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In vitro antioxidant activities of ethanolic leaf extract of *Corchorus trilocularis* L. (Tiliaceae)

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ABSTRACT

Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. The aim of this study is design to investigate antioxidant activities of ethanolic extract from leaves of *Corchorus trilocularis* in various *invitro* methods. Antioxidant properties are tested using four *invitro* methods such as 2, 2-diphenyl-1-picryl-hydrazyl (dpph) assay, superoxide radical scavenging activity, Iron chelating activity and nitric oxide radicals scavenging activity. Our results indicate that ethanolic leaf extract of *Corchorus trilocularis* has IC_{50} value of 450 µg/ml, 170 µg/ml, 310 µg/ml, 450 µg/ml of dpph, superoxide, iron chelating, nitric oxide radicals scavenging activity respectively. In all the testing, a significant correlation existed between concentrations of the extract and percentage inhibition of free radicals. The free radical scavenging activities may be attributed to the presence of adequate phenolic (6.84 mg/g of Gallic acid) and flavonoids compound (4.25 mg/g of Quercetin). These *in vitro* assays indicate ethanolic leaf extract of *Corchorus trilocularis* is a better source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Keywords: *Corchorus trilocularis*, Antioxidant activity, Total phenolic, Flavonoid content.

1. INTRODUCTION

Living cells may generate free radicals and other reactive oxygen species as a results of physiological and biochemical processes [1]. They are generally by-product of various endogenous processes that can be stimulated by external factors, such as air pollution, irradiation, smoking, stress and toxins present in food and/or drinking water. Lots of research has clearly showed that free radicals and ROS are exert oxidative damaging effects by reacting with nearly every molecule found in living cells including cell membranes, cytoplasm, ribosome, mitochondria, DNA and RNA [2]. They entail contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS [3, 4]. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural resources [5]. Plants have a good source of naturally occurring and biologically active compounds known as phytochemicals. These phytochemicals have been found to act as antioxidants by scavenging free radicals and may have therapeutic potential for free radical associated disorders [6]. Many plant extracts and phytochemicals have shown to have free radical scavenging properties but generally there is still a demand to find more information concerning the antioxidant potential of plant species. Recently, there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury [7]. Synthetic antioxidant like butylated hydroxyl toluene (BHT), butylated hydroxyanisole (BHA), propylgallate (PG) and tertiary butyl-hydroquinone (TBHQ) are known to ameliorate oxidative damages but they have been restricted due to their carcinogenic and harmful effect on the lungs and liver [8]. Therefore, investigations of antioxidants are focused on naturally occurring substances, especially plant phytochemicals. *Corchorus trilocularis* L. (Tiliaceae) is one of the most common plants in

India and is available throughout the year. The plant has been reported to possess various activities such as anti-inflammatory [9], antidiabetic [10] and demulcent properties [11]. In traditional folklore medicine in India, *Corchorus trilocularis* is also used for the treatment of syphilis [12]. Natural antioxidant compounds such as n-Hexadecanoic acid, Vitamin E, Squalene have been identified in *Corchorus trilocularis* by GC/MS analysis [13]. In fact, as far as we know, there have been no reports available in the literature on the antioxidant activity of ethanolic leaf extracts of *Corchorus trilocularis* Linn. Therefore we carry out the current investigation to examine the antioxidant activities of ethanolic leaf extracts of *Corchorus trilocularis* through various in vitro models.

2. MATERIALS AND METHODS

2.1. Collection and identification of plant materials

Fresh leaves of the selected plant *Corchorus trilocularis* were collected from Thirunelveli district, Tamil Nadu, India. The plant materials were taxonomically identified and authenticated by Dr. V. Chelladurai, Research officer - Botany (scientist C), Central council for research in Ayurveda and Siddha, Govt. of India; Thirunelveli. The plant was thoroughly washed in running tap water to remove soil particles and adhered debris and finally washed with sterile distilled water. The leaves of the plant alone were segregated and dried under shade, pulverized by a mechanical grinder into fine powder. The powdered materials were stored in air tight polythene bags till use.

2.2. Chemicals

All chemicals used for the current study were of analytical grade and purchased from Sigma, USA and SD fine, India.

2.3. Preparation of extracts

The *Corchorus trilocularis* plant powdered materials were extracted with 99.9 % v/v ethanol (70–80 ^{o}C) by hot continuous percolation method in Soxhlet apparatus for 24 hrs [14]. The extract was concentrated by using a rotary evaporator and subjected to freeze up drying in a lyophilizer till dry powder was obtained.

2.4. 2, 2- diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay [15]

A methanolic solution of 0.5 ml of DPPH (0.4 mM) was added to 1 ml of the different concentration of plant extract and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extract served as the positive control. Rutin used as standard. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

Scavenging activity (%) = A_{518} Control - A_{518} sample/ A_{518} Control X 100

Where A_{518} Control is the absorbance of DPPH radical with methanol;

A_{518} Sample is the absorbance of DPPH radical with sample extract/standard.

2.5. Superoxide radical scavenging activity [16]

Superoxide radical (O_2^-) was generated from the photo reduction of riboflavin and detects by nitro blue tetrazolium dye (NBT) reduction method. The assay mixture contain sample with 0.1 ml of nitro blue tetrazolium (1.5 Mm NBT) solution, 0.2 ml of ethylene diamine tetra acetic acid (0.1 M EDTA), 0.05ml of riboflavin (0.12 mM) and 2.55 ml of phosphate buffer (0.067 M phosphate buffer). The control tubes also set up where in DMSO was added instead of sample. The reaction mixture was illuminated for 30 minutes and the absorbance at 560 nm was measures against the control sample. Quercetin was used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculates by comparing the results of control and the test samples.

% inhibition of $O_2^{-}\bullet$ = $[A_0-A_1]/A_0$ X 100

Where

A_0 is the absorbance of control; A_1 is the absorbance of test

2.6. Iron chelating activity [17]

The principle is based on the formation of O-Phenanthroline-Fe^{2+} complex and its disruption in the presence of chelating agents. The reaction mixture containing 1 ml of 0.05% O-Phenanthroline in ethanol, 2 ml ferric chloride (200 µM) and 2 ml of various concentrations of samples ranging from 10 to 1000 µg was incubated at room temperature for 10 min and the absorbance of the same was measured at 510 nm. EDTA was used as a classical metal chelator. The experiment was performed in triplicates.

% inhibition of Fe radicals = $[A_0-A_1]/A_0$ X 100

Where

A_0 is the absorbance of control; A_1 is the absorbance of test

2.7. Nitric oxide radical scavenging activity [18]

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which was measured by the following method. The reaction mixture (3ml) containing 2ml of sodium

nitroprusside (10 mM), 0.5 ml of phosphate buffer saline (1 M) 0.5 ml of ethanolic extract were incubated at 25°C for 150 mins. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipette out and mixed with 1 ml of sulphanilic acid reagent (0.33%) and allowed to stand for 5 min for completing diazotization. Then 1 ml of naphthylethylene diamine dihydrochloride (1% NEDA) was added, mixed and allowed to stand for 30 mins. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interact with oxygen to produce nitrite ions which can be estimated by the use of Griess Illosvery reaction at 540 nm.

% inhibition of NO• = $[A_0-A_1]/A_0$ X 100

Where

A_0 is the absorbance of control; A_1 is the absorbance of test.

2.8. Determination of Total Phenolic contents [19]

The total phenol content was determined by Folin-Cio-calteu reagent method. 0.5 ml of extract (1:5dilution) and 0.1 ml of Folin-Ciocalteu reagent (0.5 N) were mixed and incubated at room temperature for 15 min. 2.5 ml saturated sodium carbonate was added, incubated for 30 min at room temperature and absorbance was measured at 760 nm. The total phenol content was expressed in terms of Gallic acid equivalent (mg/g).

2.9. Determination of Total flavonoids contents [20]

The reaction mixture (3.0 ml) that comprised of 1.0 ml of extract (1:10 dilution), 0.5 ml of aluminum chloride (1.2%) and 0.5 ml of potassium acetate (120 mM) was incubated at room temperature for 30 min and absorbance was measured at 415 nm. The total flavonoid content was expressed in terms of quercetin equivalent (mg/g)

2.10. Statistical analysis

Tests were carried out in triplicates. The mean values were calculated from the triplicate values. Values were expressed as the Mean ± SD (n=3) and differences between groups were considered to be statistically significant if $p<0.05$. Data from the test groups were compared with IC50 value of the standard which is the concentration of sample, required to scavenge 50% of DPPH, nitric oxide and iron free radicals

3. RESULTS AND DISCUSSION

Free radical is a molecule with an unpaired electron and is involved in bacterial and parasitic infections, lung damage, inflammation, reperfusion injury, cardiovascular disorders, atherosclerosis, aging and neoplastic diseases [21]. They are also involved in autoimmune disorder like rheumatoid arthritis etc. [22]. Antioxidant compounds may function as free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen development [23]. Various validations suggest that the biological actions of some natural compounds are correlated to their antioxidant activity [24]. Therefore, in the recent years the search for natural antioxidants has increased among researchers. Our results also demonstrated that the ethanolic leaves extracts of *Corchorus trilocularis* possess free radical scavenging activity *in vitro* models

3.1. 2, 2- diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH• assay has been generally used as a quick, reliable and reproducible parameter to search the *in vitro* general antioxidant activity of pure compounds as well as plant extracts [25] .Antioxidants either transfer an electron or a hydrogen atom to DPPH•, thus neutralizing its free radical character [26]. The reducing capacity of compounds could serve as indicator of potential antioxidant property [27]. In the present study, ethanolic extract of leaves of *Corchorus trilocularis* L. showed maximum DPPH radicals scavenging activity of 57.92 % at 500 µg/ml whereas for Rutin (standard) was found to be 67.37 % at 500 µg/ml. This study reports that the percentage of scavenging effect on the DPPH• radical was concomitantly increased with the increase in the concentration of ethanolic leaf extracts from 100 to 500 µg/ml (Table 1).

Table - 1: DPPH radical scavenging activities of *Corchorus trilocularis* L

S. No	Conc. (µg/ml)	Ethanolic extract of *Corchorus trilocularis*	Rutin (Std)
1	100	12.04±0.054	15.52±0.5
2	200	17.43±0.20	21.61±0.79
3	300	29.47±0.014	37.28±0.13
4	400	42.63±0.076	46.73±0.47
5	500	57.92±0.051	67.37±0.11
IC_{50} = 450 µg/ml; IC_{50} = 420 µg/ml			

*All values are expressed as mean± SEM for the three determinations.

The IC_{50} of the ethanolic extract of leaves of *Corchorus trilocularis* L, was found to be 450µg/ml which was less than the standard Rutin (IC_{50}, 420µg/ml)

3.2. Superoxide radical scavenging activity

Superoxide is a highly reactive molecule that reacts with various substances produced through metabolic processes. Superoxide dismutase enzymes present in aerobic and anaerobic organisms catalyses the breakdown of superoxide radical [28]. Percentage scavenging of superoxide anion examined at different concentrations of ethanolic leaf extract of *Corchorus trilocularis* (100, 200, 300, 400, 500 µg/ml) was depicted in **Table 2.**

Table - 2: Superoxide radical scavenging activity (% Inhibition) of *Corchorus trilocularis* L

S. No	Conc. (µg/ml)	Ethanolic extract of *Corchorus trilocularis*	Quercetin (Std)
1	100	31.37±0.051	54.82±0.21
2	200	54.95±0.23	78.69±0.042
3	300	74.28±0.028	84.43±0.022
4	400	78.12±0.024	90.52±0.013
5	500	84.27±0.16	93.35±0.011
IC_{50} = 170 µg/ml ; IC_{50} = 90 µg/ml			

*All values are expressed as mean± SEM for the three determinations.

It was revealed that Superoxide radicals were scavenged by extracts in a concentration-dependent manner. Ethanolic leaf extract of *Corchorus trilocularis* showed maximum superoxide radical scavenging activity of 84.27 % at 500 µg/ml whereas for quercetin (standard) was found to be 93.35 % at 500 µg/ml. The IC_{50} of the ethanolic extract of *Corchorus trilocularis* and quercetin were found to be 170µg/ml and 90µg/ml respectively. The IC50 of the ethanolic extract of leaves of was less than the standard quercetin

3.3. Iron chelating activity

Iron is an extremely reactive metal and catalyses oxidative changes in lipids, proteins and other cellular components, but it is essential for life because it is required for oxygen transport, respiration and activity of many enzymes [29]. It causes lipid peroxidation through the Fenton and Haber-Weiss reaction [30] and decomposes the lipid hydroxide into peroxyl and alkoxyl radicals that can perpetuate the chain reaction [31]. The iron chelating activity of ethanolic extract of *Corchorus trilocularis* and standard EDTA were tabulated in **Table 3.** Ethanolic extract of leaves of *Corchorus trilocularis* L. showed maximum iron chelating activity of 68.11% at 500 µg/ml whereas for EDTA (standard) was found to be 82.65 % at 500 µg/ml. The IC_{50} of the ethanolic extract of *Corchorus trilocularis* and EDTA were found to be 310µg/ml and 140µg/ml respectively

Table - 3: Iron chelating activity (% Inhibition) of *Corchorus trilocularis* L

S. No	Conc. (µg/ml)	Ethanolic extract of *Corchorus trilocularis*	EDTA (Std)
1	100	27.68±0.029	43.27±0.003
2	200	42.82±0.012	61.41±0.011
3	300	49.28±0.011	69.46±0.015
4	400	58.88±0.036	78.61±0.010
5	500	68.11±0.022	82.65±0.014
IC_{50} = 310 µg/ml; IC_{50} = 140 µg/ml			

*All values are expressed as mean± SEM for the determination

3.4. Nitric oxide radical scavenging activity

Nitric oxide is an important chemical mediator generated by endothelial cells macrophages, neurons, etc. which is involved in the regulation of various physiological processes. Excess concentration of nitric oxide associated with several diseases. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxy nitrite anions, which act as free radicals [32]. Ethanolic extract of leaves of *Corchorus trilocularis* L. showed maximum nitric oxide radical scavenging activity of 53.62% respectively at 500 µg/ml whereas for ascorbic acid (standard) was found to be 57.28 % at 500 µg/ml. The IC_{50} of the ethanolic extract of *Corchorus trilocularis* and ascorbic acid were found to be 450µg/ml and 400µg/ml respectively. The nitric oxide radical scavenging activity of ethanolic extract of *Corchorus trilocularis* and standard ascorbic acid were tabulated in Table 4.

Table - 4: Nitric oxide radical scavenging activity (% Inhibition) of *Corchorus trilocularis* L.

S. No	Conc. (µg/ml)	Ethanolic extract of *Corchorus trilocularis*	Ascorbic acid (Std)
1	100	20.29±0.018	24.36±0.011
2	200	34.44±0.022	41.37±0.08
3	300	39.34±0.029	47.68±0.021
4	400	44.52±0.017	49.53±0.036
5	500	53.62±0.091	57.28±0.013
IC_{50} = 450 µg/ml; IC_{50} = 400 µg/ml			

*All values are expressed as mean± SEM for the determination

3.5. Total phenolic content

Phenolic compounds are known as powerful chain breaking antioxidant [33] and they are very important a plant constituent because of their scavenging ability, which is due to their hydroxyl groups [34]. In ethanolic leaf extracts of *Corchorus trilocularis,* the total phenolic content was found to be 6.84 mg/g of Gallic acid (Table 5).

3.6. Total flavonoids content

Flavonoids are a group of polyphenolic compounds, which exhibit several biological effects such as anti-inflammatory, anti-hepatotoxic, anti-ulcer, anti-allergic, anti-viral and anti-cancer activities [35]. They are capable of effectively scavenging the reactive oxygen species because of their phenolic hydroxyl groups and so they are potent antioxidants also [36]. The total flavonoids content of ethanolic leaf extracts of *Corchorus trilocularis* was determined to be 4.25 mg/g of Quercetin (Table 5).

Table - 5: Total Phenolic and flavonoids content in *Corchorus trilocularis L*

S. No	Content	Ethanolic extract of *Corchorus trilocularis*
1	Total phenolic content	6.84±0.010 (mg/g of Gallic acid)
2	Total flavonoids content	4.25±0.0132 (mg/g of Quercetin)

* All values are expressed as mean± SEM for the three determinations

4. CONCLUSION

In the present study, ethanolic leaf extract of *Corchorus trilocularis* exhibited outstanding scavenging effect on 2, 2-diphenyl-1-picryl-hydrazyl (DPPH), superoxide radical, Iron chelating and nitric oxide radical *invitro*. Results of our study clearly showed that ethanolic extract of *Corchorus trilocularis* L. leaves is a potent scavenger of reactive species and higher antioxidant capacity. Presence of adequate amount of phenol and flavonoid compounds may account for this fact. Therefore, *Corchorus trilocularis* can be used as an accessible source of natural antioxidants and a possible food supplement, with potential application in the pharmaceutical industry. Further studies are warranted for the isolation and characterization of antioxidant compounds, and also *in vivo* studies are needed for understanding their mechanism of action as antioxidants.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest

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