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Evaluation of total phenols, total flavonoids and antioxidant activity of the leaves crude extracts of locally grown pigeon pea traditionally used in Sultanate of Oman for the treatment of jaundice and diabetes

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ABSTRACT

Objective: To determine the total phenols, total flavonoids and evaluate the antioxidant activity of crude extracts from the leaves of pigeon pea native to Sultanate of Oman by a popular method.

Methods: The powdered leaves samples from pigeon pea were used for extraction by maceration method with methanol solvent. The methanol free crude extract by maceration method was suspended in water and successively extracted with different polarities of solvents. The obtained crude extracts with different polarities were used for the determination of total phenols and flavonoids contents by using Folin-Ciocalteu reagent and aluminum chloride methods. The antioxidant activity of six crude extracts from pigeon pea was determined by α, α-diphenyl-β-picrylhydrazyl method.

Results: The different polarities leaves crude extracts showed a significant amount of total phenols content ranging from 97.80 to 256.00 mg of GAE/g of crude extract. The same leaves crude extracts also showed good amount of total flavonoids content ranging from 1.38 to 8.51 mg QE/g plant material. The six crude extracts from the leaves displayed significant α, α-diphenyl-β-picrylhydrazyl free radical scavenging activity with highest value in chloroform extract followed by methanol, butanol, ethyl acetate, hexane and water crude extracts (98.13%, 89.26%, 88.82%, 86.41%, 79.95% and 69.44%, respectively).

Conclusions: Leaves crude extracts from pigeon pea have high contents of total phenols and flavonoids. In this regards, it could be used as a medicine for the treatment of different diseases.

1. Introduction

Pigeon pea is one of the most important medicinal plants belonging to Abaceae family[1]. Its scientific name is pigeon pea, and locally it is called *Cajanus cajana*. It has different names in different countries: redgram, tur, arhar, and poilsd' Angle in Spanish[2], congo bean in English, pois de congo in French and ervilba decongo in Portuguese[3].

Tropical and subtropical countries are the suitable places for growing this plant. Sometimes, it is called a warm season's crop[4-9]. The morphological characteristics of this plant are different from others. It is a specially erect perennial, annual plant, having 1-4 m in height with deep taproot of 2 m[9-11]. Pigeon pea has a ribbed stem that is angled and pubescent[12-14]. The plant leaves are green and silvery grayish green with long hairs[9]. Leaves are spirally set around the stem and alternate[11]. The flowers of this plant are yellow with red color outside[9]. Therapeutically this plant are used for different aliments by different ethnic communities. Traditionally, it is used in human food as flour additive in some foods recipes, for example, soup

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and rice; it is also used as animal feed[15]. In addition, perennial pigeon pea is used as fuel, soil ameliorants and for live fences. In African villages such as Zumbia it is used in basket weaving and roofing[1,6-8,16]. However, therapeutically it is commonly used for the treatment of diabetes, dysentery, hepatitis, measles, varicella and superficial infection and for stabilizing the menstrual period[11,17-19]. The root is used as alexeritic, anthelminthic, expectorant, sedative and vulnerary[12,13]. The leaves of this plant are used for treatment of jaundice, diarrhoea and diabetes, and help to remove bladder stones[5]. In India, the leaves are used for curing sores, wounds, abdominal tumors, and diabetes[1,13,20]. However, in China, leaves are used in an infusion for overcoming anemia, hepatitis, urinary infection, yellow fever and ulcer[1,10,11]. In Brazil, the leaves are used for coughs, ulcers and fever[8,5]. The chemical compounds of pigeon pea include protein, fat, fiber and ash. Starch contains carbohydrates, soluble sugar, phosphorus, lysin, theonine, methionine, cysteine, calcium, magnesium, iron, copper, zinc, thiamine, riboflavin, niacin, potassium, sodium, ascorbic acid, B-carotene, vitamin A, vitexin, isovitexin, orientin, apigenin, luteolin, pinostrobin and cajaninstilbene acid[7,9,14,18]. The literature search reveals that no scientific work has been done on the Omani pigeon pea species. Therefore, the aim of this work is to prepare the crude extracts and determine the total phenols and flavonoids, and evaluate the antioxidant activity of the leaves crude extracts native to Sultanate of Oman.

2. Materials and methods

2.1. Materials

Different types of solvents such as methanol, butanol, chloroform, ethyl acetate, acetone, dimethyl sulfoxide were obtained from Sigma Aldrich Company. Gallic acid, sodium hydroxide, sodium nitrate, aluminium chloride and sodium carbonate were obtained from SDFCL Industrial Estate, Mumbai, India. Hexane, Folin-Ciocalteu reagent (FCR) and α, α-diphenyl-β-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich, Germany. Rotary evaporator Model-RE801 was from Yamato, and UV-visible spectrophotometer [Thermo Spectronic spectrophotometer (Great Britain, UK, Model No. Biomate)] was used for the measurement of absorbance.

2.2. Sample of plant

The leaves samples of pigeon pea were collected from Al Sharqea region, Oman. The samples were harvested on December 13, 2013 at 6 p.m. The morphological identification was done by a botanist. The voucher specimen (No. 005) was deposited in the Natural Product Laboratory, University of Nizwa, Nizwa, Sultanate of Oman.

2.3. Preparation of samples

The collected leaves samples of pigeon pea were washed with water and dried under shade at room temperature. The dry samples were powdered by using heavy duty machine. Then the powder samples were stored in a bottle to avoid decomposition.

2.4. Extraction procedure

A total of 150 g powdered samples of pigeon pea leaves was taken in a 2 L beaker and added with 500 mL of methanol solvent. The beaker was kept for 3 d and every day the mixture was stirred by a glass rod. The whole mixture was filtered under pressure by using Buchner funnel. The filtrate was evaporated by rotary evaporator to give methanol free crude extract. About 120 mL of water was added to the methanol crude extract and dissolved by hand. The whole water mixture was transferred to a separatory funnel for extraction by different solvents. About 30 mL of hexane solvent was added to it and shaken by hand for 20 min, and left for 30 min to separate into two layers. The whole process was repeated with 20 mL of hexane. The obtained two parts were combined together and hexane was evaporated to give the hexane crude extract. Similarly, chloroform, ethyl acetate and butanol solvents were used to prepare chloroform, ethyl acetate and butanol crude extracts. The remaining water part was evaporated to give water crude extract.

2.5. Determination of total phenols

2.5.1. FCR

A total of 10 mL FCR was taken in a 100 mL volumetric flask, to which 90 mL of distilled water was added. The concentration was 10%.

2.5.2. Sodium carbonate solution

About 3 g of sodium carbonate was taken in a 50 mL volumetric flask, to which 50 mL of distilled water was added. The concentration was 6%.

2.5.3. Preparation of gallic acid

Gallic acid was used as a standard for the calculation of total phenols. About 2 mg of gallic acid was taken in a 10 mL volumetric flask and diluted with 10 mL of methanol. Serial dilution technique was used for the preparation of 100, 50, 25 and 12.5 μg/mL of gallic acid. About 200 μL of each concentration was taken in a test tube, to which 1.5 mL of 10% FCR solution was added. All the test tube was kept for 5 min in a dark place. Then 1.5 mL of 6% Na_2CO_3 was added to each test tube and kept in a dark place for 2 h. Finally, the absorbance of the samples was measured at a fixed wavelength. The standard curve was made by plotting the obtained data from spectrophotometer.

2.5.4. Procedure for total phenols

Each prepared leaves crude extracts was used for the determination of total phenols. About 4 mg of each crude extract was taken in a separate test tube, to which 10 mL of methanol was added. About 200 μL of each samples was taken in a separate test

tube and suspended in 1.5 mL of 10% FCR, and kept for 5 min in a dark place. Finally, 1.5 mL of 6% Na_2CO_3 solution was added and covered by aluminum foil and kept for 2 h in a dark place. UV-visible spectrophotometer at fixed wavelength 760 nm was used for the measurement of absorbance of samples. Calculation of total phenols was based on the following formula from the calibration curve;

$y=0.0934x$

where y=absorbance of crude sample, and x=concentration of phenols.

2.6. Determination of total flavonoids

A total of 2.5 mg of sodium nitrate, 2 mg of sodium hydroxide and 5 mg of aluminum chloride were taken in a separate 50 mL volumetric flask and diluted with 50 mL of distilled water. The concentrations were 5% sodium nitrate, 4% sodium hydroxide and 10 % aluminum chloride.

Qurecetin was used as a standard for the calculation of total flavonoids. A total of 2 mg qurecetin was taken in a 10 mL volumetric flask and mixed with 10 mL of methanol. Serial dilution was performed for the preparation of 100, 50, 25 and 12.5 µg/mL of qurecetin concentration. About 250 µL of each concentration was taken in a test tube, mixed with 125 µL water and 75 µL sodium nitrate solution. The solution was kept for 6 min in a dark place. Then 150 µL of aluminum chloride was added to each test tube and kept in a dark place for 2 h. Finally, 500 µL of sodium hydroxide and 275 µL of water were added to each test tube. The absorbance was measured by UV-visible spectroscopy at a fixed wavelength 510 nm. The standard curve was prepared by plotting the obtained data.

A total of 250 µL of each crude samples (4 mg crude extract in 4 mL of methanol) were taken in a separate test tube. Then 125 µL water and 75 µL sodium nutrate solution were added to each test tube. The solution was kept for 6 min in a dark place. Then 150 µL of aluminum chloride was added to each test tube and kept in a dark place for 2 h. Finally, 500 µL of sodium hydroxide and 275 µL of water were added to each tube. The absorbance was measured by UV-visible spectroscopy at a fixed wavelength 510 nm. For the calculation of total flavonoid content, the following formula was used:

X=(A×mo)/(Ao×m)

Where 'X' is the flavonoid content in mg/g plant extract, 'A' is the absorption of the plant crude extract solution, 'Ao' is the absorption of the quercetin solution, 'm' is the mass of crude drug extract in mg and 'mo' is the mass of quercetin in the solution in mg.

2.7. Antioxidant activity assay

All six different polarities crude extracts from the leaves of pigeon pea obtained by maceration method were used for the determination of antioxidant potential by using DPPH method[1,15,17,19]. Two milligram of each leaves crude extract of pigeon pea such as hexane, chloroform, ethyl acetate, butane, methanol and water crude extract were taken in test tube and dissolved in 10 mL methanol. Different concentrations such as 200, 100, 50, 25 and 12.5 µg/mL were prepared by serial dilution. About 3.3 g of DPPH was taken in a 100 mL volumetric flask and dissolved with 100 mL of methanol. Then 2.5 mL of DPPH solution was added to all test tubes and shaken gently by hand and kept in dark place for one and half hour. The absorbance of the samples were measured by using UV-visible spectroscopy at wavelength of 517 nm. Finally antioxidant activity of the crude extract samples was calculated by using the following formula:

$$\%\ \text{Inhibition}=\frac{\text{Absorbance of standard}-\text{Absorbance of crude extract}}{\text{Absorbance of standard}}\times 100$$

3. Results

Total phenols of different polarities crude extracts such as hexane, chloroform, methanol, water, ethyl acetate and butanol crude extract from pigeon pea leaves are presented in Table 1. The results showed that the highest amount of total phenols was observed in chloroform extract (256 mg of GAE/g of powder crude extract) and lowest was in water extract (97.8 mg of GAE/g of crude extract) followed by ethyl acetate (215.6 mg of GAE/g of crude extract), butanal (182.9 mg of GAE/g of crude extract), hexane (143.2 mg of GAE/g of crude extract) and methanol extract (135.3 mg of GAE/g of crude extract). Gallic acid was used as a standard for the determination of total phenols content (Figure 1).

Table 1
Total phenols and flavonoids contents of different polarities leaves crude extracts.

Leaves of crude extracts	Phenols (mg of GAE/g of crude extract)	Flavonoids (mg QE/g crude extract)
Water	97.8	8.51
Chloroform	256.0	1.71
Methanol	135.3	3.80
Hexane	143.2	8.18
Ethyl acetate	215.6	6.29
Butanol	182.9	1.38

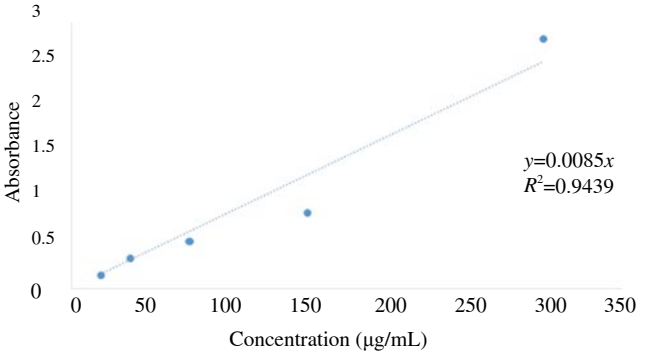

Figure 1. Gallic acid standard curve for the calculation of total phenols.

Total flavonoids content of hexane, chloroform, methanol, water, ethyl acetate and butanol crude extracts from the leaves of pigeon pea are presented in Table 1. The highest amount of total flavonoids

Table 2
Antioxidant potential of hexane, ethyl acetate, chloroform, butanol, methanol and water crude extracts from leaves of pigeon pea.

Concentration of crude extract (μg/mL)	% Inhibition					
	Water	Methanol	Hexane	Ethyl acetate	Chloroform	Butanol
200	69.44±0.09	89.26±0.23	79.95±0.07	86.41±0.15	98.13±0.11	88.82±0.41
100	57.20±0.34	67.60±0.17	59.60±0.11	66.20±0.18	91.50±0.17	79.60±0.52
50	49.90±0.17	58.90±0.41	53.00±0.44	54.10±0.22	83.20±0.14	67.10±0.17
25	41.00±0.22	44.20±0.29	44.70±0.13	46.10±0.39	73.70±0.23	54.50±0.11
12.5	39.20±0.19	39.50±0.54	38.30±0.19	41.60±0.31	64.00±0.34	47.20±0.15

was obtained from the water extract (8.51 mg QE/g plant material) and lowest was in butanol extract (1.38 mg QE/g plant material). Qurecetin was used as a standard for the determination of total flavonoids content (Figure 2).

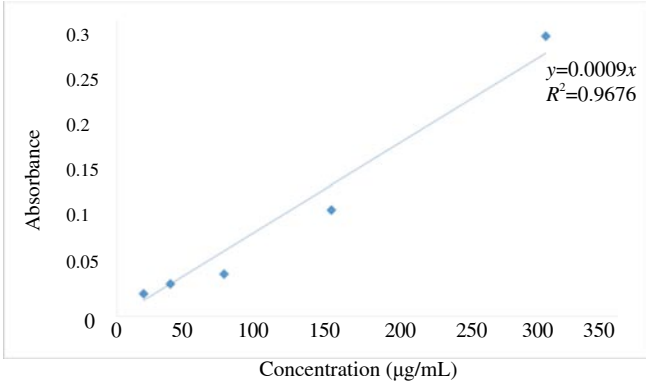

Figure 2. Qurecetin standard curve for the calculation of total flavonoids.

The % inhibition of six crude extracts from the leaves of pigeon pea against DPPH was calculated. The antioxidant potential of six crude extracts obtained was highest in chloroform extract and lowest in water crude extract. The antioxidant potential was in the following order: chloroform>methanol>butanol>ethyl acetate> hexane>water (Table 2).

4. Discussion

Almost all plant secondary metabolites and phenolic compounds are bioactive and widely present in all plant kingdom. The colour, sensory qualities, nutritional and antioxidant properties completely depends on phenolic compounds and their derivatives[15,18,20]. The total phenols contents in six crude extracts from the leaves of pigeon pea by maceration extraction method are evaluated in the present study. The contents of phenol compounds in different leaves crude extracts were different. Significant amount of phenolics compounds is present in all six crude extracts within the range of 97.8 to 256.0 mg GAE/g crude plant extract. The maximum amount of phenolics compounds was present in chloroform extract (256 mg of GAE/g of crude extract) and minimum was in water extract (97.8 mg of GAE/g of crude extract). The order of total phenolics found in the leaves crude extracts was chloroform>ethyl acetate>butanol>hexane>metha nol>water extract. However, the total flavonoids contents of the crude extracts was determined by aluminum chloride method. Significant amount of total flavonoids was present in all six crude extracts from the leaves of pigeon pea within the range of 1.38 to 8.51 mg QE/g plant material. The maximum content of total flavonoids was present in water leaves crude extract and the minimum contents was in butanol crude extract.

The free radical scavenging activities of different polarities crude extracts were investigated by DPPH method in this study. The DPPH reagent react with the crude extracts and change the colour from that of α, α-diphenyl-β-picrylhydrazyl to that of α, α-diphenyl-β-picrylhydrazine. The change of colour rate decrease indicates the scavenging potentials of the sample. In our experiment, the six different polarities crude extracts from the leaves of pigeon pea were able to change the colour of DPPH solution due to presence of different phenols compounds. According to our experimental results, among the methanol extract and its derived fractions, the highest antioxidant potential was found in chloroform crude extract and the lowest in water crude extract. The antioxidant potential was in the following order: ch loroform>methanol>butanol>ethyl acetate> hexane>water. All polarities plant crude extracts contain flavonoid, saponins tannin and aromatic compounds which are able to reduce colour change of DPPH by their hydrogen donating ability[18-21]. The results obtained from the present experiment revealed that six crude extracts from the leaves of pigeon pea possess hydrogen donating capabilities and it was acting as an antioxidant. The variation of percentage obtained in this present study might possibly be due to the use of different solvents for extraction. In addition, during the samples processing and extraction by different solvents, some volatiles active compounds have been destroyed or evaporated from the samples. In this regard, the antioxidant activity was different among the six crude leaves extracts of pigeon pea.

Further studies are designed for the isolation and identification of individual phenolic principles and also animal studies are needed for better understanding of their mechanism of action as antioxidant. Almost similar results and relationship between antioxidant activity of plant crude extracts and biochemical screening have previously been reported[1,5,11-14].

The crude extracts from the leaves of pigeon pea contain very high contents of total phenols and flavonoids. Also all six crude extracts from this plant showed very high percentage of antioxidant activity by the DPPH method. Therefore, all the

crude extracts from this plant could be used as a medicine for the treatment of different diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

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