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Original Article

Novel Extraction, Phytochemical Screening and Antibacterial Activity ofImpatiens latiflora hook F. & Thomson Leaves

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A R T I C L E I N F O

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A B S T R A C T

Impatiens latiflora hook f. & Thomson is a traditionally known medicinal plant used to heal the wounds and alleviate digestive problems. This study aimed to extract and qualitatively analyse the presence or absence of phytochemicals and determine the antibacterial activity against selected pathogens from fresh leaf extracts in methanol and hexane solvents. Standard procedures were followed for phytochemical screening and agar well diffusion technique was carried to test the antibacterial activity against four bacteria Bacillus subtilis,Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae. Screening of phytochemicals from methanol leaf extracts exhibits the presence of the compound alkaloids, terpenoids, saponins, and flavonoids, while alkaloids and steroids are present in hexane leaf extract. The antibacterial activity screening was performed at six different extracts concentrations 300, 250, 200, 150, 100, 50 mg/ml in DMSO. Ampicillin 2 mcg was utilised as standard control while DMSO haven been used as the negative control in antibacterial activity screening. The highest antibacterial activity was seen on methanol leaf extract with inhibition zone 14±1.73 mm against the bacteria Bacillus subtilis at concentration 300 and 250 mg/ml. Similarly, hexane leaf extracts showed maximum antibacterial activity with inhibition zone 14±0 mm on bacteria Bacillus subtilis at concentration 250 mg/ml. The result from our study reported the presence of significant phytochemicals and effective antibacterial activity on leaf extracts of Impatiens latiflora hook f. & Thomson against the selected bacteria and therefore itcan be used as natural antibacterial agent to treat against the pathogenic bacteria.

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INTRODUCTION

Over the centuries, humans have relied upon natural resources to carter the basic needs, one such important element is the use of the natural product in the field of medicine, particularly plants have built a sophisticated traditional medicinal system which are practiced till today.[1] Natural product plays a promising role particularly as an antibacterial agent, due to their safety and affordability.[2] The World Health Organisation (WHO) emphasizes on used of the natural medicine as a primary source of medicine, [3] it was estimated by WHO that 80% of human population requires medication obtain from plants source for the primary healthcare requirements.[4] Today 70-80% of human race particularly the developing nations practice the traditional therapeutic approach to meet the need of their primary healthcare because of its affordability with minimal side effects.[5]

Therapeutic plants makes a valuable source of the phytochemical constituents like alkaloids, saponins, flavonoids, tannins, glycosides, steroids, terpenoids etc. which are known to provide various pharmacological activities like antibacterial, anti-diabetic, antiviral, anticancer etc.[6] Traditionally used medicinal plants remains a significant source of novel product, there is rise use of active substance derived from plants with the advancement in phytochemistry and pharmaceutical industry.[7] Natural product possesses significant role in providing modern medicines to help the problems rising with the resistance of the microbes.[8] Pathogenic diseases have become a major health concern. Though bacterial infections are avoidable infections, however alarming rise in antibacterial resistance have become a major therapeutic problem.[4]Traditionally known medicinal plants contain a new source of antibacterial agents, hence there is great interest in screening such plants to verify their use as a medicinal purpose and understand the active principle.[9]

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Several compounds were isolated from the plants with the minimal toxicity to the host cell to develop new drugs to fight against bacterial pathogens.[10]Impatiens latiflora hook f. & Thomsonspecies were found in Arunachal Pradesh, Nagaland and Meghalaya,[11], Assam .[12] The leaves of Impatiens latiflora hook f. & Thomsonis a famous medicinal plant locally called as "Hoshi" which is found in moist, shady places and streams in Phek District, Nagaland, India. Traditionally, the fresh leaves are mashed and applied directly on the wounds. The leaves are also consumed by boiling in water. It tastes slimy which improves gastric, and alleviate digestive problems. The primary objective of our work is conducted to scientifically justified the usage of traditional medicinal plant *Impatiens latiflora hook f. & Thomson* leaves through extraction, screening the phytochemical constituents and examining the antibacterial activity against the selected bacteria.

MATERIALS AND METHODS

Collection of *Impatiens latiflora hook f. & Thomson* plant sample

The fresh leaves of *Impatiens latiflora hook f. & Thomson* were collected from Nagaland University Lumami campus, Nagaland, India. The plant was found in a moist area. The picture of the plant *Impatiens latiflora hook f. & Thomson* is presented in Figure 1.



Fig 1:Photographic image of plant Impatiens *latiflora hook f. & Thomson*

Preparation for *Impatiens latiflora hook f. & Thomson* leaf extraction

The fresh leaves of *Impatiens latiflora hook f. & Thomson* were extracted using Soxhlet extraction method. Each leaf of the plant was thoroughly clean with water to eliminate any traces of dirt. The leaves were cut with knives into tiny pieces and proceed for extraction. Methanol and hexane solvents were used for the extraction of *Impatiens latiflora hook f. & Thomson* leaves. 133.39 gm of fresh leaves was extracted in 250 ml of methanol and hexane solvent for 78 hours each. After the completion of extraction, the extracts were taken to rotatory evaporator to evaporate the solvents and to collect extracts (Table 1).

Phytochemical screening

Screening of phytochemical compounds of *Impatiens latiflora hook f. & Thomson* leaf extract for both methanol and hexane solvents was examined to identify the presence and the absence of compound like alkaloids, terpenoids, tannins, saponins, quinines, steroids, phenols and flavonoids. All phytochemical compounds were tested by following the standard procedure. [13-16]

Alkaloids tests

In 1 ml of the leaf extract add a couple drops of Marqus reagent (prepared from 0.5 ml formaldehyde adding 5 ml concentrated sulphuric acid). Turbidity appearance indicates alkaloid is present.[13]

Terpenoids tests

When 1 ml leaf extract was mixed to 1 ml concentrated sulphuric acid then heat for 2 minutes. Appearance of greyish colour results presence of terpenoids.[14]

Tannins tests

When 1 ml of ferric chloride mixed to 1 ml plant extract, blue, green, black colour appearance showed that tannins are present.[14]

Saponins tests

Presence of saponins is determined by 1 cm layer foam formation, 1 ml plant extract combine by 2 ml distilled water and shake well.[14]

Quinines tests

Take 1 ml of leaf extract which as mixed well to 1 ml of 1% sodium hydroxide. Formation of blue, green, red shows quinines is present.[14]

Steroids tests

1 ml of chloroform along with 1 ml concentrated sulphuric acid are combined with 1 ml of extract. Presence of steroids is shown by red colour formation of at the bottom of chloroform layer.[14]

Phenols tests

1 ml plant extract was mixed by 2 ml distilled water and 2-3 drops of 10% ferric chloride. Blue or green colour appearance signifies phenols is present.[15]

Flavonoids tests

In 1 ml of leaf extract was mixed to 1 ml of 10% lead acetate solution. Yellow precipitate appearance shows flavonoids is present.[16]

Antibacterial activity of *Impatiens latiflora hook f. & Thomson* leaf extracts

Bacterial strains

For the antibacterial activity screening study on the leaf extracts of Impatiens latiflora hook f. & Thomson, we have selected two gram-positive bacteria (Bacillus subtilis, Staphylococcus aureus) and two gram-negative bacteria (Escherichia coli, Klebsiella pneumoniae) which were maintained on a nutrient agar plate and preserved in an incubator at 37° C.

Preparation of bacterial cultures in agar media

The cultures of four bacterial strains were prepared by taking 2.24 g of nutrient agar (HiMedia) in 80 ml of distilled water. Sterilized the media in autoclave at 121° C for 25 minutes. 20 ml of sterilized agar was poured to each of four sterile plates which are allowed to cool and solidify for approximately 20 minutes. Once solidified, individual bacterial colonies were taken from maintained stock cultures using a sterilized glass rod and streaked across the agar plates surface, one strain was prepared at a time to avoid cross-contamination. The inoculated bacterial plates was further incubated for 24 hours at 37° C.

Preparation of bacterial cultures in broth media

Four bacterial broth cultures, it was prepared by measuring 0.96 g of nutrient broth (HiMedia) and dissolved in 40 ml of distilled water which was autoclaved at 121° C for 25 minutes. After the completion of autoclave, 10 ml of nutrient broth was dispensed into sterilized vials. A bacterial colony were scraped from the nutrient agar stock culture using a sterile glass rod and stirred into vails containing nutrient broth, which was incubated for 24 hours at 37° C.

Antibacterial screening of Impatiens latiflora hook f. & Thomsonleaf extracts

Antibacterial activity testing of fresh leaf extract of Impatiens latiflora hook f. & Thomsonwas accessed by following agar well diffusion method against selected bacteria. Leaf extracts was prepared at six different concentrations 300 mg/ml, 250 mg/ml, 200 mg/ml, 150 mg/ml, 100 mg/ml and 50 mg/ml DMSO (Dimethyl Sulfoxide). The antibacterial testing was conducted for both methanol and hexane solvents leaf extract. DMSO have been utilised as negative control and ampicillin 2 mcg as a standard control. The antibacterial activity screening were conducted in triplicate.

Agar well diffusion method

Antibacterial activity of *Impatiens latiflora hook f. & Thomson* leaves from methanol and hexane solvent against selected pathogenic bacteria was studied by agar well diffusion.[17] In a solidified agar petri plate, 60 µL of bacteria broth was transferred onto each labelled petri plate and spread evenly with sterilized glass rod. Sterile cork borers have been used to make 7 wells 6 mm diameter of. In respective well 15 µL leaf extract of different concentrations was poured. Prepared plates are incubated at 37 ° C for 24 hours to let extract diffuse to maximum and for the optimal bacterial growth. The result of antibacterial activity of the *Impatiens latiflora hook f. & Thomson* leaf extracts was recorded by measuring the inhibition zone (mm).

RESULT

Yield of *Impatiens latiflora hook f. & Thomson* leaf extracts

The extraction of *Impatiens latiflora hook f. & Thomson* fresh leaf through Soxhlet extraction method from methanol and hexane solvents showed the different amount of yield depending upon solvent used. The result from the methanol extract yield 4.125 gm with the extract dark green in colour while hexane extract yield 0.580 gm with yellow colour extract. (Table 2)

Table 1:The data of employed *Impatiens latiflora hook f. & Thomson* leaves for extraction

Plant species	Vernacular name	Parts plant used	Solvents used	Vol. of solvent used	Weight of fresh leaves used for extraction	Extract yield in gm	Colour of the extract	Nature of the extract
Impatiens latiflora hook f. & Thomson	Hoshi	Leaves	Methanol	250 ml	133.39 gm	4.125 gm	Dark green	Semi solid
			Hexane	250 ml	133.39 gm	0.580 gm	Yellow	

Phytochemical screening of the leaf extracts of Impatiens latiflora hook f. & Thomson

Phytochemical testing on fresh leaf extract of Impatiens latiflora hook f. & Thomson on methanol solvent reports the presence of compound like alkaloids, terpenoids, saponins and flavonoids whereas tannins, quinines, steroids, phenols were absent, while alkaloids and steroids were present in hexane solvent extract but terpenoids, tannins, saponins, quinines, phenols and flavonoids were absent (Table 2).

Table 2: Phytochemical constituents of Impatiens latiflora hook f. & Thomson leaf extracts

Tests	Solvents used	
	Methanol	Hexane
Alkaloids	+	+
Terpenoids	+	-
Tannins	-	-
Saponins	+	-
Quinines	-	-
Steroids	-	+
Phenols	-	-
Flavonoids	+	-

Key (+) represents presence of the compounds, Key (–) represents absence of the compounds

Antibacterial activity screening on Impatiens latiflora hook f. & Thomson leaf extracts

Impatiens latiflora hook f. & Thomson leaf extracts was screened for the antibacterial activity against the four selected bacteria that is Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Klebsiella pneumoniaeby agar well diffusion method.

The result revealed that, degree of bacterial growth inhibition varied depending on of solvent used for extraction and extract concentration. By estimating the inhibition zone, the leaf extracts effectiveness was recorded and expressed in millimetres (mm).

Methanol solvent extract showed a larger inhibition zone than hexane solvent extract, particularly against bacteria Bacillus subtilis with maximum inhibition zone 14±1.73 mm at 300 mg/ml and 250 mg/ml which is almost similar with comparison standard drug ampicillin 2 mcg observed against the bacteria Bacillus subtilis (Table 3).

Extracts from hexane solvent also demonstrated remarkable results against screened bacterial strains, the maximum inhibition zone observed on hexane solvent extract is from bacteria Bacillus subtilis with inhibition zone 14 ±0 at 250 mg/ml (Table 4). Antibacterial activity screening was results are observed and recorded after 24 hours of incubation.

Table 3: Antibacterial activity and mean ± standard deviation of Impatiens latiflora hook f. & Thomson leaf extracts on methanol solvent against selected bacteria

Bacteria	300 mg	250 mg	200 mg	150 mg	100 mg	50 mg	(+ ve control)	(- ve control)
Bacillus subtilis	14±1.73	14±1.73	13.33±2.51	12.66±2.51	12.66±2.51	12.33±2.51	14±1.73	0±0
Staphylococcus aureus	10±0	11±0	11.33±1	11.33±1	10.66±1.15	10.33±0.57	16.33±3.21	0±0
Escherichia coli	12.33±1.15	11.33±1.53	11.66±1.52	11.33±1.15	11±1	9.66±0.57	17.66±2.0	0±0
Klebsiella pneumoniae	12.66±2.8	11.66±2.8	12.33±3.21	13±1.73	12±2.64	9.66±0.57	16±3.6	0±0

Values are presented as mean ± standard deviation, number of the replicates (n=3)

Table 4: Antibacterial activity and mean ± standard deviation of Impatiens latiflora hook f. & Thomson leaf extracts on hexane solvent against the selected bacteria

Bacteria	300 mg	250 mg	200 mg	150 mg	100 mg	50 mg	(+ ve control)	(- ve control)
Bacillus subtilis	13±1.73	14 ±0	12.66±2.51	11.33±1.15	10.66±0.58	9±0	16.33±3.51	0±0
Staphylococcus aureus	14±2	12.66±1.15	11.33±1.52	11.33±1.15	10.33±1.53	8.66±0.58	17.33±2.3	0±0
Escherichia coli	14±1	13.33±0.57	13.66±1.15	12.66±1.15	11.33±0.57	10±0	18±1.52	0±0
Klebsiella pneumoniae	12.66±1.15	11.66±0.57	11.33±0.58	11±1	9±1	9.33±1.15	19.33±1.53	0±0

Values are presented as mean ± standard deviation, number of the replicates (n=3)

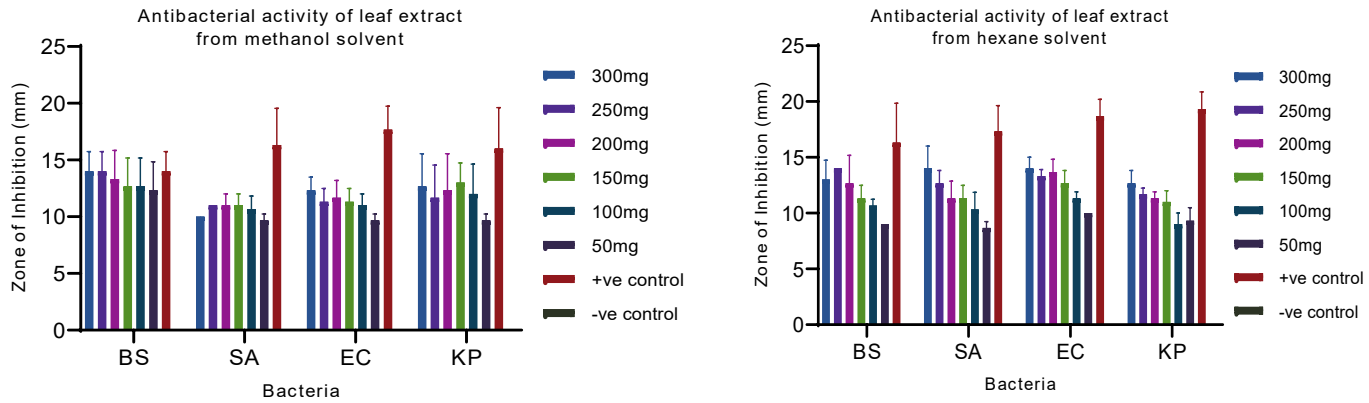


Fig 2: Antibacterial activity of Impatiens latiflora hook f. & Thomson leaf extracts on selected bacteria Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Klebsiella pneumoniae

DISCUSSION

The search for antibacterial agent from natural sources has gained significant attention, efforts have been made to discover compounds from the natural sources to replace synthetic antibiotics. Phytochemicals compounds obtained from plants source serves as fundamental source of healing with lower toxicity compared to synthetic drugs. The natural compounds provide promising solutions for controlling microbial growth and addresses the increasing issue of antibiotic resistance.[18] In present study, to validate traditional usage of the medicinal value, the fresh leaves of Impatiens latiflora hook f. & Thomson were extracted and screened for phytochemical and antibacterial activity.

The fresh leaves of the plant Impatiens latiflora hook f. & Thomson were extracted from methanol and hexane solvent by Soxhlet extraction method. 133.39 gm fresh leaves were taken for extraction for both methanol and hexane solvent. The yield of 4.125 gm was obtained from methanol and the extract was dark green in colour similarly the hexane solvent extract yield 0.580 gm with yellow colour extract. The phytochemical screening from the extract of Impatiens latiflora hook f. & Thomson leaves result showed the presence of phytochemicals, which indicated that the compounds present in plants are responsible for defence mechanism against the pathogenic bacteria.[19] Screening of phytochemicals from methanol solvent extract showed the availability of various phytochemical constituents like alkaloids, terpenoids, saponins, phenols, flavonoids, whereas hexane solvent extract exhibits the presence of compound alkaloids and steroids.

These secondary metabolites possess diverse pharmacological as well as therapeutic properties, such as alkaloids decrease the acid secretion and inhibit the gastric motility.[20] Terpenoids provides a potential gastroprotective agents.[21] Tannins are recognized for their effectiveness in treating intestinal disorders and potential anticancer properties, steroidal compounds are effective in treating an antimicrobial activity. [22] Quinines have been known to possess remarkable antimalarial activity.[23] Saponins compounds possess an effective the defence mechanism by possessing an antimicrobial activity, flavonoids exhibit antioxidant, anti-inflammatory, antimicrobial, anticancer and antiallergic reactions, phenolic groups are good antioxidant activity.[24] Phenols and flavonoid are two significant group of phytochemicals exhibiting biological properties such as anti-inflammatory, antispasmodic, antiulcer, antidepressant, antidiabetic, antitumor, antimicrobial, and antioxidant properties, on the other hand steroid compounds possess remarkable antibacterial and insecticidal properties.[25]

From our study, through antibacterial activity screening on leaf extract of Impatiens latiflora hook f. & Thomson, it has shown a higher zone of inhibition on methanol solvent leaves extracts as compared to hexane solvent extracts. Evaluation of the methanol solvent extract antibacterial activity result is shown in Table 3 and Figure 2 (a). The maximum zone of inhibition is observed in the methanol solvent extract was on the gram-positive bacteria Bacillus subtilis with zone inhibition of 14±1.73 mm at the extract concentration 300 mg/ml and 250 mg/ml, whereas the bacteria Staphylococcus aureus exhibits the maximum inhibition zone 11.33±1 mm at extract concentration of 200 mg/ml and 150 mg/ml. Similarly, highest inhibition zone reported in the gram-negative bacteria Escherichia coli is 12.33±1.15 mm at extract concentration of 300 mg/ml and maximum inhibition zone observed on Klebsiella pneumoniae is 13±1.73 mm at 150 mg/ml extract concentration.

Antibacterial activity of Impatiens latiflora hook f. & Thomson leaf extract on hexane solvent is recorded in Table 4 and Figure 2 (b). The most effective antibacterial activity was seen in bacteria Bacillus subtilis with zone inhibition of 14±0 mm at 250 mg/ml extract concentration and Staphylococcus aureus exhibits the inhibition zone of 14±2 mm at extract concentration of 300 mg/ml. Similarly, highest antibacterial activity observed in gram-negative bacteria Escherichia coli with inhibition zone 14±1 mm at extract concentration of 300 mg/ml and Klebsiella

pneumoniae exhibit the highest inhibition zone 12.66±1.15 mm at 300 mg/ml. Result shows that bacteria belonging to gram-positive shows greater inhibition zone in comparison to gram-negative bacteria, this attributes to fact that gram-positive bacteria have higher susceptibility to chemical compounds and has simpler single cell wall in comparison to that of the three-layered cell wall of gram-negative bacteria.[26]

CONCLUSION

Traditionally, medicines obtained from plant source have been as a primary source of healing, making them valuable medicinal research today. The results from our study of Impatiens latiflora hook f. & Thomson leaf extracts, the screening of the phytochemical compounds exhibits the availability of the significant compounds like alkaloids, terpenoids, saponins and flavonoids from methanol solvent, whereas alkaloids and steroids were found to be available in hexane solvent extract. From antibacterial activity screening of methanol and hexane solvent extracts against the four selected bacteria, the most effective antibacterial activity was seen on the bacteria Bacillus subtilis. Overall, this study identified the presence important compounds and remarkable antibacterial activity providing evidence in supporting the traditional use of the medicinal plant Impatiens latiflora hook f. & Thomson in treating various illness and supports the potential lead to use as a natural antibacterial agent in therapeutic applications.

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Conflict of Interest

The authors declared that they have no conflicts of interest.

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