

EVALUATION OF PHARMACOLOGICAL ACTIVITIES OF PECTIN EXTRACTED FROM APPLE AND CITRUS POMACE

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ABSTRACT

In the present study, the pectin, a natural gelling plant polysaccharide was extracted from pomace portion of apples and oranges. Further the antimicrobial, antioxidant and anti-inflammatory activities of pectin extracts were investigated. It was found that the yield of pectin content extracted was predominant in apples (20.60 %) in comparison to that of oranges (10.56%). The pectin samples extracted from apples and oranges were qualitatively evaluated for characteristic properties. Further the phytochemical constituents were qualitatively determined by conventional procedures. The results confirmed the presence of alkaloids, flavanoids, reducing sugars, steroids and cardiac glycosides in orange pectin while alkaloids, reducing sugars, steroids, cardiac glycosides and anthraquinones were present in apple pectin. Tannins, saponin and anthraquinones were absent in orange pectin while tannins, flavanoids and saponin were absent in apple pectin. In separation of pectin extracts by TLC, the R_f values of the pectin samples were found to be similar viz. Apple pectin (0.62), Orange pectin (0.62) to that of standard pectin (procured from Ranchem), 0.63. The apple pectin and orange pectin showed almost similar retention time viz. 1.821 minutes and 1.757 minutes specifically on HPLC chromatogram. The FT-IR spectra of extracted pectin samples were determined using a computerized FTIR spectrometer in the range of 4400–400 cm⁻¹ by the KBr pellet technique. Two outstanding features of the infrared spectra of pectinate are bands at 1745-1743 and at 1607-1604 cm⁻¹. One other feature which should be mentioned is a band, beyond the afore mentioned range, appearing at 1418-1417 cm⁻¹. In the spectra of polygalacturonate, the absence of carbonyl stretching bands above 1680 cm⁻¹ demonstrates that the polygalacturonate is de-esterified to a degree of <2%. The extracts were also found to have antimicrobial potential against the Methicillin resistant *Staphylococcus aureus*. It was found that apple pectin had potent antimicrobial activity against *E.coli*, Methicillin resistant strain of *S. aureus* (isolated from blood) and *Aspergillus niger* while orange pectin was found to have antimicrobial potential against *E.coli* and *Aspergillus niger*. Apple pectin extracts were found to have potent antimicrobial activity in comparison to that of orange pectin. Apple pectin extracts showed least MIC values viz. 10⁻⁴ µg/ml against *Aspergillus niger*, 10⁻⁵ µg/ml against *E.coli* and Methicilin resistant *S. aureus* (isolated from blood). Orange pectin extracts showed similar MIC values viz. 10⁻⁵ µg/ml against *E.coli* and *Aspergillus niger*. Amongst both the pectin extracts, Total phenolic content of orange pectin was found to be much more (38 µg/ml) in comparison to that of apple pectin (20 µg/ml). The results of TPC correlate the findings of Total antioxidant activity determination assay (based on absorbance) which also illustrates that orange pectin had potent antioxidant activity in comparison to apple pectin. The extracts were also screened for anti-inflammatory activities and the results were found to be satisfactory as the extracts showed promising anti-inflammatory activities too.

Key words: Pectin, pharmacological activities, active constituents, antimicrobial, antioxidant, anti-inflammatory activity.

INTRODUCTION

Pectin is a complex mixture of polysaccharides that makes up about one third of the cell wall dry substances of higher plants. Much smaller proportions of these substances are found in the cell walls of grasses. The highest concentration

of pectin is found in the middle lamella of cell wall, with a gradual decrease as one passes through the primary wall toward the plasma membrane. Apple is particularly rich in pectin, the name applied to any one of a group of white, amorphous complex carbohydrates with a high molecular weight. These water-soluble fibers are

found in plant tissues, most commonly ripe fruits such as citrus fruits crabapples, plums, currants and apples. Researchers have found that raw apples are the richest fruit sources in pectin. The characteristics structure of pectin is a linear chain of a (β -1-4)-linked D-galacturonic acid that forms the protein-backbone, a homogalacturonan. Pectin and combinations of pectin with other colloids have been used extensively to treat diarrheal diseases, especially in infants and children. Although a bactericidal action of pectin has been proposed to explain the effectiveness of pectin treating diarrhea, most experimental results do not support this theory. However, some evidence suggests that under certain *in-vitro* conditions, pectin may have a light antimicrobial action toward *Echerichia coli*. Pectin reduces rate of digestion by immobilizing food components in the intestine, this result in less absorption of food. The thickness of the pectin layer influences the absorption by prohibiting contact between the intestinal enzyme and the food, thus reducing the latter's availability (Slany *et al.*, 1981 a & b). Due to its large water binding capacity, pectin gives a feeling of satiety, thus reducing food consumption. Experiments showed a prolongation of the gastric emptying half-time from 23 to 50 minutes of a meal fortified with pectin. These attributes of pectin are used in the treatment of disorders related to overeating. Pectin hydro gels have been used in tablet formulations as a binding agent and have been used in controlled-release matrix tablet formulations (Mukhiddinov *et al.*, 2000; Sriamornsak, 1999). Therefore the study was undertaken to reveal the pharmacological activities of pectin extracted from apples and oranges.

MATERIALS AND METHODS

All the chemicals and reagents used in the experiments will be procured from C.D.H and Ranchem. Glass wares used were from Borosil. The media and broth used for microbial culture will be procured from Hi-Media Ltd., Mumbai.

Collection of sample:

The apple and orange fruit samples were collected in sterilized conditions from local gardens/retail market in sterilized polythene bags and were stored at 4 °C in a refrigerator until use.

Extraction of Pectin:

The simple procedure for extraction of pectin was designed in order to compare the yield of pectin content in each of the varieties of apple fruit (Mathur *et al.*, 2011 b). About 40 g of each of the fruit samples were washed with N-saline. Then after the fruit samples were crushed and homogenized at full speed in a blender separately. The crushed and homogenized materials obtained were allowed to dry at 60 °C in hot air oven for about 2 h till the pectin extracted turns into powder. The pectin yield was then determined in both the fruit samples. The powdered pectin of each of the varieties was sterilized with N-saline and was further dried to obtain the sterilized pectin. The sterilized pectin was kept for further use in sterilized vials. The yield of pectin extracted was determined.

Identification tests of Pectin:

Pectin extracted was qualitatively determined by the following tests as mentioned in USP monographs.

(a) *Stiff gel Test*

1g of pectin was heated with 9ml of water on a water bath till a solution is formed, on cooling stiff gel formed was taken as positive sample.

(b) *Test with 95% Ethanol*

On adding an equal volume of ethanol (95%) to 1% w/v solution of pectin sample, a translucent, gelatinous precipitate produced (distinction from most gums) was taken as positive test.

(c) *Test with Potassium Hydroxide (KOH)*

To 5ml of a 1% w/v solution of pectin sample, 1ml of a 2% w/v solution of KOH was added and set aside for 15 minutes. A transparent semi gel will be produced. When the above gel is acidified with dilute HCl and shaken well, a voluminous, colorless gelatinous precipitate is formed. This upon boiling will become white and flocculent.

(d) *Iodine test:*

To 5ml of recently boiled and cooled 2% w/v solution of sample, 0.15 ml of Iodine solution was added. No blue color presence was taken as an indicator of positive test.

(d) Test for Acidity:

An aqueous solution of pectin sample was acidic to blue litmus paper.

Phytochemical Screening for pectin extracted:

The portion of the dry extracts was subjected to the phytochemical screening using the method adopted by Trease and Evans (1983) and Harborne (1983). Phytochemical screening was performed to test for alkaloids, saponin, tannins, flavanoids, steroids, sugars, cardiac glycosides and anthraquinones (Sofowora, 1993).

Test for alkaloids:

The 0.5 g of the extract was dissolved in 5 ml of 1% HCl and kept in water bath for about 2 minutes. 1ml of the filtrate was treated with Dragendroff's reagent. Turbidity or precipitation was taken as indicator for the presence of alkaloids.

Test for Tannins:

About 0.5 g of the sample was dissolved in 10 ml of boiling water and was filtered. Few ml of 6% FeCl₃ was added to the filtrate. Deep green colour appeared confirmed the presence of Tannins (Trease and Evans, 1983).

Test for Flavanoids:

About 0.2 gm of the extract was dissolved in methanol and heated for some time. A chip of Mg metal was introduced followed by the addition of few drops of conc. HCl. Appearance of red or orange color was taken as indicator of the flavanoids.

Test for Saponin:

About 0.5 g of the extract was stirred with water in the test tube. Frothing persists on warming was taken as a evidence for the presence of saponin.

Test for Steroids:

Salkowski's method was adopted for the detection of steroids. About 0.5 g of extract was

dissolved in 3 ml of chloroform and filtered. To the filtrate, conc. H₂SO₄ was added to form a lower layer. Reddish brown color was taken as positive for the presence of steroids ring (Agarwal *et al.*, 2011).

Test for Cardiac glycosides

About 0.5 g of the extract was dissolved in 2ml of glacial acetic acid containing 1 drop of 1% FeCl₃. This was under laid with conc. H₂SO₄. A brown ring obtained at the interphase indicates the presence of deoxy sugar. A violet ring appeared below the ring while in the acetic acid layer a greenish ring appeared just above ring and gradually spread throughout this layer.

Test for reducing Sugars:

1ml each of Fehling's solutions, I and II was added to 2 ml of the aqueous solution of the extract. The mixture was heated in a boiling water bath for about 2-5 minutes. The production of a brick red precipitate indicated the presence of reducing sugars.

Test for Anthraquinones:

5ml of the extract solution was hydrolyzed with dil/conc. H₂SO₄. 1 ml of dilute ammonia was then after be added to it. Rose pink colour confirmed the presence of anthraquinones.

Estimation of Total Phenolic Content (TPC) of Pectin:

The Total phenolic content of each pectin sample was determined by the method of Singleton and Rossi (1965). The phenolic content was expressed as mg/g gallic acid equivalents. In brief 100 µl aliquots of the sample were added to 2 ml of 0.2 % (w/v) Na₂CO₃ solution. After 2 minutes of the incubation. 100 µl of 500 ml/l Follin-Ciocalteu reagent was added and the mixture was allowed to stand for 30 minutes at 25 °C. The absorbance was measured at 750 nm using a UV-VIS Systronics spectrophotometer. The blank consist of all reagents and solvents but no sample. The Total Phenolic Content (TPC) was determined using the standard gallic acid calibration curve.

Purification and Characterization of pectin extracted of both apples and oranges via

chromatographic and spectroscopic techniques:

Separation of Pectin extracted by TLC:

TLC Separation was performed for the pectin extracted from apples and oranges. Standard pectin was also taken as reference. In TLC, detection system used was Butanol: Water: Acetic acid (5:4:1). Iodine chamber was also used for detection. Rf values were determined after the appearance of spots.

High-performance liquid chromatography (HPLC):

HPLC analysis of extracted pectin samples were performed in Roorkee Research and Analytical Laboratory Pvt. Ltd., Roorkee (Uttarakhand), India using a Shimadzo LC- 2010 HPLC system (Kyoto, Japan), equipped with a Shimadzo LC 2010 UV-VIS detector with a thermostated flow cell and a selectable two wavelengths of 190 - 370 nm or 371–600nm. The detector signal was recorded on a Shimadzo LC2010 integrator. The column used was a Chiral Column block heating-type Shim-pack VP-ODS (4.6 mm interior diameter × 150 mm long) with a particle size of 5 µm. Mobile phase was used containing 50 % acetonitrile along with 50 % Phosphate buffer was used at a flow rate of 3.0 ml/min, column temperature 25°C. Injection volume was 40 µl and detection was carried out at specific wavelength having maximum absorbance as calculated by UV absorption spectra at maximum wavelength.

Fourier Transform Infrared (FTIR) studies:

The IR spectrum of purified pectin extracted from apples and oranges was recorded in Roorkee Research and Analytical Laboratory Pvt. Ltd., Roorkee (Uttarakhand), India using a computerized FTIR spectrometer (Perkin Co., Germany) in the range of 4400–400 cm⁻¹ by the KBr pellet technique.

Determination of *in vitro* antimicrobial activity of pectin extracted from apples and oranges:

The pectin extracted from apples and oranges was dissolved in N-saline (1 mg/ml) and evaluated for *in vitro* antimicrobial activity.

Culture Media:

The media used for antibacterial test is Nutrient agar/broth and Sabouraud's dextrose agar/broth of Hi media Pvt. Bombay, India.

Inoculum:

The bacterial pathogen was inoculated into nutrient broth and incubated at 37 °C for 4 h and the suspension will be checked to provide approximately 10⁵ CFU/ml. Similar procedure was done for fungal strains by inoculating in Sabouraud's dextrose broth for 6 h.

Culture medium:

The pathogenic bacterial cultures were inoculated into Nutrient broth and incubated at 37 °C for 18 h and suspension was checked to provide approximately, 10⁵ CFU/ml. The same procedure was done for fungal pathogens and there strains will be inoculated into Sabouraud's dextrose broth but the fungal broth cultures were incubated at 48-72 h.

Microorganisms used :

Pure cultures of various pathogenic bacterial and fungal strains, *E. coli* NCIM 2065, *Lactobacillus plantarum* NCIM 2083, *Micrococcus luteus* ATCC 9341, *Salmonella abony* NCIM 2257, *Candida albicans* NCIM 3471, *Aspergillus niger* NCIM 1196 and Methicillin resistant strains of *Staphylococcus aureus* (MRSA) isolated from clinical specimens viz. pus and blood of infected patients were procured with authentication for the study. The standard bacterial and fungal cultures used for the study were procured from Roorkee Research & Analytical Labs Pvt. Ltd., Roorkee (U.K), India and MRSA strains were procured from Shooloni University, H.P., India.

Determination of diameter of zone of inhibition by well diffusion method:

The agar well diffusion method (Perez *et al.*, 1993) was modified. Nutrient agar medium (NAM) was used for growth of pathogenic bacteria cultures. The culture medium was inoculated with the bacterial pathogen separately suspended in nutrient broth. Sabouraud's dextrose agar/broth was used for growth of pathogenic fungal cultures. The culture medium was inoculated with the fungus separately

suspended in Sabouraud's dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with separate endophytic fractions and solvent blanks. Standard antibiotic (Erythromycin, 1 mg/ml) was simultaneously used as the positive control. The plates were incubated at 37 °C for 18 h. The antibacterial activity was evaluated by measuring the diameter of zone of inhibition observed. For assaying, antifungal activity of endophytic fractions, Sabouraud's dextrose agar/ broth medium plates was used. The same procedure as that for determination of antibacterial property was adopted and then after the diameter of zone of inhibition was observed after 48-72 h. Fucanazole (1mg/ml) was used as standard for determination of antifungal activity. The procedure for assaying antibacterial and antifungal activity was performed in triplicates to confirm the average readings of diameter of zone of inhibition observed for each of the test organism.

Determination of MIC and MLC :

The broth dilution method (Vollekova *et al.*, 2001 and Usman *et al.*, 2007) was adopted for determination of MIC and MLC values against the pathogens. The pectin extracts (1 mg/ml) were serially diluted in different aliquots and the final volumes of the aliquots were made up to 1 ml with N-saline (0.85 % NaCl). Equal amount of the specific pathogen was added in different aliquots and the test tubes were kept for 48 h at 30 °C. The minimum dilution of the pectin extract that kills the bacterial and fungal growth was taken as MLC (Minimum lethal count) while the minimum dilution that inhibits the growth of the organism was taken as MIC.

Determination of *in vitro* antioxidant activity of pectin extracted and enzyme purified:

Determination of Total Antioxidant Activity:

Total antioxidant activities of pectin fractions and ascorbic acid were determined by the method of Pan *et al.*, 2008. An aliquot (0.1M) of these fractions was combined with 1ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were then capped and

incubated at 95 °C for 90 minutes. After that the samples were cooled at 25°C, the absorbance was measured at 695 nm against blank. The blank contained 1ml of reagent solution without sample. The total antioxidant activity was expressed as an absorbance value at 695 nm. Higher absorbance value indicates the maximum antioxidant activity.

Determination of *in vitro* anti-inflammatory potential of pectin extracted of both apples and oranges:

(A) *The human red blood cell (HRBC) membrane stabilization method:*

The method as prescribed (Gopalkrishnan *et al.*, 2009; Sakat *et al.*, 2010) was adopted with some modifications. The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid and 0.42 % NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10 % suspension will be made. Pectin extracts were prepared (in appropriate concentrations) using distilled water and to each concentrations, 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of HRBC suspension were added. It was then incubated at 37°C for 30 minutes and centrifuged at 3,000 rpm for 20 minutes and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (100 µg/ml) was used as reference standard and a control was prepared by omitting the extracts. The experiments were performed in triplicates and mean values of the three will be considered. The percentage (%) of HRBC membrane stabilization or protection was calculated using the following formula:

Percent Protection (%) = (100- OD of drug treated sample/OD of Control) X 100

(B) *Inhibition of Albumen Denaturation:*

Method as prescribed (Sakat *et al.*, 2010) was followed with minor modifications. The reaction mixture will be consisting of test extracts and 1% aqueous solution of bovine albumin fraction,

pH of the reaction mixture was adjusted using small amount at 37°C HCl. The sample extracts were incubated at 37°C for 20 minutes and then heated to 51°C for 20 minutes, after cooling the samples the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was taken as a standard drug. The experiment was performed in triplicates. Percent inhibition of protein denaturation was calculated as follows:

Percent inhibition (%) = (OD of Control-OD of Sample/OD of Control) X100

(C) Heat induced hemolysis:

The reaction mixture (2 ml) consisted of 1 ml of test sample solution and 1 ml of 10 % RBCs suspension, instead of test sample only saline was added to the control test tube. Diclofenac sodium was taken as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56°C for 30 minutes. At the end of the incubation the tubes were cooled under running tap water. The reaction mixtures were then centrifuged at 2500 rpm for 5 minutes and the absorbance of the supernatants were taken at 560 nm. The experiment was performed in triplicates for all the test samples. Percent hemolysis was calculated by the formula mentioned in the above procedure.

RESULTS

Table 2: Qualitative tests of pectin extracted from apples and oranges

S.No.	Fruit pectin	Observation	
1.	Apple pectin	Stiff gel Test	Translucent, gelatinous precipitate
		Test with 95% Ethanol	Colorless gelatinous precipitate which on heating gives white and flocculent precipitate
		Test with Potassium Hydroxide (KOH)	Colorless gelatinous precipitate is formed. This upon boiling will became white and flocculent.
		Iodine test	Absence of blue colour
	Test for Acidity	Acidic pH (4.5)	
2.	Orange pectin	Stiff gel Test	Translucent, gelatinous precipitate
		Test with 95% Ethanol	Colorless gelatinous precipitate which on heating gives white and flocculent precipitate
		Test with Potassium Hydroxide (KOH)	Colorless gelatinous precipitate is formed. This upon boiling will became white and flocculent.
		Iodine test	Absence of blue colour
	Test for Acidity	Acidic pH (4.5)	

In the present investigation the pectin extracted from apples and oranges procured from local market were used for evaluation of antimicrobial, antioxidant and anti-inflammatory properties.

Yield of Pectin extracted:

It was found that the yield of pectin content extracted was predominant in apples (20.60 %) in comparison to that of oranges (10.56%). The results are illustrated in Table 1.

Table 1.1: Percent Pectin yield of pectin extracted from apples and oranges

S.No.	Fruit pectin	Pectin yield (%)
1.	Apple pectin	20.60
2.	Orange pectin	10.56

Identification tests of pectin extracted:

The pectin samples extracted from apples and oranges were qualitatively evaluated for stiff gel formation, treated with ethanol (95%) to form translucent, gelatinous precipitate. Treated with Potassium Hydroxide (KOH) and HCl to form voluminous, colorless gelatinous precipitate which on heating gives white and flocculent precipitate. Treated with Iodine, absence of blue colour gives positive test. Pectin extracted samples converts blue litmus paper to red, showed pH 4.5 as observed by pH meter. The results are shown in Table 2.

Table-3: Phytochemical screening of the pectin extracts

Type of pectin	Phytoconstituents							
	Alkaloids	Tannins	Reducing Sugar	Flavanoids	Saponin	Steroids	Cardiac Glycosides	Anthraquinones
Orange pectin	+	-	+	+	-	+	+	-
Apple pectin	+	-	+	-	-	+	+	+

Phytochemical Screening for pectin extracted:

The portion of the dry extract of pectin obtained was subjected to phytochemical screening for qualitative examination of alkaloids, tannins, flavanoids, steroids, saponin, cardiac glycosides, reducing sugar and anthraquinones. The results confirmed that alkaloids, flavanoids, reducing sugars, steroids and cardiac glycosides were present in orange pectin while alkaloids, reducing sugars, steroids, cardiac glycosides and anthraquinones were present in apple pectin. Tannins, saponin and anthraquinones were absent in orange pectin while tannins, flavanoids and saponin were absent in apple pectin (Table-3).

Purification and Characterization of pectin extracted of both apples and oranges via chromatographic and spectroscopic techniques:**TLC profile of extracted pectin samples:**

The extracted pectin samples obtained from apples and oranges showed brown coloured spots on TLC as observed in Iodine chamber. The R_f values of the pectin samples were almost similar viz. Apple pectin (0.62), Orange pectin (0.62) and Standard pectin (procured from Ranchem), 0.63. The results are shown in Table 4 and Figure-1.

Table-4: TLC profile of pectin extracted from apples and orange

S.No.	Pectin	R _f values
1.	Apple pectin	0.62
2.	Citrus pectin	0.62
3.	Standard pectin	0.63

Figure 1. TLC profile of extracted pectin with reference to that of standard pectin**Purification of extracted pectin samples via HPLC:**

The apple pectin and orange pectin showed almost similar retention time viz. 1.821 minutes and 1.757 minutes specifically on HPLC chromatogram. The chromatograms are shown in Figure-2 (a & b).

Characterization of extracted pectin samples via FT-IR spectra:

The FT-IR spectra of extracted pectin samples were determined using a computerized FTIR spectrometer (Perkin Co., Germany) in the range of 4400–400 cm⁻¹ by the KBr pellet technique. Two outstanding features of the infrared spectra of pectinate are bands at 1745-1743 and at 1607-1604 cm⁻¹. One other feature which should be mentioned is a band, beyond the afore mentioned

Figure-2 (a): HPLC chromatogram of apple pectin

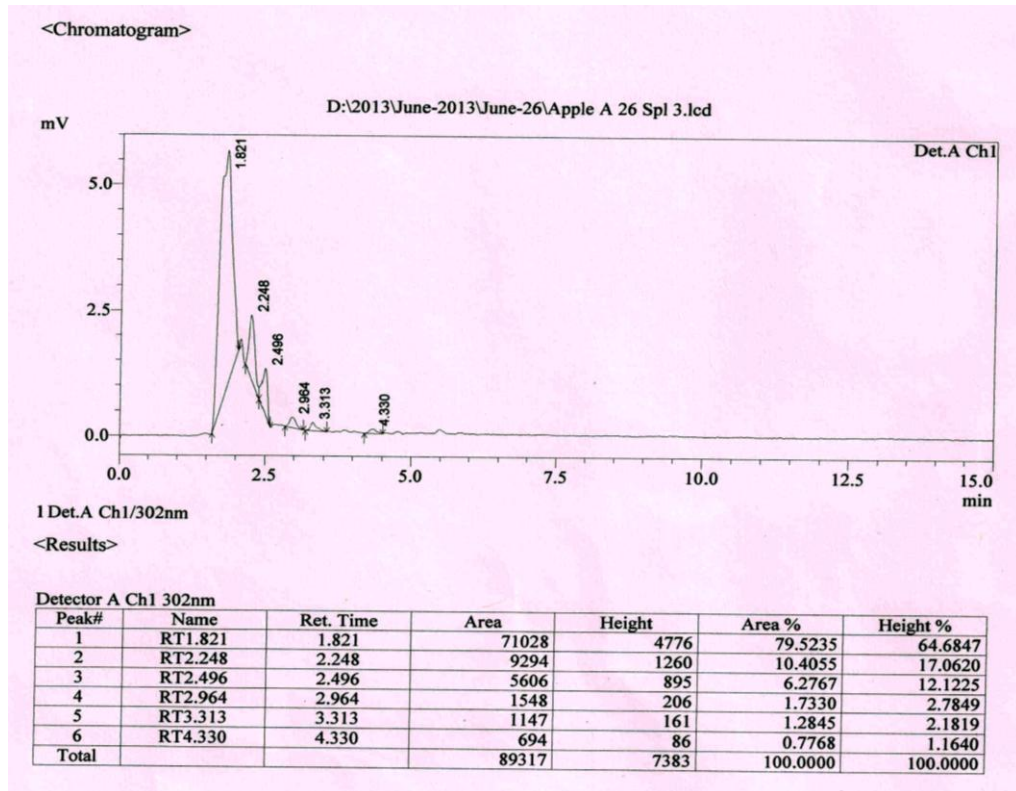


Figure-2 (b): HPLC chromatogram of orange pectin

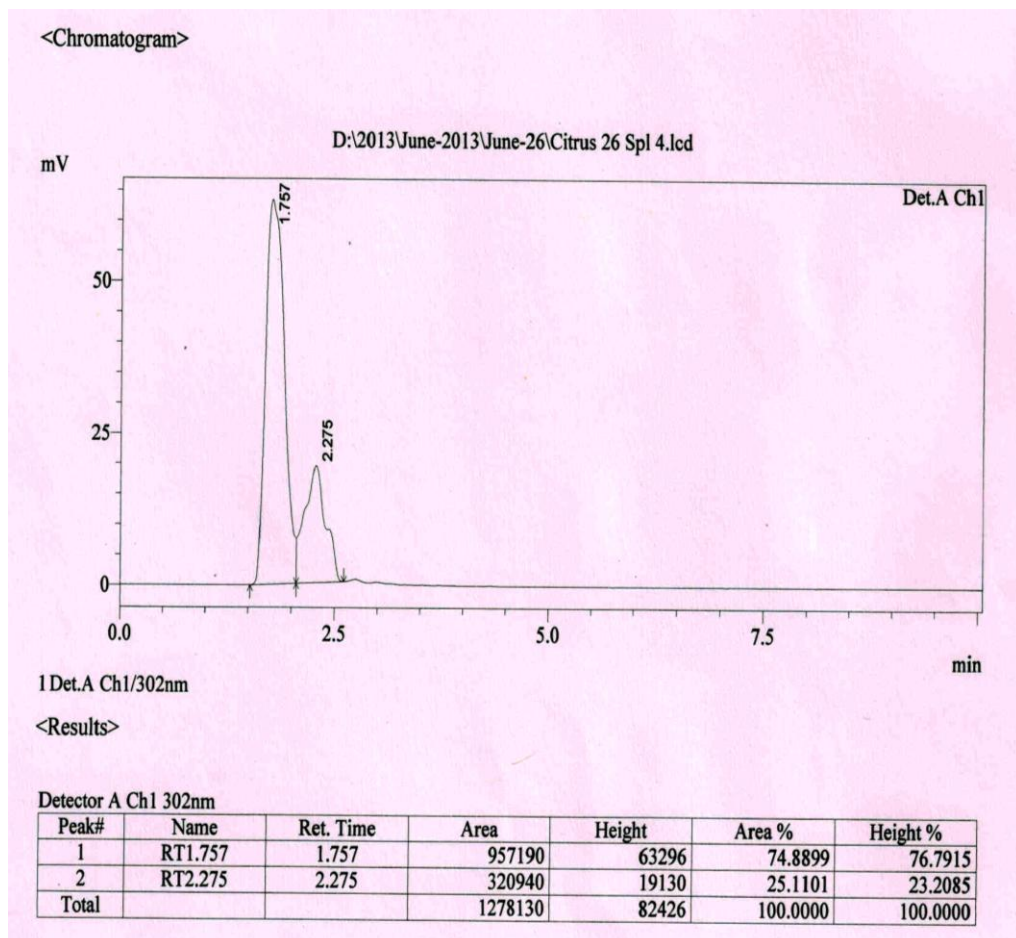


Figure-3 (a): FT-IR spectra of apple pectin

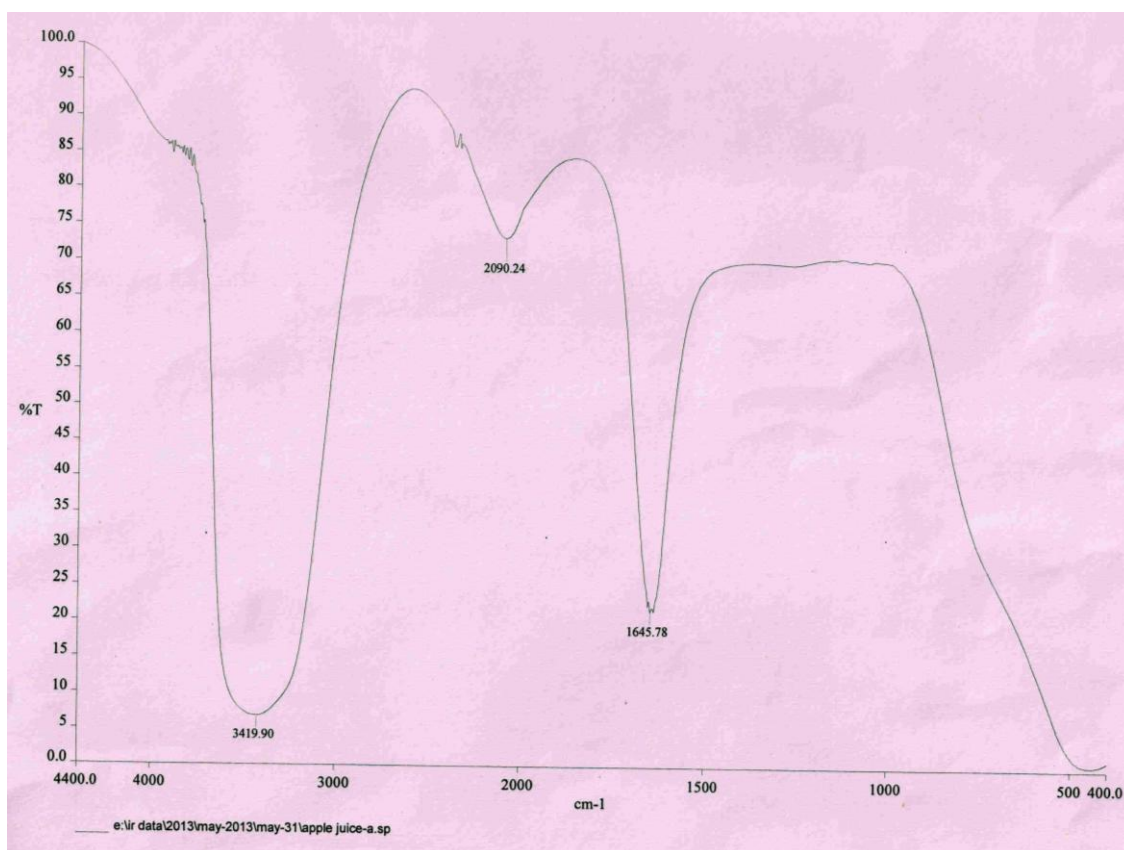


Figure-3 (b): FT-IR spectra of orange pectin

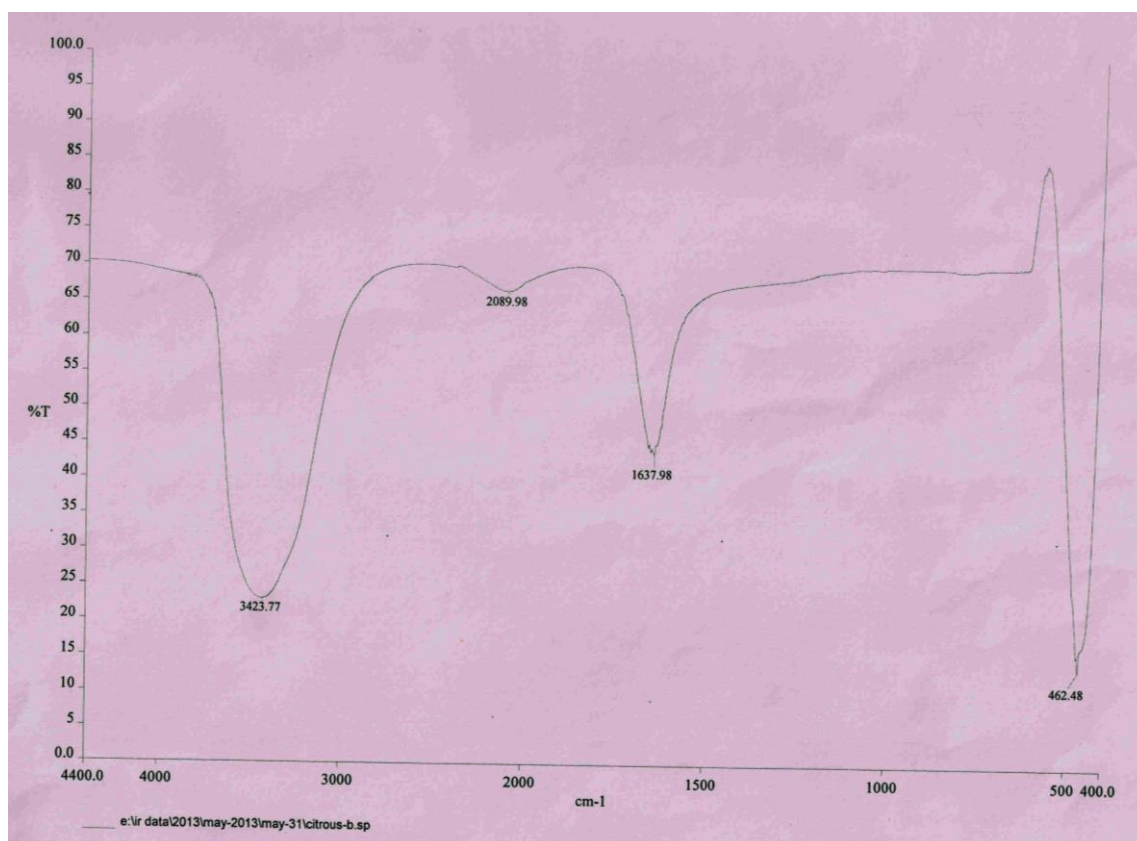


Table-5 (a): Antibacterial activity of pectin extracts

Pectin/positive control	Zone of Inhibition (mm)				MRSA (Isolated from pus)	MRSA (Isolated from Blood)
	<i>Micrococcus</i>	<i>E. coli</i>	<i>S. abony</i>	<i>L. plantarum</i>		
Apple pectin (1g/ml)	NA	17.0	NA	NA	NA	15.0
Citrus Pectin (1g/ml)	NA	15.0	NA	NA	NA	NA
Std. Pectin (1g/ml)	NA	NA	NA	NA	NA	15.0
Erythromycin (1 mg/ml)	20.0	27.0	21.0	15.0	18.0	21.0

range, appearing at 1418-1417 cm^{-1} . In the spectra of polygalacturonate, the absence of carbonyl stretching bands above 1680 cm^{-1} demonstrates that the polygalacturonate is de-esterified to a degree of <2%. The bands at X07-1604 and 1417 cm^{-1} correspond to the antisymmetric and symmetric stretching vibrations of the carboxylate groups. The spectra are shown in Figures 3 (a & b).

Antimicrobial activity and MIC values of pectin extracts:

The results showed that apple and orange pectin possessed antimicrobial potential against the pathogens causing nosocomial infections. The extracts were also found to have antimicrobial potential against the Methicillin resistant *Staphylococcus aureus*. It was found that apple pectin had potent antimicrobial activity against *E.coli*, Methicillin resistant strain of *S. aureus* (isolated from blood) and *Aspergillus niger* while orange pectin was found to have antimicrobial potential against *E.coli* and *Aspergillus niger*. The results are shown in Table-5 (a & b) and Figure-4.

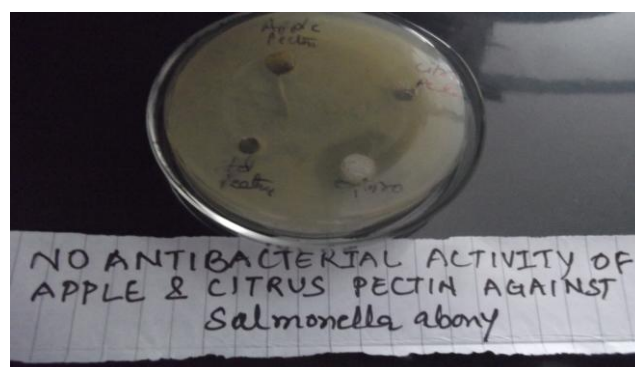
Apple pectin extracts were found to have potent antimicrobial activity in comparison to that of orange pectin. Apple pectin extracts showed least MIC values viz. 10^{-4} $\mu\text{g/ml}$ against

Aspergillus niger, 10^{-5} $\mu\text{g/ml}$ against *E.coli* and Methicillin resistant *S. aureus* (isolated from blood). Orange pectin extracts showed similar MIC values viz. 10^{-5} $\mu\text{g/ml}$ against *E.coli* and *Aspergillus niger*. The results of MIC are shown in Table-6.

Table-5 (b): Antifungal activity of pectin extracts

Pectin/positive control	Zone of Inhibition (mm)	
	<i>Aspergillus niger</i>	<i>Candida albicans</i>
Apple pectin (1g/ml)	26.0	NA
Citrus Pectin (1g/ml)	27.0	NA
Std. Pectin (1g/ml)	27.0	NA
Flucanazole (1mg/ml)	30.0	11.0

Figure-4: Antimicrobial activity of pectin extracts



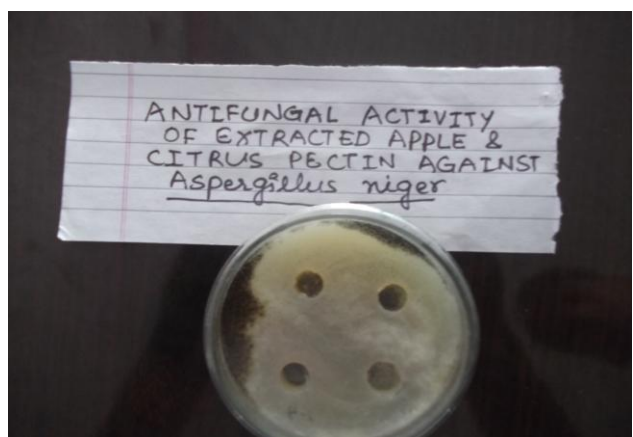


Table-6: MIC and MLC values of pectin extracts

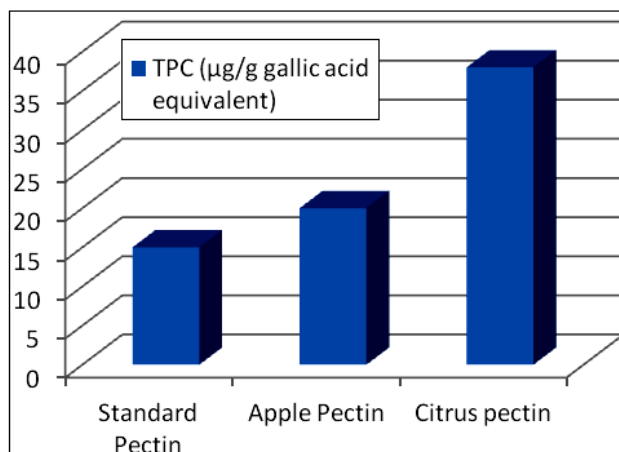
Pectin sample	MIC and MLC values	Against the pathogen
Apple Pectin	MIC = 10^{-5} $\mu\text{g/ml}$ MLC = 10^{-4} $\mu\text{g/ml}$	Against <i>E. coli</i>
Apple Pectin	MIC = 0.1 $\mu\text{g/ml}$ MLC = 10 $\mu\text{g/ml}$	Against MRSA (isolated from blood)
Apple Pectin	MIC = 10^{-4} $\mu\text{g/ml}$ MLC = 0.001 $\mu\text{g/ml}$	Against <i>A. niger</i>

Standard Pectin	MIC = 10^{-5} $\mu\text{g/ml}$ MLC = 10^{-4} $\mu\text{g/ml}$	Against MRSA (isolated from blood)
Citrus Pectin	MIC = 10^{-5} $\mu\text{g/ml}$ MLC = 10^{-4} $\mu\text{g/ml}$	Against <i>E. coli</i>
Citrus Pectin	MIC = 10^{-5} $\mu\text{g/ml}$ MLC = 10^{-4} $\mu\text{g/ml}$	Against <i>A. niger</i>

Antioxidant activity of pectin extracts:

Amongst both the pectin extracts, TPC content of orange pectin was found to be much more (38 $\mu\text{g/ml}$) in comparison to that of apple pectin (20 $\mu\text{g/ml}$). The results of TPC correlate the findings of Total antioxidant activity determination assay (based on absorbance) which also illustrates that orange pectin had potent antioxidant activity in comparison to apple pectin. The results of TPC are shown in Figure-5.

Figure-5: TPC ($\mu\text{g/g}$ gallic acid equivalents) of pectin extracts



Results of total antioxidant activity revealing maximum absorbance; maximum antioxidant activity of orange pectin ($A_{695-0.05}$) are shown in Figure-6.

Anti-inflammatory activity of pectin extracts:

(A) The human red blood cell (HRBC) membrane stabilization method:

Amongst pectin extracts, orange pectin (1g/ml) showed 88 ± 0.06 % protection of HRBC in hypotonic solution in comparison to apple pectin extracts (66 ± 0.06 %). The results were compared with standard Diclofenac sodium

which showed 90.54 ± 0.06 % protections at 120 mg/ml. The results are shown in **Table-7**.

Figure-6: Total antioxidant activity (A_{695}) of pectin extracts

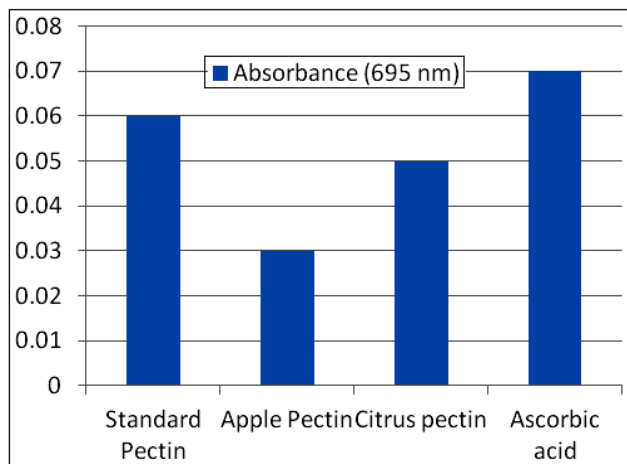


Table-7: Percent protection of HRBC membrane/membrane stabilization of pectin extracts and positive control

Pectin sample	Percent protection of HRBC membrane
Standard Pectin	56 ± 0.06
Apple Pectin	66 ± 0.06
Citrus pectin	88 ± 0.06
Diclofenac sodium (positive control)	90.54 ± 0.06

(B) Inhibition of Albumen Denaturation:

Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti inflammation activity, ability of extract on protein denaturation was studied. The orange pectin extracts were found to be effective in inhibiting heat induced albumin denaturation in comparison to apple pectin extracts at a dose of 1g/ml. Maximum percent inhibition, 68.05 ± 0.06 was observed from orange pectin extracts followed by apple pectin extracts (66.03 ± 0.06). The results were compared with standard Diclofenac Sodium which showed 93.54 ± 0.06 % inhibitions in albumen denaturation at 120 mg/ml. The results are reported in **Table-8**. Since during inflammation condition, protein of the cell gets denatured, thus here albumen protein is

used as a model whose protection in denaturation by pectin extracts was studied.

Table-8: Percent protection/Inhibition of Albumen Denaturation of pectin extracts and positive control

Pectin sample	Percent Inhibition of Albumen Denaturation
Standard Pectin	50 ± 0.06
Apple Pectin	66.03 ± 0.06
Citrus pectin	68.05 ± 0.06
Diclofenac sodium (positive control)	93.54 ± 0.06

(C) Heat induced hemolysis:

Stabilization of RBCs membrane was studied for further establishes the mechanism of anti-inflammatory action of different pectin extracts. The maximum inhibition was recorded 78.82 ± 0.06 % from citrus pectin extracts followed by apple pectin extracts (76.65 ± 0.05 %). The results were compared with standard Diclofenac Sodium which showed the maximum inhibition 85.92 ± 0.05 % at 120 mg/ml. The results are reported in **Table-9**. Heat induced hemolysis method is another method depicting HRBC membrane stabilization.

Table-9. Percent protection in heat induced hemolysis of HRBC membrane by pectin extracts and positive control

Pectin sample	Percent protection in heat induced hemolysis of HRBC membrane
Standard Pectin	56 ± 0.06
Apple Pectin	76.65 ± 0.05
Citrus pectin	78.82 ± 0.06
Diclofenac sodium (positive control)	85.92 ± 0.06

DISCUSSION AND CONCLUSION

Apples and citrus fruits rich in Vitamin C are rich sources of pectin. The pectin contains various active constituents. In the present study, the antimicrobial, antioxidant activity and anti-inflammatory activity of pectin has been

investigated. It was found that the yield of pectin content extracted was predominant in apples (20.60 %) in comparison to that of oranges (10.56%). The pectin samples extracted from apples and oranges were qualitatively evaluated for stiff gel formation, treated with ethanol (95%) to form translucent, gelatinous precipitate. Treated with Potassium Hydroxide (KOH) and HCl to form voluminous, colorless gelatinous precipitate which on heating gives white and flocculent precipitate. Treated with iodine, absence of blue colour gives positive test. Pectin extracted samples converts blue litmus paper to red, showed pH 4.5 as observed by pH meter. The portion of the dry extracts of pectin obtained was subjected to phytochemical screening for qualitative examination of alkaloids, tannins, flavanoids, steroids, saponin, cardiac glycosides, reducing sugar and anthraquinones.

The results confirmed that alkaloids, flavanoids, reducing sugars, steroids and cardiac glycosides were present in orange pectin while alkaloids, reducing sugars, steroids, cardiac glycosides and anthraquinones were present in apple pectin. Tannins, saponin and anthraquinones were absent in orange pectin while tannins, flavanoids and saponin were absent in apple pectin. The extracted pectin samples obtained from apples and oranges showed brown colored spots on TLC as observed in Iodine chamber. The Rf values of the pectin samples were almost similar viz. Apple pectin (0.62), Orange pectin (0.62) and standard pectin which showed 0.63. The apple pectin and orange pectin showed almost similar retention time viz. 1.821 minutes and 1.757 minutes specifically on HPLC chromatogram.

The FT-IR spectra of extracted pectin samples were determined using a computerized FTIR spectrometer in the range of 4400–400 cm^{-1} by the KBr pellet technique. Two outstanding features of the infrared spectra of pectinate are bands at 1745-1743 and at 1607-1604 cm^{-1} . One other feature which should be mentioned is a band, beyond the afore mentioned range, appearing at 1418-1417 cm^{-1} . In the spectra of polygalacturonate, the absence of carbonyl stretching bands above 1680 cm^{-1} demonstrates

that the polygalacturonate is de-esterified to a degree of <2%. The bands at X07-1604 and 1417 cm^{-1} correspond to the anti-symmetric and symmetric stretching vibrations of the carboxylate groups. The results showed that apple and orange pectin possessed antimicrobial potential against the pathogens causing nosocomial infections. The extracts were also found to have antimicrobial potential against the Methicillin resistant *Staphylococcus aureus*. It was found that apple pectin had potent antimicrobial activity against *E.coli*, Methicillin resistant strain of *S. aureus* (isolated from blood) and *Aspergillus niger* while orange pectin was found to have antimicrobial potential against *E.coli* and *Aspergillus niger*. Apple pectin extracts were found to have potent antimicrobial activity in comparison to that of orange pectin. Apple pectin extracts showed least MIC values viz. 10^{-4} $\mu\text{g/ml}$ against *Aspergillus niger*, 10^{-5} $\mu\text{g/ml}$ against *E.coli* and Methicillin resistant *S. aureus* (isolated from blood). Orange pectin extracts showed similar MIC values viz. 10^{-5} $\mu\text{g/ml}$ against *E.coli* and *Aspergillus niger*. Amongst both the pectin extracts, TPC content of orange pectin was found to be much more (38 $\mu\text{g/ml}$) in comparison to that of apple pectin (20 $\mu\text{g/ml}$). The results of TPC correlate the findings of Total antioxidant activity determination assay (based on absorbance) which also illustrates that orange pectin had potent antioxidant activity in comparison to apple pectin. Results of total antioxidant activity revealing maximum absorbance; showed maximum antioxidant activity of orange pectin (A_{695} -0.05) and correlates the previous findings. The results of antimicrobial and antioxidant activity correlates the previous findings (Mathur *et al.*, 2011 a & b).

Anti-inflammatory activity of pectin extracts was determined by human red blood cell (HRBC) membrane stabilization method, Inhibition of Albumen Denaturation and Heat induced hemolysis. Amongst pectin extracts, orange pectin (1g/ml) showed 88 ± 0.06 % protection of HRBC in hypotonic solution in comparison to apple pectin extracts (66 ± 0.06 %). The results were compared with standard Diclofenac sodium which showed 90.54 ± 0.06 %

protections at 120 mg/ml. Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti inflammation activity, ability of extract on protein denaturation was studied. The orange pectin extracts were found to be effective in inhibiting heat induced albumin denaturation in comparison to apple pectin extracts at a dose of 1g/ml. Maximum percent inhibition, 68.05 ± 0.06 was observed from orange pectin extracts followed by apple pectin extracts (66.03 ± 0.06). The results were compared with standard Diclofenac Sodium which showed 93.54 ± 0.06 % inhibitions in albumen denaturation at 120 mg/ml. Since during inflammation condition, protein of the cell gets denatured, thus here albumen protein is used as a model whose protection in denaturation by pectin extracts was studied. Stabilization of RBCs membrane was studied for further establishes the mechanism of anti-inflammatory action of different pectin extracts.

The maximum inhibition was recorded 78.82 ± 0.06 % from citrus pectin extracts followed by apple pectin extracts (76.65 ± 0.05 %). The results were compared with standard Diclofenac Sodium which showed the maximum inhibition 85.92 ± 0.05 % at 120 mg/ml. Heat induced hemolysis method is another method depicting HRBC membrane stabilization. Pectin extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release. Some of the NSAIDs are known to possess membrane stabilization properties which may contribute to the potency of their anti-inflammatory effect. Though the exact mechanism of the membrane stabilization by the extract is not known yet; hypotonicity-induced hemolysis may arise from

shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components.

The extract may inhibit the processes, which may stimulate or enhance the efflux of these intracellular components (Vane *et al.*, 1994). Orange pectin extracts in comparison to apple pectin extracts were effectively inhibiting the heat induced hemolysis. These results provide evidence for membrane stabilization as an additional mechanism of their anti-inflammatory effect. This effect may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. The extracts inhibited the heat induced hemolysis of RBCs to varying degree. Thus, the results thus conclude that apples and citrus fruits are best for consumption and the pectin extracted from such fruits can be utilized to formulate a potent antibiotic, potent antioxidant and anti-inflammatory agents. However further studies are needed to refine the technique.

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