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Comparison between Water Hyacinth and Waste Cooking Oils as Source of Biofuel Production

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Abstract
The problem of fossil fuel increases by time around the world, Biodiesel is an environmentally friend renewable diesel fuel alternative. The present work aims to determine the possibility for biofuel production from non-edible plant (Water hyacinth) and waste cooking oils in addition to potential uses as source for glycerol, pigments and antioxidant compound sources. this study aimed to investigate the feasibility of biodiesel production from water hyacinth using two solvent systems (chloroform: methanol, 2:1v/v and Hexane), Moreover, WCO applying one steps alkaline transesterification process using methanol, NaOH (0.25) and KOH (0.25, 0.5 and 1g) as a catalyst and hot distilled water for purification. both produced biodiesel (from Eichhornia and WCO) was characterized using FTIR and some chemical constant such as IV, SV and AV. The highest biodiesel produced from Eichhornia recorded with chloroform: methanol system when compared with Hexane. However, the highest biodiesel produced from WCO was recorded when using KOH (0.25g). Also, the obtained results reported the possibility for use glycerol produced from the both biofuel sources in body butter (makhmaria) production, Authors concluded that, the produced biodiesel from both sources was within the recommended standards of biodiesel fuel. Also, water hyacinth became useful in antioxidant compounds production, the contained pigments may be used as natural coloring substances in different fields.
Keywords: WCO; Eichhornia sp; Biodiesel; Pigments; Material balance.

1. Introduction
According to the environmental worries and the consumption of non-renewable, biodiesel is a nonpetroleum-based fuel defined as fatty acid methyl or ethyl esters derived from natural energy resources, developing alternative resources of energy as a substitute of traditional fossil fuels has been raised. Biodiesel is produced by chemically reacting a vegetable oil or animal fat with an alcohol such as methanol or mono-alkyl esters of long chain fatty acids derived from a renewable lipid feed stock, such as vegetable oil or animal fat [1, 2].

The amount of cooking oil produced every year is immense, over 15 million of tons. So, WCOs and fats cause disposal problems in many parts of the world. These problems could be changed into both economic and environmental benefits by proper utilization and management of WCO as a fuel substitute. In Egypt, millions of liters of waste cooking oil (WCO) are discarded annually into sewage systems, pollute water streams and adds to the cost of treating effluents. In an attempt to reduce the cost of biodiesel and pollution caused by WCO [3].

Biodiesel from oil crops, waste cooking oil, animal fats (first generation) and non-crop plants (second generation) cannot realistically satisfy even a small fraction of the existing demand for transport fuels. Water hyacinth was found to be one of the best sources of (renewable) biodiesel capable of meeting the global demand for fuels [4, 5]. Studies have been directed towards making use of this hydrophytes for the production of bioactive substances which exhibited antimicrobial, anticancer, antioxidant and anticorrosion of metals and alloys activities [3, 6-10].

Water hyacinth grow very fast especially in summer months in all the Egyptian water bodies and cause serious problem to navigation, irrigation and deterioration of drinking water quality [11].

Eichhornia sp represents a promising organism for fuel production in because of their high availability and high biomass yield .Moreover, the utilization of Eichhornia sp as the feedstock for bioethanol production has a number of advantages. Water hyacinth is low in lignin concentration with high content of cellulose and hemicellulose [12].

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This investigation aims to produce biofuel and other biochemical compounds from non-used biomass such as water hyacinth and waste cooking oils.

2. Materials and Methods

2.1. A-Materials

2.1.1. Chemicals and Drugs

Pure Petroleum ether, chloroform, Hexane, acetone, methanol and DMSO were purchased from E. Merck Co. (Darmstadt, Germany). Sulfarhodamine, 2, 2 diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.1.2. Plant Sample

Water hyacinth was collected during summer 2020, from El-Zomor canal, Giza, Egypt. The hydrophytes were cleaned from any epiphyte and debris by washing several times with tap water, distilled water, then it was left to air dry at room temperature, ground and kept in glass bottles till use.

2.1.3. Waste Cooking Oil

The sample of waste cooking oil (WCO) of non-edible oil grade, was collected from a local food popular restaurant (Giza, Egypt).

2.1.4. Extraction of Oil from Plant Samples

Extraction of oil was carried out using two extraction solvent systems to compare the oil content in each case and select the most suitable solvent system for the highest biodiesel yield.

2.1.5. Chloroform / Methanol (2:1, v/v) Method

A known weight of each ground dried plant species (65 g dry weight) was mixed separately with the extraction solvent mixture; chloroform / methanol (300 ml, 2:1, v/v) for 20 min using shaker. filter and the sample residue was extracted three times by 100 ml chloroform followed by filtration according to Bligh and Dyer [13].

2.1.6. Hexane Solvent Method

A known weight of each ground dried plant species (65g dry weight) was mixed with the extraction solvent mixture, Hexane kept to settle, followed by filtration according to Hossain and Salleh [14].

2.1.7. Pretreatment

Collected WCO was dried over calcium chloride ($CaCl_2$) and then filtered to remove any suspended matter, $CaCl_2$ crystals, and burned food bits, etc.

2.2. Transesterification and Biodiesel Production from WCO

The production methodology followed in this study was according to Tomasevic and Siler-Marinkovic [15] with little modification, where the alkali-catalyzed transesterification was applied. Basically, methanol was the alcohol of choice and different alkaline agents (NaOH and KOH) at different concentrations (0.25. 0.5 and 1.0 g) were used as the catalyst. Sod. Or Potassium methoxide solution (PMS) was prepared freshly by mixing a predetermined amount of methanol (≈ 12 wt % of oil) with alkaline agents at the concentration mentioned above in a container until all the catalyst dissolved. The SMS or PMS was then added to 200 g of WCO and stirred vigorously for 30 min at 30^{o}C. Then after, the mixture was carefully transferred to a separating funnel and allowed to stand for 4 h. the lower layer (glycerol, methanol and most of the catalysts) was drained out. The upper layer (methyl esters MEs, some methanol and traces of the catalyst) was transferred into another flask containing freshly prepared SMS or PMS mixed at 60 rpm under reflux at 60^{o}C for 30 min. afterwards; the mixture was carefully transferred to a separating funnel and allowed to stand there over night. The glycerol was removed by gravity settling, whereas the obtained crude esters layer was transferred into water bath to remove excess methanol at 65^{o}C and 20 kPa. The obtained crude methyl esters were then cleaned thoroughly by washing with warm (50^{o}C) deionized water, dried over anhydrous Na_2SO_4, weighted and applied for further analysis. All the steps and further analysis were in duplicates and the final reported results were the average values.

2.3. Transesterification and Biodiesel Production from Water Hyacinth

The extracted oil was evaporated under vacuum to release the solvent mixture solutions using rotary evaporator at 40- 45 °C. Then, the oil produced from Water hyacinth was mixed with a mixture of catalyst (0.25g NaOH) and 24 ml methanol, with stirring properly for 20 min. The Mixture was kept for 3hrs in electric shaker at 3000 rpm. [16]. After shaking the solution was kept for 16 hrs to settle the biodiesel and the sediment. The biodiesel layer was separated from sedimentation by flask separator carefully. Biodiesel was washed by 5% water many times until it becomes clear then Biodiesel was dried by using dryer and finally kept under the running fan for 12 h. the produced biodiesel was measured (using measuring cylinder)

2.4. Sediment Analysis (Pigments and Glycerol)

2.4.1. Pigment Determination

A pigment separated from glycerol layer was determined according to Holden [17]. 10 ml of the sediment (pigments + glycerol) was mixed with 5g of active charcoal, after 24 hr, the pigments adsorbed on charcoal was separated by filtration. Then, the pigments eluted from charcoal surfaces by acetone. The pigments were determined spectrophotometrically (CT-2200 spectrophotometer) at the indicated wave lengths and substitute in the equations:

Chlorophyll a (mg\g) = 12.3 X A (663) – 0.8 X A (645) XV \ A X 100 X W

Chlorophyll b (mg\g) = 19.3 X A (645) – 3.6 X A (663) XV \ A X 100 X W

Carotenoids (mg\g) = 4.57 X A (452) – 0.22 X Total chlorophylls

Where; A (663), A (645) and A (452) were the absorbance at these wave lengths.

V = Volume in ml.

A = Length of light path in the cell.

W = the fresh weight of alga in gram.

2.5. Qualitative Analysis of Glycerol

Dunstan 's test (Borax-Phenolphthalein test) was used as confirmation test for the presence of glycerol.

2.6. Antioxidant Activity of Pigments Extract

- DPPH Radical Scavenging Activity

The scavenging effects of pigments extract was determined by the method of Yen and Chen [18], where, 2.0 ml of 0.16 mM DPPH solution (in methanol) was added to a test tube containing 2.0 ml aliquot of sample. The mixture was vortexed for 1 min and kept at room temperature for 30 min in the dark. The absorbance of all the sample solutions and ascorbic acid as natural standard were measured at 517 nm. The percentage (%) of scavenging activity was calculated as the following:

$$\% \text{ Antioxidant activity} = (\text{Control-Sample} \times 100) / \text{Control}$$

Where: control in DPPH solution (0.16 mM).

2.7. FT-IR Analysis

To investigate the functional groups involved in Transesterification process, FT-IR analysis was carried out using Fourier transform infra-red spectrometer Perkin Elmer FTIR spectra. The spectra were collected within a scanning range of 400 – 4000/cm.

2.8. Natural Makhmaria Preparation

The glycerol production from the aqueous layer of lipids was used for production of body butter or makhmaria as the following: 2 spoons Vaseline; 2 spoons cream; 2 spoons almond oil; 2 spoons glycerin in addition to Add the favourite perfume and the makhmaria produced was evaluated by 5 expert persons on approval template includes (Odor, appearance, color.---etc).

2.9. Physico-Chemical Characterization of Produced Biodiesel

The purified product obtained from oil esterification was tested for estimating their fuel properties using the standard methods of analysis for petroleum product (ASTM Standard Methods) and compared with the standards for petro-diesel oil [7].

3. Results and Discussion

3.1. Lipid Contents and Biodiesel Production from Water Hyacinth

Table (1) recorded the lipid content, biodiesel and glycerol content as (%) from water hyacinth in addition to the color of produced biodiesel, the obtained results indicated that lipid extraction using chloroform: methanol (2:1v/v) gave the highest lipids production (9.37%) when compared with the lipids extracted using Hexane solvent (1.65%). Also, the results revealed that the highest biodiesel and glycerol were observed with chloroform: methanol (2:1) when compared with other solvent extraction (Hexane) by (6.4, 2.3 and 0.95, 0.36%, respectively). The color of both produced biodiesel from both systems are difference (as shown in table 1), the bright yellow color of the produced biodiesel from chloroform: methanol (2:1v/v) maybe due to the high carotenoids content which extracted using this solvent system when compared with the quantity of carotenoids with hexane solvent system as shown in Table (2).

Table-1. Lipid, biodiesel and glycerol content in the collected water hyacinth

Parameter	Solvent systems	
	$CHCl_3$:MeOH (2:1v/v)	Hexane
Total lipids (%)	9.37	1.65
Total biodiesel (%)	6.4	0.95
Total Glycerol (%)	2.3	0.36
Biodiesel colour	Bright yellow	light yellow

Table-2. Pigments content (as mg/g) of water hyacinth using two solvent extraction systems

Pigment	Solvent systems	
	$CHCl_3$:MeOH (2:1v/v)	Hexane
Chlorophyll a	0.66	0.10
Chlorophyll b	0.85	0.0
Total chlorophyll	1.51	0.10
Total carotenoids	5.22	2.966
Total pigments	6.73	3.066

These results were in agreement with those reported by Afify, *et al.* [19] and Shalaby, *et al.* [8] who reported that, the chloroform: methanol (2:1) system is better than Hexane: ether (1:1) when extraction of total lipids and biodiesel production, In addition to, salt stress and nitrogen starvation conditions induced dramatic increase in total carotenoids of both green microalga Dictyochloropsis splendida and the cyanobacterium Spirulina platensis.

Not only an increase in carotenoids under stress conditions was performed but also the lipid content and consequently the biodiesel production was enhanced [10].

3.2. Pigments Content of Water Hyacinth

Transesterification of the extracted lipids from *Eichhornia crassipes* produced an upper biodiesel layer and a sediment (pigments and glycerol) lower layer.

In order to make use of all the transesterification products, separation of each layer and studying their characteristics were performed.

The sediment lower layer contains both the pigments (chlorophylls and carotenoids) and glycerol.

Total chlorophylls (a and b) and total pigment content (chlorophylls and carotenoids) as shown in Table 2, the highest total pigments were recorded with chloroform: methanol (2:1) solvent system when compared with Hexane (6.73 and 3.066 mg/g respectively) while the total carotenoid contents reached to 5.22 mg/g in first solvent and 2.966 mg/g with hexane system. These results agree with the results obtained by Shanab, *et al.* [20].

3.3. Biodiesel Production from Waste Cooking Oils (WCO)

Table (3) recorded the total biodiesel and glycerol content as (%) from waste cooking oils using different type and concentrations of catalyst, the obtained results indicated that the highest biodiesel production was recorded with KOH (0.25) followed by KOH (0.5), KOH (1.0) and NaOH (0.25) by 88.9, 72.9, 68.9 and 60%.

These results were in agreement with those reported by Shalaby and El-Gendy [7] who reported that, use of potassium hydroxides better choice for transesterification of oil into biodiesel.

Table-3. Biodiesel and glycerol content production from waste cooking oils treated with different catalyst

Catalyst type	Concentration	Biodiesel%	Glycerol%
Sodium hydroxide	0.25g	60	40
Potassium hydroxide	0.25g	88.9	11
	0.5g	72.9	27
	1.0g	68.9	35

3.4. Chemical Properties of Produced Biodiesel from WCO and Water Hyacinth

The acid value measures the content of free acids in the sample, which have influence on fuel aging. It is measured in terms of the quantity of KOH required to neutralize sample. The base catalyzed reaction is reported to be very sensitive to the content of free fatty acids, which should not exceed a certain limit recommended to avoid deactivation of the catalyst, formation of soaps and emulsion [21]. Sharma, *et al.* [22] reviewed the literature and found that acid value of the feedstock for alkaline transesterification has to be reduced to less than 2 mg KOH/g (i.e. 1%), while only few examples of transesterification with feedstock acid value of up to 4.0 mg KOH/g (i.e. 2%) were found. They also reported that when waste cooking oil is used as feedstock, the limit of free fatty acids is a bit relaxed and the value a little beyond 1% (i.e. 2 mg KOH/g) did not have any effect on the methyl ester conversion.

The feedstock acid value obtained in this study was ≈ 13mL. Thus, in the light of the previous discussion on the requirements for the feedstock acid values, it could be concluded that the used WCO had values above the recommended. However, this value did not turn out to be limiting for the efficiency of the applied two-stage process, as it will be discussed along to the obtained product yield and purity later.

The acid value of the produced MEs was ranged between (0.5 to 0.1) form both sources (WCO and water hyacinth). The average percentage of acid value lowering from the feedstock to the corresponding biodiesel was about 97% (Tables 4 and 5). The recorded acid value of the produced biodiesel is very near for standard petro-diesel and meets the standards limits of EN14215 and D-6751, indicating that the free fatty acid content will not cause operational problems, such as corrosion and pump plugging, caused by corrosion and deposit formation.

Table-4. Some chemical properties of produced biodiesel from WCO

Parameter	Feedstock	Produced biodiesel				Biodiesel (EN14214)
		1	2	3	4	
Acid value (mL)	13.7	0.5	0.1	0.4	0.4	< 0.5
Iodine value (g I2/ 100 g)	28.6	20.2	14	21.8	26.4	< 120

Saponification value (mg/g)	11.39	16.83	12.62	15.14	15.98	NA

(1)NaOH (0.25g); 2)KOH(0.25g); 3)KOH(0.5g); 4)KOH(1.0g).

Table-5. Some chemical properties of produced biodiesel from Eichhornia sp using two organic solvent

Parameters	Chloroform: MeOH	Hexane	Biodiesel (EN14214)
Acid value (mL)	0.4	0.5	< 0.5
Iodine value (g I_2/100g)	18.4	19.5	< 120
Saponification value (mg KOH/g)	14.3	15.3	NA

The iodine value of the feedstock used in this study, which is a measure of unsaturation degree, was ≈ 28.6 mg I_2/100 g oil. According to JUS EN 14214:[14], MEs used as diesel fuel must have an iodine value less than 120 g I_2 per 100 g of sample. MEs obtained in this study had low iodine value ranged 26.4 to 14 mg I_2/100 g form both sources (WCO and water hyacinth). and this finding is in accordance to the fatty acid composition, i.e. the calculated total unsaturation degree of MEs. Predojevic [21], reported that, iodine value depends on the feedstock origin and greatly influences fuel oxidation tendency. Consequently, in order to avoid oxidation, special precautions must be taken during the storage of biodiesel produced from WCO, as it is known that, used frying oils have a variety of qualities, and possess properties significantly different from the neat oils. During frying process, oil is continuously or repeatedly subjected to high temperatures in the presence of air and moisture. Under these conditions a variety of degradation reactions can occur, such as auto-oxidation, thermal polymerization, thermal oxidation, isomer cyclization and hydrolysis which increase the iodine value.

Saponification value represents milligrams of potassium hydroxide required to saponify one gram of fat or oil. The obtained results indicated that in general, esters had higher saponification values than the corresponding oils. Saponification values of the feedstock and product ranged 11.39 and 16.83 mg KOH/g oil form both sources (WCO and water hyacinth)., respectively as shown in Tables (4 and 5).The average percentage of the increase in saponification value was 21%. It is known that a triglyceride has 3 fatty acid chains associated and each triglyceride will give 3 methyl esters, stoichiometrically it may be expected that the same amount of fatty acid carbon chain in neat feedstock oil and biodiesel will react with that same amount of KOH giving the soaps, i.e. their saponification values will be the same. But this assumption could be varied in case of using WCO as a feedstock, as its properties differ significantly from neat oils as a consequence of cyclization, polymerization and degradation of triglycerides that occur during the frying.

3.5. Antioxidant Activity of Pigments Extracted from Water Hyacinth

Antioxidant activity (%) of different pigments extracts (chloroform:methanol, 2:1) and Hexane against DPPH radical scavenging revealed that, pigments extracted by chloroform: methanol (2:1) recorded the highest activity (73.8%) compared to the other Hexane extract and natural antioxidant standard, Vit. C (48.3 and 91.55). these results due to the concentrations of pigments extracted by chloroform:methanols (2:1) especially the carotenoids compounds as antioxidants when compared with hexane extract (5.22 and 2.966 mg/g) as shown in Table (2). Theses results agree with the results obtained by Onofrejová, *et al.* [23] _; Liang and Su [24] who reported that the highest antioxidant activity of Haiany methanolic extract may be attributed to the pronounced contents of both total carotenoids and phenolic compounds, which are known by their potent radical scavenging efficiencies.

The infra-red spectrum of the produced biodiesel , Figures 2a and 2b, Tables 6a and 6b showed the IR bands of biodiesel produced from WCO and Water hyacinth respectively, the absorbance at 1744 for C=O ester, 2854, 2926 (CH_2, CH_3). The presence of ester group and absence of hydroxyl peak can be correlated to the transesterification process of WCO and Water hyacinth oils.

Table-6a. FT-IR data of feedstock WCO and produced biodiesel from WCO

Bond	Functional group	Wave numbers (cm $^{-1}$) of control (WCO feedstock)	Differences in wave number of produced biodiesel
O-H	Alcohol and phenols	3395	ND
H-C-H	Alkyl chain	2916 2819	+40 +35
-C=O	Carbonyl group	1736	+7

Table-6b. FT-IR data of biodiesel produced from Eichhornia using two organic solvent

Bond	Functional group	Wave numbers (cm $^{-1}$) of biodiesel produced by CHCl3:MeOH	Differences in wave number of biodiesel produced by Hexane
O-H	Alcohol and phenols	ND	ND
H-C-H	Alkyl chain	2926 2855	+30 0

Figure-1. Antioxidant activity (as %) of pigment extracted from water hyacinth against DPPH at 100 µg/ml

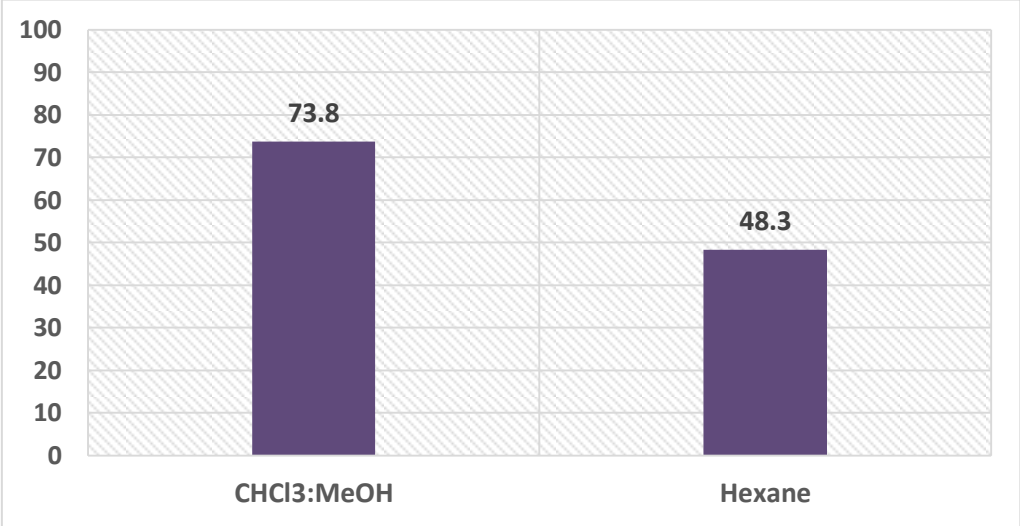


Figure-2a. FTIR chart for A): Feedstock of waste cooking oil B): Produced biodiesel from WCO

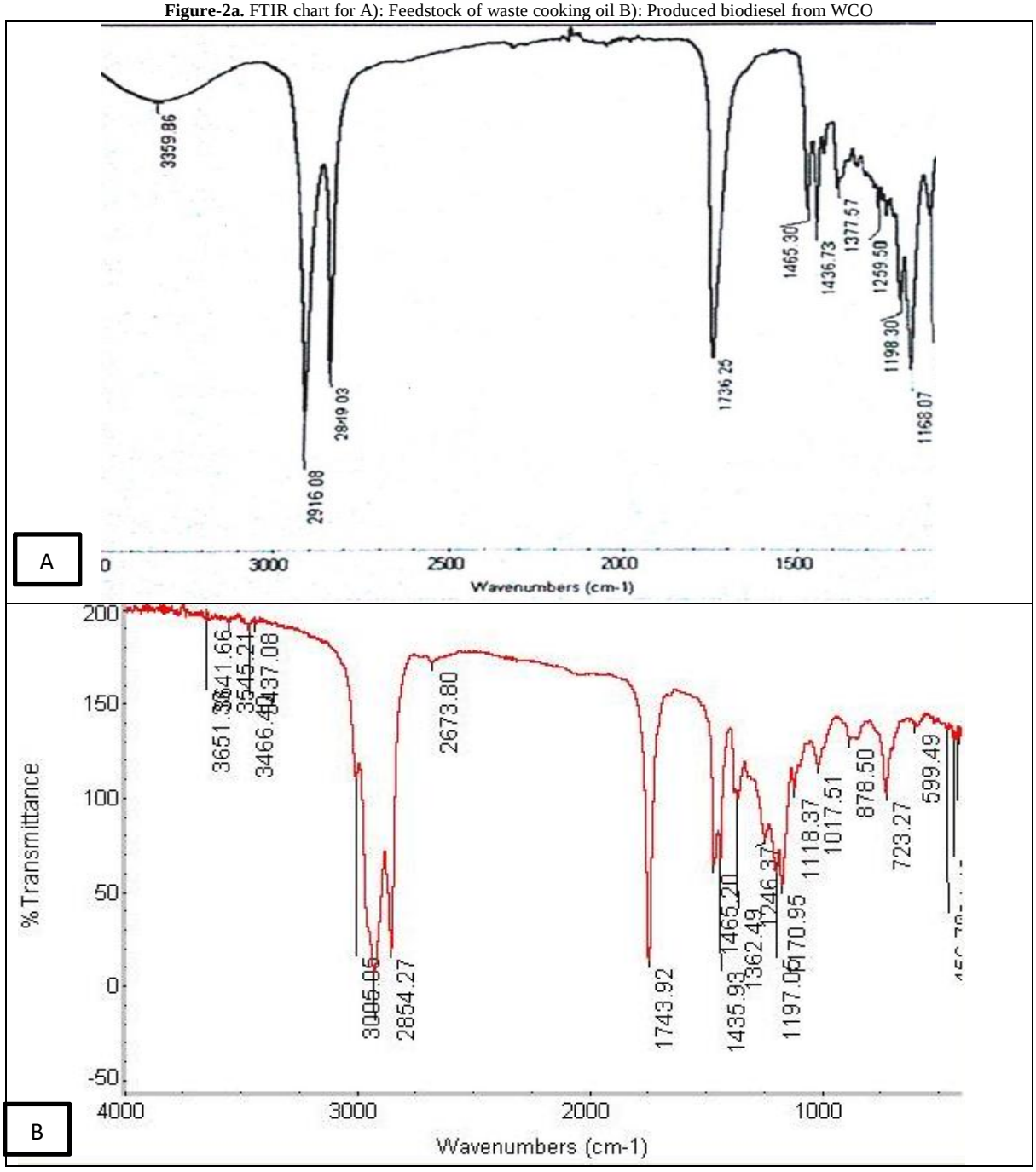
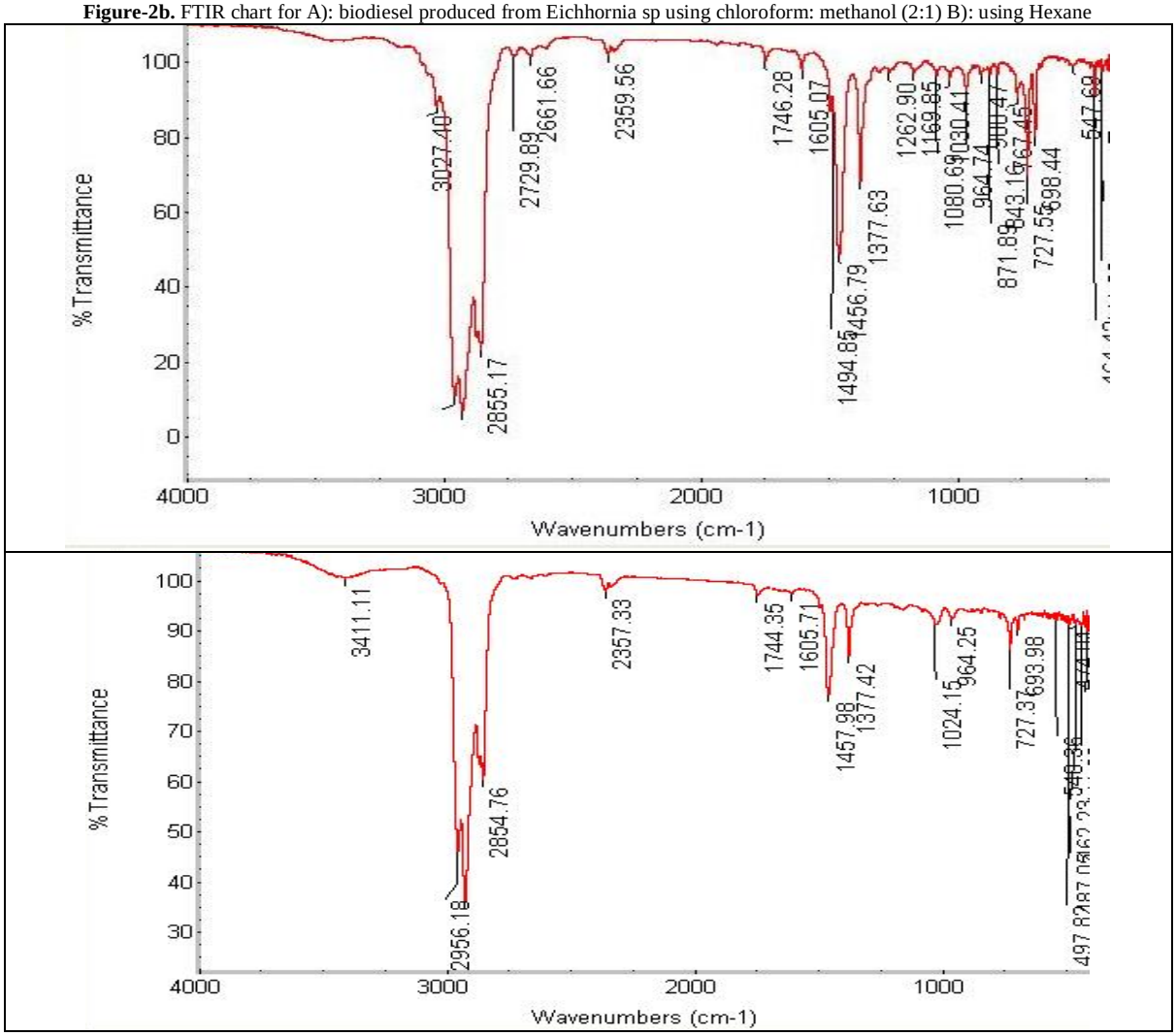


Figure-2b. FTIR chart for A): biodiesel produced from Eichhornia sp using chloroform: methanol (2:1) B): using Hexane



3.6. Production of Body Butter (Makhmaria)

Table 7 and Figure 3 represent body butter (Makhmaria) production from different glycerol layer sources (after separation of biodiesel layer), the produced makhmaria was evaluated by different expert as shown in Table (7), the obtained results reported that samples no. 3 and 5 recorded highest score when compared with remaining samples by (9.57 and 8.87) as average for all physical properties.

Table-7. The evaluation results of produced Makhmaria (body butter)

Parameter	**Sample no.**				
	1	**2**	**3**	**4**	**5**
Colour	9.4	7	9.7	6.8	9.4
Body and texture	8	5.6	9.2	7	8.4
Odour	7.2	8.2	10	8.4	8.9
Appearance	8	6.2	9.4	6.8	8.8
Average 2	8.15	6.75	9.57	7.25	8.87

Figure-3. Makhmaria (body butter) from produced glycerol as by-products for biodiesel production

4. Conclusion

It can be concluded that the pronounced results may encourage a country-wide project for not only collecting and getting rid of water Hyacinth and waste cooking oils, but also making industrial economic value in different country. Harvesting water hyacinth, not only clean the drinking water from its deleterious effect but also would be used for the production of biodiesel, glycerol and pigments; Moreover, waste cooking oils can be used for biodiesel and glycerol production in large scale.

Competing Interests

The authors declare that they have no competing interests.

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