



IN VITRO ASSESSMENT OF TOTAL PHENOLIC CONTENT AND ANTIOXIDANT PROPERTIES OF *CITRULLUS COLOCYNTHIS* ROOT EXTRACTS

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Abstract

The occurrences of natural antioxidants in plants are generating a lot of interest due to their accompanying health benefits. *Citrullus colocynthis* is a traditional medicinal plant extensively growing in arid regions and is well documented for its anthelmintic, antimicrobial, anti-inflammatory, antidiabetic and anticarcinogenic activities. The ability of root extracts to scavenge free radicals was investigated by various antioxidant assays. Phenolic content in root extracts was estimated by Folin-Ciocalteu's reagent. Methanolic extract exhibited highest percent inhibition of free radicals at all the concentrations tested. Phenolic content of extracts showed significant correlation with antioxidant properties suggesting that *C. colocynthis* extracts may serve as potential source of valuable antioxidants.

Key words: *Citrullus colocynthis*, free radicals, methanolic extract, phenolic content, antioxidant assay.

Introduction

Free radicals are highly unstable species that are continuously produced during cellular metabolism and their overproduction cause damage to several biomolecules such as membrane lipids, protein, nucleic acids and enzymes. Oxidative stress by reactive oxygen species are responsible for development of many chronic and degenerative disorders such as atherosclerosis, cardiovascular diseases, stroke, arthritis, diabetes, Alzheimer's disease and cancer (Seifried *et al.*, 2007; Chand *et al.*, 2018). Antioxidants by delaying or inhibiting the oxidation of lipids or other biomolecules can play a major role in prevention of such diseases. Synthetic oxidants although have potent free radical scavenging activity but are toxic and harmful for human health. This has generated considerable interest in the search for natural antioxidants in plants as they are safe and are less expensive. Various plant species have been documented as source of various pharmacological important compounds but the potential application of a large number of plant species for their therapeutic use is still unknown (Kapadiya *et al.*, 2016; Jagessar, 2019).

Citrullus colocynthis (L.) Schard, belonging to the family cucurbitaceae also known as bitter apple is a

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valuable medicinal plant distributed in the desert and sandy parts of the world. Traditionally it is used in the treatment of disease such as rheumatism, constipation, indigestion, oedema, fever, jaundice, intestinal parasites, leprosy, diabetes and cancer (Hussain *et al.*, 2014). Pharmacological analysis of different parts of this plant have shown the presence of many therapeutic compounds such as phenols, flavonoids, cucurbitacins, glycosides, alkaloids and saponins (Rani *et al.*, 2017; Sagar and Dumka, 2018).

The current problem was carried out to investigate *in vitro* antioxidant potential of various root extracts of *C. colocynthis*.

Material and Methods

Preparation of *C. colocynthis* extracts

Plants of *C. colocynthis* were collected from different areas of southern Haryana. Herbarium specimen of *C. colocynthis* was authenticated at FRI, Dehradun with the accession number of 10814. For the preparation of petroleum ether, benzene, chloroform, methanol and aqueous extracts, dried grounded roots were soaked in these solvents for 4-5 days. Extracts after filtering were dried at 40°C on a rotary evaporator. Percentage scavenging activity of various extracts was determined at concentration of 0.2, 0.4, 0.6, 0.8 and 1 mg/ml.

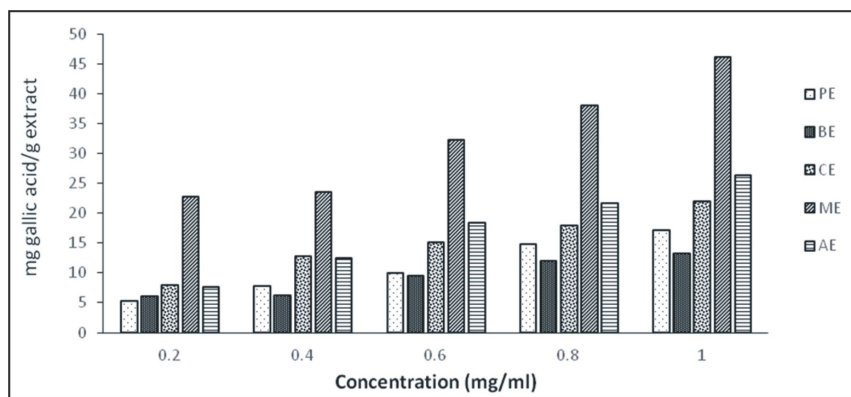


Fig. 1: Total phenolic content (TPC) of root extracts of *Citrullus colocynthis*; (PE- petroleum ether extract, BE- benzene extract, CE- chloroform extract, ME- methanol extract, AE- aqueous extract).

Assessment of total phenolic content (TPC) and antioxidant activity

Phenolic content in the root extracts was determined by modified Folin-Ciocalteu's method (Singleton and Ross, 1965). TPC was expressed in the form of mg gallic acid/g of extract.

The potential of the root extract to scavenge DPPH radical was estimated by the method of Lee *et al.*, (2003). Ascorbic acid was used as standard in range of 10-50 µg/ml solution. Superoxide anion scavenging activity was assayed by reduction of nitroblue tetrazolium (NBT) by extract as described by Liu *et al.*, (1997). BHT was used as the standard. The scavenging activity for hydroxyl radicals was measured with TBA reaction (Kunchandy and Rao, 1990) and ascorbic acid served as reference.

The percentage inhibition was analysed by the equation:

$$\% \text{ scavenging activity} = \frac{A_{(\text{control})} - A_{(\text{Standard or sample})}}{A_{(\text{control})}} \times 100$$

$A_{(\text{control})}$ = absorbance of control

$A_{(\text{Standard or sample})}$ = absorbance of standard or sample

Analysis

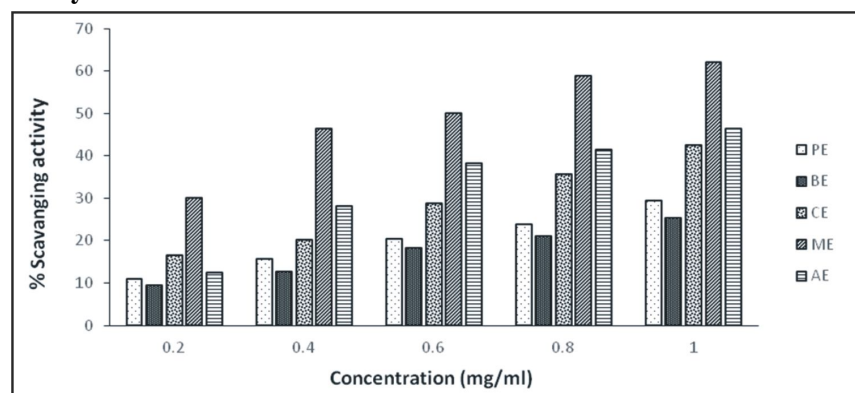


Fig. 2: DPPH free radical scavenging activity (%) of root extracts of *C. colocynthis*; (PE- petroleum ether extract, BE- benzene extract, CE- chloroform extract, ME- methanol extract, AE- aqueous extract).

The results were expressed as mean \pm standard deviation (SD) of three replicates. For estimation of the level of significance one way analysis of variance (ANOVA) was used and the difference between concentrations were analysed by Duncan's multiple range test. Correlations between TPC and antioxidant assays were determined by Pearson correlation coefficient.

Results and discussion

Total phenolic content

Plant phenolic compounds are the most extensive secondary metabolite and have the ability to scavenge free radicals. These compounds have gained much attention as antioxidants because of their capability to act as reducing agents, hydrogen donors and to chelate metals (Labiad *et al.*, 2017; Ozay and Mammadov, 2019). TPC content in different extracts is exhibited in fig. 1. At a concentration of 1 mg/ml maximum total phenolic content was found in methanolic extract (46.22 mg GAE/g). In roots total phenolic content decreased in the following order: methanol > chloroform > aqueous > petroleum ether > benzene extracts.

DPPH radical scavenging activity

For estimation of free radical scavenging activity of plant products DPPH assay is most commonly used method. Plant extracts by donating electron or H-atom neutralise the free radical character of DPPH resulting in discolouration of the solution from purple to yellow (Adebiyi *et al.*, 2017, Mahmood *et al.*, 2019). Methanolic extract of root exhibited maximum % inhibition and it was 30.12, 46.31, 50.12, 58.76 and 62.12% at 0.2, 0.4, 0.6, 0.8 and 1 mg/g concentration respectively. Ascorbic acid at a concentration of 10 µg/ml exhibited a percentage inhibition of 20.84 and for 50 µg/ml, % inhibition was 84.80. As the concentration of extract was increasing there was a proportional fading of the solution, proving increased ability of *C. colocynthis* extracts to reduce DPPH radical to corresponding hydrazine.

Superoxide radical scavenging activity

Superoxide radical damages biomolecules by formation of more potent reactive oxygen species such as H_2O_2 , OH radical and singlet oxygen species resulting in oxidative stress and aging. Besides generating other

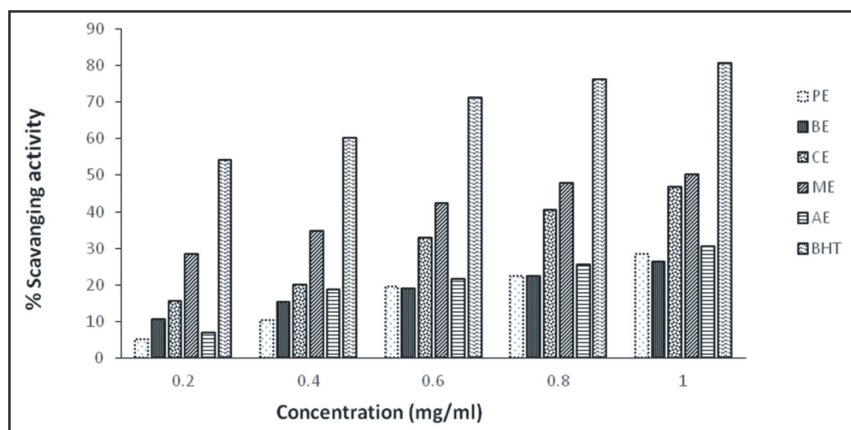


Fig. 3: Superoxide radical scavenging activity (%) of root extracts of *Citrullus colocynthis*; (PE- petroleum ether extract, BE- benzene extract, CE- chloroform extract, ME- methanol extract, AE- aqueous extract).

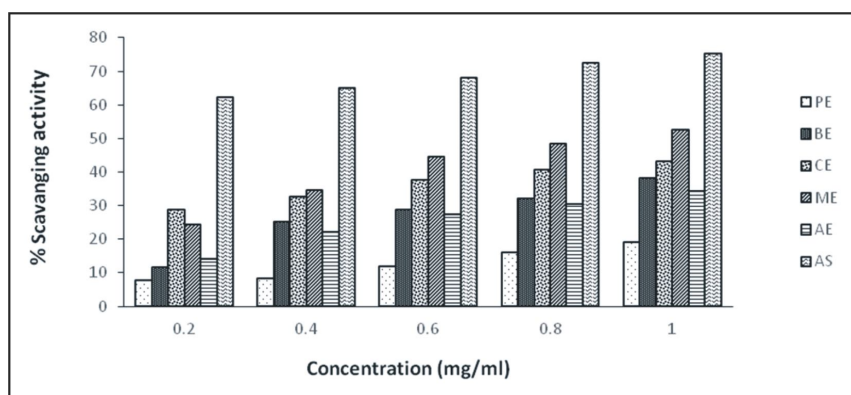


Fig. 4: Hydroxyl radical scavenging activity (%) of root extracts of *Citrullus colocynthis*; (PE- petroleum ether extract, BE- benzene extract, CE- chloroform extract, ME- methanol extract, AE- aqueous extract).

powerful radical superoxide anion directly initiates lipid peroxidation contributing to tissue damage and other diseases (Saeed *et al.*, 2012). Results prove that *C. colocynthis* show promising antioxidant activity by scavenging superoxide radical. Antioxidant potential of methanolic extract was most powerful and at 1mg/ml concentration % scavenging activity of methanolic, chloroform, aqueous, petroleum ether and benzene extracts was 50.35, 46.93, 30.75, 28.65 and 26.57% respectively (Fig. 3). However at all the tested concentrations the activity of BHT was more than methanolic extract.

Hydroxyl radical scavenging activity

Hydroxyl radicals are extremely reactive oxygen species thus causing enormous damage to biological systems. It is responsible for lipid peroxidation and capable of reacting with every molecule inside the cell thus contributing to various pathophysiological processes. Hydroxyl radical by reacting with nucleotide in DNA causes breaks in DNA chains contributing to mutations and cancer (Rajamanikandan *et al.*, 2011; Kanimozhi and Chitra, 2013). In the present study root extracts showed

the ability to prevent OH radical stimulated deoxyribose degradation into malondialdehyde. The ability to scavenge OH radical increased as the extract concentration raised (Fig. 4). The methanolic extract exhibited highest scavenging activity than other extracts and at concentration of 1mg/ml it reached up to 52.50% but it was less than reference ascorbic acid (75.39% at 1mg/ml).

Correlation between antioxidant tests and total phenolic content

Plants phenolics by scavenging free radicals are effective in many degenerative diseases caused by oxidative stress. A number of bioactive compounds have been isolated from different parts of *C. colocynthis* (root, stem, leaves and fruits). Gill *et al.*, (2011) has reported high concentration of alkaloids, steroids, flavonoids, coumarins and glycosides in *C. colocynthis* seed extracts. Similarly Sultan *et al.*, (2010) obtained a considerable amount of alkaloids, phenolic compounds, flavonoids and ascorbic acid from entire *C. colocynthis* plants. Three flavonoid glycosides isovitexin, isoorientin-3'-O-methyl ether and isosaponarin were found in *C. colocynthis* fruits extracts while flavonoid such as quercetin, have been isolated from other parts (Delazar *et al.*, 2006; Mahesh *et al.*, 2008). Medicinal properties of this plant has been reported due to presence of cucurbitacin glycosides 2-O-β-D-glucopyranosyl cucurbitacin I and 2-O-β-D-glucopyranosylcucurbitacin L in the fruit extract (Shekarchi *et al.*, 2015; Dhakad *et al.*, 2017).

Various plant species have shown a good correlation between total phenolic content and antioxidant activity advising that their antioxidant activity may be due to

Table 1: Correlation analysis between different antioxidant assays and total phenolic content in methanolic extract (1mg/g) of *C. colocynthis*.

Assays	Total phenolics in roots	
	r	R ²
DPPH radical scavenging	0.885972*	0.784*
Super oxide radical scavenging	0.948341*	0.856*
Hydroxyl radical scavenging	0.925246*	0.899*

r- correlation coefficient, R²- coefficient of determination, *significance at p<0.05.

occurrence of phenolic compounds (Shahriar *et al.*, 2012; Rebaya *et al.*, 2015; Ismail *et al.*, 2019). In case of *C. colocynthis* significant positive correlation was observed between scavenging activity for DPPH, Superoxide radical, hydroxyl radical with phenolic content and the value of r was 0.885972, 0.948341, 0.925246 and 0.951545 respectively (Table 1). These results revealed that antioxidant capacity of the *C. colocynthis* might be possible due to presence of phenolics in extracts. However, future investigations are required to recognise and isolate the components responsible for antioxidant activity.

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