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Research Article

Antioxidant Activity and Phytochemical Screening of *Costus pictus* D. Don Leaf Extracts

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ABSTRACT
The occurrence of chronic diseases is rapidly growing worldwide. Diet and nutrition are significant aspects in the promotion and maintenance of good quality health throughout the whole life span. Overproduction of liberated radicals occurred due to physiological and biochemical changes in our body and then can cause oxidative damage to biocompounds of human body (e.g. lipids, proteins, DNA). Medicinal plant based products exert beneficial properties such as antioxidant, anticancer, hypoglycemic and hypolipidaemic activities so their utilization is increased recently. This study includes preliminary phytochemical screening and quantification of phenol and flavonoid of *Costus pictus* aqueous (CPAQ) and methanolic (CPME) extracts. DPPH, FRAP, Fe^{3+} reducing power, and superoxide scavenging assay were used for the estimation of antioxidant potential of leaf extracts CPAQ and CPME. CPME gave the elevated amount of phenol and flavonoid content and antioxidant activities. The antioxidant activity of *Costus pictus* extracts significantly correlated ($P < 0.05$) with the phenolic content. Ascorbic acid used as standard compound and showed maximum antioxidant power in this study.

Keywords: *Costus pictus*, secondary metabolites, Antioxidant activities, Ascorbic acid.

INTRODUCTION

Medicinal plants have long olden times of use and they are widespread in both developing and developed countries. World Health Organization reported that, 80% of the world populations rely primarily on traditional therapies in which use of plant extracts or their active components are involved[1]. Interest in the utilization of medicinal and aromatic plants as pharmaceuticals, perfumes and cosmetics, flavoring, herbal and alternative natural products has been significantly spent in the recent years[2]. Secondary metabolites are present in abundant amount in plants. These compounds are isolated and they have various pharmacological activities like anticancer, antibacterial, analgesic, anti-inflammatory, antitumor, antiviral and other activities to a higher or lesser degree[3]. Secondary metabolites include flavonoids, phenols and phenolic glycosides, isolated saponins and cyanogenic glycosides, stilbenes, tannins, terpenoids nitrogen compounds like alkaloids, amines, betalains and some other endogenous metabolites which may contribute to the medicinal properties of plants[4].

Sometimes there is an imbalance between reactive oxygen species (ROS) generation and antioxidant activity which will result into generation of free radicals which are chemically unsteady and harm cells of human body including lipids, proteins and DNA[5]. They are known to be the causal of oxidative stress which is grossly implicated in the pathogenesis of various diseases such as cancer, diabetes, cardiovascular diseases, aging and metabolic syndrome[6,7]. Antioxidants are striking interest nowadays because of their properties to prevent the emerged toxic effects of free radicals and prevent harmful effect on fats and other foods in human body. In these kind of circumstances, natural antioxidants play an important role rather than artificial antioxidants[8]. At present, most of the antioxidants are produced artificially. The main drawback with the synthetic antioxidants is the consequences *in vivo*[9]. Some artificial antioxidants like BHA and BHT found to be harmful to our body[10]. The use of natural antioxidant has gained much attention from consumers because they are considered safer than synthetic antioxidants. Recently, there has been a worldwide trend towards the use and ingestion of natural antioxidants present in different parts of plants due to their phytochemical constituents[11].

A medicinal plant of *Costaceae* family, *Costus pictus* D. Don is a vital therapeutic plant usually identified as Spiral flag or Insulin plant. The plant is persistent in all the seasons and is a rhizomatous herb with red spiral stem and huge green leaves on it. The beauty of this plant is their yellow red striped flowers on spike at the tip on mature stems[12]. The family Costaceae has four genera and approximately 200 species. The genus Costus is the major group in the family with about 150 species that are mainly tropical in distribution. This plant is commonly used by the tribal people of Kolli hills of Tamilnadu, to treat diabetes[13]. *C. pictus* recently bring in to India from the American countries as an herbal care for diabetes and therefore

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commonly called as 'insulin plant'[14]. This is believed to be inhabitant of tropical climates of Asia, Central America, South America and Africa. Recently this plant gets very high medicinal interest due to its medicinal potential and shows a variety of pharmacological properties such as anti-diabetic, diuretic effect, anti-oxidant, anti-cancers and putative activity[15]. Particularly, in south Indian region, it is grown as ornamental plant.

The plant has not been studied for many of its pharmacological activities. As per our knowledge, scientific literature for the extraction with different solvent systems, identification of efficient solvent system and antioxidant activities correlation with phenolic content was not found for this plant. To provide a systematic basis to validate the remedial usage of the plant *C.pictus*, the present study was designed to give the details on the phytochemistry (qualitative and quantitative components) and antioxidant activities of the aqueous and methanolic extracts of the plant.

MATERIALS AND METHODS

Plant collection

The fresh plants of *Costus pictus* D.Don were collected from Green park nursery and farm, Doldha, Gujarat in August, 2014 and authentication by a Botanist, Dr. Minoo Parabia at Department of Bioscience, VNSGU, Surat. The voucher specimen (ABN/1) was deposited at CGBIT department, Maliba Campus, Bardoli, Gujarat. The plants were then planted and fresh leaves were collected. The leaves were washed 2-3 times with tap water, shade dried, then mixed in electric grinder to get fine powder and stored in refrigerator in air tight bottle. The fresh and mature leaves were used for study.

Solvent extraction

Weigh 10 g dried powdered plant materials and use them extraction using 100 ml of different solvents (Methanol, aqueous, petroleum ether, acetone, benzene, chloroform and hexane) by using Soxhlet apparatus for 6-8 hr at a temperature not more than their respective boiling points. The extracts were filtered using Whatman No.1 filter paper and then under vacuum at 40 ^{0}C concentrated by using a rotary evaporator. The residual extracts were stored 4 ^{0} C further use.

Phytochemical screening

For the identification of secondary metabolites present in plant extracts of *C.pictus*, preliminary qualitative analysis was performed[16,17].

Determination of total phenolic and flavonoid contents

Total phenol content in plant extracts were estimated spectrophotometrically according to the Folin–Ciocalteu colorimetric method[18]. For the reference drug, Gallic acid was used and results were expressed as gallic acid equivalents (GAE). Gallic acid standard curve y = 0.0322x + 0.0986, R^2 = 0.9947.

For the estimation of total flavonoid content, previously described method was used and expressed as gram of rutin equivalent (RE)/ 100 g of extract[19]. Rutin standard curve y = 0.0326x + 0.0918, R^2 = 0.9999. The absorbance for total phenol and flavonoid was measured at 756nm and 420 nm, respectively using UV-VIS spectrophotometer (Shimadzu UV-Mini1240). All results were performed and analyzed in triplicates.

1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity of *C.pictus* leaves extracts was estimated according to the method of Miliauskas[20]. Concentration of the extracts required to cease the initial concentration of DPPH by 50% i.e., EC50 values were measured. The scavenging ability of the plant on DPPH was calculated using the equation: $\%\, inhibition = (AC - AE)/AB \times 100\%$, where AC = absorbance of the control and AE = absorbance of tested samples. All results were performed and analyzed in triplicates.

Determination of superoxide radical scavenging activity

Superoxide scavenging was determined by the nitroblue tetrazolium reduction method[21] and it based on the reduction of nitro blue tetrazolium (NBT) in the presence of nicotinamide adenine dinucleotide (NADH) and phenazine methosulfate under aerobic condition. Reaction misture consisted of 1 M NBT, lM NADH with or without sample and Tris buffer (0.02 M, pH 8.0) and addition of 1 M phenazine methosulfate (PMS) to the mixture started the reaction. The change in the absorbance was recorded at 560 nm after 2 minutes. For standard and extracts, percentage inhibitions were calculated against control without standard and extracts, respectively. Following formula was used to calculate the abilities to scavenge the superoxide radical: $\%\, inhibition = (AC - AE)/AB \times 100\%$, where AC = absorbance of the control and AE = absorbance of tested samples.

Ferric Reducing Antioxidant Potential (FRAP) Assay

FRAP assay was used to determined the ferric reducing potential of CPAQ and CPME[22]. The absorbance of the reaction mixture was read at 593 nm. FRAP value was expressed in terms of mmol Fe2+/g of sample using ferric chloride standard curve Y = 0.1238x + 0.0596, R^2 = 0.992. All results were performed and analyzed in triplicates.

Reducing Power Assay

Estimation of Fe^{3+} reducing power of extracts was done by the modified method of Oyaiz[23]. The reaction mixture consist of extracts of various concentrations (100-1000µl) with 0.2 M phosphate buffer (pH 6.6) and 1%, w/v potassium hexacyanoferrate ($K_3Fe(CN)_6$), and the incubated at 50°C in a water bath for 20min. 10% trichloroacetic acid (TCA) solution was added to the reaction to stop it and centrifuged at 800 g for 10 min. Half part of the obtained supernatant was mixed with half part of distilled water and 0.1 part of ferric chloride ($FeCl_3$) solution (0.1%, w/v) for 10 min. The absorbance of reaction mixture was taken at 700 nm. Elevated absorbance value of reaction mixture indicated higher reducing power.

Statistical analysis

All the analysis were performed in triplicates and expressed as mean value ± SD. For the calculation dose-response relation of extracts, regression analysis was performed. Correlation coefficient was found out using linear regression analysis. Evaluation of statistic

significance was done statistically using *t*-test and $P < 0.05$ was considered to be significant.

RESULTS

Phytochemical analysis

Leaf extracts of *C.pictus* evaluated for identification of efficient solvent system. This screening identified the efficient solvent systems which have the highest yield of extracts. Table 1 shows the percentage of yield of crude extracts (Methanol, aqueous, petroleum ether, acetone, benzene, chloroform and hexane) of leaves of *Costus pictus*.

The 8 extracts yielded by Soxhlet extraction method have shown considerable differences. The extraction was done on the basis of polarity of the solvents. The maximum extract yield was obtained with methanol (4.05 g) and it was followed by aqueous (3.15 g), and hexane has the lowest yield (least polar solvent) (0.57 g). Methanol and aqueous extracts found to be most efficient solvent systems with highest percentage yields and further used for preliminary, quantitative analysis of secondary metabolites and antioxidant assays. Preliminary screening of *C.pictus* aqueous (CPAQ) and methanol (CPME) confirmed the presence of various secondary metabolites like, alkaloids, cardiac glycosides, flavonoids, glycosides, phenols, saponins, steroids, tannins, and terpanoids compounds (Table 2) in both the extracts.

Determination of total phenolic and flavonoid contents

The results of total phenol and flavonoid content showed that the CPME is slightly higher in total phenolic content i.e., 110.47 ± 1.02 mg GAE/g and total flavonoid content 86.87 ± 0.97 mg RE/g extracts compare to CPAQ which has 90.39 ± 0.82 GAE/g of extract and 69.28 ± 1.07 RE/g extract, respectively. Here, the presence of phenolic and flavonoid content were confirmed qualitatively as well as quantitatively in both the extracts. There is a possibility that the availability and synergistic effects of these kinds of secondary metabolites attribute antioxidant and other pharmacological activities to the medicinal plants.

DPPH radical scavenging activity

The antioxidant property of both the extracts was investigated and compared by various biochemical assays like, DPPH, superoxide scavenging assays, FRAP, reducing power assay, etc. Determination of DPPH radical scavenging capability was done by decrease in the absorbance which is induced by presence of antioxidants in extracts. In Figure 1 comparison of DPPH radical scavenging activity of standard drug ascorbic acid and extracts were shown. An efficient antioxidant concentration required to decrease the early DPPH radical concentration by 50% is known as EC50, which was determined from scavenging activity plotted graph versus different concentrations of standard drug and extracts (Table 3). Lower value of EC50 is a sign of strongest ability of extracts or drug to act as potent DPPH radical scavengers. The radical scavenging reaction of ascorbic acid with DPPH was essentially instantaneous; the reactions of DPPH with extracts were also quick but slower compared to that with ascorbic acid. Highest DPPH scavenging activity was of standard ascorbic acid with EC50 of 0.109 mg/ml. DPPH radicals scavenging activity CPAQ and CPME both extracts showed significant DPPH activity which comparable to standard drugs ascorbic acid. DPPH radical scavenging by CPAQ and CPME at 1 mg/ml was 81.25 % and 89.25 % with EC50 values were 0.468 and 0.360 mg/ml, respectively, while ascorbic acid have 99.87% activity at 1ml concentration.

Determination of superoxide radical scavenging activity

Tissue damage and other diseases in body are contributed by superoxide ($O_2^{\bullet -}$) radical which are the precursors of more reactive species and they are very toxic to cellular level.

When the plant extract have the hydroxyl radical ($^{\bullet}OH^{-}$) scavenging capacity, it can be correlate with to the antioxidant potential to the plant. Hydroxyl scavenging activity of standard drug and plant extracts were shown in Figure 2. Plant extracts CPAQ and CPME have the OH^{-}scavenging activity which is dependent on dose response. Plant extract CPME have the higher scavenging activity compared to CPAQ. Ascorbic acid as the standard drug showed the highest activity. The EC50 values were determined for CPAQ, CPME and ascorbic acid and they were 0.490, 0.412 and 0.105 mg/ml, respectively (Table 3). The radical scavenging activity graph showed the similar pattern as obtained by DPPH radical scavenging activity (Figure 2).

Ferric Reducing Antioxidant Potential (FRAP) assay

Ferric reducing antioxidant potential of the extracts CPAQ and CPME were estimated by their capability to reduce TPTZ–Fe (III) complex to TPTZ–Fe (II). Considerable FRAP activity of standard and extracts confirmed their reducing capacity. Here, the FRAP value of CPME was 190.40 equivalent mmol of Fe2+/g sample which was higher compared to CPAQ (154.60 equivalent mmol of Fe2+/g sample). These results are comparable with the FRAP value of ascorbic acid (205.49 mmol Fe (II)/g extract) (Table 3).

Reducing Power Assay

Comparisons for reducing power of standard ascorbic acid and both extracts were shown in Figure 3 as dose-response curve. The reducing power will be higher as the absorbance of the reaction mixture increase. The standard compound ascorbic acid showed the highest reducing power. CPAQ and CPME, both the extract shown increase in reducing power with increase in concentration but CPME revealed significant reducing power compare to standard drug.

Correlation between total phenolic and antioxidant activity

Total phenol content of both the extracts and their antioxidant activity showed the positive linear correlation. The correlation coefficient ranged from 0 to 1.00 indicated a positive relationship between the total phenolics and total antioxidant activity. CPME revealed a significant correlation between their antioxidant capacity and total phenolic and this showed that the phenolic compounds largely contribute to the antioxidant activities of the plant, and therefore could play a major role to give the medicinal properties of the plant.

Table 1: *C.pictus* leaves extraction for different solvents and their color, yield and yield percentage.

Solvents	Material	Extract Color	Yield (g) *	Yield percentage
Hexane	leaves	Dark green	0.57	5.7
Benzene	leaves	Dark green	1.82	18.2
Diethyl ether	leaves	light green	1.31	13.1
Chloroform	leaves	light green	0.74	7.4
Ethyl acetate	Leaves	dark green	1.19	11.9
Acetone	Leaves	dark green	2.93	29.3
Methanol	Leaves	dark green	3.15	31.5
Aqueous	Leaves	bright yellow	4.05	40.5

*10 g powder for 100 ml solvent

Table 2: Qualitative analysis of aqueous and methanolic extracts of C.pictus leaves.

Phytochemical	*C.pictus*extracts	
	CPAQ	CPME
Alkaloids	+	+
Phenols	+	+
Flavonoids	+	+
Anthroquinones	+	+
Fatty acids	+	+
Tannins	-	-
Saponins	+	+
Anthocyanins	+	+
Carbohydrates	+	+
Glycosides	+	+
Proteins and	+	+
Amino acids	+	+
Steroids	+	+
Triterpenoids	+	+

+ Presence - Absence

DISCUSSION

Plants are a very important source of potential valuable bioactive compounds for the development of new therapeutic agents. Thus, these observations have assisted in developing new drugs for the therapeutic use in human beings. However, not numerous studies are accessible on the pharmacological properties of *C.pictus*. Hence, here we evaluated *C.pictus* plant for their phytochemistry and antioxidant activities. The remedial benefit of medicinal plants is usually contributed to their antioxidant properties. For the evaluation of the total phenolic and flavonoid content and different antioxidant assay were performed for the aqueous and methanolic extracts of *C.pictus*.

Phenols have the antioxidant activity with property of free radical termination and they are the key compounds of the plants. Free scavenging activity of the plants is highly correlate and dependent on the total phenolic content present in that plant[24]. Compounds such as flavonoids, which have hydroxyl functional groups, contribute significantly for the antioxidant effect in plants[25]. Both extracts possess higher amount of phenolic content which was confirmed by quantitave analysis. Same kind of results were observed for the different extracts of *C.pictus* plant by other studies[26]. In our study we found that CPAQ and CPME both have the phenolic and flavonoid compounds in significant amount and showed the antioxidant activity with notable EC50 values and hence, this plant can be used as an important source of antioxidant.

Emerged interest in plant oxidants has emerged during recent years probably due to the appearance of the undesirable side effects of many synthetic antioxidants. In medicinal plant world, there are enormous number of diverse bioactive compounds with antioxidant activity that play significant role in terminating the generation of free radical chain reaction. So here we evaluate *C.pictus* as a new antioxidant agent by various assays. Nucleic acids, proteins, lipids or DNA can be oxidized because of the presence of free radicals. Antioxidants have the ability to trap these kinds of highly reactive free radicals and oxygen species and they can terminate the degenerative diseases in our body. Estimation of the antioxidant activity can be done by many assays, but DPPH assay is one of the best-known, precise, and commonly utilized methods[27]. DPPH has the advantage of being unaffected by certain side reactions such as metal ion chelation and enzyme inhibition[28]. A corresponding transformation was observed as the donation of H+ to the DPPH radicals occur and it will convert violet color to pale yellow color. Absorbance at 517 nm showed corresponding decrease as the DPPH scavenging activity increase. CPME have the higher phenol as well as higher antioxidant potential, so these two properties were significantly correlated with each other ($P < 0.005$).

To estimate the reducing power assays is used in which assessment of donation of electron by antioxidants are measured[29]. This assay is based on the reduction of Fe^{3+} to Fe^{2+} by the plant extracts. The antioxidants, present in the extracts reduce the ferric cyanide complex (Fe^{3+}) to the ferrous cyanide and form (Fe^{2+}). Depending on the reducing power of the compounds and their concentrations, there is a change in the solution with different shades from green to blue[30]. Blue colored complex Perl's Prussian is formed by antioxidants which have the strong reducing ability at 700 nm optical densities. Standard ascorbic acid had shown the highest reducing power ability from tested samples. From the review it has been observed that the hydrogen donating capability of the antioxidants contribute the reducing power potential to the plants[31]. So, the presence of higher amount of reductones in the methanolic extract of *C.pictus* may responsible for the higher reducing ability compared to aqueous extract. The function of reductones is to convert free radicals into more stable compound by donating an electron and stop the reactions of free radical chain.

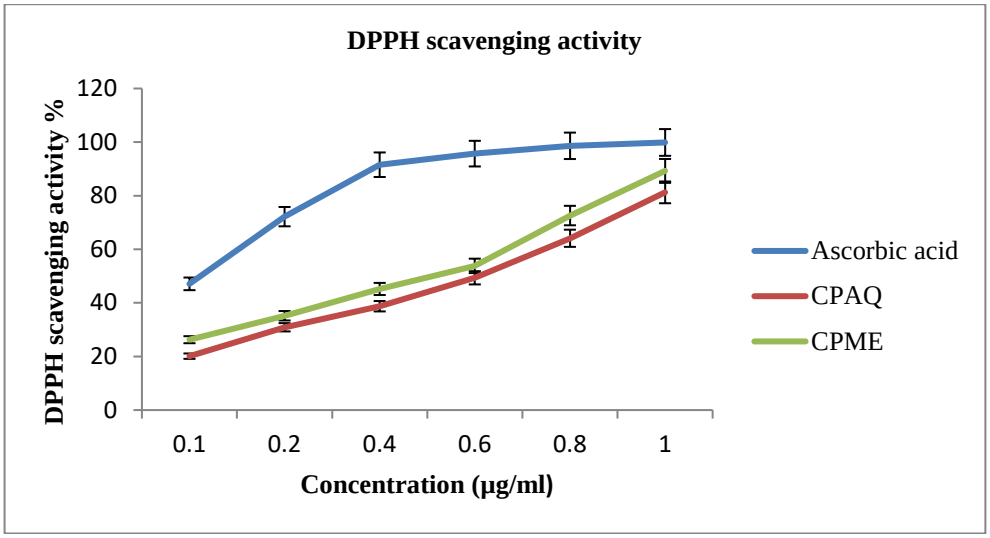

Figure 1: The graphical representation of DPPH scavenging activity.
Values are expressed as mean ±standard deviation (n = 3). Ascorbic acid was used as a standard

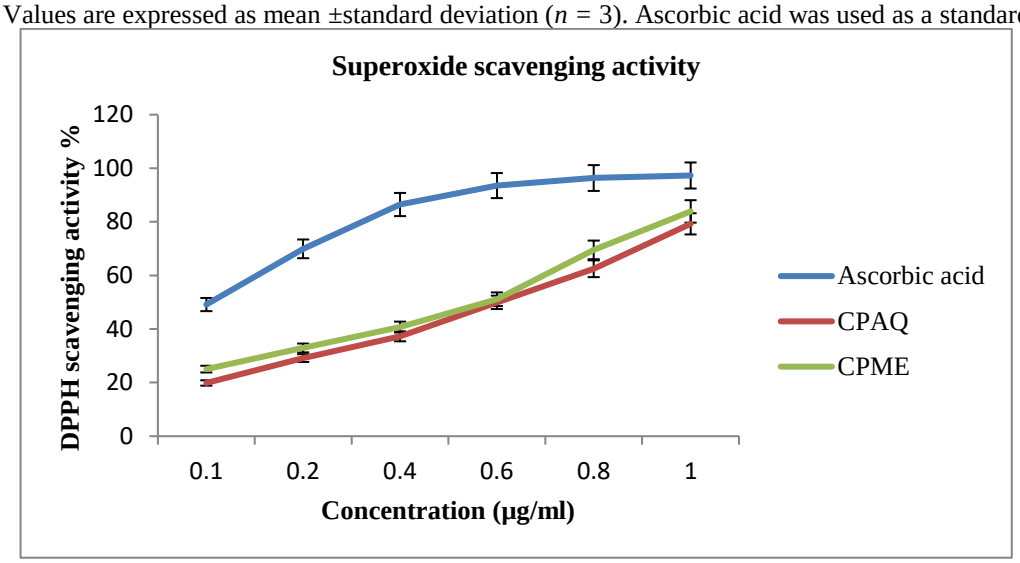

Figure 2: The graphical representation of Superoxide scavenging activity
Values are expressed as mean ±standard deviation (n = 3). Ascorbic acid was used as a standard.

Table 3: EC50 value is defined as the effective concentration of antioxidant necessary to decrease the radical concentration by 50%. FRAP values represent as equivalent mmol of Fe^{2+}/gram sample.

Antioxidant activities	EC50 (DPPH) mg/ml	EC50 (Superoxide) mg/ml	FRAP Value (mmol of Fe^{2+}/g)
CPAQ	0.468	0.491	154.60 ± 0.51
CPME	0.360	0.407	190.14 ± 0.38[a]
Ascorbic acid	0.109	0.105	205.49 ± 0.59 [a]

Means ±standard deviation (n = 3) followed by the same letter in last column are not significantly different at P≤0.05.

In FRAP assay, antioxidants react and convert ferric tripyridyltriazine (Fe3+-TPTZ) complex to a colored compound ferrous tripyridyltriazine (Fe2+-TPTZ)[32]. Antioxidants play an important role as reducing agent as they break the chains of free radicals by donating hydrogen atom[33]. The result of our study was comparable with the study of Benzie and Szeto[34], who found out that higher the phenolic content is, higher would be the reducing power of the extract and these two have the strong correlation with each other. Rice-Evans et al. stated that the phenolic compounds have the redox potential, hence have the reducing properties and can donate hydrogen atom as well as quench the singlet oxygen[33]. Redox properties of the phenol compounds play an important role for the determination of the antioxidant potential of any compound.

Superoxide are formed from molecular oxygen by oxidative enzymes as well as via nonenzymatic reactions such as auto-oxidation by catecholamines[17]. Oxidative damage at cellular level in our body can be induced by the superoxide anions which play a crucial role in the formation of various other reactive oxygen species such as

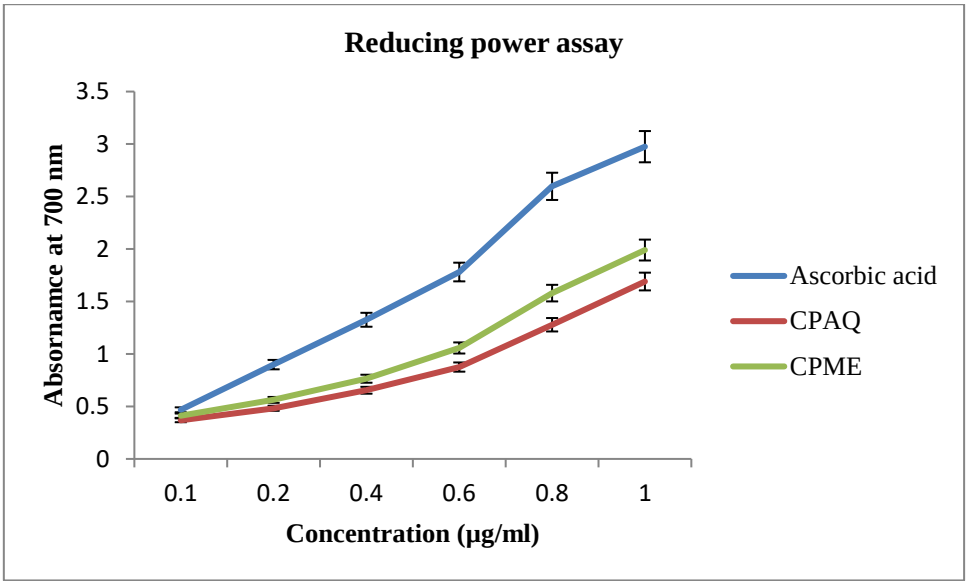

Figure 3: The graphical representation of reducing power assay.
Values are expressed as mean ±standard deviation (n = 3). Ascorbic acid was used as a standard.

hydrogen peroxide, hydroxyl radical and singlet oxygen[35]. From the results of this study it has been observed that the extracts possess the superoxide scavenging properties and have the potential to scavenge superoxide anions. Results visibly pointed out that *C.pictus* is a potent scavenger of superoxide radicals.

There are diverse mechanisms of the antioxidant activities in the different assays and due to this it is observed that there is a differential scavenging potentials of the extracts and standard drug against various assays. Here, the results of total phenolic content and different assays of antioxidant activities for the CPME and CPAQ showed that there is a strong and significant correlation among them. Stereo selectivity of the radicals or difference in solubility is the factor which is the reasons for the multiplicity in the radical scavenging properties. Crude extracts have the mixture of antioxidant compounds and synergistic effects of these compounds which give the justification to the diverse antioxidant activities of the extracts.

Inhibition of the oxidative mechanisms lead to degenerative diseases is due to the antioxidants like phenolic acids; phenols and flavonoids which scavenge free radicals such as peroxide, hydro peroxide or lipid peroxyl[36]. Nahak determined the antioxidant activity for the ethanolic extract of *Costus speciosus* rhizome using DPPH assay[37]. Higher level of free radical scavenging activity was found by this assay with 71.61± 0.02% with IC50 value 25µg/ml. Here, in our study, the methanol and aqueous extracts of *C. pictus* was tested for antioxidant activity using DPPH radical scavenging assay and other antioxidant assays and CPME showed the promising results.

Here, in the present study, an unexplored medicinal plant *Costus pictus* was evaluated for the qualitative and quantitative parameters and their antioxidant activity. Total phenol content and antioxidant activity of the plant extracts showed significant connection. With this kind of examination, it would be easier to treat and prevent the human diseases occurring due to the free radical. Methanolic extract of the *C.pictus* could be the safer and effective antioxidant source. Therefore, advance research is required for the isolation identification and characterization of the active components present in the extracts. *In vivo* studies should perform for the confirmation of pharmacological activities possess by active compounds. Our findings also provide a strong rationale for further investigation to understand the molecular mechanism of the antioxidant and as the plant is commonly known as Insulin plant, exploration for the synergistic effect of antioxidant and antiglycation is possible.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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