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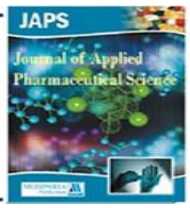

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Phytochemical investigation of *Vernonia cinerea* (Family: Asteraceae)

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ABSTRACT

Phytochemical screening of *Vernonia cinerea* (Family: Asteraceae) showed the presence of steroids, glycosides, triterpinoids & esters in the methanolic extract of stem bark and leaves of the plant. The presence of these compounds clearly indicates different medicinal properties of *V. cinerea*. NMR data also confirmed the presence of Lupeol, 12-oleanen-3-ol-3ß-acetate, Stigmasterol, ß-sitosterol in n-hexane portion.

Keywords: Phytochemical screening, plant extracts, NMR

INTRODUCTION

Vernonia cinerea (Family: Asteraceae) is a terrestrial annual erect herb. It grows up to 80 cm high. It can be found in roadside, open waste places, dry grassy sites and in perennial crops during plantation. It is located especially in different Asian countries such as India, Bangladesh and Nepal. Stems are rounded solid hairy. Leaves are alternate spiral, elliptic and the length is more than 2 cm long/wide. Flowers are bisexual grouped together in a terminal head (Gani, 2003).

V. cinerea is an important medicinal plant having application in abortion, cancer and various gastrointestinal disorders (Yusuf *et al.,* 1994). Toxicity study of the plant on mice was carried out but the results were inadequate for definite conclusion (Latha *et al.,* 2010). Chloroform extract of stem-bark and leaves of *Vernonia cinerea* showed diuresis property but methanolic extract exhibited antidiuresis (Adeboye *et al.,* 1997). Both polar and non-polar fraction of the plant extract showed analgesic, antipyretic and anti inflammatory effect (Iwalewa *et al.,* 2003). Polar extract of *V. cinerea* is found to have antidiarrhoeal activity (Ganesh *et al.,* 2011) but there is no study on non-polar fraction. Antibacterial (Rizvi *et al.,* 2011) and anti larval activity against filarial vector (Arivoli *et al.,* 2011) was reported but no information regarding antifungal and antiprotozoal activity is found. Carbon tetrachloride fraction of methanolic extract possesses significant antioxidant properties (Kumar and Kuttan *et al.,* 2009) but whether this plant extract could affect anticholinesterase and thus finally be used for treating Alzheimer disease because of antioxidant property is not reported.

Therefore, the present study was designed to investigate the phytochemical bioactive compounds of the methanolic extract of *V. cinerea* and its antioxidative, anticholinesterage property. Finally antidiarrhoeal property and antidiabetic activity of non-polar carbon tetrachloride fraction of methanolic extract were studied.

MATERIAL AND METHODS

Collection and Identification of plant materials

Fresh plant of *Vernonia cinerea* was collected from Gabtoli, Dhaka, Bangladesh in October, 2007. This plant was identified by Bangladesh National Herbarium. The reference sample for the plant was DACB Accession Number 32126.

Preparation of plant extract

The stem-bark and leaves were sun dried for 5 days. The plant materials were then oven dried for 24 hours at low temperature. 960 gm of powdered material (Stem-bark and leaves) was macerated with 7.5 L of methanol in two 4 L round bottom flask. The containers were sealed with cotton plug and aluminum foil at room temperature for 15 days with occasional shaking. The mixture was filtered through cotton and then evaporated to dryness (45^0C) under reduced pressure by rotary evaporator. The obtained crude extract was 49.54 grams. The percentage yield of the extract was calculated by using the formula below:

% yield= (weight of extract/weight of plant material) ×100%

15 gm of methanolic extract was triturated with 270 ml of methanol containing 30 ml distilled water. The crude extract was dissolved completely. It was mother solution. This solution was partitioned successfully by four solvents of different polarity. The mother solution was taken in a separating funnel. 100 ml of n-hexane was added here and the funnel was shaken and kept undisturbed. Then the organic portion was collected and repeated thrice. Carbon tetrachloride (CCl_4) and dichloromethane (CH_2Cl_2) extract was collected with the help of aqueous mother fraction adding 38 ml and 48 ml distilled water respectively keeping the other procedure unchanged. Finally *n*-hexane, CCl_4, CH_2Cl_2 and aqueous extract were obtained.

Phytochemical Screening

For preliminary phytochemical analysis the freshly prepared crude methanolic extract was tested for the presence or absence of reducing sugar, tannins, flavonoids, saponins, gums, steroids and alkaloids by using standard phytochemical procedures according to Trease and Evans (2002). Different solvent systems were used in sephadex column.

Detection of chemical compounds by NMR spectroscopy

For isolation of different types of compounds *n*-hexane soluble fraction was subjected to TLC screening. This revealed a considerable number of compounds and required further fractionation. Sephadex was soaked in a mixed solvent of *n*-hexane:Dichloromethane:Methanol at the ration of 2:5:1 for swelling. Then slurry of extract was added into glass column. The previous solvent mixture was used as initial mobile phase. The column was eluted with the same solvent mixture and finally washed with dichloromethane and methanol mixture with increasing polarity. Different fractions were collected in 28 test tubes that are rendered for evaporation to dryness. 1 to 14, 15 to 22, 23 to 26, 27 to 28 test tubes had solvent systems n-hexane: Dichloromethane: Methanol (20:50:10), Dichloromethane: Methanol (90:10), Dichloromethane: Methanol (50:50) and methanol (100%) respectively. These column fractions were screened by TLC under UV light after spraying with vanillin-sulfuric acid reagent. Fractions having significant result were selected for further investigations with small column and suitable solvent systems. Crystal found in the end was analyzed by NMR for detection of isolated compound. ^{1}H NMR spectra were recorded using a Bruker AMX-400 (400 MHz) instrument and the spectra were referenced to the residual non-deuterated solvent ($CDCl_3$) signals. Column chromatography (CC) was conducted over (Merck) (Germany) sephadex (LH-20). Spot on TLC plates were visualized under UV light (254 and 366 nm) after spraying with vanillin-sulfuric acid, followed by heating at 110^oC for 5-10 minutes.

RESULTS

Plant extraction

The yield of the methanolic extract of stem-bark and leaves of *V. cinerea* was 5.16 % (w/w) dry matter having coffee color.

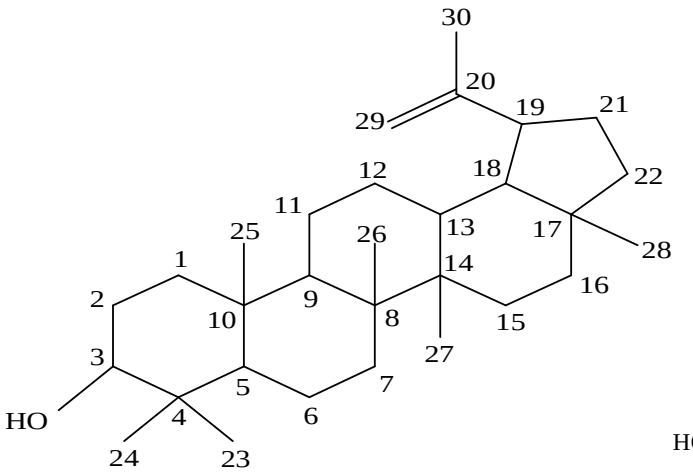
Phytochemical screening

Phytochemical screening of the stem-bark and leaves extracts of *V. cinerea* confirmed the presence of triterpinoids, glycosides, steroids, ester which are presented in Table 1.

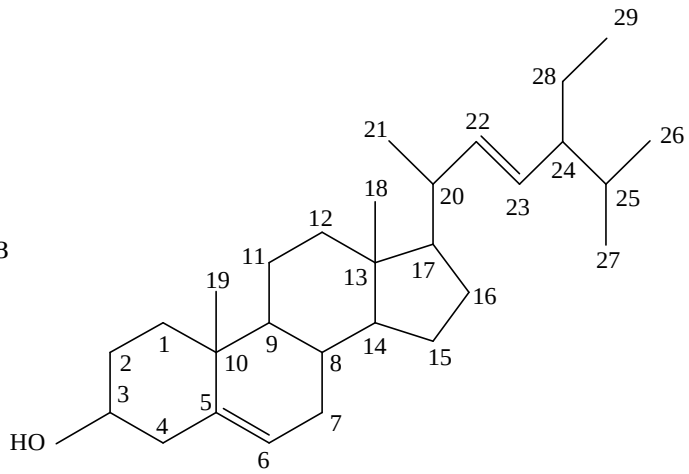
Chemical Compound

Due to significant result in test tube 6, 7, 8 and 9 these were selected for further investigation. With the help of a small column and mobile phase consisting of ethyl acetate and hexane in 14:86 ratios the column elution was separated in 22 beakers. These were kept at room temperature covered with aluminum foil to dryness. After 4 days, white crystals were found in different beakers. These crystals were then analyzed by NMR. Different fractions contained different compounds which are presented in Table 2. The structure of the compounds found after analyzing in NMR is presented in Figure 1.

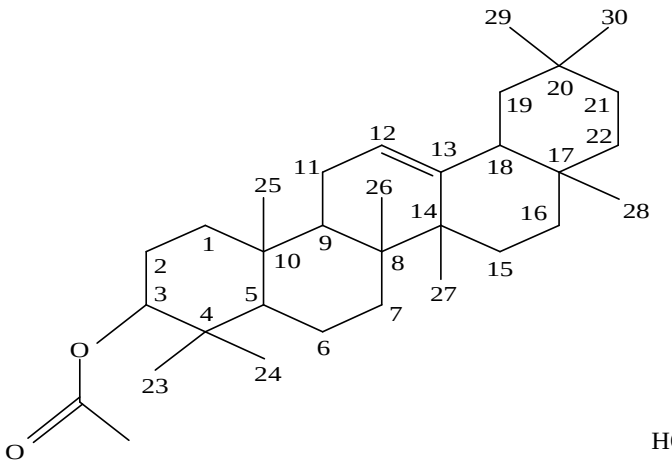
^{1}H NMR spectrum of compound 1 showed a double doublet (J = 11.5, 5.03 Hz) of one proton intensity at δ 3.21 ppm, typical of an oxymethine proton at C-3 of a triterpene. The splitting pattern of this proton confirmed the ß orientation of the C-3 oxygenated substituent. The spectrum also displayed two singlets at δ 4.68 and 4.56 ppm (^{1}H each) assignable to the vinylic protons at C-29. The ^{1}H NMR spectrum showed seven singlets at δ 0.95, 0.79, 0.83, 1.02, 0.93, 0.799 and 1.68 ppm (3H each) assignable to methyl group protons at C-4 (H_3-23, H_3-24), C-10 (H_3-25), C-8 (H_3-26), C-14 (H_3-27), C-17 (H_3-28) and C-20 (H_3-30),



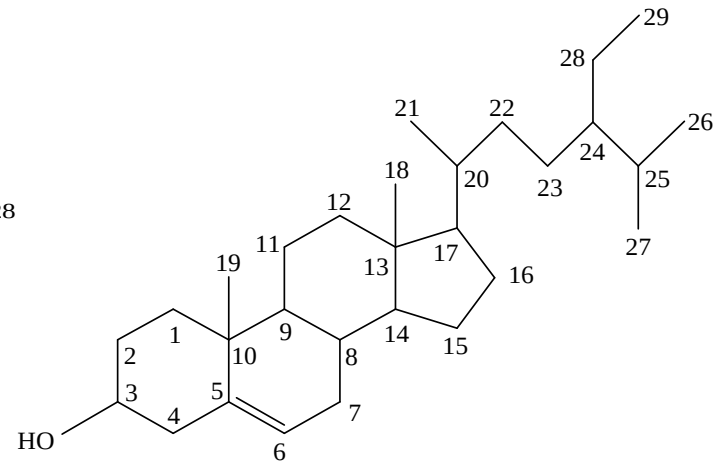
Lupeol



Stigmasterol



12-Oleanen-3-ol-3ß acetate



ß-sitosterol

respectively. By comparing the ^{1}H NMR data with previously published data (Aratanechemuge *et al.*, 2004), compound 1 was identified as lupeol. The identity of 1 was further substantiated by co-TLC with an authentic sample of lupeol.

The ^{1}H NMR spectra of compounds 2 and 3 readily demonstrated the steroidal nature of these compounds. The spectral data of compounds 2 and 3 were super imposable to the ^{1}H NMR spectral data published for ß-sitosterol (Morales *et al.*, 2003) and stigmasterol (Kolak *et al.*, 2005). Additionally, thin layer chromatographic analysis of 2 and 3 with authentic samples of ß-sitosterol and stigmasterol, respectively, also confirmed their identity.

^{1}H NMR spectrum of compound 4 displayed a proton broad singlet at δ 5.18 which indicates the presence of olefinic proton. Eight singlets each of three proton intensity at 1.12, 1.06, 1.02, 1.00, 0.97, 0.93, 0.87, 0.84 ppm (3H each) assignable to methyl group protons at C-4 (H_3-23, H_3-24), C-10 (H_3-25), C-8 (H_3-26), C-14 (H_3-27), C-17 (H_3-28) and C-20 (H_3-29, H_3-30) respectively. By comparing the ^{1}H NMR data with previously published data (Krishnaswamy *et al.*, 1975) and (Chiu *et al.*, 2008) compound 4 was identified as 12-oleanen-3-ol-3ß-acetate. Spectral analysis is shown in Table 3.

DISCUSSION

Phytochemical screening of the plant extract confirmed the presence of several bioactive compounds like glycosides, triterpinoids, esters which could be responsible for the versatile medicinal properties of this plant. NMR data indicated the presence of Lupeol, 12-oleanen-3-ol-3ß-acetate, Stigmasterol, ß-sitosterol in n-hexane portion. In order to study the effects of these compounds on biological system needs more studies as these compounds might be responsible for use of this plant in different diseases (Panda *et al.*, 2009).

Table 1: Phytochemical screening of the stem-bark and leaves extracts of *V. cinerea.*

S/L No.	Chemical Constituents	Test	Extract	Result
1.	Test for Reducing Sugar	Benedict's Test	MEVC	-
		Fehling's Test	MEVC	-
		Alpha Napthol Solution Test	MEVC	-
2.	Test for Tannins	Ferric Chloride Test	MEVC	-
		Potassium dichromate Test	MEVC	-
3.	Test for Flavonoids	Hydrochloric Acid Test	MEVC	-
4.	Test for Saponins	Foam Test	MEVC	-
5.	Test for Gums	Molisch Test	MEVC	-
6.	Test for Steroids	Libermann-Burchard Test	MEVC	+
		Sulphuric acid Test	MEVC	+
7.	Test for Alkaloids	Mayer's Test	MEVC	-
		Wagner's Test	MEVC	-
		Dragendroff's Test	MEVC	-
		Hager's Test	MEVC	-
8.	Test for Glycosides	Anthroquinone test Keller-killani test	MEVC	+
9.	Test for triterpinoids	Liebermann-Burchard test	MEVC	+
10.	Test for esters	Zeisel determination test	MEVC	+

+ Present, - Absent, MEVC: Methanolic extract of stem-bark and leaves of *V. cinerea*.

Table. 2: Name of the Compounds in different Solvent System.

Fraction no.	Developing solvent system	Name of compounds
11-12	Ethyl acetate: Hexane = 14:86	12-oleanen-3-ol-3ß-acetate
16-17	Ethyl acetate: Hexane = 14:86	Lupeol,12-oleanen-3-ol
18-19	Ethyl acetate: Hexane = 14:86	Stigmasterol
20	Ethyl acetate: Hexane = 14:86	Stigmasterol
21-22	Ethyl acetate: Hexane = 14:86	Stigmasterol, ß-sitosterol
Rest of 16	Ethyl acetate: Hexane = 14:86	Lupeol

Table. 3: Physicochemical and ^{1}H NMR spectral data of lupeol (1), *ß*-sitosterol (2), stigmasterol (3) and 12-oleanen-3-ol-3 *ß*-acetate (4) in $CDCl_3$

	1 (lupeol)	2 (*ß*-sitosterol)	3(Stigmasterol)	4(12-oleanen-3ol-3 *ß*-acetate)
Physical appearance	**Colorless crystalline mass**	**Amorphous powder**	**Colorless niddles**	
Proton position		**^{1}H NMR mult δ (ppm), *J* (Hz)**		
2				
3	3.21, dd (11.5, 5.03)	3.51, m	3.51, m	4.46, m
6		5.35, m (7.0)	5.35, m	
18		0.67, s	0.67, s	
19	2.38, m	1.01, s	1.01, s	
21		0.91, d (6.4)	0.92, d (6.0)	
22			5.14, dd (15.0, 6.5)	
23	0.95, s		5.35, dd (15.0, 9.0)	1.12, s
24	0.79, s			1.06, s
25	0.83, s			1.02, s
26	1.02, s	0.83, d (6.0)	0.84, d (6.0)	1.00, s
27	0.93, s	0.80, d (6.0)	0.82, d (6.0)	0.97, s
28	0.799, s			0.93, s
29	4.68, br. s	0.85, d (6.0)	0.82, t (6.5)	0.87, s
	4.56, br. s			
30	1.68, s			0.84, s

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