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Research Article

Investigating and Comparing the Phytochemical Screening and Potential Antibacterial Activity of Fresh and Dry Leaves Extracts of *Persicaria decipiens*

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ABSTRACT

Persicaria decipiens is a traditional medicinal plant to treat skin conditions such as ringworm and bites from snakes, dogs and insects used by the people of Nagaland, India. The study investigates and compares the phytoconstituents compounds and antibacterial activity of *P. decipiens* fresh and dry leaves extracts. Fresh and dry leaves of *P. decipiens* were extracted using hexane and methanol in a soxhlet apparatus. The extracts were investigated through phytochemical screening using standard procedures. The antibacterial efficacy of the extracts at various concentrations, 50, 100, 150, 200, 250 and 300 mg, was tested against *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and using the agar well diffusion method. DMSO was used as a negative control and ampicillin at 2 mcg was used as a standard positive control, respectively. The fresh leaf methanol and hexane extracts were more effective than the dry leaf methanol and hexane extracts against the test bacteria. The antibacterial activity of fresh leaf methanol extract was observed at 300 mg concentration with 21 ± 1 mm inhibition zone against *K. pneumoniae*, while for the dry leaf methanol extracts was observed at 300 mg concentration with a zone of inhibition of 7.67 ± 0.57 mm against *Bacillus subtilis*. The fresh hexane extracts were observed at 200 mg with a zone of inhibition of 16.66 ± 0.57 mm against *Bacillus subtilis*, while the dry leaf hexane extracts were observed at 300 mg with a 10 ± 1 mm zone of inhibition against *K. pneumoniae*. Phytochemical screening of the extracts results that both fresh and dried leaf methanol extracts contained alkaloids, tannins, saponins, phenols and flavonoids. Also, it is observed that the fresh leaf hexane extracts confirmed the presence of steroids, while the dried leaf hexane extract tested positive for tannins. These studies provide information about *P. decipiens* that contain excellent phytochemical compounds and fresh and dry leaf extracts can be used as an antibacterial agent.

INTRODUCTION

Nature, the ultimate artisan of molecular design, has produced a diverse array of molecular structures and serves as a resource for drug discovery, producing novel chemical compounds, pharmacophores, and frameworks that can be developed into treating various diseases and useful bioactive substances.^[1] For countless ages, natural remedies have been employed to enhance well-being, forming the cornerstone of modern medical advancements as many pharmaceutical breakthroughs originate from

natural sources.^[2] The World Health Organization (WHO) states that medicinal plants serve major source of numerous essential drugs that are nature's invaluable gift to humans. Approximately 80% of people use traditional medicine containing compounds extracted from medicinal plants in developed countries.^[3-6] Countless medicinal plant extracts have been acknowledged as valuable resources for their ethnopharmacological properties, which are effective in the treatment of many diseases that can be bacterial, fungal, or parasitic, among many others.

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[7,3] Throughout history, humans have depended on nature to fulfill their fundamental needs, such as medicines that can treat a wide range of diseases.^[8] Globally, infectious diseases remain the primary cause of mortality in both humans and animal in which antimicrobial agents significantly contributing in lowering the global burden of infectious diseases.^[9] Antimicrobial drugs have the most outstanding contribution to therapeutics.^[10] In many developing countries, medicine remains the mainstay of health care, employing local traditions and beliefs.^[11] The concern towards using traditional medicine and medicinal plants nowadays is given much importance in developing countries for maintaining good health, as they are effective against microorganisms.^[12] Medicinal plants are used in India, with most of the population relying on them. Although herbal medicine is widespread in India, only a few species have been screened for their biological activity.^[13] Considering the efficacy of medicinal plants as antibacterial agents, this study aims to extract, phytochemical screening and evaluate the antibacterial activity of the medicinal plant *Persicaria decipiens*, belonging to the Polygonaceae family, locally referred to as “Nampi” in the Ao dialect of Nagaland, India. It typically grows along the banks of rivers, canals, lakes, and roadsides in tropical and subtropical countries. In traditional medicine, its leaves are used to treat skin conditions such as ringworm and bites from snakes, dogs, or insects. Hence, more studies on using plants as therapeutic agents should be prioritized. The study determines the efficacy of plant extracts exhibiting antibacterial activity and phytochemical screening, which could serve as promising candidates for developing new antimicrobial agents and phytochemicals against standard microorganism strains.

MATERIALS AND METHODS

Collection of *P. decipiens* plant materials

Fresh and healthy *P. decipiens* leaves were collected from Tinpati of Kohima region, Nagaland, India (Fig. 1), in the month of May. The leaves of *P. decipiens* were washed with tap water about 2 to 3 times and placed in a cool place. Some of the leaves of the plant *P. decipiens* were air-dried for 3 weeks and were stored and labeled until further studies.

Preparation of *P. decipiens* extracts

P. decipiens fresh and dried leaves were extracted separately.

The fresh leaf of the plant *P. decipiens* was chopped into pieces with the help of a knife. The chopped leaf materials were initially packed in filter paper and were weighed using a digital weighing balance. A total of 54.303 g of chopped fresh leaves with 180 mL of hexane were



Fig. 1: *P. decipiens*.

extracted using a Soxhlet extractor and allowed to run for about 48 hours. Once the extraction was completed, the hexane solvent was evaporated using a rotary evaporator, resulting in a yield in the bottom glass flask. Similarly, the same process was reported for the fresh leaves with methanol solvents (180 mL). The extract yielded 0.484 g for the hexane solvent and 4.193 g for the methanol solvent, with a dark greenish residual mass for both the extracts. The dried leaves of *P. decipiens* were cut into fine pieces using scissors and were packed in filter paper. The dry leaves weigh a total of 12.187 g and were extracted with 180 mL of hexane solvent using a soxhlet extractor for 4 days. Once the extraction was completed, the hexane solvent was evaporated using a rotary evaporator, resulting in a yield extract (0.673 g). Similarly, the same process was followed for the dried leaves with methanol solvents (180 mL) and 0.457 g of yield was obtained.

Preparation of Various Concentrations of the Extracts

A solution of 300 mg was prepared by adding 300 mg of the extracts dissolved in 1-mL of DMSO. Each of the extracts of different concentrations, 250, 200, 150, 100, 50 mg, was prepared from the 300 mg solution.

Phytochemical Screening

Phytochemical screening for *P. decipiens* fresh and dry leaf of methanol and hexane extracts was carried out to determine the phytoconstituents, including steroids, terpenoids, tannins, alkaloids, quinines, saponins, phenols and flavonoids, using qualitative phytochemical procedures.^[14-17]

Test for Alkaloids

Take approximately 5 mL of the extract and add about 3 to 4 droplets of Marquis reagent. The Marquis reagent was prepared by adding 5 mL concentrated H₂SO₄ to 0.5 mL formaldehyde. Turbidity shows the presence of alkaloids.^[14]

Test for Terpenoids

To 1-mL concentrated H₂SO₄, add an equal volume of the extract and heat over a flame for approximately 2 minutes. A grayish color appearance shows the presence.^[15]



Table 1: Yields (g) of the produced extracts

Plant species	Local name	Conditions of the leaves	Solvent used for extraction	Extract yield (g)	Colour of the yield
<i>P. decipiens</i>	Nampi	Fresh leaves	Methanol	4.193	Dark greenish
		Fresh leaves	Hexane	0.484	Dark greenish
		Dry leaves	Methanol	0.457	Dark greenish
		Dry leaves	Hexane	0.673	Dark greenish

Table 2: Phytochemical screening of fresh and dried leaves methanol and hexane extracts

Sl. No.	Phytochemical Test	Fresh Leaf Extract		Dry Leaf Extract	
		Hexane	Methanol	Hexane	Methanol
1	Alkaloids	-	+	-	+
2	Terpenoids	-	-	-	-
3	Tannins	-	+	+	+
4	Saponins	-	+	-	+
5	Quinines	-	+	-	-
6	Steroids	+	-	-	-
7	Phenols	-	+	-	+
8	Flavonoids	-	+	-	+

(+) : presence of the compound and (-) : absence of the compound

Test for Tannins

To 1-mL FeCl₃, add an equal volume of the extract. A black color or blue-green shows the presence of tannins.^[15]

Test for Saponins

Take about 2 mL of the distilled water and add about 1-mL of the extract and shake it thoroughly. A 1-cm layer of foam shows the presence of saponins.^[15]

Test for Quinines

To 1-mL of 1% NaOH, add an equal volume of extract. The formation of a blue-green or red colour detected Quinines.^[15]

Test for Steroids

Take about 1-mL of the extract, add an equal amount of the chloroform and concentrated H₂SO₄. A red color in the lower chloroform layer detected steroids.^[15]

Test for phenols

To 2 mL distilled water, 1-mL extract was mixed and prepared, after which a few droplets of 10% ferric chloride were added to the test tube. A blue or green coloration shows the presence.^[16]

Test for flavonoids

To 2 mL of 10% lead acetate solution, add an equal volume of the extract. A yellow precipitate shows the presence.^[17]

Test microorganisms

The antibacterial activity of *P. decipiens* fresh and dry leaves extracts was assessed against four bacteria, namely *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumonia*.

Antibacterial Activity

The antibacterial activity of fresh and dry leaves of

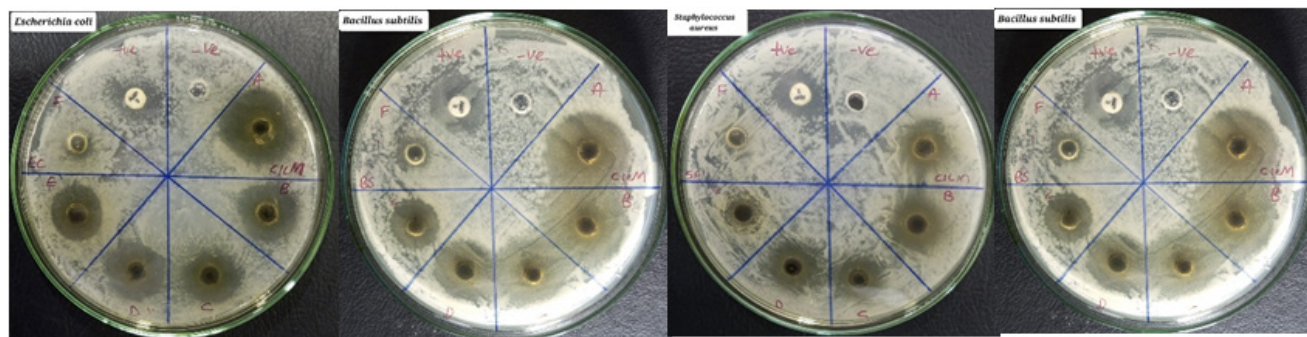


Fig. 2: Antibacterial activity of fresh leaf methanol extract in six different concentrations A: 300 mg, B: 250 mg, C: 200 mg, D: 150 mg, E: 100 mg, F: 50 mg, positive control (ampicillin) and negative control (DMSO) against *E. coli*, *K. pneumonia*, *S. aureus* and *B. subtilis* bacteria.

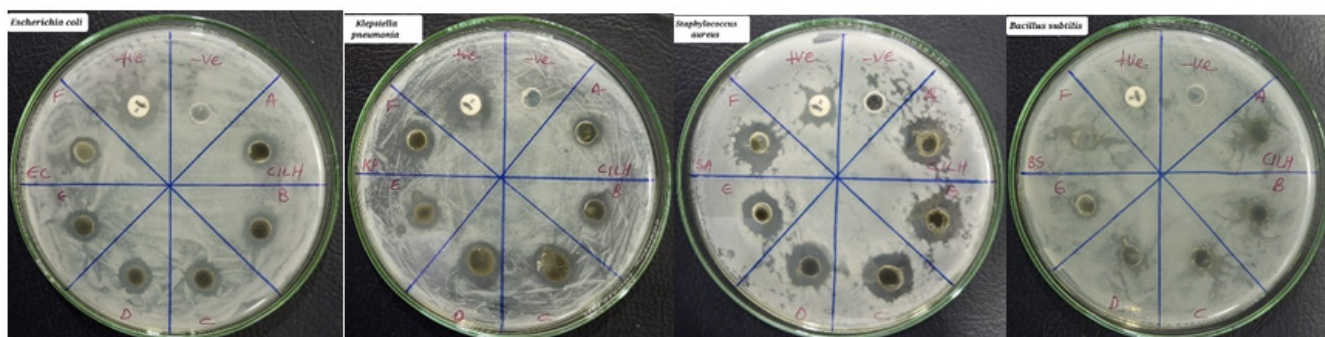


Fig. 3: Antibacterial activity of fresh leaf hexane extract in six different concentrations A: 300 mg, B: 250 mg, C: 200 mg, D: 150 mg, E: 100 mg, F: 50 mg, positive control (ampicillin) and negative control (DMSO) against *E. coli*, *K. pneumoniae*, *S. aureus* and *B. subtilis* bacteria.

P. decipiens methanol and hexane extracts against four bacterial strains was employed using the agar well diffusion procedure.^[18-19] The required quantities of nutrient agar were autoclave and prepared. After sterilization, nutrient agar was transferred to the petriplates and left to solidify for about half an hour. A sterile environment

was maintained throughout the experiment. The agar petriplate surface was inoculated by spreading 60 µL of the bacterial strains. 6 mm diameter hole was punched aseptically using the help of a sterile pipette tip, and 5 µL of the leaf extract of various concentration 50, 100, 150, 200, 250 and 300 mg is introduced into the well. Here,

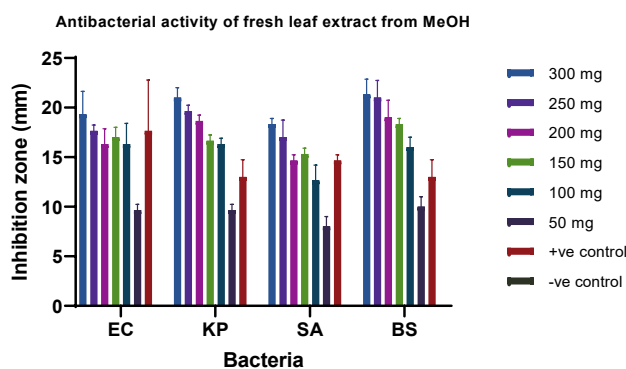


Fig. 4: Sensitivity of the four bacteria EC: *E. coli*, KP: *K. pneumoniae*, SA: *S. aureus* and BS: *B. subtilis* to fresh leaf methanol extract of *P. decipiens* at different concentrations, positive control (ampicillin) and negative control (DMSO).

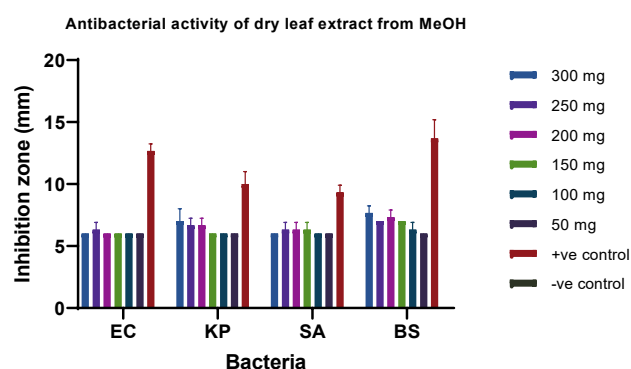


Fig. 6: Sensitivity of the four bacteria EC: *E. coli*, KP: *K. pneumoniae*, SA: *S. aureus* and BS: *B. subtilis* to dry leaf methanol extract of *P. decipiens* at different concentrations, positive control (ampicillin) and negative control (DMSO).

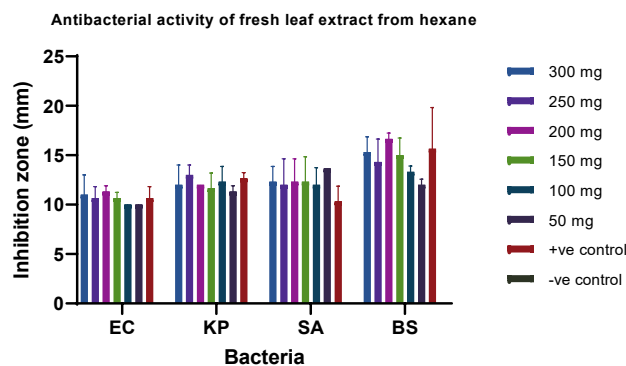


Fig. 5: Sensitivity of the four bacteria EC: *E. coli*, KP: *K. pneumoniae*, SA: *S. aureus* and BS: *B. subtilis* to fresh leaf hexane extract of *P. decipiens* at different concentrations, positive control (ampicillin) and negative control (DMSO).

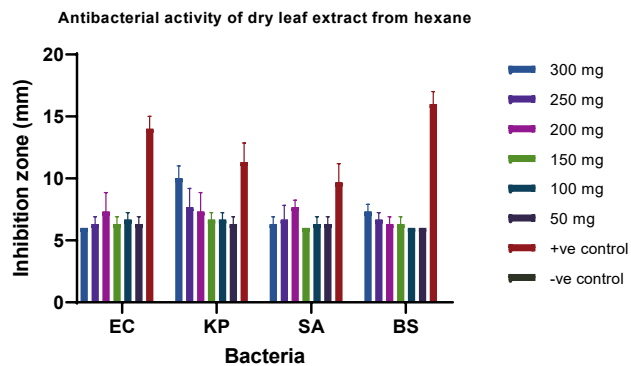


Fig. 7: Sensitivity of the four bacteria EC: *E. coli*, KP: *K. pneumoniae*, SA: *S. aureus* and BS: *B. subtilis* to dry leaf hexane extract of *P. decipiens* at different concentrations, positive control (ampicillin) and negative control (DMSO).



Table 3: Antibacterial activity observed in bacteria plates when treated with fresh and dry leaf of *P. decipiens*, methanol and hexane extracts of at different concentrations

Bacteria	Concentrations (mg)	Fresh leaf extract		Dry leaf extract		
		Methanol	Hexane	Methanol	Hexane	
EC	300	19.33 ± 2.30	11 ± 2	6 ± 0	6 ± 0	
	250	17.66 ± 0.57	10.66 ± 1.15	6.3 ± 0.57	6.33 ± 0.57	
	200	16.33 ± 1.52	11.33 ± 0.57	6 ± 0	7.33 ± 1.53	
	150	17 ± 1	10.66 ± 0.57	6 ± 0	6.33 ± 0.57	
	100	16.33 ± 2.08	10 ± 0	6 ± 0	6.67 ± 0.57	
	50	9.66 ± 0.57	10 ± 0	6 ± 0	6.33 ± 0.57	
	+ve control	17.66 ± 5.13	10.66 ± 1.15	12.67 ± 0.57	14 ± 1	
	-ve control	0 ± 0	0 ± 0	0 ± 0	0 ± 0	
	KP	300	21 ± 1	12 ± 2	7 ± 1	10 ± 1
		250	19.66 ± 0.57	13 ± 1	6.67 ± 0.57	7.67 ± 1.52
200		18.66 ± 0.57	12 ± 0	6.67 ± 0.57	7.33 ± 1.52	
150		16.66 ± 0.57	11.66 ± 1.52	6 ± 0	6.67 ± 0.57	
100		16.33 ± 0.57	12.33 ± 1.5	6 ± 0	6.67 ± 0.57	
50		9.66 ± 0.57	11.33 ± 0.57	6 ± 0	6.33 ± 0.57	
+ve control		13 ± 1.73	12.66 ± 0.57	10 ± 1	11.33 ± 1.52	
-ve control		0 ± 0	0 ± 0	0 ± 0	0 ± 0	
SA		300	18.33 ± 2.08	12.33 ± 1.52	6 ± 0	6.33 ± 0.57
		250	17 ± 1.73	12 ± 2.64	6.33 ± 0.57	6.67 ± 1.15
	200	14.66 ± 0.57	12.33 ± 2.30	6.33 ± 0.57	7.67 ± 0.57	
	150	15.33 ± 0.57	12.33 ± 2.51	6.33 ± 0.57	6 ± 0	
	100	12.66 ± 1.52	12 ± 1.73	6 ± 0	6.33 ± 0.57	
	50	8 ± 1	12 ± 0	6 ± 0	6.33 ± 0.57	
	+ve control	14.66 ± 0.57	10.33 ± 1.52	9.33 ± 0.57	9.67 ± 1.52	
	-ve control	0 ± 0	0 ± 0	0 ± 0	0 ± 0	
	BS	300	21.33 ± 1.52	15.33 ± 1.52	7.67 ± 0.57	7.33 ± 0.57
		250	21 ± 1.73	14.33 ± 2.30	7 ± 0	6.67 ± 0.57
200		19 ± 1.73	16.66 ± 0.57	7.33 ± 0.57	6.33 ± 0.57	
150		18.33 ± 0.57	15 ± 1.73	7 ± 0	6.33 ± 0.57	
100		16 ± 1	13.33 ± 0.57	6.33 ± 0.57	6 ± 0	
50		10 ± 1	13.66 ± 0.57	6 ± 0	6 ± 0	
+ve control		13 ± 1.73	15.66 ± 4.16	13.67 ± 1.52	16 ± 1	
-ve control		0 ± 0	0 ± 0	0 ± 0	0 ± 0	

Value expressed as mean diameter of inhibition zone ± standard deviation

for positive control, ampicillin at 2 mcg and for negative control, DMSO was employed. Then, the petriplates were placed in the incubator and maintained at 37°C for 12 hours duration. Antibacterial activity of the fresh and dry leaves of *P. decipiens* methanol and hexane extracts observed in the bacteria plates was measured by the zone

of inhibition (mm). The experiments were performed three times independently and were recorded.

Statistical analysis

To ensure the independence and correlation of the results, all the measurements were replicated three times. The

results were expressed as the mean diameter of the inhibition zone (mm) with the corresponding standard deviation.

RESULTS

Plant extract preparation

An enough yield of each extract was produced to be used in the phytochemical screening and antibacterial activity, with a yield of 4.193 g from fresh leaf methanol extracts, 0.484 g from fresh leaf hexane extracts, 0.673 g from dried leaf methanol extract and 0.457 g from dried leaf hexane extract, as shown in Table 1.

Phytochemical analysis

The phytochemical analysis of *P. decipiens* fresh and dried leaves extracts (methanol and hexane), which were evidenced by remarkable colour changes and formation of precipitate, is shown in Table 2. From the qualitative findings presented in Table 2, *P. decipiens* fresh leaf methanol extracts were observed to show positive for alkaloids, tannins, saponins, quinines, phenols, flavonoids, but terpenoids and steroids were absent. In contrast, for dried leaves, methanol extract phytochemical compounds such as phenols, saponins, alkaloids, flavonoids and tannins were recorded as present. Also, it is observed that the fresh leaf hexane extracts confirmed the presence of steroids, while the dried leaf hexane extract tested positive for tannins.

Antibacterial activity of extracts

The inhibitory effects of *P. decipiens* fresh and dry leaf methanol and hexane extracts at various concentrations against *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Escherichia coli* were determined as illustrated in Figs 2-7. The recorded values are presented in Table 3.

Dried methanol extracts of leaves against the tested bacterial strains showed the highest antibacterial activity at a concentration of 300 mg with an inhibition zone of 7.67 ± 0.57 mm against *B. subtilis*. 7 ± 1 mm zone of inhibition was reported at a concentration 300 mg against *K. pneumoniae*, 6.33 ± 0.57 mm of inhibition zone was observed at 200 and 150 mg concentration against *Staphylococcus aureus* and 6.3 ± 0.57 mm of inhibition zone was observed at 250 mg concentration against *E. coli* (Fig. 6). The dried hexane extracts of leaves exhibited highest antibacterial activity at 300 mg concentration with inhibition zone 10 ± 1 mm against *K. pneumoniae*, 7.67 ± 0.57 mm of inhibition zone was reported at 200 mg concentration against *S. aureus*, 7.33 ± 0.57 mm of inhibition zone was reported at 200 mg concentration against *B. subtilis* and 7.33 ± 1.53 mm of inhibition zone was reported at 200 mg concentration against *E. coli* (Fig. 7). For the fresh leaves methanol extract shows highest antibacterial activity at 300 mg concentration with inhibition zone 19.33

± 2.30 mm against *E. coli*, 21 ± 1 mm of inhibition zone was observed at 300 mg concentration against *K. pneumoniae*, 21.33 ± 1.52 mm of inhibition zone was observed at 300 mg concentration and 18.33 ± 2.08 mm zone of inhibition was reported at a concentration 300 mg against *S. aureus* (Fig. 2 and 4).

The fresh leaves hexane extract shows highest antibacterial activity at 200 mg concentration with inhibition zone 11.33 ± 0.57 mm against *E. coli*, 13 ± 1 mm of inhibition zone was observed at 250 mg concentration against *K. pneumoniae*, 16.66 ± 0.57 mm of inhibition zone was observed at 200 mg concentration against *B. subtilis*, 12.33 ± 1.52 mm of inhibition zone was reported at a concentration of 300 mg against *S. aureus* (Fig. 3 and 5). Both the fresh leaf methanol and hexane extracts show greater inhibition than that of the standard drug, ampicillin. The results indicate that both fresh and dried methanol and hexane extracts of *P. decipiens* recorded good zones of inhibition against *B. subtilis*, *E. coli*, *K. pneumoniae* and *S. aureus*.

DISCUSSION

Reports from the phytochemical screening of *P. decipiens* leaves show phytoconstituents such as quinines, tannins, flavonoids, phenols, alkaloids, and saponins were abundant in fresh leaf methanol extract. In contrast, phytochemical tests saponins, flavonoids, tannins, phenols and alkaloids were present in the dry leaf methanol extract. Also, it is observed that the fresh leaf hexane extracts confirmed the presence of steroids, while the dried leaf hexane extract tested positive for tannins. The variations in phytochemical levels between fresh and dried leaf extracts can be attributed to several factors, such as the drying process, storage conditions, and plant chemical composition. Drying can affect the stability and concentration of certain phytochemicals, leading to either an increase or a decrease in their levels.^[20] The phytoconstituents were identified as biologically active compounds that were present in both the fresh and dried methanol and hexane extracts and are responsible for various activities. Numerous studies reported that medicinal plants are rich in essential oils, flavonoids, coumarins, alkaloids, lectins, tannins, polypeptides, terpenoids, polyacetylenes, and phenolics, etc. Phenolic compounds are recognized for anti-mutagenic, antiviral, antimicrobial activities and antioxidant.^[21] Enenebeaku and colleagues conducted a scientific study demonstrating the efficacy of tannins, saponins, and flavonoids in neutralizing the lethal effects of venom through in-vivo assays.^[22] Since tannins, saponins, phenols, and flavonoids are known for their enzyme-inhibiting and protein-binding properties, they can inhibit the phospholipase A2 activity present in cobra venom.^[23] The present study also identified significant phytochemical constituents such as saponins, flavonoids and tannins in *P. decipiens* leaves extracts, in which these compounds may have



contributed to treating the infectious diseases from bites of snakes, dogs and insects. Fresh leaf methanol and hexane extracts had stronger antibacterial properties than dried leaf methanol and hexane extracts. The difference in activity between dry and fresh extracts may be due to the samples' processing, drying, or extraction procedures. Some active chemicals from the dry samples might have evaporated or been damaged during the preparation of the extracts.^[24] This explains why all the dry leaf extracts shows low inhibition zone than the fresh leaf extracts for the antibacterial activity. The result also observed that the fresh leaf methanol and hexane extracts were more effective against the bacteria than the positive control due to the fact that the extract has a mix of bioactive compounds, while the standard drug only has one pure compound.^[25]

CONCLUSION

In conclusion, the medicinal plant *P. decipiens* tested in this study possess phytoconstituents compounds such as tannins, steroids, saponins, alkaloids, phenols, quinones and flavonoids, which determine its physiological properties and medicinal value. The antibacterial study of *P. decipiens* fresh and dried leaves, methanol and hexane extracts at different concentrations, observed that the fresh leaves' methanol extract exhibits the highest antibacterial activity against the test bacteria. These studies validate that the *P. decipiens* leaves have pharmacological applications and have antibacterial activity. Additionally, plant based antimicrobials agent possess numerous medicinal potential and effectively serve the intended purpose with fewer side effects than the synthetic-based antimicrobials.

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