

Evaluation of Chemical Analysis Profile of *Citrullus colocynthis* Growing in Southern Areas of Khyber Pukhtunkhwa, Pakistan

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Abstract: *Citrullus colocynthis* (L.) Schrad. was analyzed for its chemical composition including bioactive secondary metabolites, dietary vitamins and transition elements. The results confirm the presence of these bioactive chemical constituents comprising flavonoids (1.39mg/100 g), saponins (0.52 mg/100 g), alkaloids (1.64 mg/100 g), phenolic contents (1.22 mg/100g). The medicinal plant contained riboflavin (0.61 mg/100 g), thiamine (0.26 mg/100 g) and ascorbic acid (30.12 mg/100g). The levels of some transition elements determined (Mg, Na, Fe, Cd, Pb, Mn, Cu and Cr) are 33.35, 78.34, 3.88, 0.77, 0.42, 0.069, 5.22 and 3.72 ppm respectively.

Key words: *Citrullus colocynthis* • Cucurbitaceae • Chemical Analysis.

INTRODUCTION

Cucurbitaceae is a largest family containing 120 genera and approximately 825 species [1] typically distributed in the tropical countries poorly represented in temperate regions. In Pakistan it is represented by 17 genera and 32 species [2]. Plants are mostly prostrate or climbing herbaceous annuals characterized by 5-angled stems and coiled tendrils, leaves alternate, palmately 5-lobed or divided; exstipulate and flowers are unisexual rarely bisexual. Cucurbitaceae is important as a source of food like pumpkin (*Cucurbita pepo*), melon (*Cucumis melo*), cucumber (*Cucumis sativus*), water melon (*Citrullus lanatus*), *Lagenaria siceraria* (bottle gourd) and *Luffa cylindrical* (sponge gourd).

Citrullus colocynthis (L.) Schrad. is a medicinal plant belonging to family *Cucurbitaceae*. It is a wide spread annual uncultivated plant, procumbent herb having small flowers with yellow color. The fruit is very bitter. It grows fast in the sandy soils and widespread in different parts of Saudi Arabia. This plant is used as anticancer agent in many drugs. It is also used as antipyometra in animals [3]. As there are reports on phytochemical and biological investigation of this plant.

So the present study was designed to evaluate the secondary constituents, minerals and vitamins of *C. colocynthis* commonly used in herbals.

MATERIALS AND METHODS

Plant Materials: The plant *C. colocynthis* was collected from Distric Karak, Khyber Pukhtunkhwa Pakistan, in June 2010 and was identified by Plant taxonomist. The voucher specimen has been deposited in the herbarium of our botany department Kohat University of Science and Technology Kohat (KUST). The whole plant was air-dried for 15 days and crushed into powder with electrical grinder and finally stored in airtight bottles before analysis.

Flavonoid Determination: 5 g of the plant specie was extracted repeatedly with 150 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper no. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed [4].

Alkaloid Determination: 3 g of the sample was weighed into a 250 ml beaker and 250 ml of 25% acetic acid in ethanol was added and covered to stand for 4 h. This was filtered and the extract was concentrated using a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed [5].

Determination of Total Phenols: For the analysis of the phenolic component, the fat free sample was boiled with 100 ml of ether for 30min. 10ml of the extract was pipette into a 100 ml flask, then 20ml of distilled water was added. 4 ml of ammonium hydroxide solution and 10 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 40 min for colour development. The absorbance of the solution was read using a spectrophotometer at 550 nm wavelengths [5].

Saponin Determination: 5 g of ground plant sample was dispersed in 250 ml of 25% ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 60°C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated thrice. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight. The saponin content was calculated in percentage [5].

Determination of Riboflavin: 10 g of the sample was extracted with 150 ml of 50% ethanol solution and shaken for 1 h. This was filtered into a 250 ml flask and then 15 ml of the extract was pipette into 50 ml volumetric flask. 10 ml of 5% potassium permanganate and 10 ml of 30% H₂O₂ were added and allowed to stand over a hot water bath for about 30 min. 2 ml of 40% sodium sulphate was added. This was made up to 50 ml mark and the absorbance measured at 550 nm in a spectrophotometer [6].

Determination of Thiamin: 10g of the sample was homogenized with ethanolic sodium hydroxide (100 ml). It was filtered into a 250 ml flask. 10 ml of the filtrate was pipette and the colour developed by addition of 10 ml of potassium dichromate and read at 360 nm. A blank sample was prepared and the colour also developed and read at the same wavelength [6].

Determination of Ascorbic Acid (Vitamin c): 10 g of the sample was weighed into an extraction tube and 200 ml of EDTA/TCA (2:1) extracting solution were mixed and the

mixture shaken for 30 min. This was transferred into a centrifuge tube and centrifuged at 2900 rpm for about 25 min. It was transferred into a 250 ml volumetric flask and made up to 100 ml mark with the extracting solution. 20 ml of the extract was pipette into a volumetric flask and 1% starch indicator was added. These were added and titrated with 20% CuSO₄ solution to get a dark end point [7].

Elemental Analysis: The elemental contents such as Mn, Cu, Pb, , Cr, Fe, Cd, Na and Mg of the selected medicinal plant specie were determined using atomic absorption spectrometer. The results were obtained while using a working standard of 1000 ppm for the studied specie.

Statistical Analysis: Each experiment was repeated three times. The results are presented with their means, standard deviation and standard error.

RESULTS AND DISCUSSION

The quantitative determination of secondary constituents of *Citrullus colocynthis* are tabulated in Table 1. Appreciable amount of flavonoids, saponins and alkaloids were detected. The concentration of flavonoide contents in *Citrullus colocynthis* was 1.39 ± 0.8 mg/100 g as compared to saponins whose concentration was 0.52 ± 0.2 mg/100 g. The concentration value of alkaloids in this specie was 1.64 ± 0.02 mg/ 100 g while phenolic contents were 1.22 ± 0.12 mg/100 g respectively.

Results of analysis of *Citrullus colocynthis* showed that the plant is also a rich source of vitamins. Riboflavin was found to be 0.61 ± 0.10 mg/100 g in the studied plant. Similarly thiamine was found to be 0.26 ± 0.18 mg/100 g followed by ascorbic acid having concentration of 30.12 ± 0.22 mg/100 g on dry weight basis (Table 2).

The result of the mineral assessment clearly shows that the medicinal plant is also a rich source of mineral/transition elements. This result become so important when the usefulness of such minerals like Ca, Mg, P, K and Na in the body is considered. However, the lower Na content (0.100g) is an added advantage because of the direct relationship of sodium intake with hypertension in human [8]. The elemental analysis of the medicinal plant specie showed significant concentration of each element in *Citrullus colocynthis*. Higher concentration (78.34 ppm) of Na was detected in *Citrullus colocynthis* followed by Mg (33.35 ppm) and Cu (5.22 ppm) While the analysis in case of Fe and Cr it was highest in Fe (3.88 ppm) followed by Cr (3.72 ppm).

Table 1: Phytochemical composition of the whole plant of *Citrullus colocynthis* expressed as mg/100 g dry weight

Phytochemicals	Concentration mg/100g
Flavonoids	1.39 ± 0.8
Saponins	0.52± 0.2
Alkaloids	1.64 ± 0.02
Phenolic compounds	1.22± 0.12

Results are mean of triplicate determinations on a dry weight basis ±standard deviation

Table 2: Vitamin composition of *Citrullus colocynthis* on a dry weight basis expressed as mg/100 g

Vitamins	Concentration mg/100g
Riboflavin	0.61± 0.10
Thiamine	0.26±0.18
Ascorbic acid	30.12± 0.22

Results are mean of triplicate determinations on a dry weight basis ±standard deviation

Table 3: Elemental composition of *Citrullus colocynthis*

Elemental Analysis (ppm)							
Fe	Cd	Pb	Mn	Cu	Cr	Mg	Na
3.88	0.77	0.42	0.069	5.22	3.72	33.35	78.34

The concentrations of Mn, Pb and Cd were found in trace amounts (Table 3). It has been reported that for many plant species Cr proved to be toxic at 5 mg/L. In this regard, the studied plant has very lesser concentration of Cr as compared to that of recommended level for toxicity in plants [9]. In case of Pb concentration, the suggested concentration in plant species is 2 to 6 mg/L [10], so the analyzed plant specie carries very lesser level of Pb, which further clarifies their use as food supplement or their medicinal benefits.

The secondary constituents of *Citrullus colocynthis* evaluated include the flavonoids, saponins and alkaloids. Flavonoids are the secondary metabolites that exhibit antioxidant activities, including the radical scavenging effects. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process [11]. From these finding *Citrullus colocynthis* may have antioxidant activities due the presence of flavonoids contents. Phenolic compounds are a class of antioxidant agents, which act as free radical terminators [12]. Currently number of synthetic antioxidants available but generally there is still a demand to find more information

concerning the antioxidant potential of the plant specie. Due to the presence of flavonoids which acts as anti-inflammatory, this agreed with the findings of polyphenolic compounds with known properties, which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action [13]. Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity [14]. In particular, despite widespread use of wild plants as medicines in Pakistan, there have been also found the relationship of total flavonoid and phenol contents with antioxidant activity. In the longer term, plant species (or their active constituents) identified as having high levels of antioxidant activity in vitro may be of value in the design of further studies to unravel novel treatment strategies for disorders associated with free radicals induced tissue damage [15].

Saponins are a class of chemical compounds, one of many secondary metabolites found in natural sources, with saponins found in particular abundance in various plant species [16]. There is tremendous, commercially driven promotion of saponins as dietary supplements and nutraceuticals. There is evidence of the presence of saponins in traditional medicine preparations [17].

This plant is good source of vitamins including ascorbic acid, riboflavin and thiamine. The Plant *Citrullus colocynthis* has higher concentration of ascorbic acid. Vitamin C is also a highly effective antioxidant. Even in small amounts vitamin C can protect indispensable molecules in the body, such as proteins, lipids (fats), carbohydrates and nucleic acids (DNA and RNA) from damage by free radicals and reactive oxygen species that can be generated during normal metabolism as well as through exposure to toxins and pollutants (e.g. smoking). Vitamin C may also be able to regenerate other antioxidants such as vitamin E [18]. Severe vitamin C deficiency has been known for many centuries as the potentially fatal disease, scurvy. Scurvy is rare in developed countries because it can be prevented by as little as 10 mg of vitamin C daily [19]. Ascorbic acid is used in herbal medicine for the treatment of common cold and other diseases like prostate cancer [20]. As rich source of phytochemicals, minerals and vitamins the plant can be a potential source of useful drugs.

CONCLUSION

Preservation and use of medicinal plants has taken substantial amount of interest in recent years. It has been used worldwide by the local and marginal communities for

curing various diseases from ancient times. Most of the plant species are also used as food supplement along with its oral decoctions. However, little have been done so far to verify the uses in this regard. The present research is an effort in doing so.

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