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INVITED ARTICLE

EVALUATION OF THE ANTIOXIDANT PROPERTY OF THE ETHANOLIC EXTRACTS OF CHONEMORPHA MACROPHYLLA ROOTS

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Abstract

Ethanol extracts of *C. macrophylla* roots were prepared using 50% and 95% ethanol and were named as CMRH and CMRE respectively. Preliminary phytochemical studies were performed and identified the presence of polyphenols and flavonoids. Antioxidant studies of both the extracts were performed using ABTS scavenging assay, DPPH scavenging assay, nitric oxide scavenging assay and lipid peroxidation assay methods. The IC_{50} values of CMRH, CMRE and rutin in ABTS assay were 5.63±0.74, 2.30±0.20 and 3.03±0.06 µg/ml respectively. In the DPPH assay, the IC_{50} values were 71±0.58, 72.33±2.89 and 32.08±0.88 µg/ml respectively. IC_{50} values obtained in nitric oxide scavenging assay were around 1 mg/ml for both the samples. The IC_{50} values obtained in LPO assay were 210±10.0 µg/ml and 230±2.0 µg/ml for CMRE and BHA respectively. The IC_{50} value obtained in LPO assay for CMRH was above 1 mg/ml. All of the above results indicate the antioxidant potential of both the extracts and it can possibly due to the polyphenols and flavonoid antioxidants present in the plant.

Keywords: *C. macrophylla roots, evaluation of antioxidant property, ABTS scavenging assay, DPPH scavenging assay, nitric oxide scavenging assay, Lipid peroxidation assay.*

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Introduction

Plants are being examined closely for new antioxidants, owing to the beneficial health effects of phytochemical antioxidants. Antioxidants through its free radical scavenging activity are expected to protect cells from the attack of free radicals. The consumption of antioxidants containing polyphenols and flavonoids found to decrease the incidents of diseases related to oxidative stress[1]. Excessive oxidative stress has been implicated in the pathology and complications of *Diabetes mellitus*[2]. Free radical action leading to oxidative damage of lipids and DNA is also reported[3]. According to WHO, 65 to 80% of the people of the developing countries depend on medicinal plants for treatment[4]. Use of *C. macrophylla* roots is mentioned in many Ayurvedic preparations[5]. Synonyms of C. macrophylla are *C. fragrans* and *C. grandiflora*. Thus it was decided to evaluate the traditional claims to find a sustainable, ecofriendly, nontoxic and low cost agent for humanity. The flavonoid content in the methanol fraction of the CMRH was determined by aluminium chloride colorimetric assay. The value determined of flavonoid by extrapolation from graph is 24.45 µg/ml equivalent to rutin[6].

Materials and Methods:

Two different extracts of *C. macrophylla* roots were prepared using 50% and 95% ethanol and were used for the studies[6]. The extract prepared using ethanol 50% was named as CMRH and the ethanol 95% extract was named as CMRE. The plants were identified by Dr. K V George, Professor of Botany, CMS College, Kottayam and the voucher specimen number is KRK/UCP/CMS/505.

Preparation of the test solutions:

The standard rutin and the extracts for testing were weighed accurately and separately dissolved in distilled DMSO to get stock solutions of 10 µg/ml. These solutions were serially diluted with DMSO to obtain the required lower dilutions and were used for antioxidant studies.

ABTS assay:

ABTS solution 2mM concentration was prepared in distilled water(100 ml) and added 17 mM potassium persulphate to it(0.3 ml). Potassium persulphate solution (17 mM) was prepared in distilled water. Serial dilutions of stock solution ranging from 100 µg/ml to 1.56 µg/ml of the extract were made using DMSO. The above prepared dilutions (20 µl), DMSO (100 µl) and ABTS 2 mM solution (16 µl) were taken in the wells of 96 well micro-titer plate, mixed and allowed to react. Same volume of DMSO alone was served as blank. The experiment was performed in triplicate. Absorbance was measured after 20 minutes using a micro plate reader at 750 nm against to respective blanks. Control was also performed in a similar manner omitting the test samples[7].

DPPH radical scavenging assay:

DPPH solution100 µM was prepared in methanol. Serial dilutions of stock solution ranging from 1000 µg/ml to 15.6 µg/ml of

the extract were made using DMSO. DPPH solution 200 µl was taken in the wells of the micro-titer plate, 50 µl of each of the test sample or the standard solution was added separately to the wells of the micro-titer plate. Control and blank were performed in a similar way as in the previous assay. The plates were incubated at 37°C for 30 minutes and the absorbance was measured at 490 nm, using a micro plate reader against the respective blanks[8].

Nitric oxide scavenging assay:

Sodium nitroprusside solution 10 mM was prepared in distilled water. Acetic acid 50% and 20% were used to prepare naphthyl ethylene diaminedihydrochloride solution (NEDD, 0.1%w/v) and sulphanilic acid solution (0.33% w/v) respectively. Serial dilutions of stock solutions ranging from 1000 to 15.6 µg/ml of the extract were made in DMSO. The reaction mixture containing sodium nitroprusside (10 mM, 4ml), phosphate buffer saline (PBS, pH-7.4, 1 ml) and the extract in DMSO (1ml) at various concentrations and standard were separately incubated at 25°C for 150 minutes. After incubation 50 µl of each of the reaction mixture containing nitrite ion was added to wells in a micro-titer plate, sulphanilic acid (100 µl) was added, mixed well and allowed to stand for 5 minutes for completion of diazotisation. Then 100µl of NEDD reagent was added, mixed and allowed to stand for 30 minutes in diffused light. The pink colour developed was measured at 540 nm against blanks in a microplate reader. Control experiment was also performed in the same way without the test extract[9].

Lipid peroxidation assay:

Ferric chloride solution 2mM, Trichloroacetic acid solution (50%v/v) (TCA), Thiobarbituric acid solution (0.37%w/v) (TBA) and phosphate buffer (pH-7.4) were prepared in distilled water. Egg lecithin: The egg yolk was separated and washed with acetone until the yellow colour disappeared. The solvent was evaporated to yield a creamy white powder. The powder was dissolved in phosphate buffer, pH-7.4 at a concentration of 3 mg/ml and used for further analysis. Serial dilutions of stock solutions ranging from 1000 to 15.6 µg/ml of the extract were made in DMSO. The reaction mixture containing egg lecithin (1 ml), ferric chloride solution (0.02 ml) and extract or standard (0.1 ml) in DMSO at various concentrations were incubated for 1 hour at 37°C. After incubation 15% TCA (2ml) and 0.37% TBA (2 ml) were added. Then the reaction mixture was boiled for 15 minutes, cooled, centrifuged and separated the supernatant. Transferred 100 µl each of the supernatant liquid to wells in a micro titer plate and the absorbance was measured at 540 nm using a microplate reader against respective blanks[10]. Control experiment was performed in a similar way without the test extract. All the experiments were performed in triplicate.

Statistical analysis:

All the antioxidant assays were performed in triplicate and the results were presented as mean ± standard deviation (SD).

Results:

The results of antioxidant evaluations performed by ABTS, DPPH and nitric oxide scavenging assay are depicted in table1. The IC_{50} values determined for CMRH, CMRE and the known antioxidant rutin were 5.63±0.74, 2.30±0.20 and 3.03±0.06 µg/ml respectively in the ABTS radical scavenging assay studies. In the DPPH assay, the IC_{50} values of CMRH, CMRE and the known antioxidant rutin were 71.0±10.58, 72.33±2.89 and 32.08±0.88 µg/ml respectively. IC_{50} values obtained in nitric oxide scavenging assay method for CMRH, CMRE and rutin were Ã 1000, 923.33±7.5 and 86±2.0 µg/ml respectively.

Table 1: Antioxidant activity of test samples

Samples	IC_{50} ± SD values in µg/ml by methods			
	Nitric oxide	ABTS	DPPH	LPO assay
CMRE	923.33±7.5	2.30±.0.20	72.33±2.89	210 ± 10.00
CMRH	>1000.00	5.63±0.74	71±10.58	> 1000
Rutin	86± 2.00	3.03±0.06	32.08 ± 0.88	-
BHA	-	-	-	230.00 ±2.00

n=3

In the LPO assay the IC50 values of CMRE and the known antioxidant BHA were 210±10.00 and 230±2.0 µg/ml respectively.

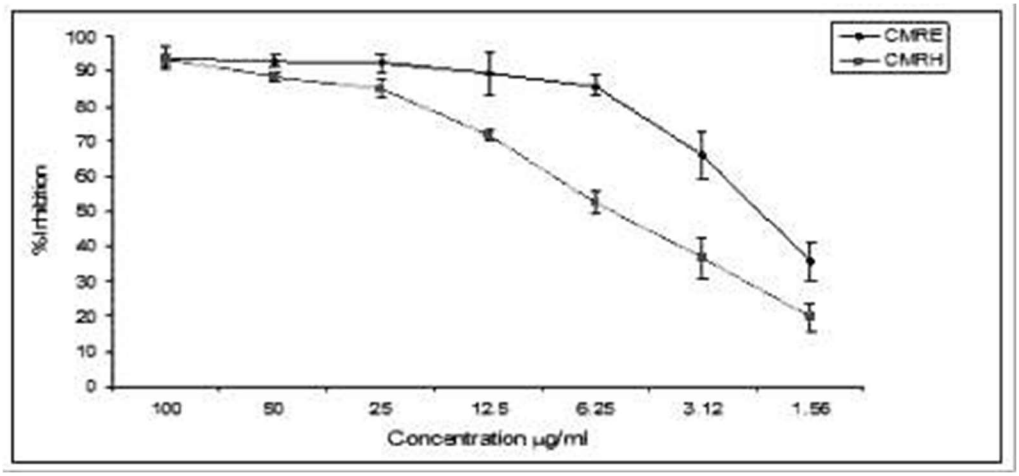

Fig.1 ABTS radical scavenging assay of CMRE and CMRH

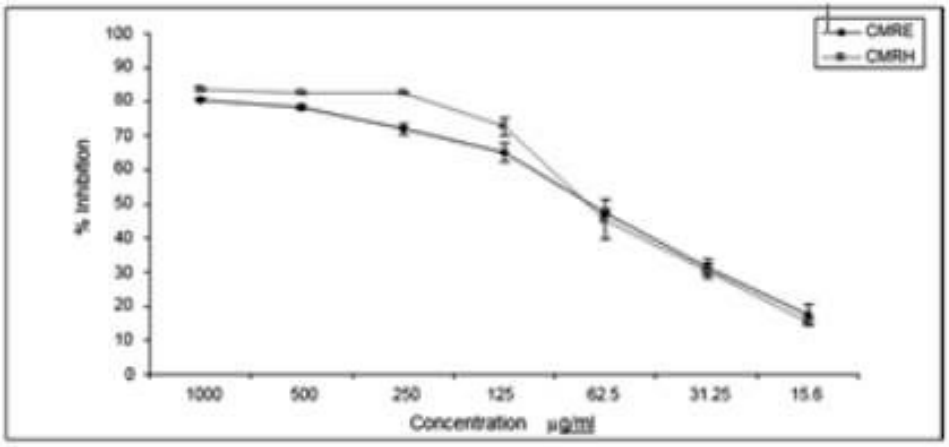

Fig.2 DPPH radical scavenging assay of CMRE and CMRH

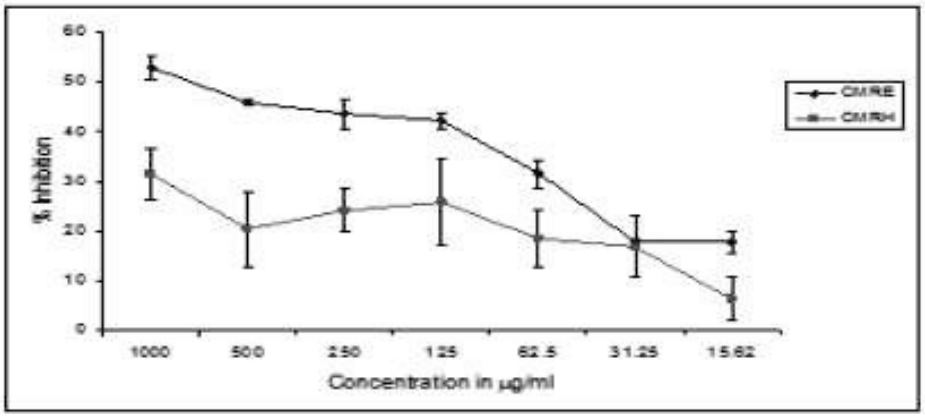

Fig. 3 Nitric oxide radical scavenging assay of CMRE and CMRH

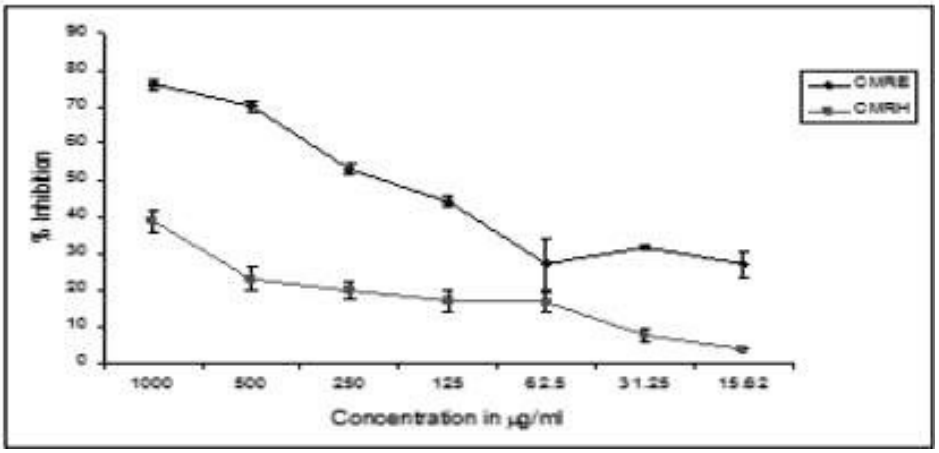

Fig.4 Lipid peroxidation inhibition assay of CMRE and CMRH

Discussion:

Polyphenols are widely distributed in plants are beneficial for human health due to its antioxidant activity through free radical scavenging[11]. Antioxidant activity determination has become one of the important evaluation methods for the neutraceutical and therapeutic effects of traditional medicines[12]. Results of several studies indicate the relationship between antioxidant activity of plant polyphenols and flavonoids[13, 14, 15]. ABTS assay and DPPH assay will work both in the aqueous and organic systems. Thus it can be used to evaluate both hydrophilic and lipophilic antioxidants. In addition to that, the ABTS assay can be performed in a wide range of pH. The hydrogen/electron transfer from the antioxidants to DPPH radical occur in the DPPH radical scavenging assay[16]. In the ethanol 50% extract of *C. macrophylla* root, phenolic and flavonoid content were detected, estimated and reported already[6]. The IC_{50} values of both the extracts and of the known antioxidant rutin were found to be less than 10 µg/ml in ABTS method. The IC_{50} value of rutin was found to be almost half the IC_{50} values of CMRE and CMRH in ABTS method. In the DPPH method, the IC_{50} values of both the extracts and of rutin were found to be less than 100 µg/ml. In the DPPH method, the IC_{50} value of CMRH was found to be less than that of rutin and was found to be more than that of CMRE. The IC_{50} value obtained in the nitric oxide scavenging assay for rutin was around 0.1 mg/ml and that of both the extracts were above 1 mg/ml. In the lipid peroxidation assay method, IC_{50} obtained for CMRE and BHA were around 200 µg/ml and that of CMRH was above 1000 µg/ml.

The results obtained from all the evaluation methods indicate the antioxidant property of both the extracts. The antioxidant effects of both the extracts were comparable with that of rutin in both, ABTS and DPPH methods. In the lipid peroxidation assay, the antioxidant activity of CMRE was almost equal to that of BHA. These results are matching well with the polyphenol and flavonoid content reported earlier[6].

Conclusion:

The results obtained in all the four different methods of antioxidant studies indicate the antioxidant property of both the extracts CMRE and CMRH. The antioxidant property demonstrated can probably be due to the polyphenols and flavonoids present in the extracts. As there are already reported ability of plant polyphenols and flavonoids to prevent DNA as well as cell damage, future studies can be performed to evaluate the anticancer and antidiabetic studies on this plant.

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