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Screening of Antioxidant and Antiulcer Potential of *Citrullus colocynthis* Methanolic Seed Extract

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ABSTRACT

Medicinal plants are the backbone of Traditional systems of medicine throughout the world. They are invaluable, incredible and traditional source for the curing various diseases in the form of medicines. The objective of our present study was to evaluate the antioxidant and antiulcerogenic property of *Citrullus colocynthis* seeds. The motivation behind this study was to highlight the importance of traditionally consumed seeds as a potent source of bioactive compounds. Phytochemical screening of methanolic seed extract showed the presence of various phytoconstituents that are responsible for pharmacological activities. Antioxidant activity of extract was studied spectrophotometrically by 1, 1-diphenyl-2-picryl hydrazyl and Hydrogen Peroxide free radical scavenging method. The methanolic seed extract of *Citrullus colocynthis* showed maximum percentage inhibition of 79.4 and 72.4% by 1, 1-diphenyl-2-picryl hydrazyl and Hydrogen peroxide method respectively at 300 μg mL^{-1}, as the seed extract showed good antioxidant potential. It was further evaluated for anti-ulcerogenic activity by pyloric ligation induced ulcers model in Wistar albino rats. *Citrullus colocynthis* (200 mg kg^{-1}) showed maximum inhibition of gastric volume, free acid and total acidity 1.68±0.18, 39.86±3.86 and 61.23±1.87 at dose 200 mg kg^{-1}. The maximum percentage inhibition of the extract by pyloric ligation was 71.57% at a dose of 200 mg kg^{-1}. The results of Present study revealed that the seed extract of *Citrullus colocynthis* possesses significant antiulcerogenic activity due to its free radical scavenging property.

Key words: *Citrullus colocynthis*, antioxidant activity, anti-ulcerogenic activity, 1,1-diphenyl-2-picryl hydrazyl, Hydrogen peroxide, pyloric ligation

INTRODUCTION

The use of natural medicine is a very rich tradition particularly among the people of India, China, Egypt and Brazil. Traditional medicinal system in India includes Ayurveda, Siddha and Unani which are based on the use of natural medicine (Borikar *et al*., 2009). Natural medicines have come from various sources like terrestrial plants, terrestrial microorganisms, marine organisms, terrestrial vertebrates and invertebrates (Newman *et al*., 2000). But medicines obtained from plant sources are highly useful. Natural medicine are also known as botanical medicine or phytomedicine which refers to use of seeds, berries, roots, leaves, bark and flowers for medicinal purposes (Hasan *et al*., 2009). Based on the literature survey and other important uses of cucurbitaceae family, it gave the idea to explore this family more and more for its therapeutic

potential. This family is commonly known for melons, gourds or cucurbits and includes crops like cucumbers, squashes (including pumpkins) and luffas and there are about 118 genera in this family which includes 825 species (Shah *et al.*, 2010). Fruits and peel part of this family have been largely explored for their pharmacological activities but the seed part has been ignored even of the fact that it has been well accepted by Ayurveda and traditional system of medicine (Toyama *et al.*, 2008). The motivation behind this study is to highlight the importance of seeds as a therapeutic agent and a cheaper source of bioactive material.

Citrullus colocynthis is also one of the plants belonging to Family Cucurbitaceae. It is a fruit commonly known as bitter apple. It is a native plant of North Africa, being common throughout Morocco, Egypt and Sudan (Al-Ghamdi *et al.*, 2009). It has been used in herbal treatment of diabetes (Karim *et al.*, 2011), edema, bacterial infection and cancer. The aqueous pulp extract of fruit is used for kidney, liver functions (Rahbar and Nabipour, 2010) and as an antidiabetic medication.

Studies have shown the significance of oxidative stress, mitochondrial dysfunction and free radicals in aging and in pathogenesis of many diseases viz. autoimmune disorder, epilepsy, depression, respiratory disorders, multiple sclerosis and peptic ulcers (Koneru *et al.*, 2011). Current studies of free radicals have confirmed that food rich in antioxidant plays an essential role for the prevention of cardiovascular diseases and cancer etc. (Siddiq *et al.*, 2005). The phenolic compounds isolated from plants are of great interest due to their antioxidative and anticarcinogenic activity. They play a very important role in absorbing and neutralizing free radicals. They contain not only minerals and primary metabolite but also a diverse array of secondary metabolite with antioxidant potential (Chanda *et al.*, 2011). Due to adverse effect of synthetic antioxidants on human body, it is logical to use antioxidants obtained from natural sources (Khanahmadi and Janfeshan, 2006).

Reactive oxygen species such as superoxides, hydrogen peroxide and hydroxyl etc are considered to be fundamental causative agent for the occurrence of lesions in stomach and duodenum through oxidative damage. The role of antioxidant against gastric mucosal damage is emphasized in their ability to prevent initiation of lipid peroxidation and by scavenging free radicals. There are also mounting evidences that free radicals are significantly involved in pathogenesis of ulcers (Oluwole *et al.*, 2007). Peptic ulcer is the most common gastrointestinary disorder in clinical practice. For over a century peptic ulcer disease has been one of the leading cause of gastrointestinal surgery, with high mortality and morbidity rates (Gill *et al.*, 2009). It may also be due to some other factors like age, inheritance, cigarette smoking and diet habits (Gill *et al.*, 2011a).Compounds obtained from plants are successfully used in treatment of this ailment without much side effects so natural products and plant extracts are considered as a safer approach for treatment of ulceration (Sood *et al.*, 2010).The development of new antiulcer drug from medicinal plants is an attractive proposition because diverse chemical compounds have been isolated from medicinal plants with antiulcer activity and have been shown to produce promising results in the treatment of gastric ulcers (Parvez *et al.*, 2010). The main objective of the current study was to investigate the antioxidant and antiulcerogenic activity of traditionally consumed *Citrullus colocynthis* methanolic seed extract.

MATERIALS AND METHODS

Plant material: *Citrullus colocynthis* seeds were collected in the month of September, 2010 from local market of Shaheed Bhagat Singh Nagar, (Pb), India. The seeds are authenticated and voucher specimen number no. 0395 has been deposited in Botanical and Environmental Science

Department, Guru Nanak Dev University, Amritsar. For examination only healthy seeds were chosen. The seeds were cleaned, washed, dried and carefully powdered in grinder at room temperature and were kept in tight containers protected from light.

Drugs and chemicals: The drugs and chemicals used were 1,1-diphenyl-2-picrylhydrazyl obtained from Sigma Chemical Co. Methanol, chloroform were purchased from SD Fine, Chem. Ltd, Mumbai. Ranitidine was obtained as free sample from Jackson Laboratories, Amritsar.

Experimental animals: The animal study was carried out during the months of Nov, 2010 to April, 2011. Thirty Wistar albino rats of either sex (Animals were divided into 5 groups each containing 6 animals of average weight 150-180 mg) were purchased from Punjab Agricultural University, Ludhiana. They were acclimated to standard animal house condition such as temperature (24.0±1.0°C), relative humidity 55-65% and 12 h light /12 h dark cycle. They were allowed free access to standard dry pellet diet. The food was withdrawn 18 h before the experiment but allowed free access of water. The experimental protocol was approved by Committee for the Purpose of Control and Supervision of Experiments on Animals.

Extraction: The seeds were dried and powdered. Seeds were then macerated in methanol for a period of 24 h and maceration was repeated for 3 times. The solvent was completely removed by rotary evaporator at 45°C (Zamani *et al*., 2007) and crude extract was obtained. This crude extract was then defatted with hexane in a separating funnel and the remaining extract was concentrated and stored in the refrigerator. This extract was further used for antioxidant and antiulcer activity.

Phytochemical screening: The methanolic seed extract was subjected to phytochemical screening for the presence of flavonoids, alkaloids, carbohydrates, phytosterols, triterpenoids, saponins, tannins according to standard procedure. Alkaloids were tested using various regents such as Dragendroff's reagent, Hager's regent, Mayer's reagent. Sterols were tested by Liebermann's test. Triterpenoids were tested by Libermann-burchard's test. The ferric chloride test was performed for tannins, Molish test for carbohydrates, and Ninhydrin test for proteins. Flavonoids were tested by concentrated nitric acid (Harborne, 1973).

Quantitative evaluation of 1, 1-diphenyl-2-picryl hydrazyl scavenging activity: The antioxidant activity of the methanolic extract was determined on the basis of scavenging activity of the stable 1, 1-diphenyl-2-picryl hydrazyl free radical (Sreejayan and Rao, 1996).

Inhibition of 1,1-diphenyl-2 picryl-hydrazyl radical was calculated using the equation:

$$I(\%) = 100 \times \frac{(A_0 - A_S)}{A_0}$$

Where:

A_0 = Absorbance of the control

A_S = Absorbance of tested sample

Quantitative evaluation of hydrogen peroxide free radical scavenging activity: Antioxidant estimation was carried according to the method of (Gill *et al*., 2011b).

Antiulcer activity

Experimental design for pyloric ligation induced ulcer model: Animals were divided into 5 groups each containing 6 animals.

Group 1: Disease control group subjected to pyloric ligation for the induction of ulcer

Group 2: Administered standard (ranitidine 50 mg kg^{-1}, p.o.) 1 h before pyloric ligation on the day of experiment

Group 3: Administered dose (100 mg kg^{-1}, p.o.) 1 h before pyloric ligation on the day of experiment

Group 4: Administered dose (150 mg kg^{-1}, p.o.) 1 h before pyloric ligation on the day of experiment

Group 5: Administered dose (200 mg kg^{-1}, p.o.) 1 h before pyloric ligation on the day of experiment

Pyloric ligation induced peptic ulcer: The pylorus was ligated according to the method of Shay *et al.* (1945).

Estimation of Gastric Volume and Free and Total Activity Changes in PL Mode.

Four hours after ligation, stomachs were dissected out and contents were collected into measuring cylinder to measure the volume of gastric contents. The gastric contents were centrifuged and subjected to titration for estimation of free and total acidity. 1ml of gastric juice is pipette out in 100 mL conical flask, 2-3 drop of topfer's reagent (Srikanth and Muralidharan, 2009) (Rajkapoor *et al.*, 2002).

Acidity (Meq/l/100 g) can be calculated by using the formula:

$$\text{Acidity} = \frac{\text{Volume of NaoH} \times \text{Normality of NaOH}}{0.1} \times 100 \text{ MEq/l/100 g}$$

where, MEq stands for milliequivalent and it is a unit.

Estimation of gastric ulcerative index changes in PL model: Ulcerative index was measured by method of Takagi *et al.* (1969).

Statistical analysis: All the results were expressed as Mean±Standard Error of Means (SEM). The data was analyzed by one way Analysis of Variance (ANNOVA) followed by turkey multiple range test. The ($p<0.01$) was considered to be statistically significant.

RESULTS

The phytochemical screening of methanolic extract of *Citrullus colocynthis* seeds revealed the presence of carbohydrates, phytosterols, triterpenoids, phenolic compounds and Coumarin glycoside (Table 1). Triterpenoids, Coumarin glycosides and flavonoids have many pharmacological activity including antiulcer and antioxidant activity.

Antioxidant activity by 1, 1-diphenyl-2-picryl hydrazyl free radical and hydrogen peroxide method: 1, 1-diphenyl-2-picryl hydrazyl is one of the free radicals widely used for testing preliminary radical scavenging activity of a compound or an unknown plant extract. In the present

Table 1: Phytochemical Screening of *Citrullus colocynthis* seed extract

Plant constituent/test	Hydroalcohalic extract	Chloroform extract	Methanolic extract
Alkaloids	+	+	+
Carbohydrates	-	+	+
Sterols	-	-	++
Saponins		-	+
Triterpenoids	-	+	+++
Flavonoids	+	+	++
Coumarin glycoside	+	+	++

(-) indicates absence, (+) indicates minor presence and (+++) indicates high presence of corresponding constituent

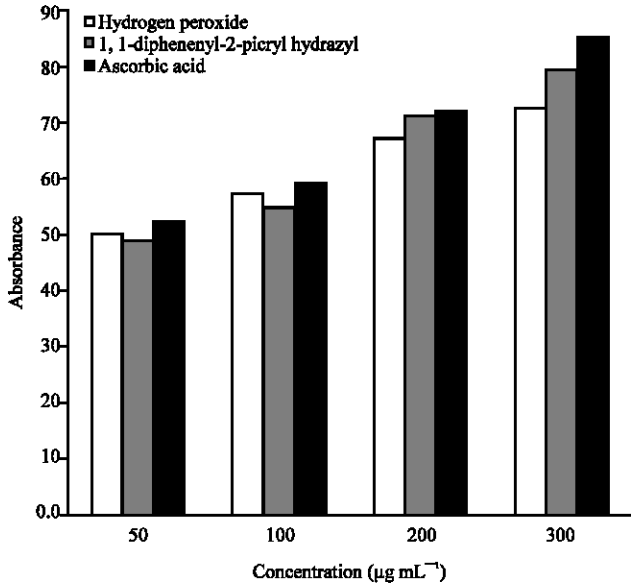

Fig. 1: Comparison of percentage inhibition of *Citrullus colocynthis* methanolic seed extract by 1, 1-diphenyl-2-picryl hydrazyl method and hydrogen peroxide with ascorbic acid

study, methanolic seed extracts of *Citrullus colocynthis* showed potential free-radical scavenging activity. The antioxidant activities of the individual compounds, present in the extracts may depend on structural factors, such as the number of phenolic hydroxyl or methoxyl groups, flavone hydroxyl, keto groups, free carboxylic groups and other structural features (Bhuiyan and Hoque, 2009). The reduction capacity of 1, 1-diphenyl-2-picryl hydrazyl radical was determined by decrease in absorbance at 517 nm induced by antioxidant. Due to hydrogen donating capacity of 1, 1- diphenyl-2-picryl hydrazyl it gets converted into 1,1 diphenyl -2- picryl hydrazine and hence shows decrease in absorbance. The antioxidant activity was also evaluated by hydrogen peroxide method. The % age inhibition was found to be maximum at 300 µg mL^{-1}. The % age inhibition of methanolic seed extract was 79.4 and 72.4% at 300 µg mL^{-1} with 1, 1-diphenyl-2-picryl hydrazyl and Hydrogen peroxide, respectively and is comparable to the standard Ascorbic acid (Fig. 1).

Antiulcer activity by pyloric ligation method: Various mechanisms are responsible for ulcer production in different experimental rat models (Parmar and Desai, 1993; Bose *et al*., 2003). Hence, it is not possible to propose a single mechanism for a particular drug. Digestive effect of the

Table 2: Effect of *Citrullus colocynthis* methanolic seed extract on ulcerative index and percentage inhibition in Pyloric ligated rat models

Groups (mg kg^{-1})	Gastric volume (mL/100 g)	Free acidity (mEq L^{-1})	Total acidity (mEq L^{-1})	Ulcerative index	Inhibtion (%)
Normal	3.67±0.23	53.00±0.35	76.00±0.85	0.0	0.0
PL	5.18±0.45	67.31±1.47	109.18±0.43	5.10±0.56	0.0
Ranitidine (50 mg kg^{-1})	1.13±0.34	21.18±2.77[a]	58.62±1.38[a]	1.10±0.37[a]	78.40±0.15
MECC (100 mg kg^{-1})	2.39±0.23	44.36±3.67[b]	68.45±1.76[b]	1.95±0.18[b]	61.76±0.45
MECC (150 mg kg^{-1})	2.01±0.28	40.92±3.46[b]	64.31±1.65[b]	1.56±0.15[b]	69.41±0.52
MECC (200 mg kg^{-1})	1.68±0.18	39.86±3.86[a]	61.23±1.87[a]	1.45±0.12[a]	71.57±0.12

Effect of methanolic extract of *Citrullus colocynthis* seed on ulcerative parameter in pyloric ligation model. Data are represented as Mean±SEM. Statistical analysis was done by one way ANOVA followed by Turkey test. [a] $p<0.01$ as compared to PL Control group. [b] $p<0.01$ as compared to ranitidine treated group

accumulated gastric juice is beheved to be responsible for the production of ulcers in pyloric ligated rats and also gastric acid secretion, reflex or neurogenic effect may play a important role in formation of ulcers (Goswami *et al.*, 1997). In the pyloric ligation method the animals were pretreated with MECC (Methanolic extract of *Citrullus colocynthis*) at different doses (100, 150, 200 mg kg^{-1}). It has been proposed that the ulcer formation in the pyloric ligation method is developed due to accumulation of gastric acid and pepsin, which lead to auto-digestion of gastric mucosa. The pyloric ligation has caused the accumulation of gastric secretion in the disease control group. The dose of 100,150 and 200 mg kg^{-1} of methanolic seed extract produced a marked reduction in the ulcer index, gastric volume, free acidity and total acidity significantly in comparison with control group. Free and total acidity estimated in control group were 67.31±1.47 and 109.18±0.43, respectively. Treatment with methanolic extract (200 mg kg^{-1}) has reduced the gastric volume (1.68±0.18), free acidity (39.86±3.86), total acidity (61.23±1.87) and ulcerative index (1.45±0.12) which is comparable to ranitidine treated group (Table 2). The results of the present study indicate that the methanolic seed extract (200 mg kg^{-1}) significantly reduces ($p<0.01$) the total volume of gastric juice, free and total acidity of gastric secretion and also has potential against gastric ulcers in rats. The control animals had ulcers and haemorrhagic streaks, whereas the animals administered with methanolic seed extract showed significant reduction in ulcer index.

DISCUSSION

Citrullus colocynthis seeds have been investigated for antioxidant and antiulcerogenic activity in our current study and the extract showed optimum activity. This may be due free radical scavenging property and presence of flavonoids in the extract which may suppress the aggressive factors which may lead to reduction of the gastric ulcers. *Citrullus colocynthis* seeds have been explored for its analgesic, anti-inflammatory activity (Marzouk *et al.*, 2010) and antihyperlipedemic activity (Zamani *et al.*, 2007). The Fruit part and fruit pulp of *Citrullus colocynthis* plant have been explored for antioxidant activity (Kumar *et al.*, 2008; Dallak and Bin-Jaliah, 2010). Aerial parts of *Citrullus colocynthis* showed maximum antioxidant activity at a dose of 500 μg mL^{-1} (Shekhawat *et al.*, 2010). Ethanolic extract of *Lagenaria breviflora* fruit (Onasanwo *et al.*, 2011) and leaves of *Wilbrandia ebracteata* (Gonzalez and Di-Stasi, 2002) showed antiulcer activity at a dose of 150 and 1000 mg kg^{-1}, respectively. The above literature review showed that not much attention has been paid toward the seed part but they are frequently used by our ancestors in traditional system of medicines. Phytochemical screening of methanolic seed extract showed the

presence of alkaloids, steroidal glycosides and flavonoids, based on phytochemical screening the extract has been further evaluated for its antioxidant activity by hydrogen peroxide and 1, 1-diphenyl-2-picryl hydrazyl method. In the presence of an antioxidant which can donate an electron to 1, 1-diphenyl-2-picryl hydrazyl, the purple colour which is typical to free 1, 1-diphenyl-2-picryl hydrazyl radical decays, and the change in absorbance at 517 nm was measured spectrophotometrically. The maximum antioxidant activity observed was 72.4 and 79.4% at 300 μg mL^{-1} by Hydrogen peroxide and 1, 1-diphenyl-2-picryl hydrazyl respectively. As the seed extract showed potent antioxidant activity, so it was further evaluated for antiulcerogenic activity because free radicals play a huge role in the formation of gastric ulceration. The extract showed decline in ulcer score with percentage inhibition of 71.57±0.12% by pyloric ligation induced ulcer method at 200 mg kg^{-1} dose as compared to standard (ranitidine). These results indicate that the extracts of *Citrullus colocynthis* seeds have effective anti-ulcer activity against stomach lesions. From the above discussion it can be inferred that methanolic seed extract of *Citrullus colocynthis* can be used as potent antioxidant and antiulcerogenic agent.

CONCLUSION

From the present investigation it can be concluded that the methanolic seed extract of *Citrullus colocynthis* showed antioxidant activity by free radical scavenging and quenching property due to which it also possessed antiulcerogenic activity and the seeds can be used as potent source of natural antioxidant and antiulcerogenic agent.

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