MEMBRANE PROCESSING, CLARIFICATION AND OPERATING CONDITIONS ON PHYTOCHEMICAL PROPERTIES OF ALOE VERA EXTRACT

A PROJECT REPORT

Submitted by

R. DIVYA (1110204010)

E. INDHUMATHI (1110204016)

C. KEERTHIKA (1110204022)

in partial fulfillment for the award of the degree

of

BACHELOR OF TECHNOLOGY

IN

BIOTECHNOLOGY

KUMARAGURU COLLEGE OF TECHNOLOGY

(An Autonomous Institution affiliated to Anna University of Technology, Coimbatore)

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BONAFIDE CERTIFICATE

Certified that this project report **"MEMBRANE PROCESSING, CLARIFICATION AND OPERATING CONDITIONS ON PHYTOCHEMICAL PROPERTIES OF ALOE VERA EXTRACT"** is the bonafide work of **"DIVYA.R , INDHUMATHI.E** and **KEERTHIKA.C"** who carried out the project work under my supervision.

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ABSTRACT

Membrane filtration is an attractive process, which have been widely used in food,dairy and biotechnology industries since it has the ability to produce very specific separations at ambient temperatures with no phase change can ,in many applications make membrane filtration much more cost-effective solution. Estimation of different phytochemicals like phenols, flavonoids present in aloe vera was carried out by conducting experiments in ultrafiltration with respect to operating conditions of various transmembrane pressure(0.2,0.4,0.6,0.8) and the values are compared. The concentration of phenols,flavanoids present in the peel extracts of aloe vera at different membrane cut off like 10kDa and 30kDa were estimated. This results had proven that the amount of phytochemicals retained were higher in the ultra-filtered permeate .The phenolic content was estimated by folin-ciocalteau assay and the flavonoid content was estimated using $Alcl_3$ method,before and after ultrafiltration .The ultra filtered permeate has more retained amount of phenols (56.81 mg/g) and flavonoids (82 mg/g).These results were also compared with Vidic *et al.*,(2014) literature values.

Keywords: Ultrafiltration, Permeate, Transmembrane pressure, Phytochemicals, Membrane cut-off .

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$Alcl_3$	Aluminium chloride
g/kg	gram/kilogram
kDa	Kilo Dalton
kPa	Kilo Pascal
L/H	Liter/hour
MF	Micro Filtration
MWCO	Molecular Weight Cut Off
NaOH	Sodium Hydroxide
OD	Osmotic Distillation
PEGMA	Polymethacrylate
PJ	Pomegranate Juice
PVPP	Polyvinylpolypyrrolidine
TMP	Transmembrane pressure
TSS	Total Soluble Solids
UF	Ultrafiltration
UFP	Ultra Filtered Permeate
UFR	Ultra Filtered Retentate
UV-Vis	UV-Visible Spectrophotometer

CHAPTER 1
INTRODUCTION

1.1 GENERAL

Filtration is defined as the separation of two or more components from a fluid stream .It refers to the separation of solid, immiscible particles from liquid or gaseous streams. Membrane filtration extends this application further to include the separation of dissolved solutes in liquid streams and for separation of gas mixtures. The primary goal of the membrane is to act as a selective barrier. It should permit passage of certain components and retain certain other components of mixture. The four developed industrial membrane separation processes are Microfiltration (MF), Ultrafiltration (UF), Reverse Osmosis (RO) and Dialysis. The range of application of the three pressure driven membrane water separation processes are Reverse Osmosis, Ultrafiltration and Microfiltration. UF and MF are basically similar in the mode of separation, which is molecular sieving through increasingly fine pores. MF membranes filter colloidal particles and bacteria from 0.1 to 10 µm in diameter. UF membranes can be used to filter dissolved macromolecules, such as proteins, from solutions. The mechanism of separation by RO membranes is quite different. In RO membranes, the membrane pores are so small, from 3 to 5Ǻ in diameter, that they are within the range of thermal motion of the polymerchains that form the membrane.

Clarifying fruit juice using membrane filtration has been common place since the late 1970's. The process has many advantages over conventional plate and frame filtration processes including: no filter aid requirements and reduced enzyme dosages.

Nevertheless, juice clarification via membrane filtration is not without its problems. During processing membranes tend to foul, greatly reducing system productivity. To reduce fouling problems, the juice is typically pre-treated using pectinases, gelatin, heat or some combination of all three.

Membrane processes are potentially attractive for processing tropical fruit juices due to the preservation of their nutritional and sensory characteristics. The product juice has extremely low haze value, devoid of microorganisms and of high quality. Since these processes are carried out at room temperature, pasteurization and sterilization at higher temperature are not required. UF and MF are generally used for clarification and reverse osmosis is used for concentration of fruit juice. (Cassano *et al*.,2003)

ALOE VERA

Aloe vera plant has been known for centuries and is used for its health, beauty, medicine and skin care properties. Aloe vera has its roots in the Arabic word 'Alloeh' meaning 'shining bitter substance', and 'vera' in Latin means 'true'. The Egyptians called Aloe 'the plant of immortality'. Chinese called it as their 'elixir of youth'. Americans called Aloe as the 'burn plant', 'medicine plant' and 'the mystery plant'. It is also known by a number of names such as 'the wand of heaven', 'heaven's blessing', 'miracle plant' and 'the silent healer'.

The botanical name of Aloe vera is *Aloe barbadensis Mill*. It is also known by different names, such as, *Aloe chinensisBak*, *Aloe elongate Murray, Aloe indica Royale, Aloe officinalisForsk, Aloe perfoliata, Aloe rubescens DC*. It belongs to Asphodelaceae (Liliaceae) family, native to northern Africa.

Aloe vera is a medicinal plant of higher economic value. It grows in different size ranging from 10 to 30 inches. It forms a dense rosette. It varies in color from light to dark green. It has long leaves, edges have points but they are not sharp. It has wary coating to keep the moisture inside. It grows in the dry regions of Africa, America, Asia and Europe. In India, it is largely found in the regions of Rajasthan, Gujarat, Andhra Pradesh, Maharashtra and Tamilnadu.

Aloe vera is a spiky cactus like xerophytes, which develops water-storage tissues in the leaves to survive in dry and low rainfall receiving areas. Clump forming perennial plant with thick fibrous root which produce basal leaves. Leaves are covered with thick cuticle, beneath which epidermis and mesophyll are present.

Aloe vera is a stemless or very short-stemmed succulent plant growing to 24 to 39 cm tall, spreading by offsets and root sprouts. The leaves are lanceolate, thick and fleshy, green to grey-green with some varieties showing white flecks on their upper and lower stem surfaces. The margin of the leaf is serrated .The flowers are produced on a spike up to 90cm tall, each flower being pendulous, with a tubular corolla 2-3cm long. Aloevera forms arbuscular mycorrhiza, asymbiosis that allows the plant better access to mineral nutrients in the soil.

There are 250 species of aloe vera grown all over the world (for example: *aloe barbadensis, aloe aborescens,* etc…).There are 200 types of molecules present in aloe vera. Aloe has 75 nutrients, 200 active compounds, 20 minerals,18 amino acids and 12 vitamins. Gel contains about 98% of water, 2% of solid content with polysaccharides,minerals,enzymes,sugars,anthraquinones,protein,lipids, lignin,saponins,sterols,salicylic acid, amino acids and phenolic acids. It also contains antioxidant, vitamins like vitamin A,C,E,B1,B2, choline and folic acids.

Aloe leaf structure contains four layers: 1) The outer protective layer called, the rind, which helps in the synthesis of carbohydrates and proteins. 2) A layer of bitter fluid called, the sap, which contains anthraquinones and glycosides. 3) The inner part of the leaf called, the mucilage gel, 4) and, the inner gel, which contains 98% water and rest contains essential amino acids, glucomannans, lipids and vitamins.

Aloe vera is a succulent plant species. Historically, Aloe vera has been used for its medicinal properties .It has a wide range of medicinal application such as wound healing, reducing blood sugar in diabetes, sooths burns, eases inpestiual problem, reduces arthritic swelling ,ulcer curatine object, stimulates immune responses against cancer. It has rejuvenating, healing, soothing properties .Anthraquinone derivatives of aloe vera plays an important role in the treatment of tumors, diabetes, ulcer and cancer.

Aloe vera has been specially used to treat patients with severe and various skin diseases, since it rejuvenates the skin. It acts as a moisturizer and hydrates the skin. It helps in treating minor vaginal irritations. It is used for mouth sores, known as stomach sores, or cold sores.

Aloe vera is a natural product that is being used widely in cosmetic industry, food as a natural flavouring, food supplements, and herbal remedies.

In pharmaceutical industry, Aloe vera is used as a laxative. It is also used to treat many conditions like constipation by increasing the bowel movements, ulcers, diabetes, headaches, arthritis, and coughs. It works in the intestinal tract to break down the food residues and help clean out the bowel. Hence it greatly reduces discomfort, bloating, and helps to relieve from stress.

ADVANTAGES

- To heel burns, cut and bruises.
- It helps diabetics who suffers from topical sores due to their disease.
- Moisturizes and hydrates the skin.
- Supports healthy digestion.
- Support a healthy immune system.
- Reduce harmful toxins.
- Increase the absorption of nutrients.
- Enhance antioxidant activity.
- Balance stomach acidity naturally.
- Soothe occasional muscle and joint pain.
- Antisephis, which can kill mold,bacteria,fungi,viruses.
- Presence of saponin,used as a cleansing agent.
- Controls dandruff, stimulates hair growth and inhibits premature graying.
- Cures dark spots, dull skin, acne scars, dry skin and skin burns.

1.2 OBJECTIVES

1. To evaluate antioxidant and other biological potential of aloe vera juice.
2. To screen and optimization of membrane processing condition for retaining biological properties of aloe vera juice.
3. To evaluate biological properties of membrane processed aloe vera juice

CHAPTER 2

REVIEW OF LITERATURE

2.1 ULTRAFILTRATION

Ultra filtration (UF) is a variety of membrane filtration in which forces like pressure or concentration gradients leads to a separation through a semi permeable membrane. High molecular weight solids are retentate, low molecular weight solutes pass through the membrane in the permeate . This separation process is used in industry for purifying and concentrating macromolecular solutions. It is different from membrane gas separation, which separates based on absorption and diffusion.

2.1.1 Principle

The basic operating principle of ultra-filtration uses a pressure induced separation of solutes from a solvent through a semi permeable membrane. The relation between the applied pressure on the solution and the flux through the membrane is described by the Darcy equation:

$$J = \frac{TMP}{\mu R_t}$$

Where, J -flux ,

TMP - transmembrane pressure ,

μ - solvent viscosity,

R_t - total resistance.

2.2 Membrane fouling

2.2.1 Concentration Polarization

When filtration occurs, the concentration of rejected material at the membrane surface increases. Increased ion concentration can develop an osmotic pressure on the feed side of the membrane. This reduces the trans-membrane pressure of the system. It shows that concentration polarization differs to fouling as it has no lasting effects on the membrane itself.

2.2.2 Types of fouling

1) Particulate deposition

This describe the mechanisms of particulate deposition on the membrane surface and in the pores:

- Standard blocking
- Complete blocking
- Cake filtration
- Intermediate blocking

2) Scaling

As a result of concentration polarization, increased ion concentrations crossed solubility of the appropriate range and precipitate on the membrane surface. These salt deposits can block pores causing decreased flux and membrane degradation

3**) Bio-fouling**

Microorganisms will attach to the membrane surface forming a gel layer-known as biofilm .The film increases the resistance to flow. In spiral-wound modules blockages formed by biofilm can form non-uniform flow distribution and increase the possibilities of concentration polarization.

2.3 Membrane Arrangements

With respect to shape and material of the membrane, different modules. In ultra filtration, modules vary according to the required hydrodynamic and mechanical stability of the system. The main modules are:

2.3.1 Tubular modules

This module has polymeric membranes on the inside of plastic components which allows for easy cleaning. They have less permeability, high volume content and low packing density.

2.3.2 Hollow fibres

A module consists of 50 to 1000 of hollow fibres .The advantage of having self-supporting membranes as is the ease of cleaning.

2.3.3 Spiral wound molecules

This kind of modules has a combination of flat membrane sheets separated by a thin meshed spacer material. These sheets are rolled around a central perforated tube,fitted into a tubular steel pressure vessel.It is limited by thin channels where feed solutions with suspended solids has effects of blockage of the pores in the membrane.

2.3.4 Plate and frame

This uses a membrane placed on a flat plate separated by a mesh like material. The feed is passed through the system from which permeate is separated and collected. This module provides low volume, membrane replacement and the ability to feed viscous solutions because of the low channel height.

2.4 Process considerations

2.4.1 Pre-treatment

Treatment of feed to the membrane is required to prevent damage to the membrane which reduces the effects of fouling. Types of pre-treatment are dependent on the type of feed and its quality. Other types of pre-treatment include pH balancing and coagulation.

2.5 Membrane Specifications

2.5.1 Materials

Most UF membranes use polymer materials, ceramic membranes, which is used for high temperature applications.

2.5.2 Pore Size

The choice of pore size is to use a membrane with a pore size one tenth that of the particle size to be separated. This reduces the amount of smaller particles entering the pores .They block the pores which allows the cross-flow velocity to dislodge them.

2.6 Operation strategy

The major kinds of operation strategies are :

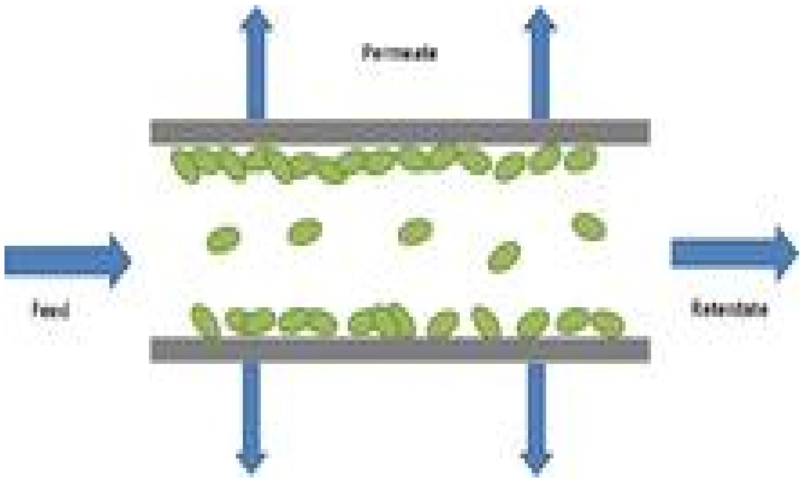

Figure 2.1 Cross flow filtration

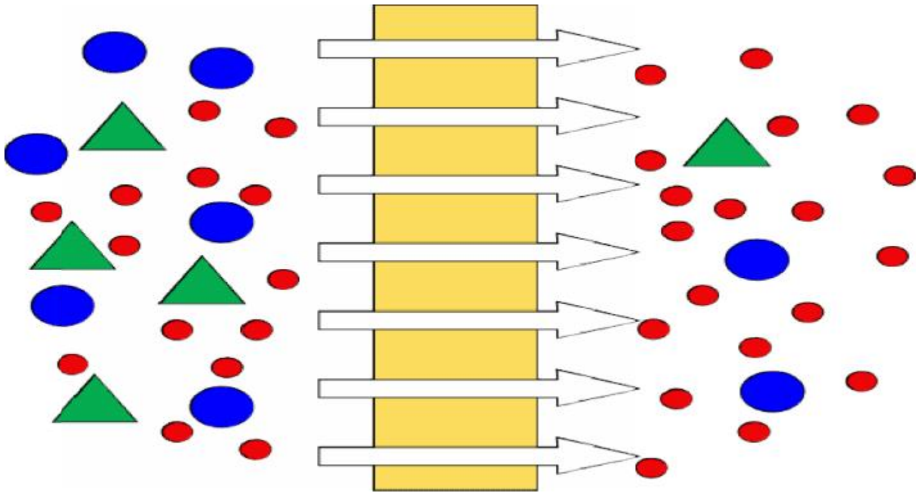

Figure 2.2 Dead end filtration

2.7 Flow Type

UF systems can be with cross-flow or dead-end flow. In dead-end filtration the flow of the feed solution is perpendicular to the membrane surface. In cross-flow filtration feed flow is parallel to the membrane surface.

2.8 Post Treatment

Post-treatment of the product is dependent on the components of the permeate and retentate .

2.9 Cleaning

Membrane cleaning is to prevent the accumulation of foulants. Backwashing is done for every 10 min to remove cake layers formed on the membrane surface. The types of chemicals used for cleaning are:

- Acidic solutions for the regulation of inorganic scale particles.
- Removal of organic compounds was done by alkali solutions.

2.10 Applications

- Manufacturing of cheese
- Removal of pathogens from milk
- Waste water treatment
- Recovery of juices
- Fruit juice clarification
- Dialysis
- Desalting

2.11 Ultrafiltration using carbosep membranes for the clarification of apple juice

Brujin *et al.*,(2003) had performed the clarification of apple juices using carbosep membrane of 15 and 50 kDa, tangential velocity of 2 and 7m/s and transmembrane pressure of 150 and 400kDa . The results revealed that there is no optimum operating conditions for the set of variables .Color, clarity and turbidity of juice improved after ultrafiltration Deterioration of juice quality during storage was clarity loss, turbidity and post-bottling sediment formation. Zero-order reaction kinetics were analyzed in order to find clarity loss and shelf-life was determined .Pasteurized and ultrafiltered apple juice had a shelf-life of at least 5 months ,when stored in glass containers at 16 ^{0}Celsius in the dark.

2.12 Effective clarification of pomegranate juice

Bagci *et al.*,(2014)This study was to introduce an effective pre-clarification step to improve performance of UF and to obtain a high quality pomegranate juice with improved clarity. The effects of various pre-clarification treatments utilizing gelatin, bentonite and polyvinyl, PVPP on UF performance were evaluated through analysis of flux behavior and membrane fouling. Quality parameters of the s like pH, total acidity phenolic content, anthocyanins ,phenolic acids ,organic acids ,antioxidant activity and color with various pre- clarification treatments were also investigated. Pre-clarification treatments which included PVPP exhibited a higher overall adsorption capacity, mainly of low molecular weight phenolics .This results revealed that both the reduction of fouling and juice clarity were achieved by application of PVPP and bentonite.

2.13 Limiting permeate flux in the clarification of untreated starfruit juice by membrane ultrafiltration

Sulaiman *et al* .,(1998)had studied about the limiting flux of untreated star fruit juice obtained during clarification using membrane filtration. In a stirred cell unit, experiments were conducted using membranes of 25,000 MWCO at 30^0Celsius for a range of juice concentration of solute in the bulk solution and the gel layer was also established. It was proved that the gel model could not deals about the clarification of star fruit juice.

2.14 Pre-concentration of longan juice extract with microfiltration

Montatip *et al.,* (2007) had pre-concentrated the longan juice extract with microfiltration and reverse osmosis .It was found that yields of the pre-concentrated longan juice were 71.63 ±1.31% and 37.69 ± 2.72% . Total yield of pre-concentrated longan juice was only 21.67 % of the dried longan. This juice in a hermetically sealed container was sterilized at 240F for 20 min providing 4.8 min of sterilizing value. Results showed that the product had 25.87 ±1.63% TSS ,Ph 5.16 ± 0.09 and 0.42 ± 0.03 % acid as citric acid .

2.15 Apple juice clarification using ultrafiltration and microfiltration polymeric membranes

The flux behavior of polyethersulfone (0.2µm) , polyvinylidene fluoride (0.2µm), cellulose (0.2µm,100 kDa,30 kDa,10kDa) membranes was examined during dead-end filtration of apple juice. Membrane with molecular weight cut-off of 30 and 100kDa had high flux performance to 0.2µm or 10 kDa membranes .A cross flow system with tubular polymeric membranes was used to clarify apple juice at a temperature of 50^0 Celsius a cross flow velocity of 3.3 m/s and a transmembrane pressure of 414 kPa. Steady state fluxes increased as the molecular weight cut-off increased The impact of filtration through the 9kDa membrane was noticed on the physical and chemical properties like lower flavanol content, and experienced minimal changes in browning and turbidity.

2.16 Study of fouling mechanism in pineapple juice clarification by ultrafiltration

Barros *et al*.,(2003) had studied the flux behavior of ceramic and polysulfone membranes was examined during cross-flow UF of pectinized pineapple juice. The effects of TMP and temperature on membrane fouling have been evaluated. The results revealed that hollow fiber membrane separation process is controlled by a cake filtration mechanism and a pore blocking fouling mechanism .The model parameter *k* was modeled as a temperature and transmembrane pressure The main properties of pineapple juice were to determine the highest permeated flux and best clarified juice.

2.17 Effect of various pretreatment methods on permeate flux and quality during ultrafiltration of mosambi juice

Rai *et al*.,(2007)had carried out the process of ultrafiltration of mosambi juice for the production of high quality juice. Different pretreatments methods were studied for clarification with high permeate flux and low membrane fouling. The various pretreatment methods were centrifugation fining by gelatin, bentonite ,enzymatic treatment. The resulting permeate juice after filtration had more than 93% clarity without deterioration of important juice quality.

2.18 Clarification of stevia extract by ultra filtration

Chhaya *et al*.,(had investigated the permeate flux and permeate quality recovery of stevioside in the permeate by ultrafiltration of four different membranes namely. The 30 kDa was found to be the most suitable one. To analyze the effects of the operating conditions transmembrane pressure drop and stirrer speed on the permeate flux and permeate quality under steady state conditions. The steady state permeate flux increased with pressure drop as well as stirrer speed .

2.19 Ceramic membrane based ultrafiltration of apple juice

Vladisavljevic *et al* (2003) had clarified the raw depectinized apple juice in ultrafiltration using ceramic tubular membranes with a molecular weight cut-off of 300000, 50000 and 30000 Da. The experiments have been carried out over a wide range of transmembrane pressures, temperatures, and feed flow rates . Permeate flux significantly decreased with time until a steady-state was established .Higher permeate flux was obtained at higher temperature .The steady state permeate flux reached a maximum to powers ranging between 0.22 and 0.31,at a transmembrane pressure of about 200 kPa.

2.20 A membrane-based process for the clarification and the concentration of the cactus pear juice

Cassano *et al*., (2007) had studied the potentiality membrane – based process for the clarification and concentration of cactus pear fruit juice. The fresh juice with a soluble solid content was clarified by an ultrafiltration. The clarified juice was concentrated by osmotic distillation up to a TSS content. To evaluate the effects of the membrane processes on the quality and composition of the juice ,the quality of the juice was analyzed with respect to total antioxidant activity, ascorbic acid, citric and glutamic acid, betalains and viscosity.

2.21 Ultrafiltration of kiwifruit juice

Cassano *et al* .,(2007) had studied the effect of transmembrane pressure(TMP),axial flow-rate and temperature on permeation flux of fresh depectinised kiwifruit juice which was clarified by UF process. Cake layer and irreversible fouling resistances gave the total resistances of 2.23% and 2.75%. A good restore of the hydraulic permeability of the membrane was observed after a cleaning treatment performed using alkaline and acid detergents .This results revealed that flux increased with temperature from 20 to 30^0C and with axial feed flow rate from 300 to 700 l/h. The flux-pressure curves showed no increase in permeate fluxes.

2.22 Clarification and concentration of pomegranate juice using membrane process

Cassano *et al* **.,**(2011)had investigated the production of concentrated pomegranate by using a two-step membrane process based on a clarification step of the fresh non-depectinized juice performed by hollow-fiber ultrafiltration (UF) membranes and a concentration step of the clarified juice by using an osmotic distillation (OD) apparatus. Both processes were performed at ambient temperature producing a clear juice and a concentrated juice with a total soluble solids of 162 g kg^{-1} and 520g kg^{-1}. The performance of UF and OD operations were evaluated in terms of productivity and quality of the processed juice. Suspended solids were removed in the clarification step.

2.23 Pulsed electric field based ultrafiltration of sugar beet juices

Loginov *et al*., (2011) had studied the ultrafiltration of sugar beet juices obtained by high temperature diffusion and novel pulsed electric field(PEF) assisted low temperature diffusion. Filtration experiments were7 carried out in the concentrated mode using a batch dead-end filtration cell and hydrophilic polyethersulfone membranes with molecular weight cut-offs (MWCO) of 10, 30 and 100 kDa. The transmembrane pressure was within the range 0.5-3.7 bar. The influence of filtration conditions on filtration rate, filtration mechanism and filtrate quality were studied.

2.24 Quantification of gel deposition by electric field enhanced ultrafiltration of synthetic fruit juice

Sarkar *et al.*, (2008) had studied the effects of applied electric field during cross-flow ultrafiltration of synthetic juice. The introduction of the electric field is effective in enhancing the permeate flux due to the electrophoresis of the gel forming pectin molecules. A model based on gel layer theory was made to quantify the flux and growth of the gel layer thickness which was also measured with high-resolution microscopy.

2.25 Clarification and concentration of citrus and carrot juices by integrated membrane processes

Cassano *et al.*, (2003) had studied the ultrafiltration process to clarify the raw juice. The RO process was used to pre-concentrate the permeate to 15-20 g TSS/100g. This results revealed that it is possible to suggest an integrated membrane process, yields concentrated juices of high quality and high nutritional value. The residual fibrous phase coming from the UF process being a stabilizing treatment and reused for the preparation of beverages enriched in fibres.

2.26 Surface functionalization by photo graft copolymerization to polyethersulfone – based ultrafiltration membranes

Susanto *et al* .,(2007) had prepared the low-fouling ultrafiltration membranes by simultaneous photo graft copolymerization of PEG, PEGMA onto a PES membrane with a cut-off of 50 kg/mol. A characterization was performed by flux measurement and sieving curve analysis, FTIR-ATR spectroscopy, contact angle and zeta potential measurement. All modified membranes showed more resistance to fouling. This study provides information for the development of low fouling ultrafiltration membranes .

CHAPTER 3
MATERIALS AND METHODS

3.1 ULTRAFILTRATION

Ultrafiltration process was performed during the course of this experiment to characterize the given membrane and to identify the amount of phenol and flavonoid present in the permeate and retentate of fed aloe vera juice. The main objective being optimization of membrane operating conditions (membrane molecular weight, the flow rate, and transmembrane pressure) to obtain high amount of phenol and flavonoid from aloe vera juice.

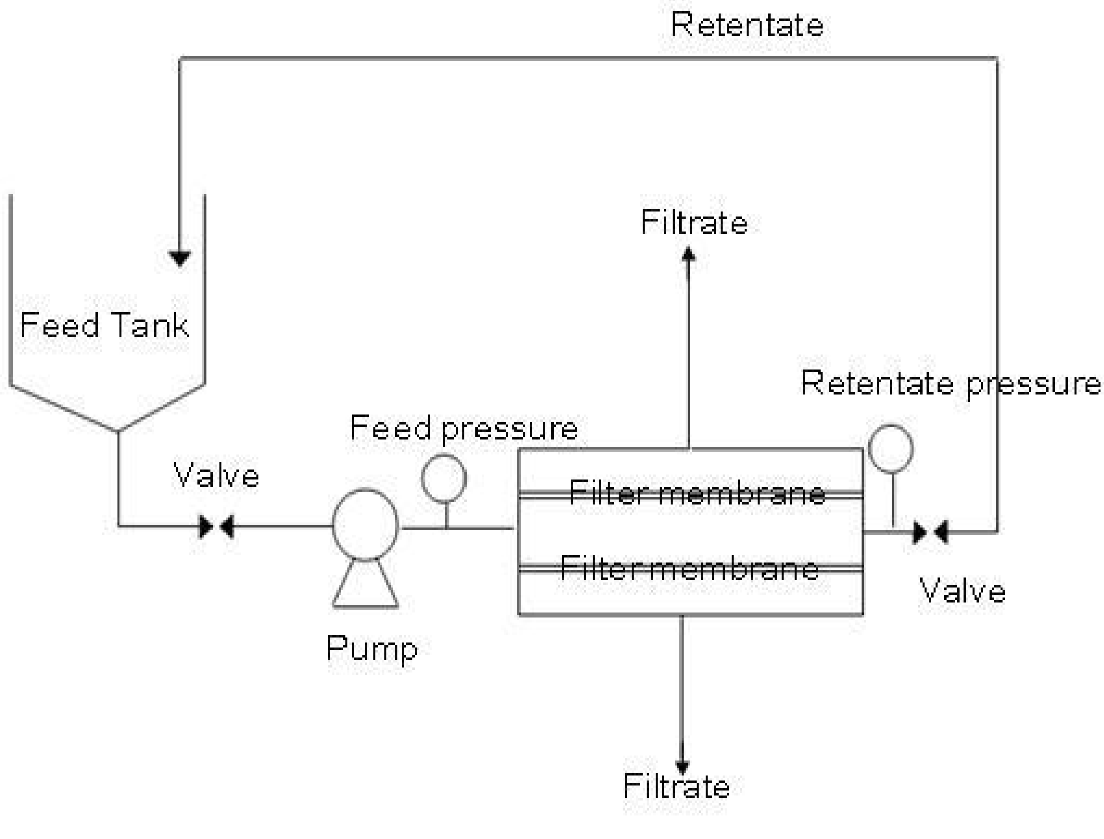

Figure 3.1 Schematic diagram of ultra filtration process

3.2 APPARATUS AND MATERIALS:

3.2.1 Cross flow ultrafiltration

- Effective membrane area (0.098 sq.m)
- Maximum surface temperature (35±2 ^{0}C)

3.2.2 UV-Vis spectrophotometer

- Elico SL159(Elico,India)
- Range 190-1100 nm

3.2.3 Weighing balance

- Shimadzu EL300 (Japan)
- Accuracy (0.01 g)

3.2.4Membrane

- Pore size (0.2 µm)

3.3 REAGENTS REQUIRED:

1) Sodium Hydroxide
2) Sodium carbonate
3) Aluminium chloride
4) Folin's ciocalteau reagent
5) Methanol

The above mentioned chemicals were purchased from Himedia, Mumbai.

3.4 PROTOCOL:

3.4.1 PREPARATION OF ALOE VERA JUICE

- Aloe Vera plant used , was obtained from the local region of Coimbatore.

- The fresh plant was washed thoroughly to remove the dust particles.
- The leaves were cut, the peel was removed and cut into pieces.
- The peel was air dried at room temperature for two days.
- The peel was then crushed into powder using a mixer.
- The powder was weighed and 0.4 g of the powder was taken.
- To the powder, 5 ml of previously heated distilled water with 60°C and 2ml of methanol was added.
- 75 ml of water was added and filtered.
- The volume was made up to 1000 ml.

3.4.2 MEMBRANE WASHING

To use the membrane for subsequent runs it should be washed. The feed tank was filled with RO water and run. The membrane was cleaned with RO water. The membrane was washed using 0.5 N sodium hydroxide solutions. To free the membrane from sodium hydroxide, it was flushed with water at gradually increasing pressures.

After the ultrafiltration process with the aloe Vera juice, the membrane was again washed with water. After washing the membrane was stored in 0.1 N sodium hydroxide solution to prevent any microbial growth on the membrane surface and for the reuse of the membrane.

3.4.3 ULTRAFILTRATION PROCESS

- The feed tank was filled to its capacity with aloe vera juice.
- The ultrafiltration membrane of specific molecular weight cut off (10KDa) was set.
- The sample before ultrafiltration was collected and it serves as crude.

- With the aloe Vera juice made into 1000ml was run for the ultrafiltration process.
- The transmembrane pressure was set at 0.2 bar.
- The velocity was noted. The volume of permeate obtained per minute was noted to calculate the flow rate.
- The permeate and retentate was collected separately.
- The total phenols and flavonoids estimation was done for the crude, permeate and retentate in triplicate.
- Again the procedure was repeated by varying the transmembrane pressure of 0.4, 0.6 and 0.8 bar.
- The phenol and flavonoid content for different transmembrane pressure was identified and compared.
- The above procedure was repeated with a membrane molecular cut off of 30 KDa with different transmembrane pressure was done.
- The amount of phenol and flavonoids obtained with the processing of 30 KDa was compared with values obtained from the membrane of 10 KDa

3.4.4 ESTIMATION OF PHENOL

- 0.1ml of aloe vera juice sample (crude, permeate, retentate) was taken.
- 3.8 ml of distilled water was added to that.
- Folin Ciocalteau Reagent of 0.5 ml was added.
- It was incubated at room temperature for 3 minutes.
- 2 ml of 20% sodium carbonate was added.
- It was kept in boiling water bath for 1 minute.
- Blue color was developed.

- The absorbance was measured using UV –visible spectrophotometer at 650 nm.
- The total amount of phenol was calculated using Gallic Acid as a standard.

3.4.5 ESTIMATION OF FLAVONOID

- One ml of aloe Vera juice sample (crude, permeate and retentate) was taken.
- To that, added 4ml of distilled water.
- 0.3 ml of 5% sodium nitrite was added.
- It was incubated at room temperature for 5 minutes.
- After 5 minutes, 0.3 ml of 10% aluminium chloride was added.
- After 5 minutes, 2ml of 1M sodium hydroxide was added.
- The volume was made into 10 ml with distilled water.
- The solution was mixed and absorbance was measured using UV-visible spectrophotometer at 510 nm.
- Total flavonoid content was expressed as mg quercetin equivalent.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 COMPONENTS ANALYSIS IN ALOE VERA

Initially, aloe Vera juice along with their major compositions was studied in terms of flux mechanisms, several techniques regarding fouling compounds were chosen. The compounds which makes up of aloe Vera juice exists in soluble and suspended forms. Compounds were also analyzed in terms of cell debris. Aloe Vera has various compositions of phytochemicals identified various phenolic acids/ polyphenols, phytosterols, fatty acids,ketones etc. Aloe Vera extract assay values will depend on many variables including aloe Vera growing location, storage conditions, time of storage and different extraction techniques.

4.2 ULTRAFILTRATION PROCESS

Thus, the ultra filtration process was carried out by using membrane cut-off 10 kDa and 30 kDa at different transmembrane pressure of about (0.2, 0.4, 0.6, 0.8 bar) and then the total phenolic, flavonoids ,anthraquinone in aloe Vera extract were estimated ,after the collection of crude , retentate and permeate.

Then, the total phenolic, flavonoids content were estimated with the corresponding standard graph values.

4.3 MEMBRANE PROCESSING OF ALOE VERA USING 10kDa

Thus, the ultra filtration process was carried out initially by 10kDa at different transmembrane pressure of (0.2, 0.4, 0.6,0.8) and the total phenolic and flavonoid content were estimated.

4.4 ESTIMATION OF TOTAL PHENOLIC CONTENT (10 kDa)

Then, the sample extract of crude, permeate and retentate were collected after ultra filtration process and the total phenolic content were estimated using folin ciocalteau reagent with gallic acid standard equivalent.

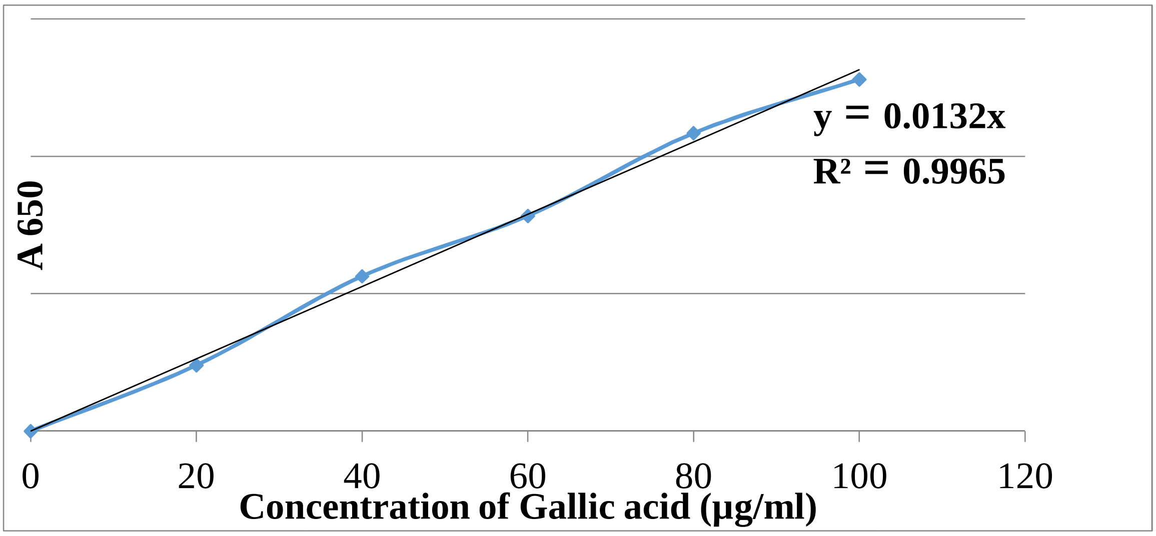

FIGURE 4.1 Standard graph for gallic acid

By using the equation: **y=0.0132x**

Substituting absorbance (y) for each value, the concentration of phenols (µg/ml) present in the sample can be found.

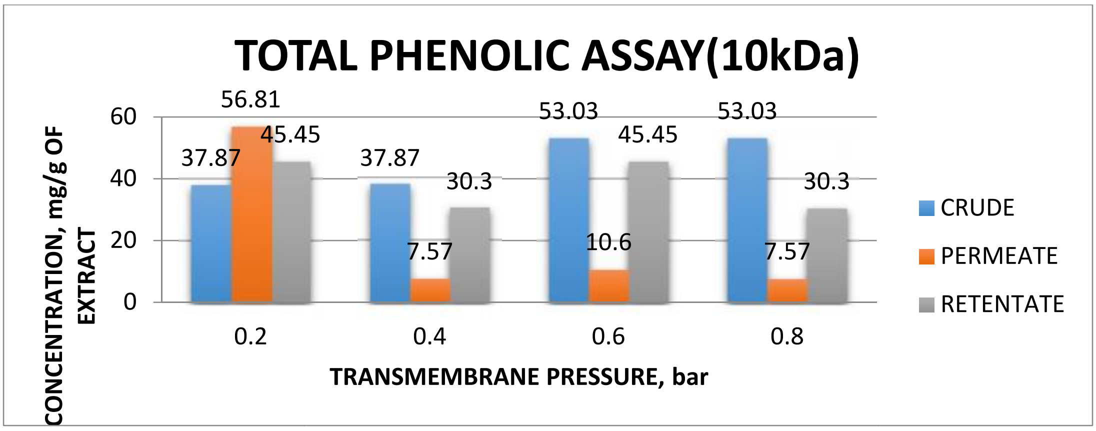

Figure 4.2 Comparison of phenolic content in terms of concentration by using membrane cut off-10kDa

With respect to different transmembrane pressure in membrane cut off -10kDa,the concentration of phenols retained in mg gallic acid equivalent/g of extract .The above shown graph depicts that more amount of polyphenols gets passes through the pores via extract and collected in the permeate and low molecular weight compounds along with little debris get retained in the retentate. At TMP of 0.2 bar
the concentration is more of 56.81mg/g of extract.

4.5 ESTIMATION OF TOTAL FLAVONOID CONTENT (10kDa)

Then, the sample extract of crude, permeate and retentate were collected after ultra filtration process and the total flavonoid content were estimated using aluminium chloride with quercetin standard equivalent.

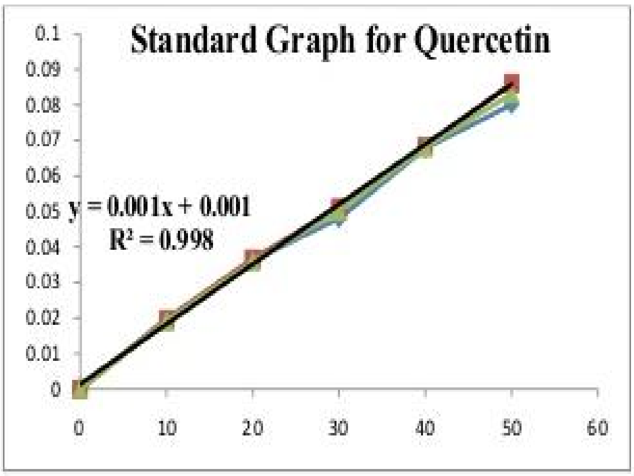

Figure 4.3 Standard graph for flavonoid assay

By using the following equation**: y=0.001x**

Substituting absorbance(y) value, the concentration of flavonoids (μg/ml) present in the sample can be found.

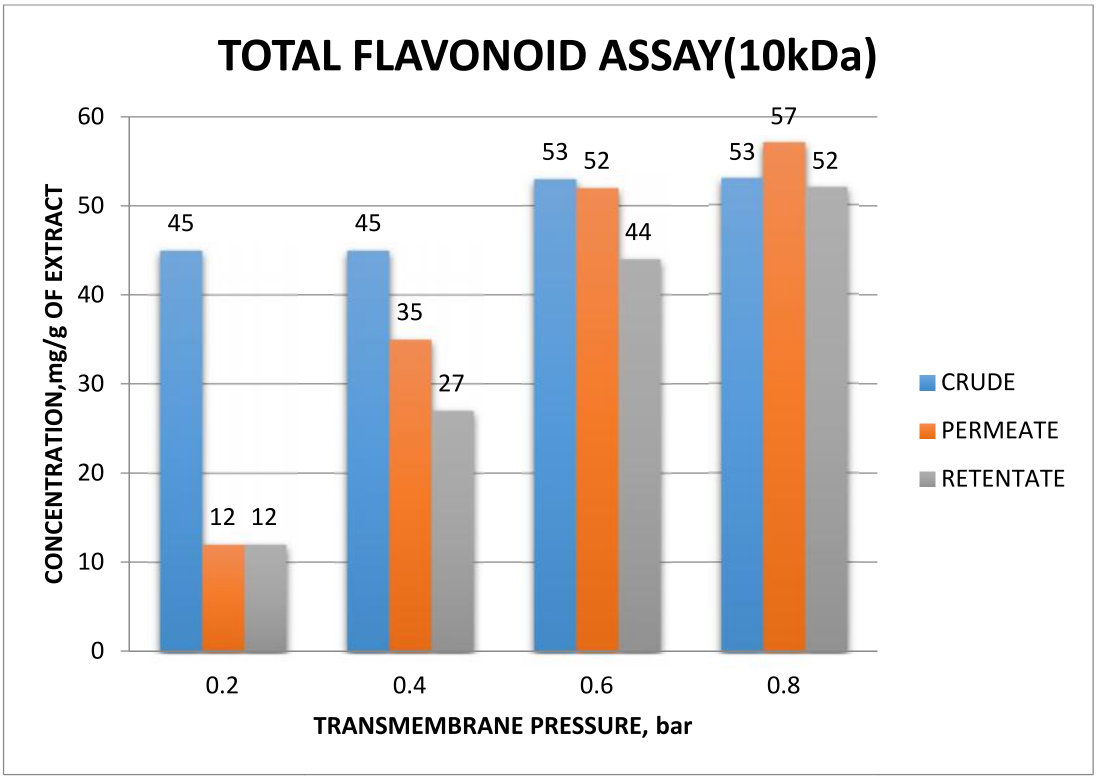

Figure 4.4 Comparison of flavonoids content in terms of concentration by using membrane cut off - 10kDa

At 10kDa membrane cut off, the concentration of flavonoids present in the permeate with respect to 0.8 TMP is more, which has a concentration range of 57 mg of quercetin equivalent/g of extract

It also depicts that the concentration gradually increases from 0.2 to 0.8 bar in retentate and permeate. But, in crude extract the concentration of flavonoids doesn't make a great difference by varying the pressure.

4.6 MEMBRANE PROCESSING OF ALOE VERA EXTRACT USING 30 kDa

Thus, the ultra filtration process was carried out initially by 30 kDa at different transmembrane pressure of (0.2, 0.4, 0.6, and 0.8) and the total phenolic and flavonoid content were estimated.

4.7 ESTIMATION OF TOTAL PHENOLIC CONTENT (30 kDa)

Then, the sample extract of crude, permeate and retentate were collected after ultra filtration process and the total phenolic content were estimated using folin ciocalteau reagent with gallic acid standard equivalent

By using the equation from the standard graph **(Fig 4.1)**

y=0.0132x

Substituting absorbance (y) for each value, the concentration of phenols (µg/ml) present in the sample can be found.

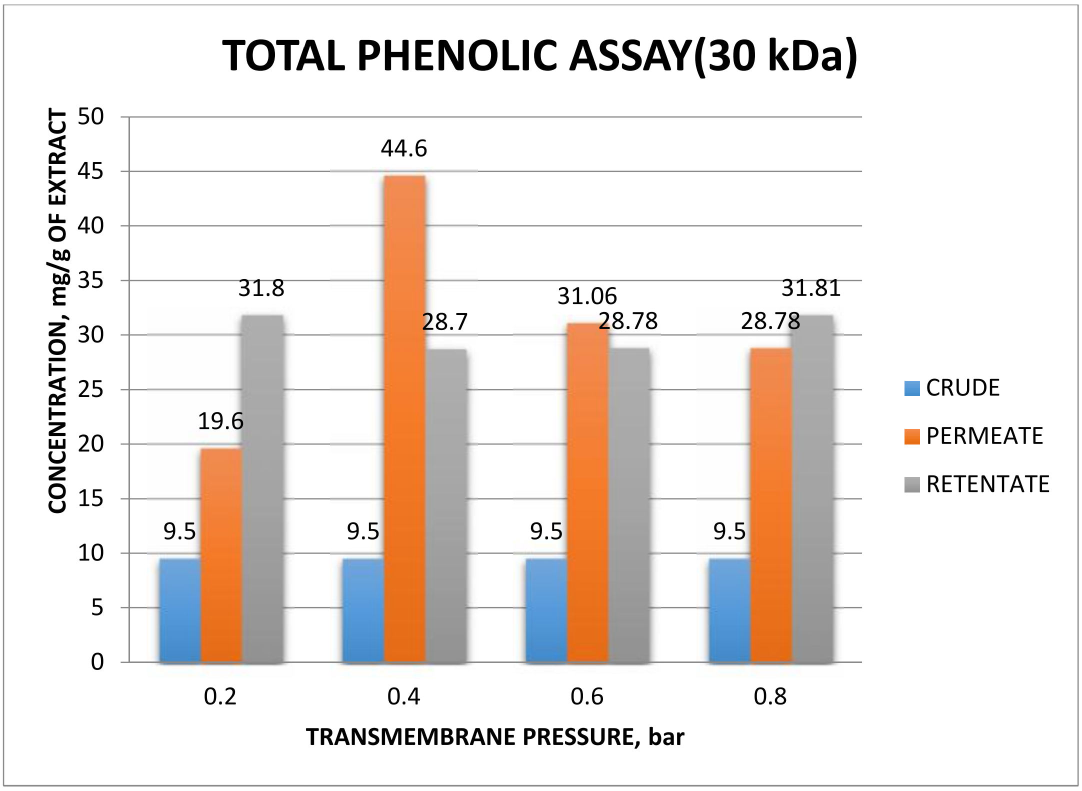

Figure 4.5 Comparison of phenolic content in terms of concentration with by using membrane cut-off - 30 kDa

With respect to membrane cut-off 30kDa ,phenols get retained in the retentae and the concentration gradually increases but in case of permeate the concentration is 44.6 mg of gallic acid equivalent /g of extract at 0.4 bar.

The crude has the same amount of concentration of phenols present in terms of gallic acid equivalent.

4.8 ESTIMATION OF TOTAL FLAVONOID CONTENT

Then, the sample extract of crude, permeate and retentate were collected after ultra filtration process and the total flavonoid content were estimated using aluminium chloride with quercetin standard equivalent.

By using the following equation: **y=0.001x**

Substituting absorbance(y) value, the concentration of flavonoids (µg/ml) present in the sample can be found.

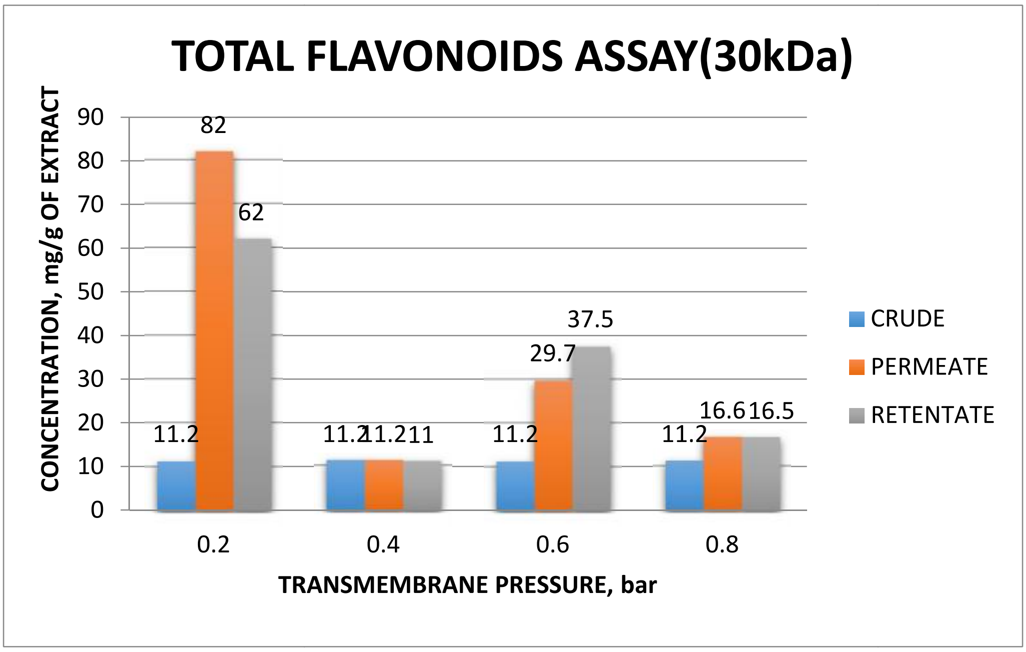

Figure 4.6 Comparison of flavonoids content in terms of concentration by using membrane cut off -30kDa

At 30 kDa,the concentration of flavonoids is very high at 0.2 bar in a range of 82 mg of gallic acid equivalent.The retentate range getgradually decreases as and when pressure increases.

Comparatively,the concentration of phenols obtained in the 10 kDa membrane (56.1 mg/g of extract) is higher than the 30 kDa (44.6 mg/g of extract) in the ultrafiltered permeate.

Similarly, the concentration of flavonoids obtained in the ultrafiltered permeate was higher in the 30kDa membrane(87 mg/g of extract) than in the 10 kDa membrane at different transmembrane pressure.

Table 4.1 Comparison of values of phytochemicals with Vidic *et al*.,(2014)

Phytochemical Properties	**Results**	**Vidic *et al*.,(2014)**
Total phenolic content	56.81mg/g	7.99mg /g
Total flavonoid content	82mg/g	9.17 mg/g

The concentration of phytochemicals (i.e) phenols and flavonoids was found to be higher in the ultra-filtered permeate rather than in various kinds of extraction in Aloe Vera peel extract ,which has been compared with the Vidic *et al*.,(2014) literature values.

CHAPTER 5

CONCLUSION

This particular study had investigated the process of ultra filtration in Aloe Vera extract. At different transmembrane pressure, the concentration of phytochemicals like phenols, flavonoids which has been retained were estimated.

The concentration of phenols and flavonoids which has more antioxidant content were obtained in the ultra filtered permeate rather than in crude and retentate.

And it is also compared with the work done by (vidic *et al)* with the proven data of the peel extracts ,which has the highest phenolic content (7.99 mg of gallic acid equivalent/g extract) and flavonoid content(9.17 mg of quercetin equivalent/g extract).

But in this study,we have proven that after ultrafiltration process the peel extracts which has high value of phenolic content of
56.81 mg/g of extract at 10kDa (0.2 bar) as comparatively greater than 30 kDa(0.4 bar) with 44.6 mg/g of extract.

The retention of macromolecules like various phytochemicals depends on transmembrane pressure as well.

The ultrafiltered permeate which was obtained with high phenolic and flavonoid content can be further undergone with purification techniques.

Then, the purified phytochemicals will be characterised for some applications purposes in food, pharmaceutical industries since it has several properties like anti-bacterial ,anti-cancerous ,laxatives,
anti-aging, anti-septic etc.

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