INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 14, No. 3, p. 416-422, 2019

RESEARCH PAPER OPEN ACCESS

A comparative study of total phenolic contents and antioxidant potential of seeds of *Peganum harmala*

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Key words: *Peganum harmala* L., Methanol, Dichloromethane, Extraction, Total phenolic content, Antioxidant activity.

http://dx.doi.org/10.12692/ijb/14.3.416-422 Article published on March 27, 2019

Abstract

Various solvents including methanol, hexane, benzene, chloroform and dichloromethane were utilized for the extraction of phenolic contents from the seeds of *pegnum harmala*. Total phenolic contents (TPC) were determined by using Folin-Ciocalteu reagent method against gallic acid as standard by UV-Vis spectrophotometer at 765 nm while DPPH free radical scavenging activity of the extracts was measure by using UV-Vis spectrophotometer and taking reading at 517 nm and ascorbic acid as standard. Total phenolic contents 27.7 mg GA/g, 22.2 mg GA/g, 26.4 mg GA /g, 30.7 mg GA /g and 17.3 mg GA /g were for dichloromethane, benzene, chloroform, methanol and hexane extracts respectively. Methanol extract showed high TPC content and high antioxidant activity (72%) followed by dichloromethane extract (67%), chloroform extract (63 %), benzene extract (52 %) and hexane extract (48 %) respectively for 50 µL of each sample. The antioxidant activity was concentrated dependent for all the solvents and high TPC contents showed higher antioxidant activity.

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Introduction

In every aspect plants are very important for the survival of human beings. Today in this modern world people are still preferring herbal medicine due to their less harmful effects then synthetic drugs. About 6000 species of plants are present in Pakistan which are medicinally significant. Scientist also discovered various kinds of secondary metabolite or active constituents form the plants which are using in many activities such as antibacterial, anticancer, antitumor and many other activities in a major or minor form (Iqbal, 2015).

Peganum harmala L. *(P. harmala)* is an important medicinal and traditional plant using in variety of disease to relief pain such as asthma, coli, jaundance and many others. It belongs to the family Zygophyllaceae and contains 250 species and 22 genera. It is commonly known as wild rue, Syrian rue and harmal and growing in arid, semiarid, sandy soil and dry region. It is mostly found in North Africa, Middle East, China, Pakistan, India and Iran.

The height of this plant is from 30 to 60 cm with short creeping roots. The length of root can reach up to 6.1 m downward. Leaves are two inches in length, conceived independently and finely separated into long limited fragment. The flowers of *Peganum harmala* L. are lone, little, light yellow or white in color and has potential to develop into fruit which is a leathery, three-valve seed capsule which is three to eight inch in diameter and contains more than fifty dark-brown, angular seeds (Asgarpanah, 2012).

The seeds of *P.harmala* contains a large number of alkaloids and β-Carboline alkaloid and used in fever, abortion, red dye, diarrhea and many other human chronic diseases (Khademalhosseini, 2015).

Plants show medicinal properties due to the presence of certain chemical substances (secondary metabolites) that are responsible for different type of impacts on the human body. Phytochemicals present in *P. harmala* are flavonoids, alkaloids, terpenoids, saponins, tannins and phenolic compounds. As these metabolites are not directly essential for normal growth, development or reproduction of an organism hence they are named as secondary metabolites. These compounds are helpful in curing various human diseases. These substances are also responsible anticancer, antibacterial, analgesic, antitumor, antioxidant and antiviral activity.

Peganum harmala seeds also comprises a large extent of total phenolic and total flavonoids component and act as strong antioxidant plant. (Kaskoos, 2014; Iqbal, 2015).

The smoke of burning seeds of *P. harmala* traditionally used in Iran and Turkey against the evil eye and as disinfectant agent. The roots of this are used to kill lice after it boiled. It is also used in India and Africa for the treatment of syphilis and fever respectively (Mazandarani, 2012).

Reactive oxygen species (ROS) are produced by living organisms as a result of normal cellular metabolism and environmental factors, such as air pollutants or cigarette smoke. These species are highly reactive molecules and can damage cell structures such as carbohydrates, nucleic acids, lipids, and proteins and alter their functions.

The shift in the balance between oxidants and antioxidants in favor of oxidants is termed “oxidative stress.” Regulation of reducing and oxidizing (redox) state is critical for cell viability, activation, proliferation, and organ function. Aerobic organisms have integrated antioxidant systems, which include enzymatic and non-enzymatic antioxidants that are usually effective in blocking harmful effects of ROS (Kasparova *et al*., 2005). However, in pathological conditions; the antioxidant systems can be overwhelmed. Oxidative stress contributes too many pathological conditions and diseases, including cancer, neurological disorders, atherosclerosis, hypertension, ischemia/perfusion, diabetes, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, and asthma (Nurul and Asmah, 2012).

The aim of this study was to quantify natural phytochemicals such as flavonoids, saponins, terpenoids and glycosides from *Peganum harmala* seed extracts and to determine their total phenolic content (TPC) as well as their antioxidant (by DPPH) and antibacterial activity.

Materials and methods

Plant material

Seeds of *Peganum harmala* L. was collected from the market of Lahore (Pakistan). These were dried under shade for 20 days. Dried seeds were ground into fine powder and kept in desiccator until extracted.

Preparation of plant extract

The dried seeds of plant (50 g) were extracted against different polarity solvents including hexane, benzene, chloroform, dichloromethane and methanol by using solvent extraction method. The samples were kept in dark in sample bottles for 2 weeks. After this time, the solvents were evaporated through rotary evaporator under reduced pressure. The concentrates were stored in glass bottle at room temperature for further analysis.

Determination of total phenolic content (TPC)

Total phenolic content of extracts were determined by Folin-Ciocalteu reagent method by following procedure of (Leamsomrong, 2009). From stock solution of Gallic acid (1M) different dilutions of 10ppm, 20ppm, 30ppm, 40ppm and 50ppm were prepared for preparation of calibration curve. After that, 1 mL of plant extract was mixed 5 ml Ciocalteu reagent (0.2 M), after keeping 3 minutes 4 mLof Sodium carbonate (75g/L) solution was added. The mixtures were then stand for 30 minutes and the samples were run to the spectrophotometer at 765 nm. Total phenolic compounds were calculated by using calibration curve of standard Gallic acid.

Determination of antioxidant activity

Antioxidant activity of different organic extract of *Peganum harmala* L. seeds were determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method.

The DPPH test was accomplished by ensuing the method of Epson (Epsin, 2000). Each sample with different concentration of 10, 20, 30, 40 and 50μg/mL were mixed with 3 ml of methanolic DPPH solution. The absorbance of the resultant solution and blank (with only DPPH) was taken after staying time of 30 minutes at ambient temperature against absorbic acids as positive control. Absorbance was checked for standard and samples at 517 nm using UV-Vis spectrophotometer. The percentage of radical scavenging activity was calculated using the following equation:

$$\% \text{ inhibition} = [(A_B - A_S) / A_B] \times 100$$

Results and discussion

Total phenolic content

In this study, quantitative total phenolic content analysis was done by using spectrophotometer from different organic extract of *P. harmala* seeds by already reported procedure of Leamsomrong, 2009. Gallic acid was used as standard, its five concentrations 10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm were prepared and their absorbance was measure at 765 nm. The linear equation with different concentration of Gallic acid at 765 nm is shown below in the fig. 1.

Table 1. Concentration of Total Phenolic Compounds in extract of *Peganum harmala* L. seeds.

Sample	Absorption	Total phenolic contents (mg GA/g)
Pegnum Harmala seeds	Methanol extract	30.9
Pegnum Harmala seeds	Dichloromethane extract	27.7
Pegnum Harmala seeds	Chloroform extract	26.4
Pegnum Harmala seeds	Benzene extract	22.2
Pegnum Harmala seeds	Hexane extract	17.3

Determination of total phenolic compounds in different organic extract of *P. harmala* seeds was carried out by spectrophotometric assay as per the method (Leamsomrong, 2009). Five different solvents like dichloromethane, benzene, chloroform, methanol and hexan with different polarity were used for the extraction. These extracts were reduced to 50% of total solid and then total phenolic contents were determined by using folin-Ciocalteu reagent and taking absorptions at 765 nm. The results are summarized in the table 1, which were measured by the standard gallic acid curve.

Table 2. Antioxidant activity of extracts of *Pegnum Harmala* seeds.

Concentration	Methanol	Dichloromethane	Chloroform	Benzene	*n*-Hexan
10 µL	33	29	26	25	21
20 µL	49	38	37	33	26
30 µL	56	52	46	40	33
40 µL	63	59	55	47	41
50 µL	72	67	63	52	48

*standard ascorbic acid showed 92% antioxidant.

The total phenlic contents of *pegnum harmala* seeds from Pakistan were compared with the other reported literature like Djarmouni *et al*., 2012 and Maroua *et al*., 2017. Our results are comparable with these reported results.

Antioxidant activity

Peganum harmala has medicinal properties for the treatment of different diseases and also used as folk medicine in different countries.

The seed extract of this plant contain large amount of phenolics and flavonoids which act as antioxidant and inhibit the production of oxidative species produced in body. Antioxidants have the capacity to protect the body from oxidative stress damage.

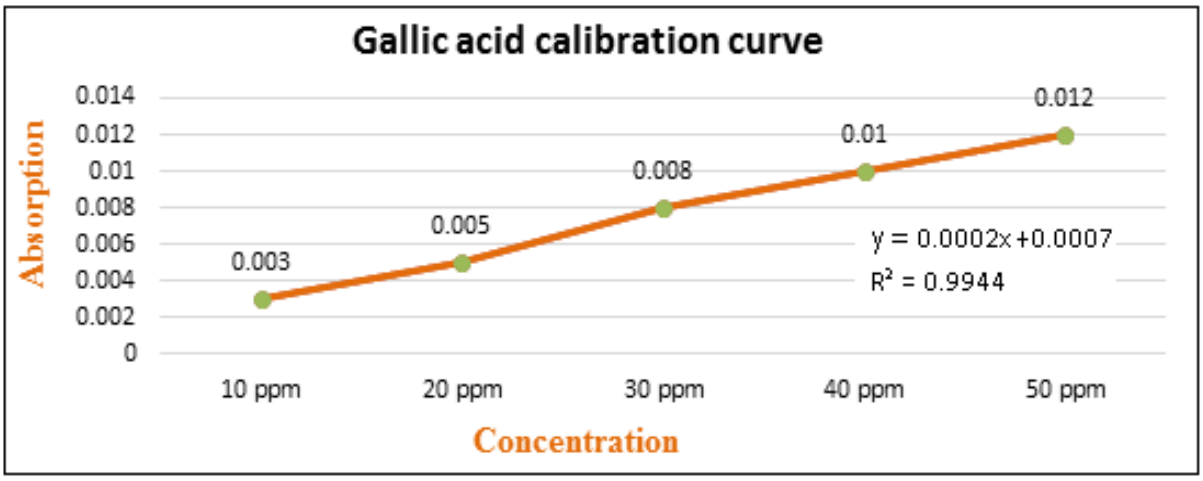

Fig. 1. Calibration curve of different concentrations of Gallic acid.

The antioxidant capacity of any artificial or natural antioxidant can be estimated though its hydrogen donating act to DPPH radical.

In this work antioxidant activity of different organic extract of *Peganum harmala* seeds was performed by using DPPH. Methanol extract of plant seed was found to have strong antioxidant activity of 72 % at 50μg/mL against ascorbic acid which show the % inhibition of 92 %. Followed by dichloromethane extract (67%), chloroform extract (63 %), benzene extract (52 %) and hexane extract (48 %). The inhibitory power of *P. harmala* seed extract to scavenge free radical depends on the dose of extract

used. Comparison of antioxidant activity of all organic extract of *P. harmala* seed is given below in table 2.

The antioxidant activity is mainly dependent on phenolic and flavonoid compounds. As, it is clear from the table 1 that phenolic contents are higher in methanol so the antioxidant activity is also higher (Table 2). Furthermore, it is also concluded that higher concentration revealed higher antioxidant activity.

Antioxidants derived from food and medicinal plants have been increasingly investigated for their various nutritional function and health benefits. In biological system, reactive oxygen species (ROS) and reactive nitrogen species (RNS),such as superoxide, hydroxyl, and nitric oxide radicals, can damage the DNA and lead to the oxidation of lipid and proteins in cells (Fang *et al.*, 2002). Normally, antioxidant system occurring in human body can scavenge these radicals, which would keep the balance between oxidation and anti-oxidation. Nonetheless, the exposure of cigarette smoking, alcohol, radiation, or environmental toxins induces the production of excessive ROS and RNS, which disrupt the balance between oxidation and anti-oxidation and result in some chronic and degenerative diseases (Li *et al.*, 2015; Wang *et al.*, 2016; Zhou *et al.*, 2016) The increment of intake of exogenous antioxidants would ameliorate the damage caused by oxidative stress through inhibiting the initiation or propagation of oxidative chain reaction, acting as free radical scavengers, quenchers of single oxygen and reducing agents (Baiano *et al.*, 2015).

Conclusion

Pegnum Harmala seeds were extracted with various solvents including methanol, hexane, benzene, chloroform and dichloromethane and their total phenolic contents (TPC) were determined by using Folin-Ciocalteu reagent method against gallic acid as standard by UV-Vis spectrophotometer at 765 nm. Furthermore, their DPPH free radical scavenging activity of the extracts was measure by using UV-Vis spectrophotometer and taking reading at 517 nm and ascorbic acid as standard. Total phenolic contents 27.7 mg GA/g, 22.2mg GA/g, 26.4mg GA /g, 30.7 mg GA /g and 17.3 mg GA /g were for dichloromethane, benzene, chloroform, methanol and hexane extracts respectively. Methanol extract showed high TPC content and high antioxidant activity (72%) followed by dichloromethane extract (67%), chloroform extract (63 %), benzene extract (52 %) and hexane extract (48 %) respectively for 50 µL of each sample. The antioxidant activity was concentrated dependent for all the solvents and high TPC contents showed higher antioxidant activity.

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