

EVALUATION OF MEDICINAL ACTIVITIES OF *COSTUS SPECIOSUS****Indu Sanadhya¹, Annika Durve¹, Meeta Bhot², Jossy Varghese² and Naresh Chandra³**¹Department of Biotechnology, Birla College of Arts, Science and Commerce, Kalyan-421304, Mumbai, India.²Department of Botany, Birla College of Arts, Science and Commerce, Kalyan-421304, Mumbai, India.³Pro-Vice Chancellor, University of MumbaiArticle Received on
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Author****Dr Indu Sanadhya**Department of Biotechnology,
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and Commerce, Kalyan-
421304, Mumbai, India.**ABSTRACT**

The aim of the present work is to evaluate the antioxidant, anti-inflammatory and an anti-viral activity of *Costus speciosus* leaves extract through *in vitro* bioassay analysis. Antioxidant activity was studied by *in vitro* biochemical assays, anti-inflammatory by human RBC membrane stabilisation *in vitro* assay and anti-viral through haemagglutination and anti-haemolysis of human RBCs methods. The results were found to be positive for all the assays and reveal the use of *Costus speciosus* as a good herbal source of antioxidant, anti-inflammatory and anti-viral agent. TLC fingerprinting data reveals that the positive results of *Costus speciosus* leaves extract for medicinal properties may be due to presence of separated phytochemicals.

KEY WORDS: *Costus speciosus*, antioxidant, anti-inflammatory, anti-viral, TLC.**INTRODUCTION**

Costus speciosus, belongs to the family Zingiberaceae is an erect herbaceous plant up to 2 m height with long lanceolate leaves and white fragrant flowers in terminal clusters. The plant flowers during the months of July and August (Sarin *et al.*, 1974). It is mentioned in Ayurvedic literature as antidiabetic plant. The plant is the main source of diosgenin, tigogenin and saponins. Also contain aliphatic hydroxyl ketones, triterpenes, starch mucilage, oxa-acids, fatty acids, abscisic acid, and corticosteroids. Medicinally it is used as anti-inflammatory, anti-arthritic, antidiabetic, anti-cholinesterase and ecbolic agent. Many

unknown and lesser known plants are used in folk and tribal medicinal practices in India. The medicinal values of these plants are not known much to the scientific world. *Costus speciosus* is one such plant used by the locals of the regions of eastern Himalayan belt (Rajesh *et al.*, 2009). In the present study, antioxidant, anti-inflammatory (RBC membrane stabilizing activity), haemagglutination inhibition activity and hydrogen peroxide induced haemolysis inhibition activities was studied.

Antioxidants are the compounds that protect the cells against the damaging effects of free radicals by scavenging them. Free radicals are the reactive oxygen and nitrogen species produced in the body due to the oxidative stress. This oxidative stress is linked with various chronic diseases such as cancer, diabetes, aging, inflammation and neurodegenerative disorders. Plants are the valuable source of antioxidants because of the presence of many free radicals scavenging phytoconstituents such as phenols, tannins, flavonoids, carotenoids, coumarins, anthocyanin, and glycosides. These constituents possess ideal structural chemistry for free radical scavenging activity (Indu *et al.*, 2013).

Inflammation is a bodily response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed physiological functions. It is triggered by the release of chemical mediators from injured tissue and migrating cells. The commonly used drug for management of inflammatory conditions are non-steroidal anti-inflammatory drugs, which have several adverse effects especially gastric irritation leading to formation of gastric ulcers (Megha *et al.*, 2013). Therefore, many researches have focused in recent years on medicinal plants considered as safe produce with fewer side-effects for patients, thus, these plants become sources of herbal anti-inflammatory agents (Kossivi *et al.*, 2014). Haemagglutination inhibition and hydrogen peroxide induced haemolysis inhibition activities *in vitro* model system represents the anti-viral activity of the plant extracts.

MATERIAL AND METHODS

Preparation of plant extract

Costus speciosus plant was collected from the forest area of Jhabua District of Madhya Pradesh. Methanol extract of fresh leaves of *Costus speciosus* was prepared using cold extraction method by continuous shaking in orbital shaker at 100 rpm (25°C). After 24 hours, the extract was filtered and residue of the sample was re-extracted by the same procedure using methanol as the solvent for the period of one week. After one week, the collected

filtrates were concentrated by evaporating the methanol at 50°C. Dried residue of the sample was re-dissolved in methanol and used for the further analysis (Indu and Annika, 2014)

Antioxidant study

Total antioxidant activity

Total antioxidant activity of methanol extract of *Costus speciosus* leaves was determined according to the method of Prieto *et al.* An aliquot of leaf extract of 1.0 ml (1.0 mg/ml) was combined with 1.0 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated in a boiling water bath at 95 °C for 90 min. Then, the samples were cooled to room temperature and the absorbance was measured at 695 nm against blank prepared in the same conditions by replacing sample with 1.0 ml of solvent. All the analyses were performed in triplicate and the results were averaged. Antioxidant capacity was expressed as Ascorbic acid equivalents (mmol/mg) (Prieto *et al.*, 1999).

Ferric reducing antioxidant power

The reducing power of methanol extract of *Costus speciosus* leaves and Ascorbic acid was determined according to the method of Oyaizu (1986). 2.0, 4.0, 6.0, 8.0 and 10.0 mg/ml of the extracts and 0.2, 0.4, 0.6, 0.8 and 1.0mg/ml of standard (ascorbic acid) was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture was incubated at 50°C for 20 minutes. 2.5 ml of trichloroacetic acid (10%) was added to the 2.5 ml of the reaction mixture, which was then centrifuged at 3000 g for 10 minutes. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%) and the absorbance was measured at 700 nm against blank prepared in the same conditions by replacing sample with 1.0 ml of solvent. All the analyses were performed in triplicate and the results were averaged. Increased absorbance of the reaction mixture indicated increasing reducing power.

Anti-inflammatory activity (RBC membrane stabilising activity)

Preparation of red blood cells (RBCs) suspension

Fresh whole human blood (10 ml) was collected and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of blood was measured and re-constituted as 10% v/v suspension with normal saline (Megha *et al.*, 2013).

Heat induced haemolytic assay

The reaction mixture (2 ml) consisted of 1 ml of methanol extract of *Costus speciosus* leaves (re-dissolved in DMSO) at different concentrations (2.0, 4.0, 6.0, 8.0 and 10.0 mg/ml) and 1 ml of 10% RBCs suspension. Positive control was made using saline while negative control consists of DMSO. Aspirin was taken as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56°C for 30 min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. All the analyses were performed in triplicate and the results were averaged (Megha *et al.*, 2013). The percentage of haemolysis of HRBC membrane can be calculated as follows (Seema *et al.*, 2011): % Haemolysis = (Optical density of Test sample / Optical density of Control) X 100 The percentage of HRBC membrane stabilisation can be calculated as follows: % Protection = 100 – [(Optical density of Test sample / Optical density of Control) X 100]

Haemagglutination inhibition activity

Haemagglutination activity of methanol extract of *Costus speciosus* leaves were tested against human erythrocyte blood by the method of Saha *et al* (2001). Fresh blood was collected from healthy persons, centrifuged and the erythrocytes were separated. About 4% erythrocyte suspension was prepared in phosphate buffer (pH 7.4). About 1 mL of the plant extract at different concentration of 2.0, 4.0, 6.0, 8.0 and 10.0 mg/ml (re-dissolved in DMSO) was taken with 1 mL of 1% erythrocyte and incubated at 4°C for 10 mins. After incubation, the results were noted. Smooth button formation in bottom indicated negative activity, while a rough granular deposition at bottom showed positive activity. The intensity of haemagglutination was determined from the extent of deposition.

Anti-haemolytic assay

Inhibition of H₂O₂ induced red blood cell (RBC) haemolysis of methanolic extract of *Costus speciosus* leaves was examined by the *in vitro* method of Tavazzi *et al* (2001). The erythrocytes from human blood were separated by centrifugation and washed with saline or isotonic sodium phosphate buffer (pH 7.4) until the supernatant was colourless. The erythrocytes were then diluted with saline or phosphate buffer to give a 4% suspension. Varying amounts of sample (2.0, 4.0, 6.0, 8.0 and 10.0 mg/ml, re-dissolved in DMSO) with saline or buffer were added to 2 mL of the suspension of erythrocytes and the volume was

made up to 3.5 mL with saline or buffer. This mixture was pre-incubated for 120 min and then 0.5 mL H₂O₂ solutions of appropriate concentration in saline or buffer were added. The concentration of H₂O₂ in the reaction mixture was adjusted so as to bring 90% haemolysis of blood cells after 120 min incubation. Incubation was concluded after these time intervals by centrifugation during 5 min at 1 000 g and the extent of haemolysis was determined by measurement of the absorbance at 540 nm corresponding to haemoglobin liberation. Anti-haemolytic activity was expressed as the inhibition percentage and was calculated using the following formula:

$$\text{Anti-haemolytic activity (\%)} = (\text{Control } 540 \text{ nm} - \text{Sample } 540 \text{ nm}) \times 100 / \text{Control } 540 \text{ nm}$$

Where, Sample 540 nm was the absorbance of the sample and Control 540 nm was the absorbance of the control.

TLC fingerprinting

Methanol extract of *Costus speciosus* leaves was analysed by TLC for the presence of different components. Samples were prepared according to the method of Wagner and Bladt (1996). Silica gel G₆₀ F₂₅₄ TLC precoated plate (Merck) was used as adsorbent. The plate was developed using chloroform: glacial acetic acid: methanol: water (5:2:2:1) as mobile phase. The number of bands present in the samples was detected by spraying the plate with Anisaldehyde sulphuric acid reagent.

RESULT AND DISCUSSION

Antioxidant Study

Total antioxidant activity

Total antioxidant activity is a quantitative assay, since the antioxidant activity is expressed as the number of equivalents of Ascorbic acid. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH with the maximal absorption at 695nm (Prieto *et al.*, 1999). The linear equation of ascorbic acid for total antioxidant activity was found to be $y=2.676x$ with $r^2=0.9979$ (Fig. 1). The antioxidant activity of leaf extract was found to be 0.22 ± 0.02 mM Ascorbic acid/mg extract of *Costus speciosus* leaves.

Ferric reducing antioxidant power

The reducing ability of a compound generally depends on the presence of reductants which have been exhibited antioxidative potential by breaking the free radical chain, donating a

hydrogen atom (Saha *et al.*, 2008). The presence of reductants (i.e. antioxidants) in plant extract causes the reduction of the Fe³⁺/ferricyanide complex to the ferrous form. Therefore, the Fe²⁺ can be monitored by measuring the formation of Perl's Prussian blue colour at 700 nm. Increase in absorbance at 700 nm reflects an increase in reductive ability (Duh *et al.*, 1999). As the concentration of *Costus speciosus* leaves extract and Ascorbic acid increases the ferric reducing antioxidant power increases (Fig. 2). The reducing power of extract of was very potent and it increases as the quantity of sample increases.

Anti-inflammatory activity (RBC membrane stabilising activity)

Heat induced haemolytic assay

The inhibition of heat induced HRBC membrane lysis i.e, stabilisation of HRBC membrane was taken as a measure of the anti-inflammatory activity. Methanolic extract of *Costus speciosus* leaves are effective in inhibiting the heat induced haemolysis of HRBC at different concentrations (2.0-10.0 mg/ml. It showed the maximum stabilisation of HRBC membrane 80.0% at 10mg/ml. With the increasing concentration the membrane haemolysis is decreased and membrane stabilisation / protection is increased (Table 1). Hence anti-inflammatory activity of the extracts was concentration dependent.

The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may well stabilize lysosomal membranes. Stabilization of lysosomal is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bacterial enzymes and proteases, which causes further tissue inflammation and damage upon extra cellular release. The lysosomal enzymes released during inflammation produce a various disorders. The extra cellular activity of these enzymes are said to be related to acute or chronic inflammation. The anti-inflammatory constituents act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane (Govindappa *et al.*, 2010).

Haemagglutination inhibition activity

Many viruses attach to molecules present on the surface of RBCs. Haemagglutination inhibition assay was performed to investigate the receptor binding affinity of the compounds present in the crude methanolic extract of *Costus speciosus* leaves on human erythrocytes or there receptors may present in viruses. It was found that methanolic extract of *Costus speciosus* leaves have the binding affinity to the receptors of erythrocytes and prevent

agglutination (rough granular deposition at bottom) (Table 2 and Fig. 3). The results showed possible benefits of extract as an antiviral therapeutics (Repon *et al.*, 2013).

Anti-haemolytic assay

Methanolic extract of *Costus speciosus* leaves showed concentration dependent anti-haemolysis of RBCs. As the concentration increases anti-haemolysis activity of extract increases and it was to be 83.33% at 10 mg/ml of concentration (Table 2). Biomembranes may be most susceptible to free radical attacks due to its content of polyunsaturated fatty acids. The lipid oxidation by the presence of hydrogen peroxide leads to the formation of free radicals that leads to the haemolysis of the erythrocyte and expulsion of its haemoglobin content. The content of expelled haemoglobin is monitored spectroscopically. This test tests the antioxidant levels in a sample by its potential to prevent haemolysis (Repon *et al.*, 2013).

TLC Fingerprinting

In TLC finger printing six bands were separated in the methanol extract of *Costus speciosus* leaves with the R_f values of 0.56, 0.45, 0.40, 0.32, 0.22 and 0.15 (Fig. 5). TLC fingerprinting helps in the separation of phytoconstituents present in the extract. Positive results for antioxidant, anti-inflammatory, haemagglutination inhibition and anti-haemolytic activities may be due to the presence of these phytoconstituents.

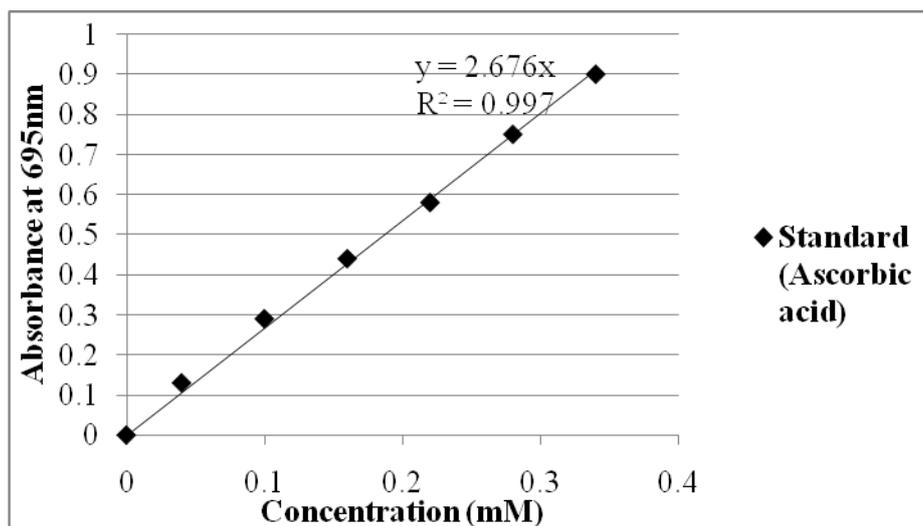


Figure 1: Standard calibration curve of Ascorbic acid for total antioxidant activity analysis of *Costus speciosus* leaves extract

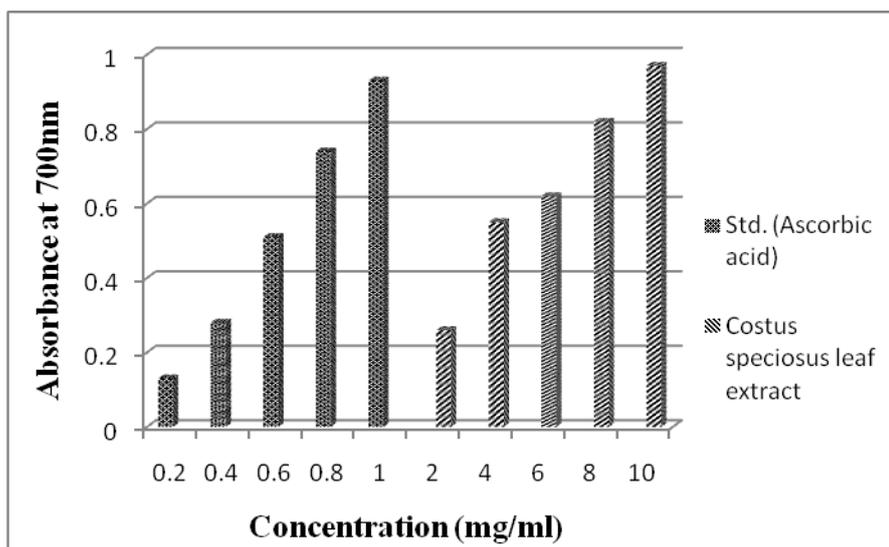


Figure 2: Ferric reducing antioxidant power of *Costus speciosus* leaves extract and Ascorbic acid

Table 1: Effect of *Costus speciosus* leaves extract and Standard (Aspirin) on HRBC membrane haemolysis and membrane stabilization

Concentration (mg/ml)	% Hemolysis of <i>C. speciosus</i>	% Stabilisation of <i>C. speciosus</i>	% Hemolysis of Aspirin	% Stabilisation of Aspirin
2.0	80.0	20.0	45.16	54.84
4.0	38.18	61.81	24.12	75.88
6.0	32.72	67.27	19.7	80.30
8.0	29.09	70.91	15.6	84.40
10.0	20.0	80.0	7.35	92.65

Table 2: Haemagglutination inhibition and Anti-haemolytic activities of *Costus speciosus* leaves extract

Concentration (mg/ml)	Haemagglutination inhibition activity of <i>C. speciosus</i>	Anti-haemolytic activity of <i>C. speciosus</i>
2.0	+	33.0
4.0	+	50.0
6.0	+	66.70
8.0	+	75.0
10.0	+	83.33
Control	-	-

-: Smooth button formation in bottom indicated no haemagglutination inhibition activity

+: rough granular deposition at bottom indicated haemagglutination inhibition activity



Figure 3: Haemagglutination inhibition activity of *Costus speciosus* leaves extract, A= rough granular deposition at bottom indicated haemagglutination inhibition activity of extract, B= Smooth button formation in bottom indicated no haemagglutination inhibition activity in control (without extract)

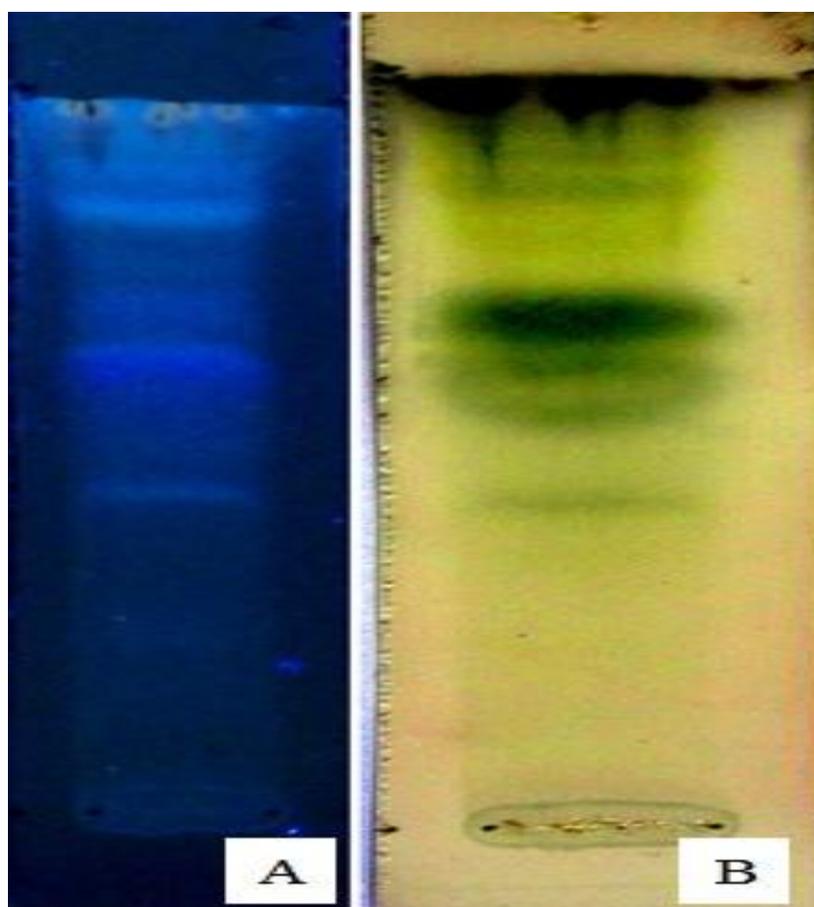


Figure 4: TLC fingerprinting of Methanol extract of *Costus speciosus* leaves extract A= chromatogram at 366 nm before spraying with Anisaldehyde sulphuric acid reagent, B= chromatogram in visible light after spraying with Anisaldehyde sulphuric acid reagent

CONCLUSION

The methanol extract of *Costus speciosus* leaves showed potent antioxidant, anti-inflammatory, haemagglutination inhibition and anti-haemolytic activities, may be due to the presence of different phytoconstituents. Thus, the *Costus speciosus* can be introduced as safe source of herbal antioxidant, anti-inflammatory and anti-viral drug. Further studies can be done on the isolation of bioactive compound from the leaves extract of *Costus speciosus*.

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