ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022

Determination Of Total Flavonoid Content, Total Steroids Content, Total Tannin Content And Antioxidant Activity Of Fruits Of Hydro Alcoholic Extract Of *Piper Longum* Linn.

Dr. Swati Jogdand[1], Dr. Narendra Gowekar[1], Mrs. Sujata Shinde[1], Dr Rahul Dumbre[1], Mr. Shyam Awate[2], Dr. Neelam Dashputre[3], Dr Savita Yadav[4], Dr. Naitik Trivedi[5]

1. CAYMET's Siddhant college of Pharmacy, Pune
2. IVM'S. Indrayani Education and Research, Pune
3.Institute of pharmacy Bhujbal Knowledge city Adgaon nashik Maharashtra India
4. Bharati Vidyapeeth (Deemed to be University), Poona College of Pharmacy, Pune, Maharashtra.
5.A R College of Pharmacy, Anand, Gujarat

***Corresponding Author:**
Dr. Swati Jogdand
CAYMET's Siddhant College of Pharmacy, Sudumbre,
Pune-410501, Maharashtra, India. Email: swati.18sept@gmail.com

ABSTRACT

In current study, aim was to estimate the total flavonoid, steroids and tannin content as well as antioxidant activity of hydro alcoholic extract of *Piper longum* Linn. The hydro alcoholic extract was prepared by using alcohol:water concentration. The total flavonoid content was 5.45± 0.06 mg E/g, total steroid content was 338.25± 0.83 mg E/g and total Saponin content 42.57 mg E/g was found in extract. The total flavonoid content was determined by Aluminium chloride colometric complex method using Quarcetin as standard, total steroid content was determined by ferric–chloride complex method using Diosgenin as standaard, total Saponin content was determined by Vanillin sulphuric acid complex method using Diosgenin as standard. The antioxidant activity of hydro alcoholic extract of *Piper longum* was evaluated using 2,2-diphenyl-1- picyhydrazyl (DPPH) assay method. The hydro alcoholic extract of *Piper longum* Linn. exhibited % radical scavenging activity for 1000 µg/ml was found to be 26.56 % .

KEYWORDS: *Piper longum* Linn., DPPH, Total flavonoid, Total steroid, Total Saponin

INTRODUCTION

Different varieties medicinal plants have been used by existence human civilization. Since ancient archives, human uses medicinal plants have been found such as *Piper longum* Linn. in India, Nepal, Indonesia, Malaysia and Sri Lanka. (Satyavati et al., 1987) According to

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WHO (World Health Organization), 80% of individuals universal were assessed depend on herbal medicines. (Ekor et al., 2014) Furthermore, about 21,000 plants species have the probability or abilities for being used as medicinal plants (Joy *et al.* 2001). Because of their pain-relieving and curative capabilities, medicinal plants have been appreciated and trusted on in about 75% of our medicines until at the moment (Chevallier, 2012). In spite of the advances and advantages of existing medicines, medicinal plants deal the profits that pharmaceutical drugs frequently lack, assisting to maintain the body's power to recover good health. (Rukayah et al. 2006) Chemical dynamic substances lies in medicinal plants exhibits medicinal value which produce a certain spiritual action on the human body and the furthermost chief bioactive constituents of plants are steroids, tannins, flavonoid and phenolic compounds (Rajendra *et al.* 2011).

Piper longum Linn. Belongs to family Piperaceae is mostly cultivated in central Himalayas to Assam, Khasi and Mikir hills, lower hills of West Bengal and evergreen forests of Western Ghats from Konkan to Kerala. (Satyavati et al., 1987) A small aromatic plant trailing to the ground, also climbing with erect and thin branches. (Dorman & Deans, 2000) Piperine is the major chemical bioactive constituent of the plant. (Khandhar et al., 2010) Also seeds of *Piper longum* gave sesamin, sylvatine and dieudesmin. The components like fatty acids of seeds were reported to presence of palmitic, hexadecenoic, stearic, linoleic, oleic, higher saturated acids, arachidic and benhenic acids. The leaves gave hentriacontane, triacontanol, and β-sitosterol. (Kirtikar & Basu , 1980 and Rastogi & Malhotra, 1993) *Piper longum* is used as anticancer, hepatoprotective, anti iflammatory, immunomodulatory, antimicrobial, anti platelet, anti hyperlipidemic, analgesic, adulticidal, melanin inhibiting, larvicial, cardioprotective activities, etc.(Kumar,et. al, 2011)

Plant material

Fruits of *Piper longum* Linn. were collected from Pirangut area in Pune, India. Plant authentication was approved by Dr. C. R. Jadhav, Botanist, Botanical Survey of India, Ministry of Environment, Forests and Climate Change, Koregoan Road, Pune. A receipt sample (BSI/WRC/IDEN.CER./2029/H3) of the plant was placed in Siddhant college of Pharmacy, Pune.

Soxhlet Extraction

The plant substantial was dried out in ordinary circumstances to sustain its dynamic

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principles and reduced into fine powder with blender. These ground fruits (1000gm) were deposited in fitted container. Powdered fruits of *Piper longum* Linn.were extracted distinctly with 1000ml of diluted alcohol (70:30-Alcohol:Water) by heating under reflux on water bath for 6 hours at 55°C. The mixture was then sieved and marc was extracted two times. The filtrates as of every extracted stage were combined and concentrated under vacuum using a rotary vacuum evaporator. Afterward whole extraction method, the extract was deposited in sterile containers in refrigeration conditions, for additional usage.

DPPH Radical Scavenging Assay

1,1-diphenyl-2-picrylhydrazine(DPPH) radical scavenging assay is one of the greatest comprehensively method of antioxidant assay. DPPH is a steady free radical that reacts through compounds that can provide a hydrogen atom. This technique is founded on the scavenging of DPPH in addition of a radical species or an antioxidant which changes color in the DPPH solution. The antioxidant potential is then noted by the decrease in absorption at 517 nm. (Kedare SB, 2011)

A solution of 300µM DPPH solution was set in methanol. 1.5 ml of this mixture added to 1.5 ml of various concentrations of Ascorbic acid (2, 4,6,10,15,20µg/ml) liquefied in methanol. Incubated this mixture in shady for 20mins and then noted the absorbance at517nm. In sample extract, a stock solution of 1000µg/ml was set by dissolving 10 mg in10ml of water, sonicated for 30 mins and the same practice was recurring using the overhead revealed steps. The percentage of Radical Scavenging activity (RSA%) using the following equation

$$RSA\% = \frac{Ao - As}{Ao} \times 100$$

Where,

Ao = DPPH Control

As= Sample or standard

Quantitative Analysis

Quantification of Total Flavonoid

Total flavonoid content was restrained using a spectrophotometric assay based on aluminium chloride complex formation. Methanolic solutions of Quercitin in was considered as reference standard to get a calibration graph. The analytical process for measuring total flavonoids was achieved as follows:

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Make a stock standard solution of 1000µg/ml by dissolving 2mg in 2ml of methanol. Make a sequences of working reference solutions by proper dilution of the stock standard solution with methanol to give a attentiveness range of 6–40 µg/ml (6.0; 8.0; 10.0;15.0;20.0; 40.0 µg/ml). Mixed 1 ml of each one of the reference solutions, with 0.5 ml 5% AlCl3(w/v) in a volumetric flask and made up the volume to 10ml with methanol. Permitted the mixture to sit for 30 mins and noted the absorbance at 425 nm. For blank, used 1ml methanol in stead of Quercetin. For sample extract, a stock solution of 2000µg/ml was made by dissolving 20 mg in 10ml of water, sonicated for 30 mins and the same protocol was repeated. The sample extract was analyzed in triplicates To obtain the regression, absorbance was plotted against concentration. (M Madhu, 2016)

Quantification of Total Steroid

To measure Total steroid content a spectrophotometric assay built on ferric-ferrous complex formation was used. Calibration graph was obtained using Ethanolic solutions of Diosgenin as reference standard. The analytical technique for measuring total steroid content was achieved as follows:

A stock standard solution of 1000µg/ml was prepared by dissolving 2mg in 2ml of ethanol. A sequence of working reference solutions made by suitable dilution of the stock standard solution with ethanol to give a concentration range of 20–150µg/ml (20.0;30.0;50.0;75.0;100.0; 150.0 µg/ml). 1ml of test extract of steroid solution was placed into10ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were mixed, followed by potassium hexacyano ferrate (III) solution (0.5%w/v, 0.5 ml). The mixture was kept in a water-bath maintained at 70°C for 30 minutes with random shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm. For sample extract, a stock solution of 1000µg/ml was made by dissolving 10 mg in10ml of water, sonicated for 30 mins and the same procedure was recurrent using the above mentioned steps. (M Madhu, 2016)

Quantification of Total Saponin

Total saponin content was obtained by using a spectrophotometric assay established on vanillin-sulphuric acid assay technique. Calibration graph was obtained using Ethanolic solutions of Diosgenin as reference standard. The analytical process for noting saponin content was did as mentioned below:

Stock standard solution of 1000µg/ml was prepared by dissolving 2mg in 2ml of

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ethanol. A series of working reference solutions was prepared by appropriate dilution of the stock standard solution with ethanol to give a concentration range of 25-300µg/ml (25.0; 50.0; 75.0;100.0;200.0; 300.0 µg/ml). Incubated 0.25 mL of standard or reagent blank with 0.25 mL of 8% (w/v) vanillin in ethanol and 2.50 mL of 72% (v/v) sulphuric acid in water for 15 min at 60°C in a water bath. After cooling at ambient temperature for 5 mins, the absorbance were measured at560nm. For sample extract, a stock solution of 1000µg/ml was prepared by dissolving 10 mg in10ml of water, sonicated for 30 mins and the same protocol was repeated using the above mentioned steps. Appropriate dilution factors considered to maintain the calibration range. Regression is obtain by was plotting absorbance against concentration. The saponin content was found to be42.57mgE/g of Diosgenin or 4.26% inplant extract. (M Madhu, 2016)

Results

Quantification of Total Flavonoid

Standard curve (Figure 1) was found using quarcetin, the total flavonoid equivalence content was found to be (5.45mgE/gm) (Table 1) in Hydro alcoholic extract of *Piper longum* Linn.

Table 1: Total Flavonoid content of *Piper longum* Linn. Hydroalcohlic extract.

Conc (µg/ml)	Absorbance
6	0.1334
8	0.1958
10	0.2402
15	0.4162
20	0.5383
40	1.0109
Sample_Avg	0.2788
Total Flavonoid content (mgE/g of Quercetin)	5.45
% Content	0.54

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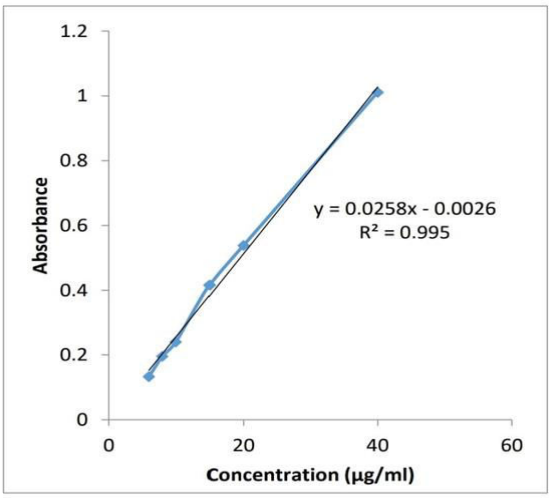

Figure 1: Standard curve of Quercitin

Quantification of Total Steroid

Standard curve (Figure 2) was found using Diosgenin, the total steroid equivalence content was found to be (33.83mgE/gm) (Table 2) in Hydro alcoholic extract of *Piper longum* Linn.

Table 2: Total Steroid content of *Piper longum* Linn. Hydroalcohlic extract.

Conc (µg/ml)	Absorbance
20	0.15298
30	0.264
50	0.40745
75	0.623
100	0.8149
150	1.202
Sample	0.5033
Total steroidcontent mgE/g of Diosgenin	338.25
% Content	33.83

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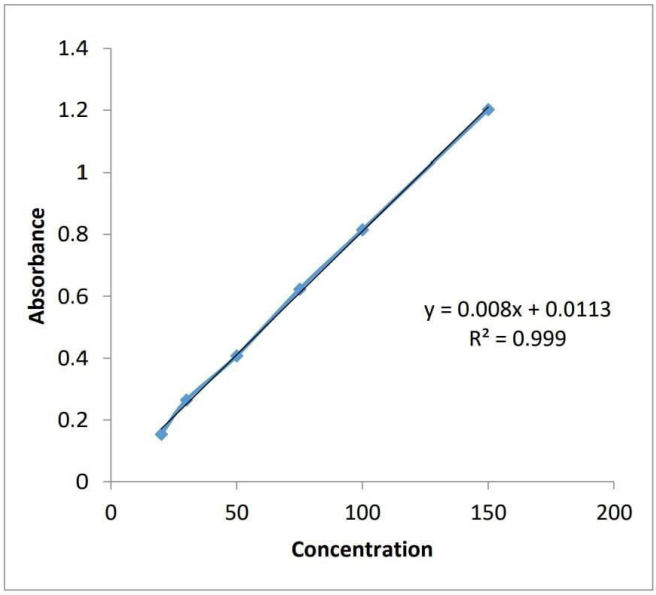

Figure 2: Standard curve of Disogenin

Quantification of Total Saponins

Standard curve (Figure 2) was found using Diosgenin, the total steroid equivalence content was found to be (42.57mgE/gm) (Table 3) in Hydro alcoholic extract of *Piper longum* Linn.

Table 3: Total Saponin content of *Piper longum* Linn. Hydroalcohlic extract.

Conc (µg/ml)	Absorbance
25	0.0735
50	0.1231
75	0.1965
100	0.2533
200	0.5066
300	0.7255
Sample_Avg	0.1148
Saponin Content mgE/g of Diosgenin	42.57
Saponin Content in %	4.26%

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Results of DPPH Radical Scavenging Activity

Delay or inhibition of oxidation processes is the ability of antioxidants. This types of reaction takes place in presence of atmospheric oxygen. Antioxidant property of plant elaborate in a defensive mechanism of (Pisochi et al., 2016)

Hydro alcoholic extract of *Piper longum* Linn. was screened for antioxidant property by DPPH radical scavenging activity. The extract was investigated in comparison with the known antioxidant such as ascorbic acid (Figure 3).The assay was performed using various concentrations of hydro alcoholic extract of *Piper longum* Linn. i.e. 2µg/ml, 4, 6, 10, 15, 20 µg/ml and percent DPPH scavenged was found to increase with increasing concentration.(Table 4)

The % radical scavenging activity for 1000µg/ml extract was found to be 26.56% and the concentration of extract needed to scavenge the free radicals by 50% was calculated as 1.8mg/ml by using formula.

Table 4: DPPH Radical scavenging activity of *Piper longum* Linn.

Conc (µg/ml)	Absorbance	% DPPH Activity Remaining	% DPPH Scavenged
Control	**0.6914**	**100**	**0**
2	**0.6237**	**90.21**	**9.79**
4	**0.5507**	**79.65**	**20.35**
6	**0.4657**	**67.35**	**32.65**
10	**0.3886**	**56.24**	**43.80**
15	**0.1636**	**23.66**	**76.34**
20	**0.0439**	**6.35**	**93.65**
Sample	**0.5077**	**73.43**	**26.57**

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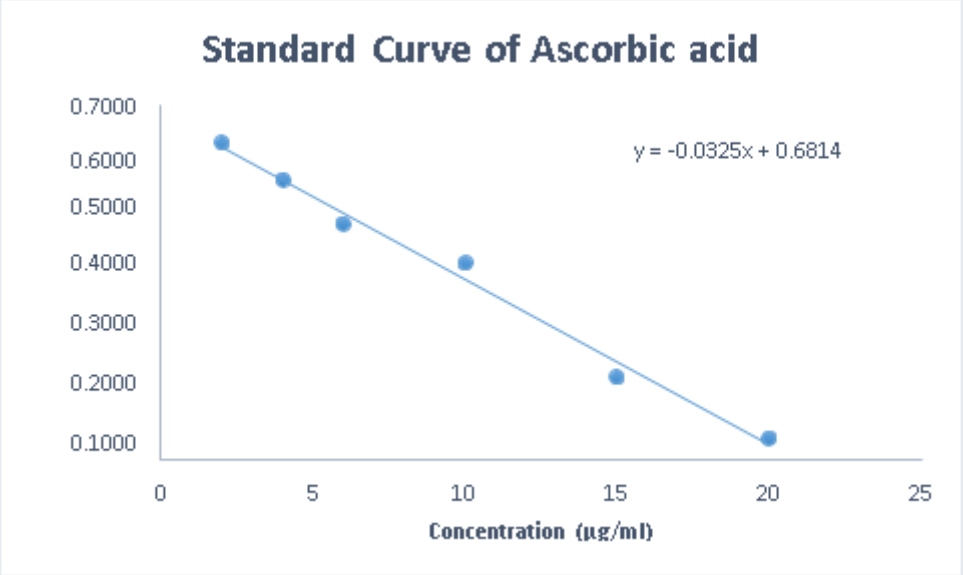

Figure 3: Standard curve of Ascorbic acid

CONCLUSION

Piper longum Linn. exhibited presence of flavonoids (5.45. mgE/gm), steroids (33.83mgE/gm), saponins (42.57mgE/gm) in equivalence by quantitative analysis technique and DPPH antioxidant potential was identified.

ACKNOWLEDGMENT

The authors are thankful to Siddhant college of Pharmacy, Pune for providing laboratory facility and Botanical survey of India for plant identification.

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