Phytochemical screening and antioxidant activity of a edible, medicinally important plant taxa *Mollugo pentaphylla* L. (Molluginaceae)

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**Abstract:** *Mollugo pentaphylla* L. is a medicinally important plants in India. Many rural peoples are consumed this plants as a food mainly leaf and stem portion. But the other parts like stem and root of this plant is also has a great medicinal value. The objective of the present study to evaluate the identification of phytochemical constituents that are present on this plants. In qualitative analysis, the phytochemical compounds such as carbohydrates, proteins, polyphenols, carotenoid, ascorbic acid, alkaloide, tannin, flavonoid, glycoside, volatile oil etc. Besides this, these plants also have antioxidant potential in high concentration.

**Keywords:** Antioxidant activity; Medicinal Importance; Phytochemical Screening.

**Introduction**

*Mollugo pentaphylla* Linn. commonly known as carpet weed (English), Pitta saga (Oriya) is a perennial herb belonging under the family Molluginaceae, found throughout India, Ceylon, Malacca, China, Japan, Fiji etc. Roots are creepier and adventitious, leaves are trifoliate small oval shape; flowers are white, pentameric and bisexual. The urban people used this plant medicinally in paste form orally and externally for treatment of skin allergic condition, antimicrobials etc. (Rama and Gurjar., 1990; Evans and Evans, 2002; Chopra and Chopra, 1956). Many of the plant extracts have proven to possess pharmacological action (Pawar et al., 2001) due to the presence of various phyto-chemicals. It is also a traditionally used medicines, which commonly practiced in many villages of India due to its various beneficial effects. An erect slender annual herb, the herb is conceded stomachic, aperients and antiscptic. It is used in stomach ache and to promote local discharge flowers and tender shoos in decoction have a diaphoretic effect roots are used in gout and rheumatism The leaves are employed in the preparation of poultices for sorelegs. Decoctions of roots are used to treat eye diseases. Highly esteemed by Hindus as a bitter vegetable which they eat occasionally on account of its stomachic, aperient and antiscptic properties (Kirtikar and Basu, 1999). There is a growing interest in the pharmacological evaluation of various planta used in Indian traditional system of medicine (Malhotra and Singh, 2006). Historically plants have been used in folk medicine to treat various diseases and are rich natural sources of antioxidants. Many researchers have examined the effect of plants used traditionally by indigenous people to support liver function and treat diseases of the liver. In most cases, research has confirmed traditional experience by discovering the mechanism and mode of action of these plants. Hot H₂O extract of dried entire plant in India used for whooping cough and in cases of atrophy in human (Singh, 1980) and Decoction of dried entire plant used to treat hepatitis in Taiwan. *M. pentaphylla* is a component in an important folk medicine named “Peh-Hue-Juwa-Chi-Gao” in Taiwan, which is used as an anticanicer, antitoxic and diuretic agent (Lin et al., 2004). Eaten as a pot-herb, it is also used medically for mouth infections. The original scientific studies on the plant reported to possess active antifungal activity, antibacterial activity, spermicidal and spermistastic effect, anti-inflammatory and hepato protective activity and antioxidant activity (Ghosh, et al., 1984). The plant is reported to contain Flavones such as Apigenin and Mollupentin, Mollugogenol A, ananfungul triterpenoid, Mollugogenol B, Mollugogenol D, Oleanolic acid and a steroid Beta–Sitosterol (Hamburger, et al., 1989).

**Materials and Methods**

The present study was carried out within the different intersection is in Midnapore Zone, one of the important holy cities in India and is extended from latitude 22°41’61” in the north to longitude 87°38’44” in the east with a tropical climate during the time period of March, 2016 to May, 2016. The sites were selected for the present study includes Forest Area, Urban Area and Industrial Area. The plant found in wet, damp or moist soil vigorously.
For the present study, fresh leaves, stem, root of *Mollugo pentaphylla* collected from the experimental site beside the road of Vidyasagar University during the month of March, 2016.

**Plant Extract Preparation for Chemical analysis:**
The entire plant was separated and dried in Hot Air Oven. The dried plant material were ground to coarse powdered. The powdered plant was extract with methanol and water then it was preserved in freez for further chemical test. Take 0.2 gm of the powdered drug in test tube, add 2ml of the solvent and shake it for 1minute (Harborne, 1973). Filter through cotton wool plug over a glass funnel. If required the solvent with the powder may be heated on a water bath for 2 min before filtration (Brinda, et al., 1991).

**Test for Carbohydrate**

**Procedure**

- Add a two drops of Milisch’s reagent (5% 1-naphthol in alcohol) to about 2ml of test solution and mix well. Incline the tube and add about 1ml of concentrated sulphuric acid along the sides of the tube. Observe the colour at the junction of two liquids.
- To 1ml of Fehling’s solution ‘A’, add 1ml of Fehling’s solution ‘B’ and a few drops of the test solution. Boil for a few minutes.
- To 2ml of Benedict’s reagent add five drops of the test solution. Boil for five minutes in a water bath. Cool the solution.

**Test for Protein**

**Procedure**

- To 2ml of the test solution add 2ml of 10% NaOH. Mix. Add two drops of 0.1% CuSO₄ solution.
- To 4 ml of the solution which should be at neutral pH add 1 ml of 0.1% freshly prepared ninhydrin solution. Mix the contents and boil for a couple of minutes. Allow to cool.
- Add 2ml of glacial acetic acid to 2ml of the test solution. Then add about 2ml of conc. H₂SO₄ carefully down the sides of the test tube. Observe the colour, change at the junction of the two liquids.

**Test for Iodine:** Add a few drops of iodine solution to about 1ml of the test solution. Appearance of blue colour is due to the formation of starch iodine complex.

**Test for Glycosides:** Take 0.5 ml of alcoholic extract of the drug add 1ml of water and 1ml of sodium hydroxide solution. A yellow color indicates the presence of glycosides.

**Test for Saponins:** Weight 0.1 gm of the drug and place it in a clean dry test tube. Add 10ml of D/W put a stopper and vigorously shake for 30 minutes allow the tube to stand in a vertical position and observe for a period of 30 minutes if a honeycomb forth more than 3cm above the surface of the liquid persists for 30 minutes, the drug is presumed to contain saponins.

**Test for volatle Oil:** To the thin section of the drug add alcoholic solution of Sudan 111; Red color indicates presence of volatile oil.

**Test for Flavonoid:** Add 5ml Lead acetate to about 100mg of plant extract.

**Test for Alkaloid:** Add 10 ml HCl to the 250 plant extract and then the solution filtered. 2ml of filtrate material add with the 2ml of picric acid.

**Antioxidant Analysis**

**Radical scavenging effect of extracts in DPPH radicals:** DPPH (1,1-diphenyl-2-picrylhydrazyl) can make stable free radicals in aqueous or ethanol solution (Jung et al., 2005). When DPPH reacts with an antioxidant compound, it is reduced the change of color (from purple to light yellow) was measured at 517nm on UV-VIS spectrophotometer. The radical scavenging potential was calculated as RSC (% of inhibition). Where,

\[
\% \text{ RSC} = \frac{OD_{\text{control}} - OD_{\text{sample}}}{OD_{\text{control}}} \times 100
\]

**Hydrogen peroxide Decomposition:** The assay mixture contained 4 ml of H₂O₂ solution (80mM) and 5mL of phosphate buffer (pH 7.4). One milliliter of each extract dissolved in water (25mg/mL) was rapidly mixed with the reaction mixture by a gentle swirling motion at room temperature (Veeru, et al., 2009). 1 mL portion of the reaction mixture was then blown into 2 mL of dichromate /acetic acid reagent at 60 s intervals. The decomposition of H₂O₂ was determined based on the standard plot for H₂O₂ was determined based on the standard plot for H₂O₂ and the monomolecular velocity constant K was determined by using the formula:

\[
K=\frac{1}{t \log_{10} S_0/S}
\]

Where, S₀ is the initial concentration and S is the final concentration of H₂O₂.
Results and Discussions
The study of the chemical constituents and the active principles of the medicinal plants have acquired a lot of importance all over the world, the present study including screening of Phytochemical Analysis the Mollugo pentaphylla subjected to preliminary qualitative chemical analysis. The dried powdered were used for analysis. The qualitative chemical test for the extracts was performed. The preliminary phytochemical analysis was conducted for the presence of bioactive compounds. The Qualitative analysis revealed the presence of carbohydrates, protein, saponins, tannins, terpenoids, flavonoids, steroids, phenols, proteins, alkaloids and glycosides, volatile oils, in the whole plant of Mollugo pentaphylla (Table.1). In the present study the antioxidant activity of M. pentaphylla was determined by DPPH assay, H₂O₂ assay. This all of the assay for antioxidant properties in Mollugo pentaphylla showed highest total antioxidant capacity (Fig.1). The presence of the above phytoconstituents in Mollugo pentaphylla, have medicinal importance for its wide spectrum of pharmacological activity which includes antiseptic, anti-inflammatory, anti-rheumatic and antioxidant activities (Table.2) and made it as a handy herbal remedy in treating wounds, inflammation, fever, stomach ache and joint pains (Doss and Anand, 2012).

Table 1: Phytochemical Tests:

<table>
<thead>
<tr>
<th>Phytochemical tests</th>
<th>Water extract</th>
<th>Methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
<td>Stem</td>
</tr>
<tr>
<td>1. Carbohydrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Felling's test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>b. Benedict test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>c. Molish's test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>d. Seliwanoff's test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>e. Barfoed's test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2. Protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Biuret test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>b. Ninhydrin test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>c. Glyoxyl reaction</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>f. for Trpophan</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3. Tannin test: Ferric chloride test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4. Glycoside test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5. Iodine</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6. Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7. Alkaloid: Hanger's test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8. Flavonoid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9. Volatile oil</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 2: Antioxidant potential of fresh tissue of Mollugo pentaphylla.

<table>
<thead>
<tr>
<th>Name of Plant</th>
<th>Parts</th>
<th>DPPH Test I.C. 50 Value</th>
<th>H₂O₂ Test I.C. 50 Value</th>
<th>Radical Scavenging I.C. 50 Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mollugo</td>
<td>Leaf</td>
<td>0.662</td>
<td>0.174</td>
<td>0.038</td>
</tr>
<tr>
<td>pentaphylla</td>
<td>Stem</td>
<td>0.532</td>
<td>0.074</td>
<td>0.034</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>0.739</td>
<td>0.070</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Figure 1: The graphs shows antioxidant activity of Mollugo pentaphylla in Hydrogen peroxide assay (A); the antioxidant activity by DPPH assay (B).

Conclusion
In the present study showed the qualitative of the phytochemical constituents in the methanolic and water extract of the whole plant of Mollugo pentaphylla clearly reveals the presence of various biologically active compounds useful in treating various ailments in humans. Since, the detected phytochemicals are known to have biological activity; these plants can be used for their extraction for medicinal use. Antioxidants are used for preventing the deleterious consequences of oxidative stress; hence, there is increasing interest in the protective functions of natural antioxidants from plant source. The soluble antioxidants which prevents oxidative damage to the cell membrane induced by aqueous radicals (Sudhahar et al., 2007). Antioxidants act as a scavenger of ROS to prevent, or at least alleviate, the deleterious effects caused by ROS. Acting as a chain breaking antioxidant, it impairs with the formation of free radicals in the process of formation of intercellular substances through the body, including collagen, bone matrix and tooth dentine (Veeru et al., 2009). To conclude, the phytochemicals are either the product of plant metabolism or synthesized for defense purposes which are known to have bioactivity. The occurred bioactive phytochemical and the antioxidant potential of these Mollugo species can be used for medicinal purpose. This study also leads to the further research in the way of isolation and identification of the active compound from the plants using chromatographic and spectroscopic techniques.
Acknowledgement

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