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RESEARCH ARTICLE

ISOLATION AND STRUCTURE ELUCIDATION OF A FLAVONE AND TANNIC ACID FROM SUDANESE *SONCHUS OLERACEUS* PLANT

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ABSTRACT

Sonchus oleraceus (moleita) from the family Asteracae was investigated for its antioxidants activities. The ethyl acetate extract of the plant showed 89% radical scavenging activity (RSA). The extract was subjected to column chromatography. Solvent mixtures were used from least to highest polarities (petroleum ether till methanol). 12 fractions showed high antioxidant activity. Radical scavenging activity was tested with DPPH (1,1-Diphenyl-2-picrylhydrazyl). The structures were identified with modern spectrum techniques as 1H NMR, ^{13}CNMR, 2D proton NMR(cosy),HSQC (heteronuclear single quantum correlation) and IR frequencies. Compound (1) is 7-hydroxy, 6 –C-glycoside, 3' hydroxyl pyridine-flavon (0.530 gm). Solvent system for isolation was (Tol: Eth.Acet: Formic Acid). Compound (2) is tannic acid dimer (0.11 gm). Solvent system for isolation was Ethyl Acetate: Methanol (4:6).

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INTRODUCTION

A common feature among the different ROS types is their capacity to cause oxidative damage to proteins, DNA, and lipids. These cytotoxic properties of ROS explain the evolution of complex arrays of nonenzymatic and enzymatic detoxification mechanisms. ROS are continuously produced as by-products of various metabolic pathways localized in different cellular compartments. Under physiological steady state conditions these molecules are scavenged by different antioxidative defense components that are often confined to particular compartments. Nonenzymatic antioxidants include the major cellular redox buffers ascorbate and glutathione (GSH), as well as tocopherol, flavonoids, alkaloids, and carotenoids (Klaus and Heribert, 2004). An antioxidant is a molecule stable enough to donate an electron to a free radical and neutralize it. Antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property (Halliwell, 1995). Polyphenols are considered to be the most effective antioxidants, they can also intensify the activity of other antioxidants soluble in lipids vitamins, and also vitamin C (Elbieta *et al.,* 2008).

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Tannins are high molecular polyphenolics, they are subdivided into hydrolysable and condensed tannins. Condensed tannins are polymeric flavonoids. Hydrolysable tannins are derivatives of gallic acid (3,4,5 trihydroxylbenzoic acid). Flavonoids and their conjugates form a very large group of natural products. They are found in many plant tissues, where they are present inside the cells or on the surfaces of different plant organs. The chemical structures of this class of compounds are based on a C6-C3-C6 skeleton. They differ in the saturation of the heteroatomic ring C, in the placement of the aromatic ring B at the positions C-2 or C-3 of ring C, and in the overall hydroxylation patterns. The flavonoids may be modified by hydroxylation, methoxylation, or *O*-glycosylation of hydroxyl groups as well as *C*-glycosylation directly to carbon atom of the flavonoid skeleton (Erich Grotewold.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).

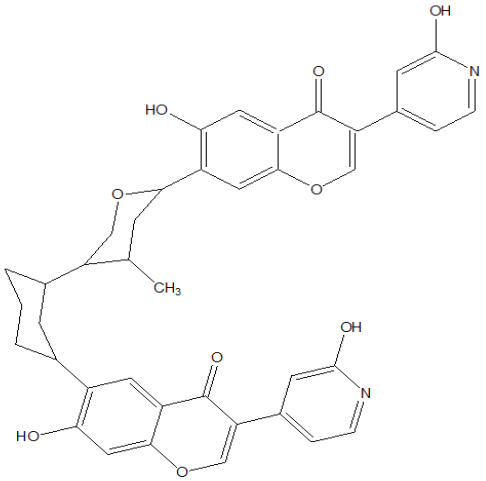

Fig. 1.1. Flavone

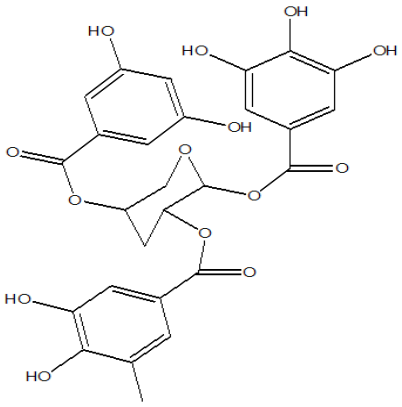

Fig. 1.2. Tannic acid

and evaporation of solvent. The collected extract was left to dry in glass sample bottles. (Hilmi *et al*, 2014).

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 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 K, Fujikawa, 1992).

Ethyl acetate extract

250 gms methanolic crude extract were dissolved in 500 ml distilled water and 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 ethyl acetate extract

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.

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	

MATERIALS AND METHODS

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 from the dicloromethane. When plant dried was re-extracted with methanol for five days with continuous filtration

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 3.2. IR functional group identification of the flavone compound

IR frequency(cm^{-1})	Indication
3360	Medium N-H- stretch
2929	CH- stretch
1714	Carbonyl stretch
1602	Double bond and aromatic resonance stretch.

Table 3.3. Proton NMR of flavone in CD_3OD as a solvent

^1H (ppm) δ	Indication
8.5	proton shift next to N in pyridine ring C3
8.1	single shift next to electron rich centre
7.7	is proton shift in C5 adjacent to the fused ring.
6.9	proton shift in C8 singlet (no-neighboring protons)
6.5	Proton shift of a phenol
6.2-6.0	Ortho-substituted protons from a phenol
5.8	is a chemical shift of ph-O-Ar
5.3	a proton in conjugated double bond (singlet)
2.8	is methylene proton between phenyl group and a sugar molecule.

Table 3.4. 2D proton NMR (cosy) of flavones

2D proton shifts	Indication
8↔6	-OH- in the pyridine ring
7.7↔6	-OH- in fused ring
5.5↔2.8	$-CH_2-$ correlated to –C=C-
1.9↔1.5	methyl group proton in a hexyl glucose molecule

Table 3.5. ^{13}C NMR and HSQC chemical shifts of flavone in CD_3OD as a solvent

position	13C (ppm) δ	Indication	HSQC shifts	Indication
C3''	145	Downfield shift due to phenol	130↔5.5	double bonds correlated with aromatic ring
C5''	130	Aromatic carbon next to N atom	110↔6.1 Singlet	C-OH in a conjugating system and no neighbouring protons.
C6	129	Aromatic carbon	100↔6.8	C-OH in a conjugating system next to N atom
C2'	112	Bouble bond	30↔1.9	CH_3 proton next to OH group of a sugar
C3'	103	Ring C. -C-O-carbon		
	30-25	CH_2 of the sugar units.		

Compound 2 Tannic acid

Table 3.6 Infra red frequencies (cm^{-1}) of tannic acid in CD_3OD as a solvent

IR frequency cm^{-1}	Indication
3436	Phenol stretch band (broad)
1588	Aromatic stretch
1415	Carboxylate ion bending

Table3.7 Proton NMR of chemical shifts of the tannic acid in CD_3OD

Proton NMR(ppm)δ	Indication
7.8	chemical shifts of resonating aromatic rings. (1 H on to ring
7.4	2H j is small ,substitution is in meta position
7.2---6.5	sharp characteristic signals representing phenolic structures
6.2	identical douplet indicating –COOH-CH attachment of carboxylate to sugar
3.9	is proton chemical shifts of –CH2-O .

Table 3.8. 2D proton NMR of Tannic acid

2D proton NMR chemical shifts	indication
3.6------3.8	Proton shifts of two adiacent protons to OH groups
3.5------1.5	OH in cyclohexane of sugar

Table 3.9 ^{13}C NMR chemical shifts of Tannic acid

^{13}C chemical shifts ppm	indication
180	Carbon shift of carboxylic acid
146	Aromatic carbons
145	Aromatic carbons
116	Conjugating carbon of carboxyl
105	Conjugation in double bond area
75	C—OH in sugar molecule

DISCUSSION OF RESULTS

7-hydroxy, 6 –C-glycoside, 3' hydroxyl pyridine-flavone: The high molecular weight of the compound -7-hydroxy,6 –C-glycoside ,3' hydroxyl pyridine-flavone (652m/z) indicates repeated units of the monomer. The infra red frequencies of the isolated flavone indicated the presence of medium N-H stretch (3360 cm^{-1}),-CH-stretch (2929), carbonyl group stretch band (1714) and bouble bonds and aromatic resonance (1602). Proton NMR for the flavones were wide signal at 8.5 ppm for a proton meta to N atom (J=2) .Sharp single signal at 8.1 next to an electron rich centre.7.7ppm signal for fused rings.6.5 and 6.7 are signals of meta substituted phenols. 6.2, 6.0 singlets are protons ortho to OH (J=9).5.3 signal at double bond area.5.8 doublet of doublet Ar-O-CH- proton in a conjugating double bond area. 2D NMR chemical shifts for the isolated flavones indicated proton correlation between H and N at 8 and 6 chemical shifts. Sharp splits when CH_2 correlated to C=C and CH-Ar bonding at (5.5and 2.8) ppmThe correlation between 1.9 and 1.5 shifts indicates methyl proton in hexyl sugar molecule. ^{13}C NMR for the isolated flavones is 145 of phenol carbon. 130 ppm is a shift for aromatic carbon.112 ppm double bond carbon shift and 103 is –C-O- on ring C.

Lastly for HSQC 130↔5.5 double bonds correlated with aromatic ring.
110↔6.1 is a correlation of C-OH in a conjugating system .
100---6.8 carbon in resonating area and a proton downfield due to N atom.

The 1H-NMR spectrum from cited research of the isolated flavone showed signals at chemical shifts of aromatic region δ 6-8 ppm. The 1H-NMR spectrum of the isolated compound displayed two meta-coupled doublets at δ 6.4ppm (d, J = 2.0 Hz, 1H) and δ 6.21 ppm (d, J = 2.0 Hz, 1H) respectively for aromatic protons H-6 and H-8 on the ring A. The position of H-6 and H-8 are meta to each other. This is evidenced by the coupling constant values (J) 2.0 Hz, which is Jmeta 1-3 Hz This also proves the ring A contain two substituents at position C-5 and C-7. Two doublet signals at δ 6.92 ppm (d, J = 9.1 Hz, 1H) and δ 7.86 ppm (d, J = 9.1 Hz, 1H) showed the orientation of the ortho proton signal for the proton H-3', 5' and H-2', 6' ring B with the substituents at position C-4'. A singlet signal at δ 6.61 ppm (1H, s) showed proton H-3 in ring C has isolated proton. The 1H-NMR spectrum exhibited proton signals characteristic of flavone nucleus. (Sovia Lenny *et al*., 2013). Infra red frequencies of the isolated tannic acid dimer indicated a broad phenolic OH stretch at 3434 cm^{-1}. An aromatic stretch at 1588cm^{-1}.A carboxylate bending at 1415 cm^{-1} Proton NMR showed a chemical shift at 7.8 of resonating aromatic rings. 7.4 (2H j is small substitution is in meta position).

There are sharp symmetrical signals at, 7.2, 7.0, 6.8, 6.6 and 6.5 representing phenolic structures .There is identical duplet at 6.2 indicating –COOH-CH_2,attachment of carboxylate to sugar structureAlso there is a proton shift at.3.9 indicating –CH_2-O. Concerning 2D NMR combination of proton chemical shifts: 1.5↔3.5 is (hydroxyl in cyclohexane i.e. sugar molecule). Correlation of 3.6 with 3.8 (proton shifts of two adjacent protons with –OH- groups). For HSQC correlation indication of 5.2 proton shift with 105 carbon shift (5.2↔105) of conjugated double bonds.

Conclusion

Purification, isolation and spectroscopic analysis of the EtOAc. Active fractions, led to the isolation of two compounds 7-hydroxy, 6 –C-glycoside, 3' hydroxyl pyridine-flavone. And Tannic acid Dimer.

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