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Polyphenolic composition and invitro evaluation of radical scavenging and anti diabetic properties of *Canthium parviflorum*

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ABSTRACT

The main objective of the present study was to investigate the nutraceutical properties of the bioactive components from the extracts of *Canthium parviflorum* seeds (CPS). The anti-oxidant property of CPS extracts was carried out by estimating the total phenolic, flavonoid contents, flavonols, flavonols and radical scavenging properties. The results of these findings show that the aqueous extract of CPS rich in polyphenols (87.5 $\pm$ 0.2 mg GAE /g) and flavonoids (50.1 $\pm$ 0.2 mg QE/g) compounds than organic extracts. The effective inhibitory activity of CPS against α- amylase and α- glucosidases enzyme related to type- 2 diabetes was evaluated and compared. The aqueous extract was exhibited the maximum α-amylase and β-glucosidase inhibition of 91.1 $\pm$ 0.4 and 90.5 $\pm$ 0.1 % respectively. The seed extracts show a maximum of 25.5 % of protein and 6.4 mg/100g of zinc. The seed extract was exhibited maximum anti-oxidant property with beta carotene content of 5.4 mg/100 g of seed. The present work may be the platform to use to develop high-value nutritional compounds by unutilized plant materials. The natural anti-oxidant and polyphenols present in the extracts were showed great nutraceutical value, and it may be used to develop nutraceutical to enhance a healthy diet.

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INTRODUCTION

Increasing evidence viewing the contribution of oxidative stress induced by free radicals on the progress of various disorders, studies on polyphenols and antioxidant abundantly present in plants have consider to be one of the important thrust area of nutraceuticals and agriculture research (Re *et al.*, 1999). Natural antioxidants are playing a pivotal role in helping prevention of cell damage in the human body. Polyphenols are enhancing health, and show a effective protection against diseases such as neurogenic disorders, cardiovascular diseases and cancers (Sepahpour *et al.*, 2018). The extract of

Canthium parviflorum has shown powerful antioxidative property and the ulcero-protective activity. The seeds of *Canthium parviflorum* have been used as anti-fever, demulcent and antidiabetic in many traditional medicine May and lamiaa (2018). The inception of non insulin dependent type II diabetes affects with the body's capability to metabolize carbohydrates for energy, resulting to hyper levels of blood sugar (Khan *et al.*, 2017).

The plant is exhibiting anti diabetic activity, which is similar to that of biguanides (*e.g.* metformin) that has limited efficacy to lower hyperglycemia and exert its effect by decreasing carbohydrate absorption from the intestine (Kabra and Patel, 2018). Starch is the main form of dietary, digestible carbohydrate and this will get absorbed only when the digestive enzymes like amylase and other, secondary enzymes are broken down (Ibrahimi and Hajdari, 2020). The inhibitors of amylase and glucosidase are also termed as starch blockers because they contain molecules that inhibit the dietary starch for rapid absorption in the body. Recently, it has been shown that phenolics are secondary compound and it play a pivotal role in enhancing amylase inhibition and therefore it has the ability to contribute to type II diabetics management (Moyo *et al.*, 2012). *Canthium parviflorum* can be a best candidate for developing antioxidant and antidiabetic rich health supplements in low cost. So the current study focused to investigating antioxidant property and anti-diabetic activity of the extracts of *Canthium parviflorum* plant seed.

MATERIALS AND METHODS

Sample preparation

The healthy seeds were collected from the region of Cauvery delta near Thiruthuraipoondi, Tamilnadu, India and dried in a fluidized bed drier at 45°C for 30 minutes.10 g of seeds were homogenized and the fat was separated with 50ml n-Hexane using a magnetic stirrer and filtered. The seed extracts were prepared using different solvents as water (60 ° C), Ethanol, Methanol. The freeze extract dried extracts were stored at 4 °C until used.

Physio Chemical analyses

According to (Boyer and Walker, 2020) method, Crude protein was determined from the content of nitrogen by Kjeldahl method by using factor 6.25. The level of Crude fiber, moisture, Ash and carbohydrate content were evaluated by (Paez *et al.*, 2016). The saponin, steroids and other content of phytochemicals in the extracts of CPS were estimated according to the method of (Rajasree *et al.*, 2016).

Zinc content

Samples were digested by incinerating in muffle furnace at maximum of 550°C for 6 hrs. After mineralization, samples were transferred to 100ml flask and made up to mark with distilled water. The aliquots of the digested samples were analyzed for zinc metal using Atomic Absorption Spectrophotometer (AAS) (Saeed *et al.*, 2017).

Estimation of β-carotene content

100 mg of sample was extracted with 150ml of 12% methanolic-KOH in a mortar and pestle. The extract was first poured to a separating funnel and 15ml of Petroleum ether was added to it. It was well shaken and allowed to separate for 10 minutes. The petroleum ether phase was then collected and its color intensity of them were determined at 452nm by using UV spectrophotometer (Sepahpour *et al.*, 2018).

Total phenolic content

The total ployphenolic content of the CPS extracts were evaluated according to the method of (Kabra and Patel, 2018). 2.5 ml 10% Folin-Ciocalteau reagent (v/v) was added with the seed extracts and neutralized by adding 2.0 ml of Sodium Carbonate (7.5%). The mixture was thoroughly mixed and incubated at 45 °C for 40 minutes and the absorbance was taken at 725nm in the UV-Visible spectrophotometer. The total phenol content was expressed as mg of Gallic Acid Equivalent (GE)/g of seed.

Estimation of flavonoids content

The total Flavonoid content was evaluated on according to slightly modified method of (Ibrahimi and Hajdari, 2020). 500 μl of the sample extract was taken and mixed with 0.5 ml methanol, 50 μL 10% $AlCl_3$, with this solution, 0.5 ml of 1M potassium acetate and 1.4 ml of distilled water were added. The solution was mixed well and it was incubated for 30 minutes in dark and the absorbance was taken at 415nm by using myricetin as standard. The results were expressed as mg of Myricetin equivalents per gram of seed.

Total Flavonols Content

The total flavonols content were estimated according to the method of (Moyo *et al.*, 2012). 300 μl of sodium acetate (5 g/ 100 ml) and 2 ml of 2% $AlCl_3$ was prepared with ethanol. This solution was mixed with 500 μl of seed extract. The mixture was mixed well and incubated for 2.5 h at 20 °C. The absorbance was taken at 440 nm. Total flavonols content was determined as quercetin (mg/g) using the equation derived from the standard calibration curve:

Y=0.020 X, R^2 =0.99, where X is the absorbance and Y is the quercetin equivalents (mg QE/g).

Total Flavanol Content

The total flavanol content was evaluated by the vanillin reagent method with catechin as a standard (Rockenbach *et al.*, 2011). 5 ml vanillin reagent was prepared with 200 ml of 4% HCl methanol and 0.5 g of reagent. It was added to 1.0 ml of sample extract. The blank solution was prepared by adding 500 μl of 4% HCl in methanol. After 20 min incubation in dark the absorbance was taken at 500 nm. The results were expressed as mg of catechin equivalents (CE)/ g.

DPPH and ABTS free radical scavenging activity

DPPH & ABTS radical-scavenging activity was estimated by the method of (Saeed *et al.*, 2017; Re *et al.*, 1999) respectively by the following formula

$$\%\ Inhibition = \frac{(Abs_{control} - Abs_{extract}) * 100}{Abs_{control}}$$

Where, $Abs_{control}$ is the absorbance of the control (distilled water instead of the extract) and $Abs_{extract}$ is the absorbance of extract.

α - amylase inhibition assay

The α - amylase inhibition activity was evaluated by the method of (Khan *et al.*, 2017). The enzyme α -amylase (EC 3.2.1.1) was mixed with 0.5ml of 0.2 mol/L sodium phosphate buffer (pH 6.7 with 0.06 mol/L NaCl). 0.5 ml of seed extract was added with the solution and incubated at 25 °C for 30 minutes. 0.5 ml of starch solution (0.5%) in 0.02 mol/L sodium phosphate buffer (pH 6.7, with 0.006 mol/L NaCl) was added with the mixture. Then, the mixture was mixed thoroughly and incubated at 25 °C for 10 min.

The reaction was stopped by adding 1ml of Dinitrosalicylic acid (DNSA) solution, and the mixture was incubated again in a boiling water bath for 5 min and cooled at room temperature. The absorbance was evaluated at 540 nm after appropriate dilutions. The α-amylase inhibitory activity was calculated as percentage inhibition.

$$\%\ Inhibition = [(Abs_{Control} - Abs_{Samples})] \times 100/Abs_{Control}$$

Where $Abs_{control}$ is the absorbance of the control, and Abs_{sample} is the absorbance of the extract.

α -glucosidase inhibition assay

0.5 ml of sample extract of CPS with 500 μL of α -glucosidase (EC 3.2.1.20) solution (1.0 U/mL) was mixed 1 ml of 0.1 M phosphate buffer (pH 6.9), and the mixture was incubated at 27°C for 15 min. The substrate solution was prepared with 0.1 M phosphate buffer (pH 6.9) mixed with 500 μL of 5 mM p-nitrophenyl- α-d-glucopyranoside. The reaction mixtures were mixed well and incubated at 25 °C for 10 min. Then absorbance was taken at 405 nm. The α -glucosidase inhibitory activity (May and lamiaa, 2018) was calculated as percentage inhibition by the following equation

$$\%\ Inhibition = [(Abs_{Control} - Abs_{Samples})] \times 100/Abs_{Control}$$

Where $Abs_{control}$ is the absorbance of the control, and Abs_{sample} is the absorbance of the extract.

RESULTS AND DISCUSSION

The proximate analysis has been done to investigate the basic nature of the elements and biomolecules present in the aqueous seed extract. The protein content in *Canthium parviflorum* seeds analyzed, and it was observed to be 25.53 $\pm$ 1.38 %, which can act as a potential protein supplement in the daily diet. Similarly, the zinc content of the seeds was 6.469 $\pm$ 1.94 mg/100 g. It plays a vital role in immunity development and normal growth. Many biological functions like cellular integrity, protein synthesis and nucleic acid metabolism require zinc. The ash content of the sample was found to be 5.39 $\pm$ 1.02%, while the fibre content was 2.16 $\pm$ 2.89 %. The carbohydrate value was 16.10 $\pm$ 1. 37 % and Moisture content of the seeds was determined, and it was 7.20 $\pm$ 1.31% (Table 1).

Carotenoids are playing a major role in quenching of free radicals with their highly reactive conjugated double bonds and also act as free radical traps or anti-oxidants. The β-carotene content in seeds of *Canthium parviflorum* was found to be 5.2$\pm$2.61 μg/g of seed. β-carotene has certain anti-cancer activity and others which may include protection against cardiovascular disease or cataract prevention. The total phenolic content was determined in each extract as it is observed as a major source of the anti-oxidant potential in plants.

The polyphenolic content in the different crude extracts (A), (M) and (E) of CPS were found out to be 87.5, 81.9 and 75.88 GAE /gdw, respectively. The aqueous extract of CPS shows a maximum concentration of saponin, alkaloid, tannin and anthraquinone. The anti-oxidant property of seed extracts may be influenced by the presence of many of these phytochemicals. The inhibition of free radical formation might be influenced by the presence of abundant level of these phytochemicals and polyphenols. Moreover, the presence of carotenoids is influencing the level of nutraceutical properties of the seed extract.

Maximum flavonoids content was found in the aque-

Table 1: Proximate Analysis of seed extracts

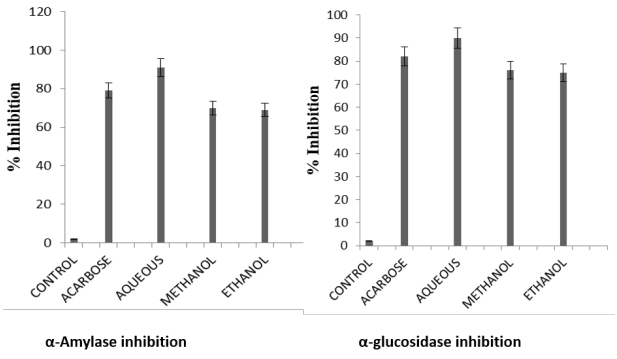
Parameters/Elements	Percentage/Content
Crude fat (%)	43.52 ± 1.5
Crude protein (%)	25.53 ± 1.38
Total ash (%)	5.39 ± 1.02
Total fiber (%)	2.16 ± 2.89
carbohydrate (%)	16.10 ± 1. 37
Moisture content (%)	7.20 ± 1.3
Zinc content (mg/100g)	6.4

Data wererereported as mean ± standard deviation of triplicates

Table 2: Polyphenolic Content and Radical Scavenging properties of seed extract

Total Polyphenols and Phytochemicals/ Radical scavenging Activity	Aqueous Extract	Methanolic Extract	Ethanolic Extract
Total phenolic content (mg GAE/ g)	87.5 ± 0.2a	81.9 ± 0.2b	75.1 ± 0.2b
Total Flavonoid content (mg ME/g)	50.1 ± 0.2b	31.2 ± 0.5b	24.2 ± 0.1a
Total Flavonols (mg QE/g)	11.4 ±0.4a	10.2 ±0.1b	9.3 ±0.2b
Total Flavanols (mg CAE/g)	15.1 ±0.1c	12.5 ±0.2b	9.3 ±0.4a
β –Carotene (mg carotenoid / 100 g)	5.2 ± 0.2b	4.8 ±0.1c	4.1 ± 0.1c
Saponin, Alkaloid, Anthraquinone & Tannin	++++	++	++
Triterpenoids & Steroids	+++	++	++
DPPH (%) CPS	91.1 ± 0.1a	89.4 ± 0.2b	85.6 ± 0.1a
ABTS (%) CPS	94.1 ± 0.2a	88.2 ± 0.4b	85.4 ± 0.1b

Means were containing different upper case (row) and lower case (column) with different superscripts are significantly different (P≤ 0.05). Data were reported as mean± standard deviation of triplicates. +=Less present, ++ = Moderate present, +++ = Strong present



Data were reported as mean ± standard deviation of triplicates.

Figure 1: Effect of CPS on α-Amylase and α-glucosidase inhibition

ous extract of CPS, and it was found to be 50.1 and comparatively higher than methanol (31.2) and ethanol (24.1) mg ME per g dw respectively (Table 2). Similarly, the aqueous extract of CPS exhibited maximum flavonols and flavonol concentration of 11.4 mg QE/g and 15.1 mg CAE/g of seed extract. Flavonoids play a pivotal role in antioxidant regulation by scavenging of free radicals and chelating metal ions (copper and iron). The flavonoids are highly influencing the inhibition of enzymes responsible for a various free radical generation.

In the initial stage of reaction, due to the occurrence of unpaired nitrogen electrons, the radical DPPH has a violet colour and, after reacting with the oxygen atom of a radical scavenger (anti-oxidant compound) reduced and formed DPPH-H which is yellow. The percentage inhibition of DPPH was highest aqueous than organic extracts.

The polyphenolic compounds are playing as singlet oxygen quenchers and hydrogen donors because of their redox properties. The highest free radical scavenging percentage of the aqueous extract was measured to be 91.1%. Similarly, the aqueous extract of CPS showed the highest percentage of free radical scavenging (94.1 %) against ABTS radicals. The natural polyphenols in CPS have the most ability to quench ABTS radicals and can donate a hydrogen atom.

Individual plant phenolics can inhibit the activity of the α-amylase enzyme and hence demonstrated therapeutic benefits such as hypoglycemic effect and are therefore useful in the dietary management of

Type II diabetes. Such promising inhibitory activity of the polyphenols on the -amylase enzyme has been the focus of attention in the management of Type II diabetes mellitus. The % α - amylase inhibition for the crude extracts aqueous, methanolic and ethanolic extracts of CPS were found, and the maximum activity (91.1%) was found for aqueous extract (Figure 1).

The effective management of diabetes mellitus, especially the non-insulin-dependent Type II diabetes involves the prevention of an excessive rise of the blood glucose level by inhibiting the digestive starch enzymes. Similarly, the results revealed that the aqueous (hot water) extract of CPS inhibit another enzyme α -glucosidase effectively, and it was found to be 90.5 %. The retardation effect of α-amylase and α -glucosidase enzyme inhibitors would be one of the most effective ways to control Type II diabetes. This result was significantly ($P<0.05$) higher than the inhibition observed in organic extracts.

CONCLUSIONS

The nutritional properties of CPS extract were investigated, and the results confirmed that the extract of seeds of CPS might be considered as a better source of polyphenols, anti-oxidants, protein, antidiabetic compounds and other micronutrients. The anti-oxidants are preventing the oxidative stress in our body and also help in controlling diabetes. The flavonoids play multiple significant roles in our diet against age-related illness, including cell damage due to oxidation, cardiovascular illness, and carcinogenic diseases due to the acceleration of different ageing factors.

The conclusion of the present investigation results has proposed that the seeds have enough potential micronutrients to meet out our daily nutrient requirements. The present study may be the platform for the development of nutritional enrichment in food for diabetic and other deficient patients with low-cost processing techniques.

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Conflict of Interest

All the authors have no conflict of interest for this study.

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