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International Journal of Current Research
Vol. 10, Issue, 01, pp.63834-63837, January, 2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

ISOLATION AND STRUCTURE ELUCIDATION OF TWO ANTIOXIDANT COMPOUNDS FROM SUDANESE *SONCHUS OLERACEOUS* PLANT

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ARTICLE INFO

Article History:
Received 14th October, 2017
Received in revised form
19th November, 2017
Accepted 03rd December, 2017
Published online 19th January, 2018

Key words:
Antioxidant activity, Radical scavenging activity, DPPH, *Sonchus oleraceus*, Ethyl acetate, Column chromatography, NMR.

ABSTRACT

Sonchus oleraceus is a wild herb that grow after the rainy season in Sudan. Chemical analysis of the herb led to the isolation of two antioxidant compounds. The ethyl acetate extract showed 89% RSA with DPPH (1,1-Diphenyl-2-picrylhydrazyl). 30 gms of the ethyl acetate extract were separated into 12 -fractions with column chromatography. Purification of fraction 1 led to isolation and identification of two compounds. The solvent mixture for fraction one separation was (chloroform: ethyl acetate) 40:60. Purification solvent mixture was (toluene: ethylacetate: formic acid) 9:1:1.The two isolated compounds were a lignan and a chalcone. Their structures were elucidated with NMR (nuclear magnetic resonance) technique.

Citation: Obeid, H. A., Saeed, A. E. M. and Khalid, H. S. 2018. "Isolation and structure elucidation of two antioxidant compounds from sudanese *Sonchus oleraceous* plant", *International Journal of Current Research*, 10, (01), 63834-63837.

INTRODUCTION

The aim of the present study is to: 1- investigate the antioxidant activity of the ethyl acetate fraction which contains flavonoids glycosides. 2-Isolation and purification of active compounds. 3-Structure elucidation of the active isolated compounds using IR (infra-red) and NMR spectroscopic techniques. Antioxidants prevent oxidative damage to lipids, proteins, enzymes and DNA that occurs when there is excess of free radicals and other reactive oxygen species (ROS) in the body (Ratnam *et al*., 2006). The genus *Sonchus* contains more than 50 species and a study of six wild *Sonchus* species showed that *S.oleraceus* possessed the highest in-vitro antioxidant activity. *S. oleraceus*leaf extracts exhibited four times the antioxidant activity of blueberry extracts, a fruit known for its high antioxidant activity (Zong *et al*., 2012). Puha (*Sonchus oleraceus L*.) is a rich source of polyphenols, and exhibits strong antioxidant activity as measured by the 2,2-diphenylpicrylhydrazyl (DPPH) assay. However, the potential of puha to protect against degenerative diseases requires that low molecular weight antioxidants (LMWA) are absorbed and active in human cells (Arlene *et al*., 2011). Plants generally contain antioxidants including Flavonoids, terpenoids and phenolic acids and have been used as traditional herb remedies in many cultures (Arlene *et al*., 2011). The genus *Sonchus* contains more than 50 species. A study of six wild sonchus species showed that *S. oleraceus* contained the highest in vitro antioxidant activity (Jie Yin *et al*., 2007). Twelve active fractions were isolated from the ethyl acetate extract, using column chromatography. The activities of flavonoids and their glycosides were evaluated with 1,1-Diphenyl-2-picrylhydrazyl. Lignans are produced by oxidative dimerisation of two phenylpropane units; they are mostly present in nature in the free form. The interest in lignans and their synthetic derivatives is growing because of potential applications in cancer chemotherapy and various other pharmacological effects (IoanaIgnat *et al*., 2011). Chalcone is a generic term given to compounds bearing the 1, 3-diphenyl-2-propen-1-one framework and belong to the flavonoid family (Maayan *et al*., 2005). Chemically they are open-chain flavonoids in which the two aromatic rings are joined by a three carbonα, β-unsaturated carbonyl system. Chalcones are abundantly present in nature starting from ferns to higher plants and a number of them are polyhydroxylated in the aryl rings (Cushman and Nagarathnamm, 1991).

In this research two compounds were isolated from fraction 1. These compounds are:

Eusiderin and a Chalcone.

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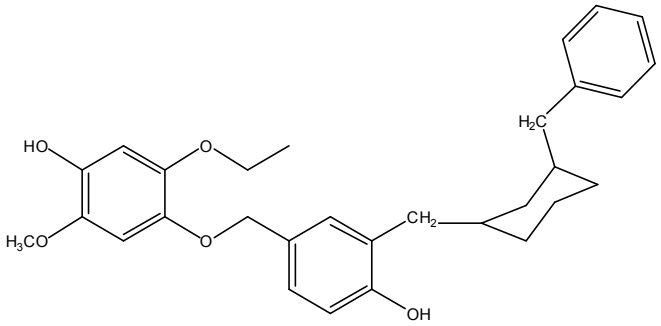

Figure 1. Eusiderin (neolignan)

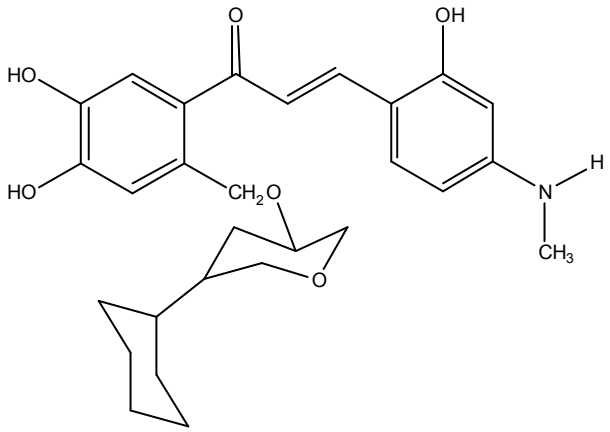

Figure 2. Chalcone

MATERIALS AND METHODS

H^1, 2D proton NMR and ^{13}C NMR spectra were recorded with 700 MHz Brucker Advance by using tetramethylsilane (TMS) as an internal standard for samples dissolved in CD_3OD (deuterated methanol). The chemical shifts (δ) were reported in ppm relative to TMS = 0. Infra- red (IR) spectra were recorded on Shimadzu spectrometer. All TLC and PTLC were performed on flurescent Silica gel GF 254.The TLC bands were visualized under UV lamp.

Preparation of plant crude extracts

3 kgs of the coarse plant powder was extracted by soaking in dichloro methane for seventy two hours with daily filtration and evaporation. Solvent was evaporated under reduced pressure to dryness using rotary evaporator apparatus. The plant residue was left to dry. When dried was re-extracted with methanol for five days with continuous filtration and evaporation of solvent. The collected extract was left to dry in glass sample bottles (Hilmi *et al*., 2014).

Ethyl acetate Extract

250 gms methanolic crude extract were dissolved in 500 ml distilled water then shacked three times successively with 500 ml ethyl acetate solvent using a separatory funnel. Ethyl acetate layers were combined together and evaporated under reduced pressure using rotary evaporator apparatus. Successive extraction was done for three days.

Column chromatography

30 gms of dry ethyl acetate fraction were introduced into column chromatography. Eluent solvents from lowest polarity of petroleum ether and increasing polarity with solvent mixtures up to methanol as most polar. Solvents used were petroleum ether, chloroform, ethyl acetate and methanol. 12 column fractions had radical scavenging activities with DPPH. Fr.1 (1.2 gm) (chloroform /ethyl act.) 4**:**6. Fr.2 (1.4gms) (chlorm/eth.cet.) 3:7. Further purification of these two fractions was done on preparative TLC plates.

Antioxidant assay for DPPH free radical scavenging activity

The DPPH radical scavenging was determined according to the modified method of (Shimada *et al*.) in 96-wells plate, the test samples were allowed to react with 1,1-Diphenyl-2-picryl-hydrazyl stable free (radical (DPPH) for half an hour at 37°C. The concentration of DPPH was kept as 300 μM. The test samples were dissolved in DMSO (dimethyl sulfoxide reagent) while DPPH was prepared in ethanol.

After 30 min. incubation, decrease in absorbance was measured at 517 nm using multiple reader UV-spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group.All the tests and analysis were run in triplicates (Shimada and Fujikawa, 1992).

RESULTS

The radical scavenging activity started at 69% when mobile phase was chloroform: ethylacetate (40:60). The activity increased to a maximum of 90% when the same solvent mixture was in the ratio 20:80. The activity showed another increase when methanol was introduced with ethyl acetate in the ratio 30:70.

Table 1. Column chromatography and radical scavenging activity

Fraction number	Eluent (mobile phase)	Weight of fraction	%RSA+SD (DPPH)	TlC solvent system
F1	Chloroform :Ethyl acetate 40 :60	1.199	69+-0.08	Toluene:Ethyl acetate:formic acid 9 :1 :1
F2	30:70	0.640	88+_0.04	8 : 2 : 1
F3	20:80	0.880	90+-0.01	
F4	10:90	0.444	89+-0.00	
F5	100%eth acet	0.152	77+-0.03	
F6	Methanol:Ethyl acetate. 10: 90	0.076	69+-0.08	
F7	20:80	0.463	82+-0.10	Tol. Eth.Ac. Meth 80. 20.20
F8	30:70.	2.931	86+-0.02	
F9	40:60	2.401	74+-0.08	60:40:10 ml
F10	50:50	2.100	57+-0.04	
F11	60meth:40eth.acet	0.838	70+-0.03	50tol:40meth:10formic
F12	70:30	0.776	90+-0.05	

DISCUSSION OF RESULTS

Eusiderin (neolignan)

The IR spectrum indicated the presence of hydroxyl group (3411 cm^{-1}), the presence of aromatic group (1598 cm^{-1}), and the presence of ether aryl bonds (1388 cm^{-1}). The proton NMR analysis for the same compound indicated two symmetrical aromatic chemical shifts at δ 7.7, 6.5 singlet indicating a phenol with no chemical shifts.

Table 2. IR functional group identification of compound brown-green - amorphous solid. (Eusiderin)

IR frequencies (cm^{-1})	Indication
3411	Broad stretch of –OH- group
2935	-CH- stretch
1598	Strong aromatic bending
1388	Aryl ether bending

Table 3. Proton NMR of Eusiderin in CD_3OD solvent

^1H (p.p.m.) δ	Explanation
7.7-7.6	Two symmetrical aromatic chemical shifts substitution in meta position, coupling (J 0.1 x700 Hz).
7.4	A proton chemical shift of –OH
6.9	Proton chemical shifts (singlet no neighbouring protons) is characteristic for Ar--O.
6.5	chemical shift is singlet and characteristic for a phenol
4.5	chemical shift is for Ar-O- R
3.6	characteristic of sugars $–CH_2OH$ units
2.5	is a a proton chemical shift for Ar-R

Table 4. ^{13}C of Eusiderin in CD_3OD solvent

Position	^{13}C (p.p.m.) δ	Explanation
C2	136	Etheritied aromatic carbon carbon
C4	132	Ar-OH
C5	129	Ar-OCH3
C7’	128	Aromatic carbon
C3	125	Aromatic carbon
C4’	121	Ar-CH2-O
C2’’	76	CH2-OH of sugar
C3’’	74	CH2-OH
C6’’	33	O-R
C5’’	14	Aliphatic carbon

Table 5. IR functional group identification of dark- brown-amorphous solid (Chalcone)

IR frequencies cm^{-1}	Indication
3388	-OH- stretch
2831	-CH- stretch
1728	-C=O- carbonyl stretch
1596	Aromatic –C=C- stretch
1112.8	Aryl $–O–CH_2$ bending

Table 6. Proton NMR of- 3’, 4’, 2’’, trihydroxy-2-methoxy glycoside, 4’’’ methylamine chalcone in CD_3OD solvent

^1H (p.p.m.) δ	Explanation
8.5	Singlet (1 H) of an amine meta substituted on an aromatic ring (J 2.8).
7.7	Protons affected by a fused ring
6.8	Protons with respect to carbonyl appear as two douplets
6.7-6.5	Sharp singlets phenol
5.5-5.3	Protons affected by a double bonds shifts region.
3.5	CH2-OH of a glucose unit
1.5	Chemical shift of a proton on a cyclohexane.

Table 7. ^{13}C of- 3’, 4’, 2’’, trihydroxy-2-methoxy glycoside, 4’’’ methylamine chalcone in CD_3OD solvent

Position	^{13}C (p.p.m.) δ	Explanation
C4’	139	AR-NH-
C4-C5	129	Ar-OH
C1’’	128	Carbonyl carbon
C3’	125	Aromatic carbon
C2’’	114	Double bond
C3’’’	70-60	CH_2-OH
C1’’’	35-30	CH_2-O-CH_2

4.3 triplet for Ar-CH3-O.ortho to –OH (J=15). 4.5 sharp signal for Ar-O (J=11, orth substituted). ^{13}C NMR indicated chemical shifts at 136 for aromatic –ether correlation.δ132 presence of a phenol. 129 shift indicates Ar-OCH_2.

3’, 4’, 2’’, trihydroxy-2-methoxy glycoside, 4’’’ methylamine chalcone

The IR spectrumfor the chalcone indicated the presence of hydroxyl group (3388 cm^{-1}), the presence of –CH- stretch (2831cm^{-1}), the presence of a carbonyl group (1427 cm^{-1}), the presence of aromatic electrons (1596 cm^{-1}) and the presence of aryl-O-C-bending (112.8 cm^{-1}). Proton NMR, 8.5δ singlet meta substituted (J 2.8). 7.7-6.7 protons, with respect to carbonyl group appear as two doublets with large J values (J=18). 6.8, 6.7. A chemical shift 6.5, sharp singlets (no neighbouring protons), is a chemical shift of -OH- on aromatic ring.(meta substituted J=3 Hz and para 6.7_H). ^{13}C NMR, 139 chemical shift at C4” of Ar-NH-.Chemical shift at 129 at C4 for ArOH. δ115 at C2” for double bonds. α and β protons of chalcones appear as two douplets with large J value ranging 6.4 -7.5 ppm. 13C values 116- 128 for α carbon and 139-145 for β carbons with respect to carbonyl (Sheik Khadar *et al*., 2015).

Conclusion

Purification, isolation and spectroscopic analysis of the active fraction 1 from ethyl acetate extract, led to the isolation of two compounds.

1) Eupisidinin lignan.
2) 3’, 4’, 2’’, trihydroxy-2-methoxy glycoside, 4’’’ methylamine chalcone.

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