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In-vitro Evaluation of Antioxidant Potency of Organic Extracts of Leaves of *Corchorus depressus* Linn.

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Authors' contributions

This work was carried out in collaboration between both authors. Author FN designed the study, managed literature searches, performed the analyses of study and statistical analysis, wrote the protocol and the first draft of the manuscript. Author SAK also managed the literature searches and revised the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: Evaluation of free radical scavenging and lipid peroxidation inhibitory activities of commercially available leaves of *Corchorus depressus* Linn. by *in-vitro* chemical analysis.
Methodology: Four different solvent extracts methanol-CDM, ethanol-CDE, ethyl acetate-CDEA and hexane-CDH were studied for their free radical scavenging activities using 1-diphenyl-2-picrylhydrazyl (DPPH) radical assay method and the results were expressed as SC_{50} values. The lipid peroxidation inhibitory activities of three different concentrations (0.1, 0.5 and 1.0 mg/mL) were evaluated using β-carotene-linoleic acid model system and the results were expressed as oxidation rate ratio (R_{OR}), antioxidant activity (A_A) and antioxidant activity coefficient (C_{AA}). L-Ascorbic acid-AA, α-tocopherol-TOC and BHT were used as reference compounds. The total polyphenol contents of these extracts were also determined using Folin-Ciocalteau reagent.
Results: The total polyphenol contents of extracts were decreased in the order of: CDM > CDE > CDEA >> CDH. In DPPH radical assay, CDM exhibited higher free radical scavenging activity

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(SC_{50}: 216.27 µg/mL) than CDE (327.84 µg/mL) and CDEA (544.28 µg/mL). In comparison with natural antioxidants AA and TOC, the overall ranking was decreased in the order of: AA > CDM > TOC > CDE > CDEA >> CDH. In β-carotene-linoleic acid model system, CDM also exhibited higher protective activity against lipid peroxidation than other extracts as it exhibited lower R_{OR} (0.4903 to 0.2752) and higher A_A (50.96 to 72.47) and C_{AA} (280.58 to 513.13) respectively amongst the studied extract. In comparison with BHT and TOC, CDM exhibited promising potency than TOC. Amongst the studied concentration 0.5 and 1.0 mg/mL were the mostly potent antioxidant against oxidation of linoleic acid.

Conclusion: CDM exhibited most propitious antioxidant activities irrespective of the method undertaken in this study. CDM and CDE had protective activity against oxidation of fatty acid as well as both efficiently scavenged free radicals.

Keywords: Corchorus depressus; leaves; free radical scavenging activity; lipid peroxidation inhibitory activity.

1. INTRODUCTION

Lipid peroxidation is a free radical chain reaction [1-2]. It proceeds in three distinct steps as initiation of autoxidation occurs when hydrogen atom at α-methylene group in double bonds of unsaturated fatty acids is removed to form a lipid radical (R•). Free lipid radicals which have been formed can combine with molecular oxygen to form peroxide free radicals (ROO•) which can react with the substrate to more lipid radicals (R•) and hydroperoxides (ROOH). During propagation, *in-vivo*, hydroperoxides and free radicals produced by its decomposition may cause damage to proteins [3-4], enzyme [5] and also generate carcinogens [6-7], atherosclerosis [8-11], and coronary artery disease, especially myocardial infarction [12]. Termination of the oxidation chain reaction occurs when the free radicals are deactivated or destroyed by antioxidants [13]: Either by scavenging free radicals, in which case the compound is described as primary or chain-breaking antioxidants, or by a mechanism that does not involve direct scavenging of free radicals, in which case the compound is secondary or preventive antioxidants which reduce the rate of chain initiation by a variety of mechanisms [2]. Leafy materials are well known as rich sources of flavonoids and phenolic acids and are recognized as a major source of flavonoids in the diet [14-15] as well as their health promoting properties in humans [14-16]. They can act as primary or chain-breaking antioxidants by donation an electron to the free radical of the fatty acid and stops the propagation steps [17-19]. Thus, this study was carried out on the leaves of *Corchorus depressus* Linn.

Corchorus depressus Linn. (Family-Tiliaceae, later merged with Malvaceae) [20-22], a perennial herb, geographically distributed mostly in tropical and subtropical region of South-Asia, and North America [23-25]. It is commonly known as Bhaufali or Bauphali or Munderi [25-27]. Locally this herb is sold under the name of "Boh Phali". Traditionally, the leaves of *C. depressus* are used as an emollient and cooling agent [28]. Mucilage is used for the treatment of gonorrhoea and it is applied as a poultice for wounds healing purposes. Decoction of seeds and leaves are used as tonic in combination with milk and sugar [25,29]. As a folk medicine, this plant is also used as ailment of aches, dysentery, in tumors [27], for liver disorder [30] and in sexual dysfunction [31,32].

Survey on phytochemical studies on this plan revealed it contained a number of flavonoids as apigenin, luteolin, quercetin and kaempferol [33], cycloartane triterpene glucosides depressoside A and B [23], bidesmosidic cycloartane-type glycosides depressosides C and D [34], monodesmosidic cycloartane triterpene glycosides, depressosides E and F, and flavonol glycosides, depressonol A and B [35]. Phytochemical screening on this plant also revealed the presence of saponin, glycosides, flavonoids and alkaloids [32]. Biological studies on this plant revealed, the whole plant exhibited antipyretic [36], analgesic and antipyretic [37], antioxidant and hepatoprotective [38-39], antimicrobial, antifungal and antioxidant [24,29,40-41], aphrodisiac [42-43], and antimalarial [44] activities. Based on the folk medicinal uses and literature reported bioactivities of *C. depressus*, the present study was carried out to evaluate antioxidant potency in terms of free radical scavenging and lipid peroxidation inhibitory activities of different organic solvent extracts of *C. depressus* leaves and their relation with concentration of

antioxidants and duration of *in-vitro* chemical reaction.

2. MATERIALS AND METHODS

2.1 Plant Materials and Preparation of Extracts

The samples of dried leaves of *Corchorus depressus* Linn. were authenticated by local herbalist and bought from registered herbs selling shop located in Sharjah, UAE. A herbarium voucher specimen is deposited in the Dubai Pharmacy College for future reference. For aiding of grinding process, the collected samples were further dried in an oven at 40 to 50℃ for 22 hours, and then ground into powder form. The extractions of powdered samples were carried out separately using hexane, ethyl acetate, methanol and ethanol solvents respectively. The extraction process was conducted in a Soxhlet extractor for a period of 30 hours. Following completion of extractions, the solvent was evaporated to dryness using a rotary vacuum evaporator. The yields of extracts were recorded as 1.66% for hexane (CDH), 1.82% for ethyl acetate (CDEA), 2.16% for ethanol (CDE) and 2.91% for methanol (CDM) solvent respectively. All the extracts were refrigerated until further use.

2.2 Chemicals and Reagent

Analytical grade absolute ethanol, ethyl acetate, methanol, hexane and spectroscopic grade methanol were purchased from Merck, Darmstadt, Germany. 1,1-Diphenyl-2-picrylhydrazyl (DPPH•) radical, Folin-Ciocalteau reagent, anhydrous sodium carbonate, trans-β-carotene, linoleic acid, tween 40, butylated hydroxytoluene (BHT), (+)-α-tocopherol (TOC), L-ascorbic acid (AA) and quercetin were all purchased from Sigma Aldrich Chemical Co. (USA).

2.3 Analysis of Extracts for their Total Polyphenols Content

Diluted Folin-Ciocalteau reagent (1:10) was used in the determination of total polyphenol contents of extracts of *C. depressus* as described by Nessa et al. [45]. All the extracts were dissolved in methanol and sonicated for 5 minutes. Different concentration of reference compound (quercetin) solution was prepared in methanol for establishing the calibration curve. 100 µL of each extract and quercetin solution was then transferred into the test tubes, and added 2 mL Folin-Ciocalteau reagent and 2 mL of sodium carbonate solution (7.5%) respectively and kept at room temperature for 1.5 hr. The absorbance of each solution was read at 760 nm using Shimadzu-1700 UV-VIS spectrophotometer (Japan). The results were expressed as mg of quercetin equivalent polyphenols per g of dried extract. The experiment was repeated for three times.

2.4 Evaluation of Free Radical Scavenging Activity of Extracts

The free radical scavenging activity of extracts of *C. depressus* was evaluated using DPPH• radical (1,1-diphenyl-2-picrylhydrazyl) assay method as described by Nessa et al. [45] with slight modification. Different concentration of extracts and reference compound solution were prepared in methanol. 100 µL of each solution was transferred into the cuvette and then added 2.5 mL of 25 µL/mL freshly prepared methanolic solution of DPPH radical. The measurement of absorbance at 517 nm was commenced immediately against a blank (without sample) and continued for 30 min at 2 min intervals by using data capturing software of Shimadzu-1700 UV-VIS spectrophotometer (Japan). The experiments were conducted at 25℃ and repeated for three times. The percent scavenging of DPPH radicals by samples at 30 min interval were calculated using the formula: % Scavenging of DPPH• = $[(A_{B(0)} - A_{A(t)})/A_{B(0)}]$ X 100, where, $A_{B(0)}$ is the absorbance of the blank at t = 0 min and $A_{A(t)}$ is the absorbance of the antioxidant at t = 30 min. Calibration curves were established for extracts and reference compounds and the results were expressed as SC_{50} values (concentration sufficient to obtain 50% of a maximum scavenging capacity).

2.5 Evaluation of Lipid Peroxidation Inhibitory Activities of Extracts Using β-carotene-linoleic Acid Model System

Lipid peroxidation inhibitory activities of extracts and reference compounds were evaluated using β-carotene-linoleic acid model system as described elsewhere [46-48]. β-Carotene (0.1 mg/mL), linoleic acid (20 mg/mL) and tween 40 (100 mg/mL) solution were prepared in chloroform and transferred to a round bottom flask, and then evaporated the solvent to dryness

at 30°C using a vacuum rotary evaporator. 50 mL oxygenated water was then added into the round bottomed flask and sonicated for 3 min and the resultant solution was an emulsion. 5 mL of emulsion was then transferred into the test tubes and added 200 µL of methanolic solution of extracts and reference compounds (0.1 mg/mL, 0.5 mg/mL and 1.0 mg/mL) to give a final concentration of antioxidants 20 µg, 100 µg and 200 µg respectively. α-Tocopherol and BHT were used as reference compounds. Blank solution was prepared without antioxidant solution. The experiment was repeated three times. The measurement of absorbance of all solution was commenced at 470 nm immediately against a blank and continued for 120 min at 15 min intervals using Shimadzu-1700 UV-VIS spectrophotometer (Japan). The samples were placed in water bath between measurements and temperature controlled at 50℃ to enhance bleaching of β-carotene. The antioxidant potency of samples was expressed by three different parameters as:

(i) Oxidation rate ratio (R_{OR}) = R_{sample}/R_{blank}, where degradation rates (R_D) were calculated according to the first-order kinetics: $R_D = ln(a/b) \times 1/t$; where, *ln* is natural log, *a* is the initial A_{470} (t = 0) and *b* is the A_{470} at t = 15, 30, 45, etc. min [47].

(ii) A_A (antioxidant activity) = $[(R_{blank} - R_{sample})/(R_{blank})] \times 100$, where, R_{blank} and R_{sample} were the bleaching rates of β-carotene without and with the addition of antioxidant, respectively [48].

(iii) C_{AA} (antioxidant-activity coefficient) = $[(A_{S(120)} - A_{B(120)}/(A_{C(0)} - A_{B(120)}] \times 1000$, where, C_{AA} is from 0 to 1000, $A_{S(120)}$ is the absorbance of the emulsion containing antioxidant at t = 120 min, $A_{B(0)}$ and $A_{B(120)}$ are the absorbance of the blank at t = 0 and 120 min respectively [46].

2.6 Statistical Analysis

The results of all experiments were expressed as mean (three replicates) ± standard deviation (S.D). The data of results were compared and analyzed by one-way analysis of variance (ANOVA). Tukey's test (P = .05) was performed to determine the significance of the difference in means of between groups and within groups.

3. RESULTS AND DISCUSSION

3.1 Total Polyphenols Content of Extracts

The highest amount of polyphenols was recorded in methanol extract-CDM and lowest amount in hexane extract-CDH. The overall results were decreased in the order of: CDM > CDE > CDEA >> CDH. The results were compared with each other and the mean differences of polyphenols content of four different solvent extracts were statistically significantly different (P = .05). The yield of each extract and their total polyphenol contents are presented in Table 1. The higher percent yield is recorded in CDM extract whereas lowest yield observed in CDH extract.

3.2 DPPH Radical Scavenging Activity

Free radical scavenging activities of different concentration of extracts of *C. depressus* were determined using DDPH radical scavenging assay. The stable $DPPH^{\bullet}$ radical has been widely used to test the free radical scavenging ability of various dietary antioxidant polyphenols [49-51]. This simple test can provide information on the ability of a compound to donate a hydrogen atom, and on the mechanism of antioxidant action [50]. In this assay, a compound with high antioxidant potential effectively traps this radical thereby preventing its propagation and the resultant chain reaction [49,50]. Figs. 1-4 shows the decrease in

Table 1. Total extractive values and polyphenols content of extracts of the leaves of *C. depressus*

Solvent extracts of *C. depressus* leaves	% Yield (w/w, on dried basis)	*Total polyphenols mg quercetin/g of dried extract ± S.D.
CDM (Methanol)	2.91	46.42±1.98
CDE (Ethanol)	2.16	31.16±2.58
CDEA (Ethyl acetate)	1.82	17.38±2.67
CDH (Hexane)	1.66	2.5±1.44

** Results are mean ± S.D (n = 3). S.D. = Standard deviation*

absorbance due to scavenges of DPPH radials by different concentration of solvent extracts. The faster decreases in absorbance, the most potent the antioxidant activity of the extract due to their hydrogen donating abilities [49]. Amongst the four different solvent extracts, CDM and CDE exhibited higher free radical scavenging activity than CDEA. As the concentration increases from 25 µg/mL to 500 µg/mL, the radical scavenging activity of CDM and CDE extracts also increases in addition, the steady state of chain reaction achieved within 4 to 6 min. CDEA showed moderate to poor activity against scavenging of DPPH radicals and only at higher concentration it effectively scavenged free radicals, and the steady state of the reaction was not achieved within 30 min. CDH was a very poor free radical scavenger as almost no decrease in absorbance occurred even at higher concentration and the steady state of the chain reaction was not established within 30 min. In case of L-ascorbic acid (AA), it scavenged free radicals effectively and the steady state appeared within a minute [50]. α-Tocopherol (TOC) also scavenged DPPH radical effectively and stabilized the reaction within 2 to 6 min. L-Ascorbic acid (AA) and α-tocopherol (TOC) were used as reference compounds.

The SC_{50} values of extracts and reference compounds were calculated based on 30 min of reaction time by regression analysis from its corresponding graph as presented in Fig. 5. CDM exhibited lower SC_{50} value in comparison to other tested extracts. As per ranking, the free radical scavenging activities of extracts and reference compounds were decreased in the order of: AA > CDM > TOC > CDE > CDEA >> CDH. CDH extract was not active in the studied concentration ranges. The SC_{50} values of extracts and reference compounds were compared and there were statistically significant differences (P = .05) in their mean values as shown in Table 2. The high SC_{50} value of CDEA indicated that it was a moderate to poor free radical scavenger. CDM exhibited significantly higher (P = .05) antioxidant activity than natural antioxidant TOC. The high polyphenolic contents of CDM extract might contributed to its higher free radical scavenging activity. A number of researchers reported on DPPH radical scavenging activity of *C. depressus*, where either leaves or roots or whole plants were used as raw materials. The reported IC_{50} values were: 121.3±2.33 µg/mL for ethanolic extract of whole plants [38], and 56.34±0.06, 54.93±0.06, 11.80±0.02 µg/mL were for methanol, ethanol and n-hexane extracts of leaves [29] respectively. Another study on roots, the IC_{50} values for methanol, ethanol and n-hexane extracts were 69.01±0.09, 68.04±0.12 and 19.23±0.16 respectively [24]. In comparison with our study it revealed that all the extracts were exhibited higher SC_{50} values than literature reported values. In addition, hexane extract was not active in the working concentration ranges. It seems geographical distribution of plants affect its phytochemical constituents as well as antioxidant activity.

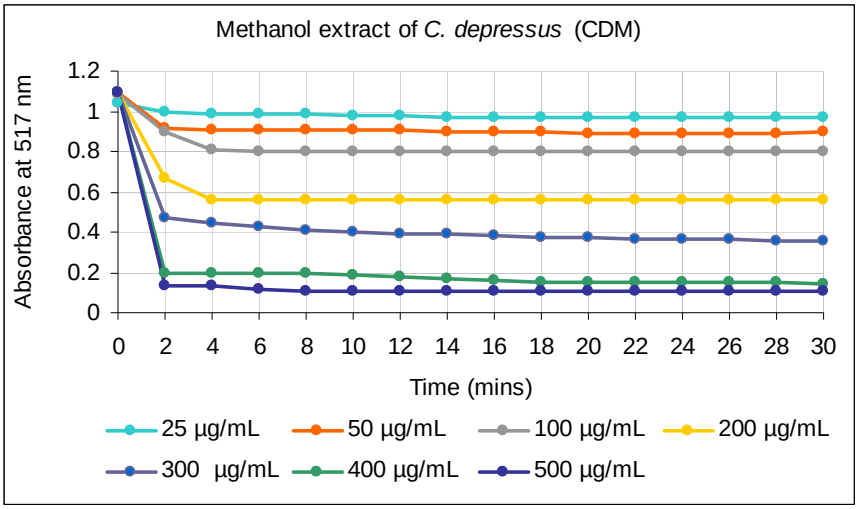

Fig. 1. Hydrogen donating abilities of different concentration of methanol extract of *C. depressus* leaves (CDM) on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

Table 2. SC_{50} values of different solvent extracts of the leaves of *C. depressus* for scavenging of free radicals as assessed with DPPH radical scavenging method

Solvent extracts of *C. depressus* leaves	*SC_{50} (µg/mL) ± S.D.	r
CDM (Methanol extract)	216.27±2.54	0.9950±0.0002
CDE (Ethanol extract)	327.84±3.12	0.9740±0.0005
CDEA (Ethyl acetate extract)	544.28±4.09	0.9715±0.0027
CDH (Hexane extract)	Not active at 25 µg/mL to 500 µg/mL	---
AA (L-Ascorbic acid)	83.77±1.38	0.9866±0.0051
TOC (α-Tocopherol)	252.32±1.89	0.9785±0.0064

**SC_{50} values were calculated from regression lines where: r = correlation coefficient. Results are mean ± S.D (n = 3). S.D. = Standard deviation*

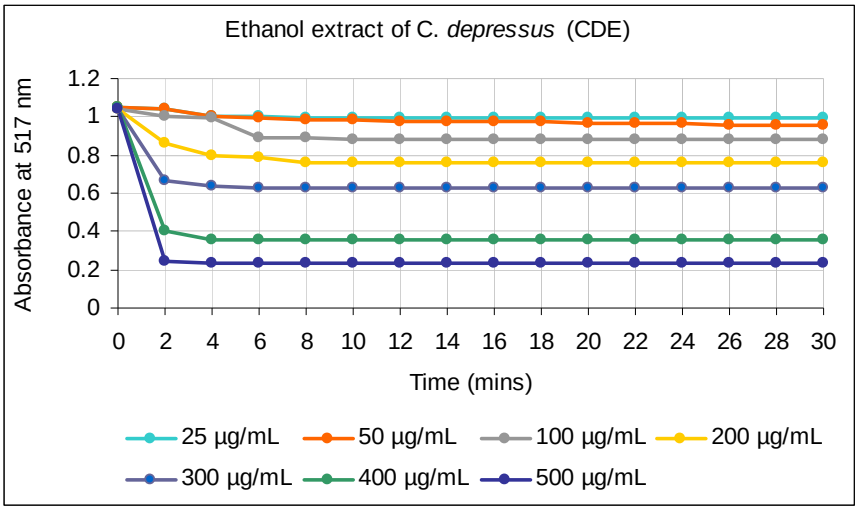

Fig. 2. Hydrogen donating abilities of different concentration of ethanol extract of *C. depressus* leaves (CDE) on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

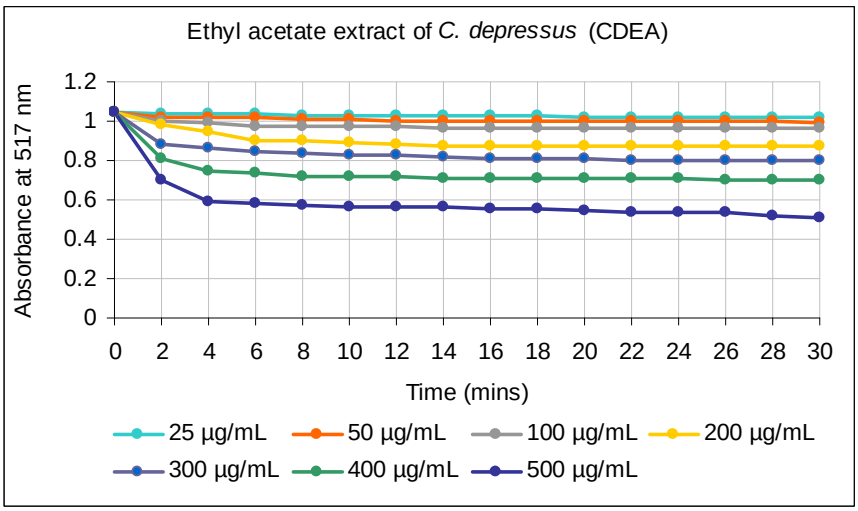

Fig. 3. Hydrogen donating abilities of different concentration of ethyl acetate extract of *C. depressus* leaves (CDEA) on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

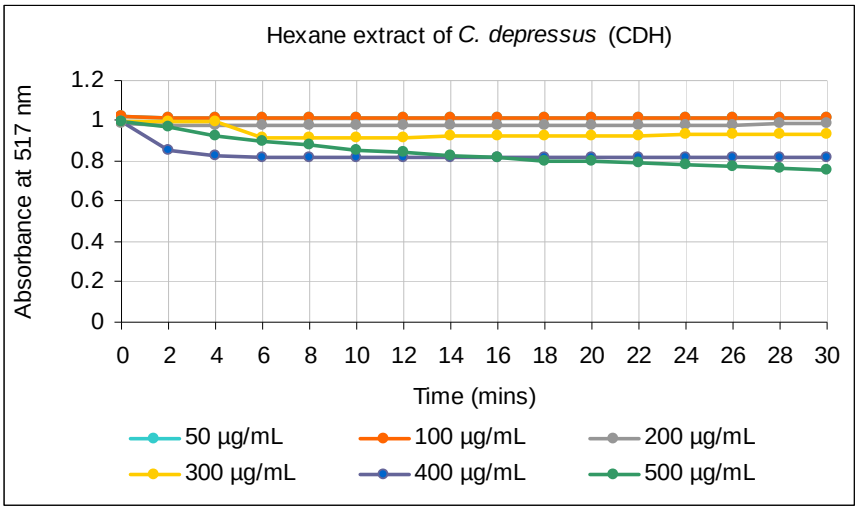

Fig. 4. Hydrogen donating abilities of different concentration of hexane extract of *C. depressus* leaves (CDH) on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

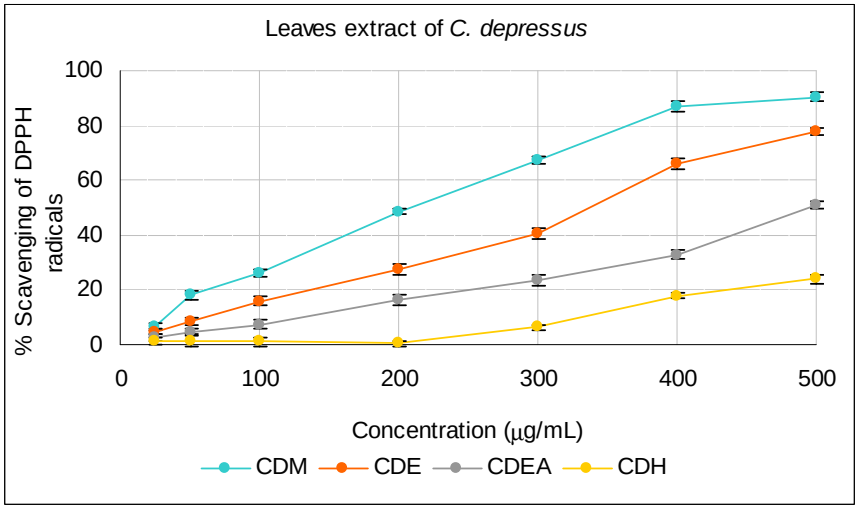

Fig. 5. Free radical scavenging activity of different solvent extracts of leaves of *C. depressus* measured at 30 min using the DPPH radical assay. Results are mean±S.D (n = 3)

3.3 Inhibition of Lipid Peroxidation

Lipid peroxidation inhibitory activities of extracts of *C. depressus* were evaluated using β-carotene-linoleic acid model system. It is based on the ability of different antioxidants to decrease the oxidative losses of β-carotene in a β-carotene-linoleic acid emulsion. Three different concentrations as 0.1, 0.5 and 1 mg/mL for each extracts (CDH, CDEA, CDE and CDM) and reference compounds (TOC and BHT) were tested. The results of the antioxidative potency of extracts against bleaching of β-carotene were expressed by three parameters as A_A, C_{AA} and R_{OR} are presented in Table 3. The bleaching rate of β-carotene by extracts are presented in Figs. 6-9. The oxidation rate ratio, R_{OR} for 0.1 mg/mL sample solution, BHT exhibited highest antioxidant activity as the R_{OR} value is increased in the order of: BHT > TOC > CDM > CDE > CDEA >> CDH. For concentration 0.5 mg/mL, CDM exhibited higher antioxidant activity than TOC and the overall ranking of R_{OR} of all samples increased in the order of: BHT > CDM > TOC > CDE > CDEA >> CDH. However, for 1.0 mg/mL, CDM and CDE exhibited higher antioxidant activity than TOC as follows: BHT > CDM > CDE > TOC > CDEA >> CDH. The R_{OR}

determine the strength of an antioxidant, the R_{OR} value is an inverse measure of the strength i.e., the lower the R_{OR} value the potent the antioxidant. As per ranking, CDM exhibited lowest R_{OR} values amongst the studied extracts. According to comparison of mean values within groups, R_{OR} values were significantly different (*P* = .05) amongst the samples except TOC where there were no statistically significant differences (*P* = .05) in between studied three different concentrations. In comparison of R_{OR} values of CDM with other extracts and reference compounds, there were no significant differences (*P* = .05) in mean values of 0.1 mg/mL (CDM), 0.5 mg/mL (CDE) and 1.0 mg/mL (CDEA); 0.5 mg/mL (CDM) and 1.0 mg/mL (CDE); 1.0 mg/mL (CDM), 0.5 mg/mL (BHT) and 0.5 mg/mL (CDE); 0.5 mg/mL (CDM) and 1.0 mg/mL (TOC) respectively. In comparison in between CDEA with other samples, the mean values of R_{OR} were not significant (*P* = .05) for 0.1 mg/mL (CDEA) and 0.5, 1.0 mg/mL (CDH). Amongst the reference compounds, the mean differences were not significantly different (*P* = .05) between TOC and 0.1 mg/mL (BHT).

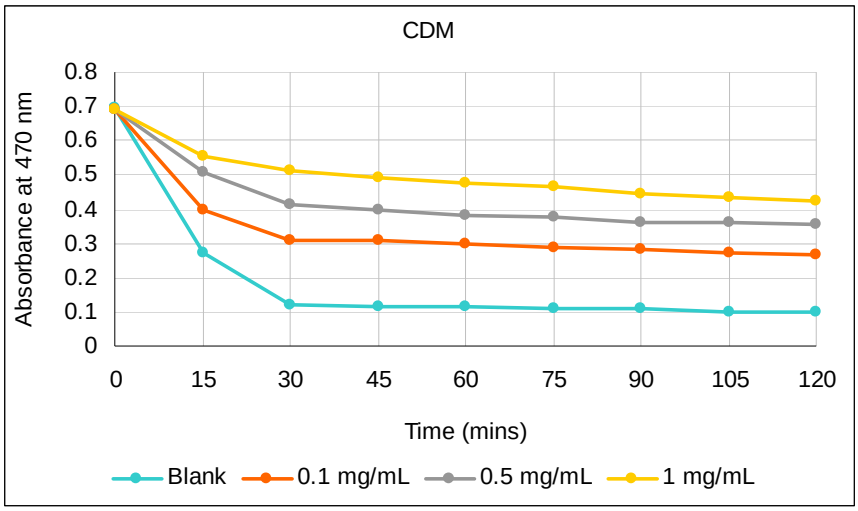

Fig. 6. Hydrogen donating abilities of 0.1, 0.5 and 1.0 mg/mL of methanol extract (CDM) of *C. depressus* leaves measured using the β-carotene-linoleic acid model system

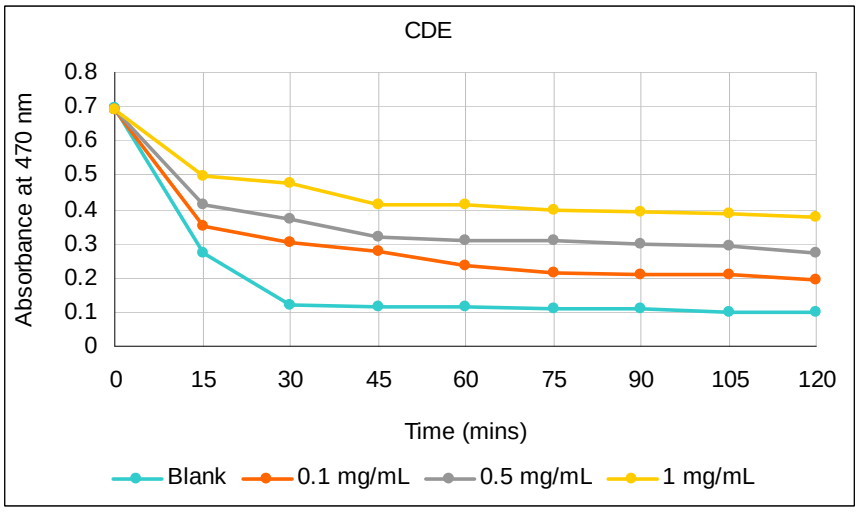

Fig. 7. Hydrogen donating abilities of 0.1, 0.5 and 1.0 mg/mL of ethanol extract (CDE) of *C. depressus* leaves measured using the β-carotene-linoleic acid model system

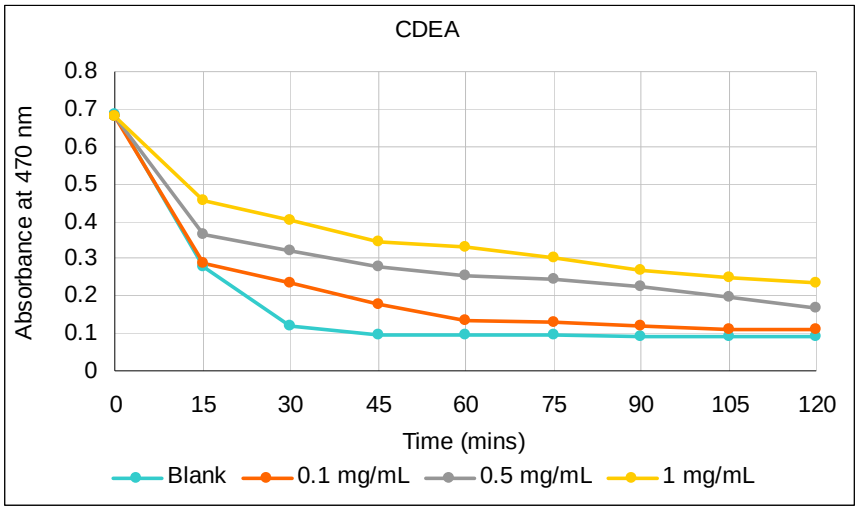

Fig. 8. Hydrogen donating abilities of 0.1, 0.5 and 1.0 mg/mL of ethyl acetate extract (CDEA) of *C. depressus* leaves measured using the β-carotene-linoleic acid model system

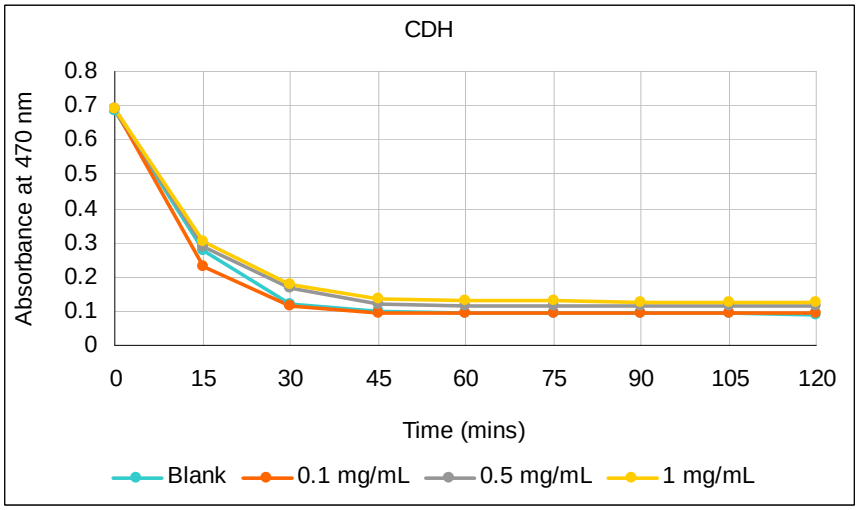

Fig. 9. Hydrogen donating abilities of 0.1, 0.5 and 1.0 mg/mL of hexane extract (CDH) of *C. depressus* leaves measured using the β-carotene-linoleic acid model system

The second parameter antioxidant activity-A_A was calculated to evaluate the potential of extracts against bleaching of β-carotene and the results were compared with reference compounds. The results are shown in Table 3. For 0.1 and 0.5 mg/mL sample solution, CDM exerted higher antioxidant activity than other extracts, however showed lower activity than BHT and TOC and the overall ranking for A_A was decreased in the order of: TOC > BHT > CDM > CDE > CDEA >> CDH. For concentration 0.5 mg/mL and 1.0 mg/mL, the ranking of A_A was in the order of: BHT >TOC > CDM > CDE > CDEA >> CDH. The A_A value for the concentration 0.1 mg/mL of CDM was not significantly (P = .05) different with 0.5 mg/mL (CDE), 0.1 mg/mL (TOC) and 1 mg/mL (CDEA) respectively. The mean values of 0.5 mg/mL (CDM) were not significantly different with 0.5 mg/mL (BHT) and 0.5, 1.0 mg/mL (TOC). In case of 1.0 mg/mL-CDM, the mean values were not significantly different (P = .05) with 1.0 mg/mL (CDE). The A_A value for the concentration 0.5 mg/mL of CDH was not significantly different (P = .05) with 0.1 mg/mL–CDEA. In addition, 0.1 mg/mL-BHT and

0.1 mg/mL-TOC exhibited similar antioxidant activity in respect of statistical analysis.

The third parameter antioxidant activity coefficient-C_{AA} was calculated based on the scale 0 to 1000. For 0.1 mg/mL concentration, TOC exhibited higher protective activity against oxidation of fatty acid and the overall antioxidant activity decreased in the order of: TOC > BHT > CDM > CDE > CDEA >> CDH. For 0.5 and 1.0 mg/mL concentrations, BHT exhibited higher antioxidant activity than TOC and the results were decreased in the order of: BHT > TOC > CDM > CDE > CDEA >> CDH. From the statistical analysis of C_{AA} mean values as presented in Table 3, for CDM, there were no significant differences (P = .05) in between of 1.0 mg/mL CDM, 0.1 mg/mL BHT and 0.5 mg/mL TOC; 0.1 mg/mL-CDM and 0.5 mg/mL-CDE respectively. 1.0 mg/mL-CDE also exhibited equivalent activities with 0.1 mg/mL-TOC. In comparison between CDE and CDEA extracts, there were no significant differences (P = .05) in between 0.1 mg/mL-CDE and 0.5 mg/mL-CDEA. CDH exhibited poorer antioxidant activity and in comparison with CDEA, however, there were no statistical significant differences (P = .05) in mean values of 0.5 mg/mL and 1.0 mg/mL-CDH. No literature was reported on β-carotene bleaching method for evaluation of lipid peroxidation inhibition activities of *C. depressus*. However, Zabben et al. [29] reported the ammonium thiocyanate assay method for determination of lipid peroxidation inhibition of leaves extracts, where, methanol (49.51±0.08%), ethanol (49.06±0.09%) and n-hexane (42.12±0.07%) extracts were equally active. In contrary, in our study, methanol and ethanol extracts exhibited promising antioxidant activity at higher concentration (1 mg/mL) and hexane extract was a very poor antioxidant.

Table 3. Parameters used to evaluate the lipid peroxidation inhibitory activity of different solvent extracts of the leaves of. *C. depressus*

Samples	Parameters used for evaluation of lipid peroxidation inhibitory activity		
	***R_{OR} (Oxidation rate ratio ± S.D.)**		
	0.1 mg/mL	**0.5 mg/mL**	**1.0 mg/mL**
CDM	0.4903±0.0223^{a}	0.3465±0.0080^{bd}	0.2752±0.0186^{c}
CDE	0.6551±0.0148	0.4779±0.01175^{a}	0.3116±0.0123^{bc}
CDEA	0.9113±0.0323^{f}	0.6941±0.0281	0.5290±0.0193^{a}
CDH	0.9800±0.0182	0.9271±0.0328^{f}	0.8618±0.0130^{f}
BHT	0.4215±0.00159^{e}	0.2876±0.0218^{c}	0.1998±0.0142
TOC	0.4301±0.0231^{e}	0.4001±0.0811^{e}	0.3821±0.0264^{ed}
	***A_A (Antioxidant activity % ± S.D.)**		
	0.1 mg/mL	**0.5 mg/mL**	**1.0 mg/mL**
CDM	50.96±2.23^{a}	65.34±0.80^{b}	72.47±1.86^{c}
CDE	34.49±1.48	52.20±1.17^{a}	68.83±1.23^{c}
CDEA	7.97±2.18^{d}	29.62±1.58	47.88±0.84^{a}
CDH	1.99±1.82	7.28±3.28^{d}	13.81±1.30
BHT	56.99±2.32^{e}	68.12±2.75^{b}	77.93±3.66
TOC	54.11±2.09^{ae}	61.45±2.62^{b}	63.81±2.66^{b}
	***C_{AA} (Antioxidant activity coefficients ± S.D.)**		
	0.1 mg/mL	**0.5 mg/mL**	**1.0 mg/mL**
CDM	280.58±19.36^{a}	427.04±9.27	513.13±16.92^{b}
CDE	158.24±9.37^{d}	291.24±10.51^{a}	466.89±15.27^{c}
CDEA	29.22±11.84^{ef}	128.72±16.02^{d}	240.58±15.30
CDH	7.30±5.92^{e}	25.85±11.96^{ef}	51.15±5.42^{f}
BHT	466.12±8.81^{b}	660.34± 9.33	837.47±7.23
TOC	480.47±12.43^{c}	530.71± 9.94^{b}	594.87±1.34

**Each value is expressed as mean ± S.D (n = 3). Means with similar small letter within column/rows for each particular parameter are not significantly different (P = 0.05)*
S.D. = Standard deviation

4. CONCLUSION

The antioxidant activity of CDM and CDE extracts is credited to their hydrogen donating properties as of their polyphenolic contents. The results of *in-vitro* antioxidant activity revealed that CDM and CDE contained higher polyphenols which turned them as potent antioxidants in terms of scavenging free radical scavenging and lipid peroxidation inhibitory activities. This extracts acted as primary antioxidants are believed to intercept the free radical chain of lipid oxidation by donating hydrogen from the phenolic hydroxyl groups of polyphenols, thereby forming a stable end product, which does not initiate or propagate further oxidation of the lipid [52]. CDM exhibited higher antioxidant activity than CDE, also indicated that the extraction with methanol not only gave high yield of the extract but also gave high antioxidant activity, which was confirmed by two methods used for the antioxidant assay. Thus, the results of the present work indicated that the selective extraction of antioxidant from natural sources by appropriate solvent was very important in obtaining fractions with high antioxidant activity. In addition, further study necessary to conform the beneficial role of these extracts.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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