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RESEARCH ARTICLE

Laboratory Assessment and Phytochemical Screening of *Mollugo spergula*

Sagar Naskar[*1,2] Upal K. Mazumder[1], Raj Biswas[2]

[1]Dept. of Pharmaceutical Technology, Jadavpur University, Kolkata, India

[2]Bengal College of Pharmaceutical Sciences and Research, Durgapur, India.

ABSTRACT

Medicinal plants are used for the treatment of various ailments especially in India due to its traditional systems and wide biodiversity. Less than 5% of the Indian medicinal flora has been evaluated systematically till date indicating a vast untapped potential midst of the global opportunity. The aim of the present study is the laboratory assessment and phytochemical screening of *Mollugo spergula.* The methanol extract of *Mollugo spergula* contains 3.67 µg of β-carotene and 33.9 µg of lycopene, 9.23 mg of Vitamin C per g of the extract. The extract contains polyphenols viz.14.51 µg of pyrocatechol and 2.91 µg of gallic acid per g of the extracts. From the present study it has been found that the *Mollugo spergula* is enriched with valuable phytoconstituents having antioxidant activity. Further studies are going on in our laboratory to evaluate its wider biological activity.

KEYWORDS: *Mollugo spergula;* Phytoconstituents; Lycopene; Vitamin C; Polyphenols

INTRODUCTION

Medicinal plants are used for the treatment of various ailments especially in India due to its traditional systems and wide biodiversity. Less than 5% of the Indian medicinal flora has been evaluated systematically till date indicating a vast untapped potential midst of the global opportunity. Antioxidant compounds in natural foods play an important role as a health protecting factor. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols, lycopene, beta carotene and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. There are a number of clinical studies suggesting that the antioxidants in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers. The free radical scavenging activity of antioxidants in foods has been substantially investigated and reported in the literature by Miller and Rigelhof et.al[1,2]. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, lycopene, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant. compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants. *Mollugo spergula* Linn. (synonym: *Glinus oppositifolia*, family: Molluginaceae) is an annual herb which finds application for the treatment of skin diseases in the indigenous system of medicine[3]. The aim of the present study is the laboratory assessment and phytochemical screening of *Mollugo spergula.*

MATERIALS AND METHODS:

The plant material was collected from the suburbs of South 24 Parganas, West Bengal, India. The air dried powdered plant (aerial part of *Mollugo spergula*) was successively extracted with light petroleum, chloroform and methanol under reflux conditions. The methanol extract of *Mollugo spergula* was taken for the present study.

ESTIMATION OF BETA-CAROTENE & LYCOPENE CONTENT:

Beta-carotene & lycopene content was estimated according to the method of Nagata & Yamashita[4]. The dried methanol extract (100 mg) was vigorously shaken with 10 ml of acetone-hexane (4:6) for 1 minute and then absorbance was taken at different wavelength (663, 505, and 453 nm). Beta-carotene & lycopene content (expressed in mg/g of the extract) was measured by using the following formula:

$$\text{Lycopene} = -0.0458\, A_{663} + 0.372\, A_{505} - 0.0806\, A_{453}$$

$$\text{Beta-carotene} = 0.216 A_{663} - 0.304 A_{505} + 0.452 A_{453}$$

[*]***Corresponding author:*** Sagar Naskar ***/ Email:*** sagar_n2007@yahoo.co.in

ESTIMATION OF TOTAL PHENOLIC (PYROCATECHOL AND GALLIC ACID) COMPOUNDS:

Total soluble phenolic in the extract is determined with Folin–Ciocalteu reagent (FCR) according to the method of Slinkard and Singleton 1977[5]. Briefly, 0.1ml of extract in distilled water (contains 1mg of each extract) was transferred into 100ml Erlenmeyer flask then final volume was adjusted to 46ml by addition of distilled water. Afterwards, 1ml of FCR was added to this mixture and after 3minutes. 3ml of Na_2CO_3 (2%) was added. Subsequently, mixture was shaken for 2 hours at room temperature and then absorbance was measured at 760nm. All the tests were performed in triplicate and the results averaged. The concentration of total phenolic compounds in the extract was determined as microgram of pyrocatechol equivalent by using an equation that was obtained from standard pyrocatechol graph. The equation is given below:

Absorbance = 0.001 x Pyrocatechol (μg) + 0.0033

Absorbance = 0.0053× gallic acid (μg) -0.0059

DETERMINATION OF VITAMIN C:

25.00 ml (containing 25 mg of vitamin C) of vitamin C standard solution to a 125 ml Erlenmeyer flask is taken and 10 drops of 1% starch solution is added. Titrate this ascorbic acid (made fresh) solution against iodine solution (0.125% iodine, 1% potassium iodide) until the end point is reached (first sign of blue color that persists after 20 seconds of swirling the solution).The volume of iodine solution required is noted. Then 25 ml of the extract (containing 25 mg of extracts) is titrated with iodine solution. The volume of iodine solution required is noted. The amount of vitamin C present in the extract is determined by the comparison of required iodine solution[6].

PHYTOCHEMICAL SCREENING OF *MOLLUGO SPERGULA:*

PRESENCE OF ALKALOIDS CAN BE MEASURED BY USING THE FOLLOWING REAGENTS:

Alkaloids give reddish brown precipitate with Dragendorff's reagent reagent, cream colour precipitate with Mayer's reagent reagent, reddish brown precipitate with Wagner' reagent reagent and yellow precipitate with Hager's reagent reagent.

PRESENCE OF FLAVONOIDS CAN BE MEASURED BY SHINODA TEST OR ZINC HYDROCHLORIDE TEST:

In case of Shinoda test, to the test solution add few magnesium turnings and concentrated HCl drop wise, pink scarlet, crimson red or occasionally green to blue colour appears after few minutes. In case of Zinc hydrochloride test, to the test solution add a mixture of zinc dust and conc. HCl acid. It gives red colour after few minutes.

PRESENCE OF SAPONIN GLYCOSIDES CAN BE MEASURED BY FROTH FORMATION TEST:

Place 2 ml solution of drug in water in a test tube, shake well, stable froth (foam stability is more than 20 sec) is formed.

PRESENCE OF STEROIDS CAN BE MEASURED BY LIBERMANN-BURCHARD AND SALKOWSKI TESTS:

In case of Libermann-Burchard test, the extract is treated with few drops of acetic anhydride, boil and cool. Then add concentrated sulphuric acid from the side of the test tube, brown ring is formed at the junction two layers and upper layer turns green which shows presence of steroids. In case of Salkowski test, the extract is treated with few drops of concentrated sulphuric acid, red colour at lower layer of the tube indicates, presence of steroids.

PRESENCE OF TANNINS CAN BE MEASURED BY GOLDBEATER'S SKIN TEST AND FERRIC CHLORIDE TEST:

In case of Goldbeater's skin test, add 2% HCl acid to a small piece of goldbeater's skin, rinse it with distilled water and place in the solution to be tested for five minutes. Then give wash of distilled water and transfer to a 1% $Feso_4$ solution. A brown or black colour on the skin indicates presence of tannins. In case of ferric chloride test, treat the extract with 5% $Fecl_3$ solution, blue colour if hydrolysable tannins are present and green colour appears if condensed tannins are present.

RESULTS AND DISCUSSION:

Lycopene, vitamin C and polyphenolic compounds constitute the main class of natural antioxidants present in plants, foods, and beverages. Polyphenolic compounds are usually quantified employing FCR. Vitamin C can be estimated volumetrically and lycopene by the spectroscopic method. The methanol extract of *Mollugo spergula* contains 3.67 μg of β-carotene and 33.9 μg of lycopene, 9.23 mg of Vitamin C per g of the extract. The extract contains polyphenols viz.14.51 μg of pyrocatechol and 2.91 μg of gallic acid per g of the extracts. Preliminary phytochemical screening suggests the presence of tannins, alkaloids, saponins in the methanol extract of *Mollugo spergula.*

During the last decade, there has been an escalation of interest in the role of antioxidants in health care and diseases. Antioxidants act as free radical scavengers and are found to play a significant protective role against oxidative stress in a variety of diseases such as liver cirrhosis[8,9], inflammation, atherosclerosis, diabetes, cancer[10], neurodegenerative disease[11], nephrotoxicity and also the aging process. Therefore there is a growing interest in finding naturally occurring potential antioxidants, especially from plant origin. Studies to date have shown that various common fruits and vegetables contain different promising antioxidant compounds such as Vitamin E, Vitamin C, carotenoids well as flavonoids, tannins and other polyphenolic constituents[12].

CONCLUSION:

Most of the natural plants are useful for the treatment of several biological disorders as they are enriched with valuable phytoconstituents. Phytoconstituents such as lycopene, vitamin C and polyphenolic compounds are already proved to have very good antioxidant capability. So, nutritional labeling data (vitamins, lycopene and polyphenols etc.) of natural products will aid in the interpretation of clinical results obtained from biological models for chronic disease. It is reasonable to expect that high antioxidant natural products have greater potential to reduce free radicals in the body than do low antioxidant natural products. Thus it is important to know the antioxidant phytoconstituents content of natural products, in addition to knowing the basic nutritional information such as the protein, fiber, fat, mineral and vitamin contents. From the present study it has been found that the *Mollugo spergula* is enriched with valuable phytoconstituents having antioxidant activity. Further studies are going on in our laboratory to evaluate its wider biological activity.

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