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Evaluating the Physicochemical and Antimicrobial Properties of Soybean Oil in Consort with Spectral and Heavy Metal Content Available in Chattogram, Bangladesh

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Abstract

There are numerous brands of soybean oils in the local markets of South Asian region, some of which are of low quality. This study focused on the evaluation of antimicrobial and physicochemical properties of soybean oils like as, iodine value, peroxide value, free fatty acid value and heavy metal content. Comparatively, low iodine value was noticed, free fatty acid value and peroxide value was in acceptable range but heavy metal content was in alarming array. The concentration of estimated heavy metals in oil samples was found in between 6.20-12.2 for Fe, 5.0-7.5 for Cu, 0.6-3.2 for Ni, 0.31-1.30 µg/g for Pb and Cd was in below detection limit. At room temperature, FTIR spectra showed no peaks at 2166 cm-1 and 3241 cm-1 while two additional peaks appeared in this region after several times frying. The examined soybean oils showed no inhibitory activity against *E. coli*, *S. aureus*, and *K. pneumoniae*.

***Keywords:** Edible oils; Soybean oil; Physicochemical properties; FFA; FTIR; Heavy metals; Antimicrobial activity*

Introduction

Vegetable oil is a vibrant food constituent, providing strength, essential fatty acid, and a carrier of fat-soluble vitamins. These oils are commonly used in food processing and also in cosmetic manufacturing industries [1]. Both edible fats and oils are water-insoluble substances consisting primarily of fatty acid glycerol ester or triglycerides with small quantities of nonglyceridic compounds [2]. The largest integrated sources of vegetable oils are seeds of respective plants which grow in a relatively temperate area. A significant source of energy for the human diet is vegetable oil and fat, which provides 37 kJ energy from 1 g oil [3]. Among all sorts of plants, soybean oil is dug up from the seed of soybean (Glycine max) [4]. Soybean is primarily grown in South and North America (Argentina and Brazil). It is one of the major vegetable oil that is widely used in Indian subcontinent for food manufacturing purposes, particularly in Bangladesh.

Soybean is primarily imported in crude form in Bangladesh and is then processed in domestic refineries. According to the statistical (2020) report, import and domestic consumption of soybean oil in Bangladesh was 1270 metric tons and 800 metric tons respectively in 2019/20, while world production for the same period was around 56.51 million metric tons [5,6]. However, some vegetable oils are not suitable for standards that satisfy consumers with regard to their physicochemical properties or the texture and stability of food products [7,8]. It was also found that, in soybean oil the percentage of five fatty

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acids such as palmitic, stearic, oleic, linoleic and linolenic acids were 14 ± 0.62, 4.07 ± 0.29, 23.3 ± 2.43, 52.2 ± 2.64 and 5.63 ± 3.48, respectively [9,10]. Various factors such as oil processing, the fatty acid composition of the oil, the energy of heat or light, the concentration and type of oxygen, transition metals, thermally oxidized compounds, peroxides, pigments, and antioxidants affect the oxidation of oil [11,12]. Few nonglyceride components are counterproductive to the consistency of freshness, a shelf-life, and toxicity of edible oil by evaluating a variety of trace metals [13]. Availability of trace metals such as Cu and Ni is intended to enhance the rate of oil oxidation while other metals such as Pb and Cd are very significant due to their toxicity and metabolic activity [14]. The existence of metal in soybean oil is subject to a variety of factors, such as soil, climate, plant genotype, fertilizers and metal-containing pesticides applied during the production process or contamination from metal equipment's [15,16]. One of the main aims of this analysis was therefore to establish concentration levels of Pb, Ni, Cd, Cu, and Fe in soybean oil.

Some vegetable oils show potent antimicrobial activity against pathogenic microorganisms. *S. aureus* and *E. coli* are opportunistic bacteria that are widely found in the environment and the human body. These microorganisms can cause life-threatening infections in the immune-compromised patient [17]**.** So, control of these microorganisms in the food manufacturing industries is very important. In recent times, edible oils are one of the most essential components of the diet used for cooking. One of the most common methods used for cooking food is deep frying. Repeated frying results in many oxidative and thermal reactions resulting in modifications in the oil's physicochemical, nutritional, and sensory attributes [18]. In order to determine the consistency and functionality of the oil, several researchers studied the effect of temperature on texture, stability, morphology, and numerous parameters [19-21]. In this research, we attempted to track changes in the morphological properties of oils using FTIR to assess the degree of oxidation following heating and frying.

In addition, assessment of physical and chemical parameters is also essential to ensure the quality of the oil consumed in Bangladesh. Such physicochemical parameters include moisture content, pH, density, viscosity, peroxide value, iodine value, and free fatty acid value. The desire of this study was to provide information on the quality of refined soybean oil, to compare oil quality with established standards, to increase awareness among manufacturers and to provide recommendations to the monitoring authority.

Materials and Methods

Raw materials

Hydrochloric Acid (HCl), Sodium Chloride (NaCl), Potassium di-chromate ($K_2Cr_2O_7$), Starch indicator, Phenolphthalein indicator, Sodium thiosulfate ($Na_2S_2O_3$), all were A.R Grade and purchased from Merck, USA and Merck, Darmstadt Germany. Seven different brands of soybean oils were purchased from the local market of Chattogram city, Bangladesh, in 2020.

Frying process

Potatoes, onion, and ginger were peeled and cut into different sizes and fried in oil at a constant temperature for three times. Frying experiment was conducted at home condition, where cooking pan and gas burner were used.

pH, density and viscosity measurement

The pH of the oil samples was measured using Universal Indicator. The indicator was exported from Thermo Fisher Scientific, USA. The density of oil samples was measured by a pick-now meter with a capacity of 25 mL using the following equation:

Density=Mass of the oil (g)/Volume of the oil (mL) [22].

The Ostwald Viscometer (ASTMAD-435, Japan) measured the viscosity of all oil samples at 25°C, and recorded the flow time of oil samples with the aid of a stopwatch.

Peroxide value

Peroxide value of oil is mainly the measure of peroxides contained in the sample. The PV value of oil is determined by measuring the iodine content released from potassium iodide [23]. 2 g of oil sample was weighted first in a 25 ml test tube

then 2 g of potassium iodide and 20 ml of solvent mixture ($CHCl_3$:CH_3COOH at the ratio of 1:2) added to the solution and gently shacked. The contents were then boiled for 30 seconds in a boiling water bath. The test tube was then cooled to room temperature while placing in tap water and then transferred to a 250 ml conical flask. Then 20 ml of 5% potassium iodide and 50 ml of distilled water were also added to the flask. Finally, the solution mixture was titrated against 0.002 N sodium thiosulphate solution using a starch indicator towards the endpoint [24].

Iodine value

Oil samples with known weight were treated with an excess amount of iodobromide in Glacial acetic acid. Here, untreated IBr reacted with KI which converts the iodobromide to Iodine. Then the concentration of iodine is determined by titrating with standard sodium thiosulphate using the following equation:

Iodine Value=(b-v) × N × 126.9 × 100/w × 1000

Here, b is the volume of sodium thiosulphate used as blank, v is the amount of sodium sulfate used for the sample, N is the concentration of titrating solvent w is the wt. of oil sample and finally, 126.9 is the molecular wt. of iodine [25].

Free fatty acid (Acid Value)

10 g of oil sample was weighed and dissolved in hot 100 ml neutralized ethanol. Then the solution was titrated using 0.001 N alkali (KOH) solution, where phenopthelin was used as an indicator. The test solution was shaken vigorously and kept warm during the whole titration process [26]. Finally the acid value of the sample oils were calculated by means of the given equation:

Acid value (As oleic acid)=N of alkali × ml of alkali × 56.1/wt of the sample (g) [22].

Heavy metal analysis

For heavy metal analysis, we used atomic absorption spectroscopy (AAS) (Thermo Scientific, UK, Model: iCE 3300 AA System). The analyses were carried out using respective hollow cathode Lamp for Pb, Cd, Cu, Ni, and Fe under standard conditions (TABLE 1).

TABLE 1. **Instrumental conditions of Atomic Absorption Specstrophy (AAS)**

Elements	Fuel flow (L/min)	Flame Type	Wavelength (nm)	Bandpass
Fe	0.9	Air-Acetylene	248.3	0.2
Cu	1.1	Air-Acetylene	324.8	0.5
Ni	0.9	Air-Acetylene	232.0	0.2
Pb	1.1	Air-Acetylene	217.0	0.5
Cd	1.1	Air-Acetylene	228.8	0.5

Spectroscopic analysis

A Fourier Transform Infrared Spectroscopy (PerkinElmer, Liantrisant, UK, Model: Spectrum-II) was used to record FTIR spectra for the oil samples before and after frying. It was used to evaluate the saturation and unsaturation status of heated and normal oils at room temperature for observation of the oxidation in oils.

Antimicrobial susceptibility test

The antibacterial activity of sample soybean oils against *E. coli* ATCC 25922, *K. pneumoniae*, *S. aureus* ATCC 6538 were evaluated according to CLSI-2019 guidelines [27]. In this experiment, the bacterial reference strains were cultured overnight at 37°C on Nutrient agar media (Himedia Laboratories, India). The optical density of *E. coli*, *K. pneumoniae*, and *S. aureus* were measured at 625 nm by using UV-spectrophotometer (Shimadzu, Model: UV-1800). The final populations were adjusted to 10^6 cfu/ml of each bacterial strain in a sterile saline solution. An amount of 0.5 mL of the suspensions was placed on Muller Hinton Agar media (Himedia Laboratories, India) with a sterile cotton swab. Under proper aseptic condition, blank sterilized discs (OXOID, UK, 6 mm diameter) were impregnated with 20 µl of soyabean oil and DMSO (1:1 ratio). The discs were placed on the agar surface containing bacterial suspension. A broad-spectrum antibiotic, Chloramphenicol (OXOID, 30 mg/ml) was used as positive control and a paper disc containing 10% DMSO was used as a negative control. Studies were performed in triplicate. The inhibition zone was measured after 24 hours of incubation at 37°C.

Results and Discussion

The quality of soybean oils were evaluated by analyzing their physicochemical parameters like viscosity, density, free fatty acid value, peroxide value, iodine value, heavy metal content and antibacterial activity. Results associated with these tests are tabulated and interpreted below:

Viscosity, density, moisture content and pH

TABLE 2 shows the moisture content of oil samples which ranged between 0.31%-0.49%. Moisture content is a most important parameter of oil as its presence may cause rancidity or unpleasant odor in oil. According to ASTM, recommended moisture content for soybean oil is 0.2%-0.3% [28]. The test samples contained slight more moisture than recommendation level but it is more approximate to standard level. Virtually it is observed that the density of all brands of oil exist between 0.90 – 0.95 at 30°C [29]. And the oil samples exhibit a satisfactory value of density which ranged between 0.89-0.91 at 25°C. Viscosity of oil samples were observed between 33.2-43.5 cP at 25°C. In general, oil and fats possess high viscosity due to intermolecular attraction between long chains of glyceride molecule. Viscosity increase with the increase of molecular weight but decrease with temperature. According to a work by Nuruddin et al. viscosity of soybean oil at 23.9, 37.8, 48.9°C was 54.3, 31.8, 23.3 cP [30]. At 25°C, pH of oil samples was observed around 6.5.

TABLE 2. **Moisture content, viscosity, density, and pH of tested soybean oils**

Sample content	Moisture content (%)	Viscosity (cP at 25°C)	Density (g/mL at 25 °C)	pH (at 25°C)
SO1	0.31	35.2	0.909	6.5
SO2	0.41	37.2	0.917	6.9
SO3	0.52	33.2	0.899	5.8
SO4	0.30	35.6	0.916	6.3
SO5	0.25	33.7	0.910	5.5
SO6	0.41	42.6	0.912	7.1
SO7	0.49	43.1	0.919	7.5

Free Fatty Acid value (FFA)

During storage and processing, some oxidative changes are observed in oils which are determined by the increase of free fatty acids and a decrease in unsaturation [31]. As stated in TABLE 3, the FFA value of samples was observed between 1.01-1.20 Which indicates a good quality of oil. In research by Ratanesh Kumar et al. on edible vegetable oil found that the FFA value was 0.18-0.19 for individual oil where it was 0.16-0.18 for blended oil [22]. Yangi et al. reported the effect of storage duration and condition on the FFA value of soybean which was 13.7 or 9.3% mc at 30°C and 80% RH [32]. The change in FFA content due to storage condition and time was also reported by F Anwar et al. and JO Arawande et al. [33,34]. In this study, the oil samples did not fulfill the Codex Standards for Named Vegetable Oils hence as visualized in FIG. 1 and TABLE 2. However, it needs more refining and caution during storage and transportation [35].

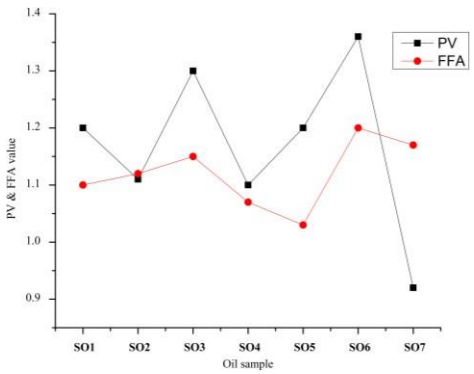

FIG. 1. **A schematic illustration of change in free fatty acid value and peroxide value of tested sample oil.**

Peroxide value (PV)

Peroxide value is the most important biochemical parameter of fats and oils which was carefully studied here. It determines the oil oxidation level during the storage period. In general, hydroperoxide has no flavor or odor of their own, but due to their instability, they break down to aldehydes and ketone which possess unpleasant odor and bad taste [36]. PV value is used to assess to what extent the rancidity took place in oil during storage. It also could be used for quality assessment and stability indication of oils and fat for industrial purposes [37]. In accordance with FIG. 1 our tested samples, the highest PV value observed was 1.42 meq/kg oil and the lowest PV value was 0.82 meq/kg oil. Here, all PV value was in the range of standard level declared by the Codex Alimentarius Commission (2001) standards [38]. Generally, oils exposed to atmospheric oxygen and light displays a sudden increase in PV value, and it dramatically increases with storage time.

TABLE 3. **Free Fatty Acid, Peroxide, and Iodine value of tested soybean oils.**

Sample Content	Free Fatty Acid value	Peroxide value	Iodine value
SO1	1.10	1.20	114
SO2	1.12	1.11	112
SO3	1.15	1.30	117
SO4	1.07	1.10	112
SO5	1.03	1.20	107
SO6	1.20	1.36	109
SO7	1.17	0.92	121

Iodine value (IV)

Iodine value is a simple chemical constant that measures the double or triple bond in oil sample. The oil sample yields high iodine value which indicates that the soybean oil contains more double bonds i.e. unsaturated. According to Bangladesh standards (1979), iodine value (IV) of soybean oil should be between 120-143 [2]. The level of IV for most commercial soybean oil is ranged between 124-136 where as it is 48-60 for Palm oil [39]. According to a research carried out by Abdulkarim et al., the IV of soybean oil was 116.9 at 25°C [40]. Here, the iodine value of most sample oil is ranged amid 107–117 which indicated a good stage of the supplied sample. However, as shown in FIG. 2 the average value of IV for oil samples was lower than that of the standard range which points out that the oil sample may be blended with a lower and cheap quality oil (i.e. Palm oil).

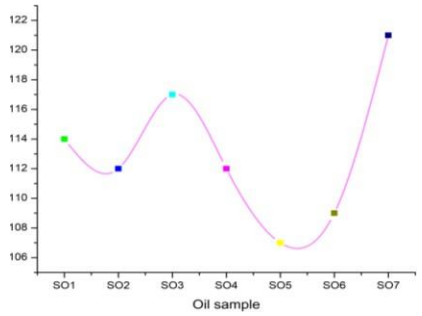

FIG. 2. **A schematic illustration of change in Iodine value of tested soybean oil.**

Heavy metal content

FIG. 3 exhibits the existence and extent of heavy metals found in the test samples. In this study, the wet digestion system was preferred and its accuracy was crisscrossed through the spike recovery system. The spike recovery values were acceptable (≥98%) for the wet digestion system and the RSD values for all tested samples were less than 5%. All metals and minerals concentration was determined on a wet weight as µg/g except Cd. The metal concentrations of tested soybean oils were found in between 6.20-12.2 for Fe, 5.0-7.5 for Cu, 0.6-3.2 for Ni, 0.31-1.30 µg/g for Pb, and Cd was below the detection limit. In general, plants intake minerals (macro nutrition's and micronutrients) from soil through its xylem and phloem tissues. But the irony of fate is that due to soil and water pollution excess amount of minerals especially heavy metals are up-taken by plants which finally absorbed by the human body through the food chain. Vegetable oils and fats are containing trace amount of

mineral depending on following factors such as, species of plant, irrigation water, soil property, fertilizer, maturity level of seed and refining system [41].

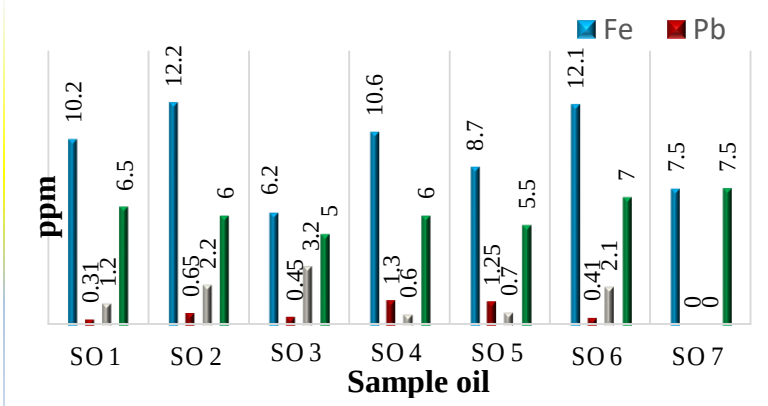

FIG. 3. **A schematic sketch of heavy metal (Fe, Pb, Ni, and Cu) content in tested soybean oil by Atomic Absorption Spectroscopy.**

The maximum dietary consumption of a particular type of oil or fat for an adult is 25.0 g [13]. According to international and local authorities, the acceptable limit of Fe in edible oil is (1-1.5 PPM).

As shown in Table 04, our test sample contains a high level of Fe than the acceptance range (>1.5 µg/g). Iron is a rudimentary component for practically all living life systems as it takes an interest in a wide assortment of metabolic procedures, including oxygen transport, DNA synthesis, electron transport, etc. Conversely, as iron can generate free radicals, its fixation in body tissues must be firmly controlled otherwise in unnecessary sums, it may also prompt tissue harm, anemia to Fe overload, and possibly to neurodegenerative maladies [42]. The value of iron in edible oil has been stated lower than our outcome in the works by Cindric et al. and PL Buldini et al. [43,44]. Pb was found in the range of 0.31-1.30 µg/g which were higher than those in previously reported [13,45,46]. According to international regulatory authorities (FAO/WHO) maximum acceptable limit of Pb in edible oil is 0.1 µg/g [47]. Pb has no advantageous part within the human digestion system framework whereas it causes vibrant toxicity [48]. Nickel content was found in tested soybean oil within the range of

0.6-3.2 µg/g which exceeded the permissible limit (<0.2 µg/g). The most severe detrimental health effects from acquaintance to nickel, such as prolonged bronchitis, abridged lung function, nasal sinus, and cancer of the lung [49]. Cd was not detected in any of the analyzed samples though it was reported in Turkish edible oil studied by Pehlivan et al. and Zhu et al. [13,50]. Copper is a common nutrient for the human body which is famous for both its vital and toxic effect. It may enter the human food chain through food processing, mineralization by crops, environmental contamination, pesticides, or fertilizers [51,52]. In our work, the maximum and minimum level of Cu content was 5.0-.5 ppm. The copper content in edible oil sample has been reported as 0.1 ppm [53]. FAO/WHO has fixed a limit for heavy metal consumption based on physique weight. For a regular adult of 60 kg physique weight, the provisional tolerable daily intake (PTDI) for iron, lead, copper, nickel, and cadmium are 48 mg, 214 µg, 3 mg, 3.6 mg, and 9.6 mg respectively [54].

TABLE 4. **Heavy metal (Fe, Pb, Ni, Cd, and Cu) content of tested soybean oils.**

Sample content	Fe (µg/g) ± SD	Pb (µg/g) ± SD	Ni (µg/g) ± SD	Cd (µg/g) ± SD	Cu (µg/g) ± SD
SO1	10.2 ± 0.03	0.31 ± 0.08	1.2 ± 0.09	BDL	6.5 ± 0.07
SO2	12.2 ± 0.08	0.65 ± 0.07	2.2 ± 0.08	BDL	6.0 ± 0.08
SO3	6.2 ± 0.05	0.45 ± 0.07	3.2 ± 0.05	BDL	5.0 ± 0.07
SO4	10.6 ± 0.06	1.30 ± 0.08	0.6 ± 0.09	BDL	6.0 ± 0.08
SO5	8.7 ± 0.05	1.25 ± 0.05	0.7 ± 0.05	BDL	5.5 ± 0.02
SO6	12.1 ± 0.04	0.41 ± 0.06	2.1 ± 0.08	BDL	7.0 ± 0.07
SO7	7.5 ± 0.02	BDL	BDL	BDL	7.5 ± 0.05

Spectral analysis

FTIR is a significant device for exploration of molecular bonds because intensities of bands in the spectrum are directly proportional to concentration and nature of bonds. In FTIR m-IR spectra have been utilized to characterize consumable oils since they separate within the concentrated and the precise recurrence at which the highest absorbance of the band displays, agreeing to the nature and composition of the test sample [55,56]. Composition and characteristics of oils affects the exact position of bands and shifts appear when additional compounds formed [23]. The FTIR spectra of soybean oils as displayed in FIG. 4a which points out the sharp peak at 3006.8-3008.30 cm-1 as C-H stretching vibration of the cis-double bond (=C-H) and 2853.76-2923.36 cm-1 shows C-H asymmetric and symmetric stretching vibrations of the aliphatic CH2, the region of double bond stretching shown at 1743.60 cm-1 which represents C=O ester carbonyl of triglycerides, bending at 1460-1463.82 cm-1 of C-H bending vibrations of the CH2 and CH3 aliphatic groups, spectra arise from stretching vibration of C-O at 1160.13-1236.3 cm^{-1}, and 722.25 cm-1 represent (-CH2), -CH=CH-overlapping of the CH2 rocking and the out of plane vibration of cis disubstituted. Percentage transmittance of almost all the peaks increased in spectra of frying oil indicating the decrease in absorbance which may be due to hydrolysis of oil during several times frying [57]. Here, FIG. 4b exhibits the spectral morphology of the fried oil at the region of 500-4000 cm^{-1}. As in sample oil at a high temperature, two additional peaks appeared which reveals that the sample oil is unstable at high temperature and alarming oxidized products may have formed.

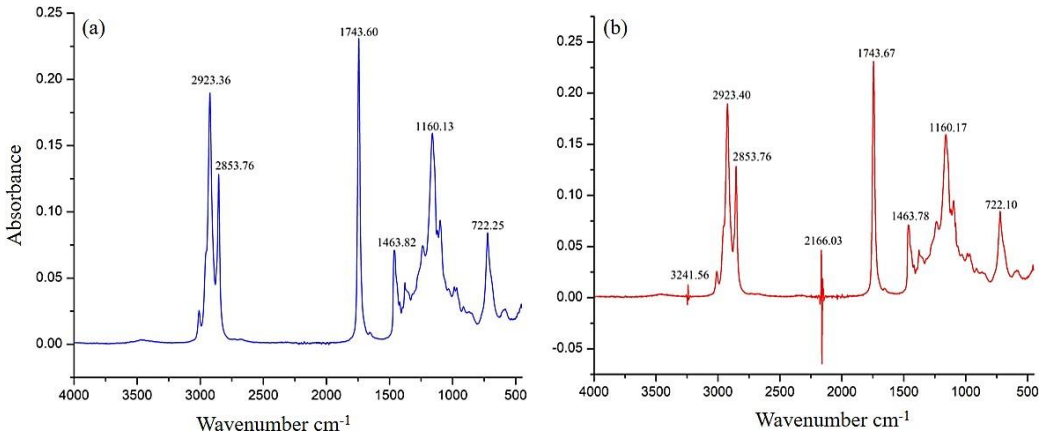

FIG. 4. **FTIR Spectra of sample oil (a) at room temperature and (b) after multiple frying for frequency range 500-4000 cm^{-1}.**

Antimicrobial susceptibility test

The antibacterial activity of seven collected soybean oils against *E. coli*, *K. pneumonia* and *S. aureus* is shown in FIG. 5. A significant and clear zone is visible around positive control but no such zone of inhibition is observed against negative control and test samples after 24 hours incubation at 37°C.

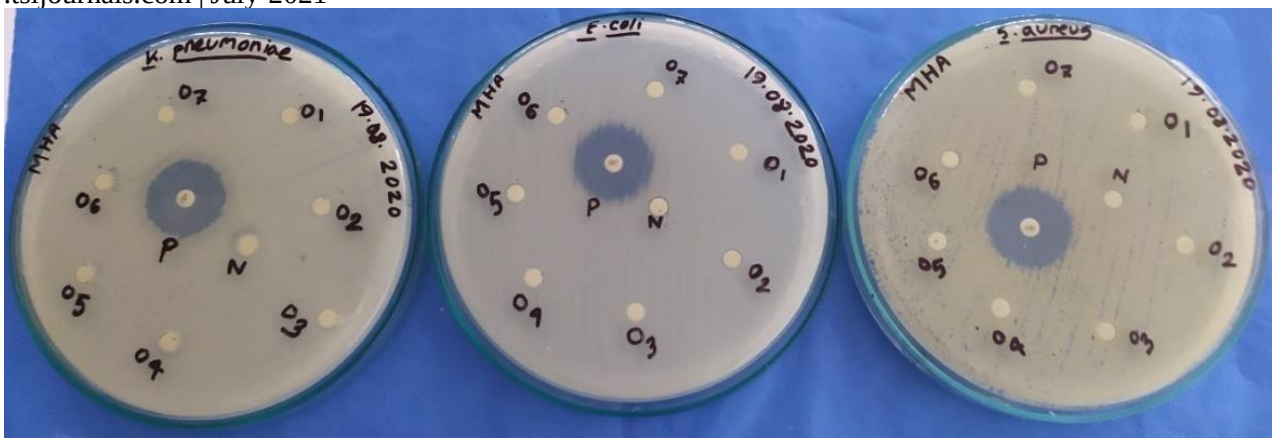

FIG 5. **A pictorial representation of antibacterial activity of soyabean oils against *K. pneumonia, E. coli,* and *S. aureus*.**

A study conducted by Tran Dang Xuan et al., in Japan reported that, three concentrations of soybean oil (0.1, 0.05, 0.01 mg/mL) exhibited significant growth inhibition zone around *E. coli*, and *S. aureus* [58]. In another work by Kamrul Islam et al., found that soybean extract of ginger displayed good antimicrobial activity against food borne pathogens *E. coli*, *P. aeruginosa*, *S. aureus* compared to the soybean alone [17]. However, the results of this study demonstrated that, the tested soybean oils showed no significant inhibitory activity against the three bacterial species.

Conclusion

The experimental characterization and analysis of different properties of oil samples lead us to some precise decision. All tested soybean oil seemed to be well refined with respect to different physical parameters and possessed a good quality except for two brands. The iodine value was comparatively poor, the free fatty acid and peroxide value were appropriate but the heavy metal content was worrying. These heavy metals may be induced in oil during processing or manufacturing steps or maybe from soil pollution. FTIR spectrum of soybean oil exhibits two additional peaks after frying, which indicates the formation of new compound due to multiple times frying. However, to increase the quality of soybean oil according to national standards, the manufacturers and the controlling authorities should take special care during refining processes.

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Conflict of Interest

None declared.

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