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Preliminary Phytochemical Analysis of *Helianthus annus* Linn of Family Asteracea

Yugal Kishor Sarle, Sadhna Goyal,*Vinod Kumar Chaurse**

Govt. J.H. College, Betul (M.P.) India, * Govt. MVM College, Bhopal (M.P.) India,

**Govt College, Athner, Dist-Betul (M.P.) India. E Mail - yugalsarle@gmail.com

Abstract- The whole plant of *Helianthus annus* Linn have been investigated for the presence of various chemical components performed with ethanol, methanol, chloroform and petroleum ether extracts using soxlet apparatus (Trease GE and Evans WC; 1989; Sofowora A; 1993). The phytochemical analysis of whole plants extracts of *Helianthus annus* Linn revealed the presence of Saponins, Tannins, Alkaloids, Flavonoids, Carbohydrates, Diterpenes, Phenol and Protein in varying properties. In this study also investigated Total Phenolic Content (TPC), Total Flavonoid Content (TFC) are obtain and Glycosides are absent. The present investigation reveals that *Helianthus annus* Linn significance of conventional medicine and the important of these phytochemical constituents were disputed with respect to the role of these plants in Ethnomedicine in india.

Key Words: *Helianthus annus* Linn, Phytochemical analysis, Ethnomedicine,

Introduction- The world is fertile with natural and medicinal plants as now more focused than ever because they have the capacity of producing many benefits to society indeed to mankind, especially in the line of medicine and pharmacological. The medicinal power of these plants lies in photochemical constituents that cause definite pharmacological on the human body (Akinmoladun AC; 2007). Infectious diseases are an important health hazard all over the world, both in developing and developed countries (Sasikumar *et al*; 2003). In the last more than three decades Pharmaceutical industries have produced more antibiotics, these drugs are increasesd resistant to the micro-organisms (Nascimento et al; 2000). There is an urgent requirment to alternative medicine for ailments. The increasing interest on traditional ethno medicine may lead to invention of novel therapeutic agents. Medicinal plants finding into cosmetics, neutraceuticles, pharmaceuticals and food supplements. The world health organization (WHO) evaluated that 80% of the population of developing countries still relies on traditional medicines, almost plant drugs, for their priliminary health care needs (Mohanasundari et al; 2007)). Plants medicinal values are lies with some chemical constituents that are responsible to change physiological change on human body. The important bioactive constituents of plants are Alkaloids, Flavonoids,

Tannins, and Phenolic compounds (Dhandapani and Sabna; 2008). This present study was to investigate the phytochemical constituents in *Helianthus annus* Linn.

Material and Method- The fresh plant of *Helianthus annus* 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 grinder machine before phytochemical screening.

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

1.2 Extraction procedure

Following procedures were adopted for the preparation of extract of powdered and shade dried whole plant material of *Helianthus annus* Linn (Khandelwal KR; 2005; Kokate CK; 1994).

1.2.1 Extraction by maceration method- 97gm of powdered whole plant material of *Helianthus annus* Linn was exhaustively extracted with different solvent (Petrolium ether, Chloroform, Aqueous and Methanol) by maceration method. Extract was obtained through evaporation to the boiling point and calculation of percentage yields from the dried extracts (Mukherjee PK; 2007).

1.3 Percentage yield determination

Percentage yield calculation

Extract of percentage yield was calculated by formula:

Percentage yield = Dry weight extract/ Dry weight of plant material x 100

1.4 Screening of phytochemical

Phytochemical examination was carried out extracts as per following standard methods.

1. Alkaloids detection: Individually extracts are dissolved in dilute Hydrochloric acid and filtered.

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

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

a) Test of Fehling: Dilute HCl is used to hydrolyze of filter, neutralized with alkali and heated with Fehling's A & B solutions and formation of red precipitate indicates the presence of reducing sugars.

3. Glycoside detection: Dilute HCl is used to hydrolyzed extract and then ready to test for 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 this was shaken in a graduated cylinder for 15minutes. 1cm layer of foam formation indicates the presence of saponins.

5. Phenols detection

a) Test of Ferric Chloride: The extract of *Helienthus annus* Linn was treated with ferric chloride solution 3-4 drops and the formation of bluish black color indicates the presence of phenols.

6. Flavonoids detection

a) Test of Lead acetate: Few drops of lead acetate solution taken in treated extract of plant. Formation of yellow color precipitate indicates the occurrence of flavonoids.

7. Detection of Proteins

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

8. Diterpenes detection

a) Test of Copper acetate: Extracts were dissolved in water and treated with few drops of Copper Acetate solution was dissolved in water with treated extracts. Diterpence presence was indicated by formation of emerald green colour (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: 2ml of extract and standards were mixed with 1ml of Folin-Ciocalteu reagent and 1ml (7.5g/l) of sodium carbonate. After vortexed the mixture it is kept for 15s and allowed to stand for 10min till colour development. The absorbance was measured using spectrophotometer at 765nm (Colufunmiso *et at.,* 2011).

1.5.2 Total flavonoids content estimation: It is calculated as Quercetin equivalent (mg/100mg) the equation based on the calibration curve: Y= 0.040X+0.009, R2 =0.999, where X is the Queercetin (QE) and Y is the absorbance.

Qualitative chromatographic analysis

Thin Layer Chromatography (TLC)

The (TLC) is based on the adsorption phenomenon. In this, mobile phase chromatography the dissolved solutes pass over the stationary phase surface (Obasi *et al.,* 2010).

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Plates Preparation

• The most frequently used stationary phase Silica gel was employed for adsorption in T.L.C.
• To reduce the band broadening the stationary phase should consist of small particles of uniform size. It provides a large surface area for interaction and a small void volume.
• To make slurry Silica Gel mixed with water.
• To make uniform layer on plate by spreading the slurry.
• Put plate for air drying in some time and then kept for activation.

Plates Activation

• For the activation of plate by heating in an oven at 100 to 110^0C for 30 minutes
• Linear movement of solutes over stationary phase is necessary for activation.

Saturation & Preparation of Chamber

1. First prepared the solvent system and pour into the chamber, then saturated the chamber.

Development of sample

• With the help of capillary tube sample were taken from few microgram to milligram applied on plate.
• Plate contains developing solvent to a depth of about 0.5 cm was placed in the chamber. Plates were removed from the chamber.
• The mobile phase front is marked by scratching the surface and the solvent was evaporated in an oven.

Calculation & Detection of R_f Value

Developed chromatogram was used for the calculation of R_f Value, the spot was calculated using the formula and results was 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

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Results

Table No. 2: % Yield of extracts of *Helianthus annuus* Linn

S. No.	Extracts	% Yield (W/W)
1.	Petrolium ether	2.33
2.	Chloroform	3.51
3.	Methanol	11.09
4.	Aqueous	12.50

Table No. 4: Result of Phytochemical screening of extracts of *Helianthus annuus* Linn

S. No.	Constituents	Pet. ether extract	Chloroform extract	Methanol extract	Aqueous Extract
1.	**Alkaloids** Wagner's Test:	-ve	-ve	-ve	+ve
2.	**Glycosides** Legal's Test:	-ve	-ve	-ve	-ve
3.	**Flavonoids** Alkaline Reagent Test: Lead acetate Test:	-ve - ve	+ve + ve	+ve + ve	+ve + ve
4.	**Diterpenes** Copper acetate Test:	+ve	-ve	-ve	+ve
5.	**Phenol** Ferric Chloride Test:	-ve	-ve	-ve	+ve
6.	**Proteins** Xanthoproteic Test:	-ve	-ve	-ve	+ve
7.	**Carbohydrate** Fehling's Test:	-ve	-ve	-ve	+ve
8.	**Saponins** Froth Test:	-ve	-ve	-ve	+ve

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Table No. 6: Calculation of R_f Value of extracts of *Helianthus annuus* Linn

***Helianthus annuus* Linn extract**		
S. No.	**Mobile phase** Toluene: Ethyl acetate Formic acid (5:4:1)	**R_f value**
1.	**(Quercetin)** Dis. travel by mobile phase= 5cm No. of spot at long UV= 1 No. of spot at short UV = 1 No. of spot at normal light= 1	 Long- 0.58 Short- 0.58 Normal- 0.58
2.	**(Chloroform extract)** Dis. travel by mobile phase= 5cm No. of spot at long UV = 8 No. of spot at short UV = 3 No. of spot at normal light= 3	 Long- 0.14, 0.46, 0.54, 0.62, 0.74, 0.8, 0.86, 0.94 Short- 0.54, 0.74,0.86 Normal- 0.54, 0.74,0.86
3.	**(Methanol extract)** Dis. travel by mobile phase= 5cm No. of spot at long UV = 6 No. of spot at short UV = 5 No. of spot at normal light= 4	 Long- 0.14, 0.42, 0.52, 0.64, 0.74, 0.84 Short- 0.32,0.44,0.52,0.66,0.76 Normal- 0.52, 0.64,0.74
4.	**(Aqueous extract)** Dis. travel by mobile phase= 5cm No. of spot at long UV = 1 No. of spot at short UV = 1 No. of spot at normal light= 0	 Long- 0.5 Short- 0.5 Normal- 0

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4.	**(Aqueous extract)** Dis. travel by mobile phase= 5cm No. of spot at long UV = 1 No. of spot at short UV = 1 No. of spot at normal light= 0	 Long- 0.5 Short- 0.5 Normal- 0

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Normal Light Short U.V Long U.V

Figure 3: Spot-1= Quercetin, Spot-2= Chloroform extract, Spot-3= Methanol extract, Spot-4= Aqueous extract

2.4 Results of estimation of total phenolic and flavonoids content

2.4.1 Total Phenolic content estimation (TPC): Total phenolic content (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: **$Y = 0.011X+0.011$, $R^2 = 0.998$,** where X is the Gallic acid equivalent (GAE) and Y is the absorbance.

Calibration Curve of Gallic acid

Table No. 7: Preparation of Calibration curve of Gallic acid

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

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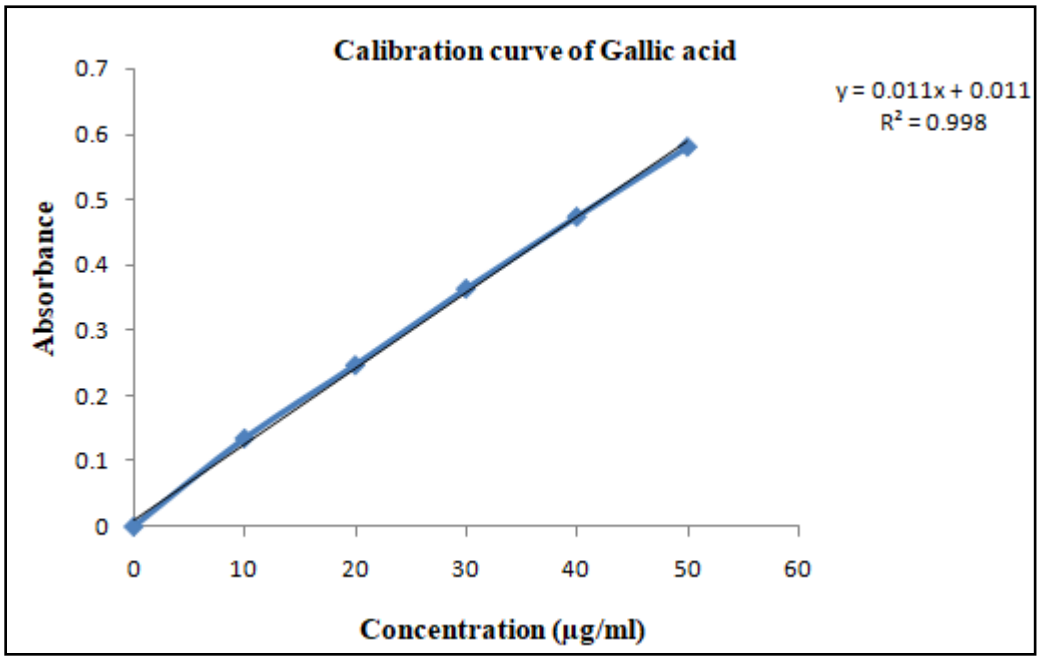

Figure 4: Graph of Calibration curve of Gallic acid

2.4.2 Total flavonoids content estimation (TFC)

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: **Y=0.032X + 0.018, R^2=0.998**, where X is the quercetin equivalent (QE) and Y is the absorbance.

Table No. 8: Preparation of Calibration curve of Quercetin

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

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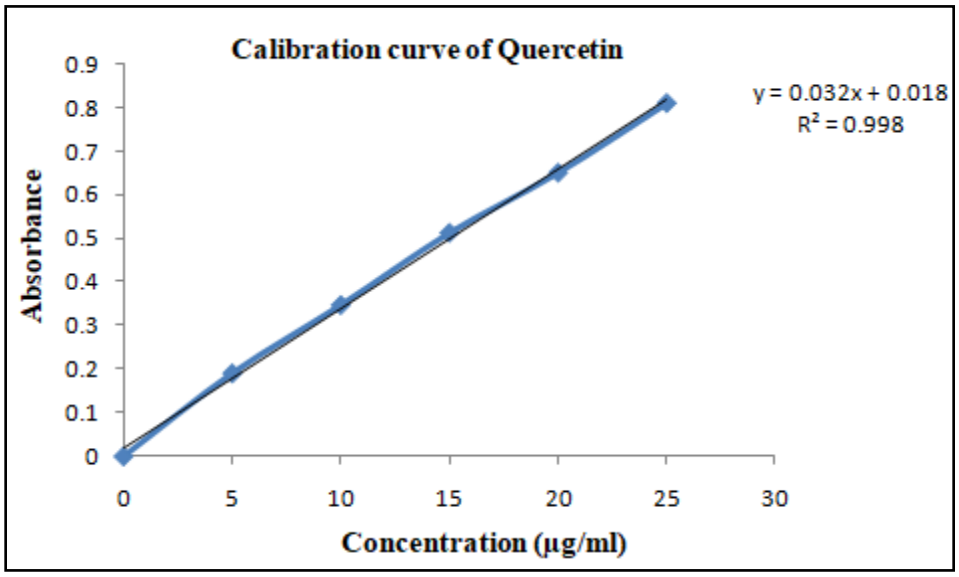

Figure 5: Graph of calibration curve of Quercetin

Table No. 10: Estimation of total phenolic and flavonoids content of *Helianthus annuus* Linn extract

S. No.	Extract	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100mg of dried extract)
1.	Chloroform	-	1.571
2.	Methanol	+ ve	1.646
3.	Aqueous	-	1.165

Discussion: The maximum value of Petroleum ether 2.33, Chloroform 3.51, Methanol 11.09 and Aqueous 12.50 were found in extracts which determine the various phytochemicals are present which shows the presence of alkaloids, glycosides, flavonoids, diterpenes, phenols, proteins, carbohydrates, saponins and tannins. Tannin has rich properties in the plant that healing of wound and inflamed mucus membrane. Flavonoids are also found in rich of the plant extracts which is the potent water soluble antioxidant and free radical scavengers which prevent oxidative cell damage, have strong anti cancerous activity (Salah *et al* 1995; Del Rio *et al* 1997 & Okwu, 2004).

Total phenolic content activity is the process to the amount of phenolic content in the samples. Phenolic compounds that contained in the plants have redox properties and the properties allow them acting as antioxidants (Shoib AB and Shahid AM, 2015; Soobrattee *et al*

2005). The results showed that the methanolic extract chloroform extract and aqueous exhibited TPC. Previous research showed that higher Phenolic content in the methanol extract it indicate more bioactivity. This extract exhibit good result in antibacterial and antioxidant activities.

The Thin Layer Chromatography profile of *Helianthus annus* Linn along with Quercetin showed one spot in long UV, Short UV and normal light as standard. Chloroform solvent in TLC also showed eight spots under long UV wavelength and three spots in normal and short UV. Methanol showed six spot in long UV, Five spot in short and four spot in normal light. Aqueous showed one spot in long and short UV and zero spot in normal light. It was indicated that specific type of ingredients are found in the plant.

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