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Article

HPLC identification of Rutin (3, 3′, 4′, 5, 7-pentahydroxyflavone-3-rhamnoglucoside) from *Costus pictus* leaves extract and evaluation of its antioxidant activity

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Abstract: Plants synthesise many secondary metabolites which are of great medicinal value. *Costus pictus* also known as Insulin plant is considered as valuable medicinal plant in preparation of many commercial pharmaceutical products. The aim of the present study is to identify one such secondary metabolite flavonoid (Rutin) in the leaves of *Costus pictus* by HPLC method and also to evaluate the antioxidant potential of Ethyl acetate extract of *Costus* using DPPH and NO scavenging assay. The Extracted material was identified as Rutin by comparing with Standard Rutin. Both the samples showed similar retention time of 2.8 minutes. The extract showed maximum DPPH free radical scavenging activity with increase in concentration and IC_{50} of Nitric acid scavenging activity was found to be more for the Extract than the standard ascorbic acid and Rutin. From this study it is concluded that *Costus pictus* leaves extract can be used as natural food supplement.

Keywords: Costus; HPLC; Rutin; antioxidant

1. Introduction

Plants have been the major source of drug discovery for the treatment of various ailments including diabetes mellitus and its secondary complications in Indian and other ancient system of medicine in the world (Atanasov *et al*., 2015). Recent decades have witnessed a resurgent interest in traditional plant treatments for diabetes, which has pervaded nutrition, pharmaceutical industry and academic research, fuelled by a growing public interest and awareness of so-called complementary and natural types of medicine.

Intake of dietary phytochemicals is associated with health benefits. Non communicable diseases such as cancer and diabetes are considered as the major cause of morbidity and mortality worldwide (Howes and Simmonds, 2014**)**. In the series of medicinal plants for the treatment of Diabetes mellitus, *Costus pictus* belonging to the family Costaceae with well-known pharmacological as well as beneficial properties is used in the recent years. *Costus pictus D. Don* is commonly called as 'Insulin plant' for its effectiveness against Diabetes Mellitus (Jose and Reddy, 2010; Modak *et al*., 2007). *Costus pictus D. Don* plant possess antioxidant, antidiabetic, anti-inflammatory, hypolipidemic effect **(**Sethumathi *et al*., 2009; Mani *et al*., 2010). Phytochemicals from natural resources open new avenues for the treatment of various diseases including diabetes (Pan *et al*., 2013). Therefore, there is a need for an active phytoingredient which possess antidiabetic and antioxidant potential without long term side effects.

Flavonoids are abundant in nature and according to their chemical structure, they are categorized into flavones, flavonols, flavanones, isoflavones, catechins and anthocyanidins. Dietary flavonoids are rich source of natural

antioxidants due to more number of target sites for free radicals (Bylka and Matlawaska, 2004) and they also possess many biological activities.

Rutin is one such flavonoid with potent antioxidant activity which strengthens arteries and veins (Baumgertel *et al*., 2003; Danila *et al*., 2007), harden bones and teeth (Horcajada *et al*., 2000), improves blood circulation. Rutin (3, 3′, 4′, 5, 7-pentahydroxyflavone-3-rhamnoglucoside) is a bioflavonoid mostly found in edible plants such as buckwheat, onions, apple, berries, tea and wine **(**Manach *et al*., 1997**)**. Till date, over 130 registered therapeutic medicinal preparations are known to contain Rutin in their formulations (Chua, 2013**)**. Rutin exerts its wide spectrum of pharmacological benefits for the treatment of various chronic diseases such as cancer, diabetes, hypertension and atherosclerosis (Sharma *et al*., 2013**)** .It acts as a potent antidiabetic compound by inhibiting alpha amylase, alpha glucosidase and aldose reductase enzymes (Dubey *et al*., 2017). Rutin may be useful for the prevention and treatment of colorectal cancer (Jantrawut *et al*., 2015).

In the present study an attempt has been made to extract Rutin from *Costus pictus* leaves and to characterize it by high performance liquid chromatography and to evaluate the antioxidant activity of ethylacetate extract of leaves.

2. Materials and Methods

2.1. Collection of specimens

Costus pictus leaves were collected from Nethra organic form, Anna Nagar, Pammal, Chennai-600075, Tamil Nadu, India during the month of September 2018. The plant was identified and authenticated by Dr. K.N. Sunil Kumar, Research Officer and Head Dept. of Pharmacognosy, Siddha Central Research Institute, Central Council for Research in Siddha, Arumbakkam, Chennai - 600106 Tamil Nadu.

2.2. Sample preparation

The Collected leaves were washed thoroughly with distilled water and dried in shade. The shade dried leaves were powdered and then used for further studies.

2.3. Soxhlet extraction

50 g of powder was defatted with (500 ml x3) petroleum ether, then extracted by Soxhlet apparatus with 250 ml of 80% ethanol till exhaustion. The extract was filtered, concentrated by evaporation under vaccum to about 10 ml. The concentrated liquid is mixed with 25 ml distilled water and extracted again with petroleum ether (50x3), and Chloroform (50x3). After extraction, the aqueous layer was collected and allowed to stand for 72 hours in cold place. A yellow precipitate is separated out of the solution. The precipitate was filtered and washed with a mixture of Chloroform: Ethyl acetate: Ethanol (50:25:25) (Ibrihem *et al.*, 2018). The residues were then dried and dissolved in ethylacetate and used for further analysis.

2.4. Identification of Rutin content by HPLC

2.4.1. Solvents and chemicals

All the chemicals used were of analytical grade. Rutin was purchased from Sigma-Aldrich. HPLC grade methanol, water, metaphosphoric acid were obtained from Merck.

2.4.2. Preparation of test and standard solution

About 100 mg of powdered extract was accurately weighed, placed in a stoppered conical flask, and 50 mL of 50% methanol was added accurately to it. This mixture was then weighed, heated under reflux for 60 min, cooled, and weighed again. The lost weight was complemented with 50% methanol and shaken well. In the similar way Standard rutin solution is also prepared.

2.4.3. Chromatographic conditions

Liquid chromatography was performed using a Shimadzu HPLC apparatus (Shimadzu LC-10A system, Kyoto, Japan) equipped with a Rheodyne injector 20 µL loop with RP-8 LiChroCart column (125 mm × 4.60 mm, 5 µm particle size). Two LC-10AD Vp pumps, an UV VIS SPD-10A Module, an SLC-10A system controller, Chromatograms were obtained and analyzed using the software Class-VP® (Shimadzu, Tokyo, Japan). The mobile phase consisted of a binary mixture of methanol-water (50:50 v/v) adjusted to pH 2.8 with phosphoric acid at isocratic flow rate of 1 ml /min with an elution time of 5 minutes. The absorbance was measured at a wavelength of 360 nm. The Chromatographic peaks of samples were confirmed by comparing their retention time with those of the reference standard Rutin.

2.5. In vitro free radical scavenging assay

The free radical scavenging activity of the extract was assayed using DPPH (Barku *et al*., 2013), Nitric oxide scavenging assay (Green *et al.,* 1982) with slight modifications.

3. Results and Discussion

The separation chromatogram of the standard, Extracted Rutin from the *Costus* is illustrated in the Figure 1.

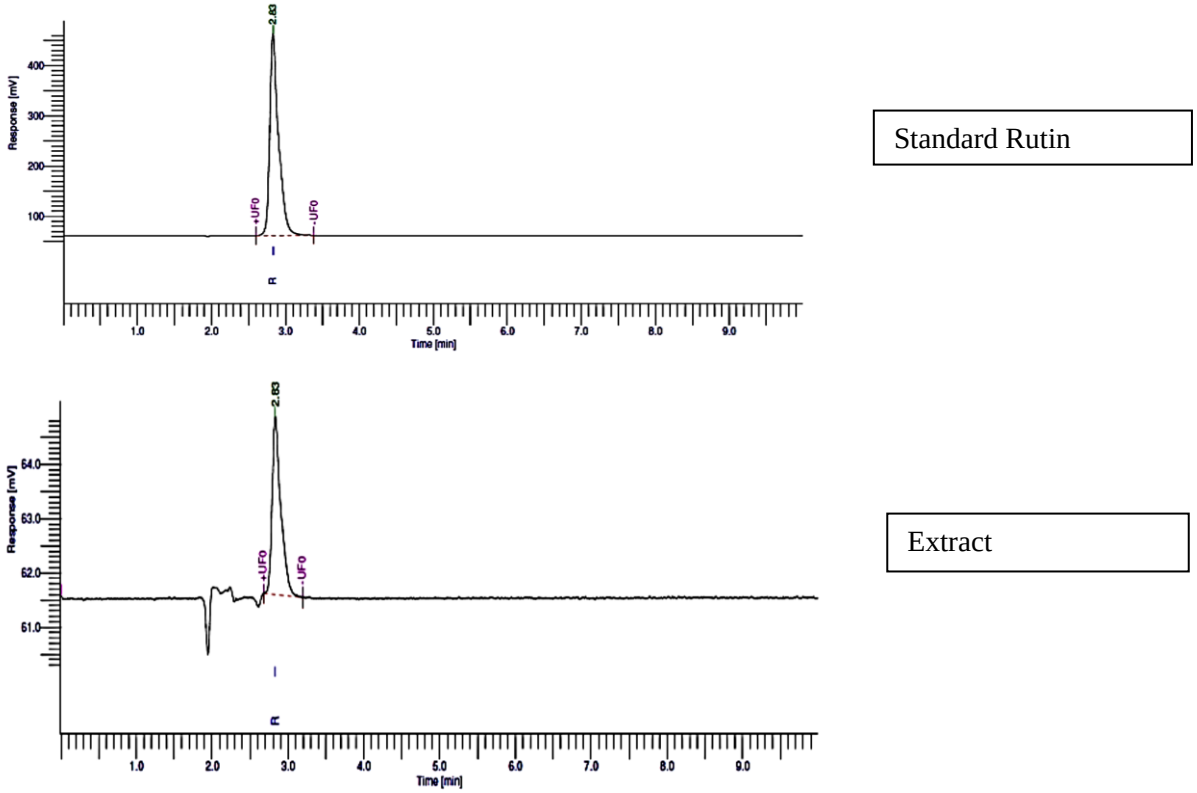

Figure 1. HPLC chromatogram of *Costus Pictus* D. Don leaves extract for Rutin identification.

The HPLC chromatogram of the sample containing Rutin fraction was compared with that of Standard Rutin. The peaks for Rutin coincided with the standard Rutin peak indicating that the Rutin is present in the extract of leaves of *Costus pictus*.
Area, Time, Area % of Standard, Extracted Rutin are provided in Table 1.

Table 1. Area, Time, Area % in HPLC chromatogram of Standard, Extracted Rutin.

Component Name	Time (min)	Area (uV*sec)	Area (%)	Tailing Factor	Theoritical Plate
Rutin	2.833	3315403.46	100.00	1.0924	6063.5086
Extracted Rutin	2.825	15304.59	100.00	1.1819	5857.5476

The HPLC analysis of both the standard Rutin and the isolated compound showed an identical retention time of 2.8 minutes, which can also be considered as a conclusive evidence that the compound was Rutin. Based on the result presented in the Table 1 it can be confirmed that Rutin is being extracted from the leaves of *Costus pictus.*

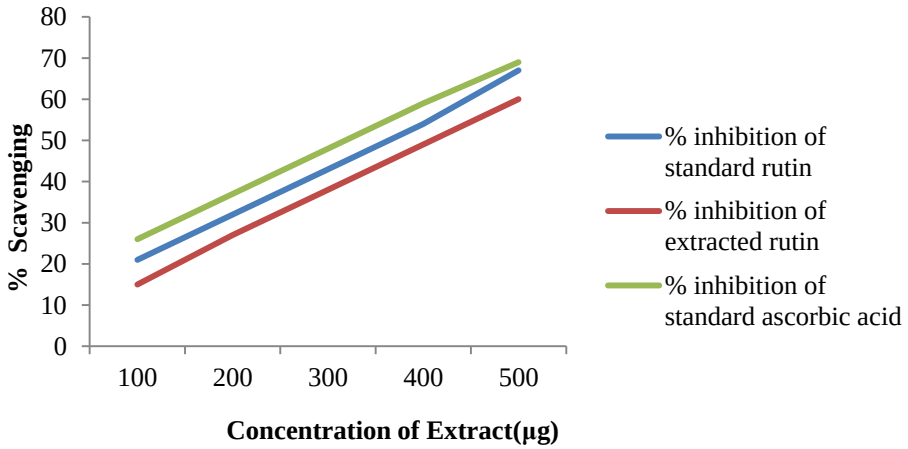

Figure 2. DPPH radical scavenging activity.

The proton scavenging action is known to be one of the important mechanism for measuring antioxidant activity. To test the radical scavenging activity of the compound or plant extracts, the commonly used free radical is DPPH. When the stable DPPH radical accepts an electron from the antioxidant compound, the violet colour of the DPPH radical was reduced to yellow coloured Diphenyl-picrylhydrazine radical which can be measured colorimetrically. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers **(**Dehpour *et al*., 2009**).** IC_{50} was determined from the plotted graph of scavenging activity against various concentrations of standard ascorbic acid, Standard Rutin, Extract. The antioxidant activity was found to be (IC_{50} - 408.9) µg for standard ascorbic acid followed by standard Rutin (IC_{50}- 357.8) µg & Extract (IC_{50}3- 20.37) µg. DPPH scavenging activity increased with the increase in concentration of the extract. The positive control, ascorbic acid showed maximum scavenging effect (Figure 2). Nitric oxide assay was carried out in the standard Ascorbic acid, Standard Rutin, Extract from a concentration of 200 to 1000µg/ml. Percentage free radical scavenging was plotted against concentration of the standards, Extract is shown in Figure 3.

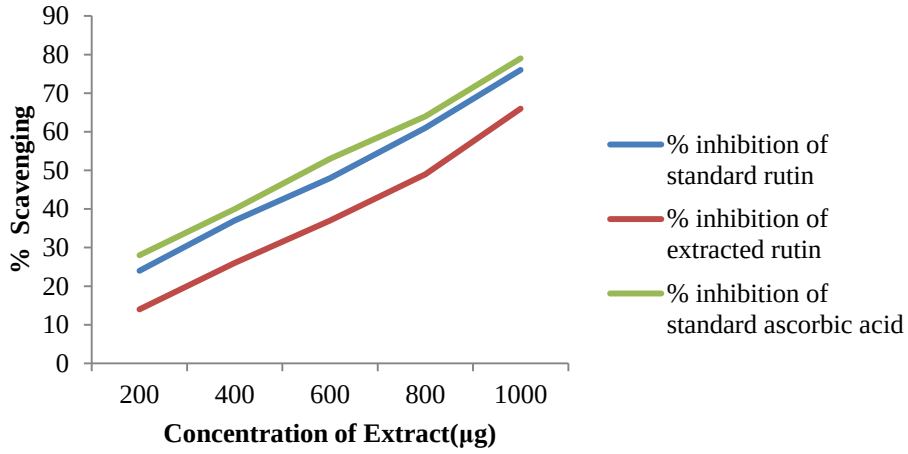

Figure 3. Nitric oxide scavenging activity.

The antioxidant activity increased with an increase in concentration of Standard ascorbic acid, standard Rutin, Extract. Standard ascorbic acid showed a maximal scavenging activity of 79% at 1000µg/ml, followed by Standard Rutin 76% and Extract (66%). The extract at a concentration of 200µg/ml showed minimum inhibition of 14%. IC_{50}value of Extract was 788.88µg/ml. Chronic exposure to Nitric oxide radical is associated with various carcinomas and inflammatory conditions including juvenile Diabetes, multiple sclerosis, arthritis and ulcerative colitis. With context to (in view of that) the study has shown that Rutin can be used as source of antioxidant.

4. Conclusions

The results of the present study revealed the presence of Rutin in *Costus pictus* leaves. The free radical scavenging activity of the extract is evident from in vitro antioxidant assays. Thus the present study suggests that *Costus pictus* can be used as potent antioxidant food supplement.

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Conflict of interest

None to declare.

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