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PHYTOCHEMICAL INVESTIGATION OF HIGH POTENTIAL MEDICINAL PLANTS FOR FRIENDLY INSECT PEST MANAGEMENT AT HERAMOSH, DISTRICT GILGIT

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ABSTRACT

The exploration of medicinal plants in Gilgit-Baltistan for pest and insect control emerges as a promising strategy for natural pest management. The research was focused on three specific plant species-*Swertia cordata, Gentiana tianschanica, and Pleurospermum candollei*-highlighting their efficacy in pest control and the conservation of biodiversity in the region. These plants boast a rich array of secondary metabolites, including alkaloids, flavonoids, steroids, triterpenoids, tannins, saponins, carbohydrates, and proteins, contributing to their pest-controlling attributes. The phytochemical investigation involved the collection of fresh plants from diverse locations, followed by air-drying and extracting plant samples for analysis. Qualitative tests on the crude extracts aimed to discern the presence of alkaloids, flavonoids, phenols, steroids, and carbohydrates. The results of the preliminary phytochemical analysis affirmed the presence of various secondary metabolites in all three plant species. Additionally, the research delved into the antioxidant potential of these plants, employing DPPH free radical scavenging assays. The extracts from different solvents of each plant species exhibited noteworthy antioxidant activity, suggesting their role as natural antioxidants. The research explores the multifaceted bioactive properties of the identified secondary metabolites, highlighting their significance in fortifying plant defense mechanisms. Alkaloids, flavonoids, steroids, triterpenoids, tannins, saponins, amino acids, and carbohydrates collectively contribute to the overall health benefits of these plants, potentially aiding in insect control and disease prevention. The study concluded by underscoring the imperative for further research to comprehensively grasp the specific uses, concentrations, and properties of these compounds for effective pest and disease control. In essence, the findings underscore the importance of harnessing medicinal plants for pest management, considering their reduced environmental impact in contrast to synthetic pesticides. The research provides valuable insights into the potential of natural compounds derived from medicinal plants, offering a sustainable approach to agricultural practices and the preservation of ecosystems.

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INTRODUCTION

The use of plants in Gilgit-Baltistan for pest and insect control highlights their potential as natural insecticides and pesticides (Hussain et al., 2023; Khan et al., 2015). *Swertia cordata*, *Gentiana tianschanica* (Jiang et al., 2021; Khaliq, 2020), and *Pleurospermum candollei* (Ahmed et al., 2022) exhibited robust capabilities in effectively managing pests. The focus on utilizing specific medicinal plants (Ali et al., 2021) for plant protection, particularly those with the highest frequency, highlights their significance in pest management (Mustafa et al., 2017). Additionally, these plants contain secondary metabolic compounds that contribute to their pest control capabilities and safeguard other medicinal plants (Khan et al., 2014). By employing these plants, not only can pests be effectively controlled, but also the biodiversity and ecosystem balance in Gilgit-Baltistan can be better preserved (Khan et al., 2014).

Traditional remedies are an essential component of modern pharmaceutical science, as they contribute significantly to the development of synthetic medicines. The presence of bioactive compounds in natural products plays a crucial role in the discovery and creation of pharmaceutical drugs (Li and Weng, 2017). Pakistan's diverse climate and geography have fostered a rich ecosystem suitable for cultivating medicinal plants. This ecological variety has provided favorable conditions for the growth and cultivation of a wide range of medicinal plants in the country (Khan et al., 2013). The crude extracts of medicinal herbs have a modest yet significant impact on the process of disease healing, which can be attributed to the synergistic actions of their components. This suggests that the combined effect of multiple ingredients in the crude extracts is more effective than isolated individual ingredients in treating diseases (Ojha et al., 2020).

Phyto-based drugs hold great significance in medical science due to their reduced toxicity compared to synthetic chemical-based drugs, which not only serve their intended biological functions but also often come with undesired side effects in the human body. Therefore, the use of phyto-based drugs is highly valued for their ability to minimize adverse effects while providing therapeutic benefits (Campos et al., 2019). The phytochemical constituents found in medicinal plants contribute to our defense mechanisms and offer protection against a wide range of diseases. These natural compounds play a crucial role in strengthening our immune system and providing a defense against various ailments (Nxumalo et al., 2021). Phytochemicals in plants are synthesized through primary and secondary metabolic pathways (Mendoza and Silva, 2018). Primary metabolites are basic molecules that participate in cellular processes and serve as a defense mechanism against predators, insects, and microorganisms in plants (Agostini-Costa et al., 2012).

The utilization of plants as medicine is gaining popularity primarily due to their lower incidence of side effects, cost-effectiveness, and higher efficacy compared to synthetic drugs (Sharma et al., 2008). This highlights the significance of active phytochemicals in addressing various health issues within the human body (Kumar and Khanum, 2012). Bioactive compounds such as alkaloids, flavonoids, tannins, and glycosides can be found in medicinal plants (Nandagoapalan et al., 2016). Each of these constituents possesses unique biological functions (Ojha et al., 2020). Alkaloids are nitrogen-containing compounds with heterocyclic structures that exhibit antimicrobial, analgesic, anthelmintic, and antidiarrheal properties (Jamil et al., 2018).

The presence of alkaloids, flavonoids, steroids, triterpenoids, tannins, saponins, carbohydrates, and proteins in plant extracts necessarily indicates that these compounds are used for the control of insect pests and diseases (Verma et al., 2019). While some of these compounds may have bioactive properties that could potentially be used for pest and disease control, it would depend on the specific plant and the concentration of these compounds in the extract (Bhalodia and Shukla, 2011). Some alkaloids have insecticidal or pesticidal properties. For example, nicotine, derived from tobacco, is an alkaloid used as a natural insecticide (Ahmed et al., 2021). Flavonoids have antioxidant properties and may play a role in a plant's defense against pests and diseases, but they are typically used directly for pest control (War et al., 2012). These compounds have various biological activities and can be used in the synthesis of certain pesticides or as precursors for the development of biopesticides (Jeran et al., 2021). Tannins can deter herbivores, but they are not commonly used as insecticides or pesticides (Daniel et al., 1999). Some saponins can have pesticidal properties and are used in biopesticides. These compounds are essential components of plant tissues but are used directly for pest or disease control. The specific glycosides matter, as some may have pesticidal properties (Ahmed et al., 2017). The effectiveness of these compounds for pest and disease control varies greatly, and their application often requires a deeper understanding of their properties, concentration, and specific uses. It is important to note that simply identifying the presence of these compounds in plant extracts does not confirm their efficacy as pest or disease control agents. The objective of the current study was to examine the phytochemical composition of three specific medicinal plants, namely *Swertia cordata*, *Gentiana tianschanica*, and *Pleurospermum candollei*.

MATERIALS AND METHODS

Fresh plants of *Swertia cordata*, *Gentiana tianschanica*, and *Pleurospermum candollei* (DC.) were collected from various locations in the Shuta and Hanuchul valleys (Figure 1 and 2). The plants were then dried in the shade until all the moisture had evaporated, and the plant material was sufficiently dry for grinding. The dried plant material was mixed and shaken thoroughly. The process involved collecting fresh plants of the three medicinal species from different areas in the Shuta and Hanuchul valleys. The plants were then air-dried in a shaded area to remove all moisture and prevent decomposition. Once the plant material was completely dry, it was ground into a fine powder. To ensure that the resulting powder was a uniform mixture, the dried plant material was shaken thoroughly to break up any clumps. This process was likely done to prepare the plant material for further analysis or use in medicinal preparations.

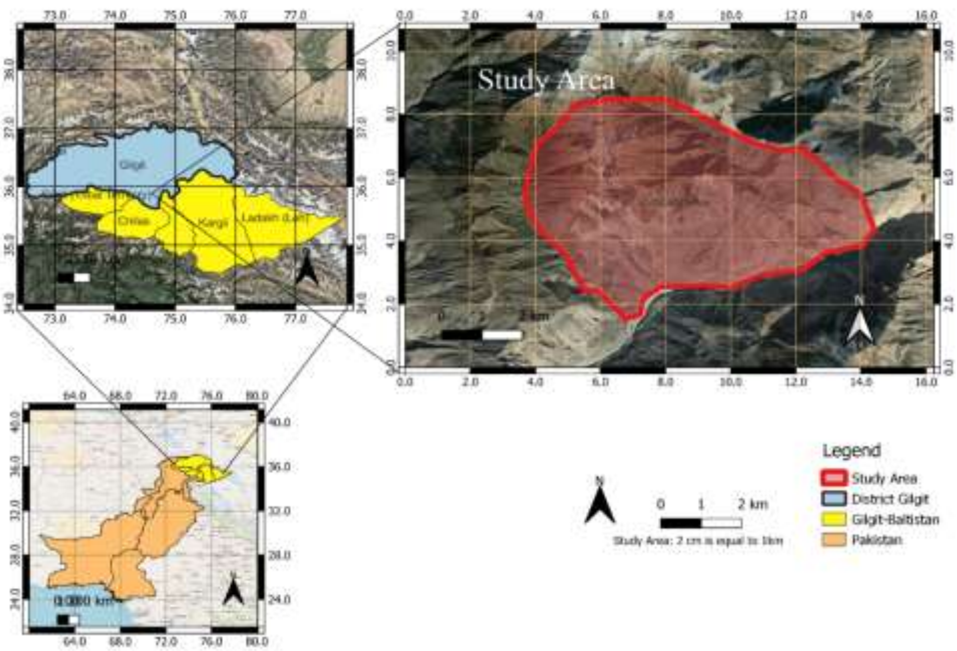

Figure 1: Map of the study area.

Figure 2: Selected medicinal plants in their wild habitats (A = *Gentiana tianschanica*, B = *Pleurospermum candollei*, C= *Swertia cordata* (Pictures were taken by Hasnain Abbas during plant specimen collection).

Extraction of plant sample

For phytochemical analysis, the aerial parts of *S. cordata* (550 g), *G. tianschanica* (6300 g), and *P. candollei* (DC.) (520 g) were extracted. The plant samples were processed by filtering and concentrating in an open environment. Subsequently, solvent-solvent extraction was performed on the methanolic extract using organic solvents and water. The concentrated methanolic extract was then fractionated with n-hexane and water, resulting in the separation of the n-hexane fraction and aqueous fraction (performed twice). To further separate the components, the aqueous layer underwent additional treatment with dichloromethane and n-hexane (two times).

Qualitative tests were conducted using different reagents on the crude extracts. The crude methanolic extract was utilized for determining the presence of alkaloids, flavonoids, phenols, steroids, and conducting the carbohydrate test using various procedures. To confirm the presence or absence of flavonoids, the alcoholic extract was treated with a few drops of sulfuric acid, and a specific color change would indicate the presence of flavonoids. In addition, to investigate phenolic compounds, a few drops of ferric chloride solution were added to 5 mg of the methanolic extract, resulting in a color change. The subsequent procedure, outlined below, was then followed.

Grinding

The dried aerial parts were ground into a fine powder using a suitable grinding apparatus to increase the surface area for efficient extraction.

Solvent Selection

A suitable solvent or solvent mixture was chosen based on the types of phytochemicals expected to be present in the plants. Commonly used solvents included methanol, ethanol, chloroform, ethyl acetate, and water.

Extraction

The powdered aerial parts were mixed with the selected solvent(s) in a suitable plant-to-solvent ratio. The mixture was then subjected to extraction using methods such as shaking, stirring, or sonication to facilitate the transfer of phytochemicals into the solvent. This step was typically performed at room temperature or under controlled conditions, depending on the extraction requirements.

Filtration

The resulting extract solution was filtered using filter paper or a filtration apparatus to remove solid particles and obtain a clear filtrate.

Concentration

The filtered extract was concentrated using methods like rotary evaporation or freeze-drying to remove the solvent and obtain a more concentrated extract.

Storage

The extracted samples are stored in appropriate containers, such as amber glass vials, to protect them from light and ensure their stability until further analysis. The aqueous layer was then treated with dichloromethane and n-hexane to separate dichloromethane and ethyl acetate (2 times) as shown in scheme-1. Qualitative tests using different reagents were performed on crude extracts (Ismail et al., 2019).

Preliminary phytochemical analysis

A preliminary phytochemical screening was carried out using the ethanolic extract of the roots by employing standard procedures (Assiry et al., 2023; Nagaraja et al., 2023).

Alkaloids

Dragendroff's Test

The presence of alkaloids in the methanolic extract was determined using Dragendroff's test. In this test, 2 mg of the extract was mixed with 5 ml of distilled water, and 2M hydrochloric acid was added until an acidic reaction was observed. To this mixture, 1 ml of Dragendroff's reagent was added. The formation of an orange or orange-red precipitate indicated the presence of alkaloids in the extract.

Mayer's Test

The presence of alkaloids in the methanolic extract was tested using Mayer's reagent. To 2 mg of the extract, a few drops of Mayer's reagent were added. If a white or pale-yellow precipitate formed, it indicated the presence of alkaloids in the extract.

Wagner's Test

The presence of alkaloids in the methanolic extract was determined by adding 1 ml of hydrochloric acid and a few drops of Wagner's reagent to 2 mg of the extract. If a yellow or brown precipitate formed, it indicated the presence of alkaloids in the extract.

Hager's Test

The presence of alkaloids in the methanolic extract was confirmed by taking 2 mg of the extract in a test tube and adding a few drops of Hager's reagent. If a yellow precipitate formed, it confirmed the presence of alkaloids in the extract.

Molisch's Test

To conduct the Molisch's test, a test tube was prepared with 2 ml of the extract. Then, 2 drops of a freshly prepared 20% alcoholic solution of α-naphthol were added to the test tube. After that, 2 ml of concentrated sulfuric acid was carefully introduced to create a layer beneath the mixture. The presence of carbohydrates was indicated by the formation of a red-violet ring at the interface of the two layers. However, this ring disappeared upon the addition of an excess amount of alkali.

Benedict's Test

By adding 0.5 ml of the extract to a test tube, followed by the addition of 5 ml of Benedict's solution, a solution was formed. The mixture was then boiled for 5 minutes. If a brick-red colored precipitate formed, it indicated the presence of carbohydrates in the extract.

Fehling's Test

By taking 2 ml of the extract, a mixture of 1 ml each of Fehling's solution A and B (in equal parts) was added to a test tube. The mixture was then boiled for a few minutes. The presence of carbohydrates in the extract was indicated by the formation of a brick-red colored precipitate.

Lead Acetate Test

To perform the Lead acetate test, 2 ml of the plant extract was mixed with 1 ml of Lead acetate solution. If a yellow precipitate formed, it indicated the presence of flavonoids in the extract.

Triterpenoids and steroids

Salkowshi Test

By treating the extract with a few drops of concentrated sulfuric acid, the presence of steroids and triterpenoids can be determined. If a red color appears in the lower layer, it indicates the presence of steroids. On the other hand, the formation of a yellow-colored lower layer indicates the presence of triterpenoids in the extract.

Proteins

Biuret's Test

To test for the presence of proteins, 1 ml of the hot extract was mixed with 5-8 drops of a 10% w/v solution of sodium hydroxide, and then 1 to 2 drops of a 3% w/v solution of copper sulfate were added. The formation of a violet-red color indicated the presence of proteins in the extract.

Millon's Test

To determine the presence of proteins, 1 ml of the extract was dissolved in 1 ml of distilled water. Subsequently, 5-6 drops of Millon's reagent were added. The formation of a white precipitate that turns red upon heating indicated the presence of proteins in the extract.

Glycosides

To analyze the free sugar content of the extract, it was hydrolyzed using mineral acids (either dilute HCl or dilute H_2SO_4). After hydrolysis, the total sugar content of the extract was determined. An increase in sugar content indicated the presence of glycosides in the extract.

Saponins

Foam Test

Shake the drug extract or dry powder vigorously with water. Persistent foam observed.

RESULTS

Table 1 illustrates the results of preliminary phytochemical tests conducted on plant extracts, specifically for three plant species: *P. Candollei*, *G. tianschanica*, and *S. cordata*. The tests aimed to identify the presence (+) or absence (-) of various secondary metabolites in each plant extract. The secondary metabolites tested include alkaloids, flavonoids, steroids and triterpenoids, tannins, saponins, amino acids, and carbohydrates. For *P. Candollei*, *G. tianschanica*, and *S. cordata*, the results indicate that all three plant species tested positive (+) for the presence of alkaloids, flavonoids, steroids and triterpenoids, tannins, saponins, amino acids, and carbohydrates. This suggests that these secondary metabolites are present in the plant extracts of all three species.

Table 1: Preliminary phytochemical tests for plant extracts.

S. No	Secondary metabolites	*P. Candollei*	*G. tianschanica*	*S. cordata*
1	Alkaloids	+	+	+
2	Flavonoids	+	+	+
3	Steroids and Triterpenoids	+	+	+
4	tannins	+	+	+
5	Saponins	+	+	+
6	Amino Acid	+	+	+
7	Carbohydrates	+	+	+

Antioxidant potential of selected medicinal plants

The DPPH free radical scavenging method was utilized to assess the antioxidant activity in four different extracts (Methanol, Ethyl acetate, DCM, and n-Hexane) of *G. tianschanica*, *S. cordata*, and *P. candollei*. The results demonstrated that all three plant species exhibit significant potency in combating free radicals. Detailed values, mean values, and percentage inhibitions for each plant extract can be found in Table 2.

For *G. tianschanica*, the mean values and percentage inhibitions varied among the different extracts. The Methanolic extract had a mean value of 0.68 with 84.88% inhibition, while the DCM extract showed a mean value of 2.708 with 39.82% inhibition. The n-Hexane extract had a mean value of 5.43 with 16.80% inhibition, and the Ethyl acetate extract demonstrated a mean value of 0.47 with 80.50% inhibition.

Similar evaluations were conducted for *S. cordata*. The Methanolic extract had a mean value of 1.47 with 90.10% inhibition, whereas the DCM extract showed a mean value of 0.28 with 79.50% inhibition. The n-Hexane extract had a mean value of 3.745 with 18.70% inhibition, and the Ethyl acetate extract exhibited a mean value of 1.14 with 78.60% inhibition.

Table 2: Antioxidant assay results of three medicinal plants.

Parameter	Antioxidant results					
	Gentiana tianschanica		*Swertia cordata*		*Pleurospermum candollei*	
	Mean values	% inhibition	Mean values	% inhibition	Mean values	% inhibition
G. Methanolic	0.68	84.88%	1.47	90.10%	0.626	86.08%
G.DCM	2.708	39.82%	0.28	79.50%	2.28	81.55%
G. n-Hexane	5.43	16.80%	3.745	18.70%	4.45	26.77%
G. Ethyl acetate	0.47	80.50%	1.14	78.60%	3.14	77.60%

Lastly, the antioxidant potential of *P. candollei* was assessed using the same methodology. The Methanolic extract displayed a mean value of 0.626 with 86.08% inhibition, while the DCM extract had a mean value of 2.28 with 81.55% inhibition. The n-Hexane extract showed a mean value of 4.45 with 26.77% inhibition, and the Ethyl acetate extract exhibited a mean value of 3.14 with 77.60% inhibition. These findings indicate the effectiveness of these plant extracts in scavenging free radicals and suggest their potential as natural antioxidants.

DISCUSSION

The results of the preliminary phytochemical tests conducted on the plant extracts of *P. Candollei*, *G. tianschanica*, and *S. cordata* revealed the presence of various secondary metabolites. Alkaloids, flavonoids, steroids and triterpenoids, tannins, saponins, amino acids, and carbohydrates were detected in all three plant species. These secondary metabolites have been widely reported for their diverse bioactive properties and potential health benefits. The presence of alkaloids suggests the potential for pharmacological activities, such as anti-inflammatory, analgesic, or antimicrobial effects, antifungal, and use for pest control. Flavonoids, known for their antioxidant and anti-inflammatory activities, can contribute to the plants' overall medicinal properties. Steroids and triterpenoids, with their potential as anti-inflammatory and anticancer agents, may further enhance the therapeutic potential of these plants. Tannins, known for their antioxidant and antimicrobial activities, can contribute to the plants, defense mechanism and health benefits. Saponins are recognized for their potential immunomodulatory and anticancer effects, which may provide additional therapeutic value. Amino acids contribute to the overall nutritional value of plants, while carbohydrates serve as a source of energy.

Earlier reports have predominantly focused on alkaloids and steroidal sapogenins (saponins) as the primary chemical substances of interest (Thakur et al., 2011). However, there are also various other naturally occurring phytochemical groups, including flavonoids, tannins, unsaturated sterols, triterpenoids, and essential oils, that have been reported (Kaur et al., 2011). The chemical structures of tannins found in plants exhibit a wide range of diversity (Razzaq et al., 2023). To facilitate research in this area, tannins can be systematically classified based on their specific structural characteristics and chemical properties (Tong et al., 2022). Generally, plant tannins can be categorized into two main groups: hydrolyzable tannins and condensed tannins (Haroun et al., 2023). This classification provides a convenient framework for studying and investigating tannins and their various properties (Singh et al., 2023).

Alkaloids have been used medicinally for centuries and are commonly known for their cytotoxic properties (Breiterová et al., 2020). Alkaloids can be categorized into three main types based on their molecular structure and biosynthetic pathway: true alkaloids (heterocyclics), protoalkaloids (non-heterocyclics), and pseudoalkaloids (Patle and Mahish, 2023). This classification is determined by the chemical structure, biochemical origin, and natural origin of the alkaloid (Gul and Hamann, 2005). Alkaloids can be derived from various biosynthetic pathways, including the shikimate pathway, ornithine, lysine, and nicotinic acid pathway, histidine and purine pathway, and the terpenoid and polyketide pathway (Gutiérrez-Grijalva et al., 2020).

Glycosides have been found to exert blood pressure-lowering effects, as indicated by numerous reports. Tannins, on the other hand, can bind to proline-rich proteins and disrupt protein synthesis. Tannins are present in a wide range of legumes, shrubs, vegetables, and fruits found across the globe (Shahin et al., 2011).

Flavonoids, phenolic substances produced by plants in response to microbial infections, have demonstrated antimicrobial activity against a wide range of microorganisms in a laboratory setting (Kebede et al., 2021). Additionally, flavonoids exhibit potent antioxidant properties and have shown promising anticancer effects (Uivarosi and Munteanu, 2017). Flavonoids constitute a diverse group of phenolic compounds or polyphenols, comprising more than 6000 unique structures (Kroll et al., 2003). In plants, flavonoids are synthesized via two biosynthetic pathways: the phenylpropanoid pathway, which produces the phenylpropanoid skeleton (C6-C3), and the polyketide pathway, which generates the precursors for the formation of polymeric C2 units (Dias et al., 2021).

Triterpenoids form a diverse group of terpenoids that are of great biological interest, encompassing a broad range of secondary metabolites with over 100 different carbon skeletons (Croteau et al., 2000). These compounds have been discovered in both terrestrial and marine organisms, exhibiting remarkable structural diversity (Fouillaud et al., 2016). Within this class of natural products, various subclasses exist, such as triterpenes, steroids, limonoids, quassinoids, and triterpenoidal and steroidal saponins (Mirjalili et al., 2009). To date, more than 30,000 triterpenoid compounds have been isolated and identified (Galappaththi et al., 2022). In *Xanthosoma robustum*, a plant commonly known as elephant ear or pata, two steroids labeled as (5) and (6) were discovered. *X. robustum* is a neotropical species native to Central America (Vil et al., 2019).

Saponins exhibit pharmaceutical importance and display amphiphilic properties (Fagbohun et al., 2023). Their biological effects, including cytotoxicity, hemolysis, and fungicidal activity, mainly stem from their capacity to disturb cell membranes (Sasidharan et al., 2023). These compounds are categorized as secondary metabolites and are present in various plants and certain marine organisms like sea cucumbers and starfish. Structurally, saponins consist of a hydrophilic glycan that comprises one or more sugar chains, linked to a hydrophobic aglycone referred to as the sapogenin (Philippe et al., 2023).

CONCLUSION

The preliminary phytochemical analysis of the three medicinal plant species, namely *Swertia cordata*, *Gentiana tianschanica*, and *Pleurospermum candollei*, revealed the presence of various secondary metabolites such as alkaloids, flavonoids, steroids and triterpenoids, tannins, saponins, amino acids, carbohydrates, and proteins. These compounds have diverse bioactive properties and play a crucial role in the plants' defense against pests and diseases. Alkaloids, such as nicotine derived from tobacco, have insecticidal or pesticidal properties and can be used as natural insecticides. Flavonoids exhibit antioxidant properties and may contribute to the plants' defense against pests and diseases. Tannins, although primarily known for their antimicrobial and antioxidant activities, could deter herbivores. Some saponins have pesticidal properties and are used in biopesticides. Glycosides, such as certain types of glycosides, also have pesticidal activity. The presence of these compounds in the plant extracts suggests their potential use as natural pest control agents. However, the effectiveness of these compounds for pest and disease control can vary depending on the specific plant species, the concentration of the compounds, and the targeted pest or disease. Further research and understanding of their properties, concentration, and specific uses are necessary for their successful application in pest and disease control.

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AUTHOR'S CONTRIBUTIONS

HA carried out the research, wrote the initial draft of the research paper; HA assisted during lab and wrote a manuscript. SWK supervised and identified the plants and MI co-supervised and facilitated the Lab work. NH, TH, SK, AN, and SH assisted in field work.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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