Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Original article https://doi.org/10.12980/jclm.4.2016J6-169

Investigation on antimicrobial activity and phytochemical screening of *Randia spinosa* (Thunb.) Poir. and *Dillenia pentagyna* Roxb.

Irulandi Kokkaiah, Geetha Sethupandian, Mehalingam Palanichamy*

Research Department of Botany, V.H.N. Senthikumara Nadar College (an autonomous institution affiliated to Madurai Kamaraj University, Madurai), Virudhunagar 626 001, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 31 Aug 2016
Received in revised form 23 Sep 2016, 2nd revised form 8 Oct 2016
Accepted 12 Oct 2016
Available online 24 Oct 2016

Keywords:
Randia spinosa
Dillenia pentagyna
Antimicrobial activity
Phytochemical analysis

ABSTRACT

Objective: To evaluate phytochemicals and antimicrobial activity of *Randia spinosa* (Thunb.) Poir. and *Dillenia pentagyna* Roxb. leaf extracts against human pathogens such as Gram positive bacteria (*Streptococcus faecalis*, *Bacillus subtilis* and *Staphyllococcus aureus*), Gram negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*), and a fungus *Candida albicans*.

Methods: Antimicrobial activity of methanol, acetone and ethyl acetate extracts of the two plants against the human pathogens was investigated by agar well diffusion method, and qualitative phytochemical screening was conducted for the presence of phytoconstituents such as alkaloids, catechins, coumarins, flavonoids, phenols, quinones, saponins, steroids, tannins and terpenoids.

Results: The qualitative phytochemical analysis showed the presence of diverse range of compounds like alkaloids, flavonoids, phenols, steroids, tannins, terpenoids and saponins. The plants exhibited broad spectrum of antimicrobial activities against all the tested bacterial and fungal pathogens. The maximum zone of inhibition was observed in methanol extracts when compared with acetone and ethyl acetate extracts. The present study demonstrated that the selected plants had promising effect on the bacterial and fungal pathogens.

Conclusions: The phytochemicals in the plants may be potentially responsible for the antimicrobial efficacy of these medicinal plants.

1. Introduction

According to World Health Organization, the majority of people in developing countries still depend on the medicinal plants for their health care needs. Generally, plant based medicines are used for their basic health care system and have no side effect[1,2]. *Dillenia pentagyna* (Dilleniaceae) (*D. pentagyna*) with many medicinal properties is being used traditionally, particularly for pneumonia, lungs and skin diseases. The decoction of barks and leaves is used to cure diarrhea and dysentery in Rema-Kalenga, Bangladesh. Bark extracts are taken internally to cure dysentery by people in Khagrachari District of Bangladesh. *D. pentagyna* bark extract is commonly used to treat diabetes, and its leaves juices are consumed by piles patients and also applied for born fracture by the tribal communities[3].

Randia spinosa (Rubiaceae) (*R. spinosa*) is a crucial medicinal plant. *R. spinosa* is medicinally important and locally used to cure various ailments. Singh *et al*. reported that the leaf, seed and bark of *R. spinosa* are used to treat diarrhoea, vomiting, fever, cancers, and induce appetite; it is abortifacient and fruits are also used for treatment of wounds, skin and stomach disorders[4].

Medicinal resources are derived from the nature, and huge number of medicinal plants are used based on traditional

*Corresponding author: Palanichamy Mehalingam, Research Department of Botany, V.H.N. Senthikumara Nadar College (an autonomous institution affiliated to Madurai Kamaraj University, Madurai), Virudhunagar 626 001, Tamil Nadu, India.
Tel: +91 94435 45338, +91 04562 280154
Fax: +91 04562 28133
E-mail: mehalingap@yahoo.co.in

Foundation Project: Supported by University Grants Commission, New Delhi under Research Award Scheme [Grant No. F.No.30-1/2013 (SA-II)/RA-2012-14-OB-TAM-1464 DATED 20.12.2013].

The journal implements double-blind peer review practiced by specially invited international editorial board members.

knowledge. Medicinal plants are used traditionally from ancient time and a number of modern scientific studies deal with the traditional knowledge[5]. In last few decades, uncontrolled growth of resistance of the microorganisms to particular antibiotics has led to emerging problems in the treatment of microbial infectious diseases[6]. However, the bacterial infection can be prevented by using natural antibiotic compounds isolated from the plants[7]. Plants are potentially used for traditional medicine and pharmaceutical medicine. Plant based phytopharmaceutical medicine highly correlated to the treatment of disease[8]. A large number of bioactive compounds from medicinal plants have lead to the discovery of novel medicinal drugs especially antibiotics[9]. A perusal of literature revealed that there is a lacuna in the preliminary screening of phytochemicals and assessing the antimicrobial activities of *R. spinosa* and *D. pentagyna*. Therefore, the present study was conducted to evaluate the antimicrobial activity and preliminary screening of phytochemicals of different extracts of these two plants.

2. Materials and methods

2.1. Plant material

The fresh leaves of *R. spinosa* and *D. pentagyna* were collected wildly from the Courtallam forest area, Tirunelveli District, Tamil Nadu, India during December 2014. The plant samples were carried to the Botany Research Laboratory. Voucher specimen numbers of VSC 823 for *D. pentagyna* and VSC 849 for *R. spinosa* were deposited in the Botany Research Laboratory, V.H.N. Senthikumara Nadar College for further references.

2.2. Preparation of leaf extracts

The dried leaf extracts were prepared by sequential extraction method using three organic solvents on the basis of polarity of solvents (acetone, ethyl acetate and methanol). Totally 30 g of the powder of dried leaves was taken in a conical flask and 200 mL of acetone was added. The conical flask was kept on the mechanical shaker for 24 h. After that, the extract was filtered through Whatman No. 1 filter paper and the pellet was allowed to dry and used for the next solvent extractions (ethyl acetate and methanol). The dried extract was recovered and stored in refrigerator at 4 ˚C for further analysis.

2.3. Recovery percentage of extract

After drying the respective extracts under oven temperature at 40 ˚C, the percentage of extracts yield was calculated using the following equation[10]:

$$\%\text{Yield} = \frac{\text{Weight of dried extracts (g)}}{\text{Weight of dried plant leaves (g)}} \times 100$$

2.4. Qualitative phytochemical analysis

The dried leaf extracts were subjected to qualitative phytochemical screening by adopting standard procedures.

2.4.1. Test for alkaloids (Mayer's test)

To 1 mL of leaf extracts, 6 drops of Mayer's reagent were added. The formation of yellowish cream precipitate indicates the presence of alkaloids[11,12].

2.4.2. Test for saponins (foam test)

One millilitre of leaf extracts were mixed with 5 mL of distilled water. The contents were heated in a boiling water bath. Frothing indicates the presence of saponins[11,12].

2.4.3. Test for tannins (Braymer's test)

A total of 1 mL of the leaf extracts were added and mixed with 2 mL of water. Then 2 drops of 5% ferric chloride solution were added. Appearance of dirty green precipitate indicates the presence of tannins[11,12].

2.4.4. Test for steroids (Salkowski test)

To 2 mL of the leaf extracts, 2 mL of chloroform was added followed by concentrated sulphuric acid. Formation of reddish brown ring at the junction shows the presence of steroids[13].

2.4.5. Test for terpenoids

Two millilitres of the leaf extracts were treated with 2 mL acetic acid. Then concentrated sulphuric acid was added. Deep red color development shows the presence of terpenoids[13].

2.4.6. Test for coumarins

Two millilitres of the extracts were taken and 3 mL of 10% sodium hydroxide was added. Formation of yellow coloration indicates the presence of coumarins[13].

2.4.7. Test for catechins

Two millilitres of the extracts were treated with few drops of Ehrlich's reagent and few drops of concentrated HCl. The pink color formation indicates the presence of catechins[13].

2.4.8. Test for phenols

A total of 1 mL of the leaf extracts were treated with 3% ferric chloride. The appearance of deep blue color shows the presence of

phenols[14,15].

2.4.9. Test for flavonoids

One millilitre of the leaf extracts were added to 1 mL of sulphuric acid. Orange color formation confirms the presence of flavonoids[14,15].

2.4.10. Test for quinones

One millilitre of the leaf extracts were treated with 5 mL of HCl. Formation of yellow precipitate indicates the presence of quinones[14,15].

2.5. Antimicrobial activity

The microorganisms such as *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Streptococcus faecalis* (*S. faecalis*), *Bacillus subtilis* (*B. subtilis*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumonia* (*K. pneumonia*) and *Candida albicans* (*C. albicans*) were procured from Department of Botany, Gandhigram Rural Institute (Deemed), Gandhigram at Dindigul, India.

The tested organisms were maintained on nutrient agar slopes and kept in a refrigerator at 4 ˚C. Then 100 mL aliquots of nutrient broth were inoculated with the culture of tested microorganisms using a loop and incubated at 37 ˚C for 24 h.

Antimicrobial activities of methanol, ethyl acetate and acetone fractions of *R. spinosa* and *D. pentagyna* were evaluated using the agar well diffusion method[16]. The stock of microorganisms was maintained on nutrient agar slant and sub-cultured in nutrient broth for incubation at 37 ˚C prior to each antimicrobial test. Mueller-Hinton agar medium was used for antibacterial susceptibility tests. The Mueller-Hinton agar medium was prepared by pouring 15 mL of molten media into sterile Petri plates. The plates were allowed to solidify, and 0.2 mL of an overnight broth culture of tested microorganisms was added to 20 mL of cooled molten agar and swabbed uniformly on the medium and allowed to dry for 5 min. For agar well diffusion method, four equidistant wells (6 mm in diameter) were made in the agar with the help of a cork-borer. Then 40 µL of leaf extracts (methanol, ethyl acetate and acetone extracts) at 0.4 mg/mL were loaded in wells (6 mm in diameter). The standard antibiotic disc of gentamicin (10 µg) was placed on the surface of the plates. The plates were incubated at 37 ˚C for 24 h. The zone of inhibition was measured around the well containing samples and standard. The experiments were performed in triplicates.

3. Results

The yields of various solvent extracts from two different plants are given in Table 1. The highest extractive yield was found in methanol extract followed by ethyl acetate and acetone extract.

Table 1
Yield of plant extracts.

Plants	Methanol extract (%, w/w)	Ethyl acetate extract (%, w/w)	Acetone extract (%, w/w)
D. pentagyna	11.66%	8.50%	8.16%
R. spinosa	10.00%	9.36%	7.36%

The results of the present investigation revealed that the extracts from the two medicinal plants exhibited antimicrobial activity against *B. subtilis*, *S. faecalis*, *S. aureus*, *P. aeruginosa*, *E. coli*, *K. pneumoniae* and *C. albicans*. Methanolic leaf extracts of *R. spinosa* and *D. pentagyna* showed potent inhibition against all the pathogens when compared to acetone and ethyl acetate extracts. The highest antimicrobial activity of *D. pentagyna* was observed in methanolic leaf extract against *P. aeruginosa* [(13.20 ± 0.29) mm], and acetone extract also showed potent activity against *P. aeruginosa* [(11.90 ± 0.81) mm] and *S. aureus* [(11.80 ± 0.72) mm] (Table 2). Methanol leaf extract of *R. spinosa* exhibited higher antimicrobial efficacy against the tested microorganisms *viz*., *P. aeruginosa* [(12.50 ± 0.50) mm], *B. subtilis* [(11.00 ± 1.00) mm], *K. pneumoniae* [(10.50 ± 0.50) mm], *C. albicans* [(10.43 ± 0.51) mm], *S. aureus* [(9.53 ± 0.50) mm], *E. coli* [(9.47 ± 0.50) mm] and *S. faecalis* [(9.33 ± 0.58) mm] when compared to acetone and ethyl acetate extract (Table 3). The results of preliminary phytochemical screening of *D. pentagyna* and *R. spinosa* were given in Tables 4 and 5.

Table 2
Antimicrobial activities of three solvent extracts from the leaves of *D. pentagyna* (mm).

Microbial strains	Zone of inhibition			
	Methanol extract	Ethyl acetate extract	Acetone extract	Gentamicin
E. coli	8.50 ± 0.50	0.00 ± 0.00	9.80 ± 0.76	20.00 ± 1.00
S. aureus	11.00 ± 1.00	10.50 ± 0.58	11.80 ± 0.72	22.10 ± 0.23
S. faecalis	9.20 ± 0.29	9.30 ± 0.58	10.70 ± 0.58	21.90 ± 0.23
P. aeruginosa	13.20 ± 0.29	10.30 ± 0.58	11.90 ± 0.81	21.20 ± 0.29
B. subtilis	12.50 ± 0.50	8.50 ± 0.50	10.50 ± 0.50	23.50 ± 0.58
K. pneumoniae	11.20 ± 0.90	9.30 ± 0.58	10.50 ± 0.50	19.30 ± 0.50
C. albicans	8.70 ± 0.58	10.50 ± 0.50	9.80 ± 0.76	20.90 ± 0.81

Values were expressed as mean ± SD of three replicates.

Table 3
Antimicrobial activities of three solvent extracts from the leaves of *R. spinosa* (mm).

Microbial strains	Zone of inhibition			
	Methanol extract	Ethyl acetate extract	Acetone extract	Gentamicin
E. coli	9.47 ± 0.50	9.17 ± 0.29	0.00 ± 0.00	20.83 ± 0.76
S. aureus	9.53 ± 0.50	9.33 ± 0.58	11.50 ± 0.50	21.83 ± 0.76
S. faecalis	9.33 ± 0.58	8.17 ± 0.29	8.50 ± 0.50	24.33 ± 0.58
P. aeruginosa	12.50 ± 0.50	9.33 ± 0.58	8.33 ± 0.58	19.83 ± 0.76
B. subtilis	11.00 ± 1.00	10.67 ± 1.15	0.00 ± 0.00	22.50 ± 0.50
K. pneumoniae	10.50 ± 0.50	11.67 ± 0.58	11.50 ± 0.50	19.17 ± 0.29
C. albicans	10.43 ± 0.51	9.00 ± 0.00	10.50 ± 0.50	19.00 ± 0.00

Values were expressed as mean ± SD of three replicates.

Table 4
Preliminary phytochemical screening of different solvent extracts of *D. pentagyna*.

Phytochemicals	Plant extracts		
	Acetone	Ethyl acetate	Methanol
Alkaloids	-	-	+
Catechins	-	-	-
Coumarins	-	-	+
Flavonoids	-	-	+
Phenols	+	-	+
Quinones	-	-	-
Saponins	-	-	+
Steroids	+	+	+
Tannins	+	-	+
Terpenoids	+		+

+: Presence of phytochemicals; -: Absence of phytochemicals.

Table 5
Preliminary phytochemical screening of different solvent extracts of *R. spinosa*.

Phytochemicals	Plant extracts		
	Acetone	Ethyl acetate	Methanol
Alkaloids	-	+	+
Catechins	-	-	-
Coumarins	+	-	+
Flavonoids	-	-	+
Phenols	+	-	+
Quinones	-	-	-
Saponins	-	-	+
Steroids	+	-	-
Tannins	+	-	+
Terpenoids	+	-	+

+: Presence of phytochemicals; -: Absence of phytochemicals.

4. Discussion

Phytochemically active compounds are derived from plant parts and extraction is performed following various techniques[17]. The isolated compounds and plant extracts possess antimicrobial properties, and they are used for treatment of various infectious diseases[18,19]. Earlier studies indicated that the medicinal plants are used against parasitic and microbial infections including bacterial, viral and fungal infections[20-26]. The present work reveals antimicrobial properties in *D. pentagyna* and *R. spinosa*. The methanolic leaf extracts of *D. pentagyna* and *R. spinosa* showed higher activity against *B. subtilis* and *P. aeruginosa*. Plant extracts showed more potent inhibitory effect on the Gram positive bacteria than the Gram negative bacteria. The present study corroborates with our previous works[27]. The plant derivatives from the extracts exhibited a good inhibitory activity against the Gram positive bacteria[28,29]. The present study showed that the methanolic, acetone and ethyl acetate extracts of leaves of *D. pentagyna* and *R. spinosa* have slight variation in the phytochemical constituents of leaves. Tannins, phenols and terpenoids are present in methanolic and acetone extracts. Contrary to the current study, Kumara and Singh[30] showed that the leaves of *R. spinosa* did not contain alkaloids but possessed steroids. Chung *et al*. reported that the terpenoids possess antimicrobial properties[31]. Tannins also have decreased the bacterial proliferation and immunostimulation, and exhibited antidiarrhoeal and antihemorrhagic properties and potent antioxidant activity[32-34]. Phenols have been reported to exhibit antioxidant properties[35]. Janani and Singaravadivel reported that saponins are crucially used as antinutrients and to decrease the high cholesterol[36]. Steroids are biologically active compounds that protect the plant system[37]. Thus, the significant antimicrobial activity against Gram positive and Gram negative bacteria may be due to the presence of phytochemicals or secondary metabolites.

In the present investigation, *D. pentagyna* and *R. spinosa* have exhibited significant antimicrobial activities with presence of phytocompounds that lead to discovering new antimicrobial agents in the pharmaceutical fields.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors are grateful to University Grants Commission, New Delhi for providing financial assistance under Research Award Scheme [Grant No. F.No.30-1/2013 (SA-II)/RA-2012-14-OB-TAM-1464 DATED 20.12.2013].

References

[1] World Health Organization. Traditional medicine. Geneva: World Health Organization; 2003. [Online] Available from: http://www.who.int/mediacentre/factsheets/2003/fs134/en/ [Accessed on 15th December, 2008]

[2] Marasini BP, Baral P, Aryal P, Ghimire KR, Neupane S, Dahal N, et al. Evaluation of antibacterial activity of some traditionally used medicinal plants against human pathogenic bacteria. *Biomed Res Int* 2015; doi: 10.1155/2015/265425.

[3] Dubey PC, Sikarwar RLS, Khanna KK, Tiwari AP. Ethnobotany of *Dillenia pentagyna* Roxb. in Vindhya region of Madhya Pradesh, India. *Nat Prod Radiance* 2009; **8**(5): 546-8.

[4] Singh NK, Mishra AK, Gupta JK, Jayalakshmi S. *Randia spinosa*: ethanobotany, phytochemistry and pharmacology-a review. *Int J Pharm Sci Rev Res* 2010; **4**(1): 127-31.

[5] Egharevba HO, Kunle FO. Preliminary phytochemical and proximate

analysis of the leaves of *Piliostigma thioniningii* (Schumach.) Mile-Redhead. *Ethnobot Leaflets* 2010; **14**: 570-7.

[6] Dizaj SM, Mennati A, Jafari S, Khezri K, Adibkia K. Antimicrobial activity of carbon-based nanoparticles. *Adv Pharm Bull* 2015; **5**(1): 19-23.

[7] Nasrullah, Suliman, Rahman K, Ikram M, Nisar M, Khan I. Screening of antibacterial activity of medicinal plants. *Int J Pharm Sci Rev Res* 2012; **14**(2): 25-9.

[8] Duraipandiyan V, Ayyanar M, Ignacimuthu S. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC Complement Altern Med* 2006; **6**: 35.

[9] Afolayan AJ. Extract from the shoots of *Arctotis artototdes* inhibit the growth of bacterial and fungi. *Pharm Biol* 2003; **41**: 22-5.

[10] Joshua M, Takudzwa M. Antibacterial properties of *Mangifera indica* on *Staphylococcus aureus*. *Afr J Clin Exp Microbiol* 2013; **14**(2): 62-74.

[11] Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. *Afr J Biotechnol* 2005; **4**: 685-8.

[12] Harbone JB. *Phytochemical methods*. 1st ed. London: Chapman and Hall Ltd.; 1973, p. 188.

[13] Rathore SK, Bhatt S, Dhyani S, Jain A. Preliminary phytochemical screening of medicinal plant *Ziziphus mauritiana* Lam. fruits. *Int J Curr Pharm Res* 2012; **4**(3): 160-2.

[14] Kokate KC. *Practical pharmacognosy*. Delhi: Vallabh Prakashan; 2000, p. 107-11.

[15] Harbone JB. *Phytochemical methods: a guide to modern techniques of plant analysis*. 3rd ed. London: Chapman and Hall Ltd.; 1998, p. 60-6.

[16] Irobi ON, Moo-Young M, Anderson WA, Daramola SO. Antimicrobial activity of bark extracts of *Bridelia ferruginea* (Euphorbiaceae). *J Ethnopharmacol* 1994; **43**: 185-90.

[17] Samy RP, Ignacimuthu S. Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats in India. *J Ethnopharmacol* 2000; **69**: 63-71.

[18] Nagesh KS, Shanthamma C. Antibacterial activity of *Curculigo orchioides* rhizome extract on pathogenic bacteria. *Afr J Microbiol Res* 2009; **3**(1): 5-9.

[19] Palombo EA, Semple SJ. Antibacterial activity of traditional Australian medicinal plants. *J Ethnopharmacol* 2001; **77**: 151-7.

[20] Kumarasamy Y, Cox PJ, Jaspars M, Nahar L, Sarker SD. Screening seeds of Scottish plants for antibacterial activity. *J Ethnopharmacol* 2002; **83**: 73-7.

[21] Stepanović S, Antić N, Dakić I, Svabić-Vlahović M. *In vitro* antimicrobial activity of propilis and antimicrobial drugs. *Microbiol Res* 2003; **158**: 353-7.

[22] Bylka W, Szaufer-Hajdrych M, Matławska I, Goślińska O. Antimicrobial activity of isocytisoside and extracts of *Aquilegia vulgaris* L. *Lett Appl Microbiol* 2004; **39**: 93-7.

[23] Behera SK, Misra MK. Indigenous phytotherapy for genito-urinary diseases used by the Kandha tribe of Orissa, India. *J Ethnopharmacol* 2005; **102**: 319-25.

[24] Govindarajan R, Vijayakumar M, Singh M, Rao ChV, Shirwaikar A, Rawat AK, et al. Antiulcer and antimicrobial activity of *Anogeissus latifolia*. *J Ethnopharmacol* 2006; **106**: 57-61.

[25] Harbourne N, Marete E, Jacquier JC, O'Riordan D. Conventional extraction techniques for phytochemicals. In: Tiwari BK, Brunton NP, Brennan CS, editors. *Handbook of plant food phytochemicals: sources, stability and extraction*. Oxford: John Wiley & Sons Ltd.; 2013, p. 399-411.

[26] Vadlapudi V, Kaladhar DSVGK. Antimicrobial study of plant extracts of *Datura metel* L. against some important disease causing pathogens. *Asian Pac J Trop Dis* 2012; **2**(Suppl 1): S94-7.

[27] Irulandi K, Geetha S, Mehalingam P. Evaluation of antioxidant properties and antimicrobial activity of *Garcinia cambogia* Hort. Ex Boerl (Clusiaceae) fruit extracts. *J Adv Appl Sci Res* 2016; **1**: 74-83.

[28] Akila P, Prince L, Bharathidasan R, Krishnapriya K. Antibacterial activity of herbal plants. *Int J Curr Microbiol App Sci* 2016; **5**(4): 681-8.

[29] Sarwar S, Sabir M, Raza SS, Malik SA. Analysis of antimicrobial activity of medicinal plant *Amaranthus viridis*. *Int J Innov Sci Res* 2016; **20**(2): 494-9.

[30] Kumara A, Singh D. Evaluation of preliminary phytochemical and pharmacognostical studies on the bark of *Randia spinosa* Thund. *Int J Res Dev Pharm Life Sci* 2012; **1**(2): 69-71.

[31] Chung PY, Navaratnam P, Chung LY. Synergistic antimicrobial activity between pentacyclic triterpenoids and antibiotics against *Staphylococcus aureus* strains. *Ann Clin Microbiol Antimicrob* 2011; **10**: 25.

[32] Dhivy R, Manimegalai K. Preliminary phytochemical screening and GC-MS profiling of ethanolic flower extract of *Calotropis gigantea* Linn. (Apocynaceae). *J Pharmcogn Phytochem* 2013; **2**(3): 28-32.

[33] Thorington RW, Ferrell KE. *Squirrels: the animal answer guide*. Baltimore: Johns Hopkins University Press; 2006.

[34] Kumar GVP, Subrahmanyam SN. Phytochemical analysis, *in-vitro* screening for antimicrobial and anthelmintic activity of combined hydroalcoholic seed extracts of four selected folklore indian medicinal plants. *Der Pharm Lett* 2013; **5**(1): 168-76.

[35] Hadzari HM, Yunus MAC, Zhari S, Rithwan F. The effects of solvents and extraction methods on the antioxidant activity of *P. niruri*. *Jurnal Teknologi* 2014; **68**: 47-52.

[36] Janani SR, Singaravadivel B. Screening of phytochemicals and GC-MS analysis of some bioactive constituents of *Asparagus racemosus*. *Int J PharmTech Res* 2014; **6**(2): 428-32.

[37] Gnanavel V, Saral AM. GC-MS analysis of petroleum ether and ethanol extracts from *Abrus precatorius* Linn. *Int J Pharm Bio Sci* 2013; **4**(3): 37-44.