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Full Length Article

Comparison of Antibacterial Potential from Leaves and Fruits of Different Herbs and Shrubs of Family Solanaceae

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Abstract

Bacterial infections are common source of illness in human, livestock and wildlife populations. Current study evaluated the antibacterial potential of leaves and fruits of *Physalis minima*, *Datura inoxia*, *Withania somnifera* and *Solanum nigrum* by Agar disc-diffusion method. Extracts of these plants were tested against *Staphylococcus aureus*, *Proteus vulgaris*, *Escherischia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Citrobacter amalonaticus*. The drug oxytetracycline was used as a positive control. The results indicate that fruits of these plants were more effective in controlling microbes in their aqueous extracts while leaves of these plants were effective in their ethanolic extracts as compared to aqueous and n-hexane extract. In comparison to leaves of these plants, the leaves of *D. inoxia* was rich source against antimicrobial agents, similarly in comparison of fruits, the fruit of *P. minima* was best in terms of antimicrobial activities. Overall these plants were most effective against *S. aureus* and least effective against *K. pneumonia* in comparison to their antibacterial potentials. © 2015 Friends Science Publishers

Keywords: Bacterial strains; Agar-disc diffusion method; Oxytetracycline; Medicinal plants

Introduction

The family Solanaceae comprises of approximately 84 genera and 3000 species, such as potato, petunia, nightshade and tobacco (Zygadlo *et al*., 1994). It is a cosmopolitan family found throughout tropical and temperate regions of the world. *S. nigrum* is a low branched annual herb, having triangular stems, alternate leaves and tiny white flowers. Fruit is green in color when immature and turns to purplish black when ripe (Kothekar, 1987). *P. minima* is also an annual herb which yields high fruit (Patel *et al*., 2011). It has small, round fruit and comprises of 150-300 seeds usually enclosed in bladder-like calyx (Avila *et al*., 2006; Peter, 2007). *W. somnifera*, commonly known as Indian ginseng and winter cherry, is an evergreen, erect, branching shrub having simple leaves and greenish or lurid yellow flowers and fruits are orange red when mature and are globose berries (Anonymous, 2007). *D. inoxia*, commonly known as devil's turnip and dhatora, also belongs to family Solanaceae (Stevens *et al*., 2001).

According to World Health Organization (WHO) more than 80% world's population relies on traditional medicine for their primary healthcare needs. Plants generally contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases (Diallo *et al*., 1999). In recent years, plant derived natural products have received much attention due to diverse pharmacological properties including antioxidant and antibacterial activity (Karthikumar *et al*., 2007; Aidah *et al*., 2014). The availability, affordability, reliability and low toxicity of medicinal plants has made them important and acceptable by all religions for implementation in medical health care all over the world (Akroum *et al*., 2009). Presently in the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also with damaging and side effects (Shariff *et al*., 2006). Therefore there is need to search for plants of medicinal value. In the present investigation extracts of *W. somnifera*, *P. minima*, *S. nigrum* and *D. inoxia* were evaluated with the objectives to determine the antibacterial potential of four species of family Solanaceae.

Materials and Methods

Bacterial Strains

Six bacterial strains were taken from bacteriology section of Bahawal Victoria Hospital and propagated in biochemistry lab at University College of Veterinary and Animal Sciences. These were *S. aureus*, *P. vulgaris*, *C. amalonaticus*, *E. coli*, *K. pneumonia* and *P. aeruginosa*. The cultures of bacteria were maintained on nutrient media and glycerol stocks were made by adding 150 µL glycerol and 850 µL cultures in the Eppendorf tubes.

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Media Preparation

Nutrient Broth: Nutrient broth was prepared by dissolving 1.3 g of it in 100 mL distilled water. Then sterilize it in the autoclave at 121ºC for 20 min.
Nutrient Agar: Nutrient agar was made by dissolving 2.8 g of it in distilled water. Then sterilize it in the autoclave at 121ºC for 20 min.

Extraction of Plant Materials

Ethanol Extract: 10 gm of dry plant powder was added to 100 mL of absolute ethanol in a conical flask and the mouth of flask was covered with aluminium foil to avoid evaporation of ethanol. After 42 h, the extract was filtered with common whatman filter paper 1.
n-Hexane Extract: 10 gm of dry plant powder was added to 100 mL of n-hexane in a conical flask and the mouth of flask was covered with aluminium foil to avoid evaporation of ethanol. After 42 h, the extract was filtered with common whatman filter paper 1.
Aqueous Extract by Freeze thaw Method: First of all, phosphate buffer saline (PBS) was made by dissolving sodium chloride (4 gm), potassium chloride (0.1 gm), potassium dihydrogen phosphate (0.1 gm), disodium hydrogen phosphate (1.16 gm) in 200 mL distilled water. 10 gm of plant powder was added in 100 mL PBS (phosphate buffer saline, pH=7.2) in a conical flask and mixed well and placed in freezer. After 24 h, the extract was thaw. The process of freeze and thaw was repeated three times. Finally, it was centrifuged at 4000 rpm and then filtered by using Whatman filter paper 1.

Cutting of Discs from Disc Diffusion Method

The discs were cut with the help of disc cutter from common whatman filter paper. The discs were dipped in respective plant extracts to test the antibacterial activity against cultured microbes. Negative controls were prepared using discs impregnated with 100 µL of the solvents i.e ethanol and n-hexane. Pre-soaked discs from commercially available antibiotic-oxytetracycline (positive control) were used as standards for comparison.

Antibacterial Assay

Bacterial strains were taken from glycerol stocks and a loop full of culture was added to the sterilized 3 mL nutrient broth. The cultures were incubated at 37ºC for 24 h at constant shaking. Observe the turbidity in the test tubes. Take OD_{600} of cultures and bring the OD_{600} at 0.4. Take 1 mL of culture and spin at 12000 rpm aspirate the supernatant and left 100 µL of supernatant on pellete. Dissolve the pellete and apply on agar.

Determination of Zone of Inhibition

The antibacterial activity assay was performed by agar disc diffusion method. The molten nutrient agar was poured into sterilized petri dishes. When the media solidified, plates were inoculated with 100 µL of the respective organism by glass spreader and incubate at 37ºC for 1 h. The pre-soaked discs (6 mm in diameter) in different extracts were placed on the agar medium seeded with respective micro-organisms. Pre-soaked discs with Oxytetracycline were used as a positive control and discs impregnated with solvent i.e ethanol and n-hexane were used as negative control. The plates were then incubated at 37ºC for 24 h to allow maximum growth of micro-organisms. The antibacterial activity of test samples was determined by measuring the diameter of zone of inhibition expressed in millimeter. The transparently cleared zones show bactericidal activity while the cleared zones containing micro-colonies showed bacteriostatic activity (Bauer *et al*., 1966; Colle and Marr, 1989).

Statistical Analysis

Values were mean±SD (standard deviation) of three replicates. All experiments were performed at least, three times (unless indicated otherwise) and were highly reproducible. Data collected was analyzed statistically by applying one-way ANOVA using statistica software and means were separated by least significant different test at $P<0.05$ (Steel *et al*., 1997).

Results

Results revealed that the tested ethanolic, n-hexane and aqueous extracts of respective plants of family Solanaceae i.e., *P. minima, S. nigrum, W. somnifera* and *D. inoxia* possess significant antibacterial activity against various bacterial strains.

Antibacterial Assay of Leaves of *P. minima*

Ethanol control exhibits maximum antibacterial activity (20 mm) against *P. aeruginosa* while n-hexane control formed largest inhibition zone (19 mm) against *S. aureus* (Fig. 1). However, *K. pneumonia* was found to be highly resistant strain. *S. aureus* was highly sensitive strain when tested against ethanolic extract of *P. minima* leaves as maximum zone of inhibition (21 mm) was produced against it whereas *P. aeruginosa, C. amalonaticus* and *E. coli* were highly resistant strains as no zone was formed against them. In case of n-Hexane extract, highest mean value (4 mm) was observed in *K. pneumonia* and *S. aureus* and minimum in *P. vulgaris* and *E. coli* while no zone was observed against *C. amalonaticus* and *P. aeruginosa*.

Aqueous extract of *P. minima* leaves formed largest inhibition zone against *P. vulgaris* (10 mm) and *E. coli* (4 mm) and formed no inhibition zone against *S. aureus* and *K. pneumonia*. Moreover, standard (positive control), oxytetracycline showed significant zone of inhibition against *S. aureus*, measured 28 mm and *P. vulgaris*, measured 26 mm respectively (Fig. 9).

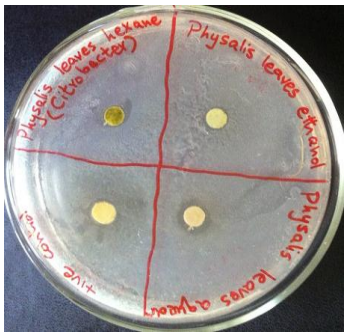

Fig. 1: Antibacterial assay of *P. minima* leaves against *C. amalonaticus*

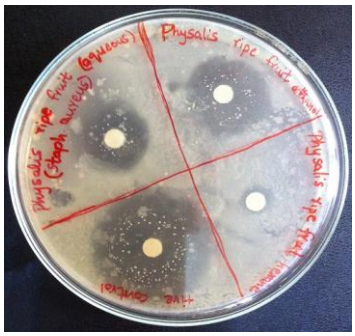

Fig. 2: Antibacterial assay *P. minima* fruit against *S. aureus*

Antibacterial Assay of Fruit of *P. minima*

Ethanolic extract of fruit of *P. minima* showed maximum zone of inhibition against *S. aureus* (13 mm) while *P. aeruginosa*, *P. vulgaris*, *C. amalonaticus* and *E. coli* were revealed as highly resistant strains as no zone of inhibition was observed against them (Fig. 2). N-hexane fruit extract showed maximum inhibition zones against *K. pneumonia* (3 mm), *E. coli* (2 mm), *P. vulgaris* (1 mm) and *C. amalonaticus* (1 mm) while *S. aureus* and *P. aeruginosa* were revealed as highly resistant strains. Aqueous extract of *P. minima* fruit showed maximum activity against *P. vulgaris* (21 mm), *S. aureus* (14 mm) and *P. aeruginosa* (9 mm) while *K. pneumonia* was found to be highly resistant strain (Fig. 10). Statistically, it has been proved that the zones produced by the fruit of *P. minima* against all bacterial strains were significant.

Antibacterial Assay of Leaves of *S. nigrum*

Ethanol control formed maximum inhibition zones against *E. coli* (20 mm), *S. aureus* (8 mm), *P. vulgaris* (6 mm) and *P. aeruginosa* (5 mm) and formed least zones of inhibition against *K. pneumonia* (2 mm) and *C. amalonaticus* (1 mm). However, n-hexane control showed maximum activity against *S. aureus* (19 mm), *E. coli* (12 mm) and *P. vulgaris* (7 mm). However, it formed no inhibition zone against *K. pneumonia* and *P. aeruginosa* (Fig. 3).

Ethanolic and n-hexane extracts of *S. nigrum* leaves was found to be highly resistant against four bacterial strains

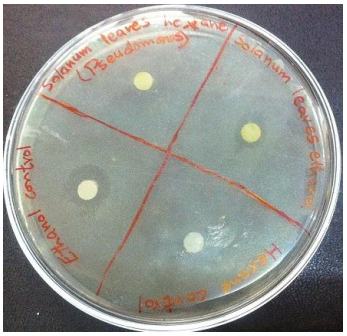

Fig. 3: Antibacterial assay of *S. nigrum* leaves against *P. aeruginosa*

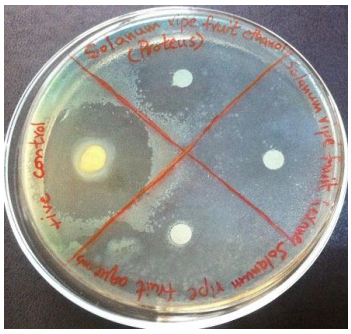

Fig.4: Antibacterial assay of *S. nigrum* fruit against *P. vulgaris*

namely *S. aureus*, *K. pneumonia* and *E. coli*. However, moderate antibacterial activity was observed against *P. aeruginosa* and *P. vulgaris* respectively (Fig. 11). Aqueous extract showed remarkable activity against *E. coli* as it formed maximum zone of inhibition (i.e. 25 mm). Least antibacterial activity (2 mm) was observed against *C. amalonaticus* while *K. pneumonia* and *P. vulgaris* exhibit no inhibition zone.

Results of Antibacterial Assay of Fruit of *S. nigrum*

Results revealed that *S. aureus*, *P. aeruginosa* and *P. vulgaris* were highly sensitive strains and *K. pneumonia*, *C. amalonaticus* and *E. coli* were highly resistant strains when tested against various extracts of fruit of *S. nigrum*. Ethanol control and n-hexane control showed maximum antibacterial activity against *E. coli*, *S. aureus* and *P. vulgaris* (6 mm) and minimum activity against *K. pneumonia* and *C. amalonaticus*, respectively (Fig. 4).

Ethanolic extract of *S. nigrum* fruit formed maximum zone against *P. vulgaris* (8 mm) and *P. aeruginosa* (3 mm) and n-Hexane extract showed strong antibacterial activity against *P. aeruginosa* (9 mm) and *C. amalonaticus* (7 mm). *S. aureus*, *K. pneumonia* and *E. coli* were highly resistant strains as no zone of inhibition when formed against them when both ethanolic and n-hexane extracts of *S. nigrum* fruits were tested (Fig. 12). Moreover, aqueous extract possess significant antibacterial activity against *S. aureus*, *P. aeruginosa* and *P. vulgaris* forming inhibition zone of 10

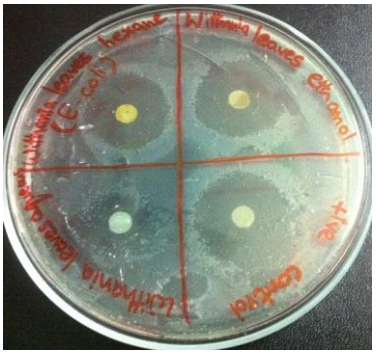

Fig. 5: Antibacterial assay of leaves of *W. somnifera* against *E. coli*

Fig. 6: Antibacterial assay of fruit of *W. somnifera* against *P. vulgaris*

Fig. 7: Antibacterial assay of leaves of *D. inoxia* against *S. aureus*

mm while *C. amalonaticus* and *E. coli* were proved as resistant bacterial strains. As standard (positive control), oxytetracycline possess antibacterial activity in all bacterial strains. Statistically, inhibition zones produced by all the bacterial strains were found to be highly significant.

Antibacterial Assay of Leaves of *W. somnifera*

Both negative controls exhibited maximum antibacterial activity (12-14 mm) against *S. aureus* and *P. vulgaris* (8.66 mm) and minimum activity against *K. pneumonia*. Ethanolic and n-hexane leaves extracts of *W. somnifera* were highly effective against *E. coli* and *S. aureus* and least effective

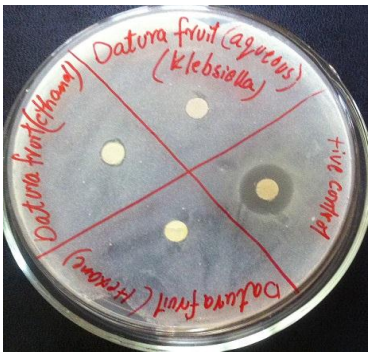

Fig. 8: Antibacterial assay of fruit of *D. inoxia* against *K. pneumonia*

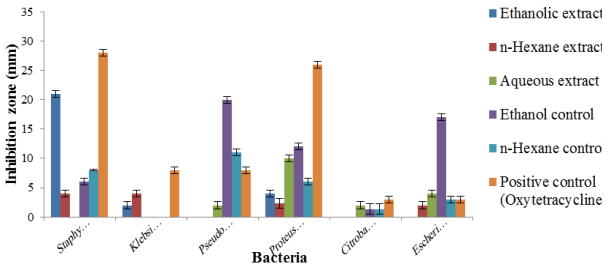

Fig. 9: Mean values of antibacterial assay of leaves of *Physalis minima*

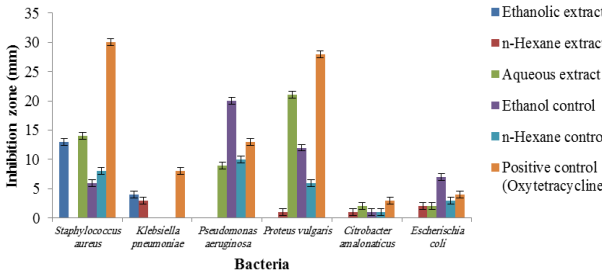

Fig. 10: Mean values of antibacterial assay from *Physalis minima* fruit

against *K. pneumonia* respectively (Fig. 5). *E. coli* and *S. aureus* were revealed as highly sensitive bacterial strains (Fig. 13). Aqueous leaf extract *W. somnifera* formed maximum zone against *P. vulgaris* (18 mm) and minimum zone against *P. aeruginosa* (2 mm). But *K. pneumonia* was found to be highly resistant when tested against all extracts of leaves of *W. somnifera* has no inhibition zone was formed against it.

Antibacterial Assay of Fruit of *W. somnifera*

Ethanol control showed maximum (13 mm) antibacterial activity against *P. vulgaris* and least (1 mm) activity against *K. pneumonia*. n-hexane control exhibited maximum (14 mm) activity against *S. aureus* and minimum against *K. pneumonia* and *E. coli*. Moreover, ethanolic fruit extracts of *W. somnifera* were highly effective against *S. aureus* (i.e. 13 mm inhibition zone) and least effective against *K.*

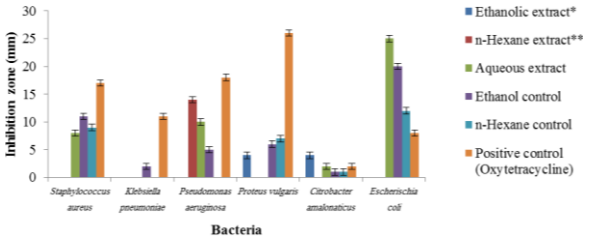

Fig. 11: Mean values of antibacterial assay from *Solanum nigrum* leaves

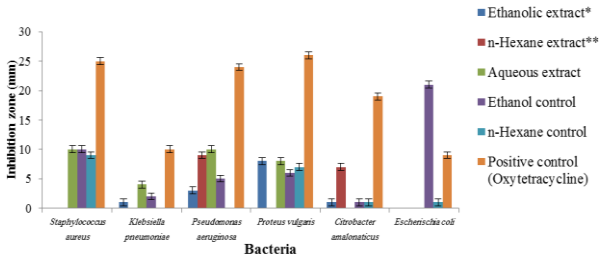

Fig. 12: Mean values of antibacterial assay from *S. nigrum* fruit

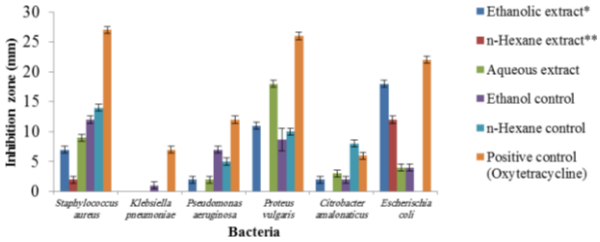

Fig. 13: Mean values of antibacterial assay of leaves of *Withania somnifera*

pneumonia, *P. vulgaris* and *E. coli* as no zone of inhibition was produced against them. However, n-hexane extract showed significant activity against *E. coli* and minimum against *S. aureus*, *K. pneumonia*, *P. vulgaris* and *C. amalonaticus* which revealed that they are highly resistant strains (Fig. 6).

Aqueous extract formed largest inhibition zone (21 mm) against *P. vulgaris* and minimum zone (1 mm) against *K. pneumonia*. But *P. aeruginosa* was found to be highly resistant as no inhibition zone was produced against it. As standard (positive control), oxytetracycline showed significant zone of inhibition against all selected bacterial strains. Largest zone (28 mm) of inhibition of positive control was observed against *S. aureus* while minimum zone (5 mm) of inhibition was observed against *C. amalonaticus* (Fig. 14).

Antibacterial Assay of Leaves of *D. inoxia*

Ethanol and n-hexane control showed maximum antibacterial activity against *P. aeruginosa* and minimum activity against *S. aureus* and *K. pneumonia* as no inhibition zone was produced against them (Fig. 7). Ethanolic leaves extract of

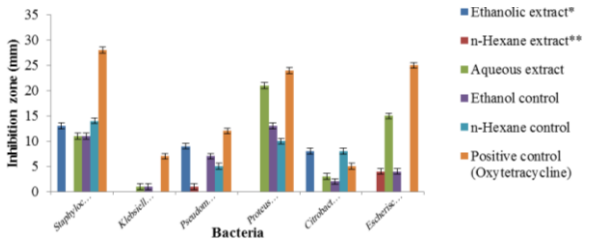

Fig. 14: Mean values of antibacterial assay of fruit of *Withania somnifera*

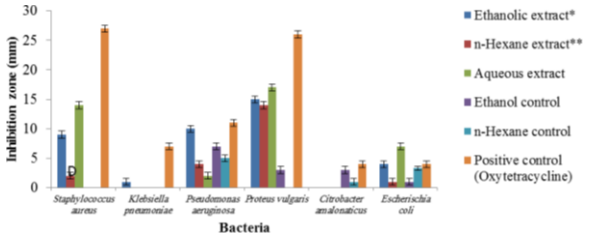

Fig. 15: Mean values of antibacterial assay of leaves of *Datura inoxia*

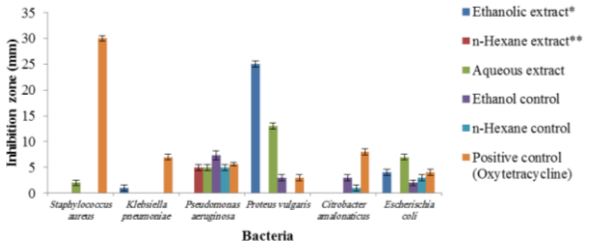

Fig. 16: Mean values of antibacterial assay of fruit of *Datura inoxia*

* = activities mentioned are the zone of inhibition produced by ethanolic extract minus zone of inhibition produced by ethanol control

**= activities mentioned are the zone of inhibition produced by n-hexane extract minus zone of inhibition produced by n-hexane control

D. inoxia was found to be highly effective against *P. vulgaris* (15 mm mean value of inhibition zone) as compared to *P. aeruginosa* (10 mm) and *S. aureus* (9 mm). *C. amalonaticus* was found to be highly resistant as no zone was produced against it. Similarly, n-hexane extract was also effective against *P. vulgaris* and *P. aeruginosa* while no inhibition zone was observed in case of *K. pneumonia*.

The largest inhibition zone formed by aqueous extract of *D. inoxia* leaves was against *P. vulgaris* (7 mm) while *C. amalonaticus* and *K. pneumonia* were found to be highly resistant species as no inhibition zone was produced against them. Moreover, oxytetracycline showed significant zone of inhibition against all selected bacterial strains (Fig. 15).

Antibacterial Assay of Fruit of *D. inoxia*

Ethanol control showed maximum antibacterial activity (3 mm) against *C. amalonaticus* and *P. vulgaris* and n-hexane control showed maximum activity against *P. aeruginosa.* However, no inhibition zones were observed against *K.*

pneumonia and *S. aureus* in both negative controls. So, *K. pneumonia* and *S. aureus* were revealed as highly resistant strains (Fig. 8).

Ethanolic fruit extracts of *D. inoxia* was found to be highly effective against *P. vulgaris* (25 mm inhibition zone) and least effective against *S. aureus*, *K. pneumonia* and *P. aeruginosa*, as no zone was produced against them. However, n- hexane fruit extract was effective against *P. aeruginosa* only. Whereas *S. aureus*, *K. pneumonia*, *P. vulgaris*, *C. amalonaticus* were found to be highly resistant strains (Fig. 16). Aqueous extract also formed maximum inhibition zone against *P. vulgaris* (13 mm) and minimum zone against *S. aureus* (2 mm). However, no zones were observed against *K. pneumonia* and *C. amalonaticus.*

Discussion

Antimicrobial compounds from plants represent a potentially novel source of antimicrobial substances and may thus have a clinical value in the treatment of antibiotic resistant antimicrobial strain (Eloff, 1998). Herbal medicines are considered as one of the most important fields of traditional medicine all over the world (Hamil *et al*., 2003). The plant extracts and their confined constituents have always been an important part of different curative systems (Vanitha and Kathiravan, 2006). Antibacterial activity was classified into highly sensitive, moderately sensitive and resistant depending on the measured values of inhibition zones. Out of different extracts, fruit extracts of *P. minima* and leaves extracts of *D. inoxia* showed most remarkable activity. Our results also correlate with the findings of Yogananth *et al*. (2012) who reported similar results on antibacterial activity of plants.

Aqueous fruit extracts of all plants were revealed as highly effective against all bacterial strains as compared to ethanolic and n-hexane extracts of selected plants. However, in case of leaves, ethanolic extracts were proved to be highly effective against all strains. Our findings are in line with the studies of Almazini *et al*. (2009); Venkatesan *et al*. (2009); Sridhar *et al*. (2011) and Shahid *et al*. (2013). Furthermore, our results are also in conformity with the findings of Nathiya and Dorcus (2012) who reported that *S. aureus* is the highly sensitive strain while *P. aeruginosa* and *K. pneumonia* is the highly resistant strain when tested by different plant extracts.

The extracts of various parts of tested plants possess a broad spectrum of activity against a panel of bacterial strains responsible for common bacterial infections. The present study forms a primary platform for further phytochemical and pharmacological investigation for the development of new potential antimicrobial compounds. In a nutshell, all these plants have a potential source of useful drugs and can be utilized in the treatment of many diseases/ailments. However, further studies are required to isolate the active principle from the crude extracts for proper drug development.

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