



## Biological Activity and Phytochemical Analysis of *Cucumis callosus* (Rottl.) Cong Underutilization Vegetable

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**ABSTARCT:** *Cucumis callosus* is a member of the cucumber family and widely many regions of world for its underutilization vegetable and medicinal purposes. The present study focuses on assess biological activity of different extracts and evaluate the presence of phytochemicals through qualitative tests. Fruit powder extracted in soxhlet extractor using different organic solvent (Chloroform, Ethanol, Methanol, Petroleum ether). All four extracts study the anti- inflammatory activity, *in vitro* alpha – amylase inhibitory activity, anthelmintic activity and antibacterial activity. The qualitative analysis of different organic solvent extracts evaluated. Four different organic solvent out of this methanol solvent extract give comparatively show better biological. activity in protein denaturation and alpha – Amylase. *C. callosus* extracts have demonstrated significant alpha-amylase inhibitory activity, which could be useful in the management of diabetes. Methanolic extracts have shown potential anthelmintic activity against various intestinal parasites, which could be useful in the treatment of parasitic infections. Fruit extracts have exhibited antibacterial activity against several bacterial strains, which could be beneficial in the treatment of bacterial infections. All organic solvent presents the alkaloid, phenol and Tannins. The presence of these phytochemicals in the plant extract may contribute to their curing of disease. The present bioactive compounds in *C. callosus* it may be potential therapeutic properties make it a promising candidate for the development of new natural medicines for various diseases and conditions. However, further studies are needed to fully understand the mechanisms of action and cytotoxicity of *C. callosus* extracts.

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**Key words:** *Cucumis callosus*; Fruit, Biological Activity; Phytochemical Analysis

### Introduction

Despite the lack of historical records, the value of traditional medicinal systems that use plants as a means of therapy is undeniable. These systems have been shown to be an effective methodology of medicinal agents, and almost 65% of the world's population has incorporated them into their primary mode of healthcare, according to the World Health Organization (WHO) [1]. The herbs contain a diverse range of chemical compounds that have therapeutic effects on the body, making them highly valued for their healing properties [2]. Phytotherapy, which is the practice of using medicinal plants to treat various illnesses and health conditions, has been utilized by traditional healers to provide healthcare to over a billion people, or roughly 80% of the world's population [3]. This demonstrates the significant role that herbal medicine plays role in global healthcare systems. The use of phytotherapy has been passed down through generations, and it continues to be an essential aspect of traditional medicinal practices.

Plants exhibit a wide range of biological activities that contribute to their growth, development,

and survival. One such activity is the production of secondary metabolites, which are non-essential compounds that play a role in plant defence, communication, and adaptation to environmental stressors. These metabolites can be classified into various groups, including alkaloids, flavonoids, terpenoids, and phenolics, and have been found to possess a diverse range of pharmacological properties. For example, flavonoids are known to have antioxidant and anti-inflammatory effects, while alkaloids such as vincristine and vinblastine have been used in the treatment of cancer. Such biological activities are not only important for the survival of plants but have also been utilized by humans for medicinal purposes and control of diseases [4].

*C. callosus* is a wild plant that is generally found in and around farmland in rural areas. *Cucumis callosus*, although historically used for medicinal and culinary purposes, has not been widely cultivated, leading to its classification as an underutilized vegetable. Kachri is typically found in arid and semi-arid regions, growing in sandy or rocky soil. It is often found in dry riverbeds, on hillsides, and in scrubland.

Kachri is typically found in arid and semi-arid regions, growing in sandy or rocky soil. It is an important wild vegetable, particularly in Rajasthan and Gujarat in India, where it is used in dishes such as kachri ki chutney and kachri ki sabzi [5]. *Cucumis callosus*, although historically used for medicinal and culinary purposes, has not been widely cultivated, leading to its classification as an underutilized vegetable. Many tribal and rural people use this fruit in their diet and breakfast as food, while others use it for medicinal purposes [6].

*Cucumis callosus*, a plant with significant medicinal value, has demonstrated several beneficial activities such as antioxidant, hyperglycaemic, and anti-inflammatory effects [7]. Due to the promising results of its pharmacological activities, I have chosen to conduct further research on this plant to investigate its potential applications in treating various health conditions. This fruit has some unique biological activities and compounds that are beneficial for humans and directly related to human health. By exploring its chemical composition and identifying its active compounds, we may be able to develop new medicines or supplements that harness the plant's therapeutic properties. This research would be a better understanding of the potential uses and benefits of *Cucumis callosus* fruit, which could have important implications for public health and nutrition.

## Material and Methods

### Collection of plant material

The fruits of *Cucumis callosus* were collected in the month of December 2022 from village Malataj, Gujarat (India). The plant was identified by Dr. Kalpesh Ishnava. Collected plant material is healthy and disease free.

### Drying and grinding

The fruit of *Cucumis callosus* was collected and wash under running tap water to remove dust particles. Then cut material in small piece with the stainless knife. The fruit pieces together with the seeds were kept to dry on trays at room temperature and. Dry conditions are essential to prevent subsequent degradation of metabolites. Protection from direct sunlight is advised to minimise chemical reaction induced by ultraviolet rays [8]. After drying, materials were powdered with a mechanical grinder. Grinding of materials into smaller particles facilitates subsequent extraction procedures, increasing the surface area. The powder was passed through 60-mesh sieve to get a fine powder.

### Preparation of plant extracts by hot extraction

The coarse powder prepare from the fruit and seeds were extract with petroleum ether (60-80°C),

chloroform (60-61 °C), methanol (60-64°C), ethanol (60- 80 °C) by hot continuous percolation process. For extraction, portion of 20 g of the dry powder were packed in filter paper thimble each time and extract in a soxhlet extractor (16 cycles) till the extractive became almost colourless. The extract was collected in big petri dish and was allow it to evaporate at oven (60 °C). After evaporation the dry extract were scrap and collected into vials and yield extract was calculate. Extracts were stored at 4°C until use for further analysis. The methanol extract of *Cucumis callosus* will be from now referred to as MECC and petroleum ether extract of *Cucumis callosus* will be from now referred to as PECC and chloroform extract of *C. callosus* will be from now referred to as CECC and ethanol extract of *C. callosus* will be from now referred to EECC.

## Evaluation of *in vitro* anti-inflammatory activity

### Inhibition of protein denaturation by BSA [9]

The assay was carried out by adopting the methods described by [9] with some modification in which the volume of each component in the reaction mixtures was reduce by half. Each of the plant extracts and the positive standards (Diclofenac sodium) were produced at a concentration of 0.1% (1.0 mg/ml). For each mixture, a reaction vessel containing 200µl of bovine serum albumin, 1400 µl of phosphate buffered saline, and 1000 µl of the test extract was create. Extracts were substitute with distil water as a negative control. The mixes were then heat. Their absorbance was measured at 660 nm after cooling. The following formula was used to determine the inhibition percentage of protein denaturation:

Denaturation inhibition percentage = (Control - Test / Control) ×100

### *In-vitro* alpha-amylase inhibitory activity

#### Modified starch-iodine method [10]

Take the various extract concentrations between 10 and 100 µg/ml were obtained. α-amylase was added to each test tube in a volume of 20 µL. At 37 °C, each test tube was incubated for 10 minutes. Each test tube received 200 µL of 1% starch solution after the incubation period. The mixture was re-incubated for 1 hour at 37 °C. Each test tube was then filling with 200 µL of a 1% iodine solution. To each test tube, 10 mL of distil water was added. The mixture's absorbance was measured at 565 nm.

% α-amylase inhibitory activity = OD of control - OD of test / OD of control ×100

### Alpha-amylase inhibition screening assay [11]

Take the 160 µl of alpha-amylase enzyme and 120 µl of bioformulation were combined and incubated at 37 °C for 45 min. The mixture was poured into the well created in the petri plate containing 3%

agar (w/v) and 1.2% starch (w/v) after incubation. Plates were left to stand at 25°C for 3 days before being filled with an iodine solution and left to stand for 15 minutes. The size of the starch hydrolysis zone was measured. The enzyme was put to the well of the plate without plant extract as a control. The percentage of inhibition was calculated by using the equation.

$$\% \text{ Amylase Inhibition} = (\text{Diameter of control} - \text{Diameter of test}) / \text{Diameter of control} \times 100$$

#### **Anthelmintic activity [12]**

The study used adult earthworms (*Eisenia fetida*) of similar lengths and tested the impact of various organic solvents' extracts and Albendazole solutions in different (10mg to 50mg) concentrations as well as a control solution of normal saline with smallest volume of DMSO. The solutions were diluted and placed in petri dishes of the same size, with three earthworms of the same size added to each dish. The worms were kept at room temperature and observed for signs of paralysis or death, and the time taken for either to occur was recorded. A worm was considered dead if it remained motionless for at least 3 minutes and lost its body colour.

#### **Bioassay for Antimicrobial activity**

##### **Agar Well Diffusion Method**

In the present study, to test antibacterial activity, twenty different plant extracts were used. The antibacterial activity was studied by agar well diffusion method [13]. From the stock, 100 mg of each plant extract were suspended in one millilitre of Dimethyl sulfoxide (DMSO). In order to make agar plates, the petri plates were thoroughly washed using detergent, dried and sterilized in autoclave at 15 lbs pressure for 15 minutes. Approximately 25 mL of sterilized selective medium was poured in to each Petri dish and solidified at room temperature. The plates were incubated at 37° C for sterility checking for overnight. Agar plates were marked and divided in to 4 equal parts, labelled for specific organism and extract number. A fresh bacterial culture of 400µl having 10<sup>8</sup> CFU/mL was spread on agar plates with glass spreader. A well of 10 mm diameter punched off at previously marked petri plates in to agar medium with sterile corkborer and then it was filled with 70µl of respective plant leaves extract. Plates were placed for 30 minutes in refrigerator for diffusion of extracts and then incubated at 37°C (or specified temperature) for 24 hours or more depending upon the organisms, until appearances of zone of inhibition. The zone of inhibition (excluding well diameter) was measured as a property of antibacterial activity. 100% DMSO were used as positive control and negative control respectively. Bioassay was performed in duplicate and repeated twice.

#### **Qualitative analysis of phytochemical screening in *C. callosus* fruit extracts**

Qualitative phytochemical analysis of fruit extracts was performed as per the standard methodology to determine the presence of Tannins, alkaloids and Phenolic compounds [14].

#### **Result and Discussion**

##### **Extractive yield (%) of *C. callosus***

Before analysing the effectiveness of various herbs, it is crucial to assess the yield of extract from each plant. It is generally observed that plants with lower extract yields are less favoured by consumers. To ensure accurate results, our study involved the calculation of extract yield. We employed four different solvents - Methanol, Chloroform, Petroleum ether and Ethanol to extract plant constituents.

The yield of all the solvent extract is mentioned in the Figure 1. *Cucumis callosus* show a maximum extractive yield 28.985 % in ethanol solvent compare to other solvent extract like chloroform, methanol, petroleum ether which show 21.70%, 28.01%, 19.41% yield respectively.

Our findings revealed that ethanol was the most effective organic solvent in eluting a significant amount of yield from the plant extract. This indicates that ethanol is a highly efficient solvent for plant extraction, which could be useful in the production of herbal products with optimal levels of active ingredients.

##### **Evaluation of *in-vitro* anti - denaturation activity of different extracts of *Cucumis callosus***

##### **Protein denaturation by using bovine albumin of leaf extracts**

The effect of different organic solvent extract of plants was evaluated against denaturation of bovine serum albumin. The results are summarized in Figure 2. The present findings exhibited a concentration dependent inhibition of protein denaturation by plant extracts throughout the range of 100 to 1000 µg/ml.

It was effective in inhibiting heat induced albumin denaturation. In BSA denaturation method at concentration of 100, 250, 500, 750,1000 µg/ml Chloroform extract (1mg/ml) showed 86.22%, 52.88%, 22.44%, 11.86% 2.56% Ethanol Extract (1mg/ml) showed 95.83%, 92.95%, 84.94%, 73.08% and 69.55% Methanol extract (1mg/ml) showed 95.51%, 87.5%, 66.67%, 64.74% and 43.58% Petroleum ether extract (1mg/ml) showed 83.65%, 70.83%, 56.09%, 44.87% and 35.89% respectively and reference drug Diclofenac sodium (1mg/ml) showed 87.82%, 67.31%, 24.04%, 9.29%, 4.08% respectively.

The present study finding that show the methanolic extract possessed maximum effect against anti-denaturation activity *in vitro*. MECC (1mg/ml)

present the phytochemical constitute of alkaloids, phenol, Tannin. It was discovered that all the extracts exhibited a high percentage of anti-denaturation properties, especially at lower concentrations. These findings align with as the concentration decreases [15].

These results suggest that the extracts may have significant potential for use in various applications requiring anti-denaturation properties, particularly at lower concentrations. All the extracts of *Cucumis callosus* have a protective effect on Bovine Serum Albumin (BSA) at a concentration of 100 $\mu$ g/ml. Specifically, the extracts were able to prevent denaturation of the BSA protein by more than 95%.

This approach is based on the principle that compounds that have a protective effect on proteins can prevent or reduce the denaturation of proteins induced by various stress factors. The use of methanolic extracts of plants has been studied for their potential anti-denaturation activity, which is typically evaluated by measuring the percentage inhibition of protein denaturation. The percentage inhibition of protein denaturation can be used as a measure of the effectiveness of the extract in protecting proteins from denaturation.

Higher percentages of inhibition indicate stronger anti-denaturation activity, which can potentially translate into therapeutic benefits for various diseases that involve protein denaturation.

In our current investigation, we explored the potential of methanolic plant extracts to prevent heat-induced protein denaturation using an *in vitro* protein denaturation assay. This approach was specifically chosen to evaluate the anti-denaturation properties of *Cucumis callosus* extract. Protein denaturation is a critical aspect of inflammatory tissue and is well known to contribute to inflammation-related diseases such as arthritis. Therefore, any agent that can protect against protein denaturation has the potential to be developed as an effective anti-inflammatory drug in the future.

The effectiveness of using plant extracts to induce anti-denaturation effects in heat-treated bovine serum albumin (BSA) has been investigated as a potential parameter for evaluating the therapeutic potential of anti-inflammatory compounds, without requiring animal testing for initial pharmacological screening [16]. As far as we are aware, there is no existing information regarding the *in vitro* anti-denaturation properties of proteins found in *Cucumis callosus*.

#### **Evaluation of *in-vitro* alpha-amylase inhibitory activity of different extract of *Cucumis callosus* Inhibition of alpha amylase by modified starch-iodine method**

Alpha-amylase is an enzyme that hydrolyses alpha bonds of large alpha linked polysaccharides like starch and glycogen to yield disaccharides like maltose which will further hydrolyze by alpha-glycosidase to yield monosaccharides like glucose [17]. The inhibitors of alpha-amylase bind to the alpha bond of polysaccharides and stop the breakdown of polysaccharides in mono and disaccharide. Hyperglycaemia is the risk factor for the development of diabetes and its complications. Therefore, control of glucose levels in the blood is a vital treatment for diabetes and the lessening of macrovascular and microvascular complications.

One way to manage diabetes is by controlling post-meal hyperglycaemia, which refers to high blood sugar levels that occur after eating. A therapeutic approach that has been found effective in achieving this goal is the use of postprandial hyperglycaemia inhibitors, which work by suppressing the breakdown of starch in the body [18]. This approach has shown promise in helping people with diabetes to better manage their blood sugar levels and prevent complications associated with the disease. In the present study, extract that prepare from a different organic solvent that shows alpha-amylase inhibitory activity and these results suggest that the extracts may have significant potential for use in various applications requiring anti-diabetic properties, particularly at lower concentrations. The present study finding that show the methanolic extract possessed maximum effect against anti-diabetic activity *in vitro*. M.E.C.C. (1mg/ml). The maximum effect in the methanol extract show 99.21 % inhibition (Figure 3) because of the starch is more available and no sugar produce that reason more helpful in the control sugar.

In this assay, a sample containing the alpha-amylase inhibitor is mixed with a solution of starch and alpha-amylase. The alpha-amylase cleaves the starch into smaller molecules, producing a blue-black color upon the addition of iodine solution. However, if an alpha-amylase inhibitor is present in the sample, it will prevent or reduce the cleavage of starch by alpha-amylase, resulting in a decrease in the intensity of the blue-black color.

#### **$\alpha$ - Amylase Inhibition Screening Assay**

This research focuses on the hydrolysis of starch by the  $\alpha$ -amylase enzyme and explores the inhibitory effects of *Cucumis callosus* samples extracted from various solvent systems on this enzyme's activity. The  $\alpha$ -amylase enzyme breaks down starch molecules into monosaccharides, which react with iodine solution to produce a blue color.

The objective of this study is to investigate the potential of different extracts for managing hyperglycemia by inhibiting  $\alpha$ -amylase activity, and

the results indicate that the extracts possess good inhibitory activity.

To compare the different solvent extracts and the control group, used the zone of clearance technique, which measures the area where starch hydrolysis did not occur. Among the solvent extracts tested, the E.E.C.C. extract demonstrated the most significant zone of clearance (Figure 4), suggesting its potential as a therapeutic agent for hyperglycaemia management.

#### **Evaluation of anthelmintic assay of different extract of *Cucumis callosus***

Inadequate management practices in third world countries have led to a high prevalence of a certain disease. Unfortunately, the development of anthelmintic resistance in helminths has become an increasing problem, leading to the need to screen medicinal plants for their anthelmintic properties. Plants are a promising source of botanical anthelmintics, as they offer a rich and diverse pool of compounds [19].

Current medicinal preparations available in the market are often ineffective or prone to resistance, resulting in recurring infections. Therefore, plant-derived drugs can serve as a model to develop more efficient and less toxic medications. The objective of this study is to investigate the potential of different extracts for anthelmintic properties.

The anthelmintic properties of the different solvent extract of *C. callosus* were studied using *Eisenia fetida*. The ethanol and methanol extract show less time for death at 50mg/ml while death is comparable with that of albendazole as death of worms was observed at 16min (Figure 5). albendazole standard drug show more time for death at same concentration.

The extract showed concentration related anthelmintic activities with all worms used in the study, with 50 mg/ml giving a shortest time of death for all worm.

Earthworm were most sensitive to the ethanol and methanol solvent extract of *C. callosus* it was confirmed that extract displayed anthelmintic properties against the worm used in study. To the best of our knowledge, there have been no prior reports on the anthelmintic activity of *Cucumis callosus*.

#### **Evaluation of antibacterial activity of different extract of *Cucumis callosus***

The experiment aimed to evaluate the antibacterial properties of different extracts of *Cucumis callosus* against two Gram-positive and two Gram-negative bacterial species using the agar well diffusion method.

Lower concentration shows the lesser activity against the strains as compare to higher. ethanolic extract of *C.callosus* shows the maximum activity in 50 mg/ml concentration against the streptococcus gram positive bacteria and shows the minimum activity against acinetobacter gram negative bacteria (Table 1).

Lower concentration shows the lesser activity against the strains as compare to higher. Methanolic extract of *C.callosus* shows the maximum activity in 50 mg/ml concentration against *Streptococcus sp.* bacteria and shows the minimum activity against bacillus albus bacteria (Table 1).

Lower concentration shows the lesser activity against the strains as compare to higher. Chloroform extract of *C.callosus* shows the maximum activity in 50 mg/ml concentration against *Streptococcus sp.* bacteria and shows the minimum activity against all bacteria (Table 1).

Lower concentration shows the lesser activity against the strains as compare to higher. Chloroform extract of *C.callosus* shows the maximum activity in 50 mg/ml concentration against all bacteria and shows the minimum activity against all bacteria (Table 1).

The results demonstrated that the ethanolic extract of *C. callosus* had the highest antibacterial activity compared to other solvent extracts of the same plant. However, all the extracts were found to be ineffective against most of the tested Gram-negative bacterial species, except for *Acinetobacter sp.* This suggests that the antibacterial compounds in *C. callosus* may have a greater effect on Gram-positive bacteria or may not be effective against some Gram-negative bacterial species.

#### **Phytochemical analysis of different solvent extract**

##### **Qualitative analysis of different solvent extract**

Table depicted the qualitative analysis of phytochemical constituents present in the plant extract, which showed the presence of alkaloids, phenols, and tannins (Table 2). The presence of these phytochemicals in the plant extract may contribute to their therapeutic potential. Alkaloids, which are known to have a significant impact on human disease control like skin disease, inflammation and fever may play a metabolic role and regulate.

#### **Statistical analysis**

Three replicates were performed for each experiment. The mean and standard deviation were used to express the data. Three replicates were performed for each experiment. The mean and standard deviation were used to express the data. ANOVA was calculated using Minitab 17 and Tukey's significant difference test was performed for pairwise comparisons between samples. Calculated ANOVA using Minitab 17 and Tukey's significant difference

test was performed for pairwise comparisons between samples.

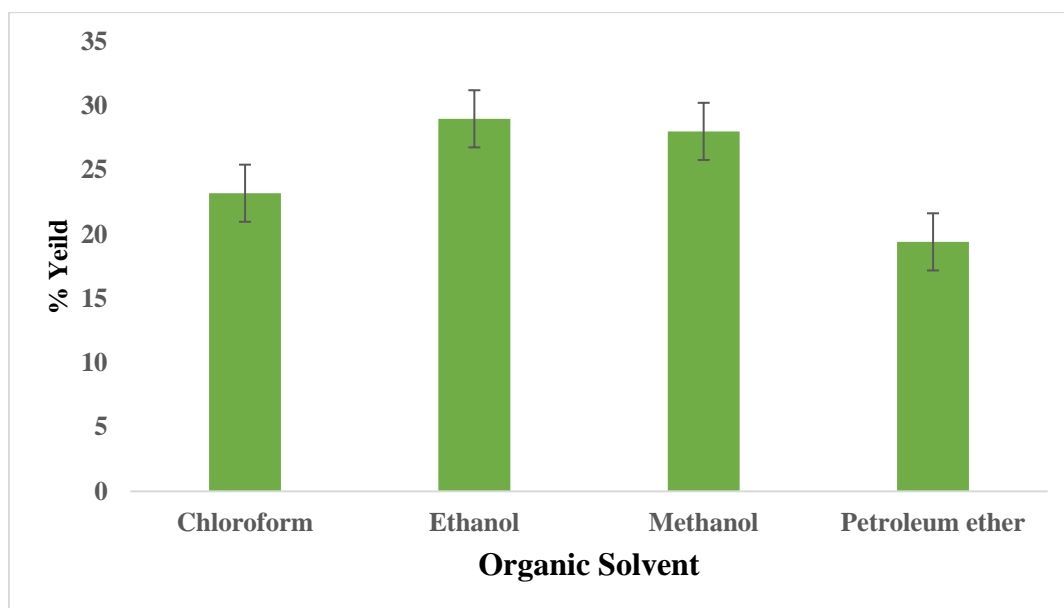


Figure 1: Extract yield of organic solvent

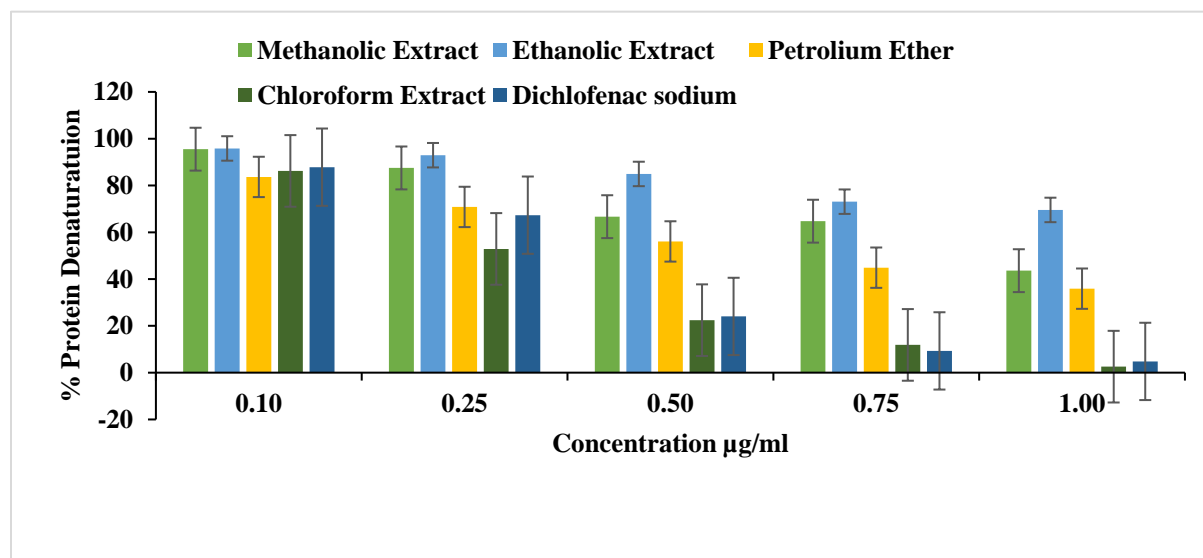


Figure 2: % Inhibition of bovine serum albumin

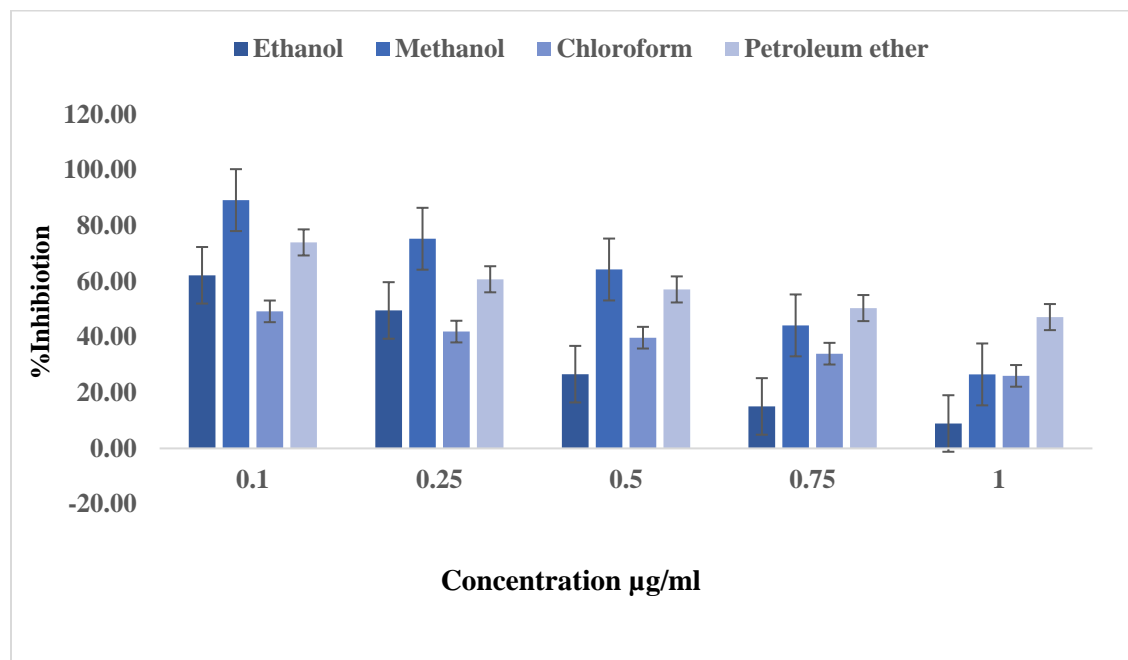


Figure 3: % Inhibition of alpha amylase

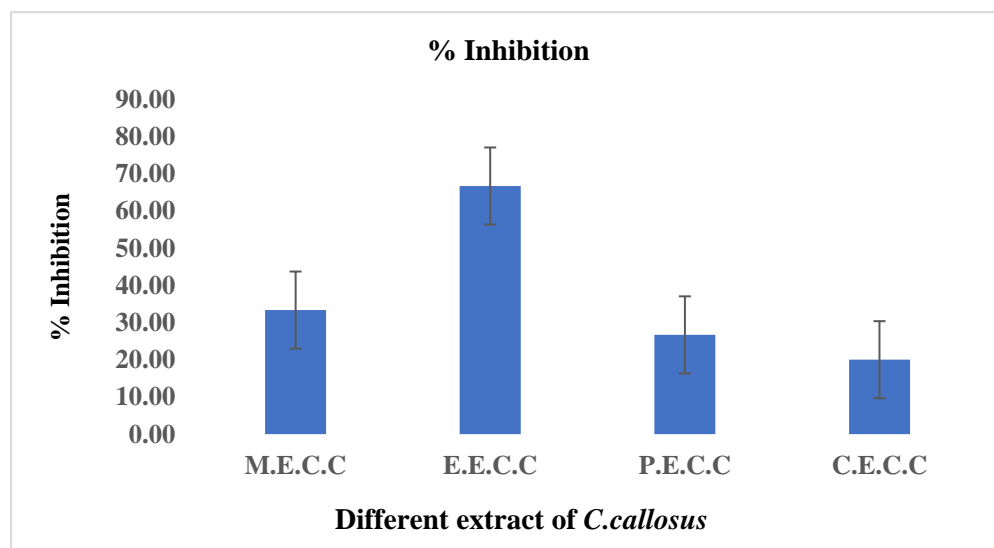


Figure 4: % Inhibition of alpha amylase

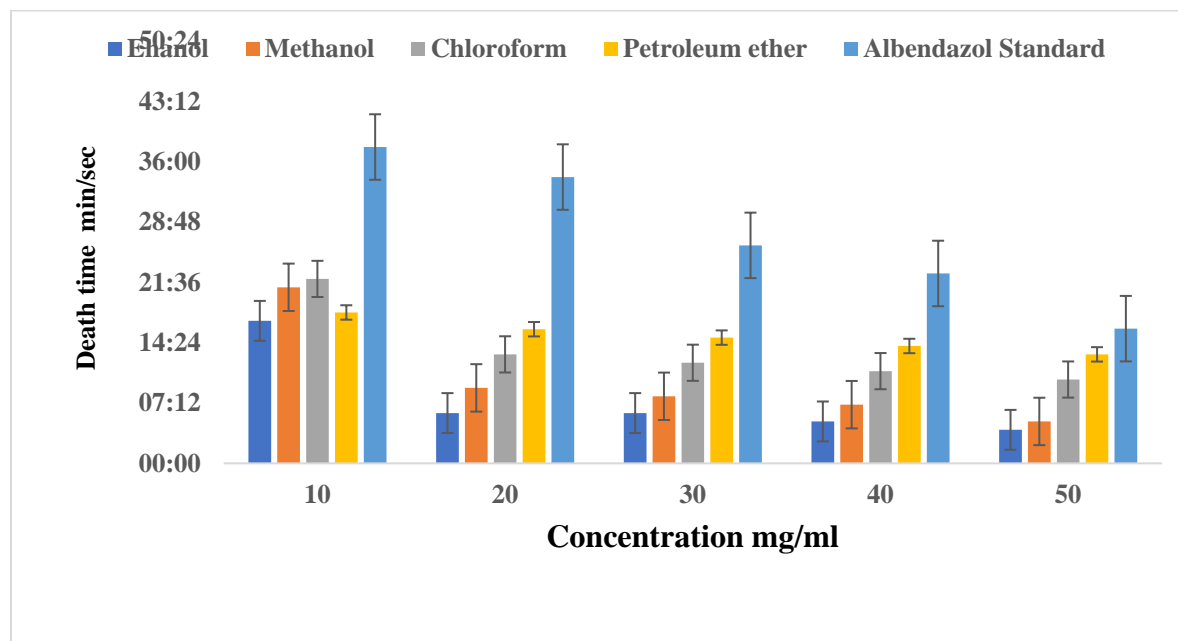


Figure 5: Anthelmintic assay of different extracts of *C. Callosus*

Table 1: Antibacterial activity of ethanolic extract of *C. callosus*

Bacterial Strain	Concentration of extract (mg/ml)	Zone of inhibition (mm)	Zone of inhibition (mm)	Zone of inhibition (mm)	Zone of inhibition (mm)
		E.E.C.C	C.E.C.C.	P.E.C.C.	M.E.C.C
<i>Acinetobacter sp.</i>	10	09 ± 0.4	09	09	09
	25	10 ± 0.20	09	11	10
	50	11 ± 0.35	10	10	11
<i>Streptococcus sp.</i>	10	09 ± 0.20	09	10	09
	25	12 ± 0.15	11	11	11
	50	13 ± 0.10	12	09	11
<i>Bacillus albus</i>	10	10 ± 0.25	09	10	09
	25	12 ± 0.5	09	11	10
	50	12 ± 0.2	10	Nil	10
<i>Morexella sp.</i>	10	00 ± 00	00 ± 00	00 ± 00	Nil
	25	00 ± 00	00 ± 00	00 ± 00	Nil
	50	00 ± 00	00 ± 00	00 ± 00	Nil



**Table 2. Qualitative analysis of different organic solvent extracts**

Sr. No	Name of the Test	C.E.	E.E.	M.E.	P.E.
1.	Alkaloids	+	+	+	+
2.	Phenol	+	+	-	+
3.	Tannins	+	+	+	+

+ = Present; - = Absent; C.E.C.C.= Chloroform extract; E.E.= Ethanolic extract;  
M.E.= Methanolic extract; P.E.=Petroleum extract

### Conclusion

Four different extracts were prepared using four different organic solvent out of this methanol solvent extract give comparatively show better biological activity. The results showed that *C. callosus* contained various phytochemical compounds such as alkaloids, tannins, phenolic compounds present. *C. callosus* extracts have shown good inhibitory effects on protein denaturation, alpha-amylase inhibitory activity, potential anthelmintic activity against various intestinal parasites and antibacterial activity against several bacterial strains. Overall, present bioactive compounds in *C. callosus* it may be potential therapeutic properties make it a promising candidate for the development of new natural medicines for various diseases and conditions. However, further studies are needed to fully understand the mechanisms of action and cytotoxicity of *C. callosus* extracts.

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### Authors' Contributions;

**Ms. Shraddha R vaghasiya:** Perform the particle and writing the MS.

**Dr. Kalpesh B Ishnava:** Supervision of the work, interpretation of the data and correction of the MS and submission.

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### Conflict of Interest:

No conflict of interest.

### Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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