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Antioxidant Properties of Prosopis Cineraria (Ghaf): Pods and Leaves

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***Abstract*-The aim of the present investigation was to evaluate the antioxidant properties of Pods and Leaves of *Prosopiscineraria* (Ghaf). Antioxidants protect biological systems against free radical damage. Insufficient levels of endogenous and exogenous antioxidants can cause oxidative stress, an imbalance of oxidants and antioxidants resulting in cellular damage or death. The antioxidant activity was assessed by DPPH scavenging activity. The methanolic extract of pods and leaves P. cineraria had shown very significant DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity. The DPPH radical scavenging activity of the extract increased with the increasing concentration of the plant extract. In DPPH free radical scavenging assay IC50 value of methanolic extract of P. cineraria pod and leaves were found to be 550 µg/mL and 480µg/mL respectively. Therefore, in vitro assays indicate that P. cineraria pods and leaves extracts are a better source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.**

***Keywords*- DPPH, diseases, antioxidant, oxidative stress, Ghaf pods.**

I. INTRODUCTION

Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anemias, asthma, arthritis, inflammation, neuro-degeneration, Parkinson's diseases, mongolism, ageing process and perhaps dementias (Oke et al, 2002).

Insufficient levels of endogenous and exogenous antioxidants can cause oxidative stress, an imbalance of oxidants and antioxidants resulting in cellular damage or death. Oxidative stress plays a significant role in diverse diseases such as cardiovascular conditions, cancer, inflammatory diseases and early ageing (progeria) (Valko et al, 2007 and Uttara et al, 2009).

It has long been recognized that naturally occurring substances in higher plants have antioxidant activity. There is growing interest toward natural antioxidants from herbal sources (Larson, 1998; Gazzani et al, 1988; Veliglu et al, 1988). Epidemiological and in vitro studies on medicinal plants and vegetables strongly have supported the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems (Cao et al, 1996; Block et al, 1992; Ness et al, 1997).

The Prosopis cineraria occurs in most of the world's hot arid and semi-arid regions as native or introduced species (Pasiecznik et al, 2001). It is a multipurpose tree of desert locally known as Ghaf and is regarded as the backbone of rural economy. Bedouin traditional lifestyle in UAE has been very much associated with the Prosopis trees and their products (Lemons et al,2003). As the country rapidly modernizing and continuous population grows, inadequate nutrient sources, exorbitant cost of animal protein is considered the main reasons for malnutrition and undernourishment among people. Due to rapid change in socioeconomic conditions of the country, less number of people get benefit by the plant as was practiced earlier. Therefore, the plant has been badly neglected for scientific studies (Islam et al, 2019). Moreover, the Prosopis sp. is considered as the national symbol of UAE (Philp, 2013).

Antioxidant components of plants are also effective in preventing many diseases (Krishnaiah et al, 2011). Plants contain various types of compounds with antioxidant activities, such as antioxidant vitamins (A, C, and E), carotenoids, coenzyme-Q, lycopenes, and phenolics (phenolic acids, flavonoids, flavonols, anthocyanins, tannins, and lignins) (Mollica et al, 2016).

DPPH assay is one of the most popular and frequently employed method among antioxidant assays. The method is simple, efficient, relatively inexpensive, and quick. However, as with most antioxidant assays, it requires a UV–Vis spectrophotometer. DPPH method was developed by Blois, 1958 and modified by Brand- Williams et al, 1995 to produce the current widely used form. DPPH is a stable free radical which possesses a deep purple colour and a strong absorption around 517 nm. The antioxidant compounds present in the medium convert DPPH radical to a more stable DPPH molecular product by donating an electron or a hydrogen atom. The colour change from purple of DPPH radical to pale yellow of reduced form of

DPPH allows the spectrophotometric determination of the antioxidant activity. The results are either expressed as SC50 (otherwise called the IC50 value), the concentration of the antioxidant causing 50% DPPH scavenging (Molyneux, 2004 and Kedare et al, 2011), or as %scavenging of DPPH at a fixed antioxidant concentration for all the samples.

In our previous study we investigated that ghaf is a potential desert nutraceutical and antimicrobial agent (AlGhais et al 2020 a, b, c). Furthermore, to continue our research to detect the potency of ghaf leaves and pods as source of antioxidative agent. With this view, the present investigation was initiated to study the antioxidant activity of methanolic extract of pods and leaves of P. cineraria which was evaluated by in vitro free radical scavenging activity. We need to spread awareness on the importance of these trees and the role they played in the functioning of a healthy ecosystem and thereby protecting the species from extinction.

II. MATERIAL AND METHODS

1. Sample Collection:
Samples of leaves and pods of ghaf (samples from five trees) were collected from trees grown on Khuzam road, Ras Al Khaimah, UAE in the month of March 2021. The leaves and pods were sun dried for 5-7 days or more and then oven dried for better grinding. The dried leaves and pods were then ground to a coarse powder using high capacity of grinding machine and then stored in airtight bottles.

2. Preparation of the extracts:
About 5 g of the coarse powder was extracted with 25.0 ml of methanol followed by continuous hot extraction method (Bhardwaj V 2021). Stirred well and kept for incubation in closed container. Then we centrifuged the tubes at 4000 rpm for 30 min. After that we transferred the supernatant extract for drying for 10 min until dry powder was obtained. We finally got residue of samples (leaves and pods). All the extracts were then stored in refrigerator at -20°C till use (Al Ghais et al 2020c).

3. Chemicals:
The chemicals used in the present investigation were of analytical grade and of high purity from Merck. Standard reagents used for analysis were purchased from Germany and USA.

4. DPPH photometric assay:
DPPH radical scavenging activity is the most commonly used method to determine the antioxidant activity of natural and synthetic materials because it is quick and simple. When antioxidant samples are mixed with DPPH reagent solution, the colour is turned from purple to yellow by time. The colour change is determined by measuring absorbance with a spectrophotometer at 517nm. The percentage of radical scavenging activity of each substance was assessed by DPPH free radical assay. The measurement of the DPPH radical scavenging activity was performed according to methodology described by Brand-Williams et al. 1995. The samples were reacted with the stable DPPH radical in a methanol solution. The reaction mixture consisted of adding A methanolic solution of 0.3ml of DPPH (0.5mM) was added to 1 mL of the different concentrations of plant extract and 3 mL of methanol allowed to react at room temperature for 30 minutes.

When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from purple to yellow) were read [Absorbance (Abs)] at 517 nm after 30 min of reaction (Spectrophotometer-Jenway 7315, Bibby scientific Ltd, UK). Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. The scavenging activity percentage (AA%) was determined according to Mensor et al. 2001:

Scavenging activity (%) = ([A517 of control − A517 of test sample] / A517 of control) × 100.

Where A517 control is the absorbance of DPPH radical+ methanol; A517 test sample is the absorbance of DPPH radical+ sample extract.

5. Determination of IC50:
The IC50 value is used to measure the antioxidant activity of test samples. It is calculated as the concentration of antioxidants needed to decrease the initial DPPH concentration by 50%. Thus, the lower IC50 value the higher antioxidant activity.

6. Statistical Analysis:
Data are expressed as mean. Pair wise comparisons were performed. Standard error mean was determined for triplicate and expressed as SEM

III. RESULTS AND DISCUSSION

1. Determination of DPPH scavenging activity:
According to our research, we found that there was a change in colour of solution in test tubes, when samples of extracts of ghaf pods and leaves are mixed with DPPH reagent solution. The colour was turned from purple to yellow by time (Figure 1). Further, the antioxidant activity of samples was determined by measuring absorbance with a spectrophotometer at 517 nm.

The percentage of DPPH scavenging activity of methanolic extract of pods and leaves of P. cineraria was presented in Table 1 and Table 2. The methanolic extract of pods of P. cineraria exhibited a maximum DPPH scavenging activity of 67.35± 0.02 % at 1000 µg/mL (Table 1) whereas methanolic extract of leaves of P.

cineraria was found to be 64.83 ± 0.025 % at 1000 µg/mL (Table 2).

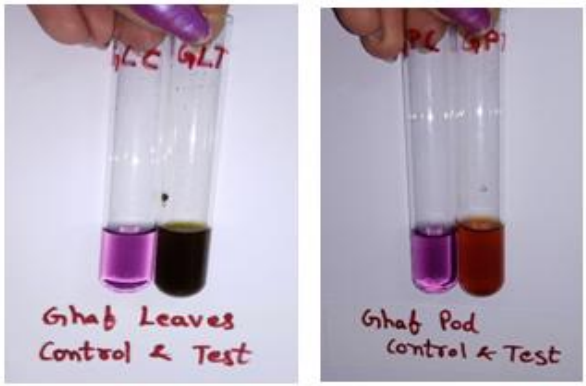

Fig 1. DPPH radical scavenging activity observed by change in the colour from purple (control) to yellow (Test).

Similar results were reported by Malik et al,2013 and Jaslin et al, 2011. The IC50 value is used to measure the antioxidant activity of test samples. It is calculated as the concentration of antioxidants needed to decrease the initial DPPH concentration by 50%. Thus, the lower IC50 value the higher antioxidant activity. The IC50 values of pod extract was found to be 550 µg/mL and leaves extract was found 480 µg/mL respectively. According to Jaslin et al, 2011, DPPH radical scavenging assay IC50 value of ethanolic extracts of Coleus spicatus was found to be 290 µg/mL and standard Rutin was found to be 480 µg/mL.

Table 1. DPPH scavenging activity of methanolic extract of ghaf pods.

SNo	Sample Concentration (methanolic extract) (µg/mL)	Scavenging activity % (±SEM) *
1	125	21.2 ± 0.05
2	250	22.17 ± 0.02
3	500	45.57 ± 0.03
4	1000	67.35 ± 0.02

*All values are expressed as mean ± SEM for triplicates

Table 2. DPPH scavenging activity of methanolic extract of ghaf leaves,

SNo	Sample Concentration (methanolic extract) (µg/mL)	Scavenging activity % (±SEM) *
1	125	27.91 ± 0.03
2	250	41.35 ± 0.05
3	500	55.41 ± 0.02
4	1000	64.83 ± 0.025

*All values are expressed as mean ± SEM for triplicates

IV. CONCLUSION

In summary, this research work demonstrate that leaves and pods of P. cineraria has remarkable antioxidant activity. According to our research analysis, we hope that the potential of P. cineraria pods and leaves could be best connected, towards a possible beneficial integration in food and pharmaceutical industry and also to spread awareness on the importance of Ghaf tree and the role they played in the functioning of a healthy ecosystem and this will also help in protecting the species from extinction.

V. ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

VI. CONSENT FOR PUBLICATION

Not applicable.

VII. AVAILABILITY OF DATA AND MATERIALS

The relevant data and materials are available in the present study.

VIII. COMPETING INTERESTS

The authors declare that they have no competing interests. All procedures followed were in accordance with the ethical standards (institutional and national).

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