Isolation and Characterization of Value Added Product from Waste *Morinda Citrifolia.L* (Noni) Seeds

Manivasagan V.[1], Saranya K.[1*], Gowtham D.[1], Mohankumar T.[1] and Ramesh Babu N. G.[1]

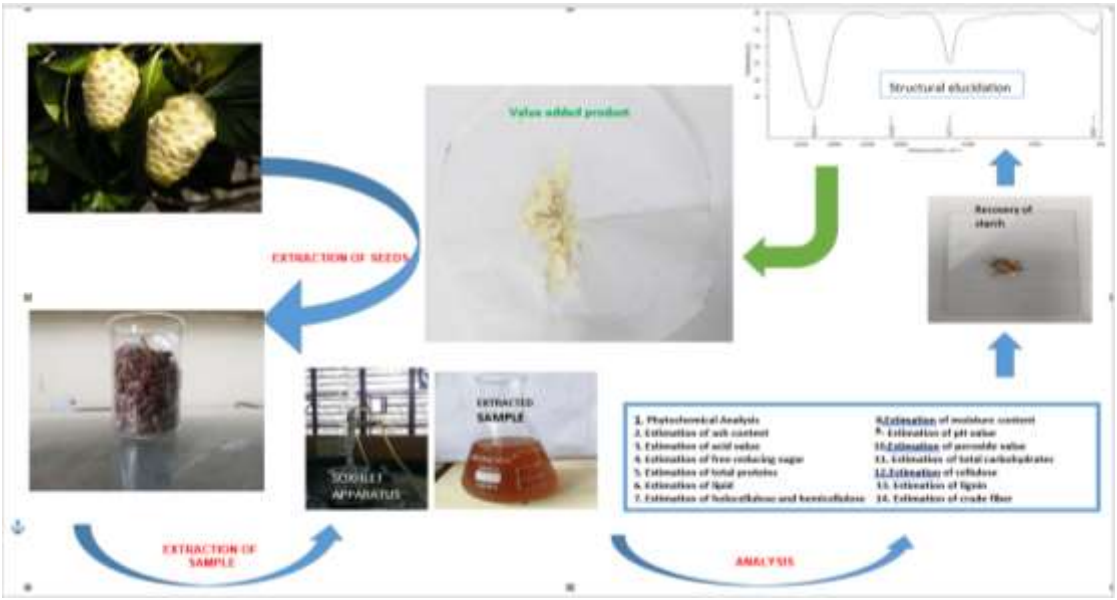
[1] *Department of Biotechnology, Adhiyamaan College of Engineering, Hosur-635 109, Tamil Nadu, INDIA*

E-mail: ksaranya31@gmail.com

Abstract

Morinda citrifolia.L fruit constitute about 18% of the waste seeds in the juice industry in India. They were characterized by the preliminary study to evaluate their possible utilization. In this study, characterizations like phytochemical analysis, FTIR, physical properties and biochemical properties of waste Morinda citrifolia.L seeds were done. The phytochemical analysis indicated the presence of sucrose, flavonoids, steroids, phenols, carbohydrates, xanthoproteis, amino acids, ketose sugars, reducing sugars. The FTIR spectra of the crude sample absorption peaks were located at about 558.99 cm^{-1} (C-Br), 1637.70 cm^{-1} (C=0), 2149.59 cm^{-1} (C=H), 3301.54 cm^{-1} (O-H). The physical properties of the waste Morinda citrifolia.L seeds like pH, acid value and peroxide value were estimated and found to be 6.12, 26.8 mg NaOH/g and 9.5 mg NaOH/g. The biochemical properties of the waste Morinda citrifolia.L seeds like moisture content, ash content, cellulose content, lignin content, lipid content, holocellulose content, hemicellulose content, carbohydrates, protein, free reducing sugars was estimated and found to be 37.4 %, 26.5 %, 50.8 %, 6.58 %, 19.4%, 55.9 %, 5.01 %, 42%, 26 %, 29 % and 35 %. The maximum amount of 32% of starch was obtained at 5°C in 0.25% concentration of NaOH. This work shows that the phytochemical analysis, physical properties, biochemical properties and extraction of starch. It also provides a footing for development of further research.

***Key words**: Morinda citrifolia.L seeds, phytochemical analysis, FTIR, physical properties, biochemical properties, starch.*



1. INTRODUCTION

The Hawaiian name for the fruit of *Morinda citrifolia.L* (Rubiaceae) is Noni. It is cultivated in Polynesia, India, Caribbean, Central, Northern South America and is a native of Southeast Asia to Australia [1]. The other names include ‘‘Indian mulberry’’, ‘‘nuna’’, or ‘‘ach’’ in India, ‘‘mengkudu’’ in Malaysia, ‘‘nhau’’ in Southeast Asia, ‘‘painkiller bush’’ in the Caribbean, or ‘‘cheese fruit’’ in Australia [2]. The noni serves as food and medicine for over 2000 years [3], the Noni fruit is claimed to prevent and cure several diseases. It as an important herb for treating some physiological disorder. It is commonly known as Mulberry or Noni in India. The genus *Morinda* including the species *Morinda citrifolia L.*, consists of around 80 species. Noni is a small tree with abundant wide elliptical leaves. The fruit is covered with small reddish-brown buds containing the seeds with spots on the surface. The Noni plant bears fruit about nine months to one year under favourable conditions and these fruits can be harvested, and the yield per tree is very low [4].

In the early 1990s, *Morinda citrifolia.L* fruit was first commercially produced in the USA. Later in 1996, it was discovered that *Morinda citrifolia.L* juice had therapeutic effects and introduced as a wellness drink. In 2003, European commission was approved by novel food of *Morinda citrifolia.L* fruit juice and limited Tahitian fruit juice was approved. The *Morinda citrifolia.L* absence for specific mechanism effects and the annual market sales to reach up to US $ 1.3 billion [5, 6]. The popularity of *Morinda citrifolia.L* is popular as a natural health and immune enhancer and functional ingredient in the dietary food supplements and the fruits contains approximately 40000 seeds in every kilo.

In this present work, characterizations like phytochemical analysis, physical properties, biochemical properties, FTIR were estimated and production of biodegradable paper. This research work shows that waste *Morinda citrifolia.L* seeds can be used to extract the valuable products such as health nutraceutical and starch.

2. Materials and Methods

2.1. Sample collection and processing

Morinda Citrifolia L., (Noni fruit) waste seeds used in this work was collected from Sipcot area, Hosur, Tamil Nadu, India. The waste seeds were dried in a hot air oven at a temperature of 40°C for 3-7 days till the weight became constant. The seeds were regularly examined to check for any fungal growth or rotting. The dried seeds were finely grounded using an electrical grinder.

2.2. Extraction

In this study, soxhlet apparatus was used for the extraction process. 10 g of waste Morinda citrifolia.L seeds were powdered, sample material was uniformly packed into a thimble and extracted with 300 ml of distilled water. The extraction process was continued for 6 hours or till the solvent in the thimble became colourless at 60ºC [7]. The extract was taken and kept on distillate unit and heated at 60ºC until the solvent evaporated. The extract of Morinda citrifolia.L seeds was stored at 4ºC for further use.

2.3. Phytochemical Analysis

Morinda citrifolia.L seeds extracts were subjected to standard phytochemical analyses to find the presence of the following phytochemical constituents sucrose, flavonoids, steroids, terpenoids, phenols,

carbohydrates, alkaloids, xanthoprotein, amino acids, ketose sugar, monosaccharide, reducing sugar [8, 9].

2.4. Estimation of moisture content, ash content

The moisture content and ash content was estimated by standard method as given by the Association of official Analytical Chemist [10, 11].

2.5. FTIR spectroscopic analysis

Fourier transform infrared spectrophotometer (FTIR) is a powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The liquid sample of solvent extract of Morinda citrifolia.L seeds were used for FTIR analysis. 1 ml of the solvent extract was encapsulated in 100 mg of potassium bromide (KBr) pellet. The liquid sample of the seed specimen was loaded in FTIR Spectroscope (Bruker spectrometer) were characterized in the range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} [12].

2.6. Estimation of pH value

2 g of the sample was added to 13 ml of hot distilled water and it was cooled in a cold water bath at 25^{o} C. The pH value were recorded [13].

2.7. Estimation of acid value

25 ml of diethyl ether and 25 ml of ethanol was mixed and the mixture was added to 10 g of sample and few drops of phenolphthalein indicator were added. The mixture was titrated with 0.1 M of NaOH and appearance of dark pink colour and the acid value was calculate by the equation 2.1 [13].

$$\text{Acid value} = \frac{56.1\ (V)\ (N)}{W} \qquad (2.1)$$

Where, V - Volume of ml in standard sodium hydroxide, N - Normality of sodium hydroxide, W - weight of the sample

2.8. Estimation of peroxide value

1 g of sample was added to 1 g of potassium iodide and the mixture was added to 20 ml of the solvent mixture was boiling with 30 seconds. The mixture was transferred into 20 ml of 5 % potassium iodide solution and the tube was washed twice with 25 ml of water and titrated with 0.1 N of $Na_2S_2O_3$, 0.5 ml of starch indicator was added and disappearance of yellow colour was seen [14, 15]. The peroxide value was calculated by the equation 2.2.

$$\text{Peroxide value} = \frac{(S)(N)(100)}{\text{g sample}} \qquad (2.2)$$

Where, S = Test - Blank, N = Normality of $Na_2S_2O_3$

2.9. Estimation of free reducing sugar

The free reducing sugars was estimated by the DNS method (Biotechnology methods / Biochemistry).

2.10. Estimation of total carbohydrates

The total carbohydrate was estimated by the standard method [16].

2.11. Estimation of total proteins

The total proteins was estimated by Lowry's method [17].

2.12. Estimation of cellulose

1 g of sample and 3 ml of acetic reagent was added to the test tube. It was kept in water bath at 100ºC for 30 minutes and centrifuged at 5000 rpm for 15 minutes. The supernatant was discarded and the residue was dried in a hot air oven at 90º C for 1 hour and weighed [18].

2.13. Estimation of lipid

1 g of sample was added to 0.8 ml of distilled water and 2 ml of chloroform and 4 ml of methanol were added. Using the homogenizer, the mixture was homogenized for 1 minute and 2 ml of distilled water was added and homogenized for 30 seconds. The mixture was centrifuged at 1000 rpm for 5 minutes at 4^0C. The supernatant was transferred to a sterile container and dried in a hot air oven at 60ºC for 1 hour and weighed [10].

2.14. Estimation of lignin

1 g of sample was added to 10 ml of 72 % H_2SO_4 and stirred for 3 hrs. The acid content was diluted with distilled water and filtered with Whatman No.1 filter paper. The filter paper was dried in a hot air oven at 60ºC for 1 hour and weighed [19].

2.15. Estimation of holocellulose and hemicellulose

1 g of sample was added to 75 ml of distilled water, 0.1 ml of acetic acid, 0.5 g of sodium chlorite were kept in water bath at 75ºC for 1 hour. 0.1 ml of acetic acid and 1 g of sodium chlorite was added and incubated at 75ºC for 1 hour and the process was repeated for 3 hours. The mixture was washed with distilled water and filtered with Whatman No.1 filter paper and the filter paper was dried and weighed [20].

2.15. Estimation of crude fiber

2 g of extract sample with 200 ml of sulphuric acid for 30 minutes was boiled and filtered through a muslin cloth and washed with water. The mixture was boiled with 200 ml of sodium hydroxide for 30 minutes and filtered through muslin cloth, wash with 25 ml of boiling 1.25 % H_2SO_4, 50 ml of three portions water and 25 ml of ethanol. The residue was transferred through the crucibles and dried with a hot air oven at 2 hours for 130^0C and crucible was cooled in a desiccator and weighed. The residue was ignited for 30 minutes at 600ºC and crucible was cooled in a desiccator and weighed [21]. The crude fiber was calculated by the equation 3.3.

$$\% \text{ crude fibre} = \frac{(W2-W1)-(W3-W1)}{\text{weight of the sample}} X\ 100 \qquad (3.3)$$

Where W_1 - empty weight of crucible, W_2 – weight of the crucible before cooling, W_3 – weight of the crucible after drying

2.16. Determination of starch

The waste Morinda citrifolia.L seeds were steeped into 200 ml of different concentrations of 0.05%, 0.1%, 0.15%, 0.2% and 0.25% NaOH at different temperatures of 3ºC, 5ºC, 7ºC, 9ºC and 11ºC for 24 hrs. The steeped seeds were washed and ground with an equal volume of water using blender for three minutes and the slurry was filtered through a 200 mesh screen. The filtered slurry was allowed to stand for 1 hour and centrifuged at 2000 rpm for 10 minutes. The top grey coloured rich protein layer was removed and excess water was added to resuspend the sample and centrifugation was done for 10 minutes. The process was repeated several times until top layer was white and the starch was dried in a hot air oven for 24 hr at 40ºC [22].

2.17. Recovery of starch

The percentage of starch recovery from waste Morinda citrifolia.L seeds extract was estimated by chemical methods. The extracted starch was dried and weighed. The starch recovery was calculated by the equation A.4 [23].

$$\text{Starch recovery (\%)} = \frac{Ws}{Wr} \text{ X } 100 \quad (2.4)$$

Where, Ws = Weight of extracted starch, Wr = weight of sample taken

3. Results and Discussion

3.1. Phytochemicals Screening

The present study investigated the presence of phytochemicals constituents in the several components of secondary metabolites of Morinda citrifolia.L seeds extract like sucrose, flavonoids, steroids, phenols, carbohydrates, xanthoproteis, amino acids, ketose sugars, reducing sugars. The study revealed the presence of phytochemicals which are used as effective nutraceutical health supplements and it is useful for pharmaceutical industries [24]. All the parts of Morinda citrifolia.L plant can be used as medicine for many diseases and health supplements and it has a number of curative properties against infectious diseases. The results were shown in the Table 3.1 and (+) indicates the presence of the constituents and (-) indicates the absence of constituents.

Table 3.1 Phytochemicals screening of Morinda citrifolia.L seeds extract

phytochemicals	observation	results
Sucrose	No blue color solution	+
Flavonoids	Yellow color appearance	+
Steroids	Reddish Brown ring formation	+
Terpenoids	Formation of red color	-
Phenols	Reddish orange percipitate	+
Carbohydrates	Violet ring formation	+
Alkaloids	Intense yellow color	-
Xanthoproteins	Blue black color formation	+
Amino acids	Purple color	+
Ketose sugar	Red color solution	+
Monosaccharide	No reddish percipitate	-
Reducing sugar	Brick red colour	+

3.2. Fourier-Transform Infrared Spectroscopy analysis (FTIR)

The FTIR characterization to identify the potential biomolecules of the Morinda citrifolia.L seeds extract and the FTIR spectra was shown in the Figure 3.1 and functional groups was shown in the table

3.2. The characterization ranges from 4000 -400 cm $^{-1}$. The functional groups of the components were separated based on their peaks values. The representative spectra of the crude sample absorption peaks located at about 558.99 cm^{-1} (C-Br), 1637.70 cm^{-1} (C=0), 2149.59 cm^{-1} (C=H), 3301.54 cm^{-1} (O-H). Several functional groups are present in the alkyl halides, ketonese, alkynes, alcohols and phenols groups. The peaks further confirms the presence of flavonoids, phenols, xanthoproteins and ketose sugar [25].

Table 3.2 FTIR frequency range and functional groups present in the Morinda citrifolia.L seeds extract

S.No	wave number (cm^{-1})	functional group present
1.	558.99	C-Br streching
2.	1637.70	C=O streching
3.	2149.59	C=H streching
4.	3301.54	O-H streching

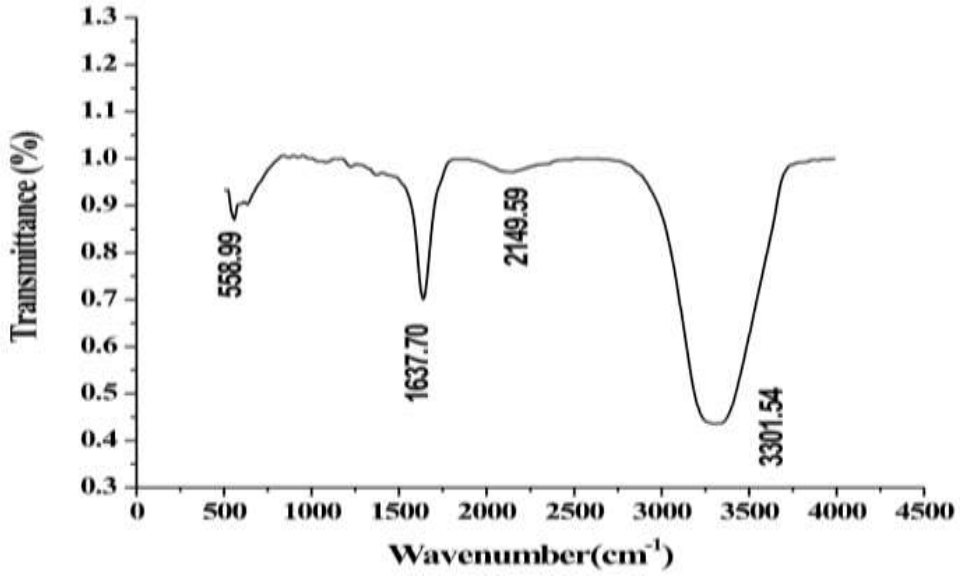


Figure 3.1 FTIR spectra for Morinda citrifolia.L seeds extract

3.3. Physical properties

The physical properties of the waste Morinda citrifolia.L seeds like pH, acid value and peroxide value was estimated and found to be 6.12, 26.8 mg NaOH/g and 9.5 mg NaOH/g and graphically shown in the Figure 3.2. The pH value is less acidic of crude sample and the acid value is higher than peroxide value, it shows that the crude extract of waste Morinda citrifolia.L seeds. The saturated fats and oil

content is higher than the unsaturated fats and oil content and the acid value should not be consumed. The acid value is an indicator for edibility of crude extract and use in industries [26, 27].

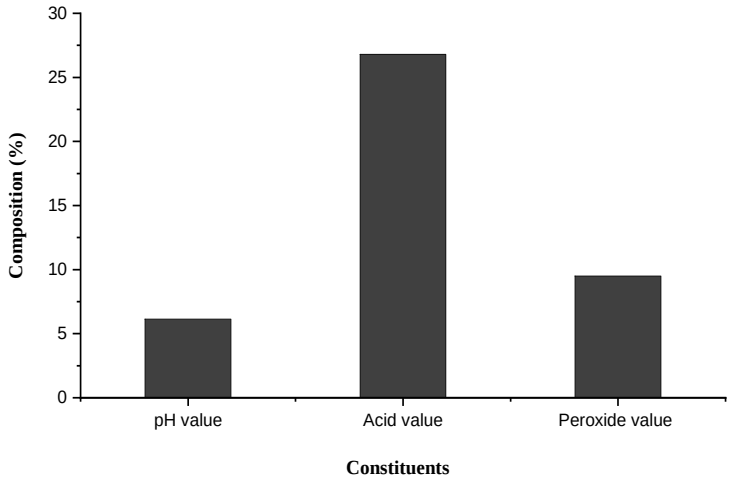


Figure 3.2 showing physical properties of Morinda citrifolia.L seeds extract

3.4. Biochemical properties

The moisture content of the Morinda citrifolia.L seeds analyzed was roughly 37.4%. The moisture content of oilseeds is industrial process such as drying, milling and oil extraction [28, 29]. The optimum moisture content is important parameter in seeds during storage which will cause damage to the maintain seed viability and seeds component [29, 30]. The ash content of the Morinda citrifolia.L seeds was 26.5% and it also mineral present in sample have special significance in proper body function [29]. The cellulose content of the Morinda citrifoli.L seeds was analysed was 50.8% is insoluble substance of plant cell walls and lignin content was 6.58% is low substance compared to the cellulose content. The holocellulose and hemicellulose content of seeds was 55.9% and 5.01% is the polysaccharide substance of simpler structure than the cellulose. Cellulose and Hemicellulose content is partially digestible but lignin is not partially digestible. The crude fiber content was analysed by 42% of waste Morinda citrifolia.L seeds. The lipid content of seeds was 19.4% of fatty acids and soluble in organic solvents but insoluble in water. Polysaccharide is an energy store carbohydrates, protein, reducing sugars is produced by most green plants content was present in 26%, 29%, 35% of Morinda citrifolia.L seeds and graphically shown in the Figure 3.3. The maximum amount of 32% of starch was obtained at 5^{o}C in 0.25% concentration of NaOH as shown in the Figure 3.4. Protein is a macronutrient essential for the supplement through diet and proper growth and it should be metabolism of human body and important constituents of food. Carbohydrates are present as physical or chemical molecules bound to other molecules and hydrates of carbon. Carbohydrates provide an important source of energy and digestible by humans and they also contribute to the appearance, sweetness and textual characteristic of many foods. Starch is a large number of glucose units consisting of polymeric carbohydrates. The free reducing sugar is a reducing agent and it also free aldehyde or ketone groups [24].

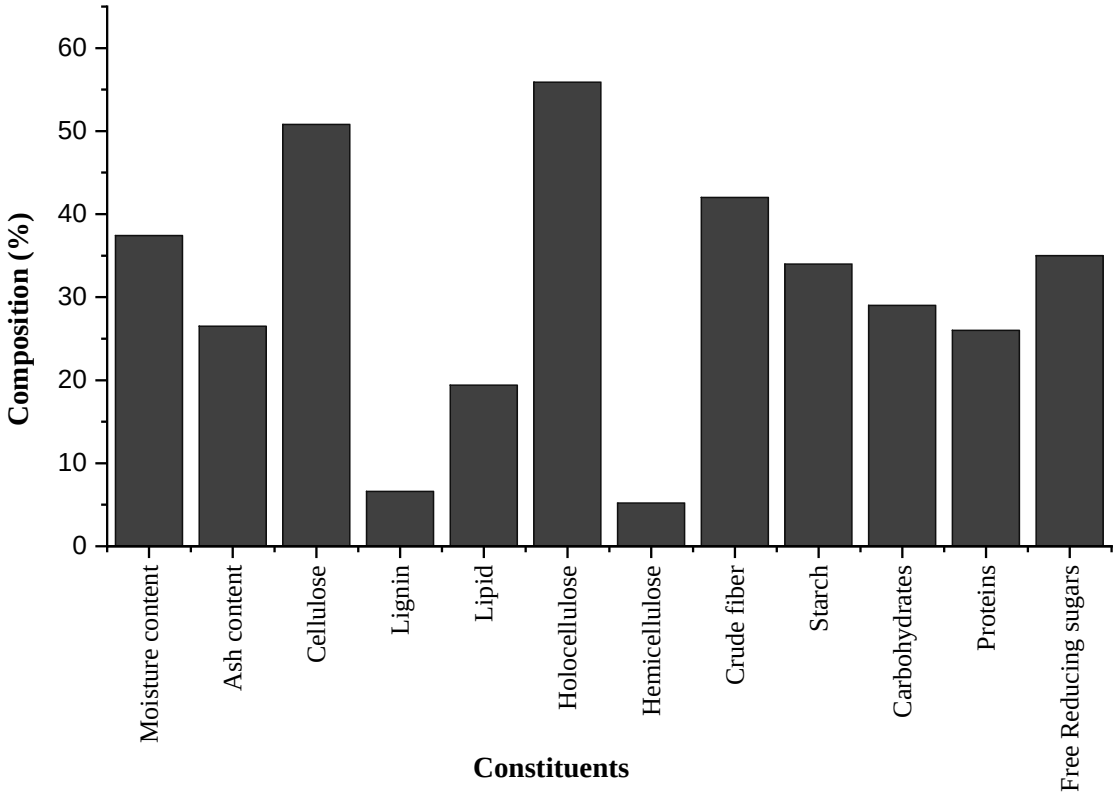


Figure 3.3 showing biochemical characterization of Morinda citrifolia.L seeds extract

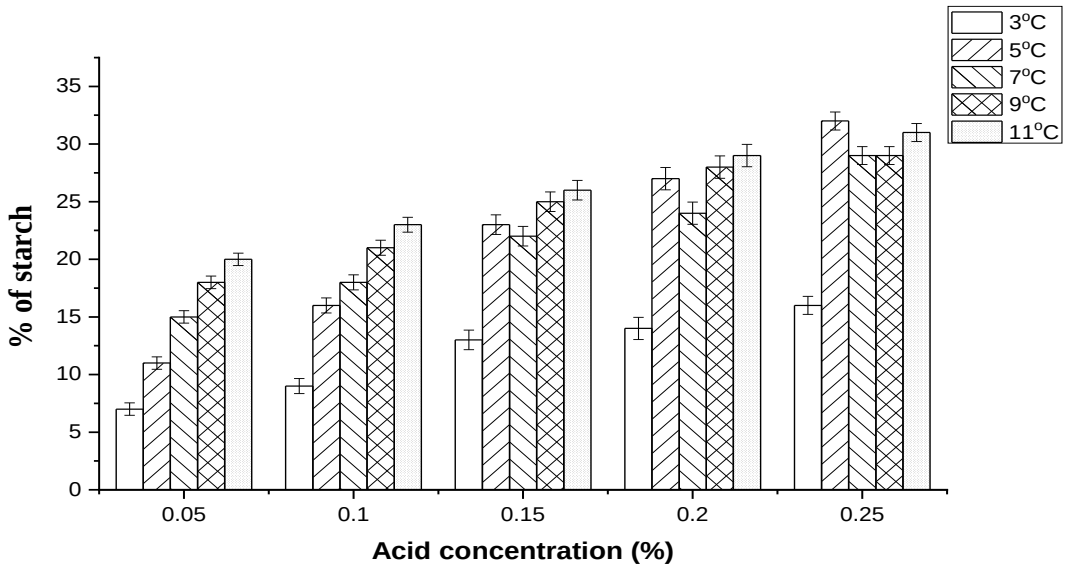


Figure 3.4 showing different concentration of starch extracted from Morinda citrifolia.L seeds

4. Conclusion

The physical properties of the waste *Morinda citrifolia.L* seeds like pH, acid value and peroxide value were estimated and found to be 6.12, 26.8 mg NaOH/g and 9.5 mg NaOH/g. The biochemical properties of the waste *Morinda citrifolia.L* seeds like moisture content, ash content, cellulose content, lignin

content, lipid content, holocellulose content, hemicellulose content, carbohydrates, protein, free reducing sugars was estimated and found to be 37.4%, 26.5%, 50.8%, 6.58%, 19.4%, 55.9%, 5.01%, 42%, 26%, 29%, 35%. The FTIR spectra of the crude sample absorption peaks are located at about 558.99 cm^{-1} (C-Br), 1637.70 cm^{-1} (C=0), 2149.59 cm^{-1} (C=H), 3301.54 cm^{-1} (O-H) and peaks further confirms the presence of flavonoids, phenols, xanthoproteins and ketose sugar. The maximum amount of 32% of starch was obtained at 5^{o}C in 0.25% concentration of NaOH .The extracted crude fiber and starch paves the way for the development of biodegradable organic cloth. The paper can also be prepared from waste *Morinda citrifolia.L* seeds. It also provides a footing for development of further research.

5. References

[1] Ross I.A., (2001) Medicinal plants of world. Chemical constituents, Traditional and Modern Medical uses. Humana press, New Jersey.

[2] Mortan, J.F., (1992) 'The ocean-going noni, or Indian Mulberry (Morinda citrifolia, Rubiaceae) and some of its "colourful" relatives' Ecological Botany, Vol. 46, pp.241-256.

[3] Earle J.E., (2001) Plantas Medicinales en el Tropico Humedo. Editorial Guayacan, San Jose.

[4] Yanine Chan-Blanco, Fabrice Vaillant, Ana Mercedes Perez, Max Reynes, Jean-Marc Brillouet, Pierre Brat (2009) 'The noni fruit (Morinda citriofolia.L): A review of agricultural research, nutritional and therapeutic properties' Journal of Food Composition and Analysis, Vol.19, pp.645-654.

[5] Potterat O., Hamburger M., (2007) 'Morinda citrifolia (noni) fruit-phytochemistry, pharmacology, safety' Planta Med, Vol. 73(3), pp.191-199.

[6] Reem Abou Assi, yusrida Darwis, Ibrahim M. Abdulbaqi, Arshad A. Khan, Lim Vuanghao, M.H. Laghari (2015) 'Morinda citrifolia (Noni): A comprehensive review on its industrial uses, pharmacological activities, and clinical trials', Arabian Journal of Chemistry.

[7] Umer Rashid, Farooq Anwar, Amer Jamil Haq Nawaz Bhatti (2010) 'Jatropha Curcas Seed Oil as a Viable Source for Biodiesel', Pakistan Journal of Botany, Vol. 42(1), pp.575-582.

[8] Edeoga1 H.O., Okwu D.E., Mbaebie B.O. (2005) 'Phytochemical constituents of some Nigerian medicinal plants', African Journal of Biotech, Vol. 4, pp.685-688.

[9] Mohamed Sham Shihabudeen. H., Hansi Priscilla. D., Kavitha Thirumurugan (2010) 'Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants', International Journal of Pharma Sciences and Research, Vol. 1(10), pp.430-434.

[10] Official Methods of Analysis of the AOAC 2000.

11] Musa A., Birnin-Yauri U.A., Muhammad C., Umar A., (2012) 'Proximate composition and mineral analysis of Nymphaea lotus seeds', African Journal of Food science and Technology.

[12] Muthukumaran Pakkirisamy., Suresh Kumar Kalakandan., Karthikeyen Ravichandran (2017), 'Phytochemical Screening, GC-MS, FT-IR Analysis of Methanolic Extract of Curcuma caesia Roxb (Black Turmeric)', Pharmacognosy Journal, Vol. 9(6), pp.952-956.

[13] Akpan U.G., Jimoh A., Mohammed A.D. (2006) 'Extraction, Characterization and Modification of castor seed oil', Leonardo Journal of Sciences, Vol. 8, pp.43-52

[14] Laboratory Handbook (1997). For oil and fat analysis (Eds L.V Cooks and C. van Rede). Academic Press, London-New York.

[15] Evwierhoma E. T., Ekop I. E (2016) 'Extraction and characterization of oils from some local seeds', International Journal of Scientific & Engineering Research, Vol. 7, pp. 1280-1294.

[16] Dubosis M., Miller K.A., Hamilton J.K., Robers P.A., Smith F. (1956) Analytical Chemistry, pp.350.

[17] Lowry O. H., Rosebrough N.J., Farr A.L and Randall R .L. (1951), Journal of Biological Chemistry, pp.193-265.

[18] Updergroff D. M. (1969), Journal of Analytical biochemistry, Vol.32, pp.420.

[19] Official Methods of Analysis of the AOAC 12th edition (1975) pp.138.

[20] Teramoto Y., Lee S.H. and Endo T. (2009), 'Cost reduction and feedstock diversity for sulfuric acid-free ethanol cooking of lignocellulosic biomass as a pretreatment to enzymatic saccharification', Bioresource technology, Vol.100, pp.4783-4789.

[21] Ranganna S. (1979), 'Man of Analysis of Friut and Veg Products', Tata McGraw-Hill Publishing Company Limited New Delhi, pp.634.

[22] Harinder Singh, Navdeep Singh Sodhi & Narpinder Singh (2009) 'Structure and Functional Properties of Acid Thinned Sorghum Starch', International Journal of Food Properties, Vol. 12(4), pp.713-725.

[23] Yograj (2017) 'Process Development from Maximum Starch Extraction rom Tikhur (Curcuma Angustifolia Roxb.) Rhizome' Department of Agricultural Processing and Food Engineering, Rajpur.

[24] Manju Mathew., Arul Sheeba Rani M. (2017) 'A comparative study on phytochemical and biochemical of the fruit and ferment juice of Morinda Citrifolia.L., (Noni)', International Journal of Advanced Science and Research, Vol. 2(3), pp.01-04.

[25] Jahnavi Alwala., Manisha D.R., Vasanth Kumar Mandla., Sreedhar Bojja., Sreelakshmi Chittamuru., Raju Nalvothula., M.P.Pratap Rudra (2014) 'Interpretative in vitro phytochemical, TLC, synthesis of silver nanoparticles and their antibacterial screening of aqueous and ethanolic extracts of Morinda Citrifolia.L (Noni) fruit and their comparative study', World Journal of Pharmaceutical Research, Vol. 3, pp.989-1007.

[26] Oderinde R A., Ajayi I A., Adewuyi A (2009) 'Characterization of seed and seed oil of Hura Crepitans and the kinetics of degradation of the oil during heating', Electron Journal of Environment and Agriculture Food Chemistry, Vol. 8(3), pp.201-208.

[27] Muhammad Bini Etsuyankpa., Muhammad Muhammad Ndamitso., Idowu Victoria Oluwatoyin., Hashim Ibrahim., Sule Philip Ivoms Ogah (2017) 'Chemical Analysis of Noni (Morinda citrofolia) Seeds and the Characterization of the Seeds Oil', American Journal of Applied chemistry, Vol. 5(4), pp. 57-61.

[28] Lubatti O.F., Bunday G., (1960) 'The Water Content of Seeds - The Moisture Relations of Seeds Peas etc', Journal of the Science of Food and Agriculture, Vol.11, pp.685-690

[29] Aman Paul., Michel Frederich., Roel Uyttenbroeck., Sandrino Filocco., Séverin Hatt., Priyanka Malik., Arnaud Monty., Frederic Francis., Christophe Blecker., Sabine Danthine., (2015) 'Proximate Analysis of seeds from some field border flowering strips', Scientific Bulletin. Series F. Biotechnologies.

[30] Gawrysiak-Witulska M., Rudzinska M., Wawrzyniak J., Siger A., (2012) 'The Effect of Temperature and Moisture Content of Stored Rapeseed on the Phytosterol Degradation Rate', Journal of the American Oil Chemists' Society, Vol.89, pp.1673-1679.