Antimicrobial Activity and Brine Shrimp Lethality of Some Selected Medicinal Plants

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Abstract

Secondary metabolites produced by plants and animals have biochemical effects in man, animals and other plants. The huge amounts of such compounds have a broad range of effects, from being acute deadly to being healthy or curative.This research project was undertaken to investigate some pharmacological properties of five medicinal plants that are used in ethnobotanical treatments in Ayurvedic medicine: *Acanthus ilicifolius*, *Euphorbia hirta*, *Pogostemon heyneanus*, *Phyllanthus reticulatus* and *Pothos scandens*. Parts of each plant used in treatments were air dried, powdered and then sequentially extracted into hexane, ethyl acetate and methanol. Brine shrimp (*Artemia salina*) lethality assay was done to determine lethal concentrations. Preliminary antimicrobial susceptibility was tested against three bacterial strains and one fungal strain. For all fifteen plant extracts, cut-well agar diffusion method was utilized on *Escherichia coli*, and agar disk diffusion method was utilized on *E. coli*, *Staphylococcus aureus*, methicillin resistant *S. aureus* (MRSA) and *Candida albicans*. The ethyl acetate extract of *P. reticulatus* was the most active (toxic) plant extract. It showed a highly toxic LC_{50} value of 22.81 ppm, which was greater than the LC_{50} value of 39.62 ppm obtained from the positive control potassium dichromate. The hexane extract of *P. reticulatus* was also highly toxic with an LC_{50} result closer to the positive control at 44.95 ppm. Ethyl acetate and methanol extracts of *E. hirta*, hexane and ethyl acetate extracts of *P. heyneanus*, and the methanol extract of *P. reticulatus* were active against both gram positive *S. aureus* and gram negative *E. coli* bacteria. Only the hexane and the ethyl acetate extracts of *P. scandens* were active against MRSA bacteria. Most of the plant extracts tested was active on the *C. albicans* fungus. The most toxic plant extract among the fifteen tested was the ethyl acetate extract of *P. reticulatus* which is more toxic than potassium dichromate. According to results obtained in microbial assay, hexane extract of *E. hirta* was the most active plant extract against *C. albicans*. All plant extracts are more active against *Candida albicans* than bacterial strains.

***Keywords*:** *Antimicrobial, Acanthus ilicifolius, Euphorbia hirta, Pogostemon heyneanus, Phyllanthus reticulatus, Pothos scandens*

1. Introduction

By providing scientific information on medicinal plants, productivity, therapeutic efficiency and competitiveness of formulations derived from these plants can be improved in the fields of medicine and pharmacy. A bioassay quantifies an amount of a chemical through the use of a living organism or living tissue as a substitute for an analytical technique or analytical instrument. Since the use of medicinal plants have become increasingly widespread, experimental screening of plant toxicity is crucial to assure the safety and effectiveness of these natural products. In this investigation, *in-vitro* analysis for antimicrobial activity against four microbial strains and brine shrimp lethality were studied as preliminary studies to identify potent plants among five selected medicinal plants.

2. Objectives

To determine the antimicrobial activity of several medicinal plants and to determine brine shrimp lethality as preliminary toxicity evaluation of those extracts.

3. Material and methods

3.1 Plant selection procedure

First 15 plants were selected, depending on their availability, from 84 plants which are nominated as ethnobotanically used plants for Asthma. The selected 15 plants were further subjected to extensive literature survey to find out previous research activities and then 8 plants were selected which had been paid

less attention by previous research activities. Finally, 5 plants were selected upon discussion with the supervisor depending on their availability, previous research works and their Family/Genus.

3.2 Collection and authentication of plant

The selected plants were collected from Matale District, Sri Lanka, since it is a rich habitat for both dry zone plants and wet zone plants. Then they were identified by a taxonomist based on herbarium records in the National Herbarium of Royal Botanical Garden, Peradeniya, Sri Lanka.

3.3 Preparation of extract

Parts of the plants that are ethnobotanically used for treatments were used in this investigation. Stem of *Pothos scandens*, leaves of *Phyllanthus reticulatus*, both stem and leaves of *Pogostemon heyneanus*, both stem and leaves of *Acanthus ilicifolius*, and whole plant of *Euphorbia hirta* were used. Each and every plant part was air dried for about 3 days and then pulverized in a mixer grinder and was sequentially extracted into separate hexane, ethyl acetate and methanol solvents.

3.4 Brine shrimp larvae assay

3.4.1 Method

Brine shrimp toxicity was determined according to the method described by Michal, Thomps and Abraliov (1956).

3.4.2 Procedure

Two thousand five hundred ppm stock solutions were prepared by dissolving 0.1250±0.0001 g of each extract in 50 ml of 1% dimethylsulfoxide (DMSO) solution (DMSO in artificial sea water). Next 1250, 625, 312.5, 156.25, 75, 37.5 and 18.75 ppm solutions were obtained by serial dilution. Potassium dichromate solution was prepared with a matching concentration series using 1% DMSO in artificial sea water to use as the positive control. A 1% DMSO solution in artificial seawater was used as the negative control. Then, 4 ml of each test solution was placed in labelled test tubes and around 10 brine shrimps with 1 ml of artificial sea water were added to each test tube. Total volume was kept at 5 ml for each test sample giving the final tests concentrations of 2000, 1000, 500, 250, 125, 60, 30 and 15 ppm. This test was carried out in triplicate for each test solution and for the positive and negative controls. All test tubes were covered with aluminium foil. After 24 hours, numbers of dead nauplii out of total shrimp in each test tube were counted using a magnifying glass and the mortality percentage was calculated. This procedure was repeated for each extract taken into the experiment and LC_{50} values were calculated using Minitab software.

3.4.3 Statistical analysis and comparison

All experiments were carried out in triplicate. The values are expressed as the mean ± standard deviation (SD). Complete Randomized Design (CRD) was used as the experimental model for analysis. Even though the 50% lethal concentration could be estimated from the dose response curve, LC_{50} values were calculated using statistical software for increased accuracy and reliability. Experimental data were analysed using the Probit analysis tool of the Minitab software program.

3.5 Antimicrobial Activity

3.5.1 Method

Antibacterial susceptibility testing was done using the agar cut well-diffusion method and agar disk diffusion method according to standards of the National Committee for Clinical Laboratory Standards with some slight modifications.

Antimicrobial susceptibility was tested against three bacterial strains and one fungal strain which were isolated from pure standard cultures of *Staphylococcus aureus*, *E. coli*, methicillin resistant *Staphylococcus aureus* (MRSA) *and Candida albicans.*

3.5.2 Procedure

Preparation of stock solution

Ten thousand ppm crude extract solutions were prepared by dissolving appropriate weights of crude extract in 100% dimethylsulfoxide (DMSO).

Media preparation and maintenance

The bacterial strains were sub cultured overnight in LB broth, which was adjusted to obtain turbidity comparable to McFarland (0.5) standard. *Candida albicans* was sub cultured overnight on Potato Dextrose Agar (PDA). Subsequently, loops of *Candida albicans* inoculum was taken from PDA plates and transferred to test tubes containing 5 ml of sterile distilled water which was further adjusted to obtain turbidity comparable to McFarland (0.5) standard.

All antimicrobial susceptible tests were conducted on Muller Hinton Agar (MHA) medium at 37°C and pH 7.3 ± 0.2. The MHA medium was prepared according to the manufacturer's recommendations and sterilized at 121°C (15 lbs.) for 15-20 minutes in an autoclave. Twenty millilitres of molten MHA medium was poured in 90 mm sterile petri dishes to give a mean depth of 4.00 ± 0.5 mm and was allowed to set at room temperature. Afterwards, the surface of the set agar medium was inoculated evenly with 2 ml of standardized inoculum by swirling and excess culture was pipetted out using a micropipette. The inoculum was allowed to be absorbed for at least 3 minutes but no longer than 15 minutes.

Cut well agar diffusion method

Using a sterile 10 mm diameter cork borer, wells were cut on each MHA plate and the bottom of each well was sealed with a drop of agar. The wells were named with code names of extracts and a separate well was used for the DMSO control. Afterwards, the plant extracts (~50 µl) were added into wells and the control well was loaded with DMSO. Three replicates were carried out for each extract. These plates were then incubated at 37°C for 24 hours and the zones of inhibition (ZOI) were observed and measured using a digital Vernier calliper.

Agar disk diffusion method

Sterile Whatman 1M filter paper disks with a 6 mm average diameter were soaked in DMSO which was used as the negative control and placed on the center of each inoculated plate. Then same type disks were soaked in plant extract stock solutions and placed on the inoculated plates at equidistance in a circle using blunt-nosed thumb forceps. Afterwards the petri dishes were incubated at 37°C for 24 h. The experiment was conducted in triplicate. A measurement of the inhibition zone diameter around each filter paper disk was taken using a digital Vernier calliper to detect antimicrobial activity.

4. Results and Discussion

4.1 Comparison and Discussion of brine shrimp lethality assay

In this experiment, some trials gave highly scattered results in relation to mean values. Therefore, one trial was rejected and two were averaged in order to obtain a minimal relative standard deviation (RSD).

The LC_{50} values for each extract are given in the Figure 1 and Table 1. According to the obtained results, the most active (toxic) plant extract among the fifteen tested was the ethyl acetate extract of *P. reticulatus*. It showed an LC_{50} value of 22.81 ppm, indicating that it is more toxic than the potassium dichromate positive control, which was measured to have an LC_{50} value of 39.62 ppm. The hexane extract of *P. reticulatus* also showed high toxicity with an LC_{50} value close to the positive control at 44.95 ppm. Only these two extracts were deemed to be highly toxic among all fifteen extracts tested with LC_{50} values between 1-100 ppm. All other extracts were less toxic than the potassium dichromate.

All three extracts of *Pogostemon heyneanus* were moderately toxic to *Artemia salina* with results of 258.05, 318.57, and 234.92 ppm for hexane, ethyl acetate, and methanol extracts respectively. Furthermore, the hexane extract of *Pothos scandens*, the ethyl acetate extract of *Acanthus ilicifolius* and methanol extracts of both *Pothos scandens* and *Acanthus ilicifolius* were moderately toxic to brine shrimp with LC_{50} values of 930.61, 573.38, 232.99, and 508.58 ppm respectively. (Figure 1)

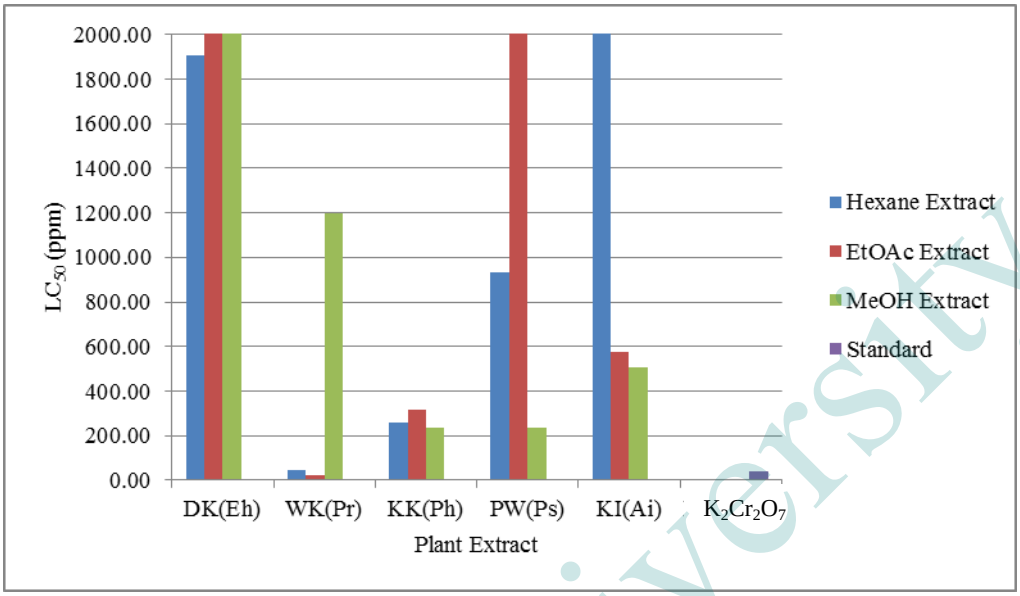

Figure 1 Brine shrimp lethality assay comparisons between tested plants

Table 1 Table of 50% lethal concentrations (LC_{50}) and their maximum efficacy

Extract Name	LC_{50} / ppm	Maximum Efficacy
Euphorbia hirta (Hexane)	1907.47	88
Euphorbia hirta (EtOAc)	2452.43	56
Euphorbia hirta (MeOH)	4056.27	20
Phyllanthus reticulatus (Hexane)	44.95	100
Phyllanthus reticulatus (EtOAc)	22.81	100
Phyllanthus reticulatus (MeOH)	1199.20	58
Pogostemon heyneanus (Hexane)	258.05	100
Pogostemon heyneanus (EtOAc)	318.57	100
Pogostemon heyneanus (MeOH)	234.92	90
Pothos scandens (Hexane)	930.61	82
Pothos scandens (EtOAc)	2920.79	43
Pothos scandens (MeOH)	232.99	100
Acanthus ilicifolius (Hexane)	4298.19	30
Acanthus ilicifolius (EtOAc)	573.38	82
Acanthus ilicifolius (MeOH)	508.58	90
Potassium Dichromate (Standard)	39.62	100

The methanol extract of *P. reticulatus*, the ethyl acetate extract of *P. scandens* and the hexane extract of *A. ilicifolius* were non-toxic to *A. salina* nauplii with statistically analysed LC_{50} results at 1199.20, 2920.79, and 4298.19 ppm respectively. All three extracts of *E. hirta* were also recorded as non-toxic with LC_{50} values beyond 1000 ppm at 1907.47, 2452.43, and 4056.27 ppm.

The Correlation between 50% lethality and the maximum efficacy is given in Figure 2. When comparing the dose response curves of all extracts taken into this experiment, the hexane and ethyl acetate extracts of *P. reticulates* proved to be the most toxic and also showed the highest maximal efficacy as indicated by their maximal attainable responses (ceiling effect). Moreover, the Hexane extract of *P. heyneanus*, the ethyl acetate extract of *P. heyneanus* and the methanol extract of *P. scandens* were moderately toxic to brine shrimp and showed their highest maximal efficacy as 100% mortality. This result

shows that even though these extracts have moderate LC_{50} values, they are effectively toxic at high concentrations.

The lowest ceiling effect was given by the methanol extract of *E. hirta*, confirming that it is non-toxic even at high concentrations. The hexane extract of *A. ilicifolius* showed the second smallest maximal efficacy while having the highest 50% lethal concentration (LC_{50}), indicating that it is also non-toxic. From these results, it can be concluded that even though some of the extracts are more potent, their maximal efficacy may be lower (National Institutes of Health, n.d.).

Another study has reported on cytotoxic effects of *E. hirta* against the Vero cell line by Perumal, Mahmud, Pillai, Lee and Ramanathan (2012). They performed serial extractions utilising hexane, dichloromethane, ethyl acetate and ethanol as solvents. According to their results, *E. hirta* extracts were not toxic to Vero cells with IC_{50} values of 126.0, 140.8, 128.3, and 119.4 ppm recorded for ethanol, hexane, dichloromethane and ethyl acetate extracts, respectively.

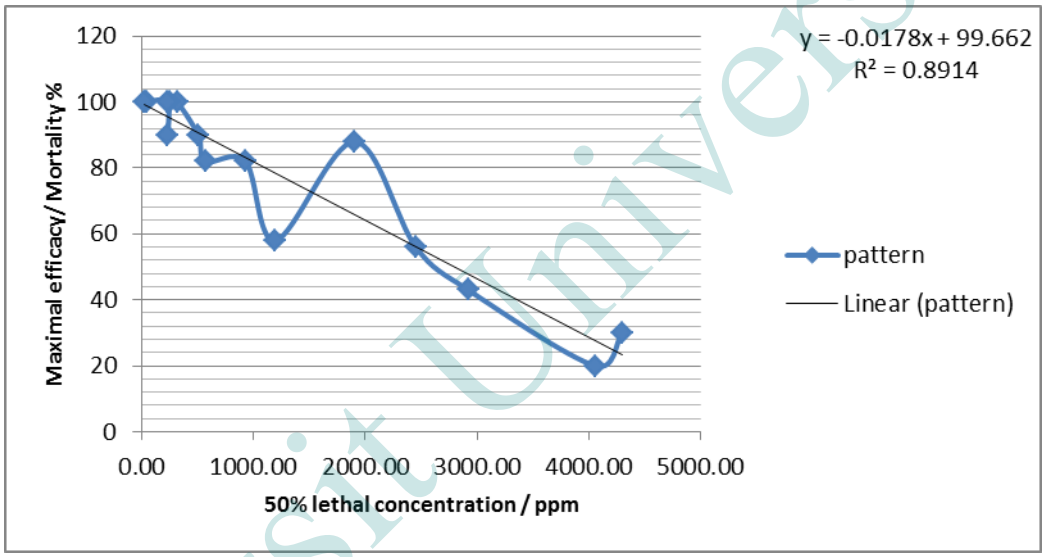

Figure 2 Correlation between 50% lethality and the maximum efficacy (ceiling effect)

Based on the US National Cancer Institute (NCI) plant screening program, a crude extract is generally considered to have *in-vitro* cytotoxic activity if the LC_{50} value (concentration that causes a 50% cell death) is less than 20 ppm. Interestingly, according to the results of Perumal *et al*. (2012), all *E. hirta* extracts exhibited no cytotoxic effects against the Vero cell line, resembling the brine shrimp assay results.

According to brine shrimp lethality experiments reported by Krishnaraju *et al*. (2005), the LC_{50} value obtained for a leaf extract of *P. reticulates* was 60 ppm (Krishnaraju *et al*., 2005). However, in their experiments, plant materials were only extracted with water/ hydro-alcohol/ alcohol as solvents. Therefore, both moderately-polar and polar compounds were extracted into the same crude extract and resulted in an averaged concentration for LC_{50} value. This can be confirmed with the results obtained in the current experiment as LC_{50} values for hexane, ethyl acetate and methanol extracts were 44.95, 22.81 and 1199.20 ppm respectively.

While LC_{50} values from the current experiment suggested the stem bark extract of *P. scandens* to be less toxic than its leaf, Yusuf *et al*. (n.d.) have reported an LC_{50} value of 14.195 µg/ml from a brine shrimp lethality assay against a methanol extract from fresh *P. scandens* leaves (Yusuf *et al., n.d.*).

4.2 Result and discussion of antimicrobial activity

In the cut-well agar diffusion experiment, ethyl acetate and methanol extracts of *E. hirta*, hexane and ethyl acetate extracts of *P. heyneanus* and the methanol extract of *P. reticulatus* showed antibacterial activity against *E. coli* strains. All other extracts did not show any significant antibacterial activity against *E. coli*. The inhibition zone diameters for cut-well agar diffusion method are given in Table 2.

In the agar disk diffusion experiment, antibacterial activity against *E. coli* strains were shown by the same extracts which showed antibacterial activity in the cut-well agar diffusion test. This result concludes that both agar dilution methods provided consistent observations. The same extracts which were active on gram negative bacteria, namely ethyl acetate and methanol extracts of *E. hirta*, hexane and ethyl acetate extracts of *P. heyneanus* and the methanol extract of *P. reticulatus*, were also active on *S. aureus* bacteria. All other extracts were not active on *S. aureus* bacteria.

Table 2 Inhibition zone diameters obtained for cut-well agar diffusion experiment against *E. coli*

Extract No	Extract Name	Inhibition zone diameter / mm			
		Echerichia coli			
		Trial 1	Trial 2	Trial 3	Avg
1	Eh (DK) –Hex	-	-	-	-
2	Eh (DK) –EtOAc	11.32	12.16	11.69	11.72
3	Eh (DK) –MeOH	11.56	11.84	11.15	11.52
4	Pr (WK) –Hex	-	-	-	-
5	Pr (WK) –EtOAc	-	-	-	-
6	Pr (WK) –MeOH	11.21	11.00	11.00	11.07
7	Ph (KK) –Hex	14.16	12.82	13.43	13.47
8	Ph (KK) –EtOAc	13.54	13.47	13.65	13.55
9	Ph (KK) –MeOH	-	-	-	-
10	Ps (PW) –Hex	-	-	-	-
11	Ps (PW) –EtOAc	-	-	-	-
12	Ps (PW) –MeOH	-	-	-	-
13	Ai (KI) -Hex	-	-	-	-
14	Ai (KI) -EtOAc	-	-	-	-
15	Ai (KI) -MeOH	-	-	-	-
	DMSO	10.00	10.00	10.00	10.00

Unlike *E. coli* and *S. aureus*, MRSA is known to be resistant to most plant extracts. Surprisingly, hexane and ethyl acetate extracts of *P. scandens* showed significant activity against MRSA. This observation might be the result of a unique mechanism of inhibition such as growth control or cell wall breaking associated with this extract. This is a significant observation and merits further research on *P. scandens* to possibly identify compounds with activity against a drug-resistant bacteria strain.

The inhibition zone diameters for agar disk diffusion method are given in Table 3 and Table 4. Most of the plant extracts taken into this experiment were active on *C. albicans*. When comparing diameters of inhibition zones, the highest activity was shown by *E. hirta.* This observation validates its use in ethnobotanical treatments to cure fungal diseases. Overall, hexane and ethyl acetate extracts of *P. scandens*, hexane and methanol extracts of *E. hirta*, the methanol extract of *A. ilicifolius*, all three extracts of *P. heyneanus*, and ethyl acetate and methanol extracts of *P. reticulatus* were active on *C. albicans.* All other extracts were not active against this fungus.

Among all fifteen extracts, the methanol extract of *P. scandens*, hexane and ethyl acetate extracts of *A. ilicifolius*, and the hexane extract of *P. reticulatus* showed no activity against any of the microbial strains tested. The antimicrobial activity of alcoholic, butanolic, and chloroform extracts of leaves and roots of the *A. ilicifolius* have been previously studied by Bose and Bose (2008). Their research showed that the alcoholic extract and the chloroform extract of *A. ilicifolius* leaves exhibit strong inhibitory action against *S. aureus* and *C. albicans*. However, in the current experiment all three extracts from *A. ilicifolius* did not show antibacterial activity against *S. aureus*. The differing results may be due to different environments from which the plants were sourced from. Nevertheless, in the current experiment the methanol extract of *A. ilicifolius* showed antifungal activity against *C. albicans*, supporting the results obtained by Bose (2008). According to results obtained by Perumal et al. (2012), dichloromethane, ethyl acetate and ethanol extracts

of *E. hirta* display anti-bacterial activity against *E. coli* and *S. aureus.* These properties were verified by the results obtained in the current experiment.

Table 3 Inhibition zone diameters obtained from the agar disk diffusion experiment against *E. coli* and *S. aureus*.

Ext. No	Extract Name	Inhibition zone diameter / mm							
		Escherichia coli				*Staphylococcus aureus*			
		Trial 1	Trial 2	Trial 3	Avg	Trial 1	Trial 2	Trial 3	Avg
1	Ps (PW) -Hex	-	-	-	-	-	-	-	-
2	Ps (PW) -EtOAc	-	-	-	-	-	-	-	-
3	Ps (PW) -MeOH	-	-	-	-	-	-	-	-
4	Eh (DK) -Hex	-	-	-	-	-	-	-	-
5	Eh (DK) -EtOAc	6.24	6.31	6.19	6.25	8.34	7.62	7.38	7.78
6	Eh (DK) -MeOH	6.46	6.74	6.61	6.60	7.13	7.46	7.29	7.29
7	Ai (KI) -Hex	-	-	-	-	-	-	-	-
8	Ai (KI) -EtOAc	-	-	-	-	-	-	-	-
9	Ai (KI) -MeOH	-	-	-	-	-	-	-	-
10	Ph (KK) -Hex	7.15	6.10	6.88	6.71	7.70	7.23	7.94	7.62
11	Ph (KK) -EtOAc	7.99	7.58	7.37	7.65	7.96	7.96	8.23	8.05
12	Ph (KK) -MeOH	-	-	-	-	-	-	-	-
13	Pr (WK) -Hex	-	-	-	-	-	-	-	-
14	Pr (WK) -EtOAc	-	-	-	-	-	-	-	-
15	Pr (WK) -MeOH	6.37	6.24	6.12	6.24	6.21	6.31	6.37	6.30

Table 4 Inhibition zone diameters obtained from the agar disk diffusion experiment against MRSA and *C. albicans*.

Ext. No	Extract Name	Inhibition zone diameter / mm							
		MRSA				*Candida albicans*			
		Trial 1	Trial 2	Trial 3	Avg	Trial 1	Trial 2	Trial 3	Avg
1	Ps (PW) -Hex	6.93	6.22	6.61	6.59	8.16	8.21	8.54	8.30
2	Ps (PW) -EtOAc	6.33	6.54	6.29	6.39	14.98	15.67	15.29	15.31
3	Ps (PW) -MeOH	-	-	-	-	-	-	-	-
4	Eh (DK) -Hex	-	-	-	-	21.34	21.76	20.96	21.35
5	Eh (DK) -EtOAc	-	-	-	-	-	-	-	-
6	Eh (DK) -MeOH	-	-	-	-	9.16	9.42	9.35	9.31
7	Ai (KI) -Hex	-	-	-	-	-	-	-	-
8	Ai (KI) -EtOAc	-	-	-	-	-	-	-	-
9	Ai (KI) -MeOH	-	-	-	-	8.95	8.17	8.51	8.54
10	Ph (KK) -Hex	-	-	-	-	18.22	18.06	18.12	18.13
11	Ph (KK) -EtOAc	-	-	-	-	18.51	18.85	18.24	18.53
12	Ph (KK) -MeOH	-	-	-	-	9.37	9.84	9.23	9.48
13	Pr (WK) -Hex	-	-	-	-	-	-	-	-
14	Pr (WK) -EtOAc	-	-	-	-	11.80	12.91	12.74	12.48
15	Pr (WK) -MeOH	-	-	-	-	11.58	12.24	11.88	11.90

The ethanol extract of *E. hirta* leaves has been analysed for antimicrobial activity, using agar well diffusion, against several bacteria species by Bhaskara, Karthik , Elumalai , Srinivasan and Gaurav (2010). They studied leaves of *E. hirta* which were collected during different time periods and reported inhibition zone diameters against *S. aureus* at 11.20, 8.23, 11.26, 16.16 mm for January to mid-March, mid-March to May end, June to mid-August, and mid-August to December, respectively. According to their results, leaves collected during mid-August to December had significantly higher antimicrobial effects compared to other time periods. Differing results observed during the current experiment may have been due to such seasonal variation. According to Ogbulie, Ogueke, Okoli and Anyanwu (2007), the ethanol extract of *E. hirta* leaves showed Minimum Inhibitory Concentrations (MIC) of 58.09 mg/ml and 22.55 mg/ml against *E. coli* and *S. aureus*, respectively, for the agar diffusion method. Their observations were similar to antibacterial activities measured for the methanol extract of *E. hirta* against *E. coli* and *S. aureus* in the current experiment.

Islam *et al*. (2014) have reported antibacterial activities of ethanol extracts, petroleum ether extracts and chloroform extracts of leaves from *P. reticulates* using the disc diffusion method. According to their results, the ethanol extract of *P. reticulates* produces a significantly large inhibition zone against *S. aureus*. In the current experiment, the methanol extract of *P. reticulates* leaves produced a clear inhibition zone against *S. aureus*, agreeing with the observations by Islam et al. (2014). Using the cut-well agar diffusion method, Sankannavar (2012) also confirmed that methanol and ethanol extracts of *P. reticulates* leaves possess antibacterial activity against *S. aureus*, further supporting the activity observations in the current experiment.

According to Vinayaka *et al*. (2009), leaves of *A. ilicifolius* displayed antibacterial activity against *E. coli* and *S. aureus* with MIC values of 450 and 250 ppm respectively. However, no such activity was observed in the current experiment for *A. ilicifolius*. The differing result may be due to different plant parts (leaves vs. stem) tested and different extraction methods (Soxhlet vs cold sonication) used.

5. Conclusion

The most toxic plant extract among the fifteen tested was the ethyl acetate extract of *P. reticulatus* which is more toxic than the potassium dichromate. The hexane extract of *P. reticulatus* also showed high toxicity and only these two extracts were deemed to be highly toxic among all fifteen extracts tested with LC_{50} values between 1-100 ppm. All other extracts were less toxic than the potassium dichromate.

Ethyl acetate and methanol extracts of *E. hirta*, hexane and ethyl acetate extracts of *P. heyneanus*, and the methanol extract of *P. reticulatus* are active against both gram positive *S. aureus* and gram negative *E. coli* while all other extracts were inactive against these strains. Only hexane and ethyl acetate extracts of *P. scandens* were active against methicillin resistant *S. aureus*. Most of the plant extracts tested was active against *C. albicans* fungus.

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