



**ANTIOXIDATIVE AND PHARMACOLOGICAL POTENTIAL OF A ZINGIBERACEOUS  
HERB *COSTUS SPECIOSUS* (KOEN)**

Sonali Sethi<sup>1</sup>, Om Prakash<sup>\*1</sup>, M. Chandra<sup>1</sup>, H. Punetha<sup>2</sup>, M. Battra<sup>3</sup> and A. K. Pant<sup>1</sup>

<sup>1</sup>Department of Chemistry, <sup>2</sup>Department of Biochemistry, College of Basic Sciences and Humanities,

<sup>3</sup>Department of Vet. Pathology, Department of Vet. Medicine, College of Veterinary & Animal Sciences,  
G.B. Pant University of Agriculture & Technology, Pantnagar, U.S. Nagar, Uttarakhand, India.

**\*Corresponding Author: Dr. Om Prakash**

Department of Chemistry, G.B. Pant University of Agriculture & Technology, Pantnagar, U.S. Nagar, Uttarakhand, India.

Article Received on 29/05/2016

Article Revised on 18/06/2016

Article Accepted on 08/07/2016

**ABSTRACT**

Rhizomes of *Costus speciosus* was analyzed for antioxidative and pharmacological activities. Rhizomes were extracted by various solvents with different polarities using various antioxidant assay systems as compared to known standards. Methanolic and petroleum ether extracts revealed good antioxidative activity in a dose dependent manner. Histopathological and biochemical evidences indicated that methanolic extracts at dose level of 500 mg/kg possess significant hepatoprotective activity against CCl<sub>4</sub> mediated tissue damage and imposed healthy effect on kidney function. The methanolic extract analyzed for antipyretic, anti-inflammatory activity and analgesic activities were noticed significant at a dose level of 100 mg/kg body wt. The methanolic extract at this level found to be comparable or slightly lesser as compared to known standard, ibuprofen. The present findings indicated that tested extracts have good source of flavonols imparting significant antioxidative and pharmacological activities.

**KEYWORDS:** Analgesic, antioxidant, antipyretic, antiinflammatory, *Costus speciosus*.

**INTRODUCTION**

*Costus speciosus* (Koen.) an important medicinal and ornamental plant of family Costaceae is widely distributed throughout the India in moist tropical evergreen forests, up to an altitude of 1200 m. Its ideal place of collection is from the Himachal sub Himalayan tracts and Western Ghats.<sup>[1]</sup> The rhizomes and roots are aphrodisiac, purgative, antihelmintic, bitter, astringent, acrid, depurative febrifuge, expectorant, tonic, improves digestion and stimulant, herb that clears toxins. Juice of the rhizomes are frequently employed for relief from headache. The alcoholic extract of *C. speciosus* was found to be promising antifungal agent.<sup>[2]</sup> An alkaloid isolated from *C. speciosus* rhizomes possess papaverine-like smooth muscle relaxant and antispasmodic activities. Its rhizomes are found beneficiary in treating pneumonia, rheumatism, dropsy, urinary diseases and jaundice. Bruised leaves and decoction of stems are used in fever and dysentery.<sup>[3]</sup>

*C. speciosus* has recently gained much importance as a commercial source of diosgenin, the latter being a suitable material for the synthesis of corticosteroids and oral contraceptives. Survey of this plant from different parts of India has been carried out by many workers.<sup>[4]</sup> Keeping these view and as part of our ongoing search on Zingiberaceae medicinal plants of India, the present study focused to evaluate antioxidant activity of various

extracts of diverse polarity from *C. speciosus* rhizomes and biochemical & pharmacological activities of methanolic extract.

**MATERIAL AND METHODS**

**Source of Plant Material**

The rhizomes of *C. speciosus* were collected from Tarai region of Pantnagar, Uttarakhand in India. The plant was identified by Dr. D.S. Rawat (Assistant Professor and plant taxonomist), Department of Biological Science, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar.

**Preparation of extracts**

The shade dried rhizomes were coarsely powdered and subjected to successive extraction using Soxhlet apparatus. The extraction was done using solvents of different polarity in their increasing order of polarity. The extracts were concentrated using vacuum rotatory evaporator at 45±5°C.

**Antioxidant activity**

**Reducing Power Activity**

The reducing power of the extracts was determined by the method reported earlier.<sup>[5]</sup> Absorbance at 700 nm is plotted against the different amount of extracts. Increase in absorbance indicates increase in reducing power.

**Effect on the chelating activity of Fe<sup>2+</sup>**

This method is based on the principle of the Fe<sup>2+</sup> chelating ability of the antioxidant by measuring the ferrous iron-ferrozine complex formed at 562 nm. [6] Chelating activity was calculated by following equation.

$$\text{Chelating activity (\%)} = \left[1 - \frac{A_t}{A_o} \times 100\right]$$

(where A<sub>t</sub> is the absorbance of the sample and A<sub>o</sub> is the absorbance of the control at 562 nm)

**2, 2'-Diphenyl picryl hydrazyl free radical scavenging activity**

This scavenging effect on the DPPH radical was determined according to the methods developed earlier. [5,7] DPPH Radical scavenging activity was calculated by following equation.

$$\text{DPPH Radical scavenging activity(\%)} = \left[1 - \frac{A_t}{A_o} \times 100\right]$$

(where A<sub>t</sub> is the absorbance of the sample and A<sub>o</sub> is the absorbance of the control at 517nm).

**Nitric oxide radical scavenging activity**

Nitric oxide (NO) was generated from sodium nitroprusside (SNP) and was measured by the Griess reagent. [8] Nitric oxide scavenging activity was calculated by following equation.

$$\text{Nitric Oxide scavenged (\%)} = \left[1 - \frac{A_t}{A_o} \times 100\right]$$

(where A<sub>t</sub> is the absorbance of the sample and A<sub>o</sub> is the absorbance of the control at 546 nm)

**Super oxide radical scavenging activity**

It is based on the method described by Wei *et al.*, [9] Super oxide radical scavenging activity was calculated by following equation.

$$\text{Superoxide radical scavenged (\%)} = \left[1 - \frac{A_t}{A_o} \times 100\right]$$

(Where A<sub>t</sub> is the absorbance of the sample and A<sub>o</sub> is the absorbance of the control at 560 nm)

**Hydroxyl radical scavenging activity**

The hydroxyl radical scavenging capacity of different polarity extracts were evaluated by the method described by Ramalingam *et al.*, [10] Hydroxyl radical scavenging activity was calculated by following equation.

$$\text{Hydroxyl radical scavenging (\%)} = \left[1 - \frac{A_t}{A_o} \times 100\right]$$

(Where A<sub>t</sub> is the absorbance of the sample and A<sub>o</sub> is the absorbance of the control at 560 nm)

**Experimental animals**

Female albino rats 2-2.5 months old weighing between 23-28 gm, were obtained from the animal facility, IVRI (Indian Veterinary Research Institute), Barielly. The animals were maintained under standard management condition and acclimatized for two weeks before the beginning of the experiment. Feed and water was given throughout the study. All the animal experiments were conducted according to the ethical norms approved by the Institutional ethical committee.

**Histopathological study**

Hepatoprotective activity through histopathological study on rat liver was performed by cutting liver into slices and properly washed in Ringer's solution and then soaked. The, slices then fixed in Carnoy's fluid I subsequently paraffin embedding. Sections were stained with haematoxylin and eosin and observed microscopically for histopathological changes.

**Evaluation of antipyretic activity**

The antipyretic activity of methanolic extract was carried by using brewer's yeast induced pyrexia method. Fever was induced by means of subcutaneously injecting 10.0 ml/kg of a 20% w/v suspension of Brewer's yeast in normal saline. Only animals whose rectal temperature increased by at least 1.0°C after 18 h of this yeast injection were included for the study. The normal rectal temperature of each animal was measured by using a flexible thermostat probe and temperature was recorded using Telethermometer. The experimental animals were divided into four groups containing six animals in each group. The control group (3) was orally administered 0.2 ml saline water while the standard group (4) was given 40 mg/kg Ibuprofen and groups 1 and 2 were given 50 mg/kg and 100 mg/kg of methanol extract of *C. speciosus*, respectively. The rectal temperature was recorded at time intervals of 1, 2, and 3 h after drug administration.

**Anti-inflammatory studies**

Paw oedema was induced by injecting 0.1 ml of 1% (w/v) carrageenan suspension into the sub planter region of the right hind paw of the rats. [11] The control group (3) was orally administered saline (0.2 ml/kg) while the standard group (4) was given Ibuprofen (40 mg/kg), and groups 1 and 2 were given 50 and 100 mg/kg of the test extract, 1 h before carrageenan injection. The measurement of paw oedema was carried out by displacement technique using plethysmometer to find out the circumference of paw oedema immediately at 0, 4 and 24 h.

**Analgesic activity**

The peripheral analgesic activity of the methanolic extract was assessed in acetic acid induced writhing experiments using rats. The standard procedure

suggested by Veerappan *et al.*,<sup>[12]</sup> was used for observing the abdominal constriction writhings resulting from intraperitoneal injection of acetic acid (10 ml/kg of 0.6% v/v glacial acetic acid solution in water). Saline (0.2ml ml/kg) was orally administered to group 3 (control group) whereas standard ibuprofen (40 mg/kg) was prescribed for group 4 and 50 and 100 mg/kg test extracts were orally administered to groups 1 and 2, respectively. Acetic acid solution was then administered to each animal after 30 min and the number of writhings counted for the next 15 min.

## RESULTS AND DISCUSSIONS

### Antioxidant activity

Different extracts (obtained in the solvents of different polarity) from *C. speciosus* were taken for phenolic assay and antioxidative potential by various methods.

**Table 1: Phenolic assay of different extracts of *C. speciosus*.**

S. No.	Extracts	T.P mg/g of Gallic acid	Flavonols mg/g of Catechin	ODP mg/g of Catechol
1	CSPE	521.81	216.84	73.42
2	CSCH	280.90	173.84	43.00
3	CSEA	253.63	143.46	56.50
4	CSCL	517.00	230.38	44.00
5	CSAC	341.81	210.38	34.50
6	CSME	404.54	285.00	65.00

CSPE = *Costus speciosus petroleum ether extract*, CSCH = *Costus speciosus cyclohexane extract*, CSEA = *Costus speciosus ethylacetate extract*, CSCL = *Costus speciosus chloroform extract*, CSAC = *Costus speciosus acetone extract*, CSME = *Costus speciosus methanol extract*, T.P=Total phenol, ODP= *o*-dihydroxyphenol

### Reducing power activity

The power of reducing activity associated with the presence of reductones, which exert antioxidant action by breaking the free radical chain through donating a hydrogen atom.<sup>[13]</sup> In this assay, ferric ions ( $Fe^{3+}$ ) are reduced to ferrous ions ( $Fe^{2+}$ ) with colour changed from yellow to bluish green at 700 nm. All the extracts exhibited good reducing power activity in a dose dependent manner with different potential. The reducing power of rhizome at 5mg/ml in decreasing order: CSPE ( $A_{700} = 0.559 \pm 0.001$ ) > CSAC ( $A_{700} = 0.480 \pm 0.001$ ) > CSME ( $A_{700} = 0.474 \pm 0.002$ ) > CSCH ( $A_{700} = 0.468 \pm 0.001$ ) > CSCL ( $A_{700} = 0.456 \pm 0.001$ ) > CSEA ( $A_{700} = 0.368 \pm 0.001$ ) as compared to known standards: BHT ( $A_{700} = 0.550 \pm 0.008$ ), catechin ( $A_{700} = 0.455 \pm 0.006$ ) and gallic acid ( $A_{700} = 0.575 \pm 0.003$ ) (Table 2). The reducing power of *C. speciosus* is probably due to the presence of di and monohydroxyl substitutions in the aromatic ring, which possess potent hydrogen donating abilities.<sup>[14]</sup>

### Effect on the Chelating activity of $Fe^{2+}$

Ferrozine, a chelating reagent, was used to indicate the presence of chelator in the reaction. Ferrozine forms a complex with free  $Fe^{2+}$  but not with  $Fe^{3+}$ . In presence of chelating agents, the complex formation between ferrous and ferrozine is disturbed, resulting in decrease the color of the complex. Measurement of color reduction therefore allows estimating the metal chelating activity

### Phenolic assay

*C. speciosus* rhizomes extracts were studied quantitatively in terms of their total phenols, flavonols and ortho dihydroxy phenol (ODP) with the help of their respective calibration curves obtained colorimetrically. The data revealed different different quantitative make up of various extracts (Table 1). The total phenol content in term of gallic acid equivalent was reported to be maximum in CSPE (521.81mg/g) along with o-dihydroxyphenol. In earlier studies phenolics have been linked with antioxidative activity and for stabilizing the lipid peroxidation.<sup>[5]</sup> Flavonols in term of catechin equivalent were observed to be maximum in CSME. Flavonols are most diverse phenolic compounds having radical scavenging activity.

of the coexisting creator.<sup>[15]</sup> Variation in the chelating activity with different dose levels of the extracts in comparison to standard antioxidants. Among the extracts it was observed that CSPE exhibited high potent activity with  $IC_{50}$  of 3.93 mg/ml followed by CSME with an  $IC_{50}$  of 6.48 mg/ml. Other extracts revealed moderate activity as compared to known standards: EDTA ( $IC_{50} = 2.132 \pm 0.065$  mg/ml) and citric acid ( $IC_{50} = 1.932 \pm 0.089$  mg/ml) (Table 2).

### Radical Scavenging Activities

#### DPPH radical scavenging activity

DPPH radical scavenging activity is a very fast method to evaluate the antioxidant activity. With this method it was possible to determine the antiradical power of an antioxidant by measurement of the decrease in absorbance of the DPPH radical with absorbance at 517nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule.<sup>[16]</sup> As comparison to hydroxyl and superoxide radical it is not being affected by side reactions such as metal ion chelation and enzyme inhibition.<sup>[17]</sup> Hence, DPPH is usually used as a substrate to evaluate the antioxidative activity of antioxidants. Dose dependent DPPH radical scavenging activity was observed in all the extracts as compared to the standard antioxidants BHT, catechin and gallic acid. Among the extracts, CSCH exhibited maximum DPPH radical scavenging activity with an  $IC_{50}$  value of 3.59 mg/ml.

DPPH radical scavenging power of extracts as comparison to known standards decreased in the order: Gallic acid>Catechin>CSCH>CSPE>CSAC>BHT>CSME>CSEA (Table 2).

#### Nitric oxide radical scavenging activity

The Nitric oxide scavenging activities of different extracts of *C. speciosus* increased with increasing concentration as compared to known standard. Maximum NO scavenging power was observed in CSME with an IC<sub>50</sub> value of 4.75 mg/ml. The IC<sub>50</sub> values of rhizome extracts of *C. speciosus* increased in the order CSME<CSEA<CSPE<CSAC<CSCH<CSCL (Table 2). The scavenging of NO radical by various extracts of revealed the potential of this important medicinal plant for treatment in anti-inflammatory disease caused by nitric oxide formation.

#### Superoxide radical scavenging activity

Results of superoxide scavenging activity of various extract of *C. speciosus* measured by PMS-NADH superoxide generating system. Among the different extracts, CSPE (IC<sub>50</sub>=4.72 mg/ml), CSME (IC<sub>50</sub>=4.93

mg/ml), and CSAC (IC<sub>50</sub>=5.91 mg/ml) exhibited strong super oxide radical scavenging activity. All the extracts exhibited different superoxide radical scavenging potential in terms of IC<sub>50</sub> values in comparison to the standard ascorbic acid (IC<sub>50</sub>=6.08 mg/ml) as reported in table 2. Superoxide anions play an important function specifically in the formation of free radicals causing oxidative damage of lipid, proteins and nucleic acids.

#### Hydroxyl ion radical scavenging activity

OH radical scavenging activity of all the extracts revealing good to moderate potential as compared to ascorbic acid. All the extracts exhibited different OH scavenging potential of their in terms of their different IC<sub>50</sub> values in comparison to the standard ascorbic acid (IC<sub>50</sub>= 2.65±0.127 mg/ml) (Table 2). CSME exhibited potent OH radical scavenging activity with a lower IC<sub>50</sub> value of 2.55mg/ml. Hydroxyl radical, an extremely reactive oxidizing radical having very short half life causing damage within a small radius. A single hydroxyl radical leads to the production of peroxides in the cell membrane leads to cell death.<sup>[18]</sup>

**Table 2: IC<sub>50</sub> values and reducing power activities of different extracts of *C. speciosus*.**

Extracts	IC <sub>50</sub> in (mg)/ml				Chelating activity	Absorbance (Reducing power) in	
	Radical scavenging activities					Dose level 5 mg/ml	At higher dose level 25mg/ml
	DPPH scavenging	NO scavenging	Super Oxide scavenging	OH scavenging			
CSPE	4.46±0.034	5.38±0.054	4.72±0.042	8.34±0.177	3.93±0.306	0.559±0.001	0.623±0.001
CSCH	3.59±0.020	8.99±0.011	9.53±0.102	3.48±0.210	7.45±0.042	0.468±0.001	0.520±0.001
CSEA	14.07±0.007	5.05±0.033	6.52±0.085	3.48±0.145	6.72±0.058	0.368±0.001	0.447±0.001
CSCL	9.98±0.003	11.75±0.009	7.76±0.114	4.30±0.703	8.00±0.194	0.456±0.001	0.554±0.002
CSAC	4.95±0.013	7.47±0.005	5.91±0.103	3.72±0.482	8.36±0.038	0.480±0.001	0.541±0.001
CSME	8.93±0.010	4.75±0.807	4.93±0.080	2.55±0.358	6.48±0.072	0.474±0.002	0.549±0.001
BHT	7.99±0.039	NA	NA	NA	NA	0.55±0.008	0.735±0.009
Catechin	1.61±0.011	NA	NA	NA	NA	0.455±0.006	0.623±0.004
Gallic acid	1.26±0.005	NA	NA	NA	NA	0.575±0.003	0.715±0.003
Ascorbic acid	NA	4.221±0.012	6.08±0.122	2.65±0.127	NA	NA	NA
EDTA	NA	NA	NA	NA	2.132±0.065	NA	NA
Citric acid	NA	NA	NA	NA	1.932±0.089	NA	NA

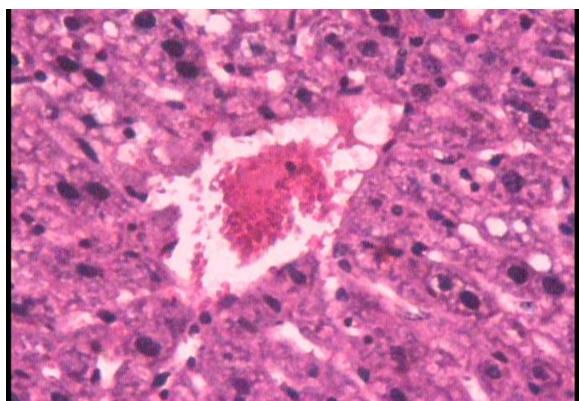
NA = not applicable

#### Histopathological study

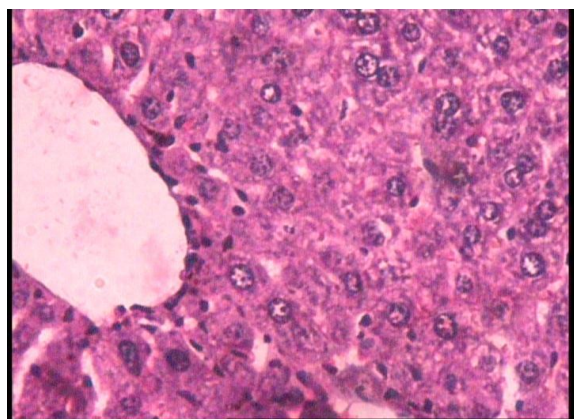
*C. speciosus* provide protection against CCl<sub>4</sub> mediated toxicity in liver and kidney as evidenced from biochemical and histopathological findings. Histopathological evidence of liver indicated that in rat treated with 250 mg/kg b.wt. had moderate congestion of blood vessel and moderate vascular degeneration and loss of sinusoidal spaces, but when dose level increases upto 500 mg/kg only mild vacuolar degeneration of hepatocyte occur (Fig1). These results indicated that methanolic extract of *C. speciosus* possess hepatoprotective role against CCl<sub>4</sub> mediated tissue damage.

The present findings indicated that at dose level of 500 mg/kg, significant hepatoprotective effect was observed in histological study which is contributed by the fact that rhizome of *C. speciosus* has rich content of saponins and glycosides.





CSME 250 mg/kg b.wt.



CSME 500 mg/kg b.wt.

Fig.1. Histopathological changes in rat liver at various test doses of *C. speciosus*

### Antipyretic effect on yeast induced pyrexia

The antipyretic effect of the methanolic extract of *C. speciosus* on yeast induced pyrexia has been presented in table 3. The test extract at dose levels of 50 and 100 mg/kg showed significant antipyretic effect as compared to control. The antipyretic effect of the extract at the dose level of 100 mg/kg was observed to be significant ( $p < 0.05$ ) to that of the control. The percentage inhibition of rectal temperature after yeast injection in the individual groups after 3 h was 8.92% in the control group, 83.87% in case of the standard drug, Ibuprofen, 77.50% in the 100 mg/kg test extract and 61.60% in the 50 mg/kg test extract. The antipyretic effect on the basis of percentage inhibition of rectal temperature after 3 h showed quite highly significant at dose level of 100 mg/kg as compared to control rather than standard drug. The test extract also exhibited dose dependent antipyretic effect which was comparable to standard at dose of 100 mg/kg. The pyrexia is an outcome of secondary impact of inflammation, tissue damage, infection and other diseased states. Flavonoids are known to target prostaglandins which are involved in pyrexia and inhibit the effect of enzymes responsible for the inflammatory process.<sup>[19]</sup>

Table 3: Antipyretic activity of methanolic extract of *C. speciosus*.

S. N.	Treatment	Dose	Body Temp. before administration of drug (°C)	Body Temp. after administration of drug (°C)				% Inhibition		
			-18h	0h	1h	2h	3h	1h	2h	3h
I	CSME	50 mg/kg	37.51±0.04	38.63±0.04	38.30±0.06 <sup>ab</sup>	38.10±0.03 <sup>ab</sup>	37.94±0.04 <sup>ab</sup>	29.46	47.32	61.60
II	CSME	100 mg/kg	37.49±0.04	38.69±0.04	38.22±0.05 <sup>a</sup>	38.00±0.05 <sup>a</sup>	37.76±0.04 <sup>a</sup>	39.16	57.50	77.50
III	Control	0.2 ml	37.50±0.04	38.62±0.04	38.58±0.03	38.54±0.02	38.52±0.02	3.57	7.14	8.92
IV	Ibuprofen	40 mg/kg	37.46±0.03	38.70±0.03	38.14±0.04 <sup>a</sup>	37.96±0.04 <sup>a</sup>	37.66±0.04 <sup>a</sup>	45.16	59.68	83.87

Values are means of three replicates ± SD. Within a column, mean values followed by the same letter are not significantly different according to Tukey's test ( $p < 0.05$ ).

### Antiinflammatory activity of *C. speciosus*

The acute anti-inflammatory effect was evaluated on the basis of circumference of paw oedema in rats induced by the carrageen agent at 50 and 100 mg/kg doses, which revealed significant decrease in inflammation with 2.62 and 2.39 mm, paw thickness, respectively ( $p < 0.05$ ) up to 24 h as compared to the control group (Table 4). The percentage inhibition of the acute inflammation in the

paw of rats was found to be comparable but a little lower in the 100 mg/kg test extract (30.12% inhibition) compared to the standard drug ibuprofen (35.67% inhibition) after 24 h of treatment. It has been reported flavonoids are able to inhibit both enzymes, as well as other mediators of the inflammatory machinery such as C reactive protein or adhesion molecules.<sup>[20]</sup>

**Table 4: Anti-inflammatory activity of methanolic extract of *C. speciosus*.**

Group	Treatment	Dose (mg/kg body weight of extract)	Change in paw thickness(in mm) after different time			% Inhibition	
			0h	04h	24h	04h	24h
I	CSME	50mg/kg	3.61±0.03	3.02±0.02 <sup>ab</sup>	2.62±0.02 <sup>ab</sup>	13.96	23.39
II	CSME	100mg/kg	3.59±0.03	2.82±0.04 <sup>ab</sup>	2.39±0.04 <sup>ab</sup>	19.67	30.12
III	Control	0.2ml	3.59±0.02	3.51±0.02	3.42±0.02		
IV	Ibuprofen	40mg/kg	3.58±0.03	2.58±0.02 <sup>a</sup>	2.20±0.04 <sup>a</sup>	26.50	35.67

Values are means of three replicates ± SD. Within a column, mean values followed by the same letter are not significantly different according to Tukey's test ( $p < 0.05$ ).

**Table 5: Analgesic activity of alcoholic extract of *C. speciosus*.**

Group	Treatment	Dose (mg/kg body weight extract)	No. of writhings	Writhing (%)	Inhibition(%)
I	CSME	50mg/kg	58.33±1.58	88.38	11.62 <sup>ab</sup>
II	CSME	100mg/kg	40.00±1.93	60.61	39.39 <sup>a</sup>
III	Control	0.2ml	66.00±1.93	100.00	
IV	Ibuprofen	40mg/kg	35.17±1.97	53.29	46.71 <sup>a</sup>

Values are means of three replicates ± SD. Within a column, mean values followed by the same letter are not significantly different according to Tukey's test ( $p < 0.05$ ).

#### Analgesic activity of alcoholic extract of *C. speciosus*

The analgesic activity was evaluated on the basis of the average number of abdominal constrictions indicated by the extension of hind paw of animals during the writhings experiment. The % writhings was observed to be significantly reduced (60.61%) at the test extract dose level of 100 mg/kg as compared with the control group, 100% writhing (Table 5). The observed peripheral analgesic effect was observed to be slightly less at the test dose of 100 mg/kg as indicated by 39.39% inhibition in writhings as compared ibuprofen which was causing 46.71% inhibition of writhing. The significant analgesic effect at the higher dose was attributed to the presence of high concentration of flavonoids which inhibited the synthesis, release or receptor responses in prostaglandin mediated effects.<sup>[21]</sup>

#### CONCLUSION

*Costus speciosus* an important indigenous traditional medicinal plant of Zingiberaceae family. Investigation on antioxidative activity and ameliorative activity of methanolic root extract has provided the new horizon for its industrial applications as well as its medicinal usages. Flavonol contents were reported to be higher in methanolic extracts. All the extracts exhibited good reducing power activity with different potential. Methanolic and petroleum ether extracts revealing good antioxidative activity in a dose dependent manner. The present findings indicated that at dose level of 500 mg/kg, significant hepatoprotective effect was observed in histological study. The methanolic extract was analyzed for antipyretic, anti-inflammatory & analgesic activities were noticed significantly at a dose level of 100 mg/kg b. wt. The significant analgesic effect at the higher dose was attributed to the presence of high concentration of flavonols.

#### ACKNOWLEDGEMENT

Financial assistance from University Grant Commission, New Delhi, India is thankfully acknowledged.

#### REFERENCES

- Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. 4th ed., Oxford, UK Clarendon Press., 2007.
- Naskar S, Islam A, Mazumdar UK, Saha P, Haldar PK, Gupta M. In vitro and in vivo antioxidant potential of Hydromethanolic extract of *Phoenix dactylifera*. J Sci Res, 2010; 2 (1): 144-157.
- Subasinghe HWAS, Hettihewa LM, Gunawardena V. Rapid onset of action of *Costus speciosus* leaf extracts on insulin resistance in experimental Wistar rats. Proceedings of the Annual Scientific Sessions of Faculty of Medical Sciences, University of Sri Jayawardenepura, Sri Lanka., 2012.
- Wei F, Jinglou C, Yaling C, Yongfang L, Liming C, Lei P. Antioxidant, Free radical scavenging, anti-inflammatory and hepatoprotective potential of the extract from *Parathelypteris nipponica* Ching. J Ethnopharmacol, 2010; 130: 521-528.
- Zak B, Dickenman RC, White EG, Burnett H, Cherney PJ. Rapid estimation of free and total cholesterol. Am J Clin Pathol, 1954; 24(11): 1307-1315.
- Kaplan A. "Urea" Kaplan, A. et al. Clin Chem the C.V. Mosby Co. St. Louis. Toronto, Princeton, 1984; 1257-1260.
- Sreedhar V, Nath LKR, Gopal NM, Nath MS. In-vitro antioxidant activity and free radical scavenging potential of roots of *Vitex trifoliata*. Res J Pharmaceutical Biological and Chemical Sci, 2010; 1(4): 1036-1044.
- Ramalingam R, Sudini S, Boddupalli BM, Anisetti RN. Antioxidant, free radical scavenging and invitro cytotoxic studies of ethanolic extracts of *Leucas indica* var. *lavandulifolia* and *Leucas indica* var.

- nagalpuramiana. Asian Pac J Trop Biomed, 2012; S1637-S1642.
9. Yamaguchi F, Ariga T, Yoshimira Y, Nakazawa H. Antioxidant and anti-glycation activity of carcinol from *Garcinia indica* fruit rind, J Agric and Food Chem, 2000; 49: 180–185.
  10. Reitman S, Frankel S, A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol, 1957; 28: 56-63.
  11. Malloy HT, Evelyn KA. The determination of bilirubin with photoelectric colorimeter. J Biol Chem, 1937; 119(7): 481-488.
  12. Verma N, Khosa RL. Development of standardization parameters of *Costus speciosus* rhizomes with special reference to its pharmacognostical and HPTLC studies. Asian Pac J Trop Biomed, 2012; S276-S283.
  13. Duan X, Wu G, Jiang Y. Evaluation of antioxidant properties of phenolics from litchi fruit in relation to pericarp browning prevention. Molecule, 2007; 12: 759-771.
  14. Hsu CL, Chen W, Weng YM, Tseng CY. Chemical composition, physical properties and antioxidant activities of yam flours as affected by different drying methods. Food Chem, 2003; 83(1): 85–92.
  15. Yen GC, Duh HY. Antioxidative properties methanolic extracts from peanut hulls. J American Oil Chem Soc, 1993; 70: 383-386.
  16. AL-Ameri NO, Azeez ZF. Morphological effects of alcoholic extract of *Costus Speciosus* Koen. on *Aspergillus* sp. that causing pulmonary infection (III). J of Nat Sci Res, 2014; 4(3): 98-101.
  17. Amarowicz R, Pegg RB, Rahimi-Moghaddam P, Weil BJA. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food chem, 2004; 84: 551-562.
  18. Srivastava S, Singh P, Mishra G, Jha KK, Khosa RL. *Costus speciosus* (Keukand): a review. Der Pharmacia Sinica, 2011; 2(1); 118-128.
  19. Binny K, Kumar SG, Thomas D. Anti-inflammatory and antipyretic properties of the rhizome of *Costus Speciosus* (Koen.). J Basic Clin Pharm, 2010; 1(3): 177-181.
  20. Gonzalez-Gallego J, Sanchez-Campos S, Tunon MJ. Antiinflammatory properties of dietary flavonoids. Nutr Hosp, 2007; 22(3): 287- 293.
  21. Gupta M, Sasmal S, Majumdar S, Mukherjee A. HPLC profiles of standard Phenolic Compounds present in medicinal plants. Int J Pharmacognosy Phytochem Res, 2012; 4(3): 162-167.