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FOURIER TRANSFORM INFRA-RED SPECTROSCOPY (FTIR) ANALYSIS AND *IN-VITRO* ANTI-INFLAMMATORY ACTIVITY OF ALMOND GUM

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ABSTRACT: Objectives: The anti-inflammatory activity of powdered almond gum (*Prunus dulcis*) was studied by using HRBC membrane stabilizing method and the bioactive compounds which are responsible for anti-inflammatory activity was analyzed by using FTIR analysis**. Methods:** To study the *in-vitro* anti-inflammatory activity Human red blood cell (HRBC) membrane stabilization method has been used and for identification of functional groups Fourier Transform infra-red spectroscopy was used. **Result and Conclusion:** Almond gum resin serves as a good anti-inflammatory agent due to the presence of saponins, steroids, terpenoids and xylan which were identified from FTIR analysis.

INTRODUCTION: Water soluble gum extrudes from the wounds on almond trees *Prunus communis* which is an almond gum [1]. It is commonly known as badam in India. From the almond tree fruits of almond that could be utilized in food. In India almond gum has received less attention, resulting in total wastage of exudate which is a rich source of various minerals including sodium, potassium, magnesium, calcium and iron [2]. Almond gum includes Aldobionic acid, L-galactose D-mannose *etc.*, it contains different component which have emulsifier, thickener, suspending pharmaceutical, adhesive, glazing agents and stabilizer. Almond gum is a natural polysaccharide is said to poses antioxidant and anti- inflammatory properties due to its polysaccharide content [3].

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Anti-inflammatory activity of *Polyalthia longifolia* seeds is measured as a decrease in paw odema [4] Almond comprises of proteins (2.45%), fats (0.85%) and carbohydrates (92.36%) carbohydrates comprise of arabinose (46.83%), galactose (35.49%) and uronic acid (5.97%) with trace of rhamnose, mannose and glucose. It is easily solvable in viscous and water solutions.

The property of a substance or treatment that reduce the inflammation is said to possess anti-inflammatory activity. Anti-inflammatory activity of medicinal plants are cuscutine, flavonoid, glucoside, bergenin and coumarin [5]. For controlling and suppressing inflammatory crisis there are various medicine such as steroids, non-steroid anti-inflammatory drugs. We need to apply the natural anti-inflammatory factors within meditation therapy to achieve increased pharmacological response and the lowest degree of unwanted side effects. Fourier transform infra-red spectroscopy is an analytical technique used for the identification of primary and secondary metabolites by observing the IR spectra [6]. It is also used to identify the

intensity of absorption spectra to find the chemical functional groups [7]. The ethanolic root extracts of extract of *Millettia pinnata* was subjected to the FTIR spectrum analysis which indicates the presence of functional groups like amides, benzene ether and halogen [8]. Aqueous extract of *Argemone Mexicana* reveals the presence of carboxylic acid and alcohol when analysed through FTIR [9].

MATERIALS AND METHODS: Almond gum was blended into powdered using a mixer. 5g of almond gum powdered was dissolved in 95% of ethanol in distilled water are kept in the shaker on overnight, then mixture was centrifuged at 4000 rpm for 10 mints after the centrifuged collect the pellet. The pellet was dissolved in distilled water and kept on magnetic stirrer for milled heat for 1hour. After 1 hour the mixture was cooled and used for masculine cloth to filter the solvent.

Preparation of the Blood Sample for Membrane: To study the *in-vitro* anti-inflammatory activity, the Human red blood cell (HRBC) membrane stabilization method has been used as a method [10]. By inhibiting lysosomal enzymes or by stabilizing the lysosomal membrane the non -steroidal drugs act [11].

From healthy human volunteer, the blood was collected, mixed with equal amount of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5 % citric acid and 0.42 %sodium chloride) in autoclave the solution. Before use all the blood samples word stored at 4°C for 24 hours. After centrifuging the blood sample at 2000 rpm for 5 minutes the supernatant was removed. The cell suspensions were was washed with sterile Isosaline solution 0.85% trisodium citrate this solution are also autoclaved 0.36% sodium chloride also separately prepared for hyposaline and centrifuged at 2000 rpm 5 min. Packed cell volume was measured till the supernatant was clear and colourless.

The cellular component was reconstituted to a 40% suspension with the phosphate buffer Saline (10 mm, pH 7.4) in this assay.

Hypotonicity Induced Human Red Blood Cell (HRBC) Membrane Stabilization Method: Almond gum powdered of different concentrations (100µl, 200µl, 300µl and 400µl) in 1ml of 0.2 M phosphate buffer and 0.5 ml of 10 % HRBC suspension, 2ml of hyposaline were incubated at 37°C for 30 min in room temperature. Hyposaline was taken in blank test tube, HRBC suspension and phosphate buffer were centrifuged at 4000 rpm for 10 minutes and the haemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Standard used was diclofenac and a control was prepared by distilled water instead of hypo saline to produce 100 % haemolysis without plant extracts. The percentage of HRBC Haemolysis and membrane stabilization or protection was calculated by optical density of drug treated sample optical density of 100.

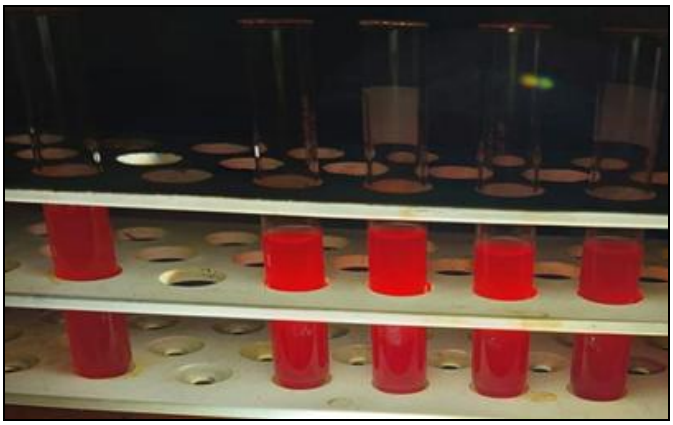

FIG. 1: POLYSACCHARIDE EXTRACTION OF POWDERED ALMOND GUM

Anti-inflammatory Activity: Anti-inflammatory assay by HRBC membrane stabilisation method **Table 1**, showing the concentration of haemolysis and protective activity of sample and standard.

TABLE 1: ANTI-INFLAMMATORY ACTIVITY BY HRBC MEMBRANE STABILIZATION METHOD

Concentration (µl)	Test sample almond gum extract (OD at 560nm)	Reference standard (Diclofenac drug) (OD at 560nm)
50	0.033	0.098
100	0.030	0.040
150	0.012	0.033
200	0.002	0.020

The erythrocyte membrane is similar to lysosomee membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. The importance of stabilizing the lysosome

membrane is to stop the inflammatory response by preventing the release of lysomal constituents of activated neutrophil such as proteases and bactericidal enzymes which cause damage and tissue inflammation upon extracellular release. Hypotonicity induced haemolysis arises due to shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components. To check the percentage of haemolysis of the HRBC cells the sample is compared with a positive control.

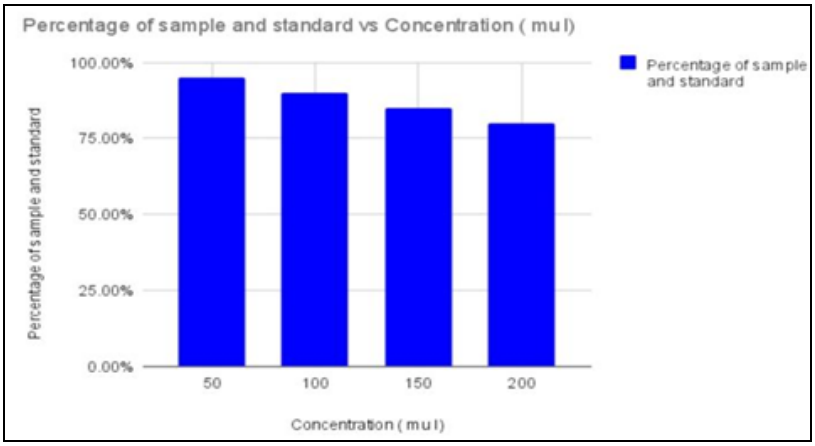

FIG. 2: SHOWING PERCENTAGE HAEMOLYSIS AND PROTECTIVE ACTIVITIES OF SAMPLE AND STANDARD

Anti-Inflammatory Assay by HRBC Membrane Stabilization: To check the percentage of haemolysis and protective of the HRBC cells, the sample is compared with a standard. When started with a minimal concentration of 50 (µl) the sample showed a haemolysis and protective activity of 95.1% and when the concentration were decreased, the activities of the sample and standard were increased. When the concentration was 200(µl) the sample and standard is 80.1% of haemolysis and protective activities.

FTIR Analysis: The FTIR spectrum of this experiment is gum of cellulose. Infrared spectrum of absorption or emission of a liquid, solid or gas can be obtained by Fourier-transform infrared spectroscopy. Wavelengths in the infrared region was measured by FTIR. This instrument covered the wavelength range from (wave number range 4000 cm^{-1} to 500 cm^{-1}). The peak which are also called absorbance band. For mid – range IR, the wave number on the infrared spectrum is plotted between 4000 to 500 cm^{-1}. **Fig. 3.**

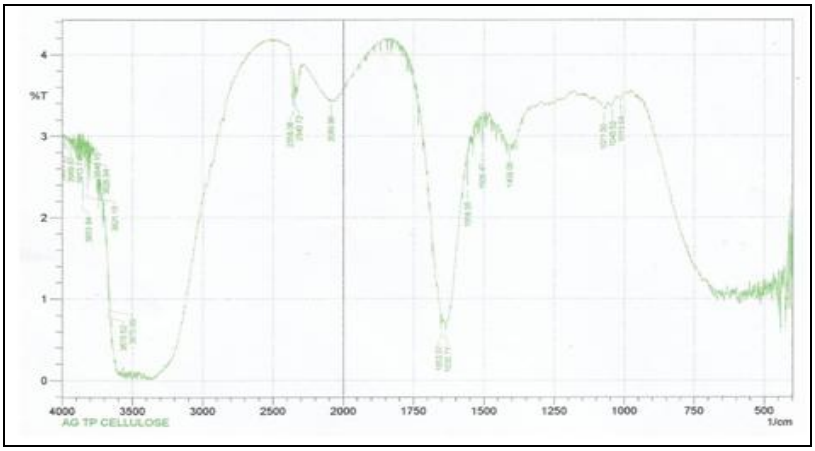

FIG. 3: FTIR SPECTRUM OF ALMOND GUM CELLULOSE

The peak is 1013.64 range of 1000 wave number and 1043.53, 1071.50 it's range of 1250 wave number of vibration spectrum so the band assignment is C-O stretching of primary alcohol and C-O-C stretching; C-O stretching. The peak is 1409.06, 1506.47, 1558.55, 1635.71, 1653.07 range of 1340 to 1720 in wave number the vibration spectrum is C- H2 deformation vibration; CH_3 symmetric deformation and C=O stretching of carbonyl, carbonyl and acetyl groups; and of

xylans. The peak is 3670.69, 3675.52, 3821.15, 3826.94, 3848.15, 3853.94, 3913.74, 3969.67, 3981.25, 3987.03, 3994.75 and 3999.57 range of 3900 wave number of vibration spectrum of band assignment is OH stretching in FTIR spectrum vibration **Table 2.**

TABLE 2: WAVE NUMBER CORRESPONDING TO BAND ASSIGNMENT

Wave number	Band assignment
~1000	C-O stretching of primary alcohol
~1170	C-O-C stretching; C-O stretching
~1340	C-H_2 deformation vibration; CH_3 symmetric deformation
~1720	C=O stretching of carbonyl, carbonyl and acetyl groups;
~3900	OH stretching

These fuctional groups confirms the presence of saponins, steroids, terpenoids and xylan. Anti-inflammatory activity could be due to these secondary metabolities such as saponins, steroids and terpenoids [12].

CONCLUSION: Due to side effects and health problem of anti-inflammatory drugs naturally occurring anti-inflammatory plant compounds are now becoming popular. From this study almond gum resin serves as a good anti-inflammatory agent due to the presence of saponins, steroids, terpenoids and xylan which were idenfied from FTIR analysis.

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CONFLICT OF INTEREST: All the authors declare that there is no conflict of interest.

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