Evaluation of TPC, TFC, and Antioxidant Activity of Extracts of *Piper longum* L.

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ABSTRACT

Medicinal plants have been crucial in treating various diseases since ancient times. This study focuses on identifying the phytochemicals in *Piper longum* L. extracts along with studying their biological activities. The powdered fruit of *P. longum* was sequentially subjected to ultrasonic extraction utilizing solvents with increasing polarity, starting from hexane and progressing through chloroform, ethyl acetate, and finally, methanol. The phytochemical analysis of extracts exhibited the presence of all tested classes of phytocompounds except saponins. The higher phenolic content (TPC) was observed in the methanol extract (53.38 mg GAE/g), whereas the chloroform extract had a TPC value of 8.51 mg GAE/g. Conversely, the chloroform extract exhibited a higher total flavonoid content (TFC) of 12.09 mg QE/g compared to the methanol extract's 7.44 mg QE/g. The antioxidant assay demonstrated the moderate antioxidant potential of the methanol extract. This study recommends further biological tests and experimental verifications to use this plant for drug discovery.

Keywords: Piper longum, Phytochemicals, TPC, TFC, antioxidant

1. INTRODUCTION

Natural compounds are physiologically active substances with a wide range of applications that come from a number of sources, such as fungi, bacteria, plants, and marine organisms [1]. These compounds are widely employed as an active component in both traditional and modern medicine to treat a variety of illnesses [2]. The World Health Organization (WHO) asserts that medicinal plants are the greatest source of a broad range of medications [3], with more than 80% dependency on traditional medicine [4]. The therapeutic value of plants is primarily attributed to the existence of bioactive compounds such as tannins, phenolics, flavonoids, glycosides, alkaloids, and terpenoids [4, 5].

Piper longum L. (*P. longum*), commonly called long pepper, derived from the Sanskrit word “Pippali”, is a flowering vine belonging to the Piperaceae family [6]. It is a readily available plant that thrives in warm climates, characterized by its dioecious nature, aromatic essence, and trailing growth pattern, featuring perennial woody roots and segmented stems [7]. The alternating leaves without stipules and flowers that grow in solitary spikes with cylindrical, blunt, and blackish-green fruits are the characteristic features [6]. The mature spikes are collected and dried as the commercial form of pippali. The unripe fruit of P. longum is cooling and sweetish whereas the ripe fruit is sweet and pungent. *P. longum* is known to be an effective remedy for various health issues, including tuberculosis, sleep disturbances, gonorrhea, menstrual pain, chronic gastrointestinal discomfort, respiratory tract infections, and arthritis [8]. It also exhibits significant anti-disease properties against a variety of

illnesses, such as cancer, diabetes, depression, and radiation toxicity [9]. Moreover, the pharmacological studies showed their potential hepatoprotective [10], anti-amoebic [11], anti-inflammatory [12], anti-fungal [13], analgesic [14], anti-depressant [15], and anti-diabetic [16]. Specifically, ripe fruit has anti-inflammatory properties that can be used to treat spleen illness, piles, bronchitis, asthma, and biliousness [17].

According to the ancient Ayurvedic verse, *P. longum* functions as a bio-enhancer, helping the body rid itself of endotoxins [18]. The fruit contains numerous alkaloids and related compounds, with piperine being the most prevalent among them, and the other phytocompounds are methyl piperine, asarinine, pipernonaline, piperettine, piperlongumine, piperlonguminine, piperundecalidine, piperderidine, and pipercide [19, 20]. The objective of this research work is to identify the phytochemicals present in the extracts of *P. longum* and study the biological activities to explore their importance in drug discovery.

2. MATERIAL AND METHODS

2.1 Chemicals

Hexane, chloroform, ethyl acetate, methanol (Fischer Scientific India), Gallic acid (Hi-media Laboratories), 2,2-diphenyl-1- picrylhydrazyl (DPPH), and quercetin (Wako Pure Chemicals, Osaka, Japan) were used.

2.2 Preparation of Plant Extracts

250 g of *P. longum* fruit was collected from the local market of the Kathmandu district and crushed into powder form by an herbal medicine disintegrator. The procedure of ultrasonic extraction was carried out to obtain various fruit extracts, namely hexane, ethyl acetate, and methanol extracts. These extracts were obtained by utilizing the solvents hexane, ethyl acetate, and methanol, respectively, through a process of solid-liquid fractionation. The percentage yield of the extracts was calculated using the formula:

$$\%\ \text{Yield} = \frac{\text{Dry weight of extract}}{\text{Dry weight of plant sample}} \times 100$$

2.3 Phytochemical Screening

The bioactive compounds present in the extract are identified by the appearance of specific colors in the phytochemical screening test, and the presence of phytochemicals was detected through chemical methods based on the methodology established by Banu and Cathrine, 2015 [21].

2.4 Determination of Total Phenolic Content (TPC)

Folin-Ciocalteu colorimetric analysis based on an oxidation-reduction reaction was used to determine the TPC of the plant extracts [22]. For the generation of the calibration curve, gallic acid was used as the standard. A 1000 µg/mL initial gallic acid solution was prepared, followed by the preparation of various concentrations ranging from 100 to 20 µg/mL through sequential dilution. 1 mL of each concentration of gallic acid solution was transferred into test tubes, to which 5 mL of 10% Folin-Ciocalteu reagent (FCR) and 4 mL of 7% sodium carbonate solution (Na_2CO_3) were added, resulting in a final volume of 10 mL. The resulting blue solution was vigorously shaken and then placed in a water bath at 40°C for 30 minutes. Lastly, the absorbance of the solution was measured at 760 nm using a spectrophotometer.

A stock solution of the extracts at a concentration of 10,000 µg/mL was prepared, and the concentration was reduced to 1000 µg/mL via serial dilution. The absorbance values were measured using the same procedure as employed for gallic acid.
The TPC of the extract was calculated using the equation,

$$\mathrm{TPC} = \frac{\mathrm{c} \times \mathrm{v}}{\mathrm{m}}$$

Here,
c = Concentration of gallic acid obtained from the calibration curve (mg/mL)
m = Dry weight of extract (g)
v = Volume of extract (mL)

2.5 Determination of Total Flavonoid Content (TFC)

Aluminium chloride colorimetric assay was used to determine the TFC of the plant extract [23]. Quercetin was used as the standard for the generation of the calibration curve. A stock solution of quercetin of concentration 1000 µg/mL was prepared, and through serial dilution, various concentrations ranging from 100 to 20 µg/mL were prepared. A 1 mL quercetin from each concentration was transferred to a 20 mL test tube containing 4 mL of distilled water. Then, 0.3 mL of 5% $NaNO_2$ was added to each test tube, followed by a 5-minute incubation period. Afterward, 0.3 mL of 10% $AlCl_3$ was introduced into the mixture, and after 6 minutes, 2 mL of 1 M NaOH was added, followed by the addition of 2.4 mL of distilled water. The resulting pink-colored solution was subjected to absorbance measurement using a spectrophotometer at 510 nm.
A stock solution of the extracts at a concentration of 10,000 µg/mL was prepared, and by the process of serial dilution, the concentration was reduced to 1000 µg/mL. The absorbance values were measured using the same procedure as employed for quercetin.
The TFC of the extract was calculated using the equation,

$$\mathrm{TFC} = \frac{\mathrm{c} \times \mathrm{v}}{\mathrm{m}}$$

Here,
c = Concentration of quercetin obtained from the calibration curve (mg/mL)
m = Dry weight of extract (g)
v = Volume of extract (mL)

2.6 Antioxidant Activity

The antioxidant potential of various extracts was assessed using 2,2-Diphenyl-1-picrylhydrazyl-hydrate (DPPH) radicals [24]. A stock solution of concentration 1000 µg/mL was initially prepared for both chloroform and methanol extracts. This stock solution was then serially diluted to prepare extract solutions from 20 to 100 µg/mL. Similarly, a 1000 µg/mL ascorbic acid solution was prepared using ethanol and subsequently diluted to produce a series of concentrations ranging from 20 ppm to 100 ppm. Each concentration of ascorbic acid solution was mixed with 2 mL of a 0.2 mM DPPH solution and allowed to incubate in darkness for 30 minutes. After incubation, the absorbance of the mixtures was measured at 517 nm using a spectrophotometer. The same procedure was applied to measure the absorbance of extracts and DPPH solution.
The percentage scavenging of DPPH free radical was evaluated using the equation;

$$\% \text{ Scavenging} = \frac{A_O - A_S}{A_O} \times 100$$

Here, (A_0) = Absorbance of the control, (A_S) = Absorbance of the test sample
The graph plot between the percentage scavenging and concentrations was used to determine the half-maximal inhibitory concentration (IC_{50}) of each extract.

3. RESULTS AND DISCUSSION

3.1 Percentage Yield

The percentage yield was found highest for the chloroform extract (1.95%), followed by methanol extract (1.9%), hexane extract (1.8%), and ethyl acetate extract (0.15%).

3.2 Phytochemical Screening

The phytochemical screening showed the presence of terpenoids, phenols, alkaloids, flavonoids, and volatile oils, as depicted in Table 1.

Table 1. Phytochemical analysis of the extracts

Class of Phytochemicals	Test	Hexane extract	Chloroform extract	Ethyl acetate extract	Methanol extract
Volatile Oil	Volatile oil test	+	+	+	+
Alkaloids	Mayer's test	-	+	+	+
	Dragendorff's test	-	+	+	+
Phenols	$FeCl_3$ test	-	+	+	+
Flavonoids	Lead acetate test	-	+	+	+
	Shinoda test	-	+	+	+
Terpenoids	Liebermann-Burchard Test	+	+	+	+
Saponin	Froth test	-	-	-	-
	Foam test	-	-	-	-

Here '+' refers presence and '-' refers absence

3.3 Analysis of Total Flavonoid and Phenolic Contents

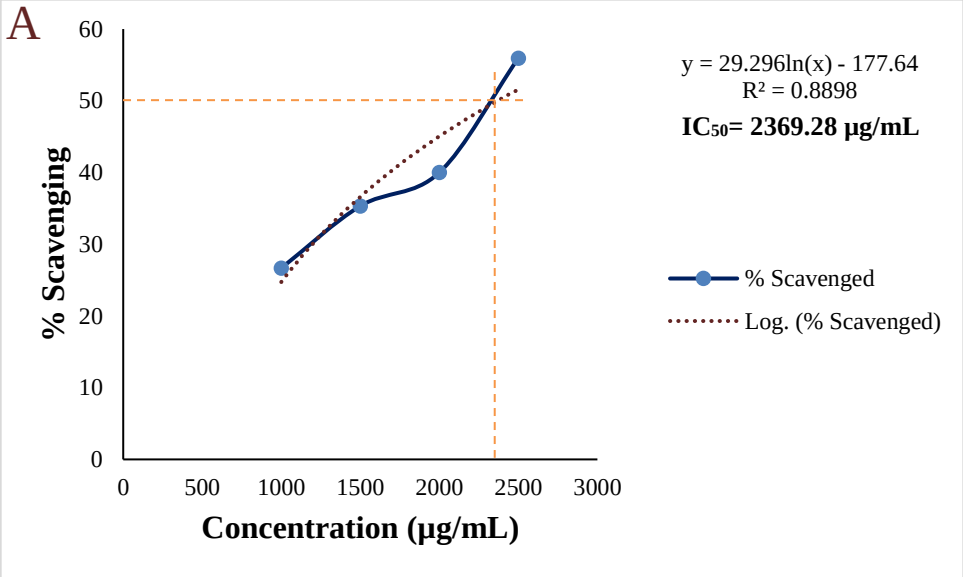
The TPC and TFC of chloroform and methanol extracts are shown in Table 2. Folin-Ciocalteu colorimetric method was used to evaluate the TPC of extracts using gallic acid as standard. The total phenolic content of chloroform and methanol extracts were found to be 8.51 mg GAE/g and 53.38 mg GAE/g, respectively. Likewise, the TFC of extracts was assessed using aluminium chloride colorimetric assay and quercetin as standard. The chloroform extracts showed a TFC of 12.09 mg QE/g, while 7.44 mg QE/g value of TFC was observed for the methanol extract.

Table 2. TPC and TFC of different extracts

Samples	TPC (mg GAE/g)	TFC (mg QE/g)
Chloroform extract	8.51	12.09
Methanol extract	53.38	7.44

3.4 DPPH Scavenging Assay

The antioxidant activity of different fruit extracts of *P. longum* was tested using the DPPH scavenging method in terms of IC_{50} value. The IC_{50} values have the inverse relation with the antioxidant potential, and the IC_{50} for chloroform extract and methanol extracts were 2369.28 µg/mL and 768.93 µg/mL exhibiting very mild antioxidant properties of methanol extract. The antioxidant activity of chloroform extract and methanol extract is illustrated in Figure 1.



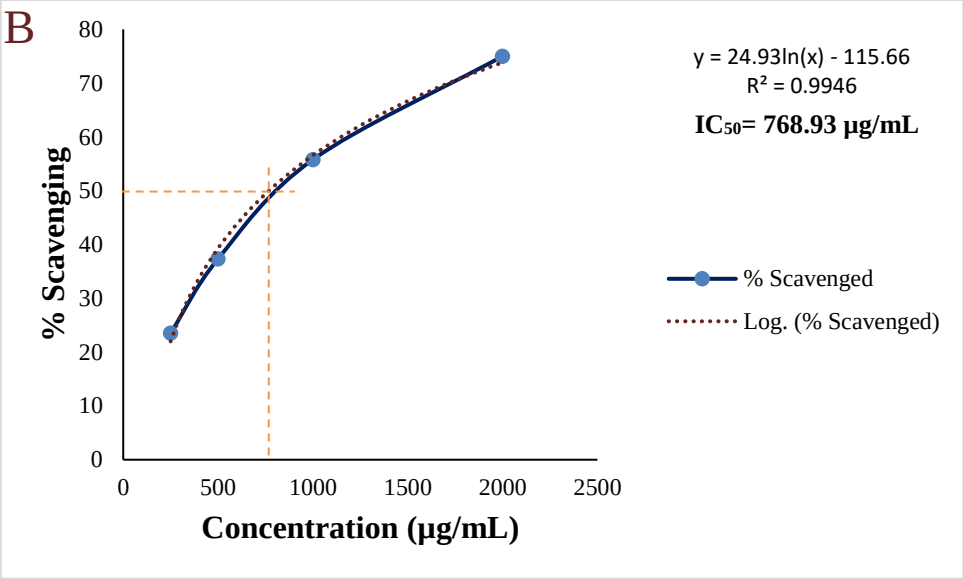


Fig. 1. Graphical representation of DPPH assay of the (A) chloroform extract and (B) methanol extract

The antioxidant property of medicinal plants is due to the presence of active constituents such as alkaloids, flavonoids, phenolics, and iridoids that reduce the reactive oxygen species [25]. These phytocompounds could exhibit various bio-activities such as antioxidant, anti-cancer, anti-diabetic, and anti-inflammatory [26, 27]. The quantitative phytochemical assessment showed a comparatively higher content of phenolics than flavonoids in methanol extract. The higher phenolics in methanol extract (Table 2) and its lower IC_{50} on DPPH assay (768.93 µg/mL) showed a correlation between the phenolic content and its antioxidant ability. The weak antioxidant nature of chloroform extract could be due to the low content of flavonoids and phenolics.

4. CONCLUSION

Phytochemical analysis of *Piper longum* fruit extracts demonstrated the presence of various phytoconstituents except saponin. The phenolic content was found to be higher in the methanol extract, while the flavonoid content was relatively low in both extracts. The methanol extract exhibited a moderate antioxidant potential in the DPPH free radical scavenging assay, whereas the chloroform extract showed a weak antioxidant activity. Hence, these research findings could be utilized for further investigation into the medicinal properties of *P. longum*.

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