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Phenolic compounds proliferation by HPLC: to find out antibacterial activities in *Ficus benghalensis* plant extract

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Abstract
Ficus benghalensis belongs to family Moraceae were investigated for in vitro Anti-bacterial activity and phytochemical analysis. The present study demonstrates the anti-bacterial activity and anti-cancerous trend of tropical tree (*Ficus benghalensis)* on the foundation of area of hindrance. For finding the various bacterial strains to check its anti-bacterial activity of a *Ficus* species three different fractions of ethanol, methanol and aqueous also used by using agar disc diffusion method. *Ficus benghalensis* reveals hinderance at the range of 0.05mg to 0.2 mg in opposition to bacteria tested. Antimicrobial activity of *Ficus benghalensis* was determined by the calculated sample of leaf, roots and fruits. These extracts were used against the four types of bacteria *Staphylococcus aureus, Escherichia coli, Pseudomonas protobacteria* and *Bacillus cereus*. The higher growth inhibition zones were reported from roots and fruit samples extract of *Ficus benghalensis*. The HPLC analysis was used for the determination of phenolic compounds, secondary metabolites and their biochemical synthesis. It was found from results that large number of phytochemicals was present in the root and fruit extracts of the *Ficus benghalensis*. It was suggested from our study that the use of roots and fruits of *Ficus benghalensis* may be helpful to produce antibacterial medicines.

Keywords: anti-bacterial, anti-cancerous, ethanol extracts, phytochemicals, HPLC, Bacterial strain

Introduction
Banyan tree is generally known as *Ficus benghalensis* related to the family Moraceae. In the moonsoon and during consecutive rain fall Ficus species growth increases very rapidly and they grow up to 35meters tall and their aerial branches suspended. Member of this family shows anti-bacterial activity against *Bacillus cereus* and *Pseudomonas aureus* which pretend the resistance against the multi drugs (Chindera *et al*. 2016) [4]. *Ficus* species belong to the Moraceae family which contain about 1000 species and included up to 2500 different kinds of woody plants, bushes and some creeping plants are present in a tropical and sub-tropical region in the world (Rønsted *et al*., 2007; Serrato *et al*., 2004) [14, 15]. For the treatment of inflammation occurring in the body stem bark of *F. racemose* was used to control the growth of the bacteria such as *Bacillus cereus*. Anthelmintic activity anti-tumor activity and antibacterial activity are shown by the fruit extracts of Ficus benghalensis. The acetone fraction shows great antibacterial activity in opposition to the gram-positive bacteria and gram -negative bacteria while the extracts which are obtained from the fruit of the *Ficus benghalensis* do not indicate the antibacterial activity. The phytochemical was analyzed by the aqueous and methanol extract of *Ficus benghalensis* represents the secondary metabolites. Plants are mainly used for the medical care of many human sicknesses which causes infection in the human body because they contain the active components such as tannins saponins, alkaloids, terpenes. *Ficus* species depicts the different potentials to control the stress full diseased such as asthma (Anil *et al*., 2016; Singh *et al*., 2014) [1, 16]. Traditional medicines are manufacture from the F. benghalensis for eye-disease, constipation, toothache, inflammation, leukodema, headache, fever, rheumatic affections, asthma cigarettes, antidote for snakebites and relieve stomachache cure (Azam *et al*., 2010) [2]. Plants are used as a medicinal source from the millions of years ago for the preventing measurements of human disease because they contain many active reagents such as (flavonoids, terpenoids, and alkaloids) which have a chemotherapist value (Natalia *et al*., 2014) [13]. About 80% people believe on the herbal medicines which are derived from the plants for the treatment of many harmful disease and many microbe resistant antibiotics are prepared from the plants (Souhila *et al*., 2019) [18]. After the progress of the microbiology many natural antibiotics derived from the stem, root, leaves, flowers, fruits of the plant these resistant antibiotics are most beneficial for the cure of many human disease. Many clinical drugs have been obtained from the natural products such as from the phytochemicals (Gopukumar and Praseetha, 2015) [10]. Many resistant bacteria are used as a therapeutic agent (Catarina, 2019) [5]. Secondary metabolites compounds exhibit inhibitory effect bacteria (Sneha *et al*., 2016) [17]. Ficus benghalensis shows Anti-helminthic Activity in which aqueous and methanolic extract is used to kill the earthworms not only use to paralyze. Anti- stress and Anti-allergic potential are shown by the bark extract of Ficus benghalensis used for the treatment of asthma and produced by leukocytosis and eosinophilia. Due to ethanolic, aqueous, ethyl acetate extract produced in the leucocytes and eosinophils in a successive amount.

Materials and Methods
Work place and collection of plant Material
Complete experimental work was done in the University of Lahore at the institute of Molecular Biology and Biotechnology (IMBB) with in the research laboratory of chemical biology and molecular biology. Fresh plant

samples of *Ficus benghalensis* such as fresh aerial roots, leaf and fruit was accumulated from the various areas of the Punjab (Lahore, Shakargarh, and Kasur). The *Ficus benghalensis* were aggregated, wipe with tap water, to clean the dust particles from the (leaves, root and fruits) and useless material then run off with distilled water. The washed sample wizened in the oven. The dried sample was crushed into small pieces. The crushed plant material is converted into the fine powder by the help of the blender. Sample are weighed and then take a jar wash with water. Weighed sample will be enter into the jar and then add ethanol into the jar. Jar will be closed with a lid or a cap.

Isolation and sample preparation

The three jars were taken in which sample of root, leaf, fruit of Ficus benghalensis and ethanol added then will cover with lid remain for 24 hours. After 24 hours the mixed solution of ethanol and plant extracts will be separated. The liquid material will be separated into another jar and residue will be remaining in the previous jar. Four different types of extracts were prepared each from *Ficus benghalensis* using acetone and water. Confirmed sample material was stored in the autoclaved bottle of 25ml at 4 refrigerators until use. The prepared autoclaved bottles were labelled exactly and stoppered carefully.

Storage of bacterial samples

The four types of bacterial strains were studied in this project. *Escherichia coli, Pseudomonas protobacteria, Staphylococcus aureus, Bacillus cereus* were poured into the freshly prepared Nutrient Agar plates and incubated for 1day. The plates were covered with aluminium foil then saved up to 5°C after that growth visible. For the better performance of the experiment organisms were freshly prepared until 24 hours culture was used. For the purity of the result bacterial culture was maintained by regular sub culturing. *Staphylococcus aureus, Escherichia coli, Pseudomonas protobacteria and Bacillus cereus* were used for antibacterial activities of plant sample extracts.

Identification of phenolic compounds by HPLC

Many phenol containing compounds were obtained from the fractions of *Ficus benghalensis* by using HPLC technique. 20 milligrams of plant extracts were mixed in the 5ml of hydrochloric acid HCl, 12ml of methanol in 18 ml of distilled water. For 2 hours obtained mixture was incubated at 90˚C then strained with 0.2mm Millipore membrane strainer before injection into HPLC.

Results

Antibacterial activity of the *Ficus benghalensis* was determined with different extracts of the acetone and aqueous. These extracts were used against the four types of bacteria *Staphylococcus aureus, Escherichia coli, Pseudomonas protobacteria* and *Bacillus cereus* under the positive control. The ethanolic and methanolic fraction show the zone of inhibition at the dose of 50 µl, 100 µl and 150µl against the various bacterial strains. The diameter shown for the various bacteria against *Staphylococcus* aureus have an inhibition zone 7.5mm, 5.4mm and 6.9mm while positive control 8.6 mm in diameter. The results showed that the extracts which were obtained from the leaves of *Ficus benghalensis* have a less antimicrobial activity against the all bacterial sample studied. The observations determined that the leaf sample also showed zone of inhibitory for the bacterial growth of *Escherichia coli* having a bacterial strain showing the diameter of inhibition zone 8.5mm, 9.6mm and 6.4mm while show the positive control 8.63 mm in diameter. *Pseudomonas protobacteria* showed the inhibition zone for the bacterial growth having a different bacterial strain showing the diameter of inhibition zone 0.6mm, 0.4mm and 1.2mm and show the positive control 0.73 mm in diameter. *Bacillus cereus* having a bacterial strain showing the diameter of inhibition zone 0.5mm, 0.6mm and 0.9mm while show the positive control 0.6mm in diameter (Figure 1).

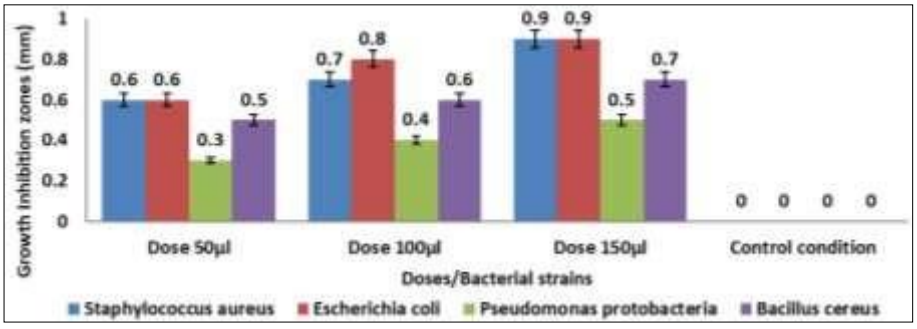

Fig 1: Antimicrobial activity for leave extract of *Ficus benghalensis*

The inhibition diameter shown for the various bacteria against *Staphylococcus aureus* have an inhibition zone 9.0mm, 8.7mm and 6.9mm while positive control 8.73 mm in diameter. The results showed that the extracts which is obtained from the fruits of *Ficus benghalensis* have a less antimicrobial activity against the all bacterial sample studied. The bacterial growth of *Escherichia coli* having a bacterial strain showing the diameter of inhibition zone 8.9mm, 7.6mm and 9.8mm while show the positive control 0.763mm in diameter. *Pseudomonas protobacteria* showed the inhibition zone for the bacterial growth having a different bacterial strain showing the diameter of inhibition zone 5.00mm, 7.4mm and 9.5mm and show the positive control 7.4 mm in diameter. *Bacillus cereus* having a bacterial strain showing the diameter of inhibition zone 2.5mm, 7.6mm and 7.7mm while show the positive control 7.6mm in diameter (Figure 2).

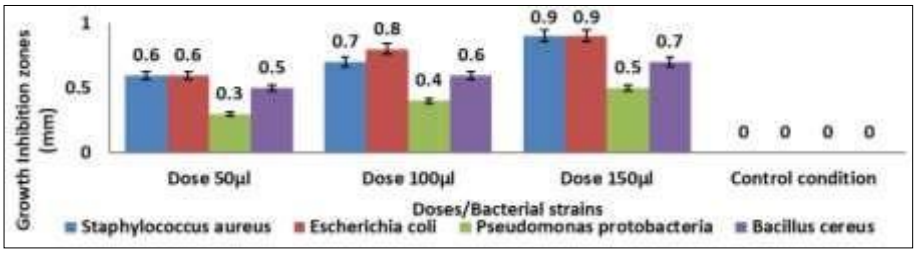

Fig 2: Antimicrobial activity for fruit extract of *Ficus benghalensis*

The inhibition diameter shown for the various bacteria against Staphylococcus aureus have an inhibition zone 0.6mm, 0.7mm and 0.9mm while positive control 0.73 mm in diameter. The results showed that the extracts which were obtained from the roots of *Ficus benghalensis* have a less antimicrobial activity against the all bacterial sample studied. The bacterial growth of *Escherichia coli* having a bacterial strain showing the diameter of inhibition zone 0.9mm, 0.6mm and 0.8mm while show the positive control 0.763mm in diameter. *Pseudomonas protobacteria* showed the inhibition zone for the bacterial growth having a different bacterial strain showing the diameter of inhibition zone 0.3mm, 0.4mm and 0.5mm and show the positive control 0.4 mm in diameter. *Bacillus cereus* having a bacterial strain showing the diameter of inhibition zone 0.5mm, 0.6mm and 0.7mm while show the positive control 0.6 mm in diameter**.**

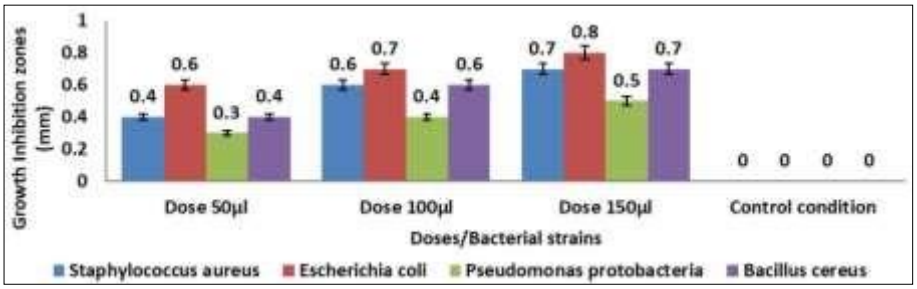

Fig 2: Antimicrobial activity for roots extract of *Ficus benghalensis*

HPLC test of roots, leaves and fruit samples from *Ficus benghalensis*

The data obtained from the HPLC test of the *Ficus benghalensis* root extracts show 7 different types of phenolic compounds such as (Lutein, Anthocyanin cyanidin 3-glucoside equivalent (CGE)), Chlorogenic acid, Caffeic acid, Quercetin, Naringenin, Kaempferol, Malondialdehyde (MDA), Morin) were identified and quantified. It was found that from roots sample methanolic extract; there were seven phenolic compounds identified as cyanidin 3- O-glucoside equivalent (CGE) (0.012mg/g), cyanidin 3-glucoside (Cy-3-Glu) (0.232mg/g),Caffeic acid (0.344 µg/g), Quercetin (2.500 mg/g),, Naringenin (2.186 mg/g),, Kaempferol (1.536 mg/g),, Malondialdehyde (1.164mg/g), (Table 1; Figure 4). It was found that from leaves sample methanolic and ethanolic extract; there were seven phenolic compounds identified as cyanidin 3- O-glucoside (Cy-3-Glu) (0.132µg/g) Caffeic acid (0.344), Quercetin (2.500), Naringenin (2.186),, Kaempferol (1.536 mg/g), Malondialdehyde (1.164mg/g) (Table 1; Figure 5). It was found that from fruit sample methanolic and ethanolic extract; there were seven phenolic compounds identified as cyanidin 3-glucoside (CGE) (0.012mg/g) 3-O-glucoside (Cy-3-Glu) (0.232mg/g) Chlorogenic acid (0.155 µg/g), quinin acid(0.344 µg/g), Quercetin (2.500 µg/g),, Naringenin (2.186 µg/g),, Kaempferol (1.536 mg/g),, Malondialdehyde (1.164mg/g), (MDA). The higher amount of phenolic acids indicated that there was presence of antibacterial activities in *Ficus benghalensis* roots and fruits (Table 1; Figure 6).

Table 1: HPLC test for root, leaves and fruit samples of *Ficus benghalensis*

Phenolic compounds	Root sample (mg/g)	Leaf sample (mg/g)	Fruit sample (mg/g)
Lutein	--	1.145	--
Anthocyanin	--	1.557	--
cyanidin 3-glucoside equivalent (CGE)	0.012	-	0.012
cyanidin 3-glucoside (Cy-3-Glu)	0.132	-	0.132
Chlorogenic acid	0.355	-	0.355
Caffeic acid	0.344	-	0.344
Rutin	--	1.041	--
Ferulic acid	--	-	--
Morin	--	-	--
Quercetin	2.500	-	2.500
Naringenin	2.186	-	-
Kaempferol	1.536	-	-
Malondialdehyde (MDA)	1.164	-	-

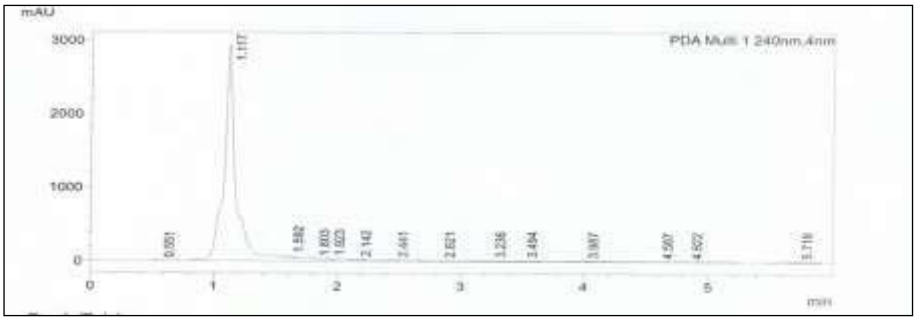

Fig 4: HPLC graphs for root sample

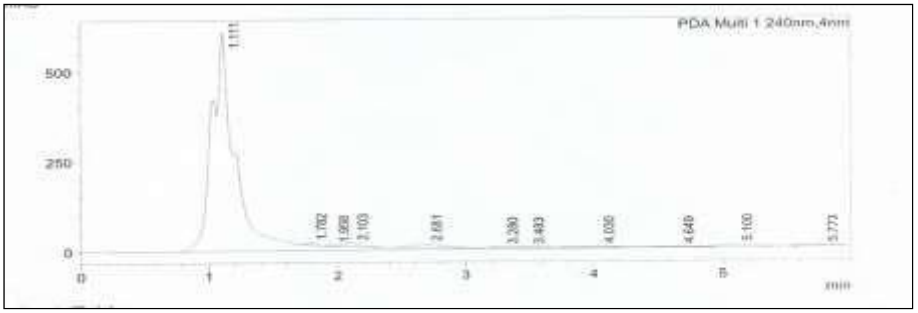

Fig 5: HPLC graphs for leaf sample

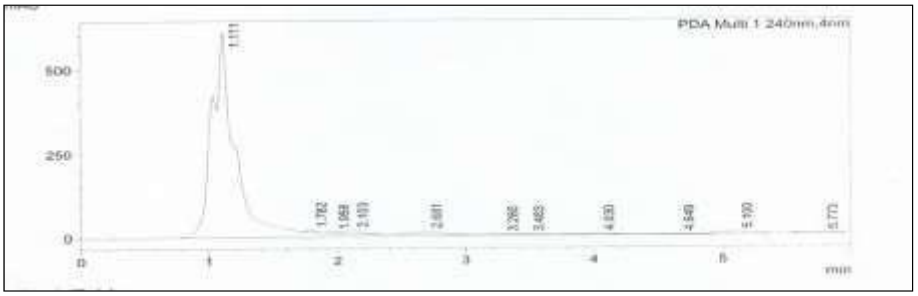

Fig 6: HPLC graphs for leaf sample

Discussions

The leaf sample of showed hindrance for a bacterial growth and *E. coli* showed the greater concentration of inhibition zone for all the extracts of roots, bark, fruit and leaf. The leafy extracts show the positive control against the *E. coli* with a greater zone of inhibition. Least zone of inhibition was shown by the *pseudomonas protobacteria* for the treatment of bacterial growth. The result from fruit extract showed that *staphylococcus aureus* having a greater sector of hindrance for two extracts pretend the positive control of inhibition diameter. The lager the inhibition diameter pretended that the extracts having an antibiotic used for the controlling of bacterial growth of required bacteria in the host cell and also used for the cure of many harmful diseases such (as asthma, cancer etc.) by bacterial strains. After the progress of the microbiology many natural antibiotics derived from the stem, root, leaves, flowers, fruits of the plant these resistant antibiotics are most beneficial for the cure of many human disease. Many clinical drugs have been obtained from the natural products such as from the phytochemicals (Farag *et al*., 2014) [9]. Many resistant bacteria are used as a therapeutic agent (Cohen, 1992). Secondary metabolites compounds exhibit inhibitory effect against bacteria (Sneha *et al*., 2016) [17]. A technique which is also known as liquid chromatography (LC) utilized for the discovering of phenolic compounds which is occurring in the *Ficus benghalensis.* Identification method was applied for the separation of flavonoids, steroids, caffeic acid, coumarin, tannins, saponins, prostaglandins and other secondary metabolites present in the plant extracts (Ephraim *et al*., 2008) [7]. This method was also applied for the biosynthesis, controlling of biological process, for the separation of antibiotics from the extracts. From our study we considered that the use of *Ficus benghalensis* roots, leaf, bark fruit extracts can be used as an antimicrobial agent for stopping the production of bacterial cells and diseases caused by these pathogens. These extracts were involved in the formulation of many medicines which can be used for the treatment of many diseases (Faiyaz *et al*., 2011; Kossive *et al*., 2018; Bhaskara *et al*., 2014; Manoj *et al*., 2008) [8, 3, 12].

Conclusion

It was found that the antibacterial and anti-cancerous activity of different extracts of *Ficus benghalensis* were shown by root, leaves and fruit extracts. It is a very low-cost effective method to control pathogenic bacteria for prevention of disease in animals and human.

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