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Pectin content as an index for screening different varieties of apple (*Pyrus Malus* L.) of Kashmir (J&K) on the basis of antimicrobial activity

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ABSTRACT

In the present investigation, pectin, the polysaccharide content in fruits is used as a basis for screening different varieties of Apple (Pyrus malus L.) of the same season of Kashmir (J&K). Different varieties of apple fruit of Kashmir (J&K) of the same season viz American, Delicious and Maharaj-ji were collected from the local gardens of Kashmir and pectin content present was extracted. The yield of pectin content was found to be maximum in Maharaj-ji (20.60 %) followed by Delicious (14.40 %) and American (11.60 %). The pectin extracted was then evaluated for its in vitro antibacterial activity against different pathogenic bacterial cultures. The results investigated that pectin extracted from Delicious variety showed potent antibacterial activity against Klebsiella pneumoniae (MIC value: 0.8 mg/ml) followed by Streptococcus pyogenes (MIC value: 0.3 mg/ml), E.coli (MIC value: 0.7 mg/ml) and Lactococcus sp. (MIC value: 0.7 mg/ml). The pectin extracted from other varieties showed no potency against any of the bacterial cultures. Further the pectin extracted from each of the variety was evaluated for in vitro antifungal activity against Aspergillus niger, Candida albicans and Saccharomyces cerevisae. It was observed that pectin extracted from any of the variety showed no potency against any of the test fungal cultures. The results of the antimicrobial activity of the pectin extracted from each of the varieties were compared with that of standard pectin and Azithromycin. It was observed that standard pectin also not showed antifungal activity similarly to that of extracted pectin. The results thus confirmed that pectin can be utilized as a potent antibacterial agent and can be utilized as an index for screening different apple varieties of Kashmir (J & K). Furthermore our study validates the use of Delicious variety for the treatment of the infections borne by the tested organisms. The pectin extracted was further assayed for cellular toxicity against sheep fresh blood erythrocytes but no hemolysis against sheep fresh blood erythrocytes was observed.

Keywords: *Pyrus malus* L., antimicrobial activity, pectin content, Delicious American, Maharaj-ji.

INTRODUCTION

Pectin is a complex mixture of polysaccharides that makes up about one third of the cell wall dry substance of higher plants. Much smaller proportions of these substances are found in the cell walls of grasses. The highest concentrations of pectin are found in the middle lamella of cell wall, with a gradual decrease as one passes through the primary wall toward the plasma

membrane [1]. At present, commercial pectins are almost exclusively derived from citrus peel or apple pomace, both by-products from juice (or cider) manufacturing. Apple pomace contains 10-15% of pectin on a dry matter basis. Citrus peel contains of 20-30% [2]. From an application point of view, citrus and apple pectins are largely equivalent. Citrus pectins are light cream or light tan in colour; apple pectins are often darker. The structure of pectin is very difficult to determine because pectin can change during isolation from plants, storage, and processing of plant material [3]. Pectin has applications in the pharmaceutical industry. Pectin favaorably influences cholesterol levels in blood. It has been reported to help reduce blood cholesterol in a wide variety of subjects and experimental conditions as comprehensively reviewed [4]. Pectin acts as a natural prophylactic substance against poisoning with toxic cations. It has been shown to be effective in removing lead and mercury from the gastrointestinal tract and respiratory organs [5]. When injected intravenously, pectin shortens the coagulation time of drawn blood, thus being useful in controlling hemorrhage or local bleeding [6]. 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* [7]. Antioxidant activity of different varieties of apple (*Pyrus malus* L.) of Kashmir (J&K) was determined on the basis of PPO activity and *in vitro* antioxidant activity [8].

EXPERIMENTAL SECTION

All the chemicals and reagents used were from C.D.H and Ranchem. Glass wares used were from Borosil. The media and broth used for microbial culture were from Hi-Media Pvt. Limited, Bombay, India.

Collection of sample

The apple fruit samples of different varieties viz. Delicious, American and Maharaj-ji belonging to the same season were collected from local gardens of Kashmir (J&K) and were stored at 4^0 C in a refrigerator.

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. About 40 g of each of the fruit samples of different varieties were washed with N-saline. Then after the fruit samples of each of the varieties were crushed and homogenized at full speed in a blender separately. The crushed and homogenized materials obtained were then allowed to dry at 60^0 C in hot air oven for about 2 h till the pectin extracted turns into powder. The pectin yield was determined in each of the varieties of apple fruit. The powdered pectin of each of the varieties was then sterilized with N-saline and was further dried to obtain the sterilized pectin. The sterilized pectin was kept for further use in sterilized vials.

Determination of *in vitro* antimicrobial activity

The pectin extracted from each of the varieties of apple was dissolved in N-saline(1 mg/ml) and was used for determination of *in vitro* antimicrobial activity.

Culture Media

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

Inoculum

The bacteria were inoculated into nutrient broth and incubated at 37^0 C for 4 h and the suspension were checked to provide approximately 10^5 CFU/ml. Similar procedure is done for fungal strains by inoculating in Sabouraud's dextrose broth for 6 h.

Microorganisms used

The test organisms *Klebsiella pneumoniae***,** *Streptococcus pyogenes***,** *E. coli***,** *Lactococcus* sp. were used as bacterial strains while *Candida albicans, Aspergillus niger* and *Saccharomyces cerevisae* were used as fungal strains**.** The organisms were procured from National Chemical Laboratory (NCL), Pune, India.

Agar well diffusion method

The agar well diffusion method [9] was modified. Nutrient Agar medium (NAM) was used for bacterial cultures. The culture medium was inoculated with the bacterial strains separately suspended in nutrient broth. Sabouraud's dextrose agar (SDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Sabouraud's dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with pectin extract (1 mg/ml) of each of the apple variety prepared in N-saline and solvent blank (N-saline) separately. Standard antibiotic (Azithromycin, concentration 1mg/ml) was simultaneously used as positive control. The bacterial plates were then incubated at 37^oC for 18 h. The antibacterial activity was evaluated by measuring the diameter of zone of inhibition observed. The same procedure was used for determining antifungal activity but in this case standard antibiotic (Fucanazole, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 37^oC for 72 h. Here also the diameter of zone of inhibition observed was measured.

Determination of MIC and MLC

The broth dilution method 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 were added in different aliquots and the test tubes were kept for 48 h at 30^0 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 of pectin extract that inhibits the growth of the organism was taken as MIC.

Determination of cellular toxicity using sheep erythrocytes

The method [10] was employed to study cellular toxicity. Briefly 10 fold serial dilution of the pectin extract (s) were made in phosphate buffered saline. A total volume of 0.8 ml for each dilution was placed in an ependroff tube. A negative control tube (containing saline only) and a positive control tube (containing tap water) were also included in the analysis. Fresh sheep erythrocytes were added to each tube, to give a final volume of 1 ml. Solutions were incubated at 37^0C for 30 minutes and all tubes were centrifuged for 5 minutes and then observed for hemolysis.

RESULTS AND DISCUSSION

In the present investigation the pectin extracted from different varieties of apple (*Pyrus malus* L.) from Kashmir (J&K) of same season was used as an index for screening different varieties on the basis of antimicrobial activity. It was found that the yield of pectin content extracted was predominant in Maharaj-ji (20.60 %) followed by Delicious (14.40 %) and American (11.60 %). The results are illustrated in **Table 1**; **Figure 1**.

Table 1: Percent Pectin yield in different varieties of apple (*Pyrus malus* L.) of Kashmir (J&K)

S.No.	Apple variety	Pectin yield (%)
1.	American	11.60
2.	Maharaj-ji	20.60
3.	Delicious	14.40

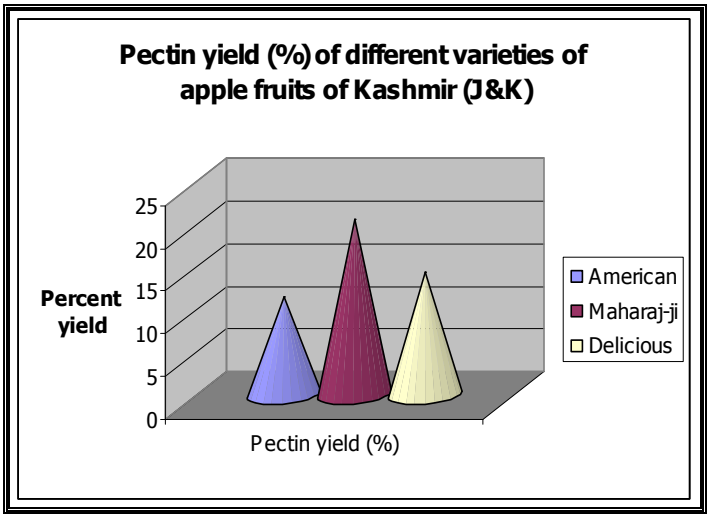

Figure 1: Percent Pectin yield in different varieties of apple (*Pyrus malus* L.) of Kashmir (J&K)

The pectin extracted was evaluated for its antimicrobial activity against the tested bacterial and fungal cultures. The results were found to be very surprising since the pectin extracted from Delicious variety only showed potent antibacterial activity while pectin extracted from other varieties showed no potency against any of the bacterial cultures. When we evaluated the antifungal activity of the pectin extracted from all the three varieties, the results were found to be negative. It means that pectin extracted from any of the variety don't possessed antifungal activity. The present investigation was performed in triplicates to assess the exact behavior of pectin towards the bacterial and fungal cultures but the results at each time were found to be similar. The results of antimicrobial activity of the test pectin extracted from each of the variety were then after compared with that of standard pectin and positive control, Azithromycin (1 mg/ml) and Fucanazole (1 mg/ml). It was found that the standard pectin showed almost similar potency to that of the pectin extracted from Delicious variety but showed no potency against any of the test fungal cultures. Positive control Azithromycin and Fucanazole were found to be potent against bacterial and fungal cultures respectively.

The Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC) of Delicious variety against the specific bacterial pathogenic culture were determined. The results of antimicrobial activity are illustrated in **Table 2 (a) & (b)**; **Figure 2 (a) & (b)**. The present investigation thus strongly emphasized the use of pectin from apples as an antimicrobial agent. Further studies are however needed to refine the method for extraction of pectin and to improvise the yield of extraction of pectin from apples. The results of the study strongly recommend the use of pectin as an antibiotic for the cure of infections caused by these pathogens. Furthermore effect of pectin on the growth of multiple drug resistant pathogens can be studied.

Table 2 (a): Antimicrobial activity of pectin extracted from different varieties of apple fruits from Kashmir (J&K) and Positive controls

S.No.	Apple variety	Diameter of zone of inhibition (mm)						
		ECO	KPN	SP	LAC	CA	AN	SC
1.	American	NA	NA	NA	NA	NA	NA	NA
2.	Maharaj-ji	NA	NA	NA	NA	NA	NA	NA
3.	Delicious	23.0	30.0	28.0	20.0	NA	NA	NA
4.	Standard Pectin	25.0	32.0	27.0	22.0	NA	NA	NA
5.	Azithromycin	38.0	35.0	39.0	35.0	NT	NT	NT
6.	Fucanazole	NT	NT	NT	NT	27.0	21.0	37.0

ECO, E.coli; KPN, K.pneumoniae; SP, S.pyogenes; LAC, Lactococcus sp.; CA, C.albicans; AN, A.niger; SC, S.cerevisae, NA, No activity; NT, Not tested

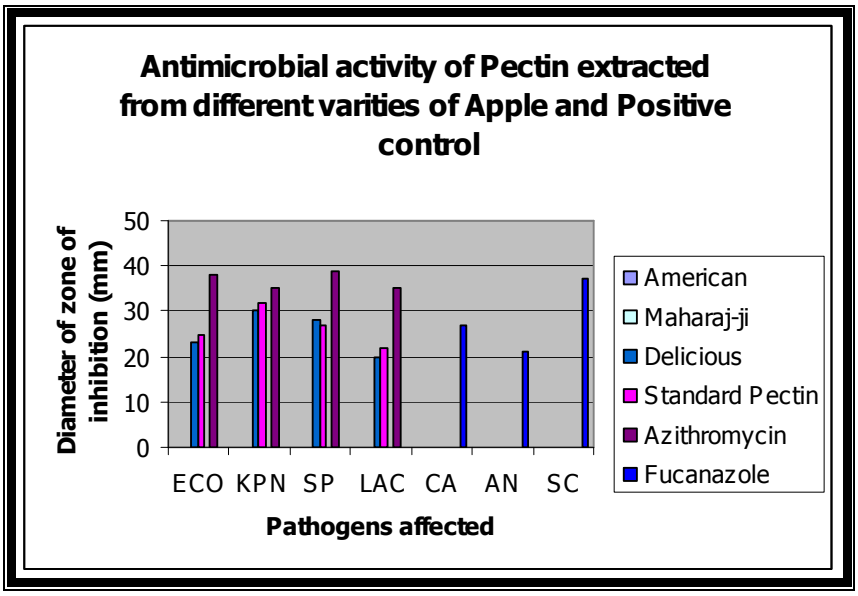

Figure 2: Antimicrobial activity of pectin extracted from different varieties of apple fruits from Kashmir (J&K) and Positive control

Table 2 (b): MIC and MLC values of pectin extracted from Delicious variety of apple from Kashmir (J&K)

Pathogens	Delicious variety of apple of Kashmir (J&K)	
	MIC (mg/ml)	MLC (mg/ml)
E.coli	0.7	0.9
Klebsiella pneumoniae	0.8	0.9
Streptococcus pyogenes	0.3	0.4
Lactococcus sp.	0.7	0.8

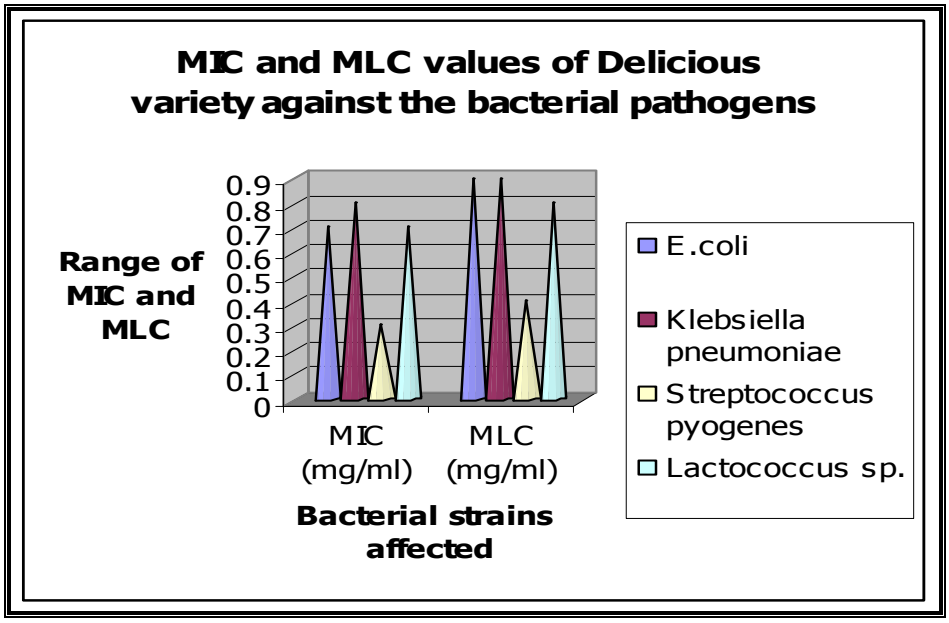

Figure 2 (b): MIC and MLC values of pectin extracted from Delicious variety of apple from Kashmir (J&K)

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