

**ANTIOXIDANT AND ALPHA-AMYLASE INHIBITORY ACTIVITY OF  
METHANOL EXTRACT OF *COLOCASIA ESCULENTA* CORM**

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**Summary**

In the present investigation antioxidant and salivary alpha-amylase inhibitory activity of methanol extract of *Colocasia esculenta* corm was evaluated. The antioxidant activity of methanol extract of *C. esculenta* (CME) was evaluated by FRAP, DPPH, and ferric reducing power assay. Similarly, total phenol content and salivary alpha-amylase inhibitory activity of CME was evaluated by Folin-Ciocalteau and DNS method respectively. The result of total phenolic content assay reveals that CME containing  $0.0137 \pm 1.33$  mg GAE/g dw of CME. The observed resultant antioxidant activity of CME in all studied models was moderate as compared with reference standard BHT and BHA. At 1 mg/ml, CME showed salivary alpha-amylase inhibitory activity with IC<sub>50</sub> value of 0.94 mg/ml. In conclusion, from the results of present study it is confirmed that antioxidant and alpha-amylase inhibitory activity of methanol extract of *C. esculenta* may contribute in its earlier observed antidiabetic potential.

**Keywords:** *C. esculenta*, Corm, Antioxidant activity, alpha-amylase inhibitory activity

## Introduction

*Colocasia esculenta* (L.) Schott. belongs to family araceae, commonly known as Taro (English). In India, It is locally recognized by its different vernacular names as Alooka or Aloopam (Sanskrit), Kachalu or Khuyya (Hindi), Alu (Marathi), Seppan-Kizhangu (Tamil), Chamadumpa (Telgu). It is a hearty succulent herb, with clusters of long heart or arrowhead-shaped leaves that point earthwards, which grows on erect stems that may be green, red black or variegated [1]. Its leaves and corm are used as food in some parts of India [2]. Corm is traditionally used as an abortifacient, to treat tuberculous ulcers, pulmonary congestion, crippled extremities, fungal abscesses in animals, and as an anthelmintic [1, 3, 4]. The young leaves are rich in Vitamin C, and also contain thiamine, riboflavin, niacin, oxalic acid, calcium oxalate and saptotoxin[1,3]. Its corm contains starch, protein and considerable amount of oxalic acid along with anthocyanins perlargonidin 3-glucoside, cyaniding 3-rhamnoside and cyaniding 3-glucoside [1, 3, 4].

Recent pharmacological studies reveals that leaves of *C. esculenta* reported to had antibacterial and antifungal [1], anthelmintic [5], anti-inflammatory [6] and antidiabetic [7] activity. Moreover,  $\alpha$ -amylase inhibitory activity [8], antidiabetic [9], in vivo antiperoxidative and antioxidant activity [10] were also attributed to tuber or root of *C. esculenta*. The earlier reported  $\alpha$ -amylase inhibitors of *C. esculenta* tuber were proteins (A-1 and B-1) [8]. In the current study we assessed the antioxidant and salivary alpha-amylase inhibitory activity of methanol extract of *C. esculenta* corm, called as CME in order to understand the role of *C. esculenta* corm in diabetes and management of its further complications due to the increased oxidative stress.

## Material and methods

### Chemicals

1-Diphenyl-2-picryl-hydrazyl (DPPH) was purchased Hi media, Mumbai, india. TPTZ (2,4,6-tripyridyl-s-triazine), Methanol, Folin-Ciocalteau reagent, gallic acid, 3,5-dinitrosalicylic acid (DNSA), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) were purchased from Molychem, Mumbai, India. All these chemicals and reagents were of analytical grade.

### Preparation of methanol extract

The corms of *C. esculenta* were collected from the local area of Sangamner, MS, India. They were washed, chopped into small pieces and shade dried at temperature for 20 days. The dried plant material was pulverized into fine powder by using a grinder. About 20 g of powdered material was soaked in 100 mL methanol with occasional shaking, at room temperature. After 4 h, the methanol soluble material was filtered off. The filtrate was concentrated to dryness under vacuum at low temperature (60 °C) using a rotary evaporator.

### DPPH radical scavenging activity

The radical scavenging effect of CME was determined by using the DPPH method [11]. A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1.0 ml of CME in methanol with different concentrations (0.5-2.5 mg/ml). The reaction mixture was mixed thoroughly and kept in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. BHT and BHA were used as references.

**Total antioxidant activity (FRAP method)**

Total antioxidant activity was determined by FRAP (ferric reducing antioxidant potential) method [12]. The stock solutions included 300 mM acetate buffer (3.1 g  $C_2H_3NaO_2 \cdot 3H_2O$  and 16 ml  $C_2H_4O_2$ ), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-*s*-triazine) solution in 40 mM HCl, and 20 mM  $FeCl_3 \cdot 6H_2O$  solution. The fresh working solution was prepared by mixing 30 ml acetate buffer, 3 ml TPTZ, and 3 ml  $FeCl_3 \cdot 6H_2O$ . The temperature of the solution was raised to 37°C before using. CME (150  $\mu$ L) was allowed to react with 2850  $\mu$ l of the FRAP solution for 30 min in the dark condition. Readings of the colored product (ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 100 and 1000  $\mu$ M  $FeSO_4$ . Results were expressed in  $\mu$ M Fe (II)/g dry mass and compared with that of BHT and BHA.

**Reducing power assay**

The reducing power of CME was assessed by our earlier adopted method [13]. Various concentrations i.e. 50-250  $\mu$ L/ml of CME in methanol (10 mg/ml) and reference standard BHT, BHA (1 mg/ml) were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanate (2.5 ml). The mixture was incubated at 50 °C for 20 min. and then trichloroacetic acid (10%, 2.5 ml) was added. This was centrifuged at 3000 rpm for 10 min. The upper layer of the reaction mixture (2.5 ml) was mixed with 2.5 ml distilled water and 0.5 ml  $FeCl_3$  (0.1%). The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

**Determination of total phenolic content**

Total phenolic content in CME was measured by modified Folin-Ciocalteu method [14]. To 500  $\mu$ L (10 mg/ml) of CME in methanol, 2.5 ml of 1:10 dilution of Folin-Ciocalteu's reagent and 2 ml of  $Na_2CO_3$  (7.5%, w/v) were added and incubated at 45 °C for 15 min. The absorbance was measured at 765 nm. Results were expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g dw).

**In vitro salivary alpha amylase inhibitory assay**

Salivary alpha amylase was prepared by diluting the saliva of healthy individual with saline and stored in cold condition. Amylase activity was assessed by 3, 5-dinitrosalicylic acid colorimetric method [15]. 500  $\mu$ L of CME of concentrations 0.05-1 mg/ml were pre-incubated with 2.5 ml of 0.2 M sodium phosphate buffer, 500  $\mu$ L of diluted saliva and 1 ml of 1% NaCl for 10 min. at 37 °C. Further, 2.5 ml of 1% starch solution was added to reaction mixture and again incubated for 15 min. at 37 °C. The enzyme reaction was terminated by adding 1 ml of 3, 5-dinitrosalicylic acid (DNS) reagent. The assay tubes were kept in a boiling water bath for 5 min, cooled under tap water. The absorbance of resultant colored solution was measured at 540 nm. Controls without inhibitor were run simultaneously. One unit of enzyme activity is defined as the amount of enzyme required to release one micromole of maltose from starch per min under the assay conditions. Inhibitory activity was expressed in terms of percent inhibition of enzyme activity.

**Statistical analysis:** Data were expressed as the mean  $\pm$  S.E.M. All the assays were performed in triplicates. Statistical analysis was carried out by using graphpad prism 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com.

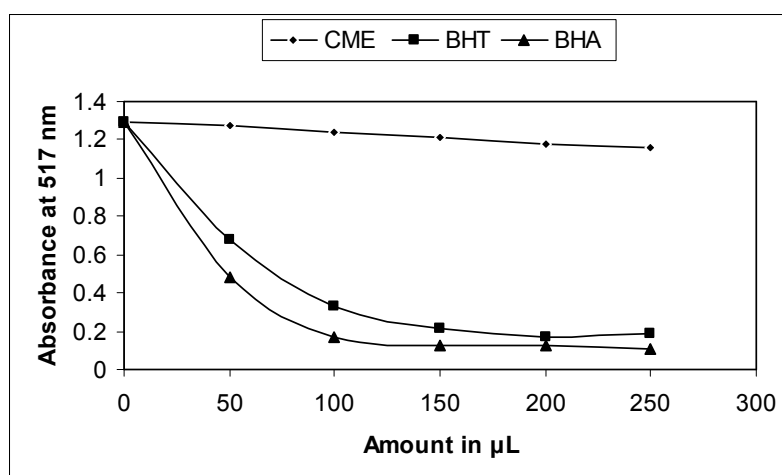
### Results and discussion

The earlier literature reports indicated that *C. esculenta* corm have potential application in the treatment of diabetes. In this study in order to understand its role in treating and management of complications of diabetes, antioxidant and salivary alpha-amylase inhibitory activity of methanol extract of *C. esculenta* corm was evaluated. The result of total phenolic content determination assay reveals that CME containing  $0.0137 \pm 1.33$  mg GAE/g dw of CME. The observed total phenolic content in CME was low. It is well known that the phenolic composition within the plants depends on chemotype and growth conditions. However, there was no any general correlation between total phenolic content and antioxidant activity [16].

Now days antioxidants are the most studied class of functional ingredients due to their protective role in various degenerative diseases such as diabetes, cancer, coronary diseases, inflammatory disorders etc., caused by increased oxidative stress by free radicals such reactive oxygen and nitrogen species (ROS/RNS). Antioxidants are known to neutralize these free radicals by donating an electron or hydrogen atom.

Fig. 1 reveals the concentration dependent DPPH radical scavenging activity of CME, BHT and BHA. The reduction capability of DPPH radical was determined by the decrease in its absorbance at 517 nm induced by antioxidants. Due to rapid hydrogen acceptable ability of DPPH, it reacts with antioxidants and gets converted into 1, 1-diphenyl-2-picrylhydrazine and hence shows decrease in absorbance [17]. At all concentrations CME exhibited moderate DPPH radical scavenging activity as compared to reference standard BHT and BHA.

**Fig. 1** DPPH free radical scavenging activity of CME (10 mg/ml), BHT and BHA (1 mg/ml)

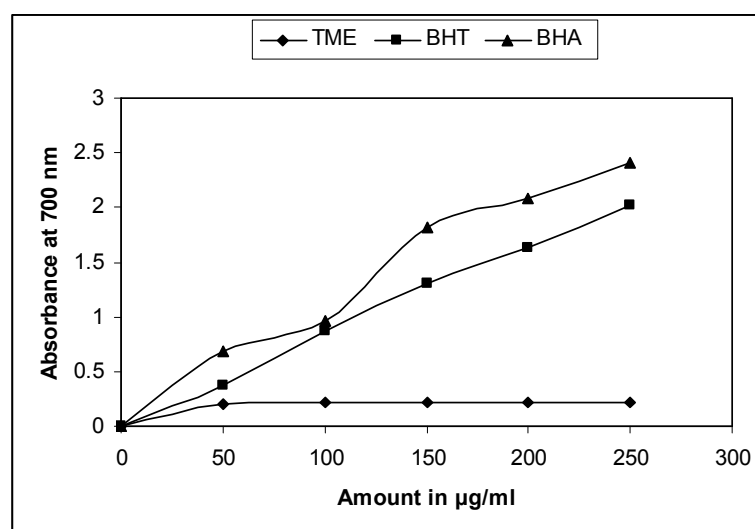


The antioxidant activity of CME, reference standard BHT and BHA was shown Table 1. In FRAP assay total antioxidant potential of CME was estimated from its ability to reduce TPRZ-Fe (III) complex to TPTZ-Fe (II) [12]. The observed FRAP value of CME was comparative lower than that of reference standard BHT and BHA.

**Table 1.** Total antioxidant activity of CME, BHT and BHA by FRAP method

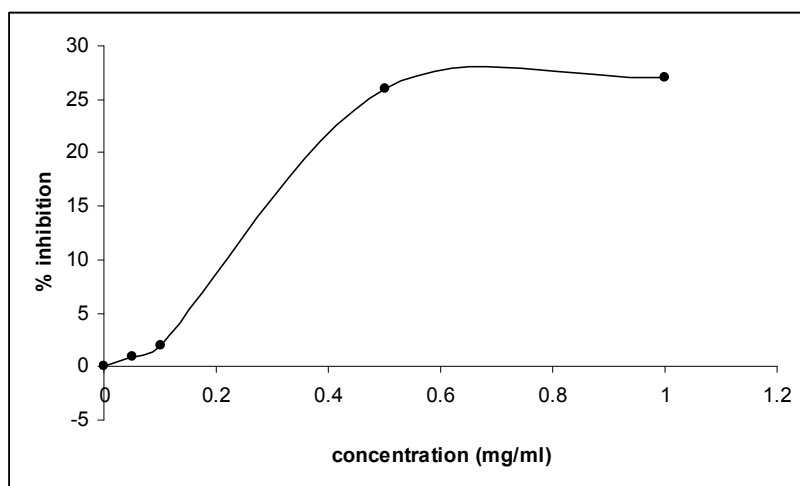
Sr. No.	Extract/Standard	FRAP value ( $\mu\text{M Fe (II)/g}$ )
1.	CME	516.6 + 7.27
2.	BHT	8666 + 7.22
3.	BHA	8333 + 7.44

Fig 2 exhibits the reducing power abilities of CME, BHT and BHA. The results of this indicated that reducing power of CME, BHT, and BHA increases with increase in concentration. The reducing power of a compound is related to its electron transfer ability and may therefore; serve as an indicator of its potential antioxidant activity [18]. The observed reducing power of CME was moderate in comparison with reference standard BHT and BHA.

**Fig. 2** Reducing power abilities of CME, BHT and BHA at different concentrations.

It is well known that amylase inhibitors prevent dietary starches from being digested and absorbed by the body. This could be useful for treating diabetes mellitus [8]. The percent salivary alpha-amylase inhibitory activity of CME at different concentration was shown Fig 3. The observed  $\text{EC}_{50}$  value i.e. the concentration of the extract, containing the  $\alpha$ -amylase inhibitor that inhibited 50% of the enzyme activity, of CME was 0.94 mg/ml.

**Fig. 3** The *in vitro* salivary alpha-amylase inhibitory activity of CME with four concentrations (i.e. 0.05, 0.1, 0.5, and 1mg/ml)



In conclusion, results of present study show that methanol extract of *C. esculenta* corm (CME) possess antioxidant potential in different studied models. However, the observed antioxidant potential of CME is moderate as compared to reference standard BHT and BHA. Result of salivary alpha-amylase inhibitory assay also reveals that CME possess salivary alpha-amylase inhibitory potential. From the results of the present antioxidant and salivary alpha-amylase inhibitory activity study of methanol extract of *C. esculenta* corm study, it is confirmed that the earlier observed antidiabetic potential of *C. esculenta* corm could be due to its antioxidant and amylase inhibitory activity.

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