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Phytochemical, Minerals and Physicochemical Properties of Watermelon Seed Oil

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Abstract:-Watermelon seed oil was extracted using the solvent extraction method using n-hexane. Citrullus lanatus well known fruit as watermelon of family Cucurbitaceae. The fruit is consumed for cooling effect. Moreover, so many literatures suggest that it contain important phytoconstituents like vitamin C, minerals etc. Seeds contain fatty acids and have phenolic and triterpinoids. The seeds extract showed so many pharmacological activities. Phytochemical screening of the seed extracts of Citrullus lanatus indicated the presence of Alkaloids, Saponins, Tannin, Phenol, Terpenoid, Amino acids in ethanol extracts. The physicochemical properties of the oil were determined. The following parameters are Oil yield, Acid value, free fatty acid, saponification value, density, specific gravity, iodine, peroxide, refractive index, viscosity value. The seed oil of Watermelon showed good physicochemical properties and could be utilized successfully as a source of edible oil for human consumption and for industrial applications.

Keywords:-Watermelon seed oil, Solvent extraction, n-hexane, ethanol.

I. INTRODUCTION

The term "vegetable" refers to all the soft, edible plant products that are usually eaten with meat, fish, or other savoury dish, either fresh or cooked Desai BB *et al.* Watermelon (*Citrullus lanatus*) is of the Cucurbitaceae family. As a member of the Cucurbitaceae, watermelon is related to the cantaloupe, squash and pumpkin and other plants that grows on vines on the ground. Fruit of Citrullus lanatus L consumed in the summer season and it gives chilling effect and reduce thirst. Watermelon plant is a spreading, hairy, tendril-bearing annual vine reaching a length of several meters. Leaves are long-stalked, oblong-ovate, 8 to 20 centimeter's long, deeply 3 to 7 lobed, with usually narrowed segments. Watermelon leaves are dark green with prominent veins. The plant is self-fertile. [1, 2] Watermelon is a good source of carotenoid and lycopene. Lycopene has been found to be protective against a growing list of cancer [3].Watermelon is also expectedly high in citrulline; an amino acid the body make use of to make another amino acid, arginine (used in the urea cycle to remove ammoniacal from the body) [4]. Watermelon is delectable, thirst-quencher which helps quench the inflammable that contributes to conditions like asthma, atherosclerosis, diabetes, colon cancer and arthritis [5]. Cucurbit seeds are source of food particularly protein and oil [6]. Watermelon fruit contained many smooth compressed seeds that thickened at the margin and of black or yellow-white colour [7].Achu, *et al*., [8] reported high lipid level in five Cucurbitaceae oil- seeds from different regions in Cameroon. Oil provides concentrated energy in diet and enhanced palatability.Njoroge G.N., Gemmill B., Bussmann R., Newton L.E., Ngumi V.W., (2007), Pollination ecology of Citrullus lanatus at Yatta, Kenya. International Journal of Tropical Insect Science, ICIPE, 24, 73-77.

II. MATERIAL AND METHODS

A. Oil Extraction

The Soxhlet apparatus used for solvent extraction where 300ml of n- Hexane was poured into round bottom flask.10 grams of powdered *Watermelon seed* was placed in the thimble and inserted in the centre of the extractor. The Soxhlet was heated at 60^{o}c. When the solvent was boiling, the vapor rises through the vertical tube into the condenser at the top. The liquid condensate drips into the filter paper thimble in the centre, which contains the oil to be extracted. The extract seeps through the pores of the thimble and fills the siphon tube, where it flows back down into the round bottom flask. This was allowed to continue for 30 minutes. It was then removed from the tube, dried in the oven, cooled in the desiccators and weighed again to determine the amount of oil extracted. The experiment was repeated by placing 5g of the *Watermelon seed into* the thimble. The weight of oil extracted was determined at 30 minutes interval. At the end of the extraction, the resulting mixture containing the oil was distilled off using simple distillation to recover solvent from the oil. The oil extracted was stored in a plastic container for further use.

Formula:

$$M_c = \frac{W_1 - W_2}{W_1} \times 100$$

B. Photochemical Screening Test[9]:

The phytochemical analysis of the two oil samples was performed to find the presence and absence of primary and secondary metabolites in seed oil. The plant metabolites such as alkaloids, phenols, sterols, terpenoids, tannins, flavonoids, cardiac glycosides, and saponins were analyzed and their test procedures were given below.

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- *Phenols:*

  Few drops of oil were treating with solution of ferric chloride. A deep bluish green precipitate indicates the presence of phenol. Ferric chloride solution is prepared by dissolving 135.2g of $FeCl_3.6H_2O$ in distilled water containing 20 ml of concentrated HCl and dilute to 1 liter.

- *Alkaloids:*

  Each one mL of oil was added to 1% aqueous HCl over water bath and filtered. The filtrate was treated with Mayer's reagent which is prepared by mixing 2g of Iodine in 6g of Potassium iodide in 100 ml of distilled water .Formation brown or reddish brown precipitate indicates presence of alkaloids.

- *Steroids:*

  One mL of oil was added to 2ml acetic anhydride and 2ml H_2SO_4. Colour change from violet to blue or green indicates the presence of steroids.

- *Terpenoids:*

  Each one mL of oil was added to 0.5ml acetic anhydride and few drops of concentrated H_2SO_4. A bluish green precipitate indicates the presence of terpenoids.

- *Tannins*

  1 mL of oil was boiled in 20ml water and filtered. A few drops of 0.1% Ferric Chloride solution were added. Brownish green or blue-black colour indicates the presence of Tannins.

- *Flavonoids*

  5ml Ammonium solution was added to aqueous filtrate of each sample solution and then few drops of concentrated H_2SO_4 is added. Yellow coloration indicates the presence of Flavonoids.

- *Saponins*

  1mL of oil was boiled with 5ml distilled water and filtered. 3ml distilled water was added to the filtrate and shaken vigorously for 5 minutes. Persistent frothing on warming indicates the presence of Saponins.

- *Glycosides*

  5ml of H_2SO_4 was added to each of the test oil in a boiling tube. The mixture was heated in boiling water for 15minutes. Fehling's solution A and B was added and the resulting mixture was heated to boiling. A brick red precipitates indicates the presence of glycosides.

- *Carbohydrates*

  5ml of the equal mixture of both Fehling's solutions A and B was added to 2ml of test oil in a boiling tube, this was heated for 2 minutes. A brick red precipitates indicates a positive result.

- *Amino acids*

  Few drops of oil were dissolved in a few ml of distilled water and 1ml of ninhydrin reagent was added to it. Development of a blue colour indicates the presence of amino acids.

C. Determination Of Mineral Elements:

- *Quantitative Analysis of Elementals* [10-14]

Elements like nitrogen, sulphur, phosphorous, potassium, sodium, calcium, magnesium, zinc, copper, iron, manganese, boron, and molybdenum were determined by following standard procedures. Ash was estimated using Gravimetric method by ASTM (1988) and the total Nitrogen estimated by the method of Micro Kjeldhal (Bremner, 1965). Sulphur was estimated with Gravimetric method (Ali Ehyaeei, 1997) and the total Phosphorous by Pemberton (1927) method. Using the Flame Photometry (Systronics mid flame 127) by following the method of Stanford and English (1949) the total amount of Potassium, Sodium, Calcium, and Magnesium were analysed. From the help of Atomic Absorption Spectroscopy (Solar-AAS2-UK made) the total amount of Zinc, Copper, Iron, Manganese, Boron, Molybdenum are estimated.

- *Estimation of mineral by Atomic Absorption spectrophotometer:*

Initially 200 ppm stock solution of the Zinc, Copper, Iron, Manganese, Boron and Molybdenum were formed by mixing required quantity of salts in distilled water for elemental analysis of seed. Perchloric-acid digestion method was used for elemental analysis (Allen, 1974) and 0.25 g of seed powder was immersed in 6.5 ml of mixed acid solution i.e. nitric acid, sulphuric acid and perchloricacid (5:1:0.1) and digested in a flask (50 ml) in fume hood on hot plate till the digestion was completed which was indicated by white fumes coming out from the flasks. Digested samples were allowed to cool and transferred in 50 ml volumetric flask, by rising volume with distilled water. Filtrate (Whatmann No. 42) was collected and concentration of each element was determined on Solar-AAS2-(UK made) atomic absorption spectrophotometer. Quantity of each element was calculated by using formula:

$$\text{Nutrient caution in plants} = (\text{ppm in extract} - \text{blank}) \times A/W \times \text{dilution factor}$$

A = Total volume of the oil (ml)
W = Weight of dry seed powder

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- *Estimation of mineral by Flame Photometry:*

The estimation for sodium, calcium, Magnesium and potassium ions was carried out using Systronics med flame 127 – flame photometer. The stock solution of Sodium was prepared by dissolving 2.542g sodium chloride in 1 litre of distilled water and it contains 1mg Na per ml (i.e. 1000 ppm). Stock solution was further diluted to give four solutions containing 10, 5, 2.5 and 1 ppm of sodium ions. The potassium stock solution was prepared by dissolving 1.909g potassium chloride in 1 litre of distilled water and resulting solution contains 1mg potassium per ml (i.e. 1000 ppm). Stock solution was again diluted to give four solutions containing 20, 10, 5 and 2 ppm of potassium ions. Calcium stock solution was prepared by dissolving 2.497 g of calcium carbonate (1000-ppm) (AR grade) into a 100-ml beaker and then transfer to a one litre volumetric flask and make up to volume with deionized-distilled water. The standards of 100, 75, 50 and 25 ppm was made by using stock solution and diluted with deionised water.

D. Determination of Physicochemical Properties[15-18]

The following physicochemical properties were determined;

- *Viscosity:*

A clean, dried viscometer with a flow time above 200 seconds for the fluid is used to test the viscosity. To eliminate dust and other solid material from the liquid sample the sample were filtered through a sintered glass crucible. The viscosity meter was charged with each of the samples by inverting the tube's thinner arm into the liquid samples and suction force was drawn up to the upper timing mark of the viscometer, after which the instrument was turned to its normal vertical position. The viscometer was placed into a holder and inserted to a content temperature bath set at 40°c and allowed approximately 10mins for the sample to come to the bath temperature at 40°c. the suction force was then applied to the timing the flow of the samples as it flow freely from the upper timing mark to the lower timing mark was recorded.

- *Density:*

The weight of an empty and dried 100ml beaker was noted. A specific volume of biodiesel was added to the 100ml beaker and the weight of the oil with beaker was noted. Then, the density of the oil is calculated by using the following formula.

Formula

$$\text{Density of oil sample} = \frac{\text{Weight of oil sample}}{\text{Volume of oil sample}}$$

- *Specific Gravity:*

An empty washed and dried as beaker was weighed on the top load weighing balance. The weight of the beaker and weighed was recorded. Exactly $50cm^3$ of the samples were recorded. The procedure was repeated with water of $50cm^3$ of water was obtained. The specific gravity was calculated by given formula.

Formula

$$\text{Specific gravity of oil sample} = \frac{\text{Weight of oil}}{\text{Weight of equal volume of water}}$$

- *Refractive Index:*

The instrument abhe refract meter with refractive indices vary from 1.4220 to 1.4859 can be help to read the fourth decimal place accurately. The temperature of the refractometer should be maintained within + 0.10^0C and it will provides the thermostically controlled water bath and to circular water through the instrument. The method of calibration was discussed in below.

➢ *Calibration of the Instrument*

The instrument of refractive index is calibrated with a glass prism of known refractive index on optical contact naphthalene or using distilled water which has a refractive index of 1.3330 at 20^0C. Alcohol and ether are used to clean the refractometer. A drop of oil or fat in case of a solid fat the temperature should be suitably adjusted by circulating hot water is placed on the prism. The prism is closed by the ground glass-half of the instrument and the dispersion screw is adjusted so that no colour line appears between the dark and illuminated halves. The dark line is adjusted exactly on the cross wires and the refractive index is read on the scale. Usually commercial instruments are constructed for with white light but are calibrated to give the refractive in terms of sodium light of wavelength 589.3nm at a temperature of 200^0C unless otherwise specified.

- *Chemical Properties [19-25]:*
  - ➢ *Saponification Value:*

One milliliter of the oil sample was added into a conical flask containing 25ml of alcoholic sodium hydroxide solution. The flask was sealed and heated in the oven for 5mins at 105°C. The excess alkali was then titrated with 0.5M HCl in the hot condition and two drops of phenolphthalein indicator was added before the titration. A blank was carried out at the same time without adding oil sample and the saponification value was calculated by following.

Formula

$$\text{Saponification value} = \frac{(B - S) \times 0.5 \times 56.10}{\text{Weight of the sample}}$$

Where,

B - Blank titer value

S - Sample titer value

- *Iodine Value:*

0.4g of the oil samples was weighed separately in 250ml conical flask and adds 20ml of carbon tetra chloride to dissolve the oil samples. Then 25ml of Dam's reagent was added to the flasks using a safety pipette in fume chamber and the stoppers were inserted then the content of the flasks were vigorously swirled. The flasks were kept in the dark for the period of 2 hours 30mins, at the end of this period, 20ml of 10% aqueous potassium iodide and 125ml of water were added to each sample using a measuring cylinder. The total contents were titrated with 0.1M sodium-thiosulphate solutions until the complete disappearance of yellow color. Then few drops of 1% starch indicator was added and the titration continued by adding thiosulphate drop wise until blue coloration disappeared after vigorous shaking. The same procedure was follows for the blank test and other samples. The iodine value is calculated by given expression.

Formula

$$\text{Iodine value} = \frac{C\,(V_1 - V_2)}{M}$$

Where,

C – Concentration of sodium thiosulphate used

V_1 – Volume of sodium thiosulphate used for blank

V_2 – Volume of sodium thiosulphate used for determination

M – Mass of the sample

- *Peroxide Value:*

Weigh the sample portion into a 250ml Erlenmeyer flask with glass stopper. Add 30ml of acetic acid- chloroform solution and swirl to dissolve the test portion. 0.5ml of saturated KI solution was added by using a suitable volumetric pipet. Allow the solution to stand for exactly 1min, thoroughly shaking the solution at least three times during the 1 min. Immediately add 30 ml of DI water and titrate with 0.1N Sodium thiosulphate, adding it gradually and with constant vigorous agitation. The titration is continuing until the yellow iodine colour has almost disappeared. Add about 0.5ml of starch indicator solution and continue the titration with constant agitation, especially near the end point, to liberate all the iodine from the solvent layer. Add the thiosulphate solution drop wise until the blue colour just disappears. The peroxide value is calculated by following given formula.

Formula

$$\text{Peroxide value} = \frac{(S - B) \times N \text{ thiosulphate } (0.1N)}{\text{Weight of oil sample } (5 \text{ ml})} \times 1000$$

Where,

S – Titration of sample,

A-Titration of blank

Results and Discussion

The result obtained for various tests carried out on *Watermelon* seed oil the analysis of essential oil.

S. NO	samples	Watermelon seed oil (ethanol extract)
1	Alkaloids	+
2	Carbohydrates	-
3	Saponins	+
4	Flavonoid	-
5	Tannin	+
6	Phenol	+
7	Glycoside	-
8	Terpenoid	+
9	Sterol	-
10	Amino acids	+

Table 1:- Phytochemical Analysis of Oil

The screening of the seeds showed the presence of alkaloids, tannins, Terpenoid, phenol, amino acids and saponins while flavonoids, glycosides, carbohydrates and sterol are absent. Tannins which are a major plant polyphenol when isolated from edible or non-edible plants have shown strong biological activity in the form of anti-tumors, anti-mutagenic, anti-bacteria. The treatment of sore throat hemorrhages and wound healing has also been linked to tannins. Alkaloids are known to be ranked the most efficient therapeutically important plant secondary metabolite and are widely used worldwide as a basic agent for analgestic, anti-plasmodic and bacterial effects. Alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly invoke a bitter taste. Glycoside is derived from glucose. Glycosides are common in plants, but rare in animals, these compounds give a permanent froth when shaken with water. They also cause heamolysis of red blood cells. A steroid is a type of organic compound that contains a characteristic arrangement of four cycloalkane rings that are joined to each other. Examples of steroids include the dietary lipid cholesterol, bile acids, and testosterone and the anti-inflammatory drug dexamethasone.

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S. No	Minerals	Watermelon seeds		
		Macro - Elements		Micro - Elements
1	Sodium	4.80	Iron	40.1
2	Potassium	3.80	Copper	0.04
3	Calcium	1.40	Manganese	7.88
4	Phosphorous	4.0	Zinc	4.85
5	Magnesium	5.75		

TABLE 2 :- Mineral Compositions

The most abundant mineral found in the sample is iron with concentration of 40.1mg/100g. Iron helps in the formation of blood and in the transfer of oxygen and carbon dioxide from one tissue to another. Iron deficiency results in impaired learning ability and interactive problems in children, and also anaemia. Manganese is the second most abundant element in the seed sample with the value of 7.88 mg/100g. Manganese plays important role in the allocation of oxygen from lungs to cells and start of enzymes reactions alarmed with carbohydrate, fat and protein metabolism. Its deficiency scarcely occur because it is present in abundance in food, however in an event of it occurring, manganese deficiency can results to retard growth and skeletal disorder. Zinc and magnesium are the subsequentrich mineral basics found in the sample of melon seeds with the values of 4.85mg/100g and 5.75 mg/100g respectively. Zinc boosts the health of our hairs, plays a role in the proper functioning of some sense organs such as ability to taste and smell. Magnesium is beneficial to blood pressure and helps to prevent sudden heart attack, cardiac arrest and stroke. The value of potassium and sodium are 5.75mg/100g and 4.80 mg/100g respectively. High concentration of potassium in the body is to increase iron utilization and beneficial to people taking diuretics to control hypertension and suffer from excessive of potassium through the body fluid. Sodium regulates fluid balance in the body and helps in the proper functioning of muscles and nerves. The concentration of calcium was found to be 1.40 mg/100g, the least of all mineral elements present in the sample. Calcium is a constituent of bones and helps the body to contract correctly, blood to clot and the nerves to convey messages. Calcium is essential for disease prevention and control and contributes to the medicinal influences of the plant. The copper content of 1.45mg/100g. It helps the body to use iron and sugar properly. It is also necessary for bone growth and nerve function. Deficiency of copper may result to anaemia.

S. No	Characterisitics	Watermelon seed oil
1	Percentage of oil Yield	35%
2	Acid Value (mg KOH/g)	5.07
3	Free Fatty Acid	2.5
4	Saponification Value (mg KOH/g)	191.6
5	Density (gcm^2)	0.867
6	Specific gravity (40^0C)	0.905
7	Iodine Value (gI_2/100g of oil)	119.29
8	Peroxide Value	4.50
9	Refractive index at 40^0C	5.43
10	Viscosity at 40^0C mm^2/s	5.2

Table 3 :- some of the properties of Watermelon seed oil.

The oil was extracted using n- hexane as solvent in the Soxhlet extractor. The extracted oil was pale yellow liquid at ambient temperature with characteristic unique odour. The oil yield of *Watermelon* was determined as 85.5 %. Acid value of oil is an important parameter which affects the transesterification of oils. High acid value in oil will produce soap during transesterification. The extracted oil was analyzed to determine its acid value; the acid value was found 5.07 mg KOH/g. The high acid value of Watermelon oil necessitated acid pretreatment of the oil before transesterification. Free fatty acids (FFA) are produced by the hydrolysis of oils and fats. FFA level is high due to time, temperature, moisture content because the oils and fats are presenting to various environments. The free fatty acid value measures the extent to which glycerides in the oil have been decomposed by lipase action. It increased significantly with increase in moisture content. The value obtained for *Watermelon seed oil* was 2.5 %. Chemical reaction involves the production of a metal salt or soap. The long chain fatty acids of fats have a low saponification value because they have a relatively fewer number of carboxylic functional groups per units mass of the fats as related to short chain fatty acids. The value for the *Watermelon seed oil* was 191.6 mg KOH/g. Density is the mass of unit volume, measured in a vacuum. It directly affects fuel performance, as some of the engine properties are strongly connected to the density. It also affects the quality of atomization and combustion. Density

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value determines for pure esters. The density of biodiesel is typically higher than that of diesel fuel and is dependent on fatty acid composition and purity. Contamination of biodiesel significantly affects its density; therefore density can also be an indicator of contamination. The density value was obtained $0.8067 gcm^{-3}$. The specific gravity of Biodiesel varies with its fatty acid composition. A denser biodiesel has higher energy content and will give better mileage and increased power. Vegetable oil will typically have a specific gravity of from 0.903 to about 0.921 depending on its fatty acid composition and temperature. There are a no. of contaminates that can alter the specific gravity of biodiesel. One of those contaminates is methanol. Biodiesel will have a range of about 0.86 to 0.90. The value obtained for *Watermelon seed oil was* 0.950 at 40ºC. The extracted *Watermelon oil* was analyzed to determine the iodine value. The iodine value was obtained as *119.29 $I_2$100g.* The low iodine value of this oil makes it suitable for biodiesel production since high iodine value leads to the formation of deposits on engines and problem during storage of the fuel. The peroxide value of Watermelon oil is *4.50* within the standard value. The value of refractive index was *5.43* at 40ºC. The viscosity of Watermelon seed oil is *5.2*.

III. CONCLUSION

In this research paper, a study on the phytochemical screening test, Minerals and physicochemical properties of watermelon seed oil for their suitability in biodiesel production as raw materials to obtain biodiesel fuel and could be suitable alternative to fossil diesel.

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