World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

Check for updates

Antioxidant activity and the estimation of total phenolic content of *Citrullus colocynthis* stem

Sushila Saini *

Department of Botany, Janta Vidya Mandir Ganpat Rai Rasiwasia College, Charkhi Dadri-127306, Haryana, India.

World Journal of Biology Pharmacy and Health Sciences, 2023, 13(03), 163–169

Publication history: Received on 03 February 2023; revised on 17 March 2023; accepted on 20 March 2023

Article DOI: https://doi.org/10.30574/wjbphs.2023.13.3.0134

Abstract

To examine the antioxidant and free radical scavenging activity of *Citrullus colocynthis* stem, five different extracts were investigated. Various *in vitro* antioxidant assay including DPPH (2,2-diphenylpicrylhydrazyl) radical, superoxide radical, hydroxyl radical scavenging activity along with reducing power and metal chelating activity were done and compared with standard antioxidants such as ascorbic acid, BHT (Butylated hydroxytoluene) and EDTA (Ethylene diamine tetra acetic acid). Total phenolic content was determined as mg/g Gallic acid equivalent and was correlated with antioxidant assays. All the five extracts showed significant antioxidant activity in dose dependent manner. Methanolic extract possesses the highest scavenging ability for all the assays and total phenolic content in it was 40.12 mg/g gallic acid equivalent. In case of methanolic extract significant correlation was observed between total phenolic content and various antioxidant assays suggesting its role as a source of valuable antioxidants against free radicals associated oxidative stress.

Keywords: *Citrullus colocynthis*; Antioxidant activity; Free radicals; Total phenolic content

1. Introduction

Many human diseases are the outcome of oxidative stress caused by free radicals generated by aerobic respiration, environmental contaminants, unhealthy food and stress. Most common oxygen derived free radicals are hydrogen peroxide (H_2O_2) superoxide anion ($O_2^{\bullet-}$) peroxyl ($ROO^{\bullet}$), reactive hydroxyl ($OH^{\bullet}$) and hypochlorous acid (HOCl) [1,2]. Free radicals generated in the cells attack biological membranes, cell organelles and DNA thereby initiating chain reactions leading to detrimental effects such as enzyme inactivation, DNA breakage and even cell death. Thus oxidative stress induced by reactive oxygen species implicated in serious health problems such as inflammatory diseases, stroke, hyperglycemia, neurodegenerative diseases, cancer and premature aging [3,4]. Plant based antioxidants could show great potential as therapeutic agents by avoiding oxidative damage thereby preventing occurrence of degenerative diseases, cancer and aging. These are secondary metabolites produced naturally in plants and can counter free radicals generation by donating their electrons thus terminating the chain reactions [5,6].

Citrullus colocynthis (L.) Schard (Cucurbitaceae) commonly called Bitter apple or tumba is a perennial viny herb widely distributed in dry regions of Africa, Asia, Arabia and Mediterranean. Its utility as a medicinal plant is well documented in indigenous system of medicine for the treatment of leprosy, diabetes, asthma, bronchitis, edema, fever, joint pain and cancer. In Indian subcontinent its fruits are used for bacterial infections, hepatic and abdominal diseases, diabetes and rheumatism [7,8]. Preliminary phytochemical analysis of different parts of *C. colocynthis* affirm the presence of tannins, flavonoids, phenolics, glycosides and triterpenoids [9,10,11] of which flavonoids and phenolics could play a role in scavenging of free radicals and thus inhibiting harmful effects resulting from oxidative stress [12]. This study was carried out to investigate the antioxidant potential of various solvent extracts of *C. colocynthis* stem.

* Corresponding author: Saini Sushila

2. Material and methods

2.1. Preparation of different extracts

Plants of *Citrullus* were collected from the dry regions of Southern Haryana and identified with voucher number 10814 at FRI, Dehradun. Stem was cut into pieces, dried in shade and grounded to fine powder. For preparation of extracts 100 gram of powder was extracted separately with petroleum ether, benzene, chloroform, methanol and water using cold percolation method. Each extract was evaporated using a rotary evaporator at 40^0C and stored for further use.

2.2. Total phenolic content and antioxidant assays

Total phenolic content was measured according to the method of Singleton and Rossi [13] with some modifications and expressed as Gallic acid equivalent (GAE). The ability of the extracts to reduce DPPH radical was assayed by decreased absorbance at 517 nm according to the method of Lee *et al* [14]. Nitro blue tetrazolium reduction method was employed for finding out superoxide radical scavenging activity [15]. The hydroxyl radical scavenging capability was evaluated by means of the deoxyribose method described by Kunchandy and Rao, [16]. Ability of extracts to chelate metal ions and to compete with Ferrozine for ferrous iron in solution was measured by method of Dinis et al [17]. Reducing power ability was assayed by observing Fe^{3+} to Fe^{2+} reduction by different extracts using the method of Yen and Duh [18]. Increased absorbance with increasing concentration indicates increased reducing power.

All the assessments were done in triplicate and to measure percentage inhibition following formula was used.

$$\% \text{ Inhibition} = A \text{ (control)} - A \text{ (sample or standard)} / A \text{ (control)} \times 100,$$

Where
A (control) = absorbance of the control
A (sample or standard) = absorbance of sample extract or standard

2.3. Statistical analysis

On way ANOVA (Analysis of Variance) followed by Duncan's multiple range tests at $p<0.05$ were used. Pearson's correlation coefficient was used to determine correlation between TPC and antioxidant assays.

3. Results and discussion

3.1. Total Phenolic Content (TPC)

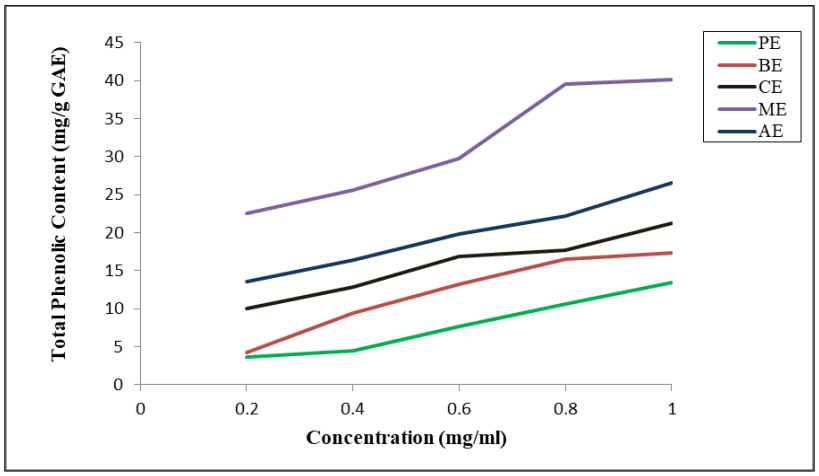

Figure 1 Total phenolic content (TPC) of stem extracts of *C. colocynthis* (PE- petroleum ether extract, BE- benzene extract, CE-chloroform extract, ME- methanol extract, AE- aqueous extract)

Phenolic compounds are the secondary metabolites present in plants having the capacity to quench free radicals. They act as reducing agents, hydrogen donors or metal iron chelators. The antioxidant activity of plant extracts can be due to phenolic compounds because of their ability to neutralize free radicals [19]. TPC was determined using the Folin-

ciocalteu reagent and expressed as Gallic acid equivalent. TPC content in different extracts is shown in Figure 1. In *C. colocynthis* stem highest total phenolic content was found in methanolic extract (40.12 mg/g GAE). Phenolic content in different stem extract was in the order: methanolic> aqueous> chloroform> benzene> petroleum ether extracts.

3.2. DPPH Radical scavenging activity

To evaluate the free radical scavenging ability of natural antioxidants DPPH assay is most widely used. DPPH radical gives a violet solution in ethanol with maximum absorption at 517 nm. As the antioxidants in the extract scavenge the DPPH radical, it resulted in discoloration of the solution which coincides with the number of electrons consumed. More bleaching of the solution from violet to yellow indicates greater efficacy of the extract to neutralize the radicals [20]. Ascorbic acid was used as a standard compound at varying concentration of 10µg-50µg/ml for determination of antioxidant activity. Table 1 exhibited the scavenging effect of different concentrations of solvents and ascorbic acid on DPPH radical. Methanolic extract has the maximum scavenging potential followed by benzene> aqueous> chloroform> petroleum extract. The IC_{50} values of different extracts were much greater as compared to ascorbic acid indicating that plant extract has weaker DPPH radical scavenging ability than reference compound.

Table 1 DPPH free radical scavenging activity (%) of stem extracts of *C. colocynthis*

Stem extracts							
Concen-tration (mg/ml)	**PE**	**BE**	**CE**	**ME**	**AE**	**Concentration (µg/ml) of AS**	**AS**
0.2	8.43±0.35^{e}	10.35±0.38^{e}	7.12±0.20^{e}	25.13±0.58^{e}	9.93±0.89^{e}	10	20.84±0.62^{e}
0.4	12.61±0.42^{d}	13.63±0.46^{d}	13.34±0.56^{d}	40.45±0.42^{d}	12.53±0.23^{d}	20	38.63±0.40^{d}
0.6	18.15±0.60^{c}	22.42±0.12^{c}	17.91±0.28^{c}	45.34±0.75^{c}	19.60±0.22^{c}	30	75.17±0.60^{c}
0.8	21.02±0.55^{b}	34.83±0.35^{b}	23.34±0.32^{b}	50.78±0.51^{b}	23.19±0.11^{b}	40	80.28±0.12^{b}
1.0	25.33±0.66^{a}	38.43±0.57^{a}	27.72±0.66^{a}	56.45±0.62^{a}	30.28±0.15^{a}	50	84.80±0.66^{a}

Values are expressed as mean±S.D., (n=3). Values with in the column not sharing common superscript letters (a-e) differ significantly at $p<0.05$ by Duncan's multiple range test. (PE- petroleum ether, BE- Benzene extract, CE- Chloroform extract, ME- Methanol extract, AE- Aqueous extract, AS- Ascorbic acid)

3.3. Superoxide radical scavenging activity

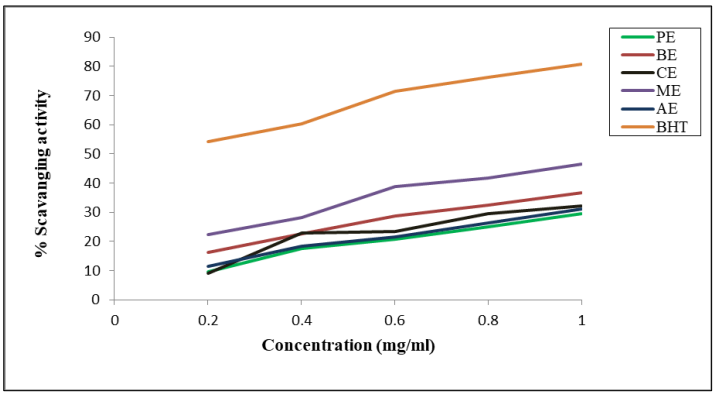

Figure 2 Superoxide radical scavenging activity (%) of stem extracts of *C. colocynthis* (PE- petroleum ether extract, BE- benzene extract, CE- chloroform extract, ME- methanol extract, AE- aqueous extract, BHT- Butylated hydroxytoluene)

Superoxide radical itself is a weak oxidant but is a precursor of H_2O_2, OH radical, singlet oxygen species which are more potent reactive oxygen species contributing to oxidative stress [21]. Superoxide anion causes lipid peroxidation, damage to biomolecules and tissues leading to the onset of various diseases. The percentage inhibition of superoxide anion generated in the PMS-NADH system was evaluated by comparing the absorbance values of control and solvents at 560 nm. In the present study all the extracts showed superoxide radical scavenging activity in a concentration dependent manner (Figure 2). In comparison with other solvents methanolic extract at the 1.0mg/ml concentration

showed highest inhibition of superoxide radical (46.62%). The IC_{50} values of methanolic extract and standard BHT (Butylated hydroxytoluene) were 1.2 and 0.18 mg/ml respectively.

3.4. Hydroxyl radical scavenging activity

Hydroxyl radicals are highly reactive radicals reacting to membrane phospholipids and causing tremendous cell damage. It is capable of damaging every biomolecule leading to mutagenesis, cytotoxicity and cancer [22]. Hydroxyl radical scavenging ability of plant extracts may inhibit the lipid peroxidation and prevent associated damage. Percentage scavenging activity of different solvents was in the order: methanolic> aqueous> chloroform> benzene> petroleum ether extracts as shown in figure 3. At 1.0mg/ml concentration percentage inhibition of hydroxyl radical generation by methanolic extract and ascorbic acid was 55.32% and 72.39% respectively, with IC_{50} of methanolic extract (1.2 mg/ml) much higher than the standard (0.094 mg/ml).

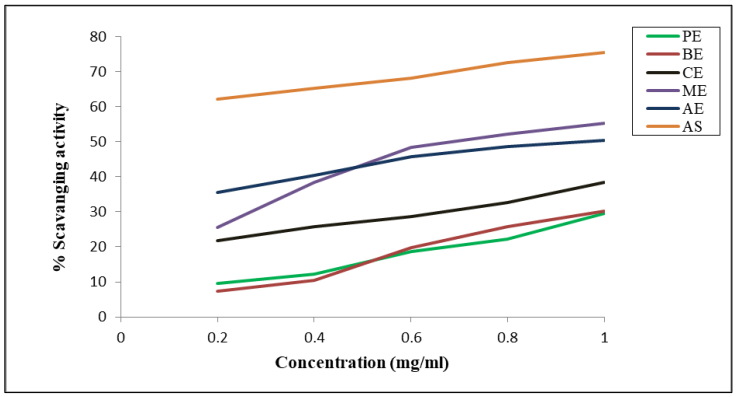

Figure 3 Hydroxyl radical scavenging activity (%) of stem extracts of *C. colocynthis* (PE- petroleum ether extract, BE- benzene extract, CE- chloroform extract, ME- methanol extract, AE- aqueous extract, AS- Ascorbic acid)

3.5. Metal chelating activity

In biological systems, transition metals lead to the generation of hydroxyl radicals by Haber-Weiss and Fenton type of reactions. Plant extracts having antioxidants can form chelates with transition metal ions resulting in the suppression of generation of hydroxyl radicals thus saving the biological molecules from peroxidation [23]. In the present study plant extracts chelate iron and thus Ferrozine-Fe^{2+} complex formation is disrupted resulting in decreased absorption at 562 nm in concentration dependent manner. The methanolic, aqueous, chloroform, benzene and petroleum ether extracts of *C. colocynthis* stem were found to possess dose dependent chelation activity as shown in results depicted in figure 4. In the stem extracts metal chelating ability was maximum in methanolic extract which varied from 25.29% at 0.2 mg/ml to 56.69% at 1.0mg/ml concentration. The mean IC_{50} value of methanolic extract (1.2 mg/ml) was higher than standard EDTA (0.19 mg/ml).

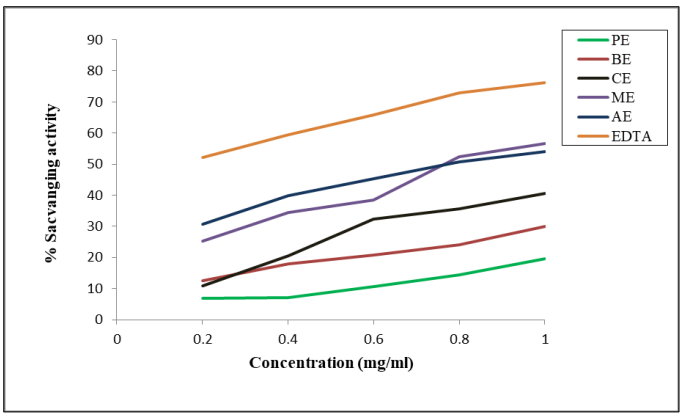

Figure 4 Metal chelating activity (%) of stem extracts of *C. colocynthis* (PE- petroleum ether extract, BE- benzene extract, CE- chloroform extract, ME- methanol extract, AE- aqueous extract, EDTA- Ethylenediamine tetra acetic acid)

3.6. Reducing power assay

Reducing power assay method depends on the presence of reductants that react with Potassium Ferricyanide and reduce it to ferrocyanide which on reaction with $FeCl_2$ form ferric-ferrous complex having maximum absorbance at 700 nm [24,25]. In this method the yellow color of the solution changes to green/blue depending on the reducing ability of extracts. Increase in absorbance with increased concentration exhibits increased reducing power of test specimens. Methanolic stem extract showed the highest reductive potential that increased with increased concentration; however it is lower than standard ascorbic acid. The reducing power ability of different solvents was in the sequence: methanolic> benzene> chloroform> aqueous> petroleum ether extracts as depicted in figure 5.

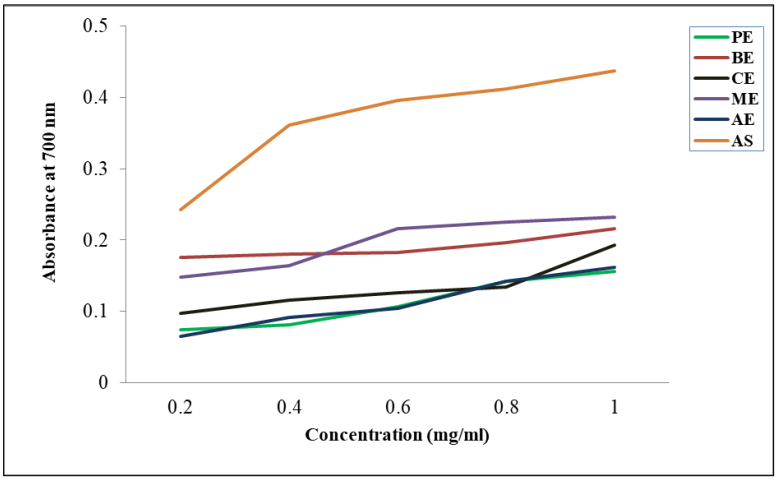

Figure 5 Reducing power assay of stem extracts of *C. colocynthis* (PE- petroleum ether extract, BE- benzene extract, CE- chloroform extract, ME- methanol extract, AE- aqueous extract, AS- Ascorbic acid)

3.7. Correlation between total phenolic content and antioxidant activity

Phenolic compounds such as phenolic acids, flavonoids, tannins and anthocyanins are secondary metabolites naturally occurring in plants having free radical scavenging ability by donating electrons or by acting as metal chelators. Several previous reports showed good correlation between antioxidant activity and total phenolic content in plant extracts [26,10]. Methanolic extract of *C. colocynthis* stem exhibited highest antioxidant activity and also showed good correlation between TPC and different antioxidant assays. Table 2 shows the value of correlation coefficient as 0.913168, 0.941537, 0.912252 and 0.928704 respectively for DPPH, superoxide, hydroxyl and metal chelating activity. These results indicate that phenolic content might be contributing towards antioxidant activity of *C. colocynthis*. From different parts of *C. colocynthis* various phenolic compounds have been previously reported [27,28]. Aqueous and ethanolic extract of *C. colocynthis* fruits, leaves and whole plant were found to contain alkaloids, flavonoids, coumarins, saponins and glycosides [29,30,31]. Different parts of *C. colocynthis* (roots, stem, leaves, and fruits) found to contain flavonoids such as quercetin, myricetin and kaempferol [32,33]. Ariel parts were found to possess flavonoids such as 8-C-p-hydroxybenzoylisovitexin, 6-C-p-hydroxybenzoylvitexin, 8-C-p-hydroxybenzoylisovitexin-4'-O-glycoside while the fruits showed the presence of isovitexin, isoorientin and isoorientin3'-o-methyl ether [34]. In nutshell, *C. colocynthis* is rich in polyphenols and flavonoids which confer to the plant antioxidant activity.

Table 2 Correlation analysis between different antioxidant tests with their respective total phenolic content at 1 mg/ml concentration in *C. colocynthis* stem methanolic extract

Assays	**Total phenolics in stem**	
	r	**R2**
DPPH radical scavenging	0.913168*	0.832*
Superoxide radical scavenging	0.941537*	0.886*
Hydroxyl radical scavenging	0.912252*	0.833*
Metal chelating assay	0.928704*	0.862*

r- correlation coefficient, R^2- coefficient of determination, *significance at $p<0.05$

4. Conclusion

On the basis of various *in vitro* assays used in present study it can be concluded that methanolic extract of *C. colocynthis* possess significant antioxidant activity. These activities may be due to phenolic compounds present in the extract thus establishing its role in preventing oxidative stress related diseases and promotion of longevity.

Compliance with ethical standards

Acknowledgments

I express my gratitude to the University Grant Commission for sanctioning Minor Research project to carry out this research.

Disclosure of conflict of interest

No conflict of interest.

References

[1] Halliwell B. Free radicals and antioxidants: updating a personal view. Nutrition reviews. 2012; 70(5): 257-265.

[2] Ye ZW, Zhang J, Townsend DM, Tew KD. Oxidative stress, redox regulation and diseases of cellular differentiation. Biochimica et Biophysica Acta (BBA)-General Subjects. 2015; 1850(8): 1607-1621.

[3] Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. The international journal of biochemistry & cell biology. 2007; 39(1): 44-84.

[4] Chand RN, Gopalan RD, Christi K. Evaluation of antioxidant properties in thirteen Fijian medicinal plants used in Alzheimer's disease and related illness. Free Radicals and Antioxidants. 2018; 8(1): 11-17.

[5] Jagessar RC. Antioxidant properties of plant extracts. Pharmaceutica Analytica Acta. 2019; 1: 18-21.

[6] Saini S, Yadav JP. In vitro assessment of total phenolic content and antioxidant properties of *Citrullus colocynthis* root extracts. Plant Archives. 2020; 20: 2927-2930.

[7] Dhakad PK, Sharma PK, Kumar S. A Review on Phytochemical Studies and biological Potential of *Citrullus colocynthis* (L.) Schrad. (Cucurbitaceae). Bioengineering and Bioscience. 2017; 5(4): 55-64.

[8] Kapoor M, Kaur N, Sharma C, Kaur G, Kaur R, Batra K, Rani J. *Citrullus colocynthis* an Important Plant in Indian Traditional System of Medicine. Pharmacognosy Reviews. 2020. 14(27):22-27

[9] Delazar A, Gibbons S, Kosari A, Nazemiyeh H, Modarresi MM, Nahar L, Sarker SD Flavoneglycosides and cucurbitacin glycosides from *Citrullus colocynthis*. DARU Journal of Pharmaceutical Sciences. 2006; 14(3): 109-114

[10] Ahmed M I, Xu X, Sulieman AA, Mahdi AA, Na Y. Effect of extraction conditions on phenolic compounds and antioxidant properties of koreeb (Dactyloctenium aegyptium) seeds flour. Journal of Food Measurement and Characterization. 2020; 14: 799-808.

[11] Li QY, Munawar M, Saeed M, Shen JQ, Khan MS, Noreen S, Alagawany M, Naveed M, Madni A, Li CX. *Citrullus colocynthis* (L.) Schrad (Bitter Apple Fruit): Promising traditional uses, pharmacological effects, aspects, and potential applications. Frontiers in Pharmacology. 2022; 12: 3848.

[12] Benariba N, Djaziri R, Bellakhdar W, Belkacem N, Kadiata M, Malaisse WJ, Sener A. Phytochemical screening and free radical scavenging activity of *Citrullus colocynthis* seeds extracts. Asian Pacific journal of tropical biomedicine, 2013; 3(1), 35-40.

[13] Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture. 1965; 16(3): 144-158.

[14] Lee SE, Hwang HJ, Ha JS, Jeong HS, Kim J H. Screening of medicinal plant extracts for antioxidant activity. Life sciences. 2003; 73(2): 167-179.

[15] Liu F, Ooi VEC, Chang ST. Free radical scavenging activities of mushroom polysaccharide extracts. Life sciences. 1997; 60(10): 763-771.

[16] Kunchandy E, Rao MNA. Oxygen radical scavenging activity of curcumin. International journal of pharmaceutics. 1990; 58(3): 237-240.

[17] Dinis TC, Madeira VM, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Archives of biochemistry and biophysics. 1994; 315(1): 161-169.

[18] Yen GC, Duh PD. Antioxidative properties of methanolic extracts from peanut hulls. Journal of the Aerican oil chemists' society. 1993; 70(4): 383-386.

[19] Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. Plants. 2019; 8(4): 96.

[20] Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, Chang CM. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of Ficus religiosa. Molecules, 2022; 27(4): 1326.

[21] Dizdaroglu M, Jaruga P. Mechanisms of free radical-induced damage to DNA. Free radical research. 2012; 46(4): 382-419.

[22] Carocho M, Ferreira IC. A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food and chemical toxicology. 2013; 51: 15-25.

[23] Santos JS, Brizola VRA, Granato, D. High-throughput assay comparison and standardization for metal chelating capacity screening: A proposal and application. Food Chemistry. 2017; 214: 515-522.

[24] Wong CC, Li HB, Cheng KW, Chen F. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. Food chemistry. 2006; 97(4): 705-711.

[25] Gülçin İ. Fe3+-Fe 2+ transformation method: An important antioxidant assay. Advanced protocols in oxidative stress III. 2015: 233-246.

[26] Hatamnia AA, Rostamzad A, Malekzadeh P, Darvishzadeh R, Abbaspour N, Hosseini M, Nourollahi K, Mehr, RSA. Antioxidant activity of different parts of Pistacia khinjuk Stocks fruit and its correlation to phenolic composition. Natural Product Research. 2016; 30(12): 1445-1450.

[27] Rezai M, Davoodi A, Asori M, Azadbakht, M. Cytotoxic activity of *Citrullus colocynthis* (L.) schrad fruit extract on gastric adenocarcinoma and breast cancer cell lines. International Journal of Pharmaceutical Sciences Review and Research. 2017; 45(1): 175-178.

[28] Rao V, Poonia A. *Citrullus colocynthis* (bitter apple): bioactive compounds, nutritional profile, nutraceutical properties and potential food applications: a review. Food Production, Processing and Nutrition. 2023; 5(1): 4.

[29] Najafi S, Sanadgol N, Nejad BS, Beiragi MA, Sanadgol, E. Phytochemical screening and antibacterial activity of *Citrullus colocynthis* (Linn.) Schrad against Staphylococcus aureus. Journal of Medicinal Plants Research. 2010; 4(22): 2321-2325.

[30] Gill NS, Supreet K, Arora R, Bali M. Screening of antioxidant and antiulcer potential of *Citrullus colocynthis* seed extract. Research Journal of Phytochemistry. 2011; 5: 98-110.

[31] Meybodi MSK. A review on pharmacological activities of *Citrullus colocynthis* (L.) Schrad. Asian Journal of Research and Reports in Endocrinology. 2020; 25: 25-34.

[32] Mahesh CM, Vidya P. Isolation and Identification of flavonoid "Quercetin" from *Citrullus colocynthis* (Linn.) Schrad. Asian journal of experimental sciences. 2008; 22(1): 137-142

[33] Meena MC, Patni V. Isolation and identification of flavonoid "quercetin" from *Citrullus colocynthis* (Linn.) Schrad. Asian journal of experimental sciences. 2008; 22(1): 137-142.

[34] Maatooq GT, El-Sharkawy SH, Afifi MS, Rosazza, JP. Cp-hydroxybenzoylglycoflavones from *Citrullus colocynthis*. Phytochemistry. 1997; 44(1): 187-190.