SCREENING, PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITIES OF PIPER LONGUM SEED EXTRACTS

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ABSTRACT

Indian spices have great potential in curing many diseases along with their spicy nature. Piper longum is known for its wide spice and medical applications. The objectives of this study are screening different solvent extracts of Piper longum and their phytochemical analysis as well as its antioxidant potential for further studies.

Various extracts of the spice Piper longum seeds like Aqueous, Methanol, Petroleum Ether and Acetone were prepared and concentrated to minimum volume. The extracts were analysed for various phytochemical quantitative analyses such as Total phenols (Folin-Ciocalteu method), Flavonoids (Aluminum chloride method), total sugars (Dubois method) and Proteins (Bradford’s method). The extracts are also subjected to analyse their antioxidant ability in different model systems like DPPH and Superoxide radical scavenging activities.

The aqueous extract contains proteins (2.5g%), flavonoids (0.8g%), polyphenols (1.1g%) and total sugars (11g%). Whereas the Acetone extract and methanol extract contain flavonoids (~0.48g%) and polyphenols (0.33g%). The petroleum ether extract contains very negligible amount of flavonoids (0.13g%) and polyphenols (0.24g%). In antioxidant model system, the aqueous crude extract showed more DPPH radical scavenging activity (35%) and showed Superoxide radical scavenging activity (21%) when compared to standards like Ascorbic acid and Alpha-tocopherol. These results indicate, the aqueous extract of Piper longum showed more activity than other extracts. This is the basis for further in vitro studies to unfold the antioxidant activity of aqueous extract of Piper longum protein.

Keywords: Piper longum, protein, polyphenols, flavonoids, sugars, DPPH.

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INTRODUCTION

There are numerous scientific studies that have focused on the pharmacological activity of bioactive components from medicinal plants and there is increasing interest on these components from scientific community as cancer suppressant [1-3]. Plants have been the source of medicines for thousands of years. Long pepper (*Piper longum*) or Indian long pepper (*Pipli*) belongs to the Piperaceae family, extensively used as spice and as medicine in South Asian subcontinent [4]. Various areas of research on *Piper longum* have been highlighted with a view to explore and isolate from various parts and identify the medicinally important phytoconstituents, which could be utilised for treating various diseases like chronic asthma, hepatotoxicity and tumours affecting the mankind [5-10].

*In vitro* study showed that the *Piper longum Linn* has a significant antioxidant activity due to the presence of important bioactive compounds (like phenolics, flavonoids, flavonols, alkaloids) and can be subjected to pharmaceutical drug formulations [6]. This study would unfold the antioxidant activity and anticancer effect of aqueous extract of *Piper longum Linn* seed proteins in order to ascertain the scientific proof for its traditional use.

MATERIALS AND METHODS

*Piper longum* seeds were procured from authentic source in Mysore, Karnataka, India. The seeds were cleaned, washed in double distilled water to remove dust particles, shade dried and powdered (100mesh British Pharmacopea), stored in glass bottle for further studies. The extracts were prepared like 10g of *Piper longum* seeds powder in 100ml of water, methanol, petroleum ether and Acetone. Vortexed for 2 hours at room temperature, filtered, and got it evaporated to concentrate the extract and then stored in shaded area.

**Chemicals:**

Standard reagents and chemicals of analytical grade were used. Bovine Serum Albumin (BSA), Quercetin, Gallic acid and DPPH were procured from Hi Media (Mumbai, India). Methanol, Acetone and Petroleum ether and other chemicals were procured from Merck and S.D. Fine chemicals India.

**Quantitative analysis:**

The various extracts of *Piper longum* such as aqueous, methanol, petroleum ether and acetone were analysed for phytochemical components such as Phenols (Folin-Ciocalteu method), Flavonoids (Aluminum chloride method), total sugars (Dubois method) and proteins (Bradford’s method).

**Estimation of total phenolic content:**

The total phenolic content was estimated by “Folin - Ciocalteu method”[11]. The estimation is based on the principle that phenolic compounds react with phosphomolybdic / phosphotungstic acid in the alkaline medium. This can be measured spectrophotometrically at 730nm. Total phenolic content was calculated using a standard Gallic acid.

**Estimation of flavonoids:**

The total flavonoids content was determined by “Aluminum chloride method” [12]. Flavonoids react with AlCl$_3$ to form acid stable complexes, which can be measured spectrophotometrically at 415nm. Quercetin was used as the standard. The concentrations of flavonoids in the test samples were calculated from the calibration plot.

**Estimation of total sugars:**

The total sugar content of the extract is estimated by “Dubois method”[13]. The sugar complexes present in the extract, react with phenol in acidic medium to form brown color, which is read immediately at 520nm. The total sugar concentration was calculated according to the standard glucose calibration curve.

**Estimation of proteins:**

Bradford assay relies on the binding of the dye Coomassie Brilliant Blue G250 to protein [14]. The dye binds most readily to arginyl and lysyl residues of proteins. Blue form of the dye which binds to protein, can be estimated by determining the amount of dye at 595nm. The concentrations of proteins in the test samples were calculated from the calibration plot. Varying concentrations of bovine serum albumin (10-100μg/μl) are used for standard calibration curve.
Antioxidant activity:

DPPH radical scavenging activity:

DPPH radical scavenging activity was assessed according to the method of Shimada et al. (1992) with minor modifications [15]. The *Piper longum* extracts at a concentration of 25 µg was mixed in 1 ml of freshly prepared 0.5 mM DPPH ethanolic solution and 2 ml of 0.1 M acetate buffer pH 5.5. The resulting solutions were then incubated at 37°C for 30 minutes and measured spectrophotometrically at 517 nm. Ascorbic acid, BHA and Curcumin were used as positive control under the same assay conditions. Negative control was without any inhibitor or *Piper longum* extracts. Lower absorbance at 517 nm represents higher DPPH scavenging activity. The %DPPH radical scavenging activity of different extracts of *Piper longum* was calculated from the decrease in absorbance at 517 nm in comparison with negative control.

Superoxide radical scavenging activity:

The Superoxide radical (O2•-) scavenging activity of spice *Piper longum* extracts was measured according to the method of Lee et al. (Lee et al., 2002) with minor modifications[16]. The reaction mixture containing 100µl of 30mM EDTA (pH 7.4), 10µl of 30mM hypoxanthine in 50mM NaOH and 200µl of 1.42mM nitro blue tetrazolium with or without *Piper longum* extracts and SOD serving as positive control at various concentrations ranging from 50-300µg. After the solution was pre-incubated at ambient temperature for 3min, 100µl of Xanthine oxidase solution (0.5U/ml) was added to the mixture and incubated for one hour at 37°C, and the volume was made up to 3ml with 20mM phosphate buffer (pH 7.4). The solution was incubated at room temperature for 20 min; absorbance was measured at 560 nm. Appropriate controls were included to rule out the artifacts induced reaction. The control was without any inhibitor. Inhibitory effect of different *Piper longum* extracts on superoxide radicals was calculated as

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\text{% Superoxide radical scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100
\]

RESULTS AND DISCUSSION

Table - 1: Quantitative analysis of various extracts of *Piper longum*

<table>
<thead>
<tr>
<th>EXTRACTS</th>
<th>QUANTITATIVE ANALYSIS OF EXTRACTS (g %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein</td>
</tr>
<tr>
<td>Water</td>
<td>2.5</td>
</tr>
<tr>
<td>Methanol</td>
<td>0</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>0</td>
</tr>
<tr>
<td>Acetone</td>
<td>0</td>
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</tbody>
</table>

The Phytochemicals analysis of the extract showed that the presence of major components like proteins, polyphenols, flavanoids and total sugars is as shown in Table-1. The aqueous extract of *Piper longum* contains more phytochemicals when compared to other extracts.

Figure - 1: DPPH radical scavenging activity of *Piper longum* seeds extracts
The DPPH radical scavenging activity showed that, at 15μg dosage, the aqueous extract showed more DPPH radical scavenging activity (38%) when compared to methanol (32%), petroleum ether (14%) and acetone (30%) extracts, and to standard antioxidants like Ascorbic acid (52%), and alpha-tocopherol (65%).

Figure - 2: Superoxide radical scavenging activity of *Piper longum* seeds extracts

The superoxide radical scavenging activity of *Piper longum* showed at 15μg dosage, the aqueous extract showed more Superoxide radical scavenging activity (28%) when compared to methanol (23%), petroleum ether (14%) and acetone (16%) extract, and to the standard antioxidants like Ascorbic acid (43%), and alpha-tocopherol (52%).

These studies are encouraging us to do further studies towards purification of the aqueous extract of *Piper longum*.

The aqueous extract of *Piper longum* have showed more significant antioxidant activity due to bioactive components like protein, total phenols, flavonoids and are subjected to pharmaceutical drug formulations. This plant is inexpensive, readily available, and effective for treating many diseases, including cancer, inflammation, depression, diabetes, obesity, and hepatotoxicity. In this study the protein concentration in aqueous extract of *Piper longum* is considerably higher than solvent extracts.
CONCLUSION
The studies showed that, the quantitative phytochemical profiling revealed that the phytochemical composition varies with the extract. In the present study, the presence of phenols, flavonoids, proteins and total sugars is more in aqueous extract than in other extracts. In the same way, the antioxidant model system shows that, the aqueous extract of *Piper longum* showed more antioxidant activity when compared to other extracts both in DPPH and Superoxide radical scavenging studies.

However, role of aqueous extract of *Piper longum* proteins as an antioxidant has not been studied till date. In this study the protein concentration in aqueous extract of *Piper longum* is considerably higher than other solvent extracts. This is the basis for further *in vitro* studies to unfold the antioxidant activity of aqueous extract of *Piper longum* protein.

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