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Studies on the Isolation, Physico-Chemical Characterization and Microbial Activities of Melon (*Cucumis melo*) Seed Oil

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ABSTRACT: Oil was extracted from melon (*Cucumis melo*) seeds by solvent extraction method using *n*-haxane, LPE (light petroleum ether, b.p. 40-60 °C) and chloroform:methanol 50:50, v/v mixtures. The oil content of the melon seed oil was found to be 28.01%. The oil was characterized with various physical and chemical properties by standard methods and compared with those of standard oils or fats. Acid value (AV), Iodine value (IV), Peroxide value, Reichert-Meissl value (RMV), Thiocyanogen value (TV), Titre value etc. were determined and found that lightly changed due to variation of storage time. Acid value and Peroxide value were increased with the increasing of storage time but RMV, TV, IV, and Titre value were decreased, which indicates that the quality of the oil deteriorates with increasing time of storage. Fatty acid composition of the oil was determined by Thin-layer Chromatography (TLC) and identified the presence of palmitic acid, stearic acid, linolenic acid and oleic acid in the oil. The low acid value of oil indicates that it can be used in edible purpose. The de-oiled seed cake of melon seed was studied for the determination of ash content, protein, moisture content and minerals (N, P, K, and Ca) quantitatively. The microbial activities of the oil sample were investigated by standard methods.

KEYWORDS: Melon, Seed, Microbial activities, Thin Layer Chromatography, Fatty Acids.

1 INTRODUCTION

Man and animals severely depend on the plant kingdom for food, clothing, shelter, medicine and many other daily necessities of life. On the other hand they also provide us many important substances such as glycosides, alkaloids, sterols, vitamins, essential oils, resins, lignins, medicine, fats and oils etc [1]. Vegetable oils differ remarkably from fish oils in containing a great variety of fatty acids especially poly unsaturated fatty acids (PUFA). Besides the fatty acids occurring both in vegetable oils and in terrestrial animals fats, namely palmitic, stearic and oleic acid. Some vegetable oils contain great proportions of linoleic acids [2]. Essential oils also known as volatile liquids, widely available in various types of plants. Examples are turpentine oil of cloves, oils of the seed of melon etc. Seeds of melon contain α-spinasterol, stigmasta-7,22,25-trienol and stigmasta-7,25-dienol which have phenol group [3]. Melon seeds are major source of essential oils [4]. Seeds are generally treated as waste; however, medicinal effects have been reported for the seeds [5], [6]. Hexane-extracted seed oil of *Cucumis melo* hybrid AF-522 was determined to contain 64 g of linoleic acid per 100 g of total fatty acids [6]. Present study is about the physico-chemical characterization of the solvent-extracted oil from the seeds of melon found in agricultural field at Chittagong area in Bangladesh and comparing the results with the data available in literature about pharmacological aspects of seeds of melon. Performance of the oil from the seeds of melon against some common microbial species is also studied.

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2 MATERIALS AND METHODS

2.1 COLLECTION OF THE SEEDS FROM FRUITS

The melon was collected from a local garden of Chittagong. Seeds were separated and preserved in desiccators in the laboratory until extraction and chemical investigation.

2.2 EXTRACTION OF OIL

Only the seeds were kept aside separately for extraction. The method employed for extraction of the oil from the seeds was solvent extraction method. Extraction was carried out in 5-L Soxhlet apparatus by using *n*-hexane and light petroleum ether (b.p. 40-60 °C) and chlorofron:methanol (50:50, v/v) mixture [7]. Oil was recovered from combined extracts by rotary evaporator.

2.3 PHYSICAL CHARACTERIZATION

All chemicals and reagents used in this study were of analytical grade unless otherwise mentioned or chemicals prepared according to the standard procedures [8], [9], [10].

Viscosity of a liquid is the measure of resistance to flow. The viscosity of the oil was determined by Oswald viscometer at 30 °C.

The refractive index of the oil sample was determined by ABBE Refractometer (MODEL; DTM-1 Atago Co. Ltd). The specific gravity of the oil sample was determined by specific gravity bottle.

The moisture content, ash content and protein content of the de-oiled seed cake of melon seed were determined by using standard methods [11], [12].

2.4 CHEMICAL CHARACTERIZATION

Various chemical properties were determined by using suitable standard methods. Saponification value, Acid value, Percentage of free fatty acid, Saponification equivalent, Iodine value, Acetyle value [13], [14], Richert-Meissl value, Polenske value, Thiocyanogen value [15], Henher value, Kirschner value, quantity of unsaponifiable matter [16] and Peroxide value [17] were determined by standard methods.

2.5 MICROBIAL EVALUATION

The microbial activities of the oil sample against four bacteria by disc diffusion method and four fungi by the food poison technique were tested respectively [18]. Nutrient agar (NA) and potato dextrose agar (PDA) were used as basal medium for bacterial and fungal activity test respectively. Chloroform was used as a solvent to prepare 1% and 10% oil solution. Proper control was maintained with chloroform.

2.6 ESTIMATION OF MINERALS

Minerals (N, P, K and Ca) of de-oiled seed cake of melon seed were determined by modified Kjeldahl method.

2.7 CHROMATOGRAPHIC EXAMINATION

The seed oil of melon was subjected to TLC examination and its fatty acid composition was identified by comparing the R_f values of different spots of chromatograms with those of standard fatty acids reported earlier in different solvent systems [9], [10].

3 RESULTS AND DISCUSSION

3.1 PHYSICAL CHARACTERIZATION

The refractive index of melon seed oil was found to be 1.4712 at 30 °C (Table-1) which is similar to soyabean oil (1.4723-1.4756). The result indicates that the oil sample is consistent with long chain unsaturated fatty acids, which supported by it's iodine value.

The specific gravity of the fats and oils does not vary as a general rule to an extent which makes this property useful in distinguishing one oil from another. The specific gravity of melon seed oil was found to be 0.9310 at 30 °C. This value has close similarity with soyabean oil (0.922-0.920) and sunflower oil (0.924-0.926) (Table-1).

The viscosity of the melon seed oil was found to be 360.24 m.p. at 30 °C. The low viscosity suggested that there are a few hydroxyl groups in the molecule which are supported by the low acetyl values of the oil (3.87) and lower refractive index of the oil.

The activation energy of the oil sample was found to be 3.532 kcal per mole. The higher value indicates the greater viscosity of the oil.

The moisture content, ash content and protein content of the de-oiled seed cake of melon seed were determined using standard methods and were found to be 1.59%, 3.01% and 14.13% respectively.

3.2 CHEMICAL CHARACTERIZATION

The saponification value is the number of milligrams of KOH required to saponify 1 g of a fat or oil. The saponification value of the melon seed oil was found to be 187.46 as shown in Table-2, which was nearest to that of soyabean oil (190-195). This comparatively lower saponification value indicates the presence of higher fatty acids in higher proportions. The saponification equivalent value of the melon seed oil was found to be 299.26.

The acid value is the number of mg of KOH required to neutralize the free acids present in 1 g of oil or fat. The acid value of melon seed oil was found to be 0.812. The relatively lower acid value makes oil more edible and nutritive. The increase in free fatty acids is generally accompanied by a rancid odor, although the odor itself is not due to the acidity. The value near to the soyabean oil (1.27-1.54), Olive oil (0.6-1.5)[19]. Low acid value is an indication of freshness of the oil and suitability of the oil for edible purpose.

Percentage of free fatty acids (%F.F.A.) of the melon seed oil was found to be 0.408 as oleic acid which was almost same as the soyabean oil (0.35-0.85) and cotton seed oil (0.4-0.9) [20], [21].

The iodine value is the number of grams of iodine that combine with 100 grams of an oil or fat. It is the measure of the degree of unstauration of a fat or oil and thus allows its classification into non-drying, drying and semi-drying types. The iodine value of melon seed oil was found to be 125.97. This value supports that the oil was moderately unsaturated.

The ester value is defined as the number of mg of KOH necessary to combine with the fatty acids which are in combination with glycerol in 1 g of fat or oil. It is determined by subtracting the acid value (A.V.) from the saponification value (S.V.). The ester value of the melon seed oil was found to be 186.65.

The Reichert-Meissl value (R.M.V.) is the number of milliliters of 0.1N KOH solution required to neutralize 5 g of a fat or oil. The R.M.V. of the melon seed oil was found to be 0.43, which was identical to the soyabean oil (0.5-2.5) and olive oil (0.6-1.5). R.M.V. is the measure of volatile water soluble fatty acids (butyric-C_4 to capric-C_{10}) present in the oil or fat. Relatively lower R.M.V. of the oil is an indication of low content steam volatile fatty acids.

The Polenske value is the number of milliliters of 1N NaOH solution required to neutralize the volatile water insoluble but alcohol soluble fatty acids distilled from 5 g of a fat or oil. The Polenske value of the melon seed oil was found to be 0.91, which indicates that the low content of the volatile alcohol soluble but water insoluble fatty acids present in the oil.

The Henher value is a measure of water insoluble fatty acids in an oil or fat. The Henher value of the melon seed oil was found to be 86.21 which was almost same as the palm oil (94.2) and cotton seed oil (94.6). It indicates that higher percentage of water insoluble fatty acid having high molecular weight is present in the oil.

Peroxide value is the amount of iodine liberated from potassium iodide by the peroxides present in the oil or fat, in terms of milliequivalents per kg or millimoles per kg. The peroxide value of the melon seed oil was found to be 81.36 meq/kg.

The acetyl value is the number of mg of KOH required to neutralize the acetic acid obtained by saponifying 1 g of acetylated fats or oil. The acetyl value of the melon seed oil was found to be 3.87, which indicates low content of free hydroxyl groups present in the oil sample.

The percentage of unsaponifiable matter (U.S.M.) of the melon seed oil was found to be 0.6021% which was almost same as the soyabean oil (0.7-1.6) and cotton seed oil (0.8-1.8). The Fat Analysis Committee of the American Chemical Society proposed that if the percentage of U.S.M. exceeds 2%, some type of foreign matter is probable to present in the oil. The foreign matter may consist of a mineral or similar hydrocarbon oil, wax or fat, spermaceti of rosin oil etc. Low U.S.M. value of the oil indicates that low content of foreign matter is present in it.

The titre value of an oil or fat is the solidifying point of the mixed fatty acids. The titre temperature is a value for characterizing oils or fats and assuming the hardness. Titre value of the melon seed oil was found to be 29 (30 °C).

The Thiocyanogen value of the oil sample was found to be 68 which is supported by Iodine value and peroxide value.

The Kirshner value of the oil sample was found to be 0.59.

3.3 ANTIBACTERIAL TEST

The antibacterial activities of the sample were studied against two gram positive and two gram negative bacteria by standard method and the results shown in Table-3. It is evident from the table that the oil sample was found to be active against all test bacteria. Highest inhibition zone (19 mm) was observed against *Salmonella typhi* for the 10% oil solution.

3.4 ANTIFUNGAL TEST

The antifungal activities of the melon seed oil were studied against four fungi and the results shown in Table-4. The oil sample was not active against the mycelia growth of *Curvularia lunata*. Except this the mycelia growth of all test fungi was inhibited by the oil sample. Highest inhibition zone (29.03 mm) was observed against *Aspergillus funiculosus* for the 10% oil solution.

3.5 ESTIMATION OF N, P, K, AND CA IN DE-OILED SEED CAKE OF MELON SEED

The people of our country have been suffering to a lot extent from protein malnutrition. From Table 5, it is proved that melon seed oil contains 2.436% of nitrogen which is well balanced in respect of essential amino acids. The percentage of phosphorus (2.974%) indicates that phospholipid may present in the oil sample. The percentage of potassium (1.17%) in the oil sample may be helpful to increase blood pressure of the people having low blood pressure. The percentage of calcium (1.432%) may help formation of rigid bone structure of the growing childs.

3.6 THIN-LAYER CHROMATOGRAPHIC (TLC) EXAMINATION OF THE MELON SEED OIL

The fatty acid methyl esters mixture obtained from the melon seed oil was subjected to TLC examination and their fatty acid composition was identified by comparing the results with the R_f values of methyl esters of standard fatty acids as reported (Table-6) in different solvent systems. It was found from the chromatograms of the oil sample that the oil gave about four to five spots. Among the spots, four spots were identified as palmitic acid, stearic acid, linoleic acid and oleic acid in the oil sample.

Table - 1: Physical properties of some important commercial oils and melon seed oil

Name of the oils	Sp. gr. at 15.5 °C	R. I. at 15.5 °C	Viscosity in m. p.	Activation energy in kcal/mole
Linseed oil	0.931-0.938	1.4479-1.48	296.0841	--
Cotton seed oil	0.912-0.922	1.4743-1.48	358.4261 at 30°C	--
Olive oil	0.915-0.919	1.4657-1.4667	466.8129	--
Sunflower oil	0.924-0.926	1.4659	331.1249 at 30°C	--
Soyabean oil	0.922-0.920	1.4723-1.4756	248-98	--
Coconut oil	0.926	1.4530	297.90	--
Tung oil	0.939-0.945	1.515-1.52	--	4.132
Castor oil	0.9561 at 27°C	1.4761	293.42	--
Palm oil	0.837	1.4510	309.24	--
Mehagini oil	0.9334 at 30°C	1.4751 at 30°C	459.32 at 30°C	3.047
Corn oil	0.921-0.938	1.4733	--	3.147
Neem seed oil	0.9192 at 30°C	1.4623 at 30°C	360.0024 at 30°C	--
Melon seed oil	**0.9310 at 30 °C**	**1.4712 at 30 °C**	**360.24 at 30 °C**	**3.532**

R. I. = Refractive index. Sp. gr. = Specific gravity. "-"= data not available.

Table-2. Chemical constants of some important commercial oils and melon seed oil.

Name of the sample	S.V.	S.E.V.	A.V.	F.F.A. (%) (as oleic)	I.V.	T.V.	Acetyl Value	Titre Value ValueºC	U.S.M. (%)	R.M.V.	P.V.	H.V.	Peroxide Value meq/kg	K.V.
Olive oil	190-195	287-295	0.6-1.5	0.25-0.60	80-88	75-83	10.04	17.26	0.5-1.5	0.6-1.5	0.5	0.6	----	----
Sunflower oil	190-194	287-295	0.6-2.4	0.15-0.45	125-140	78.4-81.3	----	17	0.3-0.9	0.5	----	----	----	----
Cotton seed oil	192-198	283-292	1.0-5.0	0.4-0.9	103-111	61-69	0.7-12.2	30.37	0.8-1.8	0.95	----	94.6	----	----
Linseed oil	189-195	287-296	4.0	0.5-0.75	175-200	----	----	----	1.0-1.5	----	----	94.8	----	----
Soyabean oil	190-195	287-295	1.27-1.54	0.35-0.85	129-137	77-85	----	22-27	0.7-1.6	0.5-2.5	0.2-1.0	----	----	----
Coconut oil	255-260	210-250	2.5-10.0	----	8.2-9.6	6.1-7.0	----	20-24	0.15-0.7	7.0-8.0	15-17	82	----	----
Palm oil	248	220-250	----	----	15-18	----	----	----	----	28	----	94.2	----	----
Melon seed oil	**187.46**	**299.26**	**0.812**	**0.408**	**125.97**	**68**	**3.87**	**29**	**0.6021**	**0.43**	**0.91**	**86.21**	**81.36**	**0.59**

S.V. = Saponification value. T.V. = Thiocyanogen value. S.E.V. = Saponification equivalent value.
U.S.M. = Unsaponifiable mater. A.V. = Acid value. R.M.V. = Reichert-Meissel value
F.F.A. = Free fatty acid value. P.V. = Polenske value. I.V. = Iodine value. H.V = Henher value.
K.V. = Kirschner value. " –" = data not available.

Table-3: Antibacterial activity of melon seed oil

Name of the bacteria	Diameter of inhibition zone in mm		
	Oil soaked in chloroform	1% oil sample	10% oil sample
Escherichia coli	17	14	17
Salmonella typhi	18	16	19
Staphylococcus aureus	13	10	12
Bacillus cereus	12	9	11

Table-4: Antifungal activity of melon seed oil

Name of the fungi	Radial growth inhibition (mm)	
	1% oil sample	10% oil sample
Aspergillus funiculosus	27.42	29.03
Fusarium equiseti	12.16	13.75
Curvularia lunata	-20.46	-22.33
Alternaria alternata	12.41	15.31

(-) means no inhibition.

Table-5: Percentage of minerals of melon de-oiled seed cake.

Name of the de-oiled seed cake	Nitrogen (%)	Phosphorus (%)	Calcium (%)	Potassium (%)
Melon seed	2.436	2.974	1.423	1.17

Table-6: The R_f values of thin-layer chromatographic examination of the melon seed oil.

Solvent System	R_f values of standard fatty acids				R_f values obtained from the spots of oil sample			
	PA	SA	LA	OA	PA	SA	LA	OA
P:E (80:20)	0.941	0.943	0.933	0.287	0.943	0.939	0.896	0.284
P:E:A (80:20:1)	0.822	0.839	0.893	0.415	0.819	0.835	0.723	0.454
H:E (80:20)	0.823	0.812	0.641	0.201	0.757	0.745	0.638	0.203

PA- Palmatic acid, SA- Stearic acid, LA- Linoleic acid, OA- Oleic acid.

4 CONCLUSION

Physico-chemical characterization and microbial studies of melon seed oil indicates that it consists of moderate amount of unsaturated fatty acid which can be used in edible purpose. Present research demonstrates the potential of the cultivation of melon seed for edible and industrial use. Presence of foreign matters such as sterols, tocopherols, vitamins A and D, is considered with respect to U.S.M % and low hydroxyl group content was confined by acetyl value of the sample. From antibacterial studies we can conclude that this work will provide valuable information about the prospect of derivation of antibiotics, pesticides and pharmaceuticals components from the seed oil of melon with further research. De-oiled seed cake of melon seed contains significant amounts of minerals (N, P, K and Ca).

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