Qualitative screening and Quantitative determination of secondary metabolites from different plant extracts of *Solanum khasianum* Clarke

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Abstract
The medicinal plants have gained significant importance in traditional medicine and pharmacy due to wide variety of curative properties. The medicinal properties of the plants rely on the presence of various phytoconstituents. The present study focused on screening of phytochemicals from different parts (leaf, stem, petiole, fruit and root) of Solanum khasianum using solvents with varying polarity like methanol, butanol, chloroform, acetonitrile and water.

The qualitative screening of phytoconstituents showed the existence of alkaloids, flavonoids, phenols, tannins, steroids, saponins and absence of quinones in all 25 extracts tested. The quantitative determination showed that root extract contains considerable amounts of total flavonoids (214.74±0.45), total phenols (183.47±0.38), total alkaloids (132.46±0.28), total antioxidants (95.66±0.27), total tannins (14.66±0.07) whereas total saponins were present in high content in fruit methanolic extract (44.99±0.33). The presence of these phytoconstituents indicates the therapeutic potential of S. khasianum and scientifically validates the traditional use of this plant.

Keywords: Solvent extracts, Phytochemicals, *Solanum khasianum*, Quantification, Standards.

Introduction

Nature is the richest source of several natural therapeutic compounds. The plants contain many bioactive constituents that determine the biological and physiological processes in humans. The medicinal value of the plants is linked with the presence of different phytochemicals in them[30,51]. These phytochemicals mainly include steroids, alkaloids, phenols, flavonoids, tannins, glycosides, triterpenoids etc. Now the majority of the world's population is relying primarily on the plant derived medicines as they are easily available, less cost effective and develop less side effects than synthetic drugs. These herbal medicines were utilized by nearly 75% of the rural people in India and about 80% population in developing countries[49,62].

The medicinal properties of the plants and their method of utilization have been focused in the ancient times in six recognized systems of medicine like Ayurveda, Homeopathy, Siddha, Unani and Naturopathy. The systems of medicine are specified in the oldest medical books of world including Ancient medicine, Charakasamhitha, Sushrusamhitha, Ebers Papyrus etc. The medicinal plants have been used in different ailments which mainly include microbial infections (like bacterial, fungal and viral). The phytochemical components existing in various plant parts offer a wide diversity of pharmacological activities. Due to these pharmacological properties, a great attention has been derived towards the utilization of these medicinal plants.

Solanaceae is one of the largest plant families with over 90 genera and 3000 species[23]. Among the different Solanaceae family members, *Solanum* is one such family with over 1500 species widespread throughout the world[2]. The members of the Solanaceae include both vegetable crops (*S. aethiopicum, S. lycopersicum, S. melongena, S. torvum and S. tuberosum*) and medicinal crops (*S. aviculare, S. capsicastrum, S. laciniatum, S. pseudocapsicum*)[7]. Such plant derived medicines are gaining much concern with regard to the effectiveness of the phytomedicine over the synthetic drugs. The family *Solanum* is popularly known for its diverse biological properties[63].

Solanum khasianum is one of the members of Solanaceae, known for its diversified medicinal values antibacterial[31], antifungal, antiandrogenic[12], anthelmintic, anti-inflammatory[6] and anticancer activity[65]. Apart from these, the plant is used traditionally to cure several ailments[33]. The phytochemical screening of the phytoconstituents was analyzed qualitatively and quantitatively. Preliminary screening for the presence of phytochemicals and their quantification signifies the benefit of utilizing the *S. khasianum* plant traditionally.

Material and Methods

Preparation of plant material: The different plant parts of *S. khasianum* (leaf, stem, petiole, fruit and root) were collected during the month of April/ May, washed thoroughly, drained and shade dried till the complete moisture content of the samples was reduced. The samples are ground to fine powder using homogenizer.

Preparation of plant extracts: *Solanum khasianum* plant material (5g each) was added to 50ml of each different solvent system like methanol, butanol, chloroform, acetonitrile and water and extracted separately by cold maceration method (by incubating in orbital shaker at 22^0C and 120 rpm for 48 hours). Subsequently, extract was

filtered using Whatmann filter paper and analyzed for preliminary screening of the phytochemicals both by qualitative and quantitative methods.

Qualitative analysis of *Solanum khasianum* Clarke: All the 5 different plant extracts were assayed qualitatively for the occurrence of diverse phytochemicals like alkaloids, flavonoids, glycosides, phenols, tannins, steroids, fats/oils, quinones and saponins (Figure 1 and table 1).

Quantitative analysis of *Solanum khasianum* Clarke: The quantification of different phytochemicals was evaluated by standard methods (Figure 2). The absorbance of the test samples (leaf, fruit and root) was noted and their concentration was evaluated by the line of regression and regression coefficient values obtained from the calibration lines (figure 3) obtained from different standards. Atropine was used as standard for quantification of alkaloids, rutin for flavonoid quantification, gallic acid for phenols, tannic acid for tannins, diosgenin for saponins and ascorbic acid for total antioxidant quantification. The concentration of the test samples was expressed as mg of the standard equivalent per gram of the plant extract.

Table 1
Qualitative tests for analysis of phytochemicals of *S. khasianum*

Qualitative analysis	Name of the test	Methodology
Alkaloids	Dragendorff's test	Plant extract (0.5ml) was mixed with HCl (2ml) and dragendorff reagent (1ml). Formation of orange red colour PPT indicates the presence of alkaloids
	Mayer's test	Plant extract (1.0ml) was mixed with few drops of mayer's reagent. Formation of pale/ cream color PPT infers the presence of alkaloids
	Wagner's test	Plant extract (10ml) was acidified by adding HCl (1.5%) and also added with few drops of wagners reagent. Formation of yellow brown color PPT indicates the presence of alkaloids.
	Hager's test	Plant extract (0.5ml) was mixed with few drops of hagers reagent. Formation of yellow color PPT indicates the presence of alkaloids.
	Tannic acid	Plant extract (0.5ml) was mixed with few drops of tannic acid (10%). Development of buff color infers the presence of alkaloids.
Flavonoids	Shinoda's test	Plant extract (1ml) was added with Mg turnings and few drops of conc.HCl. Appearance of pink/ crimpson red/ green color indicates the flavonoids.
	Alkaline reagent test	Plant extract (1ml) was treated with few drops of NaOH solution. The disappearance of yellow color after addition of dilute acid indicates the presence of flavonoids.
	Zn-HCl test	Plant extract (1ml) was added with small amount of Zinc dust and few drops of conc HCl. Formation of red color infers the presence of flavonoids.
	Lead acetate test	Plant extract (1ml) was added with aqueous lead acetate solution. Formation of bulky reddish brown PPT infers the presence of flavonoids.
	Ferric chloride test	Few drops of plant extract were mixed with few drops of $FeCl_3$ solution. Appearance of black ppt confers the presence of flavonoids
Glycosides	Legal's test	Plant extract (1ml) was mixed with few drops of pyridine and alkaline sodium nitroprusside solution. Formation of blood red colour indicates glycosides.
	Bromine water test	Plant extract (1ml) was mixed with few drops of bromine water. Formation of yellow color ppt indicates the presence of glycosides.
	Keller Killiani test	Small amount of plant extract was added with 0.4ml of glacial acetic acid with trace amounts of ferric chloride, subsequently added with conc. H_2SO_4 (0.5ml) along the walls of the tube. Appearance of blue color in the acetic acid layer indicates the presence of glycosides.
	Molish test	Plant extract (1ml) was added with few drops of alcohol α-napthol and 2ml conc. H_2SO_4 along the walls of the test tube. Formation of brown/ purple ring in the middle of two layers indicate the presence of carbohydrates.
	Conc H_2SO_4 test	Plant extract (1ml) was mixed with 1ml conc. H_2SO_4 and stood for 2 minutes. Appearance of red ppt indicates the presence of glycosides.
Tannins	Gelatin test	Plant extract (1ml) was added with few drops of 1% gelatin solution in 10% NaCl. Appearance of white ppt infers the presence of tannins.
	Alkaline reagent test	Plant extract was added with NaOH solution. Formation of yellow to red ppt infers the presence of tannins.
Saponins	Foam test	The plant extract was diluted with 20ml water and agitated for 10-15 minutes. Formation of foam layer infers the presence of saponins.
Phenols	$FeCl_3$ test	Plant extract (1ml) was mixed with few drops of $FeCl_3$. Formation of blue color indicates the presence of phenols.

	Ellagic acid test	Plant extract (2ml) was mixed with few drops of glacial acetic acid (5%) and sodium nitrate solution (5%). Appearance of niger brown PPT infers the phenols.
Steroids	Libermann Burchard test	Plant extract (1ml) was mixed with few drops of acetic anhydride, boiled and cooled to room temperature. Then conc. H_2SO_4 was added along the walls of the tube. Formation of brown ring at the junction of 2 layers and conversion of upper layer to green color indicates the presence of steroids, whereas deep red color infers the presence of triterpenoids.
	Salkowski test	Plant extract (1ml) was added with few drops of conc. H_2SO_4. Appearance of lower red color infers the presence of steroids, whereas yellow color infers triterpenoids.
Quinones	Alcoholic KOH test	Plant extract (1ml) was mixed with few drops alcoholic KOH solution. Color change from red to blue color infers the presence of quinones.

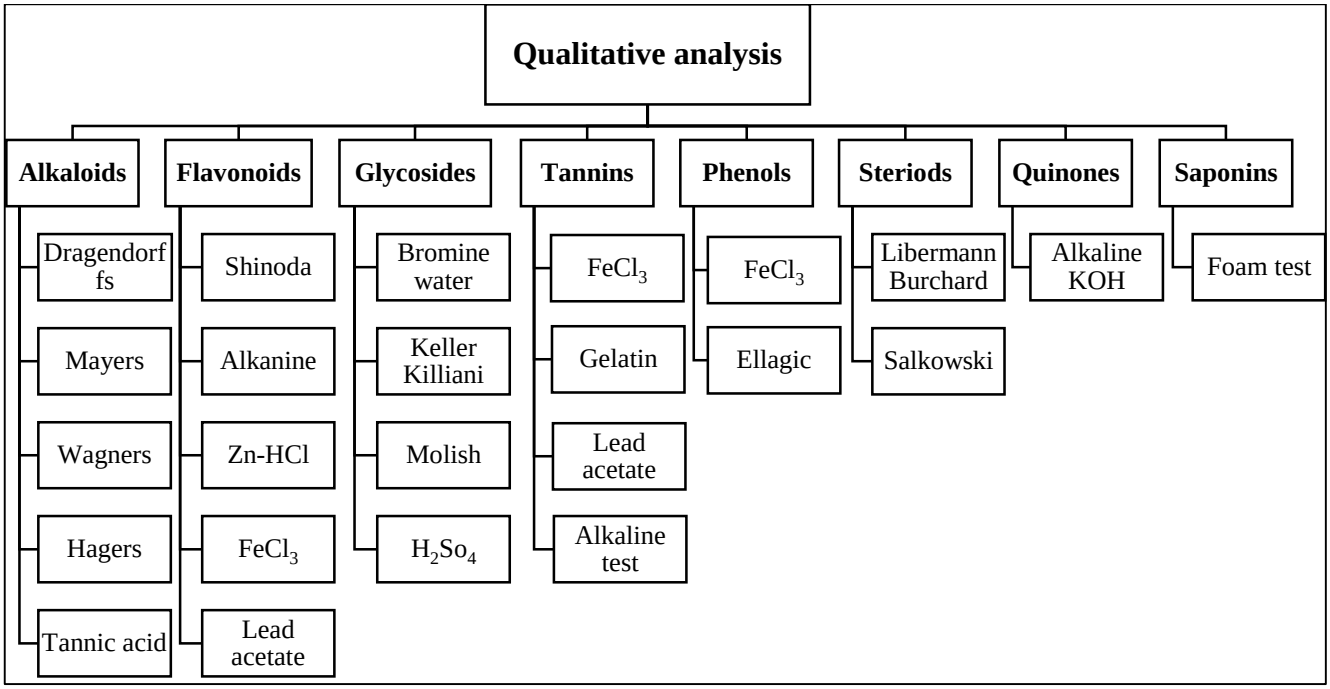

Fig. 1: Preliminary screening (Qualitative) of phytochemicals in plant *S. khasianum*

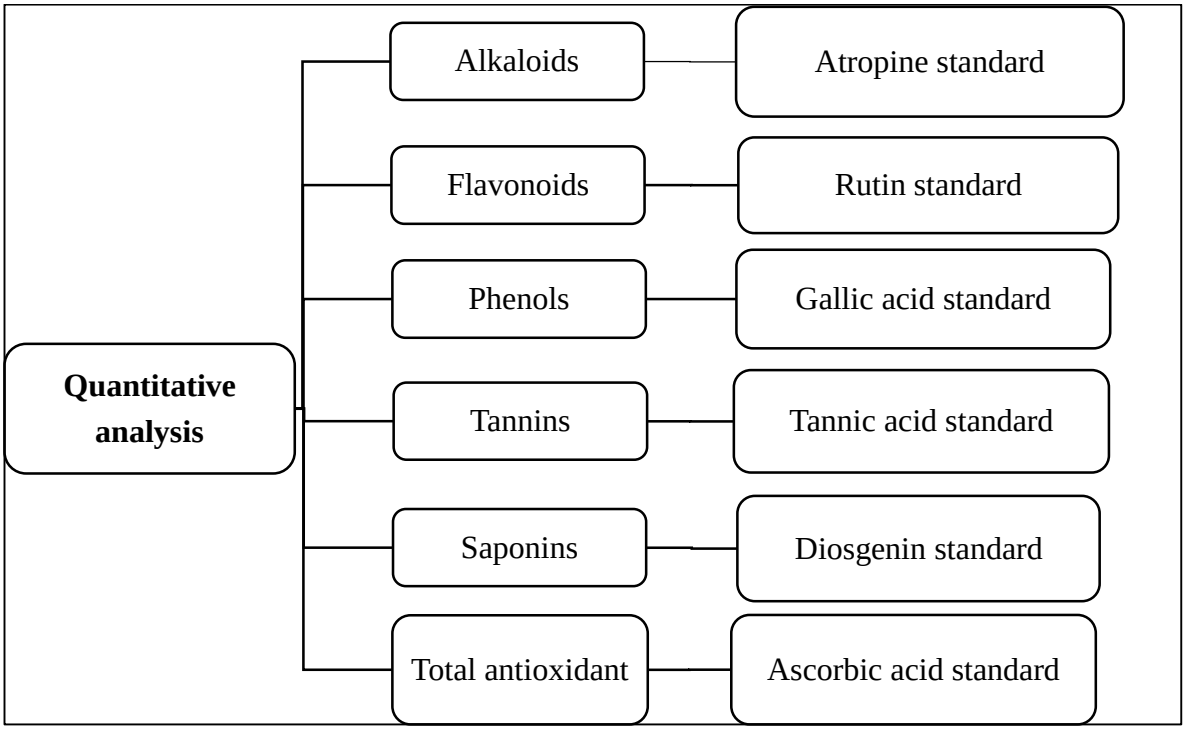

Fig. 2: Quantitative analysis of Phytochemicals of *S. khasianum*

Quantification of total alkaloid content: For the quantification of total alkaloid content, standard curve of atropine standard was used. Atropine standard was prepared at a varying concentration (50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml and 250µg/ml) and taken in different separating funnels. 5ml of phosphate buffer (pH-4.7) and 5ml BCG (Bromo Cresol Green) were added to each funnel, vigorously mixed and subsequently added with 5ml chloroform and mixed again to extract the compound into chloroform fraction. The chloroform fraction of the funnel was transferred to another flask (10ml) and the volume was made up to 10ml using chloroform. Optical density of the sample was read spectrophotometrically at 470nm.

Similarly, the quantification of the *S. khasianum* plant extracts (leaf, fruit and root) was done by dissolving the plant extracts (1mg/ml) in 2N HCl (whose pH was set to 4.7 with 0.1N NaOH). The results of the plant extracts were compared with the standard curve obtained. The total alkaloid content of the different plant extracts is expressed as atropine equivalent per gram of extract (as mg of AE/g of extract)[3,32,41].

Quantification of total flavonoid content: The quantification of total flavonoid content was analyzed spectrophotometrically by utilizing rutin as standard. Rutin standard (10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml concentration) and the plant extracts (1mg/ml) were prepared accordingly. 75µl of sodium nitrate (5%) was added and mixed thoroughly. 150µl of aluminium chloride (10% $AlCl_3$) was added and allowed to stand for 5 minutes followed by the addition of 500µl NaOH (4%) and the final volume of the reaction mixture was adjusted to 2.5ml by adding distilled water. The absorbance of the samples was measured spectrophotometrically at 510nm. The total flavonoid content of the plant extracts is expressed in terms of RE (rutin equivalent)/ gram of extract (as mg of RE/g of extract)[3,13,19].

Quantification of total phenol content: The total phenol content of samples was determined by FC (Folin Ciocalteu) reagent and gallic acid was employed as standard. Varying concentrations (10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml) of gallic acid standard and the different plant extracts (1mg/ml) were prepared. 5ml FC reagent (1:10 diluted with distilled water) and 4ml sodium carbonate (7.5 %) were added and incubated for 30 minutes at 20˚C. The absorbance of the samples was measured spectrophotometrically at 765nm. The total phenol content of the plant extracts is expressed in terms of GAE (gallic acid equivalent)/ gram of extract (as mg GAE/g of extract)[3,50].

Quantification of total tannin content: The tannin content of the samples was quantified by using tannic acid as standard and folin phenol reagent, for which varying concentrations (50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml and 250µg/ml) of tannin acid standard and different plant extracts were prepared. The samples were diluted by adding 7.5ml of distilled water and subsequently mixed with folin phenol reagent (0.5ml) and 1ml of sodium carbonate (35%). The final volume of the reaction mixture was made to 10ml by adding distilled water, vigorously shaken and incubated at room temperature for about 30 minutes. The absorbance of the samples was obtained spectrophotometrically at 725nm. The total tannin content of the plant extracts is expressed in terms of TAE (tannic acid equivalent)/ gram of extract (as mg TAE/g of extract)[24,25].

Quantification of total saponin content: For quantification of total saponins, diosgenin was used as standard. The diosgenin standard (10mg diosgenin in 20ml 80% methanol) and different plant extracts (50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml and 250µg/ml) were prepared accordingly. 250µl of the standard and test samples were added to 250µl of vanillin reagent (8%) and 2.5ml of H_2SO_4 (72%), mixed thoroughly and incubated in water bath at 60˚C for 10 minutes. Subsequently, these tubes were cooled by placing them in chilled water for 3-4 minutes. The absorbance of the samples was read spectrophotometrically at 544nm. The total saponin content of the plant extracts is expressed in terms of DE (diosgenin equivalent)/ gram of extract (as mg DE/g of extract)[3,36].

Quantification of total antioxidant content: The total antioxidant capacity of the plant extracts was analyzed by using ascorbic acid as standard. Different concentrations (50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml and 250µg/ml) of ascorbic acid standard were prepared accordingly. 300µl of the different concentrations of standard and plant extracts were taken in separate test tubes and added with 3ml of reagent solution and incubated at 95˚C for about one and half hour. The absorbance of the samples was read spectrophotometrically at 695nm[25,35].

Results and Discussion

The preliminary phytochemical screening (qualitative and quantitative) was performed to screen and analyze the different phytoconstituents present in different plant extracts of *S. khasianum.*

Qualitative analysis: The preliminary screening (qualitative) of phytochemicals present in various parts of the *S. khasianum* revealed the presence of different components like alkaloids, flavonoids, glycoside, phenols, tannins, steroids and fats whereas quinones are completely absent in the plant parts (Table 1). Among all the solvents used for extraction, methanolic extract and aqueous extracts were found to be efficient for extraction.

The qualitative analysis of leaf extract of *S. khasianum* revealed the occurrence of various phytoconstituents like alkaloids, flavonoids, glycosides, phenols, tannins, steroids/ triterpenoids and saponins in varying amounts in different solvent systems. Substantial amount of alkaloids was identified in leaf methanolic and aqueous extract followed by acetonitrile, chloroform and butanol extracts. High

amounts of phenols and flavonoids were identified in methanol and chloroform extracts and tannins in chloroform extract. The methanolic and aqueous extract showed the presence of considerable amount of saponins than other solvents while they are completely absent in butanol extract.

Moderate amounts of glycosides were detected in methanol, butanol and aqueous extract and steroids/ triterpenoids in methanol and chloroform extract. In contrast alkaloids and flavonoids are completely absent in leaf extracts of *Pistacia lentiscus*[61]. Our results are in agreement with the methanolic extracts of *S. torvum,* which showed the presence of alkaloids, flavonoids, phenols, saponins, glycosides and absence of steroids, but in contrast, tannins are absent[23] whereas copious amount of tannins is also reported in *S. nigrum*[47]. Recent reports on phytochemical screening of leaf extract of *S. khasianum* also showed the presence of alkaloids, flavonoids, glycosides, phenols, tannins, steroids and saponins[56].

The phytochemical screening of stem extracts of *S. khasianum* revealed the presence of alkaloids, flavonoids, glycosides, phenols, tannins, steroids/ triterpenoids and saponins. The alkaloids were enormously present in methanol, butanol and acetonitrile extracts. High amount of phytochemicals present was phenols in methanol and aqueous extract. Considerable amount of tannins was detected in aqueous extract followed by butanol, chloroform, methanol and acetonitrile extracts whereas flavonoids and steroids were present in all extracts.

Saponins were heavily detected in butanol and acetonitrile extract, while on contrary low amounts were detected in methanol and aqueous extracts. Glycosides were mildly detected only in butanol, chloroform and aqueous extracts. The qualitative screening of phytochemicals of stem extracts of *S. trilobatum* also revealed the presence of flavonoids, tannins, saponins and glycosides and absence of sterols and phenols but in contrast, glycosides are absent and phenols and sterols are present in *S. khasianum*[55].

The petiole extracts of *S. khasianum* also revealed the occurrence of different phytochemicals like alkaloids, flavonoids, glycosides, phenols, tannins, steroids/ triterpenoids, saponins and absence of quinones. The alkaloids were present in extensive amounts in chloroform extract, subsequent moderate levels were identified in methanol, butanol, aqueous and acetonitrile extracts whereas high amounts of flavonoids, phenols and tannins were detected in methanol extract. Moderate amount of glycosides was present in chloroform and aqueous extract while it is not detected in methanol, butanol and acetonitrile extracts.

Methanol petiole extract showed moderate amounts of steroids while on contrary, saponins were present in higher amounts in aqueous extracts. In contrast, alkaloids, flavonoids and glycosides were absent in the petiole extracts of *Pterospermum acerifolium*[42]. Similar result of presence of steroids, tannins, flavonoids and phenols was reported in petiole extract of Sedano bianco di sperlonga[59].

The preliminary screening of different solvent extracts of *S. khasianum* root showed the presence of alkaloids, flavonoids, glycosides, phenols, tannins, steroids/ triterpenoids and saponins. The results showed the occurrence of substantial amounts of phytochemicals in methanol and aqueous extracts. The methanol and butanol extracts were shown to possess high amounts of alkaloids whereas successive high concentrations of flavonoids and phenols were present in methanol extract. Copious amounts of tannins were detected in methanol and aqueous extracts, saponins in butanol and aqueous extracts. Steroids were present in moderate amounts in methanol extracts, while less amounts were present in butanol, acetonitrile and aqueous extract.

Our results are in resemblance with the results obtained in *S. xanthocarpum* root extracts, where it revealed the presence of alkaloids, flavonoids, tannins, terpenoids and absence of quinone, but in contrast, glycosides are absent[48]. Different solvent extracts of *S. khasianum* fruits revealed the presence of alkaloids, flavonoids, glycosides, phenols, tannins, steroids/ triterpenoids and saponins. Alkaloids were detected in considerable amounts in methanol, butanol and aqueous extract, whereas moderate amounts of flavonoids were present in methanol extract, glycosides in chloroform and acetonitrile extracts, phenols in methanol, butanol, acetonitrile and aqueous extracts, tannins in methanol and aqueous extracts, steroids in chloroform and acetonitrile extracts. Extensive amounts of saponins were present in methanol and butanol extracts, while moderately present in all other extracts, similar results of high saponin content of fruit extracts (oval variety) were reported in *S. melongena*[4].

Similar results of high alkaloids, flavonoids were reported in fruit extracts of *S. aethiopicum* L. *and S. macrocarpon*[28] and in contrast, steroids and saponins are completely absent in *S. macrocarpon*[10]. Our results are in disagreement with the results obtained in *S. indicum,* where the fruit extracts showed the absence of alkaloids and presence of quinones[1]. Whereas in *S. nigrum*, the fruit extracts in contrast revealed the absence of glycosides, saponins and fats/oils[7]. Gogoi et al[19,20] reported similar results of phytoconstituents in fruit extracts of *S. khasianum.* The presence of these phytoconstituents in different plant parts of *S. khasianum* shows the evidence for traditional use of *S. khasianum* in treating several ailments.

Quantitative analysis: In the present investigation, quantitative analysis of different phytochemicals like alkaloids, flavonoids, phenol, tannin, saponin and total antioxidants was evaluated by standard methods.

Determination of total alkaloid content: The total alkaloid content of the plant extracts was determined spectrophotometrically by the calibration line of atropine

standard (Figure 3A). The total alkaloid content was expressed as mg/g equivalent of atropine. From atropine standard, the standard line of regression was obtained to be $y = 0.0024x + 0.0921$ with a regression coefficient of $R^2 = 0.9921$. The total alkaloid content of the leaf, fruit and root extracts was found to be 94.96±0.54, 100.79±0.70 and 132.46±0.28 mg AE /g of extract (Table 3).

Gupta et al[22] also reported similar results of high amount of alkaloids (67.77 ± 1.24 mg/g dry weight) in the aerial parts of *S. sisymbriifolium*. The occurrence of alkaloids in *Solanum* was reported to possess anti-inflammatory, analgesic, antilipidemic, resistance towards infections[46] and antidiabetic properties[26,29].

Table 2
Phytochemical constituents of *Solanum khasianum* Clarke

Part of the plant	Component	Methanol extract	Butanol extract	Chloroform extract	Acetonitrile extract	Aqueous extract
Leaf	Alkaloids	+++	+	++	++	+++
	Flavonoids	+++	++	++	+	+
	Glycosides	++	++	-	+	++
	Phenols	+++	++	+++	++	++
	Tannins	++	+	+++	+	++
	Steroids/ Triterpenoids	++	+	++	+	+
	Quinones	-	-	-	-	-
	Saponins	+++	-	++	++	+++
Stem	Alkaloids	+++	+++	+	+++	++
	Flavonoids	+	+	+	+	+
	Glycosides	-	+	+	-	+
	Phenols	+++	+	++	+	+++
	Tannins	+	++	++	+	+++
	Steroids/ Triterpenoids	++	+	+	+	+
	Quinones	-	-	-	-	-
	Saponins	+	+++	-	+++	+
Petiole	Alkaloids	++	++	+++	+	++
	Flavonoids	+++	++	+	+	+
	Glycosides	-	-	++	-	+
	Phenols	+++	+	++	+	++
	Tannins	+++	-	++	-	++
	Steroids/ Triterpenoids	++	+	+	-	+
	Quinones	-	-	-	-	-
	Saponins	++	-	+	++	+++
Root	Alkaloids	+++	+++	++	++	++
	Flavonoids	+++	+	+	+	++
	Glycosides	+	+	-	+	++
	Phenols	+++	++	-	++	++
	Tannins	+++	+	-	+	+++
	Steroids/ Triterpenoids	++	+	-	+	+
	Quinones	-	-	-	-	-
	Saponins	+	+++	-	++	+++
Fruit	Alkaloids	+++	+++	++	++	+++
	Flavonoids	++	+	+	+	+
	Glycosides	+	+	++	++	+
	Phenols	++	++	+	++	++
	Tannins	++	+	+	+	++
	Steroids/ Triterpenoids	-	+	++	++	+
	Quinones	-	-	-	-	-
	Saponins	+++	+++	++	++	++

Table 3
Quantitative Amount of total phytochemical constituents of *Solanum khasianum* Clarke

Phytoconstituent	Amount of phytoconstituents		
	Leaf	Fruit	Root
Total alkaloid content	94.96±0.54	100.79±0.70	132.46±0.28
Total flavonoid content	210.52±0.48	169.25±0.54	214.74±0.45
Total phenol content	114.57±0.32	145.43±0.16	183.47±0.38
Total tannin content	3.33±0.05	6.93±0.06	14.66±0.07
Total saponin content	42.55±0.58	46.39±0.33	44.99±0.33
Total antioxidant capacity	87.76±0.38	61.22±0.19	95.66±0.27

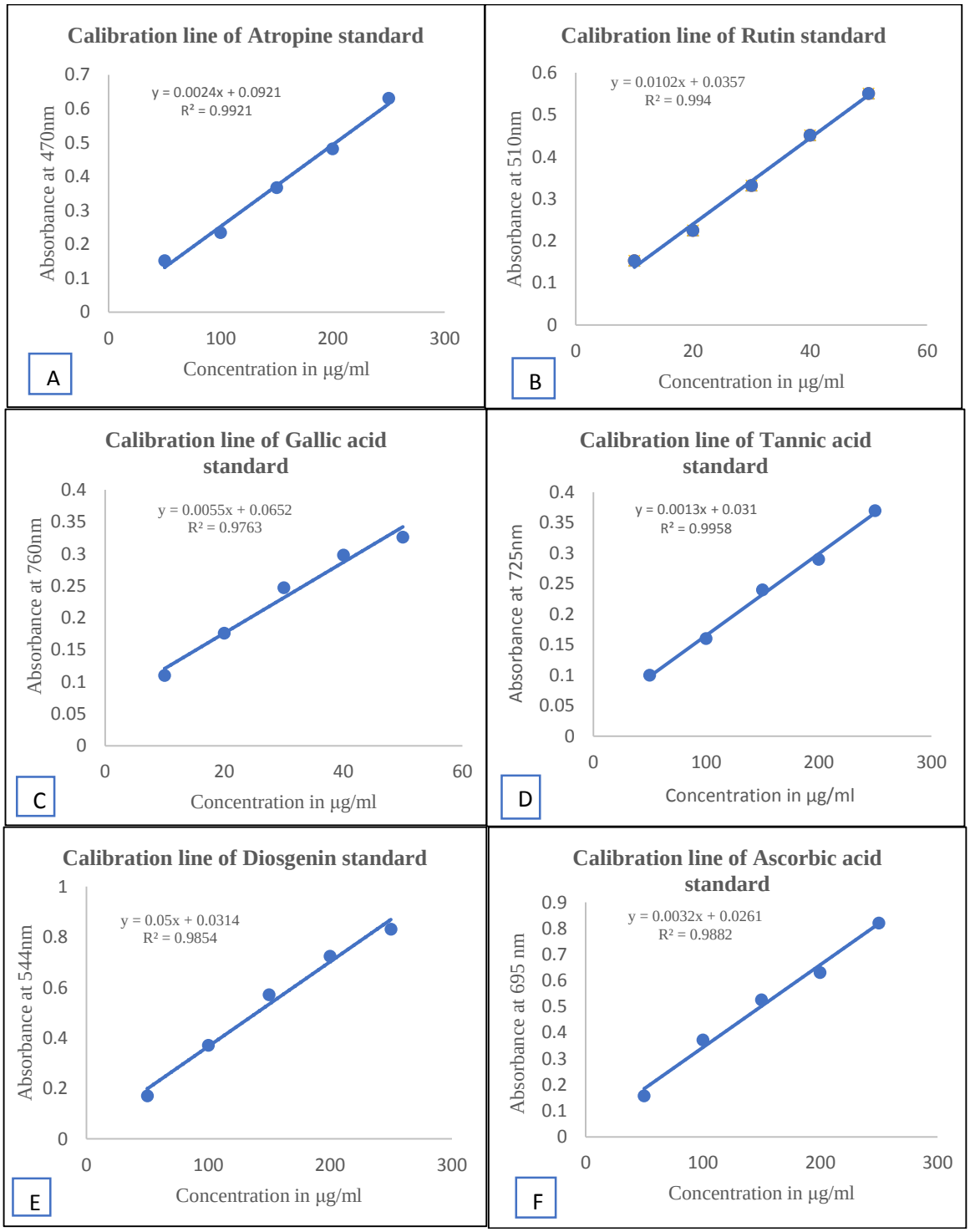

Fig. 3: Standard curves for quantitative analysis of phytochemicals.
A) Atropine. B) Rutin. C) Gallic acid. D) Tannic acid. E) Diosgenin. F) Ascorbic acid

Mahato et al[42] isolated different glycoalkaloids like solasodine, solasonine, solamargine and khasianine from the berries of *S. khasianum* and characterized them by ^{13}C NMR spectroscopy. These alkaloids also reported in other solanum members are known to possess different bioactivities like solamargine reported in *S. lycocarpum* (fruit) possessing antidiabetic, leishmanicidal, schistosomicidal; *S. sarrachoides* (leaf) possesses anticancer and *S. uporo* (root) possesses antibacterial, molluscicidal properties whereas khasianine alkaloid reported in *S. surattense* possesses anticancer and *S. xanthocarpum* possesses antibacterial, molluscicidal properties.

Similarly, solasonine reported in *S. lycocarpum* (fruit) possesses leishmanicidal, antidiabetic, schistosomicidal and solasodine reported in *S. aculeastrum* possesses anticancer, *S. torvum* possesses anti-inflammatory and *S. xanthocarpum* possesses antibacterial properties[63].

Determination of total flavonoid content: The determination of total flavonoid content of plant extracts was evaluated spectrophotometrically by the calibration line of rutin standard (Figure 3B). The total flavonoid content of the samples was expressed as mg/g equivalent of rutin. The line of regression for rutin standard was y = 0.0102x + 0.0357 with a regression coefficient of R^2 = 0.994. The total flavonoid content of the samples was found to be 210.52±0.48, 169.25±0.54 and 214.74±0.45 mg RE /g of extract (Table 3). The quantification of fruit extracts of *S. torvum* revealed high amount of flavonoids than alkaloids[58].

The members of *Solanum* are known to possess several flavonoids and they are reported to have different activities like anticancer[39,44,66], antioxidant, cancer prevention[18,38], antiviral, antidepressant[64] and hepatoprotective[46]. The presence of high flavonoid content infers the high antioxidant potentiality of the plant extract by scavenging activity and also possesses reducing power[38].

Determination of total phenol content: The total phenol content of the samples was analyzed spectrophotometrically with the calibration line of gallic acid standard (Figure 3C). The total phenol content of the samples was expressed as mg/g equivalent of gallic acid. The regression line obtained from gallic acid standard was y= 0.0055x+ 0.065 and regression coefficient of R^2 = 0.9763.

The total phenol content of the plant extracts was found to be 114.567±0.32, 145.43±0.16 and 183.467±0.38 mg GAE /g of extract in leaf, fruit and root extracts of *S. khasianum* (Table 3). Similarly, high amount of phenols (134.4±0.9 mg GAE /g of sample) was reported in *Clerodendrum serratum*[15].

In contrast, high phenolic content was identified in *Pistacia lentiscus* leaf extract than in fruit extract[23]. Ignat et al[29] reported that phenolic compounds of the plants form the considerable group of antioxidants (primary) and they also exhibit several other biological properties like anticancer, antiviral, anti-inflammatory, antispasmodic and also treat diarrhea and ulcers[8,43].

Determination of total tannin content: The quantification of the total tannin content was evaluated spectrophotometrically with reference to the calibration line of tannic acid standard (Figure 3D). The total tannin content was expressed as mg/g equivalent of tannic acid. The line of regression for the tannic acid standard was y = 0.0013x + 0.031 with a regression coefficient R^2 = 0.9958. The total tannin content of the samples was found to be 3.33±0.05, 6.93±0.06 and 14.66±0.07 mg TAE /gram of extract in leaf, fruit and root extracts (Table 3). Agoreyo et al[1] also reported similar results of high amount of tannins (12.82±0.14) in *S. melongena* (round variety). Tannins were known to possess anti-inflammatory and anticancer activity[22].

Determination of total saponin content: The determination of total saponin content of the plant extracts of *S. khasianum* was evaluated spectrophotometrically by using diosgenin standard. The total saponin content of the samples was expressed as mg/g equivalent of diosgenin. The regression line of diosgenin standard was y = 0.05x + 0.0314 and regression coefficient of R^2 = 0.9854 (Figure 3E). The total saponin content of the plant extracts was 42.55±0.58, 46.39±0.33 and 44.99±0.33 mg diosgenin equivalent /gram of sample of leaf, fruit and root extracts (Table 3). Solakhasoside, a novel steroidal saponin, was isolated from the fruit of *S. khasianum*[52].

Similarly, high quantity of saponin content was reported in the oval variety of *S. melongena*[4]. The presence of various saponins of *Solanum* was reported to possess anticancer[14,21], antifungal, antiviral[11,34,47], hepatoprotective[60] and antihypertensive[15]. The occurrence of alkaloids and saponins infers the resistance of plant towards microbial infections[27].

Determination of total antioxidant capacity: The antioxidant capacity of the *S. khasianum* plant extracts was evaluated spectrophotometrically by utilizing ascorbic acid standard. The total antioxidant content was evaluated as mg/ gram of AAE. The calibration line of ascorbic acid exhibited a regression line of y = 0.0032x + 0.0261 and the regression coefficient of R^2 = 0.9882 (Figure 3F). The total antioxidant capacity of the plant extracts was found to be 87.76±0.38, 61.22±0.19 and 95.66±0.27mg/ gram of ascorbic acid equivalent in leaf, fruit and root extracts (Table 3). In contrast, high antioxidant capacity was reported by Hasan et al[33] in fruit extracts of *Averrhoa carambola*.

The antioxidant potentiality of the plant extracts helps maintain the cell structure and metabolism by preventing damage caused by free radicals, oxidative stress and lipid peroxidation[57]. These antioxidants also help in preventing several persistent diseases like diabetes, heart diseases and cancer[68]. The screening of phytochemical constituents of different plant extracts of *S. khasianum* exhibited the high

quantity of flavonoids and phenols in root extracts that infers a remarkable antioxidant property with free radical scavenging[67], also proven to be more efficient than vitamin C, E and plant pigments (carotenoids)[53] and also possesses anticancer and anti-inflammatory activity.

Subsequently, high amount of phytoconstituents identified in root extract was alkaloids with diverse biological activities followed by saponins (fruit extract) that are well known for its anticancer properties and steroidal hormone synthesis[37]. Successive high amount of phytoconstituent evaluated was tannins in root extracts and the plants possessing tannins were known to treat inflammation, diarrhoea and skin injuries[17]. The presence of glycosides in different species of *Solanum* is known to produce cytotoxic activity[16]. The presence of these phytoconstituents indicates the bioactivity of different plant parts of *Solanum khasianum*.

Conclusion

The qualitative analysis of leaf, stem, petiole, fruit and root extracts of *Solanum khasianum* confirmed the presence of alkaloids, flavonoids, phenols, tannins, saponins, fats/ oils, steroids and absence of quinones in the different solvent extracts. The quantitative analysis of alkaloids, flavonoids, phenols, tannins, saponins and total antioxidant capacity of leaf, fruit and root extracts of *S. khasianum* revealed the presence of high amounts of flavonoids, phenols, alkaloids, antioxidant and tannins in the root extracts and high amount of saponins in the fruit extracts.

Thus, preliminary screening, quantification and subsequent determination of phytoconstituents can provide fundamental information regarding the potential healthcare benefits of *S. khasianum.* Further isolation and characterization of these phytochemicals in their pure form may be necessary for development of new drugs.

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