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Total phenols, total flavonoids contents and free radical scavenging activity of seeds crude extracts of pigeon pea traditionally used in Oman for the treatment of several chronic diseases

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PEER REVIEW

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Comments

This study done by the author is very good for the scientific community. The present study on antioxidant activity of different seeds crude extracts of pigeon pea gives a valuable brief and scientific preliminary information about this plant.

Details on Page 320

ABSTRACT

Objective: To investigate total phenols, flavonoids content of methanol crude extract of pigeon pea seeds powder and its different polarities soluble fractions and evaluate their free radical scavenging activity.

Methods: The powder seeds samples were used for extraction with methanol. Methanol crude extract from the seeds was defatted by water and fractioned by different polarities of solvents. The investigation of total phenols and flavonoids contents of total six crude extracts of pigeon pea were determined by Folin-Ciocalteu reagent and aluminum chloride calorimetric methods. Antioxidant activity of six crude extracts from the seeds of pigeon pea was determined through α, α -diphenyl- β -picrylhydrazyl method.

Results: Significant amount of total phenols content was presented in hexane crude extract among the six crude extracts of pigeon pea and the order was hexane>chloroform>methanol>ethyl acetate>butanol>water. Similarly, significant amount of total flavonoids content was presented in chloroform crude extract and the order was chloroform>methanol>ethyl acetate>butanol>water>hexane. The crude extracts displayed significant α, α -diphenyl- β -picrylhydrazyl free radical scavenging activity with highest value in hexane extract followed by butanol, methanol, ethyl acetate, chloroform and water crude extracts having value of 95.50%, 83.30%, 64.60%, 64.20%, 60.60% and 52.60%, respectively.

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

KEYWORDS

Pigeon pea, Seeds, Maceration method, Total phenols, Total flavonoids, Free radical scavenging activity, UV-visible spectrophotometer

1. Introduction

Human health is affected by the free radicals which plays an important role to the human health by triggering different chronic diseases such as heart diseases, cancer, hypertension, diabetes,

atherosclerosis and other chronic diseases[1]. Natural antioxidants from various natural sources can play an important role for the prevention of various chronic diseases[1,2]. Several previous studies showed that different phytochemicals are presented in the plants and they are considered as rich sources of phytochemicals[3]. The

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phytochemicals are having important biological properties such as antioxidant activity, antimicrobial activity, anticancer activity, antifungal activity, *etc*[1,2]. Most of the investigators have found natural antioxidants from different parts of plant[3,4]. Among them, phenols and flavonoids compounds are known as safe natural antioxidants. Also, several *in vivo* studies have shown that a high dietary intake of natural antioxidants are strongly associated with longer life expectancy[3]. All natural antioxidants are helpful to reduce risk of developing several chronic diseases, various types of cancer, diabetes, obesity and blood pressure, and improve endothelial function[3,4].

Pigeon pea is a medicinal plant which belongs to the Fabaceae family[5,6]. In Oman, it is known as *Cajanus cajana* and its scientific name is pigeon pea. Sometimes, it is also called a warm season crop[7]. Tropical and subtropical countries are the suitable places for rapid grow of pigeon pea[8]. The plant height is about 1-4 meters with deep taproot of 2 meters[9]. Pigeon pea has a ribbed stem that is angled and pubescent[9]. Normally, the leaves are green colour with long hairs[9]. Leaves are spiral set around the stem and alternate[8]. The flowers are yellow colour with outside red color[9]. In Oman, ethnic communities usually use different parts of this plant for the treatment of several chronic diseases. Mostly, it is used for human food and flour additives[10]. In addition, it is widely used as fuel wood, soil ameliorants and live fences. It is also used as basket weaving and roofing by Zumbian people[11,12]. Therapeutically, it is widely used for the treatment of several chronic diseases, such as diabetes, dysentery, hepatitis, measles, varicella and superficial infection and for stabilizing the menstrual period[1,2,13,14]. Roots of this plant are used for the treatment of alexeritic, anthelmintic, expectorant, sedative and vulnerary[13,14]. Traditionally, the most important use is to treat jaundice, diarrhoea, diabetes and help to remove bladder stones[15]. The leaves are used in India for treatment of sores, wounds, abdominal tumors and diabetes[16]. In China, the leaves are used as an infusion for overcoming anemia, hepatitis, urinary infection, yellow fever and ulcer[5-7]. However, Brazilian use it for the treatment of coughs, ulcers and fever[13,14]. Several active chemical compounds such as protein, fat, fiber, ash, carbohydrates, soluble sugar, phosphorus, lysin, threonine, methionine, cystine, calcium, magnesium, iron, copper, zinc, thiamine, riboflavin, niacin, potassium, sodium, ascorbic acid, β -carotene, vitamin A, vitexin, isovitexin, orientin, apigenin, luteolin, pinostrobin and cajanin stilbene acid are presented in pigeon pea[5,6,13,14,17-19]. To our knowledge, there is little information about antioxidant study on α, α -diphenyl- β -picrylhydrazyl (DPPH) radical scavenging activity of different crude extract of pigeon pea species[17]. However, the method of extraction and preparation of crude extracts used in their study was not the same as in the present study. Therefore, the design of this work was to prepare the crude extracts from the seeds powder

of locally grown pigeon pea and to determine their total phenols and flavonoids as well as evaluate their antioxidant activity.

2. Materials and methods

2.1. Materials

Several solvents such as methanol, butanol, chloroform, ethyl acetate, acetone, dimethyl sulfoxide were used in this study obtained from Sigma-Aldrich Company, Germany. Other chemicals such as gallic acid, sodium hydroxide, sodium nitrate, aluminium chloride and sodium carbonate for the determination of total phenols and flavonoids were obtained from S.D. Fine-Chem Ltd. Industrial Estate, Mumbai, India. Hexane, Folin-Ciocalteu and DPPH were obtained from Sigma-Aldrich Company, Germany. Rotary evaporator was used from Yamato, Model-RE801 and UV-visible spectrophotometer (UV-1800 Shimadzu spectrophotometer, Japan) was used for the measurement of absorbance.

2.2. Plant of samples

The seeds of pigeon pea were collected from local farmer at Al Sharqea region, Oman on December 13, 2013 at 6 pm. 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 seeds samples were processed for drying at 45 °C using oven. After drying the samples, seeds were used for powder by using blender machine. Then the powder samples were stored in an amber colour bottle to avoid decomposition.

2.4. Extraction procedure

The powder samples of pigeon pea seeds were used for extraction through maceration method. The seeds powder samples (100 g) was taken in a 2 L beaker and added approximate 300 mL of methanol. The whole mixture was kept at room temperature for 3 d. The mixture was up and down by glass rod every day. Then the whole mixture was filtered under pressure by using Buchner funnel. The methanol crude extract was obtained by evaporating solvent using rotary evaporator. Approximately 100 mL of water was added to the methanol crude extract and dissolved it by hand. The water mixture was transferred to a separated funnel for extraction. Different polarities solvents were used for fractionation. Firstly, 30 mL of hexane was used for extraction and shaken by

hand for 20 min and left it for 30 min. Then the upper hexane layer was collected and repeated the whole process with 20 mL of hexane. The obtained two hexane parts were combined together and evaporated hexane to obtain 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 by rotary evaporator to obtain water crude extract.

2.5. Determination of total phenols content

The total phenols content of different polarities crude extracts of pigeon pea was measured by Folin-Ciocalteu method described by Hossain *et al*[20]. The gallic acid calibration curve was used for the calculation of total phenols content.

2.5.1. Preparation of gallic acid calibration curve

Standard gallic acid was used for the calculation of total phenols. A total of 2 mg of gallic acid was taken in a 10 mL of volumetric flask and dissolved with methanol. Different concentrations of gallic acid ranging from 12.5 µg/mL to 200 µg/mL were prepared. About 200 µL of each concentration gallic acid was taken in a test tube and added 1.5 mL of 10% Folin-Ciocalteu reagent solution. All the experimental test tubes were kept at room temperature in a dark place for 5 min. Then 1.5 mL of 6% Na₂CO₃ was added to each test tube and the mixture was kept in a dark place at room for 2 h. Finally, the absorbance of each concentration of gallic acid was measure by UV-visible spectrophotometer at fixed wavelength 760 nm. Methanol was used as a blank. The standard curve was prepared by plotting concentrations verses absorbance of gallic acid (Figure 1).

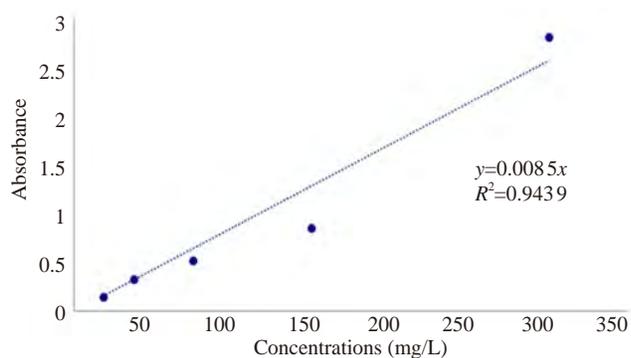


Figure 1. Gallic acid standard curve for the calculation of total phenols content.

2.5.2 Determination of total phenols content in test seeds crude extracts

Different polarities seeds crude extracts were used for the determination of total phenols. Each crude extract such as hexane, chloroform, ethyl acetate, butanol, methanol and water (1 mg) was taken in a separate test tube. Methanol (2.5 mL) was added to each

test tube and then dissolve. A total of 200 µL of each crude extract samples was taken in a separate test tube and added 1.5 mL of 10% Folin-Ciocalteu reagent. The mixture was kept at room temperature for 5 min in a dark place. Then, 6% Na₂CO₃ solution (1.5 mL) was added to each test tube and covered by aluminum foil. Finally, all the tubes were incubated at 40 °C for 2 h. The absorbance of the incubated seeds crude mixture was measured by UV-visible spectrophotometer at fixed wavelength 760 nm.

2.6. Preparation of quercetin standard

For the determination of total flavonoids, quercetin standard was used to prepare the calibration curve. A total of 2 mg of quercetin was dissolved with methanol (10 mL) and then diluted to prepare concentrations of 12.5 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL and 200 µg/mL. Each prepared concentration of quercetin standard (250 µL) was taken in a test tube added water (125 µL) and 5% sodium nitrate solution (75 µL). The mixture was kept at room temperature for 6 min. Then 10% aluminum chloride (150 µL) was added to each test tube and kept in a dark place for 2 h. Finally, it was diluted with sodium hydroxide (500 µL) and water (275 µL). The absorbance of different concentrations of quercetin standard was measured by UV-visible spectrophotometer at fixed wavelength 510 nm. The standard calibration curve was prepared by plotting concentrations verses absorbance of quercetin (Figure 2).

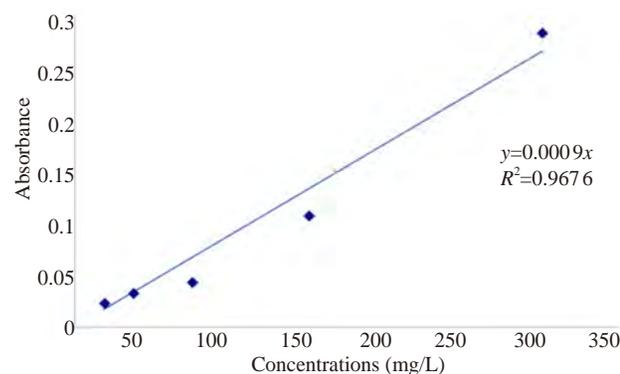


Figure 2. Quercetin standard curve for the calculation of total flavonoids content.

2.7. Determination of total flavonoids content in test seeds crude extracts

Different polarities seeds crude extracts of pigeon pea were used for the determination of total flavonoids content. Each seeds crude extract of pigeon pea (4 mg) was diluted with methanol (4 mL). Each stock crude sample (250 µL) was taken in a separate test tube. Then 125 µL of water and 75 µL of sodium nitrate solution were added to each test tube. The mixture was kept at room temperature for 6 min and then added aluminum chloride (150 µL) to each test tube and kept for another 2 h in a dark place. All the working test tubes were

diluted with sodium hydroxide (500 μ L) and water (500 μ L). The absorbance was measured by UV-visible spectrophotometer at fixed wavelength 510 nm. Finally, total flavonoids content of the crude extract samples was calculated by using established formula.

2.8. Antioxidant activity assay

The antioxidant activity of the prepared crude extracts from pigeon pea was measured through DPPH method according to the method of Hossain *et al*[21]. All crude extracts were dissolved in methanol and assayed in triplicate. The crude extracts such as hexane, chloroform, ethyl acetate, butane, methanol and water (2 mg) was taken in test tube and dissolved with 10 mL of methanol. Five concentrations such as 12.5 μ g/mL, 25 μ g/mL, 50 μ g/mL, 100 μ g/mL and 200 μ g/mL were prepared through dilution method. DPPH (3.3 mg) was taken in a 100 mL volumetric flask and dissolved with methanol. Each prepared concentration (1.5 mL) was taken in a test tube and added 2.5 mL of DPPH solution. The prepared mixture was shaken gently by hand and kept in dark place for 90 min. The absorbance of the samples was measured by using UV-visible spectrophotometer at wavelength 517 nm. Finally, antioxidant activity of the crude extract samples was calculated by using the following formula:

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

3. Results

Plants crude extracts contain different classes of phenols compounds, which are soluble in the selective solvents. Different polarities crude extracts of pigeon pea seeds used for the determination of total phenols content was presented in Table 1. According to the Table 1, the maximum content of total phenols was found in hexane (153.00 mg of GAE/g of powder crude extract) and the minimum was presented in water (35.50 mg of GAE/g of powder crude extract). The following order of total phenols content presented among the six seeds crude extracts of pigeon pea was hexane>chloroform>methanol>ethyl acetate>butanol>water.

Table 1

Total phenols and flavonoids contents of different polarities seeds crude extracts through maceration method.

Seeds crude extracts	Total phenols (mg of GAE/g of powder crude extract)	Total flavonoids (mg of QE/g of dry plant material)
Water	35.50 \pm 0.09	0.18 \pm 0.11
Chloroform	86.30 \pm 0.14	1.58 \pm 0.19
Methanol	74.00 \pm 0.22	1.14 \pm 0.29
Hexane	153.00 \pm 0.17	0.16 \pm 0.13
Ethyl acetate	57.40 \pm 0.32	0.89 \pm 0.07
Butanol	42.50 \pm 0.44	0.76 \pm 0.18

Similarly, the total flavonoids content among six different polarities

crude extracts of pigeon pea seeds was presented in Table 1. The results showed that the maximum amount of flavonoids content obtained from the seeds of pigeon pea was in chloroform extracts (1.58 mg of QE/g of dry plant material) and the minimum was in water (0.18 mg of QE/g dry of plant material).

Different polarities crude extracts such as hexane, chloroform, ethyl acetate, methanol, butanol and water obtained from the seeds of pigeon pea through maceration extraction method were used for the determination of antioxidant activity by established DPPH method[21]. The antioxidant activity of crude extracts was calculated through established formula. The maximum amount of activity among the six crude extracts obtained from seeds was presented in hexane extract and minimum amount was presented in water crude extract. The following order was found among the six seeds crude extracts hexane>butanol>methanol>ethyl acetate>chloroform>water (Table 2).

Table 2

Antioxidant activity of different polarities crude extracts from seeds samples of pigeon pea.

Concentration of crude extract (μ g/mL)	% Inhibition					
	Water	Methanol	Hexane	Ethyl acetate	Chloroform	Butanol
12.5	31.60 \pm 0.44	26.70 \pm 0.23	29.40 \pm 0.14	33 \pm 0.08	31.30 \pm 0.29	30.40 \pm 0.55
25	31.70 \pm 0.17	32.30 \pm 0.34	35.10 \pm 0.16	33.20 \pm 0.45	34.10 \pm 0.63	47.50 \pm 0.41
50	35.10 \pm 0.21	35.90 \pm 0.15	53.30 \pm 0.39	40.30 \pm 0.22	41.60 \pm 0.44	59.90 \pm 0.37
100	41.10 \pm 0.17	48.40 \pm 0.18	77.20 \pm 0.12	48.90 \pm 0.19	50.40 \pm 0.15	76.50 \pm 0.18
200	52.60 \pm 0.11	64.60 \pm 0.09	95.50 \pm 0.77	64.20 \pm 0.26	60.60 \pm 0.23	83.30 \pm 0.08

4. Discussion

The extraction of phenol compounds is depended by various parameters such as temperature, time of extraction and solvent polarity[11]. Selection of the most appropriate conditions and solvents are required for the extraction of the highest amount of total phenols compounds. Almost all phenol compounds are considered as bioactive compounds and they are widely present in all parts of the plant[21]. Due to the bioactive phenol compounds, almost all crude extracts showed different biological activities[15,21,22]. In our present study, the amount of total phenols content among the six crude extracts from the seeds of pigeon pea through maceration method were presented. The concentration of total phenol compounds among the six seeds crude extracts were not the same. The difference of phenols content depends on various parameters such as temperature, time of extraction and solvent polarity[20]. Among the six seeds crude extracts, the significant amount of phenol compounds was presented in hexane crude extracts. However, the range of total phenols content among the six seeds crude extracts was within the range from 35.50 to 153.00 mg of GAE/g of powder crude extract. The following order of total phenols obtained among the six crude extracts was hexane >chloroform>methanol>ethyl acetate>butanol>water. On the other hand, the determination of total flavonoids content among the crude extracts obtained through maceration method was determined by

aluminum chloride method. Significant amount of total flavonoids content was presented in chloroform crude extract among six crude extracts of pigeon pea. There is very good correlation between the total phenols and flavonoids contents among the six crude extracts obtained from the seeds of pigeon pea.

The antioxidant activity of six crude extracts was evaluated through DPPH method[20-23]. The percentage of inhibition among the six crude extracts obtained from the seeds of pigeon pea through DPPH method was presented. The inhibition percentage among the six crude extracts was not the same. The difference of inhibition of the crude extracts was depends on the bioactive compounds such as phenols and flavonoids presented in seeds crude extracts[24]. The antioxidant activity is their interaction with crude extracts to produce oxidative free radicals. All the crude extracts do not have the same capacity to produce oxidative free radicals. The antioxidants of seeds crude extracts reacted with DPPH stable free radical and changed its colour from DPPH to α,α -diphenyl- β -picrylhydrazine. The decreasing colour change indicated the scavenging activity of the crude sample antioxidant. In this study, the six different polarities crude extracts from seeds by maceration extraction method were able to change the colour of DPPH solution. It could be due to the presence of phenol and flavonoids compounds in the crude extracts. It showed that the maximum antioxidant activity among the seeds crude extracts was in hexane extract and the minimum amount was presented in water crude extract. The following order was found among the six seeds crude extracts: hexane>butanol>methanol>ethyl acetate>chloroform>water. Crude extracts from plants contains secondary metabolic compounds such as flavonoids, phenols, saponins, tannin and aromatic compounds has been already reported by several authors[21-24]. They are able to reduce colour change by DPPH by their hydrogen donating capability[21-24]. The results presented showed that all crude extracts from the seeds of pigeon pea have hydrogen donating capabilities. They are acting as an antioxidant. However, the hydrogen donating capabilities were not the same among the six crude extracts. Therefore, the inhibition percentage was difference among the crude extracts due to secondary metabolics. In addition, the difference percentage obtained in this present study might possibly due to the various parameters such as temperature, time of extraction and solvent polarity.

The above results showed that further investigation related to the isolation and identification of individual bioactive compound are needed. Also, animal studies are needed for better understanding their mechanism of antioxidant action. Similar results and relationship related to phenols and flavonoids contents were obtained and antioxidant activity of the seeds crude extracts have previously been reported[3-7].

The seeds crude extracts from the pigeon pea through maceration method showed very high content of total phenols and flavonoids

contents. Also, the crude extracts from seeds showed very high percentage of antioxidant activity through DPPH method. Therefore, all the crude extracts from this plant could be used as a medicine for the treatment of different chronic diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are grateful to Prof. Dr. Nafsiah Binti Shamsudin, Dean, College of Pharmacy and Nursing, University of Nizwa, Sultanate of Oman, for her continuous encouragement during the work. Thanks to University of Nizwa, Nizwa, Sultanate of Oman for providing all chemicals and other expenses from their internal fund to carry out this project. The authors wish to express sincere gratitude to the Central Instrument Laboratory, College of Agriculture and Marine Sciences, Sultan Qaboos University, Sultanate of Oman, where the test were confirmed (Grant Ref No. SOP/ADO/72/2014).

Comments

Background

Human health is affected by the free radicals which play an important role to the human health by triggering different chronic diseases such as heart diseases, cancer, hypertension, diabetes, atherosclerosis and other chronic diseases. Pigeon pea is a tropical medicinal plant belongs to the Fabaceae family. In Oman, it is known as *Cajanus cajana* and its scientific name is pigeon pea. Therapeutically, pigeon pea is widely used for the treatment of several chronic diseases, such as diabetes, dysentery, hepatitis, measles, varicella and superficial infection and for stabilizing the menstrual period.

Research frontiers

This study is to prepare different polarities crude extracts from the seeds of pigeon pea and evaluate their antioxidant potential activity and determinate the total phenolics and flavonoids contents.

Related reports

The literature search reveals that there is no research on Omani species.

Innovations & breakthroughs

It is a new medicinal plant grown worldwide. The data generated from this plant will be useful to the scientific community.

Applications

This plant is a medicinal plant and the main use of it is for the treatment of several chronic diseases such as diabetes, dysentery, hepatitis, measles, varicella and superficial infection and for stabilizing the menstrual period.

Peer review

This study done by the author is very good for the scientific community. The present study on antioxidant activity of different seeds crude extracts of pigeon pea gives a valuable brief and scientific preliminary information about this plant.

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