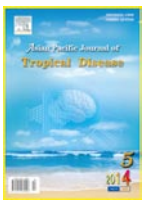
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Document heading doi:10.1016/S2222-1808(14)60591-0

Comparative study of total phenolics, flavonoids contents and evaluation of antioxidant and antimicrobial activities of different polarities fruits crude extracts of *Datura metel* L.

Mohammad A. Hossain*, Marwah Salim Ali Al Kalbani, Shaima Abdullah Juma Al Farsi, Afaf Mohammed Weli, Qasim Al-Riyami

School of Pharmacy, College of Pharmacy and Nursing, University of Nizwa, P.O. Box 33, Postal Code 616, Nizwa, Sultanate of Oman

PEER REVIEW

Peer reviewer

Dr. SM Mizanur Rahman, Professor, Department of Chemistry, University of Dhaka, Bangladesh.
E-mail: smmrdu@yahoo.com

Comments

The present study on total phenol and flavonoids contents of various fruits crude extracts of *Datura* species and evaluation of their antioxidant and antimicrobial study is giving the valuable brief and scientific information about this plant.
Details on Page 382

ABSTRACT

Objective: To determine the total phenolics and flavonoids and to evaluate antioxidant and antimicrobial activities of different extracts from fruits of *Datura metel* (*D. metel*).
Methods: Different crude extracts from the fruits of *D. metel* were subjected to determination of total phenolics, flavonoids, antioxidant and antimicrobial activities by established methods.
Results: The total phenolics results showed that ethyl acetate extract was the most efficient (60.26%) compared to hexane, chloroform, butanol and methanol extracts which had phenolic contents of 50.08, 35.50, 52.54 and 26.49%, respectively. Almost similar results were obtained from the fruits crude extracts for total flavonoids and results found that methanol crude extract was the highest (1.71%) compared to other crude extracts. The antioxidant activity results showed that methanol extract acted the highest activity compared to other extracts and in the order of methanol>ethyl a cetate>hexane>chloroform>butanol extract. All extracts were displayed moderate antibacterial potential against the tested bacteria such as *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonus aeruginosa* in the range of 0%-10%.
Conclusions: The results of this present study clearly showed that the crude extracts of *D. metel* demenstrated antimicrobial and antioxidant activities and it may act as potential antioxidant sources for human biological system.

KEYWORDS

Datura metel, Crude extracts, Total phenol, Total flavonoids, Antioxidant activity, Antimicrobial capacity

1. Introduction

Secondary metabolite compounds *e.g.*, tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides and other volatiles or essential oils were found in fruits, vegetables and plants[1]. These secondary metabolites have been related to mortality rates of many cure and incurable diseases such as cancer and cardiovascular disease[2-4]. Fruits and plants are the main sources to reduce hypertension, increase the immune system, clean the contaminants and pollutants, and reduce inflammation[5,6]. All crude extracts from fruits and plants represent a rich source of antioxidant agents, antifungal and antibacterial[7-11]. Due to the effects and resistance of bacterial microorganisms against antibiotics, recently many scientists and researchers have drawn attention to

*Corresponding author: Mohammad A. Hossain, School of Pharmacy, College of Pharmacy and Nursing, University of Nizwa, P.O. Box 33, Postal Code 616, Nizwa, Sultanate of Oman.
Tel/Fax: +96899708496
E-mail: hossainabi@gmail.com
Foundation Project: Supported by Central Instrument Laboratory, College of Agriculture and Marine Sciences, Sultan Qaboos University, Sultanate of Oman (Grant No. 507/SOP/OB/1/2013).

Article history:
Received 27 Apr 2014
Received in revised form 5 May, 2nd revised form 18 May, 3rd revised form 23 May 2014
Accepted 19 Jun 2014
Available online 3 Jul 2014

the crude extracts and biologically active pure compounds isolated from plant species used in herbal remedies[12-15].

Datura metel (*D. metel*) is an important flowering medicinal plant belongs to the family Solanaceae. The name comes from Sanskrit Dustura or Dahatura[16]. Its height is about 1.5 m. The powder samples of fruits are used in the form of a capsule covered with short spines. *D. metel* is an erect branched undershrub with long white flowers and spiny spherical fruits[16]. Traditionally its fruit is used for treatment of diarrhoea, gonorroea and bronchitis[17,18]. It is also used for the treatment of catarrh, epilepsy, insanity, hysteria, rheumatic pains, hemorrhoids, painful menstruation skin-ulcers and wounds[4,5]. Recently the fruit is used to calm cough and to treat laryngitis and treacheries due to tannin[9]. Different types and groups of phytochemicals have been found in the fruit of *D. metel*. Nowadays, it is essential to isolate, identify and characterize the phytochemicals of locally grown fruits by herbalists for the treatment of different diseases. There are some proposals that have been untaken on the integration of traditional fruits in health care program in Sultanate of Oman. Also there is a need to investigate the antimicrobial activities of locally available fruits and medicinal plants and to develop the fruits and plants as potential sources of antioxidant and therapeutic agents[15-18]. Previously some authors have extensively studied and documented on total phenol, flavonoid, antioxidants and antimicrobial activities of this fruits in other countries[8,9]. However, very little information is known about the phytochemicals such as phenol, flavonoids *etc*. in the fruits and their antioxidant and antimicrobial activities. The literature search also reveals that still no works has been done on the fruits of Omani *D. metel* species. Therefore, the aim of this present study was carried out to determine the total phenolics and flavonoids and to evaluate the antioxidant and antimicrobial activities of the fruit crude extracts of *D. metel* L. collected from Nizwa, Sultanate of Oman.

2. Material and methods

2.1. Chemicals and materials

The chemicals used such as hexane, chloroform, ethyl acetate and acetic anhydride were purchased from Scharlau, European Union. 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid and qucrectin were obtained from Sigma-Aldrich, Germany. The methanol and butanol solvents were obtained from Emsure, Germany. A grinder (Super Deluxe, India) and rotary evaporator (Yamato, Rotary Evaporator, Model-RE 801) were used for samples preparation. The UV spectroscopy (UV-1800 Shimadzu spectrophotometer, Japan) was used to measure the absorbance of the samples. Ammonia was obtained from Appli Chem, Germany. Sodium hydroxide and sulphuric acid were obtained from Ohilip Harris, England. Whatman filter papers were used as discs in this present study (GE Healthcare Companies, China, Catalogue number: 1001090). The bacterial strains such as *Staphylococcus aureus* (*S. aureus*) , *Escherichia coli* (*E. Coli*) and *Pseudomonus aeruginosa* (*P. aeruginosa*) were collected from Nizwa Hospital, Nizwa, Sultanate of Oman.

2.2. Plant materials

The fruit samples of *D. metel* were collected on November 10, 2012 at 8:30 am from Al Jabal Akhdar, Nizwa, Sultanat of Oman. Collected fruits samples were instantly packed in polyethylene bags to avoid contamination and decomposition of the chemical compounds. During the fruit collection time the outside temperature was 27 °C. The collected fruit samples were transported to the Natural Products Laboratory, University of Nizwa and kept at room temperature for cleaning, drying and extracting.

2.3. Preparation of samples

The collected fresh fruits of *D. metel* samples were washed twice with water to remove the dust and insect. The fruit samples were separated from the affected one. The healthy fruit samples were cut into small pieces with a knife. The small pieces samples (500 g) were dried under shade at room temperature for 7 d. The dried fruit samples (80.33 g) were ground into powder using a grinder machine (Jaipan, Super Deluxe, India).

2.4. Extraction procedure

The powder samples (80.33 g) were extracted with polar methanol solvent (360 mL) using a Soxhlet extractor at fixed temperature for 72 h. The polar solvent was completely evaporated by a rotary evaporator (Yamato, Rotary Evaporator, Model-RE 801) to give solvent free crude extract. The solvent free crude extract (28.68 g) was defatted with water (100 mL) and shaken by hand until the crude extract dissolved. The diluted crude extract was transferred to a separatory funnel and successively extracted with organic solvents of different polarities such as hexane, chloroform, ethyl acetate and butanol to give hexane, ethyl acetate, chloroform and butanol and residual methanol fractions, respectively. The whole process was repeated twice until complete extraction. The crude extracts were combined together, concentrated and dried under vacuum pressure.

2.5. Determination of total phenolics by Folin-Ciocalteu reagent method

Folin-Ciocalteu reagent with gallic acid external calibration curve method was used to determine the total phenolics content of different organic crude extracts from fruit of *D. metel*[19]. Each crude extract *i.e.*, methanol, hexane, chloroform, ethyl acetate and butanol extracts (1 mg) was taken in a test tube and 1 mL of methanol solvent was added. The different concentrations of each sample such as 0.30, 0.20, 0.10, 0.05 and 0.025 mg/L were prepared using serial dilution technique. The same procedure was followed for gallic acid standard. Each concentration of each crude extract samples (200 μL) was taken in a separate test tube and mixed with Folin-Ciocalteu reagent (1.5 mL). The sample test tubes were kept in a dark place for five minutes. Then sodium carbonate (1.5 mL) was added to the samples tubes and mixed together by hand. Again the test tubes were kept in the dark to complete reaction for two hours. The absorbance was measure by using a UV spectrophotometer at a fixed wavelength 760 nm. The determination of total phenol in different polarities was carried out in triplicate and the obtained results were averaged.

2.6. Determination of total flavonoids by aluminum chloride colorimetric method

Colorimetric method with minor modifications was used to determine the total flavonoid contents in different polarities crude fruit extracts of *D. metel* as described by Hossain[19]. The crude extract (0.5 mL) was taken in a test tube and then 10% aluminum chloride solution (0.1 mL), 1 mol/L potassium acetate (0.1 mL) and distilled water (4.3 mL) was added to it. The whole mixture was mixed together by vertex and the samples were incubated at room temperature for 30 min. The absorbance of the samples was measured at a fixed wavelength 415 nm by using a UV spectrophotometer. Quercetin standard was used to prepare the calibration cruve. The determination of total flavonoids in different polarities of crude extracts was carried out in triplicate and the obtained results were averaged.

2.7. Evaluation of antioxidant activity by DPPH method

The antioxidant activity of different concentration of plant crude extracts from the fruit of *D. metel* was estimated by the standard conventional DPPH method with slight modification[20]. The different crude extracts of *D. metel* at different concentrations (0.012, 0.025, 0.05, 0.10 and 0.20 mg/L) were taken in a separate test tubes. One milliliter of DPPH solution (0.1 mmol/L) dissolved in methanol was added to each test tubes and shaken vigorously. After the addition of DPPH solution, all the test tubes were shaken gently and allowed to stand at 27 °C in a dark place for 45 min. The blank control was prepared in the same way without any crude extract. The absorbance of the prepared samples was measured using UV spectroscopy at wavelength 517 nm. Each method in this experiment was replicated three times. Radical scavenging activity of the tested crude extracts samples was estimated as an inhibition percentage and was calculated by using the following formula:

$$\% \text{ Inhibition} = \frac{A_{control} - A_{extract}}{A_{control}} \times 100$$

Where $A_{control}$ represent the absorbance of the blank control and $A_{extract}$ represent the absorbance of the extracts.

2.8. Antibacterial activity assay

The evaluation of antibacterial activity was carried out by agar disc diffusion method[21]. Negative control was prepared using the same solvents employed to dissolve the samples. Each extract was subjected to serial dilution technique by using dimethyl sulphoxide as a solvent to give 2 mg/mL, 1 mg/mL, 0.5 mg/mL and 0.25 mg/mL solutions. The previously prepared different concentrations of different polarities crude extracts of *D. metel* was tested for its antimicrobial activity against one Gram positive bacteria (*S. aureus*) and two Gram negative bacteria (*E. coli* and *P. aeruginosa*) on nutrient agar plates using disc diffusion method. Whatman No. 1 sterile filter paper discs (5 mm diameter) were impregnated with all crude extracts of *D. metel* and placed on the inoculated agar. The plates were incubated at 37 °C for 24 h. The evaluation of antibacterial activity was to measure the diameter of the zones of inhibition against the three tested bacteria. Each method in this experiment was replicated for three times.

3. Results

3.1. Extracts of D. metel fruit

The amorphous solid were obtained by completely evaporation of methanol. The methanol dry crude extract was defatted with water and then extracted successively from non-polar solvent with increasing polarities (Table 1).

Table 1
Amounts of different crude extracts from the fruit of *D. metel*.

Extracts	Amounts (g)
Hexane	0.20
Ethyl acetate	0.21
Chloroform	0.12
Butanol	0.35
Methanol	4.54

3.2. Total phenolic contents

The total phenolic contents of different crude extracts were determined by Folin-Ciocalteu method with gallic acid standard (Table 2). Among the five crude extracts, ethyl acetate extract was containing highest amount of phenolic compounds (60.26 mg GAE/100 g dry extract) followed by butanol extract, hexane extract, chloroform and methnol extract.

3.3. Total flavonoid contents

The total flavonoid contents of different fruit crude extracts of *D. metel* ranged from 1.18 to 1.71 mg quercetin/g dry weight (Table 2). Among the five extracts, methanol crude extract was containing highest amount of flavonoid compounds (1.71 mg quercetin/g dry weight) followed by butanol extract, chloroform extract, ethyl acetate and hexane extract.

Table 2

Total phenolics and flavonoids contents of different fruits crude extracts of *D. metel*.

Crude extracts	Total phenolics (mg GAE/100 g dry extract)	Total flavonoids (mg quercetin/g dry weight)
Hexane	50.08	1.18
Ethyl acetate	60.26	1.29
Chloroform	35.50	1.37
Butanol	52.54	1.55
Methanol	26.49	1.71

3.4. Antioxidant activity

In this study, the evaluation of antioxidant activity of five different polarities *D. metel* fruit crude extracts was investigated through *in vitro* models such as radical scavenging activity using DPPH method. The results of antioxidant activity of different polarities crude extracts was in the order of methanol>ethyl acetate>hexane>chloroform>butanol extract (Figure 1).

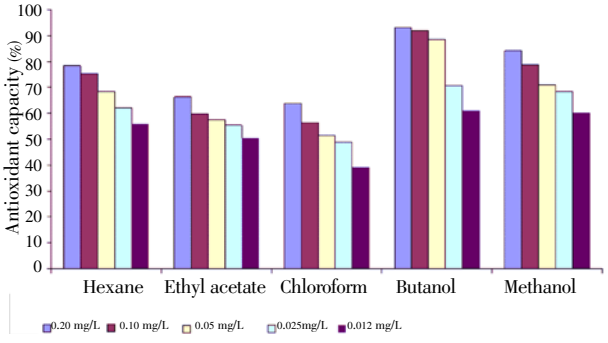


Figure 1. Antioxidant capacity (%) of fruit crude extracts of *D. metel* by DPPH method.

The five extracts from the fruits of *D. metel* were able to change DPPH colour and the free radical scavenging potentials of the extracts of were found to be in the order of methanol extract>ethyl acetate extract>hexane extract >chloroform extract>butanol extract.

3.5. Antibacterial activity

The evaluation of *in vitro* antibacterial activity of methanol crude extract and the subfractions of *D. metel* fruit against the employed bacteria were qualitatively assessed by the presence or absence of inhibition zones. The different organic fruit crude extracts of *D. metel* exhibited antibacterial activity against one Gram positive (*S. aureus*) and two Gram negative (*E. coli* and *P. aeruginosa*) bacteria at different concentrations as 2.00 mg/mL, 1.00 mg/mL, 0.50 mg/mL and 0.25 mg/mL dilution with the solvent dimethyl sulphoxide. Most of the fruit crude extract of *D. metel* showed moderate potential of antibacterial activity against *E. coli*, *P. aeruginosa* and *S. aureus* bacteria (Table 3).

Table 3

Antimicrobial activity of different crude extracts of *D. metel* against *E. coli*, *P. aeruginosa* and *S. aureus*.

Extracts	Concentrations	*E. coli* (mm)	*P. aeruginosa* (mm)	*S. aureus* (mm)
Hexane	2.00 mg/mL	8.00±0.11	9.00±0.37	nd
	1.00 mg/mL	8.00±0.17	nd	nd
	0.50 mg/mL	8.00±0.21	nd	nd
	0.25 mg/mL	nd	nd	nd
	Amoxicillin	25.00±0.22	23.00±0.11	27.00±0.28
Ethyl acetate	2.00 mg/mL	8.00±0.13	nd	nd
	1.00 mg/mL	7.00±0.20	nd	nd
	0.50 mg/mL	7.00±0.45	nd	nd
	0.25 mg/mL	nd	nd	nd
	Amoxicillin	23.00±0.12	24.00±0.22	25.00±0.15
Chloroform	2.00 mg/mL	10.00±0.07	8.00±0.10	7.00±0.26
	1.00 mg/mL	8.00±0.37	7.00±0.29	nd
	0.50 mg/mL	nd	nd	nd
	0.25 mg/mL	nd	nd	nd
	Amoxicillin	24.00±0.32	20.00±0.27	23.00±0.30
Butanol	2.00 mg/mL	7.00±0.55	nd	7.00±0.15
	1.00 mg/mL	7.00±0.15	nd	6.00±0.28
	0.50 mg/mL	nd	nd	6.00±0.77
	0.25 mg/mL	nd	nd	6.00±0.35
	Amoxicillin	26.00±0.09	24.00±0.32	25.00±0.36
Methanol	2.00 mg/mL	7.00±0.19	7.00±0.29	nd
	1.00 mg/mL	6.00±0.62	7.00±0.16	nd
	0.50 mg/mL	nd	7.00±0.53	nd
	0.25 mg/mL	nd	nd	nd
	Amoxicillin	22.00±0.17	23.00±0.18	27.00±0.28

nd: Not detectable; Values are represented as the mean±SD of three experiments.

4. Discussion

The total phenol content among the five crude extracts such as ethyl acetate extract was containing highest amount of phenol followed by butanol extract>hexane extract>chloroform>methanol extract. The antioxidant activity result among the five different polarities crude

extracts showed that methanol crude extracts presented the highest antioxidant activity compare to other crude extracts. So the present study showed that there were no relation between the total phenol and antioxidant activity. Most of the studies have reported the correlation between the total phenol and antioxidant active but our result does not agree these reports[4,5]. Also some other studies reported no correlation between the total phenol and antioxidant activity[22].

The free radical scavenging activity of the fruit crude extracts of *D. metel* were tested through DPPH method. The role of antioxidants is their interaction with oxidative free radicals. The principle of DPPH method is that the antioxidant agents react with the DPPH and produce stable free radicals. Accordingly DPPH change its own colour gradually. The gradually colour change indicates the sample contains antioxidant agents. In the present study the five extracts from the fruits of *D. metel* were able to change DPPH colour and the free radical scavenging potentials of the extracts of were found to be in the order of methanol extract> ethyl acetate extract>hexane extract >chloroform extract>butanol extract. The literature search reveals that the phytochemicals cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds (hydroquinone, pyrogallol, *etc*.), and aromatic amines (p-phenylene diamine, p-aminophenol *etc*.) were able to reduce and decolourise DPPH by their hydrogen donating ability bronchitis[9-13]. It appears that all five crude extracts from the fruits of *D. metel* had hydrogen donating capabilities to act as antioxidant. So far we know, this is the first report that have investigated the antioxidant activity of Omani *D. metel*. Hence the fruits of *D. metel* could be a good source of antioxidant agents.

The *in vitro* antibacterial activity of the methanol extract and methanol subfractions of *D. metel* against the employed three bacteria was qualitatively assessed by the presence or absence of inhibition zones. The methanol extract of *D. metel* and its subfractions revealed a very small potential of antibacterial activity against one Gram-positive and two Gram-negative bacteria at the concentration of 2.00 mg/mL, 1.00 mg/mL, 0.50 mg/mL and 0.25 mg/mL with their respective diameter zones of inhibition of 0-10 mm. The methanol crude extract of *D. metel* showed a very small potential of antibacterial activity against *E. coli* and *P. aeruginosa* bacteria, at the concentration of 2 mg/mL and 1.00 mg/mL. Butanol crude extract showed a moderate antibacterial effect against *S. aureus* at all concentration but *E. coli* showed antibacterial effect only at 2.00 mg/mL and 1.00 mg/mL. Hence, the active ingredients were more dissolved in the butanol crude extract and thus responsible for zone of inhibition of this study. However, butanol crude extract did not show any activity against *P. aeruginosa* at any concentration. On the other hand, chloroform, hexane and ethyl acetate subfractions did not show any antibacterial effects against most of the tested bacteria. The residual of methanol crude extract did not show any activity against all the tested bacterial strains. This could be due to the presence of dissolved active ingredients at very low concentrations in the fruits crude extracts. The control also did not inhibit the growth of the tested bacteria.

Based on these results, it is possible to conclude that the crude extracts from the fruits of *D. metel* could be used as antioxidant, antimicrobial agents. Further studies are needed for the isolation and identification of individual phenolic compounds and also *in vivo* studies are needed for better understanding their mechanism of action as antioxidants.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are grateful to University of Nizwa, Nizwa, Sultanate of Oman for providing all chemicals and other expenses from their internal fund to carry out this project. The authors are also grateful to Prof. Dr. Nafsiah Binti Shamsudin, Dean, College of Pharmacy and Nursing, University of Nizwa, Sultanate of Oman for her continuous encouragement during the work. Thanks to Khaloud Ali Said Al-Alawi and Ahlam Rashed Alabri, Lab Technicians, Natural Product Lab, University of Nizwa for their continuous help during the experiment. The authors wish to express sincere gratitude to the Central Instrument Laboratory, College of Agriculture and Marine Sciences, Sultan Qaboos University, Sultanate of Oman where the test were confirmed (Grant No. 507/SOP/OB/1/2013).

Comments

Background

D. metel is an important flowering medicinal plant belongs to the family Solanaceae. Traditionally this fruits are used for treatment of diarrhoea, gonorroea and bronchitis. It is also used for the treatment of catarrh, epilepsy, insanity, hysteria, rheumatic pains, hemorrhoids, painful menstruation skin-ulcers and wounds. However, very little information is known about the fruits and their antioxidant and antimicrobial activities. The literature search also reveals that still no works has been done on the fruits of Omani *D. metel* species.

Research frontiers

The aim of this present study was carried out to determine the total phenolics and flavonoids contents and to evaluate the antioxidant and antimicrobial activities of the different crude extracts.

Related reports

Literature search reveals that no work yet has been reported on Omani *Datura* species. The other parameters of this plant has been done by other researcher.

Innovations & breakthroughs

The experimental work done by the authors is scientific and it gives valuable new information to the scientific community.

Applications

This plant is used worldwide as a medicine. The output of the paper gives a information that there are so many bioactive compounds which can be used to prepare medicine.

Peer review

The present study on total phenol and flavonoids contents of various fruits crude extracts of *Datura* species and evaluation of their antioxidant and antimicrobial study is giving the valuable brief and scientific information about this plant.

References

[1] Bhattacharjee I, Chatterjee SK, Chandra G. Isolation and identification of antibacterial components in seed extracts of *Argemone mexicana* L. (Papaveraceae). *Asian Pac J Trop Med* 2010; **3**(7): 547-551.

[2] Bharathi B, Sharmiladevi R, Daniel GS. Studies on antibacterial activity and phytochemical analysis of *Datura metel* L against bacterial pathogens associated with HIV. *Adv Biotech* 2010; **10**(3): 21-25.

[3] Bhimba BV, Meenupriya J, Joel EL, Naveena DE, Kumar S, Thangaraj M. Antibacterial activity and characterization of secondary metabolites isolated from mangrove plant *Avicennia officinalis*. *Asian Pac J Trop Med* 2010; **3**(7): 544-546.

[5] Cowan MM. Plant products as timicrobial agents. *Clin Microb Rev* 1999; **12**: 564-582.

[6] Dabur R, Ali M, Singh H, Gupta J, Sharma GL. A novel antifungal pyrrole derivative from *Datura metel* leaves. *Pharmazie* 2004; **59**: 568-570.

[7] Darabpour E, Motamedi H, Nejad SMS. Antimicrobial properties of *Teucrium polium* against some clinical pathogens. *Asian Pac J Trop Med* 2010; **3**(2): 124-127.

[8] Anju D, Ratan L. Phytochemical and pharmacological status of *Datura fastuosa* Linn. *Int J Res Ayurveda Pharm* 2011; **2**(1): 145-150.

[9] Okwu DE, Igara EC. Isolation, characterization and antibacterial activity of alkaloid from *Datura metel* Linn leaves. *Afr J Pharm Pharmacol* 2009; **3**(5): 277-281.

[10] Dorman HJ, Deans SG. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J Appl Microbiol* 2000; **88**: 308-316.

[11] Gul H, Qaisrani RN, Khan MA, Hassan S, Younis N. Antibacterial and antifungal activity of different extracts of *Datura stramonium* (branches and leaves sample). *J Biotechnol Pharm Res* 2012; **3**(9): 141-148.

[12] Harborne JB. *Phytochemical methods: a guide to modern techniques of plant analysis*. 2nd ed. London: Chapman and Hall; 1984, p. 54-84.

[13] Irudayaraj V, Janaky M, Johnson M, Selvan N. Preliminary phytochemical and antimicrobial studies on a spike-moss *Selaginella inaequalifolia* (hook. & grev.) Spring. *Asian Pac J Trop Med* 2012; **3**(12): 957-960.

[14] Jarrar N, Abu-Hijleh A, Adwan K. Antibacterial activityof *Rosmarinus officinalis* L. alone and in combination with cefuroxime against methicillin-resistant *Staphylococcus aureus*. *Asian Pac J Trop Med* 2010; **3**(2): 121-123.

[15] Johnson M, Wesely EG, Zahir HMI, Selvan N. *In vivo* and *in vitro* phytochemical and antibacterial efficacy of *Baliospermum montanum* (Willd.) Muell. Arg. *Asian Pac J Trop Med* 2010; **3**(11): 894-897.

[16] Nisar M, Kaleem WA, Qayum M, Hussain A, Zia-Ul-Haq M, Ali I, et al. Biological screening of *Zizyphus oxyphylla* Edgew stem. *Pak J Bot* 2011; **43**(1): 311-317.

[17] Rizwan K, Zubair M, Rasool N, Riaz M, Zia-Ul-Haq M, de Feo V. Phytochemical and niological studies of *Agave attenuata*. *Int J Mol Sci* 2012; **13**(5): 6440-6451.

[18] Zia-Ul-Haq M, Ahmad M, Mehjabeen, Jehan N, Ahmad S, Qayum M, et al. Antimicrobial screening of selected flora of Pakistan. *Arch Biol Sci* 2011; **63**(3): 691-695.

[19] Hossain MA, Shah MD, Sang SV, Sakari M. Chemical composition and antibacterial properties of the essential oils and crude extracts of *Merremia borneensis*. *Journal of King Saud University-Science* 2012; **24**: 243-249.

[20] Hossain MA, Rahman M. Total phenolics, flavonoids and antioxidant activity of tropical fruit pineapple. *Food Res Int* 2011; **44**: 672-676.

[21] Hossain MA, Shah MD, Charles G, Iqbal M. *In vitro* total phenolics, flavonoids contents and antioxidant activity of essential oil, various organic extracts from the leaves of tropical medicinal plant *Tetrastigma* from Sabah. *Asian Pac J Trop Med* 2011; **4**: 717-721.

[22] Bajpai M, Pande A, Tewari SK, Prakash D. Phenolic contents and antioxidant activity of some food and medicinal plants. *Int J Food Sci Nutr* 2005; **56**(4): 287-291.