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Phytochemical evaluation and antioxidant assay of ethanolic extracts of traditional medicinal plants (*ferula assafoetida, Saussurea costus, Peganum harmala)* in Oman

S Hemadri Reddy[1]*, Aysha Al-Weheibi[1], Halima Al Shekaili[1] and Rehab Al-Naabi[1]

***Corresponding author:**

S Hemadri Reddy

[1]Biology section, Department of Applied Sciences, Higher College of Technology, P O Box 74, Postal code 133,Sultanate of Oman, Muscat.

Abstract

Ferula assafoetida, Saussureacostus and *Peganumharmala* are considered an important traditional folk medicines in Oman, extracts of these plants were screened for assessing bioactive phytochemical constituents and their antioxidant property. Total phenolic and flavonoid contents were evaluated to explore the reliable and potential sources of novel natural antioxidants. Preliminary phytochemical screening of different plant extracts and phytochemical investigation showed variations in the presence and amount of active ingredients. All the fractions of *P.harmala*, *S.costus and F. assafoetida have* flavonoid and phenolic compounds, whereas, the highest amount was found in *F.assafoetida which was* 0.370, 0.053 mg/ml respectively. Under antioxidant study the H_2O_2 free radical scavenging potential and Phosphomolybdate assay were studied *in vitro* for the determination of antioxidant activity. However *S. costus* extract showed the highest scavenging percentages 89%. In Phosphomolybdate assay *Ferula assafoetida* has a very good antioxidant potential due to phenolic and flavonoid components.

Keywords: Phytochemical evaluation, antioxidant study, phosphomolybdate assay, free radical scavenging

Introduction

The role of free radicals in many disease conditions has been well established. Several biochemical reactions in our body generate reactive oxygen species and these are capable of damaging crucial bio-molecules. If they are not effectively scavenged by cellular constituents, they lead to disease conditions [1].The harmful action of free radicals can be blocked by antioxidant substances, which scavenge the free radicals and detoxify the organism. Several plant extracts and different classes of phytochemicals have been shown to have antioxidant activity [2-4].

Peganum harmala was first found indry area of central Asia and southern USA [5,6] has a wide spectrum of pharmacological actions like monoamine oxidase inhibition [7], binding to benzodiazepin receptors [8] and antioxidant property [9].

Ferula genus from the family of *Umbelliferae* has been found to be a rich source of gum-resin [10]. In Iranian traditional medicine, *F. assafoetida* gum extract has been used as a remedy for abdominal pain, constipation and diarrhea and as an antihelminthic [11].

The root of *Saussurea costus* is anodyne, antibacterial, antispasmodic, aphrodisiac, carminative, skin, stimulant, stomachic, tonic and vermifuge. It is used internally in the treatment of abdominal distension and pain, chest pains due to liver problems and jaundice, gall bladder pain, constipation associated with energy stagnation, and asthma [12].The plants possess potent bioactive compounds capable of preventing and treating most oxidative related diseases[13,14]and have often been used in folkloric medicine [15].

In developing countries, the use of medicinal plants in the treatment of infectious disease is rife and reasons include the high cost of effective drugs [16]. However, potential indigenous plants exploited for medicinal purposes have to undergo basic phytochemical screening and bioassay as first step towards the ultimate development of drugs [17].Therefore, the present study aimed to investigate the potential role of *P. harmala, F. assafoetida and S. costus* as a source of antioxidants, nutritional and the rapeutic purposes.

Materials and Methods

Plant material collection and extraction

Materials of gum-resin of Ferula and costus root powder were purchased from local herb store in al-Khuwair, Sultanate of Oman during February 2014. *Peganum harmala* samples were collected

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during February 2014 from Bidyha , al-sharqiya region Sultanate of Oman, The leaves of *Peganum harmala* were shade dried and powdered with mechanical grinder, and well preserved for further use.

Maceration method

100g of each dried powder of three plants were mixed with 200ml of ethanol and were kept in rotary shaker at 100 rpm speed for 72 hours at room temperature. The extracts were filtered using Whatmann number 1 filter paper and filtrate is collected for further analysis.

Soxhalation method

100g of each dried powder of three plants were subjected to ethanolic extraction using soxhlet apparatus for 6 hours at 50 C.

Preliminary phytochemical screening

The filtrate was subjected for solvent evaporation in Rota evaporator for 2 hours at 60°C, under reduced pressure and constant temperature [18]. Phytochemical screening of extracts obtained from plant materials were carried out to determine presence of phytoconstitueunts according to [19]. This finding aimed to trace out various preliminary phytoconstituents like Alkaloids, carboxylic acid, coumarins, flavanoids, phenol, quinines, resins, saponins, steroids, tannins, glycosides, carbohydrate, protein and vitamin C to study their potential role.

Determination of total phenolic content

Total phenolic compound contents were determined by the Folin-Ciocalteau method [20]. 0.5 ml of each individual extracts were mixed with 2.5 ml of 0.2 N Folin-Ciocalteau reagent for 5 min and then 2.0 ml of 75 g sodium carbonate were added. The absorbance of the reaction was measured at765 nm in UV-visible spectrometer against the blank after 2 h of incubation at room temperature. Gallic acid was used as the positive control and values are evaluated as the mg equivalent of gallic acid per g of extract.

Determination of total flavonoid content

Total flavonoids were estimated using the method of Ordonez et al. [20]. 0.5 mL of each ethanol plant extracts were individually mixed with 1.5 mL of ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water and left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 510 nmin a UV-VIS spectrophotometer. The total flavonoid content was expressed as the mg equivalents of quercetin (QE) per g of extracts using standard curve.

Antioxidant assays

Hydrogen peroxide scavenging activity

Hydrogen peroxide solution at 2 mM/L was prepared with phosphate buffer (pH 7.4). 1 ml (25µg/ml) of extracts in distilled water was added to 0.6 ml of hydrogen peroxide solution. Absorbance was determined at 230 nm after 10 min of incubation against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging activity at 25µg/ml of extracts was determined and the % hydrogen peroxide scavenging activity was compared with the standard vitamin E [21]. H_2O_2 scavenging activity was calculated by following formula:
Hydrogen peroxide scavenging activity=

$$\frac{(\text{absorbance of control} - \text{absorbance of sample})}{\text{absorbance of control}}\ 100$$

Phosphomolybdate assay

This assay was performed according to the procedure of Umamaheswari and [22]. 200 µl of each sample was mixed with 2 ml working reagent (0.6 M H_2SO_4, 28 mM sodium phosphate and 4 mM ammonium molybdate) and the mixture was incubated in boiling water bath for 90 min at 95°C. The mixture was allowed to stand at room temperature for 30 minutes; absorbance was read at 765 nm against a blank. Ascorbic acid was used as standard. Antioxidant activity was determined according to following formula:
Antioxidant effect (%)

$$=\frac{(\text{absorbance of control} - \text{absorbance of sample})}{\text{absorbance of control}}\ 100$$

Results

Phytochemical analysis

Preliminary phytochemical screening of *F. assafoetida, S. costus*, *P. harmala* revealed the presence of various bioactive compounds like: phenolics, tannins, saponins, coumarins, resin, glycosides, steroids, carboxylic acid, carbohydrate, vitamin C and alkaloids, the results of phytochemical test has been summarized in table 3.1.

Table 3.1-Preliminary Phytochemical analysis of *Ferula assafoetida, Saussurea costus*, *Peganum harmala* extracts.

Name of the extracts	H .M	H .S	F .M	F.S	C .M	C .S
Carboxylic acid	+	++	++	+++	++	+++
Coumarins	+++	+++	++	++	-	-
Alkaloid	+++	+++	-	-	++	++
Phenol	+++	+++	-	-	++	++
Quinines	-	-	-	-	-	-
Resin	++	++	++	++	++	++
Tannins	+++	+++	++	-	++	++
Glycosides	+++	+++	++	++	++	++
Steroids	+++	+++	-	-	++	++
Saponins	+++	+++	-	-	-	-
Carbohydrate (Benedict's test)	++	++	-	-	-	-
Carbohydrate (Molisch's test)	++	++	++	-	+++	+++
Vitamin C	-	-	+++	++	++	+++
Protein	-	-	+	-	+	-

Note: Values expressed in the table are the mean values of three replicates, (+++: highest, ++: moderate, +: low, - : absence). Note: (H.M :*Peganum harmala* Maceration extraction , H .S: *Peganum harmala* soxhletion extraction, F .M :*Ferula assafoetid* Maceration extraction , F.S *Ferula assafoetid* soxhletion extraction, C .M: *Saussurea Costus* Maceration extraction , C .S *Saussurea Costus* soxhlation extraction.

Saussurea costus, *Peganum harmala* extracts

Total phenolic content

The measured absorbance values at 765 nm for the indicated concentration of gallic acid solutions are in the range of 0.08 to 1.16. Within this range of concentrations (0.001 to 0.05 mg/ml), the calibration curve of gallic acid has clearly exhibited linearity. Total phenolic content of six ethanolic extracts was varied ranging from 0.008 mg/ml to 0.053 mg/ml gallic acid equivalent per g dry weight with reference of standard curve(y = 23.254x,R = 0.9779).

Table 3.2: Total phenolic content of plant extracts.

Name of the plant	Absorbance at765 nm	Total phenol Content (mg/ml)
Peganumharmala by soxhlation	1.2395	0.029
Peganumharmala by maceration	1.067	0.028
Saussureacostus by soxhlation	0.6725	0.029
Saussureacostus by maceration	0.227	0.008
Ferula assafoetida by soxhlation	0.6835	0.046
Ferula assafoetida by maceration	0.641	0.053

Total flavonoid contents

The calibration curve for 6 sequentially and independently prepared stock standard solutions of quercetin that depicts the concentration of quercetin against the absorbance, as presented in Figure 3.3.

Table 3.3: Total flavonoid content of different plant extracts.

Name of the plant	Absorbance at 510 nm	Flavonoid Content (mg/ml)
Peganum harmala by soxhletion	0.569	0.164
Peganum harmala by maceration	0.593	0.171
Saussurea costus by soxhletion	1.0655	0.308
Saussurea costus by maceration	0.13	0.038
Ferula assafoetida by soxhletion	0.525	0.152
Ferula assafoetida by maceration	1.28	0.370

The absorbance value increased proportionally upon increasing the concentration of quercetin from 0.05 to o.6 mg/ml. A slight deviation from the linearity was found to be occurred at the higher concentration region of quercetin calibration plot. Total flavonoid content of six ethanolic extracts was varied ranging from 0.038mg/ml to 0.370mg/ml quercetin equivalent/g dry weight with reference of standard curve(y = 3.4597x,R = 0.9103).

Antioxidant assays

Hydrogen peroxide scavenging assay

The scavenging ability of six ethanol extracts are varied according to capacity of each extract in removing H_2O_2 .among the tested plant extracts *S. costus* shown highest % of hydrogen peroxide scavenging.

Table 3.4: Hydrogen peroxide scavenging activity of different plant extract.

Sample	Concentration	Mean Absorption	%hydrogen peroxide scavenging activity
Ferula assafoetida by maceration	25 µg/ml	1.110	61 %
Ferula assafoetida by soxhlation	25 µg/ml	0.924	67 %
Peganum harmala by maceration	25 µg/ml	0.935	67 %
Peganum harmala by soxhlation	25 µg/ml	0.963	66 %
Saussurea costus by maceration	25 µg/ml	0.307	89 %
Saussurea costus by soxhlation	25 µg/ml	1.056	62 %
Vitamin E	25 µg/ml	0.065	97.7 %

Note: All the values expressed in the given table are the mean values of three replicates

Phosphomolybdate assay

Phosphomolybdate is another assay that is performed to assess the overall antioxidant activity of the extract.

Table 3.5: Total antioxidant capacity % of six extracts through Phosphomolybdate assay.

Sample	Absorbance (nm)		% TAC
	Control	Sample	
Peganum harmala by soxhletion	2.088	1.99	4.69 %
Peganum harmala by maceration		1.77	15.2 %
Saussurea costus by soxhletion		1.5675	24.928 %
Saussurea costus by maceration		1.435	31.27 %
Ferula assafoetida by soxhletion		0.955	54.26 %
Ferula assafoetida by maceration		1.0485	49.78 %

Note: % of antioxidant capacity expressed in table are the mean values of three replicates.

Discussion

The pharmacological action of crude drugs of and other therapeutic uses are due to their therapeutically active constituents. For that, preliminary phytochemical analysis reviewed pronounced importance because the crude drugs possess varied composition of phytochemical constituents. These active constituents of plants play a vital role in the treatment of different diseases such as tannins possess anti-inflammatory and anticancer activity [23,24] among the six different extracts, harmala contain the highest

amount of tannins and flavonoids which are antioxidant, anti-inflammatory and anticancer agents [25].

Coumarins are antioxidant, maintain blood pressure and inhibit lipid peroxidation [26] it is found more in harmala extract followed by ferula extract. Furthermore, alkaloids possess ant leukemic and anticancer activity [27]. Harmala and costus extracts contain good amount of alkaloids whereas it isnot found in ferula extract. Saponin is antimicrobial agent and maintains the blood cholesterol level [28] foundat good concentration in Harmala extract.

It has been recognized that flavonoids show antioxidant activity and their effect on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process [29,30]. Phenolic compounds are a class of antioxidant compounds which act as free radical terminators [31]. The compounds such as flavonoids, which contain hydroxyl functional groups, are responsible for antioxidant effect in the plants [32,33]. All the fractions of *P.harmala*, *S.costus and F. assafoetida have* flavonoid and phenolic compounds. As the result show the highest amount found in *F. assafoetida which was* 0.370, 0.053 mg/ml respectively.

Hydrogen peroxide is a weak oxidizing agent and it is not very reactive, can cross biological membranes. Because of the possible involvement of hydrogen peroxide in the generation of hydroxyl radicals, this property places hydrogen peroxide in a more prominent role to initiate cytotoxicity than its chemical reactivity. Thus removing H_2O_2 is very important for the protection of living systems. Extracts scavenged hydrogen peroxide which may be attributed the presence of phenolic groups that could donate electrons to hydrogen peroxide, thereby neutralizing it into water [34].

Table 3.4 shows % hydrogen peroxide scavenging activity of the ethanol extracts (*P. harmala*, *S. costusand F. assafoetida)* by maceration andsoxhlation. *S.costus*extract caused a strong dose-dependent inhibition of hydrogen peroxide which is closest to standard. Whereas *F. assafoetida by* maceration caused a weak dose-dependent inhibition of hydrogen peroxide. All the extracts of *P. harmala*, *S. costus and F. assafoetida* scavenged hydrogen peroxide at a concentration of 25 μg/ml, the scavenging percentages were 61, 89 for *F. assafoetida by* maceration , *S.costus* by soxhlation respectively.

In the presence of antioxidant sample Mo (VI) reduced to Mo (V) with subsequent formation of green colored phosphomolybdenum V complex exhibiting maximum absorbance at 765nm. Our findings demonstrate that *Ferula assafoetida* has a very good antioxidant potential that was due to the collective contribution of phenolic and flavonoid components of the plant.

Conclusion

The results of the present investigation reported that *Ferula assafoetida, Saussurea costus, Peganum harmala* possess strong antioxidant potential that due to contribution of its phenolics and flavonoid contents, and it would be advantageous to use the plant antioxidant in therapeutic drugs for the implications of human health. We conclude that harmala has strong antioxidant followed by ferula than costus. In which it showed the most bioactive constituents found in harmala extract (coumarins, alkaloid, phenol, tannins, glycosides, steroids, saponinsetc).Natural product antioxidants significantly contribute in preventions of pathological consequences caused by free radicals. Furthermore plant derived antioxidant are safer and cheaper than their synthetic counterparts. Further studies are needed for the isolation and identification of bioactive compounds more specifically responsible for antioxidant activity.

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