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PHARMACOGNOSTICAL, PHYTOCHEMICAL AND *IN VITRO* ANTIOXIDANT POTENTIAL OF *SACCOPETALUM TOMENTOSUM* LEAF EXTRACTS

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Abstract

Biologically active compounds from natural sources are of interest as possible new drugs for different diseases. Over many centuries humans have been mining the bounties of nature for discovering natural products that have been used for the treatment of all human diseases. The genus Miliusa (Annonaceae) has over 60 species that are native to India, Bhutan, Australia, and New Guinea, but are mostly found in numerous Asian nations such as Vietnam, Thailand, and China. *Saccopetalum tomentosum* (Annonaceae, *S. tomentosum*) is also known as hoom, kari. It is a huge deciduous tree that may reach a height of 20 metres. Further synonymous name for *Saccopetalum tomentosum* is Uvaria *tomnentosa* or *Miliusa tomnentosa*. *S. tomentosum* are used as antibacterial, anticancer, anthelmintic, antiparasitic and pesticidal agents. The objective of this study was to investigate pharmacognostical, phytochemical features and *in vitro* antioxidant activities of leaf of *S. tomentosum* collected from Bhopal region of Madhya Pradesh. The different pharmacognostical parameters were evaluated as per standard protocols with some modifications. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic and flavonoids was carried out by Folins Ciocalteau reagent method and aluminium chloride method respectively. The *In vitro* antioxidant activity of chloroform, ethyl acetate and ethanolic extract of the leaf was assessed against DPPH free radical scavenging assay, SOS activity and reducing power assay method using standard protocols. Phytochemical analysis revealed the presence of carbohydrates, glycosides, alkaloids, flavonoids, triterpenoids and steroids ect. The total phenolics content of leaves ethanolic extract was (323.667mg/100mg), followed by flavonoids (448.333mg/100mg). The activities of all leaves extracts against DPPH, SOS and reducing power assay method were concentration dependent. The diverse array of phytochemicals present in the plant thus suggests its therapeutic potentials which may be explored in drug manufacturing industry as well as in traditional medicine.

Keywords: *Saccopetalum tomentosum*, Pharmacognostical, Phytochemical, Antioxidant, DPPH, SOS, Reducing power assay.

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Introduction

There has been intense interest recently among the public and the media in the possibility that increased intake of dietary antioxidants may protect against chronic diseases, which include cancers, cardiovascular, and cerebrovascular diseases. Antioxidants are substances that, when present at low concentrations, compared with those of an oxidizable substrate, significantly prevent or delay a pro-oxidant-initiated oxidation of the substrate [1]. A pro-oxidant is a toxic substance that can cause oxidative damage to lipids, proteins, and nucleic acids, resulting in various pathological events or diseases. Examples of pro-oxidants include reactive oxygen and nitrogen species (ROS and RNS), which are products of normal aerobic metabolic processes. ROS include superoxide ($O_2^{-}\cdot$), hydroxyl (OH·), and peroxyl (ROO·) radicals, and hydrogen peroxide (H_2O_2). RNS include nitric oxide (NO·) and nitrogen dioxide ($NO_2\cdot$) [2, 3]. There is considerable biological evidence that ROS and RNS can be damaging to cells and, thereby, they might contribute to cellular dysfunction and diseases. The existence and development of cells in an oxygen-containing environment would not be possible without the presence of a complicated antioxidant defense system that includes enzymatic and nonenzymatic components. The nonenzymatic antioxidants, most of which have low molecular weights and are able to directly and efficiently quench ROS and RNS, constitute an important aspect of the body's antioxidant system components [4]. The interaction among these antioxidants and the difficulty in measuring all of them individually prompted the development of assays for measuring total antioxidant capacity. The measurement of total antioxidant capacity of all these nonenzymatic antioxidants is necessary and important in evaluating in vivo antioxidant status in many clinical and nutritional studies. During the last decade, there was a growing demand for natural plants having diverse activities towards diseases especially chronic ones that need long term management [5]. Annonaceae is a pantropical plant family that includes shrubs, trees, and lianas. There are around 130

genera and 2300 species in the family. Although Annonaceae's location within the Angiosperms and order Magnoliales, as well as its family circumscription, is unambiguous and undisputed [6]. Annonaceae plants are utilised as antibacterial, anticancer, anthelmintic, antiparasitic, and pesticidal agents [7]. The genus *Miliusa* (Annonaceae) has around 40 species that grow in the tropical rainforests of India, Thailand, South China, and North Australia [8]. Miliusa species range in size from tiny to huge trees and may be found in a variety of rainforest ecosystems. In Australia, only three species of the genus Miliusa exist, all of which are indigenous to the country and contain two essential oils [9]. The herb is used in traditional medicine to treat a variety of symptoms, including gastropathy and glomerulonephropathy [10]. *S. tomentosum* oil has been discovered to have antibacterial and analgesic effects in Chinese traditional medicine [11]. Knowledge of plant chemical ingredients is desirable since it will be useful in the production of complicated chemical molecules [12]. Two novel isoquinoline alkaloids, 2,10-dimethoxy-3,11-dihydroxy-5,6-dihydroprotober -berine and 1,9-dihydroxy-2,11-dimethoxy-4,5-dihydro-7-oxoaporphine, were recovered from ethanolic preparations of M. cuneata (Graib) stem and leaves, together with thirteen recognized alkaloids [13]. *S. tomentosum* is one of them, and while its traditional uses are unknown, its fruits are eaten in some parts of India, and its tree produces a pale yellow gum known as karee gum [14]. The aim of this work was to determine the quality (types), quantity (amount) of bioactive compounds and in vitro antioxidant activity of leaf of *S. tomentosum* in Bhopal region of Madhya Pradesh.

Materials and Methods

Plant material

The Pinnacle Biomedical Research Institute (PBRI), near the Bharat Scout and Guides Campus, Shanti Marg, Shyamla Hills Road, Depot Chouraha, Bhopal, Madhya Pradesh 462003, India, collected the leaves of *S. tomentosum*. Botanist Dr. Saba Naaz from the Saifia College of Science in Bhopal's Department of Botany carried out the plant's identification and authentication. For future use, a voucher specimen with the number 287/Saif./Sci./Clg/Bpl for *S. tomentosum* was conserved in the department of botany at Saifia College of Science, Bhopal.

Chemical reagents

The Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India), and SRL Pvt. Ltd. (Mumbai, India) provided all the chemicals used in this work. The investigation only employed analytical-grade compounds.

Pharmacognostical evaluation

Total ash value

Accurately weighed 5gms of powdered leaves of *S. tomentosum* were taken in a dried silica crucible. It was incinerated at 600 ˚C temperature, until free from carbon and then cooled. The weight of ash was taken and the percentage of it was calculated with reference to the air-dried sample. The percentage of total ash was calculated with reference to the air-dried powder [15].

$$\% \text{ Ash content} = \frac{\text{Weight of crucible + ash} - \text{Weight of crucible}}{\text{Weight of crucible + sample} - \text{Weight of crucible}} \times 100$$

Loss on drying

Accurately weighed 5gms of powdered leaves of *S. tomentosum* were taken in a crucible. It was kept in a hot air oven at 105-110 ˚C, until free from moisture. The percentage of moisture content was then calculated with reference to the air-dried sample.

$$\text{LOD \%} = \frac{\text{Wt. of petridish + crude drug} - \text{After drying Wt. of petridish + sample}}{\text{Weight of crude drug}} \times 100$$

Water soluble ash

The total ash obtained was boiled with 25 ml of water for few minutes, filtered and the insoluble matter was collected on ashless filter paper. Then, it was washed with hot water, ignited in silica crucible for 15 minutes at temperature not exceeding 450˚C, cooled and weighed the obtained residue. The difference in weight represents the water soluble ash. Finally, the percentage of water soluble ash was calculated with reference to the air-dried sample [15].

$$\% \text{ Water soluble ash} = \frac{\text{Weight of crucible + ash} - \text{Weight of crucible}}{\text{Crude drug weight}} \times 100$$

Acid insoluble ash

The total ash obtained was boiled for 5 minutes with 25 ml of 2 N HCl, filtered and the insoluble matter was collected on ashless filter paper. Then, it was washed with hot water, ignited in silica crucible for 15 minutes at temperature not exceeding 450 ˚C, cooled and weighed the obtained residue. The percentage of acid insoluble ash was calculated with reference to the air-dried sample [15].

$$\% \text{ Acid soluble ash} = \frac{\text{Weight of crucible + ash} - \text{Weight of crucible}}{\text{Crude drug weight}} \times 100$$

Alcoholic extractive value

Macerated 5 gm of the air dried coarsely leaves powder with 100 ml of 95 % ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter, it was filtered rapidly taking precautions

against loss of the solvent. Evaporated 25 ml of the filtrate to dryness in a tarred flat bottom shallow dish dried at 105^0 C and weighed. The percentage of ethanol soluble extractive was calculated with reference to the air dried drug [16].

Alcohol soluble extractive value = Weight of reside /Weight of the drug ×100

Water soluble extract

Macerated 5 gm of the air dried coarsely leaves powder with 100 ml of chloroform water in a closed flask for 24 hours. Shaking frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Evaporated 25 ml of the filtrate to dryness in a tarred bottom flat bottom shallow dish dried at 105°C and weighed. The percentage of water soluble extractive value was calculated with reference to the air dried drug [16].

Water soluble extractive value = Weight of reside /Weight of the drug ×100

Hot soxhlet extraction method

This technique involved gathering, correctly washing, and properly rinsing the blossoms of *S. tomentosum*. They were mechanically pulverised after being shade-dried. The plant material from *S. tomentosum*, either whole or coarsely powdered, was successively extracted using solvents such as petroleum ether, ethyl acetate, chloroform, and ethanol in increasing polarity order for various lengths of time. The Soxhlet apparatus' chamber was filled with powder using a "thimble" design. The solvent used for extraction was heated in flasks, and its vapours were then condensed in a condenser. The powder is extracted by touch when the condensed extractant is dropped into the thimble holding it. The liquid inside the chamber syphon drops into the flask when the liquid level in the chamber reaches the top of the syphon tube. This procedure was continued until an evaporated drop of solvent from the syphon tube did not leave any residue. The resulting extract was filtered, dried by concentration, weighed, and stored for later use [17]. The following formula is used to determine the extract's yield.

Yield (%) = Weight of the residue obtained×100

Weight of the plant material taken

Phytochemical screening of the extract

A variety of phytoconstituents, including alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids, and flavonoids were qualitatively analysed in the leaves extracts of *S. tomentosum* [18, 19] .

Quantification of secondary metabolites

For the purpose of estimating the quantity of phytoconstituents contained in plant extracts, quantitative analysis is a crucial instrument. TPC and TFC are established for this. TPC and TFC levels were determined using a conventional technique using extracts from the leaves of *S. tomentosum*.

Total phenolic content estimation

The Folin Ciocalteu reagent was used to calculate the total phenolic content of the extracts. Gallic acid concentration (20-100 µg/ml) was produced in methanol. Concentrations of 100 µg/ml of plant extract were also made in methanol, and 0.5 ml of each sample was added to the test along with 4 ml of 7.5% sodium carbonate and 2 ml of a 10 fold diluted folin Ciocalteu reagent. The tubes were parafilm-covered, and after 30 minutes of intermittent shaking at room temperature, the absorbance at 760 nm was measured using methanol as a blank. Gallic acid's conventional regression curve was used to compute the total phenol content, and the results were given in milligrammes per gramme (mg/g) of gallic acid [20].

Total flavonoid content estimation

Rutin (20 to 100µg/ml) was produced in methanol at various concentrations. Test samples with a polarity of 100µg/ml or close to it were created. A sample that had been diluted to 0.5 ml was combined with

2 ml of distilled water before being added to 0.15 ml of a 5% NaNO2 solution. After waiting for 6 minutes, 0.15 ml of a 10% AlCl3 solution was added. The combination was then given 5 minutes to stand before receiving 2 ml of a 4% NaOH solution. After reducing the final volume to 5ml with distilled water, the mixture was let to stand for an additional 15 minutes. At 510 nm, the absorbance was calculated using water as the reference. The standard regression curve of quercetin and rutin was used to compute the total flavonoid content [20].

In vitro anti oxidant activity of plant extract

DPPH assay

Free radical scavenging activity of the extracts of *S. tomentosum* leaves, based on the scavenging activity of the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined by the method of Ali et al [21]. Different volume of extracts/standard (20-100µg/ml) was taken from stock solution in a set of test tubes and methanol was added to make the volume to 1 ml. To this, 2 ml of 0.1mM DPPH reagent was added and mixed thoroughly. Absorbance at 517 nm was determined after 30 min and the percentage inhibition activity was calculated by using the equation: % scavenging activity=[(A0-A1)/A0]×100. Where A0 is the absorbance of the control and A1 is the absorbance of the extract. Lower the absorbance, the higher is the free radical scavenging activity. The curves were prepared and the IC50 value was calculated using linear regression analysis.

Superoxide anion radical scavenging activity

1 ml of nitroblue tetrazolium (NBT) (100 µl of NBT in 100 mM phosphate buffer, pH 7.4), 1 ml of NADH (468 µl in 100 mM phosphate buffer, pH 7.4), solution as well as varying volumes of extracts of *S. tomentosum* leaves (20, 40, 60, 80 and 100 µg/ml), were mixed well with methanol. The reaction was started by the addition of 1 ml of phenazine methosulfate (PMS) (60 µl/100 mM phosphate buffer, pH 7.4). The reaction mixture was incubated at 30°C for 15 min. The absorbance was measured at 560 nm in a spectrophotometer. Incubation without the sample (extract) was used as a blank sample. Ascorbic acid was used as the standard in comparing the different sample. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity [22]. The percentage scavenging was calculated by using the formula shown below:

% Inhibition = [(Ab of control- Ab of sample/ Ab of control x 100]

Reducing power assay

Preparation of standard solution

3 mg of ascorbic acid was dissolved in 3 ml of distilled water/solvent. Dilutions of this solution with distilled water were prepared to give the concentrations of 20, 40, 60, 80 and 100µg/ml.

Preparation of extracts

Stock solutions of extracts of *S. tomentosum* leaves were prepared by dissolving 10 mg of dried extracts in 10 ml of methanol to give a concentration of 1mg/ml. Then sample concentrations of 20, 40, 60, 80 and 100µg/ml were prepared.

Protocol for reducing power

According to this method, the aliquots of various concentrations of the standard and extracts of *S. tomentosum* leaves (20 to 100µg/ml) in 1.0 ml of deionized water were mixed with 2.5 ml of (pH 6.6) phosphate buffer and 2.5 ml of (1%) potassium ferricyanide. The mixture was incubated at 50°C in water bath for 20 min after cooling. Aliquots of 2.5 ml of (10%) tri-chloroacetic acid were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution 2.5 ml was mixed with 2.5 ml distilled water and a freshly prepared 0.5 ml of (0.1%) ferric chloride solution. The

absorbance was measured at 700 nm in UV spectrometer (Systronic double beam-UV-2202). A blank was prepared without adding extract. Ascorbic acid at various concentrations (20 to 100µg/ml) was used as standard [22].

Results

Pharmacognostical evaluation

Table 1: Pharmacognostical evaluation of plant sample

Parameters	Value in percentage (%)
	S. tomentosum
Total ash value	5.41
Loss on drying	12.23
Water soluble ash	11.58
Acid insoluble ash	0.88
Water extractive value	2.98
Alcoholic extractive value	13.72

Plant extraction

The plant material was extracted by soxhlet extraction method and the percentage yield calculated by the following formula:-

$$\% \text{ yield} = \frac{\text{Actual Yield} \times 100}{\text{Theoretical yield}}$$

Table 2: Results of percentage yield of leaves extracts

S. No.	*S. tomentosum* Extract	% Yield
1	Pet. ether extract	1.61
2	Chloroform extract	5.84
3	Ethyl acetate extract	12.45
4	ethanolic extract	14.67

Phytochemical analysis

Table 3: Phytochemical evaluation of *S. tomentosum* leaves

S. No.	Experiment	Result		
		Ethyl acetate	**Chloroform**	**Ethanol**
Test for Carbohydrates				
1.	Molisch's Test	+	+	+
2.	Fehling's Test	+	+	+
3.	Benedict's Test	+	+	+
4.	Bareford's Test	+	+	+
Test for Alkaloids				
1.	Mayer's Test	+	+	+

2.	Hager's Test	-	+	+
3.	Wagner's Test	-	+	+
4.	Dragendroff's Test	-	+	+
Test for Terpenoids				
1.	Salkowski Test	+	-	-
2.	Libermann-Burchard's Test	+	+	-
Test for Flavonoids				
1.	Lead Acetate Test	+	-	+
2.	Alkaline Reagent Test	-	-	+
3.	Shinoda Test	-	+	+
Test for Tannins and Phenolic Compounds				
1.	$FeCl_3$ Test	+	+	+
2.	Lead Acetate Test	+	-	+
3.	Gelatine Test	-	-	-
4.	Dilute Iodine Solution Test	+	+	+
Test for Saponins				
1.	Froth Test	+	+	+
Test for Protein and Amino acids				
1.	Ninhydrin Test	-	+	+
2.	Biuret's Test	-	-	-
3.	Million's Test	-	-	-
Test for Glycosides				
1.	Legal's Test	-	-	+
2.	Keller Killani Test	-	-	+
3.	Borntrager's Test	-	-	+

Quantitative phytochemical analysis

Total phenolic content (TPC) estimation

Table 4: Total Phenolic content (TPC) of extract of *S. tomentosum* leaves

	Total phenolic content (mg/gm equivalent to Gallic acid)		
Extracts	**Chloroform**	**Ethyl acetate**	**Ethanol**
Absorbance Mean±SD	0.172±0.003	0.144±0.008	1.325±0.024
TPC	47.167	123.333	323.667

Total flavonoid content (TFC) estimation

Table 5: Total Flavonoid content (TFC) of extract of *S. tomentosum* leaves

	Total flavonoid content (mg/gm equivalent to rutin)		
Extracts	**Chloroform**	**Ethyl acetate**	**Ethanol**
Absorbance Mean±SD	0.236±0.004	0.324±0.003	0.456±0.005
TFC	128.333	216.333	448.333

***In vitro* anti oxidant activity**

Table 6: DPPH assay of ascorbic acid, ethyl acetate, chloroform and ethanolic extract

S. No.	**Conc. (µg/ml)**	**Ascorbic acid (% Inhibition)**	**Ethyl acetate Extract (% Inhibition)**	**Chloroform Extract (% Inhibition)**	**Ethanolic Extract (% Inhibition)**
1.	**20**	55.04	48.50	36.46	50.44
2.	**40**	59.65	51.50	40.71	55.44
3.	**60**	65.13	55.58	49.73	58.65
4.	**80**	75.75	58.41	53.81	64.88
5.	**100**	84.25	66.19	60.35	71.66
IC 50 Value		**11.80**	**31.56**	**65.98**	**20.69**

Table 7: Superoxide anion radical scavenging activity

S. No.	**Conc. (µg/ml)**	**Ascorbic acid % Inhibition**	**Ethyl acetate % Inhibition**	**Chloroform % Inhibition**	**Ethanol % Inhibition**
1	20	57.66	49.39	42.84	53.72
2	40	65.02	53.83	52.52	56.95
3	60	73.28	65.42	62.39	64.41
4	80	83.77	77.21	65.82	71.27
5	100	92.23	83.77	71.16	82.05
	IC 50	**4.51**	**25.47**	**34.48**	**15.85**

Table 8: Result of reducing power assay

S. No.	Conc. (µg/ml)	Absorbance of Ascorbic acid	Absorbance of Ethyl acetate extract	Absorbance of Chloroform extract	Absorbance of Ethanolic extract
1.	20 µg/ml	0.127	0.091	0.051	0.102
2.	40 µg/ml	0.184	0.15	0.084	0.167
3.	60µg/ml	0.268	0.226	0.141	0.247
4.	80µg/ml	0.374	0.254	0.181	0.273
5.	100µg/ml	0.452	0.298	0.232	0.338

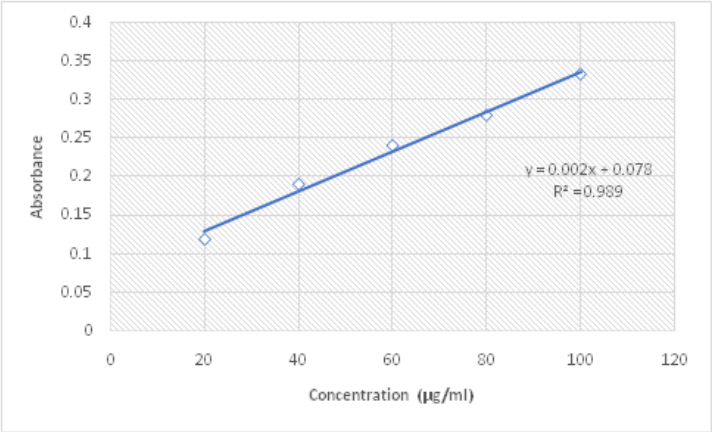

Figure 1: Graph represent standard curve of Gallic acid

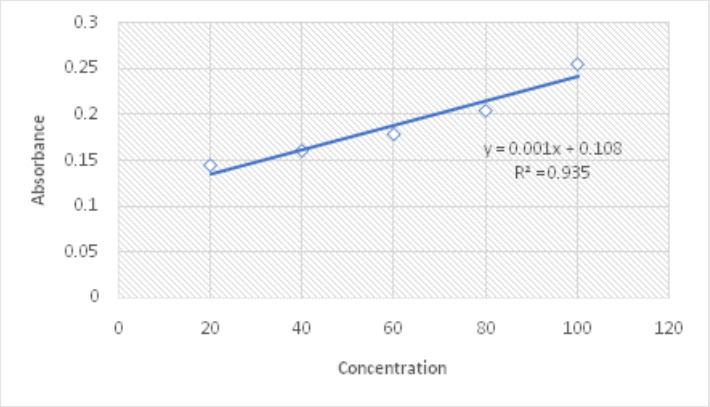

Figure 1: Graph represent standard curve of Rutin

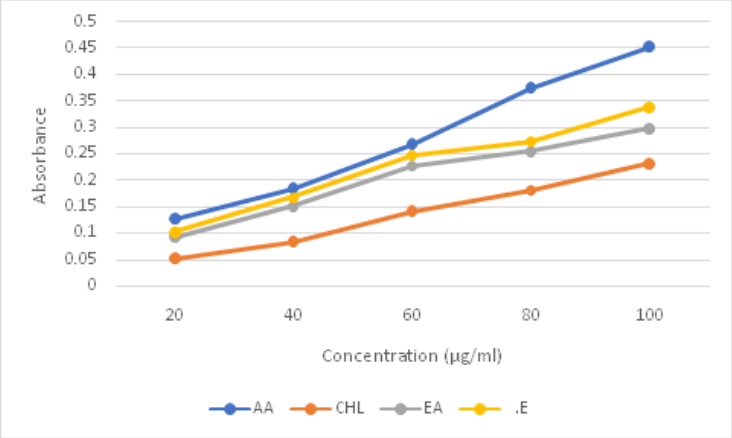

Figure 3: Reducing power assay

Discussion

The present study deals with the studies on pharmacognostic, phytochemical, and antioxidant activity on leaves of *S. tomentosum*. Raw materials were analyzed for identity, quality and purity as per the standards prescribed by WHO and Ayurvedic Pharmacopeia of India. The loss on drying of dry powder of *S. tomentosum* was 12.23%. The ash value was determined by three different forms viz., total ash, water soluble ash and acid insoluble ash. The total ash of crude powder of *S. tomentosum* was found to be 5.41%, water soluble ash was 11.58 and acid insoluble ash was 0.88%. The water and alcoholic extractive value of crude powder of *S. tomentosum* was found to be 2.98 and 13.72% Table 1. The crude extracts so obtained after each of the successive hot soxhlet extraction process were concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The percentage yield of extraction is very important in phytochemical extraction in order 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 the leaves of the plants using petroleum ether, ethyl acetate, chloroform and ethanol as solvents are depicted in the Table 2. The results of qualitative phytochemical analysis of the crude powder of leaves of *S. tomentosum* are shown in Table 3. Ethyl acetate, chloroform and ethanolic extracts of leaves sample of *S. tomentosum* showed the presence of carbohydrates, glycosides, alkaloids, flavonoids, triterpenoids and steroids ect. Phytochemicals present in plant act as the source for the treatment of different health problem. Different phytochemical have different therapeutic value. Total phenolic content (TPC) was measures by using Folin-ciocalteau's reagent method and total flavonoid content (TFC) of *S. tomentosum* was measured by Aluminum chloride method. The TPC and TFC of the extracts were expressed as milligram of gallic acid equivalent per gram of extracts i.e. mg GAE/g extract and milligram of rutin equivalent per gram of extract i.e. mg RE/g extract respectively. The TPC and TFC in ethyl acetate and ethanolic extract were found to be 123.333,323.667mg/gm and 216.333, 448.333mg/gm respectively Table 4, 5 & Figure 1, 2. Antioxidant plays a major role in protecting our body from disease by reducing the oxidative damage to cellular component caused by reactive oxygen species. Recent investigations suggest that the plant origin antioxidants with free-radical scavenging properties may have great therapeutic importance in free radical mediated diseases like diabetes, cancer, neurodegenerative disease, cardiovascular diseases, aging, gastrointestinal diseases,

arthritis, and aging process. The antioxidant activity of plant extracts were determined by different *in vitro* methods such as the DPPH free radical scavenging assay, SOS activity, and reducing power assay. DPPH radical scavenging activity of ethanolic extract of *S. tomentosum* exhibited percent inhibition % 71.66and its IC50 value was found to be 20.69µg/ml Table 6. Similarly, SOS scavenger activity of ethanolic extract of *S. tomentosum* exhibited percent inhibition 82.05% and its IC 50 value was found to be 15.85µg/ml Table 7. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Dietary antioxidant such as ascorbic acid was used for comparison. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants. The extract of *S. tomentosum* showed good reducing capacity Table 8 & Figure 3.

Conclusion

It can be concluded that from present investigation the observed level of phytoconstituents revealed that *S. tomentosum* is a rich source of antioxidant compounds proved by in vitro studies. Currently available synthetic antioxidants are suspected to cause or prompt negative health effects, hence strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. The broad range of activity of the extracts suggests that multiple mechanisms are responsible for the antioxidant activity. The multiple antioxidant activity of extract demonstrated in this study clearly indicates the potential application value of the *S. tomentosum.* However, the in vivo safety of *S. tomentosum* needs to be thoroughly investigated in experimental rodent models prior to its possible application as an antioxidant ingredient, either in animal feeds or in human health foods. Further studies, on the use of above plants for their antioxidant role in various systems may provide potential natural antioxidants.

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