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KEY WORDS: *Sesuvium portulacastrum* ,halophyte, Column Chromatography , TLC , HPLC.

Chromatographic separation of phytoconstituents from *Sesuvium portulacastrum.*

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ABSTRACT

The bioactive components were separated from *Sesuvium portulacastrum* (Aizoaceae family) a halophyte known to be used as a traditional medicine.The present study focuses on Chromatographic characterisation of the halophyte using TLC ,Column chromatography and HPLC. Evaluation of solvent system for chromatographic separation revealed that Ethyl acetate : acetic acid :water (5:1:1) is the most appropriate solvent system, for separation of bioactives. From the crude extract of halophyte, 20 fractions eluted by column chromatography and were confirmed by TLC to have specific Rf value . The 3 different eluted fractions, were further characterized by HPLC which illustrates highest peak values at 2.885 , 3.027 ,2.093.

Introduction:

Mangroves are facultative halophytic plants found in tropical and sub-tropical areas of inter tidal zones [18]. Mangrove and mangrove associates contain biologically active antiviral, antibacterial and antifungal compounds. They provide a rich source of steroids, triterpenes, saponins, flavonoids, alkaloids and tannins[1].

Sesuvium portulacastrum (L.) L. (seapurslane) is halophyte used on the Senegal coast as a haemostatic and its decoction is the best known antidote for stings of venomous fish [13] .The essential oil extracted from its leaves have notable antibacterial activity against both Gram-positive and Gram-negative bacteria and displayed significant antifungal and antioxidant activity[14]. Sesuvium portulacastrum ,molecular phylogenetic studies using 18S rRNA gene sequence has revealed, its closely reltated to Perkesia aculata (Cactaceae) [19].

But there is a need for isolation characterization, determination of bioactivity of the lead compound for its pharmaceutical exploitation. Analytical methods play important roles in the discovery, development and manufacture of pharmaceuticals [14].

Isolation and separation of bioactive compounds for further analysis are highly necessary. Analytical methods like the column chromatography; thin layer chromatography (TLC) and bioautography are the economical methods in the field of pharmacognosy. In the present study, attempt has been made to fractionate and isolate the biologically active compound using different chromatographic techniques and development of suitable solvent system.

2. Materials and Methods

2.1 Collection of Plant materials : Sesuvium portulacastrum was collected from Kelva beach, Thane District Maharashtra and was identified with the help of manual published by Kathiresan K [10]

2.2 Plant extract preparation: Plant powder was initially diluted with different solvents, with solvent to sample ratio of 10:1 (v/w). The diluted plant sample was kept at room temperature undisturbed for 24 h. Then, it was kept in the orbital shaker for next 48 h. Solvents ethanol, methanol, acetone, hexane, and diethyl ether were used for the extraction process.

The diluted sample was filtered through Whatman No. 1 filter paper to obtain crude extracts. The extract was concentrated at 40°C using rotaevaporator. The dried crude concentrated extracts were weighed to calculate the yield percentage and stored at room temperature until further analysis.

2.3 Thin Layer Chromatography: The TLC plates (precoated TLC plates Silica Gel G) were trimmed to strips and the position of the origin marked by a straight line. The extract of Sesuvium portulacastrum was spotted on the origin and put in tank containing a solvent system. The procedure was followed with other strips and various solvent-solvent ratios (N hexane : Ethyl acetate : water in 12:3:5, 12:1:1, 10:1:1, and 5:1:1) until good resolution was noticed. It was viewed by spraying with silver nitrate and iodine vapour and visualized under UV light[11,17]. The retention factor (Rf) values of all the spots were determined by the following formula:

Retention factor = Distance traveled by the plant extract/distance traveled by the solvent system.

2.4 Column chromatography : Column Chromatography was performed on all the extracts obtained from Sesuvium portulacastrum using various solvent system. The column (2 cm X 25 cm) was packed with silica gel till it is about three-fourth filled ,activated with methanol and was kept overnight. The plant extract was loaded on the top, eluted with various solvent combination. The column fractions were again tested with TLC chromatogram and the Rf values were determined. The fractions with similar Rf value were combined together and kept for further screening [23].

2.5 High Performance Liquid Chromatography: Analysis of methanol extracts of leaf of Sesuvium portulacastrum was performed by HPLC. The HPLC system consists of LC-20AT binary gradient liquid chromatograph pump and PDA UV-Vis detector and Rheodyne type injector fitted with 20 ul capacity fixed loop all from Shimadzu Prominence Modular HPLC .The column used was Shim Pack C18 at ambient temperature. The output signals were monitored and processed using spinchrom CFR software. The solvent system optimized for the analysis was methanol:water in the ratio 40:60. The flow rate was 1 mL/minute and detection wave length was set at 232 nm.

Result and Discussion:

The crude extracts of Sesuvium portulacastrum were subjected to chromatographic characterization to separate and isolate active copmponents.To characterize it, different types of chromatographic techniques viz. TLC, column chromatography along with HPLC studies were undertaken. Prior to the chromatographic separation, proper solvent system was determined.

The initial task was of optimizing the solvent sytem , for that purpose ten solvent systems taking different polar and non polar solvents such as water, methanol, acetic acid, chloroform, ethyl acetate, formic acid n-butanol, n hexane an petroleum ether in varying ratios were used , for a particular extract for separation of compounds by thin layer chromatography (Table 1). The result showed that Ethyl acetate : acetic acid :water in the ratio 5:1:1 was most appropriate solvent system for all the plant extracts. Further, thin layer chromatography separation and Rf value determination of the crude extracts using this solvent system were undertaken (Table 2). Among the five extracts, better separation of the active compounds was achieved in case of the methanol extract by the

solvent system and was used for further characterization study.

Table 1. Selection of solvent system for chromatographic separation.

Solvent System	Ratio	*Sesuvium portulacastrum*				
		H	EA	E	M	AQ
Petroleum ether : n hexane	5:1	-	-	-	-	-
N hexane : Chloroform	5: 1	+	-	-	-	-
Chloroform : Ethyl acetate	9:1	+	+	-	-	-
Chloroform : Methanol	9:1	-	+	+	+	-
Ethyl acetate : acetic acid:water	10:1:1	-	+	+	-	-
N butanol:acetic acid : water	5:5:1	-	+	-	-	-
Acetic acid: Formic acid : water	5:1:1	-	-	-	-	-
Ethyl acetate : acetic acid:water	5:1:1	+	+	+	+	+
Ethyl acetate : acetic acid:water	12:3:5	+	+	-	+	+
Ethyl acetate : acetic acid:water	12:1:1	-	-	+	+	-

Table 2 TLC of methanol extract of leaf of Sesuvium portulacastrum in different ratios of solvent system.

Solvent System	No of bands	RF value
Ethylacetate:aceticacid:water (10:1:1)	-	-
Ethylacetate:aceticacid:water (12:3:5)	4	A=0.11,B=0.29,C=0.34,D=0.67
Ethylacetate:aceticacid:water (5:1:1)	2	A=0.31,B= 0.79
Ethylacetate:aceticacid:water (12:1:1)	2	A=0.12,B= 0.43

Table 3. TLC of methanol extract of leaf of Sesuvium portulacastrum in different ratios of solvent system.

Solvent System	Column fractions	No of bands	RF value
Ethylacetate:aceticacid:water (5:1:1)	1	2	A=0.14,B=0.24
	2	1	B=0.33
	3	No band	-
	4	2	A=0.11,B=0.37
	5	2	A=0.1,B=0.72
	6	1	A=0.25
	7	No band	-
	8	No band	-
	9	1	A=0.4
	10	No band	-
	11-20	No band	-

The extract was fractionated by column chromatography and the different fractions were checked by TLC for the presence of active compounds following similar Rf values as determined before (Table 4). Out of 20 fractions, six fractions showed clear spots/bands with different Rf values in the TLC plate. These spots were assessed for HPLC studies along with standards.

The HPLC chromatogram of the fractions2,4,5 are shown in the figure 1, 2,3 respectively.The HPLC chromatogram of figure 1 indicates(fraction 2 of column chromatography) gave 2 peaks with retention times 2.885,2.991. The highest peak was at 2.885. The HPLC chromatogram of figure2 indicates(fraction 4 of column chromatography) gave 5 peaks with retention times 2.47, 2.706, 3.027, 3.204, 4.416, the highest peak was at 3.027.The HPLC chromatogram of figure3 indicates(fraction 5 of column chromatography) gave 3 peaks with retention times 2.903, 3.017, 4.412 ,the highest peak was at 2.093.

To characterize the bioactive compounds several techniques were used among which chromatographic techniques were extensively used. Different polar and non polar solvents such as water, methanol, acetic acid, chloroform, n-butanol, toluene, ethyl acetate and formic acid in different combinations were used to carry out TLC. Except n-butanol, acetic acid and water no solvent system gave successful results in chromatographic separation of the components. The successful separation of biomolecules by chromatographic technique depends upon suitable solvent system and its optimization [22].

The phytochemical profiling of Sesuvium porulacastrum using GCMS , FTIR shows the presence of phenolics and flavanoids[20] .The current study focused on isolation of phytoconstituents by using analytical chromatographic techniques .

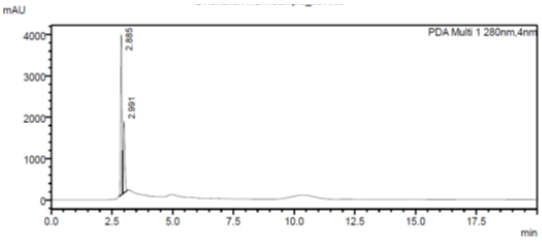

Figure1: HPLC Chromatogram of fraction 2

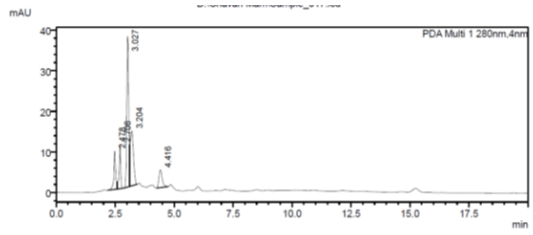

Figure2: HPLC Chromatogram of fraction 4

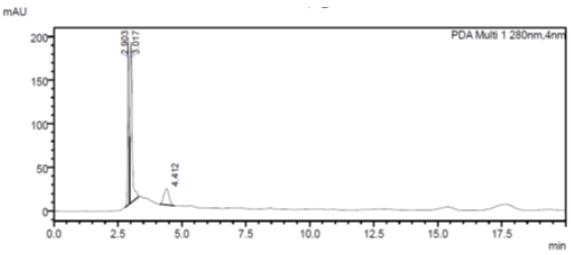

Figure3: HPLC Chromatogram of fraction 5

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