IJPSR (2015), Vol. 6, Issue 8 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 23 December, 2014; received in revised form, 11 February, 2015; accepted, 05 May, 2015; published 01 August, 2015

EFFICIENCY OF DIFFERENT SOLVENTS ON PHYTOCHEMISTRY PROFILE OF WATER MELON (*CITRULLUS VULGARIS* SCHRAD.) SEED EXTRACTS

M. Angeline Christie Hannah and S. Krishnakumari *

Department of Biochemistry, Kongunadu arts and science college, Coimbatore, Tamil Nadu, India

Keywords:

Citrullus vulgaris Schrad., Metabolites, Phytochemistry, Soxhlet extraction, Yield percentage, Watermelon seeds

Correspondence to Author:
Dr. S. Krishnakumari

Associate Professor, Department of Biochemistry, Kongunadu Arts and Science College, Coimbatore - 641029, Tamil Nadu, India

E-mail: krishnashanmugambc@gmail.com

ABSTRACT: *Citrullus vulgaris Schrad.* has occupied a popular place in the indigenous system of folk medicine because of its high nutritional and antioxidant properties. The study aims to elucidate the extractive yield percentage and screen the seed extracts of watermelon using different solvents for its phytochemical profile. The solvents selected were Methanol, Ethanol, Chloroform, Petroleum ether, Ethyl acetate, Acetone and Hot aqueous extracts. The shade dried powdered seed material was subjected to Soxhlet extraction using different solvents and Pressurized hot water extraction. The resultant extracts were concentrated and the preliminary screening for phytochemical profiling was carried out using standard laboratory protocols. Analysis of the extractive yield percentages using different solvent extracts and pressurized hot water extract showed hot aqueous seed extract to have the highest yield percentage. The seed extracts with different solvents and pressurized hot water extract were screened and tabulated with the presence and/or absence of various phytochemical constituents such as the primary metabolites and the secondary metabolites and various other bioactive elements which are thought to be responsible for its antioxidant properties.

INTRODUCTION: According to the World Health Organization (WHO), as many as 80% of the world's people depend on traditional medicine for their primary health care needs. The greater part of traditional therapy involves the use of plant extracts or their active principles. The pharmacological treatment of disease began long ago with the use of herbs [1]. Methods of folk healing throughout the world commonly used herbs as part of their tradition. Plants have been major sources of medicine and plant secondary metabolites have been attributed for most plants' therapeutic activities [2-3].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(8).3396-00

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(8).3396-00

Phytomedicines have shown great promise in the treatment of intractable infectious diseases[4]. Traditional medicine practice is an important part of healthcare delivery system in most of the developing world [5] and is a source of primary health care [6]. Traditional medical knowledge of medicinal plants and their use by indigenous culture are not only useful for conservation of cultural traditions and biodiversity but also for community healthcare and drug development now and in the future[7-8].

Phytochemical screening is of paramount importance in identifying new source of compound having medicinal significance, to make the best and judicious use of available natural wealth[9]. Plants continue to serve as possible sources for new drugs and chemicals derived from various parts of plants[10-11]. *Citrallus vulgaris Schrad.* (Water melon) produces a fruit that is about 93% water,

hence the name "water" melon. The seeds are obovate to elliptical, flattened, 0.5–1.5 cm × 0.5–1 cm, smooth, yellow to brown or black, rarely white[12-13]. Every aspect of the fruit of watermelon has nutritional value, including the rind and the seeds. The pulp and seeds are prepared in a number of different ways for eating[14].

The flat brown seeds have a much higher food value than the flesh and have a nice nutty taste. The seeds of watermelon fruit are a good source of various bioactive compounds and its importance being much unaware among the society. The seed is used in the treatment of urinary tract infections, bed wetting, dropsy and renal stones, alcohol poisoning, hypertension, diabetic and diarrhoea[15-16].

Thus the therapeutic activities of the watermelon seeds are due to the presence of the phytochemical and bioactive compounds present which aids them to exert antioxidant properties against various disorders.

MATERIALS AND METHODS:

Plant Material:

The watermelon fruits were purchased from Coimbatore market, Tamilnadu and the seeds from them were collected. The taxonomic identification of the fruit was done with the help of Dr. V.S. Ramachandran, Professor, Bharathiar University, Tamilnadu, India. The seeds collected were washed and shade dried. The dried seed samples were powdered using mechanical grinding mortar for effective extraction with solvents.

Extraction:

The shade dried powdered seed material was extracted using seven different solvents namely Methanol, Ethanol, Chloroform, Petroleum ether, Ethyl acetate, Acetone and Aqueous (hot). The extraction using solvents was carried out in Soxhlet extractor and hot aqueous extraction in pressurized extractor at the ratio of 10g seed powder with 100 ml solvent. The extracts were then concentrated to dryness under reduced pressure and controlled temperature (40 – 50^{o}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^{o}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 seed 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,

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

Where W_1 is the weight of the extract in grams and W_2 is the weight of the sample taken for extraction.

Phytochemical Profiling:

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 topreliminary phytochemical profiling using the standard laboratory protocols[17-23]. The tests that are performed for each phytoconstituent are represented in **Table 2**.

RESULTS: The results that are obtained are given in tables as follows under each of its respective topics. All the experiments performed were under standard laboratory conditions and in a proper way with standard protocols.

Extractive yield:

The extractive yield percentage of the seed extracts with different solvents and hot aqueous extract was calculated using the formula and listed in **Table 1**. The solvents used were Methanol, Ethanol, Chloroform, Petroleum ether, Ethyl acetate, Acetone and Hot aqueous extract. Among the seven different solvents used, the hot aqueous seed

extract was found to exhibit the highest extractive yield percentage. 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 seeds.

TABLE 1: EXTRACTIVE YIELD PERCENTAGE OF *CITRULLUS VULGARIS SCHRAD.* SEED EXTRACTS

S. No	Extracts	Yield Percentage (w/w)
1.	Methanol extract	19.8
2.	Ethanol extract	21.2
3.	Chloroform extract	2.5
4.	Petroleum ether extract	6.8
5.	Ethyl acetate extract	7.2
6.	Acetone extract	5.2
7.	Hot aqueous extract	42.6

Phytochemical Profiling:

The preliminary phytochemical profiling of the seed extracts using different solvents showed 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 phytonutrients that are screened becomes a platform for the synthesis of refined chemical structures which are essential for therapeutic purposes.

Thus **Table 2** lists the presence or absence of the phytoconstituents in the seed extracts with different solvents. This may be useful for the understanding the mechanism behind the physiological response of the body against diseases.

TABLE 2: PHYTOCHEMICAL SCREENING OF WATERMELON (*CITRULLUS VULGARIS SCHRAD.*) SEED 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	Libermann buchard 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	Brontrager's test	–	–	–	+	+	+	–
		Hydrochloric acid test	++	–	+	–	+	–	–
14.	Acids	Sodium bicarbonate test	++	+	+	+	++	++	++
15.	Thiols	Sodium nitoprusside test	+	++	+	+	+	+	++
16.	Terpenoids	Salkowski test	++	+++	+++	+++	++	+++	++
17.	Triterpenoids	Libermann buchard test	+	–	–	–	–	–	+
18.	Coumarins	Sodium hydroxide test	++	–	+	–	+	+	++
19.	Resins	Turbidity test	+++	++	+++	++	+	+	+

20.	Quinones	Sulphuric acid test	+++	++	+++	++	++	++	+++
21.	Oxalate	Acetic acid test	++	_	_	++	_	+	+
22.	Anthocyanin	Hydrochloric acid test	+	_	++	++	_	+	++
23.	Anthraresinoids	Brontrager's test	++	++	++	+	++	++	++
24.	Emodins	Ammonium hydroxide test	++	_	+	_	+	_	_
25.	Chalcones	Ammonium hydroxide test	++	_	+	_	+	_	_
26.	Anthocyanoside	Sodium hydroxide test	+	+	_	+	_	++	+
27.	Phlobtannins	Ammonia test	+	_	_	_	+	+	+
		Hydrochloric acid test	++	_	+	_	+	_	_
28.	Gum and Mucilages	Swelling test	+++	++	+++	+	++	++	+++
29.	Glycosides	Modified brontrager's test	++	_	+	_	+	+	++
30.	Cardiac glycosides	Keller Killiani test	++	_	+	_	+	+	++
		Legal's test	+++	+	++	+++	_	_	+

(+) Low Presence, (++) Medium Presence, (+++) High Presence, (-) Absence

DISCUSSION: The extractive yield percentage may be useful in the analysis of solvent selection that would provide a higher amount of phytoconstituent extraction. This may provide knowledge on the usage of appropriate solvent and thus leading to a good extraction process. Further the phytochemical screening may aid in the detection of the bioactive elements that are responsible for the therapeutic properties of watermelon seeds. This may be then used for the quantitative estimation of the phytonutrients. The phytochemicals 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.

The present study 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, phlobtannins, glycosides, terpenoids and triterpenoids etc.

Anthocyanins help the immune system to function more effectively against viral infections [24]. Coumarin is found to be a potential antioxidant that efficiently scavenges the free radicals [25]. Terpenoids and tannins are attributed to analgesic and anti-inflammatory properties. It also contributes to the property of astringency [26]. Saponins are also found to have beneficial health effects [27]. Fixed oils possess antiviral and antibacterial activity [28]. Thus the presence of almost all of these phytonutrients in watermelon seeds aids in therapeutic response against various diseases.

CONCLUSION: Thus the watermelon seed extracts are found to possess an excellent source of phytonutrients that provides them with 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.

Phytochemical screening of 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 pharmacological properties of watermelon seeds may therefore yield to the conclusion that it may be due to the presence of various phytoconstituents in a good amount that are adequate enough to fight against infection and major ailments. The quantitative estimation of the screened phytochemicals may pay a way for the further analysis of 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.

ACKNOWLEDGEMENT: 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-Feb-2014.

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How to cite this article:
Hannah MAC and Krishnakumari S: Efficiency of Different Solvents on Phytochemistry Profile of Water Melon (*Citrullus Vulgaris* Schrad.) Seed Extracts. Int J Pharm Sci Res 2015; 6(8): 3396-00.doi: 10.13040/IJPSR.0975-8232.6(8).3396-00.