International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(5): 407-410
www.biochemjournal.com
Received: 01-02-2024
Accepted: 07-04-2024

Tuybia Bilal
Faculty of Forestry, SK University of Agricultural Sciences & Technology of Kashmir, Srinagar, Jammu & Kashmir, India

PA Sofi
Faculty of Forestry, SK University of Agricultural Sciences & Technology of Kashmir, Srinagar, Jammu & Kashmir, India

SA Gangoo
Faculty of Forestry, SK University of Agricultural Sciences & Technology of Kashmir, Srinagar, Jammu & Kashmir, India

Ishtiyaq Ahad
Division of Entomology, FoA, SK University of Agricultural Sciences & Technology of Kashmir, Jammu & Kashmir, India

SA Mir
Division of Agricultural Statistics, SK University of Agricultural Sciences & Technology of Kashmir, Jammu & Kashmir, India

SN Zaffar
Faculty of Forestry, SK University of Agricultural Sciences & Technology of Kashmir, Srinagar, Jammu & Kashmir, India

M Iqbal Jeelani
Faculty of Forestry, SK University of Agricultural Sciences & Technology of Kashmir, Srinagar, Jammu & Kashmir, India

Tawseef Ahmad Mir
Department of Botany, BFIT Group of Institutions, Suddhowala, Dehradun, Uttarakhand, India

Benatul Behar
Faculty of Forestry, SK University of Agricultural Sciences & Technology of Kashmir, Srinagar, Jammu & Kashmir, India

Irtizah Mushtaq
Faculty of Forestry, SK University of Agricultural Sciences & Technology of Kashmir, Srinagar, Jammu & Kashmir, India

Humera Gulzar
Faculty of Forestry, SK University of Agricultural Sciences & Technology of Kashmir, Srinagar, Jammu & Kashmir, India

Corresponding Author:
Tuybia Bilal
Faculty of Forestry, SK University of Agricultural Sciences & Technology of Kashmir, Srinagar, Jammu & Kashmir, India

Phytochemical analysis and insecticidal potential of pyrethrum (*Chrysanthemum cinerariaefolium*) Extracts: A sustainable approach to pest management

Tuybia Bilal, PA Sofi, SA Gangoo, Ishtiyaq Ahad, SA Mir, SN Zaffar, M Iqbal Jeelani, Tawseef Ahmad Mir, Benatul Behar, Irtizah Mushtaq and Humera Gulzar

DOI: https://doi.org/10.33545/26174693.2024.v8.i5e.1118

Abstract
The study explores the phytochemical composition and insecticidal potential of different parts of the pyrethrum plant (*Chrysanthemum cinerariaefolium*). Pyrethrum, known for its natural insecticidal properties derived from pyrethrins, is considered a safer alternative to synthetic insecticides. Flowers, leaves, and roots were collected from experimental fields of Forest Products and Utilization, Faculty of Forestry SKUAST-K, and subjected to methanol extraction to obtain crude plant extracts. These extracts were then screened for alkaloids, flavonoids, saponins, phenols, and tannins. Results showed varying secondary metabolite concentrations among plant parts. Results indicated varying concentrations of secondary metabolites among plant parts, with flowers exhibiting high alkaloids, flavonoids, and tannins, leaves showing moderate alkaloids and flavonoids but lacking tannins, and roots possessing significant alkaloids and tannins but fewer flavonoids, saponins, and phenols. The presence of these compounds suggests insecticidal potential, with alkaloids in flowers and roots combating aphids, flavonoids enhancing insecticidal effects, and tannins acting as feeding deterrents. Phytochemical analysis illuminates pyrethrum's insecticidal efficacy, contributing to sustainable pest management knowledge and promoting botanical resources for eco-friendly pest control.

Keywords: Pyrethrum, phytochemicals, insecticide, secondary metabolites

Introduction

Pyrethrum (*Chrysanthemum cinerariaefolium*), a member of the Chrysanthemum genus within the *Asteraceae* family, is a perennial plant found in temperate regions. It bears petite, daisy-like white flowers, from which the natural insecticides known as pyrethrins are extracted. Historically, pyrethrum was cultivated in numerous African nations, employing manual labor for planting, harvesting, and drying the crop (Davis, 2001; Gulzar *et al.*, 2024; Wandahwa *et al.*, 1996) [1, 5, 6]. The initial endeavor to introduce pyrethrum species in India dates back to 1931, undertaken by the Forest Department of Jammu and Kashmir (Panda, 2005) [2]. Besides their aesthetic appeal, pyrethrum flowers have been extensively utilized as a source material for insecticide production. Due to their non-toxic nature to humans, pyrethrums are particularly suited for household insect control. Nonetheless, their application in formulating horticultural dusts, sprays, and livestock repellents is equally widespread (Gnadinger, 2001) [4]. One of the most notable attributes of pyrethrum, setting it apart from other insecticides, is its remarkably low toxicity to mammals. This quality renders pyrethrum the sole insecticide deemed safe for human use, while synthetic alternatives pose significant health risks to humans, domestic animals, and wildlife (Panda, 2005) [2]. Approximately two centuries ago, an individual residing in central Asia stumbled upon the discovery that dried, crushed flowers from specific chrysanthemum varieties were toxic to insects. During the Napoleonic Wars (1804-1815), this "insect powder" was utilized by French soldiers to combat flea and body lice infestations. Since then, pyrethrum has been employed in various forms for efficient insect control with minimal toxicity.

The growing recognition of the hazards associated with synthetic insecticides, coupled with the rapid development of resistance among insect populations, has bolstered the preference for pyrethrins, which do not exhibit these drawbacks. Consequently, the demand for pyrethrum flowers is on the rise in the global market, presenting India with promising prospects to meet this escalating demand, particularly as pyrethrum cultivation can thrive in regions like the Kashmir valley. Pyrethrum is employed as an insecticide in multiple forms including powder, spray, cream, aerosol, coils, and ointment (Bilal *et al.*, 2022; Panda, 2005) [3, 2].

Materials and Methods

Plant collection and extraction technique

Pyrethrum extracts from flowers, leaves, and roots were gathered from the experimental field of Forest Products and Utilization, Faculty of Forestry SKUAST-K. These plant parts were oven-dried until a constant weight was achieved. Subsequently, pyrethrum plant extracts were meticulously prepared by measuring out 100 grams each of dried flowers, leaves, and roots separately.

Method of plant extraction

Solvent extraction

Using the Soxhlet extraction technique, a crude plant extract was prepared. Twenty grams of powdered plant material were placed in a thimble, and 250 ml of methanol solvent was added for extraction. Each extraction was conducted independently. The extraction process was maintained for 24 hours, during which the solvent was continuously circulated through the plant material in the thimble via a siphon tube in the extractor until the solvent turned colorless. Subsequently, the extract was collected in a beaker. The solvent was then evaporated from the extract at a temperature range of 30 °C to 40 °C on a hot plate until all the solvent was removed. The resulting dried extract was stored in a refrigerator at 4 °C for future use in phytochemical analysis.

Methods of phytochemical analysis

In Table (1) standard technique is given and utilized along with procedure of phytochemical analysis shown in Figure (1), the Flower, Root and Leaf extracts were tested for presence of bioactive compounds:

1. **Test for Alkaloid:** For each sample, 3 ml of flower extract was mixed with 1 ml of dilute hydrochloric acid, followed by filtration. Subsequently, from the Mayer's reagent two drops were added to the filtrate. The formation of a yellow-colored precipitate revealed the presence of alkaloids. This procedure was replicated for the root and leaf extracts as well.
2. **Test for flavonoids:** For each sample, 3 ml of flower extract was combined with two drops of sodium hydroxide solution. The development of an intense yellow color, which subsequently turned colorless upon addition of dilute acid, indicated the presence of flavonoids. This process was replicated for the leaf and root extracts as well.
3. **Test for saponins:** For each sample, 3 ml of flower extract was mixed with 2 ml of water and shaken vigorously. The formation of a foamy lather indicated the presence of saponins. This process was repeated for the leaf and root extracts.
4. **Test for phenols:** For each sample, 3 ml of flower extract was mixed with 1% ferric chloride solution and distilled water. The formation of a bluish-black color indicated the presence of phenols. This procedure was repeated for the root extracts and then to leaf extracts as well.
5. **Test for tannins:** For each sample, 3 ml of flower extract was combined with 1% gelatin solution containing NaCl. The formation of a white precipitate hinted about the presence of tannins. This process was repeated for the root extracts followed to leaf samples also.

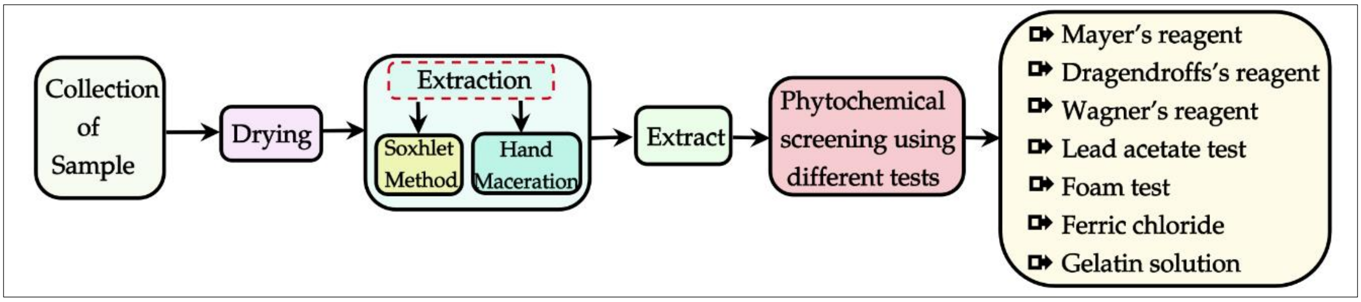

Fig 1: Procedure of phytochemical analysis

Table 1: Phytochemical analysis procedure

Phytochemical	Test procedure	Observation (Showing results)
Alkaloid	Filtrate + Mayer's reagent	Absence of Yellowish or white precipitate indicated the absence of alkaloid in the extract.
	Filtrate+ Dragendroff's reagent	Absence of Orange or orange-red precipitate indicated the absence of alkaloid in the extract.
	Filtrate + Wagner's reagent	Absence of cream coloured precipitate indicated the absence of alkaloid in the extract.
Flavonoid	Filtrate + Alkaline reagent	Development of intense yellow & then colour less indicated the presence of flavonoid in extract.
	Filtrate + Lead acetate solution	Formation of yellow colour precipitate indicated the presence of flavonoid in extract.
Saponins	Foam test	Development of stable foam confirms the presence of saponins in the extract.
Phenols	Extract + distilled water + ferric chloride	Formation of bluish black colour indicated the presence of phenols.
Tannins	Extract + gelatin solution + sodium chloride	Formation of white precipitate revealed the presence of tannins.

Table 2: Secondary metabolites in flower, leaf and root extracts of pyrethrum (*Chrysanthemum cinerariaefolium*)

Secondary Metabolite	Presence or absence in plant parts		
	Flower	Leaves	Root
Alkaloids	+++	++	++
Flavonoids	+++	++	+
Saponins	++	+	+
Phenols	+	+	+
Tannins	+++	___	++

+++: Present in high amount +: Present in little amount
++: Present in average amount —: Absent

Quantitative analysis of phytochemicals

1. **Alkaloids:** Begin by placing 5 grams of the plant sample into a beaker, followed by the addition of a solution comprising 200 ml of ethanol and 10% acetic acid. After thorough stirring, allow the mixture to sit undisturbed for a duration of 4 hours before filtering it. Next, heat the filtrate in a water bath until its volume reduces to 1/4 of the original, effectively concentrating the extract. Gradually incorporate concentrated ammonium hydroxide until precipitation ceases, then dissolve the resultant solution to collect the precipitate. Rinse the precipitate with diluted ammonium hydroxide, filter, and air-dry it. Finally, determine the weight of the dried alkaloid, which is expected to undergo sublimation.
2. **Flavonoids:** The method entails combining 10 grams of the plant specimen with 100 ml of an 80% methanol solution in water at ambient temperature. After thorough mixing, the entire mixture is filtered through filter paper to eliminate any solid residues. The resulting filtrate is subsequently moved to a water bath for solvent evaporation until complete dryness is attained. The sample is then weighed iteratively until a consistent weight is achieved.
3. **Tannins:** The quantity of tannins is determined using the spectrophotometer method. Initially, 0.5 grams of the plant sample is weighed into a 50 ml plastic bottle, followed by the addition of 50 ml of distilled water. The mixture is then agitated for one hour before being filtered into a 50 ml volumetric flask and brought up to the mark. Next, 5 ml of the filtered sample is pipetted into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 M HCl and 0.008 M $K_4Fe(CN)_6.3H_2O$. The absorbance of the solution is measured using a spectrophotometer at a wavelength of 395 nm within a timeframe of 10 minutes.
4. **Phenols:** The quantity of phenols is determined using the spectrophotometer method. Initially, the plant sample is boiled for 15 minutes with 50 ml of $(CH_3CH_2)_2O$. Following this, 10 ml of distilled water is added to the boiled sample in a 50 ml flask. Subsequently, in the mixture, 2 ml of NH_4OH solution and 5 ml of concentrated $CH_3(CH_2)_3CH_2OH$ are added. The sample is then made up to the mark and left for 30 minutes to allow for color indication. Finally, the absorbance of the sample is measured using a spectrophotometer at a wavelength of 505 nm.

Results and Discussion

The analysis of various components in different segments of the pyrethrum plant (flowers, leaves, and roots) unveiled the existence of phenols, saponins, alkaloids, flavonoids, and tannins, as outlined in Table (1). The study findings demonstrated that various pyrethrum parts contain distinct quantities of secondary metabolites, characterized by differences in color intensity. Specifically, pyrethrum flower extracts exhibited the highest levels of alkaloids, flavonoids, and tannins, with an average amount of saponins and a lesser quantity of phenols. In contrast, pyrethrum leaf extracts displayed average levels of alkaloids and flavonoids, a minimal amount of saponins and phenols, and the absence of tannins. Pyrethrum root extracts showcased higher levels of alkaloids and tannins, alongside minimal quantities of flavonoids, saponins, and phenols.

Extraction and subsequent phytochemical analysis are pivotal in deciphering the medicinal properties of plants and pinpointing the active compounds behind their biological effects. An array of extraction techniques, including maceration, percolation, infusion, digestion, decoction, and hot continuous extraction (solvent extraction), are utilized to extract phytochemicals. Various solvents such as water, ethanol, methanol, acetone, ether, benzene, chloroform, etc., are employed in these extraction processes.

In our study, we employed the soxhlet method to extract phytochemicals from pyrethrum's flowers, leaves, and roots. The methanolic extract obtained underwent various tests, including Mayer’s, Wagner’s, Dragendroff’s, lead acetate, alkaline, and foam tests, to identify specific phytochemicals. Results indicated the absence of certain alkaloids and the presence of flavonoids and saponins. This thorough approach not only identifies pyrethrum's phytoconstituents but also sheds light on their potential medicinal properties. The choice of extraction method and solvent is crucial for obtaining a representative extract, enhancing our understanding of the plant's therapeutic potential.

In the present study, extracts from pyrethrum's flowers, leaves, and roots as given in Table (2) revealed the presence of various bioactive compounds: alkaloids, flavonoids, phenols, tannins, and saponins. Concentrations varied among plant parts, with each demonstrating a unique profile. Flower extracts exhibited the highest concentrations of alkaloids, flavonoids, and tannins, with moderate levels of saponins and minimal phenols. Leaf extracts showed average levels of alkaloids and flavonoids, low saponins and phenols, and an absence of tannins. Root extracts had average alkaloids and tannins, lower saponins, flavonoids, and phenols. This variance underscores pyrethrum's distinct chemical composition, emphasizing the importance of considering specific plant parts for phytochemical extraction and analysis. These findings align with the research conducted by Haouas *et al.*, (2008) [7], which documented the utilization of pyrethrum plant extracts and its phytoconstituents for anti-feedant, repellent, and insecticidal purposes.

The presence of alkaloids in pyrethrum flower and root extracts is highlighted as a significant factor contributing to the observed anti-aphid activity. Alkaloids are crucial natural substances known for their pivotal role in plant defenses, forming part of the plant's mechanisms against phytophagous insects. This defensive role aligns with their reported significance in plant defense, alongside other compounds such as terpenoids, phenols, flavonoids, tannins, and steroids. The anti-aphid activity observed in pyrethrum leaf extracts, despite the relatively modest amounts of phytochemicals present, suggests that these compounds, including alkaloids, may exhibit insecticidal properties even at lower concentrations. This observation is consistent with the findings reported by Rattan (2010) [10], who noted that insecticidal substances can be potent even when present in low concentrations due to their varied mechanisms of action. Several of these compounds target acetylcholine receptors within the nervous system or the membrane sodium channels of nerves.

The presence of flavonoids in pyrethrum extracts is suggested to play an important role in the observed anti-aphid activity. Flavonoids, renowned for their cytotoxic properties and their capacity to interact with various enzymes through complexation, are considered to contribute to the deterrent effect against aphids. This is consistent with the research conducted by War *et al*., (2012) [8], who highlighted that both flavonoids and isoflavonoids possess the ability to safeguard plants from pests by affecting the behavior, growth, and development of insects. Additionally, Shripad *et al*., (2003) [11] documented the insecticidal and antimicrobial properties of flavonoids extracted from *Ricinus communis* against *C. chinensis*. They noted that the anti-aphid activity observed in pyrethrum extracts could stem from the synergistic interactions of different phenolic compounds, including flavonoids. This highlights the complex interplay of phytochemicals in plant defense mechanisms against pests.

The presence of average levels of tannins in both pyrethrum flower and root extracts suggests a significant role in the observed anti-aphid activity. Tannins are known to strongly affect phytophagous insects, aligning with their potential in aphid control. These findings resonate with the observations made by Sharma *et al*., (2009) [12], who highlighted the effects of tannins on insect growth by their ability to bind to proteins, thereby reducing nutrient absorption and inducing midgut lesions. Tannins, characterized by their astringent and bitter properties, serve as feeding deterrents to numerous insect pests, consistent with Roitto *et al.*, (2009) [13], who emphasized their role in decreasing plant nutritive value to herbivores.

Conclusion

The study comprehensively analyzed the phytochemical composition of pyrethrum (*Chrysanthemum cinerariaefolium*) extracts from flowers, leaves, and roots, revealing varying concentrations of alkaloids, flavonoids, saponins, phenols, and tannins. These compounds indicate the insecticidal potential of pyrethrum, with alkaloids combating aphids, flavonoids enhancing insecticidal effects, and tannins acting as feeding deterrents. Pyrethrum emerges as a sustainable alternative to synthetic insecticides due to its low mammalian toxicity and eco-friendly nature. By harnessing the insecticidal properties of pyrethrum, sustainable pest management strategies can be developed, minimizing environmental impact and promoting biodiversity conservation.

References

1. Davis JM. Study of the flower feasibility of Pyrethrum (*Chrysanthemum cinerariaefolium* Vis.) as a new crop for North Carolina. NCSU Horticulture Science. 2001.
2. Panda H. Herbs cultivation and medicinal uses. National Institute of Industrial Research, Delhi: 2005:1-9.
3. Bilal T, Mushtaq T, Ahmad PI, Gangoo SA, Behar B, Ayoob B, *et al*. Botanicals their use as antimicrobial, antifungal and anti-insecticides. The Pharma Innovation. SP-11 (5). 2022:1521-8.
4. Gnadinger, C.B. Pyrethrum Flowers. Vedams eBooks (P) Ltd New Delhi 110 034, India. 2001;xvi: 380
5. Gulzar H, Sofi PA, Pala NA, Peerzada IA, Shah IA, Nazir S, Kaif M. Effect of spacing and shading intensity on growth performance of Podophyllum hexandrum Royle: A critically endangered plant species of Western Himalaya. Ecological Frontiers. 2024 Feb;44(1):105-11.
6. Wandahwa P, Van Ranst E, Van Damme P. Pyrethrum (*Chrysanthemum cinerariaefolium* Vis.) cultivation in West Kenya: origin, ecological conditions and management. Industrial Crops and Products. 1996 Dec 1;5(4):307-22.
7. Haouas D, Halima-Kamel MB, Hamouda MH. Insecticidal activity of flower and leaf extracts from Chrysanthemum species against *Tribolium confusum*. Tunisian Journal of Plant Protection. 2008;3(2):87-93.
8. War AR, Paulraj MG, Ahmad T, Buhroo AA, Hussain B, Ignacimuthu S, *et al*. Mechanisms of plant defense against insect herbivores. Plant signaling & behavior. 2012 Oct 1;7(10):1306-20.
9. Cox C. Insecticide fact sheet: Pyrethrins/Pyrethrum. Journal of Pesticide Reform, 2002:22(1):14-20.
10. Rattan RS. Mechanism of action of insecticidal secondary metabolites of plant origin. Crop protection. 2010 Sep 1;29(9):913-20.
11. Upasani SM, Kotkar HM, Mendki PS, Maheshwari VL. Partial characterization and insecticidal properties of *Ricinus communis* L foliage flavonoids. Pest Management Science: Formerly Pesticide Science. 2003 Dec;59(12):1349-54.
12. Sharma HC, Sujana G, Manohar Rao D. Morphological and chemical components of resistance to pod borer, *Helicoverpa armigera* in wild relatives of pigeonpea. Arthropod-Plant Interactions. 2009 Sep;3:151-61.
13. Roitto M, Rautio P, Markkola A, Julkunen-Tiitto R, Varama M, Saravesi K, *et al*. Induced accumulation of phenolics and sawfly performance in Scots pine in response to previous defoliation. Tree Physiology. 2009 Feb 1;29(2):207-16.