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RESEARCH ARTICLE

Isolation and Characterization of a Water-soluble Disaccharide from Methanolic Extract of *Arisaema utile* and Its Evaluation for Antioxidant and Antifungal Activity

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ABSTRACT

The aim of this study is to isolation, identification and characterized the bioactive compounds from the rhizomes of *Arisaema utile*. Preliminary phytochemical screening of the rhizome extract of *A. utile* revealed the presence of steroids, terpenoids, flavonoids, alkaloids, saponins, carbohydrates, and fatty acids. The air-dried rhizomes were pulverized to a powder, subjected to Soxhlet extraction and compound isolation. The isolated compound was colorless crystalline, which was further subjected to IR, ^{13}CNMR, and ^{1}HNMR for proper characterization and elucidation of the structure. The compound was concluded as water-soluble disaccharide–Sucrose (2R,3R,4S,5S,6R)-2-[(2S,3S,4S,5R)-3,4-dihydroxy-2,5-bis(hydroxymeth-yl)oxolan-2-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol, antioxidant activity of the isolated compound was measured by 2,2-Diphenyl-1-picrylhydrazyl assay under *in vitro* condition. The isolated compound showed the most promising radical scavenging activity at a concentration of 10 µg/ml. Antifungal activity of the isolated compound was investigated against five fungal strains (*Candida albicans*, *Candida glabrata, Candida paropsilosis*, *Candida tropicalis,* and *Candida krusei*), using MIC_{80} assay. The most susceptible strains were *Candida paropsilosis* (MIC_{80} = 128 µg/mL).

Keywords: Antifungal activity, antioxidant activity, *Arisaema utile*, spectroscopyc

INTRODUCTION

A substantial part of all drugs is still based on compounds originally isolated from nature. The plant kingdom is a treasure house of potential drugs, and in recent years, there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects. *Arisaema* is a genus of about 150 species in the flowering plant family Araceae, native to Eastern Africa, Central Africa, Asia, and Eastern North America. Asiatic species are often called cobra lilies, while western species are often called jack-in-the-pulpit. It can be found growing on rocky slopes at an altitude of 2400–4600 m. It grows in shady, moist, well-drained, and fertile soil. *Arisaemas* are tuberous perennials that die back to the ground in winter. *Arisaema utile* emerges in spring. Most of the species from genus Araceae have a history of use in folk medicine for the treatment of various infectious diseases. Rhizomes of few species of *Arisaema* have a long history of use in traditional medicine, especially in Asian countries such as *Arisaema jacquemontiana* for Muscular strength and Skin infections and *Arisaema propinquum* for Skin eruption or rashes.[1] Many medicinal plants have been screened extensively for their antimicrobial potential worldwide.[2-4] Further, plant phenolic compounds have been found to possess potent antioxidant,[4-9] antimicrobial, and anticancer activities.[10,11] As an individual plant, *A. utile* is used for treating various infections in the blood, liver, and bile which correlates to the signs and symptoms of parasitic and microbial infections, cancer and inflammatory conditions. A lectin was also purified from tubers of Himalayan cobra lily *A. utile*.[12] One of the most exciting properties resulting out of the interaction of lectins with lymphocytes is mitogenicity, i.e., the triggering of quiescent and non-dividing lymphocytes into

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a state of growth and proliferation. The discovery of first mitogenic lectin Nowell[13] led to the detection of many other such lectins, most notably concanavalin A,[14] wheat germ agglutinin,[15] and pokeweed mitogen.[16] The crude extracts of this plant as mentioned above showed significant antimicrobial, antioxidant, and prominent cytotoxic activities against few cancer cell lines. The plant has reports of being used in traditional medicines by the tribal people of Jammu and Kashmir for curing various diseases. Keeping in view global and national scenario of medicinal plants, encouraged by these finding, we carried out in-depth phytochemical isolation and further investigated the antifungal and antioxidant activities of the isolated compound from *A. utile,* especially existing at high altitudes of Jammu and Kashmir with proven folklore medicinal claim.

MATERIALS AND METHODS

Collection of plant material and processing

The *A. utile* plant material was collected in the month of July from Gulmarg area of district Budgam of Jammu and Kashmir state, India. Voucher specimen of *A. utile* bearing specimen no 27911 was identified and deposited at KASH herbarium in center of biodiversity and plant taxonomy, University of Kashmir, Srinagar, Jammu and Kashmir, India. The rhizomes of the plant were shade dried, then pulverized into powder with the aid of grinder. The powder obtained from the plant was then used for the isolation of constituents using Soxhlet extraction and column chromatography.

Extraction and purification

About 800 g of the powdered rhizome of *A. utile* was subjected to sequential extraction using Soxhlet apparatus from nonpolar to polar solvents such as *n*-hexane < ethyl acetate < methanol. The solvent was recovered under reduced pressure using rotary evaporator under vacuum condition, and the residue was stored in the refrigerator. Methanolic extract was chromatographed on a silica gel column and eluted with solvent mixtures of increasing polarity, composed of hexane, ethyl acetate, and methanol. All the fractions were monitored on TLC. Fractions collected with methanol:ethyl acetate (40:60) were pulled together as these fractions showed a single spot on TLC. Further, these combined fractions were kept in the refrigerator overnight for crystallization which resulted in the formation of crystalline compound 3. The structure of the isolated compound was established on the basis of elemental analysis and spectroscopic evidence (IR,^{1}HNMR, and ^{13}CNMR). The structure was simulated using ACD/NMR program to obtain the chemical shifts of both proton and carbon.

Spectroscopic characterization of compound 3

The various spectroscopic methods such as FT-IR, DEPT,^{1}H-NMR, and^{13}C-NMR were used to elucidate the structure of isolated compounds. The Fourier transform-infrared (FTIR) spectroscopy was carried out on a PerkinElmer FT-IR fitted with Spectrum software version 10.3.2 using a liquid sampler.^{1}H-NMR (400MHz) and ^{13}C-NMR (400MHz) were recorded using $CDCl_3$ as a solvent in MeOD on Bruker, Avance (400MHz) NMR spectrometer.

Determination of antioxidant activity of compound 3

The *in vitro* antioxidant potential of the isolated compound 3 was measured in terms of hydrogen donating or free radical scavenging ability using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the standard procedure.[17] 0.1 mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 1.0 ml of the test solution in methanol at different concentrations of isolated compounds (2, 4, 8, 10, and 12 µg/mL). The reaction mixture was incubated at 37°C for 30 min in darkness. The absorbance of the sample at 517 nm was measured and then compared with that of a control solution containing the reaction mixture amended methanol instead of isolated compounds. Ascorbic acid (2, 4, 8, 10, and 12 µg/mL) was used as the standard reference compound, and the percentage of DPPH free radical scavenging activity was calculated using the following equation:

% Scavenging activity = $((A_0-A)/A_0\times100)$.

Where A_0 was the absorbance of the control (blank, without compound) and A was the absorbance of the reaction mixture. All the tests were performed

in triplicate, and the graph was plotted with the mean values.

Antifungal activity of compound 3

Microtiter assay

All the fungal strains of *Candida* used in this study were grown for 48 h at 30°C to obtain single colonies, which were resuspended in a 0.9% normal saline solution to give an optical density at 600 nm (OD_{600}) of 0.1. The cells were then diluted 100-fold in YNB (yeast nutrient broth) medium containing 2% glucose and the respective auxotrophic supplements. The diluted cell suspensions were added to the wells of round-bottomed 96-well microtiter plates (100 µl/well) containing equal volumes of medium (100 µl/well) and different concentrations of isolated compound 3. A drug-free control was also included. The plates were incubated at 30°C for 48 h. The MIC test endpoint was evaluated both by eye and by reading the OD_{620} in a microplate reader and is defined as the lowest drug concentration that gave >80% inhibition of growth compared with the growth of the drug-free controls (the MIC at which 80% of isolates are inhibited [MIC_{80}]).[18]

RESULTS AND DISCUSSION

FTIR spectroscopy

The IR absorption spectrum of compound 3 showed absorption peaks at 3429.2 cm^{-1} (O-H stretching.); 2996.8 cm^{-1} and 2914.8 cm^{-1} (aliphatic C-H stretching); 1654.9 cm^{-1} and 1431.3 cm^{-1} (C=C absorption peak); 1312.0 cm^{-1} (OH def) and 1013.8 cm^{-1} (Ether); and 954.2 cm^{-1} (C-H bond) [Figure 1]. The compound is a colorless crystalline compound. On subjection to IR spectroscopic analysis, absorptions bands appeared between 3570.36 and 3186.51 cm^{-1} that is characteristic of O-H stretching, 2997.8 and 2914.8 cm^{-1} are due aliphatic or C-H stretching or (CH_3), 1654.9 and 1431.3 cm^{-1} due to double (C=C) stretching, 1043.7 cm^{-1} due to Ethers or (C-O). Other absorption frequencies include 1379.1cm^{-1} are a bending frequency for cyclic $(CH_2)_n$. The absorption frequency at 864.7 cm^{-1} signifies (CH deformation) cycloalkane. These absorption frequencies resemble the absorption frequencies observed for sucrose as resembled data published by Mohamed *et al*.[19]

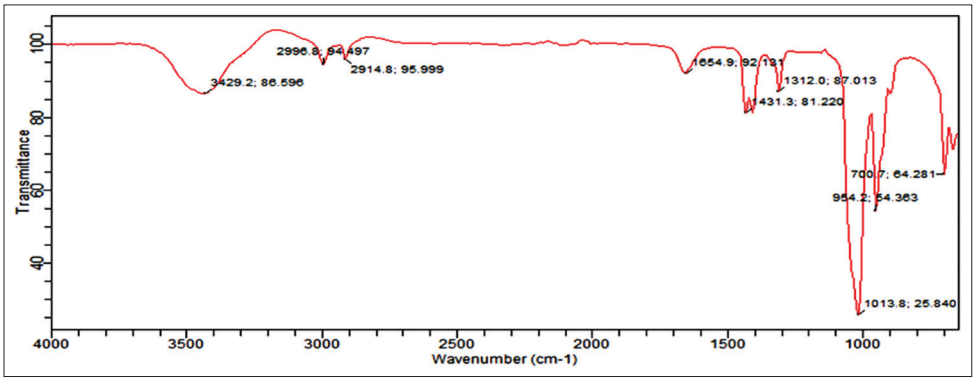

Figure 1: Infrared absorption spectra of Compound-3

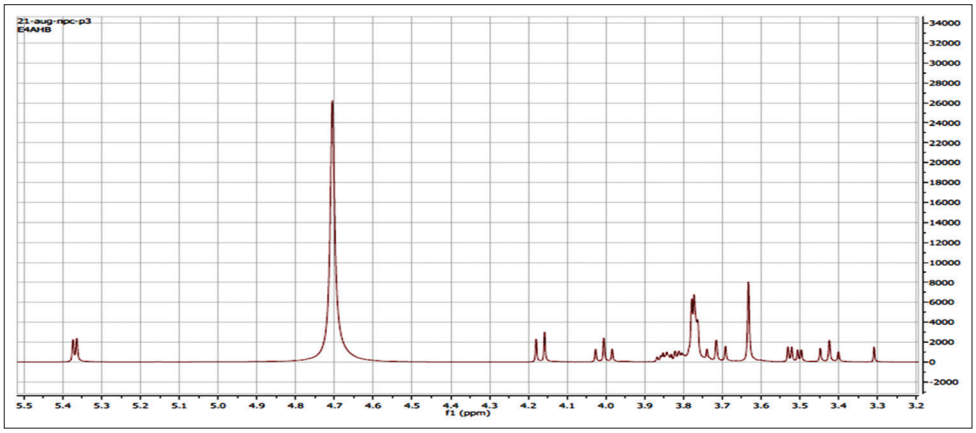

Figure 2: ^{1}H-NMR absorption spectra of Compound-3

Nuclear magnetic resonance (NMR) spectroscopy

^{1}HNMR ($CDCl_3$, 400MHz) of compound 3 has given signals at δ 4.01 (1H, m, H-1), 3.62 (1H, m, H-2), 3.98 (1H, m, H-3), 4.71 (1H, m, H-4), 5.37 (1H, m, H-5), 5.36 (d, H-6), 4.18 (1H, m, H-9), 3.69 (2H, m, H-10), 4.16 (2H,m, H-11), 4.03 (2H, m, H-12) ppm [Figure 2].

The chemical shift of the H-l resonance of the α-D-glucopyranosyl moieties is *δ* 4.01 for Compound-3. The chemical shifts of the H-3,4 resonances of the *β*-D-fructofuranosyl moieties are diagnostic, namely, 3.98 (d) and 4.71 (t) for compound-3. Identification on the basis of these limited "H-n.m.r." data are realistic. The above spectral features are in closed agreement to those observed for sucrose according to Jung *et al*.[20] and De Bruyn A and Van Loo.[21]

^{13}CNMR and DEPT ($CDCl_3$, 100MHz) of compound 3 has given signal at 103.7(C-6), 92.2 (C-5), 81.2 (C-9), 76.52(C-7), 74.07 (C-8), 72.64 (C-3), 72.46 (C-1), 71.13 (C-4), 69.29 (C-2), 62.41 (C-12), 61.45 (C-10), 60.20 (C-11) [Figures 3 and 4].

The presence of reducing D-glucose or D-fructose is indicated by the characteristic resonances

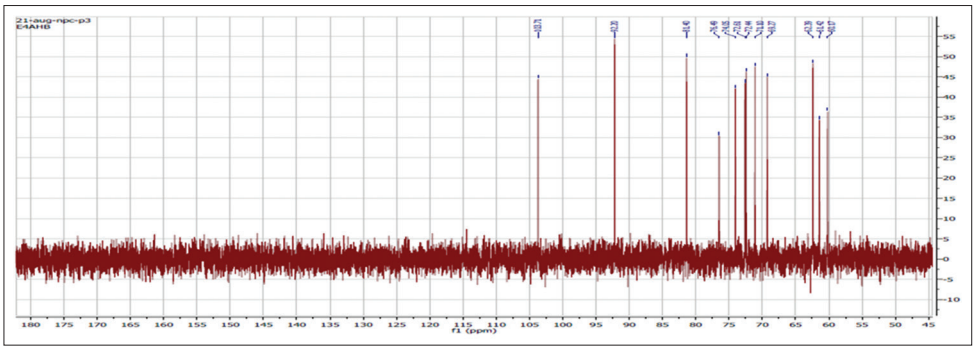

Figure 3: ^{13}C-NMR absorption spectra of Compound-3

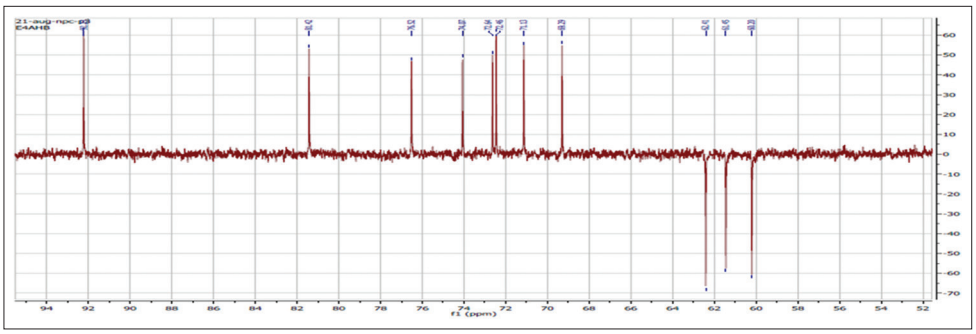

Figure 4: DEPT absorption spectra of Compound-3

Table 1: ^{1}H, ^{13}C-NMR chemical shift values for Compound-3 in comparison with those reported in literature

Carbon atom	^{13}C Reported in literature	^{13}C Reported in literature	^{13}CNMR (Our report)	^{1}HNMR Reported in literature	^{1}HNMR Reported in literature	^{1}HNMR Our report)	Nature of carbon
C-1	70.7	72.62	72.8	4.01	5.20 t	4.01	CH
C-2	70.4	69.26	69.2	3.32	5.08 t	3.62	CH
C-3	70.9	72.45	72.6	3.93	4.96 dd	3.98	CH
C-4	71.9	71.13	71.1	4.75	4.59 d	4.71	CH
C-5	90.2	92.24	92.2	5.48	4.26 dd	5.37	CH
C-6	104.7	103.74	103.7		4.11 dd	5.36	C
C-7	75.5	76.39	76.4	5.46	3.68 m		CH
C-8	74.0	74.02	74.07	5.45	3.2 br s		CH
C-9	78.5	81.44	81.4	4.17	2.08 s	4.18	CH
C-10	63.6	61.35	61.45	3.55	2.05 s	3.69	CH_2
C-11	62.7	60.14	60.17	4.28, 4.54	2.02 s	4.16	CH_2
C-12	64.1	62.43	62.41	4.28, 4.39	2.00 s	4.03	CH_2

Table 2: Antioxidant activity of Compound-3

Concentrations	2 μg/ml	4 μg/ml	8 μg/ml	10 μg/ml	12 μg/ml
% Age inhibition Compound 3	27.5	19.16	31.66	37.5	26.66
% Age inhibition ascorbic acid	53.3	58.3	64.16	68.33	62.5

Table 3: Antifungal activity of Compound-3

Strains drugs	*C. albicans*	*C. glabrata*	*C. paropsilosis*	*C. tropicalis*	*C. krusei*
Compound-3	256	>256	128	>256	>256
FLC	4	4	4	4	64

C. albicans: Candida albicans, C. glabrata: Candida glabrata, C. paropsilosis: Candida paropsilosis, C. tropicalis: Candida tropicalis, C. krusei: Candida krusei

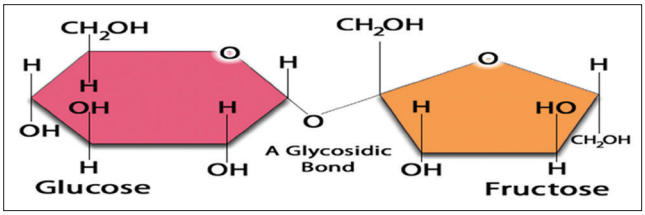

Figure 5: Structure of sucrose

at δ 96.7 and 92.9 for C- 1 in a- and β-D-glucopyranose, respectively, and at δ 102.3 and 98.9 for C-2 of β-D-fructopyranose and β-D-fructofuranose, respectively. These results are in according to the previous data of De Bruyn A, Van Loo[21] Table 1 shows the ^{1}H-NMR and ^{13}C-NMR values in comparison with the previous data available.[20,22,23]

Test for carbohydrates: Molisch's test

To 2 ml of extract 2–3 drops of alpha naphthalene solution in alcohol was added, shaken for 2 min and 1 ml of concentrated sulfuric acid was added slowly from the sides of the test tube. A deep violet color at the junction of two layers indicates the presence of carbohydrates.

In this paper, we report the isolation of one compound, a disaccharide-sucrose [Figure 5]. This compound has earlier been isolated from other plant species but being reported from *A. utile* for the first time. Isolation of the compound was effected through the chromatographic technique, and its structure was established based on NMR spectroscopic data together comparison with other existing data [Table 1]. The isolated compound is soluble in methanol/DMSO and is colorless crystalline in nature.

Based on the related data (FT-IR, DEPT, ^{1}H-NMR, and ^{13}C-NMR) also on comparison the experimental data matched with the simulated data, the structure of the isolated compound is proposed as:

BIOLOGICAL ACTIVITIES

Antioxidant activity

DPPH free radical scavenging capacity of the compound-3 was measured by DPPH assay under *in vitro* conditions. The ability of the examined compound 3 and its derivatives to act as a donor for hydrogen atoms in the transformation of DPPH radical into its reduced form $DPPH_2$ was investigated. The examined samples were able to reduce the stable purple colored DPPH radical into yellow colored $DPPH_2$ [Table 2]. Compound-3 showed the most promising radical scavenging activity at a concentration of 10 μg/ml. These results are plotted in the form of a graph [Figure 6]. Antioxidant activity of various disaccharides has already been reported by a number of workers. Antioxidant activity of O-glycosides shows significant inhibition,[24] antioxidative activity of porcine plasma protein-sugars was reported by Benjakul *et al.*,[25] and antioxidative activities of water-soluble disaccharide chitosan derivatives were successfully carried out and reported by Lin and Chou.[26]

Antifungal activity

In this study, the antifungal activity of the compound-3 isolated from methanol extract of *A. utile* was tested. Antimicrobial activity was investigated against five fungal strains (*C. albicans, C. glabrata, C. paropsilosis, C. tropicalis,* and *C. krusei*), using MIC_{80} assay. The compound-3 displayed weak antifungal activity against these fungal strains except for *C. paropsilosis* which was found to be most susceptible among these

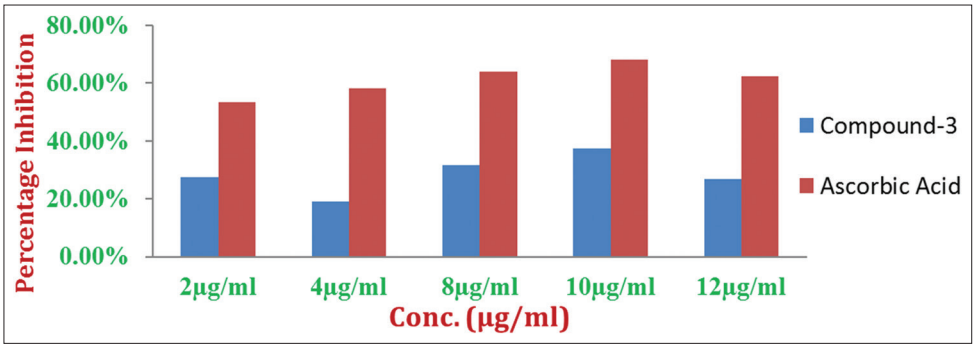

Figure 6: 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity of Compound-3

strains. As shown in Table 3, these strains were found to be susceptible strains but at higher concentrations of the compound-3 as compared to the control drug used. The most susceptible strains were *C. paropsilosis* (MIC_{80} = 128 µg/mL) and for *C. albicans C. glabrata, C. tropicalis, and C. krusei* the MIC_{80} value was recorded at concentrations of 256 µg/mL and above.

MIC_{80} values (µg/ml) for each sample FLC is the positive control fluconazole

Antimicrobial activity of various disaccharides has already been reported by a number of researchers. Antimicrobial activity of water-soluble quaternary disaccharide chitosan N-betainates was determined against *Escherichia coli* and *Staphylococcus aureus* by Holappa *et al*.[27] Antibacterial activity of the water-soluble N-alkylated disaccharide chitosan derivatives against *E. coli* and *S. aureus* was reported by Manoharan *et al*.[28] The effects of mono- and disaccharides on the antimicrobial activity of the lactoperoxidase (LPO) system against *Salmonella Enteritidis* were investigated by Al-Baarri *et al*. The results clearly reveal that most of the sugars inhibit the antimicrobial activity of the LPO system and sucrose was the weakest inhibitor.[29]

CONCLUSION

In our study, a water-soluble disaccharide was isolated for the first time from rhizomes of *A. utile* and its antifungal and antioxidant potential was evaluated. The structure of the isolated compound was identified as sucrose on the basis of spectroscopic methods and by comparing their physical properties reported in literature. The isolated compound exhibited weak free radical scavenging activity using DPPH assay and weak antifungal activity against tested fungal strains except for *C. paropsilosis* which was found to be most susceptible among these tested strains. These strains were found to be susceptible strains but at much higher concentrations of the isolated compound – sucrose as compared to the control drug used. Sugar is a common foodstuff traditionally used for its sweetening properties, which might be accompanied by antioxidant properties arising from molecules (polyphenols, Maillard products) other than sucrose of the cane brown sugars. These findings provide a reason for weak antioxidant activity of isolated sucrose hence justifies our work on the antifungal activity.

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REFERENCES

1. Ahmed S, Ajaz T. An ethnobotanical survey of medicinal plants used by Gujjar and Bakerwal community of Tehsil Manjakote of district Rajouri J & K State (India). Int J Eng Sci 2017;7:4142.
2. Kaur GJ, Arora DS. Antibacterial and phytochemical screening of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi*. BMC Complement Altern Med 2009;9:30.
3. Mothana RA, Lindequist U, Gruenert R, Bednarski PJ. Studies of the *in vitro* anticancer, antimicrobial and antioxidant potentials of selected Yemeni medicinal plants from the island Soqotra. BMC Complement Altern Med 2009;9:7.
4. Adedapo AA, Jimoh FO, Koduru S, Masika PJ, Afolayan AJ. Assessment of the medicinal potentials of the methanol extracts of the leaves and stems of

Buddleja saligna. BMC Complement Altern Med 2009;9:21.
5. Adesegun SA, Fajana A, Orabueze CI, Coker HA. Evaluation of antioxidant properties of *Phaulopsis fascisepala* CB Cl. (Acanthaceae). Evid Based Complement Alternat Med 2009;6:227-31.
6. Hutadilok-Towatana N, Chaiyamutti P, Panthong K, Mahabusarakam W, Rukachaisirikul V. Antioxidative and free radical scavenging activities of some plants used in Thai folk medicine. Pharm Biol 2006;44:221-8.
7. Wang KJ, Yang CR, Zhang YJ. Phenolic antioxidants from Chinese toon (fresh young leaves and shoots of *Toona sinensis*). Food Chem 2007;101:365-71.
8. Gacche RN, Dhole NA. Antioxidant and possible anti-inflammatory potential of selected medicinal plants prescribed in the Indian traditional system of medicine. Pharm Biol 2006;44:389-95.
9. Chang HC, Huang GJ, Agrawal DC, Kuo CL, Wu CR, Tsay HS. Antioxidant activities and polyphenol contents of six folk medicinal ferns used as "Gusuibu". Bot Stud 2007;48:397-406.
10. Alcaráz LE, Blanco SE, Puig ON, Tomás F, Ferretti FH. Antibacterial activity of flavonoids against methicillin-resistant *Staphylococcus aureus* strains. J Theor Biol 2000;205:231-40.
11. Cushnie TP, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents 2005;26:343-56.
12. Goldstein IJ. What should be called a lectin? Nature 1980;285:66.
13. Nowell PC. Phytohemagglutinin: An initiator of mitosis in cultures of normal human leukocytes. Cancer Res 1960;20:462-6.
14. Harris H, Robson EB. Precipitin reactions between extracts of seeds of *Canavalia ensiformis* (Jack bean) and normal and pathological serum proteins. Vox Sang 1963;8:348-55.
15. Aub JC, Sanford BH, Wang LH. Reactions of normal and leukemic cell surfaces to a wheat germ agglutinin. Proc Natl Acad Sci 1965;54:400-2.
16. Brittinger G, König E. Lymphocytenstimulierung durch Pokeweed-Mitogen (PWM). Klin Wochenschr 1969;47:1307-13.
17. Braca A, De Tommasi N, Di Bari L, Pizza C, Politi M, Morelli I, *et al.* Antioxidant principles from *Bauhinia tarapotensis*. J Nat Prod 2001;64:892-5.
18. Mukhopadhyay K, Kohli A, Prasad R. Drug susceptibilities of yeast cells are affected by membrane lipid composition. Antimicrob Agents Chemother 2002;46:3695-705.
19. Mohamed GF, Shaheen MS, Khalil SK, Hussein AM, Kamil MM. Application of FT-IR spectroscopy for rapid and simultaneous quality determination of some fruit products. Nat Sci 2011;9:21-31.
20. Jung MH, Kim HS, Sangdoo A, Kim CT, Jin MH, Yim YH, *et al*. Complete assignment of the $^ H1 $ and $^{13} C $ NMR spectra of a sucrose ester from *Euphorbia lathyris* L. J Korean Magn Reson Soc 2000;4:125-32.
21. De Bruyn A, Van Loo J. The identification by 1H- and 13C-n.m.r. Spectroscopy of sucrose, 1-kestose, and neokestose in mixtures present in plant extracts. Carbohydr Res 1991;211:131-6.
22. De Bruyn A, Van Beeumen J, Anteunis M, Verhegge G. 1H-NM R study of some D-aldohexapyranosyl-D-fructos (id) es in D2O. Bull Soc Chimiques Belges 1975;84:799-811.
23. Bock K, Thøgersen H. Nuclear magnetic resonance spectroscopy in the study of mono-and oligosaccharides. In: Annual Reports on NMR Spectroscopy. Vol. 13. London: Academic Press; 1983. p. 1-57.
24. Rajaganesh R, Jayakumar J, Sivaraj C, Raaman N, Das TM. Synthesis and antioxidant activity of a novel class of 4, 6-O-protected O-glycosides and their utility in disaccharide synthesis. Carbohydr Res 2010;345:1649-57.
25. Benjakul S, Lertittikul W, Bauer F. Antioxidant activity of Maillard reaction products from a porcine plasma protein-sugar model system. Food Chem 2005;93:189-96.
26. Lin HY, Chou CC. Antioxidative activities of water-soluble disaccharide chitosan derivatives. Food Res Int 2004;37:883-9.
27. Holappa J, Hjálmarsdóttir M, Másson M, Rúnarsson Ö, Asplund T, Soininen P, *et al*. Antimicrobial activity of chitosan N-betainates. Carbohydr Polym 2006;65:114-8.
28. Manoharan KP, Benny TK, Yang D. Cycloartane type triterpenoids from the rhizomes of *Polygonum bistorta*. Phytochemistry 2005;66:2304-8.
29. Al-Baarri AN, Hayashi M, Ogawa M, Hayakawa S. Effects of mono- and disaccharides on the antimicrobial activity of bovine lactoperoxidase system. J Food Prot 2011;74:134-9.