Pharmacognostical Assessment and Phytochemical Screening of Young Shoot of Aquatic Medicinal Herb *Sagittaria sagittifolia* L. (Arrowhead)

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Abstract

Objective: The present study aimed to evaluate the pharmacognostical and phytochemical screening conducted in the young shoot of *Sagittaria sagittifolia* Linn. Pharmacogonastical study including the organoleptic, physico-chemical and Fluorescence analysis through standard procedure of WHO of young shoot powder of *Sagittaria sagittifolia* L. Phytochemical and physico-chemical analysis through standard method of Harborn. The result revealed that the organoleptic character shows light and dark green colour, characteristic odour and slightly bitter taste. The Fluorescence behavior carried out under, normal light (visible light) and UV-light by using different chemical reagent, it shows different colour changes. The presence of secondary bioactive compounds viz. tannins, saponin, alkaloids, terpenoid, phenol, glycoside and steroid were confirmed through phytochemical screening. The pH value, loss on drying, total ash, water soluble ash, acid insoluble ash, and swelling index values are (5.9, 9.81±0.11, 3.75±0.05, 0.21±0.03, 2.45±0.02, 1.11) respectively summarized in table. The study provides us the additional source of therapeutic drug.

Key Words: Pharmacognostical, physico-chemical, Secondary bioactive compounds,

Sagittaria sagittifolia.

Introduction

Traditionally the quality assessment and pharmacognostical study of herbal medicine helps to evaluate the Pharmacognosy that is based on morphology, microscopic and physical characteristics of crude drug. Pharmacognosy is an easy technique to be used for complete information of the drug. Now a days the pharmaceutical industry is mostly dependent on natural resource for supplement of raw material for extraction of therapeutic, medicinal compounds. *Sagittaria sagittifolia* L. which belongs to the family Alismataceae is a perennial submersed- floating aquatic plant of temperate and tropical fresh- water wetlands, lakes, ponds, rivers, estuaries native to Asia and Europe, commonly known as Arrowhead. It has been widely gathered for its large nutritious tuber, leaves and young shoot in Chinese traditional medicine to provide alternative therapy for the treatment of many ailment and diseases. Aquatic medicinal plants constitute an effective source of both traditional and modern medicines. It contains diverse groups of bioactive compounds that possess enormous therapeutic properties. These are much safer than synthetic drug and show lesser side effects (3). Traditionally the plant has been used in India and china for several important medicinal and therapeutic purposes. The present study was therefore initiated to evaluate the Physicochemical, phytochemical, microscopic, fluorescence and organoleptic properties of *Sagittaria sagittifolia* L. var. Arrowhead besides their uses as food item. It provides a new additional source of therapeutic crud drug (2).

Material and Methods

Fresh young shoot of plant were collected at pre- flowering stage from natural watershed reservoir in and around Maheshra Tal and Chilua Tal of Gorakhpur and authenticated by Herbarium Department of Botany, DDU Gorakhpur University Gorakhpur. The plant materials were thoroughly washed with running distilled water to remove extraneous unwanted matter and then air dried under shade till the constant weight. After that the drying plant sample was grinded in mixture with the help of pistil mortar and mechanical grinder afterword the powder sample was kept in small plastic bottle and bags with proper labeling and used for biologically active compound and phytochemical analysis and activity.

Preparation of Plant Extract

The quality of phytochemicals extracted from plant depends on the nature of the plant material, its origin, moisture content and the particle size. The variation in different extraction procedure affect quantity and secondary metabolite composition and extract due to time taking during extraction, type of extraction, concentration of solvents, its polarity, nature of solvents and temperature. Plant extracts mostly occur in plant as a mixture of various types of bioactive compounds or phytochemical with different polarities, thus their separation will be more challenging for the process of identification and characterization of biologically active components.

Soxhlet Extraction

The crude extract of plant material (Leaves, Tubers, Stems, Root) was prepared by soxhlet extraction method. About 10-20 gram powdered plant material was uniformly packed in to thimble and extracted with 200 ml of absolute or 80 % of organic solvents (Absolute Ethanol, Ethanol 80 %, Absolute Methanol, Methanol 80%, Distilled Water, Acetone and Chloroform, petroleum ether) separately about 48 hours or till the solvent in siphon tube of an extractor become colorless. After that the extract was filtered through a paper filter (whatmann no. 1) and evaporated under reduced pressure and controlled temperature (45^0C -$50\ ^0$C) by the rotator evaporator to make more concentrate and it was stored in dark glass bottle at 4^0 C for further analysis.

Extractive Value of Plant Sample

The extractive value or the yield percentage of the plant sample is calculated before and after extraction process using the formula:

$$\text{Extract Yield \%} = \frac{W1}{W2} \times 100$$

Where,

W1= Net weight of powder in gram after extraction

W2 = Total weight of powder in gram taken for extraction

Screening of Phytochemicals:

The Phytochemical screening of crude extract of young shoot carried out through standard protocol to trace out the secondary active constituents in different organic solvent. The active constituents that is tannin, saponins, flavonoids, phenols, steroids, glycosides, protein, amino-acids, starch, terpenoid and alkaloids on the basis of colorations and precipitation on chemical reaction by Harborne, 1973.(10)

Pharmacognostic Studies

Physicochemical analysis

Ash Values

Ash values were determined by incinerating the plant crude sample at the temperature possible to remove all the carbon and find out the total amount of inorganic solutes present in sample. Higher temperature may result in changes carbonate to oxides.

Total Ash

Weigh about five gram of plant sample and taken into a silica crucible (pre- heated) which are place over the burner. The sample till the fumes is no longer produce and then places the crucible in muffle furnace and heat at 550^{o} C for 4 to 6 hour, after complete heating cool down the material in desiccator, then weight the material on ash less filter paper.

Calculation

$$\text{Ash \%} = \frac{\text{Weight of the ash}}{\text{Weight of the sample}} \times 100$$

Water Soluble Ash

For determination of water soluble ash, the hot distilled water 25 ml was used. The water soluble ash is a part of total ash which is soluble in hot water. The obtained total ashes are boiled in 25 ml distilled water for 5 minutes then filter through whatmann filter paper no.1. The insoluble matter was washed and ignited in ash less filter paper and obtained ash was weight and calculates the value of water soluble ash with respected to original plant sample.

Acid Insoluble Ash

Determination of acid insoluble ash, the obtained total ash was taken in a boiling tube having 20 ml of distilled hydrochloric acid and boiled on water bath and then filter using ash less filter paper. The insoluble matter which was collected on filter paper is wash with hot distilled water, ignited the residue on hot plate and cooled in desiccators then weight. Calculate the portion of acid insoluble ash with reference to the dried crude material.

Swelling Index

Swelling properties of drug having specific utility. According to WHO about 1 or 2 gm of plant material (powdered) was accurately weight and placed into 25 ml of distilled water, shaken the mixture thoroughly in every 10 min for 1 hour at room temperature. Measure the volume in ml occupied by plant sample and calculate swelling index in relation to one or two gm of crude plant sample.

Foaming Index

The Foaming capability of plant extracts is measured in terms if foaming index (FI). About one gm of powdered the plant material was weighed accurately and transferred to a 500 ml conical flask containing 100 ml of boiled distilled water. Then it was boiled for 30 min, cooled the extract and filtered into volumetric flask (100 ml). Dilute the volume using sufficient dist.water through the filter paper. After that the decoction was poured into 10 stoppered test tubes in successive portions of 1 ml, 2 ml etc. up to 10 ml. The volumes the test tubes were made up to the mark (10 ml) with water. Then the test tubes were stoppered and shaken. It was allowed to stand for 15 minutes and height of the foam measured and foam index calculated using the formula:

Foaming Index $\frac{1000}{a}$

Moisture content

Moisture content was determined by the method of AOAC (Association of official analytic chemists) using hot air- oven. Initially the amount of 5 gm of plant sample (powdered) was taken in dry and pre- weight, petriplate in triplicate. The sample was uniformly spread in petridishes, weight and transfer place into the oven for 8 hours at 105 ^{o} C. After drying place the petriplates into desiccator, cool and reweight the petriplates and its dried sample. Than calculate the moisture content.

Calculation

$$\text{Moisture \%} = \frac{W1 - W2}{W1} \times 100$$

Where –

W1 = Wight of the sample before drying

W2 = Wight of sample after drying

Fluorescence analysis

The powdered plant sample were subjected for fluorescence analysis. Small quantity of powder placed on microscopic slide, treated with few drops of freshly prepared reagents and after one to two minutes the slide was observed under day light and ultraviolet radiation (365 nm).

Organoleptic characters

The organoleptic characters were observed by different sensory parameter such as odour, colour, consistency and test of finely grounded powdered young shoot of *Sagittaria sagittifolia* L.

Result and Discussion

The result shows that the pharmacognostical estimation of the shoot powder have been studied under following parameters.

Extractive values:

The extractive values important for the evaluation of bioactive constituents present in the crude drug and also for the analysis of specific components soluble in a particular solvent. The values are higher when polarity of the solvent is higher. In this investigation the ethanol extractive are higher than aqueous extract. (Table 1)

Physico-chemical analysis:

The value of physico-chemical determination is summarized in (table 3). It includes pH, Loss on Drying, Total ash value, Acid insoluble ash, Water soluble ash, Foaming index and swelling index.

Phytochemical Screening:

Shoot powder of *Sagittaria sagittifolia* L. were subjected for phytochemical screening. There are five different solvents were used (Ethanol, Methanol, Acetone, Ethyle acetate, Chloroform and dist. Water).[4] The extracts have been applied for phytochemical quality test for identifying the active constituents by using standard Protocols. The results are expressed in table 2. The results are dependent on colour intensity, precipitation reaction and expressed as + for light color, ++ for moderate and +++ for more instant color.[10]

Organoleptic characters:

The powder of dried shoot of *S. sagittifolia* L. is subjected for organoleptic characters and results are summarized in table 4.

Fluorescence Analysis:

The fluorescence behavior of shoot powder were analyses under ordinary light and UV light from different polarity solvents extracts and crude powder. The results are given summarized in table 5. It gives different color variability with identical reagents. (8)

Table 1: Percentage Yield of Young Shoot of *Sagittaria sagittifolia* L.

Solvents	Extractability
Ethanol	36.19
Methanol	26.23
Acetone	20.05
Ethyl acetate	11.31
Chloroform	21.08
Dist. Water	31.52

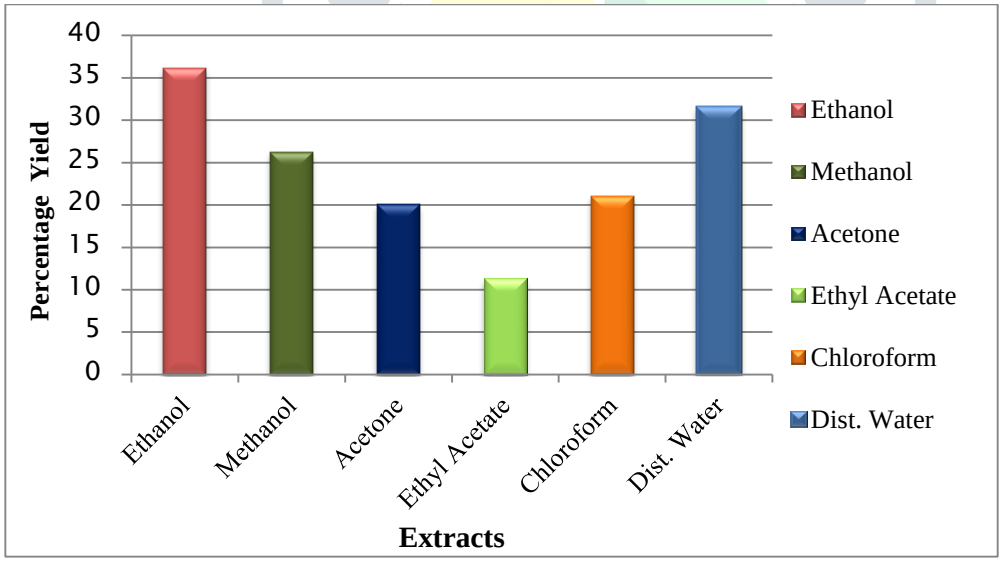

Figure 1: Percentage Yield of Different Extracts of *Sagittaria sagittifolia* L.

Table 2: Phytochemical Screening of Young Shoot of *Sagittaria sagittifolia* L.

Phytochemicals	Test	Extracts of Young Shoot					
		EE	ME	AE	EA	CE	DWE
Saponins	Foam	+++	+++	++	+	+	+
Tannins & Phenols	Ferric chloride	++	+++	+	+	+	++
Carbohydrates	Fehling's test	++	++	++	+	+	++
	Benedict's	+	++	++	+	++	+
Steroid/ Terpenoids	Salkowski test	+	++	+	++	+	+
	Libermann	++	+++	++	+	-	+
Glycosides	Borntragers	++	+++	+	+	+	+
	Keller-killiani	+	++	++	+	+	+
Protein and Amino acids	Biuret test	++	+	+	+	+	++
	Ninhydrin test	++	+++	++	++	++	+
Alkaloids	Hager's	++	+++	+	+	+	+
	Mayer's	+	++	++	+	+	+
	Wagner's	+	++	+	+	+	+

Table 3: Physico-chemical parameters of Young Shoot of *Sagittaria sagittifolia* L.

Parameters	Value
pH	5.9
Loss on Drying	9.81±0.11
Total Ash Value	3.75±0.05
Acid insoluble ash	0.21±0.03
Water soluble ash	2.45±0.02
Foaming Index	1.11
Swelling index	No significant result

Table 4: Organoleptic Characters of Young Shoot of *Sagittaria sagittifolia* L.

Solvents	Color	Consistency	Test	Odor
Aqueous	Light Green	Solid	Slightly bitter	Characteristic
Ethanol	Light Green	Solid	bitter	Characteristic
Methanol	Dark Green	Solid	Slightly bitter	Characteristic
Chloroform	Dark Green	Solid	bitter	Characteristic
Acetone	Dark Green	Semi Solid	Slightly bitter	Characteristic

Table 5: Fluorescence analysis of Young Shoot of *Sagittaria sagittifolia* L.

Powder Treatment	Ordinary light	UV-Light
Powder + Ethanol	Yellowish Green	Dark Green
Powder + Methanol	Light Green	Dark Green
Powder + Glacial acetic acid	Light Green	Fluorescent Green
Powder + Benzene	Greenish	Dark Green
Powder + Conc. HNO_3	Dark Green	Fluorescent Green
Powder + Conc. HNO_3 50%	Dark Green	Fluorescent Green
Powder + Conc. H_2SO_4	Dark Green	Dark Green
Powder + Conc. H_2SO_4 50%	Dark Green	Dark Green
Powder + Conc. HCl	Pale Green	Dark Green
Powder + Conc. HCl 50%	Pale Green	Dark Green
Powder + Conc. $FeCl_3$ 5%	Brownish Green	Dark Green

Figure 2: *Sagittaria sagittifolia* L. A- Whole Plant, B- Young Shoot, C- Powdered form of Shoot

Conclusions

The study revealed the presence of medicinally important biologically active constituent in the plant. It contains large number of health promoting chemical substance and phytonutrients which are essential for human nutrition and therapeutic purposes. There are no more detailed standardized work has been reported in literature for this plant. It is concluded that the isolation of important bioactive compounds of this plant should be done and should be used for further studies and exploration of its medicinal value for humankind.

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