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Original Article

In-vitro Antioxidant Activity of Hydroalcoholic Extract of Leaves of Colocasia Esculenta Linn

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Abstract

Context: Although *Colocasia esculenta* Linn. (Araceae) commonly known as patarveliya in Guajarati possesses several medicinal properties, a little is known about its antioxidant activity. **Objective:** The current research was designed to examine the antioxidative potential of hydroalcoholic extract of leaves of *Colocasia esculenta* (HECE) for the first time using several *in-vitro* analytical methods. **Materials and methods:** The antioxidant activity of HECE was evaluated using reducing power ability, nitric oxide scavenging assay and the estimation of total phenolic contents. **Results and Discussion:** The HECE gave an IC50 value of 45.75±0.38 µg/ml. The reducing power was investigated by Fe3+-Fe2+ transformation in the presence of extract tested using ascorbic acid as standard. The HECE showed increase in reducing ability with increase in concentration at 700nm. The total antioxidant capacity by nitric oxide scavenging method is expressed as curcumin equivalents. The content of total phenolics in HECE was found to be 49.21 ìg gallic acid equivalent/mg. **Conclusions:** Based on the above results, the higher the phenolic content, the higher the antioxidant capacity was very well observed with HECE. Hence the dried leaves of *C. esculenta* could be considered for preparation of neutraceuticals with potent antioxidant effect suitable for prevention of human disease. The results obtained in the current study indicate that HECE is a potential source of natural antioxidants.

Keywords: Antioxidant activity, *Colocasia esculenta*, Nitric oxide scavenging assay, Reducing power ability, Total phenolic contents

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Introduction

Natural products have been our single most successful source of medicines. Each plant is like a chemical factory capable of synthesizing unlimited number of highly complex and unusual chemical substances whose structures could otherwise escape the imagination forever. Although clinical trials and experiments involving whole animals are important in natural product screening but because of financial, ethical and time limitations, importance of *in-vitro* screening is gaining popularity [43]. Oxidative modifications of DNA, proteins, lipid, and small cellular molecules by reactive oxygen species (ROS) play a role in a wide range of common diseases and age-related degenerative conditions [5]. These include cardiovascular disease, inflammatory conditions, and neurodegenerative disease such as Alzheimer's, mutations, and cancer [3, 21, 37, 8, 24, 28]. Furthermore, antioxidants are also believed to play a cardinal role in the oxidative deterioration of cosmetics, foodstuffs, and pharmaceutical preparations. There is an increasing interest in natural antioxidants, namely polyphenols, present

in medicinal and dietary plants that might help prevent oxidative damage. The antioxidant activity of several plant materials has recently been described [42, 30, 39, 6, 11, 19, 27, 12] and a number of plant products, including polyphenols, flavonoids, and terpenes, exert an antioxidant action.

C. esculenta Linn. (Araceae) is widely distributed throughout India. Poi, a starchy paste produced from *C. esculenta* showed the Anti-Cancer effects on colonic adenocarcinoma cells *in vitro* [7]. Ethanolic extract of leaves showed an inhibitory effect on leukocyte migration and a reduction on the pleural exudates as well as reduction on the granuloma weight in the cotton pellet granuloma method thus showed anti-inflammatory activity [41]. Cyanoglucoside extract from *C. esculenta* showed hypoglycemic activity [18]. Arabinogalactan, dietary fiber from *C. esculenta* showed hypolipidaemic effect by decreasing hepatic production of VLDL [4]. Tarocystatin protein from *C. esculenta* showed strong antifungal activity on some ubiquitous phytopathogenic fungi [50]. Methanol and aqueous crude extract of different parts of *C. esculenta* showed antimicrobial activity against one or more species of bacteria [49]. Leaf juice is stimulant, expectorant, astringent, appetizer, and otalgia. The juice expressed from the leaf stalks are used with stalks is used with salt as an absorbent in cases of inflamed glands and buboes.

Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which are commonly used in processed foods, are known to have toxic and carcinogenic effects on human health [23, 29, 31]. Therefore, screening of plant species to identify new antioxidants has become critically important in recent years. There are so far no reports related to antioxidant activity of HECE. The purpose of this study was to determine the antioxidant capacity and phenolic contents of HECE.

Methods

Plant Material

Leaves of *C. esculenta* were purchased from local market. The plant was identified and authenticated by Botanical Survey of India, Jodhpur. A voucher specimen (SU/DPS/Herb/05) of the same has been deposited in the Department of Pharmaceutical Sciences, Saurashtra University for the future reference.

Chemicals

All chemicals were analytical grade and chemicals required for all biochemical assays were obtained from Sigma Chemicals Co., USA.

Preparation of crude extract

Leaves were dried in shade, moderately grinded by electric grinder. The powdered materials (leaves) were subjected to qualitative tests for the identification of various phytoconstituents like alkaloids, glycosides, steroids, terpenoids and flavanoids. Then the powder was macerated with hydroalcoholic solvent (ethanol: water - 50:50) for 7 days with intermittent shaking. On 8th day the macerate was filtered through muslin cloth and solvent was removed under reduced pressure and the hydroalcoholic extract was then obtained (yield-9.8% w/w). The extract was stored at 5 ^{o}C until use.

Phytochemical screening

Preliminary phytochemical investigations conducted as per the procedures described by Kokate [26] and Trease and Evans [48].

Reducing power ability

The reducing power capacity of the extracts was assessed as described by Oyaizu [33]. The Fe2+ can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. One ml of the extract (20-100 ìg/ml), 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide were incubated at 50°C for 30 min and 2.5 ml of 10% trichloroacetic acid was added to the mixture and centrifuged for 10 min at 3000g. About 2.5 ml of the supernatant was diluted with 2.5 ml of water and is shaken with 0.5 ml of freshly prepared 0.1% ferric chloride. The absorbance was measured at 700 nm. Ascorbic acid was used as the standard. All tests were performed in triplicate and the graph was plotted with the average of the three determinations.

Nitric oxide scavenging method

Nitric oxide generated from sodium nitroprusside was measured by the Griess reagent by the method described by Rao [35]. Various concentration of the extract and sodium nitroprusside (10 mM) in phosphate buffer saline and 150 µl of each dose level

by dilution with methanol was incubated at room temperature for 15 min. After the incubation period, 5 ml of Griess reagent (1% sulphanilamide, 2% o-phosphoric acid, 0.1% napthyl ethylenediamine dihydrochloride) was added. The absorbance of the chromophore formed was read at 546 nm using UV-visible spectrophotometer, shimadzu, UV-1700, Japan. The inhibition of nitric oxide generation was estimated by comparing the absorbance values of control with that of treatments. Curcumin was used as standard values are reported as mean ± SEM of three determinations.

Estimation of total phenolic content

Total soluble phenolics of the extract were determined with Folin-Ciocalteu reagent using Gallic acid as the standard [29]. An aliquot of 0.1 ml suspension of 1 mg of the extracts in water was totally transferred to a 100 ml volumetric flask and the final volume was adjusted to 25 ml by the addition of distilled water. Folin-Ciocalteu reagent (1 ml) was added to this mixture, followed by 4 ml of 20% sodium carbonate 5 min later. Subsequently, the mixture was shaken for 30 min at room temperature and the absorbance was measured at 760 nm using UV-visible spectrometer shimadzu, UV-1700, Japan. The concentration of total phenolic compounds in the extracts was determined as ìg gallic acid equivalent by using the standard gallic acid graph.

Statistical analysis

All results are expressed as mean ± S.E.M. Linear regression analysis (Origin 6.0 version) was used to calculate the IC50 values.

Results

Phytochemical investigations

Preliminary phytochemical tests of HECE show the presence of glycoside, phytosterol, phenolic compounds, saponin and flavonoids as predominant active constituent.

Reducing power ability

Antioxidants have an important role in preventing a variety of diseases and aging because they inhibit or delay the oxidation process by blocking the initiation or propagation of oxidizing chain reactions [1, 44]. The reducing ability of a compound generally depends on the presence of reductants [34], which have exhibited antioxidative potential by breaking the free radical chain, donating a hydrogen atom [17]. The presence of reductants in the extracts causes the reduction of the Fe3+-ferricyanide complex to the ferrous form. Therefore, the Fe2+can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Figure 1 shows reducing capacities of HECE

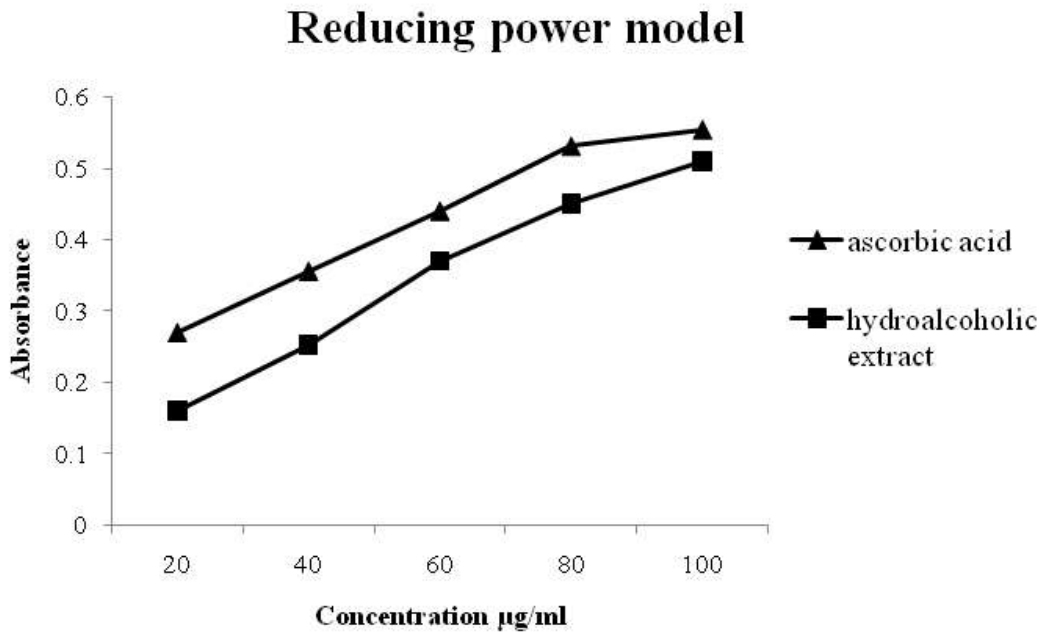

Fig.1: Antioxidant activity of different concentrations of hydroalcoholic extract and ascorbic acid in reducing power method. Each value represents mean ± SEM

compared with ascorbic acid. The reducing power of HECE and standards increased with increasing concentration of samples. The leaf extract showed the highest reducing ability. However, the activity was less than the standard, ascorbic acid.

Nitric oxide scavenging method

Active oxygen species and free radicals are involved in a variety of pathological events. In addition to ROS, nitric oxide is also implicated in inflammation, cancer and other pathological conditions. A potential determination of oxidative damage is the oxidation of tyrosine residue of protein, peroxidation of lipids, and degradation of DNA and oligonucleosomal fragments. Nitric oxide or reactive nitrogen species formed during its reaction with oxygen or with superoxide such as NO2, N2O4, N3O4, nitrate and nitrite are very reactive. These compounds alter the structure and function of many cellular 81 components. Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of this damage. IC50 value of leaves extract was found to be 45.75 ± 0.38 ìg/ml (Table 1).

Table 1: Comparison of IC50 values of hydroalcoholic extract with standard in nitric oxide scavenging method

Model	IC50 value of HECE (µg/ml)	IC50 value of standard (µg/ml)
Nitric oxide scavenging method	45.75 ± 0.38	28.25 ± 1.07

Total phenolic content

The amount of total phenolic compounds was investigated in HECE. Phenols are very important plant constituents because of their scavenging ability which is due to their hydroxyl groups [13]. The total amount of phenolic compounds in the plant extracts was determined as micrograms of gallic acid equivalent by using an equation that was obtained from standard gallic acid graph. HECE (1 mg) was equivalent to 49.21 ìg gallic acid.

Discussion

There are many reports that support the use of antioxidant supplementation in reducing the level of oxidative stress and in slowing or preventing the development of complications associated with diseases [32, 38, 46]. Many synthetic antioxidant components have also shown toxic or mutagenic effects, which have shifted attention toward the naturally occurring antioxidants. Numerous plant constituents have been proved to show free radical scavenging or antioxidant activity [2, 45]. In this respect, flavonoids and other polyphenolic compounds have received greatest attention. Plant tissues contain a network of compounds that control the level of reactive oxygen species, including phenolic compounds, vitamins C and E, glutathione, and several enzymes. Phenolic compounds widely distributed in the natural plant tissues include flavonoids, tannins, hydroxycinnamate esters, and lignins [36]. Furthermore, interest in employing antioxidants from natural sources to increase the shelf life of foods is considerably enhanced by consumer preference for natural ingredients and concerns about the toxic effects of synthetic antioxidants [15, 40, 47]. *C. esculenta* seems to be a good source of plant species containing large amounts of flavonoids and phenolic compounds, so it is considered to be a promising source of natural antioxidants [25].

In the current study, the antioxidant activities of HECE were determined by using different antioxidant tests. It has been reported that reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity [51, 9]. HECE exhibited comparatively similar reducing power as ascorbic acid suggesting that it had strong electron-donating capacity.

Nitric oxide is a free radicals product in mammalian cells, involved in the regulation of various physiological processes. However, excess production of NO is associated with several diseases [22]. In the present study the nitrite produced by the incubation of solutions of sodium nitroprusside in standard phosphate buffer at 25ÚC was reduced by the HECE. This may be due to the antioxidant principles in the extract which compete with oxygen to react with nitric oxide thereby inhibiting the generation of nitrite.

It has also been reported that the antioxidant activity of plant materials was well correlated with the content of their phenolic compounds [14, 16, 10].

Estimation of total phenolics using the Folin-Ciocalteu reagent and gallic acid as a standard revealed that *C. esculenta* is a good source of polyphenol.

Conclusion

On the basis of the results obtained in the current study, we conclude that the HECE possesses high antioxidant activities that might be helpful in preventing or slowing the progress of various oxidative stress–related diseases. The HECE studied here could also be considered as good candidates for food preservation or functional foods, as well as for pharmaceutical and natural plant–based products. Further investigations on the isolation and identification of antioxidant components in the plants may lead to chemical entities with potential for clinical use.

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