

PHYSICOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF *TRICHOSANTHES ANGUINA* AND *SWIETENIA MAHAGONI* SEEDS

M.A. Ali¹, M.A. Sayeed^{2*}, M.S. Islam², M.S. Yeasmin³, G.R.M.A.M. Khan³ and Ida I. Muhamad¹

¹Department of Bioprocess Engineering, Faculty of Chemical Engineering, Universiti Teknologi Malaysia, UTM Skudai, 81310 Johor, Malaysia

²Department of Applied Chemistry and Chemical Engineering, Rajshahi University, Rajshahi-6205, Bangladesh

³BCSIR Laboratories, Rajshahi-6206, Bangladesh

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ABSTRACT. The core objective of this research was to determine the characteristics of oils, nutritional composition and antimicrobial activities of *T. anguina* and *S. mahagoni* seeds. Physicochemical characteristics of oils implied higher degree of unsaturation in *T. anguina* whereas, *S. mahagoni* oil contained appreciable level of unsaturation. Tri-acyl-glycerols and neutral lipids were found to be most abounded components recorded to 86.2 and 91.3% for *T. anguina* and 87.0 and 89.4% for *S. mahagoni*, respectively. GLC analysis showed the presence of fatty acids from series C_{16:0} to C_{18:3} for *T. anguina* and C_{16:0} to C_{20:0} for *S. mahagoni* in which principal fatty acids accounted as punicic (45.1%) in *T. anguina* and linoleic (30.1%) in *S. mahagoni* seed oils. Of the major energy producing nutrients, the seed samples contained large amounts of lipid (36.1% in *T. anguina* and 57.9% in *S. mahagoni*) and protein (20.9% in *T. anguina* and 13.0% in *S. mahagoni*) and potentially useful amounts of other nutrients. The knowledge from the nutritional analysis could be important to its appropriate industrial use and for improvement in the nutritional value. Assessment of the anti-microbial studies reported herein revealed the crude extracts of *T. anguina* and *S. mahagoni* seeds were not significantly inhibition against most of the tested bacteria and fungi. This study may lead to further ethno-pharmacognostic investigations to identify new compounds with therapeutic promise.

KEY WORDS: Seed oil, Fatty acids, Nutritional composition, Antimicrobial activities

INTRODUCTION

Trichosanthes anguina Linn., belongs to the family Cucurbitaceae, cultivated in India for its fruits which has been disseminated in several parts of South East Asia and East Africa. It is a warm-seasoned quick-growing crop but, depending upon locality, it can be grown more or less throughout the year in the plains [1]. The seeds are used as an anthelmintic and antidiarrhoeic and also used for the treatment of biliousness and syphilis [2]. From the nutritional point of view, the fruits from *T. anguina* are important sources of nutrients necessary for human and animal health [3]. On the other hand, *Swietenia mahagoni* Jacq. is an evergreen to semi-evergreen hardwood timber species of the family Meliaceae, having seeds chestnut brown in colour, 4-5 cm long, compressed, crested and extended into a wing at the attachment end [4]. *S. mahagoni* is widely planted in homesteads, roadsides and marginal lands. The seed extracts have been accounted to possess cytotoxic [5] and anti-microbial properties [6]. It may also be used as a potential agent for diabetic's therapy due to its agonistic activity [7]. The seeds of *S. mahagoni* are a good agricultural product and have been found potentially rich in fat (64.9%) as reported by Kleiman and Payne-Wahl [8]. Several works have been conducted on the physicochemical properties and anti-microbial activities of *T. anguina* [2, 9, 10] and *S. mahagoni* [5-8].

The present work generates special data on the analysis of *T. anguina* and *S. mahagoni*, including physicochemical characteristics of oils, their nutrient contents and anti-microbial

*Corresponding author. E-mail: drmdabusayeed@gmail.com

activities of various extracts of seeds of *T. anguina* and *S. mahagoni*. This investigation will be useful to identify the bio-active compounds of the seeds, which may be responsible for the therapy of several infectious and metabolic diseases of man and animals.

EXPERIMENTAL

Plant materials and chemicals

The seeds of *T. anguina* were purchased from the local market of Rajshahi city and ripe fruits of *S. mahagoni* were harvested from the plants grown in Rajshahi University campus, Bangladesh. The seeds of *S. mahagoni* were separated from the fruits manually, and washed several times with water to remove the foreign materials. Afterwards, seeds from both the plants were dried in sunlight for four consecutive days and then in an electric oven at 45 °C until the seeds reached to a constant weight. The seeds were ground to a fine powder, packaged, and stored in a refrigerator at 4 °C prior to analysis. Solvents were obtained from Merck (Germany) and BDH (England). Silica gel (60-120 mesh) and Silica gel (HF₂₅₄) were products of Merck (Germany). Esters 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 oil from the powdered seeds of each of the plants was extracted separately with light petroleum ether (40-60 °C) in a soxhlet apparatus for about 24 h and the solvent was removed by rotary vacuum evaporator (Buchi Labortechnik AG, Postfach, Switzerland). The percentage of oil content was computed.

For anti-microbial studies, powder from each of the plant materials 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 and the combined extract was filtered and concentrated under a vacuum evaporator. Extraction was carried out successively with ethyl acetate and methanol from the residue left after extraction with petroleum ether applying the same procedure as mentioned above.

Physico-chemical characteristics of the oils

Specific gravity of the oil was determined at 34 °C with the help of a pycnometer (Cole-Parmer, Illinois, USA). Refractive index of the clear oil was determined at 27 °C using Abbe Refractometer (ATAGO T-series, Model-3T, Texas, USA) following IUPAC [11] method. ASTM [12] testing methods were followed for determining pour, flash, fire and cloud points. Smoke point was estimated according to the method of AOCS [13]. Iodine value (Wijs) and unsaponifiable matters were determined by the methods depicted by Ranganna [14] while the saponification value, saponification equivalent, acid value, percentage of free fatty acid (FFA), ester value, and peroxide value were determined according to the methods described by Williams [15].

Separation of acylglycerols

The oil was separated into mono-, di- and tri-acylglycerols 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.* [16]. For quantitative determination of acylglycerol classes, the sample (675 mg in 3 mL petroleum ether) was adsorbed on the top of the column; tri-acylglycerols were eluted with benzene, di-acylglycerols with a mixture of diethyl ether and benzene (1:9, v/v), and mono-

acylglycerols with di-ethyl ether. Approximately 1.5-2 mL/min fractions were collected. Elution was monitored by thin layer chromatography (TLC). Purities of the separated fractions were confirmed by TLC, using silica gel and hexane-di-ethyl ether 80/20 (v/v) as solvent system. Spots were visualized with chromic-sulphuric acid at 180 °C.

Fractionation of lipids

A total of 700 mg lipid extracted from the seeds of both plants by the method of Bligh and Dyer [17] was fractionated into three major lipid groups: neutral lipid, glycolipid, and phospholipid by silica gel column chromatography [18]. Neutral lipids were eluted with chloroform, glycolipids with acetone and phospholipids with methanol. Approximately 0.5-1.0 mL fractions were collected per minute and elution was monitored by TLC. Solvents were evaporated in vacuum rotary evaporator and percentages of these fractions were determined by gravimetric method.

Fatty acid composition of oils

Fatty acid composition of *S. mahagoni* seed oil was determined as their methyl esters prepared by boron-trifluoride methanol complex method [19]. A GCD PYE Unicam gas chromatograph (PYE Unicam Ltd., Cambridge, UK) equipped with a flame ionization detector was used to determine the fatty acid methyl esters. Nitrogen carrier gas was used at a flow rate of 30 mL per min. Fatty acids were separated on a 1.8 m × 2 mm i.d. glass column packed with 6% BDS (butanediol succinate polyesters) on solid support, Anakorm ABS (100/120) mesh. Analysis was carried out at isothermal column temperature 190 °C, injector and detector temperatures for GLC analysis were 230 °C. On the other hand, the oil from *T. anguina* seed was converted to methyl esters with NaOMe-MeOH and analysed by GLC (Model 5890, Hewlett Packard) equipped with a flame ionization detector and SE-30 column at 190 °C. The identification of punicic acid in *T. anguina* seed oil was achieved according to the methods described elsewhere [10, 20].

Analysis of T. anguina and S. mahagoni seeds

Moisture, ash, and crude fiber contents were determined by the methods depicted by Ranganna [14]. Lipid content was estimated by the method of Bligh and Dyer [17] using a solvent mixture of chloroform and methanol (2:1 v/v). The crude protein was determined by the micro-Kjeldahl method and a conversion factor of 6.25 was used to quantify the crude protein content [21]. Water soluble protein was determined by the method of Lowry *et al.* [22] using bovine serum albumin as the standard. Determination of starch content was based on analytical method outlined elsewhere [23]. Free sugar content was determined by colorimetric method [24] and total carbohydrate was calculated by the difference [25].

Anti-microbial screening

Various solvent extracts such as petroleum ether, ethyl acetate and methanol of *T. anguina* and *S. mahagoni* seeds were tested against 6 pathogenic bacteria and fungi each by the standard disc diffusion method [26-28]. Nutrient agar medium was used for determining anti-bacterial activity whereas potato dextrose agar medium (PDA) was selected for anti-fungal activity. Kanamycin (30 µg/disc) and fluconazole (50 µg/disc) were used as standard for comparison in anti-bacterial and anti-fungal tests, respectively.

The crude extracts were dissolved in sufficient amount of the respective solvents, so that each 10 µL of solutions contained 300 µg of the test materials for anti-microbial activity. The

anti-microbial 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 anti-biotic disc used.

RESULTS AND DISCUSSION

The solvent extracts of *T. anguina* and *S. mahagoni* seeds yielded 31.0 and 49.0% oil, which are almost near to the reported values 29.1% [29] and 47.5% [6], respectively. The information on detailed characteristics of seed oils and nutritional compositions of seeds from same species are too scanty for meaningful comparisons.

Physico-chemical characteristics

As can be seen from Table 1, the specific gravities of *T. anguina* and *S. mahagoni* seed oils were determined to be 0.9276 and 0.9169 at 34 °C, being higher than 0.8640 at 40 °C [30] for *Trichosanthes cucumerina* and lower than 0.9334 at 30 °C for *S. mahagoni* seed oils [6]. Refractive indices were evaluated to be 1.4878 at 27 °C for *T. anguina* and 1.4683 at 27 °C for *S. mahagoni* seed oils that are lowered compare to the value 1.4979 at 25 °C for *T. anguina* seed oil reported elsewhere [29]. The specific gravity and refractive index of *S. mahagoni* seed oil are lower than those in the present sample of *T. anguina*. Specific gravity and refractive index are very stable parameters and should be used for checking the identity of oils. Smoke point of *S. mahagoni* seed oil could not be determined due to the oil low burning characteristics, soaking tendency, fluidity, etc. Flash and fire points as determined from *T. anguina* seed oil (331 and 361 °C) were higher than those of the experimental sample *S. mahagoni* (90 and 100 °C) and of tobacco seed oil (142 and 162 °C) [31]. Cloud and pour points of *S. mahagoni* seed oil (6 and -6 °C) appeared to be higher than the values estimated from the experimental sample *T. anguina* (1.10 and -7 °C) and also from the tobacco seed oil (-15 and -18 °C) detected by Abbas Ali *et al.* [31]. Smoke, fire and flash points of the fatty material are measured of its thermal stability when heated in contact with the air. Fatty acids are much less stable than acylglycerols; hence the smoke, fire and flash points of ordinary oils depend principally upon their content of free fatty acids [32].

Table 1. Physical characteristics of *T. anguina* and *S. mahagoni* seed oils.

Characteristics	<i>T. anguina</i>	<i>S. mahagoni</i>
Specific gravity at 34 °C	0.9276 ± 0.0023	0.9169 ± 0.0054
Refractive index at 27 °C	1.4878 ± 0.0041	1.4683 ± 0.0048
Cloud point (°C)	1.1 ± 0.10	6.0 ± 0.50
Pour point (°C)	-7.0 ± 0.20	-6.0 ± 0.30
Flash point (°C)	331.0 ± 3.0	90.0 ± 1.1
Fire point (°C)	361.0 ± 2.8	100.0 ± 1.0
Smoke point (°C)	210.0 ± 1.6	*

Values are mean ± standard deviation of three experiments. *Smoke points could not be determined due to their low burning characteristics, soaking tendency, fluidity, etc.

As depicted in Table 2, iodine value of the *T. anguina* seed oil was found to be 127.6; being same to the value 127.3 reported by Adebooye [33], but much higher than the value 38.0 [30] for *Trichosanthes cucumerina* seed oil. Iodine value estimated for *S. mahagoni* seed oil (92.5) was lower than the value 109.7 for *Swietenia macrophylla* seed oil [34] and was consistent with the value 94.7 for the same oil [6]. Therefore, the sample from *T. anguina*, in contrast to the

sample of *S. mahagoni* under investigation has greater tendency to become rancid by oxidation. Saponification values were 187.7 and 200.3 while saponification equivalents calculated to be 298.8 and 280.3 for *T. anguina* and *S. mahagoni* seed oils respectively. The saponification value of *T. anguina* was lower than the values 195.6 and 127.3 for *Trichosanthes cucumerina* [30, 33] and that of *S. mahagoni* was higher compared to 191.8 for *Swietenia macrophylla* [34] seed oils. This comparatively low saponification value as estimated from *T. anguina*, indicated the presence of a higher proportion of higher fatty acids compared to the *S. mahagoni* seed oil. The percentages of free fatty acids (4.2 in *T. anguina* and 5.6 in *S. mahagoni*) are higher than the values 2.5 of *Trichosanthes cucumerina* [30] and 0.60 of *Swietenia macrophylla* [34] seed oils. The present value from *S. mahagoni* was higher than the value 3.2 cited in the literature [6] for the same oil. Ester values of the samples were calculated as 179.3 for *T. anguina* and 198.2 for *S. mahagoni* seed oils from acid value and saponification value, which were differed from each other.

Table 2. Chemical characteristics of *T. anguina* and *S. mahagoni* seed oils.

Characteristics	<i>T. anguina</i>	<i>S. mahagoni</i>
Iodine value (Wijs)	127.6 ± 1.7	92.5 ± 1.3
Saponification value (mg KOH/g)	187.7 ± 1.0	200.3 ± 2.6
Saponification equivalent	298.8 ± 1.7	280.3 ± 2.1
Acid value (mg KOH/g)	8.4 ± 0.41	11.1 ± 0.22
Free fatty acids (%) as oleic	4.2 ± 0.31	5.6 ± 0.25
Ester value	179.4 ± 1.7	198.2 ± 1.8
Unsaponifiable matter (%)	1.5 ± 0.31	1.9 ± 0.23
Peroxide value (meq/kg)	2.2 ± 0.61	2.6 ± 0.34

Values are mean ± standard deviation of three experiments.

The unsaponifiable matter contents were found to be 1.5% in *T. anguina* and 1.9% in *S. mahagoni* seed oils. The present value for *S. mahagoni* was higher than the values 1.1% for *Swietenia macrophylla* seed oil [34] and 0.52% for the same seed oil [6]. Comparatively higher value for unsaponifiable matters as obtained in the sample of *S. mahagoni* indicate higher amount of hydrocarbons, higher alcohols and sterols than those contained in seed oil of *Trichosanthes anguina*. The seed oils of *T. anguina* and *S. mahagoni* displayed the peroxide values of 2.2 and 2.6 meq/kg, respectively, which were determined in normal laboratory conditions. The peroxide value of *T. anguina* was slightly higher than the value 2.9 for *Trichosanthes cucumerina* [33] seed oil. Present experimental results revealed that both the seed oils are quality oil.

Acylglycerol and lipid composition

As shown in Table 3, tri-acylglycerols of the total weight of oil accounted as 86.2% for *T. anguina* and 87.0% for *S. mahagoni*. The total recovery of acylglycerol of *T. anguina* and *S. mahagoni* seed oils were 92.5 and 91.4% (average), respectively that indicated the seed oils contained higher amount of non-acylglycerol than that of found in rice bran oil [35]. Of the two samples, seed oil of *T. anguina* contained higher percentage of mono-acylglycerol that can be separated easily by column chromatography and used as emulsifier. Neutral lipids account for 91.3% in *T. anguina* and 89.4% in *S. mahagoni* of total lipids while only 4.5 % in *T. anguina* and 4.8% in *S. mahagoni* glycolipids were detected. Phospholipids make up only 2.4% in *T. anguina* and 3.5% in *S. mahagoni* of total lipids. Results indicated that neutral lipids were found to be most abundant component of seed lipid constituted to over 91.0% for *T. anguina* and 89.0% for *S. mahagoni* of the total weight of the lipid. In this study, the seeds from both

samples were found to contain lower amount of neutral lipids compared to the results obtained for *Sesamum indicum* [36] and *Basella rubra* [37] seed lipids.

Table 3. Acylglycerol and lipid composition (wt %) of *T. anguina* and *S. mahagoni* seeds.

Parameter	Composition	<i>T. anguina</i>	<i>S. mahagoni</i>
Acylglycerol	Monoacylglycerol	2.2 ± 0.60	1.4 ± 0.23
	Diacylglycerol	4.1 ± 0.21	3.0 ± 0.13
	Triacylglycerol	86.2 ± 0.44	87.0 ± 0.61
Lipid	Neutral lipids	91.3 ± 0.61	89.4 ± 1.27
	Glycolipids	4.5 ± 0.55	4.8 ± 0.34
	Phospholipids	2.4 ± 0.11	3.5 ± 0.30

Values are mean ± standard deviation of three experiments.

Fatty acid composition

Results of fatty acid composition shown in Table 4 reflected to be fatty acid range C_{16:0} to C_{18:3} in *T. anguina* and C_{16:0} to C_{20:0} in *S. mahagoni* seed oils containing saturated and unsaturated fatty acids. The fatty acids found were palmitic, stearic, oleic, linoleic and punicic for *T. anguina* and palmitic, stearic, arachidic, oleic, linoleic and linoleinic for *S. mahagoni* seed oils. These results partially agree with the findings of other [8, 10, 29]. The differences in our results from them could be explained by variations in soil and climatic conditions. The linoleic acid enriched in fatty acid profile (20.1% in *T. anguina* and 30.1% in *S. mahagoni*), may be the precursor of prostaglandins (known to occur in accessory genital gland, seminal plasma and lung tissue of human body) and play a vital role in human health [37]. Regarding the unsaturated fatty acids in *T. anguina*, punicic acid was the major one, its percentage being 45.1, which was higher than the value 42.80% detected by Chisholm and Hopkins [29] and was lower than the value 46.30% reported by Lakshminarayana *et al.* [10] with the same seed oil. The punicic acid content in *T. anguina* was lower than the value 51.7% in *Trichosanthes nervifolia*, but higher than the value 41.8% of *Trichosanthes bracteata* seed oils reported by Lakshminarayana *et al.* [38]. Polyunsaturated fatty acids are very important for human nutrition. Linoleinic acid was found to be 13.5% in *S. mahagoni* seed oil. In the saturated fatty acids profile, stearic acid was the highest estimated as 11.6% in *T. anguina* and 15.8% in *S. mahagoni* seed oils. GLC data also indicated that the seed oils contained mainly unsaturated fatty acids 82.0% and 69.5%, while saturated fatty acids were found to be present at 18.0% and 30.5% for *T. anguina* and *S. mahogany* seed oils, respectively.

Table 4. Fatty acid composition (%) of *T. anguina* and *S. mahagoni* seed oils.

Fatty acids	<i>T. anguina</i>	<i>S. mahagoni</i>
Palmitic acid (C _{16:0})	6.4 ± 0.14	13.6 ± 0.11
Stearic acid (C _{18:0})	11.6 ± 0.50	15.8 ± 0.26
Arachidic acid (C _{20:0})	nd	1.1 ± 0.10
Oleic acid (C _{18:1})	16.8 ± 0.21	25.9 ± 0.19
Linoleic acid (C _{18:2})	20.1 ± 0.67	30.1 ± 0.16
Linoleinic acid (C _{18:3})	nd	13.5 ± 0.12
Punicic acid (C _{18:3})	45.1 ± 0.45	nd

Values are mean ± standard deviation of three experiments. nd = not detected.

Nutritional composition

As shown in Table 5, the moisture contents of *T. anguina* and *S. mahagoni* seeds were found to be 7.5 and 15.2%, respectively, being higher than the value 3.1% for *Trichosanthes cucumerina* seed [30]. The lipid contents ranged from 36.1% in *T. anguina* to 57.9% in *S. mahagoni* seeds; these are much higher than the value 4.0% in *Cassia fistula* seed [39]. Ash contents were estimated as 4.4% in *T. anguina* and 2.8% in *S. mahagoni*. Total protein (N×6.25) of *T. anguina* seeds was estimated to be 20.9% of which 6.9% was water soluble, and this value for total protein was higher than *Trichosanthes nervifolia* (16.7%) and *Trichosanthes bracteata* (18.8%) seeds reported by Lakshminarayana *et al.* [10], but lower than *Trichosanthes cucumerina* (27.2%) seeds reported by Adebooye [33]. Total protein (N×6.25) of *S. mahagoni* seeds was 13.0% of which 7.5% was water soluble. The protein content in *S. mahagoni* seeds agrees with the value 13.5% reported by Kleiman and Payne-Wahl [8] for the same seed oil. Findings in the present results revealed that the *T. anguina* seed is qualified as protein-rich to satisfy the protein needs of the consuming population. Starch and free sugar contents were estimated to be 7.3 and 2.9% for *T. anguina* and 4.2 and 1.9% for *S. mahagoni* seeds. The present values for starch contents are much lower than the value 22.7% for tiger nut [15]. Crude fiber contents estimated as 1.9% in *T. anguina* and 1.4% in *S. mahagoni* were lower than the value 8.0% [30] for *Trichosanthes cucumerina* seeds. Carbohydrate contents were determined to be 29.2% for *T. anguina* and 9.7% for *S. mahagoni* seed oils.

Table 5. Nutritional composition of *T. anguina* and *S. mahagoni* seeds.

Parameters (g/100 g)	<i>T. anguina</i>	<i>S. mahagoni</i>
Moisture	7.5 ± 0.13	15.2 ± 0.14
Lipid	36.1 ± 0.39	57.9 ± 1.2
Ash	4.4 ± 0.19	2.8 ± 0.12
Total protein	20.9 ± 0.78	13.0 ± 0.23
Water soluble protein	6.9 ± 0.14	7.5 ± 0.11
Starch	7.3 ± 0.27	4.2 ± 0.56
Free sugar	2.9 ± 0.23	1.9 ± 0.11
Crude fiber	1.9 ± 0.10	1.4 ± 0.45
Total carbohydrate	29.2	9.7

Values are mean ± standard deviation of three experiments.

Anti-microbial activities

As shown in Table 6, petroleum ether, ethyl acetate and methanol extracts of *S. mahagoni* seeds have been shown to be mild to moderately effective against most of the tested bacteria. The gram negative bacteria such as *Shigella shiga* were resistant against the crude extracts ethyl acetate and methanol whereas methanol extract did not show any inhibitory effect against *Shigella boydii*. The results were compared with those of kanamycin as a standard antibiotic. Petroleum ether extract showed higher activity against *Staphylococcus aureus* (12 mm) whereas, ethyl acetate displayed maximum towards *Streptococcus β-haemolyticus* (13 mm) and *Bacillus subtilis* (14 mm). The activities were more than two-third to that of standard. But the activities, on overall consideration of the extracts against gram positive bacteria were better as compared to those against gram negative bacteria. All of the three extracts (petroleum ether, ethyl acetate and methanol) from *T. anguina* seeds displayed weak activities against most of the tested bacteria (Table 7). Petroleum ether extract was found to be active against all the tested bacteria. Ethyl acetate extract showed maximum antibacterial effect against *Bacillus subtilis* with inhibition diameter 14 mm that is two-third to that of standard.

Table 6. Antibacterial activities of different extracts of *S. mahagoni* seeds.

Test organisms	Diameter of zone of inhibition in mm			
	PES	EES	MES	STK
Gram positive				
<i>Staphylococcus aureus</i>	12	8	9	22
<i>Streptococcus β-haemolyticus</i>	10	13	8	23
<i>Bacillus subtilis</i>	9	14	10	21
Gram negative				
<i>Klebsiella species</i>	8	7	6	22
<i>Shigella shiga</i>	7	0	0	23
<i>Shigella boydii</i>	8	8	0	21

PES = petroleum ether extract (300 μ g/disc); EES = ethyl acetate extract (300 μ g/disc); MES = methanol extract (300 μ g/disc) and STK = kanamycin (30 μ g/disc).

Table 7. Antibacterial activities of different extracts of *T. anguina* seeds.

Test organisms	Diameter of zone of inhibition in mm			
	PES	EES	MES	STK
Gram positive				
<i>Staphylococcus aureus</i>	8	7	7	22
<i>Streptococcus β-haemolyticus</i>	6	7	9	23
<i>Bacillus subtilis</i>	11	14	0	21
Gram negative				
<i>Klebsiella species</i>	9	0	8	20
<i>Shigella shiga</i>	8	0	6	23
<i>Shigella boydii</i>	7	6	6	21

PES = petroleum ether extract (300 μ g/disc); EES = ethyl acetate extract (300 μ g/disc); MES = methanol extract (300 μ g/disc) and STK = kanamycin (30 μ g/disc).

Table 8 demonstrate that all the crude extracts (petroleum ether, ethyl acetate and methanol) obtained from *S. mahagoni* seeds displayed weak activities against most of the tested fungi. Methanol extract did not show any activity against *Trichoderma viride* and *Helminthosporium sativum*. Petroleum ether extract showed maximum anti-fungal effect against *Helminthosporium sativum* with inhibition diameter 14 mm that is more than that of standard. From Table 9, it is evident that ethyl acetate and methanol extracts showed little activities against all of the tested fungi. The inhibitory effect of petroleum ether extract was maximum (14 mm) against *Aspergillus niger* that is equal to that of standard anti-fungal agent fluconazole. The fungus *Aspergillus flavus* did not show any effect towards any crude extracts.

Table 8. Antifungal activities of different extracts of *S. mahagoni* seeds.

Test organisms	Diameter of zone of inhibition in mm			
	PES	EES	MES	STF
<i>Penicillium sp.</i>	12	10	8	16
<i>Aspergillus niger</i>	10	9	6	14
<i>Trichoderma viride</i>	7	6	0	13
<i>Aspergillus flavus</i>	7	6	6	12
<i>Candida albicans</i>	8	6	9	14
<i>Helminthosporium sativum</i>	14	8	0	13

PES = petroleum ether extract (300 μ g/disc); EES = ethyl acetate extract (300 μ g/disc); MES = methanol extract (300 μ g/disc) and STF = fluconazole (50 μ g/disc).

Table 9. Antifungal activities of different extracts of *T. anguina* seeds.

Test organisms	Diameter of zone of inhibition in mm			
	PES	EES	MES	STF
<i>Penecillum sp.</i>	10	8	9	16
<i>Aspergillus niger</i>	14	8	6	14
<i>Trichoderma viride</i>	8	9	0	13
<i>Aspergillus flavus</i>	0	0	0	12
<i>Candida albicans</i>	8	9	6	14
<i>Helminthosporium sativum</i>	11	9	8	13

PES = petroleum ether extract (300 µg/disc); EES = ethyl acetate extract (300 µg/disc); MES = methanol extract (300 µg/disc) and STF = fluconazole (50 µg/disc).

The anti-microbial activities of different extracts reported herein were not significantly enough against most of the tested organisms. During screening, it was found that the crude methanol extracts of *T. anguina* and *S. mahagoni* displayed lower activities against most of the tested organisms compared to those of rest extracts. The ethyl acetate extracts of both plants showed maximum activities against *Bacillus subtilis* whereas the fungi *Aspergillus flavus* was found resistant against all the extracts of *T. anguina* seeds.

CONCLUSIONS

The physicochemical characteristics of the seed oils from *T. anguina* and *S. mahagoni* can be helpful to identify the quality of oil and oil products for possible industrial or commercial uses. The seed oils of *T. anguina* contained higher percentage of unsaturated fatty acids as compared to saturated fatty acids which is the characteristics of vegetable oils. From the quality point of view the seed oils reported herein, are comparable to other oils and can be utilized in the paint, varnish and ink industries and also recommended for *T. anguina* seed oil to human consumption after properly refining. The findings also imply that *T. anguina* seeds represent potentially useful and important nutritional sources for the people of Bangladesh. Protein content also commends *T. anguina* seed as a nutritive complement. On the other hand, the under utilized seeds of *S. mahagoni* contained higher amount of lipid which makes a good source for industrial uses. On the basis of data obtained from microbiological investigations, conclusion may be drawn that the crude extracts from *T. anguina* and *S. mahagoni* seeds may be used as drug to treat the disease 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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