Antimicrobial potentials of different solvent extracted samples from *Physalis ixocarpa*

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Abstract: The present study investigates the antimicrobial activities of different solvent extracted samples isolated from different parts of *Physalis ixocarpa* through disc diffusion assay using three different concentrations. Statistical analysis of the data revealed that different parts of the plant showed varying degree of inhibition against different bacteria at different concentrations. Different solvent extracted samples from the calyx showed inhibitory activity against most of the bacteria under study. Extracts from leaf and fruit samples showed activity against *S. aureus* and *K. pneumoniae* and extracts from the stem tissues were effective to control the growth of *E. coli* and *K. pneumoniae.* Crude methanolic extract from the stem and n-butanol extracted samples from fruit exhibited strong inhibitory activity against *Klebsiella pneumoniae* at highest concentrations. Antifungal activity was observed only in crude methanol extract from the leaf against *Rhizopus stolinifer*, *Aspergillus niger* and *Penicillium chrysogenum.*

Keywords: Antimicrobial activity, disc diffusion assay, *Physalis ixocarpa,* solvent extracts.

INTRODUCTION

Medicinal and aromatic plants contribute to major portion of the flora. The plant materials obtained from these plants are used in the pharmaceuticals, cosmetics, and drug industries. Approximately 20% of the world flora has been tested for their pharmacological or biological activities (Suffredini *et al*., 2004). It is estimated that 80% of the population in developing countries relies on traditional plant based medicines for their health issues (WHO, 1991). Even many of the modern medicines are based on raw materials obtained from medicinal plants due to their easy availability, least side effects, low prices, and lasting curative property. Extracts of many plants possesses potent antimicrobial activities (Bakht *et al*., 2011 a, b, c and d; 2012; 2013 a, b; 2014a,b, c; 2015; Nasir *et al.,* 2015; Ullah *et al.,* 2015; Khan and Zakia, 2014; Ahmad *et al*., 2015; Ashraf *et al*., 2015; Karabuluti and Sule, 2015). Different parts of medicinal plants extracts, infusions, decoctions, powders are used to treat different illness in humans, animals and plants (Nostro *et al*., 2000). Antimicrobial properties of the plants are associated with plants compounds, which were first documented in the late 19th century (Zaika, 1975). These compounds include wide variety of secondary metabolites such tannins, terpenoides, alkaloids and flavonoid with *in vitro* antimicrobial properties (Cowan, 1999).

Physalis ixocarpa brot. belong to the genus *Physalis*, which contains about 100 species of annual and perennial herbs (Willis, 1966). It is related to both tomato and potato. It is native to South American continent. Mexico and Guatemala is popular for the cultivation of tomatillo and its wild and domesticated version can also be found here. *Physalis* species are used as folk medicine for treating cancer, leukemia, hepatitis and other diseases. The fruit of *P. ixocarpa* is used as eyewash, to treat gastrointestinal problems and respiratory diseases (Caceres *et al*., 1991). It is used as tonic, diuretic, laxative, applied in inflammations, enlargement of the spleen, ascites and as a helpful remedy in ulceration of the bladder. The leaves are crushed and applied over snakebite site (Karthikeyani and Janardhanan, 2003). Fruits of *P. minima* are used to for the treatment of spleen disorders. Nathiya and Dorcus (2012) reported that a number of secondary metabolites were found in different parts of *P. minima* including alkaloids, anthraquinones, flavonoids, cardiac glycosides, phenols, quinones, reducing sugars, saponins, steroids, starch, tannin and terpenoids. They also observed antibacterial activity of different extracts of *P. minima* against *B. cereus, B. subtilis, Citrobacter* sp., *E. aerogenes, E. coli, K. pneumoniae, P. aeruginosa, P. fluorescens* and *S. aureus* using agar well diffusion method. Limited research work has been carried out on the pharmacological activities of *P. ixocarpa*, therefore, the aim of the present research to investigate the *in vitro* antimicrobial activity of different solvent extracted from various parts of *P. ixocarpa.*

MATERIALS AND METHODS

Collection and identification of plant materials

The present research work was conducted at the Institute of Biotechnology and Genetic Engineering, The Agricultural University Peshawar, KPK Pakistan. Two kg of fresh plant material was collected from Baylay Baba Mountains of District Shangla, KPK Pakistan during 2013. The plant specimen was identified by plant

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taxonomist Prof. Dr. Farrukh Hussain, Chairman Department of Botany University of Peshawar and deposited with voucher number 326 in the herbarium of Botany department, University of Peshawar, KPK Pakistan. The plant material (stem, leaves, fruit and their calyx) were shade dried separately and milled to fine powder by tissue homogenizer (Infinigen™ Tissue Mixer Mill).

Preparation of crude extracts and their fractions

The powdered plant material (500g) was macerated in 97% methanol 7 days. The solution was stirred regularly for 7 days at room temperature. The methanol soluble compound was filtered through What man filter paper (What man ^{TM}). Fresh methanol was added to the remaining plant material and filtered again and this process was repeated three times. The resulting methanolic solution was subjected to rotary evaporator for evaporation (Rotavapor ^{R}-R 210/R215; BUCHIL Labortechnik AG). Methanol was removed at 45°C under vacuum pressure and semi-solid extract was obtained (crude extract).

Fractionation of crude

The crude extract was weighted and divided into two portions. One portion (15 g) was tested as crude methanol extract, while the other portion was dissolved in 300ml distilled water and transferred to separatory funnel for further fractionation with various solvents. Distilled n-hexane (200ml) was added, shaken gently and the upper layer containing n-hexane soluble compound was removed. The lower layer was extracted again with n-hexane and this process was repeated three times. All fractions of n-hexane were pooled, dried through rotary evaporator to obtain semi-solid material. The semi-solid fraction was dried in china dish at 45°C on water bath and kept in glass vials until used. The same fractionation process was carried out for ethyl acetate and butanol. The lower aqueous phase was dried through rotary evaporator and water bath. The same procedure was followed for the calyx, stem, leaf and fruits samples of the *Physalis ixocarpa*.

Culture media and its preparation

Nutrient agar media (HiMedia Laboratories Pvt. Ltd.) was used for the culturing and growth and nutrient broth for shaking incubation and standardization of different microorganisms. Media was prepared as described in Bakht *et al*. (2 011 a, b, c, d; 2012; 2013 a, b; 2014 a,b,c).

Microorganisms tested

Antibacterial and antifungal activity of different solvent extracted samples from stem, leaf, calyx and fruit was tested against different bacterial and fungal strains (table 1).

Disc diffusion susceptibility method

The antibacterial activity of different solvent extracted samples of *P. ixocarpa* was tested by disc diffusion assay according to the methods of Bauer *et al*. (1966) and antifungal activity by Ramdas *et al*. (1998). Three concentrations of the extracts (100, 200 and 300 ppm) in volume of 6µl were applied to the disc. Antibiotic and antifungal drugs used as positive control for Gram positive, Gram-negative bacteria and fungus were Erythromycin, Ciprofloxacin and Clotrimazole respectively.

For Gram positive bacteria: Erythromycin 50µg 6 μl^{-1}
For Gram negative bacteria: Ciprofloxacin 50µg 6 μl^{-1}
For Fungi: Clotrimazole 50µg 6 μl^{-1}

STATISTICAL ANALYSIS

Data are presented as mean values of three replications. MSTATC computer software was used to carry out statistical analysis (Russel and Eisensmith, 1983). The significant difference among means was compared using Least Significant Difference (LSD) test (Steel *et al*., 1997).

RESULTS

The antibacterial activity of different solvent extracted samples from the calyx of *P. ixocarpa* against *B. subtilis* is shown in fig. 1. Crude methanol and aqueous extracted samples were not effective to control the growth of *B. subtilis* and recorded 0% zone of inhibition (ZI). Ethyl acetate and n-hexane reduced the activity of *B. subtilis* at all concentrations. However, maximum reduction in growth was observed at higher concentration of both samples. Moreover, maximum activity was noted by ethyl acetate extracted samples against *B. subtilis* (35% ZI) at higher concentration compared with n-hexane (29% ZI) at the same concentration. Butanol extracted samples showed activity against *B. subtilis* measuring 28% ZI at higher concentration, however, at lower concentration, did not inhibit the growth of *B. subtilis* and showed 0% ZI when compared with positive and negative controls. fig. 2 revealed that crude extracted samples were not effective at all concentrations to control the growth of *E. coli* measuring 0% ZI. Aqueous extracted samples were more effective to inhibit the activity of *E. coli* at all concentrations compared with other solevnt extrated samples, maximum ZI being achieved at higher concentration (65%). Ethyl acetate and n-hexane extracted samples did not inhibit the growth of *E. coli* at lower concentration measuring zero percent ZI, however, at higher concentrations, both of the extracts reduced the growth of *E. coli.* fig. 3 indicates data regarding antimicorbial activity of diferent solvent extracted samples from the calyx of *P. ixocarpa* against *X. oryzae*. Crude extracts, n-hexane and aqueous extracted samples did not reduce the activity of *X. orayzae* at lower concentration, however, at higher concentrations these extracts inhibit the growth of *X. Oryzae.* Maximum activity was observed for butanol extracted samples

followed by ethyl acetate extracted fraction and minimum inhibition was revealed by aqueous extracted at lower concentration.

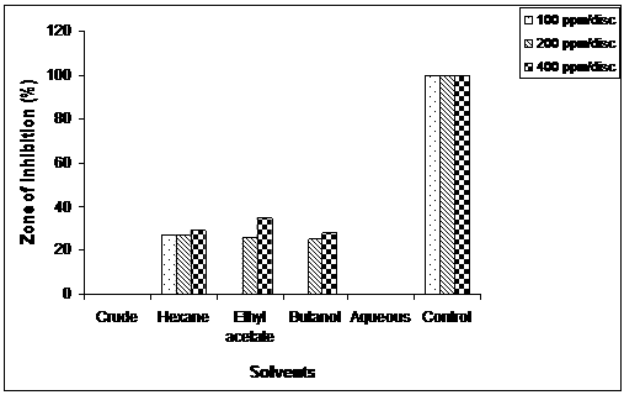

Fig. 1: Antibacterial activity of crude (methanol), n-hexane, ethyl acetate, butanol and aqueous extracted samples from *Physalis ixocarpa* calyx tissues against *B. subtilis* by disc diffusion assay.

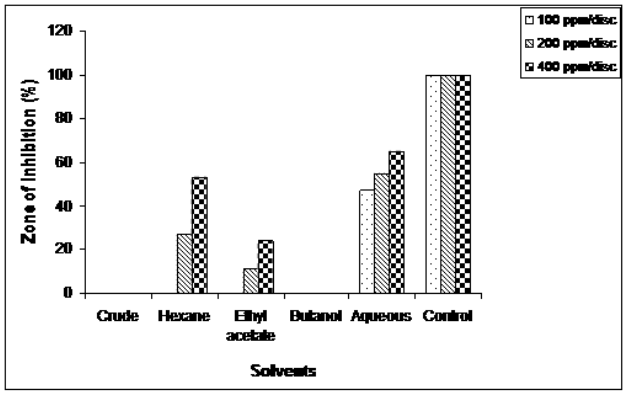

Fig. 2: Antibacterial activity of crude (methanol), n-hexane, ethyl acetate, butanol and aqueous extracted samples from *Physalis ixocarpa* calyx tissues against *E. coli* by disc diffusion assay.

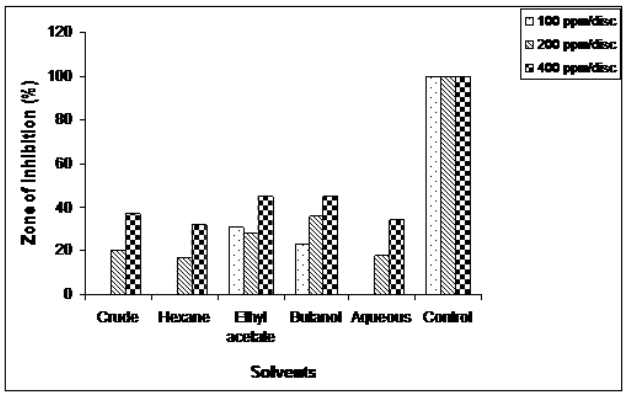

Fig. 3: Antibacterial activity of crude (methanol), n-hexane, ethyl acetate, butanol and aqueous extracted samples from *Physalis ixocarpa* calyx tissues against *X. orayzae* by disc diffusion assay.

The data shown in fig. 4 shows the antimicrobial activity of different solvent extracted samples from the calyx of *P. ixocarpa* against *S. aureus.* All samples were effective to control the growth of *S. aureus* at all concentration except butanol extracted samples at lower concentration. The data revealed that maximum inhibition was noted in aqueous extracted samples (34% ZI) at higher concentration followed by n-hexane extracted samples (32% ZI) at the same concentration. Minimum reduction in the growth of *S. aureus* was observed by butanol at lower concentration. fig. 5 present data concerning antimicrobial activity of different solvent extracted samples against *K. pneumonia.* Butanol and ethyl acetate extracted samples from *P. ixocarpa* did show activity against *K. pneumonia* at all concentration revealing 0% ZI. Similarly, crude extracts, n-hexane and aqueous extracted samples did not reveal activity against *K. pneumonia* at lower concentration. Crude, n-hexane and aqueous extracted samples inhibited the growth of *K. pneumonia* at higher concentrations. Maximum reduction in the growth of *K. pneumonia* was observed due to crude and aqueous extracted samples at higher concentrations.

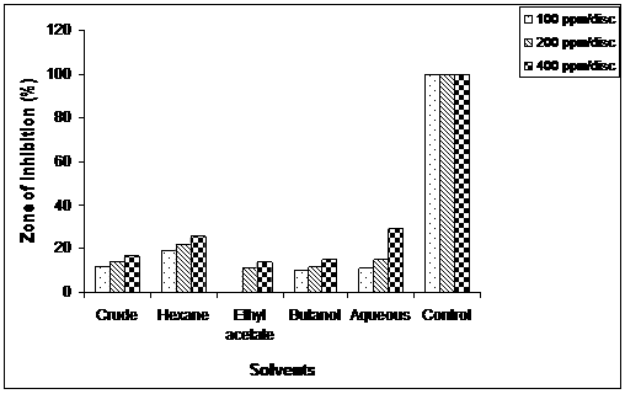

Fig. 4: Antibacterial activity of crude (methanol), n-hexane, ethyl acetate, butanol and aqueous extracted samples from *Physalis ixocarpa* calyx tissues against *S. aureus* by disc diffusion assay.

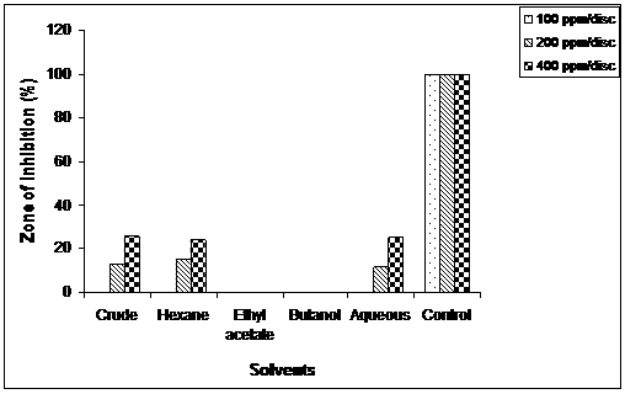

Fig. 5: Antibacterial activity of crude (methanol), n-hexane, ethyl acetate, butanol and aqueous extracted samples from *Physalis ixocarpa* calyx tissues against *K. pneumonia* by disc diffusion assay.

Fig. 6 indicates results regarding antimicrobial activity of different solvent extracted samples against *B. subtilis*.

Butanol, n-hexane and aqueous extracted samples from the leaves of *P. ixocarpa* did not show activity against *B. subtilis* at all tested concentrations revealing 0% ZI. Ethyl acetate extracted leaf samples were effective to control the growth of *B. subtilis* and maximum reduction in growth was noted at higher concentration. Similarly, crude leaf extract showed some activity against *B. subtilis* at higher concentration and no activity were recorded at lower concentration against the same microbe. N-hexane butanol and aqueous extracted samples from leaf of *P. ixocarpa* did not reduce the growth of *K. pneumonia* at any tested concentration (fig. 7). Crude and ethyl acetate leaf extract showed activity against *K. pneumonia* at all test concentration and butanol extracted leaf samples were effective at higher concentration only. Fig. 8 shows data concerning antimicrobial activity of leaf extracts against *S. aureus.* Our results indicated that different solvent extracted leaf samples were active against *S. aureus* except aqueous extracts at all concentrations. Crude leaf extract was more effective to reduce the growth of *S. aureus* followed by ethyl acetate extracts at higher concentrations. N-hexane and butanol extracted leaf samples were equally effective to inhibit the growth of *S. aureus* at lower concentration. The data also revealed that crude leaf extracts and their fractions did not inhibit the growth of *X. Oryzae* at any concentration. Analysis of the data also revealed that n-hexane and ethyl acetate samples extracted from the stem tissues of *P. ixocarpa* inhibited the growth of *E. coli* at all tested concentrations (fig. 9). Crude extracts from stem tissues showed activity *E. coli* at higher concentrations and revealed zero percent ZI at lower concentration. N-butanol and aqueous extracted from stem showed no activity against *E. coli* at all the tested concentration recording 0% ZI. Maximum inhibition in *E. coli* growth was observed at higher concentration of n-hexane extracted samples from stem tissues and minimum activity against *E. coli* was noted by ethyl acetate extracted samples from stem. The growth of *K. pneumonia* was reduced by all the tested solvent extracted samples from stem tissues at all concentrations except aqueous and butanol extracted samples where no activity was noted against *K. pneumonia* (0% ZI). Our results revealed that maximum activity against *K. pneumonia* was measured when crude extracted at higher concentration from stem tissues was applied followed by hexane extracted samples at the same concentration. Minimum activity was noted against the same microbe by hexane extracts when tested at lower concentration (fig. 10). Our results revealed that no activity was recorded by all stem extracts at any concentration against, *S. aureus*, *R. stolonifer* and *X. orayzae*.

Fig. 11 presents data concerning antimicrobial activity of different solvent extracted samples from the fruits of *P. ixocarpa* against *K. pneumonia.* The data indicated that ethyl acetate, butanol and hexane extracted samples from the fruits of *P. ixocarpa* did not show any activity against *K. pneumonia* at all tested concentrations measuring 0% ZI. Crude and aqueous extracted samples of fruit tissues reduced the growth of *K. pneumonia* at all tested concentrations. The results indicated that aqueous extracts of fruit tissues measure maximum zone of inhibition at higher concentration followed by crude at the same concentration. Minimum reduction in the growth of *K. pneumonia* was observed by n-hexane extracted fruit samples at lower concentration. Crude extracts and aqueous extracted samples from fruit tissues were effective to reduce the growth of *S. aureus* at all tested concentrations. N-hexane, butanol and ethyl acetate extracted samples from fruit tissues did not inhibit the growth of *S. aureus* at all concentration measuring 0% ZI. The data also indicated that crude extracts and aqueous extracted samples from fruit tissues were equally effective to control the growth of *S. aureus* (fig. 12). Our results also revealed that different solvent extracted samples from fruit tissues were not effective to control the growth of *X. orayzae*, *E. coli* and *B. subtilis* at all tested concentrations.

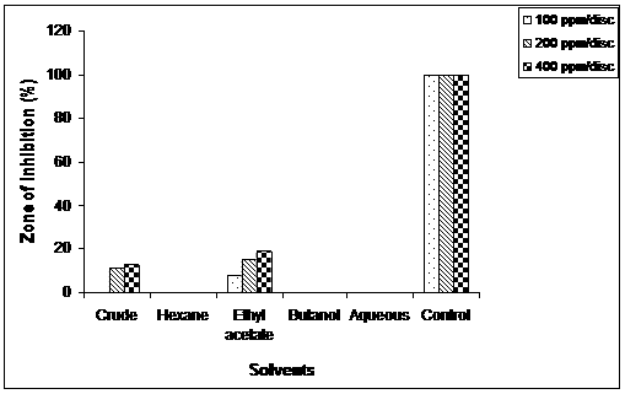

Fig. 6: Antibacterial activity of crude (methanol), n-hexane, ethyl acetate, butanol and aqueous extracted samples from *Physalis ixocarpa* leaf tissues against *B. subtilis* by disc diffusion assay.

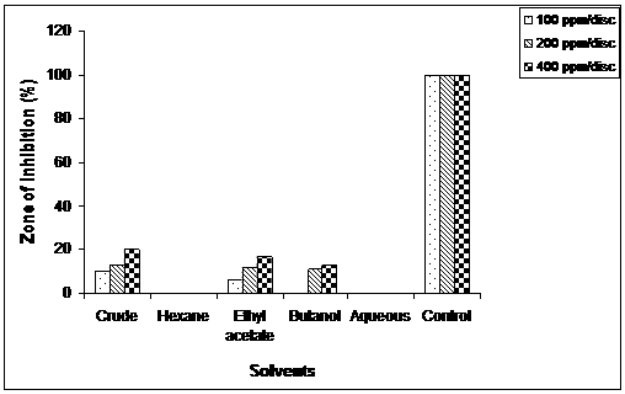

Fig. 7: Antibacterial activity of crude (methanol), n-hexane, ethyl acetate, butanol and aqueous extracted samples from *Physalis ixocarpa* leaf tissues against *K. pneumonia* by disc diffusion assay.

Fig. 13 shows data regarding antifungal activity of different solvent extracted samples from leaf tissues against *R. stolonifer*. The data indicated that crude extract was more potent to reduce the growth of *R. stolonifer* at all tested concentrations. N-hexane, ethyl acetate, butanol and aqueous extracts did not inhibit the growth of *R. stolonifer* at all concentrations measuring 0% ZI. Our results also revealed that different solvent extracted samples from the leaf tissue were very effective to inhibit the activity of *A. niger* at all tested concentrations except aqueous extracted samples which did not show any activity against this fungus measuring 0% ZI (fig. 14). Data regarding antifungal activity of *Penicillium chrysogenum* by different solvent extracted samples from the leaves of *P. ixocarpa* is shown in fig. 15. The data indicated that crude extracted samples showed good activity against *Pencillium* at all tested concentrations. Maximum activity was revealed when crude extracted was tested at higher concentration. N-hexane, ethyl acetate, butanol and aqueous extracted leaf samples did not show activity against the same fungus revealing 0% ZI. Our results also revealed that different solvent extracted from leaf did not inhibit the growth of *Rhizopus oryzae* and *A. alternatum* at any concentration.

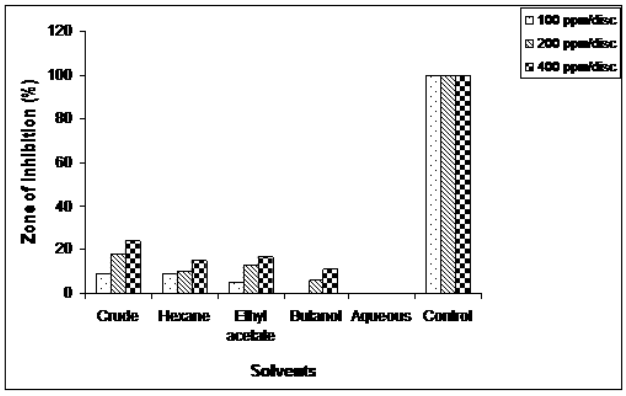

Fig. 8: Antibacterial activity of crude (methanol), n-hexane, ethyl acetate, butanol and aqueous extracted samples from *Physalis ixocarpa* leaf tissues against *S. aureus* by disc diffusion assay.

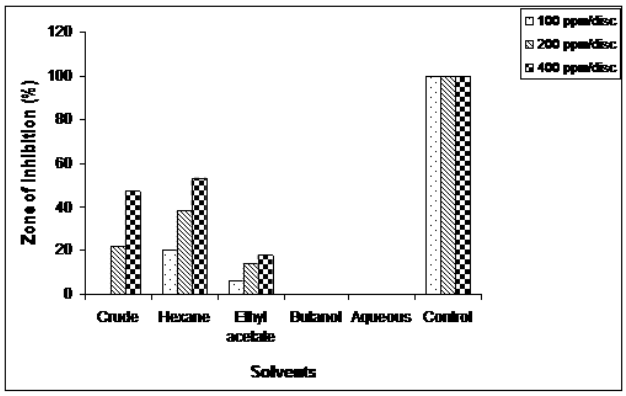

Fig. 9: Antibacterial activity of crude (methanol), n-hexane, ethyl acetate, butanol and aqueous extracted samples from *Physalis ixocarpa* stem tissues against *E. coli* by disc diffusion assay.

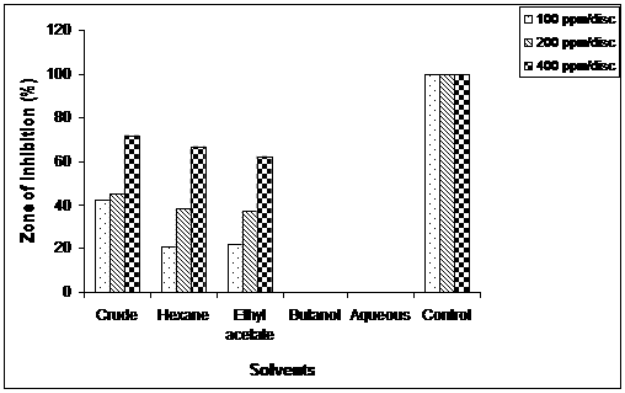

Fig. 10: Antibacterial activity of crude (methanol), n-hexane, ethyl acetate, butanol and aqueous extracted samples from *Physalis ixocarpa* stem tissues against *K. pneumonia* by disc diffusion assay.

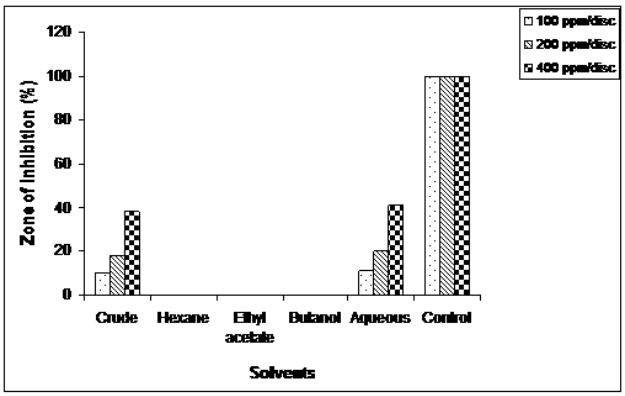

Fig. 11: Antibacterial activity of crude (methanol), n-hexane, ethyl acetate, butanol and aqueous extracted samples from *Physalis ixocarpa* fruit tissues against *K. pneumonia* by disc diffusion assay.

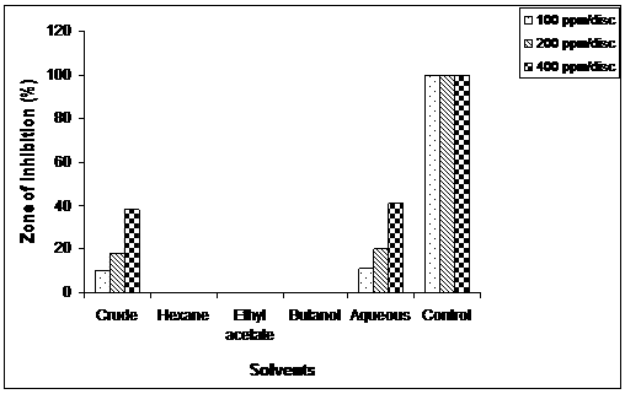

Fig. 12: Antibacterial activity of crude (methanol), n-hexane, ethyl acetate, butanol and aqueous extracted samples from *Physalis ixocarpa* fruit tissues against *S. areus* by disc diffusion assay.

DISCUSSION

The antibacterial activity of different solvent extracted samples from the calyx of *P. ixocarpa* indicated that crude methanol and aqueous extracted samples did not inhibit the growth of *B. subtilis*. Ethyl acetate and n-hexane extracted samples were effective to control the activity of *B. subtilis* at the tested concentrations. Highest growth inhibition was measured by ethyl acetate extracted samples against *B. subtilis* at higher concentration. Butanol extracted samples also showed activity against *B. subtilis* measuring at higher concentration, however, no activity was noted at lower concentration when compared with positive and negative controls. These results agree Prasad *et al*. (2009) and Gavimath *et al*. (2012), Bakht *et al*. (2011 a, b, c and d; 2012; 2013 a,b; 2014) Malik *et al.* (2015). The data suggested that crude extracted samples were not effective at all concentrations to control the growth of *E. coli* and aqueous extracted samples were effective to reduce the activity of *E. coli* at all concentrations compared with other solvent extrated samples. Ethyl acetate and n-hexane extracted samples did not inhibit the growth of *E. coli* at lower concentration, however, at higher concentrations, both of the extracts reduced the growth of *E. coli.* These results agree with Prasad *et al*. (2009) and Gavimath *et al*. (2012). Our results indicated that crude extracts, n-hexane and aqueous extracted samples did not inhibit the activity of *X. orayzae* at lower concentration, however, at higher concentrations these extracts were effective to reduce its activity. Highest growth reduction was noted for butanol extracted samples followed by ethyl acetate extracted fraction. All tested samples inhibited the growth of *S. aureus* at all concentration except butanol and ethyl acetate at lower concentration. Maximum inhibition was noted in aqueous extracted samples at higher concentration followed by n-hexane extracted samples at the same concentration. Similar results are also reported by Caceres *et al*. (1991) and Mahesh and Satish (2008). These results agree with Prasad *et al*. (2009). Butanol and ethyl acetate extracted samples from *P. ixocarpa* did not show any activity against *K. pneumonia* at all concentration. Similarly, crude extracts, n-hexane and aqueous extracted samples did not reveal activity against the same microbe at lower concentration, however, were effective at higher concentrations. Maximum reduction in the growth of *K. pneumonia* was noted for crude and aqueous s extracted samples at higher concentrations.

The results also showed that butanol, n-hexane and aqueous extracted samples from the leaves of *P. ixocarpa* did not reduce the growth of *B. subtilis* at all tested concentrations. Ethyl acetate extracted leaf samples were more effective to control the growth of *B. subtilis* causing maximum reduction in growth at higher concentration. Similarly, crude leaf extract showed some activity against *B. subtilis* at higher concentration and no activity at lower concentration against the same microbe. These results agree with Mahesh and Satish (2008), Prasad *et al*. (2009) and Gavimath *et al*. (2012). N-hexane and aqueous extracted samples from leaf tissues of *P. ixocarpa* did not inhibit the growth of *K. pneumonia* at any tested concentration. Crude and ethyl acetate extracted samples showed activity at all test concentration and butanol extracted samples were effective at higher concentration only. These results agree with Shariff *et al*. (2006), Mahesh and Satish (2008) and Gavimath *et al*. (2012). Our results also suggested that different solvent extracted samples leaf tissues were active against *S. aureus* except aqueous extracts at lower concentration. Crude leaf extract was more effective to control the activity of *S. aureus* followed by ethyl acetate extracts at higher concentrations. N-hexane and butanol extracted samples were equally effective to reduce the growth of *S. aureus* at all concentrations. Similar results are also reported by Mahesh and Satish (2008), Helvac *et al*. (2010), Patel *et al*. (2011), Gavimath *et al*. (2012) and Nathiya and Dorcus (2012). The data also revealed that crude leaf extracts and their fractions did not reduce the growth of *X. Oryzae* at any concentration. N-hexane and ethyl acetate extracted samples from the stem tissues of *P. ixocarpa* reduced the growth of *E. coli* at all tested concentrations. Crude extracts showed activity against *E. coli* at higher concentrations only. Butanol and aqueous extracted measured no activity against *E. coli* at all the tested concentrations. Maximum inhibition in *E. coli* growth was observed at higher concentration of n-hexane extracted samples from stem tissues. These results agree with Prasad *et al*. (2009). The growth of *K. pneumonia* was reduced by all solvent extracted samples at all concentrations except aqueous and butanol extracts where no activity was noted against *K. pneumonia*. Our results revealed that maximum activity was measured when crude extracted at higher concentration from stem tissues was applied followed by ethyl hexane extracted samples at the same concentration. These results agree with Mahesh and Satish (2008). Our results revealed no activity by all stem extracts at any concentration against *S. aureus, R. stolonifer* and *X. orayzae*.

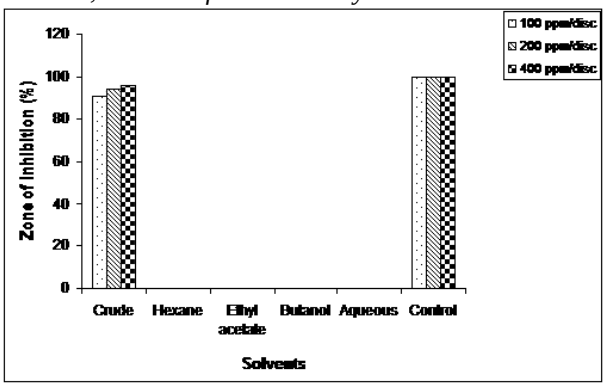

Fig. 13: Antibacterial activity of crude (methanol), n-hexane, ethyl acetate, butanol and aqueous extracted samples from *Physalis ixocarpa* leaf tissues against *R. orayzae* by disc diffusion assay.

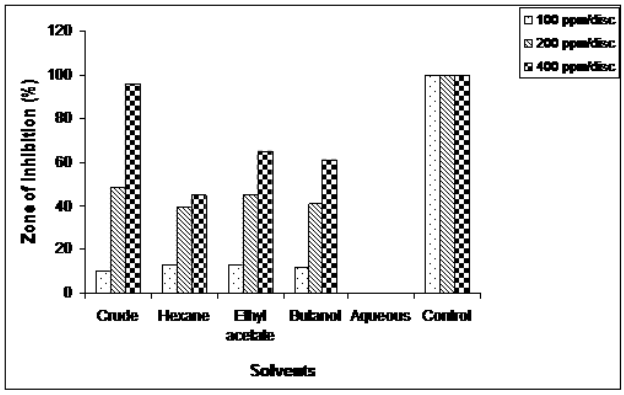

Fig. 14: Antibacterial activity of crude (methanol), n-hexane, ethyl acetate, butanol and aqueous extracted samples from *Physalis ixocarpa* leaf tissues against *A. niger* by disc diffusion assay.

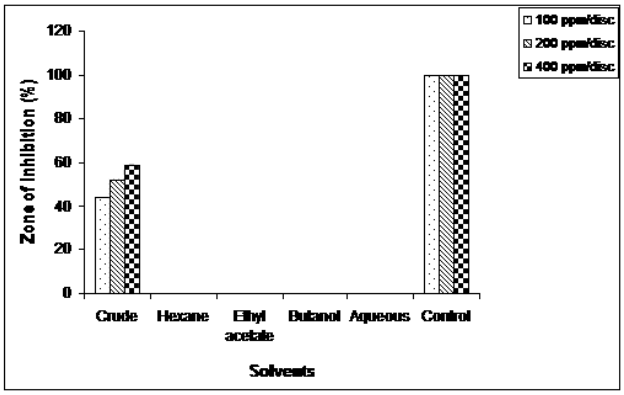

Fig. 15: Antibacterial activity of crude (methanol), n-hexane, ethyl acetate, butanol and aqueous extracted samples from *Physalis ixocarpa* leaf tissues against *Pencillium chrysogenum* by disc diffusion assay.

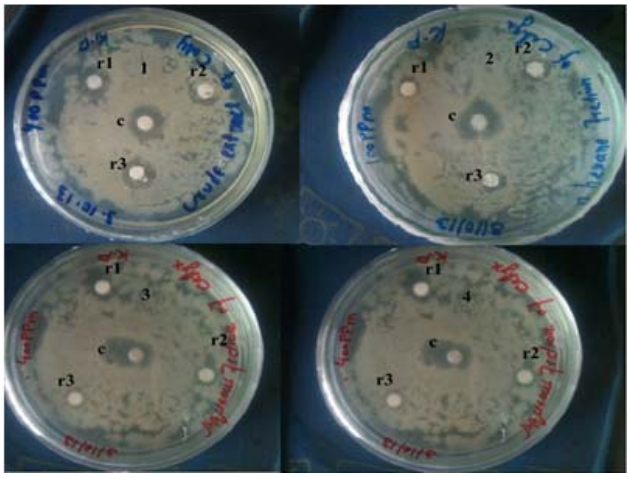

Plate 1: Antibacterial activity of calyx extracts against *K. Pneumoniae.*

The data further indicated that ethyl acetate and aqueous extracted samples from the fruits of *P. ixocarpa* did not reduce the growth of *K. pneumonia* at all tested concentrations. Crude, n-hexane and butanol extracted samples inhibited the growth of *K. pneumonia* at all tested concentrations. The results indicated that crude extracts of fruit tissues measure maximum zone of inhibition at higher concentration followed by butanol and n-hexane at the same concentration. Crude extracts and aqueous extracted samples from the fruit tissues were effective to reduce the growth of *S. aureus* at all tested concentrations. Hexane, butanol and ethyl acetate extracted samples did not inhibit the growth of *S. aureus* at all concentration measuring. The data also revealed that crude extracts and aqueous extracted samples from fruit the tissues were equally effective to control the growth of *S. aureus*. Our results also suggested that different solvent extracted samples from the fruit tissues were not effective to control the growth of *X. orayzae*, *E. coli* and *B. subtilis* at all tested concentrations. It is reported that *Physalis* genus contains several physalin (13,14-seco-16, 24-cycloergostane) compounds (Sen and Pathak, 1995), and some of them revealed anti microbial activity (Pietro *et al*., 2000).

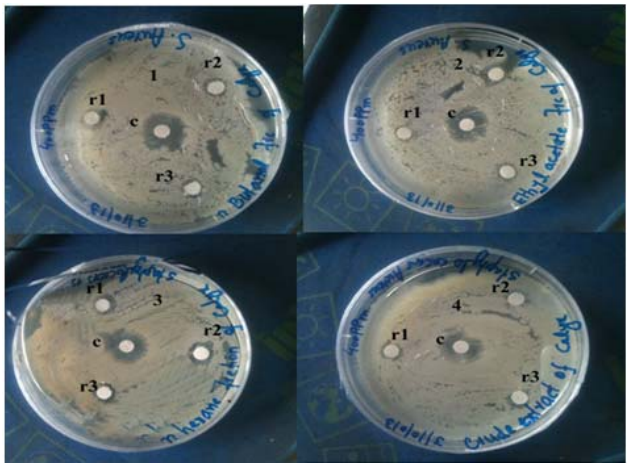

Plate 2: Antibacterial activity of calyx extracts against *S. aureus.*

Data regarding antifungal activity of different solvent extracted samples from the leaf tissues against *R. stolonifer* indicated that crude extract was more potent to reduce the growth of *R. stolonifer* at all tested concentrations. N-hexane, ethyl acetate, butanol and aqueous extracts did not reduce the growth of *R. stolonifer* at all concentrations. Our results also indicated that different solvent extracted samples from the leaf tissue were very effective to reduce the activity of *A. niger* at all tested concentrations except aqueous extracted samples. These results agree with Prasad *et al*. (2009). The data indicated that crude extracted samples showed good activity against *Pencillium chrysogenum* at all the tested concentrations. Maximum activity was revealed when crude extracted was tested at higher concentration. Similar results are reported by Shariff *et al.* (2006). Hexane, ethyl acetate, butanol and aqueous extracted leaf samples did not reduce the growth of the same fungus. Our results also revealed that different solvent extracted

Table 1: Microbial strains used in this research work

Microbial species	Gram strain type	Detail of microbial strains used
Bacillus subtilis	Positive	Clinical isolates obtained from Microbiology Department Quaid-E-Azam University Islamabad Pakistan
Escherichia coli	Negative	ATCC 25922
Klebsiella pneumonia	Negative	Clinical isolates obtained from Microbiology Department Quaid-E-Azam University Islamabad Pakistan
Staphylococcus aureus	Positive	ATCC 6538
Xanthomonas oryzae	Negative	Department of Plant Pathology, The University of Agriculture Peshawar Pakistan
Aspergillus niger		Department of Plant Pathology, The University of Agriculture Peshawar Pakistan
Pencillium chrysogenum		Department of Plant Pathology, The University of Agriculture Peshawar Pakistan
Rhizopus stolonifer		Department of Plant Pathology, The University of Agriculture Peshawar Pakistan
Rhizopus oryzae		Department of Plant Pathology, The University of Agriculture Peshawar Pakistan
Acromonium alternatum		Department of Plant Pathology, The University of Agriculture Peshawar Pakistan

samples from leaf tissues did not inhibit the growth of *Rhizopus oryzae* and *A. alternatum* at any concentration.

Our results were innovative for two reasons when compared with the findings of other researchers. Firstly, we first isolated the crude extracts and then fractionated the crude with different solvents from in ascending polarity starting from less polar to more polar, which demonstrated effective isolation of different bioactive compounds whereas other researchers used crude extract in different solvent. Secondly, we report for the time the antifungal activity in *P. ixocarpa*, not investigated and reported earlier.

CONCLUSION

From these results it can be concluded that different solvent extracted samples from various parts of *P. ixocarpa* measured varying degree of growth inhibition of different bacteria at different concentrations. Calyx extracts reduced the growth of most of the bacteria under study. Extracts isolated from leaf and fruit samples showed activity against *S. aureus* and *K. pneumoniae* and extracts from the stem tissues were effective against *E. coli* and *K. pneumoniae.* Crude methanolic extract from the stem and butanol extracted samples from fruit exhibited strong activity against *K. pneumoniae* at highest concentrations. Antifungal activity was observed only in crude methanol extract from the leaf against *R. stolinifer*, *A. niger* and *P. chrysogenum*.

REFERENCES

Ahmad N, Zebta KS, Javid H and Razia P. (2015). Phytochemical, antibacterial and antioxidant investigation of *Alhagi maurorum* Medik. *Pak. J. Bot*., **47**: 121-124.

Ashraf A, Raja AS, Farooq A, Shaukat AS and Khalid MA. (2015). Chemical composition and biological activities of leaves of *Ziziphus mauritiana* L. native to Pakistan. *Pak. J. Bot.,* **47**: 367-376.

Bakht J, Tayyab M, Ali H, Islam A and Shafi M (2011a). Effect of different solvent extracted samples of *Allium sativum* on bacteria and fungi. *Afr. J. Biotechnol.,* **10**: 5910-5915.

Bakht J, Islam A, Tayyub M, Ali H and Shafi M (2011b). Anti-microbial potentials of *Eclipta alba* by disc diffusion method. *Afr. J. Biotechnol.,* **10**: 7668-7674.

Bakht J, Ali H, Khan MA, Khan A, Saeed M, Shafi M, Islam A and Tayyab M (2011c). Antimicrobial activities of different solvents extracted samples of *Linum usitatissimum* by disc diffusion. *Afr. J. Biotechnol.,* **10**: 19825-19835.

Bakht J, Islam A and Shafi M (2011d). Antimicrobial potential of *Eclipta alba* by well diffusion method. *Pak. J. Bot.,* **43**: 161-166.

Bakht J, Azra and Shafi M (2012). Anti-microbial activity of *Nicotiana tobaccum* using different solvent extracts. *Pak. J. Bot.,* **44**: 459-463.

Bakht J, Khan S and Shafi M (2013a). Anti-microbial potentials of fresh *Allium cepa* against gram positive and gram negative bacteria and fungi. *Pak. J. Bot.,* **45**: 1-6.

Bakht J, Azra and Shafi M (2013b). Anti-microbial potential of different solvent extracts of tobacco (*Nicotiana rustica*) against gram negative and positive bacteria. *Pak. J. Bot.,* **45**: 643-648.

Bakht J, Shehla K and Shafi M (2014a). *In vitro* anti-microbial activity of *Allium cepa* (dry bulbs) against

Gram positive and Gram-negative bacteria and fungi. *Pak. J. Pharma. Sci.,* **27**: 139-145.

Bakht J, Shaheen S and Shafi M (2014b). Antimicrobial potentials of *Mentha longifolia* by disc diffusion method. *Pak. J. Pharma. Sci*., **27**: 939-945.

Bakht J, Gohar N and Shafi M (2014c). *In vitro* antibacterial and antifungal activity of different solvent extracted samples of *Alhagi maurorum*. *Pak. J. Pharma. Sci*., **27:** 1955-1961.

Bakht J, Fatema S, Shafi M. (2015). Screening of *Vinca rosea* for their antibacterial and antifungal activity by disc diffusion assay. *Pak. J. Pharma. Sci.,* **28**: 833-839.

Bauer AW, Kirby WMM, Sherris JC and Turck M (1966). Antibiotic susceptibility testing by standardized single disk method. *Am. J. Clin. Pathol.,* **45**: 493-496.

Caceres A, Alma VA, Ana EO and Blanca ES (1991). Plants used in guatemala for the treatment of respiratory diseases. 1. Screening of 68 plants against gram-positive bacteria. *J. Ethnopharm*., **31**: 193-208.

Cowan MM (1999). Plant products as an antimicrobial agents. *Clin. Microbiol. Rev*., **12**: 564-582.

Gavimath CC, Kulkarni SM, Raorane CJ, Kalsekar DP and Gavade BG (2012). Antibacterial potentials of *Solanum indicum*, *Solanum xanthocarpum* and *Physalis minima*. *Int. J. Pharma. Appl.*, **3**: 414-418.

Helvac S, Kokdil G, Kawai M, Duran N and Guvenc A (2010). Antimicrobial activity of the extracts and physalin D from *Physalis alkekengi* and evaluation of antioxidant potential of physalin D. *Pharma. Biol*., **48**: 142-150.

Karabuluti G and Sule O. (2015). Antifungal activity of *Evernia prunastri, Parmelia sulcata, Pseudevernia furfuracea* var. Furfuracea. *Pak. J. Bot.,* **47**: 1575-1579.

Karthikeyani TP and Janardhanan K (2003). Indigenous medicine for snake, scorpion and insect bites/stings in siruvani hills, Western ghats, South India. *Asi. J. Microbiol. Biotechnol. Environ. Sci*., **5**: 467-470.

Khan AY and Zakia L. (2014). Screening of medicinally natural extracts for their antibacterial activity against *Salmonella* species. *Pak. J. Bot.,* **46**: 2269-2275.

Mahesh B and Satish S (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogens. *World J. Agric. Sci*., **4**(S): 839- 843.

Malik R, Tasveer ZB, Muhammad FS, Uzma Y, Muhammad IH and Imran AK. (2015). Antimicrobial activity of *Nerium oleander* L. and *Nicotiana tabacum* L. : A comparative study. *Pak. J. Bot.,* **47**: 1587-1592.

Nasir A, Dawood A and Bakht J (2015). Antibacterial activity of different solvent extracted samples from the flowers of medicinally important *Plumeria obstusa*. *Pak. J. Pharma. Sci*., **28**: 195-200.

Nathiya M and Dorcus D (2012). Preliminary phytochemical and anti-bacterial studies of *Physalis minima* Linn. *Int. J. Curr. Sci*., **32**: 24-30.

Nostro A, Germano MP, Angelo V, Marino A and Cannatelli MA (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Lett. Appl. Microbiol*., **30**: 379-348.

Patel T, Shah K, Jiwan K and Neeta S (2011). Study on the antibacterial potential of *Physalis minima* linn. *Indian J. Phram. Sci*., **73**: 111-115.

Pietro RC, Kashima S, Sato DN, Januario AH and Franca SC (2000). *In vitro* antimycobacterial activities of *Physalis angulata* L. *Phytomed*., **7**: 355-358.

Prasad SHKR, Swapna NL, Rajasekhar D, Anthonamma K and Prasad M (2009). Preliminary phytochemical and antimicrobial spectrum of cultured tissue of physalis minina (L.). *Int. J. Chem. Sci.,* **7**: 2719-2725.

Ramdas K, Suresh G, Janardhana N and Masilamani S (1998). Antifungal activity of 1,3-disubstituted symmetrical and unsymmetrical thioureas. *Pest Sci*., **52**: 145-151.

Russel DF and Eisensmith SP (1983). MSTAT-C. Crop Soil Science Department, Michigan State University USA.

Sen G and Pathak HD (1995). Physalin L, a 13,14-seco-16, 24 cyclosteroid from *Physalis minima*. *Phytochem*., **39**: 1245-1246.

Shariff N, MS Sudarshana, S Umesha and P Hariprasad (2006). Antimicrobial activity of *Rauvolfia tetraphylla* and *Physalis minima* leaf and callus extracts. *Afr. J. Biotechnol*., **5**: 946-950.

Steel RGD, Torrie JH and Dickey DA (1997). Principles and Procedures of Statistics. A Biometrical Approach, 3rd Ed. McGraw Hill Book Co. Inc. New York USA. pp.172-177

Suffredini JB, Sader HS, Goncalves AG, Reis AO, Gales AC, Varella AD and Younes RN (2004). Screening of antimicrobial extracts from plants native to the brazilian amazon rainforest and atlantic forest. *Brazil. J. Med. Biol. Res*., **37**: 379-384.

Ullah R, Bakht J and Shafi M (2015). Antibacterial and anti-oxidant potential of *Periploca hyaspidis*. *Bangladesh J. Pharmacol.,* **10**: 645-651.

WHO (1991). The Use of Essential Drugs. 8th report of the WHO Expert Committee. World Health Organization, Geneva, Ackerknecht, E.H. Therapeutics: From the Primitives to the Twentieth Century.

Willis JC (1966). A dictionary of the flowering plants and ferns. Cambridge Univ. Press, Cambridge, UK.

Zaika LL (1975). Spices and herbs: Their antimicrobial activity and its determination. *J. Food and Saf.,* **9**: 97-18.