Full Length Research Paper

Effects of various extracts from *Pistacia eurycarpa* Yalt. on growth duration of *Mycobacterium tuberculosis*

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The aim of this study was to investigate the effects of various extracts obtained from *Pistacia eurycarpa* Yalt. on the exact time of appearance of the colonies *of Mycobacterium tuberculosis* on solid media. For this purpose, the fatty acid compositions of the fixed oils were analyzed using Gas Chromatography – Mass Spectrometry (GC-MS) in the fruit and peels. The fixed oils and the water-soluble extracts obtained from the plant as well as the fatty acids were added either separately or together on the surfaces of the Löwenstein Jensen and Middlebrook 7H11 agar media. Their effects on growth time of 40 strains of *M. tuberculosis* complex were investigated. Therefore, all of water-soluble extracts accelerated the growth of the mycobacteria in a statistically significant manner in both media. It was observed that the fixed oils and the fatty acids individually did not cause any significant effect on growth duration of the *M. tuberculosis* strains. Finally, the water-soluble extracts of *P. eurycarpa* fruit and its peel significantly accelerated the growth of *M. tuberculosis* by shortening the growth duration of *M. tuberculosis* at least by one third.

Key words: *M. tuberculosis*, *Pistacia eurycarpa*, gas chromatography – mass spectrometry, growth time.

INTRODUCTION

Tuberculosis control can be achieved if individuals with the disease receive adequate and timely treatment. Currently, the rapid diagnosis of tuberculosis has gained important public health significance due to the increasing incidence of tuberculosis and the advent of multidrug-resistant *Mycobacterium tuberculosis* strains.

In 2011, 1.4 million people died from TB, including almost one million deaths among HIV-negative individuals and 430 thousand among people who were HIV-positive (World Health Organization, 2012; Lawn and Zumla, 2011; Lönnroth et al., 2010). In order to prevent the spread of tuberculosis and start the specific treatment

immediately, rapid diagnosis is of a great importance. Presently *mycobacterial* culture method is the most accepted method in the diagnosis of tuberculosis. However, 3 to 6 weeks are required for the diagnosis by the conventional culture method. It is possible to reduce this period to 10 to 15 days using liquid culture systems (Cruciani et al., 2004; Winn et al., 2006). Diagnostic molecular microbiological methods which provide faster results with higher sensitivity and specificity are also used in the diagnosis of tuberculosis (Kaul, 2001). Unfortunately, there are various problems concerning these techniques (Lucke, 2011). Therefore, the

techniques which are more reliable, labor and cost effective, and easier to implement and providing faster results are needed.

Pistacia eurycarpa Yalt. is a member of Anacardiaceae family (Yaltirik, 1976; Kafkas, 2002). Various researches have shown that this plant, an endemic plant in Turkey, is rich in fat content and contains high levels of fatty acids (Demirci et al., 2001; Kafkas et al., 2007). It was determined that the extracts obtained from this plant have antimicrobial and antifungal effects. There are soaps which used in the area of cosmetics are made from *P. eurycarpa* oils (Kordali et al., 2003; Alma et al., 2001). Mycobacteria possess a complex lipid-rich cell wall therefore this could be a reason for the slow growth. Hence it is considered the extracts of *P. eurycarpa* that contains high levels of lipid could be increase the growth rate of mycobacteria. This study was an analysis on whether the addition of various extracts of *P. eurycarpa* into Löwenstein Jensen (LJ) and Middlebrook 7H11 agar (7H11) medium has an effect on the acceleration of growth rate of *M. tuberculosis*.

MATERIALS AND METHODS

A total of 38 clinical strains defined as *M. tuberculosis* complex and 2 standard strains of *Mycobacterium tuberculosis* (H37Rv, H37Ra) were included in this study. Twenty strains were isolated from the clinical specimens submitted to the clinical microbiology laboratory of Gazi Hospital and 18 strains were isolated from the clinical specimens in the Tuberculosis Laboratory of Refik Saydam Hygiene Center in Ankara, Turkey. Clinical specimens like sputum were processed using NALC-NaOH and cultured in BACTEC MGIT 960 tubes with PANTA. The samples taken from the tubes which detected positive for growth were stained. The bacteria identified as acid fast positive were re-cultured with and without NAP. The mycobacteria strains inhibited by NAP were accepted as *M. tuberculosis* complex (Winn et al., 2006; Babady and Wegnenack, 2012). The growth duration of every strain in LJ medium was measured and recorded. Effects of the plant extracts on the growth rate of *M. tuberculosis* were determined using LJ Medium (Biomerieux) and 7H11 (BBL Seven H11 Agar Base) with 10% fresh OADC added (Winn et al., 2006). The fruits belonging to the plant selected for the analysis were collected from the trees growing in vicinity of Siirt province in October, 2010. There is no requirement or ethical approval for this *in vitro* research.

Identifying fatty acid components of the plant

The peels and fruits of the plant were pounded in a mortar and its fixed oils were exposed to extraction for 6 h with *n*-hexane in the Soxhlet apparatus**.** After the organic phase was evaporated at 40°C in reduced pressure, the remaining portion was incubated for 10 min after adding 0.5 N methanolic sodium hydroxide reagent and then, was exposed to saponification.

Subsequently, for methyl *trans*-esterification reaction, 5 ml of 14% BF_3 with methanol was added to the oils obtained and concentrated by boiling in a hot water bath for 2 min. Five milliliter (5 ml) of *n*-hexane was added to this component and boiled for 1 min. It was left to cool down at room temperature and after addition of saturated NaCl. After cooling, it was put into 25 ml volumetric flask and completed to 25 ml with the addition of saturated NaCl and transmitted to separator funnel and then upper phase was

removed for analysis (Kan et al., 2009).

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis was carried out in order to determine fatty acid methyl ester contents of the oil (Kan et al., 2009; Blomberg et al., 2002). Fatty acid composition analysis was achieved by Agilent 6890 Network GC system combined with Agilent 5975C VL MSD (Flame ionization detector, FID) detector and DB Waxetr column (60.0 mm × 0.23 mm × 0.25μm) was used. Helium with 3.3 ml/min flow rate and 1 μl injection capacity was used as the carrier gas. FID temperature was 250°C and initial flow rate was 3.1 ml/min. The MS parameters were applied under electron impact ionization (70 eV) with 35-450 atomic mass units.

Identifying the peaks

The retention times of the oils of the fruit and peel samples and mass weights of fatty acids that were obtained with GC, respectively were identified with GC-MS and a comparison was made using the mass spectra in Wiley and Nist databases for the detection of peaks. As an authentic sample, Fame mix Supelco-1891-1AMP that is composed of methyl esters of palmitate, stearate, oleate, linoleate, linolenate, and arachidic acid was used.

Preparation of peels and fruits of *P. eurycarpa*'s water-soluble extract samples and addition to media

The water-soluble extracts of the peels and fruits of *P. eurycarpa* were obtained using the cold infusion technique. To achieve this, 15 g of each of the peel and fruit of *P. eurycarpa* were pounded in a sterilized mortar and 15 ml of distilled water was added in each and pounded for 15 min more. Water (45 ml) was added to each extract obtained and mixed for one hour and the upper liquid was sterilized using a 45 μm millipore filter (Singh, 2008). Various concentrations (2, 5, 8, and 10 μl) of extracts were spread on the surface of the solid media and observed that 5 μl optimally filled the surface without overflowing and thus it was decided to add 5 μl for each application. Each of these extracts was taken for 5 μl and they were spread to surface of 7H11 and LJ media.

Preparation of the fixed oils

The fixed oils obtained with Soxhlet extraction from the fruit and peels of *P. eurycarpa* were sterilized using millipore filters and 5 μl from each of oil samples were spread onto surface of 7H11 and LJ media.

Addition of individual fatty acids into medium

Fatty acids to be analyzed were used in 0.5 μg/ml concentration which was found to have no antibacterial effect by Alma et al. (2004). Myristic acid (Sigma), palmitoleic acid (Sigma P9417), stearic acid (Sigma S4751), linoleic acid (Sigma L1376), linolenic acid (Sigma L2376), arachidic acid (Sigma A3631), and methyl *cis*-11-eicosenoic acid (Sigma E6885) were obtained from their corresponding commercial suppliers. In preparation of the solutions, myristic acid was dissolved in ether, while other fatty acids were dissolved in isoamyl alcohol. Final concentrations of 0.5 μg/ml were prepared and 5 μl from each was spread to the surfaces of LJ and 7H11 media. For every strain of bacteria, 12 LJ and 12 7H11 media were used and media were enumerated from 1 to 12. Media numbered as 12 were reserved for growth control media and

nothing was added to these media.

Cultivation of bacteria

7H11 medium was poured into 12-well tissue culture test plates (Orange Scientific), 5 ml for each well. Commercially available LJ media were used in the study. The loopful colonies of the bacteria from 3-4 weeks-old *M. tuberculosis* cultures grown on LJ medium were suspended in sterile tubes with glass beads containing 5 ml of Middlebrook 7H9 broth with OADC (Becton Dickinson). The preparations were vortexed and supernatants were removed after 30 min. After centrifuging at 3200 rpm, the pellets were washed and the optical densities were adjusted to 0.05. An aliquot of 0.005 ml, containing $6x10^4$ cfu was inoculated into each medium (Hedegecock, 1970). We obtained good results with these dilutions in the preliminary experiments. Inoculated media were incubated at 37°C and controlled daily for any growth. The exact time of appearance of the colonies were recorded.

Duplicate inoculations were made for each sample, and bacteria were isolated generally with one or two days of deviation.

Statistical analysis

While investigating the effect of each substance on the growth rate of bacteria, in the face of each variable, the difference between the average day of growth of the control strains and all other strains of bacteria was analyzed using the Wilcoxon Signed Ranks Test.

RESULTS

Percentage of the fruit oil yield was calculated as 24.86% and the peel as 54.51% (w/w). 99.98 and 99.96% of the fatty acid compositions of the fruit oil and fruit peel oils, respectively was identified by GC-MS technique. We found that the fruit oil composition was consisted of mostly oleic, palmitic, linoleic and stearic acids (58.55, 22.85, 11.03, and 3.4%, respectively) and to a lesser amount linolenic, palmitoleic, arachidic, eicosenoic and myristic acids.

When the daily growth times for the substance-free LJ and 7H11 media were compared, it was found that the growth in 7H11 medium (in 12.3 days) took place earlier than it was in LJ medium (in 15.9 days) and the difference was found to be in statistically significant levels.

In this study, no fatty acid was found to have a remarkable effect on growth rate of bacteria in terms of growth times measured. In the experiments made in LJ medium, it was found that the average growth time is 15.9 days for the tested strains. The average growth day for bacteria in LJ medium with the oil extracted from the peel was found to be 13.6 days. It was observed that this provides an advantage of 2.3 days in terms of growth time of bacteria.

The growth time was 9.8 days in the presence of the water soluble peel extract, 10.45 days in the presence of the fruit extract, in LJ medium. In each of the two conditions, growth time has accelerated in significant levels in comparison with the control media. It was

observed that growth time of bacteria has been shortened a more than one third.

However, this time was 7.6 days in the presence of water-soluble peel extract, 8.5 days in the presence of fruit extract, in 7H11 medium. Again, in the statistical evaluations, this shortening of the time of growth was found to be significant levels for each of the two factors.

The average growth day of the tested 40 strains of bacteria in LJ and 7H11 media in the presence of various substances are presented in Tables 1 and 2. The data presented in Tables 1 and 2 are summarized in Tables 3 and 4 with some statistically parameters.

The average growth day for bacteria in the presence of each substance was compared with those in substance-free media. It was observed that the water-soluble extracts of the fruit and peel had significantly increased the growth rate of bacteria in two separate media (p<0.05). These extracts are shown as Arabic numbers in Table 1. It was also observed that other substances whose effects were analyzed did not have any remarkable effect on the growth rates of bacteria.

DISCUSSION

The increase of multiple resistant strains to antituberculosis drugs named MDR and XDR have made the infection more dangerous (Nathanson et al., 2010; Marvar et al., 2011). Early diagnosis of tuberculosis makes it possible for specific treatment to begin without delay. Besides, early diagnosis is of vital importance in terms of preventing the spread of the disease. Various molecular techniques that facilitate early diagnosis of tuberculosis have been developed and are presently in use. However, molecular test such as polymerase chain reaction (PCR) and other alternative amplification methods are difficult to apply or have low sensitivity (Fakruddin et al., 2013; Nikam et al., 2013; Derese et al., 2012; Lucke, 2011; Washington State Department of Health, 2011; Levy et al., 1989).

M. tuberculosis strains grow approximately in 15-45 days in solid media (Winn et al., 2006). This time loss causes delay in treatment and may lead to further spread of the disease. Intensive efforts have been made and different media have been developed for faster growth of *M. tuberculosis* (Satti et al., 2010; Kotian et al., 1983). However, no reasonable and viable steps that can solve the problem have been taken yet. There are diverse molecular microbiological tests which get result within hours for identifying *M.tuberculosis* infections. New ones are added to them every day. Yet these tests does not eliminate the need for culture. The use of broth media reduces the growth time of *M.tuberculosis*. *M.tuberculosis* strains grow within 15-45 days in LJ media, whereas this period lessens to 10-12 days in broth media (Crucianini et al., 2004; Lucke, 2011). However LJ medium still commonly used in several laboratories. While the broth media generally based on radiometric or fluorometric

Table 1. The growth time (day) of 40 strains of *M.tuberculosis* complex in LJ medium in the presence of 11 different substances.

M.tbc	1	2	3	4	5	6	7	8	9	10	11	12
B1	4	5	6	5	7	6	7	6	5	7	7	7
B2	9	7	9	9	14	9	12	9	7	9	9	9
B3	6	11	8	9	15	9	17	9	7	8	7	7
B4	12	14	19	19	20	18	20	18	20	20	20	19
B5	9	6	12	17	12	15	17	15	13	12	17	12
B6	7	6	12	17	12	11	12	11	11	12	12	12
B7	12	12	24	24	22	23	22	23	23	22	22	23
B8	17	12	17	18	22	27	26	27	25	27	27	27
B9	12	15	12	12	17	12	12	12	12	12	12	12
B10	12	12	13	12	12	12	17	12	12	12	14	13
B11	14	14	15	17	22	27	23	27	28	22	23	28
B12	15	12	17	17	18	15	19	15	12	18	19	19
B13	7	12	20	21	22	23	21	23	24	22	21	24
B14	12	12	12	17	15	12	18	12	16	15	18	16
B15	6	10	12	17	16	14	14	14	14	16	14	14
B16	10	11	17	17	16	18	17	18	17	18	17	17
B17	12	9	12	18	15	13	14	13	15	15	14	14
B18	7	10	12	12	12	12	17	12	13	12	17	12
B19	6	12	12	14	17	20	22	20	22	17	22	21
B20	7	7	8	9	12	13	13	13	12	12	13	13
B21	6	11	7	7	15	7	17	7	8	8	7	7
B22	12	14	19	19	20	20	20	20	18	20	20	19
B23	7	12	20	21	19	23	21	23	24	19	21	24
B24	10	11	17	17	16	18	16	18	16	17	17	17
B25	6	10	12	17	16	14	18	14	13	16	18	14
B26	17	12	17	16	24	24	27	24	26	27	26	27
B27	12	12	12	17	17	16	18	16	16	17	18	16
B28	9	6	12	17	12	12	17	12	11	12	17	12
B29	12	15	16	17	14	12	13	12	15	14	13	12
B30	7	6	12	17	12	11	13	11	11	12	13	12
B31	12	12	24	24	22	23	22	23	21	22	22	23
B32	6	12	12	14	22	22	22	22	12	22	22	21
B33	12	12	15	14	13	13	13	13	12	13	13	13
B34	7	7	8	9	12	13	13	13	13	12	13	13
B35	14	14	15	17	25	27	26	27	29	25	26	28
B36	7	10	12	12	12	12	12	12	12	12	12	12
B37	12	9	12	18	15	12	14	12	14	15	14	14
B38	15	12	17	17	18	19	19	19	17	18	19	19
B39	4	5	6	5	7	6	7	6	7	7	7	7
B40	9	7	9	10	14	9	12	9	7	9	10	9
Mean	9.8	10.45	13.6	15.0	16.9	15.5	17.0	15.5	15.2	16.4	16.6	15.9

1-Water soluble extract of peel, 2-Water soluble extract of fruit, 3- Peel lipid extract, 4- Fruit lipid extract, 5- Linoleic acid, 6- Linolenoic acid, 7- Stearic acid, 8- Myristic acid, 9- Palmiteloic acid, 10- Arachidic acid, 11- Eikosenoic acid, 12- Growth control.

detection systems, they are more expensive. If any accelerating effects on the growth rate of extracts detected in this study, it is supposed this extracts could be used in all types of media.

There are unique lipid forms in the cell walls of mycobacteria, which make up the 60% of the cell wall

Table 2. The growth time (day) of strains of *M.tuberculosis* complex in 7H11medium in the presence of 11 different substances.

M.tbc	1	2	3	4	5	6	7	8	9	10	11	12
B1	4	4	4	4	11	7	10	7	7	6	7	6
B2	9	13	13	14	14	13	13	13	13	13	14	13
B3	7	7	8	9	9	9	9	9	9	9	9	9
B4	6	6	8	10	9	9	9	9	10	9	9	9
B5	6	12	12	12	9	11	12	11	12	12	12	12
B6	7	6	7	8	8	8	9	8	9	8	9	9
B7	9	9	9	9	15	14	14	14	15	15	15	15
B8	9	9	13	14	15	14	15	14	15	15	14	15
B9	6	6	8	9	12	12	13	12	12	12	13	12
B10	7	7	12	9	13	13	13	13	12	13	14	13
B11	17	17	19	20	26	27	27	27	27	27	27	28
B12	7	7	9	9	11	11	12	11	12	11	12	10
B13	7	6	13	14	13	13	14	13	13	12	13	13
B14	5	5	8	7	9	9	9	9	9	9	9	9
B15	5	6	7	7	9	8	9	8	9	9	9	9
B16	9	9	10	11	11	11	11	11	11	11	11	11
B17	9	12	10	9	14	13	13	13	13	13	12	13
B18	12	12	14	13	15	15	15	14	15	15	15	15
B19	6	9	9	8	10	9	10	9	9	10	10	9
B20	6	9	9	9	9	9	9	9	9	9	8	9
B21	7	7	8	9	9	10	9	10	9	9	9	9
B22	6	6	9	10	9	9	8	9	9	9	9	9
B23	7	6	13	14	12	13	10	13	12	12	10	13
B24	9	9	12	12	13	14	12	12	13	13	12	13
B25	5	6	7	7	9	9	9	9	9	9	9	9
B26	9	9	20	21	24	22	24	23	24	24	24	25
B27	5	5	10	7	9	9	9	9	10	9	9	9
B28	6	12	12	12	12	12	14	12	13	12	14	13
B29	6	6	9	9	12	12	12	12	11	12	12	12
B30	7	6	7	8	9	8	10	8	9	9	10	9
B31	9	9	9	9	13	12	12	13	13	13	12	13
B32	6	9	9	8	8	9	10	9	9	8	10	9
B33	6	7	7	10	10	10	10	10	9	10	10	10
B34	6	9	9	9	10	9	9	9	9	10	9	9
B35	17	17	19	20	27	27	27	27	27	27	27	27
B36	12	12	13	12	14	13	14	15	13	13	13	14
B37	9	12	6	9	14	13	14	13	12	13	13	13
B38	7	7	9	9	11	11	12	11	12	11	12	10
B39	4	4	4	4	11	7	10	7	7	6	7	6
B40	9	14	12	11	13	13	13	14	13	12	13	13
Mean	7.65	8.5	10.2	10	12.2	11	12.1	11.9	12.1	11.9	12.1	12.3

(Winn et al., 2006). As the synthesis of these complex lipids is complicated and time consuming, it is possible that late growth of bacteria was due to this process. This study was performed to analyze the effects of the oil extracts and the water-soluble extracts isolated from *P. eurycarpa*, whose lipid richness has been demonstrated in various studies, on the growth rate of *M. tuberculosis.*

In this study, GC-MS method has demonstrated that there are 8 different fatty acids in fruit peel and 9 fatty acids in the fruit. Myristic acid was found only in the fruit. Composition of fatty acids found within the scope of this research was revealed to be in conformity with the profiles found by Kafkas et al. (2007).

It is determined that *M. tuberculosis* strains grow 2.3

Table 3. The mean of growth times (day), confidence intervals, and some statistically parameters for strains of *M.tuberculosis* complex in LJ medium in the presence of 11 different substances .

LJ Medium	1	2	3	4	5	6	7	8	9	10	11	12
Mean	9.8	10.45	13.6	15.0	16.9	15.5	17.0	15.5	15.2	16.4	16.6	15.9
95% Confidence Intervals	8.7-10.9	9.5-11.3	12.1-14.9	13.6-16.6	14.6-17.5	13.6-17.4	15.4-18.5	13.6-17.4	13.2-17.2	13.9-17.3	14.5-18.0	14.0-17.9
Standard Deviation	3.5	2.8	4.4	4.6	4.3	5.9	4.3	4.0	6.1	5.3	5.4	6.0
Standard Error	0.5	0.4	0.7	0.7	0.7	0.9	0.7	0.9	0.9	0.8	0.8	0.9
Median	9.50	11.50	12.00	17.00	15.50	13.50	17.00	17.00	13.50	15.00	17.00	14.00
Minimum	4	5	6	5	7	6	6	7	5	7	7	7
Maximum	17	15	24	24	25	27	26	27	28	27	28	28
Significance (P 0.05)*	0.0001	0.0001	0.0001	0.517	0.960	0.059	0.039	0.059	0.020	0.052	0.229	-

*Statistically significance of the differences between the means growth time in the control media and media with various substances.

Table 4. The mean of growth times (day), confidence intervals, and some statistically parameters for strains of *M.tuberculosis* complex in 7H11 medium in the presence of 11 different substances.

7H11 Medium	1	2	3	4	5	6	7	8	9	10	11	12
Mean	7.65	8.5	10.2	10.0	12.2	11.0	12.1	11.9	12.1	11.9	12.1	12.3
95% Confidence Intervals	6.7-8.5	7.5-9.6	8.9-11.3	9.2-11.5	10.8-13.6	10.5-13.3	10.9-13.7	10.5-13.4	10.6-13.5	10.4-13.4	10.6-13.6	10.5-13.6
Standard Deviation	2.8	3.2	3.6	3.7	4.4	4.4	4.4	4.5	4.5	4.6	4.5	4.8
Standard Error	0.4	0.5	0.5	0.6	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Median	7.00	8.00	9.00	9.00	11.00	11.00	12.00	11.00	12.00	11.50	12.00	11.50
Minimum	4	4	4	5	8	7	8	7	7	6	7	6
Maximum	17	17	20	21	27	27	27	27	27	27	27	28
Significance (P 0.05)*	0.0001	0.0001	0.0001	0.0001	0.517	0.180	0.960	0.059	0.973	0.166	0.600	-

*Statistically significance of the differences between the means growth time in the control media and media with various substances.

days earlier with peel lipid extracts and 1 day earlier with fruit extracts in LJ medium than the control growth tubes. It was obtained 1-2 day earlier growth time by adding some substances to conventional media (Lu et al., 2002; Anargyros et al., 1990). Nevertheless it does not provide a major benefit to diminish the growth time 2 days in a period of 1-1.5 month-long growth time in LJ medium. The isolation periods of *M. tuberculosis* was found approximately 13.2 days by Bactec MGIT 960, 15.2 days by Bactec 460 and 25.8 days in LJ medium from clinical samples. It is known that mycobacteria grow rapidly in broth media than solid media (Cruciani et al., 2004). Since the bacteria strains used in this study isolated earlier, the average growth time was found as 15.9 days in LJ medium. It is known that mycobacteria in clinical samples can be determined

within hours by molecular microbiological methods (Lucke, 2011). However, LJ and MBA media are still used for isolation of *M.tuberculosis* from clinical samples in several laboratories. Consequently, these two media were used in this study. Assuming that a positive effect provided in any medium, it can be also applicable to all media. Some researchers have analyzed accelerating effects of growth of mycobacterium by adding various substances to the tuberculosis media. Kotian et al. (1983) showed that addition of nicotine into the medium has a slight contribution to the acceleration of growth of mycobacterium. Hedgecock (1970) have managed to obtain faster and better rate of growth of mycobacteria by adding triton into the medium. Triton solubilizes turbid fatty acids and, thus, reduces the toxic effects of fatty acids. In another article, it has been argued that mycothiol is present in mycobacterium cell wall, reduces the effects of oxidizing agents and some antibacterial substances, causing to faster and better growth of mycobacteria (Sareen et al., 2003). During the course of the study, it has been observed that some fatty acid combinations prevent growth of bacteria especially in 7H11 medium. It was considered that this prevention effect developed due to the fact that the fatty acids were put into wide-surface medium, exposing them to oxygen, and were kept at 37°C for a long time, leading to oxidization and resulting in a toxic effect on the bacteria. Additionally, it is possible that these lipids formed a thin layer between the air and the surface of the medium, preventing to obtain oxygen requirement of the bacteria. In this type of experiments, adding triton to medium may remove these delays (Hedgecock, 1970).

Tested bacteria were inoculated by streaking them onto the media since commercially available solid media were used. We used the solid media because these types of media were employed in most of the mycobacteriological studies in our country.

The fruit and peel water-soluble extracts of *P. eurycarpa* accelerate the growth and development of mycobacterium. In this sense, it is possible to state that this is a first step in finding the existence of a substance of economic value which can be added to media formulas.

In our future experiments, we will study the effects of the water-soluble extracts on the liquid media. Additionally, we will continue to study the identification of the active components in the extracts that affect the acceleration of the growth time of the mycobacterium.

In conclusion, the fruit and fruit peel water-soluble extracts of *P. eurycarpa* reduce growth time of mycobacterium approximately by one third. Identification of effective substances in these extracts and addition of these extracts into mycobacterium medium will accelerate growth time of mycobacterium by means of culture method.

The diagnosis of the disease in the tuberculosis patients and the mycobacterial susceptibility tests will thus be quickened. We think that the result obtained is a significant step in solving the problem of late diagnosis of tuberculosis by means of culture.

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