2249-5746
International Journal Of Ayurvedic And Herbal Medicine 3:3 (2013)1173:1183

Journal Homepage http://interscience.org.uk/index.php/ijahm

ISOLATION OF ACTIVE COMPONENTS DERIVED FROM RHIZOME OF EUPHORBIA WALLICHII HOOK

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Abstract

The objective of the present investigation was to isolate the active components present in rhizome of euphorbia wallechii. The plant material were extracted with various solvents (pet. ether, methanol and water), water was found to be more active among them. Preliminary phytochemical investigation of the aqueous extract of the rhizome of euphorbia wallechii showed that the saponins, steroids, and triterpenoids were found more active among the active constituents. Whereas flavonoids, Cardiac Glycosides showed the less positive nature. The aqueas water extract of Euphorbia wallichii was undergone column chromatography with different solvent fractions. Fraction E which was obtained after purification as a cream color powdered material with the elution of 10 % ethyl acetate in hexane: chloroform and methanol: water from the silica gel column loaded methanol soluble fraction of the plant. It was noticed that melting point apparatus measured 210-212 °C. Hence, it was concluded that fraction E was found very close to the multiflorenol (multiflorane) class based up on the spectral analysis the compound was named as Pentacyclic tri-terpenoid saponin. The structure of the isolated compound was characterized by using IR, CNMR, HNMR and Mass spectrophotometric methods. Therefore, further biological investigations needs to be carried out on isolated compounds present in this plant.

Introduction

The plants have been used as medicines since the beginning of human civilizations (Hill, 1952) and have been a source of treatment of the common day ailments. The Plant Euphorbia Wallichii belongs to family Euphorbiaceae. It grows on higher altitudes and is traditionally used for respiratory troubles, skin infections and is anti-inflammatory in action. . Three flavonols namely quercetin, kaemferol and myricetin from

Euphorbia wallichii in three different solvents have been isolated and it was concluded that Quercetin was found to be the most abundant flavonol present in Euphorbia wallichii. (Taskeen et al. 2009)

Therefore, the objective of the present investigation was to isolation of active components derived from rhizome of Euphorbia wallichii by using IR, NMR and Mass spectrophotometric methods.

Materials and Methods

Plant material The plant material was collected from the Danwas hills of Tangmarg Tehsil, District Baramulla of Jammu and Kashmir, India. Taxonomic identification was done from Centre of Plant Taxonomy and Biodiversity, University of Kashmir J&K India. The rhizome of *Euphorbia wallichii* was dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Extraction Soxhlet apparatus was used for the extraction of selected plant material using different solvents in increasing order of polarity. They are n-hexane, petroleum ether, chloroform, ethanol and distilled water. The Extraction was performed for 48 hours at 42-45^0 C or 8 cycle's .The crude extracts were concentrated by using vacuum evaporator operated at 40^0 C at 600-700 mm vacuum. Taxonomic identification was done from Centre of Plant Taxonomy and Biodiversity, University of Kashmir J&K India.

Preliminary phytochemical investigation of the aqueous extract was done by using various phytochemical tests including Dragendroff and Mayer's tests for alkaloids, alkaline reagent test for flavonoids and Kellar-Killiani test, Froth formation test, Salkowski test for cardiac glycosides, glycosides saponins, and steroid-terpenoid, respectively. Table 1.

Separation and Purification Techniques:

For purification of the compound from the plant extract two types of Chromatographic techniques were used:

(1) Column Chromatography

(2) Thin layer Chromatography

Column Chromatography Technique:

Seventeen grams of the fraction obtained from precipitation was suspended in 30 ml. chloroform and adsorbed in 20gm. of silica gel (silica gel 60-120 mesh). The chloroform was removed under reduced pressure and a dry silica adsorbed sample remained, Silica gel (95 gm) slurry was made using 90% ethanol and packed into a column pre-plugged with a small piece of cotton at the bottom and fixed in a clamp. Silica adsorbed sample was transferred to the column. The column was then filled with eluting solvent and allowed to run at a rate of 40 drops per minute. The column was eluted with 90% ethanol to facilitate the elution of pigment materials. Ethyl acetate: Methanol (4:1), Chloroform: Ethanol: Water (4:1:1) and Chloroform: Methanol: Water (20:10:2) were used. Finally, methanol was used to elute the rest components.

The column was run continuously and the fractions were collected sequentially in labeled flasks. The solvent was removed under reduced pressure, and components of each chromatographic elute was analyzed by thin layer chromatography. Solvent and column chromatographic fractionation is shown in Table 2.

Thin layer Chromatography:

For the thin-layer chromatography (TLC), sample from the column chromatographic elute was dissolved in alcohol and spotted on to TLC plates produced from (Silica Gel No.60 F254) by means of a micropipette. The plates were placed allowed to run in pre-saturated glass tank containing Chloroform: Methanol: Water (6:4:1). This solvent was chosen for its better resolution of maximum number of constituents with clear separation. The plates were then air dried and examined under UV light of wavelength 256nm and 360nm. Fluorescent spots were en-circled with a pencil. The plates were subsequently sprayed with freshly prepared Dragendroffs reagent and heated for 10 minute at 110^{o}C to facilitate the development of colored spots. The position of the sports on the TLC plates was notes by calculating the retention factor (Rf), the distance of components traveled divided by the distance, solvent traveled from the base, as shown in Table 3.

Isolation of the compound from the purified Materials:

Isolation of the compound from the purified materials was carried out by using (1) Acid hydrolysis (2) Methylation

(1) Acid hydrolysis: The collected purified fraction (10 ml) was mixed with 2 ml of methanolic HCL (10%) and was refluxed for 4 hrs. in vacuum evaporator at the temperature of 40^0C. After 4 hrs, the mixture was again diluted with 3 ml of distilled water and then evaporated to remove methanol. The aqueous solution was extracted with chloroform. The aqueous layer was neutralized with 10% NaOH and concentrated under reduced pressure. Then the purified fraction was collected for further methylation.

(2) Methylation:

The purified crude extract obtained from acid hydrolysis was further purified by methylation. Methanol was added to it and left for evaporation until it was converted to crystal form.

Spectral analysis of the fractions:

Fractions were crystallized and send to Sophisticated Analytical Instrumentation Facility (SAIF), Central Drug Research Institute Lucknow for IR, ^{13}CNMR, ^{1}HNMR and MASS spectral analysis.

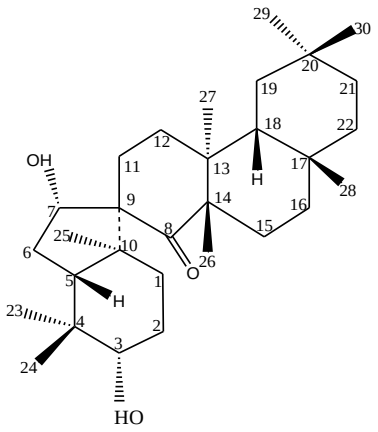
Results and Discussion

Structural elucidation

Fraction E which was obtained after purification as a cream color powdered material with the elution of 10 % ethyl acetate in hexane: chloroform and methanol: water from the silica gel column loaded methanol soluble fraction of the plant. It was noticed that melting point apparatus measured 210-212 °C. The IR

spectrum characteristics of fraction E_2 was observed at 3500, 2365 and 1217 cm^{-1} which was due to the presence of hydroxide (OH), C=C and $–HC=CH_2$ groups, respectively Table 4.

The ^{13}CNMR spectrum of fraction E_3 displayed all together 29 Carbon signals will be formulated C-13 in the molecule. These 29 carbon signals were displayed at methyl, 10 methylenes, 4 methines and remaining 8 quaternary carbons. The signal at δ 73.10 in the broad spectrum was resolved into 2 signals as the methyl and methylene. Thus, finally a tri-terpene nature was reconfirmed. The carbonalic carbon resonated at δ 69.49 (C-3) and δ 76.09 (C-7) a down field signal at δ 101.37 was due to the ketonic function in the molecule. The detailed ^{13}CNMR spectral data are given in the Table 5.The proton ^{1}HNMR spectrum of fraction E_4 exhibited 8 methyl singlets between δ 0.73 to 2.693. Thus, gave an idea of triterpene nature in addition to methyl singlet. The same spectrum showed 2 significant signals at δ 2.56 as a double doublet due to carbonyl proton. H-3 and H-7, respectively. The remaining signals have been given in Table 6. The molecular mass of fraction E_5 was determined by using a JEOL-AccuTOF JMS-T100LC mass spectrum at M^2 458 and the formula of corresponding peak was depicted via high resolution electron impact mass spectrum as $C_{30}H_{50}O_3$ .When these spectral data was compared with the various authentic penta cyclic tri-terpenoid compound obtained from Phyto Lab. Germany (2003), and with the books entitled “spectrometric identification of organic compound” by Silverstein et al. (1974) and “Glossary of Indian Medicinal Plants with active principles by Asolkar et al. (2005). Hence, it was concluded that fraction E was found very close to the multiflorenol (multiflorane) class based up on these spectral analysis the compound was named as Pentacyclic tri-terpenoid saponin and the structere was assigned to the fraction isolated from the *Euphorbia wallichii is* given below.



Pentacyclic triterpenoid

The phytochemical investigation of the aqeous extract of *euphorbia wallechii* showed that the saponins, steroids, and triterpenoids were found in strongly positive nature.Where as flavonoids ,Cardiac Glycosides showed the positive nature .But the alkaloids were abesent in the test sample as shown by both Dragandroffs and Mayers tests.

Conclusion

Table 1: Showing preliminary phytochemical screening of selected plant extract

S. No.	**Presence of Components**		**Name of the test performed**	***Euphorbia wallichii***
1	Alkaloids		Dragendroff's reaction	_
			Mayer's reaction	_
2	Flavonoids		Alkaline reagent test	+
3	Glycosides	Cardiac Glycosides	Keller-Killiani test	+
		Saponin Glycosides	Froth formation test	++

		Steroids and triterpenoids	Salkowski test	++

Positive (+), Strong positive (++), Negative (-).

\Table 2. Showing Column Chromatography of selected plant extract

Euphorbia wallichii	Chloroform:Methanol 15:09	A	60	Yellowish brown
	15:08:01	B	27	Yellowish
	14:08:01	C	31	Greenish
	13:07:01	D	43	Dark brown
	12:06:01	E	57*	Light brown

*This fraction was used for spectral analysis viz. IR, UV, CNMR, HNMR & Mass.

Table 3 .Thin Layer Chromatography of plant extracts.

Plant extracts	**Solvents systems used**	**Obtained Spots**	**Color characteristics**			**Rf value**
			Visual	**Iodine**	**Uv light**	
Euphorbia wallichii	Chloroform: methanol: water (12:06:01)	Spot -1	Brown	Dark brown	Blackish Brown	0.59
		Spot -2	Yellow	Dark yellow	Brown	0.83
		Spot - 3	Green	Dark green	Blackish green	0.95

Table 4 IR spectrum of *Euphorbia wallichii.*

No. of peaks	**Peak range in cm^{-1}**	**Assigned group**

1. Single sharp	3500	OH group
2. Single blunt	2365	>C=C<
3. Single sharp	1217	$CH\text{-}CH_3$ group
4. Single blunt & large	765	$CH\text{-}CH_2$ group

Table 5 ^{13}CNMR spectrum of *Euphorbia wallichii.*

No. of Peaks	Peaks range (100 MHz)
1	**101.37**
2	**76.09**
3	**75.87**
4	**75.49**
5	**73.10**
6	**69.60**
7	**69.49**
8	**60.62**
9	**101.37**
10	**76.09**
11	**75.87**
12	**75.49**
13	**73.10**
14	**69.60**
15	**69.49**
16	**60.62**
17	**76.09**
18	**75.87**
19	**75.49**
20	**73.10**

21	**69.60**
22	**69.49**
23	**60.62**

Table 6 ^{1}HNMR spectrum of *Euphorbia wallichii.*

No. of Peaks	Peaks range (400 MHz)	Hz
1	**4.23**	**dd, J = 7.1, 7.1**
2	**3.48**	**dd, J = 9.8, 6.1**
3	**2.69**	**dd, J 14.3, 6.0**
5	**2.04**	**m, Ha**
6	**1.59**	**m**
7	**1.54**	**m, H_b**
23	**1.22**	**S**
24	**1.07**	**S**
25	**0.92**	**S**
26	**0.90**	**S**
27	**0.82**	**S**

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