COMPARATIVE STUDY AND PHYSIOCHEMICAL CHARACTERIZATION OF *DIPLOKNEMA BUTYRACEA* SEED OIL WITH EDIBLE OILS

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Abstract: *Diploknema butyracea* (chiur) seed oil is used as edible in Uttarakhand Himalayan region. It has several medicinal properties; in the present study is focus on physiochemical study such as saponification value, acid value, iodine value and peroxide value of three different oils. Oil extract from chiur seed is isolated by mechanically and Soxhlet apparatus. Extracted oil was light brown, buttery taste, buttery in odour and soluble in n-hexane and chloroform. Result show different physiochemical value of chiur, such as acid value (13.01), Iodine value (23.94), Peroxide value (5.00) and Saponification value (156.24). Also compare these values of available edible oil such as cow ghee and mustard oil.
***Key words*: *Diploknema butyracea,* saponification value, Acid value, Iodine value, Peroxide value.**

I. INTRODUCTION

Diploknema butyracea (chiur) commonly known as 'Indian butter tree' belongs to the family Sapotaceae **(Devkota et al, 2012)**. There are 7 species of genus *Diploknema* which are spread in India, Bhutan and semi deciduous forests of Philippines and Andaman Island **(Bandyopadhyay, 2017)**. It is also called as "Madhuca" in Hindi and 'Honey tree' or 'Honey flower' when translated in English **(Dhakal et al, 2014)**. Chiur tree has broad leaves and is usually 22-30 m in height. Flowers of this plant are bisexual and flowering takes place during cold season and fruit ripens in summer (June-July), which is yellow in colour **(Jackson et al, 1994; Puri et al, 2017)**. It is a deciduous tree that produces edible fruits and fruits having economic importance **(Manmohan et al, 2009)**. Fruits are oval shaped, enclosing 1-3 seeds inside **(Devkota et al, 2012)**. *D. butyracea* is a latex yielding plant which occurs in Himalayan region and is distributed in Arunanchal Pradesh, Bhutan, Darjeeling, Garhwal, Himachal Pradesh, Kumaun, Uttarakhand and adjacent parts of North-East region including semi-deciduous forest of Andaman island (**Kureel et al, 2008; Rajkumar and Parthasarathy2008**). It grows on steep slopes of Sub- Himalayan tracts at an altitude of 300-1500 m **(Majumdar et al, 2012)**. Temperature ranges in between 240C to 270C is natural habitat of *D. butyracea* **(Zargar and Kumar 2018)**.

Seeds are the most valuable part of this plant as it contains nearly 65% oil/fat, which is known as "chiur ghee" or "Phulwara butter" locally **(Tamta and Tewari 2018)**. Oil/fat acts as energy reserve which is stored in the form of triacylglycerols in seed and helps in seed germination **(Baud and Lepiniec 2008)**. The major components identified in seed fat are palmitic acid methyl ester, oleic acid methyl ester, linoleic acid methyl ester and stearic acid methyl ester **(Devkota et al, 2012)**. Its seeds acts as the source of edible oil for local people **(Tamta and Tewari 2018)**.The edible oil/butter is utilized in preparation of medicines and is used in burning lamps **(Tag and Tsering 2012)**. It is also used as raw material in production of soap and candles **(Majumdar et al, 2012)**. Oil cake could be used as fish poison, fertilizer and for protection of crop from insects **(Shakya 2000)**. After processing of chiur, the cake is used as manure **(Kureel et al, 2008)**.

D. butyracea plant is used in treatment of ulcers, rheumatic pain, hemorrhage, inflammation of tonsils, diabetes and leprosy **(Tsering and Tag 2014)**. *D. butyracea* oil is used for treatment of paralysis and sprains **(Rashmi and Tyagi 2015)**. Seed fat cures headache, pimple, boils and juice of its bark can treat diseases like asthma and indigestion (**Devkota et al, 2012**). The flowers are rich source of nectar which is used to produce jiggery which has high economic value in Uttarakhand. 17% tannin is present in its bark and is used in tanning and dyeing. Its leaves are used as fodder and branches can be used as fuel wood **(Bahar 2011)**. In the present study is based on physiochemical comparison between edible oil/ghee present in market with *D. butyracea* seed oil/fat.

II. MATERIALS AND METHODOLOGY

2.1 Collection of Plant Material

The seeds of *Diploknema butyracea* (chiur) were collected from forest region of Pithoragarh, Uttarakhand, India and authenticated by Botany department of K.L D.A.V (PG) college, Roorkee, Uttarakhand.

2.2 Extraction of oil

The oil of chiur (*D. butyracea*) seeds was extracted mechanically as well as with different solvents in soxhlet apparatus. 25g of seed powder was extracted in soxhlet apparatus with 250ml of each solvent like n-hexane, chloroform: methanol (2:1). The respected solvents were further evaporated with the help of heating mantle. Concentrated oil was obtained and stored at 4°C for further use **(Morshed et al, 2012)**.

2.3 Determination of physical properties of fatty oil

2.3.1 Colour and flavor

The colour and flavor of oil/fat samples was determined by observation using several independent individuals and with the help of colour charts oil/fat colour were correlated.

2.3.2 Solubility of fatty oil in different solvents

A small amount of fatty oil samples was taken in 3 test tubes and 3ml of n-hexane was added to each of it to determine solubility. Solubility test was further performed by using different solvents- Chloroform (b.p- 61.2°C), petroleum ether (b.p- 42 -62°C) and benzene (80.1°C) solvent by following same procedure **(Morshed et al, 2012)**.

2.3.3 Determination of melting and boiling points

The melting and boiling point of the oil/fat samples were determined by using a thermometer ±1° C. The boiling point of oil depends on the degree of unstauration of fatty acids **(Zahir et al, 2014)**.

2.3.4 Determination of moisture content

To determine moisture content, three beakers were weighed and 10g of oil/fat sample was added in each. The samples were dried in oven at 105°C to constant weight, cooled in desiccators and weighed. The procedure was repeated 3 times and average value was calculated **(Birnin-Yauri, 2011)**.

2.3.5 Determination of ash content and organic matter content

A crucible was pre-heated in a muffle furnace at 500°C, then it was cooled in desiccators and 2g of the seed sample was transferred into the crucible and weighed. The crucible along with its content was kept in the muffle furnace at 525°C for 12 hours until white ash was obtained. Ash content was calculated by weighing the crucible and difference was determined. The organic matter content in the seed sample was calculated by subtracting the percent ash content from 100.

2.4 Determination of chemical properties of oil

2.4.1 Determination of saponification value

To determine the saponification value, 1g of oil/fat sample was weighed in a conical flask to which 25ml of 0.5 N alcoholic KOH was added. It was heated under reserved condenser for 30-40 minutes until sample was fully dissolved. After cooling the sample, few drops of phenolphthalein indicator was added and titrated against 0.1 N HCl until pink end point was obtained. Similarly a blank was determined **(Morshed et al, 2012)**. The saponification value was calculated from the following equation.

$$\text{Saponification value} = \frac{(\text{Blank} - \text{Test}) \times \text{Normality (Hcl)} \times 56.1}{\text{Weight of sample (g)}}$$

2.4.2 Determination of acid value

5 grams of oil sample was weighed in a conical flask, to which is added 25ml diethyl ether, 25ml alcohol (ethanol) and 1ml of phenolphthalein indicator. It was neutralized with 0.1 M Sodium Hydroxide solution with continuous shaking until a pink colour was obtained **(Morshed et al, 2012)**. The acid value was calculated from the following equation:

$$\text{Acid value} = \frac{\text{Titration (ml)} \times 5.61}{\text{Wt (g) of sample used}}$$

2.4.5 Determination of Iodine value

Exactly 0.212g of oil sample was taken in iodine flask. To is was added 25ml of chloroform and 25ml of wiz solution. 5ml of 10% KI solution was added on the cap of iodine flask and left for 30 minutes. After this the cap was opened and 10ml of KI solution and 5-6 drops of starch indicator were added. At last 30ml of distill water was added and it was titrated against 1 N Sodium thiosulphate solution **(Hasan et al, 2016)**. The iodine value was calculated using the following equation:

$$\text{Iodine value} = \frac{(\text{Blank} - \text{Sample}) \times 12.69 \times 0.1\ (N)}{0.212\ (\text{wt of the sample})}$$

2.4.6 Determination of peroxide value

1 gram of oil sample was taken in a conical flask; to it 30ml of solution (18ml acetic acid + 12ml chloroform) and 0.5ml of KI solution was added. It was shaken for 1 minute and after this 30ml of distill water and 0.5ml of starch indicator was added to it. At last it was titrated against 0.1 N sodium thiosulphate solution **(Hasan et al, 2016)**. Blue colour disappears as an end point. The peroxide value was calculated using the following equation:

$$\text{Peroxide value} = \frac{\text{Burette reading} \times 1000 \times N}{\text{Sample wt}}$$

III. RESULTS AND DISCUSSION

The results show comparative analysis of seed extract of *Diploknema butyracea* (chiur) with edible oil such as cow ghee and mustard oil on the basis of physiochemical study.

3.1 Oil extraction

Oil was extracted mechanically as well as with solvents using soxhlet apparatus. The maximum yield 16 g of oil was obtained using n-hexane as a solvent, While mechanical method and chloroform : ethanol solvent show less yield of oil 4g and 12.74g respectively **(Table 1)**.

Table 1: Different method used to extract seed oil

Method	Yield of oil
Mechanical method	4 g of oil
n-hexane (solvent)	16 g of oil
Chloroform : ethanol (2 : 1) (solvent)	12.74 g of oil

3.2 Colour and flavor

The colour of the chiur ghee is light brown, while cow ghee and mustard oil show yellowish and dark yellow (reddish) colour respectively. Chiur ghee and Cow ghee have buttery taste and odour with solid state, while mustard oil has pungent taste and odour due to the presence of allyl isothiocyanate and activator of TRPA1 channel with liquid state **(Table 2).**

Table 2: Colour, Taste , Odour and physical state of oil/fats

Oil/fat	Colour	Taste	Odour	State
Chiur ghee	Light brown	Buttery (little bitter)	Buttery	Solid
Cow ghee	Yellowish	Buttery	Buttery	Solid
Mustard oil	Dark (reddish) yellow	Pungent	Pungent	Liquid

3.3 Solubility of oils in different solvent

Small volumes of fatty oils (3 ml) were taken in test tubes and small volume of solvent (3-4 ml) was added to each. Clear solution was obtained in each solvent. Solubility of oil samples was performed with n-hexane, chloroform, petroleum ether and benzene solvent. All three oils were found to be soluble in each solvent **(Table 3)**.

Table 3: Selected oil solubility in different solvents

Solvents	Chiur oil	Cow ghee	Mustard oil
n-hexane	soluble	Soluble	soluble
chloroform	soluble	soluble	soluble
Petroleum ether	soluble	soluble	soluble
benzene	soluble	soluble	soluble

3.4 Melting and boiling points

Melting and Boiling point of the oil/fat samples was measured using thermometer. The boiling point of mustard oil was found highest 302°C compare to chiur and cow ghee. Mustard oil found in liquid form so no melting point exist while chiur and cow ghee were found 38°C and 42°C respectively **(Table 4).**

Table 4: Melting point and Boiling point of selected oil/fats

Oil/fat	Melting point	Boiling point
Chiur oil	38°C	260°C
Cow ghee	42°C	270°C
Mustard oil	_	302°C

3.5 Moisture content

Mustard oil and cow ghee have moisture content of 0.14% and 0.94% as lowest and highest values respectively. The higher is the moisture content in oil, the greater the value used for baking, frying, food texturing and in industries for manufacturing of detergents, soaps and cosmetics **(Table 5, Figure 1)**.

Table 5: Moisture content of selected oil/Fats

Fat/oil sample	Moisture content (%)
Chiur ghee	0.90
Cow ghee	0.94
Mustard oil	0.14

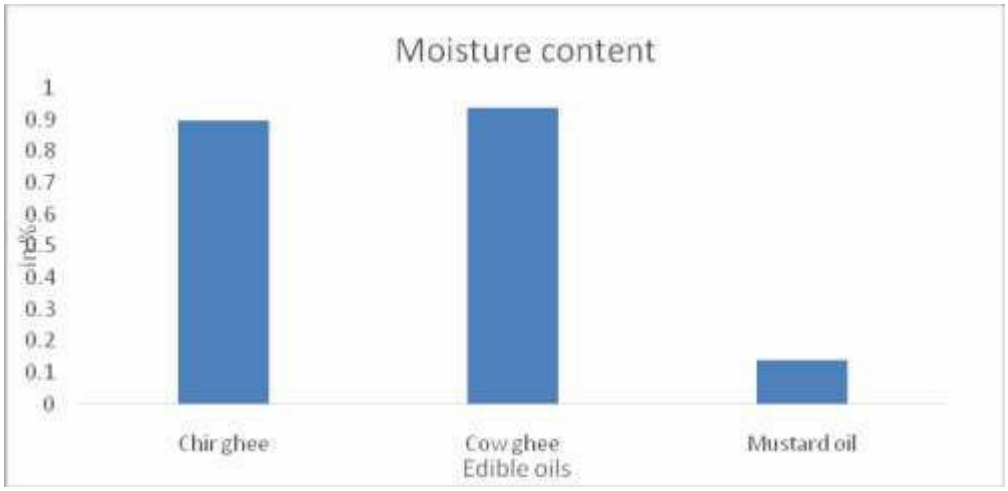

Figure 1: Moisture content of selected fats/oil

3.6 Ash content and organic matter content

Ash content of seed powder was determined with the help of muffle furnace at 525°C temperature. The organic matter content in the seed was calculated by subtracting the percent ash content from 100. *D. butyracea* seeds have ash content of 2.4% and 97.6% organic matter content.

Weight of empty beaker (b1) = 47.282 g

Weight of empty beaker + sample (b2) = 49.282 g

Weight of empty beaker + dry ignited sample (b3) = 47.330 g

$$\text{Ash\%} = \frac{b3 - b1}{b2 - b1} \times 100$$

$$= 2.4\%$$

Organic matter content = 100 – 2.4

= 97.6%

3.7 Chemical properties of oil and its comparative analysis with cow ghee and mustard oil

3.7.1 Saponification value

Saponification value of oil/fat gives the information about the average chain length hence the molecular weight of fatty acids in oil sample. Higher the saponification value of oil sample, the shorter the average chain length of fatty acids and lower the saponification value, the longer the average chain length of fatty acids. The saponification value of the oil samples obtained shows that 185.69 mg KOH/g for cow ghee is highest value and 148.10 mg KOH/g for mustard oil is lowest value **(Table 6, Figure 2)**.

Table 6: Saponification value of selected oil/fats

Oil/fat	Saponification value
Chiur ghee	156.24 mg KOH/g
Cow ghee	185.69 mg KOH/g
Mustard oil	148.104 mg KOH/g

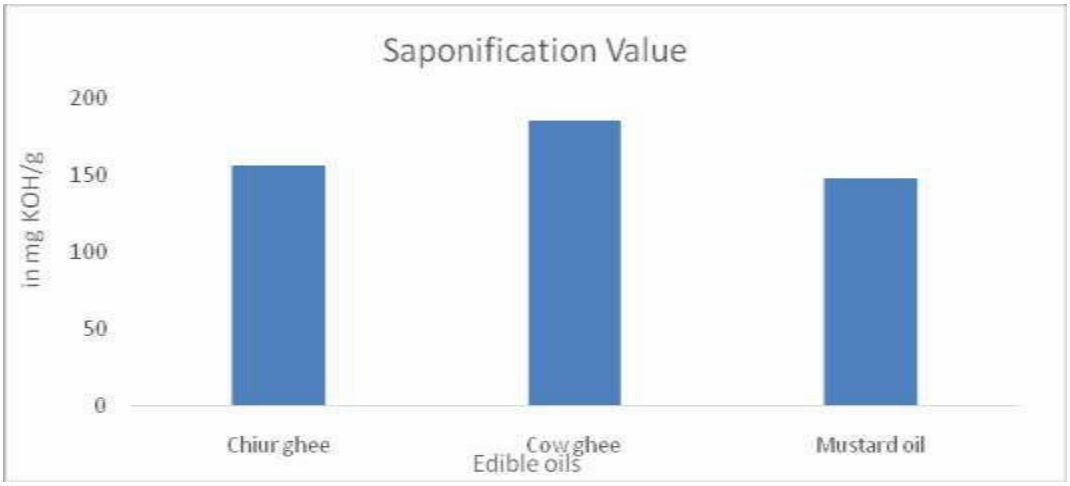

Figure 2: Saponification value of selected fats/oil

3.7.2 Acid value

Acid values of oil/fat determine the amount of free fatty acids present in the oil/fat sample. It is use to find the purity of oils. The higher and the lower value was obtained for chiur ghee and cow ghee and were 13.01 and 1.79 respectively. Higher acid value shows that triglyceride of oil is converted into glycerol and fatty acids which are responsible for rancidity of oil **(Table 7, Figure 3)**.

Table 7: Acid value of selected oil/fats

Oil/fat	Acid value
Chiur ghee	13.01
Cow ghee	1.79
Mustard oil	2.35

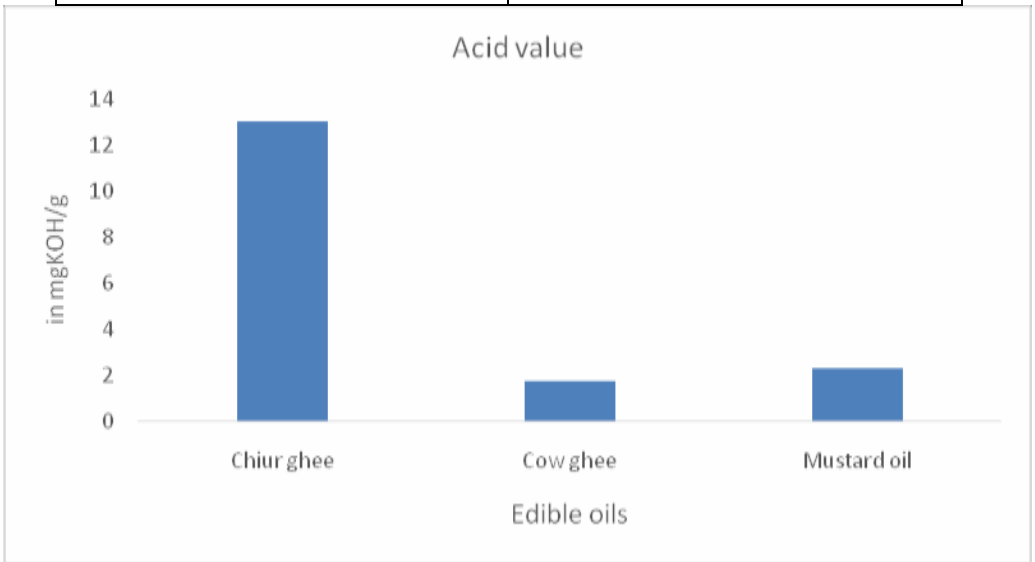

Figure 3: Acid value of selected fats/oil

3.7.3 Iodine value

Iodine value determines the degree of unsaturation in oil/fat sample. It determines the stability of fat/oil sample to oxidation and allows quantitative analysis of overall unsaturation of the fat. The higher and lower iodine values were obtained for mustard oil and cow ghee and were 110.73 and 11.97. The lower iodine value of oil indicates that nearly 95% of fatty acid in oil is saturated so they have less C=C double bonds and therefore minimum unsaturation whereas higher iodine value indicates higher unsaturation of oil sample. Oil having lower iodine value shows greater oxidative storage stability **(Table 8, Figure 4)**.

Table 8: Iodine value of selected oil/fats

Oil/fat	Iodine value
Chiur ghee	23.94
Cow ghee	11.97
Mustard oil	110.73

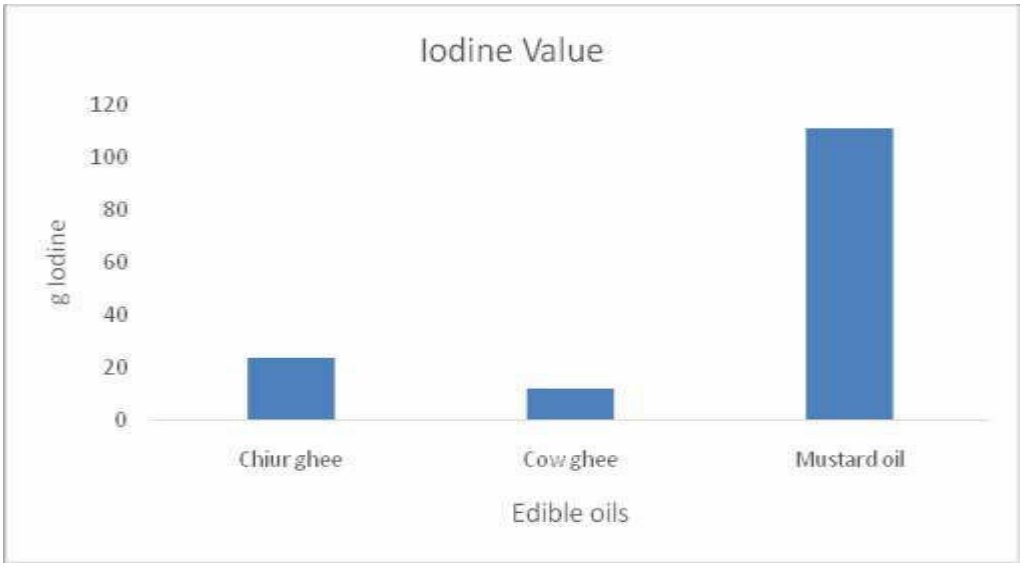

Figure 4: Iodine value of selected fats/oil

3.7.4 Peroxide value

Peroxide values were determined after heating oil/fat at 200° C for 30 minutes. Peroxide value of oils indicates the quality and stability of fat/oil. It is used as a measure of the extent to which rancidity reactions have occurred during storage. In the study chiur ghee and cow ghee showed the lower peroxide values 5 meq O_2/kg which indicates good quality of both these fats/oils.

Table 9: Peroxide value of selected oil/fats

Oil/fat	Peroxide value
Chiur ghee	5
Cow ghee	5
Mustard oil	10

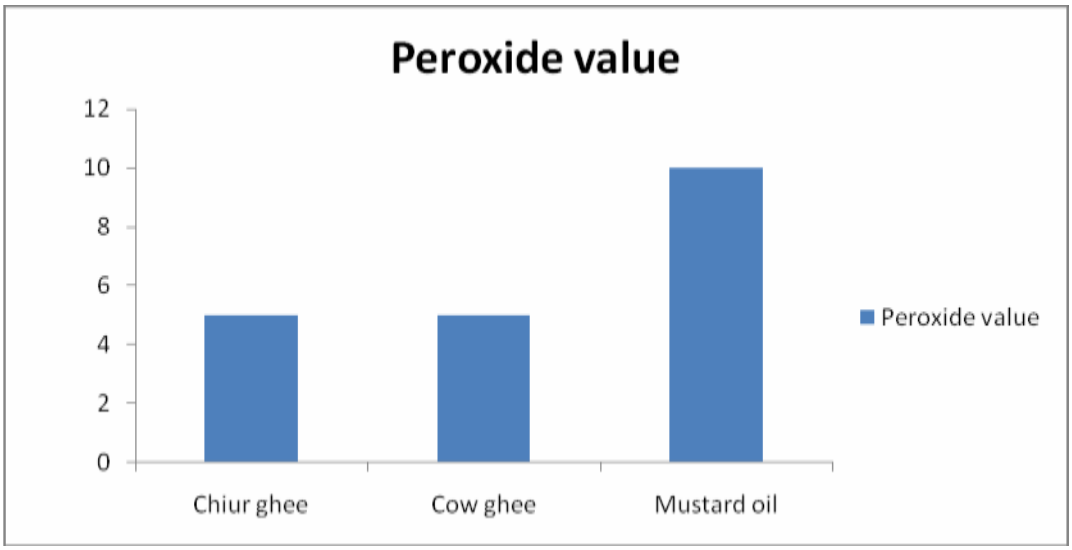

Figure 5: Peroxide value of selected fats/oil

The physiochemical properties of oil/fat samples of Chiur ghee, Cow ghee and Mustard oil are presented in above tables. The saponification values of chiur ghee, cow ghee and mustard oil were 156.24, 185.69 and 148.10 respectively. The obtained value of mustard oil was little lower than the values reported by **Sharif (2017)**, who found the saponification value of three verities of mustard oil to be, 157.60, 157.90 and 159.40. Acid values of chiur ghee, cow ghee and mustard oil were 13.01, 1.79 and 2.35. The highest value was found in chiur ghee and lowest was reported in cow ghee. The acid value could get increased due to contamination while sample preparing and handling. Iodine values of chiur ghee, cow ghee and mustard oil was 23.94, 11.97 and 110.73. The highest value was found in mustard oil and lowest value was reported in cow ghee. The obtained value of mustard oil was quite similar to the values reported by **Singh (2018)**, who found the iodine value of mustard oil, 109.4.Peroxide values were 5, 5 and 10 for chiur ghee, cow ghee and mustard oil which was obtained after heating the oil/fat sample at 200^0C for 30 minutes, to check the effect of temperature on peroxide value of samples. The increase in value could be due to the oxidation of fatty acids due to high temp. poor storage or poor handling.

IV. CONCLUSION

The observation results concluded that we should consider the physicochemical properties for selecting good type edible oil. Result show iodine value is high, the peroxide value is also high but not vice versa. It is also concluded that oils with low free fatty acids have a tendency to have high boiling point and low peroxide value which are good quality of ideal edible oils**.** Chiur oil and cow ghee have same peroxide value, while chiur ghee show high iodine and acid value compare to cow ghee. So it is edible oil/fats in many region of India.

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