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Phenolic content and *in vitro* bioactivities of Chinese Potato (*Plectranthus rotundifolius*) tuber extracts

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Abstract

Plectranthus rotundifolius, commonly known as Chinese potato in India, is a perennial herbaceous plant of the Mint family *Lamiaceae* and is native to the tropical Africa. These are found to be rich in nutrients and have great medicinal properties. The tubers contain several secondary metabolites that are of therapeutic and pharmaceutical importance. The tubers in the dry and cooked form were analysed for the phenolic content and the *in vitro* bioactivities including antioxidant, anti-inflammatory and anti-diabetic activities by various biochemical assays. The tubers were also subjected to cooking to find out its effect on the phenolic content and antioxidant activities. The study revealed that cooked tubers have greater phenolic content and antioxidant, anti-inflammatory and antidiabetic activities than the raw tubers and it indicates that cooked form of tuber is better to consume than the raw tuber.

Keywords: Chinese potato, bioactivity, phenolics, flavonoids

Introduction

Roots and tuber crops play a substantial role in food security and nutrition. Most of the tuber crops are potential sources of bioactive phytochemicals including phenols and flavonoids (Farombi et al*.,* 2000, Champagne et al*.,* 2011). Apart from cassava and sweet potato, there are several minor root and tuber crops which are rich sources of bioactive phytochemicals and Chinese potato is one among them. Chinese potato (*Plectranthus rotundifolius* or *Solenostemon rotundifolius*) is a perennial herbaceous plant of the Mint family *Lamiaceae.* These plants are native to tropical Africa and are called in different names such as Native Potato, Country Potato, Hausa Potato or Sudan Potato. In India, these are known as Chinese Potato.The coleus potatoes contain reducing sugar, protein, crude fat and crude fibre (Anbuselvi et al., 2013) and are of great medicinal value and it lowers the blood cholesterol as well as fends off the fungal and viral infections in humans. The mature tubers are used as a substitute for potatoes. There are several secondary metabolites present in these tubers with potential therapeutic and pharmaceutical applications. The leaves of these plants are used in traditional medicines for the treatment of dysentery, treatment of blood in urine, eye disorders etc. Several species of *Plectranthus* are used as folk medicine for skin irritations, antiseptics, vermicide and nausea (Narukawa et al., 2001).

The bioactivity of Chinese potato tubers is mostly due to the phenolic compounds present in them. These are soluble in polar organic solvents. In human perspective, dietary phenolics are useful to human health, possibly by acting as antioxidants, anticarcinogens and cardioprotective agents. The antioxidant activities of these compounds are responsible for the health effects including the

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prevention of certain cancers and coronary heart diseases (Pietta, 2000). Flavonoids are the phenolics substances that are widely found in the fruits and vegetables. Studies reveals that the ingestion of flavonoids reduces the risk of cardiovascular diseases, metabolic disorders, and certain cancers. These effects are due to the physiological activity of flavonoids in the reduction of the oxidative stress, inhibiting low-density lipoproteins oxidation and platelet aggregation and acting as vasodilators in blood vessels. The antioxidant activity of the tubers of *Plectranthus rotundifolius* was analysed by DPPH assay (Chen and Ho, 1995) and ABTS assay (Re et al., 1999). Phytochemical analysis was performed in the leaves of the *Plectranthus* species, *Plectranthus amboinicus*. The leaves of *P. amboinicus* are rich in phytochemicals and secondary metabolites such as steroids, tannins, flavonoids and alkaloids, which are probably responsible for its medicinal properties (Ware et al*.,* 2019).The species of *Plectranthus* are known to possess anti-inflammatory activities also. *Plectranthus amboinicus* inhibits the pain induced by acetic acid and formalin and the inflammation caused by carrageenan. The *in vitro* anti-inflammatory activity of the extract was determined by the method of the inhibition of protein denaturation (Padmanabhan and Jangle, 2012; Elias et al*.*, 1988). Manikandan et al., (2016) reported that *Plectranthus rotundifolius* showed the presence of different functional groups such as alcohol, phenols, amines, alkanes, aldehydes, carboxylic acid, isocyanides, alkynes, isocyanates, ketones, tertiary and primary alcohols and chloro compounds. Previous studies showed that extracts of *Plectranthus rotundifolius* possess *in vitro* anti-tumour activity (Singh et al*.*, 2013), antioxidant activity (Sandhya et al., 2001) and acts as cancer chemopreventing agent (Nugraheni et al*.,* 2011).

The processing techniques may affect the bioactivity of Chinese potato tubers, since phenolics are highly soluble in water and are sensitive to temperature, pH conditions etc. Since the tubers are largely used for edible purpose, it is important to understand the phenolic content and antioxidant activity of the cooked tubers. Hence, the objectives of the present study were to estimate the phenolics and flavonoid contentsin Chinese potato tubers in relation to the *in vitro* antioxidant, anti-inflammatory, and antidiabetic activitiesand to study the effect of cooking on the phenolic content and its bioactivity.

Materials and Methods

Materials

The fresh tubers of Chinese potato were collected from the experimental farm of ICAR-CTCRI. Methanol (99.5%) and 2, 2- diphenyl-1-picryl hydrazyl (DPPH) were purchased from Merck India Pvt. Ltd. (Mumbai, India). Gallic acid was procured from Sigma-Aldrich Corporation, St. Louis, MO, USA.

Preparation of tuber extract

The fresh Chinese potato tubers were washed thoroughly with running water to make free of the adhered soil and then peeledand sliced into equal pieces. About 200g of the tuber slices were lyophilized. Another 200g of the raw tuber was boiled in 300ml of water for about 25-30 min till the tubers were cooked.The cooked tuber was then transferred into a beaker, cooledand lyophilized. The raw and cooked tubers were then ground into a powder by using a mill (IKA all basic mill). Then, 5g each of the sample was weighed andthen homogenised with methanol. It was then centrifuged, and the supernatant was transferred to a bottle. The process was repeated until the yellow colour of the supernatant was completely removed. The collected supernatants of both raw and cooked tubers were concentrated by using a Rotary flash evaporator (Buchi Multivapor). The residue after concentration was dissolved in methanol and transferred to vials and used for further analysis.

Estimation of total phenolic content

The total phenolic content of the tuber extract was determined by Folin-Ciocalteu assay using Gallic acid as standard (Malick and Singh, 1980). Briefly, to a test tube containing 0.5 ml of the extract, 2 ml of methanol and 0.5 ml of Folin-Ciocalteu reagent were added and is followed by the addition of 2 ml of 20% sodium carbonate (Na_2CO_3) solution. All the tubes were thoroughly shaken and covered with aluminium foil and were kept in dark for 1 hour. After the incubation period, the samples were centrifuged, and the supernatant was separated. The absorbance was measured at 765 nm using a spectrophotometer (Perkin Elmer, Lambda-25, Switzerland) with methanol as the blank. Triplicate analyses were performed for both the extracts. Gallic acid (trihydroxybenzoic acid) was used as the standard. The total phenolic content was expressed as milligrams of gallic acid equivalents (mg of GAE g^{-1} of sample) in the calibration curve.

Estimation of total flavonoid content

The total flavonoid content in the tuber extracts was determined by the aluminium chloride colorimetric method (Patel et al., 2010). To a test tube, 1.5 ml of the extract was taken and 1.5 ml of 5% $AlCl_3$ solution was added. It was mixed well, and the tubes werecovered with aluminium foil and incubated for 60 min at room temperature. After incubation, the absorbance was measured at 420 nm against a mixture of 1.5 ml of methanol and 1.5 ml of $AlCl_3$ as the blank. Triplicate analyses were performed for both the raw and cooked extracts of the tuber. A calibration curve was plotted using Quercetin as the standard. The total flavonoid content was expressed as milligrams of quercetin equivalents (mg of quercetin g^{-1} of sample).

In vitro bioactivity studies

In vitro antioxidant activity

The antioxidant activity of the extracts was determined by the DPPH and ABTS assays. Two hundred µl of both the extracts were diluted to 2 ml using methanol and were used for the assays.

DPPH assay

The free radical scavenging capacity of the extracts of Chinese potato tubers was evaluated according to the method of Chen and Ho (1995) with slight modifications. Briefly, 0.5 mM DPPH solution was prepared by weighing 0.0197 g (19.7 mg) of DPPH (2,2-diphenyl-1-picryl hydrazyl) and making up to a volume of 100ml using methanol. From the diluted extracts of raw and cooked tubers, different concentration of the extracts ranging from 5 µl-160 µl (5,10,20,40, 80 and 160 µl) were taken in different test tubes and all the samples were made up to 2 ml with methanol. Then, 1 ml of DPPH solution was added to all the tubes. The contents were thoroughly mixed, covered and were kept in dark for 30 min at room temperature. The absorbance of the violet coloured solution was measured by using a uv-visible spectrophotometer (Perkin Elmer) at 517 nm against methanol as the blank. Gallic acid was used as the standard. The percentage (%) inhibition was calculated as follows:

$$\%\,\text{Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100 \ldots \text{Eqn 1}$$

ABTS assay

The free radical scavenging activity of the extract was determined by ABTS radical cation decolourization assay using gallic acid as the standard (Wojdyło et al., 2007). Different concentrations of the diluted extracts of raw and cooked Chinese potato tubers (5 µl, 10 µl, 20 µl, 40 µl, 80 µl, and 160 µl) were taken in different test tubes and were made to a volume of 2 ml using methanol. To these tubes, 1 ml of ABTS solution was added and mixed well. All the tubes were covered with aluminium foil and kept in dark for 20 min at room temperature. The absorbance of the green coloured solution was measured spectrophotometrically at 734 nm against methanol as the blank. The % inhibition was calculated as follows:

$$\%\,\text{Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100 \ldots \text{Eqn 2}$$

Anti-inflammatory activity: Inhibition of protein denaturation

The anti-inflammatory activity of the extracts was determined by the protocol described by Padmanabhan and Jangle (2012) with slight modifications. Different concentrations of the raw and cooked tuber extract (5 µl, 10 µl, 20 µl, 40 µl, and 80 µl) were taken in different test tubes and 0.2 ml of egg albumin was added to all the tubes.The volume of each test tube was made up to 3 ml by using 0.2 M phosphate buffer of pH 6.6. The tubes were well vortexed and then incubated in a boiling waterbath for 10-15 min which resulted in the protein denaturation. The tubes were then cooled to room temperature and the activity of each mixture was measured at 660 nm using a spectrophotometer with buffer as the blank. Aspirin (Acteylsalicylic acid) was used as the standard. The anti-inflammatory activity was determined as :

$$\%\,\text{Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100 \ldots \text{Eqn 3}$$

Antidiabetic activity

The antidiabetic activity of the Chinese potato tuber extracts was determined by DNS (3,5-Dinitrosalicylic acid) method to determine the α-amylase inhibitory activity, by quantifying the reducing sugar (glucose equivalent) liberated under the assay condition (Thakkar and Patel, 2010; Chen et al., 2001). Different concentrations of the raw and cooked Chinese potato tuber extracts (5 µl, 10 µl, 20 µl, 40 µl, 80 µl, and 160 µl) were taken in test tubes and 250µl of 0.02M sodium phosphate buffer (pH 6.9) containing α-amylase enzyme (240 U/mL) was added to each tube and then incubated for 20 min at 37°C. Then, 250 µl of 1% starch solution, prepared in 0.02 M sodium phosphate buffer was added to the test tubes. The tubes were shaken well and incubated for 15 min at 37°C. Then 1 ml of 1% dinitrosalicylic acid (DNS) was added to all the tubes and then incubated in a boiling water bath for 10 min. To the cooled mixture, 2 ml of distilled water was added and the absorbance was measured at 540nm using thephosphate buffer as blank. The % inhibition was determined as follows:

$$\%\,\text{Inhibition} = \frac{(AC - AC_b) - (AS - AS_b)}{(AS - AS_b)} \times 100 \ldots \text{Eqn 4}$$

Where, AC was the absorbance of control, AC_b was the absorbance of control blank, AS was the absorbance of the sample, and AS_b was the absorbance of the sample blank.

Results and Discussion

Total phenolic content

The total phenolic content in the raw tuber was found to be 1027 mg 100 g^{-1} and that in the cooked tuber was 6068.8 mg 100g^{-1} on fresh wt. basis (Table 1). It was found that the total phenolic content was higher in the cooked tubers and it was about 5 times greater than that in the raw tubers. According to Bhave and Dasgupta (2019), the cooked sample of *Plectranthus amboinicus* exhibited higher phenolic content (10 mg GAE g^{-1}) than the raw sample (8 mg GAE g^{-1}). The results of the present study are in agreement with this report. The percent gain in the total phenol content during cooking might be due to the breakdown of tough cell walls and release of trapped phenolic compounds (Oboh et al., 2007). Navarre et al., (2010) assessed the targeted phytonutrients survival upon cooking of potatoes by different methods and noticed that the total phenolics, chlorogenic acids, flavonols and vitamin C did not significantly decrease after cooking by any of the methods. Cooking typically resulted in an increase in the recoverable amounts of the phenolic compounds. Supporting this finding is that antioxidant capacity also showed a corresponding increase. However, in another study by Gumul et al. (2017), loss in polyphenolic compounds after cooking was observed in the tubers of some of the potato varieties and it negatively influenced their antioxidant activity. But some exception was also observed in some other varieties, where antioxidant activity was not altered by cooking process, in spite of lowered level of total polyphenols, including flavonoids.

Potato nutrients and bioactive components appears to be influenced by cooking methods. Vinita and Punia (2018), reported a variety of effects such as destruction, release and structural transformations of the phytochemicals taking place during cooking process. The total phenolic content of the potato and carrot were found to be 25.23 and 19.14 mg GAE 100 g^{-1} on fresh weight basis and the total flavonoid content were 18.71 and 12.27 mg GAE/100 g respectively. An increase of 83% in the total phenolic content was observed in the boiled potatoes and they reported that there is an overall increase in the TPC and TFC content of the cooked potato and carrot with a significant increase in antioxidant activity also. Bembem et al., (2013) also reported that TPC and TFC were less affected by cooking of potato tubers. The TPC increased in all the cooking processes and DPPH activity of the cooked tuber was higher compared to that of raw potato tuber. A study by Blessington et al., (2010) showed an increase in most of the individual phenolic compounds after baking, frying and microwave processing when compared to the uncooked tuber samples.

Total flavonoid content

The flavonoid content in the cooked Chinese potato tubers was 7072.8 mg 100 g^{-1} and that of the raw tuber was 1400 mg 100 g^{-1} on fresh wt. basis (Table 1). According to Murthy et al., (2018), the total flavonoid content in the *Plectranthus rotundifolius* tubers in the methanolic extract and was about 22.59 mg g^{-1} and this agrees with our results. The flavonoid content was found to be higher in cooked tuber as compared to the raw tubers. In the work of Vinita and Punia (2018), the total flavonoid content was found to be increased for the cooked potato and carrot. According to Muhamad et al., (2019), the TPC and TFC in the ethanolic extract of *Plectranthus amboinicus* tubers was significantly higher in the boiled samples than in the control and was correlated with the antioxidant activity. This suggested that boiling can be used as a method to enhance the antioxidant activity. According to Bhave and Dasgupta (2019), the cooked sample of *Plectranthus amboinicus* exhibited higher flavonoid content than that in the raw sample. In the cooked samples, the flavonoid content was found to be 12.6mg QEAC g^{-1} and in the raw sample, it was 7.2 mg QEAC g^{-1}. All these studies suggest that cooking enhances the TPC, TFC and the antioxidant activity.

Table 1. Total phenolic and flavonoid contents in Chinese potato tuber

Sample	Total Phenolics (mg 100 g^{-1} of fresh tuber)	Total Flavonoids (mg 100 g^{-1} of fresh tuber)
Raw tuber extract	1027.0	1400.0
Cooked tuber extract	6068.8	7072.8

In vitro bioactivity studies

DPPH scavenging activity

DPPH (2,2-diphenyl-1-picryl hydrazyl) radical scavenging assay is the most widely used method for the screening of the antioxidant activity of the plant extracts (Kedare and Singh, 2011). The scavenging activity was expressed as percentage inhibition and IC_{50}. The Chinese potato tuber possessed significant radical scavenging activity in comparison to the standard, Gallic acid (Table 2). The cooked tuber extract exhibited significantly higher antioxidant activity towards DPPH radicals than the raw tuber extract. The inhibition of DPPH was 96.8±2.75% at a concentration of 160 μg ml^{-1} for the cooked tubers and 91.4±2.70% in the case of raw tuber extractat the same concentration (Table 2). The percentage inhibition increased with increase in concentration of the extract. The results

Table 2. The DPPH radical scavenging activity of Chinese potato tuber extracts and Gallic acid

Sample	Concentration (μg ml^{-1})	Percentage of Inhibition (%)	IC_{50} (μg ml^{-1})
Gallic acid	2	13.9±1.01	7.87
	4	26.7±1.15	
	8	59.9±1.84	
	16	91.3±2.64	
Raw tuber extract	10	23.7±1.01	35.97
	20	40.2±1.44	
	40	67.8±2.14	
	80	79.1±2.19	
	160	91.4±2.70	
Cooked tuber extract	10	26.1±1.12	26.38
	20	42.8±1.72	
	40	74.1±1.88	
	80	84.7±2.21	
	160	96.8±2.75	

*Values are the mean of three replications ± standard deviation

showed that these tuber extracts have very high potential as natural antioxidants. The IC_{50} was also lower forthe extracts of cooked sample indicating more activity. According to Murthy et al. (2018), the DPPH scavenging activity of the tubers of *Plectranthus rotundifolius* increased with increase in concentration and the greater ability to scavenge the free radical was shown by the methanolic extract with an EC_{50} of 15.9 μg ml^{-1}. The methanolic extracts of *Plectranthus hadiensis* (Forssk). Schweinf. spreng showed higher antioxidant activity as compared to the standards *viz*., ascorbic acid and BHT (Butylated Hydroxy Toluene) (Menon et al., 2012). Bembem et al., (2013) reported that cooking increased the antioxidant activity of potato. According to them, the DPPH activity of the raw and processed samples ranged from 16.13% in raw potato to 32.48% in sauteed potato. According to Navarre et al. (2010), cooking resulted in a recoverable amount of phenolic compounds and thus showed increase in antioxidant activity. Bhave and Dasgupta (2019) reported that the total phenolics, total flavonoids and the DPPH scavenging activity are higher for the cooked sample of *Plectranthus amboinicus* when compared to that of the raw sample. Vinita and Punia (2018) reported that the DPPH scavenging activity of the cooked potato and carrot were higher than that in the raw potato and carrot.

$ABTS^+$ Radical scavenging assay

ABTS radical scavenging assay of the tuber extract revealed that the raw tuber and cooked tuber showed percentage inhibition of 93.7±3.25% and 98.6±3.84%, respectively at a concentration of 160 μg ml^{-1} and the IC_{50} values were 14.47 and 4.73, respectively. The IC_{50} value of the standard, gallic acid was found to be 1.83 (Table 3). From the above results, it was understood that the extract of the cooked tuber exhibited significantly higher antioxidant activity than the raw tuber and similar result were obtained for DPPH radical scavenging assay also. Bellumori et al*.,* (2017) reported that the boiled pink and violet-fleshed potatoes showed the highest efficacy as radical scavengers in the ABTS test. There was a better antioxidant activity against ABTS in case of *Plectranthus stocksii* (Muniyandi et al., 2017).

Table 3. ABTS radical scavenging activity of Chinese potato tuber extract

Sample	Concentration (μg ml^{-1})	Percentage Inhibition	IC_{50} (μg ml^{-1})
Gallic acid	1.2	33.8±1.51	1.83
	1.6	43.5±1.62	
	2	51.6±2.11	
	2.4	62.8±2.81	
	2.8	82.4±3.10	
Raw tuber extract	10	31.1±1.32	14.47
	20	52.1±1.54	
	40	74.4±1.85	
	80	82±1.89	
	160	93.7±3.25	
Cooked tuber extract	10	33.4±1.48	4.73
	20	55.5±1.59	
	40	79.5±2.24	
	80	87.2±2.09	
	160	98.6±3.84	

*Values are the mean of three replications ± standard deviation

Anti-inflammatory assay by protein denaturation

The method of anti-denaturation of egg albumin was chosen to evaluate the anti-inflammatory property of the Chinese potato tuber extract. The cooked tuber extract had better anti-inflammatory activity than the raw tuber extract and the percentage inhibition of the raw tuber and cooked tuber extracts were 90.5±2.76%

and 94.5±2.81%, respectively at a concentration of 80 μg ml^{-1} (Table 4). The IC_{50} values were found to be 22.97 and 19.44 for extracts of raw and cooked tuber, respectively.

Table 4. Anti-inflammatory activity of the Chinese potato tuber extract

Sample	Concentration (μg ml^{-1})	Percentage Inhibition	IC_{50} (μg ml^{-1})
Aspirin	5	25.1±1.15	16.21
	10	41.2±1.52	
	20	65.0±2.15	
	40	87.4±2.52	
	80	97.1±3.11	
Raw tuber	5	20.0±1.12	22.97
	10	35.2±1.81	
	20	60.0±2.08	
	40	79.1±2.13	
	80	90.5±2.76	
Cooked tuber	5	22.1±1.12	19.44
	10	40.2±1.49	
	20	61.5±2.03	
	40	82.6±2.31	
	80	94.5±2.81	

*Values are the mean of three replications ± standard deviation

Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation (Sadique et al., 1989). The methanolic extract of *Coleus forskohlii* showed good activity towards the BSA anti-denaturation assay (Menon and Latha, 2011). When BSA is heated, it undergoes denaturation and expresses antigens associated with type III hypersensitive reaction related to disease like serum sickness. Several species of *Plectranthus* are known to possess the anti-inflammatory activities.

Antidiabetic assay: Inhibition of α-amylase

The tuber extracts exhibited a dose dependent inhibition of α-amylase activity and the percentage inhibition increased with increase in the concentration of the extract. The percentage of inhibition shown by the raw tuber and cooked tuber was 93.0±3.11% and 96.7±2.11%, respectively at a concentration of 80 μg ml^{-1} indicating the higher inhibitory activity of cooked tuber extract against the α-amylase compared to the raw tuber extract (Table 5). The IC_{50} values of the raw and cooked tubers were found to be 23.01 and 16.20, respectively. Prathibha et al*.,* (1995) reported that the coleus tuber possessed high anti-amylase activity. When the tubers were processed by the pressure cooking there was a significant reduction or complete elimination in the inhibitory activity.

Table 5. Inhibition of α-amylase by Chinese potato tuber extract

Sample	Concentration (μg ml^{-1})	Percentage Inhibition	IC_{50} (μg ml^{-1})
Raw tuber	5	21.1±1.13	23.01
	10	35.3±1.81	
	20	57.1±1.79	
	40	79.5±2.13	
	80	93.0±3.11	
Cooked tuber	5	26.1±1.18	16.20
	10	41.4±1.50	
	20	65.8±2.13	
	40	84.1±1.91	
	80	96.7±2.11	

*Values are the mean of three replications ± standard deviation

Conclusions

There are several secondary metabolites present in Chinese potato (*Plectranthus rotundifolius*) tubers with potential therapeutic and pharmaceutical applications. The bioactivity of the tubers is mostly due to the phenolic compounds present in them. Since the tubers are largely used for edible purpose, it is important to understand the phenolic content andantioxidant activity of the cooked tubers. The total phenolic content andtotal flavonoid content was higher for the cooked Chinese potato tubers compared to the raw tubers. Also, the antioxidant assays including DPPH and ABTS assays performed revealed that the cooked tuber contains greater activity than the raw tuber. Anti-inflammatory and antidiabetic assays also revealed that cooked tuber was having greater activity than raw tuber. The study reveals the importance of including this tuber in our everyday diet.

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