

## **Antibacterial, antioxidant and antiglycation potential of *Costus pictus* from Southern region, India**

**Mala Majumdar and Prachi S. Parihar**

*Department of Biotechnology, Center for Post Graduate Studies, Jain University, Bangalore, India*

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### **ABSTRACT**

Methanol and aqueous extracts of *Costus pictus* was tested *in vitro* for their antibacterial and antioxidant activities. An antibacterial activity analysis was carried out using agar well diffusion and broth dilution techniques. The antibacterial assay of leaf, root, stem and flower extracts of *C. pictus* against four bacteria are presented. The minimal inhibitory concentrations (MICs) of all the extracts of *C. pictus* were found to be between 20-30µg/ml. Antioxidant activity of the extracts was determined by DPPH radical scavenging capacity assay. Methanol extracts showed the highest antioxidant activity. The total phenolics in the extracts were determined colorimetrically by using the Folin-Ciocalteu reagent. The total flavonoid content of the extracts was evaluated by a spectrophotometric method. The total phenolic content ranged from  $148.67 \pm 0.83$  to  $233.83 \pm 0.44$  µg of gallic acid/g of plant extracts. The flavonoid content varies from  $370.08 \pm 0.96$  µg of quercetin/ g of plant extracts. The reduction of NBT by Amadori products was inhibited by incubating glucose/ BSA system with different parts of *C. pictus* (leaf, root, stem and flower) and aminoguanidine, and every parts showed a stronger inhibitory effect than aminoguanidine. The ability of the test samples to inhibit NBT reduction was in the order of stem methanol > flower methanol > root methanol > leaf methanol. The present study reveals that the selected plant would exert several beneficial effects by virtue of its antioxidant and antiglycation activity and could be harnessed for drug formulation.

**Keywords:** *Costus pictus*, *In vitro*, antioxidant, antiglycation, AGEs.

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### **INTRODUCTION**

Infectious diseases are leading cause of death worldwide. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug- resistant pathogens [1]. Synthetic chemicals have their side effects and the development of bacterial resistance [2] to the presently available antibiotics has necessitated the search for new antimicrobial agents, which led to the screening of several medicinal plants [3, 4, 5]. Plant-based antimicrobials [6] represent a vast untapped source of medicines and are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [7]. More than 30% of modern drugs are derived from plants [8]. Systematic screening of folk medicines may result in the discovery of novel compounds [9].

Recently much attention has been focused on reactive oxygen species and free radicals which lead to oxidative stress [10,11]. Oxidative stress is an important contributor to a variety of pathological conditions including cardiovascular dysfunction, atherosclerosis, inflammation, carcinogenesis, drug toxicity, reperfusion injury, cancer, diabetes and neurodegenerative diseases [12]. This has prompted investigations in the use of natural antioxidants as a complementary therapeutic approach. The antioxidant properties of plant extracts have been attributed to their

polyphenolic content which have gained considerable importance due to their potential as antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and anti-inflammatory activities [13-16]. In this regard, some phenolics have shown antioxidative action in biological acting as scavengers of singlet oxygen and free radicals [17]. Many have reported that phenolic compounds [18] are the most active antioxidant due to their structure-activity relationships and are responsible for the observed antimicrobial activity [19]. Proteins are also modified by glucose through the glycation reaction, resulting in the formation of advanced glycation end (AGEs) which has received considerable attention in recent years. Advanced glycation end-products (AGEs) are generated in the diabetic mellitus as a result of chronic hyperglycemia and enhanced oxidative stress [20,21]. Via direct and receptor-dependent pathways, AGEs promote the development and progression of diabetic complications, including neuropathy, nephropathy, and cardiovascular disease [22,23]. AGEs can accumulate at many sites of the body in diabetes, including the heart and large blood vessels. Because the abundance of AGEs has direct relevance to the pathogenesis of diabetic complications, a clear understanding of the factors contributing to AGE formation may help in ameliorating tissue damage. Recent reports have suggested that metal-catalyzed oxidation reactions play a major role in accelerating the rate of AGE formation [24]. Therefore, agents with antiglycation and antioxidant properties may retard the process of AGE formation by preventing further oxidation of Amadori products and metal-catalyzed glucose oxidation.

*Costus pictus* D.Don, is one of the medicinally important plants, belongs to family Zingiberaceae [25]. It is commonly called as 'Insulin plant', as its leaves are proved to produce antidiabetic effects [26,27]. It is a newly introduced plant in India; originated probably in Mexico. The rhizomes of these plants possess several biologic activities like antioxidant, cytotoxic and antitumour [28]. Powdered leaves of the medicinal plant *Costus pictus* known to possess therapeutic effect, when supplemented to streptozotocin induced diabetic rats, is found to reduce blood glucose level by 21% after 15 days of supplementation [29]. The methanolic leaf extract of *Costus pictus* is used to lower blood glucose level in alloxan induced diabetic rats [26]. The antihyperglycemic and insulin secretory activity of an aqueous extract of *Costus pictus* leaf is investigated in streptozotocin induced diabetic rats[30]. Toxicity studies and antidiabetic activity of methanolic extract of this plant has been reported previously[28]. With this background, the antimicrobial, antioxidant and antiglycation activities of different parts of *Costus pictus* were analyzed. According to literature survey, very few reports are present related to the antiglycation activity of *C. pictus*. The relative influence of bioactive components like total phenol and flavonoid in the antioxidant activity was also studied.

## MATERIALS AND METHODS

### Ethnomedical Information and plant collection

Fresh plant parts were collected randomly from Dept. of Biotechnology, Horticulture Department, Hulimavu, Bangalore, Karnataka and voucher specimen were identified and authenticated at National Ayurveda Dietetics Research Institute, Bangalore, India.

### Extraction

Dried parts of the plants were ground to a fine powder with a grinder at room temperature. The powdered plant material (10 g) was then extracted using a Soxhlet extractor with 100 mL of methanol (MeOH) at 60 °C for 6 h. The extract was filtered and evaporated to dryness in a vacuum at 40 °C with a rotary evaporator. After determination of the yield, the extract was dissolved in methanol for further study [31].

Aqueous extraction was performed by soaking 100 g of the dry powder of plant materials in distilled water (500 ml) and shaken for 3 h by electric shaker. The suspension was filtered through muslin gauze and the filtrate kept in deep freezer for 24 h, which was then lyophilized. The lyophilized dry powder was then collected in stoppered sample vial, weighed and kept in desiccators to avoid absorption of water until used [32].

### Test Organisms

Bacterial isolates used in this work included *Shigella* sp., *Klebsiella* sp., *Bacillus subtilis* and *Escherichia coli* which were obtained from the Biotechnology department of Jain University, Bangalore, India. All the bacterial strains were suspended in nutrient broth and incubated at 37°C for 24h. Nutrient agar was used for testing the antibacterial activity.

**Determination of antibacterial activity**

The antibacterial activity was tested using standard agar well diffusion and broth dilution methods [32,33].

**Determination of Total Phenolic Content**

The total phenolic content of the crude aqueous and methanol extracts were determined using the method of McDonald *et al.*, 2001 with slight modifications [34]. The calibration curve was prepared by mixing ethanol solution of gallic acid (1ml; 0.025-0.400mg/ml) with 5ml Folin-Ciocalteu reagent and sodium carbonate (4ml, 0.7M). Absorbance values were measured at 765nm and the standard curve was drawn. 1ml of crude methanol extract (5g/L) was also mixed with the above reagents and after 30 min the absorbance was measured to determine the total phenolic content. All determinations were carried out in triplicates. The total phenolics concentration in the extract in gallic acid equivalents (GAE) was calculated by using the following formula:

$$T=CV/M$$

Where T=total phenolic contents, milligram per gram plant extract in GAE; C=the concentration of gallic acid established from the calibration curve, milligram per milliliter (mg/ml); V=the volume of extract, milliliter; M=the weight of methanol plant extract, gram.

**Determination of Total Flavonoid Content**

Total flavonoid contents were measured with the aluminium chloride colorimetric assay [35]. Aqueous and methanolic extracts has been adjusted to linearity range *i.e.* (400µg/ml) and different dilution of standard solution of quercetin (10-100µg/ml) were added to 10ml volumetric flask containing 4ml of distilled water. To the above mixture, 0.3ml of 5% NaNO<sub>2</sub> was added. After 5min, 0.3ml of 10% AlCl<sub>3</sub> was added. After 6min, 2ml of 1M NaOH was added and the total volume was made to 10ml with distilled water. Then the solution was mixed well and the absorbance was measured against a freshly prepared reagent blank at 510nm. Total flavonoid content of the extracts was expressed as percentage of quercetin equivalent per 100g dry weight of sample.

**Antioxidant activity (DPPH free radical scavenging activity) determination**

The antioxidant activity of the plant extracts was examined on the basis of the scavenging effect on the stable DPPH free radical activity [36]. Ethanol solution of DPPH (0.05 mM) (300 µl) was added to 40 µl of extract solution with different concentrations (0.02 - 2 mg/ml). DPPH solution was freshly prepared and kept in the dark at 4°C. Ethanol 96% (2.7 ml) was added and the mixture was shaken vigorously. The mixture was left to stand for 5 min and absorbance was measured spectrophotometrically at 517 nm. Ethanol was used to set the absorbance zero. A blank sample containing the same amount of ethanol and DPPH was also prepared. All determinations were performed in triplicate. The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following equation.

$$\text{Percent (\%)} \text{ inhibition of DPPH activity} = [(AB - AA) / AB] \times 100$$

Where AA and AB are the absorbance values of the test and of the blank sample, respectively. A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and represented as IC<sub>50</sub> value for each of the test solutions.

**Non-enzymatic protein glycation**

Experiments were performed according to the method of Wu *et al.*, 2009 with slight modification [37]. Briefly, bovine serum albumin (BSA, 20 mg/ml, 10 ml) was mixed with glucose (500 mM, 5 ml) and 0.02% sodium azide in phosphate buffer (200 mM, pH 7.4). The sample with different concentration dissolved in phosphate buffer (200 mM, pH 7.4, 5 ml) was added to the reaction mixture, and then the mixture was incubated for 30 days at 37 °C to obtain glycated materials. Aminoguanidine was used as a positive control. Some of the glycated materials were taken from the system for the following experiments in 0, 7th, 14th, 21st and 28th day after incubation.

**Spectrophotometric analyses of nitro-blue tetrazolium (NBT) reductive assay**

The procedure of NBT reductive assay followed the method of Baker *et al.*, 1994 with slight modifications [38]. The glycated material (0.5 ml) and NBT reagent (0.3 mM, 2.0 ml) in sodium carbonate buffer (100 mM, pH 10.35) were incubated at room temperature for 15 min, and the absorbance was read at 530 nm against a blank.

## RESULTS AND DISCUSSION

The antibacterial assay of leaf, root, stem and flower extracts of *C. pictus* against four bacteria are presented in Table 1. From the present data it is quite evident that both aqueous and methanol extracts of the plant parts exhibited inhibitory activity on the growth of the four tested microbes. According to Table, the methanolic extracts of leaf, stem and root of the plant showed better zone of inhibition against *Shigella flexneri* (16.33±3.2mm), *Klebsiella pneumoniae* (18.11±2.4mm), *Bacillus subtilis* (17.67±2.4mm), and *Escherichia coli* (16.33±2.3) at the concentration of 150 µg/ml respectively. All the results were compared with the standard antibiotics such as streptomycin and carbenicillin. For both aqueous and methanol extracts, a two way ANOVA test was conducted. The test revealed that extract concentrations have significant effect ( $P < 0.05$ ) on the inhibition of the three bacteria (*E. coli*, *Shigella flexneri* and *Bacillus subtilis*), however, the concentrations did not have significant effect ( $P > 0.05$ ) on the inhibition of *Klebsiella pneumoniae*. The inhibitory effect produced by the methanol extract of the leaf methanol on the three bacteria were higher than the effect produced by the aqueous extracts except for root methanol which showed maximum zone of inhibition against *Shigella flexneri*.

Data analysis obtained from the antimicrobial assay of the different extracts of *C. pictus* were in accordance to the similar findings of Adejumbi *et al.*, 2008 [39]. Earlier work on *C. pictus* [27], showed marked activity against *Bacillus cereus*, *Enterobacter faecalis*, *Salmonella paratyphi*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, *Streptococcus faecalis* and *Klebsiella pneumoniae* (22-40mm/50µl inhibition zone). The plant extracts prepared with methanol as solvent provided more consistent antimicrobial activity, as also reported earlier [40,41]. None of the aqueous extracts of *C. pictus* produced better zones of inhibition in the Kirby-Bauer analysis. This might have resulted from the lack of solubility of the active constituents in aqueous solutions [42]. Similar results were exhibited earlier [43] where aqueous extracts did not show much significant activity while the organic extracts (petroleum ether and methanol) showed the highest activity against the test bacteria. The results of minimum inhibitory concentrations are shown in the table 2. The result showed that methanolic extract of root is highly sensitive against *E. coli* and *Shigella flexneri* (MIC-30 µg/ml), leaf methanol extract is highly sensitive against *E. coli* and *Bacillus subtilis* (MIC-30µg/ml and 25µg/ml respectively). Flower extract is highly sensitive against *Klebsiella pneumoniae* (MIC- 20µg/ml). Significant antioxidant properties of the *C. pictus* have been recorded by DPPH methods with phytochemicals like phenols, flavonoids, terpenoids that are necessary for the reduction in the occurrences of many diseases [44].

Unlike other free radicals such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions such as metal ion chelation and enzyme inhibition [45]. DPPH assay is known to give reliable information concerning the antioxidant ability of the tested compounds. Fig 1 demonstrates a significant reduction in the concentration of DPPH due to scavenging ability of methanolic extract of all the parts of *C. pictus* [28]. Table - 3 depicted the total phenolic and flavonoid contents of extracts of *C. pictus*. The flower extract of *C. pictus* was found to contain a noticeable amount of total phenols and flavonoids (233.83 ± 0.44µg/g; 930.00±0.00µg/g) which play a major role in controlling antioxidants. It is reported that the phenolics are responsible for the variation in the antioxidant activity of the plant [46]. From the Table 4, the reduction of NBT by Amadori products was inhibited by incubating glucose/ BSA system with different parts of the plant and aminoguanidine. The ability of the test samples to inhibit NBT reduction was in the order of stem methanol > flower methanol > root methanol > leaf methanol. The above results were in support with Liang *et al.*, 2011 which also showed a significant reduction of NBT with increasing time [47]. The BSA- glucose model adopted in this study provides a useful tool for assessing the effects of various extracts on the non-enzymatic glycation process.

**Table 1: Antibacterial activity of *Costus pictus* by the well diffusion method**

Microorganism	Leaf		Root		Stem		Flower	
	Concentration (150µg/ml)							
	Aq.	Meth	Aq.	Meth	Aq.	Meth	Aq.	Meth
<i>Shigella flexneri</i>	12.33±2.0	13.67±2.2	8.33±1.6	16.33±3.2	11.7±1.9	15.33±2.2	6.70±1.5	12.7±2.0
<i>Klebsiella pneumoniae</i>	10.66±1.9	18.11±2.4	7.0±1.5	15.3±2.3	10.7±1.9	16.7±2.37	8.33±1.6	11.7±2.0
<i>Bacillus subtilis</i>	12.33±2.0	17.67±2.4	7.68±1.6	16.0±2.3	11.7±1.9	13.33±2.1	10.7±1.9	11.7±1.9
<i>Escherichia coli</i>	9.66±1.8	15.67±2.3	9.33±1.7	15.3±2.3	11.7±1.9	16.33±2.3	8±1.63	10.7±1.9

Values are mean ± SE (n=3) at  $P < 0.05$

Table 2: The MIC values ( $\mu\text{g/ml}$ ) of *Costus pictus* against bacteria tested in the broth dilution assay

Microorganism	Leaf		Root		Stem		Flower	
	Concentration (5-65 $\mu\text{g/ml}$ )							
	Aq.	Meth	Aq.	Meth	Aq.	Meth	Aq.	Meth
<i>Shigella flexneri</i>	55	40	45	30	50	50	50	40
<i>Klebsiella pneumonia</i>	50	25	40	35	45	30	35	20
<i>Bacillus subtilis</i>	50	25	50	30	40	35	50	45
<i>Escherichia coli</i>	55	30	55	30	45	35	45	45

Table 3: Total phenolic and flavonoid content of *C. pictus*

Plant parts	Total Phenol Content( $\mu\text{g/g}$ )	Flavonoid Content( $\mu\text{g/g}$ )
Leaf	148.67 $\pm$ 0.83	370.08 $\pm$ 0.96
Root	148.33 $\pm$ 0.33	405.41 $\pm$ 0.22
Stem	228.33 $\pm$ 0.44	426.33 $\pm$ 0.68
Flower	233.83 $\pm$ 0.44	930.00 $\pm$ 0.00

Each value in the table was obtained by calculating the average of three experiments  $\pm$  SE

Table 4 Effect of methanolic extracts (100 $\mu\text{g/ml}$ ) of *C. pictus* and aminoguanidine

Plant Parts	% Glycation	% Inhibition
BSA+ Glucose	100	0
Leaf methanol	46.4	53.6
Stem methanol	30.4	69.6
Root Methanol	37.7	62.3
Flower Methanol	31.9	68.1
Aminoguanidine	55.1	44.9

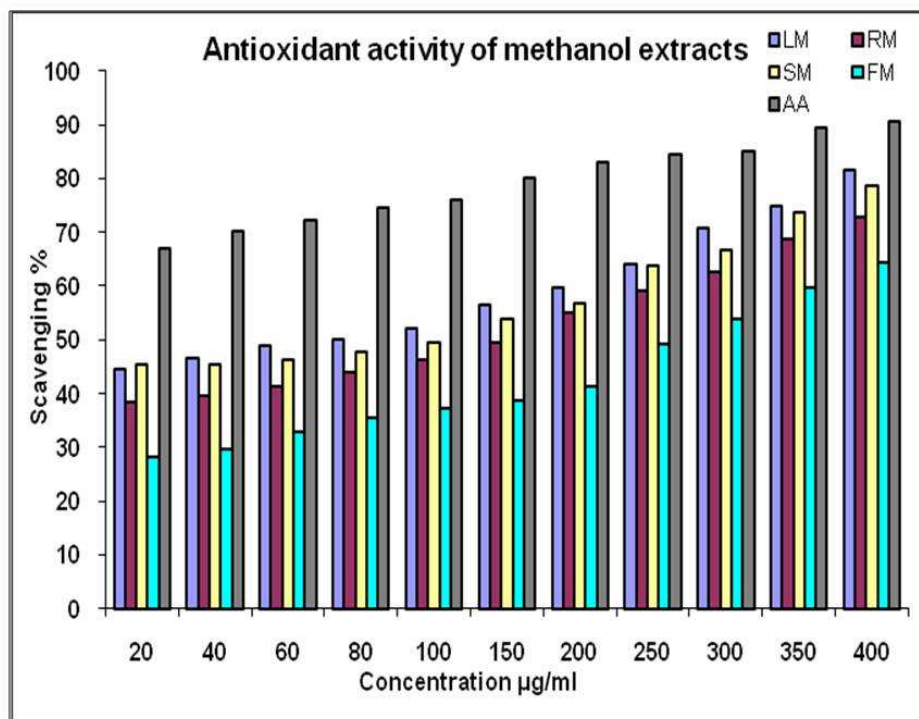


Fig. 1 Antioxidant activity of methanol extracts

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**CONCLUSION**

In the present study, we evaluated the antimicrobial activity of the different parts (leaf, flower, stem and root) of *Costus pictus* which exhibited pronounced activity against all tested microorganisms and the activity was quite comparable with the standard antibiotics screened under similar conditions. The remarkable antibacterial activity exhibited by the methanolic extracts can be attributed to the synergic effect of the antimicrobial agents present in it. In conclusion, the data presented here indicate that the methanolic extracts of flower and stem of *C. pictus* possess *in vitro* antioxidant activity against oxidative protein damage and should be considered as new sources of natural antioxidants. In this study, *C. pictus* extracts showed protective effects against glucose-induced protein modifications, significantly inhibiting the AGEs formation. Our findings also provide a strong rationale for further investigation to understand the molecular mechanism of the antiglycation activity of the extracts with both high antioxidant and high antiglycation activities and explore the possible synergistic antiglycation between them.

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