



# QUANTITATIVE DETERMINATION OF TOTAL PHENOLS AND FLAVONOIDS CONTENT IN *CITRULLUS LANATUS* FRUITS

Harinderjit Singh<sup>1\*</sup> and Hayat M. Mukhtar<sup>2</sup>

<sup>1</sup>\*Research Scholar, Department of Pharmaceutical Sciences,  
I.K.Gujral Punjab Technical University, Jalandhar (Punjab), India.

<sup>2</sup>Natural Product Laboratory, Department of Pharmacognosy and Phytochemistry,  
Amritsar College of Pharmacy, Amritsar (Punjab), India.

## Abstract

The *Citrullus lanatus* (Thunb) is commonly known as watermelon belonging to family Cucurbitaceae. Traditionally, the plant has been used as anthelmintic, 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 Folin Ciocalteu'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.

**Key words:** *Citrullus lanatus*, Flavonoid, Phenol, Watermelon.

## Introduction

The *Citrullus lanatus* (Thunb) 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 (Varghese *et al.*, 2013). *Citrullus lanatus* seed contains phytochemical constituents like alkaloids, flavonoids, tannins, amino acids, sugars, cardio glycosides, terpenoids, steroids, carotenoids, oils and fats (Varghese *et al.*, 2013). The amino acid citrulline was first extricated from watermelon and broke down. The various pharmacological activities have been scientifically reported fro this potential plant such as antibacterial, antifungal (Braide *et al.*, 2012;

Thirunavukkarasu and Ramanathan, 2010), antioxidant (Rahman *et al.*, 2013), anti-inflammatory activity (Madhavi *et al.*, 2012), gastroprotective (Sharma *et al.*, 2014), anti-hepatotoxic (Altas and Kizil, 2011), anti-atherosclerotic (Poduri and Rateri, 2013) and analgesic (Kumari *et al.*, 2013).

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

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

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

Results are presented as mean ± S.D. of three observations.

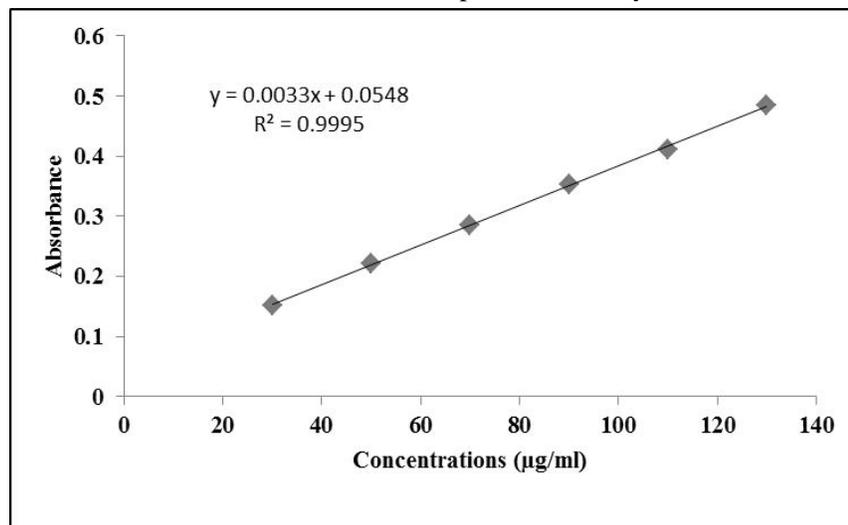
research work were collected from wild regions of different states such as Rajasthan, Punjab and Haryana. The botanical identity of collected dried fruits of *Citrullus lanatus* was confirmed before starting the research work by Department of Botanical and Environmental Sciences, Guru Nanak Dev University, 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

**Fig. 1:** Standard plot between absorbance and gallic acid at different concentration.

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 respective ethanol extract using reflux technique as per standardized procedure available online (Richa *et al.*, 2017). The various extracts and fractions were subjected to preliminary phytochemical testes for identification of phytochemical nature (Farnsworth, 1966).

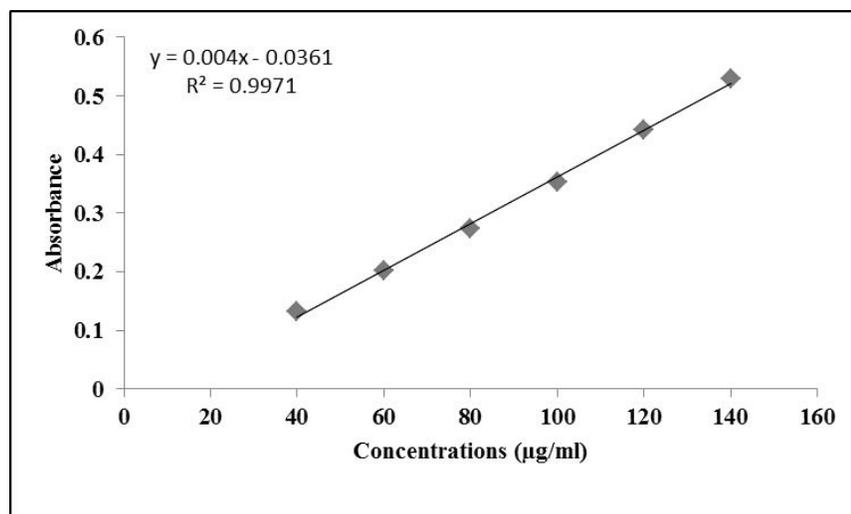
#### Quantitative determination of total phenols and flavonoids content

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, (Kumar *et al.*, 2014). 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 ± 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 (Kumar *et al.*, 2014). The results of total phenols and flavonoids content of test samples are depicted in table 1 & fig. 1, 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.



**Fig. 2:** Standard plot between absorbance and quercetin at different concentration.

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.

Phenolic constituents goes about as potential trademark operator for malignant growth anticipation and by and large scattered in plant drugs (Thaipong *et al.*, 2006; Pourreza, 2013). Flavonoids and other phenolic blends show diverse pharmacological activities, *e.g.*, quieting, antiatherosclerotic, antitumor, antiviral, antifungal, antimicrobial, cell fortification, hepatoprotective, antiulcer, antidiabetic and cardioprotective (Tapas *et al.*, 2008). Free radicals show basic occupation in pathogenesis of diabetes and diabetes related bothers (Oberley, 1988). An expansive research work has been finished on polyphenols which show promising antidiabetic properties (Mohan and Nandhakumar, 2014).

*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, antiangiogenic, hepatoprotective, against prostetic hyperplasia and atherosclerosis (Chinmay *et al.*, 2015). 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.

## Acknowledgement

Authors duly acknowledge Research Scholar, Department of Pharmaceutical Sciences, I.K.Gujral Punjab Technical University Jalandhar, Punjab, India and Amritsar College of Pharmacy, Amritsar, Punjab, India for providing research facilities.

## Reference

- Altas, S. and G. Kizil (2011). Protective effect of Diyarbakır watermelon juice on carbon tetrachloride-induced toxicity in rats. *Food Chem. Toxicol.*, **49**: 2433-8.
- Braide, W., I.J. Odiong and S. Oranusi (2012). Phytochemical and antibacterial properties of the seed of watermelon (*Citrullus Lanatus*). *Prime J. Microbiol. Res.*, **2**: 99-104.
- Chinmay, D.D., J. Anurekha and S.T. Mukul (2015). Phytochemical and pharmacological profile of *Citrullus lanatus* (Thunb). *Biolife.*, **3**: 483-8.
- Farnsworth, N.R. (1966). Biological and phytochemical screening of plants. *J. Pharm. Sci.*, **55**: 225-76.
- Kumar, D., A. Jamwal, R. Madaan and S. Kumar (2014). Estimation of total phenols and flavonoids in selected Indian traditional plants. *J.P.T.R.M.*, **2**: 329-338.
- Kumari, A., J. Rao and J. Kumari (2013). Analgesic activity of aqueous extract of *Citrullus Lanatus* peels. *Adv. Pharmacol. Sci.*, **1**: 135-8.
- Madhavi, P., R. Maruthi and V. Kamala (2012). Evaluation of anti-inflammatory activity of *Citrullus lanatus* seed oil by *in-vivo* and *in-vitro* models. *Int. Res. J. Pharm. Appl. Sci.*, **2**: 104-8.
- Mohan, S. and L. Nandhakumar (2014). Role of various flavonoids: Hypotheses on novel approach to treat diabetes. *J. Med. Hypotheses Ideas.*, **8**: 1-6.
- Oberley, L.W. (1988). Free radicals and diabetes. *Free Radic.*

- Biol. Med.*, **5**: 113-124.
- Poduri, A. and D.L. Rateri (2013). *Citrullus lanatus* 'sentinel' (watermelon) extract reduces atherosclerosis in LDL receptor-deficient mice. *J. Nutrit. Biochem.*, **24**: 882-6.
- Pourreza, N. (2013). Phenolic compounds as potential antioxidant. *Jundishapur J. Nat. Pharm. Prod.*, **8**: 149-150.
- Rahman, H., K. Manjula and T. Anoocha (2013). *In-vitro* antioxidant activity of *Citrullus lanatus* seed extracts. *Asian J. Pharm. Clin. Res.*, **6**: 152-7.
- Richa, D. Kumar and S. Kumar (2017). Screening of antidepressant activity and marker based standardization of *Baptisia tinctoria* (L.) R. Vent. *Indian J. Pharm. Sci.*, **79**: 395-401.
- Sharma, S., V. Dave, S. Paliwal, J. Dwivedi and S. Jain (2014). Gastroprotective activity of reconstituted red fruit pulp concentrate of *Citrullus lanatus* in rats. *An. Sci. Life.*, **34**: 103-8.
- Tapas, A.R., D.M. Sakarkar and R.B. Kakde (2008). Flavonoids as nutraceuticals: A review. *Trop. J. Pharm. Res.*, **7**: 1089-1099.
- Thaipong, K., U. Boonprakob, K. Crosby, L. Cisneros-Zevallos and D. Hawkins Byrne (2006). Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. *J. Food Compos. Anal.*, **19**: 669-675.
- Thirunavukkarasu, P. and T. Ramanathan (2010). Screening of antimicrobial effects in watermelon. *J. Biol. Sci.*, **10**: 682-5.
- Varghese, S., R. Narmadha and D. Gomathi (2013). Phytochemical screening and HPTLC fingerprinting analysis of *Citrullus lanatus* (Thunb.) seed. *J. Acute Dis.*, **5**: 122-6.