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Preliminary phytochemical evaluation of *Sphaeranthus indicus* Linn of Asteraceae family

Yugal Kishor Sarle[1], Sadhna Goyal[2], Vinod Kumar Chaurse[3]
[1]Govt. J.H. College, Betul (M.P.) India,[2]Govt. MVM College, Bhopal (M.P.) India,
[3]Govt College, Athner, Dist-Betul (M.P.) India.

Abstract: Phytochemical analysis of whole plants of *Sphaeranthus indicus* Linn was performed using petroleum ether, chloroform, methanol and aqueous extracts using Soxhlet apparatus standard methods outline (Trease GE and Evans WC, 1989; Sofowora A, 1993) were followed *Sphaeranthus indicus* Linn is a very useful medicinal plant of asteraceae family and common annual spreading herbs found in rice field throughout India. The presence of Alkaloids, Flavonoids, Glycosides, Diterpenoids, Tennins, Proteins, Carbohydrates and Total Phenolic Content (TPC) estimation was analysed by Soxilation method. Traditional medicines of the plants are great significance of the ethnomedicine in India.
Keywords: *Sphaeranthus indicus* Linn, Phytochemical analysis, Proximate analysis.

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1. Introduction

In recent years plants has been in the main stream as an economically and commercial alternatives. The therapeutic evaluation of these plants and studies on their biological constituents has been the main focus of research in development countries (Edeoga HO *et al*, 2005). The practice of plants in therapeutic drug system for examples Ayurveda, Unani and Siddha are well conceded. Approximately 3000 plants are authoritatively documented for their therapeutic worth and over 6000 plants are used in established herbal and old used in established, herbal and old drug organization in india (Prakash UNK, 2014). This fertile world is with natural and medicinal plants, and have the capability many benefits of society for the humanity and pharmacological. Medicinal importance of these plants lies in constituents of photochemicals that is responsible for changes in the human body (Akinmoladun AC *et al*, 2007). Whole herb contain obtained by steam distillation of ocimen, alfa-terpine, bita ionone, d-codinene, alfa-ionone, alfacitral, geranion, p-methoxycinnamaldehyde (Basu NK *et al*, 1946) and alkaloid sphearanthine (Gupta RK *et al*, 1996). The alcoholic extract of powered Capitula contains stigmasterol, bitasistosterol, hentriacontane, sesqueterpinelactone (Gogat MG *et al*, 1986), sphaerathanolide (Yadav RN and Kumar S, 1998) flavone and isoflavone glycoside. The potential of plant has producing new drugs of great benefit to human being. *Sphaeranthus indicus* Linn was found to possess powerful medicinal properties to cure skin infections, diseases of the liver, jaundice, bronchitis, In view of the medicinal importance of *Sphaeranthus indicus* Linn. The system, it was decided to work on the phytochemical investigations on *Sphaeranthus indicus* Linn.

Material and Method- The fresh plant of *Sphaeranthus indicus* Linn was simultaneously collected from cultivated farms and open field of Betul district M.P. India. A fresh part of the plant was identified for phytochemical analysis. After 15 days plants was completely dried and it was grinded into powdered with 1mm size by using Grider machine before phytochemical screening.

1.1 Preparation of extracts- Four solvents were used to extraction of arial part of *Sphaeranthus indicus* Linn. The solvents are Petroleum ether, Ethanol, Chloroform and Aqueous 30gm of the powdered of *Sphaeranthus indicus* Linn were extracted with different solvents in soxlet apparatus in 250ml of each solvent separately for 48hours (Harbone JB, 1973).

1.2 Extraction Method

Preparation for extract, there are following method was adapted - Powdered and shade dried whole plant material of *Sphaeranthus indicus* Linn was taken (Khandelwal KR, 2005; Kokate CK, 1994).

1.2.1 Extraction by maceration method- 97gm of powdered whole plant material of *Sphaeranthus indicus* Linn was exhaustively extracted with different solvent through maceration method (Petrolium ether, Chloroform, Aqueous and Methanol). For the calculation of percentage yield dried extract was obtained from evaporation of extract (Mukherjee PK, 2007).

1.3 Percentage yield determination

Percentage yield calculation

Extract of percentage yield was calculated by formula:

Percentage yield = Extract's dry weight / Dry weight of plant material x 100

1.4 Screening of phytochemical

For the screening of phytochemicals following standard methods was carried out.

1. Alkaloid detection: Individual extract was dissolved in dilute HCl and filtered it.

a) Test of Hager: Reagent of Hager was used in treatment of filters (Hager's: Solution of saturated picric acid). For the confirmation of Alkaloids it is detected by the formation of yellow coloured precipitate.

2. Carbohydrate detection: Extract was filtered and individually dissolved in 5ml distilled water. For the presence of carbohydrate filtrates were used as a test.

a) Test of Fehling: Dilute HCl is used to hydrolyzed of filter and neutralization was done by alkaline treatment and warm with Fehling's A & B solutions. Red colour precipitate formation indicates the presence of reducing sugars.

3. Glycoside detection: Dilute HCl is used to hydrolyzed extract and ready for the test of glycosides.

a) Test of Legal's: Sodium nitropruside is used in treatment of extracts in pyridine and sodium hydroxide. Cardiac glycoside presence indicating with pink and red colours .

4. Saponins detection

a) Froth's Test: 20ml of distilled water was taken, diluted with extract and for fifteen minutes was shaken in a graduated cylinder. Formation of foam layer one centimeter indicates the presence of saponins.

5. Phenols detection

a) Test of Ferric Chloride: The extract *Sphaeranthus indicus* Linn was taken with 3-4 drops of $FeCl_2$ solution. Bluish black color formation indicates the presence of phenols.

6. Flavonoids detection

a) Test of Pb $(CH_3COO)_2$ Lead acetate: Lead acetate solution taken with few drops in treated extract of plant. Yellow color precipitates indicate the occurrence of flavonoid.

7. Proteins Detection

a) Test of Xanthoprotein: Few drops of concentrate Nitric acid were taken to treated extract of yellow color indicates the presence of proteins.

8. Diterpenes detection

a) Test of Cu$(CH_3COO)_2$ Copper acetate: Extracts were treated with few drops of Copper Acetate solution. Green colour occurrence indicated diterpenes (Audu *et al*, 2007; Roopashree *et al*, 2008; Obasi *et al*, 2010).

9. **Tannin detection** - Pottasium Ferrocynide method was used (Van-Burden and Robinson, 1981).

1.5 Quantitative Studies of Phytoconstituents

1.5.1 Total Phenol Content (TPC) estimation: Folin-Ciocalteu reagent 1ml and sodium carbonate 1ml (7.5g/l) mixed with 2ml extract and standard. For the colour development mixture was vortexed for few seconds and allowed it stand 10min for colour development. The absorbance was taken from spectrophotometer at 765nm (Colufunmiso *et at*, 2011).

1.5.2 Total flavonoids content (TFC) estimation: It is calculated as Quercetin equivalent (mg/100mg) equation for calibration curve: $Y= 0.040X+0.009$, $R^2 =0.999$, where X = Queercetin (QE) and Y = absorbance.

Qualitative chromatographic analysis

Thin Layer Chromatography (TLC)

This method is based on adsorption. In mobile phase dissolved solutes pass over the stationary phase (Obasi *et*

al, 2010).

Calculation & Detection of R_f Value

Developed chromatogram was used for the calculation of R_f Value using formula and result was given in the table.

Mobile phase used for Thin Layer chromatography

Compound	Mobile phase	Visualization
Gallic acid	Toluene: Ethyle acetate: formic acid (5:4:1)	Normal light, short UV* & long UV*
Quercetin	Toluene: Ethyle acetate: formic acid (7:5:1)	Normal light, Short UV* and long UV*

Short UV-254nm, Long UV- 365nm

Results

Table No. 1: % Yield of whole plant material of *Sphaeranthus indicus* Linn.

S.No.	Extract	Amount Found (g)	% yield(w/w)
1	Petrolium ether	1.49	1.54
2	Chloroform	2.11	2.18
3	Methenol	2.51	2.59
4	Aqueous	5.19	5.35

Table 2:

Phytochemical analysis of the extracts *Sphaeranthus indicus* Linn arial part.

S.No.	Components	Petroleum Ether Extract	Chloroform Extract	Methanol Extracts	Aqueous Extracts
1.	Tannins	Negative	Negative	Negative	Positive
2	Alkaloids Hager's Method	Negative	Negative	Negative	Positive
3	Glycosides Legal's Method	Negative	Negative	Positive	Negative
4	Flavonoids Lead acetate Method	Negative	Negative	Positive	Positive
5	Diterpenes Copper acetate Method	Negative	Negative	Positive	Positive
6	Phenol Ferric Chloride Method	Negative	Negative	Positive	Positive
7	Proteins Xanthproteic Method	Negative	Negative	Positive	Positive

8	Carbohydrates Fehling's Method	Positive	Positive	Positive	Positive
9	Saponins Froth Method	Negative	Negative	Positive	Positive

Table N.3**:** Percentage of crude Alkaloids, Phenols, Tannins, Flavonoids and Saponins in *Sphaeranthus indicus* Linn.

S.NO.	Constituents	Percentage (%)
1	Alkaloids	0.30±0.21
2	Phenols	0.41±0.19
3	Flavonoids	0.11±0.26
4	Tannins	0.58±0.28
5.	Saponins	5.08±0.2

5. 4 Estimation of Total Phenolic(TPC) and Flavonoid content (TFC)

5.4.1 Total phenolic content estimation (TPC)

Total phenolic content (TPC) was 1mg/100mg of Gallic acid equal dry extract of sample using the equation obtained from the calibration curve: Y=0.011X+0.011,

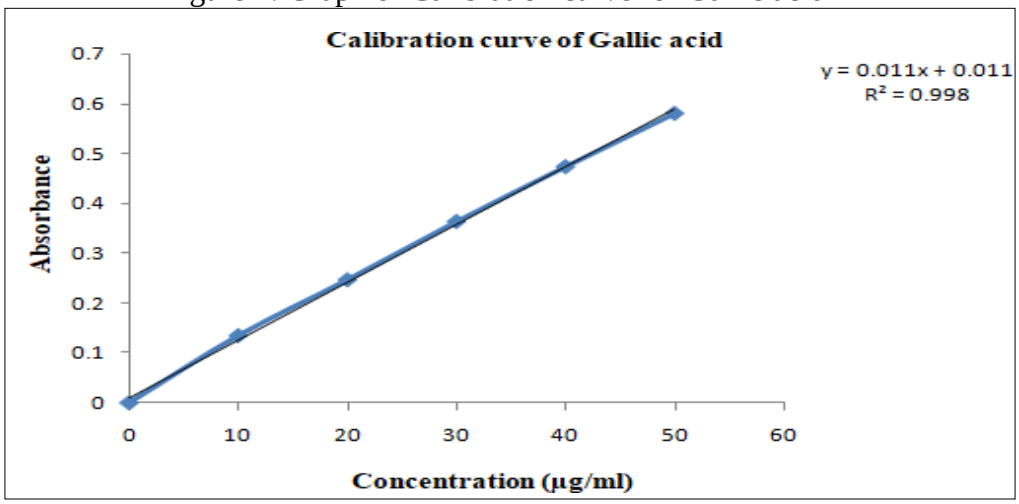
R^2 = 0.998, where X = Gallic acid equivalent (GAE) and Y = absorbance.

Gallic acid Calibration curve

Table No. 4: Gallic acid calibration curve preparation.

S.No.	Concentration (ug/ml)	Absorbance
1	10	0.135
2	20	0.247
3	30	0.364
4	40	0.474
5	50	0.581

Figure 1: Graph of Calibration curve for Gallic acid



Total flavonoid content (TFC) estimation: It is calculated as Quercetin equivalent (mg/100mg) using the equation based on the calibration curve: Y= 0.040X+0.009, R^2 =0.999,

Where X = Queercetin (QE) and Y=absorbance.

Table No.5: Quercetin calibration curve preparation

S. No.	Concentration (µg/ml)	Absorbance (OD)
1.	5	0.191
2.	10	0.348
3.	15	0.514
4.	20	0.652
5.	25	0.812

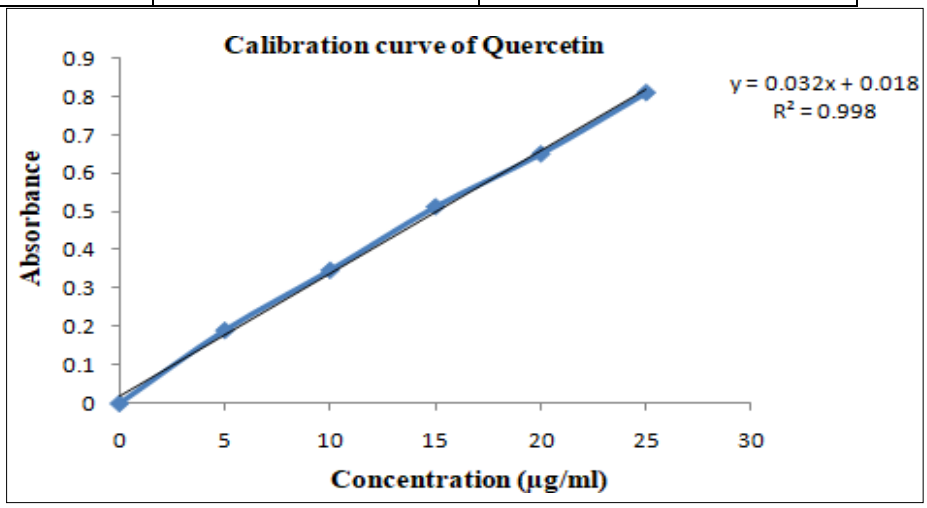


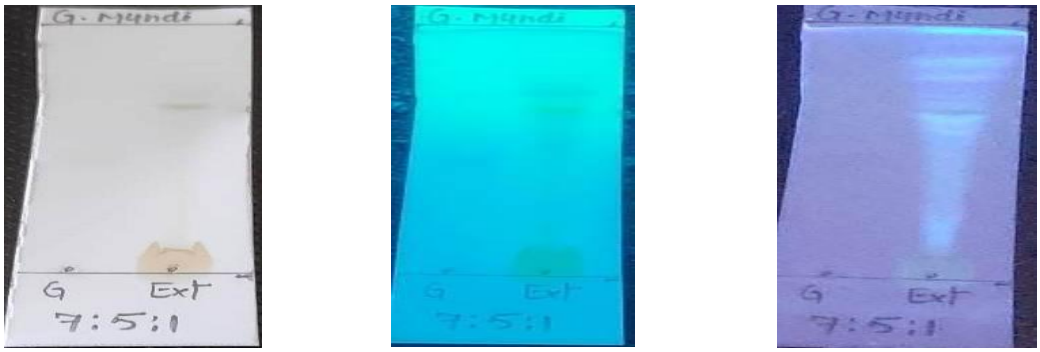
Figure 2: Graph of calibration curve of Quercetin

Table No.6: Estimation of total phenolic and flavonoids content in different extracts of *Sphaeranthus indicus* Linn.

S.No.	Extract	Absorbance	
		Total phenolic content (mg/100mg QE)	Total flavonoids content (mg/100mg QE)
1	Methanol	0.062	0.252
2	Aqueous	0.039	0.167

Result of thin layer chromatograph

Normal Light Short UV Long UV



TLC of Gallic acid

Thin layer chromatography of Quercetin

Results: Thin layer chromatography of Gallic acid

Sample Extract **Methanol extract of *Sphaeranthus indicus* Linn**

Mobile Phase	Toluene: Ethyl acetate: Formic acid (ratio 7:5:1)
Plate Tag	I
Distance travelled by mobile phase	5.0 cm
	Number of spots
Normal Light	2
Short Wavelength	4
Long Wave Length	5
Visibility	Considerable
Any Match with Gallic acid Standard	Yes (0.58cm.)
	Spot distance
Normal Light	3.2, 3.8
Short Wavelength	3.2, 3.3, 3.8, 4.1
Long Wave Length	2.9, 3.2,3.3, 4.0, 4.4
	Rf Values of each spot (from bottom to top)
Normal Light	0.64, 0.76
Short Wavelength	0.64, 0.66, 0.76, 0.82
Long Wavelength	0.58, 0.64, 0.66, 0.8, 0.88
Spot Sequence (Left to right)	
First	Gallic acid
Second	Methanol extract of *Sphaeranthus indicus* Linn.

Results: Thin layer chromatography of Quercetin

Sample Extract **Methanol extract of *Sphaeranthus indicus* Linn**

Mobile Phase	Toluene: Ethyl acetate: Formic acid (ratio= 5:4:1)
Plate Tag	I
Distance travelled by mobile phase	4.0 cm

	Number of spots
Normal Light	3
Short Wavelength	2
Long Wave Length	5
Visibility	Considerable
Any Match with Quercetin Standard	Yes (0.60cm.)
	Spot distance
Normal Light	2.1,2.4,2.4
Short Wavelength	2.7, 3.3
Long Wave Length	2.1,2.4, 2.7, 3.3,3.9
	R_f Values of each spot (from bottom to top)
Normal Light	0.52,0.6, 0.67
Short Wavelength	0.60,0.82
Long Wavelength	0.52, 0.60, 0.67, 0.82,0.97
	Spot Sequence (Left to right)
First	Quercetin
Second	Methanol extract of *Sphaeranthus indicus* Linn

Discussion- This study was focused on examining preliminary phytochemical and Thin Layer Chromatography studied of whole plant of *Sphaeranthus indicus* Linn. The maximum extractive value in percent was found in Petrolium ether 1.54, Chloroform 2.18, Methanol 2.59 and Aqueous 5.35. All the extracts were subjected to detecting the various phytochemical constituents that revealed the presence of Alkaloids, Glycosides, Flavanoid, Diterpenes, Phenol, Proteins, Carbohydrates, Saponins and Tannins. Phytochemical analysis indicated a high percentage of Saponins and this is the reasons behind the hypolipidemic activity of the medicinal plant. There are phytochemical constituents which are Alkaloids are negative in all solvents including Petrolium ether, Chloroform, Methanol, Aqueous and Glycosides are also negative in all solvents except Methanol, Flavanoid, Diterpenes, Saponins and Phenols are positive in 50% solvent. Protein shows negative and Carbohydrate shows positive in all cases and absence of Alkaloids and presence of Carbohydrate indicate the inhance microbial growth. Phytochemical screening methods were also done the confirmation of secondary metabolites and conclude that *Spheranthus indicus* Linn has a sufficient antimicrobial activity.

Phenolic compound in the plant have redox properties and the properties allow them acting as antioxidants (Shoib AB and Shaid AM, 2015; Soobrattee *et al*., 2005). The result shows that Chloroform extract exhibited higher phenolic contant as compare to Methanolic extract. Aqueous extract shows moderate value and higher phenolic contant in Methanol extract is for bioactivity that is why we can expect to exibit good result for antioxidant and antimicrobial activity.

Total Flavonoid Content (TFC) extracts are reported as Chloroform 1.537, Methanol 0.252, and Aqueous 0.167. Profile of Methanolic extract and aqueous material showed yellow colour spot under UV wavelength. It indicates the presence of Isoflavonoid compound.

The Thin Layer Chromatography profile along with Quercetin showed one spot in long UV, Short UV and normal light as standard. Chloroform, Methanol and Aqueous solvent in TLC also showed yellow colour spot under UV wavelength. TLC analysis of *Spherenthus indicus* Linn showed seven spots in long UV, six spots in short UV and also six spot in normal light with chloroform extracts. It was indicated the presence of Eugenol, Geraniol, Iodin and citral. *Spharanthus indicus* Linn is a very much reputed drug used in Ayurvedic field (Venkatachalam DP, 2018).

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