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HPLC analysis of flavonoid compounds and antioxidant action of Nirmali (*Strychnos potatorum*) seeds

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Abstract

There is a growing interest for the plant-based medicines in pharmaceutical industry. Plant derived Antioxidants have gained huge importance regarding their medicinal value. *Strychnos potatorum* Linn (*S. potatorum*) seeds are used in the Indian traditional system of medicine for the treatment of leucorrhoea, gastropathy, bronchitis, chronic diarrhoea, hepatopathy, nephropathy, gonorrhoea, strangury, renal and vesicle calculi, diabetes and eye diseases. The present study was carried out to determine the *in vitro* antioxidant activity and HPLC analysis of chloroform, ethanol and aqueous seeds extract of *S. potatorum*. Antioxidant activities of ethanolic extract of *S. potatorum* was evaluated by DPPH scavenging assay and quercetin was detected in extract of *S. potatorum* under study by using RP-HPLC analysis. In DPPH assay, antioxidant activity was increased dose dependently when compared with standard drug ascorbic acid and IC₅₀ value of ethanolic extract and standard was found to be 19.93 and 34.80 µg/ml. Quercetin content was highest in ethanolic extract, was quantified as 0.9286µg/ml than chloroform extract 0.6813 µg/ml and in aqueous extracts 0.3583µg/ml. Results obtained indicated that *S. potatorum* exhibits good potential to prevent diseases caused by the overproduction of free radicals and it might also be used as a potential source of natural antioxidant agents. Which needs to be investigated further for their medicinal and/ cosmaceutical applications.

Keywords: *Strychnos potatorum* Linn, antioxidants, DPPH assay, quercetin, HPLC analysis

Introduction

Plants have an almost limitless ability to synthesize aromatic substances mainly secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. In many cases, these substances serve as the molecules of plant defense against predation by microorganisms, insects, and herbivores. Further, some of which may involve in plant odour (terpenoids), pigmentation (tannins and quinines), and flavour (capsacin). However, several of these molecules possess medicinal properties^[1]. Numerous crude extracts and pure natural compounds from plants are reported to have antioxidant and radical-scavenging activities and intensive research has been carried out, either to characterize the antioxidant properties of extracts and/or to isolate and identify the compounds responsible for those activities seeking the development of natural antioxidant formulations in the areas of food, medicine and cosmetics^[2-5]. *S. potatorum* Linn. (Loganiaceae) commonly known as Katakam in Ayurveda and Tetan-kotai in Tamil is a moderate-sized tree found in southern and central parts of India, Srilanka and Burma^[6]. The ripe fruit is emetic, diaphoretic and alexiteric; it cures inflammation, anemia, jaundice and causes biliousness^[7]. In ayurvedic system of medicine, the seeds are used in vitiated conditions of kapha and vata, hepatopathy, nephropathy, gonorrhoea, leucorrhoea, gastropathy, bronchitis, chronic diarrhoea, dysentery, strangury, renal and vesicle calculi, diabetes, burning sensation, dipsia, conjunctivitis, scleritis, ulcers and some eye diseases^[8]. The ripe seeds are used for clearing muddy water. They are reported to be very effective as coagulant aids. Alum aided by the seeds has been found to be very effective in removing the suspended impurities from coal-washery wastes. The clarification is due to the combined action of colloids and alkaloids in the seeds^[9]. Presence of diaboline (major alkaloid) and its acetate^[10], sitosterol, stigmasterol, oleanolic acid and its 3acetate, saponins containing oleanic acid, galactose and mannose^[11], triterpenes^[12], mannogalactans (polysaccharides)^[13, 14] etc., have been reported. In the present study, quantification of quercetin by HPLC technique has been made in relation to its antioxidant which was not investigated previously.

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Material and Method

Plant material

S. potatorum plant material was procured from local market of Bhopal (M.P.)

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), SigmaAldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All other chemicals and solvents used were of HPLC and analytical grade.

Extraction

The dried seeds sample was powdered by mechanical grinder and sieved to give particle size 40-100 μ m. The powder was stored in polythene bags at room temperature. *S. potatorum* dried and powdered seed material (100 gm) was extracted with hot continuous percolation method (Soxhlet extraction). The temperature was maintained at 70°C. The extraction was carried out using chloroform, ethyl acetate, ethanol and water as a solvent. The extract was filtered through a Whatman, No.1 and evaporated to dryness under reduced pressure by the rotary evaporator. The obtained crude extract was stored in dark glass bottles for further processing. % Yield of the extract obtained was calculated by formula as mentioned below:

$$\text{Extractive yield value} = \frac{\text{Weight of concentrated extract}}{\text{Weight of plant dried powder}} \times 100$$

Phytochemical study

In previous paper [15] the preliminary phytochemical and quantitative phytochemical assay screening of the crude extract revealed the presence of flavonoids compound. The crude extract was partitioned (gradient) with different solvents like chloroform, ethyl acetate, ethanol and water. After conducting preliminary *in vitro* DPPH free radical scavenging assay on these fractions, ethanolic extract of *S. potatorum* has highest ethanolic extractive percentage compare to other extracts, maximum antioxidant activity (bioactive fraction) was selected and screened with different *in vitro* antioxidant methods.

In vitro anti-oxidant screening

DPPH free radical scavenging assay

DPPH scavenging activity was measured by the spectrophotometer. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10- 100 μ g/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm [16].

$$\% \text{ Reduction} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

Quantification of flavonoid compounds by HPLC technique

For HPLC investigation of flavonoid compounds the ethanolic, chloroform and aqueous extracts of *S. potatorum* under study were used as a preliminary assessment of various compounds. The HPLC apparatus used for analysis was composed of a waters equipped with a UV dual detector and generated data were analyzed using Waters Ace software. For chromatographic separation Thermo C18 column (250X4.6mm, 5 μ m) was applied. The chromatographic analysis was performed at ambient temperature on a RP-C18 analytical column with a mobile phase composed of Acetonitrile: Methanol (50:50 v/v) and was isocratically eluted at a flow rate of 1 mL/ min. A small sample volume of 20 μ L was used for each sample run, being injected into the HPLC system. The chromatogram was monitored with UV detection at a wavelength of 256 nm. Analysis time was 15min for both, standards and samples used for analysis. A quercetin was used as standards. A thermospectronic model of Labindia 3000 + UV/VIS Spectrophotometer with 1cm. matched quartz cells was used for determination of λ_{max} .

Results and Discussion

The crude extract so obtained after the Soxhlet extraction process, extract was further concentrated on water bath evaporation the solvent completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from different samples using chloroform, ethyl acetate, ethanol, aqueous as solvents are depicted in the Table 1. The results showed that maximum yield was found in alcoholic extract. It may due to the solubility of principle contents presence be higher in case of alcoholic solvent, thus it has been accepted that it is a universal solvent for the extraction of plant constituents.

Table 1: Results of percentage yield of plant material

Solvents	<i>S. potatorum</i>
Chloroform	1.3%
Ethyl acetate	1.6%
Ethanol	3.4%
Aqueous	2.8%

DPPH radical scavenging activity of ethanolic extract was shown in Table 2 & Fig 1. The extract exhibited potent radical scavenging activity against DPPH methods in concentration dependent manner. The IC₅₀ value of the extract was comparable to the standards used (Ascorbic acid). The HPLC chromatogram of standard quercetin and ethanolic extract are shown in Fig 2 and the values are expressed in ppm. The retention time for standard and extracts was found approx same. Characteristics parameters for standard quercetin were given in Table 3 and results of quantitative estimation of quercetin in extracts were given in Table 4.

Table 2: Results of DPPH radical scavenging assay

Conc. (µg/ml)	Ascorbic Acid (% Inhibition)	Ethanolic extract (% inhibition)
10	40.79273	38.45
20	54.99587	45.65
40	58.87696	50.23
60	69.36416	62.12
80	75.39224	70.32
100	84.9711	74.65
IC ₅₀ Value	19.93	34.80

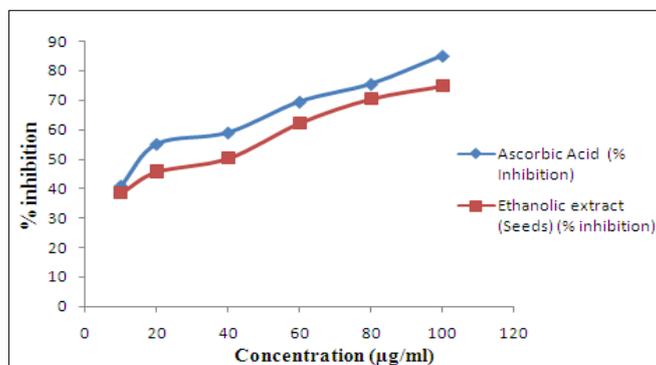


Fig 1: Results of antioxidant activity using DPPH method

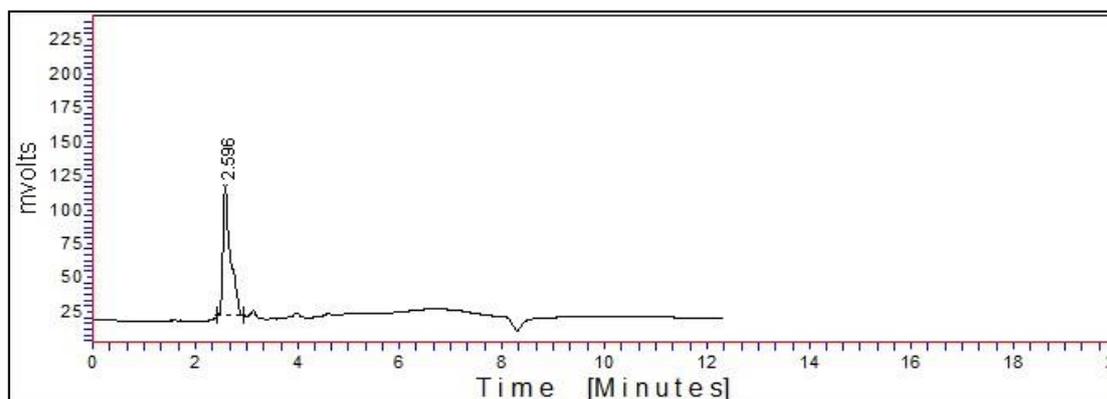


Fig 2: Chromatogram of standard quercetin

Table 3: Characteristics of the analytical method derived from the std. calibration curve

Compound	Linearity range µg/ml	Correlation co-efficient	Slope	Intercept
Quercetin	5-25	0.999	94.39	-30.43

Table 4: Quantitative estimation of quercetin in extracts

Extract	% Assay
Ethanollic	0.9286
Chloroform	0.6813
Aqueous	0.3583

Conclusion

The *S. potatorum* extracts has shown significant antioxidant. The active fractions contain flavonoid in the plant which includes quercetin with already established antioxidant activities. Thus *S. potatorum* can be a good candidate for novel phytomedicine that can be used to treat several diseases. The future study shall be directed towards the identification of bioactive compounds and quality standards for developing a potential drug.

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