in Himalayan regions. It is a perennial herbaceous plant, which prefers moist humus-rich soil under partial or full shade. The plant grows from 10 to 100 cm tall with rhizome thickness of 1 to 2.5 cm. The rhizome grows very slowly and due to its high medicinal value and over exploitation, this plant is now in the edge of extinction. Recently, Danu *et al* reported vegetative propagation technique to conserve this endangered plant². In another study, Raomai *et al* performed thin cell layer culture technique and developed mini-rhizomes with increased

steroidal saponins³. The whole plant is primarily used to treat cancer and also it acts as a febrifuge. The dried rhizomes of *Paris polyphylla* called Rhizoma Paridis have been used as a traditional medicine for the treatment of microbial infection, snake bite, convulsions, fractures, throat swelling, diarrhoea and liver cancer⁴⁻⁶. Wang *et al*, reported the antiviral activity of *Paris polyphylla* against enterovirus 71 and coxsackievirus B3 and also its immune-modulating activities⁷. Nearly 98 compounds were identified from the rhizome, which includes more than 30 steroidal saponins⁸. Steroidal saponins are believed to be the main active ingredients in this species that showed antitumour, antibacterial, platelet agonist and immune-stimulating properties⁹⁻¹¹. The steroidal saponins of stems and leaves of *Paris* possess antimicrobial activity¹². In the current study, the presence of phytochemical compounds was determined by preliminary phytochemical analysis.

MATERIALS AND METHODS

Chemicals

All the chemicals and reagents used in the study were of analytical grade and high quality.

Collection of Plant Materials

The rhizomes of *Paris polyphylla* were collected from Arunachal Pradesh. The collected plants were identified and authenticated by Dr. K. R. Murugeswaran, Botanist, Regional Research Institute of Unani Medicine, Chennai, Tamil Nadu, India.

Preparation of Extracts

The rhizomes were dried and crushed into fine powder. Extraction of dried rhizome powder was carried out in five different solvents with high, medium and low polarity. The solvents used were water, methanol, ethanol, ethyl acetate and chloroform. The sample was soaked in the above respective solvents in the ratio 1:5 and kept in shaker for 48 hours at 28°C. After 48 hours, the samples were filtered using Whatman no. 1 filter paper. The solvents were then evaporated by using a hot water bath and the crude extracts obtained were stored in sterile glass bottles for further screening and analyses.

Phytochemical Screening

The aqueous, methanol, ethanol, ethyl acetate and chloroform extracts of *Paris polyphylla* were screened for the presence of phytochemical compounds using standard procedures¹³⁻¹⁶.

Test for alkaloids

The extracts were treated with 2N HCl and heated gently on hot water bath for 10 minutes. After boiling, the solution was filtered and each filtrate was taken in two test tubes to detect the presence of alkaloids.

Mayer's test: To each filtrate, few drops of Mayer's reagent were added and observed for the formation of turbidity or dull-white precipitate.

Dragendorff's test: To each filtrate, few drops of Dragendorff's reagent were added and observed for the formation of turbidity or reddish-brown precipitate.

Test for flavonoids

NaOH test: The extracts were treated with 10% NaOH followed by 5N HCl and observed for a colour change from yellow to colourless.

Test for saponins

Foam test: The extracts were shaken vigorously with few mL of distilled water for 15 minutes and observed for the formation of stable foam.

Lead acetate test: The extracts were treated with 1% lead acetate solution and observed for the formation of white precipitate.¹⁷

Test for carbohydrates

Benedict's test: The extracts were boiled with few mL of Benedict's reagent and observed for the formation of red precipitate which indicates the presence of reducing sugars.

Molisch's test: The extracts were treated with few drops of Molisch's reagent and shaken well. Then, concentrated H₂SO₄ was added along the sides of the test tube and observed for the appearance of red or dull-violet ring at the interface.

Test for proteins and amino acids

Ninhydrin's test: The extracts were boiled with few drops of 0.2% Ninhydrin solution and observed for the formation of blue or violet colour.

Biuret's test: The extracts were treated with equal volume of 1% NaOH followed by few drops of 4% CuSO₄ and observed for the formation of violet or purple colour.

Test for phenols and tannins

Lead acetate test: The extracts were treated with 10% lead acetate and observed for the formation of white precipitate.

Test for quinones

Alkaline reagent test: The extracts were treated with 1% NaOH and observed for the formation of bluish-green or red colouration.

Acid reagent test: The extracts were treated with few drops of concentrated HCl and observed for the formation of yellow precipitate.

Test for glycosides

Fehling's test: The extracts were treated with 1% HCl and then 10% NaOH was added to neutralise the mixture. Equal amount of Fehling's A and B solutions were added in drops and observed for the deposition of brick-red precipitate.

Test for anthraquinone glycosides

Borntrager's test: The extracts were boiled with 10% HCl and then filtered. To the filtrate, equal amount of chloroform was added on cooling and shaken. The chloroform layer was separated and 10% NH₃ was added to it and observed for the appearance of pink to cherry-red colour in the ammonical layer which indicates the presence of anthraquinone glycosides.

Test for cardiac glycosides

Keller-Kelliani's test: The extracts were treated with glacial acetic acid and few drops of 10% FeCl₃. Then, concentrated H₂SO₄ was added along the sides of the test tube and observed for the formation of brown ring at the interface which indicates the presence of deoxy sugar characteristic of cardenolides. A violet ring may appear below the ring and in the acetic acid layer a greenish ring may form.

Test for terpenoids

Salkowski's test: The extracts were treated with chloroform followed by few drops of concentrated H₂SO₄ and observed for the formation of reddish-brown colour at the interface.

Test for sterols

Liebermann-Burchard's test: The extracts were treated with chloroform followed by acetic anhydride and heated gently. The test samples were cooled and concentrated H₂SO₄ was added along the sides of the test tube and observed for the formation of reddish-brown precipitate.

Test for anthocyanin

NaOH test: The extracts were treated with 2M NaOH and observed for the formation of bluish-green colour.

RESULTS AND DISCUSSION

The phytochemical constituents of various extracts of the rhizomes of *Paris polyphylla* were summarised in Table 1. Flavonoids, carbohydrates, cardiac glycosides, terpenoids and sterols were present in all the extracts. Alkaloids were present only in ethyl acetate and chloroform extracts. Saponins, phenols and tannins were present in all the extracts except ethyl acetate extract. Quinones were present in methanol, ethanol and chloroform extracts. Glycosides were present only in chloroform extract. These phytochemicals exhibit various medicinal and pharmacological activities.

Flavonoids are polyphenolic compounds which possess antihepatotoxic, anti-inflammatory, antitumour, anti-osteoporotic and anti-ulcer activity¹⁸. Alkaloids are important secondary metabolites produced by plants. The

Table 1: Phytochemical screening of *Paris polyphylla* rhizome

S.No	Phytochemical Test	Extracts				
		Water	Methanol	Ethanol	Ethyl Acetate	Chloroform
1	Alkaloids test					
a	Mayer's test	-	-	-	+	+
b	Dragendorff's test	-	-	-	+	+
2	Flavonoids test					
a	NaOH test	+	+	+	+	+
3	Saponins test					
a	Foam test	+	+	+	-	+
b	Lead acetate test	+	+	+	-	+
4	Carbohydrates test					
a	Benedict's test	-	-	-	-	+
b	Molisch's test	+	+	+	+	+
5	Proteins and amino acids test					
a	Biuret's test	-	-	-	-	-
b	Ninhydrin's test	-	-	-	-	-
6	Phenols and tannins test					
a	Lead acetate test	+	+	+	-	+
7	Quinones test					
a	Alkaline reagent test	-	+	-	-	+
b	Acid reagent test	-	+	+	-	+
8	Glycosides test					
a	Fehling's test	-	-	-	-	+
9	Anthraquinone glycosides test					
a	Borntrager's test	-	-	-	-	-
10	Cardiac glycosides test					
a	Keller Kelliani's test	+	+	+	+	+
11	Terpenoids test					
a	Salkowski's test	+	+	+	+	+
12	Sterols test					
a	Leibermann-Burchard's test	+	+	+	+	+
13	Anthocyanin test					
a	NaOH test	-	-	-	-	-

+=Present; -=Absent

pharmacological activities of alkaloids include antimalarial, antitumour, antimicrobial, anti-asthma and antihyperglycemic. Alkaloids are mainly used for drug manufacturing. Saponins are natural plant glycosides with strong foam-forming properties. They possess medicinal activities like antitumour, antiviral, antifungal, anti-allergic, immune-modulating, hypoglycemic and molluscicidal activities¹⁹. Yan *et al* reported the antitumour activity of the steroidal saponins of the rhizome of *Paris polyphylla* on LA795 lung adenocarcinoma in T739 inbred mice²⁰.

Glycosides are most important plant compounds which is used in the treatment of congestive heart failure and to reduce blood pressure. Cardiac glycosides are group of compounds which include digoxin, digitoxin, digitalis and ouabain. They increase the force of contraction of the heart and are used in the treatment of congestive heart failure and cardiac arrhythmia²¹. Terpenoids are the largest group of plant compounds which act as therapeutic and preventive agents. They exhibit

cytotoxicity against a number of tumour cell lines²². Phytosterols are similar to cholesterol and are mainly used to lower the plasma cholesterol level. Other activities include antitumour, antioxidant, anti-inflammatory and antimicrobial effects. Phenols and tannins act as primary antioxidants. Phenolic compounds possess anti-aging, antitumour, anti-inflammatory and cardiovascular protection properties²³. Tannins act as potential antimicrobial and antitumour agents. Quinones are oxidised derivatives of aromatic compounds which exhibit antimicrobial, antiparasitic and antitumour activities.

CONCLUSION

The rhizome of *Paris polyphylla* is a valuable source of phytochemicals that have been used traditionally for various ailments. The phytochemical analyses of aqueous, methanol, ethanol, ethyl acetate and chloroform extracts revealed the presence of alkaloids, flavonoids, saponins, carbohydrates, glycosides, cardiac glycosides, terpenoids,

sterols, quinones, phenols and tannins in the rhizome. Many *invitro* and *invivo* studies proved the antitumour activity of the rhizome. Further research work should be carried out to isolate, purify and characterise the phytochemical compounds responsible for the pharmacological activities. The phytochemical compounds of *Paris polyphylla* and their derivatives can be used as a potential source for the discovery of new drugs.

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