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Original Article

PHYTOCHEMICAL PROFILING OF SUCCESSIVE EXTRACTS OF FRUIT AND STEM BARK OF *SOLANUM PUBESCENS*

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ABSTRACT

Objective: *Solanum* (L) is the most representative genus of *Solanaceae*, *Solanum* species are a rich source of bioactive compounds which are known to possess a variety of biological activities. *Solanum pubescens* is a wild plant growing abundantly as weed in forest and the hills of South-Eastern Ghats in Andhra Pradesh. This study purposed to investigate the phytochemical constituents present in different extracts of *Solanum pubescens*.

Methods: Solvents such as hexane, chloroform, ethyl acetate and ethanol, were used to isolate the bioactive compounds from fruit and stem bark. Fluorescence analyses of the plant powder and of different successive extracts were carried out under normal light and UV light.

Results: The preliminary phytochemical screening of fruit and stem bark has revealed that oils & fats, alkaloids, flavonoids, carbohydrates, saponins, coumarins and phenolics are present in different extracts. Interestingly, alkaloids are present only in the ethanol extract. The quantitative analysis revealed that *Solanum pubescens* is very rich in phenolics followed by flavonoids, alkaloids, saponins, carbohydrates and oils, which gives a very strong reason to select this plant for future evaluation for its pharmacological properties. It is interesting to note that this is the first report showing a thorough qualitative and quantitative analysis of fruit and stem bark of *Solanum pubescens.*

Conclusion: This study certainly helps us to detect specifically the cytotoxic compounds, which are presumed to play an important role in exerting curative properties of various ailments.

Keywords: *Solanum pubescens,* Fluorescence, Phytochemical screening, Alkaloids, Flavonoids.

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities in general. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Many of the indigenous medicinal plants are used as spices and food plants [1].

Plants synthesize a vast array of secondary metabolites that are important for human life. For therapeutic purpose, phytochemical investigation of plants is an interesting area of research, leading to the isolation of several new compounds. Therefore, in recent years research is more oriented towards folk medicine, searching for new leads for the development of better drugs against infectious diseases [2] and other common ailments.

Several plants belonging to the family *solanaceae* exhibited strong cytotoxic and antitumor properties. *Solanum trilobatum, Solanum incanum, Solanum capsicastrum, Solanum indicum, Solanum sodomaeumand, Solanum nigrum* are few species reported to possess potential antitumor activities. Reports indicate that steroidal alkaloids from the Solanum species possess strong cytotoxic and antitumor properties [3][4][5]. Solamargin from *Solanum nigram*, incanumine from *Solanum sodamaeum*, solasoinine from *Solanum crinitum* and several other steroidal alkaloidal glycosides are known to possess these properties [5]. Hence, based on these reports the present study was undertaken to screen the active constituents of*Solanum pubescens.*

Solanum pubescens is a wild plant. It is an annual erect, unarmed shrub growing upto 1.5m tall abundantly growing as weed of forest and the hills of South-Eastern Ghats in Andhra Pradesh and commonly known as Ushtichettu, Kasivuste and pajarito in Telugu and Kaattu sundai kaai in Tamil, flowering and fruiting is in the month of July to February. *Solanum pubescens* is a traditional medicine plant for the treatment of headache,menstrual pain, rheumatoid arthritis, tuberculosis, ulcers, etc.[6] and it has been used in the treatment of whooping cough and of certain other diseases [7]. Furthermore, it has been used in the treatment of hypoglycaemia and topical application for skin infections. Similarly, in scientific literature there are very few reports on evaluated pharmacological properties like antidiabetic[8], hepatoprotective[9], gastroprotective[10], anti-inflammatory[11], Anti-anxiety, Anti-depressants, Myorelaxant[12], and Antidiarrheal[13]. It has not been extensively used in the traditional medicine may be for its bitterness which may acts as cytotoxic agent. Thus for, *Solanum pubescens* has not been explored for a through quantitative and qualitative phytochemical analysis. There are no reports on its complete phytochemical contents except a few indicating the existence of flavonoids and alkaloids in leaf extract [14] [15] [16]. Hence, it is imperative take up a thorough phytochemical analysis to understand the major groups of phytoconstituents present in this plant. Keeping this in view, the present study was aimed to systematically screen the phytochemicals of this plant. It is believed that this study may pave the way for further high throughput analysis of the plant in terms of chemical characterization followed by the evaluation of important pharmacological properties.

MATERIALS AND METHODS

Plant material collection

Unripe fruits of *Solanum pubescens* and stem bark were collected from the surrounding hills of Rayadurg jurisdiction of Eastern Ghats, Anantapur Dist. Andhra Pradesh, India. The plant was confirmed by referring the Phytographia [17] followed by the authentication of a taxonomist Prof. Pullaiah, Dept. of Botany, Sri Krishnadevaraya University, Anantapur, Andhra Pradesh. The specimen is deposited at Department of Biotechnology, Kuvempu University, Shankarghatta, Karnataka.

Chemicals

Hexane, chloroform, ethyl acetate, ethanol and all the chemicals used for phytochemical analysis were purchased from Merck and Himedia. The chemicals and solvents used are of analytical grade.

Powder analysis

Fresh aerial parts of *Solanum pubescens* were collected and washed under running tap water to remove adhered filth. The fruit and stem bark material was then washed with distilled water, blotted and shade dried. The samples were pulverized in a mixer, sieved with a fine mesh sieve and used for organoleptic and fluorescence analysis.

Organoleptic evaluation

The taste and smell of the environment are important to humans in everyday life and are of particular relevance for the selection of medicinal versus non-medicinal plant species [18]. Organoleptic evaluation refers to the assessment of the selected plant drug by colour, odour, taste and texture, etc. The organoleptic characters of the samples were evaluated according to the methods of Jackson and Snowdown [19].

Fluorescence analysis

In the course of routine analysis, it is sometimes advantageous to apply rapid tests for the identification of purity of materials. Many substances such as alkaloids like quinines in diluted sulphuric acid when suitably illuminated emit light of different wavelength or colour from that which falls on them. This emitted light (fluorescence) ceases when the exciting light is removed [20]. Fluorescence analysis of *Solanum pubescens,* both the plant powder and different successive extracts as such and extracts dissolved in vehicle solvents were used for the analysis under ordinary and UV light adopting the method proposed by Kokoshi, and Chase [21] [22].

Soxhlet extraction

The fruits and stem bark were air dried and powdered and subjected for successive extraction in a Soxhelet extractor using solvents of increasing polarity i.e. Hexane, chloroform, ethyl acetate and ethanol. The extracts were concentrated to dryness under reduced pressure in a rotary evaporator to yield dried extracts.

Pilot solubility tests of plant extracts

Solubility tests were carried out for the analysis of solubility of crude extracts in different solvents like, hexane, chloroform, ethyl acetate, acetone, DMSO, ethanol, methanol, water, 1N NaOH, and 1N HCl.

Preliminary phytochemical screening of plant extracts

Phytochemical screening assay is a simple, quick, and inexpensive procedure, an important tool in bioactive compound analyses like carbohydrates, saponins, oils, fats, flavanoids, terpenoids, alkaloids etc. protocol described by Harborne[23]was followed with minor modifications for phytochemical screening.

Quantitative estimation of phytochemicalss

Total alkaloid estimation

Alkaloid determination was done using Harborne method [23]. 5 g of sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and precipitate was collected, washed with dilute ammonium hydroxide and then filtered. The crude alkaloids were dried and weighed.

Total saponin estimation

Saponin determination was done using Obadoni and Ochuko (2001) method [24]. 10 g of sample powder was taken into a conical flask and 100 ml of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue was re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously for purification. The aqueous layer was recovered, the purification process was repeated. Then 60 ml of n-butanol was added, the combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride where the solvent layer was recovered and heated on a water bath. After evaporation the samples were oven dried to a constant weight and the total saponin content of *Solanum pubescens* was calculated as percentage yield per gram.

Total phenolic content estimation

The concentration of total phenolics in the *Solanum pubescens* extracts were determined according to the protocol described by Chandler and Dodds (1993) [25]. 1 mL of each *Solanum pubescens* extract was mixed in a test tube containing 1 mL of 95% ethanol, 5 mL of distilled water and 0.5 mL of 50% Folin–Ciocalteu reagent. The resultant mixture was allowed to react for 5 min and 1 mL of 5% sodium carbonate was added, mixed thoroughly and placed in dark for 1 h. The absorbance was read at 725 nm using the UV–VIS spectrophotometer. The total phenolic contents in *Solanum pubescens* were expressed as gallic acid equivalents in microgram per gram of the extract.

Total flavonoid estimation

Aluminium Chloride Colorimetric method of Woisky *et al.,* (1998) [26] was followed for the determination of total flavonoid concentration of different extracts. 80% ethanolwas used to dissolve 10 milligrams of quercetin and diluted to 100 to 500 µg/ml to make the calibration curve. The diluted standard solutions (0.5 mL) were separately mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1M Potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. The amount of 10% aluminium chloride was substituted by the same amount of distilled water in blank. Similarly, 0.5 mL of different extracts which have shown positive results in preliminary phytochemical analysis were reacted with aluminum chloride for determination of flavonoid content as described above.

Total carbohydrate estimation

Total carbohydrate was estimated by Anthron method [27]. Dextrose was used for standard gradient preparation of 100- 500 µg. 400 µl of all the extracts were taken and the volume was made up to 1 ml to which 4 ml of anthron reagent was added. The reaction mixture was incubated in boiling water bath for 10 min. The absorbance was red at 620 nm.

Statistical analysis

Data are expressed as Mean ± S. E. All the assays were analysed by one-way analysis of variance. (ANOVA)

RESULTS

Organoleptic characters studies

Organoleptic study forms an important part of plant material analysis and is a method for the qualitative evaluation based on the study of morphological and sensory profiles of whole drugs. Its efficacy in plant profiling has been reported in many studies [28] [29]. The present investigation of the organoleptic characters of aerial parts of *Solanum pubescens* plant powder indicated the characters like colour, odour and taste, texture and size. The data is tabulated in Table1.

Table 1: Organoleptic study of the aerial parts of *Solanum pubescens*

Unripe fruit Stem bark		
Parameters	**Observation**	
Condition	Dried and crumpled	Dried
Colour	Shiny Brown	Light green
Odour	Pungent	Strong Aromatic
Taste	Peppery	Bitter
Texture	Smooth	Smooth with minute hairs
Size	5-8 mm	1-2 mm

Fluorescent analysis of plant material

The fluorescence characteristics of aerial parts of *Solanum pubescens* were studied under UV light adopting the method proposed by Kokoshi *et al.,* (1959)[21]. The behaviour of the samples with different chemical reagents was studied and fluorescence characters were analysed in normal and UV light at 366nm and tabulated in Table (2). Among the different solvents tested.

Fruit material with petroleum ether and hexane did not show any fluorescence. Other reagents showed characteristic colouration in both fruit as well as stem bark powder.

Table 2: Fluorescent analysis of aerial parts of *Solanum pubescens*

S. No.	Particulars of the treatment	Unripe Fruit		Stem bark	
		Normal light	UV light	Normal light	UV light
1.	Powder as such	Br	GW	LY	WG
2.	Powder + 1N NaOH (aqueous)	YBr	G	Y	G
3.	Powder +1N NaOH (alcoholic)	Y	LG	Y	G
4.	Powder + 50% HCl	R	G	Y	G
5.	Powder + 50%H2SO4	YR	G	Y	G
6.	Powder + 50%HNO3	O	G	Y	G
7.	Powder + Ammonia	YO	G	Y	G
8.	Powder + Iodine	YO	R	YO	R
9.	Powder + 5% FeCl3	O	R	Y	Br
10.	Powder + Nitric acid + Ammonia	O	G	Y	R
11.	Powder + Petroleum ether	T	T	T	LG
12.	Powder + Chloroform	LY	C	LY	R
13.	Powder + Methanol	Y	W	Y	W
14.	Powder + Hexane	T	T	T	LG
15.	Powder + Ethyl Acetate	LY	W	LY	LR
16.	Powder + Ethanol	LY	W	LY	C

G: Green, Y: Yellow, O: Orange, Br: Brown, LG: Light Green, R: Red, LR: Light red, T: Transparent, LY: Light yellow, YBr: Yellowish brown. W: White, C: Cream

Soxhlet extraction

300 gram of the powdered material of fruit and stem bark was refluxed separately with 1/10 (w/v) hexane, chloroform and ethanol in a soxhlet apparatus for 48 h. The percentage yield of hexane, chloroform, ethyl acetate and ethanol extracts from fruit and stem bark were calculated.

Among the fruit extracts the ethanolic extract has maximum yield of 15.45% followed by hexane (4.94 %), chloroform (3.41%) and ethyl acetate extracts (1.171%). Similarly, in stem bark extracts, ethanol extract showed maximum yield of (upper liquid, 11.59% and bottom crystals0.836 % followed by ethyl acetate, hexane and chloroform with 3.55%, 1.24% and 1.08% respectively. The results are tabulated in Table 3 and 4.

Table 3: Percent yield of *Solanum pubescens* fruit extracts (w/v)

Solvents	Material (g)	Nature of the extract	Yield %
Hexane	300	Light Greenish colour oil	4.94
Chloroform	300	Dark brown powder	3.41
Ethyl acetate	300	light yellow sticky mass with pungent smell	1.71
Ethanol	300	Dark brown sticky mass	15.45

Table 4: Percent yield of *Solanum pubescens* stem bark extracts (w/v)

Solvents	Material (g)	Nature of the extract	Yield %
Hexane	300	Light Greenish colour solid	1.24
Chloroform	300	Dark brown powder	1.08
Ethyl acetate	300	Light yellow sticky mass with pungent smell	3.55
Ethanol	300	Dark brown sticky mass (U L)	11.59
		Dark brown sticky mass with characteristic crystals (B C)	0.836

UL: Upper Liquid, BC: Bottom Crystals

Pilot solubility tests

Solubility tests of all extracts were performed using different solvents where all the extracts were soluble in DMSO followed by methanol, ethanol, ethyl acetate, chloroform, acetone and water, whereas, hexane extracts were soluble only in hexane. Furthermore, all the extracts have shown good solubility in 1N NaoH and 1N HCl but has shown very poor phytochemical results. The observations are tabulated in Table 5.

Selection of vehicle solvents

Among the solvents tested for pilot solubility analysis, those solvents that have fewer effects in *invivo* system are taken as vehicle solvents for further drug formulations as represented in Table 6.

FH was dissolved in ethanol, FC, FEA, BH, BC and BEA were dissolved in DMSO, whereas, both the ethanolic extracts of fruit and stem bark were dissolved in water.

Fluorescent studies of successive extracts and extracts with vehicle solvents

Fluorescent features of successive extracts and extracts dissolved in vehicle solvents were analysed as described above, and the results are tabulated in Tables 7 and 8.

Preliminary phytochemical screening

Preliminary phytochemical results in *Solanum pubescens* revealed the presence as well as absence of certain photochemical

constituents in the extracts such as alkaloids, flavonoids, steroids, tannins, and coumarins, carbohydrates, saponins, oils and fats etc. in fruit and stem bark extracts (Table 9).

The phytochemical evaluations of the extracts showed that among the crude extracts of fruit, the hexane extract contains only steroids, oils and fats. Flavonoids, tannins, steroids and coumarins are the only compounds present in the chloroform extracts, whereas, ethyl acetate contains carbohydrates, flavonoids, steroids, tannins and coumarins. However, the ethanol extract is very rich in alkaloids, carbohydrates, flavonoids, saponins, tannins and coumarins. It is noteworthy that the alkaloids and saponins are present only in the ethanol extract. Starch content is present in all the extracts of fruit except hexane extract.

Similarly, phytoconstituents of stem bark extracts revealed that the hexane extract and chloroform extract have shown the presence of flavonoids and phenolic content, whereas, ethyl acetate extracts has shown positive results for flavonoids, tannins and coumarins and it showed positive results for alkaloids only with Dragendorff's reagent. Furthermore, the two fractions of ethanol extract are rich in alkaloids followed by flavonoids, saponins and coumarins.

Table 5: Pilot solubility tests of all the extracts of *Solanum pubescens.*

S. pubescens **extracts**	**Solvents**									
	H	**C**	**EA,**	**A,**	**D,**	**EOL,**	**M**	**W**	**NaOH**	**Hcl**
FH	**+**	**-**	**-**	**+**	**-**	**++**	**-**	**-**	**-**	**-**
FC	**-**	**-**	**-**	**-**	**++**	**-**	**-**	**-**	**++**	**-**
FEA	**-**	**+**	**++**	**++**	**++**	**-**	**++**	**+**	**++**	**+**
FEol	**-**	**-**	**-**	**-**	**++**	**++**	**++**	**++**	**++**	**++**
BH	**+ Δ**	**++ Δ**	**++ Δ**	**+ Δ**	**++ Δ**	**+ Δ**	**+ Δ**	**-**	**+ Δ**	**+ Δ**
BC	**-**	**++**	**+ Δ**	**+ Δ**	**++ Δ**	**+ Δ**	**+ Δ**	**-**	**+ Δ**	**-**
BEA	**-**	**++**	**++**	**++**	**++**	**+**	**++**	**+ Δ**	**++**	**++**
BUL	**-**	**-**	**-**	**-**	**++**	**++**	**++**	**++**	**++**	**++**
BBC	**-**	**-**	**-**	**-**	**++**	**++**	**++**	**++**	**++**	**++**

H: hexane, C: chloroform, EA: ethyl acetate, A: acetone, D: DMSO, EOL: ethanol, M: methanol, W: water, 1N NaOH, and 1N Hcl, +: Partially soluble, ++: Complete soluble, **Δ**: on heating

Table 6: Preferred solubility of extracts for pharmacological evaluation

Fruit Extracts	**Solvent**	**Stem Bark Extracts**		**Solvent**
FH	Ethanol	**BH**		DMSO Δ
FC	DMSO	**BC**		DMSO Δ
FEA	DMSO	**BEA**		DMSO
FEol	Water	Ethanol	**B UL**	Water
			B BC	Water

FH: fruit hexane, FC: fruit chloroform, FEA: fruit ethyl acetate, FEol: fruit ethanol, BH: bark hexane, BC: bark chloroform, BEA: bark ethyl acetate, BUL: bark upper liquid, BBC: bark bottom crystals

Table 7: Fluorescent analysis of *S. Pubescens* extracts.

S. No.	**Extract**	**Under Normal Light**	**Under UV Light Short Wavelength**	**Under UV Light Long Wavelength**
01.	**FH**	Dark Green	Dark Green	Orange
02	**FC**	Dark Brown	Green	Green
03.	**FEA**	Reddish Brown	Greenish Black	White and Green
04.	**FEol**	Reddish Brown	Greenish Black	Brownish Red
05.	**BH**	White & Light Green	White & Light Green	Red
06.	**BC**	Brown	Dark Green	Brownish Red
07.	**BEA**	Yellowish Brown	Greenish Black	Red and Green
08.	**BUL**	Yellowish Brown	Greenish Brown	Red and Green
09.	**BBC**	Brown	Dark Green	Reddish Brown

Table 8: Fluorescent analysis of *S. pubescens* extracts dissolved in vehicle solvents.

S. No.	**Extract**	**Under Normal Light**	**UV Light Short Wavelength**	**UV Light Long Wavelength**
01.	**FH**	Light Green	Green	Orange
02	**FC**	Light Brown	Dark Green	Creamish white
03.	**FEA**	Light Brown	Green	Light Green
04.	**FEol**	Bark Brown	Dark Green	Light Green
05.	**BH**	Light yellow	Dark Green	Creamish White
06.	**BC**	Yellow	Dark Green	White
07.	**BEA**	Yellow	Light Green	Light Brown
08.	**BUL**	Brown	Dark Green	Rad
09.	**BBC**	Yellow	Green	Whitish green

Quantitative estimation of phytochemicals of *Solanum pubescens*

The phenolics, flavonoids, and carbohydrates in *Solanum pubescens* extract were quantified spectrophotometrically. The analysis of total phenolic content in fruit extracts was estimated, where the ethanol extract was rich in phenolics followed by ethyl acetate, hexane and chloroform extracts with a quantity of 186.59±1.13, 142.82±0.15, 95.36±0.81 and 91.63±0.2mg/g respectively. Likewise, in stem bark extracts, ethyl acetate extract was rich in phenolic content (189.57±0.1mg/g), followed by chloroform (98.4±0.21mg/g) and

hexane extract (60.63±0.18mg/g). In ethanolic extract fractions, phenolic content was high in bottom crystals followed by upper liquid showing 243.71±0.14 and 127.82±0.46 mg/g of extracts respectively (Fig 1).

Table 9: Preliminary qualitative tests for phytochemicals in fruit and stem bark extracts of *S. pubescens.*

Tests	Fruit extracts				Stem bark extracts				
								Ethanolic	
	F H	F C	F EA	F EoI	B H	B C	B EA	U L	B C
Alkaloids									
a) Dragendorff's test	-ve	- ve	-ve	+ ve	- ve	-ve	+ ve	+ ve	+ ve
b) Hager's test	-ve	- ve	-ve	+ ve	- ve	-ve	- ve	+ ve	+ ve
c)Wagner's test	-ve	- ve	-ve	+ ve	- ve	-ve	- ve	+ ve	+ ve
d) Mayer's test	-ve	- ve	-ve	+ ve	- ve	-ve	- ve	+ ve	+ ve
Carbohydrates									
a) Anthrone test	-ve	- ve	+ve	+ ve	- ve	- ve	- ve	- ve	- ve
c) Fehling's test	-ve	- ve	+ve	+ ve	- ve	- ve	- ve	- ve	- ve
Flavonoids									
a) Ferric chloride test	+ve	+ve	+ve	+ ve	+ ve	+ ve	- ve	- ve	- ve
b) Alkaline reagent test	+ve	+ve	+ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
c) Lead acetate solution test	+ve	+ve	+ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
Triterpenoids									
a)Liebermann- Burchard's test	-ve	-ve	-ve	- ve	- ve	-ve	- ve	- ve	- ve
Proteins									
a) Biuret test	-ve	-ve	-ve	- ve	- ve	-ve	- ve	- ve	- ve
Saponins									
a) Foam test	-ve	-ve	-ve	+ ve	- ve	-ve	- ve	+ ve	+ ve
Steroids									
a)Salkowaski reaction	+ ve	+ ve	+ ve	- ve	- ve	- ve	- ve	- ve	- ve
b) Liberman test	+ ve	+ ve	+ ve	- ve	- ve	- ve	- ve	- ve	- ve
Tannins and Phenolics									
a)$Fecl_3$ test	- ve	+ve	+ ve	+ ve	+ ve	+ ve	+ ve	- ve	- ve
b)Lead acetate test	- ve	+ ve	+ ve	+ ve	+ve	- ve	+ ve	- ve	- ve
Coumarins	- ve	+ ve	+ ve	+ ve	- ve	- ve	+ ve	+ ve	+ ve
Oils and fat	+ve	-ve	-ve	- ve	- ve	-ve	- ve	- ve	- ve
Starch	–ve	+ ve	+ ve	+ ve	-ve	-ve	- ve	- ve	- ve

FH: fruit hexane, FC: fruit chloroform, FEA: fruit ethyl acetate, FEol: fruit ethanol, BH: bark hexane, BC: bark chloroform, BEA: bark ethyl acetate, UL: upper liquid, BC: bottom crystals, +: Presence, -: Absence,

Similarly, quantification of flavonoids in all the extracts was investigated. In fruit extracts, hexane has high amount of flavonoids followed by ethyl acetate, chloroform and ethanol extracts yielding 188.05±0.09, 105.48±0.2, 82.04±0.14 and 81.7±0.19 mg/g respectively. In stem bark extracts ethyl acetate showed highest amount of flavonoid (97.1±0.11mg/g) followed by chloroform (86.33±0.25mg/g) and hexane extract (76.08±0.09mg/g), in stem bark ethanolic extract fractions, bottom crystals showed about 85.65±0.09mg/g of flavonoids followed by upper liquid 56.45±0.05mg/g of extracts (Fig 1).

However, alkaloid and saponins content of *Solanum pubescens* material was analysed by direct method. In this assay, the stem bark is shown to possess high amount of alkaloids (49.6±0.31mg/g) followed by fruit (39.5±0.29 mg/g). Interestingly, fruit material was rich in saponins (37.86±0.09 mg/g) followed by stem bark (12.13±0.19 mg/g) (Fig 2).

Furthermore, the quantification of carbohydrates was assessed based on the preliminary phytochemical screening in which only fruit extracts have shown positive results. Hence they were selected for the quantification. In which ethanol extract have about 712.68±0.01mg/g of carbohydrates followed by chloroform, ethyl acetate then hexane extract yielding 617.83±0.15, 286.46±0.11 and 246.77±0.21 mg/g respectively (Fig 2).

DISCUSSION

The genus *Solanum* L. consists of over 2000 species distributed worldwide is the largest in Solanaceae and is one of the largest among all flowering plants [30]. The species are medicinal herbs [31] and contain unique alkaloids and other biochemical constituents used for the treatment of diverse ailments [32]. The identification and characterization of components derived from herbal or medicinal plant extracts has been gaining more attention. Hence, in the present investigation *Solanum pubescens* an indigenous medicinal plant which is not very well explored for its medicinal properties has been subjected for extraction.

Organoleptic study is the preliminary step in the standardization of crude drugs, and forms an important part of powder analysis and is a method for the qualitative evaluation based on the study of morphological and sensory profiles of plant material. The study revealed the characteristic colour, odour, taste and nature of fruit, and stem bark of *S. pubescens.* The peppery taste, pungent and strong aromatic smell indicates the presence of prominent secondary metabolites in fruit and stem bark. Interestingly, this plant is bitter in taste and it was observed that even the grazing animals will not feed on the plant. It is believed that the heavy content of alkaloids flavonoid and saponins present in this plant may be responsible for its extreme bitterness, which is generally a prominent feature of some of the plants belonging to Solanaceae family.

Fluorescence analysis of the drug powder with different reagents/solvents is an important pharmacognostic tool in finding out the various chromophores of the chemical constituents present in the drug under study [33]. This method is adequately sensitive and enables the precise and accurate determination of the analyte over a satisfactory concentration range [34]. The pharmaceutical and neutraceutical industries are currently confronted with adulteration and cheating [35]. The observation of the salient features of the plant is inevitable in this field to circumvent adulteration and substitution. Further, the fluorescence analysis of *S. pubescens* material and extracts under different UV wavelength helped us to trace that the extracts are surely having very important phytochemicals, and pave the way for further phytochemical exploration.

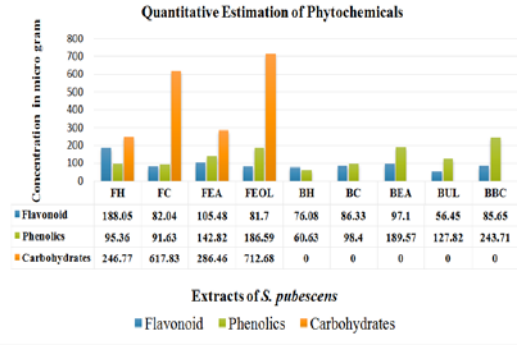

Fig. 1: Concentrations of flavonoids, phenolics and carbohydrates in *Solanum pubescens* fruit and stem bark extracts.

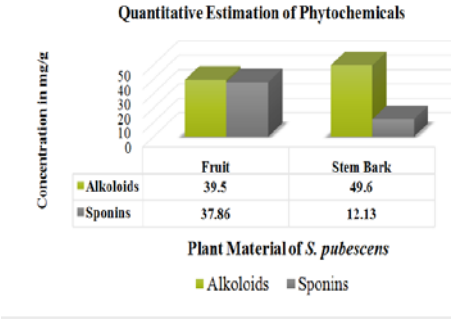

Fig. 2: Concentrations of alkaloids and saponins in *Solanum pubescens* fruit and stem bark.

In the present investigations, the successive extraction of both fruit and stem bark material with ethanol extract showed highest percentage of yield. The fruit hexane extract gave dark green oil. Interestingly, ethanol stem bark extract showed the formation of two separate layers as upper liquid (UL) and bottom crystals (BC).

It is noteworthy that essential oils from *Solanum* species are less frequent [36], in which the plant *Solanum nigrum* berries contains about 3.50±0.17% of crude oil [37]. Whereas essential oil from the aerial parts of *S. nigrum* var. *virginicum* was obtained by hydrodistillation with an yield of 0.56% (v/w), on a dry weight basis [38], *Solanum elaeagnifolium* seeds have about 2.95± 0.35% of oil [39], *Solanum melongena* unripe fruit has 1.65±0.62% [40]. Similarly, the dried fruits of *Solanum erianthum* gave yellowish liquid oil of 0.05 % v/w yield, and the 0.05 % v/w of essential oil recorded in the fruits is comparatively higher than those previously reported in *S. hypomalacophyllum* and *S. pseudocapsicum* [36]. Furthermore, it is remarkably interesting to report that the unripe fruits of *Solanum pubescens* contains about4.94±0.1% of crude oil, and it is found that this is the first report of the presence of highest amount of essential oils when compared to the other species of Solanum genus.

The study further channelled into exploring the extracts for the presence of phytochemicals, in which the first step was to understand the nature of solubility and to select proper vehicle solvent for further analysis. Among the nine different solvents as well as reagents used in this study, water, DMSO and ethanol were preferred as vehicle solvents for drug formulation for further pharmacological studies. The phytochemical screening is must for the detection of chemical groups which be useful in the identification of the bioactive principles and subsequently may lead to drug discovery and development. Similar studies for phytochemicals like alkaloids, phenols, tannins, oils and fats, flavanoids, saponins, steroids and quinones, are reported in related species like *Solanum pseudocapsicum*[41] and *Solanum nigrum* Linn. [42].

The preliminary phytochemical studies have clearly demonstrated that the plant *Solanum pubescens* is a rich source of essential oils, alkaloids, flavonoids, saponins, steroids, tannins and coumarins. Among the extracts the ethanolic and ethyl acetate extract is found to have more phytoconstituents. It is presumed that the presence of these constituents together could be attributed to the presence of curative abilities. Currently, investigations are underway to isolate, and characterize bioactive compounds to evaluate the beneficial pharmacological properties.

The quantitative analysis has revealed that *Solanum pubescens* is very rich in phenolics followed by flavonoid, alkaloid, saponins, carbohydrates and oils, which gives a very strong reason to select this plant for future evaluation of cytotoxic intern anticancer and other pharmacological properties. This is the first report on the qualitative and quantitative analysis of fruit and stem bark of *Solanum pubescens.*

CONCLUSION

Pharmaceutical preparation derived from natural resources often contain compounds that contribute the defence systems and apparently play a role in the fortification against degenerative diseases. The phytochemical investigations of *Solanum pubescens* concludes that in both the materials, ethanolic extracts are rich source of secondary metabolites followed by ethyl acetate, chloroform and hexane extracts. Thus, this investigation gives a clear indication of the presence of probable bioactive compounds in the form of oils, alkaloids, tannins, and flavonoids in the different crude extracts of *S. pubescens* supporting the traditional use of the plant in a few cases. Further, this is the first systematic analysis on the chemical constituents of the plant *S. pubescens* as there are no reports available thus far. Certainly, future investigations would throw much light on the beneficial properties, which could open new avenues to economically exploit the plant as a rich bioresource of essential oils, alkaloids and flavonoids for pharmaceutical industry.

CONFLICTS OF INTEREST

Authors declare that there are no conflicts of interest.

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