BIOTECHRONZZ

THE TECHNICAL MAGAZINE

2019 - 2020

DEPARTMENT OF BIOTECHNOLOGY

SRI SHAKTHI

INSTITUTE OF ENGINEERING AND TECHNOLOGY

(AN AUTONOMOUS INSTITUTE)

(Approved by AICTE, New Delhi, Affiliated to Anna University, Chennai)
L & T Bypass Road, Coimbatore - 641 062, Tamil Nadu, India.

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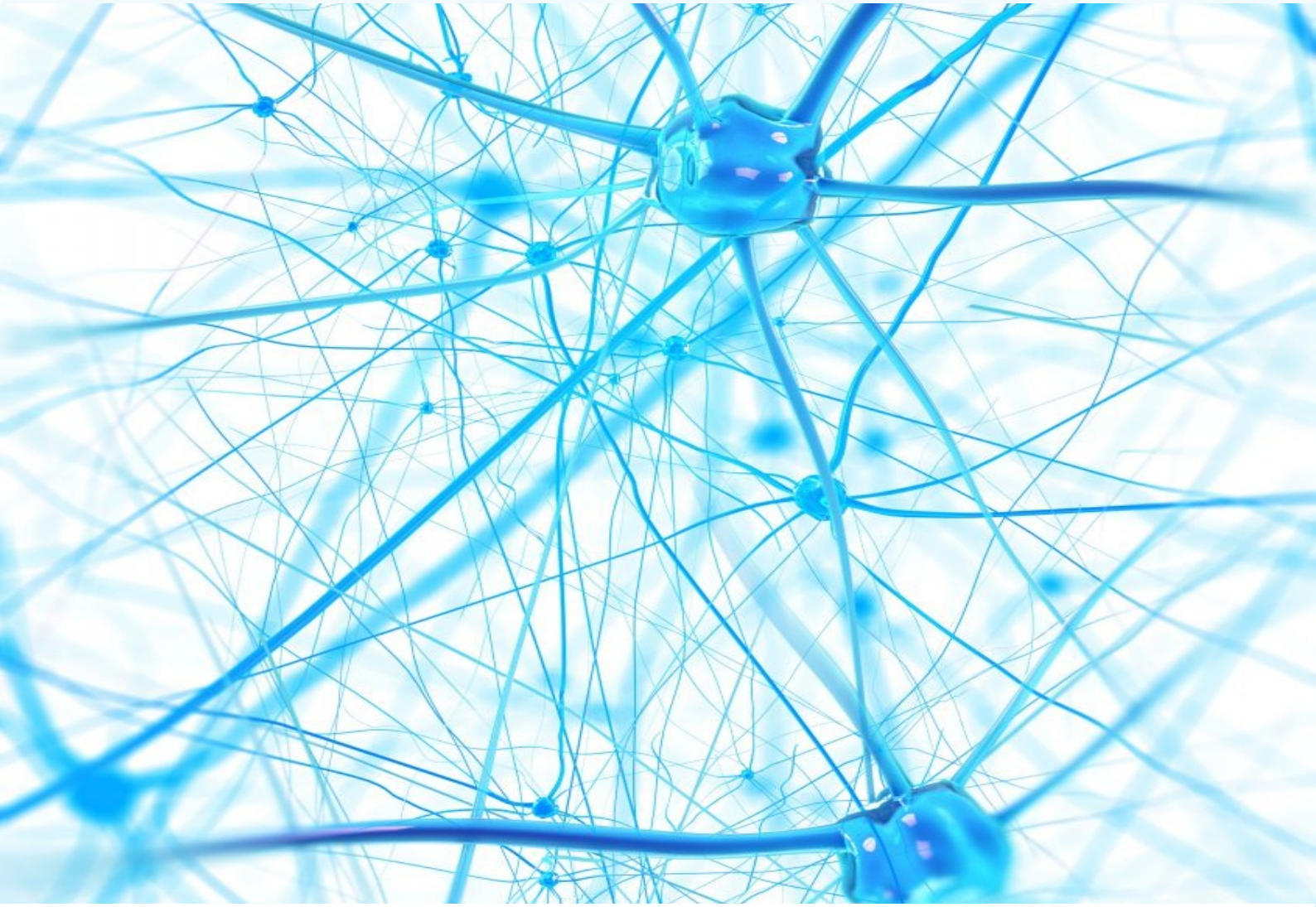

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PREFACE

Biotechronzz is the official technical magazine of the Department of Biotechnology, Sri Shakthi Institute of Engineering & Technology, a souvenir that showcases the myriad of talents possessed by the students. This year is the 2nd Edition and we are proud to introduce the 2nd Volume of Biotechronzz, a tradition that had taken roots since the first volume.

Right from the outset, we have been very meticulous with our planning, from the content we have received, to the layout and other particulars, to the final copy, we have all put in our hardest efforts to express the flair for science and language, shown by our students. Despite all the issues that arose throughout this roller coaster ride, we have made sure that the magazine fittingly reflects the magnificence that it deserves. This physical copy is the culmination of countless discussions, diligent efforts and the timely dedication of everyone involved. We hoped to achieve and maintain the highest calibre of the contents, and hope we did justice.

We hope this serves as yet another interesting edition. Our sincerest thanks to the Design Team for their committed efforts to breathe life to the vision we had in mind. Our heartfelt gratitude to our faculty advisors, staff, and teachers for supporting us in bringing together this edition, successfully.

We wish our readers a joyful reading experience. We hope you enjoy reading through it and draw as much inspiration from it as we did.

Editorial Board

EDITORIAL BOARD

ASSOCIATE EDITORS

TABLE OF CONTENT

Article: A new source for biodiesel – caustic fish

Pooja T, T R Shivanni, Harshini. M
Department of Biotechnology, Sri Shakthi Institute of Engineering and Technology, Coimbatore.

Abstract: Limited availability of edible oils prevents it from being used as viable source of Biodiesel. Low cost and abundantly found fish oil produced from soap stock could be a better option for biodiesel processing. Such type of fish oil contains a higher amount of moisture and FFA and requires a pre-treatment prior to biodiesel production. The use of animal fat to produce bio-diesel is not a new technology; however the adaptability of this technology to aquatic resources has only attracted public interest recently. The stress on land based products to produce biofuels is becoming quite significant and will be even more so in years to come. Therefore looking at aquatic resources for energy production makes not only ecological sense but economic sense too. The conversion process is simple after the fish oil has been produced from the left over waste of the fishing industry the oil is cleaned purified and with the addition of some caustic soda and methanol the bio-diesel is produced. 1kg of fish waste can produce up to 1.13lts of bio-diesel.

Keywords: Biodiesel, Caustic, fish

Development of biodiesel which is a renewable alternative energy source, could easily reduce global dependence on petroleum, and could also help to reduce air pollution. Worldwide production of biodiesel is mainly done by utilizing edible oils such as soybean, sunflower and canola oils. Since, India is not self-sufficient in edible oil production, hence, some non-edible oil seeds available in the country are required to be tapped for biodiesel production The bio-diesel produced from fish waste would be anon-toxic and fully biodegradable renewable fuel that can easily be adapted without any modification to current diesel engines (Raheman H*et al.,* 2007). Bio-diesel is particularly

good for the environment as opposed to standard fuel or diesel because it reduces the air toxins, CO_2, particulates, black smoke and other hydrocarbons. The fish oil is similar to a vegetable oil or animal oil and it reacts with an alcohol (methanol), the catalyst used is generally caustic soda. This produces a pure bio-diesel or B100 (100% bio-diesel) with a valued by product glycerin. Glycerin is an important by-product, and is currently further being enhanced and could become a new source of income for bio-diesel producers. It is a colourless, odorless, slimy liquid which is used for pharmaceutical, food and cosmetic purposes. Up to now market conditions have impeded this valuable by product to be sold commercially, however, worldwide researchers and experts are looking at ways to enhance the product and find more ways to utilize it in order to make it economically and commercially viable. Some fish oils contain essential fatty acids like omega 3, which is a highly valued commodity especially in the

pharmaceutical industry. Therefore care has to be taken on which types of fish are used when producing the fish oil. Below you will find a table of fish species and their content of Omega 3 fatty acids per 100 gr. One of the lowest in Omega 3 content but high in oil is catfish. One other note of care is the acid content of the oil extracted. or example, salmon oil is high in acid and this acid needs to be removed. Therefore an additional step in removing this acid is required. Sulfuric acid is added to reduce the acid value of the oil. Once this has been done the process of trans-esterification can begin.

Biodiesel is a fatty acid methyl ester (FAME). It is produced from a chemical reaction called trans esterification (Jose M*et al.*, 1999). When entering the biodiesel processor, the raw material is heated up to 40-50 degrees Celsius. During heating, methanol is mixed effectively with the catalyst for the trans-esterification operation. Search for stable and continuous source/feedstock for biodiesel production is important to utilize the benefits of biodiesel as an alternative to diesel fuel. Many of the researchers have tried edible as well as non-edible oils for biodiesel processing, but due to their higher prices, it is not feasible to produce biodiesel from them. Low cost and abundantly available fish oil prepared from soap stock could be a viable option. Fish oil is produced in large quantity by fish-processing industry. The viscera, eyes, fins, tails, and other discarded parts of fish are used as soap stock in the manufacturing processes of various fish products. The weight proportion of soap stock is about 25% of the fishery production. The soap stock of marine fish including mackerel, salmon, tuna, and cod is frequently ground into fish meal for aquaculture, livestock, and pet food, and Costs little.

Table 1: Fish species and their Omega 3 fatty acid content

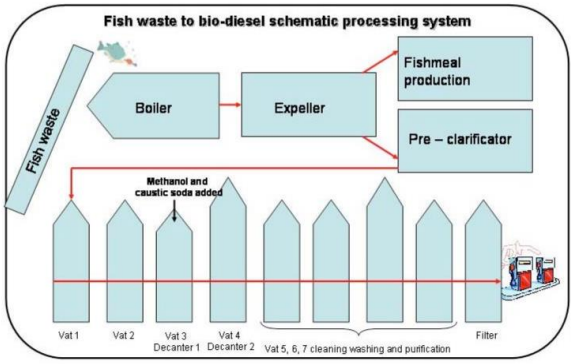
Fish species	Omega 3 (EPA+DHA) content (g) per 100 g of fish
Tuna (fresh)	0.28-1.51
Atlantic salmon	1.28-2.15
Mackerel	0.4-1.85
Atlantic herring	2.01
Rainbow trout	1.15
Sardines	1.15-2
Halibut	0.47-1.18
Tuna (canned)	0.31
Cod	0.28
Haddock	0.24
Catfish	0.18
Flounder or sole	0.4
Oyster	0.44
Shrimp	0.32
Scallop	0.2
Cod liver oil capsule	0.19
Omacor (Pronova)	0.85

Source: adapted from the guidelines of the American Heart Association.

In India, fishing industries could play a bigger role in giving rise to the new industrial sector of biodiesel production by supplying its by –product(soap stock) to the biodiesel processing industries (Dorado, M.P *et al*, 2002).Hence, biodiesel production from fish may leads to the control of solid waste generated from fish industries and helps in improving Indian economy. Although there is great potential for the use of fish-oil as biodiesel for transportation sector or as a power source, research in this field is limited.

The technology used in the production of bio-diesel from fish waste is adaptable and transferable in many other parts of the world including developing regions in Africa, Asia and Latin America as well as small fishing communities and small islands who rely heavily on oil imports. It can provide labor, and produce local energy free from greenhouse gases and emissions. With little

investment in already existing fishing communities local energy can be produced at very little cost (Masjuki HH *et al.*, 2001).

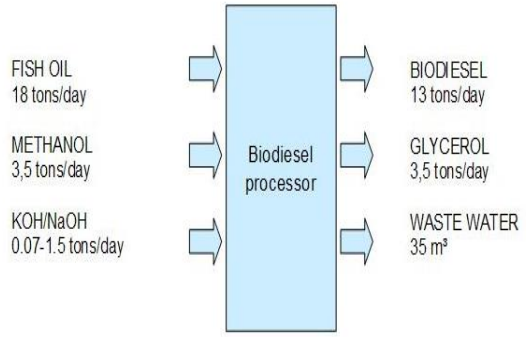


Pic : Fish waste as it arrives at the bio-diesel plant

The refined fish oil was first filtered by filter paper mainly to remove the remaining dirt and other inert materials and then placed in a conical flask equipped with magnetic stirrer and water condenser (Steigers JA, 2002).The base catalyzed trans esterification process for biodiesel production was optimized. Under agitation, the raw oil was heated closer to its boiling point to remove the water contaminants present in the oil. After that, the oil was allowed to cool down under room temperature. The treated oil was then taken for biodiesel production. The above treated oil was again agitated and heated up to a desired temperature on the hot plate. A fixed amount of freshly prepared sodium hydroxide–methanol olution was added to the oil, this moment was considered as the starting time of the reaction (Demirbas A, 2003). When the reaction reached the preset reaction time, heating and stirring were stopped. The products of reaction were allowed to settle for three hours. During settling, two distinct liquid phases were formed: crude ester phase at the top and glycerol phase at the bottom. The crude ester phase separated from the bottom glycerol phase was then washed by warm de-ionized water several times until the washed water became clear.

The fome was dried using anhydrous sodium sulphate and then there maining excess methanol and water in ester phase were removed by evaporation under atmospheric condition. In this way neem methyl ester was prepared. The final biodiesel was then filtered to remove remaining sediments with the help of filter paper. The reactions were investigated step by step. The optimal value of each parameter involved in the processes was determined keeping the rest of the parameters as constant. After optimal value of each parameter was attained, the value was adopted for the optimization of the next parameter (Ma, F, *et al.*, 1999).

Thus, crude fish oil from soap stock contains a higher amount of initial FFAs, which could not be directly employed for biodiesel processing. Caustic stripping is the cheapest and convenient way for the reduction of FFAs from fish oil while acid esterification darkens the fish oil (Reyes JF *et al.*, 2006). Base catalyzed trans esterification is a faster and an economical way of biodiesel processing with its optimized parameters which leads to the achievement of desired quality of biodiesel fuel (within the limits prescribed by ASTM,IS and EN standards).



The challenges are the heterogeneity of fish waste, remoteness of fish processing plants in NL, and fish oil is high in free fatty acids (FFA). The remoteness of the plants means that producing the biodiesel for export is likely not feasible. Further, the high FFA content and high rate of degradation of the fish oil exaggerates the export problem. High FFA content translates to an extra pre-treatment step. The most likely option for fish biodiesel is on-site production for blend in the diesel engine for energy.

Reports conclude though that there is potential to take the tonnes of waste from the salmon farming industry and turn it into a fuel to be used in diesel engines, the challenges notwithstanding. More study and research will no doubt continue and the salmon aquaculture industry will watch closely.

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Article: Biotechnology- A Commercial perspective

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Abstract: The world's historic religions emerged within the context of agriculture and primitive biotechnology, and as one might expect they are at home in that context, for instance through their affirmation of agricultural festivals. By the time of Charles Darwin (1809–1882), plant and animal breeders were deliberate and highly successful in applying techniques of selective breeding to achieve specific, intended results. Darwin's theory of evolution is built in part on his observation of the ability of animal breeders to modify species. Biotechnology is a set of techniques by which human beings modify living things or use them as tools. In its modern form, biotechnology uses the techniques of molecular biology to understand and manipulate the basic building blocks of living things. The earliest biotechnology, however, was the selective breeding of plants and animals to improve their food value. This was followed in time by the use of yeast to make bread, wine, and beer. These early forms of biotechnology began about ten thousand years ago and lie at the basis of human cultural evolution from small bands of hunter-gatherers to large, settled communities, cities, and nations, giving rise, in turn, to writing and other technologies.

Keywords: Biotechnology, DNA, GMO, Agriculture

One is often spoilt for choice when faced with the task of selecting a field in which he wants to build his career. So many new and innovative fields of study, which were unheard of just a few decades ago, are emerging fast and offering scope for tremendous growth. In this vast sea of choices, there are many options which sound fancy and attractive but are not yet well understood. One such common example is biotechnology. Sounds familiar right? The moment you hear this term; you get a strange feeling of familiarity, yet you are not able to say what exactly it

is. This is a dilemma faced by many young minds, who want to know if this field is a good fit or not and whether there is a scope for success if they choose to pursue it. Let us help you in this regard by answering some of the questions that we ourselves had at the start of our journey in this field. Yes, now you have deciphered that we are someone associated with biotechnology, but still wondering what it is, aren’t you?

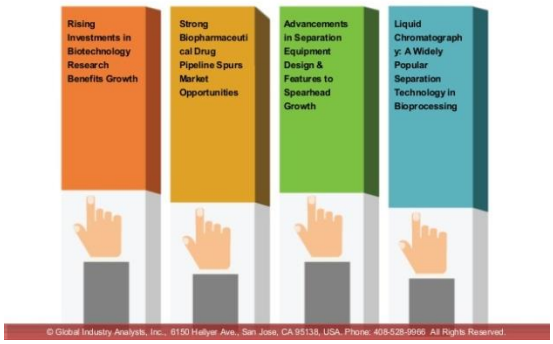

Fig.1 Snapshot of Biotechnology Market trends

In simple terms, biotechnology is the application of the available technology to support and enhance the ability to survive and grow. In this field, you will be in direct interaction with living organisms in one form or the other - be it the tiny microbes or the mighty blue whales, all forms of life are a part of this field. The main aim of any person in this field is to exploit the various processes and mechanisms found in different life forms for potential applications in our daily life. One simple example is animal breeding - we grow animals for the purpose of obtaining various products from them like milk, meat, eggs, skin, horns etc.

Biotechnology comprises of many different fields like microbiology, pharmaceutical technology, biomedical engineering, environmental technology, molecular biology, genetics and stem cell technology just to name a few. Just as there are different fields in biotechnology, so are its applications - be it the use of genetic engineering to obtain high yielding variety of seeds or the use of stem cells to regenerate damaged organs. Yes, you read it right - it is now possible to regenerate entire organs freshly from a single stem cell. Though the number of such possible applications is really huge, here we have tried to touch upon a few to give a brief view into the scope of this field.

Let me start with microbiology, as the name suggests, this field deals with microorganisms. Various properties and characteristics of microbes like bacteria and fungi are used to synthesize products and services that benefit humans. The most common and ancient application is the process of brewing. Practiced since time unknown, it is the process of manufacturing fermented foods and drinks from raw materials which are rich in sugars. According to statistics, production by unrecorded or unorganized manufacturers amounted for more than 60% of alcohol consumption in India [1]. Only 38% of the alcohol consumed worldwide is manufactured by branded labels, according to the alcohol-industry funded International Centre on Alcohol Policies (ICAP) [2]. In the year 2005, the total net revenue/turnover of the 26 major alcoholic beverage industries stood at a whopping $155 billion, with total operating profit of $26 billion [3].

Pharmaceutical technology deals with the design, synthesis and marketing of pharmaceutical compounds. These compounds may be sourced from microbes, plants and animals or synthesized chemically. According to the WHO, the global pharmaceuticals market is worth US$300 billion a year, a figure expected to rise to $400 billion within three years. The 10 largest drugs companies control over one-third of this market, several with sales

of more than $10 billion a year and profit margins of about 30% [4].

Genetic engineering is the process of manipulating the genes of an organism to alter its traits and characteristics. According to the report **"**Genome Editing / Genome Engineering Market **by Application - Global Forecast to 2019", t**he global market for genome editing was valued at $1845 million in the year 2014 and is expected to reach $3514 million by the year 2019, with a cumulative annual growth rate (CAGR) of 13.75% [5]. The market for genetic engineering is expected to be driven by increased R&D expenditure, growth of biotechnology and pharmaceutical industries, increased funding for genomics research, technological advancements, increasing demand for synthetic genes and global rise in production of genetically modified crops.

Stem cell technology deals with the ability to synthesize the necessary cells and organs from a single undifferentiated cell, called the stem cell. Depending on the potency of a stem cell, one or more different types of cells/organs can be synthesized from it. The various products offered by the stem cell market currently are therapies for diseases such as haematological cancers, cord blood banking and stem cell based assays. Various therapies which are being developed for myocardial infarctions, heart failure and other diseases have reached phase 3 trials successfully. According to the report "Stem Cell Technologies and Applications: World Industry and Market Outlook 2015-2025", the current value of stem cell market is $7.2 billion and will reach $12 billion by the year 2018 [6].

The dairy industry deals with the production of milk and milk products like cheese, butter etc. The global milk production is estimated to be 735 billion litres per annum. The European Union is the largest producer with a capacity of 156 billion litres per annum, followed closely by India with a capacity of 131 billion litres per annum. The top eight milk producing countries account for 407 billion litres per annum, which is equivalent to 55% of the global production [7]. The total dairy export value was pegged at $2.88 billion during the year 2014-15 [8].

The poultry and meat industry deals with the production of meat and eggs from animals like chicken, cow, pig, ducks etc. There is a rapid increase in the demand for animal proteins globally, due to increased prosperity and rapid growth in the urban population. The global poultry production is expected to double by the year 2030 in order to cater to this demand [9]. In July 2014, the United States department of agriculture had predicted that the world poultry production and consumption will grow at 31.6% to reach 119.4 million metric tons (MMT) by 2023 from 97 MMT in 2012 [10].

In conclusion, one can easily say that the field of biotechnology offers huge potential for growth and development. It is not an overstatement to state that this field finds its application in all aspects of life; be it the food we eat, the dress we wear, the house we live and so on.

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Batch and Continuous Extraction of Bromelain Enzyme from pineapple juice (*Ananas comosus*)

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Abstract: Bromelain is one of the vegetal proteases found in pineapple plant. It has numerous applications in food and pharmaceuticals. This study was focused on industrial large-scale bromelain purification technique (Reversed micelles) which will assist in determining the effect of processing conditions on the purification efficacy. The purification was carried out in batch extraction and a COCO simulation for continuous extraction. The cationic micellar solution was made of CTAB as a surfactant, isooctane as a solvent and hexanol as a co-solvent. For the batch process, a best purification factor at the values of surfactant agent, co-solvent and KBr concentrations, pH of the back and forward extractions were, 152.18 mM, 10.53 % v/v, 1.24 M, 2.67 and 7.90, respectively. For the continuous operation, optimal process was determined using simulation. This optimal point led to a productivity of 96.128% and a purification fold of 10.87.

Keywords: Reversed micelles, Bromelain, pineapple, Optimization, Response surface, COCO, Purification fold.

Bromelain is one of the protease enzymes found in the pineapple plant (*Ananas comosus*). Stem bromelain is the major protease present in extracts of pineapple stem while fruit bromelain is the major enzyme fraction present in the juice of the pineapple fruit (Kelly et al., 1996). Some other minor cysteine endopeptidases (ananain, comosain) are also present in the pineapple stem bromelain (SBM). Although the fruit bromelain (FBM) was discovered much earlier than stem bromelain, the biochemical characterization of the latter enzyme has been described in more detail (Harrach et al., 1998). The numerous industrial and therapeutic applications of enzymes necessitated their production. In fact, they represent about 60% of all commercial enzymes worldwide. They are widely used in food, pharmaceutical and detergent industries (Feijoo-Siota et al., 2010). It has been extensively used in food industry for meat tenderization, baking processes, protein hydrolysate production, and beer clarification, as food supplement and in prevention of browning of apple juice (Tochi et al., 2008). Similarly, SBM is a highly accepted phyto therapeutic agent. In fact, it had obtained universal acceptability as therapeutic drug. This is owing to its history of effectiveness and safety. It was firstly introduced as a therapeutic compound in 1957. It is not a licensed medical product, thus, it, is freely available to the general public in health food stores and pharmacies in the USA and Europe (Brien et al., 2004). Additional clinical applications of bromelain include modulation of tumor growth, third degree burns and improvement of antibiotic action. It is also used as a drug for the oral

systemic treatment of inflammatory, blood-coagulation-related diseases and some 3 malignant diseases. Furthermore, bromelain is involved in the reversible inhibition of platelet aggregation, sinusitis, bronchitis, angina pectoris, thrombophlebitis, surgical traumas, pyelonephritis and improved absorption of drugs especially antibiotics (Maurer et al., 2001). Bromelain has also been successfully used as digestive enzyme in many intestinal disorders. It has been shown that the enzyme serves as adequate replacement of pepsin and trypsin in case of deficiency(Wu et al., 2012).

Pulp, stem and Peels of Pineapple was crushed, filtered and the filtrate was obtained. The filtrate contains the crude bromelain enzyme. The filtrate samples were deep frozen at -5ºC. The cationic micellar solution was made of CTAB (surfactant), isooctane/butanol as a solvent and hexanol as a co-solvent (Hasmann, 2000). The solution for backward extraction should contain the counter ions for CTAB. So, potassium bromide (KBr) was used as counter ion and their concentration used for the process was based on the design of experiments. The samples of pineapple juice were subjected to determine bromelain enzyme activity and the total protein concentration. Pineapple juice and micellar solution were evenly mixed (5 mL each). The mixture was stirred in a glass tube until homogenization (emulsion) occurs. The separation of the phases was performed by centrifuging it at 8000 rpm for 5 minutes. The light phase (micellar) 22 was taken for the backward extraction of the bromelain (Hasmann et al., 1999 and 2000). COCO (CAPE-OPEN to CAPE-OPEN) is a CAPE-OPEN compliant steady state simulation environment. Based on results of the batch extraction process, the input values (Table 4) were entered for continuous extraction process on simulation tool.RMS extracted solution was freeze dried at -20atm for 5hours using ALPHA 1-2 LD plus freeze dryer. The dry sample of BML was subjected for obtaining maximum absorbance using ultra-violet spectrophotometer (LAMBDA 35). The peaks were found at 280nm. Protein content by Lowry's method (Lowry et al., 1951) at 280 nm. A FT-IR spectrometer with 2 cm−1 resolution was used for sample analysis. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with standard/extracted enzymes (bromelain). A 20 µL aliquot of the sample obtained from RMS extraction was subjected to analytical high performance liquid chromatography (HPLC) performed on an Agilent Technologies ® apparatus with a UV/vis LC-20A detector. The overall continuous extraction of bromelain form pineapple juice was desinged using coco (cape open to cape open) simulation. The input for the continuous extraction was obtained from the best fit of the RSM of batch extractions. The conditions were optimized in the continuous system using various tools of coco simulation.

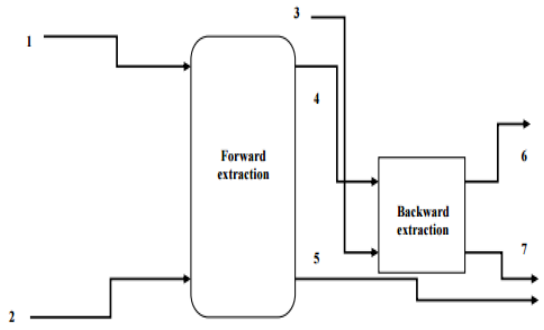

Figure 1: Block diagram for continuous bromelain extraction Where, 1- Pineapple juice; 2- Micellar solution inlet; 3- backward extraction solution inlet; 4- Light phase outlet (raffinated); 5- Heavy phase outlet (extracted); 6- solvent waste; 7- bromelaine outlet.

In order to get maximum yield theoretically, the necessary conditions were altered in coco simulation software. The efficiency of the product varies as the process changes from batch to continuous mode and it decreased considerably in the due course of conversion (Hebbar et al., 2011, Hebbar et al., 2012). But, on using the coco simulation tool of extraction design, the conversion efficiency can be considerably increased. By 38 altering the conditions, the required output efficiency can be altered and the result shows that the increase in efficiency of the continuous process. It was obtained as 96.128% on comparing the cent batch process. The following UV visible spectro photometric graph shows the presence of peak for both the extracted and standard bromelain at same wavelength (280nm). (Swaroop et al., 2013)

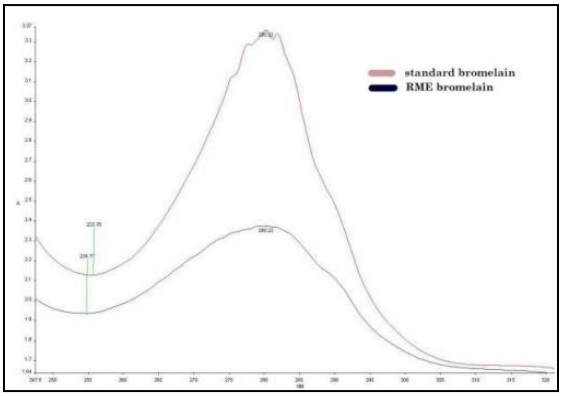

Figure 2: UV-Visible spectrophotometric result

The FTIR spectrum of freeze-dried bromelain is shown in Figure . The characteristic C–N stretch vibration frequencies of mono alkyl guanidinium are assigned to the observed IR bands at 1638, 1425– 1256 and 1053 cm−1 . The band at 1760-1670 cm−1 (s) shows the presence of C=O groups (amides at ~1639 cm−1). This confirms the presence of amino acids that contain amine groups in their side chain, i.e., aspargine and glutamine (Devakate et al., 2009). Bands at 3428 cm−1 for valine are assigned to the N-H stretching mode.

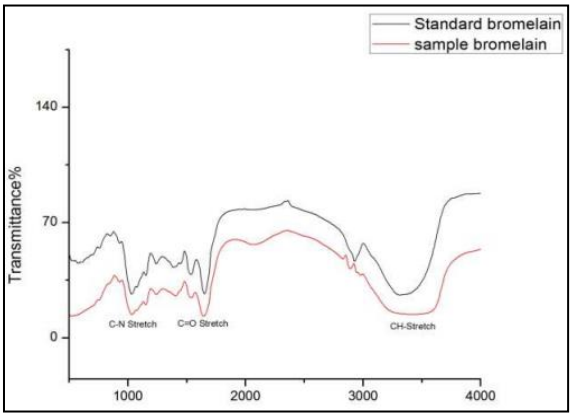

Figure 3: FTIR spectrum of freeze-dried purified bromelain powder

Thus, the crude pineapple juice was subjected to batch extraction in order to obtain bromalein. The presence of bromelain was confirmed using necessary qualitative techniques and the amount of the enzyme along with its activity was estimated. After the execution of batch process, the extraction was subjected to continuous process using the same inputs. Based on the method of coco simulation, it was found that the efficiency of the continuous extraction was nearly equal to that of batch process and the obtained result was 96.128% of the cent batch process. This shows increased efficiency of the continuous process of bromelain extraction when compared to the currently existing methods.

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Article: A new biorefinery process boosts the biofuel production from algae

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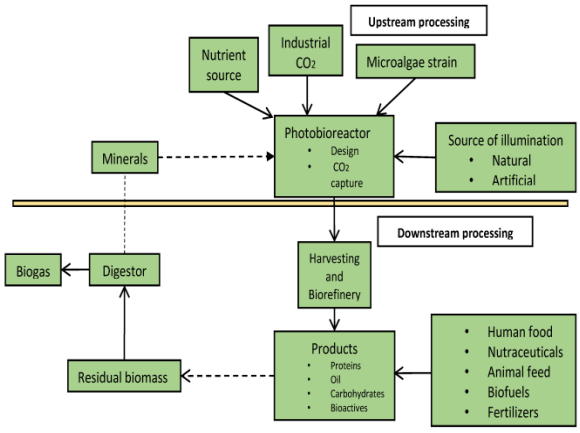
Abstract: A new biorefinery process developed by scientists at the Energy Department's National Renewable Energy Laboratory (NREL) has proven to be significantly more effective at producing ethanol from algae than previous research.In that work, scientists examined two promising algal strains, Chlorella and Scenedesmus, to determine their applicability as biofuel and bioproduct producers. They concluded Scenedesmus performed better in this process with impressive demonstrated total fuel yields of 97 gallons gasoline equivalents (GGE) per ton of biomass. Cost of algal biofuel production is still a major challenge and the Energy Department has made reducing the costs of both algae production and conversion of algal intermediates to fuels significant goals. In traditional processes, the algae produce lipids that get converted into fuels. These results led to the conclusion that the novel CAP process is capable of reducing the cost of algal biofuel production by nearly $10/GGE compared to a "lipids only" process, taking the modeled cost down to $9.91/GGE. While this is not nearly low enough to compete with petroleum, this approach can be combined with reduced costs for biomass production to provide a path forward to achieve that goal.

Keywords: biorefinery , Chemical reactor, Catalyst

In recent years, the rapid depletion of fossil fuels, increase in energy demand, global warming, increase in price of fossil fuels depends on economic and political behaviors increased orientation to alternative energy sources. In this context, biodiesel that is one of the renewable alternative energy sources draws attention because of its useful features such as easily biodegradable and environmentally friendly. However, biodiesel production from oil crops does not meet the required demand of vehicle fuel, and recently it is not economic and feasible. It needs to be

improved to produce more economically to be able to compete with diesel in the market. Vegetable oils and crops which biodiesel produced from are a kind of human food sources and the shortage on food source cause to go up prices and make the biodiesel high-priced. To meet the requirements, the interest on algae is increased day by day since this technology has potential to meet global demand. Microalgae have higher productivity per area and no need for farm field to grow as opposed to oil crops and animal fat. Microalgae use sunlight to reduce CO2 to biofuels, foods, fertilizers, and valuable products. Furthermore, microalgae can be used to get different types of biofuels. Using microalgae as fuel source is not a novel idea but recently the prices of diesel and global warming hit this solution

to the top.The other significant feature is that algae can grow everywhere and every season in a year since there are thousands of algae species that have different adaptations and different properties. They can grow in saltwater, freshwater, lakes, deserts, marginal lands, etc. In addition to biodiesel production, algae can be also used as feedstock to produce different valuable products such as fertilizer, energy, neutraceuticals, protein, animal feed etc. The other significant property is that microalgae can remove some heavy metals, phosphorous, and nitrogen from water during its growth. Algae also clean up the water. Moreover, microalgae sequester lots of carbon by photosynthesis. Utilization of carbon dioxide by algae is significantly lowering the risk for greenhouse gas effects. Lastly, usage of microalgae for biodiesel almost cancels out the carbon dioxide and sulfur release to atmosphere (N.D. Weiss *et al.*, 2009).



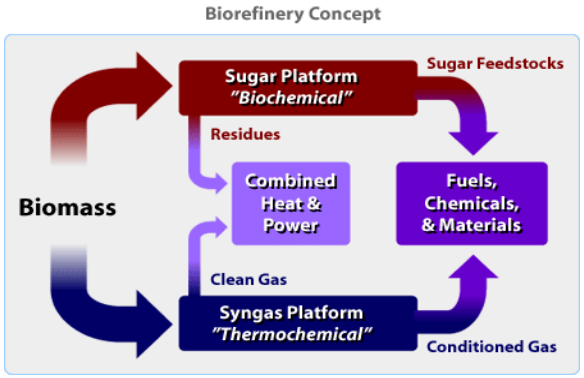
A new project to develop technology to drive forward an algae-based biorefinery which aims to develop and validate technological processes designed to obtain biodiesel through algae cultivation. Cost of algal biofuel production is still a major challenge and the Energy Department has made reducing the costs of both algae production and conversion of algal intermediates to fuels significant goals. In traditional processes, the algae produce lipids that get converted into fuels. However, simply increasing the amount of lipids in algae isn't expected to bring costs down enough(T. Dong*et al.*, 2015). NREL determined further progress could be made by more completely using all algal cellular components instead of just relying on the lipids. By applying certain processing techniques, microalgal biomass can produce carbohydrates and proteins in addition to lipids, and all of these can be converted into co-products.

Chemical composition (% dry matter basis) of selected microalgae (Bruton et al., 2009).

	Protein	Carbohydrate	Lipids	Nucleic acid
Freshwater algal species				
Scenedesmus obliquus	50–56	10–17	12–14	3–6
Scenedesmus quadricauda	47	–	1.9	–
Scenedesmus dimorphus	8–18	21–52	16–40	–
Chlamydomonas rheinhardii	48	17	21	–
Chlorella vulgaris	51–58	12–17	14–22	4–5
Chlorella pyrenoidosa	57	26	2	–
Spirogyra sp.	6–20	33–64	11–21	–
Euglena gracilis	39–61	14–18	14–20	–
Spirulina platensis	46–63	8–14	4–9	2–5
Spirulina maxima	60–71	13–16	6–7	3–4.5
Anabaena cylindrica	43–56	25–30	4–7	–
Marine algal species				
Dunaliella bioculata	49	4	8	–
Dunaliella salina	57	32	6	–
Prymnesium parvum	28–45	25–33	22–38	1–2
Tetraselmis maculata	52	15	3	–
Porphyridium cruentum	28–39	40–57	9–14	–
Synechoccus sp.	63	15	11	5

In their initial work, NREL researchers determined that through the use of a solid-liquid separation process, the carbohydrates can be converted to fermentable sugars, which can then be used to produce ethanol. However, as much as 37 percent of the sugars were lost during that process. Those trapped sugars "cannot be used for

fermentation without a costly washing step, resulting in a loss of overall fuel yield," according to the Algal Research report.In their most recent work, NREL researchers hypothesized the amount of ethanol could be significantly increased by simplifying the processing. By skipping the solid-liquid separation process and exposing all algae components directly to fermentation conditions, both ethanol (from the carbohydrate fraction) and lipids can be recovered simultaneously. Using Scenedesmus and the CAP, and after upgrading the lipids to renewable fuels, scientists were now able to produce a total fuel yield estimated at 126 GGE per ton. That's 88 percent of the theoretical maximum yield and 32 percent more than the yield from lipids alone (A. Sluiter*et al.*, 2008).The NREL researchers also were able to recover 82-87 percent of the lipids from the CAP, even after ethanol fermentation and distillation, indicating that the initial fermentation of sugars in the pretreated biomass slurry doesn't significantly impede lipid recovery. These results led to the conclusion that the novel CAP process is capable of reducing the cost of algal biofuel production by nearly $10/GGE compared to a "lipids only" process, taking the modeled cost down to $9.91/GGE. While this is not nearly low enough to compete with petroleum, this approach can be combined with reduced costs for biomass production to provide a path forward to achieve that goal.



An integrated algal biorefinery process, termed CAP, is successfullydemonstrated by Y. Chisti, 2007. The algal slurry after acid pretreatment is a sufficientmedium for cultivating yeast to produce ethanol without any additional nutrients. Almost all the fermentable sugars were utilized in CAP. The utilization efficiency of carbohydrates is significantly improved (ethanol yield of 31 GGE/ton biomass) compared to previous refinery design cases (ethanol yield of 20 GGE/ton biomass). Lipid yield is not adversely affected by fermentation and ethanol removal, reaching 87% of FAME recovery. CAP can further reduce microalgal biofuel cost by 9% achievinga modeled energy yield of 126GGE/ton of total fuelproducts with$9.91/GGE from *S. acutus* biomass (J. Shekiro *et al.*, 2012). Removing an additional SLS reduced capital and operating cost resulting in a simplified and robust process. It is likely that a number of high-value co-products, such as PUFA and protein residue, may also be produced via the CAP processing concept, resulting from the process' relatively non-destructive nature of fractionating whole algal biomass to individual component constituents. High-value co-product opportunities possess potential to significantly reduce the high

cost of algal biofuel production, especially from the extracted stillage fraction, which, in our current analysis, is relegated to anaerobic digestion for biogas production. The NREL lab used a solid-liquid separation process to convert the carbohydrates to fermentable sugars, which can then be used to produce ethanol. A substantial part of the sugars, up to 37%, were lost during that process, though. The new research skips the separation process and exposes all algae components directly to fermentation conditions. Thus both ethanol and lipids can be recovered simultaneously. After upgrading the lipids to renewable fuels, scientists achieved a total fuel yield of 126 gallons gasoline equivalents (GGE) per tonne of biomass. Increasing ethanol yield by using all algal components instead of just the lipids can help decrease the cost for algal biofuel production. The goal is to develop sustainable, cost-competitive biofuels from feedstocks, including algae, which can help to reduce dependence on foreign oil, reduce greenhouse gas emissions, and create economic opportunities.

Microalgae are the untapped resource with more than 25,000species of which only few are in use. The overarching goal of microalgal biotechnology is to improve the productivity of these organisms in order to meet the demands of a rapidly growing market. Large-scale open ponds had lower productivity than required for economic deployment, probably due to low night temperatures in the areas where these open ponds were tested. Studies are being carried out with methyl acetate as a substrate which avoids glycerol formation and lipase inhibition. Unsaturated fatty acid content is high in algal oils and their presence lowers esterification yields.

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Article: Extraction of Flavanoids from *Albizia amara* for commercial application.

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Abstract: *Albizia amara* commonly called as *Arapu* is a plant of Ayurvedic and traditional importance as indigenous tribes used different parts of the plant because of its medicinal values. The plant is widely used owing to its remarkable properties like reducing body temperature, etc. In addition the bark, pods and roots have been found to contain anti-cancer and anti-ulcer activities. In this study, the ethanolic extract of *Albizi aamara* plant was obtained and filtered followed by treatment with lead acetate and concentrated hydrochloric acid to obtain crude filtrate. Then the filtrate was subjected to lyophilisation. The final lyophilized product was treated with ethyl alcohol and subjected to flavanoid extraction by TLC and HPLC. Flavonol glycosides (quercitin and isoquercitin) present in albizia found to have properties such as body temperature reduction and control of hairfall. Then the extract was mixed with coconut oil and formulated as albizia hair oil possessing the properties of controlling hairfall and reducing dandruff.

Keywords: *Albizia amara*, Flavanoids, HPLC, Oil Formulation, Hair fall.

Dandruff is a major cosmetic problem that poses very great public health concern both in developed and developing countries (Gulfraz *et al.,* 2006). No population in any geographical region would have passed through freely without being affected by dandruff at some stage in their life (Chun*et al.,* 2005). The word dandruff (dandruff, dandriffe) is of Anglo-Saxon origin, a combination of "tan" meaning "tetter" and "drof" meaning "dirty" (Harborne *et al*.,, 1986). Dandruff is a chronic scalp condition characterized by scaling, itching and redness of the scalp. It occurs when scalp sheds epidermal cells in large clumps. The skin of scalp renews itself about once a month. Usually, scalp sheds dead cells in nearly invisible way, but

sometimes cell turnover becomes unusually rapid and dead cells are shed as visible flakes called dandruff (Goswami *et al*., 2004).Plant-derived substances have recently become of great interest owing to their versatile applications in treating hair fall and dandruff . *Albizia Amara* which is known to be a traditional plant found in south east region. This is used for most of the medical treatments like hair fall, antidandruff. Albizia plants are widely used therapeutically in treating insomnia, irritability, wounds as antiseptic, antitubucular, antidysentric etc., Albizia leaf extract also contains flavonoids, alkaloids, trepenes, saponinsin which *Albizia Amara* contains two flavonol glycosides (quercitrin and iso quercitrin). Plant based natural constituents can be derived from any part of the plant like

bark, leaves, flowers, roots, fruits, seeds, i.e. any part of the plant may contain active components (Ghafour et al., 2010). Plants contain different types of compounds such as resins, rubbers, gums, waxes, dyes, flavours, fragrances proteins, amino acids, bioactive peptides, phytohormones, sugars, flavonoids, and bio pesticides. Flavonoids are a group of about 4000 naturally occurring poly phenolic compounds, found universally in foods of plant origin. These are primarily recognized as the pigments responsible for the colours of leaves, especially in autumn. According to the IUPAC nomenclature, they can be classified into: flavonoids, (examples: quercetin, rutin), iso flavonoids, neo flavonoids. The three flavonoids classes above are all ketone-containing compounds. Flavonoids are also important for human health. Like vitamins, these compounds are not produced endogenously by the body and must be supplied either through the diet or nutritional supplements (Winkel 2002).

Albizia Amara plant leaf is collected from forest region of south east and shade dried in room temperature, and then it is crushed to small pieces using pestle and mortar and finely powdered in an electric grinder. Flavonoids extraction will be done by immersing 100 gram of *Albizia amara* leaf powder in ethyl alcohol in room temperature for 24 hours using magnetic stirrer and the obtained extract will be further concentrated using vacuum rotary evaporator. The crude concentrated extract obtained from the vacuum rotary evaporator will then be filtered using what man No 1 papers and the process will be repeated again using the remaining residue with 300 ml ethyl alcohol in order to ensure the complete extraction during each time. The two filtrates that obtained on filtration with the whatman No1 filter and treated with 100 ml of 1% lead acetate for 4 hrs and allowing the mixture to undergo precipitation. The mixture will then be filtered once again after the precipitation process. To the obtained filtrate a mixture of 250 ml acetone and 30ml of concentrated hydrochloric acid (HCl) will be added and filtered. The resulting pellet will be finally subjected to lyophilization (Freeze- drying) at -50ºC under vacuum for 12 hrs of time period. The extract will be dissolved in ethyl alcohol. The extraction process will be done once again for 1hr and filtered to produce a red coloured filtrate .Finally, the filtrate will be placed in a clean and dry Petri dish away from light at room temperature until deep red brown coloured powder is obtained. The crude extract obtained from the flavonoid extraction process will be dissolved in ethanol and spotted on TLC plates (5x 20 cm) coated with silica gel. These plates are developed in chromatography chamber containing solvent mixture of (butanol, acetic acid and water (70:25:5, v/v/v) and let to stand for 1 hr. The developed plates will be air dried and visualized under ultraviolet light (UV light). The plates will be then placed in a chamber that has been saturated with ammonia vapours that has the ability to observe the colour of spot and plates will also be placed in a chamber saturated with I2 vapours to observe the colour of spot. Rf values will be calculated for the obtained isolated sample. HPLC analysis is done to find the presence of flavonoids in the *Albizia amara* solvent extract which contains partially of methanol and ethanol. Before injecting the sample system wash

has been done using distilled water. Column lines are purged and A line is connected with methanol and B line is connected with Acetonitrile in the ratio of 7:3. Then the process is set for 30min at the flow rate of 0.5ml/min. solvent sample is filtered using membrane suction from the 20ul of the sample is injected to the column by using photo diode detector. Then the variable wavelength is detected at339nm.

The *Albizia amara* leaf extract obtained from the ethanolic extract (10g of *Albizia amara* leaf powder and 150ml of ethanol) followed by which the crude extract (6.8g) was obtained on lyophilizing (freeze drying) the earlier obtained extract has been confirmed to contain flavonoids, by the final results of qualitative and various quantitative tests such as Phyto chemical Screening ,TLC and HPLC. The appearance of yellow colour on addition of 1% KOH to the alcoholic extract obtained from *Albizia amara* leaf powder confirms the presence of the flavonoids in the sample.

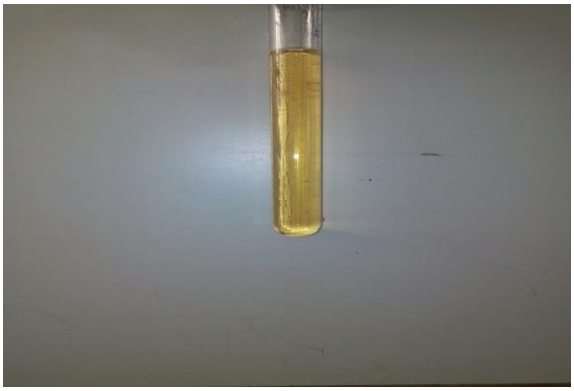

Figure 2: Qualilative analysis of Flavanoids

The separation of the flavonoid compounds present in the *Albzia amara* leaf extract has been sotted in the TLC plates that has been coated with the silica gel. Now these spots over the TLC plates of size 5x20 confirm the presence of the flavonoids.

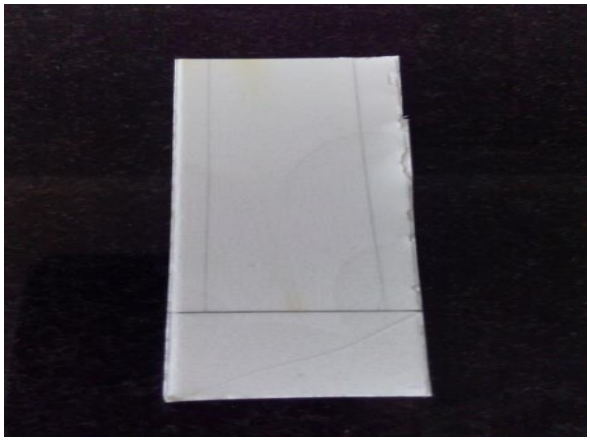

Figure 2: TLC Results

The High Performance Liquid Chromatography (HPLC) technique has been done to detect the presence and quantify the types of flavonoids present in the *Albizia amara* leaf extract. The HPLC graph shows the presence of three different types of flavonoids present in the crude sample such as rutin, isoquercitin and quercetin. The solvent system that has been used for the HPLC analysis of the crude sample is methanol and acetonitrile.

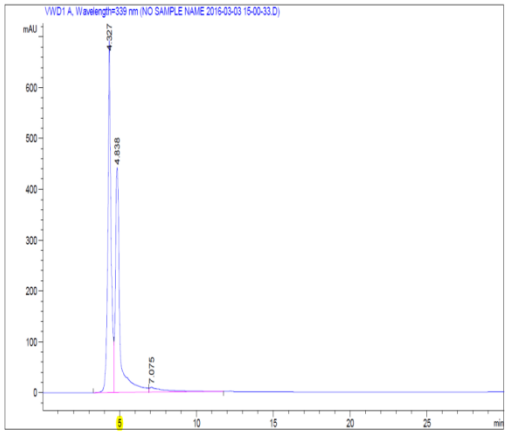

Figure 3: HPLC graph showing presence of flavanoids

From 10 g of *Albizia amara* leaf, 6.8g of crude extract was obtained.The amount of flavanoids present in the ethanolic crude extract of albizia amara leaves were found to be 3.42, 3.13 & 0.25g of rutin,

isoquercitin and quercitin respectively. This indicates that flavanoids are active compounds and present in large amount. Finally the purified flavanoids are mixed with pure coconut oil and formulated as medicated oil. This formulated product can be used for commercial applications. It reduces the hiarfall and controls dandruff.

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