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Qualitative and Quantitave Phytochemical Screening of Secondary Metabolites in Seeds of *Schleichera Oleosa* (Lour.) Oken

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ABSTRACT

The medicinal plants are extensively used by tribal people worldwide since the time immemorial. *Schleichera oleosa* (Lour.) Oken is one of such plant used to treat many diseases from the time of Ayurveda. The present study includes the qualitative estimation of phytoconstituents such as carbohydrates, proteins, saponins, phenolic compounds and tannins, alkaloids, glycosides, phytosterols, steroids, terpenoids, coumarins and flavonoids as well as quantitative estimation of secondary metabolites such as saponin, alkaloids, flavonoids, terpenoids and tannins in the seeds of *S. oleosa*. The study confirmed the presence of phytocompounds in seeds of *S. oleosa.* The presence of these secondary metabolites can revolutionize the nutraceutical and Industrial sector to cope up the health and nutritional problems of human beings.

Keywords: Carbohydrates, Terpenoids, alkaloid, Saponin, *Schleichera oleosa*

I. INTRODUCTION

Phytochemicals are naturally occurring biochemical compounds in plants that are responsible for giving a characteristic color, odor, taste and texture to the plants. Along with it, the phytochemicals are also helpful in inhibiting pathogenic microorganisms and in prevention of various diseases like cancer and cardiovascular diseases [1]. *Schleichera oleosa* (Lour.) Oken of family Sapindaceae is commonly known as "Kusum" used in the Indian Medicinal System since the time immemorial. The bark of plant is reported to have antioxidant [2] and antimicrobial [3][4] activities. It is used in arthritis, headache, nostalgia, inflammations, malaria and ulcers. Muthukrishnan and Sivakumar worked on the pharmacognostical investigation and phytochemical analysis of leaves of the plant [5]. The primary metabolites include proteins, carbohydrates, starch and lipid are required for the proper growth and development of the plant. The secondary metabolites are synthesized during the secondary metabolism of the plants and potential sources of drugs. The phytoconstituents such as saponins, alkaloids, flavonoids, tannins and cardiac glycosides are the most important secondary metabolites [6]. These secondary metabolites possesses a wide range of pharmacological activities such as analgesic, anti-inflammatory, antiviral, antimicrobial, antispasmodic and anticancer activities [7][8][9][10]. Therefore, the *S. oleosa* has been analysed for the qualitative and quantitative estimation of secondary metabolites of seeds.

II. MATERIALS AND METHODS

A.

A. Plant Material Collection and Identification

The part of the plant material used in the present work is the seeds of *Schleichera oleosa* (Lour.) Oken. The seeds were collected from the Kushmi forest, Gorakhpur region during the year 2016-17. The Herbarium of *S. oleosa* was prepared and authenticated from Department of Botany, DDU Gorakhpur University, Gorakhpur. Dried plant materials were finely powdered and then stored in air tight container at room temperature.

B. Preparation of plant extract and Qualitative Phytochemical Screening

Twenty gram of the powdered plant material was taken in a beaker and extracted in various solvents (aqueous, ethanol, methanol, acetone, ethyl acetate, petroleum ether and chloroform). Then, the extract is filtered through whatman filter paper and the filtrate is used for phytochemical screening. The extracts were tested for the presence of various bioactive compounds such as phenolic compounds and tannins, saponins, flavonoids, glycosides, terpenoids, steroids, phytosterols, coumarins, alkaloids, carbohydrates and proteins using the standard methods of Sofowara 1993, Harborne, 1998[11] [12].

C. Determination of Saponin Content

The saponin content was determined by the method of Obadoni and Ochuko, 2001[13]. Twenty gram of the finely ground powdered sample was weighed and put into a conical flask. 100 ml of 20% aqueous ethanol was added into the sample. The sample was then, heated over a hot water bath for four hours with continuous stirring at about 55˚C. The mixture was then filtered and re-extracted with another 200 ml of 20% ethanol. The combined extract was reduced to about 40 ml over a hot water bath at 90˚C. The concentrate was transferred into 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 ml of n-butanol was added. The combined extract was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was, then, dried in the oven to a constant weight. The amount of saponin content in the sample was calculated as percentage.

D. Determination of Alkaloid Content

The alkaloid content of the samples was determined by the procedure of Harborne (1973) [14]. Five gram of the finely ground plant sample was mixed with 50 ml of 10% acetic acid in absolute ethanol and the solution was allowed to stand for 4 hours. The mixture was then, filtered through Whatman No.1 filter paper and the filtrate was concentrated to 1/4th of its original volume on a water bath maintained at 90˚C. The alkaloid was precipitated from the sample using concentrated ammonium hydroxide (NH_4OH) and then, allowed to sediment. The precipitate was then filtered and washed with concentrated NH_4OH and the, dried in a hot air oven. The residue is alkaloid and can be calculated as:

$$\text{Alkaloid \%} = \frac{W_2 - W_1}{W} \times 100$$

Where,

W_1=Initial weight before drying
W_2=Final weight after drying
W= Weight of Sample

E. Determination of Flavonoid Content

The total flavonoid content in the plant sample was determined by the method described by Kim *etal.*, 2003[15]. The 100 mg of the plant sample was extracted with 100 ml of the methanol at room temperature and filtered through Whatman No. 1 filter paper. Aliquots of 50µl was appropriately diluted to 250 µl of distilled water and then, 150µl of 5% $NaNO_2$ solution was added to the solution and the mixture was allowed to react for 5 minutes. Following this, 30µl of 10% $AlCl_3$ was added. The mixture was then incubated at ambient temperature (25ºC) for an additional 5 minutes. Finally, the reaction mixture was treated with 100µl of 1M NaOH. The mixture was immediately diluted by the addition of 55µl of the distilled water. The mixture was vigorously shaken and the absorbance was taken at 510 nm against a blank prepared similarly by replacing the extract with double distilled water. The total flavonoid content was calculated from calibration curve using Catechin equivalents (CTE).

F. Determination of Terpenoid Content

About Ten gram of the powdered plant sample was taken and soaked in alcohol for 24 hours. The extract was filtered and the filtrate was extracted with petroleum ether. This extract was treated as total terpenoid (Ferguson, 1956) [16].

G. Determination of Tannin Content

Five hundred mg of the accurately weighed plant sample was transferred into 250 ml plastic bottle. Fifty ml of the distilled was gradually added and

shaken vigorously for an hour in a mechanical shaker. Then, the solution was filtered into a 50 ml volumetric flask and made up to the mark. Then, 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 minutes. The amount of tannin content in the sample was calculated from the calibration curve using Tannic acid as standard. The result was expressed in terms of mg per gram Tannic acid equivalent (mg/g TAE) [17].

H. Physicochemical Analysis

The physicochemical analysis (i.e., moisture content total ash content, water soluble ash content, acid insoluble ash content, sulphated ash content) of the plant sample was carried out using the method adopted by United States Pharmacopoeia-National Formulary (2003)[18].

Statistical Analysis

All the analyses were done in triplicates and the results were statistically analyzed and expressed as mean± Standard Deviation (S.D.).

III. RESULT AND DISCUSSION

Primary and secondary metabolites of seeds of *S. oleosa* were qualitatively and quantitatively analysed (Table 1 and 2). Experimental evaluation indicated the *S. oleosa* rich in primary carbohydrates, proteins as well as secondary phytoconstituents such as saponins, phenolic compound and tanins, alkaloids, glycosides, phytosterols, terpenoids, coumarins and Flavonoids. Out of the seven seed extracts, the ethanolic extract showed maximum number of plant constituents such as carbohydrates, proteins, saponins, phenolic compounds and tannins, alkaloids, glycosides, phytosterols, terpenoids, coumarins and flavonoids followed by aqueous extract (carbohydrates, proteins, saponins, phenolic compounds and tannins, alkaloids, glycosides, terpenoids, coumarins and flavonoids); methanolic extract (carbohydrates, proteins, saponins, Phenolic compounds and tannins, terpenoids, coumarins and flavonoids), acetone (Phenolic compounds and tannins, terpenoids, coumarins and flavonoids), ethyl acetate (phenolic compounds and tannins, terpenoids and flavonoids), petroleum ether (phenolic compounds and tannins) and chloroform (terpenoids) extract. The content of saponin in seed is 1.38±1.97%, alkaloid 0.73±0.29%, terpenoid 0.86±0.08%, flavonoid 2.43±0.06 mg/g and tannin 0.29±0.16 mg/g (Table.2).

The total moisture content in the powdered seeds of this plant was found to be 12.17±0.45%. The total ash value and water soluble ash contents is 6.12±1.34% 3.16±0.71%. It was found that most of the total ash is soluble in acid. Therefore, the acid insoluble value of ash content is low i.e., 1.83±0.91%. The sulphated ash content was found to be 1.56±0.34%. The results of moisture content, total ash, water soluble ash, acid insoluble ash and sulphated ash analyses along with extraneous matter and pH of the seed extract are given in Table. 3 and Table. 4.

The seeds of *S. oleosa* are full of primary as well as secondary metabolites. There has been an increasing interest in research on saponin from plant sources due to their versatile health benefits such as immunomodulating, antiphlogistic, antihepatotoxic,antibacterial, hypoglycemic, antifungal, molluscicidal and antiallergic activities [19]. The alkaloids also exhibit a broad range of biological activities including anti-inflammatory, antihepatofibrogenetic, immunomodulatory, neuroprotective, antibacterial, antifungal, antidiarrheal, hypolipidemic and anticancer properties [20]. Flavonoids have received numerous attention due to their antioxidative, free radical scavenging, hepatoprotective, anticancer and antiviral activities. They also play an important role in combating oxidative stress [21]. Similarly, terpenoids are the large group of natural products with more than 4000 individuals that exhibits cytotoxicity against cancer cells having promising potential in liver cancer therapy [22] The secondary metabolites are of particular interest in nutraceutical [23], Pharmaceuticals [24] and many other R & D industries. Its utilization, conservation and propagation should be taken and the exploitation of these secondary metabolites from the *Schleichera oleosa* is to be taken in the national programme of Ayush in order to meet the requirements and necessity of the country.

Table. 1: Phytochemical screening of bioactive constituents in extracts of seeds of *Schleichera oleosa* (Lour.) Oken.

S.N.	Phytochemicals	Test Performed	Seed Extracts in Different Solvents						
			AE	EE	ME	ACE	EAE	PE	CE
1.	Carbohydrates	Benedict's test	++	+	+	-	-	-	-
		Molisch's test	+	+	-	-	-	-	-
2.	Proteins	Biuret test	++	+	+	-	-	-	-
		Xanthoproteic test	+	+	-	-	-	-	-
3.	Saponins	Foam test	++	++	++	-	-	-	-
4.	Phenolic compound and Tanins	Ferric chloride test	++	++	++	+	+	+	-
5.	Alkaloids	Hager's test	-	+	-	-	-	-	-
		Dragendroff's test	+	-	-	-	-	-	-
6.	Glycosides	Legal's test	+	+	-	-	-	-	-
		Keller Killiani test	-	+	-	-	-	-	-
7.	Phytosterols	Salkowski test	-	+	-	-	-	-	-
8.	Steroids	Libermann Burchard test	-	-	-	-	-	-	-
9.	Terpenoids	Libermann Burchard test	+	+	+	+	+	-	+
10.	Coumarins	Fluorescence test	+	+	+	+	-	-	-
11.	Flavonoids	Alkaline Reagent test	++	++	++	+	+	-	-

+ = Present, - = Absent. AE= Aqueous Extract, EE= Ethanol Extract, ME=Methanol Extract, ACE= Acetone extract, EAE= Ethyl acetate Extract, PE= Petroleum Ether Extract, CE=Chloroform Extract.

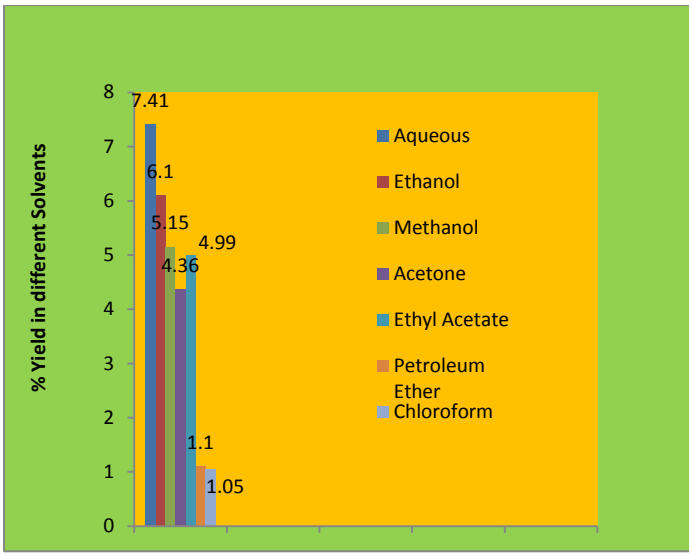

Figure. 1: Percentage yield of seeds of *S. oleosa* (Lour.) Oken in different solvents.

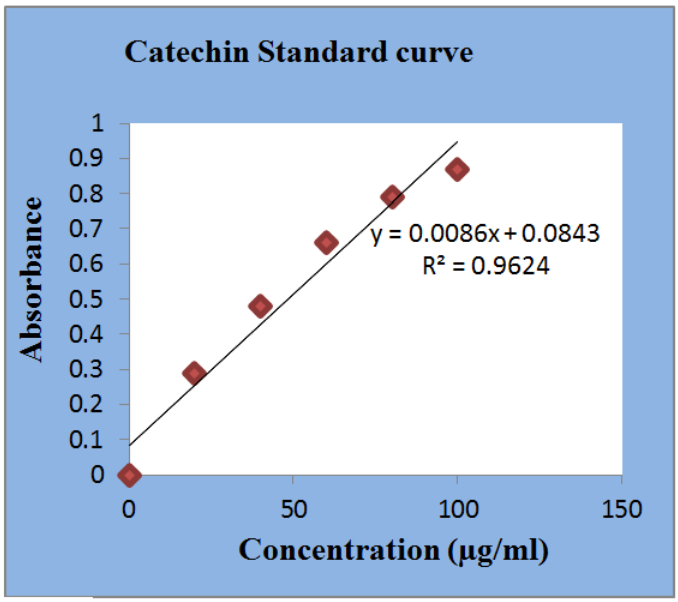

Figure. 2: Catechin Standard Curve

Table. 2 Quantitative Estimation of Secondary Metabolites of *S. oleosa* (Lour.) Oken

S.No.	Secondary Metabolites	Value±S.D.
1.	Saponin (%)	1.38±1.97
2.	Alkaloid (%)	0.73±0.29
3.	Terpenoid (%)	0.86 ±0.08
4.	Flavonoid (mg/g)	2.43±0.06
5.	Tannin (mg/g)	0.29±0.16

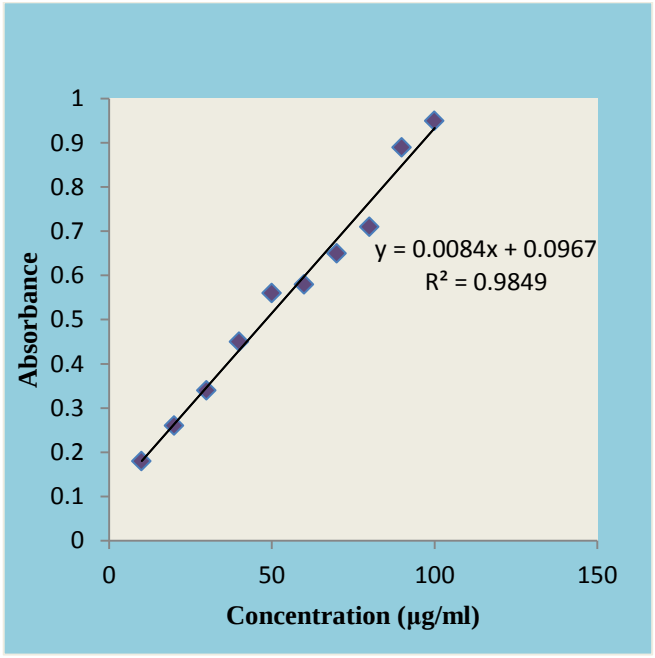

Figure. 3: Tannin acid Standard Curve

Table. 3: Analysis of Total moisture content, total Ash, water soluble, acid insoluble ash and sulphated ash.

S.No.	Parameter	(%) Mean±S.D.
1.	**Moisture Content**	12.17±0.45
2.	**Total Ash**	6.12±1.34
3.	**Water Soluble Ash**	3.16±0.71
4.	**Acid insoluble ash**	1.83±0.91
5.	**Sulphated ash**	1.56±0.34

Table. 4: Analysis of Foreign (Extraneous) matter and pH of seeds of *S. oleosa*.

S.No.	Parameter	Value
1.	**Foreign matter**	Nil
2.	**pH**	6.26±0.05

IV. CONCLUSION

The preliminary phytochemical study of the different seed extracts indicated the presence of various bioactive phytoconstituents i.e., carbohydrates, proteins as well as secondary metabolites i.e., phenolic compounds and tannins, saponins, terpenoids, glycosides, flavonoids, etc. Thus, the seeds of *S. oleosa* can be used to cure various ailments as well as have a great potential to be in pharmaceutical industries for preparation of herbal drugs. The study also leads to the further research for the isolation and the identification of active compounds in the seeds of the plant.

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