

Original Research Article

Evaluation of the antimicrobial activity of different solvent extracts of *Ipomoea littoralis* (Blume): a naturally growing medicinal plant in Sri Lanka.

ABSTRACT

Aims: The emergence of drug-resistant microbial pathogens has become a global health burden. Hence there is a timely need to discover novel anti-microbial agents. The aim of the current study was evaluate the anti-microbial potential of different extracts of *Ipomoea littoralis* against some pathogens causing gastro-intestinal tract infections.

Study design: Experimental study

Place and Duration of Study: Department of Basic Sciences at Faculty of Allied Health Sciences and Research Laboratory at Faculty of Medicine, General Sir John Kotelawala Defence University, Ratmalana, Sri Lanka, between July 2018 and November 2018.

Methodology: The aqueous, methanol, acetone and hexane extracts were prepared with the leaves, roots and stem of the plant *Ipomoea littoralis* separately. The agar well diffusion method and broth dilution method were applied in order to screen the anti -microbial activity of each test extract against the *Escherichia coli*, *Salmonella enterica*, *Shigella dysenteriae*, *Candida albicans* and *Staphylococcus aureus*. Statistical comparisons were made using Duncan's new multiple range test. Significance was set at $P = .05$.

Results: The zone of inhibition of most of the test extracts showed a significant ($P = .05$) difference when compared with the negative control, suggesting that majority of the extracts of the selected plant material are active against the tested pathogens. The observed lowest MIC value was 31.25 mg/ml, while the highest MIC value was 250 mg/ml. Aqueous and acetone extracts of stem showed the lowest MIC value against *E. coli*, while methanol and acetone leaves extracts showed highest inhibition against *S. enterica*. The MIC value was 31.25 mg/ml against *S. aureus* by aqueous stem, hexane leaves and methanol stem extracts. The aqueous stem, hexane roots and the acetone leaves extract showed the lowest (31.25 mg/ml) MIC value against *C. albicans*. The MIC value was 31.25 mg/ml for methanol leaves and stem extract against *S. dysenteriae*.

Conclusion: The anti- microbial potency of different solvent extracts of the plant *I. littoralis* is varied against different pathogens causing gastro-intestinal tract infections.

Keywords: *Ipomoea littoralis*, Gastro-intestinal tract infections, Agar well diffusion method, Broth dilution method

1. INTRODUCTION

A Microbe is an organism that is microscopic which is too small to be seen by the naked eye. They include a massive range of organisms including bacteria, fungi, viruses, algae, archaea and protozoa. They exist almost everywhere on earth and play a critical role in maintaining the balance of the ecosystems [1]. Microbes are found in and outside of the human body and they are closely related to every aspect of human life. Some microbes are beneficial to humans which act as the key element in food preparation, purification of waste water, reducing atmospheric nitrogen and transform it to ammonia important for agriculture, etc. Certain microbes provide a source of antibiotics and vaccines, which are important to maintain the healthy life [2]. The species which live in association with human body surfaces are called the normal flora and involve in, protection of the host from infections or promoting nutrition and health. Thus majority of them are harmless while some of them are detrimental to the human life. They are called pathogens which invade the human body and cause infectious diseases which lead to the mortality of millions of people every year [1].

Antibiotics is a group of chemicals that used to treat bacterial infections. There are two main mode of actions of antibiotics. The agents which kill bacteria are called "bactericidal", while the antibiotics that stop the growth of pathogen are called "bacteriostatic". Usually they are synthesized in nature by soil bacteria and fungi. There are several mechanisms that the antibacterial chemicals exert their activity [3].

When pathogens are no longer inhibited by an antibiotic to which they were previously sensitive, it is known as antibiotic resistance. Bacteria become drug-resistant due to over-use or misuse of antibiotics by humans. The emergence and spread of new antibacterial-resistant bacteria continue to grow everyday all around the world. Therefore, currently it has become a global health threat as well as an economic burden [3].

Hence, there is a timely need for the discovery of new agents which are able to suppress the growth of drug-resistant pathogenic bacterial strains. Thus researchers focus their interest towards the investigation for new natural sources, which can provide promising anti-bacterial active chemicals [3]. World Health Organization has been stated, plants as a reliable source to discover novel therapeutic agents which are cheaper as well as lack of side effects [4]. The scientific findings revealed that, plants contain phytochemicals which are responsible for the curative properties of many diseases [5].

Plants have been used to treat various diseases and maintain the human health from ancient times. Due to the medicinal value of many plants, they have been incorporated in to the medicines prepared by traditional medical practitioners [6]. Thus plants played a major role in traditional medicine systems around the world. The plants are using to treat different ailments in humans, which are prepared in different forms, including decoctions, ointments, etc. [7].

Sri Lanka is a hotspot of biodiversity, which possess number of plants with different medicinal values which provide a huge natural source for the investigation of new medicinal agents. Sri Lankan Traditional Medicine practitioners have been used different herbs in order to treat and control different types of infections. The current study was designed to screen the antimicrobial activity of a free growing medicinal plant in Sri Lankan rural areas. The different solvent extracts of leaves, roots and stem of *Ipomoea littoralis* were screened for the anti -microbial activity against the pathogenic species causing gastro-intestinal tract infections including *Salmonella enterica*, *Staphylococcus aureus*, *Escherichia coli*, *Shigella dysenteriae* and *Candida albicans*.

Majority of *E. coli* strains are harmless. But some kinds of *E. coli* can cause diarrhea, urinary tract infections, respiratory illness, pneumonia and other illnesses [8]. *Salmonella* spp. is a group of gram-negative rod shape bacteria, cause diseases such as salmonellosis, gastroenteritis, typhoid fever and paratyphoid fever in humans [9]. *Shigella* is also a gram-negative, facultative anaerobic, nonspore-forming, non-motile, rod-shaped bacterial group, which cause severe diarrhea associated with passage of watery stool mixed with blood [10]. *Staphylococcus aureus* is a sphere-shaped (coccal), gram-positive bacterium, which often cause skin infections, pneumonia, heart valve infections, and bone infections. Some strains produce toxins that can cause staphylococcal food poisoning, toxic shock syndrome, or scalded skin syndrome [11]. *Candida albicans* is an opportunistic fungus, usually living in normal flora along the digestive tract. *Candida* infection entered in to the bloodstream can spread all over the human body and affect the kidneys, heart, lungs, eyes, or other organs causing high fever, chills, anemia, and sometimes a rash or shock [12].

Ipomoea littoralis is a medicinal plant belongs to family convolvulaceae. It is a free growing vine in rural areas of Sri Lanka, which is commonly consumed by the rural inhabitants, as a green vegetation as well as a medicinal plant [13]. Mainly leaves and roots are used for the ayurvedic treatments to cure gastritis, cough, asthma, kidney disease and liver disease [13].

Previous studies on *Ipomoea* spp reported that they possess many different bioactivities including anti-microbial activity. *I. batatas* (sweet potato) is a medicinal plant which uses to treat the inflammation and oral disease. Investigators revealed that leaves of the plant contain phytochemicals such as triterpenes, steroids, alkaloids, coumarins, flavonoids, saponins, tannins and phenolic acids. *I. batatas* leaves extracted with 70% ethanol showed a promising anti-microbial activity against *S. mutans*, *S. mitis*, *S. aureus* and *C. albicans* [15]. The leaves of *I. carnea* also contain various phytochemicals such as glucosides, alkaloids, flavonoids, fatty acid, alcohol and tannins. Bio synthetic method used to find out the activity against some pathogens indicated that the crude acetone extraction was active against *Proteus vulgaris*, while crude ethanol extracts elucidate anti-microbial activity against *Pseudomonas aeruginosa*. The ethyl acetate, acetone, ethanol and acetone fractions of leave extraction showed active against *Salmonella typhi* and *Proteus vulgaris*, *Alternaria alternate* and *Curvularia lunata* [16].

Fresh leaves, stem and seeds of *I. Obscura* contain steroids, alkaloids, phenolics, and flavonoids. Chloroform, acetone, alcohol, water and ether extractions are active against *Staphylococcus aureus*, *Bacillus subtilis* and *Rhodococci* while not active against *E-coli*, *Proteous vulgaris*, *Pseudomonas* and *Salmonella* [17]. Roots of *I. aquarica* showed medicinal effects on liver disease, eye disease, and constipation. Diuretic activity has been proved in *I. aquatic* when investigated in the swiss albino mice [18]. Aqueous extract from *I. cairica* showed Anti-Respiratory syncytial virus activity *in vitro*. The ethanolic extract of this plant presents an anti-nociceptive effect [18]. Methanolic extracts from the seeds of the *I. indica* showed biological activity against Herpes simplex. Methanolic acid/aqueous extract from the seeds of this species were also reported for anti-bacterial activity against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E. coli*. [18].

Although the other species belong to the genus *Ipomoea* were extensively studies, *I. littoralis* was overlooked. Therefore the current study was designed to screen the anti-microbial activity of different solvent extracts of the plant *I. littoralis* against common pathogenic bacteria and fungus, in order to validate its medicinal properties.

2. EXPERIMENTAL DETAILS

2.1 Collection of Plant Material

Different parts of the plant were collected from Kurunagala and Kegalle district, Sri Lanka during the period between July 2018 and August 2018. The plant materials were authenticated by National Herbarium, Peradeniya, Sri Lanka and the voucher specimen (KDU/FAHS/2018/0102) is deposited in the herbarium of Faculty of Allied Health Sciences, Kotelawala Defence University.

2.2 Preparation of Extracts

The plant materials were dried in open air separately and powdered using an electrical grinder. Each sample was macerated for 7 days in different solvents including distilled water, methanol, acetone and hexane. The plant extracts were freeze dried and stored under 8 °C until using for experiments.

2.3 Screening for anti-microbial activity

Salmonella enterica (ATCC 14028), *Shigella dysenteriae* (ATCC 11835), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Candida albicans* (ATCC 10231) were used to screen the anti-microbial activity of plant samples.

2.3.1 Qualitative Screening- Agar well diffusion Method

The stored samples were re-suspended in respective solvents and used for the experiments. Standard bacterial inocula were prepared by direct colony method. The each inoculum was prepared by making a direct saline suspension of isolated colonies selected from an 18 - 24 h agar plate. Then the suspension was adjusted to achieve a turbidity equivalent to a 0.5 McFarland turbidity standard. This results in a suspension containing approximately $1 - 2 \times 10^8$ CFU/ml.

Then each bacterial suspension (50 ul) were spread on the agar plate surface using a sterile spreader. Four wells with a diameter of 5 mm were punched aseptically on each agar plate. Gentamycin was used as a positive control. The solvent used to prepare each extract was used as the respective negative control. These wells in each plate were filled with (100 ul) of test extract (250 mg/ml), positive control and respective solvent. After 24 h incubation at 37°C the diameter of the zone of inhibition around each well was measured using a vernier caliper. This procedure was performed for all the selected microbial species. The procedure was repeated for 3 time for each test extract.

2.3.2 Quantitative Screening - Broth dilution Method

A two-fold dilution series of each test extract was prepared (500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml and 31.25 mg /ml) using freeze dried samples. Five sets of dilution series of each test extract were prepared one for each microbial species. Broth without antimicrobial agent was prepared for each test organism as the growth control tube. Gentamycin (0.1 mg/ml) was used as the positive control. Within 15 minutes of preparation,

the standardized inoculum of each pathogen was diluted using the broth so that, after inoculation, each tube contains approximately 5×10^5 CFU/ml.

The adjusted inoculum (1 ml) was added to each tube containing 1 ml of each test extract in the dilution series and mixed. The tubes were closed with loose screw-caps, plastic or metal closure caps, or cotton plugs and incubated at 37°C for 24 h. The Minimum Inhibitory Concentration (MIC) is the lowest concentration of antimicrobial agent that completely inhibits growth of the organism in the tubes as detected by the unaided eye. The turbidity of the suspension of each tube containing the antibiotic dilution series was compared with the respective growth-control tubes.

2.4 Statistical Analysis

The results were given as mean \pm SEM. Data analysis was performed by SPSS version 21.0. Statistical comparisons were made using Duncan's new multiple range test. Significance was set at $P = .05$.

3. RESULTS AND DISCUSSION

3.1 Agar well diffusion for different solvent extracts of *I. littoralis*

According to the results (Table 1) the highest diameter of zone of inhibition against *S. dysenteriae* was shown by methanol extract of leaves. Except few extracts, most of the test extracts showed a significant inhibition ($P = .05$), compared to the negative control. Observed inhibition among test extracts was not significant ($P > .05$) different between them. All the extracts showed a significant difference ($P = .05$), compared to the positive control.

Table 1. Diameter of zone of inhibition (mm) for different solvent extracts of *Ipomoea littoralis* against *S. dysenteriae*

| Part of the plant | Extraction | Negative control | Test extract | Positive control |
|-------------------|------------|------------------|----------------------|--------------------|
| Root | Methanol | 5.02 ± 0.01 | $5.79 \pm 0.38^{*a}$ | $13.59 \pm 0.48^*$ |
| | Aqueous | 5.01 ± 0.02 | 5.39 ± 0.32^a | $14.99 \pm 0.26^*$ |
| | Acetone | 5.02 ± 0.02 | $5.85 \pm 0.39^{*a}$ | $15.12 \pm 0.24^*$ |
| | Hexane | 5.03 ± 0.01 | $5.79 \pm 0.38^{*a}$ | $14.98 \pm 0.18^*$ |
| Leaves | Methanol | 5.03 ± 0.02 | $5.99 \pm 0.50^{*a}$ | $13.52 \pm 0.48^*$ |
| | Aqueous | 5.02 ± 0.01 | 5.05 ± 0.02^a | $14.99 \pm 0.17^*$ |
| | Acetone | 5.03 ± 0.01 | $5.79 \pm 0.36^{*a}$ | $14.39 \pm 0.14^*$ |
| | Hexane | 5.01 ± 0.02 | $5.72 \pm 0.33^{*a}$ | $13.58 \pm 0.39^*$ |

| | | | | |
|------|----------|-------------|---------------------------|---------------|
| Stem | Methanol | 5.02 ± 0.01 | 6.52 ± 0.23 ^{*a} | 14.59 ± 0.34* |
| | Aqueous | 5.02 ± 0.02 | 5.05 ± 0.02 ^a | 14.79 ± 0.12* |
| | Acetone | 5.01 ± 0.03 | 5.85 ± 0.39 ^{*a} | 14.99 ± 0.25* |
| | Hexane | 5.01 ± 0.02 | 5.05 ± 0.02 ^a | 13.72 ± 0.33* |

* Significant compared to negative control (P = .05), ^a Significant compared to positive control (P = .05).

When considering the observed values for the diameter of zone of inhibition (Table 2), the methanolic and acetone leaf extracts showed the maximum anti-microbial activity against *S. enterica*. Majority of test extracts showed a significant inhibition (P = .05) against the pathogen. But when compared the observed zone inhibition values among the test extracts against the pathogen there was no significant (P > .05) difference between the values. When compared to the observed values for positive control (Gentamycin), all the extracts showed a significant difference (P = .05).

Table 2. Diameter of zone of inhibition (mm) for different solvent extracts of *I. littoralis* against *S. enterica*

| Part of the plant | Extraction | Negative control | Test extract | Positive control |
|-------------------|------------|------------------|---------------------------|------------------|
| Root | Methanol | 5.03 ± 0.02 | 5.97 ± 0.54 ^{*a} | 14.70 ± 0.12* |
| | Aqueous | 5.02 ± 0.02 | 5.97 ± 0.53 ^{*a} | 13.04 ± 0.30* |
| | Acetone | 5.01 ± 0.01 | 5.77 ± 0.38 ^{*a} | 13.50 ± 0.12* |
| | Hexane | 5.02 ± 0.03 | 5.04 ± 0.01 ^a | 13.57 ± 0.06* |
| Leaves | Methanol | 5.02 ± 0.02 | 6.10 ± 0.55 ^{*a} | 13.30 ± 0.25* |
| | Aqueous | 5.02 ± 0.01 | 5.37 ± 0.33 ^a | 13.57 ± 0.06* |
| | Acetone | 5.03 ± 0.01 | 6.12 ± 0.07 ^{*a} | 12.91 ± 0.19* |
| | Hexane | 5.01 ± 0.03 | 5.64 ± 0.31 ^{*a} | 14.57 ± 0.14* |
| Stem | Methanol | 5.02 ± 0.01 | 5.90 ± 0.45 ^{*a} | 14.50 ± 0.46* |
| | Aqueous | 5.03 ± 0.03 | 5.90 ± 0.48 ^{*a} | 14.64 ± 0.31* |
| | Acetone | 5.03 ± 0.01 | 5.77 ± 0.37 ^{*a} | 14.70 ± 0.24* |

| | | | |
|--------|-------------|--------------------------|---------------|
| Hexane | 5.01 ± 0.02 | 5.04 ± 0.01 ^a | 14.11 ± 0.29* |
|--------|-------------|--------------------------|---------------|

Significant compared to negative control (P = .05), ^a Significant compared to positive control (P = .05).

According to the obtained results, the diameter of zone of inhibition against *S. aureus* for all the test extracts were significantly different (P = .05) from the values obtained for negative control as well as the positive control. Among them the highest activity was shown by hexane extract of leaves and aqueous extracts of stem. There was no significant difference (P > .05) between the values of inhibition diameter among the active extracts.

Table 3. Diameter of zone of inhibition (mm) for different solvent extracts of *I. littoralis* against *S. aureus*

| Part of the plant | Extraction | Negative control | Test extract | Positive control |
|-------------------|------------|------------------|---------------------------|------------------|
| Root | Methanol | 5.01 ± 0.03 | 6.88 ± 0.20 ^{*a} | 15.21 ± 0.29* |
| | Aqueous | 5.02 ± 0.02 | 6.01 ± 0.47 ^{*a} | 13.81 ± 0.12* |
| | Acetone | 5.01 ± 0.02 | 5.88 ± 0.48 ^{*a} | 15.01 ± 0.19* |
| | Hexane | 5.03 ± 0.01 | 6.41 ± 0.16 ^{*a} | 14.75 ± 0.27* |
| Leaves | Methanol | 5.01 ± 0.03 | 6.61 ± 0.08 ^{*a} | 13.94 ± 0.60* |
| | Aqueous | 5.03 ± 0.01 | 6.35 ± 0.19 ^{*a} | 14.41 ± 0.15* |
| | Acetone | 5.02 ± 0.02 | 6.35 ± 0.07 ^{*a} | 13.88 ± 0.18* |
| | Hexane | 5.01 ± 0.03 | 7.21 ± 0.27 ^{*a} | 14.48 ± 0.20* |
| Stem | Methanol | 5.01 ± 0.03 | 7.01 ± 0.38 ^{*a} | 15.28 ± 0.09* |
| | Aqueous | 5.02 ± 0.02 | 7.21 ± 0.27 ^{*a} | 14.21 ± 0.29* |
| | Acetone | 5.01 ± 0.02 | 6.35 ± 0.16 ^{*a} | 14.55 ± 0.40* |
| | Hexane | 5.03 ± 0.01 | 5.75 ± 0.32 ^{*a} | 14.41 ± 0.14* |

Significant compared to negative control (P = .05), ^a Significant compared to positive control (P = .05).

All the test extracts exerted a significant inhibition (P = .05) against *E. coli* (Table 4) except the aqueous and hexane extracts of the roots. Among them the highest activity was shown by aqueous extract of stem. But there was no significant difference (P > .05) between the values of inhibition diameter among the active extracts. However, when compared to the

observed values for respective positive control, all the extracts showed a significant difference (P =.05).

Table 4. Diameter of zone of inhibition (mm) for different solvent extracts of *I. littoralis* against *E.coli*

| Part of the plant | Extraction | Negative control | Test extract | Positive control |
|-------------------|------------|------------------|---------------------------|------------------|
| Root | Methanol | 5.02 ± 0.01 | 6.25 ± 0.19 ^{*a} | 14.91 ± 0.20* |
| | Aqueous | 5.01 ± 0.02 | 5.45 ± 0.39 ^a | 14.98 ± 0.16* |
| | Acetone | 5.00 ± 0.03 | 5.85 ± 0.39 ^{*a} | 15.18 ± 0.30* |
| | Hexane | 5.02 ± 0.02 | 5.05 ± 0.02 ^a | 14.24 ± 0.27* |
| Leaves | Methanol | 5.02 ± 0.01 | 6.11 ± 0.56 ^{*a} | 15.18 ± 0.26* |
| | Aqueous | 5.02 ± 0.01 | 6.05 ± 0.52 ^{*a} | 14.31 ± 0.19* |
| | Acetone | 5.01 ± 0.03 | 6.31 ± 0.05 ^{*a} | 13.98 ± 0.34* |
| | Hexane | 5.03 ± 0.02 | 6.11 ± 0.05 ^{*a} | 14.91 ± 0.20* |
| Stem | Methanol | 5.01 ± 0.01 | 5.90 ± 0.45 ^{*a} | 14.50 ± 0.46* |
| | Aqueous | 5.02 ± 0.02 | 6.98 ± 0.16 ^{*a} | 14.84 ± 0.19* |
| | Acetone | 5.03 ± 0.02 | 6.91 ± 0.35 ^{*a} | 14.98 ± 0.20* |
| | Hexane | 5.02 ± 0.02 | 6.38 ± 0.14 ^{*a} | 14.51 ± 0.14* |

* Significant compared to negative control (P =.05), ^a Significant compared to positive control (P =.05).

The highest inhibition against *C. albicans* (Table 5) was shown by acetone extract of leaves. Most of the test extracts exerted a significant inhibition (P =.05) except the methanol extracts of the roots and leaves, when compared with the negative control. But there was no significant difference (P > .05) between the values of inhibition diameter among the active extracts. However, when compared to the observed values for respective positive control, all the extracts showed a significant difference (P =.05).

Table 5. Diameter of zone of inhibition (mm) for different solvent extracts of *I. littoralis* against *C. albicans*

| Part of the plant | Extraction | Negative control | Test extract | Positive control |
|-------------------|------------|------------------|----------------------------|------------------|
| Root | Methanol | 5.02 ± 0.02 | 5.41 ± 0.33 ^a | 14.07 ± 0.60* |
| | Aqueous | 5.02 ± 0.01 | 6.14 ± 0.05 ^{**a} | 14.67 ± 0.58* |
| | Acetone | 5.03 ± 0.02 | 6.34 ± 0.21 ^{**a} | 13.60 ± 0.03* |
| | Hexane | 5.01 ± 0.03 | 6.81 ± 0.33 ^{**a} | 15.01 ± 0.20* |
| Leaves | Methanol | 5.01 ± 0.02 | 5.47 ± 0.40 ^a | 14.54 ± 0.49* |
| | Aqueous | 5.02 ± 0.02 | 5.80 ± 0.38 ^{**a} | 13.41 ± 0.20* |
| | Acetone | 5.01 ± 0.03 | 6.94 ± 0.16 ^{**a} | 13.60 ± 0.57* |
| | Hexane | 5.02 ± 0.01 | 6.54 ± 0.05 ^{**a} | 13.07 ± 0.31* |
| Stem | Methanol | 5.03 ± 0.01 | 6.47 ± 0.21 ^{**a} | 14.60 ± 0.50* |
| | Aqueous | 5.02 ± 0.02 | 6.74 ± 0.33 ^{**a} | 14.80 ± 0.21* |
| | Acetone | 5.02 ± 0.01 | 5.74 ± 0.30 ^{**a} | 14.00 ± 0.31* |
| | Hexane | 5.03 ± 0.01 | 6.00 ± 0.51 ^{**a} | 12.24 ± 0.30* |

^a Significant compared to negative control (P = .05), * Significant compared to positive control (P = .05).

3.2 Broth dilution Method for different solvent extracts of *I. littoralis*

According to the obtained results, the observed lowest MIC value was 31.25 mg/ml, while the highest MIC value was 250 mg/ml (Table 6). Aqueous and acetone extracts of stem showed the lowest MIC value of 31.25 mg/ml against *E. coil*, suggesting that mainly the stem of the plant possess the maximum inhibitory action against *E. coil*.

The lowest MIC value (31.25 mg/ml) was showed against *S. enterica* by methanol and acetone leaves extracts, indicating highest anti-bacterial effect on *S. enterica* is exerted by the leaves extracts. The MIC value was 31.25 mg/ml against *S. aureus* by aqueous stem, hexane leaves and methanol stem extracts. This reports that both stem and leaves may contain the anti-bacterial compounds which are active against *S. aureus*.

The aqueous stem, hexane roots and the acetone leaves extract showed the lowest (31.25 mg/ml) MIC value against *C. albicans* suggesting that all three parts of the plant possess anti-microbial effect against *C. albicans*. The lowest MIC Value (31.25 mg/ml) was showed by methanol leaves and methanol stem extract against *S. dysenteriae*, indicating that the solvent methanol extracts the potent anti-bacterial agents from the plant materials.

Table 6. Observed MIC values for different solvent extracts of *I. littoralis* against tested pathogens

| Extract | Part of the plant | Micro organism | | | | |
|----------|-------------------|--------------------------|-------------------------------|-------------------------------|-----------------------------|----------------------------------|
| | | <i>E-coli</i> (mg/ml) | <i>S. enterica</i> (mg/ml) | <i>C. albicans</i> (mg/ml) | <i>S. aureus</i> (mg/ml) | <i>S. dysenteriae</i> (mg/ml) |
| Aqueous | Leaves | 62.5 | 250 | 125 | 125 | 125 |
| | Roots | 125 | 125 | 62.5 | 125 | 125 |
| | Stem | 31.25 | 62.5 | 31.25 | 31.25 | 250 |
| Hexane | Leaves | 62.5 | 125 | 62.5 | 31.25 | 62.5 |
| | Roots | 250 | 250 | 31.25 | 62.5 | 62.5 |
| | Stem | 62.5 | 250 | 62.5 | 250 | 250 |
| Methanol | Leaves | 62.5 | 31.25 | 125 | 62.5 | 31.25 |
| | Roots | 62.5 | 62.5 | 125 | 62.5 | 62.5 |
| | Stem | 125 | 62.5 | 62.5 | 31.25 | 31.25 |
| Acetone | Leaves | 62.5 | 31.25 | 31.25 | 125 | 62.5 |
| | Roots | 125 | 125 | 62.5 | 250 | 62.5 |
| | Stem | 31.25 | 62.5 | 62.5 | 125 | 62.5 |

Different parts of the same plant may contain different chemical constitutions [5]. Therefore different parts such as leaves, stem, roots, flowers and fruits of the same plant may show

variability in their bioactivities. The current study screened the anti-microbial effect in leaves, stem and roots of the selected medicinal plant.

The different crude extracts of the same element of a selected plant may contain different composition of phytochemicals according to the solvent they have been extracted [5]. This variability is caused by the chemical properties of each solvent such as the polarity. Therefore the current study tested the anti-microbial activity of the different solvent extracts including aqueous, hexane, methanol and acetone extracts, of the different parts of the selected plants.

The microbial pathogens possess various virulent factors which are involved in the pathogenesis of the infectious diseases. The virulent factors as well as cellular properties of different pathogens are varied. Therefore the mechanism of pathogenesis is different from each pathogen. Hence, a particular pathogen is responsible for the causing an infection which is unique to it. Therefore the anti-microbial agents which are active against a particular pathogen may vary from other pathogens. The current study was designed to screen the anti-microbial activity of the selected plant materials on different species of microbes, including *Salmonella enterica*, *Staphylococcus aureus*, *Escherichia coli*, *Shigella dysenteriae* and *Candida albicans*.

The current study used the results of the agar well method for the first screening of the antimicrobial activity of the test extracts. According to the observed results, the zone of inhibition of most of the test extracts showed a significant ($P = .05$) difference when compared with the negative control. This observation suggests that majority of the extracts of the selected plant material are active against the tested pathogens. But when compared the observed zone inhibition values among the test extracts against each microbial species, there was no significant ($P > .05$) difference between the values. Therefore the results do not provide detailed information on the extract with higher antimicrobial activity against each tested pathogen. To identify the most active extracts against the tested pathogens, the broth dilution method was performed as the second screening test. This test provided the minimum inhibitory values for each extract against the pathogens, which provided a better quantitative information on the effect of test extracts.

When compared to the observed values for respective positive control, all the extracts showed a significant difference ($P = .05$) between values. This indicated that the activity of the test extracts was not potent compared to the standard drug gentamicin. This may be, because the test extracts are the crude extracts which contain plenty of chemicals and therefore the antimicrobial activity of a particular active compound may diluted. But as the gentamicin is a pure compound, it may show a potent activity. Therefore the higher concentrations of the test extracts may show more activity than the activity observed in present study. May be the higher concentrations of the test extract may show a better anti-microbial activity. Also if the bioactive compound are identified and purified, they may also show a potent activity than the crude extracts.

As already mentioned that the previous studies on *Ipomoea spp* reported that they possess anti-microbial activity. *I. batatus* leaves extracted with 70% ethanol showed a promising anti-microbial activity against *S. mutans*, *S. mitis*, *S. aureus* and *C. albicans* [15]. Bio synthetic method used to find out the activity against some pathogens indicated that the crude acetone extraction was active against *Proteus vulgeries*, while crude ethanol extracts elucidate anti-microbial activity against *Pseudomonas aeruginosa*. The ethyl acetate, acetone, ethanol and acetone fractions of leave extraction showed active against *Salmonella typhi* and *Proteus vulgeries*, *Alternaria alternate* and *Curvularia lunata* [16]. Fresh leaves, stem and seeds of *I. Obscura* were active against *Staphylococcus aureus*, *Bacillus subtilis* and *Rhodococci* while not active against *E-coli*, *Proteous vulgeries*, *Pseudomonas* and *Salmonella* [17]. Methanolic acid/aqueous extract from the seeds of this species were also

reported for anti-bacterial activity against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E. coli* [18]. The current study proved that different parts of the plant *I. littoralis* also possess anti-microbial activity against selected pathogens.

4. CONCLUSION

The results of the present study revealed that the tested parts of each plant possess anti-microbial activity against tested pathogens. Among them some of them showed considerably higher activity against the each pathogen. The in - depth studies on these extracts may leads to discover of novel anti-microbial agents against tested pathogens.

CONSENT

Not Applicable

ETHICAL APPROVAL

Not applicable

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ABBREVIATIONS

ATCC - American Type Culture Collection

CFU – Colony Forming Units

MIC – Minimum Inhibitory Concentration

DEFINITIONS

Minimum inhibitory concentration (MIC): the lowest concentration of a chemical, usually a drug, which prevents visible growth of a bacterium

Zone of inhibition: If an antibiotic stops the bacteria from growing or kills the bacteria, there will be an area around the medium where the bacteria have not grown enough to be visible