Original Article

Anti-Bacterial And Anti-Fungal Activity Of Wild Plant Trillium Undulatum Against Multi-Drug Resistant Pathogens

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Abstract

Finding novel medications to combat the threat of drug-resistant strains is crucial to the effectiveness of chemotherapy. T. undulatum extracts were tested for their antibacterial and antifungal efficacy, and the presences of phytochemicals responsible for its biological activity were identified. Secondary metabolites such phenols, terpenoids, quinones, cardiac glycosides, and alkaloids were found by phytochemical study. Using antibiotics as a standard, the well diffusion test was used to determine whether or not plant composites have antibacterial activity against a number of pathogens known to cause severe urinary tract infections. T. Undulatum was tested for its antibacterial properties against a variety of bacteria, including Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Salmonella typhi, using both ethanolic and aqueous extracts. Different plant extracts shown varied antibacterial activity. Aqueous plant extracts were more effective than ethanolic ones as antibacterial agents against gram-positive and gram-negative bacteria. The aqueous extracts of T. Undulatum showed the greatest antibacterial potential, inhibiting 26 mm of S. typhi, whereas the ethanolic extract showed 25 mm of inhibition. Several strains of fungi were killed by the plant, demonstrating its potent anti-fungal properties. When tested against the fungal strain Mycosphaerella citri, the aqueous and ethanolic extracts showed the highest zone of inhibition to be 17 mm. The findings provide credence to the traditional use of extracts from these plants as medicine.

1. INTRODUCTION

Since the beginning of time, plants have been used to provide important nutrition and to promote both physical and mental health growth in human (Savriama, 2018). They are recognized to lower stress and enhance wellbeing due to their ornamental value or for use in horticulture therapy (Chalmin-Pui et al., 2021). Despite the fact that many plants are toxic, they are nonetheless used in certain sociocultural settings, including superstitious and magical rituals as well as fishing and hunting gear. However, plants provide a wide range of health benefits and essential goods for humans (Takase et al., 2015).

T. undulantum is a member of the Melanthiaceae, that belongs to Trilliaceae family (Jackes, 2010). The plants species of this genus have so far yielded a number of steroidal sapiens that have been extracted and purified

(Busia, 2016). Numerous preclinical investigations have supported the widespread use of Trillium-genus plant species as a treatment for a range of illnesses in various traditional healing systems (Süntar, 2020). In traditional medicine, the rhizomes of T. erectum, also known as Beth roots, have been used to treat hemorrhages from the uterine, urinary system, and lungs. At least a thousand years have passed since T. tschonoskii was first utilized in China (Li et al., 2018). This plant's dried rhizomes have been utilized as herbal treatments to cure hypertension, neurasthenia, elation, headaches, get rid of carbuncles, and lessen discomfort. n-BuOH extract has also been shown to have anticancer and pro-apoptotic effects on human lung cancer cells (Lee et al., 2018). The ethanol, ethyl acetate, and butanol extracts of T. tschonoskii significantly decreased the edoema of rat hind paw edoema brought on by injection of carrageenan. Finally, T. tschonoskii enhanced the production of anti-oxidase enzymes in rats, which improved learning and memory (Ur Rahman et al., 2017).

The usage plant-based medications and dietary supplements have grown recently. Scientists working on natural products, ethnopharmacologists, botanists, and microbiologists are scouring the planet for phytochemicals and "leads" that might be used to treat infectious illnesses (Rout and Issue). None of the present medications, which make up between 25 and 50 percent of the market, are antimicrobials. The use of antimicrobial and other medications derived from plants is finding more acceptance in mainstream medicine as standard antibiotics lose their efficacy and new infections, especially viral ones, continue to be resistant to this class of medication (Anand et al., 2019).

The current increase in interest in plant antimicrobials has been fueled in part by the fast loss of plant species. Natural product chemists and microbiologists share the belief that a large number of potentially beneficial phytochemical structures that may be chemically produced are in danger of being lost forever (Scalbert et al., 2011). However, it has long been known that exposure to antimicrobial medications may function as a selection pressure for the spread of genes or other genetic components that provide bacteria the ability to "resist" the harmful or inhibiting effects of the treatments. Therefore, ironically, the usage of antibiotics may eventually make them useless against their intended populations (Morris, 2014). E. coli, K. pneumoniae, Haemophilus, and many other b-lactamase producers have spread across the globe, creating a significant treatment challenge. Hospitals are rife with MDR strains of E. coli and K. pneumoniae, which are also increasingly being identified from illnesses acquired in the population. A nosocomial pathogen called Candida albican has been shown to be responsible for 50–70% of cases of invasive candidiasis (Ahmed and Sciences, 2021). Alarmingly, nosocomial candidemia has been more common during the last ten years. All of this has had serious repercussions, including rising medical costs and patient death. Therefore, additional research on using plants as medicinal agents should be prioritized, particularly studies relevant to the management of antibiotic-resistant bacteria. This study's goal was to assess the effectiveness of plant compounds and extracts on multidrug-resistant bacteria and fungus (Fair and Tor, 2014).

2. METHODOLOGY

2.1. Collection of plant specimen

The plant specimen was collected from local area of Shogran Valley district Mansehra in June 2021, and was identified with the help of Flora of Pakistan website. Leaves, stems, and flowers were preserved in FAA (Formalin, Acetic Acid, and Alcohol) and sent to the Department of Botany at Hazara University in Mansehra for further research.

2.2. Preparation for powder drug

To remove contaminants, tap water was used to wash the plant. Separated plant pieces were washed and dried in the shade for four to seven days. The components were dried, and then ground using an electric grinder. For further analysis, the powder was placed in a plastic container (Abubakar et al., 2020).

2.3. Plant morphology identification

The plant morphology was identified based on their color, length, surface, structure and fruits of leaves and stem (Barthlott et al., 2017).

2.4. Preparation of plant extract

In order to prepare the plant extract for the bactericidal test, ethanol and distilled water were employed as solvents. 250–400 mL of solvent was used to hydrate 10 mg of powder and was preserved for 7 days. Through filter paper, the extract was filtered. After the crude extract was dried using air dry oven at 60°C and these semi-solid extract were used for the further investigation (Ahmed et al., 2014).

2.5. Anatomy of Leaves and stem

The transverse section of leaves and stems were cut using sharp blade (microtome) into small thin pieces for anatomical studies (Metusala, 2017).

2.6. Phytochemical Screening

Two different solvents—ethanol and distilled water—were used for the phytochemical analysis. Shaking 10 grammes of powder that had been steeped in 100 millilitres of solvent for 7 days Filter paper was used to purify the extract. The materials were then put to use in the ongoing phytochemical study (Solihah et al., 2012).

2.6.1. Carbohydrates investigation

2 mL of plant extract and iodine solution was used in equal proportion in test tube. The purple or blue color indicates the presence of carbohydrates (Borah and Biswas, 2018).

2.6.2. Tannins investigation

1 mL of plant extract was added to the test tube, followed by 2 mL of ferric chloride. Pale blue or dark greenish color is the indication of tannis (Tambe et al., 2021)

2.6.3. Saponins investigation

The saponins test was also performed by adding 2ml of plant extract in 2ml of distilled water and shaking continuously for 15 minutes. The presence of saponins can be seen in the formation of the fourth layer (Syahputra et al., 2021).

2.6.4. Flavonoids investigation

2 mL of plant extract was placed in a test tube, followed by 2 mL of NaOH, which was mixed with 2 mL of dilute HCl, resulting in a yellow color, which indicates the presence of flavonoids in the plant extract (Tyagi and Agarwal, 2017).

2.6.5. Alkaloids investigation

2 mL of plant extract and concentrated HCL was mixed with Mayer's reagent. The ceramic precipitate shows the existence of Alkaloids (Arora et al., 2017).

2.6.6. Quinones investigation

1 mL of plant extract and H_2SO_4 were added in a test tube for the quinones test. The presence of quinones is indicated by the reddish tint (Kaur et al., 2019).

2.6.7. Glycoside investigation

Plant extracts (2 mL) were combined with 3 mL of chloroform, agitated, and then ammonia solution (10 %) was added to the lower chloroform layer. Glycosides are responsible for the pink color (Balamurugan et al., 2019).

2.6.8. Cardiac Glycosides investigation

A mixture of 1 mL of H2SO4 and glacial acetic acid was used to identify cardiac glycosides. The provided plant extract then had a few drops of 5% ferric chloride added to it (Panchal and Parvez, 2019).

2.6.9. Phenols investigation

Few drops of 10% ferric chloride were added to a mixture containing 1 mL of plant extract and 2 mL of water. Phenol is indicated by a colour that is blue-green (Tyagi and Agarwal, 2017).

2.6.10. Coumarins investigation

A plant extract volume of 1 mL was combined with 1 mL of 10% NaOH. The presence of coumarins is indicated by a yellowish color (Roghini and Vijayalakshmi, 2018).

2.6.11. Paleobotanies investigation

For Paleobotanies, 1mL of plant extract was placed in test tube, and then 2 % HCL was added drop by drop. The appearance of reddish color is the indication of paleobotanies (Jones, 2014).

2.6.12. Anthraquinones investigation

1 mL of plant extract and NaOH were mixed together. The presence of anthraquinones is indicated by violet /pink color (Yusuf et al., 2014).

2.7. Bacterial and fungal strains collection

Five bacterial strains in which four were gram negative (E. coli, S. typhi, K. pneumoniae and P. aeruginosa) and only one was gram positive (S. aureus) while three fungal strains (Penicillium, M. citri and Rhizopus stolonifer) were collected from microbiology department, Hazara University Mansehra (Bammou et al., 2020).

2.8. Antibacterial activity of plant extract (T. Undulatum)

The plant extract (T. Undulatum) activity was checked using well diffusion technique. First nutrient agar media was prepared and sterilized. Four wells were created on the bacterial lawn using nutrient agar plates. The plant extracts prepared in ethanol and aqueous solution were added in the two wells respectively while antibiotic (Ciprofloxacin) was poured into 3rd well and DMSO was added into the 4th well. The plates were then overnight incubated for observation of ZOI (Nouri-Aiin, 2022).

2.9. Anti-fungal activity

The lawn of the three fungal strains (Penicillium, M. citri and Rhizopus stolonifer) were made on the SDA media. Two wells were then created in which in the 1st well ethanolic plant extract were added and in the 2nd well aqueous plant extract were added. The plates were then incubated for 24 hours for the observation of ZOI (Ncama et al., 2019).

3. RESULTS

3.1. Morphological features of T. Undulatum plant

T. Undulatum was identified based on its morphology, which includes characteristics such as its leaf colour, arrangement, size, shape, margins, and venation. The leaves were oblong in form, silver in colour, with pinnate venation and an entire border. The traits of fruits and flowers were also studied (Table 1).

Table 1. Characteristics and morphology of T. Undulatum plant

S. No.	Features	Observations
1	Leaf arrangements	Whorled
2	Type	Simple
3	Margin	Entire
4	Venation	Pinnate
5	Leaf size, type and color	3-6 inches, simple and green
6	Fruit length and width	< 1 inch
7	Fruit color	Red/Burgundy
8	Flower color	White
9	Flower character	Solitary/ Showy

3.2. Fractures of leaves and stems of T. Undulatum

Through organoleptic investigation the fractures of leaves and stems of T. Undulatum plant was recorded and explained in table 2.

Table 2: Characteristics of Leaves and stems of T. Undulatum

S. No.	Features	Observations
1	Color	Green
2	Odor	Pleasant
3	Texture	Sessile (leaves) and smooth (stem)
4	Taste	Edible

3.3. Phytochemical Screening of T. Undulatum

Ethanol and distilled water were used to analyse the phytochemical content of T. Undulatum plant extracts. Paleobotanies, saponins, tannins, cardiac glycosides, and coumarins were not detected in ethanolic or distilled water, according to phytochemical analysis. In addition, terpenoids, alkaloids, carbohydrates phenolic compounds, quinones, and flavonoids were found (table 3).

Table 3: Phytochemical test of Powder of T. Undulatum plant

S. No.	Phytochemicals	Ethanol	Distilled Water
1	Phenols	+	+
2	Anthraquinones	-	-
3	Terpenoids	+	-
4	Tannins	-	-

5	Saponins	-	-
6	Fixed oil	+	+
7	Coumarins	-	-
8	Paleobotanies	-	-
9	Carbohydrates	+	+
10	Cardiac Glycosides	+	-
11	Glycosides	-	-
12	Quinones	+	+
13	Flavonoids	+	+
14	Alkaloids	+	+

Key: + Present, - Absent

3.4. Antibacterial activity of T. Undulatum ethanolic and aqeuous extract

T. Undulatum plant extract, has been tested against a number of harmful bacteria, including E. coli, S. aureus, P. aureginosa, K. pneumoniae, and S. typhi. Despite aqueous extract showed largest zone of inhibition (26 mm) against S. typhi and lower (14 mm) against S. aureus, while positive findings were also tested against S. typhi (25 mm) by ethanolic extract but less potent against K. pneumonia (figure 1 & 2). Additionally, the increased zone of inhibition against P. aureginosa was shown by antibiotics (table 3.2).

Table 4: Antibacterial activity of T. Undulatum

Bacterial strains	Ethanolic	Aqueous	Antibiotics
E. coli	20±2.64AB	25±1.73A	29±2AB
K. pneumonia	15.66±0.57C	17±2BC	21.66±3.05C
P. aureginosa	19.33±1.52BC	18±1B	32.33±3.05A
S. typhi	25±1A	26±3A	28.33±2.08BC
S. aureus	16.33±1.52C	14±2.64C	27.33±3.05BC

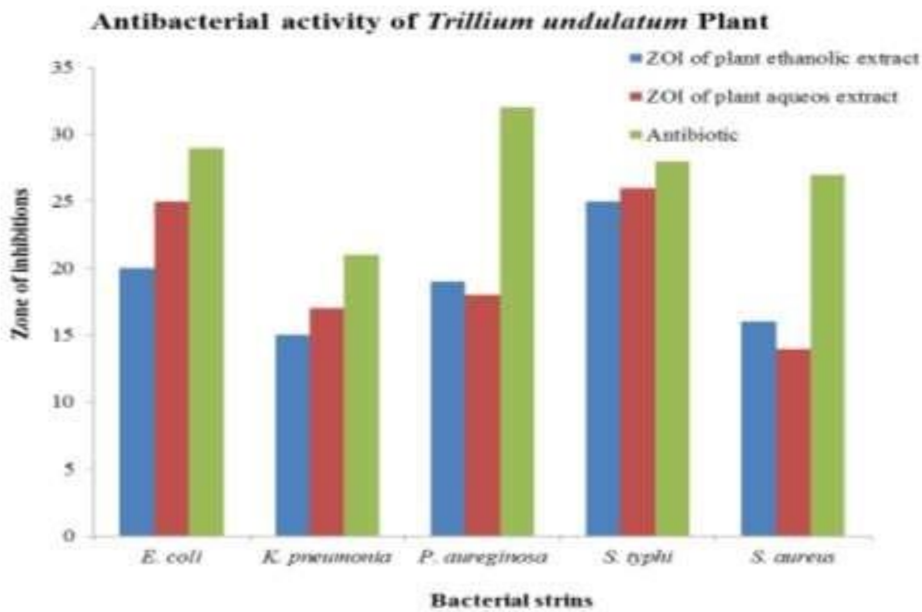

Figure 1: Antibacterial studies of T. Undulatum against different bacterial strain

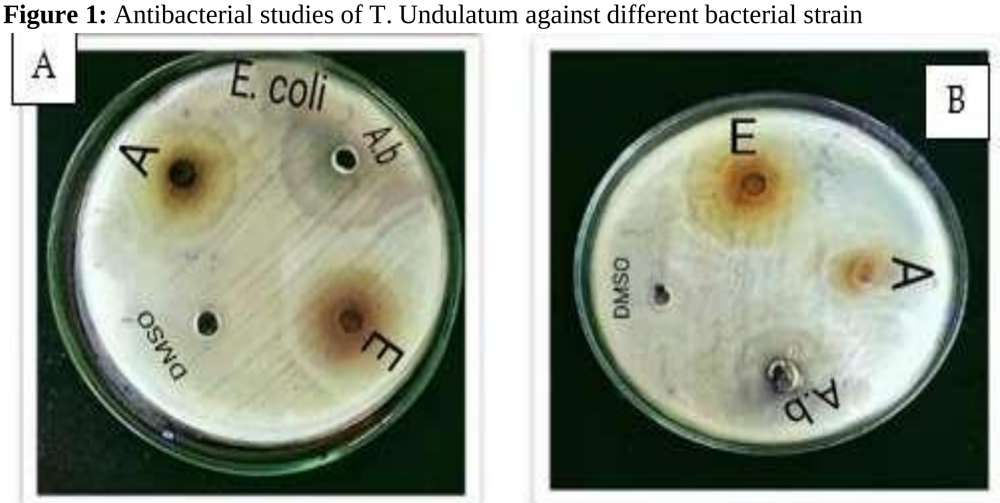

Figure 2: Antibacterial activity of plants extracts (A) E. coli (B) P. aeruginosa

3.5. Antifungal activity of T. Undulatum

The higher zone of inhibition (17.66±2.08), and lower zone of inhibition (11.66±1.5) was found at aqueous extract denoted by (A) against M. citri, and Penicillium strains respectively. In case of ethanolic extract denoted by (C), the high zone of inhibition was recorded against Rhizopus stoloniferas strains, but there were no significant change in ethanolic extract against fungal strains. In case plant parts, the ethanolic showed higher antifungal activity against Rhizopus stoloniferas and Penicillium strains compare to aqueous extract while, aqueous showed higher antifungal activity against M. citri strains than ethanolic extract.

Table 5: Antifungal activity of T. Undulatum

S. No.	Fungal strains	Ethanolic	Aqueous
2.	Penicillium	14.66±1.15A	17.66±2.08A
3.	Rhizopus stolonifer	17±3A	11.66±1.52B
1.	M. citri	14.66±2.51A	14.66±0.57AB

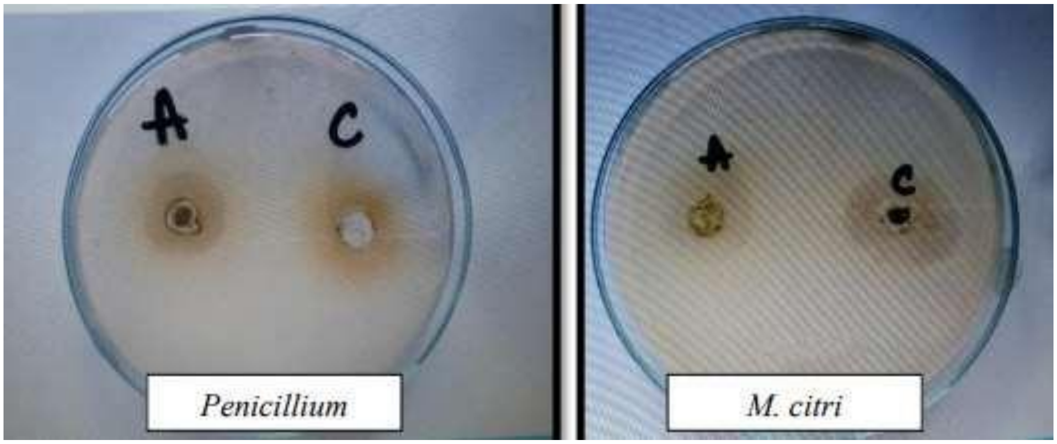

Figure 3: Antifungal activities of plant extract against fungus

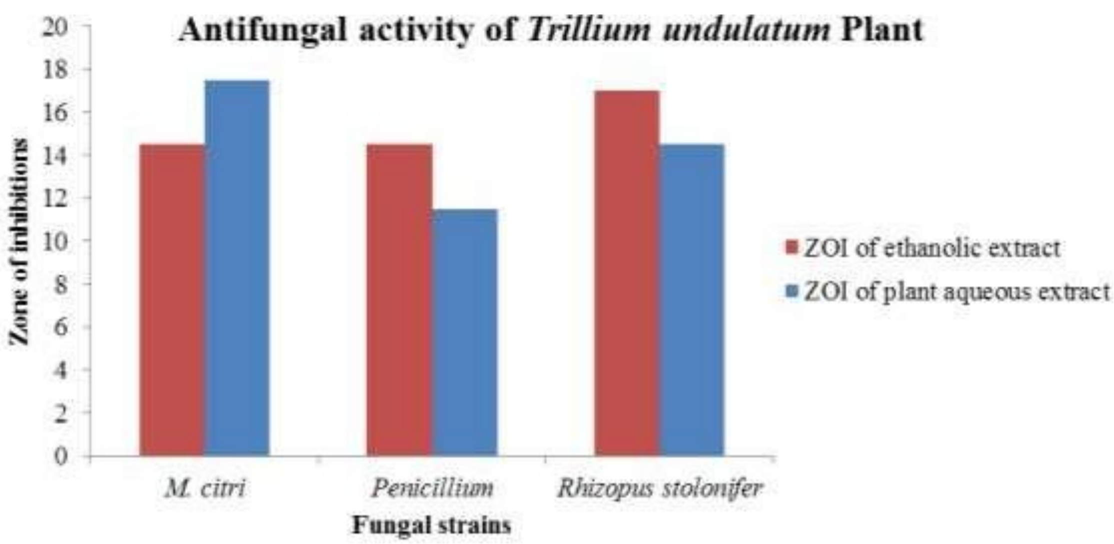

Figure 4: Antifungal studies of T. Undulatum against different fungal strains

4. DISCUSSIONS

The significant impact that medicinal plants have had on human society. Most things found in nature come from plants. T. Undulatum is a member of the Melanthiaceae family, which is often used for its therapeutic qualities (Soleimani et al., 2022). There are many different plant species, but only a few of them have been characterised in terms of their pharmacological and phytochemical properties. However, there has been zero research on the phytochemical, antibacterial, and antifungal properties of plants. The therapeutic value of this plant has so been determined via the use of many factors (Hussein and El-Anssary, 2019).

Phytochemical analyses, as well as tests for bacteria and fungi, were performed for this study. Identifying and verifying the quality of a plant requires a microscope (Munshi et al., 2021). Phytochemical elements of several plant extracts were shown to have significant therapeutic effects (Kumar et al., 2021). The secondary metabolites found in T. Undulatum include sugars, coumarins, alkaloids, and terpenoids, according to phytochemical analyses. Alkaloids found in plant extract shown similarities across several plant species (Lans et al., 2018). Secondary compounds in plants are responsible for their healing abilities. As a result, our findings show that plants are a valuable resource for secondary metabolites that may be used to fight off many pathogens responsible for illness. Plants' therapeutic effects are due to secondary chemicals (Mattosinhos et al., 2022). These results demonstrate that plants are a valuable resource for the development of therapeutics targeting pathogens responsible for a wide range of disorders. Medicinal plants are very significant to human culture. Few species have had their pharmacological and phytochemical qualities studied and recorded (Ismail et al., 2012).

The plant's secondary metabolites are responsible for its antibacterial effect. However, the antibacterial properties of the plant T. Undulatum have not been studied until recently. Five different pathogenic bacteria, including E. coli, S. aureus, P. aureginosa, K. pneumonia, and S. tyhpi, were tested against the aqueous and ethanolic extract of the plant T. Undulatum (Lans et al., 2018). Both plant extracts were able to kill bacteria, although the aqueous extract was more effective against S. typhi (maximum zone of inhibition, 26 mm), while the ethanolic extract was more effective against S. typhi (maximum zone of inhibition, 25 mm). The plant is effective against S. typhi, a gram-negative bacterium. Antibiotics were shown to have a larger zone of inhibition against test bacteria strains than both ethanolic and aqueous extracts (Polash et al., 2017). The zone of inhibition against P. aureginosa and S. aureus was greater in the ethanolic extract than in the aqueous extracts, but the zone of inhibition against E. coli and S. typhi was greater in the aqueous extracts. Many secondary metabolites in the plant have antibacterial effects (Frempong et al., 2021).

Plant extracts were tested for their antifungal effects against Penicillium, M. citri, and R. stolnifer, among others, in both aqueous and ethanolic media. Maximum zone of inhibition against M. citri in the aqueous medium was 17 mm, but in the ethanolic extract it was only 14.66 mm. This study demonstrates the potential importance of plants as a source of antifungal activity (López-Malo et al., 2020).

CONCLUSION

Studies on T. undulatum's morphology and anatomy provide very strong evidence that it can be distinguished from other members of the genus. Studies on the phytochemistry of T. undulatum revealed that the plant contains active secondary metabolites such as phenol, alkaloids, lipids, and carbohydrates. Plants may be utilised to make different antibiotics and medicines to treat ailments, according to antibacterial and antifungal activity of plant composites. According to the extract's antibacterial properties, the plant may be used to produce further antibiotics and drugs to cure disorders in the future.

The biological effects of this plant, including its anti-analgesic, sedative, and insecticidal properties, may be studied in the future. To ensure their continued usage, people should be encouraged to conserve these delicious plants.

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