CHEMICAL ANALYSIS AND BIOACTIVITY ASSESSMENT OF *ERIOBOTRYA JAPONICA* FRUITS AND LEAVES

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ABSTRACT

Fruits and vegetables are considered good sources of many nutrients and may help to control many human infections. The photochemicals compounds like flavonoids, ply phenols, tannins saponins minerals and vitamins contained antioxidant capacity and prevent cells from oxidative damage.*Eriobotryajaponica* belongs to the family Rosaceae is considered is an important fruit in Pakistan. A present study will be conducted to evaluated leaves, seeds and fruit of *Eriobortrya japonica* for their phytochemicals specially flavonoids and polyphenol. The antioxidants activities of various extracts will be assessed by using DPPH, and ABTS assays. Where asantimicrobialactivitieswas evaluated against two bacterial and fungal strains.

Key words; *Eriobortrya japonica*, Fruits, Leaves, Chemical analysis Antioxidant, Antimicrobial actiavities

INTRODUCTION

Study of the antioxidant activity of fruits, vegetables and their chemical compounds are in increasing interest of scientists. These natural compounds may replace the use of synthetic antioxidants in food and pharmaceutical products. These substances act on free radicals, substances related to aging, senescence, and disease onset, slowing or inhibiting their action on human, plant, and animal cells (Agbafor and Nwacjuku,2011) .

These phytochemicals includes carotenoides, lignans, terpenoids, polyphenolics, sulfides, curcumins, saponins, phthalides, plant sterols, flavonoids, coumarins and alkaloids.Flavonoids are the famous group of nutrient due to its antioxidant and anti-inflammatory health benefit (Omoregie and Osagie, 2012).

Eriobotryajaponica locally called loquat and belongs to family Rosaceae.*Eriobotrya japonica* has antioxidant activity and leaves help the body to release anti-oxidants, the great anti-aging agents. It really very beneficial and widely used to prevent from a numerous illnesses, help in increasing immunity, add life-span and also deactivate toxic compounds.

. The *Eriobotrya japonica* leaf creates alots of acids that have an anti-viral effect. These types of acids create antigens that are anti-viral agents. A couple of these types of chemical substances are recognized as megastimane glycosides as

well as polyphenolic components that are recognized to create viral antigens(Aiyegoro and Okoh, 2010). Therefore keeping in view the importance of *Eriobotrya japonica* study was conducted for Quantification of various phytochemicals from leaves, fruits and seeds of *Eriobotrya japonica*, Assessment of antioxidants and antimicrobial activity of various extracts

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METERIAL AND METHOD

QUALITATIVE PHYTOCHEMICAL ANALYSIS

Qualitative analysis of plant extracts were carried out by using different analytical methods AOAC (1984). Those were further modified for current analysis

Test for carbohydrates: Plant extract was mixed with 2ml of Benedict's reagent and then boiled this mixture, red brown precipitates were appeared which means that carbohydrates were present in samples.

Test for iodine: When crude extract was mixed with 2ml of iodine solution , dark blue color appeared which means that carbohydrates were present in samples.

Test for phenols and tannins: When crude extract was mixed with 2ml of FeCl2 (2% solution of FeCl2), black or blue green color appeared which means that tannins and phenols were present in the samples.

Test for glycosides: Crude extract was mixed with 2ml of chloroform. Now added 2ml of sulphuric acid carefully then shaken this mixture carefully, reddish brown color appeared which means that glycone portion of the glycoside were present in the samples.

Test for saponins: Plant extract was mixed with 5ml of distilled water and then shaken the mixture vigorously, stable foam was formed which means that saponins were present in the samples.

Test for flavonoids: Crude extract was mixed with 2ml of NaOH solution (2% solution of NaOH), intense yellow color appeared this intense yellow color became colorless with the addition of few drops of diluted acid which means that flavonoids were present in the samples

Test for alkaloids: Crude extract was mixed with 2ml of 2%HCl. Now heated this mixture gently then added Wagner‘s and Mayer‘s reagents were added in the mixture, turbidity formed which means that alkaloids were present in the samples.

Test for terpenoids: Plant extract was mixed with 2ml of chloroform and allowed this mixture to dry. Now added 2ml of concentrated H_2SO_4 in this mixture and then heated this for 2 minutes, grayish color appeared which means that terpenoids were present in the samples.

ESTIMATION OF ANTIOXIDANT ACTIVITY

DPPH Free Radical scavenging Assay

The scavenging ability of plant extracts were assessed by 1,1diphenyl 1-2-picryl-hydrazyl(DPPH) assay. This was processed according to Moon and Shibamoto (2009) method. Made DPPH solution by mixing 3.4 in 100 ml of methanol. The assay was processed in absence of light. Made the stock solution of 20mg/ml. Made the dilutions of 20,40,60,80,100.Picked 100 µl from each. The reaction mixture was incubated for 45 mins at room temperature and the colour of reaction mixtures were changed from violet to yellow. Absorbance of each sample was measured at 517nm on UV spectrometer. The percentage scavenging was measured by given formula;

Scavenging% = (OD of control) – (OD of sample) /Absorbance of control ×100

ABTS scavenging assays

The scavenging ability of plant extracts were also assessed by 2,2-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assay. 5mg of crude extract of samples were mixed in 1ml of methanol in order to make the stock solution. 2mM ABTS mixed with 1.5mM of potassium persulphate in 100ml of distilled water in order to make ABTS stock solution. The absorbance of ABTS solution was adjusted to 0.748 at 734nm. Made the dilutions of 20,40,60,80,100. Picked the 200µl from each dilution mixed it with 2ml of ABTS. The reaction mixture was incubated for 15 mins at room temperature. Absorbance of each sample was

measured at 734nm on UV spectrometer. . The percentage scavenging was measured by given formula;

Scavenging% = (OD of control) – (OD of sample) /Absorbance of control ×100

ESTIMATION OF ANTIMICROBIAL ACTIVITY;

Antibacterial assay was carried out by disc method with slightly modifications. Two strains were used for the detection of antimicrobial activity .One was *E.coli* and other one was *Staphylococcus aureus* whereas ciproflaxin is a strong antibiotic and was used as a standard. Bacterial cultures were refreshed with the help of LB media in incubator at 37°C for 24 hours.Then take O.D at 430nm now this mixture was ready for the processes.

RESULTS AND DISCUSSION

QUALITATIVE ANALYSIS

Phytochemical analysis of *Eriobotraye japonica* fruits and Leaves were given intable 1. Qualitative analysis shows the presence of many phytochemicals such asFlavonoid , alkaloids, phenols, saponins, tannins, terpenoids , glycosides in the plantextracts. These phytochemicals were detected and used for therapeutic purpose because due all these phytochemicals plants has an antioxidant activity.

Table 1. Phytochemical analysis of leaves and Fruits

PHYTOCHEMICLS	FRUIT EXTRACT	LEAVES EXTRACT
Alkaloids	+	+
Flavonoids	+	+
Phenols	+	+
Saponins	+	+
Glycosides	+	+
Tannins	+	+

Terpenoids	+	+
Carbohydrates	+	+

+, present; - , absent

ANTIOXIDANT ASSAY

Data in table 2, described the minimum and maximam scavenging of DPPH by samples and these were compared to standard ascorbic acid which showed lower values than present study. Tables 3 was also described minimum and maximum scavenging of ABTS by samples and these were compared to standard ascorbic acid.

Table2. Percentage inhibition of DPPH by the plant extract and Ascorbic acid

Test	DPPH radical	Scavenging activity	at 517nm
Plant conc (µg/ml)	Leaves extract	Fruit extract	Ascorbic Acid
20 µg/ml	5.5± 0.351	1.6± 0.321	15.4± 0.264
40 µg/ml	19.5± 0.305	4.5± 0.351	24.4± 0.305
60 µg/ml	24.3± 0.251	6.6± 0.321	29.5± 0.404
80 µg/ml	25.6± 0.264	9.5± 0.351	36.4± 0.305
100 µg/ml	26.3± 0.152	20.4± 0.360	40.3± 0.321

Ascorbic acid IC50=127.8, Leaves 1C50= 185.1, Fruit 1C50=254.7,

Table3. Percentage inhibition of ABTS by the plant extract and Ascorbic acid

Plant conc (µg/ml)	Leaves extract	Fruit extract	Ascorbic Acid
20 µg/ml	30.5± 0.416	16.6± 0.360	40.3± 0.493
40 µg/ml	33.5± 0.4	22.3± 0.529	45.3± 0.3

60 µg/ml	41.5± 0.41	33.3± 0.251	51.5± 0.3
80 µg/ml	49.6± 0.321	43.5± 0.4	54.5± 0.351
100 µg/ml	50.5± 0.458	45.5± 0.416	57.2± 0.152

Ascorbic acid IC50=61.1, LeavesIC50=91.8, Fruit IC50=104, Seed IC50= 91.8

DETERMINATION OF ANTIMICROBIAL ACTIVITY

Data given in table 4. described the antimicrobial activity of methanolic and ethanolic extracts of samples against *E.coli* and *S.aureus* Methanolic extract showed more activity

Table 4. Antimicrobial activity of various methanolic extracts of fruits, leaves and seeds of *Eriobotrya japonica* against *E.coli* , *S.aureus*

Sample	Leaves extract	Fruit extract	Antibiotic (ciproflaxin)
E.coli	5.4± 0.25	4.6± 0.30	12.1± 0.1
S.aureus	1.3± 0.25	3.4± 0.40	10.1± 0.15

Primary metabolites such as nucleic acid, carbohydrates, proteins, fats and proteins which are essential for the plant manufactured from very small and simple molecules for examples

inorganic salts, carbohydrates, water, carbon dioxide and nitrogen containing hydrocarbons (Ettebong and Nwafor,2009; Fleuriet and Macheix,2003; Gbolade, 2009; Gulcin *et al*., 2002). Phytonutrients such as flavonoids, saponins, terpenoids, tannins, alkaloids, tannins, triterpenoids, glycosides and volatile oil are synthesize from these basic pimary metabolites. These phytonutrients are called secondary metabolites. Drugs can be prepared from these secondary metabolites because these have tendency to cure diseases and less toxic effect to living organisms (Ashafa *et al*., 2010; Brain *et al*., 1985; Chang *et al*., 2002 and Dastmalch *et al*., 2007).

QUALITATIVE ANALYSIS

Phytochemical analysis of *Eriobotraye japonica* fruits and Leaves are given in tables 5 and 6. Qualitative analysis shows the presence of many phytochemicals such as Flavonoids, alkaloids, phenols, saponins, tannins, terpenoids, glycosides in the plant extracts

(Harborn,1998;Halliwell and Gutteridge, 2007; Kesser *et al.,*2003). These phytochemicals were detected and used for therapeutic purpose because due all these phytochemicals plants has an antioxidant activity (Kim and Lee,2003; Liu, 2003;Loden and Andersson,1996; Mensor *et al*., 2001). Present study shows that all these phytochemicals present in *Eriobotrya japonica* plant and from experimental data it also shows that all these phytochemicals are present.

DETERMINATION OF ANTIMICROBIAL ACTIVITY;

The *Eriobotrya japonica* seeds, fruits and leaves extract of two regions were made in methanol and ethanaol in order to study antioxidant activity. These extracts were tested against *S.aureus* and *E. coli*. Methanol and ethanol were used because the extracts which was prepared in these solvent showed positive response. The tables 18 and 19 showed results. Disk method was adapted in order to study antimicrobial activity and for the measurement of inhibitory zones in mm.

The phytochemicals present in *Eriobotrya japonica* fruits, leaves and seeds due which plant showed increasing activity against bacteria.Methanolic extracts of *Eriobotrya japonica* seeds, fruits and leaves showed maximam activity against *E.coli* and *S.aureus* while the methanolic extracts againt *S.aureus* showed more good results than *E.coli* in the results (Moon and Shibamoto,2009; Nagavani *et al*.,2010 ; Nair and Chanda, 2007; Ndukwe *et al*., 2007; Okhwe, 2001). The ethanolic extracts of all these samples also showed effective killing against *E.coli* and *S.aureus* while the ethanolic extracts against *S.aureus* showed more good results than *E.coli*. Organic extracts showed more effective antibacterial activity than aqueous extract. This

may be due better solubility of the active components in organic solvents (Kaur and Arora, 2009).These results demonstrated that methanolic extracts of all samples have all highest activity. The study of study also support traditional application of plant and suggests that the plant extracts possess compounds with antibacterial properties that can be used as antibacterial agent for preparations of drugs (Ashafa *et al*., 2010; Oloyed,2005; Omoregie and Osagie, 2012; Ruberto *et al*.,2000; Ruch *et al*.,1989)

Table 5. Antimicrobial activity of various methanolic extracts of fruits, and leaves of *Eriobotrya japonica* against *E.coli*, *S.aureus*

Samples	**Leaves extract K**	**Fruit extract K**	**Seed extract K**	**Leaves extract H**	**Fruit extract H**	**Antibiotic (ciproflaxin)**
E.coli	3.1 ± 0.25	4.6 ± 0.30	6.1± 0.1	3.3 ± 0.3	3.2 ± 0.26	12.1± 0.1
S.aureus	1.3 ± 0.25	3.4 ± 0.40	4.2 ± 0.25	2.1 ± 0.3	2.4± 0.4	10.1± 0.15

Table shows that zone of inhibition in mm

ANTIFUNGAL ACTIVITY OF EXTRACTS OF *ERIOBOTRYA JAPONICA*

The *Eriobotrya japonica* seeds, fruits and leaves extract of two regions were made in methanol and ethanaol in order to study antifungal activity .These extracts were tested against *Aspergillus niger* and *Candida albican*. The results are presented in tables 20 and 21 which

indicates antifungal activity of ethanolic and methanolic extracts of all these samples against two fungal strains *Aspergillus niger*and *Candida albican* showing zone of inhibition (Soforwora, 1993; Surapaneni and Vishnu, 2009; Von Maydell, 1990; Wagner *et al*., 1994; Wenna *et al*.,2015).

Results shows that methanolic extracts of all these samples have maximum antifungal activity than ethanolic extracts. Methanolic extracts against *Aspergillus niger* showed more antifungal activity than *Candida albican*. The ethanolic extracts of all these samples also showed effective killing against *Aspergillus niger* and *Candida albican* while the ethanolic extracts against *Aspergillus niger* showed more good results than *Candida albican*. The results obtained in present study are contrary to results reported earlier. It is predicted that due to phytonutrients steroids and tannins that were best phenolic plant exhibited such type of activity.

Table 6. Antifungal activity of various methanolic extracts of fruits , leaves and seeds of *Eriobotrya japonica* against *Aspergillus Niger* and *Candida albican*

Samples	Leaves extract K	Fruit extracts K	Seed extract K	Leaves extract H	Fruit extracts H	Seed extracts H	Antifungal (Fluconazole)
Aspergillus	4.5± 0.3	4 ± 0.2	3.4± 0.2	6.1± 0.4	2.1± 0.1	4.1± 0.3	20± 0.1

Niger							
Candida albican	2.3± 0.4	-	1.1± 0.3	2± 0.1	-	2.1± 0.3	25.3± 0.3

-, absent , Antifungal activities of various extracts

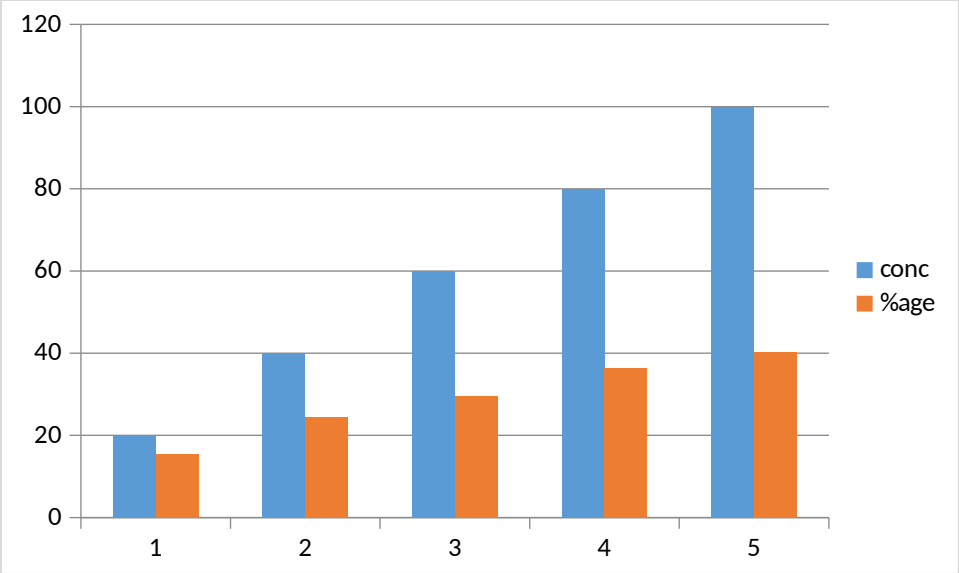


Figure 1. Inhibition of DPPH by Asorbic acid

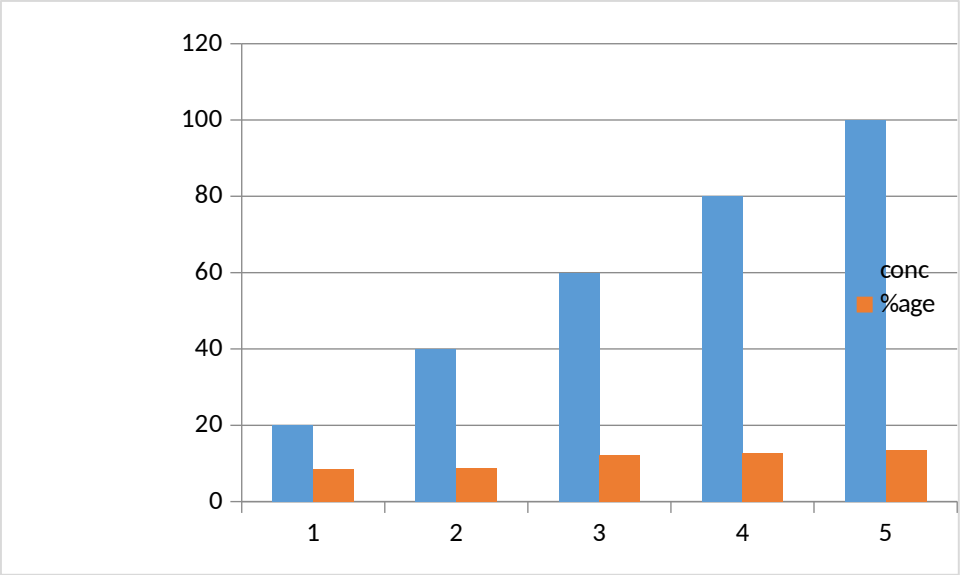


Figure 2. Inhibition of DPPH by fruit extract

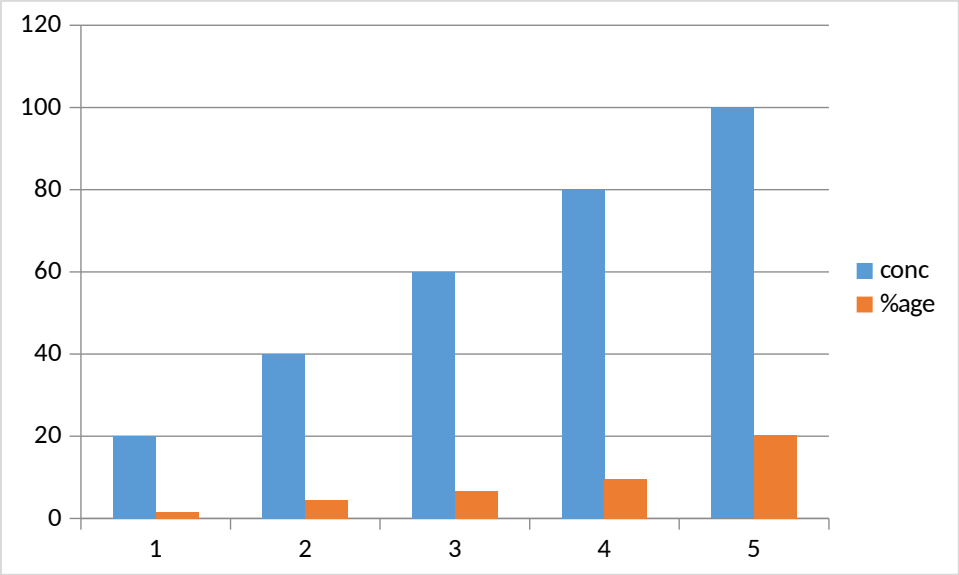


Figure 3. Inhibition of DPPH by fruit extract

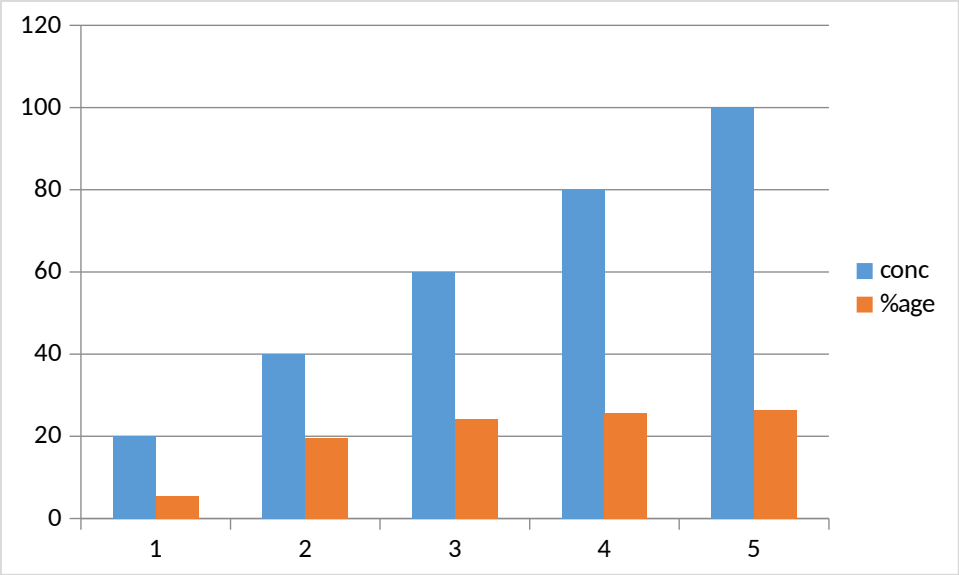


Figure 4. Inhibition of DPPH by leaves extracts

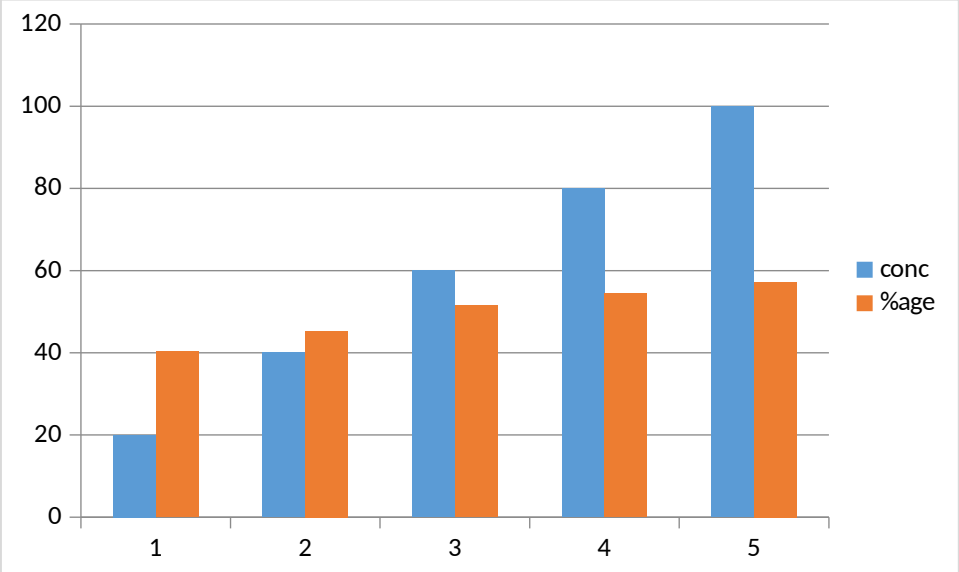


Figure 5. Inhibition of ABTS by Ascorbic acid

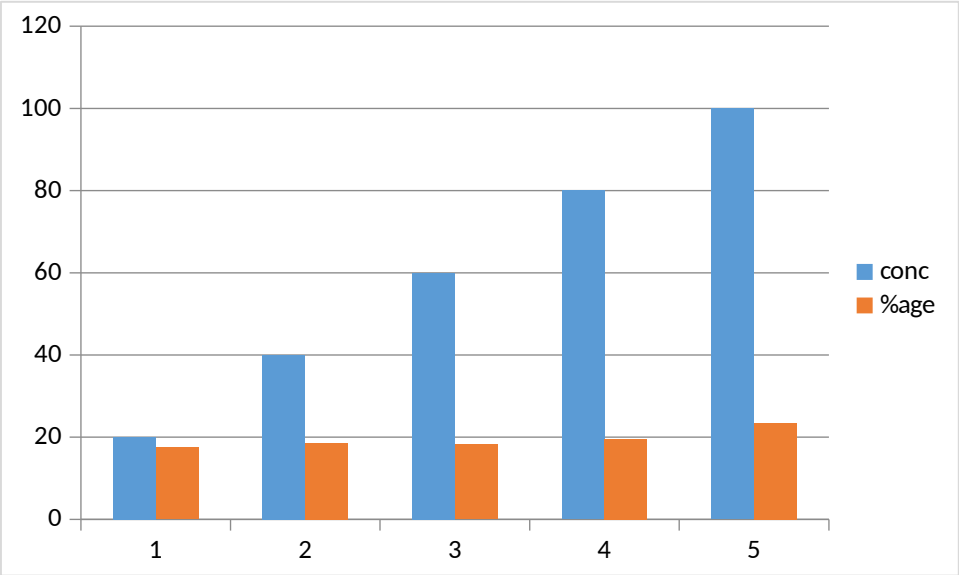


Figure 6. Inhibition of ABTS by fruit extracts

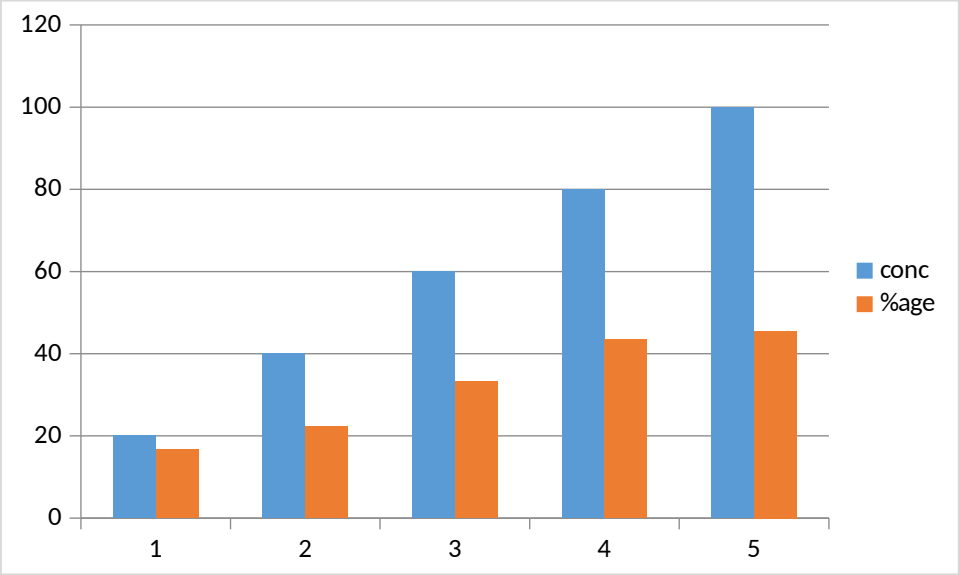


Figure 7. Inhibition of ABTS by leaves extracts

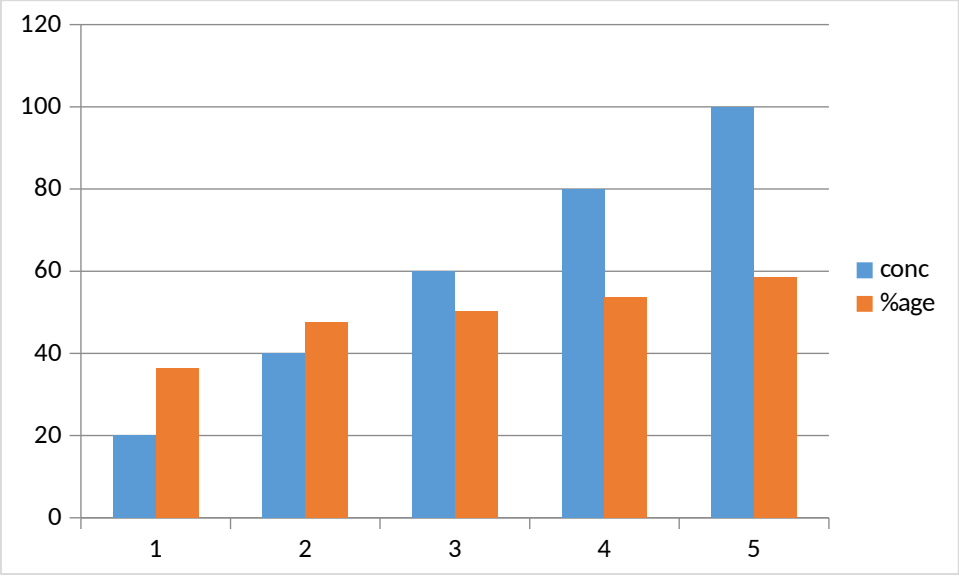


Figure 8. Inhibition of H_2O_2 by Ascorbic acid

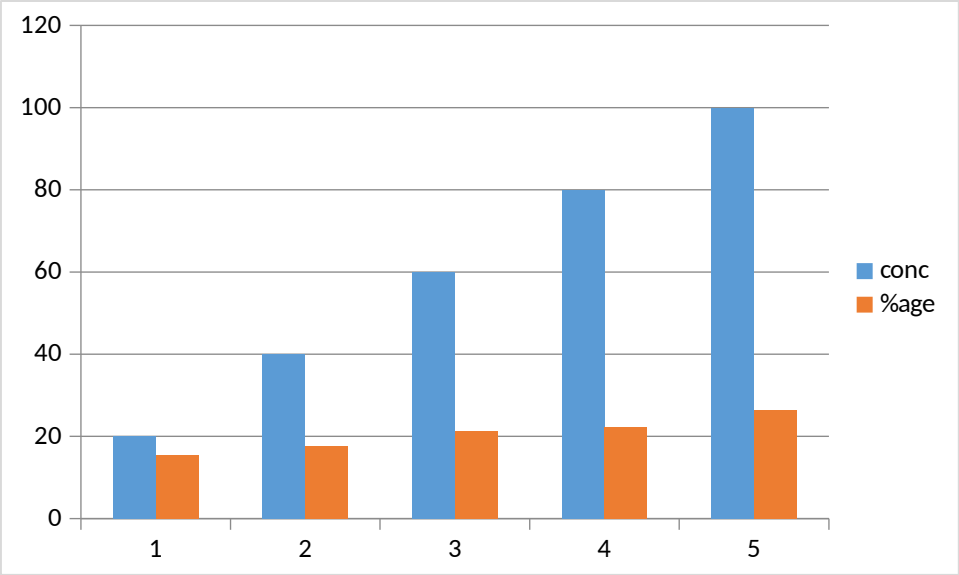


Figure 9. Inhibition of H_2O_2 by fruit extracts

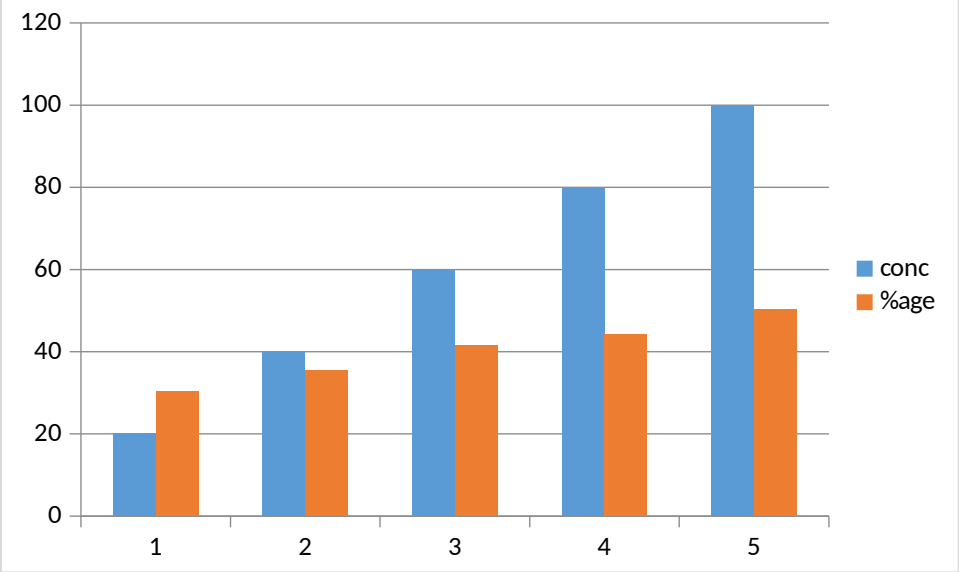


Figure 10. Inhibition of H_2O_2 by leaves extract

ANALYSIS OF QUERCETIN (FLAVONOIDS) BY USING HPLC

In order to analyse quercetin content in *Eriobotrya japonica* leaves and fruits HPLC was used.

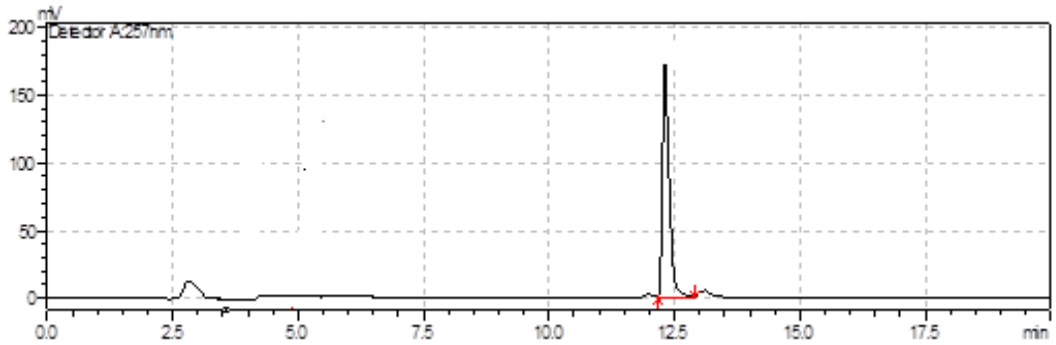
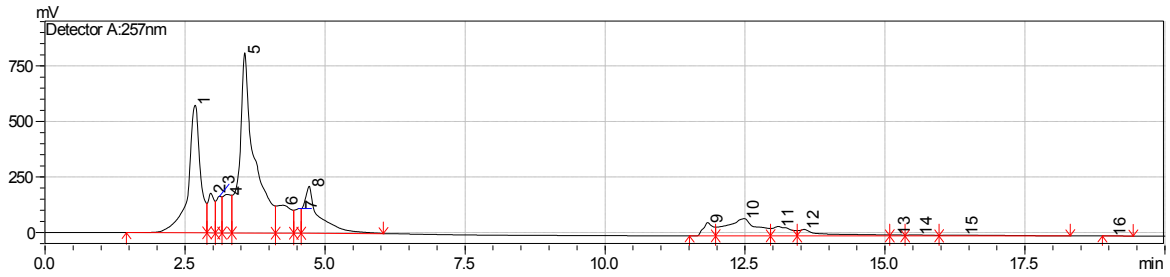


Figure 11. Quercetin Standard Peak



AREA	CONCENTRATION (ug/ml)
293868	8.761836

Figure 12. Analysis of quercetin from fruit (*Eriobotyrya Japonica*).

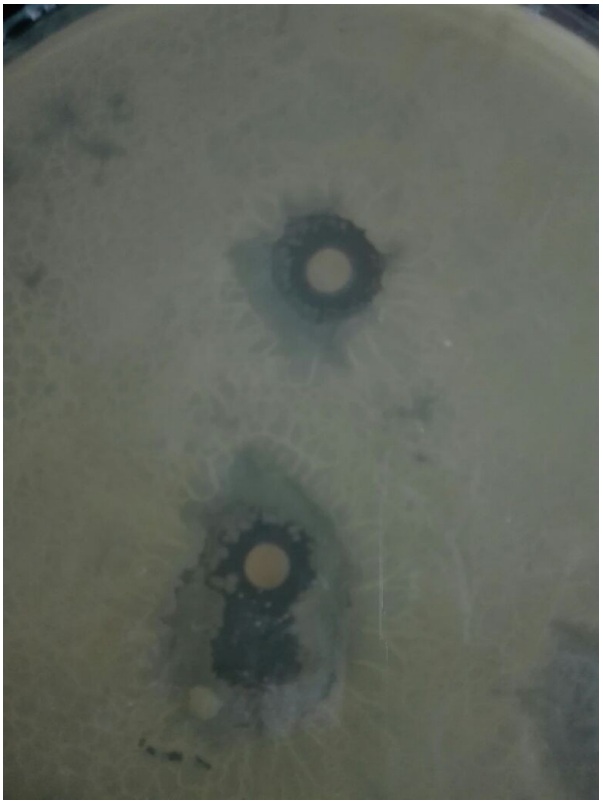


Figure 13. Antimicrobial activity of ciproflaxin (antibiotic) against *E.coli*

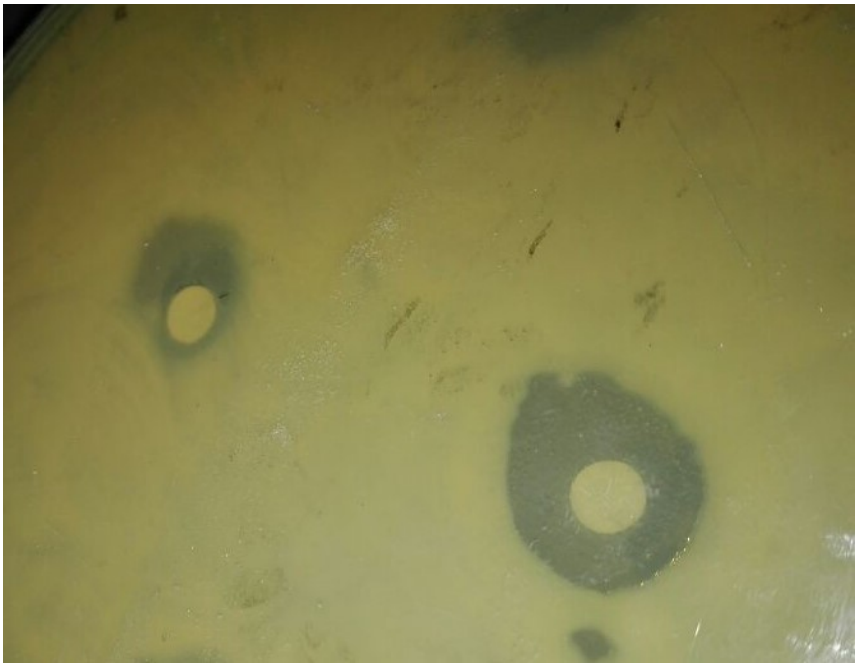


Figure 14. Antimicrobial activity of ciproflaxin (antibiotic) against *S.aureus*

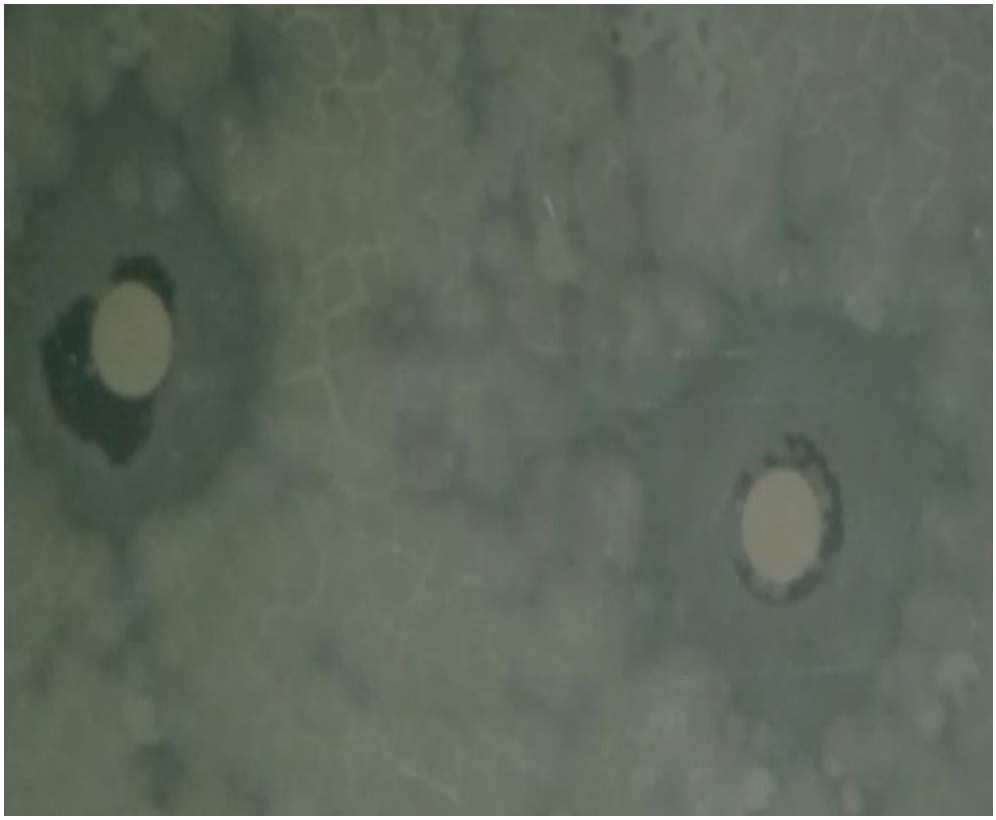


Figure 15. Antimicrobial activity of *Eriobotrya japonica* fruit against *E.coli*

Figure 16. Antimicrobial activity of *Eriobotrya japonica* fruit against *S.aureus*

Figure 17. Antimicrobial activity of *Eriobotrya japonica* leaves against *S.aureus*

SUMMARY

Eriobotrya japonica is a plant that belongs to family Rosaceae. That plant contains fruit which is commonly called loquat so it is a fruit containing plant which has a lot of beneficial effects. The fruits 0f *Eriobotrya japonica* are full grown in late winter or early spring and in the autumn or early winter, the flowers may appear which are 2 cm in diameter. The flowers are

white in colour with five petals which are produced in stiffpanicles of three to ten flowers.*Eriobotrya japonica* high in vitamin A, low in saturated fats, dietary fibers, manganese, sodium and potassium. *Eriobotrya japonica* seeds and youngleaves are basically slightly poisonous because it contain small amount of cynogenic glycosides (including amygdalin) that produce cyanide when it is digested by the consumer. Due bitter taste it cannot be eaten by the consumer due to which consumer may prevent from harm.

It observed that *Eriobotrya japonica* extracts contained significant amount of fats, protein fibers and carbohydrates. Plant was also contained phytochemical due which plant exhibited very good antioxidant activity. Due to antioxidant activity plant prevented the body from many chronic diseases such as high blood pressure or hypertension, some kind of cancers, higher sugar level in blood and many heart related problems (Wagner *et al*.,1994; Liu,2003; Kessler *et al*., 2003).Plant was also inhibited the activity of bacterial and fungal strain in order to cure many diseases which was caused by these strains due to its antibacterial and antifungal activity.

Plant also contained a very good content of qurecetin which was analysed by HPLC and also have cytotoxicity activity . Due to very beneficial effects on body it can be used in many pharmaceutical industry in order to cure the diseases

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