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Profiling of Lipid and Vitamin contents in the extract of Watermelon (*Citrullus vulgaris Schrad.*) seeds

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Abstract

There is a growing interest all over the world for discovering the untapped reservoir of medicinal plants. Many plants have been known to produce biologically active substances, some of which are related to special flavor or taste and others are found to be useful as antioxidants and/or antimicrobial agents. The therapeutic effects of several medicinal plants are usually attributed to their antioxidant phytochemicals. The current study investigates the lipid and vitamin profiling in the aqueous hot extract of watermelon (*Citrullus vulgaris Schrad.*) seeds. The lipid profile includes parameters such as total cholesterol, triglycerides, free fatty acids and phospholipids. The vitamin profile includes parameters such as Vitamin A, E, C, β carotene and Thiamine. Other than these parameters the essential oil content was also investigated to extend its role played in aiding therapeutic properties. All the parameters were quantified using standard laboratory protocols. The study results suggest the efficacy of the hot aqueous seed extract of *Citrullus vulgaris Schrad.* To be used as a good nutritional source for vitamin deficiency and also for prevention and treatment of various other disease conditions.

Keywords: Reservoir, Antioxidant, Vitamins, Lipid, Essential oil, Watermelon seeds

1. Introduction

The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants [1]. According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. However, such plants should be investigated to better understand their properties, safety, and efficiency [2]. Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, seeds [3]. Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances [4-6].

According to a survey, 75-80% of the world's population relies over such plants as they are famous for healing several diseases and are considered as a healthy source for life [7-19]. Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity. The search for eternal health and longevity and for remedies to relieve pain and discomfort drove early man to explore his immediate natural surroundings and led to the use of many plants, animal product and minerals, etc. and the development of variety of therapeutic agents. Lipids are organic compounds that include fats, waxes, phospholipids, glycolipids and sterols. All of them are present in almost every living cell. Lipids are insoluble in water but soluble in organic solvents like alcohol and chloroform [20].

Plants synthesize a wide range of hydrophobic compounds, generally known as lipids. Glycolipids in higher plants mainly consist of steryl glucosides, sphingo-glycolipids, and glycero-glycolipids [21]. These glycolipids are widely distributed in edible plants such as cereals, legumes, vegetables, and fruits. The higher amount of plant lipid can be used as essential oils, spice, oleoresins and natural food colors. Plant lipids have developed products that work with diverse requirements, as culinary, medicinal and cosmetics. In this investigation the bioavailability of bioactive lipids and its constituents from selected botanicals have been made to achieve nutrient adequacy and to prevent and treat different diseases as medicaments and food supplements. For this purpose valued natural medicinal plants were explored for the screening of natural lipids in the Phytotaxa as suggested by Sreelathadevi [22].

Essential oils, likewise other secondary biologically active substances, are characteristic only for some smaller or larger systematic groups of plants: families, genera, and even species. Vitamins are organic compounds required as vital nutrients in tiny amounts by an organism. They generally cannot be synthesized by mammalian cells and, therefore, must be supplied in

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the diet. An organic chemical compound is called a vitamin when the organism cannot synthesize the compound in sufficient quantities, and must be obtained through the diet. Essentially important, traditional medicine has not only survived, but thrived in the transcultural environment and intermixture of many ethnic traditions and beliefs despite the 'aging' or 'vanishing' of folk phytotherapy in the sense that the wealth of knowledge of medicinal plants resides mostly in elderly rural people with modest tuition [23]. Since very old times, herbal medications have been used for relief of symptoms of disease [24]. Despite the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care.

Watermelon (*Citrullus vulgaris Schrad.*) is a type of melon and one of the most economically important fruit in the Cucurbitaceae family [25, 26]. It is cultivated extensively for its pleasant-tasting fruit and grows as a trailing vine. Its original habitat was tropical Africa and today it is cultivated throughout the world [25, 27].

Scientific Classification

Kingdom	:	Plantae
Phylum	:	Embryophyta
Class	:	Dicotyledoneae
Order	:	Cucurbitales
Family	:	Cucurbitaceae
Genus	:	<i>Citrullus</i>
Species	:	<i>C. vulgaris</i>



Watermelon's Seeds, the dried seeds (dark flat) of the fruit are used as snacks when salted and roasted. 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.

Hence various bioactive compounds present in watermelon seeds could aid in its medicinal properties to act as a natural antioxidant source against various disorders. This current study investigates to determine the lipid and vitamin profile in the hot aqueous watermelon seed extracts.

2. Materials and Methods

Collection and Processing of Sample:

The watermelon fruits were purchased during the month of April to June 2014 from Coimbatore market, Tamilnadu and the seeds from them were collected. The seed samples were washed and shade dried at room temperature. 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.

Sample preparation using 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 obtained concentrated seed extracts were then stored and used for the profiling of lipid and vitamin contents.

Lipid profiling

Lipids are essential to life and that they are essential fats that play a very important role in the human body. Lipid profiling is an emerging strategy that needs to be extended so that routine lipid profiling in plants will cover usual essential additional lipids. All the estimations were carried out in triplicates and results are expressed as mg/g sample.

Free fatty acids

Free fatty acids in the seed sample were estimated using the method of Horn and Mehanan [28]. Taken 0.2 ml lipid extract of the seed sample and 6.0 ml of Chloroform-Heptane-Methanol (CHM) mixture and 200 mg of activated silicic acid were added, mixed well, centrifuged and supernatant collected. Standards and blank were also made to 6.0 ml with CHM mixture. To all these tubes, 2.0 ml of copper nitrate - TEA solution was added and mixed for 20 min. Centrifuged and transferred 2.0 ml of upper phase to another tube. 1.0 ml of the color reagent (Diphenyl carbazide) was added, shaken well and color developed was read at 430 nm against a reagent blank.

Phospholipids

Estimation of Phospholipids was done by the method of Rousser *et al.* [29]. To 0.1 ml lipid extract of the seed sample, 1.0 ml of perchloric acid was added and digested on a sand bath until it becomes colorless. Standards in the range 5-20 µg were also taken and 0.8 ml of perchloric acid was added. All the tubes were made up to 5.0 ml with water. 0.5 ml of ammonium molybdate was added followed by 0.5 ml of ascorbic acid solution and mixed well. Heated in boiling water bath for 6 min and the color developed were read immediately at 710 nm. Phosphorus content was multiplied by a factor 25, which gave the weight of phospholipids.

Total Cholesterol

Estimation of total cholesterol content was done using the method of Parekh and Jung [30]. 0.1 ml of dried lipid extract seed sample and standards (40-200 µg) were taken in series of test tubes. 3.0 ml of Ferric chloride - uranyl acetate reagent was added to the test tubes. Added 2.0 ml of the acid reagent

by keeping tubes in ice bath and the absorbance was read at 540 nm after 15 minutes.

Triglycerides

Triglycerides estimation was done by the method of Rice [31]. Taken 0.1 ml of the dried lipid extract of the seed sample and made up with 4.0 ml of isopropanol. Mixed well and added 400 mg of silicic acid. Placed in mechanical shaker and centrifuged. To 2.0 ml supernatant, added 0.6 ml of saponification reagent and incubated for 15 min. After cooling, added 1.0 ml of sodium metaperiodate and 0.5 ml acetyl acetone reagent, mixed well and incubated at 50 °C for 30 min. Absorbance was read at 405 nm. Standards tripalmitin (20-100 µg) was also treated similarly.

Extraction and Estimation of Essential oil

Weighed 50 g seed sample and grounded well. The seed meal was kept inside thimble. The thimble was placed inside soxhlet extraction apparatus. The sample was extracted with petroleum ether for 6 hours without interruption by gentle heating. Allowed to cool and the extraction flask was dismantled. The ether was evaporated and flask was weighed. The essential oil was estimated using the formula

$$\text{Oil in sample (\%)} = [\text{Weight of oil (g)} / \text{weight of sample (g)}] \times 100$$

Vitamin profiling

Vitamins serve as biocatalysts in many chemical reactions and precursors to various body factors and are required for a variety of biological processes. The vitamins that were being profiled are Vitamin A, β carotene, Vitamin B₁ (Thiamine), Vitamin C and Vitamin E. All the estimations were carried out in triplicates and results are expressed as mg/g sample.

Vitamin A estimation

Estimation of Vitamin A in the seed sample was estimated by the method of Nedd and Pearson [32]. 0.5 ml of the sample extract was mixed with 0.5 ml of chloroform and 2.0 ml of Trifluoro acetate (TFA) reagent. Absorbance was determined at 600 nm.

β carotene estimation

β carotene was estimated by the method of Nadd and Pearson [32]. Taken 1.0 ml of sample and added 1.0 ml of saponification mixture (2N KOH in 90% alcohol), heated at 60°C for 20 min. Added 25.0 ml of distilled water after cooling and transferred to a separating funnel. Extracted thrice using 25, 15, 10 ml of petroleum ether (40 °C - 50 °C). Pooled and washed with 50 to 100 ml of distilled water. Repeat until it is free of alkali. The extract was dried by adding anhydrous sodium sulphate. The volume was noted and 3.0 ml of petroleum ether phase was transferred to a cuvette and read at 420 nm against the reagent blank.

Vitamin B₁ (Thiamine) estimation

Estimation of Thiamine was estimated by the method of Sadasivam and Manickam [33]. Weighed 5.0 g of seed sample in a conical flask and added 100 ml of 0.1N H₂SO₄ solwly. Allowed to stand overnight and shaken vigorously the next

day and filtered. Discarded 10 ml of filtrate and pipetted out 10 ml extract in 100 ml separating funnel. Pipette out 10 ml of working standard. Added 3.0 ml of 15% NaOH to each funnel separately and 4 drops of ferricyande solution and was shaken for 30 sec. Added 15 ml iso-buatnol, shook vigorously for 60 sec and allowed the layers to separate and discarded the bottom layer. Added one spatula of sodium sulphate, swirled gently, clarified and collected clean extract into test tubes. Prepared set of sample blank and 10 ml of extracts, above steps were followed omitting the ferri cyanide addition. The absorbance was read at 366 nm and thiamine content was calculated using the formula.

$$\text{Thiamine } (\mu\text{g}/100 \text{ g}) = [(0.25 \times 10)/ a-a^1] \times [(b-b^1) \times 100/ 10] \times [10/5]$$

Where a - reading of standard; a¹ - reading of standard blank
b - reading of sample; b¹ - reading of sample blank

Vitamin C estimation

Vitamin C in the seed sample was estimated using the method of Omaye *et al.* [34]. 0.5 ml of the sample extract was mixed with 0.5 ml of distilled water and 0.2 ml of DTCS reagent. It was incubated at 37 °C for 3 hours. 1.5 ml of ice-cold 65% sulphuric acid was added mixed well and the solutions were allowed to stand at 37 °C for 30 min. Absorbance was determined at 520 nm.

Vitamin E estimation

Estimation of Vitamin E was carried out by the method of Varley *et al.* [35]. 1.5 ml of sample extract (test), 1.5 ml of the standard and 1.5 ml of water (blank) were taken respectively into stopped centrifuge tubes. To the test and blank added 1.5 ml of ethanol and to the standard added 1.5 ml of water. Added 1.5 ml of xylene to all the tubes mixed well and centrifuged. Transferred 1.0 ml of xylene layer and 1.0 ml of 2, 2'- dipyridyl reagent was added to each tube. Mixed and pipetted out 1.5 ml of the mixture into spectrophotometer cuvettes and absorbance of test and standard was read against the blank at 460 nm. Then added 0.33 ml of ferric chloride solution, mixed well and after exactly 15 min test and standard OD were read against the blank at 520 nm. The amount of vitamin E can be calculated using the formula.

$$\text{Vitamin E } (\mu\text{g/g}) = \frac{(\Delta A_{520\text{nm}} - \Delta A_{460\text{nm}}) \times \text{conc } [S] \times 0.29 \times \text{Total volume}}{\Delta A_{520\text{nm}} \times \text{Vol for experiment} \times \text{Weight of sample}}$$

Statistical analysis

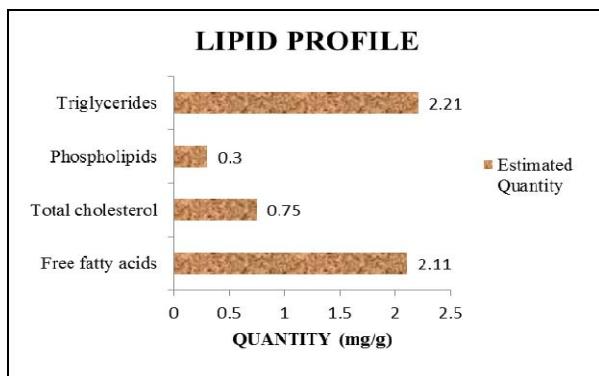
All the estimations were done in triplicates and the results were analyzed statistically. It was expressed as mean (n=3) ± standard deviation.

3. Results

The results that are obtained are given in tables and/or figures as follows under each of its respective topics. All the experiments performed were under standard laboratory conditions with standard protocols.

Estimation of Lipid profile

The lipid profiling in the hot aqueous seed extract of watermelon was carried out and depicted in Fig.1. Quantitative analysis of lipid contents in the hot aqueous seed extract of watermelon from Fig 1. Shows that Triglycerides content was found high (2.21 ± 0.27 mg/g) followed by free fatty acids (2.11 ± 0.24 mg/g) and then total cholesterol (0.75 ± 0.39 mg/g) and phospholipids (0.30 ± 0.15 mg/g).



Values are expressed by mean \pm SD of three samples

Fig 1: Lipid Profile of *Citrullus vulgaris Schrad.* seed extracts

Estimation of Oil and Vitamin contents

The essential oil and vitamin contents in the hot aqueous seed extract of watermelon were profiled and tabulated in Table 2 and 3. The term vitamin is conditional upon the circumstances and the particular organism. Vitamins have diverse biochemical functions. The activity of the compounds contained in the volatile aromatic mixture called the essential oil has the greatest importance.

Essential oil content in watermelon seeds from Table 1 was found to be $40.30 \pm 0.16\%$. Vitamins estimation in the hot aqueous seed extract of watermelon from Table 2. Shows that Vitamin E content was found high (4.06 ± 0.12 mg/g) followed by Vitamin C (0.34 ± 0.08 mg/g) and then Vitamin A (0.30 ± 0.15 mg/g) followed by β carotene (0.19 ± 0.13 mg/g) and Thiamine (0.11 ± 0.08 mg/g).

Table 2: Essential oil in *Citrullus vulgaris Schrad.* seeds

Parameter	Estimated Quantity (%)
Essential oil	40.30 ± 0.16

Table 3: Vitamin contents in *Citrullus vulgaris Schrad.* seed extracts

Vitamins	Estimated Quantity (mg/g)
Vitamin A	0.30 ± 0.15
β carotene	0.15 ± 0.13
Vitamin B ₁	0.05 ± 0.08
Vitamin C	0.34 ± 0.08
Vitamin E	4.06 ± 0.12

Values are expressed by mean \pm SD of three samples

4. Discussion

Large numbers of plant are constantly being screened for their chemical and pharmacological properties [36]. The therapeutic effects of several medicinal plants are usually attributed to their antioxidant phytochemicals. It has been suggested that there is an inverse relationship between dietary intake of antioxidant rich food and incidence of human disease [37].

The present study investigates the estimated amounts of lipid profile (Free fatty acids, Total cholesterol, Phospholipids and Triglycerides) and vitamin contents (Vitamin A, β carotene, Vitamin B₁ (Thiamine), Vitamin C and Vitamin E) present in the hot aqueous watermelon seed extract. This may give an idea on watermelon seeds which can be used as natural diet source and explains their therapeutic properties by contribution of their role in healing the deficiency disorders of vitamins.

Lipids are the major form of carbon storage in the seeds of many plant species. Lipids are the most effective source of storage energy, function as insulators of delicate internal organs and hormones and play an important role as the structural constituents of most of the cellular membranes. Fatty acids are important for thermal and electrical insulation, and for mechanical protection. Triglycerides acts as energy reserve when stored as adipose tissue also acts as insulator, shock protection. Phospholipids have an essential general role of maintaining cell order and integrity [38]. Essential oils are the main active components of many essential oil raw materials. This is the most numerous group of medicinal raw materials, which has a big tradition and still a wide application in therapeutics. Oil raw materials are obtained from natural stands and from crops.

Vitamin A is a micronutrient essential to most mammalian species. Vitamin A is necessary in vision, growth and development [39], gene transcription [40, 41], immunity, dermatology [42] etc. Carotenoids possess a range of important and well documented biological activities, and they are also reported to be potent antioxidant and scavengers of free radicals [43] and singlet oxygen [44]. β carotene and carotenoids in general are isoprenoid compounds which are not synthesized in animals but biosynthesized by plants and micro-organisms. Some dietary carotenoids, such as β carotene, serve as an important source of vitamin A, which is the major known function of carotenoids in humans.

Vitamin B has complex of vitamins in which Vitamin B₁ (Thiamine) because of its high oxidative metabolism, thiamine deficiency particularly targets the nervous system and the heart [45]. Vitamins B₃ such as niacin are required for biological processes such as mental alertness and Vitamin C for resistance to infections. Vitamin C (Ascorbic acid) is a vitamin for humans, but not for most other animal organisms. They also function as antioxidants (e.g., vitamin E and sometimes vitamin C). Vitamins have diverse biochemical functions. Some, such as vitamin D and some forms of vitamin A, have hormone-like functions as regulators of mineral metabolism, or regulators of cell and tissue growth and differentiation.

5. Conclusion

Profiling of lipid and vitamin contents of medicinal plants is very important in identifying the underlying therapeutic and nutritional sources of compounds in watermelon seeds. The nutritional properties of watermelon seeds gives a conclusion that it may be due to the presence of good amount of lipid and vitamin content that are adequate enough to fight against deficiency disorders, infection and other ailments

Thus the watermelon seed extracts becomes a natural diet source of antioxidants and are found to provide them with an ability to be used as an indigenous folk medicine for minor ailments and deficiencies by traditional and modern healers.

This can further be investigated in a wide scale for the estimation of other nutritionally important compounds which helps in the drug industry as nutraceuticals.

The quantitative estimation of the lipid and vitamin profiles may pave a way for the further analysis of other essentially available compounds to improve the health and physiology of the human life. It can also be a platform for prevention and treatment against malnutrition and other deficiency symptoms. In depth analysis of these nutritionally important compounds help the pharmaceutical industries to form natural therapy for curing diseases.

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