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Der Pharmacia Lettre, 2016, 8 (16):52-56
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ISSN 0975-5071
USA CODEN: DPLEB4

Phytochemical screening and quantitative analysis of hexane, acetone, methanol & water extracts of *Salicornia virginica* by UV-spectrophotometry

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ABSTRACT

The powdered plant material subjected to extraction using acetone, methanol, hexane and water. The extracts of plant were used for the phytochemical analysis to find out the phytochemical screening and quantitative analysis by UV method. The tests confirm the presence of various phytochemicals like Alkaloids, Saponins and Flavonoids in four extracts. The quantitative results suggest that the amount of alkaloid and flavonoids found are 1.836mg and 0.773mg respectively.

Key words: *Salicornia virginica* , phytochemical screening, quantitative analysis, UV–Spectrophotometry.

INTRODUCTION

The value of medicinal plants in drug discovery is known to us well and human being used them for various purposes from the beginning of the human history [1]. Medicinal plants contain some organic compounds which produce definite physiological action on the human body and there bioactive substances include tannis, alkaloids, carbohydrate, terpenoids, steroids and flavonoids [2–3]. Medicinal plants are of great importance to the health individuals and communities [2]. Many of these natural products have vital role as mediators of Ecological interactions; that is, they have functions in ensuring a continued survival of particular organism in often ensuring in often hostile environments where there is competition with other organisms [3]. Salicornia is from two greek words ”Salt” and “horn” they are saline plant with horn like branches. Virginia tells us that the first specimen was collected from that. *Salicornia virginica* (American glass wort, pickle weed) is a halophytic perennial dicot. This grows in various zones of intertidal salt marshes and can be found in alkaline flats. It is native to various regions of Northern Hemisphere including both coasts of North America Canada to mexico.[4] Fig. 1.

Fig. 1. *Salicornia virginica* plant image

Litarature review reveils that, Biofuels are a viable option for sustaining long-term renewable energy needs because they satisfy the criteria to be considered green ecologically friendly energy sources—halophytes are alternative,

renewable and sustainable, *Salicornia virginica* as a biofuel source by conducting a series of experiments utilizing various growth and salinity conditions.[5, 6, 7, 8 and 9].

MATERIALS AND METHODS

Collection and preparing plant materials:
The plant was collected from coastal area, bapatla. The collected plant was dried in shady conditions, the dried plant is taken and powered, the powdered plant is then stored in the suitable conditions (air tight, light resistant containers).

Chemical Reagents:
All the chemicals used were of Analytical grade and were purchased from Merk chemicals private limited, Mumbai.

Instrumentation:
Soxhlet apparatus is used for the extraction of phyto-constituents from the plant powder. TECHCOMP Double beam UV-Visible Spectrophotometer with Hitachi software, standard Quartz cuvettes with lid is used for measuring the absorbance. All the chemicals and reagents used were LR grade and were purchased from Merk chemicals PVT LTD, Mumbai.

Preparation of plant extracts for phyto chemical analysis:
25 gms of the powdered material was weighed and is subjected to soxhlet extraction using hexane, acetone, methanol and water in successive mode respectively for 48 h. The solvent was then recovered using Rotary Vacuum Evaporator and the concentrated extract was further evaporated to get dry powder. The dried powder was preserved in an airtight bottle. The crude extracts thus obtained were used for further investigation of phytochemical screening.

Phytochemical screening:
The extracts of the powdered leaves of *Salicornia virginica* analysed for the presence of various phyto constituents like steroids, triterpenoidal, saponins, alkaloids, carbohydrates, flavonoids, glycosides and phenolic compounds by using standard phytochemical procedures as described by Harborne [10].

Test's for steroids:
i. Salkowski test: Few drops of conc.H_2SO_4 is added to the plant extract shaken and on standing if the lower layer turns red in color then it indicates the presence of steroids.
ii. Libermannburchards test: To the chloroform solution of extract , few drops of acetic anhydride is added from the sides of test tube if a reddish brown ring is observed at the junction of the two layers indicates the presence of steroids.

Test's for tri terpenoids:
i. Salkowski test: Few drops of conc.H_2so_4 is added to the plant extract shaken and on standing if lower part turn golden yellow in color indicates the presence of tri terpenoids.
ii. Libermannburchards test : To the chloroform solution of extract , few drops of acetic anhydride is added from the sides of test tube if a reddish brown ring is observed at the junction of the two layers indicates the presence of tri terpenoids.

Test's for saponins:
Foam test: Small amount of extract is shaken with little quantity of water, and then if foam is produced and persists for 10 min. It confirms the presence of saponins.

Test's for alkaloids:
i. ***Wagner's test:*** The acid layer when mixed with few drops of Wagner's reagent (solution of iodide in potassium iodide) if it gives brown to red precipitate indicates the presence of alkaloids.
ii. ***Hager's test:*** The acid layer when mixed with few drops of Hager's reagent (saturated solution of pricric acid) if it gives yellow coloured precipitate indicates the presence of alkaloids.

Test's for carbohydrates:
i. Fehilings test: The extract when heated with Fehling's A & B solution if it gives an orange red ppt showing the presence of reducing sugar.

ii. *Benedicts test*: If the extract is heating with Benedict's reagent, if brown ppt is observed indicates the presence of sugar.

Test's for flavonoids:
i. Ferric chloride test: Alcoholic solution of leaf extract react with freshly prepared $Fecl_3$ if it gives black fish green color indicates the presence of flavonoids.
ii. Lead acetate test: Alcoholic solution of leaves extract with 10% lead acetate solution and gives white precipitate indicates the presence of flavonoids.

Test's for glycosides:
i. Anthraquinone test: if leaf extract powdered and extracted with either ammonia or caustic soda. If aqueous layer shows pink color indicates the presence of glycosides**.**
i. Keller-killiani test: This is for cardiac glycosides. Chloroform extract of plant and glacial acetic acid with ferrous chloride and 0.5 ml of conc. H_2SO_4. If acetic acid layer shows blue color indicates the presence of glycosides.

Test's for phenolic compounds:
Ferric chloride test: Treat the extract with ferric chloride solution if blue color appears then indicates the presence of hydrolysable tannins and green color appears indicates the presence of condensed tannins.

Quantitative estimation of phyto-constituents
A. For Alkaloids:
To 1ml of Methanolic extract 5 ml pH 4.7 phosphate Buffer was added and 5 ml BCG solution and shake a mixture with 4 ml of chloroform. The extracts were collected in a 10-ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without extract. Atropine is used as a standard material and compared the assay with Atropine equivalents.

B. For Flavonoids:
1ml of the plant extract was mixed with 1.5 ml of methanol (95%), 0.1ml of Aluminium chloride (10%), 0.1 ml of 1M potassium acetate and 2.8ml of diluted water. After incubation at room temperature for 30min, the absorbance of the reaction mixture was measured at 415 nm with UV/VIS spectrophotometer. Rutin was used as standard flavonoid and results were expressed in terms of Rutin equivalent.

RESULTS

The phytochemical characteristic of the Salicronia virginica plant investigations are summarized in Table 1. The results shown the presence of medicinally active constituent in the four extracts studied. From Table1, steroids, Triterpenes, Carbohydrates and Phenolic compounds are absent in all four extracts. Alkaloids are present in all four extracts. Flavonoids are present in only water and methanol extracts and Saponins are present in only water extract.

Table. 1. Results showing qualitative phytochemical secreening of plant Salcornia Virginica

S.No	*Secondary* Metabolites	Test names	Hexane	Acetone	Water	Methanol
1.	Steroids	A)Salkowski test B)Lieberman-Buchard's Test:	**-ve**	**-ve**	**-ve**	**-ve**
2.	Triterpenes	A)SalkowskiTest: B)Lieberman-uchard's	**-ve** **-ve**	**-ve** **-ve**	**-ve** **-ve**	**-ve** **-ve**
3.	Saponins	A)Foam test:	**-ve**	**-ve**	**+ve**	**-ve**
4.	Alkaloids	C)Wanger's Test: D) Hager's Test	**+ve** **+ve**	**+ve** **+ve**	**+ve** **+ve**	**+ve** **+ve**
5.	Carbohydrates	A)Fehling's Test: B)Benedict's Test	**-ve** **-ve**	**-ve** **-ve**	**-ve** **-ve**	**-ve** **-ve**
6.	Flavonoids	A) Ferric chloride test B) Lead acetate test	**-ve** **-ve**	**-ve** **-ve**	**+ve** **+ve**	**+ve** **+ve**
7	Phenolic compounds	A) Ferric cyanide test	**-ve**	**-ve**	**-ve**	**-ve**

Quantitative Estimation of Alkaloids in Hexane, Acetone, Methanol & Water Extracts:
The extracts were tested for the quantitative estimation of the amount of alkaloids present in the sample by fallowing BCG method. Atropine was used as standard alkaloid for the estimation of the amount of alkaloids present in the extract. Standard graph and standard absorbance results were shown in figure 2 and Table 2.

Table.2 Results of standard Atropine		
S.No	**Concentration in µg/ml**	**Absorbance**
1	5	0.258
2	10	0.366
3	15	0.461
4	20	0.547
5	25	0.663
6	30	0.756
7	35	0.849
8	40	0.939

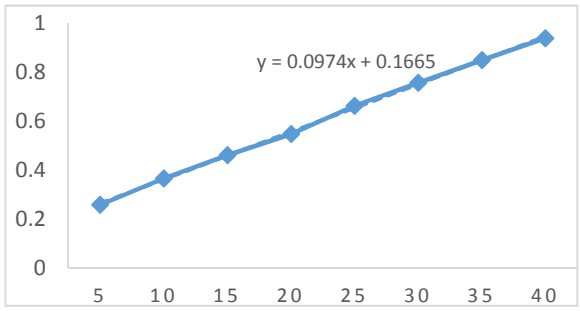

Figure-2: Linearity graph for standard Atropine

By substituting the absorbances of the samples in the standard equation, it was the amount of alkaloids present in the hexane, acetone, methanol and water extracts are found to be 0.523 mg, 0.245 mg, 0.425 mg, 0.643 mg respectively.

Quantitative Estimation of Flavonoids in the Methanol and Water Extract:

The amount of Flavanoids present in methanol and water extracts were estimated by Aluminium Chloride method. Rutin was used as standard Flavanoid and results were expressed in Rutine equivalents. Standard values were shown in Fig. 3 and Table-3. Same procedure was applied for the sample extract. Results of the extract analysis shows that the extract contain 0.423 mg, 0.350 mg amount of Flavanoids in the 300mg of methanol and water extracts of the leafs of *Salicornia virginica* .

Table.2 Results of standard Rutin		
S.No	**Conc. in µg/ml**	**Absorbance**
1	2	0.221
2	4	0.359
3	6	0.502
4	8	0.669
5	10	0.812
6	12	0.975

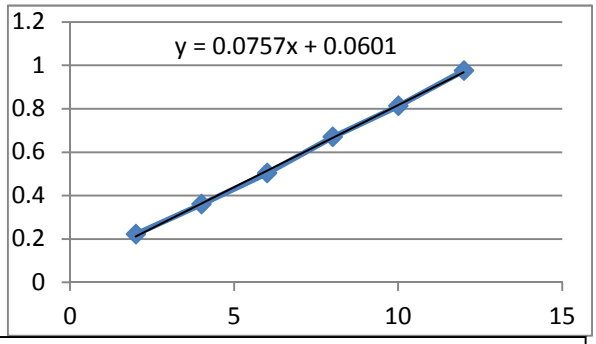

Fig-3 linearity graph for standard Rutin

The quantitative estimation of percentage yield of the extracts of Alkaloids and Flavonoids in the *Salcornia Virginica* plant studied and summarized in Table 4.

Table. 4. Results showing amount of Phytochemical constituent present in the plant *Salcornia Virginica*			
S.No	**Name of the Phyto-constituent**	**Standard Compound**	**Amount found in the extract**
2	Alkaloids	Atropine	1.836 mg
3	Flavanoids	Rutin	0.773 mg

DISCUSSION

Results of the quantitative estimation of phyto-constituents present in the leaf extract of *Salicornia virginica* show high amount of chemical constituents. Among the chemicals under the study alkaloids were found to be high amount. Summary results of the quantitative study were shown in Table-4

CONCLUSION

The phytochemical investigation showed the presence of alkaloids and flavonoids were identified and the extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent. It can also be concluded that the leaves of *Salicornia virginica* are the good sources of antioxidants and surely helpful in treating the disease associated with oxidative stress. Due to rich source of phytochemicals, this plant may be used for herbal medicine and useful for food and drugs.

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