In vitro antioxidant, Anti-diabetic, Anti-inflammatory of *Cheilocostus speciosus* rhizome extracts

Shalet Varghese, Baskaran. K*, Haseera. N, Nirmala Devi.N

Department of Biochemistry, Sree Narayana Guru College, Coimbatore, Tamilnadu, India.

***Correspondening Author:** Dr. K.Baskaran, *Assistant Professor, MSC, M.Phil., Ph.D.
Department of Biochemistry, Sree Narayana Gure College, Coimbatore-641105 Tamil Nādu, India
E-mail: baskar.bio86@gmail.com. Cell number: 91+8760302579

ABSTRACT

Costus speciosus is an erect perennial herb belonging to family Coastaceae, an important medicinal plant widely used in several indigenous medicinal formulations. Presently, these plants could be collected from wild habitat only. Due to indiscriminate collection from natural habitat it has become endangered. In the present investigation, the phytochemical and antioxidant activity of the rhizome extracts of *Costus speciosus* were evaluated.Secondary metabolites such as carbohydrates, alkaloids,. The DPPH, ABTS, nitric oxide anti scavenging activity of water, methanol, ethanol, ethyl acetate and chloroform and five extract for in vitro anti diabetic and anti-inflammatory activity. The present findings for flavonoids and minerals suggested that their contents are responsible for significant antioxidant activity in all extracts.

Keywords: *Costus speciosus,* anti diabetic, anti-inflammatory activity, antioxidant activity.

INTRODUCTION

Costus speciosus is a perennial rhizomatous herb with erect or spreading stems commonly called as crepe ginger or spiral flag in English [1]. It is an erect plant up to 2.7 m high with tuberous root stalk, a sub-woody stem at the base flowers are larger, white, in thick, cone like terminal spikes, with bright red bracts. *Costus speciosus* is native to the Malay peninsula of the south-east Asia. In India, the plant naturalizes in sub-Himalayan tract of central India and parts of Western Ghats of Maharashtra, Karnataka and Kerala [2]. It is known as Keukand, Keu, Kust (Hindi), Pakarmula (Gujrathi), Penva, Pushkarmula (Marathi), Kustha (Sanskrit) and Kostam (Tamil). The rhizomes are bitter and show antihelminthic, astringent, depurative and expectorant properties [3-6]. It has antioxidant, antifungal, antituberculosis and oestrogenic activity.

Figure 1. *Costus speciosus* (A) flowers (B) rhizomes (C) whole plant of *C. speciosus*

The rhizome extract is used as tonic and useful in reliving burning sensation, constipation, asthma, bronchitis, leprosy, anaemia and other skin alignments [7]. The rhizome of *Cheilocostus speciosus* has hepatoprotective properties[8]. Rhizome paste is used for treating boils and used as contraceptive [9]. Rhizome possesses antifertility, anticholinestrase, anti-inflammatory, antipyretic and antihelminthic activities [10.11]. Steroid saponins and sapogenins from *Cheilocostus speciosus* exhibited antifungal activity [12]. In south-east Asia, it is used to treatboils, constipation, diarrhoea, dizziness, headache, ear, eye and nose pain, and used to stop vomiting. Japanese used the rhizome extract to control syphilis[13]. Pharmacological studies showed that the rhizomes of *Cheilocostus speciosus* possess cardiotonic, hydrochloretic, diuretic and CNS depressant activity [14].

The demand of nutraceuticals is increasing day-by-day, so herbs can be a better option for the replacement of synthetic antioxidant agents. Keeping the above facts in view, this study deals with the in vitro antioxidant, anti-diabetic, anti-inflammatory activity of *cheilocostus speciosus* rhizome extracts.

Materials and Methods

The fresh rhizome and roots of *Cheilocostus speciosus* were collected from the herbal garden at Kerala University. The medicinal plants were identified, confirmed and authenticated by CMS college Kottayam, Kerala. Freshly collected samples were washed in running water and then air dried for further analysis.

Preparation of the plant extract

Fresh rhizomes of *Cheilocostus speciosus* (Hellenia *speciosa*) were sun dried for 7 days and finally autoclaved in an electric oven below 60 °C for 23 hours. The rhizomes were dried and crushed into fine powder. Extraction of dried rhizome powder was carried out in five different solvents with high, medium and low polarity. The solvents used were water, methanol, ethanol, ethyl acetate and chloroform. The sample was soaked in the above respective solvents in the ratio 1:5 and kept in shaker for 48 hours at 28°C. After 48 hours, the samples were filtered using Whatman no.1 filter paper. The solvents were then evaporated by using a hot water bath and the crude extracts obtained were stored in sterile glass bottles for further screening and analyses. Preparation of plant extracts with different polarity can be achieved using different solvents and extraction methods.

DPPH (1, 1-diphenyl-2-picryl hydrazyl) radical scavenging assay

The radical scavenging activity of *CS* five different extracts (water, methanol, ethanol, ethyl acetate and chloroform) against DPPH• was determined spectrophotometrically in a dark room by the method [15]. DPPH• is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH• reacts with an antioxidant compound that can donate hydrogen and gets reduced. The change in colour (from deep violet to blue) was measured. The intensity of the yellow colour developed was depends on the amount and nature of radical scavenger present in the sample. 1 ml of various concentrations *CS* extract was taken, 1 ml of DPPH was added and this made up to 3 ml with water. The blue colour developed was read at 517 nm and AA was used as a standard.

$$\% \text{ Scavenging activity} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

+

ABTS (2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid assay)

+

The total antioxidant activity of the samples was measured by ABTS• radical cation + decolourization assay according to the method [16]. ABTS• was produced by reacting 7 + mmol ABTS aqueous solution with 2.4 mmol potassium persulfate in the dark for 12-16 h at room temperature. The radical was stable in this form for more than two days when stored in the dark at room temperature. Then, 2 ml of diluted ABTS• solution was added to the sample varying concentrations of *CS* three different (water, methanol, ethanol, ethyl acetate and chloroform) extract. The blank contained water in place of *CS* extract. After 30 min of incubation at room temperature, the absorbance was recorded at 734 nm and compared with standard AA. Percentage of inhibition was calculated.

$$\% \text{ Scavenging} = \frac{\text{Control OD - Test OD}}{\text{Control OD}} \times 100$$

Nitric oxide scavenging activity

Nitric oxide scavenging activity was determined according to the method [17]. Nitric oxide generated from sodium nitroprusside in an aqueous solution at physiological pH interacted with oxygen to produce nitrite ions, which can be estimated by using the Griess reagent. Scavenging of nitric-oxide act against oxygen, leading to reduced production In brief, 3.0 ml of 10 mmol sodium nitroprusside in phosphate-buffered saline was mixed with different concentrations of the *CS* extract and incubated at 25 °C for 150 min. 0.5 ml of the incubated solution was taken and mixed with 0.5 ml of Griess reagent. The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-1-naphthylethylenediamine dihydrochloride was measured at 546 nm. AA was used as the reference standard.

Quantitative analysis of metabolites a**. Estimation of total alkaloids**

The total alkaloid contents in the bark samples were measured using 1,10phenanthroline with slight modifications.100mg bark powder was extracted in 10ml 80% ethanol. This was filtered through muslin cloth and centrifuged at 5000rpm for 10 min. Supernatant obtained was used for the further estimation total alkaloids. The reaction mixture contained 1ml plant extract, 1ml of 0.025M $FeCl_3$ in 0.5M HCl and 1ml of 0.05M of 1,10phenanthroline in ethanol. The mixture was incubated for 30 minutes in hot water bath with maintained temperature of 70 ± 25ºC. The absorbance of red coloured complex was measured at 510nm against reagent blank. Alkaloid contents were estimated and it was calculated with the help of standard curve of colchicines (0.1mg/mL, 10mg dissolved in 10ml ethanol and diluted to 100mL with distilled water) [18].

b. Estimation of Total Carbohydrate (Anthrone Method)

Weigh 100 mg of the sample into a boiling tube. Hydrolyze by keeping it in a boiling water bath for three hours with 5 ml of 2.5 N HCl and coal to room temperature. Neutralize it with solid sodium carbonate until the effervescence ceases. Make up the volume to 100 ml and centrifuge. Collect the supernatant and take 0.5 and 1ml aliquots for analysis. Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard. 0 serves as blank. Make up the volume to 1 ml in all the tubes including the sample tubes by adding distilled water. Then add 4 ml of anthrone reagent. Heat for eight minutes in a boiling water bath. Cool rapidly and read the green to dark green color at 630 am. Draw a standard graph by plotting concentration of the standard on the x-axis versus absorbance on the y-axis. From the graph calculate the amount of carbohydrate present in the sample tube.

Anti-diabetic activity (Alpha Amylase Inhibition Assay)

Property of plant extracts to act as inhibitors for alpha amylase was tested as measure of its anti-diabetic property.Different concentration of extract was taken into different test tubes. Make the volume to 0.5ml with phosphate buffer of pH 6.9, Control was prepared by taking 0.5ml of phosphate buffer. The solutions was then treated with 0.5ml of alpha amylase (0.5mg/ml). The solution was incubated at 25ºC for 10 minutes. Added 0.5ml of 1% starch solution in 0.02 M sodium phosphate buffer of pH 6.9 to all the tubes, and then incubate at 25ºC for 10 minutes. The reaction was stopped by adding 1.0 ml of DNS and the reaction mixture was kept in boiling water bath for 5 minutes, cooled to room temperature. The solution was mixed with 8 ml distilled water. Blank was measured by taking 1 ml of phosphate buffer. Then a set of test control tubes was prepared with the sample. To the tubes take different concentrations of the samples dilute the samples with phosphate buffer. Read the absorbance of the solution in calorimeter at 540 nm against blank solution.

% inhibition= (OD of Control- OD of Test)/ OD of Control x100

Anti-inflammatory activity

HRBC Method

The anti-inflammatory activity of plant extracts was assessed by in vitro HRBC membrane stabilization method. Fresh whole human blood (10ml) was collected and transferred to the heparin zed centrifuged tubes. The collected blood was mixed with an equal volume of Alsever solution (dextrose 2%, sodium citrate 0.8%, citric acid 0.05%, sodium chloride 0.42%, and distilled water 100 mL) and centrifuged with saline (0.85 %, dissolve

8.5g NaCl in water. Autoclave 15 min at 121°C. Cool to room temperature). To 1mL of HRBC suspension, an equal volume of plant extracts in three different concentrations 10, 50 and 100µL) was added. All the assay mixtures were incubated at 37°C for 30 minutes and centrifuged. The haemoglobin content in the supernatant solution was estimated by using a spectrophotometer at 560 nm. The percentage of protection was calculated then by the formula as given below:

Percent of protection = (100-OD of test)/OD of control ×100

Protease inhibitory activity

Prepare the protease enzyme solution by dissolving it in the buffer solution at a concentration of 0.1 mg/mL. Prepare the substrate solution by dissolving BAEE in the buffer solution at a concentration of 0.2 mM. Prepare a series of test tubes, each containing 1 mL of the substrate solution and a different concentration of the test compound (protease inhibitor).

Include a control tube with no inhibitor. Add 100 µL of the protease enzyme solution to each tube, and mix well. Incubate the tubes at 37°C for 30 minutes. Stop the reaction by adding 2 mL of 10% acetic acid to each tube, and mix well. Measure the absorbance of each tube at 253 nm using a UV spectrophotometer. Calculate the percentage inhibition of protease activity using the following formula:

% inhibition= (OD of Control- OD of Test)/ OD of Control x100

Where A, is the absorbance of the sample, A, is the absorbance of the product control and A, is the absorbance of the positive control.

Egg albumin denaturation assay

The anti-inflammatory activity of unknown crude extracts can be determined in vitro for inhibition of the denaturation of egg albumin (protein). 0.2 mL of 1% egg albumin solution, 2 mL of sample extract or standard, and 2.8 mL of phosphate-buffered saline (pH 7.4) will be mixed to form a reaction mixture of a total volume of 5 mL. The control will be made by mixing 2 mL of triple distilled water, 0.2 mL 1% egg albumin solution, and 2.8 mL of phosphate buffered saline to make a total volume of 5 mL. The reaction mixtures will be then incubated at 37±2°C for 30 min and will be heated in a water bath at 70±2°C for 15 min. After cooling, the absorbance will be measured at 280 nm by a suitable UV/Vis spectrophotometer using triple distilled water as the blank(Chandra et al., 2012). The percentage inhibition will be calculated using the relationship: % inhibition= (OD of Control- OD of Test)/ OD of Control x100

Statistical analysis

All the assays were carried out in triplicate. Experimental results are expressed as mean ± standard deviation. The results were analyzed using one-way analysis of variance and the group means were compared using Duncan's multiple range tests using SPSS version 16.

RESULTS AND DISCUSSION

Different pharmacological properties of *Costus speciosus*

Plant antioxidants when supplemented to animals have to go through many physiological and pharmacological changes, while getting absorbed, distributed, metabolized, stored, and then excreted out by the animal's body and also have to deal with several other factors such as their bioavailability, presence or absence of different transition metals, and co-antioxidant compounds. Therefore, parallel to the in vitro investigations, in vivo studies must also be undertaken, while they establish the therapeutic values of the bioactive components of the plants.

DPPH

Studies show that these compounds not only defend against ROS but also provide protection from the breaking of DNA strands [19]. To date, various in vitro studies have been conducted on different parts of *C. speciosus* like roots, stems, and leaves that have evidently established the tremendous antioxidant potential. The relative solubilities of different phytochemicals are different and follow the basic motive behind the extraction, i.e., extraction done on the basis; whether the plant matrices contain polar, nonpolar compounds or have intermediate polarity, many different extracts like methanol, ethanol, petroleum ether, n-hexane, benzene, pure acetone, and acetone–water mixture have been used to isolate a diverse variety of active secondary metabolites from *C. speciosus*. Based on evaluative studies done using antioxidant analysis methods like total antioxidant scavenging activity, 1,1-diphenyl-2-picrylhydrazyl (DPPH), it was reported that the antioxidant activity is mainly due to the phenolic contents present in different parts of the plant. In addition, the analysis of methanolic extracts of the rhizomes of *C. speciosus* showed the presence of various phytochemicals like steroids, glycosides, gracillin, methylprotogracillin, protogracillin, dioscin, methyl protodioscin, prosapogenin B of dioscin, diosgenin 3-O-β-D′ Glucopyranosyl, (1 → 3)-β-glucopyranoside. Furthermore, when assayed with DPPH and nitric oxide scavenging methods, methanolic extracts of *C.speciosus* showed much powerful nitric oxide and free radical scavenging activities as compared to the ascorbic acid[20].

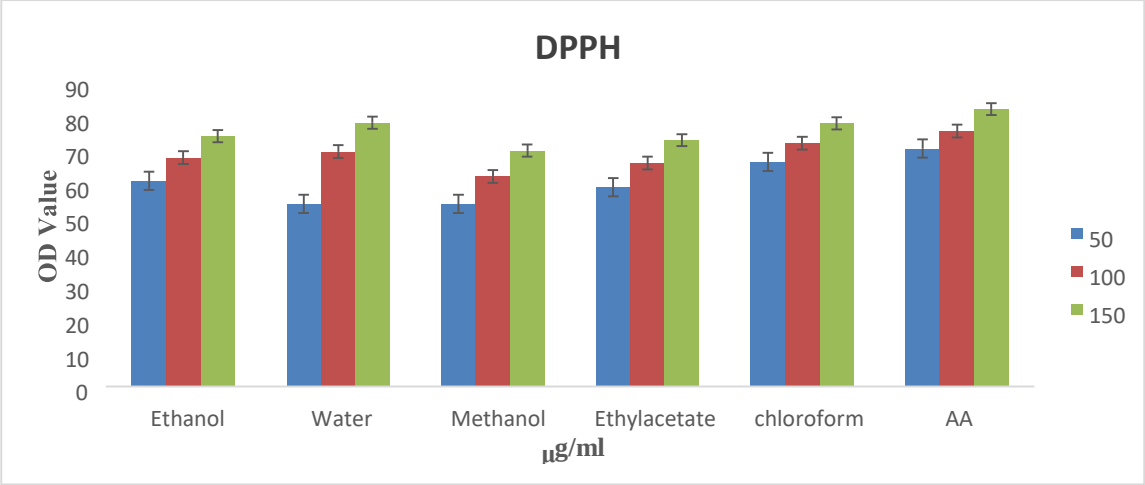


Fig.2. Shows the effect DPPH assay of different *C. speciosus root* extract

In the present study, *CS* showed a maximum % of inhibition at 1000 µg/ml concentration and these are slightly lower than that of the standard AA, (Fig. 3). The results of the present study indicate that the extract of green seaweed exhibited higher ABTS radical activity. The results indicated that acetone extract has a significant effect on the scavenging of ABTS radicals. However, the limitations of ABTS assay, such as the capability of a sample to react with ABTS radical rather than to inhibit the oxidative process and the slow reaction of many phenolics necessitate a compatible evaluation of antioxidant activity using other assays as well [21].

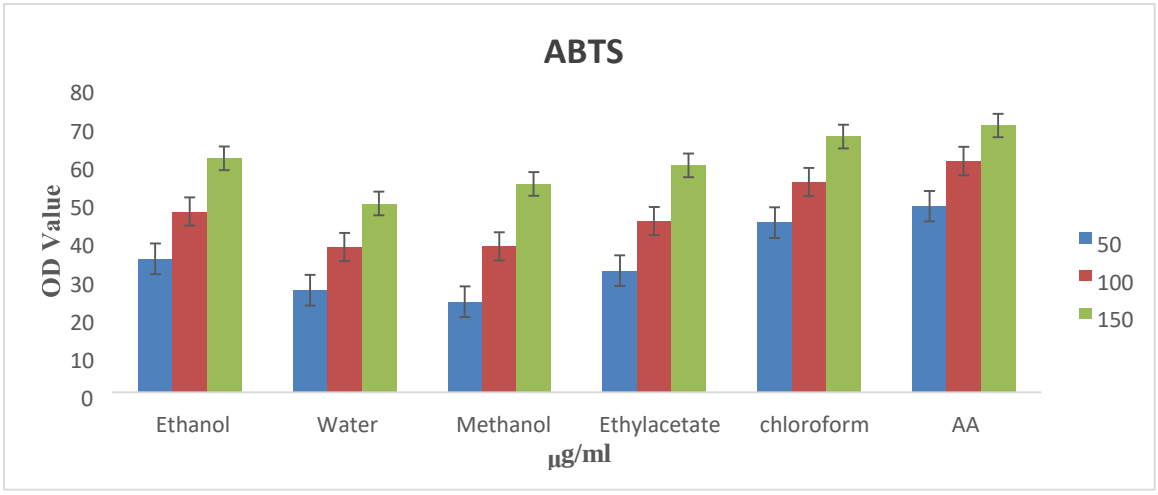


Fig.3. Shows the effect ABTs assay of different *C. speciosus root* extract

NITRIC OXIDE

The results showed that *CS* had scavenging activity and this value is comparably lower than that of the standard AA, (Fig. 4). The IC_{50} values of three different extracts (Hexane, Ethyl acetate and Acetone) of *CS*. It was also found that the IC_{50} value of the algal extracts was lower than that of AA.

The suppression of nitric oxide release may be partially attributed to direct scavenging by the extracts of *CS*, which decrease the amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro*. Recently, seaweed extracts and fractions have been considered to be a rich source of antioxidants and different types of antioxidants have been isolated from various species of seaweeds.

The potential antioxidant compounds were identified as some pigments (fucoxanthin, astaxanthin, carotenoid e. g.) and polyphenols (phenolic acid, flavonoid, tannins e. g.), which are widely distributed in seaweeds and are known to exhibit higher antioxidative activities, which have been reported through various methods of reactive oxygen species scavenging activity and the inhibition of lipid peroxidation [22,23].

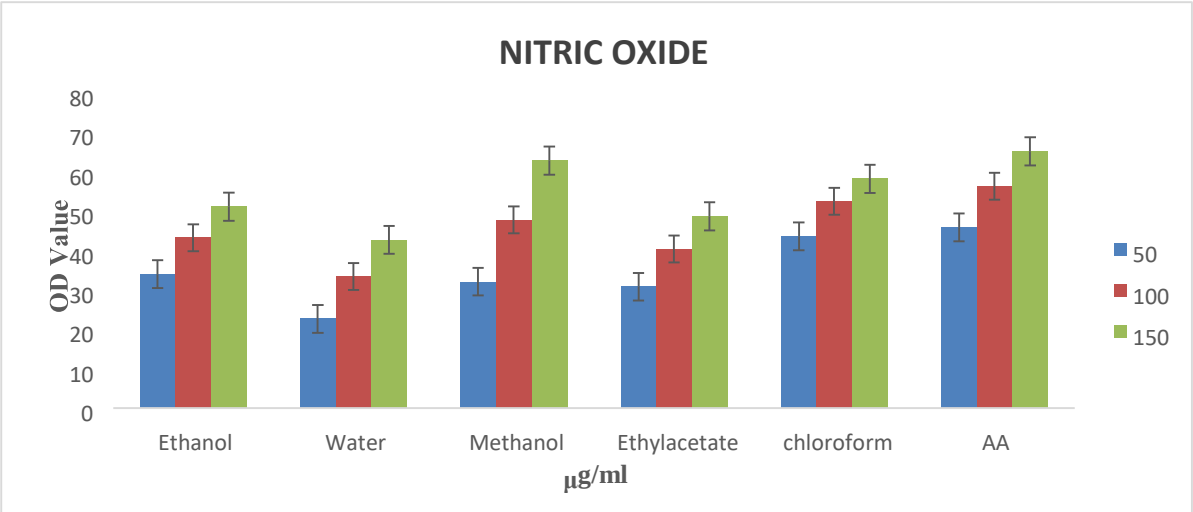


Fig.4. Shows the effect nitric oxide assay of different *C. speciosus root* extract

Total alkaloids

The phytochemical screening and quantitative estimation of chemical components of the plants studied showed that the leaf and stem were rich in flavonoids, saponins, amino acids, proteins, diterpines. These phytochemicals has medicinal and physiological activity [24]. Phytochemicals are the chemical constituents present in plants which show physiological action on the human body [25].

Alkaloids, flavonoids, phenols, diterpines, carbohydrates, proteins, glycosides, and essential oils are some of the important bioactive phytochemicals [26]. A number of report are available that represent the phytochemical presence in plant such as flavanoids, glycosides, tannins, alkaloids phenols, proteins in medicinal plants [2729]. Major phytochemicals are reported in O. sanctum are flavonoids, glycosides, Alkaloids, proteins, tannins and phenols [30] . It has been determined that antioxidant activity present in flavonoid component and its effect on human nutrition and their health. The mechanism of action of flavanoids is through scavenging or chelating process.

Flavonoids are the common antioxidants present in various medicinal plnats [31,32]. The present work was carried out to analyse the phytochemicals and flavonoids component in medicinal plant Ocimum sanctum. This study showed more flavonoid component present in leaves of Ocimum senctum. However, flavonoid component was more in methanol extract of Ocimum sanctum leaves and ethanol extract of Ocimum senctum stem. While methanol extract of Ocimum sanctum stem show less flavonoid content and ethanol extract of Ocimum sanctum leaves does not show any result. Various reports also available that indicate O.sanctum as a good source of flavonoids [33]. Since, phenolic and flavonoid components present in high amount in Ocimum species. Thus, widely used in traditional medicine system [34].

Fig 5. Standard graph for total alkaloids

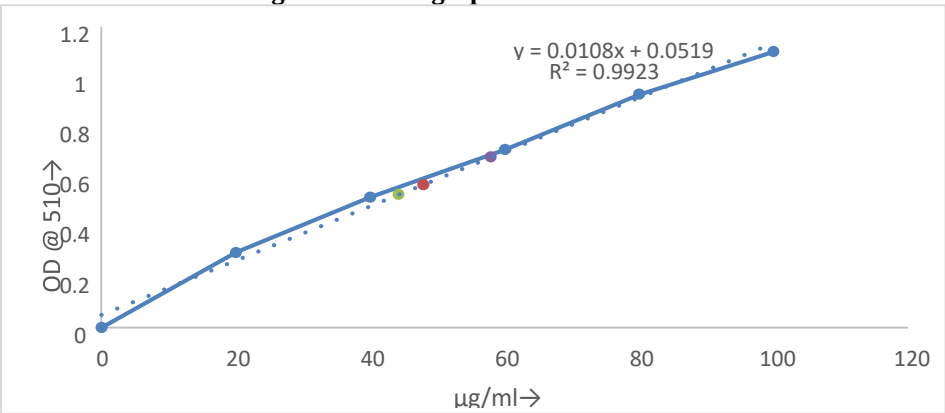
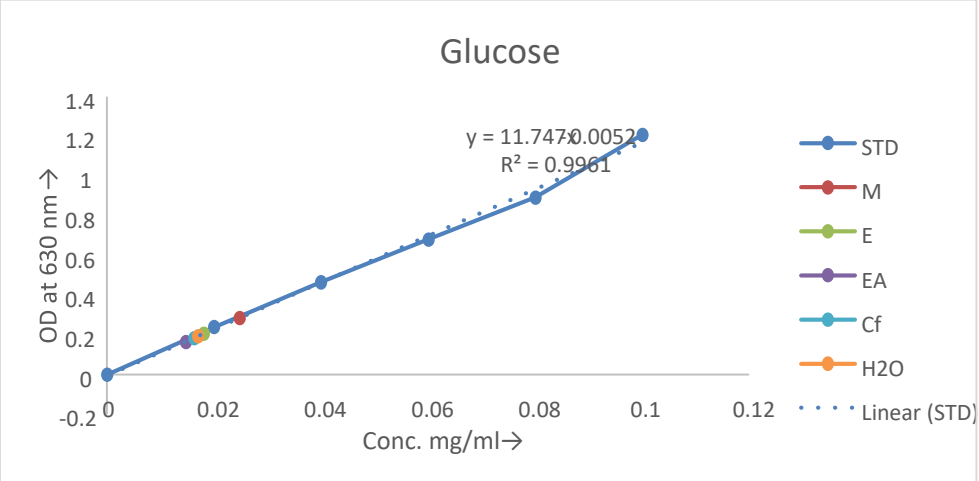


Fig 6. Standard graph for Carbohydrate



ANTIDIABETIC

There are the other numbers of investigations done by various workers to evaluate the antidiabetic potential of *C. speciosus* and alterations caused in the biochemistry of the treatment group; for example, α-glucosidase and α-amylase were inhibited by the leaf extract (methanolic extract) of the plant, which ultimately leads to the decrease in the plasma protein glycation by delaying the digestion of carbohydrate and decreasing the absorption of glucose. Other studies revealed that the antidiabetic component, costunolide, has a stimulatory effect on the beta cells that led to the secretion of insulin.Various effects of active constituents like eremanthin and costunolide derived from *C.speciosus* are shown in Fig. 7.

In diabetes high postprandial blood glucose leads to micro vascular complications include retinopathy, nephropathy, neuropathy, and macrovascular complications refer to increased atherosclerosis-related events such as myocardial infarction and stroke. One of the therapeutic approaches for controlling postprandial hyperglycemia in diabetic patient is to prevent or decreasing absorption of carbohydrate after food intake. Complex starches, oligosaccharides, and

disaccharides must be broken down into monosaccharides by αamylase and α-glucosidases before they are absorbed in the duodenum and upper jejunum [35]. Recent advances in understanding the activity of intestinal enzymes helped in the development of newer pharmacological agents [36] α-glucosidase inhibitors reduce intestinal absorption of starch, dextrin, and disaccharides by inhibiting the action of α-glucosidase in the intestinal brush border.

Inhibition of this enzyme slows the absorption of carbohydrates from the GI tract and decreases the rate of rise of postprandial glucose (PP hyperglycemia). This delay digestion and breakdown of starch may have beneficial effects on insulin resistance and glycemic index control in people with diabetes [37]. Acarbose is α-glucosidase inhibitor which reduces digestion of complex carbohydrates and slows their absorption from the gut [38]. These drugs also increase the release of the glucoregulatory hormone glucagonlike peptide-1 into the circulation, which may contribute to their glucose-lowering effects [39]. However, they may causes side effect such as malabsorption, abdominal pain, flatulence, and diarrhea which lead to a high discontinuation rate. Acarbose and miglitol should not be prescribed in individuals with renal impairment. Acarbose should be used with caution in patients with hepatic diseases because it may cause reversible elevation of hepatic enzymes [40]

Experimental results showed that both extracts significantly inhibited the αglucosidase and α-amylase enzymes. Aqueous extract showed better α-glucosidase inhibitory activity than the α-amylase inhibitory action. Ethanolic extract showed better α-amylase inhibitory activity than the α-glucosidase inhibitory action. Ethanolic extract showed more inhibitory activity than aqueous extract. Strength and Limitations There are no previous reports, to the best of our knowledge, about the inhibitory activity of this plant on in vitro αglucosidase and α-amylase. This experiment was conducted only with aqueous and ethanolic leaf extracts. Further studies are required to identify the bioactive compounds that are responsible for the inhibition of α-glucosidase and α-amylase activity

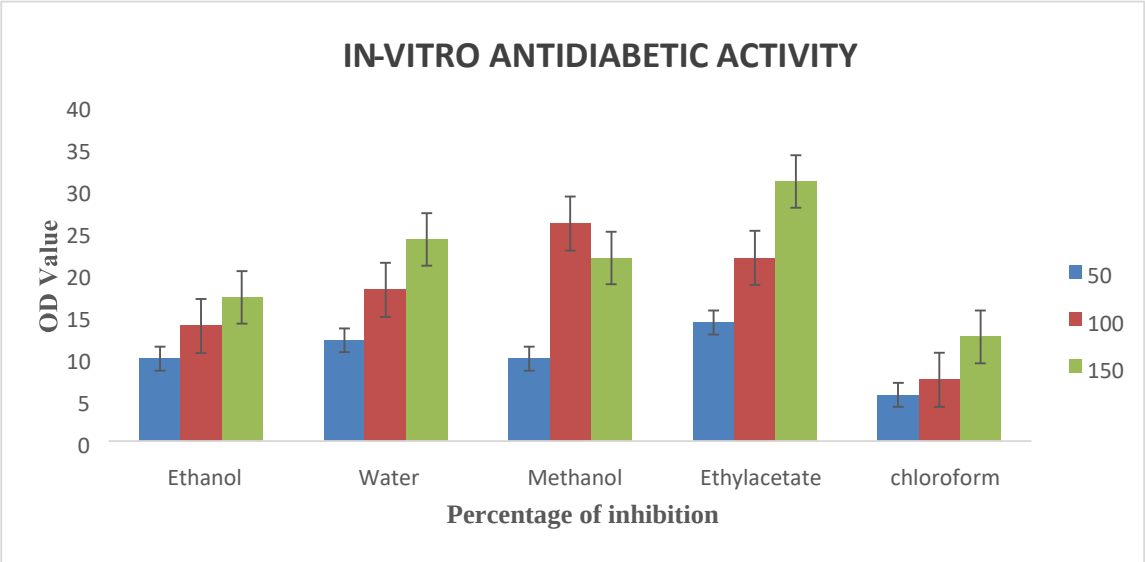


Fig.7. Shows the effect antidiabetic of different *C. speciosus root* extract

In Vitro Anti-Inflammatory Assay (Egg Albumen Denaturation Method).
Denaturation of proteins is a well-documented cause of inflammation and rheumatoid arthritis. Several anti-inflammatory drugs have shown concentration dose- dependent ability to inhibit thermally induced protein denaturation. Plant extract to inhibit thermal denaturation of protein (egg albumin) is a reflection of its anti-inflammatory activity. Ethanol and hexane extracts and aspirin (standard drug) displayed concentration dependent anti-inflammatory activity at test concentrations of 5000 to 1000 μg/mL.ethanol and hexane extracts exhibited anti-inflammatory activity.

However, at test concentrations for ethanol and hexane extracts, the anti-inflammatory activity was observed to be significantly higher ($P < 0.01$) than for aspirin (reference drug). Inflammatory activity of the extracts can be attributed to the presence of phenols, flavonoids, glycosides, terpenoids, and steroids present in *C. speciosus*, which have been reported to exhibit anti-inflammatory activity. Flavonoids are known to inhibit enzymes as well as mediators of the inflammation process such as C-reactive protein or adhesion molecules.

Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of plant extract

to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation. Maximum inhibition of 71% was observed at 500 µg/ml. Aspirin, a standard antiinflammation drug showed the maximum inhibition 68% at the concentration of 100 µg/ml compared with control (Table 1).

Neutrophils are known to be a rich source of serine proteinase and are localized at lysosomes. It was previously reported that leukocytes proteinase play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors [41]. *Cheilocostus speciosus* exhibited significant antiproteinase activity at different concentrations as shown in Table 2. It showed maximum inhibition of 53% at 500µg/ml. Aspirin showed the maximum inhibition 55% at 100µg/ml.

The HRBC membrane stabilization has been used as a method to study the invitro anti inflammatory activity because 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 [42,43]. 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 non steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane [44] .

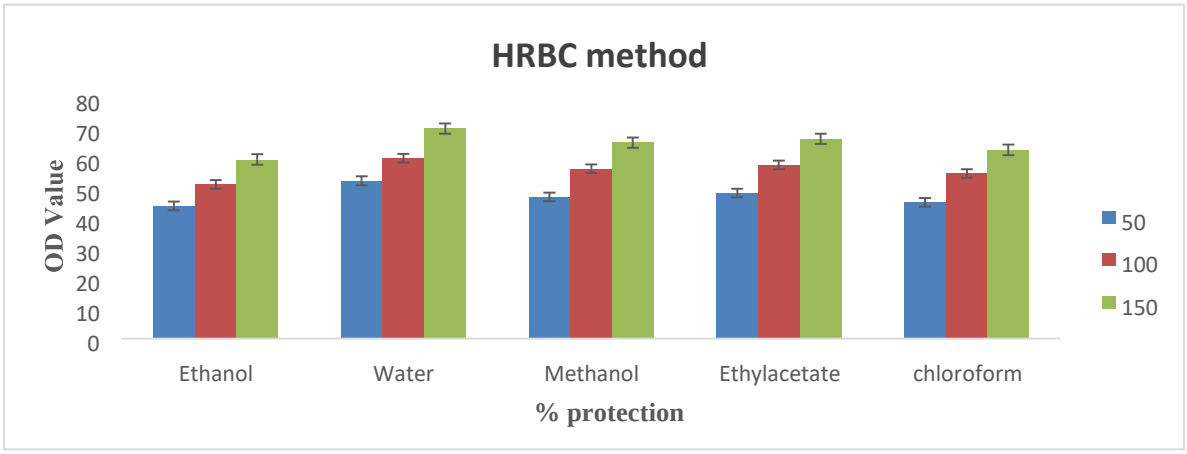


Fig.8. Shows the effect HRBC of different *C. speciosus root* extract

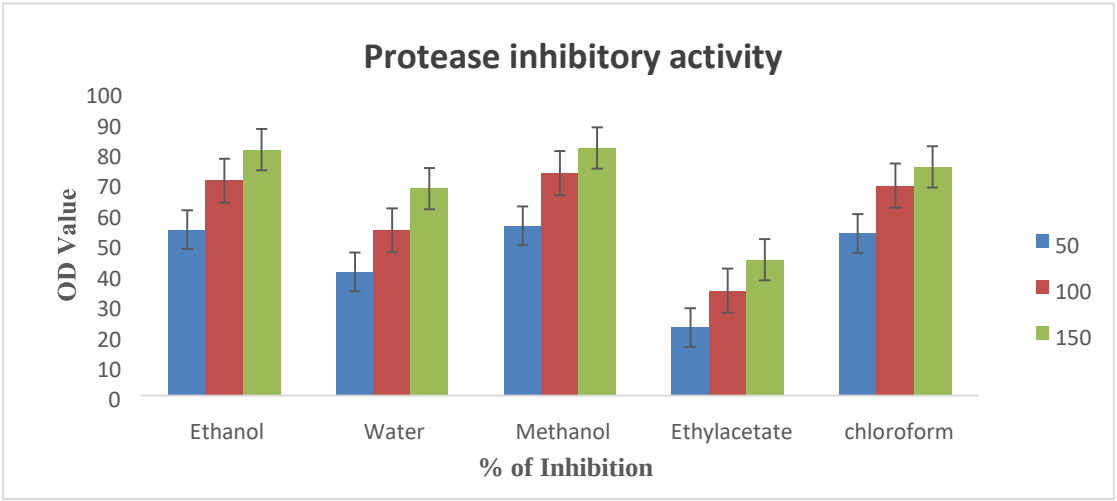


Fig.9. Shows the effect Protease inhibitory activity of different *C. speciosus root* extract

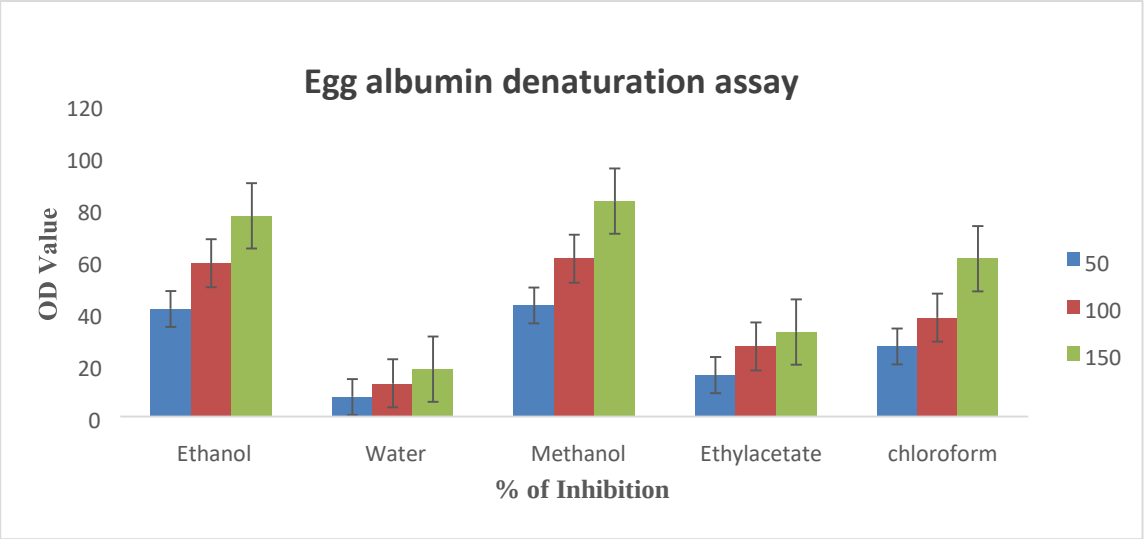


Fig.10. Shows the effect Egg albumin denaturation assay of different *C. speciosus root* extract

CONCLUSIONS

In the present study, results indicate that the methanol extracts of *Cheilocostus speciosus*ll are possess anti-inflammatory properties. These activities may be due to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, and phenols, The extract fractions serve as free radical inhibitors or scavenger or acting possibly as primary oxidants and inhibited the heat induced albumin denaturation, proteinase activity and stabilized the Red Blood Cells membrane. *Cheilocostus speciosus*also reduced the activity of lipoxygenase. Purification of each bioactive compound is necessary and this purified form of the compound can be used which may show increased activity. This study gives on idea that the compound of the plant *Cheilocostus speciosus*l are can be used as lead compound for designing a potent anti-inflammatory drug which can be used for treatment of various diseases such as cancer, neurological disorder, aging and inflammation.

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