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PHYTOCHEMICAL SCREENING AND TOTAL FLAVONOID CONTENT ASSAYS OF VARIOUS SOLVENT EXTRACTS OF SEEDS OF *STRYCHNOS POTATORUM* LINN.

Usma Gangwar*, Anita Choubey

Dept. of Chemistry, Govt. M. L. B. Girls College, Bhopal (M.P.)

*Corresponding Author's E mail: minigangwar90@gmail.com

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ABSTRACT

The objective of this research was to conduct the preliminary phytochemical screening and total flavonoid contents assays of various solvent extracts of seeds of *Strychnos potatorum*. Phytochemical screening was carried out according to the method of CK Kokate and total flavonoid content was measured by the aluminium chloride colorimetric assay. Preliminary phytochemical screening reveals the presence of flavonoids, carbohydrates and diterpenes in chloroform extract, carbohydrate in ethyl acetate extract, glycosides, flavonoids, saponin, carbohydrate, proteins amino acids and diterpens in ethanol extract and glycosides, flavonoids, saponin, carbohydrate, proteins amino acids and diterpens in aqueous extract. Ethanolic extract has the richest content of flavonoids i.e. (1.74mg QE/100mg) respectively, and aqueous extract was the least i.e. (1.5mg QE/100mg). The generated data has provided the basis for its wide use as the therapeutant both in the traditional and folk medicines and can be further subjected for isolation of the therapeutically active compounds with anticancer activity and also for further pharmacological evaluations.

Keywords: Phytochemical screening, Total flavonoids, *Strychnos potatorum*

INTRODUCTION

The value of medicinal plants in drug discovery is known to us well and the human being used them for various purposes from the beginning of the human history. [1] Traditional folk remedies from plants have always guided scientists to search for new medications in order to maintain and promote healthy life for human and animals.[2]

Strychnos potatorum is an important medicinal plant, used in Ayurveda, Unani, Siddha, and in folk medicine for treating several ailments including microbial infections, diarrhoea and diabetes. *Strychnos potatorum*, family Loganiaceae, is a medium-sized, glabrous tree of height 12-13 m. belonging to Bengal, Central and plentiful in southern India. Seeds contain no strychnine but Brucine is present. Seeds are alternative tonic, stomachic and demulcent. They are non-poisonous. Seeds are used to clarify foul and muddy water.

Alkaloids mainly diaboline, and four triterpenes viz., isomotiol, sitosterol, stigmasterol and compesterol were reported from seeds and leaves of *Strychnos potatorum*, respectively. [3,4] Further, twenty four alkaloids including diaboline were reported from the root of *Strychnos potatorum*. [5] Whereas, Adinolfi et al. (1994) have studied galactomannan and galactan (1:1.7), the mixture of the main polysaccharide component of seed. [6]

Active constituent presence in the plants materials might be responsible to the beneficial of human health. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds.[7] Flavonoids are particularly beneficial, acting as antioxidants and giving protection against cardiovascular disease, certain forms of cancer and age related degeneration of cell components. Their polyphenolic nature enables them to scavenge injurious free radicals such as super oxide and hydroxyl radicals.[8] Therefore, the objective of this research was to conduct the preliminary phytochemical screening and total flavonoid contents assays of various solvent extracts of fruits of *Strychnos potatorum*.

MATERIALS AND METHODS

Reagents

The reagents and solvents used for the extraction, phytochemical analyses and total flavonoid contents assays were analytical grade reagents.

Collection and identification of plant

Strychnos potatorum plant material was procured form local market of Bhopal. Madhya pradesh & Further plant material was identified. The dried seeds sample was powdered by mechanical grinder and sieved to give particle size 40-100 mm. The powder was stored in polythene bags at room temperature before extraction.

Preparation of extract

Strychnos potatorum dried and powdered seed material (50 g) 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 paper filter (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:

Extractive yield value = Weight of concentrated extract/Weight of plant dried powder × 100

Qualitative phytochemical analysis of plant extract

The different qualitative chemical tests were performed for establishing profile of given extract for its chemical composition. The extract was examined for the presence of various phytoconstituents such as carbohydrate, alkaloids, glycosides, saponins, phenolic compound, tannins and flavonoids. All tests were done as per the procedure given in the standard book.[9]

Total flavonoids determination

The total flavonoids assay was conducted according to Katasani Damodar.[10] Total flavonoids content was determined by using aluminium chloride colorimetric method. Aqueous extract (0.5 ml) was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 510 nm using UV-Visible spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at concentrations 0 to 25 µg/ml in methanol. The total flavonoids content was expressed as milligrams of Quercetin equivalents per gram of dried sample.

RESULTS AND DISCUSSION

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 choloroform, ethyl acetate, ethanol and water as solvents are depicted in the table 1.

Table 1: Percentage yield extracts of fruits of *Strychnos potatorum* Linn

S. No.	Extract	% Yield (w/w)
1.	Choloroform,	1.3%
2.	Ethyl acetate	1.6%
3.	Ethanol	3.4%
4.	Water	2.8%

A small portion of the dried extracts were subjected to the phytochemical test using Kokate methods to test for alkaloids, glycosides, tannins, saponins, flavonoids and steroids separately for extracts samples. Small amount of each extract was suitably resuspended into the sterile distilled water to make the concentration of 1 mg per ml. The outcomes of the results are discussed separately in the table 2.

Table 2: Results of phytochemical screening

S. No.	Chemical Tests	A	B	C	D	Observation
1.	**Alkaloids**					
	Hager's reagent	-	-	-	-	Yellow coloured precipitate
	Dragendorff's reagent	-	-	-	-	Reddish coloured precipitate
2.	**Glycosides (+ve)**					
	Legal's test	-	-	+	+	Pink to blood red colour
3.	**Phenols/Tannins**					
	Ferric chloride	-	-	-	-	Bluish black coloured
4.	**Flavonoids**					
	Lead acetate test	-	-	+	-	Yellow Coloured precipitate
	Alkaline reagent test	+	-	+	+	Colourless
5.	**Saponins**					
	Foam test	-	-	+	+	Layer of foam
6.	**Carbohydrates**					
	Fehling's solution test	+	+	+	+	Red coloured
7.	**Protein Amino acids**					
	Xantoprotein Test	-	-	+	+	Yellow coloured
8.	**Diterpenes**					
	Copper acetate	+	-	+	+	Emerald green

A=Choloroform, B=Ethyl acetate, C=Ethanol and D=Water

Preliminary phytochemical screening reveals the presence of flavonoids, carbohydrates and diterpenes in chloroform extract, carbohydrate in ethyl acetate extract, glycosides, flavonoids, saponin, carbohydrate, proteins amino acids and diterpens in ethanol extract and glycosides, flavonoids, saponin, carbohydrate, proteins amino acids and diterpens in aqueous extract.

The phytochemical screening was performed to identify the classes of chemical compounds present in the extracts. The phytochemical profile results showed that the plant extract has molecules with high technological potential for the development of new drugs with application in the treatment and prevention of various diseases.

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: **$Y=0.040X + 0.009$, $R^2=0.999$**, where X is the absorbance and Y is the quercetin equivalent (QE).

Table 3: Preparation of calibration curve of Quercetin

S. No.	Concentration	Absorbance
0	0	0
1	5	0.216
2	10	0.425
3	15	0.625
4	20	0.815
5	25	1.021

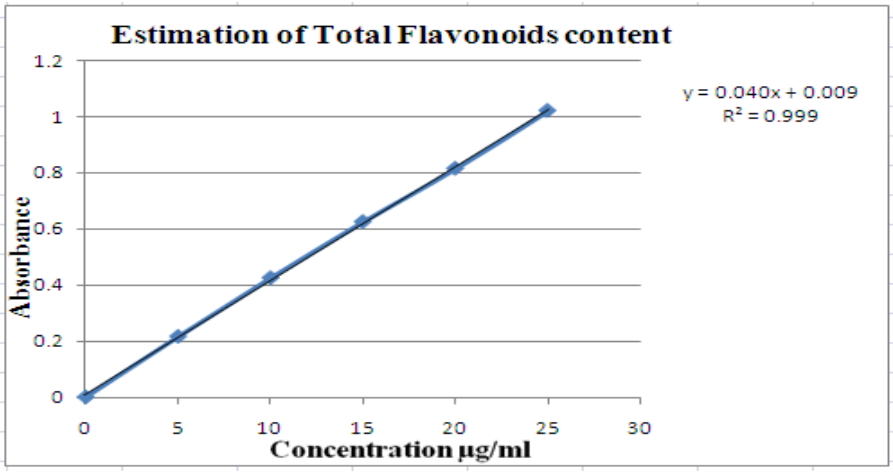

Figure 1: Graph of estimation of total flavonoids content

Table 4: Estimation of total flavonoids content

S. No.	Extracts	Total flavonoids Equivalent to Quercetin mg/ 100 mg of dried extract
1.	Choloroform	1.62
2.	Ethanol	1.74
3.	Water	1.5

Flavonoids seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. Ethanolic extract has the richest content of flavonoids i.e. (1.74mg QE/100mg) respectively, and aqueous extract was the least i.e. (1.5mg QE/100mg).

In the determination of total flavonoids, the results showed that the ethanolic solvent was having richest content of flavonoids than chloroform and aqueous solvent which may be explained by its good polarity and solubility for phenolic compounds extracted from plants. [11, 12]

The data generated from these experiments have provided the chemical basis for the wide use of this plant as therapeutic agent for treating various ailments. However, there is need to further carry out advanced hyphenated spectroscopic studies in order to elucidate the structure of these compounds.

CONCLUSION

The phytochemical screening showed that the *Strychnos potatorum* seeds extract contain a mixture of phytochemicals as glycosides, carbohydrates, flavonoids, phenolic compounds and diterpenes. The quantitative total flavonoids screening indicated that the ethanolic extract has the highest contents of flavonoids which can be an excellent choice for biological and chemical analysis and can be further subjected for the isolation of the therapeutically active compounds with medicinal properties.

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