

ISOLATION AND CHARACTERIZATION OF *PEDALIUM MUREX* SEEDS FOR ITS ANTIOXIDANT ACTIVITY

Surinderjeet Kaur*, Dr. Naresh Singh Gill

Department of Pharmaceutical Chemistry, Rayat Institute of Pharmacy, Railmajra

ABSTRACT

Pedaliium murex has been traditionally used for the treatment of various diseases like puerperal diseases, digestive tonics, ulcers, fevers, wounds other ailments and general debility. The present investigation was intended to evaluate the preliminary phytochemical characters as well as antioxidant properties of the plant. Phytochemical studies promotes new discovery for the synthesis of more potent drugs. Antioxidant activity of plant extract was evaluated by Hydrogen peroxide (H₂O₂) and Nitric Oxide (NO) method. Alkaloids and triterpenoids were detected in the plant. With the increase in concentration of seed extract, the antioxidant activity increased proportionally to the maximum activity of 79.22% at 100µg/mL and 81.22% at 100µg/mL with H₂O₂ and NO respectively.

KEYWORDS: Puerperal diseases, phytochemicals, *Pedaliium murex*, potent drugs. triterpenoids.

INTRODUCTION

Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend against attack from predators such as insects, fungi, herbivorous mammals. The chemical compounds in plants mediate their effect on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs. Plants contain a variety of bioactive compounds with antioxidant capabilities including flavonoids, phenolics, sterols, alkaloids, carotenoids and glucosinolates. [1]

Natural antioxidants are mostly derived from plant sources such as fruits, vegetables, herbs and spices. The efficacy is determined by plant species, variety, extraction, processing methods and the growing environment. The mode of action for these substances will vary depending upon the source material, the presence of synergists and antagonists, and of course the food matrix applied to. [2] Most of the natural antioxidants are particularly rich in phenolic compounds, vitamins and carotenoids. Rosaceae, Empetraceae, Ericaceae, Grossulariaceae, Juglandaceae, Asteraceae, Punicaceae and Zingiberaceae are families of plants that contain compounds with high antioxidant activities. [3] Antioxidants that are present in plants are capable of trapping free radicals from our body which are potentially harmful. Free radicals are species that exist independently having one or more unpaired electrons with which they react with other molecules by taking or supplying electrons and thus results in many pathological conditions. Researchers suggest that oxidative and free radical generated reactions are a prime contributor in degenerative processes like aging and various diseases like cancer, diabetes, atherosclerosis, etc. By enhancing the body's natural antioxidant defence or by providing supplementary dietary antioxidants, the risk of occurrence of many chronic diseases and their progression can be prevented. [4] It was surveyed that from last few years there are many families that had been investigated for their antioxidant activities but some families are yet to be explored. So our target is to explore and inspect the families out as well as checking the antioxidant activity of those families.

MATERIAL AND METHODS

COLLECTION OF PLANT MATERIAL

The seeds of *Pedaliium murex* were collected from local market of Nurpur Bedi District Ropar in Punjab. The seeds were washed, cleaned which is then shade dried and powdered by kitchen grinder. The healthy seeds were selected for authentication from NISCAIR (National Institute of Science Communication and Information Resources) New Delhi.

EXTRACTION OF PEDALIUM MUREX SEEDS

The healthy seeds of *Pedaliium murex* were selected and shade dried. The dried seeds were weighed and subjected to cold maceration for 5 days with methanol.

The extraction procedure is given below:

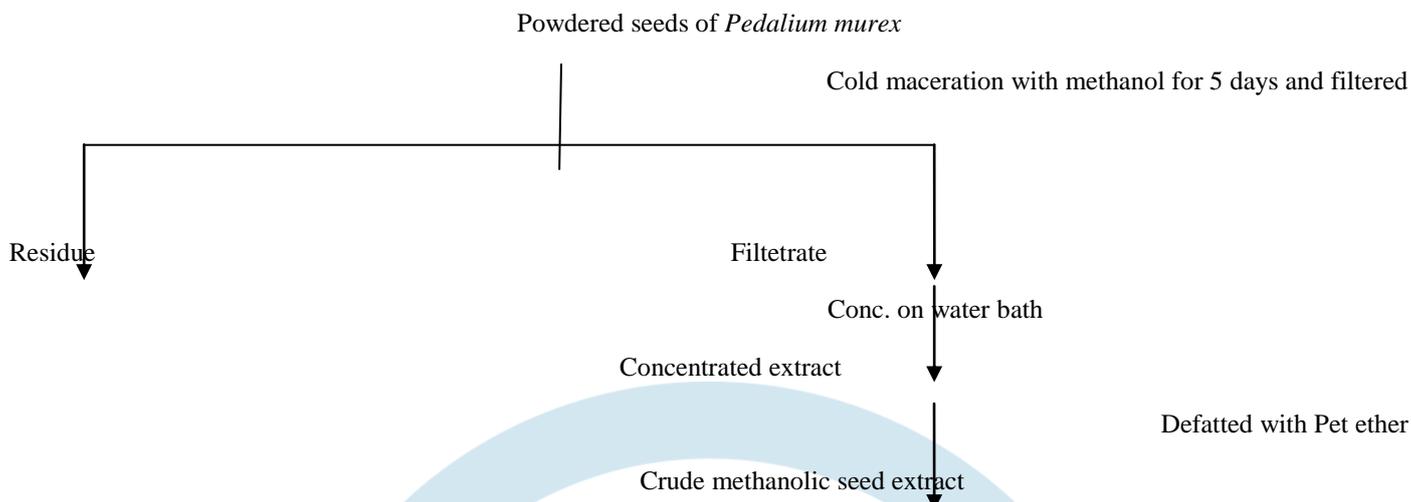


Figure 1: Flow chart for seed extraction

PHYTOCHEMICAL SCREENING OF EXTRACT

Photochemical screening of *Pedalium murex* seeds extract showed the presence of various chemical constituents like flavonoids, alkaloids, triterpenoids, cardiac glycosides.

ANTIOXIDANT ACTIVITY

In vitro evaluation of free radical scavenging activities is done by following 2 methods:

H₂O₂ Method

NO Method

Quantitative Evaluation of H₂O₂ Free Radical Scavenging Activity

Scavenging of hydrogen peroxide was measured by the following described method. A solution of hydrogen peroxide (40mM) was prepared in 0.1 M phosphate buffer (pH 7.4). 1mL of each methanolic extract of *Pedalium murex* at different concentrations (25-400 µg/mL) was added to 0.6 mL of 40 mM hydrogen peroxide solution. Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank containing phosphate buffer without hydrogen peroxide. The percentage scavenging of hydrogen peroxide of plant extract and reference standard ascorbic acid was calculated using the following formula.

Mixture of 0.1 M Dipotassium hydrogen phosphate and Potassium Dihydrogen phosphate were used as buffer.

Requirements

Chemicals and Reagents: H₂O₂ (40mM), Phosphate buffer (0.1 M), Ascorbic acid (0.05mM).

Blank: Methanol

Control: H₂O₂ (40mM) solution

Percentage Inhibition (%) = $(A_0 - A/A_0) \times 100$

A₀ = Absorbance of control

A = Absorbance of test/ standard

Quantitative Evaluation of NO Free Radical Scavenging Activity Reagents

10 mM sodium nitroprusside

Phosphate buffered saline pH 7.4

2 % sulphanilamide in ortho-phosphoric acid

0.1 % naphthylethylenediamine dihydrochloride

Procedure

To 1ml of sodium nitroprusside, 2.5 ml, phosphate buffered saline pH 7.4 was added and mixed with 1 ml of extract at various concentrations (µg/ml), then the mixture was incubated at 25° C for 30 minutes. From the incubated mixture 1.5 ml was taken. To it, 1ml of sulphanilamide in phosphoric acid and 0.5 ml of naphthyl ethylenediamine dihydrochloride were added and the absorbance was measured at 546 nm. Ascorbic acid was used as a standard. The percentage inhibition of nitric oxide radical generated was calculated by using the following formula:

RESULT AND DISCUSSIONS

The phytochemical characteristics of medicinal plant tested were summarized in the table. The results revealed the presence of medically active compounds in the plant studied. From the table, it could be seen that alkaloids, triterpenoids, flavonoids, cardiac glycosides and volatile oils were present in the plant. Alkaloids present in *Pedalium murex* are used as antibacterial agents. [5] Presence of flavonoids and mucilage in the extract of *P. murex* made the plant a valuable antiulcer drug. [6] Cardiac glycosides are effective in treatment of cardiac failure, also investigated for their antitumor activity [7, 8].

Results of phytochemical screening

S.NO.	PHYTOCONSTITUENT	INTENSITY
1.	ALKALOIDS	++
2.	STEROIDS	-
3.	TRITERPENOIDS	+++
4.	FLAVONOIDS	+
5.	AMINO ACIDS	-
6.	CARDIAC GLYCOSIDES	+
7.	CARBOHYDRATES	+
8.	PROTEINS	-
9.	TANNINS	-
10.	VOLATILE OILS	+

(+) indicates presence and (-) no presence of constituents, (++) indicates higher content

IN VITRO ANTIOXIDANT ACTIVITY

Pedaliium murex seeds show antioxidant activity by H₂O₂ method and NO method.

1. Quantitative Estimation of Antioxidant Activity Using Hydrogen Peroxide Method

Hydrogen peroxide (H₂O₂) is an oxidant that is being formed continuously in living tissues as a result of several metabolic processes. It reacts with Fe²⁺ (Feton type reaction) and generate extremely reactive oxygen species, including hydroxyl free radical, thus, its detoxification is necessary. Therefore, measurement of H₂O₂ scavenging activity is one of the useful methods for determining the ability of antioxidants to decrease the level of such pro-oxidants.

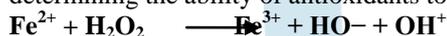


Table 1: H₂O₂ Scavenging activity of *Pedaliium murex* seeds

S.NO.	Concentration (µg/ml)	Mean Absorbance (test)	% Inhibition (test)	% Inhibition (standard)
1	25	0.0702	62.05	68.56
2	50	0.0552	67.18	72.97
3	75	0.0460	71.06	76.45
4	100	0.0385	79.22	83.24

Values are taken as average of triplicate experiments and represented as Mean± SEM

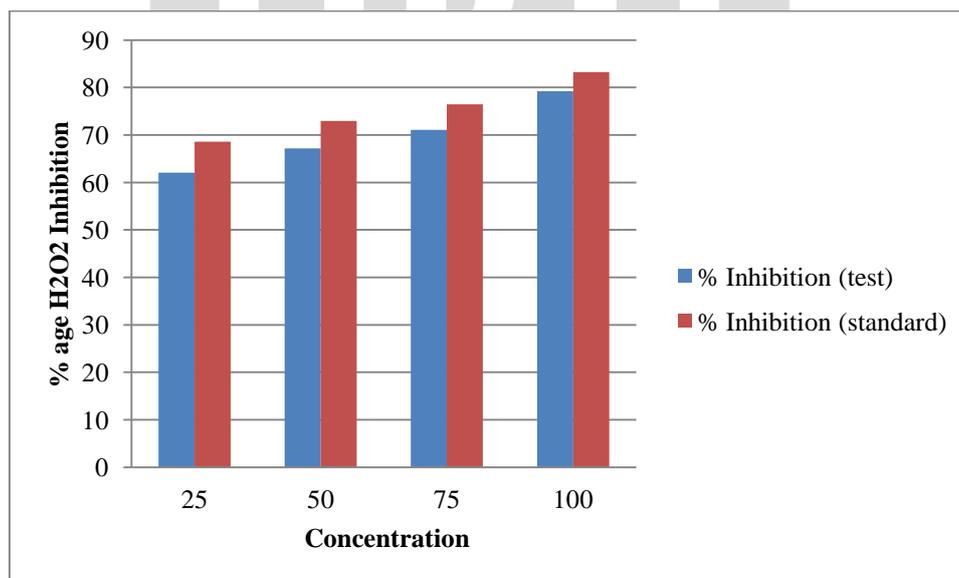


Fig. 2 Graphical representation of H₂O₂ scavenging activity

Methanolic extract of *Pedaliium murex* seeds exhibited H₂O₂ scavenging activity. With the increase in concentration of seed extract, the antioxidant activity increased proportionally with the maximum activity of 79.22% at 100µg/ml. The absorption decreased proportionally with the increase in the concentration of extract. The maximum absorption was found to be control

sample excluding drug. The percentage inhibition of test and standard samples was calculated by subtracting their absorption from the absorption of control sample

2. Radical scavenging activity by NO method

Nitric oxide scavenging activity was determined according to the method reported by Green *et al.*, 1982. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interact with oxygen to produce nitrite ion, which can be determined by the use of the Griess-Ilosvoy reaction. The nitrite ion produced diazotizes sulphanilamide and then the diazonium salt react with N, N-Naphthyl ethylene diaminedihydro chloride to give a pink color chromophore which has a maximum absorption at 546nm.

Table 2: NO scavenging activity at *Pedaliium murex* seeds

S.NO.	Concentration (µg/ml)	Mean Absorbance (test)	% Inhibition (test)	% Inhibition (standard)
1	25	0.0491	62.89	65.54
2	50	0.0417	68.47	72.45
3	75	0.0340	74.32	76.72
4	100	0.0249	81.22	84.56

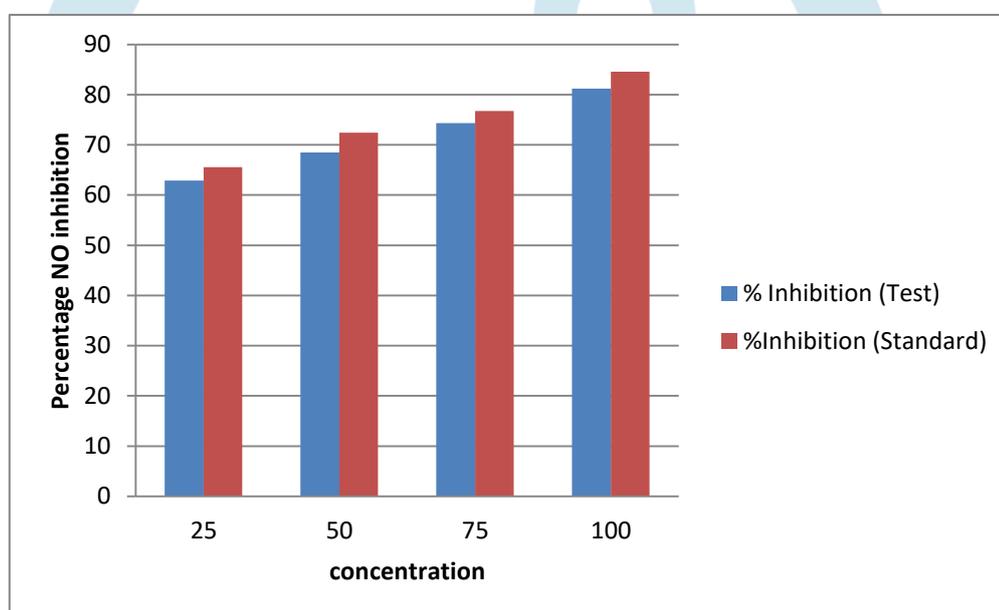


Fig. 3 Graphical representation of NO scavenging activity

Methanolic extract of *Pedaliium murex* seeds exhibited NO scavenging activity. With the increase in concentration of seed extract, the antioxidant activity increased proportionally with the maximum activity of 81.22% at 100µg/ml. The absorption decreased proportionally with the increase in the concentration of extract. The maximum absorption was found to be control sample excluding drug. The percentage inhibition of test and standard samples was calculated by subtracting their absorption from the absorption of control sample.

CONCLUSION

The present investigation on exploring the therapeutic potential of *Pedaliium murex* revealed that this plant is a rich source of antioxidant. This study also provided a scientific support to the effectiveness of *Pedaliium murex* seeds in free radical mediated diseases. The results acknowledged the presence of medicinally necessary constituents in the plants studied. Many justifications gathered from earlier studies which verified the recognised phytochemicals to be bioactive. Various studies confirmed the presence of these phytochemicals contribute medicinal and physiological properties to the plant studied in the treatment of several ailments.

REFERENCE

1. Tapsell LC., Hemphill, Cobiac L., (2006) "Health benefits of herbs and spices the past, the present, the future" Med. J. Aust, (4 Suppl);185: S4-24.
2. Said S., Allam M., Moustafa H., Mohamedz A., (2002) "A thermal stability of some commercial natural and synthetic antioxidants and their mixtures. J Food Lipids." 9:277-293.
3. Bansal S., Choudhary S., Sharma M., Kumar S., Lohan S., Bhardwaj V., Syan N., Jyoti S., (2013) "Tea: A native source of antimicrobial agents. Food Res. Int." 53, 568-584.
4. Asif M., (2015) "Chemistry and antioxidant activity of plants containing some phenolic compounds Chemistry International."1(1):35-52.
5. Rajashekar V, Rao EU, Srinivas P., (2012) "Biological activities and medicinal properties of Gokhru (*Pedalium murex* L.).Asian Pac J Trop Biomed"2012; 2(7): 581-5.
6. Banji D, Singh J, Banji OJ, Shanthamurthy M., (2010) "Scrutinizing the aqueous extract of leaves of *Pedalium murex* for the antiulcer activity in rats"; 23(3): 295-9.
7. Farnsworth N.F., (1966) "Biological and phytochemical screening of plants. Journal of Pharmaceutical Sciences" 55:225-276.
8. Doskotch R.W. (1972) "Antitumor agents V: Cytotoxic cardenolides from *Cryptostegia grandiflora* R. Br. Journal of Pharmaceutical Sciences".61:570-573.

