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RESEARCH ARTICLE

COMPARATIVE STUDIES ON THE PHYSICOCHEMICAL CHARACTERISTICS, NUTRITIONAL VALUE AND ANTIMICROBIAL ACTIVITIES OF TWO VARIETIES INDIGENOUS MULBERRY (*MORUS ALBA* L. AND *MORUS NIGRA* L.) SEEDS

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Abstract

The core objective of this research was to investigate the characteristics of oils, nutritional composition and antimicrobial activities of mulberry seeds (*Morus alba* L. and *Morus nigra* L.). The physicochemical properties, glycerides, lipids and fatty acid composition of mulberry seeds oil have been studied. It was observed that *M. alba* L. and *M. nigra* L. contain 31.06% and 29.10% yellow colored oil, respectively. The oil was fractionated into mono, di and triglycerides by silicic acid column chromatography. *M. alba* L. and *M. nigra* L. contain 90.10% and 90.85%, 4.05% and 3.15%, and 1.46% and 1.95% triglycerides, diglycerides and monoglycerides, respectively. The total lipids were fractionated into three major lipid groups, neutral lipids, glycolipids and phospho-lipids by silicic acid column chromatography. The neutral lipids, glycolipids and phospholipids were 91.90% and 93.04%, 2.1% and 2.34%, and 1.75% and 1.93%, gradually, of the total oil of the lipid applied of the two plant varieties. GLC analysis was showed the presence of fatty acids series from $C_{16:0}$ to $C_{12:2}$ for *M. alba* L. and *M. nigra* L. of which the principle fatty acids accounted as linoleic acid 74.29% and 71.14%, respectively. The knowledge from the nutritional analysis could be important to its appropriate industrial use and for improvement in the nutritional value. The seed meal samples contained huge amount of lipid (29.04% in *M. alba* L. and 27.15% in *M. nigra* L.) and protein (20.20% in *M. alba* L. and 21.50% in *M. nigra* L.) and potentially useful amounts of other nutrients. Assessments of the antimicrobial studies reported that all the extracts obtained from *M. alba* L. and *M. nigra* L. seeds showed moderately effective against most of the tested gram negative bacteria.

Keywords: Mulberry (*Morus alba* L. and *Morus nigra* L.) seed oils, glyceride and lipid compositions, fatty acid composition, nutritional composition, antimicrobial activities.

Introduction

The plant mulberry (*M. alba* L. and *M. nigra* L.) commonly known as 'Tut' in our country is cultivated in about all the districts of Bangladesh. It belongs to the family Moraceae and genus Morus. In Bangladesh mulberry is planted during October-November. The tree flowers in February- March and fruits mature by May-June. Mulberry is grown extensively for leaves used for rearing silkworms as mulberry leaves are the only source of food for silkworms. Mulberry leaves are considered diaphoretic and emollient. A decoction of leaves is used as a gargle in inflammations of the throat. The fruit is cooling and laxative; it is used for sere throat, dyspepsia and melancholia. The seeds of the two varieties contain 25-35% yellow colored drying oil (Anonymous, 1959). Linoleic acid is an essential fatty acid of the two plant varieties (*M. alba* L. and *M. nigra* L.) seed oils which cannot be produced by

the human body and is accepted as being an anticarcinogenic substance (Dini et al., 2008). Mulberry seeds oil is very rich in linoleic acid and it may be a valuable source of dietary fat.

No worth mentioned work is yet to be known about the nutritional composition analysis of Bangladeshi mulberry deoiled seed meal. Treatment of diseases with extracts of seeds, roots, rhizomes, barks and leaves of plants have been a common phenomenon from time immemorial. So it is essential to find out new antimicrobial agents from plants to relief from killer disease in perspective of Bangladesh.

The present work has been done on the physicochemical characteristics of the two plant varieties of mulberry seeds oils and nutritional value of seeds meals. Also investigate the antimicrobial activities of these seeds extracts to identify the bioactive compounds of the seeds which may be responsible for the therapy of several infectious and metabolic diseases of human beings and animals.

Materials and methods

Plant materials and chemicals

Ripe fruits of mulberry (*M. alba* L. and *M. nigra* L.) used in this work were collected from the *Bangladesh Sericulture* Research & Training Institute (BSRTI), Rajshahi, *Bangladesh*. Seeds were washed with water to remove foreign materials, dried in the sunlight and finally dried at 105°C for three hours by Forced Convection Oven (FC-610, Toyo Seisakusho Co., Ltd.). Dried seeds were ground into powder using a disk mill (model: FFC-15) and dried in an oven at a temperature of 105°C for an hour and kept in airtight container at 4°C in a refrigerator prior to analysis. Solvents were obtained from Merck (Germany) and BDH (England). Silica gel (60-120 mesh) and Silica gel (HF_{254}) were products of Merck (Germany). Ester of fatty acids, bovine serum albumin (Sigma Chemical Co.USA) and other chemicals were of analytical grade unless otherwise specified and results were expressed on dry weight basis.

Extraction procedure

The oils from the powdered seeds of each of the plants were extracted separately with n-hexane in a soxhlet apparatus for about six hours and the solvent was removed by rotary vacuum evaporator. The percentages of oils were calculated.

For antimicrobial studies, powder from each of the plant material was extracted separately at room temperature using petroleum ether (40-60°C) with gentle stirring for seven days (three times within this period). The resultant extracts were combined, filtered and concentrated under a vacuum pump, respectively. Extraction was carried out successively with chloroform and methanol from the residue left after extraction with petroleum ether applying the same procedure mentioned above.

Physicochemical characteristics of the oils

The specific gravity of the oils were calculated at 29°C with the help of a Pycnometer. Refractive index of the clear oils were estimated at 29°C using Abe Refractometer following (IUPAC, 1979) method. Iodine value of the oils were determined by the hanus method, unsaponifiable matters were determined by the method depicted by (Devine et al., 1961), while the saponification and peroxide values and percentages of free fatty acid (FFA) were determined according to the methods described by (Williams, 1966) .

Separation of Glycerides

The oils were separated into mono-, di and tri-glycerde on silicic acid by silica gel (60-120 mesh) column chromatography. The solvent systems used to elute the column were similar to those described by (Gofur et al., 1993). For quantitative determination of glyceride classes, the sample (1 gm oil dissolved in 15 mL of chloroform) was absorbed on the top of the column, triglycerides, diglycerides and monoglycerides were eluted with benzene, mixture of di-ethyl ether and benzene (1:9 v/v), and 200 mL of diethyl ether, respectively. The elution was controlled of a flow role of 1.5-2.0 mL min^{-1}. The elution of each fraction was checked by thin layer chromatography (TLC) to ensure uniformity of separation of each class of glyceride. The eluted solvents were collected in a weighted flask. The fractions thus obtained were evaporated in a rotary vacuum evaporator and the percentages of these fractions were determined by gravimetric method.

Separation of Lipids

Total lipids extracted from the seeds of both plants by the method of (Bligh and Dyer, 1959) was fractionated into three major lipid groups neutral lipid, glycolipid and phospholipid by silicic gel

column chromatography (Gofur et al., 1993) on about 150 mg seed oil lipids. Neutral lipid was eluted with chloroform, glycolipid with acetone and phospholipids with methanol. Approximately 0.50-1.0 mL/min fractions were collected per minute and elution was monitored by TLC. Solvents were evaporated in a rotary vacuum evaporator and percentage of these fractions was determined by gravimetric method.

Fatty Acid Compositions of Oils

Fatty acid compositions of the both seeds oils were determined as their methyl esters which prepared by the Boron trifluride methanol complex method (Rahman et al., 2007). A GCD PYE Unicam gas chromatograph (PYE Unicam Ltd. Cambridge, UK) equipped with a flame ionization detector was used to determine nthe fatty acid methyl esters. Nitrogen as carrier gas was used at a flow rate of 30 mL/min. Fatty acids were separated on a 1.8 m × 2 mm i.e. glass column packed with 6% BDS (Butanediol succinate polyesters) on solid support, Anakrom ABS (100/120) mesh. Analysis was carried out at isothermal column temperature of 190°C; injector and detector temperatures for GLC analysis were 230°C. The peaks were identified by comparison with standard methyl esters with respect to retention times by plotting the log of retention time against equivalent carbon length (ECL). The peak areas were determined by multiplying peak height by width at half height. The percentage of each peak was calculated as the percentage of the total area of all the peaks.

Nutritional value of Oils

Nutritional value analysis of *M. alba* L. and *M. nigra* L. seeds, moisture, ash and crude fiber content were determined by the methods depicted by (Rangana, 1986). Lipid content was estimated by the method of (Bligh and Dyer, 1959) using a solvent mixture of chloroform and methanol (2:1 v/v). The total protein was determined by the micro-kjeldahl method (Rangana, 1986) and conversion factor of 6.25 was used to quantify the total protein content. The nitrogen free extracts (NFE) were considered as total carbohydrate and were calculated by the following equation (Khan et al., 1999).

Carbohydrate (g/100g) = 100- (moisture + ash + crude fiber + lipid + protein).

Antimicrobial screening

Various solvent extracts such as petroleum ether, chloroform and methanol of *M. alba* L. and *M. nigra* L. seeds were tested against three pathogenic bacteria each by the standard disc diffusion method (Bauer et al., 1966). Nutrient agar medium was used for determining antibacterial activity and kanamycin (30µg/Disc) was used as standard for comparison in antibacterial tests.

The crude extracts were dissolved in sufficient amount of the respective solvents, 20µl of solutions combined 400µg of the test materials for antimicrobial activity. The antimicrobial activities were determined by measuring the diameter of the inhibitory zones in mm using a transparent scale. The diameters of the zones of inhibition by the samples were then compared with the diameter of the zone of inhibition produced by the standard antibiotic disc used.

Results and discussion

The solvent extraction of mulberry seeds yielded 31.06% (*M. alba* L.) and 29.90% (*M. nigra* L.) yellow colored oils (Table 1). The results were almost similar to (Xiaolan et al., 1998) and (Umit et al., 2011). The specific gravity and refractive index are very sTable parameters and should be used for checking the identity of oils. It was observed that the specific gravity and refractive index of two plant verities of the two mulberry seeds oils were comparable with other important vegetable oils (Hilditch et al., 1949). The high iodine and saponification values were more or less similar with safflower seed oil (Rafiquzzaman et al., 2006). Peroxide value of non-edible oil was in the range of 4.36-9.82 m.eq./kg (Cynthia et al., 2012). Oils having high percentages of peroxide are unsTable and grow rancid easily (Sodeke, 2005). The peroxide values of the mulberry seeds oils (Table 1) were in comparable to other vegetable oils. The % FFA values were in good agreement with black mulberry (*Morus nigra* L.) seed oil (Umit et al., 2011). From the results it's evident that two verities of mulberry seeds oils contain small amount of unsaponifiable matter such as steroids tocopherols and hydrocarbons etc.

As shown in Table 2, mono, di and triglyceride contents were estimated to be 1.46, 1.95, 4.05, 3.15, 90.10 and 90.85%, respectively. Mulberry seeds oils were separated into three major lipid

Table 1. Physicochemical characteristics of the two mulberry verities *M. alba* L. and *M. nigra* L. seeds oils.

Characteristics	***M. alba* L.**	***M. nigra* L.**
Oil (%)	31.16	29.10
Specific gravity at 29°C	0.917	0.914
Refractive index at 29°C	1.462	1.465
Saponification value (mg KOH/g)	185.04	190.58
Iodine vale	147.35	144.51
Free fatty acids (%) as oleic	2.10	2.04
Unsaponifiable matter (%)	1.13	0.95
Peroxide value (meg/kg)	1.85	2.05

Mean value of three experimental results

Table 2. Glyceride and lipid compositions of *M. alba* L. and *M. nigra* L. seeds oils (%).

Parameters	**Composition**	***M. alba* L.**	***M. nigra* L.**
Glyceride	Monoglyceride	1.46	1.95
	Diglyceride	4.05	3.15
	Triglyceride	90.10	90.85
Lipid	Neutral lipid	91.90	93.04
	Glycolipid	2.15	2.34
	phospholipid	1.75	1.93

Mean value of three experimental results

Table 3. Fatty acid compositions of *M. alba* L. and *M. nigra* L. seeds oils

Fatty acid	***M. alba* L.**	***M. nigra* L.**
Myristic acid ($C_{14:0}$)	0.07	0.07
Palmitic acid ($C_{16:0}$)	10.60	11.82
Stearic acid ($C_{18:0}$)	5.61	6.53
Linoleic acid ($C_{18:0}$)	74.29	71.14

Table 4. Nutritional compositions of *M. alba* L. and *M. nigra L.* seeds meals

Parameters (%)	***M. alba* L.**	***M. nigra* L.**
Moisture	3.96	4.60
Ash	6.05	5.10
Crude fiber	23.41	25.72
Total lipid	29.04	27.15
Total protein	20.20	21.50
Total carbohydrate	47.58	43.17

Mean value of three experimental results

Table 5. Antimicrobial activities of different extracts of *M. alba* L. seeds

Test	**Diameter of zone of inhibition in mm**				
	DMSO	**Pet ether**	**Chloroform**	**Methanol**	**Kanamycin**
Gram positive					
Sarina lutea	0	0	0	0	23
Gram negative					
Escherichia coli	15	0	13	9	21
Shigella dysenteriae	10	0	9	14	25

Table 5. Antimicrobial activities of different extracts of *M. nigra* L. seeds

Test organisms	Diameter of zone of inhibition in mm				
	DMSO	**Pet ether extract (400µg/Disc)**	**Chloroform extract (400µg/Disc)**	**Methanol extract (400µg/Disc)**	**Kanamycin (400µg/Disc)**
Gram positive					
Sarina lutea	0	0	0	0	20
Gram negative					
Escherichia coli	17	0	12	11	24
Shigella dysenteriae	13	0	10	8	22

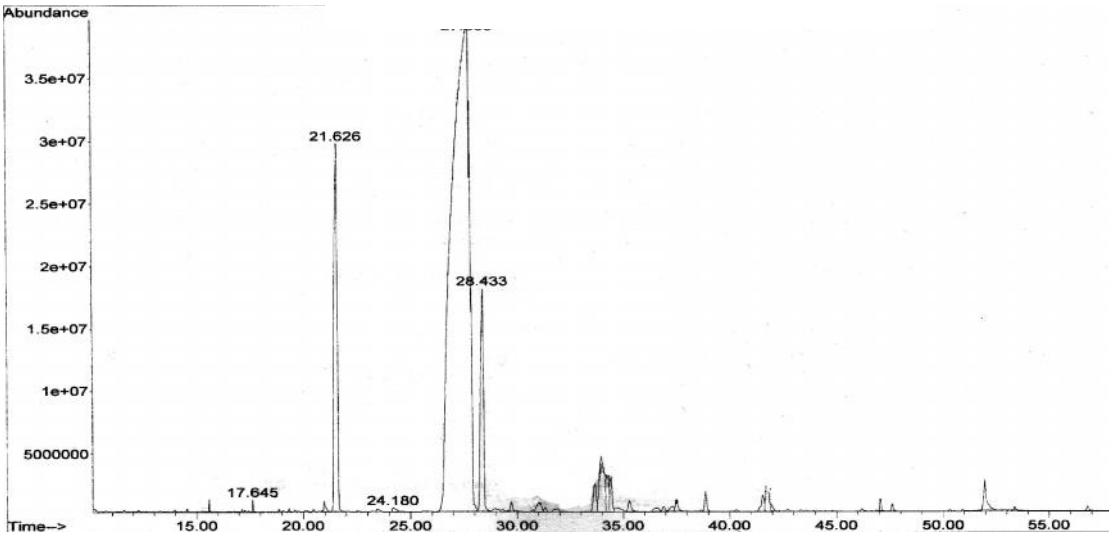

Figure 1. Gas liquid Chromatogram of methyl esters of fatty acids in *M. alba* L. seeds oil.

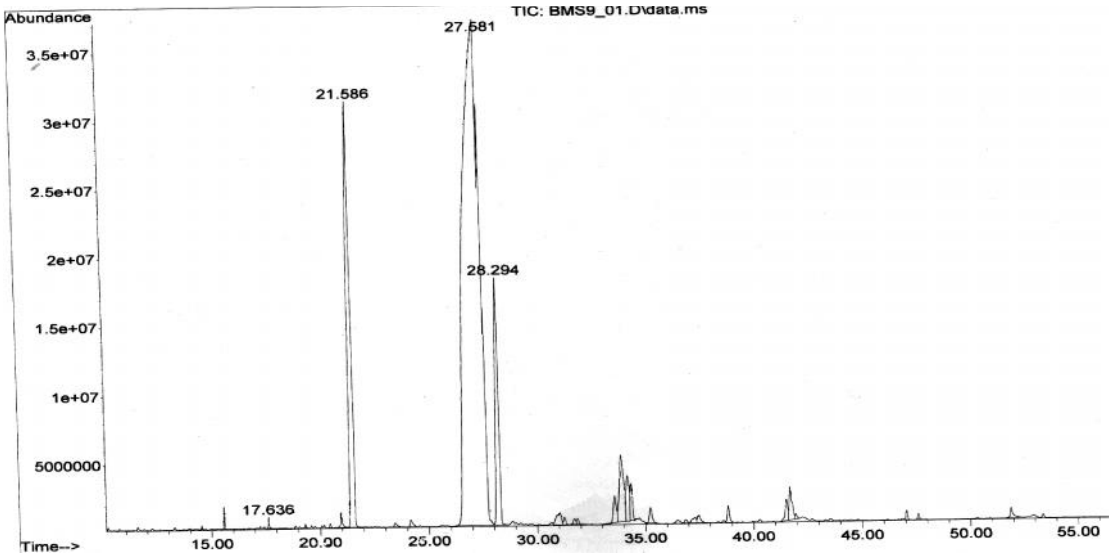

Figure 2. Gas liquid Chromatogram of methylesters of fatty acids in *M. nigra* L. seeds oil.

groups' e.g. neutral lipids, glycolipids and phospholipids as given in Table 2.

The fatty acid compositions of the oils were determined by GLC depicted in Figure 1and Figure 2 and documented in Table 3. It was indicated that the unsaturated fatty acids present in the oils of two mulberry verities were mainly linoleic (C18:2) acid 74.29% and 71.14% being almost similar to safflower seed oil (Rafiquzzaman et al., 2006) and also black mulberry (*M. nigra* L.) seed oil (Umit et al., 2011). Thus the two verities seeds oils are good source of the essential fatty acid, linoleic acid. The saturated fatty acids present in the oils of two mulberry verities *M. alba* L.and *M. nigra* L. were myristic ($C_{14:0}$) 0.07%, 0.07%; palmitic ($C_{16:0}$) 10.60%, 11.82% and stearic ($C_{18:0}$) 5.61%, 6.53% as shown in Table 3 and Figure 1 and Figure 2, respectively, which were concordant with black mulberry (*M. nigra* L.) seed oil oil (Umit et al., 2011). As shown in Table 4 the moisture contents of *M. alba* and *M. nigra* L. seeds were found to be 3.96 and 4.60%, respectively, approximately similar to the value 3.10% for *Trichosanthes cucumerina* seed (Yusuf et al., 2007). Ash contents were estimated as 6.05% in *M. alba* L. and 5.10% in *M. nigra* L.. This finding was higher than the ash content of grape seed i.e. 2.2% reported by (Kamel and Dawson, 1985). Plant absorbs minerals during growing season. The ash content depend not only on variety but also on the growing conditions e.g. soil, geographical condition. Crude fiber content estimated as 23.41% in *M. alba* L. and 25.72% in *M. nigra* L. were much more higher than the value 8.0% (Yusuf et al., 2007) for *Trichosanthes cucumerina* seeds. The lipid content was 29.04% in *M. alba* L. and 27.15% in *M. nigra* L. being much higher than 4.0% in *Casia fistula* seed (Zakas et al., 1988). Protein content of the seeds was in agreement with the results of previous studies on mulberries (Umit et al., 2011 and Xiaolan et al., 1998) in which 21.2% and 29.4% crude proteins were found, respectively. The protein content of the grape seeds was 8.2% (Kamal et al., 1985) which are lower than mulberry seeds found in present study. Also the mulberry seeds of two verities contain protein content similar to cotton seed which was found to be 22.32% by (Sawan et al., 1989). The total carbohydrate of two plant verities is nearly similar to black mulberry (*M. nigra* L.) seed oil (Umit et al., 2011).

As observed in Table 5, all the extracts obtained from *M. alba* L. seeds showed moderately effective against most of the tested bacteria. DMSO extract showed inhibitory effect only against gram negative *Escherichia coli* and *Shigella dysenteriae*. Petroleum ether extract was found to be inactive against the entire organism. Chloroform extract revealed maximum activity against *Escherichia coli* whereas methanol extract displayed higher activity towards *Shigella dysenteriae.* But the standard antibiotic Kanamycin was found to be strongly active against the gram negative *E. coli* and *Shigella dysenteriae.*

From Table 6, it is found that DMSO extract showed activity against gram negative *E. coli* and *Shigella dysenteriae.* Pet-ether extract was found to be inactive against all the microorganisms. Chloroform extract showed moderate activities against *E. coli* and *Shigella dysenteriae* whereas methanol extract displayed higher activity towards *E. coli.* But the standard antibiotic Kanamycin was found to be strongly active against all the tested organisms.

Conclusion

The physicochemical characteristics of the seeds oils of the two mulberry varieties can be helpful to indentify the quality of the oil and its products for possible industrial or commercial uses. The mulberry seeds oils contained high percentage of unsaturated fatty acids as compared to saturated fatty acids which is the characteristics of vegetable oils. From this quality point of view the seeds oils reported herein are comparable to other oils and can be utilized in paint, varnish and ink industries and also recommended for human consumption after properly refining. The seeds of two varieties are important nutritional sources for the human beings and animal. Protein content also recommended the seeds as a nutritive complement and higher amount of lipids major them a good sources for industrial uses. On the basis of data obtained from microbiological investigations, conclusion may be drawn that the crude extract from *M. alba* L. and *M. nigra* L. seeds may be used as drug to treat the diseases caused by those organisms, which are sensitive to the above mentioned samples. But before use in human being isolation of pure compound, toxicological study and clinical trial in animal model should be carried out thereafter.

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