

***In vitro* parameters used in evaluation of flavonoids and phenols contents of Citrullus species fruits.**

Harinderjit Singh¹, Hayat M Mukhtar*²

¹I.K.Gujral Punjab Technical University, Jalandhar,144001 Punjab, India

²Amritsar Pharmacy College, Amritsar,143001, Punjab, India

Abstract

The *Citrullus lanatus* is commonly known as watermelon belonging to family *Cucurbitaceae*. Traditionally, the plant has been used as anticancer, antibacterial, demulcent and diuretic. The review of literature available online suggested that geographical variation wise quantitative determination of total phenols and flavonoids in plant material has not been carried out. Thus, it was planned to quantify total phenols and flavonoids content in this plant collected from different geographical places of India such as Rajasthan, Punjab and Haryana. The various extract and fractions were prepared separately using Soxhletion and reflux techniques, respectively, as per standardized procedure. The extracts/fractions were subjected to preliminary phytochemical testes. The various crude extracts/fractions were subjected to quantitative determination of total phenols and flavonoids content using well established procedures known as FolinCiocalteu's assay and aluminium chloride assay, respectively. The ethyl acetate fractions of *Citrullus lanatus* fruits obtained from different geographical sites contained higher content of total phenols and flavonoids than their respective acetone extract and ethanol extract. Amongst various extracts/fractions of tested samples of plant, Rajasthan sample contained higher content of total phenols and flavonoids followed by Punjab and Haryana samples. As preliminary phytochemical screening of ethanol and its ethyl acetate fractions showed presence of phenolic and flavonoids constituents. Thus, it can be also concluded that these compounds may be responsible for scientifically reported pharmacological profile of this plant.

Keywords: Citrullus Species, Phenol, Watermelon.

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Introduction

The *Citrullus lanatus* is commonly known as watermelon belonging to family *Cucurbitaceae*. Traditionally, the plant has been used as anthelmintic, anticancer, antibacterial, demulcent and diuretic. In ancient times, seeds are used for hypotensive activity. Greasy oils in the seeds are used for tapeworms and roundworms infections. In Northern Sudan, it is frequently utilized for swellings, stiffness and gout. *Citrullus lanatus* seed contains phytochemical constituents like alkaloids, flavonoids, tannins, amino acids, sugars, cardio glycosides, terpenoids, steroids, carotenoids, oils and fats [1]. The amino acid citrulline was first extricated from watermelon and broke down. The various pharmacological activities have been scientifically reported from this potential plant such as antibacterial, antifungal [2-3], antioxidant [4], anti-inflammatory activity [5], gastroprotective [6], anti-hepatotoxic [7], anti-atherosclerotic [8] and analgesic [9].

The review of literature available online suggested that geographical variation wise quantitative determination of total phenols and flavonoids in plant material has not been carried out. Thus, it was planned to quantify total phenols and

flavonoids content in this plant collected from different geographical places of the India such as Rajasthan, Punjab and Haryana.

Materials and Methods

Authentication of plant materials

The exhaustive dried fruits of *Citrullus lanatus* for research work were collected from wild regions of different states such as Rajasthan, Punjab and Haryana. The botanical identity of collected dried fruits of *Citrullus lanatus* was confirmed before starting the research work by Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, Punjab, India with registration number of certificate 1165 dated 11/01/2019.

Chemicals, reagents and solvents

The laboratory grade chemicals, reagents and solvents of E Merck, Delhi, India and S.D. Fine Chemicals, Mumbai, India were used in extraction and preliminary phytochemical studies.

The analytical grade chemicals, reagents and solvents of E Merck, Delhi, India and S.D. Fine Chemicals, Mumbai, India were used in spectrophotometric studies.

Preparation of crude extracts and fractions

The various extract such as petroleum ether (defatting purpose), acetone and ethanol extracts of *Citrullus lanatus* fruits collected from different parts of country were prepared separately using Soxhletion technique as per standardized procedure available online. Similarly, the ethyl acetate fraction of each plant sampe was prepared separately from their resptive ethnaol extract using reflux technique as per standardized procedure available online [10]. The various extracts and fractions were subjected to preliminary phytochemical testes for identification of phytochemical nature [11].

Quantitative determination of total phenols and flavonoids content

The various crude extracts such as acetone and ethanol extracts and ethyl acetate fractions obtained from ethanol extract of plant obtained from different regions was subjected to quantitative determination of total phenols and flavonoids content using well established procedures known as Folin Ciocalteu's assay and aluminium chloride assay, respectively [12]. The absorbance of test samples was taken using UV/VIS spectrophotometer (Schimadzu, Japan). The results obtained from the present research work presented in the form of mean \pm standard deviation (S.D). The raw data of observations for each test sample were collected in triplicate.

Results and Discussion

The acetone extract, ethanol extract and ethyl acetate fraction of *Citrullus lanatus* fruits obtained from wild areas of different states Rajasthan, Punjab and Haryana were subjected to estimation of total phenols and flavonoids content using standardized procedures Folin Ciocalteu's assay and aluminium chloride assay, respectively [12]. The results of total phenols and flavonoids content of test samples are depicted in Table 1 and Figures 1 and 2.

The linear standard plot of gallic acid was prepared in the concentration ranges from 30, 50, 70, 90, 110 and 130 mg/ml for estimation of total phenols content and coefficient of regression was found to be 0.9995. The linear standard plot of quercetin was prepared in the concentration ranges from 40, 60, 80, 100, 120 and 140 mg/ml for estimation of total flavonoids content and coefficient of regression was found to be 0.9971.

The ethyl acetate fractions of *Citrullus lanatus* fruits obtained from wild areas of different states Rajasthan, Punjab and Haryana contained higher content of total phenols and flavonoids than their respective acetone extract and ethanol extract. Amongst various extracts and fractions of tested samples of *Citrullus lanatus* fruits, Rajasthan sample contained higher content of total phenols and flavonoids followed by Punjab sample and Haryana sample. As preliminary

phytochemical screening of ethanol and its ethyl acetate fractions showed presence of phenolic and flavonoids constituents. Therefore, ore results are in agreement with preliminary phytochemical screening.

Table 1. Total phenol and flavonoid contents of various extracts of *Citrullus lanatus* fruits.

Plant material	Test sample	Total phenols content	Total flavonoids content
		(% mg GAE/g)	(% mg QE/g)
<i>C. lanatus</i> (Rajasthan)	Acetone extract	3.25 \pm 0.18	1.10 \pm 0.10
	Ethanol extract	15.12 \pm 0.40	4.10 \pm 0.52
	Ethyl acetate fraction	21.25 \pm 0.65	6.44 \pm 0.45
<i>C. lanatus</i> (Punjab)	Acetone extract	2.75 \pm 0.25	0.98 \pm 0.15
	Ethanol extract	14.55 \pm 0.34	3.47 \pm 0.66
	Ethyl acetate fraction	20.22 \pm 0.45	5.88 \pm 0.78
<i>C. lanatus</i> (Haryana)	Acetone extract	2.08 \pm 0.10	0.45 \pm 0.59
	Ethanol extract	14.11 \pm 0.48	3.11 \pm 0.73
	Ethyl acetate fraction	19.58 \pm 0.99	5.09 \pm 0.54

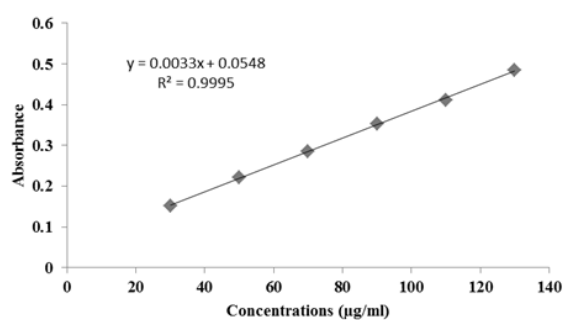


Figure 1. Standard plot between absorbance and gallic acid at different concentration.

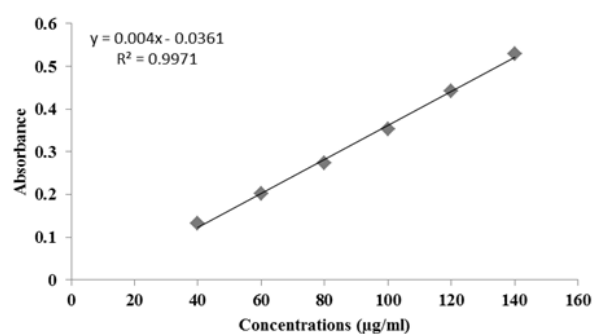


Figure 2. Standard plot between absorbance and quercetin at different concentration.

Phenolic constituents goes about as potential trademark operator for malignant growth anticipation and by and large scattered in plant drugs [13,14]. Flavonoids and other phenolic blends show diverse pharmacological activities, e.g., quieting,

antiatherosclerotic, antitumor, antiviral, antifungal, antimicrobial, cell fortification, hepatoprotective, antiulcer, antidiabetic and cardioprotective [15]. Free radicals show basic occupation in pathogenesis of diabetes and diabetes related bothers [16]. An expansive research work has been finished on polyphenols which show promising antidiabetic properties [17].

Citrullus lanatus is a medicinal plant widely used traditionally in the treatment of various disorders in world. The plant has been extensively studied by various scientist and researchers for its pharmacological activities and therapeutic approaches such as antibacterial, antifungal, antimicrobial, antiulcer, antioxidant, anti-inflammatory, gastroprotective, analgesic, laxative, anti-giardial, hepatoprotective, against prostetic hyperplasia and atherosclerosis [18]. As ethyl acetate fraction of both selected species contained higher content of total phenols and flavonoids, therefore, it can be concluded that most of the traditional activities of both plants are due to phenolic and flavonoid compounds.

Conclusion

The final conclusion of present research work was summarized on the basis of above mentioned results as Rajasthan variety of *Citrullus lanatus* fruits contained higher content of total phenols and flavonoids Punjab sample and Haryana. Thus, it can be also concluded that phenolic and flavonoids may be responsible for scientifically reported pharmacological profile of this plant.

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*Correspondence to

Harinderjit Singh

IK Gujral Punjab Technical University

Jalandhar, 144001

Punjab, India

E-mail: harindersidhu10@gmail.com