ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH



Vol 8, Issue 4, 2015

Research Article

QUALITATIVE PHTOCHEMISTRY PROFILE OF WATERMELON (CITRULLUS VULGARIS SCHRAD) RIND EXTRACTS WITH DIFFERENT SOLVENTS

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Received: 10 February 2015, Revised and Accepted: 13 March 2015

ABSTRACT

Introduction: *Citrullus vulgaris Schrad.* is known to contain bioactive compounds such as cucurbitacin, triterpenes, sterols and alkaloids, vitamins, minerals and has also proved to contain antioxidant and therapeutic properties.

Objectives: The study focus to elucidate on the percentage of extractive yield and to screen the watermelon rind extracts using various solvents and hot aqueous extract for its phytochemical profile.

Methods: The solvents used were methanol, ethanol, chloroform, petroleum ether, ethyl acetate, acetone and hot aqueous extracts. The rind samples were shade dried and powdered. It was then subjected to soxhlet extraction using the different solvents and pressurized hot water extraction. The obtained extracts were concentrated and screened for preliminary phytoconstituents using standard laboratory protocols.

Results: The rind extracts with different solvents and pressurized hot water extract showed different yield percentages and on analysis, hot aqueous extract was found to have the highest extractive yield. The preliminary screening of the rind extracts with different solvents and pressurized hot water extract were tabulated and showed the presence and/or absence of various bioactive constituents including the primary and secondary metabolites.

Conclusion: Thus, the phytochemical constituents such as the primary metabolites and the secondary metabolites and various other bioactive elements that are identifies are thought to be responsible for its antioxidant and therapeutic properties.

Keywords: Citrullus vulgaris Schrad., Metabolites, Phytoconstituents, Soxhlet extraction, Yield percentage, Watermelon rind.

INTRODUCTION

Medicinal plants are the nature's gift to human beings to make disease free healthy life. Over one and a half million traditional healers use a wide range of medicinal plants for treating ailments of both humans and livestock across the length and breadth of the country. In India, different parts of several medicinal plants or their extracts are used for the treatment of various diseases.

In India, with more than 75% of the population residing in rural areas [1] close to the natural resources, rich traditions of utilizing medicinal plants have existed among indigenous peoples for age. The curative properties of medicinal plants are mainly due to the presence of various complex chemical substances of different composition which occur as secondary metabolites [2]. Medicinal plants form a large group of economically important plants that provide the basic raw materials for indigenous pharmaceuticals [3].

The medicinal value of plants lies in some chemical substances that produce a definite physiologic action on the human body. The phytochemical research based on ethnopharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants [4].

Phytochemical which possess many ecological and physiological roles are widely distributed as plant constituents. Woody plants can synthesize and accumulate in their cells, a great variety of phytochemicals including alkaloids, flavonoids, tannins, cyanogenic, glycosides, phenolic compounds, saponins, lignins, and lignans [5]. Phytochemicals exhibit a wide range of biological effects as consequences of their antioxidant properties. Plants contain many bioactive chemical substances that produce definite physiological and

biochemical actions in the human body. These bioactive constituents are alkaloids, tannin, flavonoids, phenolic compounds, etc. [6,7].

Watermelon (*Citrullus vulgaris Schrad.*) is a warm season crop in the Cucurbit family. Watermelon rind is the area of white-colored flesh between the colored flesh (usually red or yellow) and the outer skin. Watermelon is rich in carotenoids some of which include lycopene, phytofluene, phytoene, beta-carotene, and lutein. An average watermelon contains about 30% of rind, 68% of flesh or pulp, and 2% of seeds. The rind is usually discarded, it may be applied to feeds or used as fertilizer; but it is also edible and may be used as a vegetable [8].

The inner portions of the rind which is usually light green or white contains many hidden nutrients and is also edible; however, most times it is avoided due to its unappealing flavor. It contains mainly citrulline which is a known stimulator of nitric oxide [9]. The rind has been shown to contain alkaloids, saponin, cardiac glycosides, flavonoids, phenol, moisture, lipid, protein, fiber, and carbohydrates [10].

Thus, the bioactive and phytochemical constituents present in the watermelon rind may contribute to the medicinal properties exerted by the rind against various disorders.

METHODS

Plant material

The watermelon fruits were purchased from Coimbatore market, Tamil Nadu during its season and the rinds were peeled off and collected. The taxonomic identification of the fruit was done with the help of Dr. V S Ramachandran, Professor, Bharathiar University, Tamil Nadu, India. The collected rinds were cut into smaller pieces and shade dried. The dried

rind sample pieces were powdered using mechanical grinding mortar for effective extraction with solvents.

Extraction

The shade dried powdered rind material was extracted using seven different solvents namely methanol, ethanol, chloroform, petroleum ether, ethyl acetate, acetone, and hot aqueous extract. The extraction using solvents was carried out in soxhlet extractor and hot aqueous extraction in pressurized extractor at the ratio of 10 g rind powder with 100 ml solvent. The extracts were then concentrated to dryness under reduced pressure and controlled temperature (40-50°C) using rotary evaporator. The principle behind the soxhlet extraction was based on the process of evaporation of the solvent with the volatile compounds of the sample and condensation of it back into the apparatus and the pressurized hot extraction was based on the process that heating water at 180°C under pressurized condition becomes supercritical water and act with the property of alcohol (ethanol/methanol) and extraction occurs in the pressurized extractor. The obtained concentrated extracts were then stored and used for the estimation of the extractive yield percentage and screening of phytochemical profile.

Extractive yield

The extractive yield percentage of the sample using different solvents was calculated using the formula,

Extract yield percentage (%) = $(W_1/W_2) \times 100$

Where, $\mathbf{W}_{_1}$ is the weight of the extract in grams and $\mathbf{W}_{_2}$ is the weight of the sample taken for extraction.

Screening for phytoconstituents

Plants are endowed with various phytochemical molecules such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, citrulline, lycopene, and other metabolites, which are rich in antioxidant activity.

The crude extracts obtained were used for the preparation of stock solution at the ratio of 100~mg extract with 10~ml of respective solvents. The stock solutions were then subjected to preliminary phytochemical profiling using the standard laboratory protocols [11-17]. The tests that are performed for each phytoconstituent are represented in Table 1.

RESULTS

The results that are obtained are given in Tables 1 and 2 as follows under each of its respective topics. All the experiments performed were under standard laboratory conditions and with standard protocols.

Extractive yield

The rind extracts using different solvents were subjected to yield percentage analysis was calculated using the formula and listed in Table 2. The solvents used were methanol, ethanol, chloroform, petroleum ether, ethyl acetate, acetone, and hot aqueous extract. The hot aqueous rind extract was found to exhibit the highest extractive yield percentage, among the seven different solvents used. The extractive yield percentage represents the extent of the extraction process to provide a yield rich in almost all of the phytoconstituents that are present in the watermelon rind.

Phytoconstituents analysis

The phytoconstituent analysis of the rind extracts using different solvents revealed the presence of various bioactive constituents like alkaloids, flavonoids, saponins, tannins, phenols, etc. that comes as an evidence for its biological activity as a phytomedicine. These phytoconstituents that are screened becomes a platform for the synthesis of refined chemical structures which are essential for therapeutic purposes.

The presence or absence of the phytoconstituents in the rind extracts with different solvents is listed in Table 1. This may be useful for the

understanding the mechanism behind the physiological response of the body against diseases.

The phytochemicals that are listed in the Table 1, each has its own role to be played in the defense mechanism of the body to scavenge the free radicals that are formed during disease process.

DISCUSSION

The solvent selection analysis can be easily carried out using the method of calculating the extractive yield percentage. This may be useful to provide a higher amount of phytoconstituent extraction and may provide knowledge on the usage of appropriate solvent and thus leading to a good extraction process.

Further, the analysis of phytoconstituents may provide a wide knowledge on bioactive elements detection that could be responsible for the medicinal properties of watermelon rind. This may be further be used for the quantitative estimation of the phytoconstituents identified.

The present study on watermelon rind extracts revealed the presence of the primary metabolites such as carbohydrates, proteins and amino acids, fatty acids and fixed oils, volatile oils, sterols, and steroids in an adequate amount in hot aqueous extract. It also showed the presence of the secondary metabolites like alkaloids, flavonoids, tannins, saponins, phenols, phlobatannins, glycosides, terpenoids, and triterpenoids, etc.

Anthocyanins help the immune system to function more effectively against viral infections [18]. Coumarin is found to be a potential antioxidant that efficiently scavenges the free radicals [19]. Terpenoids and tannins are attributed to analgesic and anti-inflammatory properties. It also contributes to the property of astringency [5]. Saponins are also found to have beneficial health effects [20]. Fixed oils possess antiviral and antibacterial activity [21]. Thus, the presence of almost all of these phytonutrients in watermelon rind aids in therapeutic response against various diseases.

CONCLUSION

Hence, the abundance of phytoconstituents in watermelon rind extracts provides them with an excellent source of antioxidants that has an ability to be used as an indigenous folk medicine by traditional healers. This can further be investigated in a wide scale for the purpose of drug development against various diseases.

Phytoconstituents analysis of the medicinal plants is very important in identifying new sources of therapeutically and industrially important compounds. It is imperative to initiate urgent steps for screening of plants for secondary metabolites.

The qualitative estimation of the analyzed phytoconstituents may focus on further quantitative analysis and finally on the role that they play against any pathological process. And further studies on the isolation and characterization of the bioactive compounds may also lead to interesting research process.

The medicinal properties of watermelon rind may therefore yield to the conclusion that it may be due to the presence of a good amount of various phytoconstituents that are adequate enough to act as antioxidants and scavenge free radicals thereby treating various ailments.

ACKNOWLEDGMENTS

The author wishes to thank and acknowledge University Grants Commission, New Delhi and Ministry of Minority Affairs (MOMA) for providing financial assistance in the form of fellowship under the scheme of Maulana Azad National Fellowship for Minority Students with the Award letter no. F1-17.1/2013-14/MANF-2013-14-CHR-TAM-29154 (SA-III/WEBSITE) dated 06-February-2014.

Table 1: Phytochemical screening of watermelon (C. vulgaris Schrad.) rind extracts

S. no	Parameters	Test	Methanol extract	Ethanol extract	Chloroform extract	Petroleum ether extract	Ethyl acetate extract	Acetone extract	Hot aqueous extract
1	Carbohydrates	Benedict's test	+++	++	+	+	++	+	+++
		Molisch's test	++	+	-	++	+	++	++
2	Protein and amino acids	Biuret	++	+	-	+	-	++	+++
		Ninhydrin	+++	++	-	-	-	+	+++
		Xanthoproteic test	++	++	-	-	+	+	++
3	Fatty acids	Spot test	+++	++	++	-	++	+	+
4	Fixed oils	Spot test	+++	++	++	-	++	+	+
5	Volatile oils	Smell test	++	+++	+++	++	+	+	-
6	Sterols	Salkowski test	+++	+++	+++	+++	+++	++	++
7	Steroids	Liebermann-Burchard test	_	_	++	++	+	+	+
8	Alkaloids	Meyer's test	++	++	-	+	++	++	++
		Hager's test	++	++	++	++	+	+	++
9	Flavonoids	Lead acetate test	++	++	+	-	+	+	+++
		Shinoda test	+++	+	+	+	+	++	++
10	Saponins	Foam test	+	+	-	-	+	_	++
		Froth test	++	++	+	+	++	++	++
11	Tannins	Lead acetate test	++	++	+	-	+	+	+++
		Gelatin test	+	++	-	-	+	_	+++
12	Phenols	Lead acetate test	++	++	+	-	+	+	+++
		Ferric chloride test	+++	++	++	++	+	++	+++
13	Anthraquinone	Borntrager's test	_	-	-	+	-	+	-
		Hydrochloric acid test	+	_	-	-	-	+	++
14	Acids	Sodium bicarbonate test	+	+	-	-	+	_	++
15	Thiols	Sodium nitroprusside test	+	-	+	+	++	+	+
16	Terpenoids	Salkowski test	+++	+++	+++	+++	+++	++	++
17	Triterpenoids	Libermann-Burchard test	_	-	++	++	+	+	+
18	Coumarins	Sodium hydroxide test	++	++	-	-	++	+++	++
19	Resins	Turbidity test	_	+	-	_	-	+	+++
20	Quinones	Sulfuric acid test	+++	+++	-	+	++	++	+++
21	Oxalate	Acetic acid test	_	+++	-		+++	+++	+
22	Anthocyanin	Hydrochloric acid test	+	_	_		-	+	+
23	Anthrax resinoids	Borntrager's test	++	+	-		-	+++	++
24	Emodins	Ammonium hydroxide test	++	_	_		-	_	++
25	Chalcones	Ammonium hydroxide test	++	-	-		-	-	++
26	Anthocyanoside	Sodium hydroxide test	++	++	-		++	+++	+
27	Phlobatannins	Ammonia test	+	-	-		-	+	+
		Hydrochloric acid test	++	-	-		-	-	++
28	Gum and mucilages	Swelling test	+	++	+		++	+	+++
29	Glycosides	Modified borntrager's test	++	++	_		++	+++	++
30	Cardiac glycosides	Keller Killiani test	++	++	_		++	+++	++
		Legal's test	+	_	+		++	+	+

^{+:} Trace, ++: Plenty, +++: Abundance, -: Absence, C. vulgaris: Citrullus vulgaris

Table 2: Extractive yield percentage of *C. vulgaris Schrad.* rind extracts

S. no	Extracts	Yield percentage (w/w)
1	Methanol extract	16.5
2	Ethanol extract	19.5
3	Chloroform extract	2.9
4	Petroleum ether extract	8.1
5	Ethyl acetate extract	7.9
6	Acetone extract	6.1
7	Hot aqueous extract	40.8

C. vulgaris: Citrullus vulgaris

REFERENCES

- Anonymous. Census of India, Primary Census Abstract, Government of India; 1991.
- Karthikeyan A, Shanthi V, Nagasathaya A. Preliminary phytochemical and antibacterial screening of crude extract of the leaf of *Adhatoda Vasica L*. Int J Green Pharm 2009;3(1):78-80.
- 3. Augusti KT. Therapeutic values of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.). Indian J Exp Biol 1996;34(7):634-40.
- 4. Duraipandiyan V, Ayyanar M, Ignacimuthu S. Antimicrobial activity of

- some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Complement Altern Med 2006;6:35.
- Okwu DE, Josiah C. Evaluation of the chemical composition of two Nigerian medicinal plants. Afr J Biotechnol 2006;5(4):357-61.
- Hill AF. Economic Botany. A Textbook of Useful Plants and Plant Products. 2nd ed. New York: McGraw-Hill Book Company Inc.; 1952.
- Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol 2005;4(7):685-8.
- Pons L. Exploring Important Medicinal Uses for Watermelon Rinds. United States Department of Agriculture. Agricultural Research Service. Available from: http://www.ars.usda.gov/is/pr/2003/030221. html. 2003. [Last accessed on 2014 May 14].
- Rimando AM, Perkins-Veazie PM. Determination of citrulline in watermelon rind. J Chromatogr A 2005;1078(1-2):196-200.
- Erukainure OL, Oke OV, Daramola AO, Adenekan SO, Umanhonlen EE. Improvement of the biochemical properties of watermelon rinds subjected to Saccharomyces cerevisae solid media fermentation. Pak J Nutr 2010;9(8):806-9.
- Harborne JB. Phytochemical Methods. London: Chapman and Hall, Ltd.; 1973. p. 49-188.
- Kokate CK. Practical Pharmacognosy. New Delhi: Vallabh Prakashan; 2000. p. 218.
- Gibbs RD. Chemotaxonomy of Flowering Plants. Vol. 1. Montreal and London: McGill Queen's University Press; 1974.
- 14. Ayoola GA, Coker HA, Adesegun SA, Adepoju-Bello AA, Obaweya K,

- Ezennia EC, et al. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in South Western Nigeria. Trop J Pharm Res 2008;7:1019-24.
- 15. Treare GE, Evans WC. Pharmacognosy. 7th ed. London: Bahive Tinal; 1985. p. 149.
- Kumar AR, Ilavarasn T, Jayachandran M, Decaraman P, Aravindhan N, Padmanaban N, Krishnan MR. Phytochemical investigation on a tropical plant. Pak J Nutr 2009;8:83-5.
- 17. Rizk AM. Constituents of plants growing in Qatar. Fitoterapia 1982;52:35-42
- 18. Liu AL, Shu SH, Qin HL, Lee SM, Wang YT, Du GH. In vitro anti-
- influenza viral activities of constituents from Caesalpinia sappan. Planta Med 2009;75(4):337-9.
- 19. Tseng A. Chemoprevention of tumors in MTV-H ras transgenic mice
- with coumarins. Proc Am Assoc Cancer Res 1991;32:2257.
 20. Shi J, Arunasalam K, Yeung D, Kakuda Y, Mittal G, Jiang Y. Saponins from edible legumes: Chemistry, processing, and health benefits. J Med Food 2004;7(1):67-78.
- 21. Al Ashaal HA, Farghaly AA, Abd El Aziz MM, Ali MA. Phytochemical investigation and medicinal evaluation of fixed oil of Balanites aegyptiaca fruits (Balantiaceae). J Ethnopharmacol 2010;127(2):495-501.