JOURNAL OF COMPLEMENTARY MEDICINE RESEARCH, 2020
VOL 11, NO. 5

RESEARCH ARTICLE

Open Access

In vitro evaluation of antimicrobial activity and estimation of Epicatechin from the fruit extract of Prunus armeniaca L using HPTLC technique

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ABSTRACT

In current time, recent era of science and medication advancement and the need to discover new fixes reignited enthusiasm for a portion of the antiquated customs just as to investigate the quickly growing field of phyto-pharmacology and phyto-science notwithstanding current improvements in present day clinical science. Apricot (Prunus armeniaca L) so named by Romans is an exceptionally awesome and sound organic product that gives various medical advantages. The current investigation is to assess the antimicrobial movement and estimation of Epicatechin from the natural product concentrate of Prunus armeniaca L utilizing HPTLC technique.In this examination, we have made an endeavor to evaluate Epicatechin present in the Prunus armeniaca organic product remove by HPLC strategy and furthermore to screen the antibacterial action as it is known for its restorative advantages . The Prunus armeniaca natural product separate was chromatographed on silica gel GF254 plates with Toluene: EA: FA: MeOH (3: 3: 0.4: 0.1) as versatile stage for epicatechin estimation. Location and measurement were performed by densitrometric scanning,at 280 nm. The counter bacterial action was screened utilizing plate dissemination strategy and the zone of restraint was estimated in mm distance across. The concentrate indicated noteworthy antibacterial movement against the bacterial strains tried. The normal recuperation of Epicatechin was seen as 0.81 %. The HPTLC technique has given a decent goals of Epicatechin from different constituents present in the ethanolic extract.

ARTICLE HISTORY

Received October 09, 2020
Accepted November 12, 2020
Published December 10, 2020

KEYWORDS

Prunus armeniaca L, Epicatechin, HPTLC, disc diffusion technique,

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INTRODUCTION

The act of natural medication over the globe has a solid history that goes back to 2000 BC. Home grown Medicine is an antiquated act of utilizing plants to forestall and fix ailment. Herbs contain various metabolites which have a wide scope of restorative exercises.(Mukeshwar Pandey,2011) various examination has been done on the compound constituents of therapeutic plants to a superior comprehension of their activities. In current time, present day science and medication advancement and the need to discover new fixes reignited enthusiasm for a portion of the old conventions just as to investigate the quickly extending field of phyto pharmacology and phyto science notwithstanding current improvements in current clinical science. It is generally essential to comprehend that natural medication isn't just another option, yet a mentality and a methodology that can enhance all parts of medicinal services.(Williamson ,2001). Every herb has an extraordinary arrangement of properties and activities all of which add to the general wellbeing status of the individual

Apricot (Prunus armeniaca L.) likewise called Jardalu, Khubani and Khumani, has a place with family Rosacea, perhaps the biggest family having in excess of 3000 species including almonds, peaches, apples, plums, fruits and berries.(Donmez , 2000) Apricot, so named by Romans is a superb and solid organic product that gives various medical advantages. Prunus armeniaca is a deciduous Tree developed in atmospheres with very much separated seasons. Its leaves are wide, cordate, interchange, basic, exchange, having reticulate pinnate venation, toothed on the edge, with 2 little organs at base of edge. The fowers are , basic, sessile, pentamerous, bisexual, white or pinkish, 2 to 5 blossoms for every group. The consumable organic product is a meaty drupe, smooth and smooth with an enormous pit (stone), yellow or ruddy in shading. Their flavor is practically musky, with a black out pungency which is more when the natural product is dried.(Biswajit Das,2011) Fruit isn't just expended in new structure, yet its dried and solidified structures are likewise accessible in the market.

Apricot is exceptionally nutritious loaded up with sound nutrients, minerals, flavonoids, polyphenols and other wellbeing advancing mixes. Apricots contain various powerful cell reinforcements.(Hudina , 2000) They are acceptable wellspring of both nutrient A (from beta-carotene) and nutrient C. Apricots are likewise rich in polyphenolic cell reinforcements like flavonoids. Chlorogenic corrosive (5-caffeoylquinic corrosive) is the prevailing phenolic compound in apricots.(Svetlana ,2017) The other phenolic mixes decided in apricots are neochlorogenic corrosive, caffeic corrosive, p-coumaric corrosive, ferulic corrosive and their esters. (+)- Catechin and (-)- epicatechin are likewise decided in apricot foods grown from the ground products.(Mratinic,2011) Flavonols in apricots happen generally as glucosides and rutinosides of quercetin and of kaempferol.

Apricots are nutritious and contain numerous basic nutrients and minerals which consults the natural product with numerous helpful exercises (Callistus Bvenura,2018). An assortment of pharmacological impacts of apricot and its portion have been accounted for which included enemy of parasitic, anticancer, antiaging, against atherosclerating, hostile to anginal, cardio defensive, hepato defensive, reno defensive and cell reinforcement. Apricots are a decent wellspring of dietary fiber, which help in stomach related wellbeing. Solvent fiber present in it assists with controlling blood cholesterol levels. Apricot is one of the uncommon organic products with superb wellsprings of beta carotene, lutein, zeaxanthin and nutrient C and Vitamin E and normally wealthy in cancer prevention agent. These supplements secure eyes against harm and the skin by bringing down danger of wrinkles and sun consume. They are likewise wealthy in potassium which may help in forestalling hypertension and decrease danger of stroke. The high water content in the organic product keeps the individual hydrated.

Various exploration propose that apricots forestall liver harm in view of their high cancer prevention agent content.(Otakar Rop,2009) Apricots, having a low glycemic list sustainedly affect the glucose levels. The organic product is likewise plentiful in nutrient E, which goes about as a cancer prevention agent that improves glucose levels. As per a report by the Arthritis Foundation, apricots are wealthy in beta-cryptoxanthin, a substance that can forestall osteoarthritis and other fiery types of joint pain. The magnesium present in the organic product can likewise ease provocative torment. Apricots are likewise acceptable wellsprings of iron, which helps treat sickliness. Iron improves hemoglobin creation, and this upgrades the nature of blood. The current investigation is to assess the antimicrobial action and estimation of Epicatechin from the organic product concentrate of Prunus armeniaca L utilizing HPTLC procedure.

MATERIALS AND METHODS

Plant material

The fruit extract of Prunus armeniaca L was obtained commercially and used for the study. The extracts were prepared in the following concentrations in sterile water. 5mg/ml and 10mg/ml and 20mg/ml. 50µl of extract of different concentrations were loaded on sterile filter paper

discs measuring 6mm in diameter, so that the concentration of the extract on each disc was 250µg, 500 µg and 1000ug respectively. The discs were dried and kept aseptically

Micro organisms

The organic product concentrate of Prunus armeniaca L was gotten monetarily and utilized for the examination. The concentrates were set up in the accompanying fixations in sterile water. 5mg/ml and 10mg/ml and 20mg/ml. 50µl of concentrate of various focuses were stacked on sterile channel paper plates estimating 6mm in measurement, with the goal that the grouping of the concentrate on each circle was 250µg, 500 µg and 1000ug individually. The plates were dried and kept aseptically .

An aggregate of 7 bacterial societies (Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus mirabilis and Bacillus cereus) were utilized in this examination. The way of life were put away on supplement agar inclines at 4°C for bacterial strains and SDA inclines for contagious strains in Department of Microbiology, Saveetha Dental College and were sub-refined before antimicrobial testing.

Antibacterial Procedure

Stock culture of the test creatures contrasted with Mac Farland's standard 0.5 were readied. Garden culture of the test living beings were made on the Muller-Hinton agar [MHA-M1084] plates utilizing sterile q-tip and the plates were dried for 15 minutes. Channel paper circles stacked with various groupings of the concentrate were put on the separate plates. The plates were brooded at 37°C short-term and the zone of restraint of development was estimated in millimeter distance across.(Collins,CH,2001) The plates were hatched for the time being and the zone of hindrance of development was estimated in mm width. Standard anti-toxin plates of Penicillin G(30mcg/circle) was utilized as positive control. All the test were done in triplicate to limit the test blunder.(Connie R.Mahon,2006)

Estimation of Epicatechin by HPTLC technique: HPTLC Fingerprinting

The finger printing has been finished utilizing the accompanying chromatographic conditions. (Ankita Jain,2014). Chromatography was performed on a10x10 cm pre-actuated HPTLC silica gel 60F 254 plate. Tests were applied to the plate as 6mm wide band with a programmed TLC tool Linomat 5 with N2 stream (CAMAG, Switzerland), 8mm from the base. Densitometric filtering was performed on CAMAG scanner III. The plates were prewashed by methanol and enacted at 600 C for 5 minutes before chromatography. The cut measurement was kept at 5 minutes x 0.45 minutes and 20 minutes filtering speed was utilized. The portable stage was picked subsequent to running concentrate in various versatile periods of shifting extremity (Toluene, Toluene: Ethyl acetic acid derivation and Ethyl acetic acid derivation: Methanol and so on ;). Straight climbing improvement was done in 20cm x 10cm twin glass chamber soaked with the portable stage and 10 ml of versatile stage was utilized per chromatography.

Chromatographic Analysis

The plant concentrate and standard of required fixation have been set up in methanol
furthermore, were spotted utilizing CAMAG implement. The technique was enhanced by choosing fitting versatile stage for the plant separate and individual mixes and created in a twin trough chamber, 10 x 10cm at 25°C. The plates were dried by hair dryer. The created plates were examined at suitable frequency utilizing CAMAG TLC scanner 3 and photograph archived utilizing CAMAG REPROSTAR 3. (figure 1,2)

Preparation of stock solutions

Preparation of Epicatechin standard solution

Evaluation of the standard Epicatechin (100 mcg/ml) and the example (50 mg/ml) Apricot remove were set up in methanol and assessed with the assistance of Toluene: EA: FA: MeOH (3: 3: 0.4: 0.1as the portable stage . Silica gel GF254 was utilized as the fixed stage. 2 µl, 4 µl, 6 µl , 8 µl and 10 µl of the standard Epicatechin were run in track 1-5 individually and test apricot extricate in track 6,7 and 8 having 2 µl, 4 µl, 6 µl , utilizing climbing advancement mode and the created plates were examined at 280 nm utilizing CAMAG TLC scanner 3 and photograph reported utilizing CAMAG REPROSTAR 3 and the outcome is given in the figure 3 and 10 and the pinnacle territory acquired from various tracks.

RESULT

The antibacterial activity of the apricot extract at different concentrations were screened by disc diffusion technique and the zone of inhibition was measured in mm diameter. The results are given in the table 1. The extract at different concentration exhibited antibacterial activity against all bacterial strains tested. The ethanolic extract was more effective against E.coli, Proteus mirabilis and Bacillus cereus, with a zone of inhibition of 33mm, 31mm and 30 mm diameter (at conc1000µg.) respectively and was least effective against Pseudomonas aeruginosa with zone of inhibition of 17mm (at conc. 1000 µg). Among the other

bacterial species studied Staphylococcus aureus, Klebsiella pneumoniae and Enterococcus faecalis showed a zone of inhibition of 26mm , 24mm and 25mm diameter (at conc. 1000 µg.) respectively.

The high performance thin layer chromatography seems to be a highly reliable technique for isolation and estimation of plant components.12,13,14 The finger printing following the chromatographic condition showed a comparable peak of epicatechin in the extract as that of the standard epicatechin in every track 6,7,8 (Figure 8,9,10). The epicatechin percentage was calculated from the peak area and was found to be 0.81 % with an Rf value of 0.34.

Table 1: Anti-bacterial activity of the extract on bacterial strains (zone of inhition in mm diameter)

Conc of extract (µg)	B1	B2	B3	B4	B5	B6	B7
250	12	14	15	13	12	-	13
500	19	21	23	22	19	11	23
1000	25	26	31	33	24	17	30
Standard Penicillin G	30	32	28	29	25	28	29

(B1-Enterococcus faecalis, B2-Staphylococcus aureus, B3-Bacillus cereus, B4- Escherichia coli, B5- Klebsiella pneumoniae, B6-Pseudomonas aeruginosa, B7 Proteus mirabilis

Photo document

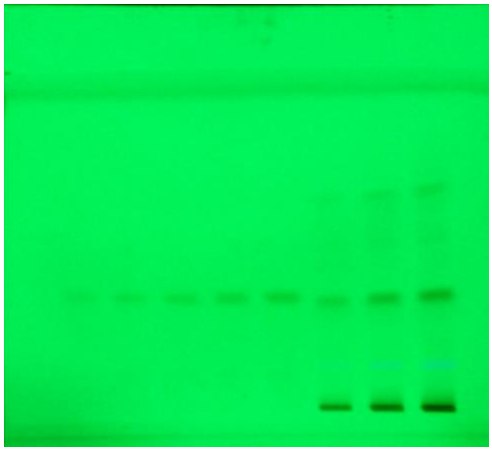

Figure 1: Apricot extract at 254nm

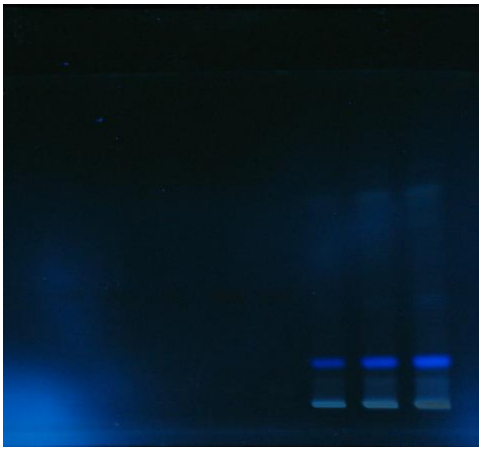

Figure 2: Apricot extract at 366nm

Peak area

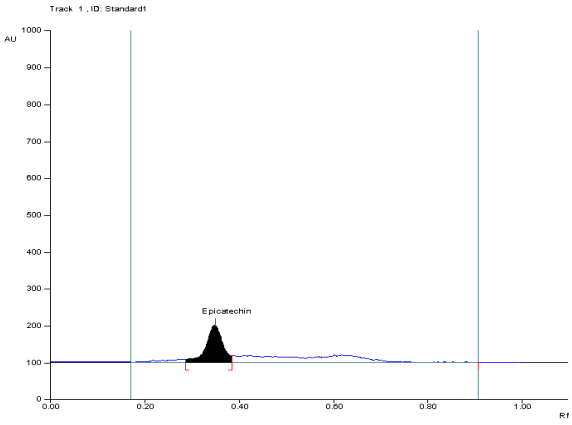

Figure 3: Track 1(Standard 2 µl)

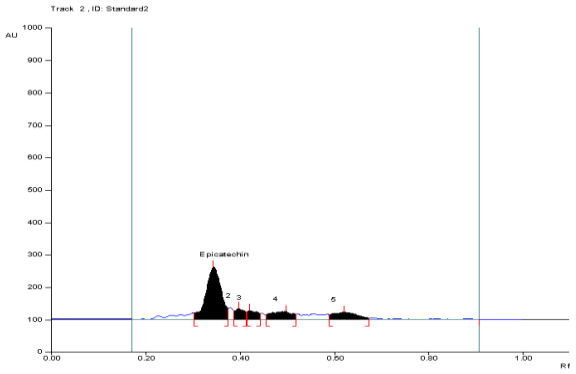

Figure 4: Track 2 (Standard 4 µl)

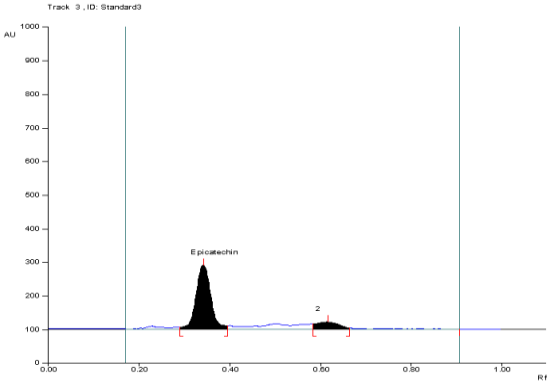

Figure 5: Track 3 (Standard 6 µl)

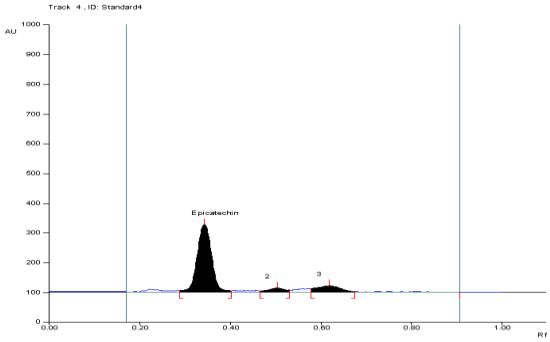

Figure 6: Track 4(Standard 8 µl)

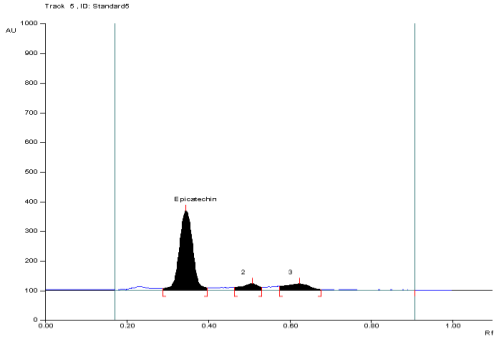

Figure 7: Track 5 (Standard10 µl)

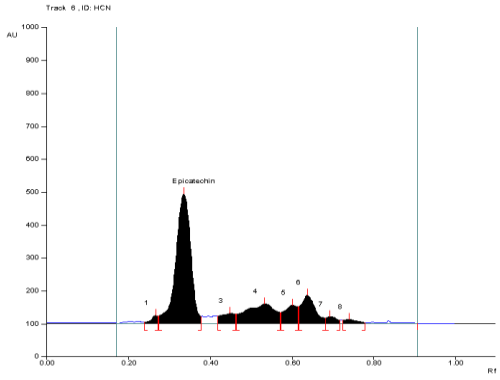

Figure 8: Track 6 (Test Extract2 µl)

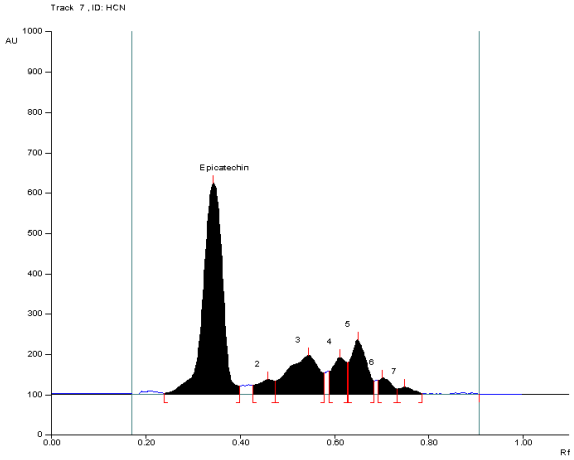

Figure 9: Track 7(Test Extract 4 µl)

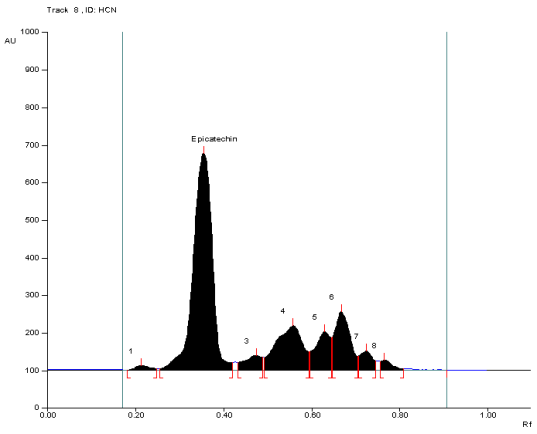

Figure 10: Track 8(Test Extract 6 µl)

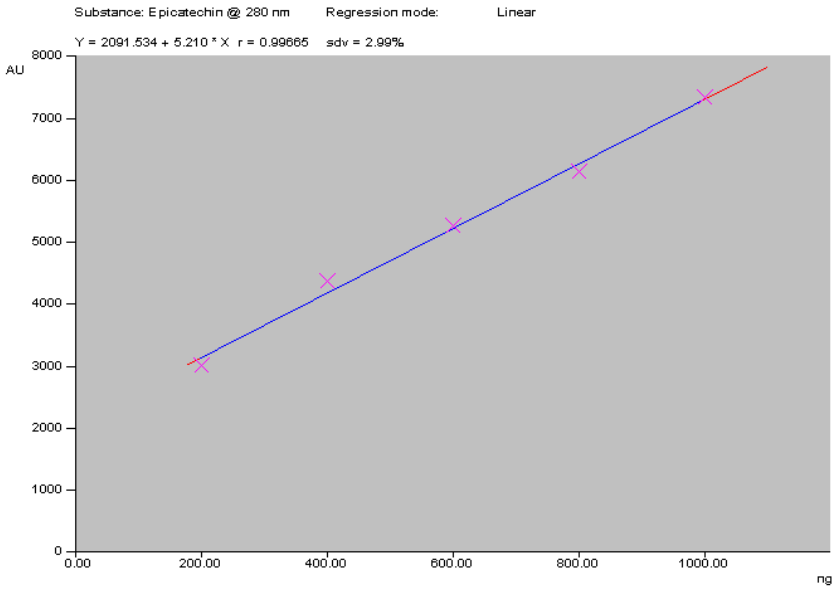

Figure:11 Standard Calibration Curve

DISCUSSION

Herbs contain an assortment of optional metabolites, for example, saponin, alkaloids, polyphenols, glycosides and terpenes. They are liable for the shading astringency, harshness, flavor and smell of the herbs. They are additionally wealthy in common cancer prevention agents. Epicatechin are flavonoids that are a piece of the polyphenol gathering. Broad exploration has been completed on epicatechin for its assorted activities on human wellbeing and as helpful operators in the treatment of different human diseases, particularly safe developing and reappearing infections.(Shawna Rekshmy dharan,2013) They are powerful cancer prevention agents, with antiviral, antimalarial and anticarcinogenetic properties. Utilization of epicatechin has been demonstrated to have medical advantages.(Das , 2013) Epicatechins have demonstrated to lessen danger of diabetes and cardiovascular maladies. These flavonoids can be utilized as restorative operators independently or in mix with other manufactured medications and anti-toxins to deliver another age of phytopharmaceuticals. (Wagner,2009) In our current investigation the organic product concentrate of apricot has seen as rich wellspring of epicatechin.

Anti-infection obstruction is turning into a disturbing worry for open which has made numerous scientists scan for new antimicrobial atoms from nature.(Shyur ,2008) Improper utilization of anti-toxins, expanded development of individuals over the world, transmission of medication safe microscopic organisms starting with one spot then onto the next has expanded the pervasiveness of medication safe microbes. The fast spread of medication safe strains among human has represented a genuine general wellbeing concern which has cleared path back to pre anti-infection period (Rabe ,1997) The difficulties looked by clinical individual against Methicillin Resistant S. aureus (MRSA), Vancomycin Resistant Enterococci (VRE), ESBL (expanded range β-lactamase), multi sedate safe tuberculosis(MTB) has made to scan for elective medication to battle tranquilize safe life form. Analysts are keen on plant extricates as meds as they are undisputable replacement for anti-toxins. The natural product concentrate of apricot displayed great enemy of microbial movement on bacterial strains tried.

CONCLUSION

The current outcomes along these lines offer a logical reason for conventional utilization of Prunus armeniaca L The utilization of herbs in people medication proposes that they speak to a financial and safe choice to treat irresistible sicknesses. It is obvious from the outcomes that, the concentrate goes about as a decent wellspring of epicatechin and furthermore has huge antimicrobial action against different bacterial pathogens tried The counter bacterial exercises could be improved if other dynamic parts are cleaned and sufficient dose decided for appropriate administration

CONFLICT OF INTEREST

Nil

Source of Funding
Nil

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