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NUTRACEUTICAL AND PHYTOCHEMICAL EVALUATION OF *SCIRPUS KYSOOR ROXB.*

Hemangi Datar[1*] and Dr. Ajit Datar[2]

[1]*Research Student, Guru Nanak Institute of Research and Development, Guru Nanak Khalsa College, Mumbai, Maharashtra, India.

[2]Adjunct Professor and Research Guide, Guru Nanak Institute of Research and Development, Guru Nanak Khalsa College, Mumbai, Maharashtra, India.

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***Corresponding Author**
Hemangi Datar
Research Student, Guru Nanak Institute of Research and Development, Guru Nanak Khalsa College, Mumbai, Maharashtra, India.

ABSTRACT

The present work was designed to evaluate the phytochemical, proximate, nutraceutical and mineral composition of *Scirpus kysoor roxb*. using standard analytical procedures. Study of antioxidant potential and heavy metal analysis was also carried out by using standard methods. The phytochemical screening of ethyl acetate extract revealed the presence of phenolic compounds, flavonoids, cardiac glycosides, phytosterols, terpenoids, saponins, carbohydrates and proteins. Total phenolic content and total flavonoid content was found as 17.23 mg/g and 58.34 mg/g respectively. The antioxidant potential $IC_{50}$was calculated as 48.50. Result of analysis showed presence of moisture (10.20%), total ash (3.875%), carbohydrates (57.33%), fats(0.258%), total proteins (23.75%) and crude fibre (3.36%). The mineral composition analysis showed substantial amount of elements in order of K>P>Mg=Na>Ca>Fe. Heavy metals like As, Cd and Pb were not detected. The conclusion of the study suggests that *Scirpus kysoor roxb*. has very good medicinal as well as nutraceutical potential and also meet the standard requirements for drug as well as nutraceutical formulation.

KEY WORDS: *Scirpus kysoor roxb*., phytochemical analysis, proximate analysis, nutraceutical analysis, elemental analysis.

INTRODUCTION

Wild edible fruits, vegetables, tubers etc. play a significant role in rural areas by providing nutrient supplementary diet .They can be considered as rich sources of various vitamins, minerals, fibers and polyphenols which provide health benefits.[1-3] Consumption of wild fruits and vegetables reduces the risk of several diseases like diabetes, cancer, coronary heart disease, neurodegenerative ailment.[1,4,5] Hence a scientific investigation of wild edible fruits, tubers, vegetables, etc. is needed to evaluate their potential which would be utilized as a source of food material as well as medicine for an ever increasing population. Therefore in the present study such an underutilized edible specie *Scirpus kysoor roxb*.is selected to evaluate its nutritional as well as medicinal potential and phytochemical properties.

Kaseru (*Scirpus kysoor roxb.*) belongs to family Cyperaceae. Its common name is Water chest nut and generally it is found on the margins of ponds and swampy places throughout India.[6,7] This tubers are oval to cylindrical, black coloured roots with rounded scars. These are black in colour externally and cream coloured internally having aromatic odour and bitter taste.[6,7] In Ayurveda, it is described as of Madhur Rasa, Madhur Vipaka, Shit(Cold) Virya. Its karmas are described as Pittaghna, Dihaghna, shukrakara, Stanyakara, Rucikara.[6,7] Its therapeutic uses are in Aruci, Atisara, Diha, Daurbalya, Netraroga, and Stanyakshaya.[6,7] It is a good source of progesterone, starch and sugar.[6-8]

Thus it is medicinally and nutritionally important but very little work has been reported on the medicinal and nutritive potential of Kaseru.[9,10] Hence it is chosen to study for present work.

Fig. 1: Tubers of Kaseru.

Fig. 2: Plant of Kaseru.

MATERIALS AND METHODS

All the chemicals reagents were procured from LobaChemie, India Pvt. Ltd. and microbiological media were obtained from Hi-media Laboratories.

Collection and Authentication of the Plant Material

Scirpus kysoor Roxb. tubers were collected from western ghats of Maharashtra. It was authenticated from Agharkar Research Institute, Pune.

Extraction

Tubers of *Scirpus kysoor roxb.* were shade dried and kept in oven at 37°C. It was ground to fine powder and sieved through sieve of mesh no.44. Sieved powder was extracted by kinetic maceration method on rotary shaker for 24 hours using ethyl acetate. This extract is used for analysis.

Qualitative Phytochemical Analysis[11,12]

Using standard protocol, primary phytochemical analysis of macerated ethyl acetate extract of *Scirpus kysoor roxb*. tubers was carried out.

Total phenolics[13]

The estimation of the total phenolic content was done using Folin – Ciocalteu method. The results were expressed as Gallic Acid Equivalents (GAE, mg/g of weight of extract). The absorbance of the standard (Gallic acid) and the extract was measured colorimetrically at 660 nm.

Total Flavonoids[13]

Aluminium chloride colorimetric method was used for the determination of the total flavonoid content. The absorbance of the standard (Quercetine) and the sample extract was measured colorimetrically at 420 nm, the total flavonoid content was expressed in mg of Quercetine equivalents per g of weight of extracts (QE, mg/g of weight of extract).

Determination of free radical scavenging potential[14]

Free radical scavenging activity of *Scirpus kysoor roxb*. was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay reagent. Ascorbic acid was used as a standard for the assay. Dried extract of *Scirpus kysoor roxb.*dissolved in methanol was used as a test solution at a concentration range of 20-100 µg/ml. 3 ml each of the solutions of the test extract was mixed with 1 ml of prepared DPPH(0.1 mM DPPH) reagent and allowed to react in dark for 30

minutes at room temperature. Afterwards, the absorbance of all the test mixtures was measured using spectrophotometer (JASCO V-630) at 517 nm. Methanol was used as a blank and methanol (without plant extract) added with DPPH reagent was used as a control for the assay. The free radical scavenging activity was calculated by following formula:

$$\%\ \text{DPPH reduction} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

The IC_{50} values of test extract and standard Ascorbic acid were calculated using linear regression analysis and compared.

Proximate Analysis[15]

The procedures recommended as per World Health Organization (WHO) guidelines were followed to calculate the parameters like moisture content, total ash, water-soluble ash and acid-insoluble ash.

Nutraceutical Analysis

Determination of fat,[16] protein[17] carbohydrate[18] and crude fibre[18] were estimated by standard methodology.% Fat was evaluated by Soxhlet method, %Carbohydrates by Anthrone method and % protein was estimated by biuret method.

Calorific (nutritive) value was determined by the following formula,[19]

Calorific value = [Carbohydrate x 4] + [Fat x 9] + [Protein × 4]

Elemental Analysis[20]

Estimation of Na, K, Fe, Ca, Mg and P was performed by Inductively Coupled Plasma - Atomic Emission Spectrometer (ICP-AES) method. Analysis was carried out at Sophisticated Analytical Instrument Fascility (SAIF),IIT, Bombay.

Heavy Metal analysis[20]

Analysis of heavy metals viz.Lead, Cadmium and Arsenic was carried out at Sophisticated Analytical Instrument Fascility (SAIF), IIT, Bombay. Analysis was carried out by the method of Inductively Coupled Plasma—Atomic Emission Spectrometry (ICP-AES) method.

RESULT AND DISCUSSION

The medicinal and nutritional potentials of *Scirpus kysoor roxb*. were evaluated in this study through phytochemical, antioxidant, proximate, nutraceutical and mineral analysis.

Results of phytochemical analysis are described in Table 1.

Table -1: Phytochemical Analysis.

Phytochemical	*Scirpuskysoor*
Alkaloids	-
Phenol	+
Tannins	-
Flavonoids	+
Terpenoids	+
Saponins	+
Sterols	+
Glycosides	+
Proteins	+
Carbohydrates	+
Essential oil	-

Results of phytochemical analysis revealed the presence of array of phytochemical groups such as phenols, flavonoids, terpenoids, sterols, saponins, glycosides, etc.and absence of alkaloids,tannins and volatile oils. Presence of these phytoconstituents supports its application as a nutraceutical and relevance of traditional medicinal use as per ethanobotanical reference. [21]

(+) = present, (-) = absent

Total phenolics

The total phenolic content was found to be 17.93 mg/g of dried extract (in terms of gallic acid equivalents).

Fig. 3 shows the calibration curve and linear regression analysis obtained for gallic acid standard used.

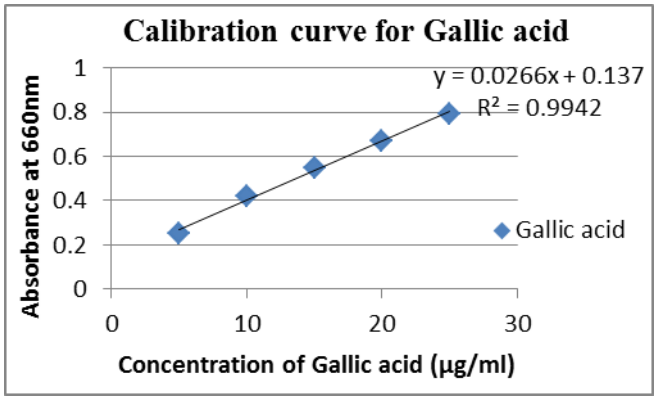

Fig. 3: Calibration curve for Gallic acid standard.

Total Flavonoids

The total flavonid content was found to be 58.34mg/g of dried extract (in terms of quercetin equivalents). Fig. 2 shows the calibration curve and linear regression analysis obtained for quercetin standard used.

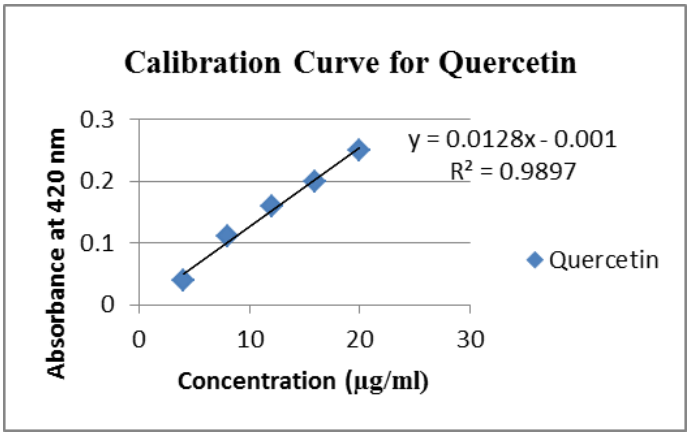

Fig.4: Calibration curve for Quercetin standard.

Free radical scavenging potential

Graph of % inhibition Vs concetration obtained in DPPH assay for for *Scirpus kysoor roxb*.and Ascorbic acid was given by fig.3 and fig.4 respectively.Value of IC_{50} for for *Scirpus kysoor roxb*.and Ascorbic acid was found to be 48.50 and 22.65 respectively. % Inhibition for *Scirpus kysoor roxb*. at 100 ppm was calculated as 86.06%. and that of Ascorbic acid was 90.38% at 35ppm.

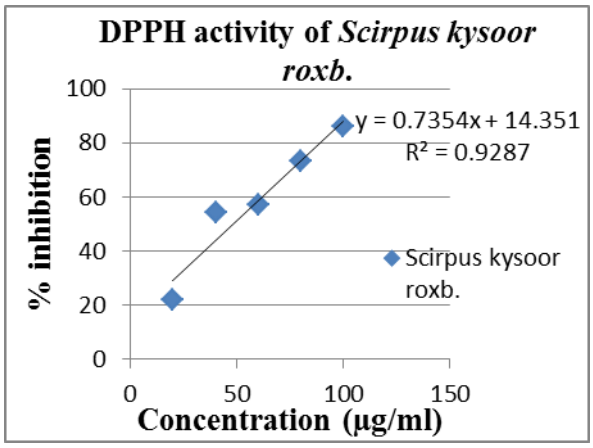

Fig. 5: DPPH assay of *Scirpus kysoor roxb*.

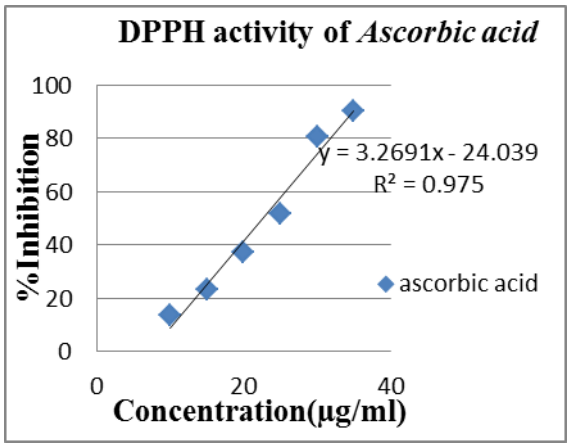

Fig. 6: DPPH assay of Ascorbic acid.

The study of anti-oxidant activity and total phenolic and flavonoid content suggests that the plant is an important source of natural antioxidant, which can be useful as a protective agent against oxidative stress.[22]

Proximate analysis and Nutraceutical analysis

Results of proximate analysis and nutraceutical analysis are mentioned in Table 2 and Table 3 respectively.

Table 2: Proximate Analysis

Parameter	Mean Value(%)
Loss on Drying	10.20+/-0.57
Total Ash	3.875+/-0.123
Acid Insoluble Ash	0.625+/-0.105
Water Soluble Ash	1.29+/-0.10

Table 3: Nutraceutical Analysis

Parameter	Mean Value(%)
Protein	23.75+/-0.00
Carbohydrates	57.33+/-1.44
Fats	0.258+/-0.02
Dietary Fibre	3.36+/-0.24

The percentage of total ash is related to the presence of mineral content which may be one of the rationale for therapeutic potential. In the present study, lower percentage of ash content (3.875%) implies the higher organic content and fairly low inorganic content of the plant.[23] The acid-insoluble ash and water soluble ash are found to be 0.625 %and 1.29% respectively. Lower value of acid insoluble ash revealed lower content of silica like substance. It also revealed that a large portion of the ash content is acid soluble and hence may be physiologically important as salts in the body when consumed. It is also indicative of high digestibility of the plant when eaten.[24] The moisture content observed is 10.20 % which infers the moderate shelf life.

The high value of protein and carbohydrate suggest its nutritional quality. Protein, fat and carbohydrate are sources of energy in diet and the amount was found to be 23.75%, 0.258%, 57.33%, respectively. Plant food that provides more than 12% of their calorific value from protein is a good source of protein.[25]Thus, *Scirpus kysoor roxb*.can be considered as good source of proteins. The plant is a moderate source of carbohydrate when compared with the Recommended Dietary Allowance (RDA) of 130g.[26]

Hence calorific value was estimated as (57.33 × 4) + (0.258 × 9) + (23.75 × 4) =326.642 Kcal. An average person requires 2000-3000 kcal per day. The plant can contribute to the caloric requirement of the body.[26]

The crude fibre content in this plant is observed to be 3.36%. Even though this is a low level but still considered as appropriate, because it aids absorption of glucose and fat. Although crude fibre enhances digestibility, its presence in high level can cause intestinal irritation, lower digestibility and decreased nutrient usage.[26]

Elemental Analysis

The elemental composition of *Scirpus kysoor roxb*.is given in Table 4.

Table 4: Elemental analysis.

Element	Ca	Fe	K	Mg	Na	P
mg/g value	0.94	0.15	10.59	1.09	1.09	2.59

Potassium (K) is present in high concentration than the other elements in *Scirpus kysoor roxb*. Potassium (K) content of 10.59 mg/g (10,590ppm) is more than half of the daily requirement for humans, which is 18.0 mg/g.[23] Concentration of Ca and P is 0.94 and 2.59mg/g (940 and 2590ppm) respectively.Ca and P are very essential elements for maintenance of bones and teeth. Magnesium (Mg) and sodium (Na) both are present in the concentration of 1.09 mg/g (1090ppm). Na is the major element of the extracellular fluid and is a key factor in retaining body fluid. In conjunction with K, through the creation of electrical potential, nerve impulses are conducted and the contraction of muscles is enabled. The presence of Ca, Mg and K were collectively well known to reduce high blood pressure as well as used in the prevention and treatment of it.[23] Thus, the presence of these major elements are indicative of the nutritional importance of *Scirpus kysoor roxb*.

Heavy metal analysis

Results of heavy metal analysis of *Scirpus kysoor roxb*.are mentioned in Table 5. Contamination of medicinal plant materials with heavy metals are attributed to many problems including environmental pollution and traces of pesticides. All heavy metals were found within the prescribed limits as per FSSAI.[27]

Table 5: Heavy metal analysis.

Element	**% value**	**FSSAI limit (ppm)**
Cd	ND	1.5
As	ND	1.1
Pb	ND	10.0

ND = ND means not detected.

CONCLUSION

Significant data generated in present study revealed that *Skirpus kysoor roxb.* can be a potential source of food (nutraceutical) as well as natural medicine. It justifies the ethanobotanical medicinal uses of the plant. Hence further isolation, purification and identification of antioxidant or bioactive compounds with its structure elucidation is essential to obtain useful chemotherapeutic agent. The data obtained in this research also provides a strong base to nutraceutical product formulation.

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