Evaluation of antioxidant and xanthine oxidase inhibitory activity of different solvent extracts of leaves of *Citrullus colocynthis*

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ABSTRACT

Background: *Citrullus colocynthis* is a folk medicinal plan of United Arab Emirates. Several studies on this plant reported and focused on the biological and toxicological profile of fruits pulp. The present study focused on the antioxidant potency of leaf extract of this plant. **Aim:** To evaluate the antioxidant and xanthine oxidase (XO) inhibitory activities of *C. colocynthis* by chemical method. **Materials and Methods:** Four different solvent extracts (methanol-CCM, methanol: water (1:1)-CCMW, chloroform-CCC and hexane-CCH) of leaves of *C. colocynthis* were investigated for their free radical scavenging activity using DPPH radical as a substrate, lipid peroxidation (LPO) inhibitory activity using a model system consisting of β-carotene-linoleic acid, superoxide radical scavenging activity (enzymatically/nonenzymatically) and XO inhibitory activity. A dose response curve was plotted for determining SC_{50} and IC_{50} values for expressing the results of free radical scavenging activity and XO inhibitory activities respectively. **Results:** The high polyphenolic content of CCM and CCMW extract showed highest antioxidant activity irrespective the method used for this investigation. The overall results decreased in the order of: CCM > CCMW > CCC > CCH. CCH extract was inactive towards chemically generated superoxide radical and poor DPPH radical scavengers. The results of LPO inhibitory activities of leaves extract (0.1, 0.5 and 1.0 mg/mL) also decreased in the order of: CCM > CCMW > CCC > CCH. Overall 1.0 mg/mL leaves extract showed highest antioxidant potency amongst the studied concentration. **Conclusion:** CCMW and CCM extract of *C. colocynthis* exhibited promising antioxidants and XO inhibitory activities.

Key words: *Citrullus colocynthis*, extracts, free radical and lipid peroxidation, superoxide radical, xanthine oxidase

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INTRODUCTION

Citrullus colocynthis L. (Cucurbitaceae) a desert plant, locally it is known as "Handal" and in English as Colocynth. It is a perennial herbaceous creeping plant, with angular and rough stems. Leaves are rough, 5 to 10 cm long, 3 to 7-lobed and fruits are nearly globular, 4 to 10 cm in diameter about the size of a small orange.[1] It is geographically distributed in the desert of North Africa, Southern Europe and Asia.[1] This plant is wildly grown in United Arab Emirates. Traditionally, the poultice of colocynth is used to combat rheumatic pain. The leaves have been used for painful menstruation and in the treatment of asthma. The fruit pulp is purgative, used as diuretic, cathartic and also used against gonorrhoea.[1,2] A literature survey on its phytochemical constituents reveals fruits of Iranian species *C. colocynthis* contained a number of flavone glucosides, isosaponarin, isovitexin and isoorientin 3'-O-methyl ether and cucurbitacin glucosides, 2-O-β-D-glucopyran osylcucurbitacin and 2-O-β-D-glucopyranosylcucurbitac in,[3] C-p-Hydroxybenzoylglycoflavanones,[4] cucurbitacin derivatives,[5,6] fatty acids.[7] Quercetin also isolated from leaves, root and fruit of Indian species.[8] Pharmacological survey on this plant revealed most of the works were done on the fruit pulp of *C. colocynthis*. As fruit extract exhibited free radical scavenging activity,[9] hypoglycemic activity,[10-16] protective ability against oxidative stress in the RBC's of alloxan induced diabetic rats,[17] antihyperlipidemic and antioxidant actions in alloxan induced diabetic rats,[18,19]

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insulinotropic and immunostimulating activities.[12,13,20-22] Fruits and aerial parts of *C. colocynthis* also exhibited antimicrobial,[23,24] anti-inflammatory activity.[25] The leaf extract showed the larvicidal and ovicidal properties.[26] Several researchers reported the case of bloody diarrhea after consuming repeated doses of colocynth.[27-30] Due to variable geographic distribution of plants, chemical constituents of plants also vary greatly as well as their biological activity. Therefore, the present study was undertaken to obtain a fairly comprehensive data about the antioxidant potential of *Citrullus colocynthis* species grown in Dubai, United Arab Emirates by using several in-vitro chemical techniques.

MATERIALS AND METHODS

Plant materials and extraction procedure

Fresh leaf of *Citrullus colocynthis* were collected from the desert of Dubai, UAE. The plant was identified by Prof. Dr. Saeed A. Khan, Medicinal and Pharmaceutical Chemistry Dept. of Dubai Pharmacy College and a harberium voucher specimen is deposited in the Dubai Pharmacy College. Freshly collected leaf were oven dried (40°C) for 5 days and then milled into powder. The powdered leaf (300g) was extracted subsequently with hexane, chloroform and methanol (2.5 liters each) in a Soxhlet extractor for 15 h each. The solvent was evaporated to dryness at 45°C using a rotary vacuum evaporator. The yield of extracts was 1.11, 3.2 and 8.12% for hexane (CCH), chloroform (CCC) and methanol (CCM) solvent extracts respectively. 100g of oven dried leaves were also extracted with methanol: water (1:1) separately. The extract was filtered and then evaporated to dryness in vacuum at 60°C in a rotary evaporator and the yield was 7.24% for methanol: water (1:1) extracts (CCMW). All the extracts were kept in refrigerator until further use.

Chemicals and reagent

Analytical grade hydrochloric acid (37%), hexane, chloroform, methanol, absolute ethanol and spectroscopic grade methanol were purchased from Merck, Darmstadt, Germany. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, *trans*-β-carotene, linoleic acid, tween 40, butylated hydroxytoluene (BHT), allopurinol, quercetin (QN), (+)-α-tocopherol (TOC), Folin-Ciocalteau reagent, phenazine methosulfate (PMS), nitroblue tetrazolium (NBT), β-nicotinamide-adenine dinucleotide (β-NADH, disodium salt), sodium phosphate dibasic 12 hydrate, anhydrous potassium phosphate monobasic, anhydrous sodium carbonate, xanthine and xanthine oxidase (XO) from bovine milk (Grade IV, ammonium sulphate suspension, 0.3unit/mg protein) were all purchased from Sigma Aldrich Chemical Co. (USA).

Determination of total polyphenols content of extract

The total polyphenolic content of *C. colocynthis* leaf extract was determined using Folin-Ciocalteau reagent.[31] The extracts (CCH, CCC, CCM and CCMW) were dissolved in water: methanol (1:1) mixture, sonicated and then centrifuged for 10 min at 10,000 *g*. 100 µL of supernatant portion of each sample (three replicates) were transferred into the test tubes and then added 2 mL of Folin-Ciocalteau reagent (diluted 1:10) and 2 mL of 7.5% Na_2CO_3 respectively. The test tubes were then kept at room temperature (25°C) for 1.5 h and read the absorbance at 760 nm. Calibration curve was prepared with different concentration of quercetin solution (water: methanol 1:1). Total polyphenol values were expressed as milligrams of quercetin equivalent per gram of dried extract.

Determination of free radical scavenging activity using the DPPH radical scavenging method

The free radical scavenging activity of different organic extracts of *C. colocynthis* leaves were evaluated using a stable radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH•) in a methanol solution described by several authors.[32-37] 2 mL of 25 µg/mL methanolic solution of DPPH radical was placed in a cuvette and 50 µL methanolic solution of the antioxidant was added. Absorbance was measured immediately at 517nm and the decrease in absorbance was determined continuously with data capturing at 2 min intervals for 30 min with a Shimadzu-1700 UV-VIS spectrophotometer (Japan). A blank sample (without antioxidant) containing the same amount of methanol and DPPH radical was prepared and measured daily. The determinations were repeated for three times and the percent scavenging of DPPH radicals was calculated according to the formula: % Inhibition = $[(A_{B(0)} - A_{A(t)})/A_{B(0)}] \times 100$, 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. The results were expressed as SC_{50} values[37] (concentration sufficient to obtain 50% of a maximum scavenging capacity).

Assay for inhibition of Lipid Peroxidation using the β-carotene-linoleic acid model system

The lipid peroxidation inhibitory activity of different solvent extracts of *C. colocynthis* was determined using the β-carotene-linoleic acid model system.[38-41] β-Carotene (0.1mg/mL), linoleic acid (20 mg/mL) and Tween 40 (100 mg/mL) were all dissolved in chloroform and transferred to a round bottomed flask. The solvent was removed using a rotary evaporator under vacuum at 30°C and 50 mL oxygenated distilled water was then added to the flask. The mixture was sonicated for 2 min in an Elamasonic (Elma, Germany) to form an emulsion. Three different concentrations 0.1, 0.5 and 1.0 mg/mL

of extracts and reference compounds were prepared in methanol. Extract solution 200 μL of each and reference a compound was mixed with 5 mL of emulsion, giving a final concentration of 20, 100 and 200 μg of antioxidants in the mixture. α-Tocopherol and BHT were used as reference compounds. Blank solution consisted of 200 μL methanol instead of antioxidants solution. The absorbance of all samples were measured at 470 nm immediately ($t = 0$) and at 15 min intervals for 120 min ($t = 120$ min) on a Shimadzu-1700 UV-VIS spectrophotometer (Japan). The samples were placed in a water bath at 50°C between measurements. Antioxidant potency was determined using three different parameters as antioxidant activity (A_A), oxidation rate ratio (R_{OR}) and the antioxidant activity coefficient (C_{AA}) using following formulas: [38-41]

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

$R_{OR} = R_{sample}/R_{control,}$ The R_{OR} determine the strength of an antioxidant, the R_{OR} value is an inverse measure of the strength.

$C_{AA} = [(A_{S\,(120)} - A_{B\,(120)}/(A_{C\,(0)} - A_{B\,(120)}] \times 1000$, where C_{AA} is an antioxidant-activity coefficient ranging from 0 to 1000, $A_{S\,(120)}$ is the absorbance of the sample containing antioxidant at $t = 120$ min, $A_{B\,(0)}$ $A_{B\,(120)}$ is the absorbance of the blank at $t = 0$ and 120 min respectively. All determinations were performed in triplicate.

Determination of xanthine oxidase inhibitory activity

The xanthine oxidase (XO) inhibitory activities with xanthine as the substrate were measured spectrophotometrically.[31] The assay mixture consisted of 1.0 mL of test solution (prepared in 12% v/v DMSO), 1.9 mL phosphate buffer (pH 7.5), 0.1 mL of enzyme solution (0.045 units/mL) and 1.0 mL 100 μM xanthine solution. The resulting solution was incubated for 15 min at 25°C. The enzyme reaction was stopped with 1M HCl (1 mL) and the absorbance of the reaction mixture was measured at 295 nm against blank solution prepared the same way without addition of enzyme solution. XO inhibitory activity was expressed as the percent inhibition of XO was calculated as follows: $(1–A_S/A_B) \times 100$; where, A_S is the absorbance of the sample and A_B is the absorbance of the blank. Different concentrations of samples were analyzed and then the half-maximal inhibitory concentration (IC_{50}) was calculated by regression analysis.

Determination of superoxide radicals scavenging activity (Enzymatic)

The superoxide radicals scavenging activity (enzymatic) was determined by using the method described by Nessa *et al.*[31] The reaction mixture comprised of 1mL of test solution, 0.9 mL phosphate buffer (0.1M, pH 7.5) 0.1 mL of XO (0.045 units/mL), 1 mL of 100 μM of xanthine, 1 mL of 600 μM NBT (0.1M phosphate buffer pH 7.5), was kept at 25°C for 10 min and the absorbance was measured at 560 nm against blank solutions which did not contain the enzyme. The percentage of scavenging activities (%) was calculated as follows: % scavenging of the superoxide radical = [1 – (absorbance of sample at 560nm)/(absorbance of blank at 560 nm)] × 100. The IC_{50} values were determined for extracts and reference compounds using regression lines. The experiment was repeated for three times.

Determination of superoxide radicals scavenging activity (Non-Enzymatic)

The scavenging activity against chemically generated superoxide radicals of different solvent extracts and reference compounds was determined spectrophotometrically.[41] The reaction mixture contained 1 mL of test solution, 1.9 mL 0.1M phosphate buffer, pH 7.4, 1 mL of 20 μM PMS, 156 μM NADH and 25 μM of NBT in phosphate buffer, pH 7.4. After 5 min of incubation at 25°C the absorbance of reaction mixture was measured at 560 nm against blank samples that contained no PMS. Here, superoxide anions were generated in a non-enzymatic (PMS/NADH) system. The percentage of scavenging activities (%) was calculated as follows: Scavenging activities % (capacity to scavenge the superoxide radical) = [1 – (absorbance of sample at 560 nm)/(absorbance of blank at 560nm)] × 100. The IC_{50} values were calculated by regression analysis.

Statistical analysis

The results were expressed as mean ± standard deviation (S.D). The data of different sources were subjected to a one-way analysis of variance (ANOVA). Tukey's test ($P < 0.05$) was performed to determine the significance of the difference between means.

RESULTS

Total polyphenol content

The total flavonoids content of *C. colocynthis* leaves extract was decreased in the order of: CCM (96.34 ± 1.45) > CCMW (76.34 ± 2.16) > CCC (14.54 ± 0.17) > CCH (3.12 ± 0.43). The highest concentration of polyphenol was present in the CCM (methanol) and CCME (methanol: water) extract and the lowest concentration was recorded in CCH (hexane) and CCC (chloroform) extract. There was a

statistically significant difference ($P < 0.05$) in polyphenol contents between the extracts.

DPPH radical scavenging activity

Free radical scavenging activity of different solvent extracts of *C. colocynthis* was determined according to the DPPH radical scavenging method and is shown in Figure 1. According to this method, a compound with high antioxidant activity effectively bind with the radical hence prevent its propagation and the resultant chain reaction.[34,37,42,43] Different concentrations of four different extracts i.e. CCM, CCMW, CCC and CCH extracts were analyzed. The results were expressed as SC_{50} values and calculated from regression lines as presented in Table 1.

CCM extract showed higher free radical scavenging activity than CCMW and CCC extract. CCM and CCMW extract scavenges free radical very slowly at lower concentration, as the concentration increases from 0.5 to 2.5 mg/mL, the reaction rate was increased and the reaction reached in a steady state within 2 min. The pattern of free radical scavenging ability of CCM and CCMW was similar, whereas CCH extract was inactive towards scavenging of free radicals at concentration ranges at 1 to 2.5 mg/mL. The lower SC_{50} values for CCM and CCMW is attributed to their higher polyphenolic content.

The results were compared with quercetin (QN) and tocopherol (TOC). QN exhibited highest free radical scavenging activity and the overall ranking of the tested extracts and reference compounds were in the order of: QN > TOC > CCM > CCMW > CCC. The mean difference between extracts and reference compounds was significant at the 0.05 level.

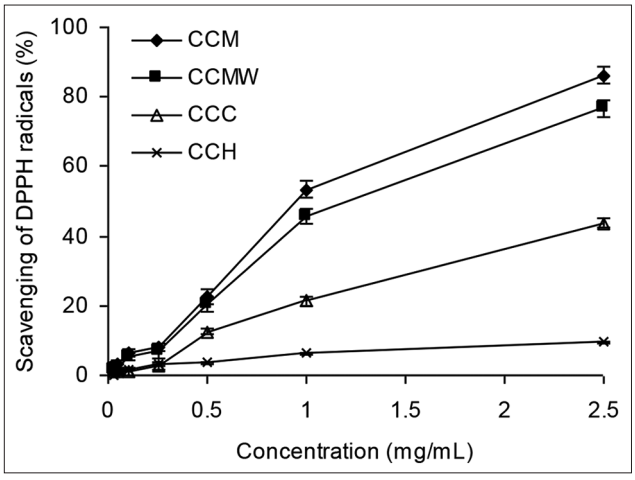

Figure 1: Free radical scavenging activity of different solvent extracts [Hexane (CCH), Chloroform (CCC), Methanol (CCM) and methanol:water (CCMW)] of *C. colocynthis* leaves measured using the DPPH-assay. Results are mean ± S.D ($n = 3$)

Lipid peroxidation inhibitory activity

During lipid peroxidation (LPO) various reactive species (radicals) are formed and they have been proved to be the main cause for the oxidative damage and aging. Hence, it is important to evaluate antioxidants on the basis of their ability to inhibit lipid peroxidation. The LPO inhibitory activity of *C. colocynthis* leaf extracts are shown in Figure 2 and the data are presented in Table 2.

For 0.1 mg/mL concentration, CCM extract exhibited highest antioxidant activity comparatively than other extracts and the results were compared with α-tocopherol (TOC) and butylated hydroxyl toluene (BHT). The mean values of CCM were not significantly ($P < 0.05$) different from TOC and BHT. The overall results were decreased in the order of: BHT ≈ CCM ≈ TOC > CCMW > CCC > CCH. In case of 0.5 mg/mL concentration, the results are decreased as follows: BHT ≈ CCM > CCMW > TOC ≈ CCC > CCH. Here, the mean values of BHT, CCM were not significantly ($P < 0.05$) different and showed highest activity than TOC. The mean values of CCC extract and TOC also were not significantly different ($P < 0.05$). For 1 mg/mL concentration extracts, CCM extract showed significantly ($P < 0.05$) highest activity in comparison with BHT and TOC. CCMW extract also exhibited higher antioxidant activity than TOC and CCC extract exhibited equal activity as TOC. In comparison amongst three different concentrations of tested samples, 1.0 mg/mL exhibited the highest antioxidant activity.

In comparison between mean values of antioxidant activity coefficients (C_{AA}), CCM extract exhibited highest activity amongst the studied extracts and the mean values were compared with TOC and BHT. At lower concentration (0.1mg/mL), the mean values of CCM and TOC were not significantly different ($P < 0.05$), on contrary at higher concentration (1.0 mg/mL), CCM exhibited significantly ($P < 0.05$) higher activity than TOC and lower

Table 1: SC_{50} values of different solvent extracts of leaves of *C. collocynthis* and reference compounds for scavenging of DPPH free radicals

Samples	*SC50 (mg/mL) ±S.D.	*r*
CCM	1.31±0.013	0.9550±0.013
CCMW	1.51±0.040	0.9616±0.005
CCC	2.76±0.055	0.9804±0.004
CCH	Not active at 1 to 2.5 mg/mL	
TOC (α-Tocopherol)	0.255±0.008	0.9903±0 0.003
QN (Quercetin)	0.045±0.001	0.9602±0.002
Statistical significant: Tukey-HSD ($P<0.05$)	Significant	

*The sufficient concentration to obtain 50% of a maximum scavenging capacity. SC_{50} values were calculated from regression lines; *r*=correlation coefficient. Results are mean±S.D ($n=3$)

activity than BHT. The mean values of C_{AA} for 0.5 mg/mL were significantly ($P < 0.05$) different from each other.

The oxidation rate ratio (R_{OR}) values of each extract were also compared with TOC and BHT. In case of 0.1 mg/mL concentration, the mean values of R_{OR} for CCM, BHT and TOC were not significantly different ($P < 0.05$). As the concentration increases from 0.5 to 1.0 mg/mL, the R_{OR} value of CCM extract decreased significantly than TOC and BHT. 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. CCH extract showed highest oxidation rate ratio amongst the studied extract. It seems, CCM extract were the most potential antioxidant and more protecting ability of beta-carotente from oxidation than natural antioxidant tocopherol as well as synthetic antioxidant BHT as assayed by beta-carotene and linoleic acid model system.

Xanthine oxidase (XO) inhibitory activity and scavenging of superoxide radicals produced enzymatically and non-enzymatically

The enzyme XO catalyses the oxidation of hypoxanthine and xanthine to uric acid and during reoxidation of XO, molecular oxygen acts as an electron acceptor, producing superoxide radical ($O^{2\bullet-}$) and hydrogen peroxide (H_2O_2).[44,45] As a result, XO is considered an important biological source of uric acid and superoxide radicals and inhibition of XO is an effective therapeutic approach for hyperuricemia causing gout and kidney stones.[46] Evidence suggests flavonoids and flavonoids rich extracts are potent XO inhibitor and superoxide scavengers.[31,47] Therefore, we evaluated the XO inhibitory activity of *C. colocynthis* leaf extracts as well as scavenging of enzymatically (xanthine/XO system) and non-enzymatically (PMS/NADH) produced superoxide radicals. In this study four different extracts (CCM, CCMW,

Table 2: Parameters used to evaluate the lipid peroxidation inhibitory activity of different solvent extracts of the leaves of *C. colocynthis*

Samples	Parameters used for evaluation of lipid peroxidation inhibitory activity		
	0.1 mg/mL	0.5 mg/mL	1.0 mg/mL
*A_A (Antioxidant activity %)			
CCH	b25.82±2.72D	ab30.32±2.00D	a33.35±1.39E
CCC	c49.36±1.44C	b60.38±0.974C	a66.79±0.571C
CCM	c57.44±1.16A	b68.59±0.796A	a82.24±0.681A
CCMW	c44.44±1.25B	b63.59±0.512B	a77.54±1.106B
BHT	c58.16±1.41A	b71.16±1.05A	a78.99±1.07B
TOC	b57.35±1.33A	ab60.15±1.21C	a62.77±1.25D
*C_{AA} (Antioxidant activity coefficients)			
CCH	b65.15±6.12E	ab79.69±8.24F	a91.91±7.86F
CCC	c174.52±9.71D	b290.86±9.61E	a381.03±7.05E
CCM	c366.027±11.74B	b490.40±9.77C	a680.17±10.66B
CCMW	c244.49±18.62C	b439.23±16.65D	a636.10±12.31C
BHT	c455.56±13.27A	b672.21±15.18A	a845.09±10.73A
TOC	c489.33±12.66A	b535.15±14.52B	a599.55±13.17D
*R_{OR} (Oxidation rate ratio)			
CCH	b0.7417±0.0272D	ab0.6967±0.0200D	a0.6664±0.0139D
CCC	c0.5063±0.0144B	b0.3961±0.0097C	a0.3321±0.0057C
CCM	c0.4255±0.0116A	b0.3141±0.0079A	a0.1776±0.0068A
CCMW	c0.5555±0.0125C	b0.3641±0.0051B	a0.2245±0.0111B
BHT	c0.4295±0.0013A	b0.2963±0.0023A	a0.2094±0.0012B
TOC	c0.4258±0.0014A	b0.4105±0.0044C	a0.3912±0.0011C

*Each value is expressed as mean±S.D (n=3). Means with similarsmall letter (a,b,c-within the row) and capital letter (A,B,C-within the column) are not significantly different (P<0.05). CCM=Methanol extract, CCMW=Methanol: water (1:1) extract, CCC=Chloroform extract and CCH=Hexane extract; BHT=Butylated hydroxyl toluene; TOC=Tocopherol

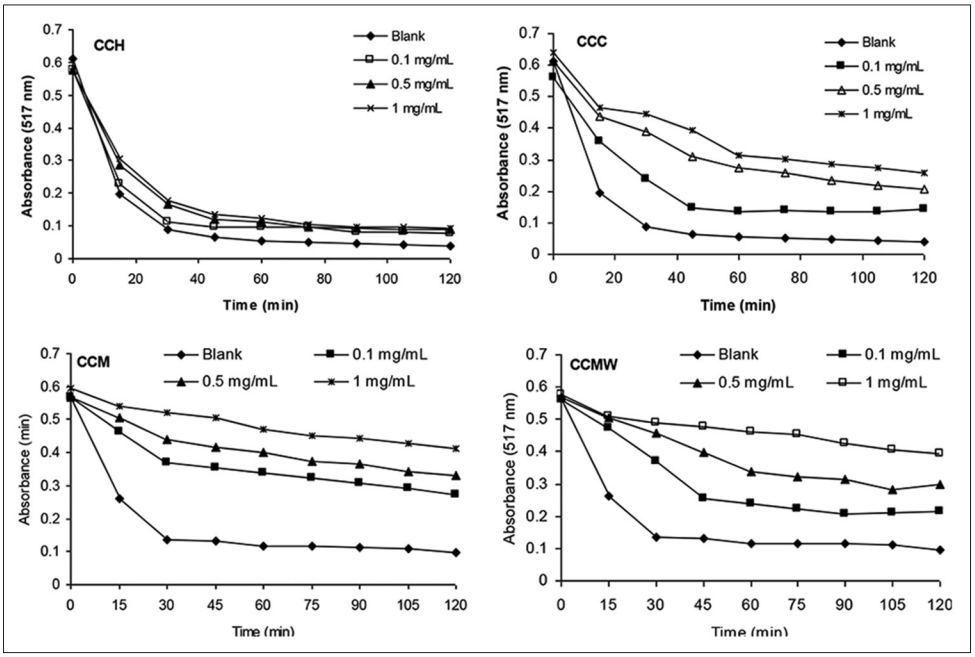

Figure 2: Hydrogen donating abilities of different solvent extracts [hexane (CCH), chloroform (CCC), methanol (CCM) and methanol-water (CCMW] of *C. colocynthis* leaves measured using the β-carotene-linoleic acid model system

CCC and CCH) and three reference compounds (AP, AA and QN) were tested and a dose-response curve was plotted as shown in Figures 3 and 4. The results were calculated from regression lines and expressed as IC_{50} values. The IC_{50} values of extracts (mg/mL) and reference compounds (μg/mL) are presented in Table 3.

CCM extract was exhibited highest XO inhibitory activity than CCMW. CCH extract was inactive towards XO as well as scavenging of superoxide radicals produced enzymatically and non-enzymatically. CCC extract was poorly active against inhibition of XO as well as against scavenging of enzymatically produced superoxide radicals, as it exhibited higher IC_{50} [Table 3]. In contrary, it scavenged non-enzymatically produced superoxide radicals effectively as it exhibited lower IC_{50} value (3.76 mg/mL) than enxymatically produced superoxide radicals (4.88 mg/mL). It seemed that CCC extract was a moderate superoxide radical scavenger and a poor XO inhibitor.

The IC_{50} values for CCM and CCWM as superoxide (X/XO) scavengers were 0.145 and 0.361 mg/mL respectively which were significantly lower than the IC_{50} values of XO inhibition. It seemed CCM and CCWM were potent superoxide scavengers than inhibition of XO. However, CCM and CCMW extract also exhibited lower IC_{50} value against scavenging of non-enzymatically produced superoxide radicals than enzymatically produced one. The overall IC_{50} value of each extract was compared statistically and the mean differences of all values were insignificant at the 0.05 level and it was decreased in the order of: CCM > CCMW > CCC. In this study quercetin (QN), L-ascorbic acid (AA) and alopurinol (AP) were used as reference compounds. All the reference compounds exhibited activity at μg/mL level, whereas extract exhibited activity at mg/mL level. Therefore, the IC_{50} values of extracts were not compared with the reference compounds. Amongst the reference compounds, AP-a potent XO inhibitor efficiently inhibited XO as well as scavenged superoxide radicals. QN- a natural known potent antioxidant[31] also exhibited lower IC_{50} value than natural antioxidant AA against scavenging of superoxide radicals. Though AA was inactive towards XO, but it efficiently

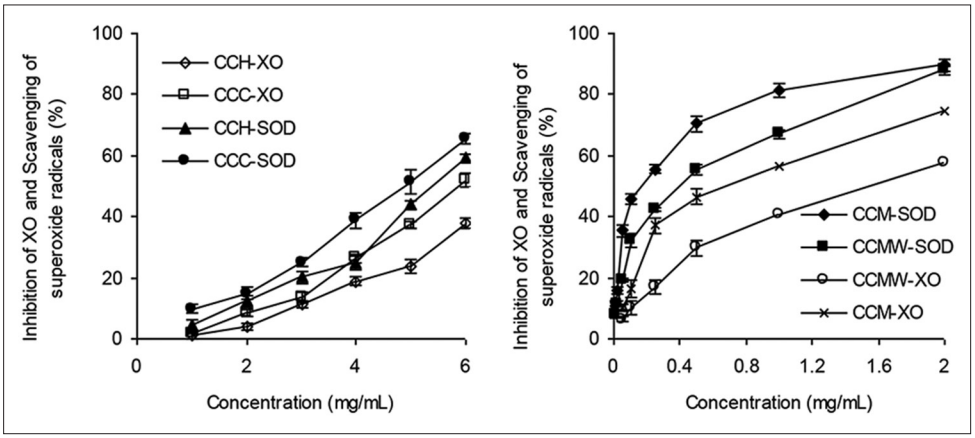

Figure 3: The different concentrations of *C. colocynthis* leaf extract (CCM, CCMW, CCH and CCC) versus the inhibition of xanthine oxidase and scavenging of superoxide radicals (enzymatically generated). Results are mean ± S.D (*n* = 3)

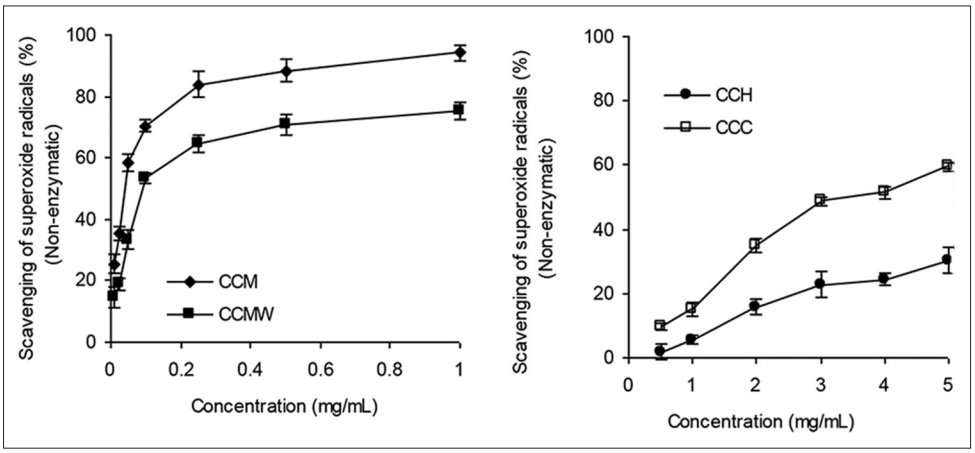

Figure 4: The different concentrations of *C. colocynthis* leaf extract (CCM, CCMW, CCH and CCC) versus the scavrnging of non-enzymatically (phenazine methosulfate - NADH) generated superoxide radicals. Results are mean ± S.D (*n* = 3)

Table 3: IC_{50} values of extracts of the leaves of *C. colocynthis* for inhibition of xanthine oxidase and reduction of superoxide level produced enxymatically and non-enzymatically

	Uric acid		Superoxide radicals (Enzymatic)		Superoxide radicals (Non-enzymatic)	
	*IC_{50} (mg/mL±S.D.)	*r*	*IC_{50} (mg/mL±S.D.)	*r*	*IC_{50} (mg/mL±S.D.)	*r*
C. Collocynthis leaf extract						
CCM	0.578±0.059	0.9841±0.007	0.145±0.002	0.9787±0.004	0.042±0.005	0.95±0.002
CCMW	1.802±0.243	0.9369±0.013	0.361±0.003	0.9707±0.008	0.108±0.013	0.9532±0.010
CCC	5.96±0.336	0.9672±0.002	4.88±0.125	0.9791±0.017	3.763±0.050	0.9368±0.009
CCH	Inactive at 1-6 mg/mL		5.68±0.170	0.9483±0.008	Inactive at 0.1-4.0 mg/mL	
Statistical significance Tukey HSD *P*<0.05	Significant		Significant		Significant	
Reference compounds						
AA	Inactive at 1-20 µg/mL		5.63±0.341	0.9514±0.002	6.57±0.233	0.9576±0.011
QN	0.882±0.112	0.9311±0.003	0.836±0.034	0.9501±0.004	0.923±0.056	0.9558±0.005
AP	0.132±0.096	0.9814±0.009	0.135±0.018	0.9621±0.012	-	-
Statistical significance Tukey HSD *P*<0.05	Significant		Significant		Significant	

*IC_{50} values were calculated from linear or log linear regression lines where: x=tested compound concentration and y=percent inhibition of enzyme activity; *r*=correlation coefficient. Results are mean±S.D. (*n*=3). CCM=Methanol extract, CCMW=Methanol: water (1:1) extract, CCC=Chloroform extract and CCH=Hexane extract

scavenged superoxide radicals. The overall ranking of XO inhibitory activity and scavenging of superoxide radicals of extracts and reference compounds were decreased in the order of: AP > QN > AA > CCM > CCMW > CCC.

DISCUSSION

There is considerable evidence that free radicals induce oxidative damage to biomolecules and play an important role in cardiovascular disease, aging, cancer, inflammatory diseases and a variety of other disorders.[48-50] Antioxidants that scavenge free radicals are now known to possess preventive as well as therapeutic potential in free radical-mediated disease conditions.[50-52]

The free radical-scavenging activity of the extracts is ascribed to their hydrogen donating ability.[53] Antioxidants are believed to intercept the free radical chain during oxidation and to donate hydrogen from the phenolic hydroxyl groups, thereby forming a stable end product, which does not initiate or propagate further oxidation of the lipid.[54] The data of this study reveal that the CCM and CCMW extracts of *C. colocynthis* leaves are free radical inhibitors and acted as primary antioxidants that react with free radicals.

The antioxidant activities of extracts are associated with their polyphenolic content. To justify the antioxidant potential of extracts of *C. colocynthis*, polyphenolic content of each extract was determined. The highest polyphenol content was recorded in CCM and CCMW extract. Whereas, CCC extract contained very lower amount of polyphenol and in CCH was the least. The results showed that CCM and CCMW extract were good antioxidant and moderate XO inhibitor irrespective of test done to evaluate the antioxidant activity. Both extracts exhibited highest antioxidant activity in LPO assay procedure than in DPPH assay. In addition, both extracts scavenged superoxide radicals effectively produced by non-enzymatic procedure rather than produced by enzymatically. It seemed both extracts were potent antioxidants rather than inhibitor of XO. However, CCM extract was more potent than CCMW extract as it could be ascribed due to their high polyphenolic contents. CCH extract was mostly inactive in all assays, though CCC extract was active but exhibited activity at higher concentration, which might not be safe for human consumption as this plant/fruit extract exhibited some toxicity in human.[27-30] Though CCM and CCMW exhibited high polyphenolic content but their content was not as high as other studied medicinal plant.[31]

CONCLUSION

Several methods were employed for the evaluation of antioxidant potential of *Citrullus colocynthis* leaves extract. It can be seen that various extracts of *C. colocynthis* leaves prepared by different solvents exhibited various degrees of antioxidant activity; the difference in the antioxidant activity and XO inhibition of the different solvent extracts may be attributed to their different polyphenolic compositions. In view of overall discussed results, it was concluded that methanol and methanol/water extract of *C. colocynthis* were good antioxidant and moderate type XO inhibitor.

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