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Quantitative estimation of plant metabolites in the hot aqueous seed extract of watermelon (*Citrullus vulgaris Schrad*.)

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Abstract
Plants are integral part of nature. Nature is a unique source of structures of high phytochemical diversity, many of them possessing interesting biological activities and medicinal properties. The use of traditional medicines is increasing and getting popularity throughout the developed and developing world. The medicinal properties of plants are due to the presence of active principles such as alkaloids, phenolics, tannins and flavonoids which constitute of many pharmacologically active compounds. Quantification of these compounds is very essential for identifying the medicinal properties present in the plants. The current study aims to elucidate the quantification of the primary and secondary metabolites in the aqueous hot extract of *Citrullus vulgaris Schrad*. (Watermelon) seeds. The primary metabolites such as proteins, total soluble carbohydrates, total amino acids and secondary metabolites such as total phenols, alkaloids, flavonoids and tannins were quantified using standard laboratory protocols. The results from the study reveal that the hot aqueous seed extract of *Citrullus vulgaris Schrad* has a good amount of nutritional and therapeutic properties based on the estimated quantitative value of the primary and secondary metabolites. Thus the finding suggests that watermelon seeds could be a potential source of natural antioxidant and can be used in preventing various diseases.

Keywords: *Citrullus vulgaris Schrad*, Phytochemicals, Primary metabolites, Secondary metabolites, Watermelon seeds.

1. Introduction
World plant biodiversity is the largest source of herbal medicine and still about 60 –80 % world population rely on plant based medicines which are being used since the ancient ages as traditional health care system. Plants continue to be used world-wide for the treatment of disease and novel drug entities continue to be developed through research into their constituents. It is now clear that, the medicinal value of these plants lies in the bioactive phytochemical constituents that produce definite physiological effects on human body. These natural compounds formed the base of modern drugs as we use today [1-3].
The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents [4]. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds [5]. These phytochemicals constitute the antibiotic principals of plants [6]. They are found to be distributed in plants [7], leaves, roots, flowers, whole plants, seeds and stems have being examined in many research projects, few reports refers to seeds as sources for pharmaceutical [8]. Chemical compounds including alkaloids, lectins and phenolic compounds such as lactones, tannins and flavonoids are present in watermelon seeds and seed coat [8], and they probably function in the protection of seeds from microbial degradation until conditions are favorable for germination [9, 10].
Plant cells produce two types of metabolites. Metabolites are compounds synthesized by plants for both essential functions, such as growth and development (primary metabolites), and specific functions, such as pollinator attraction or defense against herbivores (secondary metabolites). Primary metabolites are involved directly in growth and metabolism. Secondary metabolites are considered products of primary metabolism and are generally not involved in metabolic activity.

Watermelon (*Citrullus vulgaris Schrad*.) is a type of melon, member of the gourd family, cultivated extensively for its pleasant-tasting fruit, is one of the most economically important fruit in the Cucurbitaceae family [11, 12]. Watermelon grows as a trailing vine. Its original habitat was tropical Africa and today it is cultivated throughout the world [11, 13]. It is a large, sprawling annual plant with coarse, hairy pinnately-lobed leaves and white to yellow flowers.

Scientific Classification

Kingdom : Plantae
Phylum : Embryophyta
Class : Dicotyledoneae
Order : Cucurbitales
Family : Cucurbitaceae
Genus : Citrullus
Species : *C. vulgaris*

The fruit has a smooth hard and thick rind that are green or striped and the watery flesh is usually red in color and contains many dark, flat seeds [11, 12]. Watermelon's Seeds, the dried seeds (dark flat) of the fruit are used as snacks when salted and roasted in China, Israel, etc. In Africa, the seeds are made into coarse flour or oil may be extracted from them and use for domestic consumption. Watermelon was said to possess high level of antioxidants (Phytochemical property) which decreases the risk of kidney stone and bone loss due to old age, and it is a powerful diuretic diet.

Thus the medicinal properties of the watermelon seeds are due to the presence of various primary and secondary metabolites which could act as a natural antioxidant source against various disorders. This study aims to quantify the primary and secondary metabolites present in the hot aqueous watermelon seed extracts

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 of Botany, Bharathiar University, Tamilnadu, India.

Processing of seed samples

The seeds collected were washed and shade dried. The dried seed samples were powdered using mechanical grinding mortar for effective extraction. The shade dried powdered seed material was subjected to pressurized hot aqueous extraction.

Pressurized Hot water extraction

It was carried out in pressurized extractor at the ratio of 10g seed powder with 100 ml distilled water. The extracts were then concentrated to dryness under reduced pressure and controlled temperature (40 – 50 °C) using rotary evaporator. The principle behind 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 seed extracts were then stored and used for the quantitative estimation of primary and secondary metabolites.

Quantification of Primary metabolites

Primary metabolites comprise many different types of organic compounds, including, but not limited to, carbohydrates, lipids, proteins, and nucleic acids. They are found universally in the plant kingdom because they are the components or products of fundamental metabolic pathways Because of the importance of these and other primary pathways in enabling a plant to synthesize, assimilate, and degrade organic compounds, primary metabolites are essential.

Estimation of total proteins

Protein content was estimated by the method of Lowry *et al*. [14]. 1 ml of sample was mixed with 0.5 ml of 0.1 N sodium hydroxide and 5 ml of alkaline copper reagent. The mixture was incubated in room temperature for 30 minutes. Folin–Ciocalteau reagent, 0.5 ml was added and incubated again for 10 minutes at room temperature. The absorbance was read at 660 nm against a reagent blank. The estimation was done in triplicates and the results were expressed mg/g sample.

Estimation of total soluble carbohydrates

The total soluble carbohydrate content was estimated by the method of Hedge and Hofreiter [15]. 1 ml of sample was mixed with 4 ml of anthrone reagent. It was then incubated in boiling water bath for 8 minutes and the absorbance was read at 630 nm against a reagent blank. The estimation was done in triplicates and the results were expressed as mg/g sample.

Estimation of total free amino acids

Total free amino acid (ninhydrin method) was estimated by the method of Moore and Stein [16]. 1 ml of the sample was mixed with 1 ml of Ninhydrin and kept in boiling water bath for 20 minutes. Added 5 ml of diluent (equal volume of water and n-propanol) and incubated at room temperature for 15 minutes. The absorbance was read at 570 nm against a reagent blank. The estimation was done in triplicates and the results were expressed as mg/g sample.

Quantification of Secondary metabolites

Secondary metabolites largely fall into three classes of compounds: alkaloids, terpenoids, and phenolics. The distinction between a primary or secondary metabolite is based not only on its chemical structure but also on its function and distribution within the plant kingdom.

Many thousands of secondary metabolites have been isolated from plants, and many of them have powerful physiological effects in humans and are used as medicines. Research has focused on the role of secondary metabolites in plant defense.

Estimation of flavanoids

Flavanoids was estimated by the method of Jia *et al*. [17] 1 ml of the extract was mixed with 0.075 ml of 5% Sodium nitrite solution and incubated at room temperature for 10 minutes. 10% aluminum chloride was then added and incubated at room temperature for 6 minutes. Then 1 N sodium hydroxide was added. The absorbance was read at 510 nm against a reagent blank. The estimation was done in triplicates and the results were expressed as mg catechin equivalent/g sample.

Estimation of Alkaloids

The estimation of alkaloids was done by method of Harborne [18]. Homogenized 10 mg of plant material in a motor and pestle and 20 ml mixture of methanol: ammonia in the ratio 68:2 was added. The ammoniacal layer was decanted and fresh methanolic ammonia mixture was added after 24 hrs. The procedure was repeated thrice and extracts were pooled. The extracts were evaporated using a flash evaporator. The residue was treated with 1 N HCl and kept overnight. The acidic solution was extracted with 20 ml of chloroform thrice; the organic layers were pooled and evaporated to dryness. Basified the acidic layer with concentrated sodium hydroxide to pH 12

and extracted with 20 ml of chloroform thrice and pooled the chloroform layers. Evaporated to dryness over absorbent cotton. The fraction that contains alkaloids was weighed and expressed as mg/100 g.

Estimation of tannins

Estimation of tannins was done by the method of Bray and Thorpe [19]. 1 ml of the sample was mixed with 5 ml of vanillin hydrochloride reagent and incubated at room temperature for 20 minutes. The absorbance was read at 500 nm against a reagent blank. The estimation was done in triplicates and the results were expressed as catechin equivalents.

Estimation of total phenols

Total phenolic content (Folin - Ciocalteau method) was estimated by the method of Bray and Thorpe [19]. Added 1 ml of sample with 0.5 ml of Folin phenol reagent and incubated at room temperature for 3 minutes. Added 2 ml of 20% Na_2CO_3 after 3 minutes, mixed well and incubated in boiling water bath for 1 minute. Rapidly cooled and the absorbance was read at 650 nm against reagent blank. The estimation was done in triplicates and the results were expressed as mg/g sample.

Statistical analysis

All the estimations were done in triplicates and the results were analyzed statistically. It was expressed as mean (n=3) ± standard deviation.

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 with standard protocols.

Quantitative estimation of Primary metabolites:

Primary metabolites have a key role in survive of the species, playing an active function in the photosynthesis and respiration. Quantitative analysis of primary metabolites in the hot aqueous seed extract of watermelon from Table 1. Shows that carbohydrate content was found high (2.00±0.09 mg/g) followed by amino acid (1.74±0.37 mg/g) and then protein (1.66±0.24 mg/g). The quantification of the primary metabolites in the hot aqueous seed extract of watermelon was carried out and tabulated in Table.1.

Table 1: Quantification of primary metabolites in *Citrullus vulgaris Schrad.* seed extracts

Primary Metabolites	Estimated Quantity (mg/g)
Total Proteins	1.66±0.24
Total soluble Carbohydrates	2.00±0.09
Total free Amino acids	1.74±0.37

Values are expressed by mean ± SD of three samples

Quantitative estimation of Secondary metabolites

Secondary metabolites are organic molecules that are not involved in the normal growth and development. Quantitative analysis of secondary metabolites in the hot aqueous seed extract of watermelon from Table 2. Shows that total phenols content was found high (1.28±0.19 mg/g) followed by flavonoids (1.18±0.21 mg/g) and then alkaloids (0.82±0.02 mg/g) followed by tannins (0.76±0.23 mg/g). The quantification of the secondary metabolites in the hot aqueous seed extract of watermelon was carried out and tabulated in Table 2.

Table 2: Quantification of secondary metabolites in *Citrullus vulgaris Schrad.* seed extracts

Secondary Metabolites	Estimated Quantity (mg/g)
Flavonoids	1.18±0.21
Alkaloids	0.82±0.02
Tannins	0.76±0.23
Total Phenols	1.28±0.19

Values are expressed by mean ± SD of three samples

Discussion

The quantitative estimation of the primary and secondary metabolites may be useful in the analysis of the compounds that would be essential for the growth and development of the plant cell and also the nutritive and therapeutic properties present in the watermelon seed.

The current study provides the estimated amounts of the primary metabolites (total proteins, total soluble carbohydrates and total free amino acids) and secondary metabolites (alkaloids, flavonoids, tannins, total phenols) present in the hot aqueous seed extract of watermelon. This may provide knowledge on the growth of the seeds in watermelon and its biological activities. Further the quantitative phytochemical screening may aid in the detection of the bioactive elements that are responsible for the therapeutic properties of watermelon seeds.

Phytochemicals accumulate in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds [20]. Proteins are the primary components of living things. The presence of higher protein level in the plant points towards their possible increase food value or that a protein base bioactive compound could also be isolated in future [21]. Plant sugars can be used as artificial sweetener and they can even help in diabetes by supporting the body in its rebuilding [22].

Phenols are plant secondary metabolites, and they have an important role as defence compounds. Phenolics exhibit several properties beneficial to humans and its antioxidant properties. Flavonoids are the largest group of plant phenols and the most studied [23]. Flavonoids have been reported to exert multiple biological property including antimicrobial, cytotoxicity, anti-inflammatory, antibacterial, antiviral, anti-allergic [24-26], antitumor and cytotoxic, gastroprotective, treatment of neurodegenerative diseases, vasodilatory action [27-30].

Tannin contributes various medicinal properties such as antimicrobial, anti-inflammatory and astringent activity. They have been also reported to have anti-viral [31], antibacterial [32, 33] and anti-parasitic effects. In medicine, especially in Asian (Japanese and Chinese) natural healing, the tannin-containing plant extracts are used as astringents, against diarrhoea, as diuretics and against stomach and duodenal tumors [34]. Alkaloids protect against chronic diseases. Alkaloids are naturally synthesis by a large numbers of organisms, including animals, plants, bacteria and fungi. Alkaloids have many pharmacological activities including antihypertensive effects, antiarrhythmic effect [35] and antimalarial and antimicrobial activity by inhibiting DNA topoisomerase.

Conclusion

Thus the watermelon seed extracts are found to possess an excellent source of basic primary and secondary metabolites 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.

Quantitative phytochemical estimation of medicinal plants is very important in identifying new sources of therapeutically

and industrially important compounds. The pharmacological properties of watermelon seeds may therefore yield to the conclusion that it may be due to the presence of various primary and secondary metabolites in a good amount that are adequate enough to fight against infection and major ailments. Phytochemicals are not essential nutrients and are not required by the human body for sustaining life, but have important properties to prevent or to fight some common diseases.

The quantitative estimation of the screened phytochemicals may pave 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.

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