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Journal of Drug Delivery and Therapeutics

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Research Article

Antioxidant Potential of *Psoralea corylifolia* and *Psoralea esculenta* seeds: Comparative Study

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ABSTRACT

The *Psoralea corylifolia* L. & *Psoralea esculenta* seeds are traditionally used herbal medicine, but its comparative antioxidant activity has not been studied. The methanolic crude extracts of *Psoralea corylifolia* & *Psoralea esculenta* seeds were screened for their free radical scavenging properties using ascorbic acid as standard antioxidant. Free radical scavenging activity was evaluated using 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical. The overall antioxidant activity of *Psoralea corylifolia* was found to be the strongest. The IC_{50} values of the extracts found 0.14 ± 0 & 0.05 ± 0 mg/l respectively. The ascorbic acid levels found19.3 ± 0.10 &11.7 ± 0.49 mg/100g andthe carotenoids content were observed 28.65 ± 0.24 &16.82 ± 1.16 mg/100g in plant extracts. The highest total phenols content were found to be in *Psoralea corylifolia* with the value 31.2 ± 0.24 mg/g.The present study reveals that the selected plants would exert several beneficial effects by virtue of their antioxidant activity and may be taken for drug formulation.

Keywords: 1,1-diphenyl-2-picryl hydrazyl, antioxidant, phenol, radical scavenger.

Article Info: Received 11 April 2019; Review Completed 02 June 2019; Accepted12 June 2019; Available online 26 June 2019

Cite this article as:

Singh N, Kumar P, Gautam GK, Bihari B, Antioxidant Potential of *Psoralea corylifolia and Psoralea esculenta* seeds: Comparative Study, Journal of Drug Delivery and Therapeutics. 2019; 9(3-s):740-743

http://dx.doi.org/10.22270/jddt.v9i3-s.2985

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INTRODUCTION

It has been established that oxidative stress is among the major causative factors in induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others[1]. A great number of aromatic, medicinal, spice and other plants contain chemical compounds exhibiting antioxidant properties. Oxidative process is one of the most important routes for producing free radicals in foods, drugs and even in living systems[2]. The most effective path to eliminate and diminish the action of free radicals which cause the oxidative stress is antioxidative defence mechanisms. Antioxidants are those substances which possess free radical chain reaction breaking properties. Recently there has been an upsurge of interest in the therapeutic potential medicinal plants as antioxidants in re-antioxidants in reducing oxidative stress-induced tissue injury[3]. Among the numerous naturally occurring antioxidants; ascorbic acid, carotenoids and phenolic compounds are more effective[4]. They are known to inhibit lipid peroxidation (by inactivating lipoxygenase), to scavenge free radicals and active oxygen species by propagating a reaction cycle and to chelate heavy metal ions[5]. The study done on medicinal plants and vegetables strongly supports the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems[6]. On continuation of our experimental work for the comparative study of antioxidant activity of medicinal plants, we studied extracts of seeds of medicinal plants. The free radical scavenging activity against 1,1-diphenyl-2-picryl hydrazyl (DPPH) was evaluated during the course of work. The ascorbic acid, carotenoids and total phenol contents with antioxidant activity were also determined. The assessment of such properties remains an interesting and useful task, particularly for finding new sources for natural antioxidants.

MATERIALS AND METHODS

Plant materials

The medicinal plantsseeds were collected by self from the general market and were authenticated from National

Botanical Research Institute Lucknow. The plant materials seeds were cleaned and powdered.

Extraction

The powdered plant materials were air-dried in shed at room temperature (25°C) for 2 weeks. Methanol extracts were prepared by soxhalation of 100g each of the dry powdered plant materials in 1 litre of methanol for 6 h. The extracts were filtered first through a cotton wool and then with the help of Whatmann filter paper No. 4. The extracts were concentrated using a rotary evaporator with the hot water bath set at 40°C. The percentage yields of extracts were 9 & 5% (w/w) respectively.

Antioxidant activity (DPPH free radical scavenging activity)determination

The antioxidant activity of the plant extracts was examined on the basis of the scavenging effect on the stable DPPH free radicalactivity[7]. Ethanolic solution of DPPH (0.05 mM) (300 _l) was added to 40 _l of extract solution with different concentrations(0.02 - 2 mg/ml). DPPH solution was freshly prepared and kept in the dark at 4°C. Ethanol 96% (2.7 ml) was added and the mixture was shaken vigorously. The mixture was left to stand for 5min and absorbance was measured spectrophotometrically at 517nm. Ethanol was used to set the absorbance zero. A blank sample containing the same amount of ethanol and DPPH was also prepared. All determinations were performed in triplicate. The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the followingequation[8].

Percent (%) inhibition of DPPH activity = [(AB – AA) / AB] x 100

Where AA and AB are the absorbance values of the test and of the blank sample, respectively. A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and represented as IC50value for each of the test solutions.

Determination of ascorbic acid

The ascorbic acid was determined according to Cakmak and Marschner[9] with some modification. Each plant extract (0.5 ml of 1:5 g/ml) in methanol was separately mixed with 5 ml of 5% meta-phosphoric acid, and centrifuged at 4000 rpm for 30 min. The reaction mixture contained 0.2 ml aliquot of the 4000 rpm supernatant, 0.5 ml 150 mM phosphate buffer (pH 7.4) containing 5 mM EDTA, 0.1 ml (10 mM) DTT (1,4-dithiothreitol) and 0.1 ml (0.5%, w/v) N-ethylmaleimide (NEM) to remove excess DTT. The color was developed after addition of the following reagents in the reaction mixture: 0.4 ml (10%) trichloroacetic acid (TCA), 0.4 ml (44%) ortho-phosphoric acid, 0.4 ml (4%) 2, 2'-bipyridine in 70% ethyl alcohol, and 0.2 ml (3%) FeCl3. The mixture was then incubated at 40°C for 40 min, and the absorbance was measured at 525 nm. Ascorbic acid was used as a standard in the range of 0 to 100 g/ml.

Determination of carotenoids

Total carotenoids were determined by the method of Jensen[10]. 10ml sample was extracted with 100 ml of 80% methanol solution and centrifuged at 4000 rpm for 30 min. The supernatant was concentrated to dryness. The residue was dissolved in 15 ml of diethyl ether and after addition of 15 ml of 10% methanolic KOH the mixture was washed with 5% ice-cold saline water to remove alkali. The free ether extract was dried over anhydrous sodium sulphate for 2 hr. The ether extracts were filtered and its absorbance was measured at 450 nm by using ether as blank.

Determination of total phenol

Total phenols were recorded by Folin Ciocalteu reagent[11]. A dilute extract of each plant extract (0.5 ml of 1:5 g/ml) or Gallic acid used as standard was mixed with Folin Ciocalteu reagent (5 ml, 1:5 diluted with distilled water) and aqueous Na_2CO_3 (5 ml, 1M). The mixture was allowed to stand for 10 min and the absorbance was measured by colorimetry at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg/l solutions of Gallic acid in methanol: water (50:50, v/v). Total phenol contents were expressed in terms of Gallic acid equivalent (mg/g of dry mass), which is used as a reference compound.

Statistical analysis

The statistical significance between free radical scavenging activity values of the extracts was analyzed with a Mann-Whitney U test. P values less than 0.05 were considered to be statistically significant.

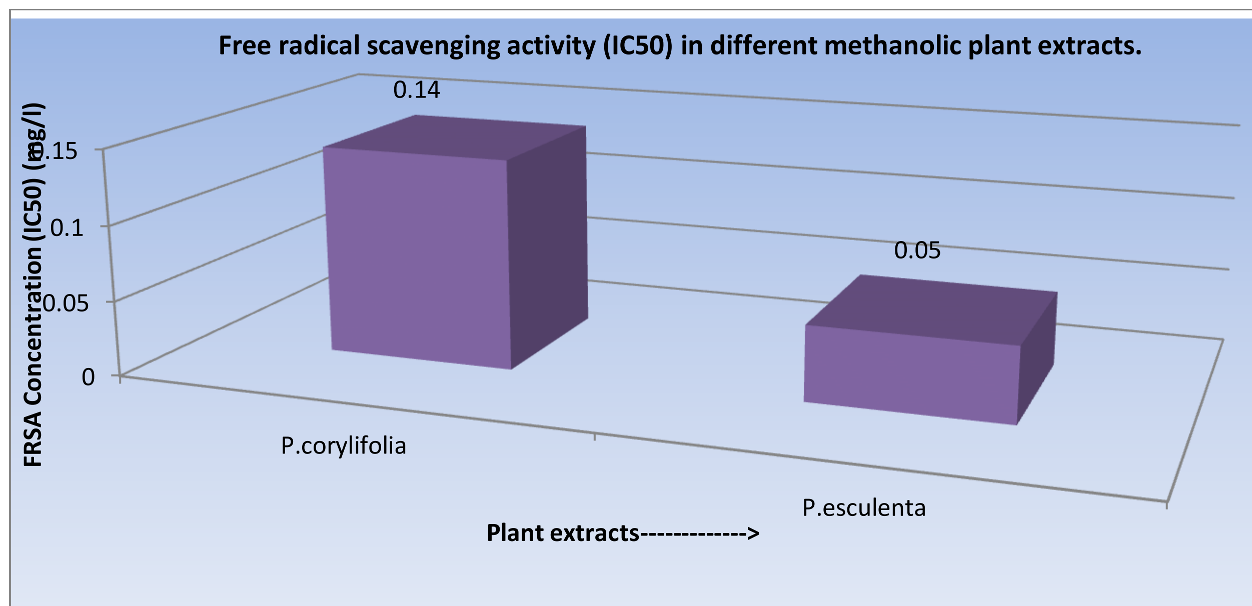

Figure:1

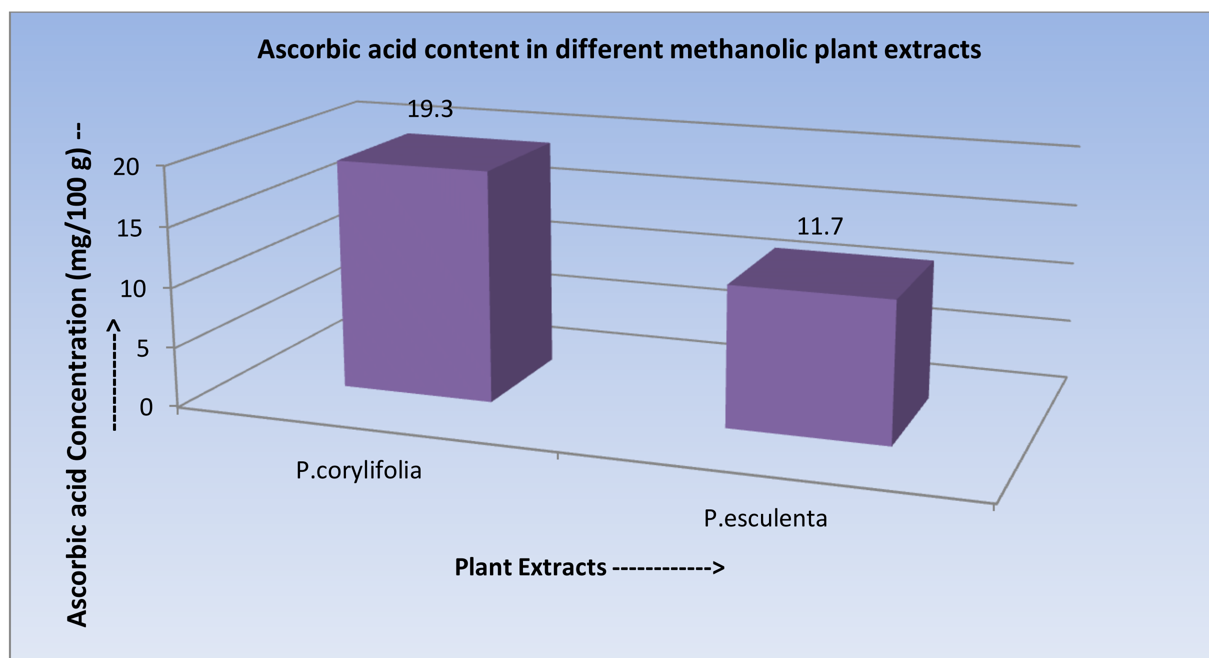

Figure:2

RESULTS AND DISCUSSION

In the present study biochemical constituents and free radical scavenging activities of medicinal plants seeds were evaluated. Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants due to their scavenging activity are useful for the management of those diseases. DPPH stable free radical method is a sensitive way to determine the antioxidant activity of plant extracts[12&13]. Figure 1 shows the amount of each extract required for 50% inhibition of DPPH activity(IC50). The free radical scavenging action of methanol extracts of *Psoralea corylifolia was stronger than Psoralea esculenta.* The therapeutic potential of natural medicinal plants asan antioxidant in reducing such free radical induced tissue injury, suggests that many plants have antioxidant activities that can be therapeutically useful[14].

Ascorbic acid acting as a chain breaking antioxidant impairs with the formation of free radicals in the process of formation of intracellular substances throughout the body, including collagen, bone matrix and tooth dentine[15&16]. The quantitative determination of ascorbic acid in plant extracts shows that they are good source of ascorbic acid. High quantity of ascorbic acid was found to be 19.3 mg/100g in *Psoralea corylifolia* & 11.7 mg/100g in *Psoralea esculenta* (Figure 2). As triking pathological change resulting from ascorbic acid deficiency is the weakening of the endothelial wall of the capillaries due to a reduction in the amount of intercellular substance. Therefore, a clinical manifestation of scurvy hemorrhage from mucous membrane of the mouth and gastrointestinal tract, anaemia, pains in the joints can be related to the association of ascorbic acid and normal connective tissue metabolism[17]. Figure 3 demonstrate the analysis of carotenoid contents in both the medicinal plants.

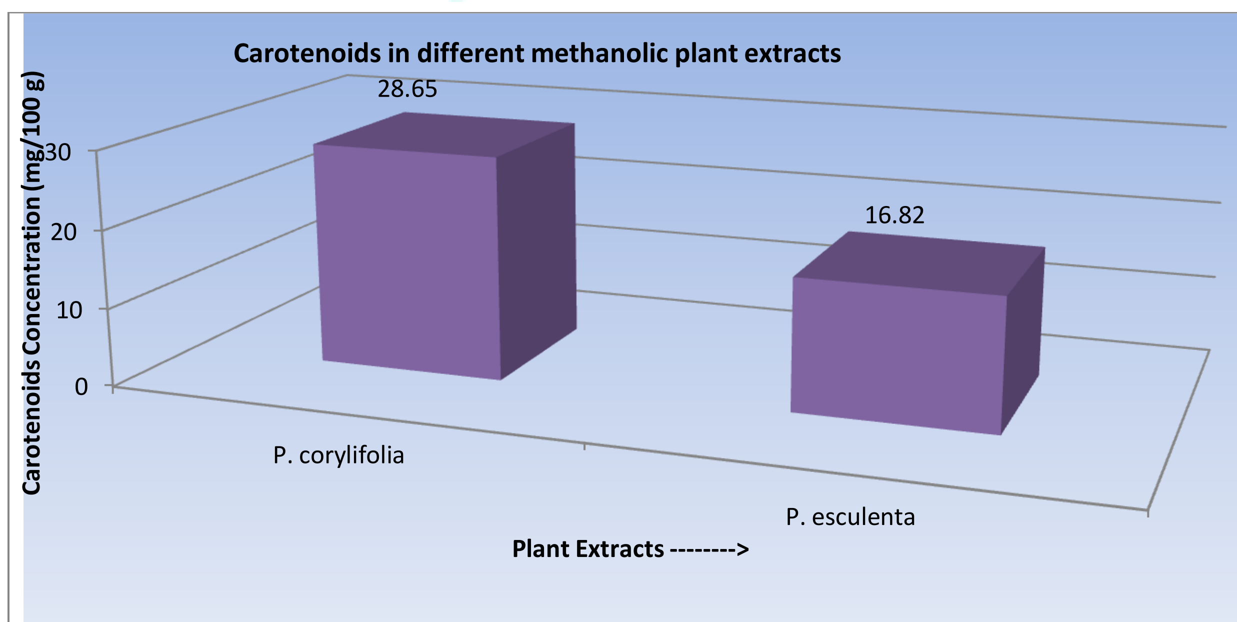

Figure:3

The carotenoid content was more in *Psoralea corylifolia* (28.65 mg/100g) while *Psoralea esculenta had* significantly lower values as 16.82 mg/100g. It was proved that carotenoids have a positive role on the epithelisation process and influence the cell cycle progression of the fibroblasts[18]. Carotenoids act as photoprotective agents and may reduce the risk of sunburns, photo-allergy and even some types of skin cancer[19]. The examined result shows that *P. Corylifolia* is a strong source of carotenoids and it can be a promising plant for use in pharmacological products designed for antioxidant activity. So far as plant phenolic constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators, it was reasonable to determine their total amount in the plant extracts[20].

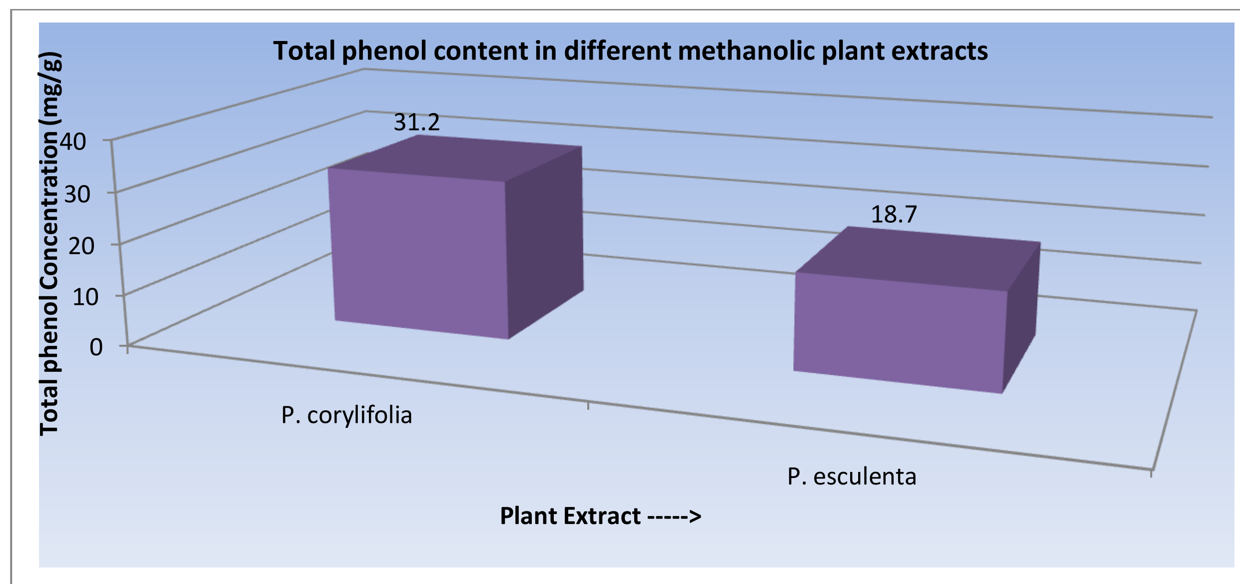

Figure:4

The content of total phenols in methanolic extracts expressed in Gallic acid equivalents (GAE) was found as 31.2 mg/g in *P. corylifolia*& 18.7 mg/g in *P. esculenta*(Figure 4). The phenols contain hydroxyls that are responsible for the radical scavenging activity mainly due to redox properties[21]. According to our study, the high phenolic content in *P. corylifolia* can explain its high free radical scavenging activity. This comparative study reveals that *P. corylifolia* have significant antioxidant activity and free radical scavenging activity. The result of the present study suggests that selected plants can be used as a source of antioxidants for pharmacological preparations which is very well evidenced by the present work.

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