IJBPAS, June, 2013, 2(6): 1260-1269

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

PHYTOCHEMICAL CONTENT AND ANTIBACTERIAL ACTIVITY OF *ATRIPLEX NUMMULARIA* EXTRACTS

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ABSTRACT

Atriplex nummularia was evaluated for its secondary metabolite contents and its anti-bacterial activity. Six compounds were isolated from this plant (four flavonoids and two steroids. Total extract and different plant fractions were screened for their anti bacterial activity. Beta sitosterol glucoside, Stigmasterol glucoside, quercetin, quercetin 3-O-*β*-glucoside, isorhamnetin 3-O-*β*-glucoside and rutin were isolated from *A. nummularia.* The total ethanol extract of *A. nummularia* and diethyl ether, chloroform, ethyl acetate and butanol fractions showed antibacterial activity against *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis,Klebsiela pneumonia, Pseudomonas eruginosa* and *E. coli.* The highest activity appeared against *S. pyogenes* by using diethyl ether and chloroform extracts.

Keywords: *Atriplex nummularia*, Flavonoids, Antibacterial Activity

INTRODUCTION

Genus *Atriplex* belongs to the family Chenopodiaceae (Goosefoot) and includes 225 species, *Atriplex nummularia* (old man saltbush) is a halophyte shrub that grows to an average height of 2.0 m. **[1]**. Various species of *Atriplex* have been used for their important medicinal values, for example; *A. semibacata* and *A. vestita* have been used as an antifungal agent and in the treatment of bronchitis **[2]**. Serum alanine aminotransferase, aspartate

aminotransferase, blood urea and serum creatinine were increased in sheep fed on *A. lentiformis* for 45 days, also reduction in live body weight occurred **[3]**.

A. hortenisis has been reported as a source of vitamin A. *A. inflata* has been mentioned to have antifungal activity in the non-polar extract against number of plant fungal pathogens. *A. inflata* and *A. parv*ifolia extracts were found to be effective against Herpes simplex viral infection **[4]**. Several species have been evaluated for their antidiabetic effects, such as *A. halimus* **[5]**. In traditional medicine, a cocktail of minerals in *A. halimus* is used to benefit glycemic control in diabetic patients **[6]**. *A. halimus* produce the polyphenols and other bioactive substances potentially useful for medicinal properties and as natural food preservation **[7]**.

The nonpolar extract of *A. lindleyi* could be medically used as antibacterial agent while its polar extract which is rich inflavonoids and flavonoid glycosides is medicinally useful as antidiabetic andantioxidant drug **[8]**.

Two phytoecdysteroids, 20-hydroxyecdysone (1) and polypodine B were isolated frommethanol extract of the seeds of *A. nummularia* **[9]**. Quercetin-4′-methoxy-7-glucorhamnoside, kaempferol-4′-methoxy-3-glucorhamnoside, quercetin -6, 4′ - dimethoxy -3 – glucorhamnoside, (scopoletin, scopolin were isolated from *A. farinosa* **[10]**. Quercetin, 3′-methoxy, 3-gluco-rhamnoside, Quercetin,3′-methoxy, 3 galacto-rhamnoside-xyloside and Quercetin,3 ′ -methoxy, 3 galacto-rhamnoside-apioside were isolated as potent estogenic flavone glycoside from *A. semibaccata* **[11]**.

The present study was designed to investigate the phytochemical contents of *A. nummularia* and to evaluate its antibacterial activity.

MATERIALS AND METHODS

Plant Material

Atriplex nummularia was collected from North Western Coast, Egypt (2010). The collected plant was kindly authenticated by Dr. Ahmed Morsy Ahmed, Prof. of plant eco-physiology,Desert Research Center, Egypt. A voucher specimen has been deposited in the herbarium of Desert Research Center, Cairo, Egypt. Plant material was air-dried in shade, reduced to fine powder.

Extraction

One kg of the dried powder of *Atriplex nummularia* (aerial parts) was extracted by percolation in 70% aqueous ethanol. The ethanol extract of the plant was filtered and the marc was re-percolated for three times. Total ethanol extract was concentrated under reduced pressure at a temperature not exceeding 35^{o}C to yield a dry extract of 91g.

Total ethanol extract was suspended in distilled water and extracted successively with diethyl ether, chloroform, ethyl acetate and n-butanol to give diethyl ether, chloroform, ethyl acetate and n-butanol fractions, respectively. Each fraction was dried over anhydrous sodium sulfate and the solvent was distilled off.

Antimicrobial Activity

Test Organisms

The microorganisms used for the experiments were obtained from the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt.

Gram-Positive Bacteria: *Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes* (ATCC 19615), *Bacillus subtilis* (ATCC 9372) and *Micrococcus luteus* (NRRL B-4375).

Gram-Negative Bacteria: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumonia* (MTCC 618) and *Enterobacter aerogenes* (ATCC 13048).

Antibacterial Testing

In vitro antibacterial activity of the total ethanol extract and fractions was studied against some Gram-negative and Gram-positive bacteria by the agar well diffusion method **[12]**. All the bacterial strains were grown and maintained on nutrient agar slants at 4°C. The total ethanol extract and fractions were dissolved in dimethylsulfoxide (DMSO) to obtain 100 and 50 mg/mL, respectively. The extracts were filtered over Whatman No 1 filter paper. The filtrates were sterilized by membrane filtration using 0.45 µm pore size filters DMSO was taken as a negative control. 100 µL of bacterial cultures species (incubated for 12-16 h) were mixed in molten Mueller Hinton Agar medium (Merck) and poured in pre-sterilized petri plates. A cork borer (6 mm diameter) was used to punch wells in the solidified medium and filled with 50 µL of extracts and control compound. The plates were incubated at 37°C for 24 h and the diameter of the zone of inhibition produced by each agent was measured in mm. Each sample was assayed in triplicate and the mean values were observed. The antibacterial activity was interpreted from the size of the diameter of zone of inhibition measured to the nearest mm as observed from the clear zones surrounding the wells.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC values of total extract and the fractions (diethyl ether, chloroform, ethyl acetate and n-butanol) were determined by agar well diffusion method against the *S. pyogenes*. Concentrations of 10, 7.5, 5.0, 2.5, 1.0 and 0.5 mg/ mL of (diethyl ether,

chloroform, ethyl acetate and n-butanol) fractions were prepared separately. The lowest concentration that inhibited visible growth of the test organisms on the agar plate after 24 h incubation at 37°C was identified as the MIC.

RESULTS

The diethyl ether and chloroform fractions (companied together) (5g) were applied on silica gel column chromatography (150 g) and gradiently eluted with *n*-hexane and *n*-hexanecontaining increasing proportions of chloroform. Similar fractions were combined together. Each collected fractions was reapplied on silica gel column and gradiently eluted with *n*-hexane and *n*-hexanecontaining increasing proportions of chloroform. Further purification was carried to offer compounds **1–3**.

Also ethyl acetate fraction (6.5 g) was applied on silica gel (170 g) for column chromatography and gradiently eluted with ethyl aetate and ethyl aetate containing increasing proportions of methanol. Similar fractions were combined together, each collected fractions was reapplied on silica gel column and gradiently eluted with ethyl acetate containing increasing proportions of methanol. Further purification was carried out using Chromatotron (preparative, centrifugally accelerated, radial, thin-layer chromatograph) model 7924T on silica gel 60 PF_{254}containing gypsum (2mm) and eluted with ethyl acetate and ethyl acetate containing increasing proportions of methanol to offer compounds **4–6**.The structures of the isolated compounds (1-6) **(Figure 1)** were established through chromatography, as well as conventional chemical and spectroscopic methods of analysis **[13, 14]**.

Compound (2):colorless amorphous, ^{1}HNMR (500 MHz, Pyridine): δ 5.33 (1H, *t, J* = 4.67 & 1.8 Hz, H-6),2.38 (1H, *t, J* = 11.3 & 11.5 Hz, H-7a), 2.10 (1H, *m*, H-8), 1.88 (1H, *m*, H-7b),0.97 (3H, *d*, *J* = 6.4 Hz, CH_3- 21), 5.20 (1H, *dd, J* = 15.2 & 8.8 Hz, H-22), 5.10 (1H, *dd, J* = 15.2 & 8.8 Hz, H-23), 4.89 (1H, *d*, *J* = 7.9 Hz, H-1`), 3.86(1H, *t*, *J* = 7.7& 8.6 Hz, H-2`),4.14 (1H, *m*, H-3`), 4.058 (1H, *m*, H-4`), 4.40 (1H, *dd*, *J* = 2.4 & 11.3Hz, H-6`a ),3.83 (1H, *m*, H-5`), 4.23 (1H, *dd*, *J* = 5.3 & 11.7 Hz, H-6`b), 0.65 (3H, *s*, CH_3-18); 0.92(3H, *s*, CH_3- 19), 0.88 (3H, *d*, *J* = 6.8 Hz, CH_3-26), 0.82 (3H, *d*, *J* = 6.4 Hz, CH_3-27), 0.80 (3H, *t*, *J* = 7.1Hz, CH_3-29), ^{13}C-NMR (125 MHz, Pyridine): δ37.2 (C-1) 28.5 (C-2), 78.21 (C3), 38.9 (C-4), 141.0 (C-5), 122.10 (C-6), 32.4 (C-7), 32.1 (C-8), 50.21 (C-9), 36.7 (C- 10), 20.9 (C-11), 39.8 (C-12), 42.8 (C-13), 56.6 (C-14), 24.8 (C-15), 39.0 (C-16), 56.2(C-17), 12.1 (C-18), 19.6 (C-19), 36.2 (C-20), 19.10 (C-21), 137.4 (C-22), 128.2 (C-23),

46.3 (C-24), 26.4 (C-25), 20.0 (C-26), 19.4 (C-27), 29.9 (C-28),12.3 (C-29), 101.9 (C-1`), 74.9 (C-2`), 78.3 (C-3`), 71.4 (C-4`), 79.8 (C-5`), 62.9 (C-6`).

Compound (3): yellow needles, ^{1}H NMR using (500 MHz, DMSO-d6): δ 7.73 (1H d *J* = 2.5 Hz H-2'), 7.63 (1H dd*J* = 8.5 & 2.5 Hz H-6`), 6.88 (1H d *J* = 8.5 Hz H-5'), 6.39 (1H d *J* = 2.5 Hz H-6) and 6 18 (1H d *J* = 2.5 Hz H-8). ^{13}C NMR (125 MHz,DMSO-d6): δ ppm 158.2 (C-2), 138.2 (C-3), 177.4 (C- 4), 162.8 (C-5), 99.1 (C-6),165.5 (C-7),94.2 (C-8), 149.1 (C-9), , 104.5 (C-10), 148.1 (C-4'), 146.2 (C-3'), , 124 1 (C-6'), 121.6 (C-1'), 116.3(C-2'), 115.9 (C-5')

Compound (4): yellow crystals:^{1}H NMR (500 MHz, DMSO-d6): *δ* 6.15 (1H, *d*, *J* = 1.84 Hz, H-6), *δ* 6.36 (1H, *d*, *J* = 2.0 Hz, H-8), *δ* 7.57 (1H, *d*, *J* = 2.0 Hz, H-2′), *δ* 6.82 (1H, *d*, *J* = 9.16 Hz, H-5′), *δ* 7.56 (1H, *dd*, *J* = 1.84, 9.0 Hz, H-6′), *δ* 5.44 (1H, *d*, *J* = 7.2 Hz, H-1″), *δ* 3.05-3.53 (6H, *m*, H-2″, H-3″, H-4″, H-5″, H-6″); ^{13}C NMR (125 MHz, DMSO-d6) δ 156.1 (C-2), 133.3 (C-3), 177.4 (C-4), 161.2 (C-5), 98.7 (C-6), 164.3 (C-7), 93.5 (C-8), 156.3 (C-9), 103.9 (C-10), 121.1 (C-1′), 115.2 (C-2′), 144.8 (C- 3′), 148.5 (C-4′), 116.2 (C-5′), 121.6 (C-6′), 100.8 (C-1″), 74.1 (C-2″), 76.5(C-3″), 69.9 (C-4″), 77.5 (C-5″), 60.9 (C-6″).

Compound (5): Yellow crystals, ^{1}H NMR (500 MHz, DMSO-d6): *δ* 6.08 (1H, *d*, *J* = 1.8 Hz, H-6), *δ* 6.29 (1H, *d*, *J* = 2.0 Hz, H-8), *δ* 6.80 (1H, *d*, *J* = 8.24 Hz, H-5′), *δ* 7.50 (1H, *dd*, *J* = 1.84, 9.0 Hz, H-6′), *δ* 7.53 (1H, *d*, *J* = 2.0 Hz, H-2′), *δ* 5.45 (1H, *d*, *J* = 7.32 Hz, H-1″), *δ* 3.01-3.68 (6H, *m*, H-2″, H-3″, H-4″, H-5″, H-6″), *δ* 3.86 (3H, s, OCH_3); ^{13}C NMR(DMSO, 125 MHz): 147.2(C-2), 136.4(C-3), 176.3(C-4), 160.5(C-5), 99.0(C-6), 162.9(C-7), 94.8(C-8), 155.9(C-9), 104.8(C-10), 122.1(C-1′), 111.9(C-2′), 149.2(C-3′), 147.6(C-4′), 115.8(C-5′), 121.1(C-6′), 100.2(C-1″), 78.6(C-2″), 76.6(C-3″), 69.7 (C-4″), 77.4 (C-5″), 60.8(C-6″), 55.8 (O-CH3).

Compound (6): yellow crystals,^{1}H-NMR (500 MHz, DMSO-d6): δ6.20 (1H, d, *J* = 2 Hz, H-6),6.38 (1H, d, *J* = 2 Hz, H-8),7.71 (1H, d, *J* = 2 Hz, H-2` ), 6.87 (1H, d, *J* = 8 Hz, H-5`), 7.64 (1H, dd, *J* = 8, 2.1 Hz, H-6`), 5.12 (1H, d, *J* = 7.50 Hz, H-1``), 4.54 (1H, d, *J* = 1.3Hz, H-1```), 3.82 (1H dd, *J* = 8.4, 2 Hz, H-6``), 3.62 (1H, dd, *J* =3.5 Hz, H-2```), δ 3.47-3.87 (6H m, sugar protons) and δ 1.25 (3H d *J* = 6 Hz, CH_3). ^{13}C-NMR (125 MHz, DMSO-d6O): δ ppm 159.31 (C-2), 135.65 (C-3), 179.34 (C-4), 166. 07 162.90 (C-5), 100.0 (C-6),(C-7), 94.93 (C-8), 149.82 (C-9),104.80 (C-10), 123.07 (C-1'), 117.73 (C-2`), 144.8 (C-3'), 148.1 (C-4'),116.07 (C-5'),123.62 (C-6'), 104.8 (C-1``), 75.73 (C-

2``), 78.15 (C-3``), 72 (C-4``), 77.2 (C-5``), 61.3 (C-6``), 102.43 (C-1```), 72.09 (C-2```), 71.36(C-3```), 72.22 (C-4```), 69.72 (C-5```), 17.93 (C-6```).

Antimicrobial Activity

The total ethanol extract of *A. nummularia* and its fractions were investigated for their antibacterial potentiality against 8 clinically important bacterial strains. The results are cited in **Table 1**. The total extract and the fractions showed activity against the tested bacteria; *Streptococcus pyogenes*> *Staphylococcus aureus*> *Bacillus subtilis*>*Klebsiell peneumonia*, while low activity against the remaining tested bacteria was observed. MIC was determined for each fraction **(Table 2)**. The most effective fractions were A1=A2 (MIC=0.5 mg/ml) >A3 (MIC= 1 mg/ml) >A4 (MIC= 2.5 mg/ml) result are shown in **Table 1** and **2**.

DISCUSSION

Six compounds were isolated from *A. nummularia*. Compounds were identified examining their ^{1}H-NMR, ^{13}C-NMR and as well comparison with the published data. Acid hydrolysis and TLC of the sugar part (ethyl acetate-methanol-acetic acid-water (65:15:10:10) revealed that compounds 3, 5 contain glucose, compound 6 contain glucose and rhamnose.

Compound (1): was identified as β-sitosterol glucoside by comparing their data with pervious published data **[10, 15 and 16]**.

Compound (2): was identified by comparing its ^{1}H NMR and ^{13}C NMR spectra with authentic samples and published data. It compared with Stigmasterol glucoside and its data were in agreement with this published for this compound **[16 and 17]**.

Compound (3): was identified by comparing its ^{1}H NMR and ^{13}C NMR spectra with authentic samples and published data. It compared with quercetin and its data were in agreement with this published for this compound **[18 and 19]**.

Compound (4): was identified by comparing its ^{1}H NMR and ^{13}C NMR spectra with authentic samples of quercetin-3-*O*-glucoside and its data were in agreement with this published for this compound **[20]**.

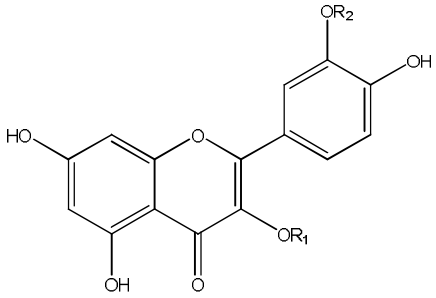
Compound (5): This compound wascompared with the reported data in literature [14], all data were identical to those of isorhamnetin-3-*O*-*β*-glucoside.

Compound (6): It is identified as rutin, ^{1}H-NMR and ^{13}C-NMR spectral data were identical with those reported for rutin **[10 and 18]**.

Antimicrobial Activity

The tested extract and fractions showed varying results against the bacterial strains. The total ethanol extract and fractions showed

antibacterial activity against most of the tested gram positive bacteria more than gram negative one. This observation supports the earlier reports that plant extracts are more active against Gram-positive bacteria than Gram-negative bacteria **[21-23]**. In addition the antibacterial activity of the extract and fractions against negative bacteria was less. The current work indicates that *A. nummularia* is a potential source of gram positive antibacterial agents especially the diethyl ether and chloroform extracts, which needs more investigation to identify the pure active agent which responsible for this activity. The current work has shown that *A. nummularia* is a potential source of gram positive antibacterial agents.



Compound	R1	R2
3	H	H
4	Glucose	H
5	Glucose	$-CH_3$
6	Gluco-rhamnoside	H

Fig.1: Isolated Compounds From *Atriplex nummularia*

Table 1: The Antibacterial Effect of the Total Ethanol Extract of *A. nummularia* and its Fractions

Microorganisms	Total extract	A1	A2	A3	A4	DMSO (Control)
S. aureus	**18±0.21**	**23±0.21**	**22±0.19**	**22±0.21**	**10±0.13**	**-**
S. pyogenes	**29±0.24**	**41±0.23**	**42±0.22**	**37±0.23**	**38±0.22**	**-**
B. subtilis	**15±0.17**	**22±0.21**	**21±0.18**	**19±0.13**	**18±0.21**	**-**
M. luteus	**8±0.11**	**10±0.15**	**11±0.19**	**-**	**-**	**-**
E. coli	**9±0.20**	**12±0.20**	**10±0.20**	**-**	**-**	**-**
P. aeruginosa	**9±0.18**	**11±0.15**	**12±0.17**	**10±0.12**	**-**	**-**
K. pneumonia	**11±0.20**	**15±0.19**	**12±0.16**	**11±0.10**	**9±0.11**	**-**
E. aerogenes	**9±0.16**	**13±0.20**	**12±0.12**	**-**	**-**	**-**

NOTE: Values are Mean of Three Replicates, Cork Borer Diameter = 6 mm

Table 2: Determination of MIC Value of Fractions of *A. nummularia* Against *S. pyogenes*

Concentrations (mg/ mL)	Fractions			
	A1	A2	A3	A4
10.0	**-**	**-**	**-**	**-**
7.50	**-**	**-**	**-**	**-**
5.00	**-**	**-**	**-**	**-**
2.50	**-**	**-**	**+**	**+**
1.00	**-**	**-**	**+**	**+**
0.50	**+**	**+**	**+**	**+**

NOTE: (-): Absence of growth, (+): Presence of growth

ACKNOWLEDGEMENT

The author appreciates the assistance of Prof. Dr. Mohamed E. Zain, College of Science, King Saud University, KSA and Mohamed A. El Sakhawy, College of Medical and Applied Sciences, Salman Bin Abdulaziz University, KSA, in antibacterial part.

REFERENCES

[1] Atiq-Ur-Rehman JB, Mackintosh JA and Fortune BE, Can the voluntary feed intake of wheat straw in sheep be improved by mixing with saltbush pastures? Proceedings, Aust. Soc. Anim. Prod., 20, 1994, 175-177.

[2] Boughalleb N, Trabelsi L and Harzallah-Skhiri F, Antifungal activity from polar and non-polar extracts of some Chenopodiaceae wild species growing in Tunisia, Nat. Prod. Res., Part A: Structure and Synthesis, 23, 2009, 988-997.

[3] Sabry GM, Fahmy AA and Donia, AMA, The Nutritive Value of *Atriplex lentiformis* as Affected by Drought and its Impact on Sheep Performance, J. Agric. Sci. Mansoura Univ., 28 (9), 2003, 6631-44.

[4] Ben Sassi A, Harzallah-Skhiri F, Bourgougnon N and Aouni M, Antiviral activity of some Tunisian medicinal plants against Herpes simplex virus type 1, Nat. Prod. Res., Part A: Structure and Synthesis, 22, 2008, 53-65.

[5] Rodriguez SA, Murray AP, Antioxidant activity and chemical composition of essential oil from *Atriplex undulata*, Nat. Prod. Commun., 5, 2010, 1841-1844.

[6] Day C, Hypoglycaemic compounds from plants, in: C.J. Bailey, P.R. Flatt (Eds.), New Antidiabetic Drugs, Smith-Gordon, London, 1990, 267.

[7] Benhammou N, Fawzia AB and Tatjana KP, Antioxidant activity of methanolic extracts and some bioactive compounds of *Atriplex halimus*, C. R. Chimie, 12, 2009, 1259-1266.

[8] Mohammed R, El-Hawary SS and Abo-youssef AA, Biological investigation of some wild Aizoaceae and Chenopediaceae species growing in Egypt, J. Nat. Pro., 5, 2012, 193-206

[9] Keckeis K, Sarker SD, and Dinan LN, Phytoecdysteroids from *Atriplex nummularia*, Fitoterapia, 71 (4), 2000, 456-458.

[10] Donia AM, Soliman GA, Zaghloul AM, Al qasoumi SI, Radwan AM and Basodan OA, Chemical constituents

and protective effect of *Ficus ingens* (Miq.) Miq. on carbon tetrachloride-induced acute liver damage in male Wistar albino rats, J. Saudi Chem. Soc., 17, 2013, 125-133.

[11] Shaker KH and Mostafa MM, Potent estogenic flavone glycoside from *Atriplex semibaccata*, Bull. Pharm. Sci., Assiut University, 27 (1), 2004, 127-132.

[12] Nair R, Kalariya T and Chanda S, Antibacterial Activity of Some Selected Indian Medicinal flora, Turk. J. Biol., 29, 2005, 41-47.

[13] Harborne JB and Baxter H, The Handbook of natural flavonoids, John Wiley & Sons, 1999.

[14] Agrawal P, Carbon 13- NMR of Flavonoids, Elsevier Science, New, York, 1989.

[15] Hassanean HA and Desoky EK, An acylate disorhamnet in Glucoside from *Zygophyllum simplex*, Phytochemistry, 31, 1992, 3293-3294.

[16] Maridass M and Ramesh U, Investigation of Phytochemical constituents from *Eulophiaepidendraea*, Inter. J. Biol. Tech., 2010, 1-7.

[17] Khatun M, Billah M and Abdul Quader MD, Sterols and Sterol Glucoside from Phyllanthus Species, Dhaka Univ. J. Sci., 60 (1), 2012, 5-10.

[18] El-Askary HI, Terpenoids from *Cleome droseri folia* (Forssk.) Del. Molecules, 10, 2005, 971-977.

[19] Mabry TJ, Markham KR and Thomas MB, Systematic identification of flavonoids Springer-Verlag, Berlin, 1970, 198.

[20] Donia AM, Al qasoumi SI, Awaad AS and Craker L, Antioxidant activity of *Covolvulus hystrix* Vahl and its chemical constituents, Pak. J. Pharm. Sci., 24 (2), 2011, 143-147.

[21] El-Toumy SA, El-Sharabasy FS, Ghanem HZ, El-Kady MU and Asmaa FK, Phytochemical and Pharmacological Studies on *Zilla spinosa*, Aus. J. Basic and Appl. Sci., 5 (8), 2011,1362-1370.

[22] Rabe T and Van Staden J, Antibacterial activity of South African plants used for medicinal purposes, J. Ethnopharmacol., 56, 1997, 81-87.

[23] Vlietinck AJ, Van Hoof L, Totte J, Lasure A, Vanden Berghe D, Rwangobo PC and Mvukiyuniwami

J, Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties, J. Ethnopharmacol., 46, 1995, 31-47.