Assessment of antioxidant potential of β-carotene and some edible fruits

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Abstract

Gac fruit (Momordica cochinchinensis), watermelon (Citrullus lanatus), and tomato fruit (Solanum lycopersicum) are high in natural antioxidants and have anticancer properties. Gac is a rich source of lycopene. These are commonly used in cancer, hypertension, dyslipidemia, inflammation, wound healing, anti-wrinkle products, moisturizers, and ulceration. In the present study, the freeze-dried powder of juices from the fruits of Momordica cochinchinensis (MC), Citrullus lanatus (CL), and Solanum lycopersicum (SL), as well as β-carotene (BC), are evaluated, using ascorbic acid (AA) as a reference standard. Three herbal products wise Sample-A MC, Sample-B CL, Sample-C SL, and β-carotene as sample-D were evaluated for invitro antioxidant activity. Free radical scavenging properties assessed by five invitro methods are DPPH, hydrogen peroxide (H_2O_2), superoxide, ferrous reducing antioxidant capacity assay (FRAC), and nitric oxide radical-scavenging activity. All the products were found to have potent antioxidant activity, which was significant ($P < 0.05$) compared to ascorbic acid. All the assays were performed in triplicate, and the results are represented as mean SD. P values are calculated by ANOVA at a 95% confidence interval by GraphPad Prism 8.0.2.

Keywords: Antioxidant; fruits; *in vitro*; edible; DPPH

1. Introduction

The fruit of MC has the potential to prevent breast and melanoma cell cancer[1]. MC is a plant of the Cucurbitaceae[2], the extract and various phytochemicals from its fruit has anti-gastritis, wound healing[3], anti-inflammatory, anti-proliferative[4], Anti-hypertensive[5], Anti-hyperlipidemia[6,] anthelmintic[7], anti-oxidant[8] even MC extract exhibited greater antioxidant activity compared to Vit-A and C. A skin cream containing MC extract demonstrated a significant moisturizing smoothness and antiwrinkle effect in clinical trials[9]. Hypoglycemic property, improve fertility by preventing testicular and epididymal damages[10], and nutritional value[11,12].

MC fruit are rich in carotenoid content; the lycopene content is 380µg/gm of seed membrane, which is 10-fold that of a well-known source of lycopene[13], relative to mass MC fruit contains 70 times the lycopene found in SL and up to ten times the β-carotene found in carrot[2]. MC fruit is an excellent source of dietary supplements rich in multi-phytochemicals[14]. Vitamin C, vitamin E, omega-3 and omega-6 fatty acids [15], Carotenoids[16] such as lutein, zeaxanthin, and cryptoxanthin, β - carotene, cis- and trans-β-carotene, cis- and trans- lycopene, fiber, Protein, and calcium[2]. are all important

phytonutrients found in MC fruit. Carotenoids from MC are more bioaccessible than those from carrots and SL[17]. Another fruit of CL, this is a member of the Cucurbitaceae family[18]. Lycopene, beta-carotene, vitamin C, citrulline, polyphenols like phenolic acids, flavonoids, stilbenes,[19] and lignans, carbohydrate (sucrose, glucose, and fructose)[20], and ascorbic acid[21] are all found in CL.

CL juice exhibits anti-inflammatory[22,23] anti-hyperlipidemic[23], antioxidant[24] anti-hyperglycemic activities[24,25]. Extracts of pulp have a gastroprotective, antiulcerative effect[26], reduces atherosclerosis[27] and have analgesic properties [28]. They also exhibit anti-urolithiatic and diuretic activity[29]. Citrulline (content of CL juice) supplements relieve fatigue or muscle soreness and lower the rating of perceived exertion (RPE)[30]. CL flesh extract increases sexual potency or sustains erections[31]. Supplementation of CL juice prevents increased post-exercise BP in females[32]. Daily CL consumption is useful for atherosclerosis (decreased vascular cell adhesion molecule-1)[33]. ethanolic extract of CL rind is cytotoxic and has been evaluated taking 7 human cancer cell lines: A549, Caco-2, H1299, HCT116, Hep2, HepG2, and MCF- 7[34]. CL juice has been shown to have hepato-neuroprotective[35], and improve muscle endurance-support[36].

The other content of my product is SL, which belongs to the family Solanaceae[37]. Lycopene, β-Carotene, Phenol, Flavonoids, Vitamin C [38], Lutein, Phytoene, Phytofluene[39], γ- Carotene and Vitamin A[40] are the main components of SL. The fruit of SL has proven therapeutic effectiveness in patients with cardiovascular dysfunction, obesity, and diabetes [41]. SL juice has anticarcinogenic[42], antioxidant and antiplatelet aggregation properties [43,44]. Reduce the risk of cardiovascular diseases by reducing the concentration of inflammatory molecules (adhesion molecules) related to atherosclerosis[45]. The SL fruit has nutritional and medicinal value as an antioxidant; it helps reduce cholesterol, prevents prostate cancer, reduces heart disease risk, improves liver health by detoxifying, and improves vision[46].

Reduced gastric acidity is clinically achieved by the SL dietary supplement in gastroesophageal reflux disease[47]. SL juice is effective in reducing systolic and diastolic blood pressure[48,49], it decreases LDL cholesterol[50] and benefit male infertility by improving sperm motility[51]. consumption of SL juice modifies clinical asthma outcomes[52] and reduce blood pressure in hypertensive pregnant women[53].

2. Materials and methods

Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH), ethanol, 0.5 mM Nitroblue Tetrazolium (NBT), 100 M phenazine methosulphate, Tris-HCl buffer (pH 8.0), 50 mM phosphate buffer (pH 7.4), 2 mM H_2O_2, and nicotinamide adenine dinucleotide (NADH), phosphate buffer (0.2 M, pH 6.6) potassium ferricyanide, trichloroacetic acid, ferric chloride, sodium nitroprusside, Griess reagent, and Ascorbic acid.

Plant Material

All three-plant materials ware procured from *IdoBio (Xi'an) Phytochem Co., Ltd., Xi'an, Shaanxi, China*, and carotenoid from *Research-Lab Fine Chem Industries, Mumbai, India*.

Statistics: The statistical significance were determined by one way ANOVA using GraphPad Prism 8 at 95% confidence interval. All the tests were performed in triplicate and graph were plotted using the mean value.

A) DPPH radical scavenging activity

The assay was carried out in accordance with the method of Blios M.S. 1958[54] with minor modification. DPPH antioxidant activity is performed by taking increasing concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/ml) of plant products and β-carotene. 1 ml of a 0.1 mM solution of DPPH in ethanol was taken from a freshly prepared stock solution and added to this 1 ml of sample solution in distilled water. Ascorbic acid is used as a reference standard. 30 minute later absorbance ware measured at 517 nm. Lower absorbance indicates higher scavenging activity. The radical scavenging activity of the sample was expressed as a percentage inhibition activity, which was calculated as follows:

Where A_0 represents the absorbance of a blank (control) containing no sample, and A_t represents the absorbance of the sample. Every test was performed in triplicate, and a graph

$$\%\ inhibition = \left(\frac{A_0 - A_t}{A_0}\right) \times 100$$

was plotted using the mean values.

B) Superoxide radical scavenging activity

Radicals of the superoxide anion (SO) are produced in a mixture of 2.0 ml of Tris-HCl buffer (16 mM, pH 8.0) with 2.0 ml of nitroblue tetrazolium (NBT, 0.3 mM) and 2.0 ml of nicotinamide adenine dinucleotide solution (NADH, 0.936 mM). Then, 1 ml of the sample solution (10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/ml) was added to this combination. 2.0 ml of phenazine methosulfate solution (PMS, 0.12 mM) was then added to the mixture to start the reaction, which was then incubated for 5 minutes at 250 °C. Absorbance was measured at 560 nm using a blank consisting of 2.0 ml Tris-HCl buffer, 2.0 ml NBT, 2.0 ml NADH solution, 4.0 ml water, and 2.0 ml PMS solution. Ascorbic acid was treated as a reference in the same way that sample solutions were. The result was expressed as a percentage inhibition, which was calculated as follows:[55]

Where A_0 is absorbance of blank (control) not containing sample, A_t is absorbance of sample.

C) Hydrogen peroxide scavenging activity

$$\%\ inhibition = \left(\frac{A_0 - A_t}{A_0}\right) \times 100$$

The free radical scavenging ability of the sample and β-carotene was determined according to the method explained by Ruch, R.J., with some modifications. Sample solutions (10-100 µg/ml) were made with 50 mM phosphate buffer (pH 7.4), to which 0.6 ml of 2 mM H_2O_2 solution was added, and the final volume was made up to 2 ml by adding 50 mM phosphate buffer. incubated for 40 minutes, and absorbance was measured at 230 nm. Ascorbic acid is used as a reference standard.[56]

The scavenging activity of the sample was calculated as a percentage inhibition using the following equation:

$$\%\ inhibition = \left(\frac{A_0 - A_t}{A_0}\right) \times 100$$

Where A_0 represents the absorbance of a blank (control) containing no sample and A_t represents the absorbance of the sample. Every test was performed in triplicate, and a graph was plotted using the mean values.

D) Ferrous reducing antioxidant power -FRAP

2 ml of samples of various concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µg/ml) were mixed with 2 ml of phosphate buffer (0.2 M, pH 6.6) and 2 ml of potassium ferricyanide (10 mg/ml). This mixture is incubated at 50^o C for 20 minutes. cooled to room temperature, and to this 2 ml of trichloroacetic acid (100 mg/l) was added. After centrifuging at 3000 rpm for 10 minutes, 1 ml of the supernatant was mixed with 1 ml of distilled water and 0.2 ml of 0.1% (w/v) ferric chloride and incubated for 10 minutes. The absorbance of the sample was measured at 700 nm by a spectrophotometer against a blank. A positive control is ascorbic acid. Higher reducing power results in greater absorbance.[57]

E) Nitric oxide radical-scavenging activity

0.5 ml of sample with various concentrations from 10 µg/ml to 100 µg/ml were added to 2 ml of 10 mM sodium nitroprusside in phosphate buffered saline. and incubated at room temperature for 150 minutes. A volume of 0.5 ml of the Griess reagent (1% sulphanilamide, 2% orthophosphoric acid, and 0.1% naphthylethylenediamine dihydrochloride) was added, and the mixture was incubated at 25° C for 5 min. scavengers inhibit the generation of nitric oxide radicals from sodium nitroprusside in phosphate buffered saline. The diazotization of nitrite with sulphanilamide, followed by coupling with napthylethylenediamine, results in a light pink to deep purple coloured chromophore in the reaction mixture, which is measured at 546 nm. The positive and negative controls were prepared in the same manner as the sample, with the exception that the solvent was used as the negative control and ascorbic acid was used as the positive control.[58,59] The decreased absorbance as a percentage of inhibition was calculated using the following formula:

Where A_0 represents the absorbance of a blank (control) containing no sample and A_t represents the absorbance of the sample. Every test was performed in triplicate, and a graph was plotted.

$$\% \, inhibition = \left(\frac{A_0 - A_t}{A_0}\right) \times 100$$

3. Results and Discussion

Results are represented as the mean of tests performed in triplicate. All the results were expressed as mean SD, calculated by one-way ANOVA, and were found statistically significant at $P < 0.05$. When compared to ascorbic acid, the GF, WM, TF, and BC have potent antioxidant activities. Overall, the results show that GF has the highest antioxidant potential for all types of free radicals tested in this study; GF's ability to scavenge free radicals was greater than that of TF and WM. The free radical scavenging activity increased as the concentration of the test sample increased. The correlation of responses with the concentration (µg/ml) was significant at $P < 0.0001$.

For all approaches, the GF IC_{50} was consistently found to be between 48.72 and 55.95. The R square and IC_{50} values are shown in the following table.

Table 1. IC_{50} values of MC, CL, SL and BC by DPPH, Superoxide, H2O2, and Nitric Oxide Activity

Sample	IC_{50}			
	DPPH	**Superoxide**	**H_2O_2**	**Nitric Oxide**
MC	51.11	48.72	55.95	54.30
CL	80.91	80.83	67.52	84.18
SL	59.02	63.08	53.08	59.86
BC	41.15	51.37	39.60	50.81
AA	32.72	34.90	30.15	41.95

DPPH radical scavenging activity

The ability of sample to remove stable DPPH ion was calculated as percentage inhibition. All sample has acceptable ion preventing ability out of this the lowest scavenging ability was found for WM and the highest for GF. IC50 and percentage inhibition of DPPH ion represented in table. Ascorbic acid used as standard. Out of all the test samples highest antioxidant activity was observed by GF at the dose of 100 µg/ml.

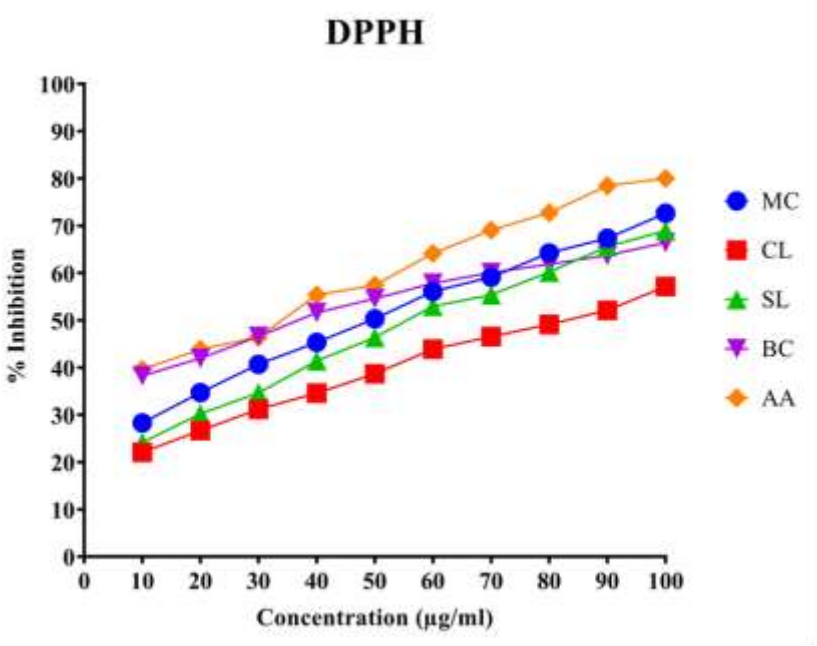

Fig. 1. DPPH radical scavenging activity of the samples (MC, CL, SL and BC), Data are expressed as mean ± SD (n = 3, p <0.0001) for all samples.

Superoxide radical scavenging activity

The discoloration of the mixture brought on by the removal of superoxide causes the absorbance to drop as sample concentration rises. 10 Sample concentration at 110-100µg/ml assayed in triplicate and SD and IC_{50} calculated. In this test the lowest inhibition activity from 10 to 100 µg/ml seen for WM was from 21.94 to 57.16%. For beta carotene the lowest inhibition was 33.28% and highest 63.62%.

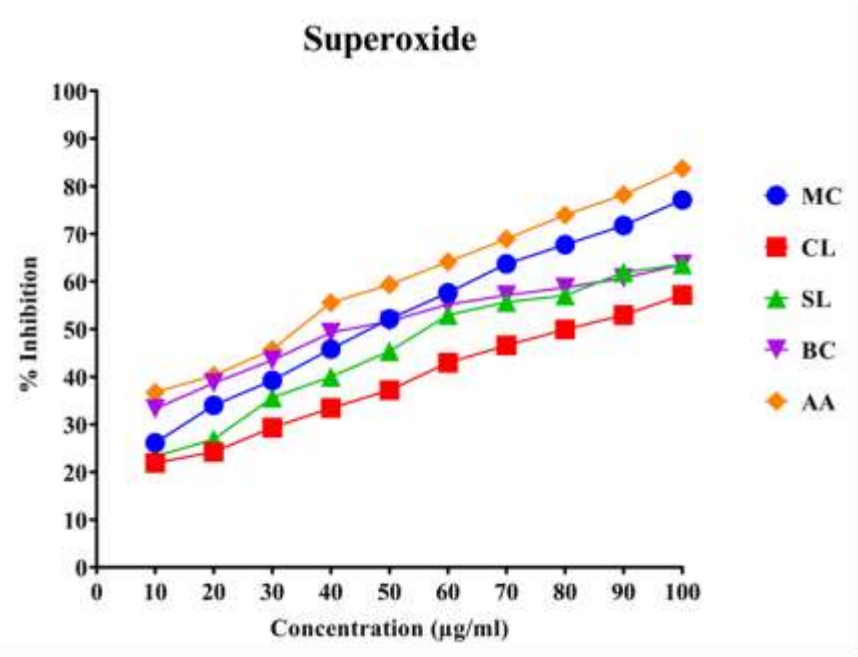

Fig. 2. Superoxide radical scavenging activity of the samples (MC, CL, SL and BC), Data are expressed as mean ± SD (n = 3, p <0.0001) for all samples.

Hydrogen peroxide scavenging activity

for all the test sample and the compound of samples showed increase inhibition activity as the concentration increased from 10 to 100 µg/ml. the highest activity was found for GF, 71.87% Inhibition of Hydrogen peroxide. For WM it was lowest comparing to other test samples. From all the test the H_2O_2 radical scavenging activity was highest 76.68% for BC.

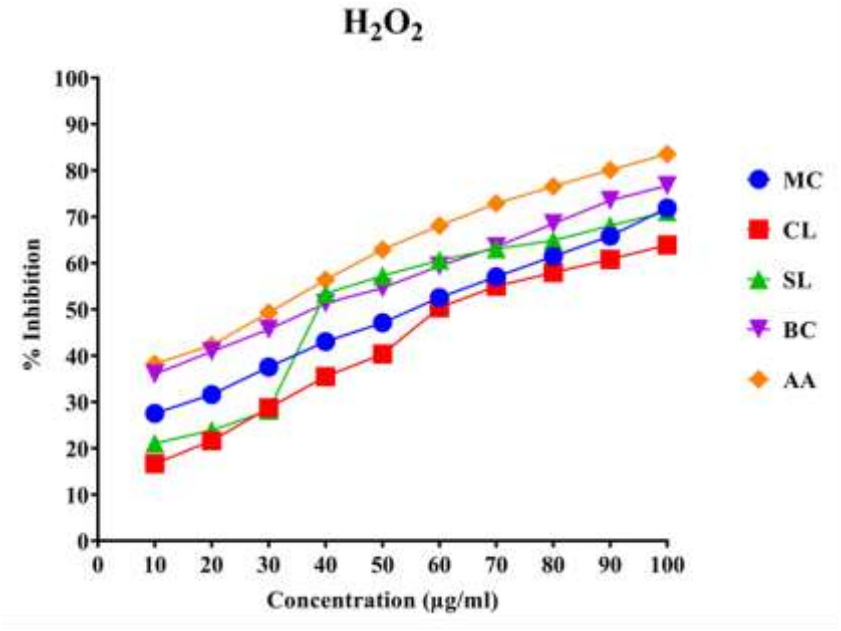

Fig. 3. Hydrogen peroxide scavenging activity of the samples MC, CL, SL and BC, Data are expressed as mean ± SD (n = 3, p <0.0001) for all samples.

Ferrous reducing antioxidant power – FRAP

Higher absorbance indicates greater radical scavenging activity, in this test the absorbance was gradually increase by increasing concentration with a significance of P <0.0001 in

relation to concentration versus absorbance. The highest absorbance was 0.099 for BC after TF (0.095). for GF and WM absorbance was 0.081 at 100 µg/ml.

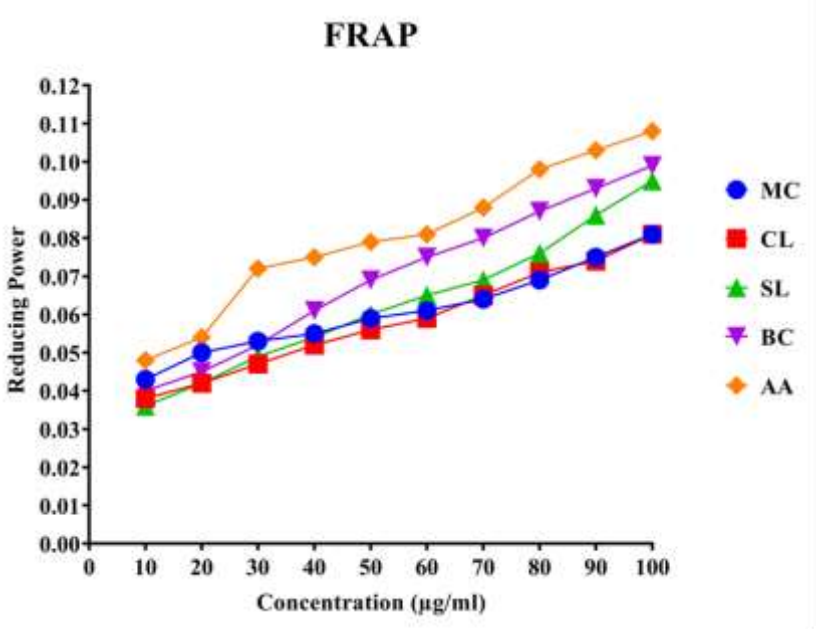

Fig. 4. Ferrous reducing antioxidant power of the samples MC, CL, SL and BC, Data are expressed as mean ± SD (n = 3, p <0.0001) for all samples.

Nitric oxide radical-scavenging activity

Nitric oxide scavenging potential was again lowest for the WM (55.08) than GF (68.55), TF (62.66) and BC (67.55) at the concentration of 100 µg/ml. The highest potential reported by GF ranging from 29.20 to 68.55. the scavenging property of BC was close to ascorbic acid.

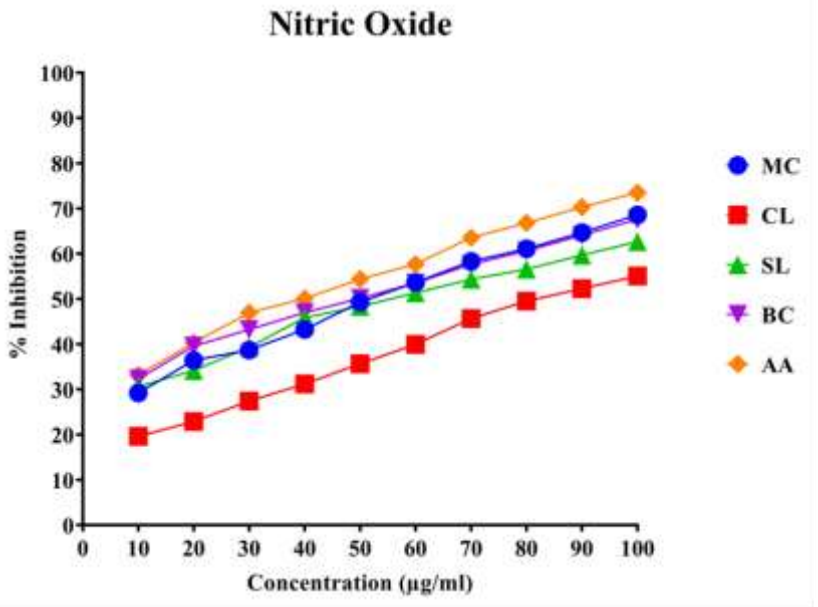

Fig. 5. Nitric oxide radical-scavenging activity of the samples MC, CL, SL and BC, Data are expressed as mean ± SD (n = 3, p <0.0001) for all samples.

4. Conclusion

All test results indicated that DPPH, H_2O_2, superoxide, FRAP, and nitric oxide had potential free radical inhibiting properties. The parts of the plant selected are rich in carotenoid pigments, which play an important role as antioxidants in biological fluids. The polyherbal

formulation can be efficiently used as a potent antioxidant. All the results are statistically significant. The selection of plant parts was based on an extensive literature study to make the formulation for oral submucous fibrosis. The investigation's earlier findings, which included the identification of phytoconstituents, along with the therapeutic results, show that it has great potential for a wide range of clinical applications. It can be used successfully to treat oral submucous fibrosis, with added clinical and nutritional benefits.

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