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Comparative study on the Physico-chemical and antibacterial characteristics of *Tamarindus indica* and *Momordica charantia* seed lipid

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Abstract
Seeds of *Tamarindus indica* and *Momordica charantia* were crushed by mechanical grinder and soxhlet extractor was used for lipid extraction using petroleum ether as solvent. Both cruse lipids were liquid in state and dark brown in color. *Momordica charantia* seed is a better source of lipid than *Tamarindus indica* seed as compared to their crude lipid content. *Momordica charantia* seed lipid can be better raw material than *Tamarindus indica* seed lipid for soap industries. Both seed lipids fall in the nondrying oil category indicating the lipids do not harden when they are exposed to air. *Tamarindus indica* seed lipid is high acidic than *Momordica charantia* seed lipid. *Tamarindus indica* seed lipid has a greater tendency for rancidity than *Momordica charantia* seed lipid. *Momordica charantia* seed lipid is suitable and *Tamarindus indica* seed lipid is not suitable for edible purposes. Both lipids are viscous in nature. Both seed lipid can be used as a mild antibacterial substance for skin infection, food poisoning, typhoid fever.

Keywords: *Tamarindus indica*, *Momordica charantia*, seed lipid, soxhlet extraction, physico-chemical test, antibacterial test

Introduction
Lipids are classes of compounds which are one of the main ingredients of our daily diet. They belong to the family of ester [1]. They are triglycerides of higher chain fatty acid. Fats are chiefly triglycerides of saturated fatty acid like lauric acid ($C_{11}H_{23}COOH$), palmitic acid ($C_{15}H_{31}COOH$), stearic acid ($C_{17}H_{35}COOH$), linolenic acid ($C_{17}H_{29}COOH$) etc. [2]. The oils and fats are chief raw materials for making bathing soaps. Soaps are generally the potassium or sodium salts of higher chain fatty acids. The conversion of these fatty acids to their potassium or sodium salts by treating with alkalis like potassium hydroxide or sodium hydroxide is known as saponification, or better, it is the alkaline hydrolysis of oils or fats [3]. An awareness of saponification value (Koettstorfer number) is an important parameter in the preparation of soap. Saponification value is also a measure of the molecular weight of the triglycerides in oils and fats. The triglycerides with high value of saponification value are considered to make better quality soaps than those with low saponification value. Saponification value is the presents and also to neutralize the free acid present in one gram of the fat or oil [3]. The long chain fatty acids founds in fats have low saponification value because they have relatively fewer number of carboxylic functional groups per unit mass of the fats, as compared to short chain fatty acids [4]. The acid value is the indirect measure of the amount of free acid. The higher the amount of fatty acid value, the higher the deterioration or rancidity of the oils and fats are undergone deterioration or rancid. As the rancidity is the hydrolytic or oxidative cleavage of triglycerides causing the formation of free acids in oils or fats [5]. Ester value is another important parameter when oils are considered. It is defined the number of milligrams of KOH required to combine with fatty acids presents in the glycerides from in 1 gm of oils or fat or it is a measure of actually how much amount of glycerides is present in a sample of oil, which is saponifiable [6]. In this paper we proposed to go through a comparative look into saponification values, acids values and ester values of sample lipids obtained from *Tamarindus indica* and *Momordica charantia* seed powder.
Tamarindus indica Linn (Commonly known as Tentul in Bangladesh) is a very delicious fruit. It has sweet and sour taste. Tamarind seed is a by-product of the commercial utilization of the

fruit [7]. The fruits are flattish, bean-like, irregularly curved and bulged pods and are borne in great abundance along the new branches and usually vary from 2 to 7 inches long and from 3/4 to 1 1/4 inches (2-3.2cm) in diameter. The seed comprises the seed coat or testa (20-30%) and the kernel or endosperm (70-75%). The seeds are hard, glossy-brown, and squarish in form, 1/8 to 1/2 in (1.1-1.25cm) in diameter and each is enclosed in a parchment like membrane [8]. The uses of tamarind are widely distributed throughout the world. The red outer cover of the tamarind seed cures diarrhea and dysentery effectively. Extracts of tamarind seeds are topically used for treating minor skin rashes. Its anti-inflammatory property eases out joint pain, especially in people suffering from arthritis. This also removes stain caused by tea, coffee, soda and smoking. Tamarind seed juice is a natural remedy to cure indigestion and increase bile production. Its rich dietary fiber helps digestion and is a great natural appetizer [9].

Momordica charantia (Bitter melon), it is used as a vegetable in many countries but since time immemorial, it is also used for administration of numerous ailments comprising ulcer, [10] diabetes mellitus [11, 12] and inflammation [13, 14] etc. It is innate to subtropical and tropical areas in Asia and some other parts of the globe. It belongs to family cucurbitaceous and in Bangladeshi cuisines it is usually pronounced as korolla. All portions of the *Momordica charantia* are palatable in nature but frequently grown for the fruit that is bitter of all [15]. The plant is herbaceous that grows around 5 m and bears simple/alternate leaves of 4-12cm with 3-7 deeply separate lobes. It is similar to a small cucumber, usually rectangle and oblong in shape and eaten green. Bitter gourd is filled with pulp and large flat seeds, which surrounding a comparatively thin layer of flesh [16, 17]. *Momordica charantia* also rich in various bioactive components containing minerals, alkaloids, vitamins, steroidal saponins, polypeptide, and aromatic volatile oil, apart from its usage as vegetable. It has ability to fight against numerous life style associated disorders, due to the presence of bioactive components [15, 18].

Advances of modern medicine, many infectious diseases once considered incurable and lethal are now amenable to treatment with antimicrobial agents [19]. The remarkably powerful and specific activity of antimicrobial drugs is due to their selectively for highly specific targets that are either unique to microorganisms. In this paper investigation also carried out on the action of those two seed lipids on *Salmonella typhi, Staphylococcus aureus* and *Pseudomona aurigonisa* with respect to a standard antibiotic.

Materials and Methods

Sample collection

The sample seeds of *T. indica* and *Momordica charantia* were collected freshly from Gollamary Seeds Vander, Gollamary, khulna, Bangladesh. Then the seeds were washed with distilled water then dried in sunlight for 7 days and then in an electric oven for 3 days at 40 ^{o}C. After removing the seed pod, the dried seeds were grinded by hand mortar followed by mechanical grinder to have powder. These seed powder samples was used for the extraction of lipid. Then the lipid was stored at Chemistry Discipline, Khulna University, and Khulna, Bangladesh for analysis.

Extraction of lipid from seed samples

Here, the lipid was extracted by soxhlet extractor. Fig 1 represents a soxhlet extractor in operation indicating its different parts. In this method the dried sample is placed in a packet of markin cloth, a porous cellulose thimble. The thimble is placed in an extraction chamber, which is suspended above a round bottom flask containing the solvent (petroleum ether) and below a condenser. The flask is heated by heating mental and the solvent evaporates and moves up into the condenser where it is converted into a liquid that trickles into the extraction chamber containing the sample. The extraction chamber is designed so that when the solvent surrounding the sample exceeds a certain level it overflows and trickles back down into the boiling flask. This process was continued for 36 hours. At the end of the extraction process, the round bottom flask containing the solvent and lipid is removed and the mixture containing petroleum ether and lipid was kept in a dry beaker that is allowed to keep in a steam bath at 65 ^{0}C until all the petroleum ether evaporates and left the dry lipid in the beaker. The mass of the remaining lipid is measured.

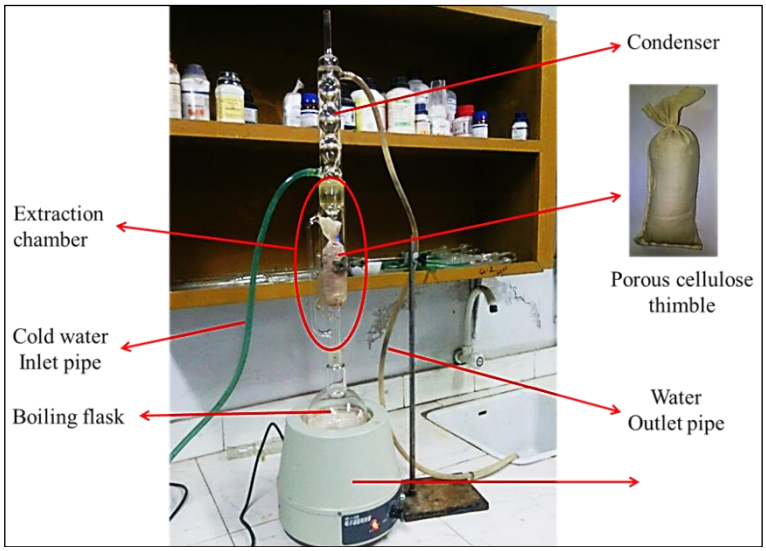

Fig 1: Lipid extraction is in progress by soxhlet extractor

Determination of total lipid content of seed samples

The percentage of lipid can be calculated by following equation-

$$\text{Percentage of lipid} = \frac{\text{Weight of lipid}}{\text{Weight of powder seed sample}} \times 100$$

Determination of saponification value of seed lipids

1.0 g of sample lipid was taken in a round bottom flask. 25 ml alcoholic KOH (0.5 M) was taken in it. The flask was connected to a reflux condenser and the mixture was reflux for half an hour with occasional shaking. When the mixture became homogeneous and no lipid remains either at the bottom or on the surface of the flask, the saponification was taken to be completed. The excess alkali was titrated with standard 0.25 M HCl, using phenolphthalein as indicator. 0.25 M HCl solution was prepared and was standardized with standard Na_2CO_3 solution using methyl orange as indicator. A blank experiment (without lipid) was performed with 25 ml alcoholic KOH (0.5 M). The difference between two titrations gives the amount of alkali required for saponification [20].

$$KOH + HCL \rightarrow KCL + H_2O$$

$$\text{Saponification value} = \frac{(V_1 - V_2) \times S \times 56.1}{W}$$

Where, V_1 is the volume of HCl solution used in blank test; V_2 is the volume of HCl solution used for test of lipid; S is the molarity of the standardized HCl solution used and W is weight of sample lipid in grams.

Determination of iodine value of seed lipids

1.0 g Oil was weighted in a conical flask. 10 ml of $CHCl_3$ was added and shake till the oil dissolved completely. 20 ml of Hanus solution was added. The flask was covered and was kept in the dark for 30 minutes with occasional shaking. Then 10 ml of 10% KI solution was added and stirred and then 100 ml of distilled water was added. The excess I_2 was titrated with 0.1 M $Na_2S_2O_3$ solution using starch as indicator near the end point [20]. A blank experiment (without oil) was performed in the same way as above [20]. The supplied $Na_2S_2O_3$ solution was standardized with standard $K_2Cr_2O_7$ solution.

$$2S_2O_3^{2-} + I_2 \rightarrow S_4O_6^{2-} + 2I^-$$

$$\text{Iodine value} = \frac{(V_1 - V_2) \times S \times 127 \times 100}{1000 \times W}$$

Where, V_1 is the volume of $Na_2S_2O_3$ solution required for blank experiment; V_2 is volume of $Na_2S_2O_3$ solution required for test of lipid; S is the molarity of the standardized $Na_2S_2O_3$ solution used and W is the weight of lipid in grams.

Determination of acid value and percentage of free fatty acid of seed lipids

1g seed lipid was placed in a dried conical flask. 25mL of absolute ethanol and 2-3 drops of phenolphthalein indicator was added. The mixture was shaken in water bath at 65˚C for 10 minutes. Then the mixture was cooled and was titrated against 0.1 M KOH until pink color appear at the end point [20].

$$RCOOH + KOH \rightarrow RCOOK + H_2O$$

$$\text{Acid value} = \frac{V \times M \times 56.1}{W}$$

Where, V is the volume of KOH used for test lipid; M is the molarity of KOH solution and W is the weight of lipid in grams.

The molar mass of oleic acid is 282 g. The percentage of free fatty acid with respect to oleic acid can be calculated by following equation [20].

$$\text{The \% of free fatty acid of seed lipid} = \text{Acid value} \times 0.503$$

Determination of ester value and percentage of glycerin of seed lipids

The ester value and percentage of glycerin can be calculated from the saponification Value (SV) and the acid Value (AV) [20].

$$\text{Ester value} = \text{Saponication value} - \text{Acid value}$$

$$\text{\% of glycerin} = \text{Ester value} \times 0.054664$$

Antibacterial test of seed lipids

Here, crude petroleum ether extract of seed lipids were taken as sample. Both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Salmonella typhi* and *Pseudomona aurigonisa*) bacterial strains were taken for the test. The antibacterial activity was evaluated by disc diffusion assay. It was done by following two steps. In first step: 2 mL of nutrient broth was taken in each screw capped vial where all of them were marked according to their strains. Two vials were taken for each strain. Then each vials containing nutrient broth were inoculated by the inoculating loop which looped single bacterial colony from the sub culture. These tasks were done in the laminar air flow cabinet. Then all the vials were allowed to incubate at 3-4 hours at 37 ºC. This was done to get the bacteria in their log phase at which the growth rate is very high. In the second step: The sterilized agar medium was poured into sterilized Petri dishes in such a way as to give a uniform layer of depth of approximately 4mm. After the medium became cooled at room temperature, it was stored in a refrigerator (4 ºC). Incubated vials log phase were removed from the incubation. Test tubes were shaken by rotation to get a uniform suspension of bacteria. Then 5µL nutrient broth containing bacteria was taken into corresponding solidified medium containing Petri dishes. The Petri dishes were rotated several times, first clock wise then anticlockwise to assure homogenous distribution of the test organisms. Thus the seeded plates were ready for disc diffusion assay. These tasks were done in the laminar air flow cabinet. All tasks were done in the laminar air flow cabinet.

0.0080gm each of lipid samples of were accurately measured and taken into screw capped vials. Then 10 mL of ethanol was added in the vial to get the sample of 800µg/mL and 400µg/mL. Filter papers previously sterilized were perforated by punch machine thus tiny discs of 5 mm diameter were obtained. Then the discs were taken in a blank Petri dish. 20µL of sample solution were applied on the discs with the help of micropipette in an aseptic condition from the stock solution. Then the discs were allowed to dry for a few minutes under the laminar to remove the solvent as it has some antimicrobial activity which can bias the evaluation. These were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antibacterial agent with that produced by test samples. Ciprofloxacin (5 µg/disc) standard disc were used as the reference for positive control. Blank discs were used a negative control. They ensure that the residual solvents (left over the discs even after air drying) and the filter paper were not active themselves.

After proper incubation, the antimicrobial activity of the test agent was determined by measuring the diameter of zone of inhibition in term of millimeter with a transparent scale. Fig 2 represents the antibacterial activity of two seed lipids against *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomona aurigonisa* with respect to Ciprofloxacin as positive control.

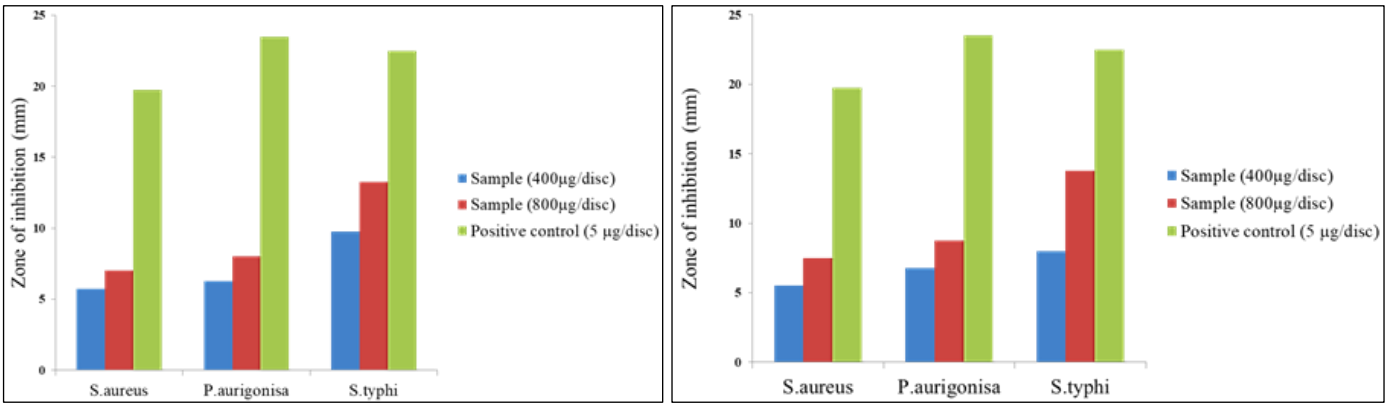

Fig 2: Graph for Evaluation of antibacterial activity of T. indica seed lipid (left) and *Momordica charantia* seed lipid (Microorganisms vs Zone of inhibition).

Results and Discussion

Both crude lipids were liquid in state and dark brown in color. The chemical parameters are examined and the values obtained is tabulated (Table-1).

Table 1: Chemical parameters for seed lipids

Parameter	*Tamarindus indica* seed lipid	*Momordica charantia* seed lipid
Lipid content	7.78%	15.82%
Saponification value	251.87	273.43
Iodine value	72.97	67.28
Acid value	8.94	2.02
% FFA	4.5%	1.01%
Ester value	242.93	271.41
% Glycerine	13.27%	14.84%

During experiment, it was seen that it takes hard labor to grind *T. indica* seed than *Momordica charantia* seed and from the Table 1, it is seen that the lipid content is more in case of *Momordica charantia* seed than that for *T. indica* seed. So, *Momordica charantia* seed is a better source of lipid than *T. indica* seed.

The saponification value indicates the amount of base required to neutralize the acid present in lipid. Higher Saponification value indicates high proportion of lower fatty acids since saponification value is inversely proportional to the average molecular weight or chain length of the fatty acids. The saponification value of *T. indica* and *Momordica charantia* seed lipid were 251.87 mg and 273.43 mg of KOH. The result indicates the presence of appreciable amount of low molecular weight fatty acids in lipid and can be used as a good raw material for soap industries [20] as well as it can be also said that *Momordica charantia* seed lipid can be better raw material than *T. indica* seed lipid for soap industries.

The fatty acid present in lipid may be saturated or unsaturated. Based on this the lipid is classified as drying, semidrying and nondrying. The presence of double or triple bonds present in fatty acids in lipid makes the lipid liquid (oil). The higher the iodine value the greater the number of double and triple bonds present in fatty acids. From the present study it was seen that the iodine value of *Momordica charantia* seed was 67.28 and that for *T. indica* seed was 72.97 g of I_2. So, *Momordica charantia* and *T. indica* seed lipids fall in the nondrying oil category because its value is bellow 90 [20]. So, it can be said that both lipids contain fatty acids is unsaturated having one double bond and does not harden when it is exposed to air.

The acid value is a measure of the free fatty acids present in lipid and it also indicates the degree of purity and extent of rancidity. A high acid value indicates a high tendency to become rancid. A high percentage of fatty acid (above 1.15%) indicates that the lipid is not suitable for directly edible purpose. The acid value of *T. indica* seed lipid and *Momordica charantia* seed lipid was found to be 8.94 and 2.02 mg of KOH respectively, which indicates that *T. indica* seed lipid is high acidic and *Momordica charantia* seed lipid is low acidic as well as it can also be said that *T. indica* seed lipid contains high amount of organic acidic substances than *Momordica charantia* seed lipid and *T. indica* seed lipid has a greater tendency for rancidity than *Momordica charantia* seed lipid [20].

The percentage of free fatty acid (%FFA) of *T. indica* seed lipid and *Momordica charantia* seed lipid was found to be 4.5% (higher than 1.15%) and 1.01% (lower than 1.15%) respectively. Comparing the values with the standard value (1.15%) [20] it can be said that *Momordica charantia* seed lipid is suitable and *T. indica* seed lipid is not suitable for edible purposes.

The ester value of *T. indica* seed lipid and *Momordica charantia* seed lipid was found to be 242.93 and 271.41 respectively. This high value was supported by the saponification value and acid value. The parcentage of glycerine was found to be 13.27% and 14.84% for *Momordica charantia* seed lipid and *T. indica* seed lipid respectively. This high value makes the lipid viscous [20].

The microbial activity was determined against *Salmonella typhi*, *Staphylococcus aureus* and *Pseudomona aurigonisa* with respect to ciprofloxacin as standard. There, ciprofloxacin was taken as 5µg/disc where the sample was taken as 800µg/disc and 400 µg/disc. The inhibition zone created by *T. indica* seed lipid and *Momordica charantia* seed lipid was very close. The inhibition zones obtained against all bacterias for sample was compared to the ciprofloxacin. The amount of sample were much higher than ciprofloxacin but both samples showed small inhibition zone than ciprofloxacin. Both samples showed very low effect on *Salmonella typhi*, *Staphylococcus aureus* and *Pseudomona aurigonisa* as compared to ciprofloxacin. So, it is clear that *T. indica* seed lipid and *Momordica charantia* seed lipid can be used as a

mild antibacterial substance for skin infection, food poisoning, typhoid fever.

Conclusion

Tamarindus indica and *Momordica charantia* seeds were crushed by mechanical grinder and the lipids were extracted by soxhlet extractor using petroleum ether as extracting solvent. Both cruse lipids were liquid in state and dark brown in color. It takes hard labor to grind *T. indica* seed than *Momordica charantia* seed and the lipid content is more in case of *Momordica charantia* seed than that for *T. indica* seed. So, *Momordica charantia* seed is a better source of lipid than *T. indica* seed. *Momordica charantia* seed lipid can be better raw material than *T. indica* seed lipid for soap industries which is confirmed by their saponification values. The iodine values suggested that *Momordica charantia* and *T. indica* seed lipids fall in the nondrying oil category and both lipids contain fatty acids is unsaturated having one double bond and does not harden when it is exposed to air. *T. indica* seed lipid is high acidic and *Momordica charantia* seed lipid is low acidic. *T. indica* seed lipid contains high amount of organic acidic substances than *Momordica charantia* seed lipid and *T. indica* seed lipid has a greater tendency for rancidity than *Momordica charantia* seed lipid which is confirmed by the acid values. From the percentage of free fatty acids, it is clear that *Momordica charantia* seed lipid is suitable and *T. indica* seed lipid is not suitable for edible purposes. The ester values and percentages of glycerin content indicates that viscous nature of both lipids. The antibacterial test suggests that *T. indica* seed lipid and *Momordica charantia* seed lipid can be used as a mild antibacterial substance for skin infection, food poisoning, typhoid fever.

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