



Pomegranate's Ethnobotanical Significance especially in Maternal Health Care in Kotmomin, District Sargodha as well as its Antimicrobial and Enzyme Inhibition Activities

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Abstract

Plants are considered a divine blessing, with 80,000 species deemed essential for medicinal use due to their crucial role in human welfare and environmental balance. Ethnobotanical research underscores the economic, biological, and cultural ties between people and plants, aiding in biodiversity conservation and rational resource utilization. Medicinal plants, constituting 18.9% of total flora, have been pivotal in traditional medicine, addressing ailments and combating infectious diseases, alongside synthetic drugs. Pomegranate, an ancient fruit with therapeutic potential dating back millennia, exemplifies traditional remedies, utilized for various ailments such as dysentery, diarrhea, and microbial infections through decoctions and folk medicine practices.

The survey revealed that *Punica granatum* (pomegranate) is commonly used for medicinal purposes by local people. Traditional uses include treating influenza, digestive disorders, cancer, diabetes, and various other ailments. Different parts of the plant, including the fruit, leaves, and seeds, are utilized for medicinal purposes. Traditional uses of pomegranate during and after pregnancy include consuming teas, decoctions, juices, and pastes for various health benefits.

Extracts from different parts of *Punica granatum* were tested for their antimicrobial activity against bacterial and fungal strains. The leaf, stem, peel, and seed extracts exhibited varying degrees of antibacterial and antifungal activities against different strains. The antimicrobial activity was assessed using minimum inhibitory concentration (MIC) values, with ethanol, methanol, and water as solvents. Results showed promising antibacterial and antifungal properties of *Punica granatum* extracts, suggesting their potential as natural antimicrobial agents.

The study also evaluated the enzyme inhibition activity of *Punica granatum* extracts against urease. Extracts from the root, leaves, stem, and seeds were tested for their ability to inhibit urease activity. The root and stem extracts showed significant antiurease activity, with

varying inhibitory concentrations. Ethanol extracts generally exhibited stronger antiurease activity compared to methanol and water extracts.

These findings demonstrate the diverse medicinal properties of *Punica granatum*, supporting its traditional use in healthcare systems and highlighting its potential for further pharmaceutical research and development.

INTRODUCTION

Among all naturally occurring biotic resources plants are considered as everlasting blessing of Allah almighty which play immense role in the life of living organisms (Marwat *et al.*, 2011).

For welfare of human being and also for environment, plants considered as major sources which play vital role among all living organisms (Mukhija *et al.*, 2010). The biological estimation of a plant species are considered as 250,000 in which 80,000 plants have being considered as more essential for remedial of diseases has welfare for economically use in society due to dependence of three quarter world population globally on plants (Joy *et al.*, 1998).

Ethnobotany is an economic, biological and cultural inter-relationship study among plants and people of an area in which they occur. Ethnobotanical studies concentrated on contributing to knowledge of plant biodiversity on one hand and take this knowledge for more scientific and social mediations on the other hand. Ethnobotanical research also helps in creation of priorities of local community to certify that the local values are converted into rational use of resources and effective conservation of biological diversity (Ali *et al.*, 2006). In the developed as well as other countries, the use of medicinal plants has been increasing extremely. The recorded uses of plants as medicinal dates back over 5000 years to the earliest known civilizations, the Sumerians in southern Mesopotamia, modern day Iraq (UNESCO, 1994). More than 3.3 billion people in the non-developed countries use medicinal plant regularly because of its characteristic of backbone of traditional medicines (Singh R.2015). According to ancient system of medicine (Ayurvedic, Unani, and Chinese) various diseases can be cured by using plants. In different ways the new drugs, general field of references and encouraging situations have been delivered by studies of ethno medicine. In the medicinal industry Medicinal plants are a fundamental constituent of research advancement. According to the current studies, classes of medicinal plants have been extended up to 50,000 which comprises of 18.9% of total flora (Amjad *et al.*, 2017). All the rural and urban areas are well familiar about the importance of medicine for curing of various diseases especially in Pakistan; this way is known as Unaini Tib (Ghani *et al.*, 2012). Instead of Pakistan all the western and Asian countries are also accepting, admiring and characterizing ethnobotany (Jeruto *et al.*, 2008).

Synthetic drugs have their unique importance for curing of all types of diseases, (Zaika 1975). But all the local indigenous rulers people don't have believe on synthetic drug. Customary they only use to cure the diseases by focal plants due to less accumulation of chemicals and useful natural occurring compounds. These compounds are present the form of alkaloids , secondary metabolites, phenolic compounds, and antioxidant , and anti-inflammatory compound by the help of extract we can get these compound easily (Ashwat *et al.*, 2007), (Chanda *et al.*, 2011).

Reneshya, (2011) stated that all types of industries especially herbal industries depending to medicinal plants due to presences of various kinds of compound like antimicrobial, scented compound and alkaloid compounds etc. for treatment of many infectious diseases. Systematic trials on the antimicrobial possessions of plant components were first recognized in the late 19th century. Due to transferable ailments, the rate of mortality has increased. As a result of world record, it is concluded that about 50,000 people died due to transferable ailments. The most common transferable ailments initiating agents are known as *Escherichia coli*, *Salmonella spp*, *Staphylococcus aureus*. Drugs are used against these viral and bacterial infections (Alavijeh *et al.*, 2012). Now these day due to vast discoveries of antibiotics etc., or antimicrobial drugs infectious rate become reduce but in spite the all the relative every living organism try it level best to survive at the lost moment of its level. Same situation have been observed due to more resistance against microbial disease medicine because of high resistance properties of microorganisms against synthetic drugs, doctor are again recommending and focusing plant therapeutic agents against microorganism (Chanda and Rakholiya, 2011).

One of the ancient comestible fruits is pomegranate, *Punica granatum* belongs to family puniceae (Meerts *et al.*, 2009). For several thousands of years it has been extensively expended by numerous beliefs. From the time of Biblical era use of pomegranate has been observed its therapeutic potentials have echoed all over the millennia (Longtin 2003). The pomegranate seed considered as a negotiator of renaissance by the Babylonians, by the Persian seeds of pomegranate deliberated in vulnerability on battle fields while the Chinese believes that seeds of pomegranate signified as permanency and immortality (Aviram *et al.*, 2000). The pomegranate fruit was used for management of tapeworm and few other parasitic infections by the Egyptians in the ancient Egyptians culture. This fruit was also used as an anthelmintic and vermifuge to cure ulcers, aphthae, diarrhea, acidosis, dysentery, hemorrhage, microbial infections and respiratory pathologies. It was also used as antipyretic (Larrosa *et al.*, 2010; Lee *et al.*, 2010).

The foremost use of pomegranate in folk medicine displays its strong astringency making it eminent all over the world in the form of aqueous decoction(i.e. boiling hulls in water for 10;40 minutes), for remedial dysentery, diarrhea and also for stomatitis. The decoction can be drunk and can also be used as mouth wash, douche or enema (Boukef *et al.*, 1982; Caceres *et al.*, 1987; Nagaraju and Rao, 1990).

MATERIALS AND METHOD

ETHNOBOTANICAL STUDY:

Study area:

Ethno botanical data was congregated from forty locals of study area from 120 males and 120 females by using questionnaire method. The data was collected arbitrarily from herbal expert and common person at the age range (25-40, 40-55 and 55-70). The outcome of the results were rechecked and compare with the available literature.

ANTIMICROBIAL ACTIVITY:

The fully grown plant parts (root, stem, leaf and peel) of *Punica granatum* were collected. All plants samples were washed with tap water. Then all parts of both plants were dried in shadow. These dried plants samples were grinded in the electric grinder. Pomegranate and

loquat powder was equally divided into four parts each contained 24gm of sample and dissolved into 200 ml of ethanol, methanol and water separately making the 12 solutions. After a day and age of one week the concentrates were separated by utilizing channel paper. The filtrate that was acquired was dissipated with the assistance of rotary evaporator. Completely dried residue was subject to UV spectrophotometry and FTIR investigation.

Antimicrobial study:

Data collection:

The *Punica granatum* extracts were checked against following bacterial and fungal strains.

Bacterial strains:

1. *Pasteurella multocida* (gram negative)
2. *Staphylococcus aureus* (gram positive)
3. *Bacillus substillus* (gram negative)
4. *Escherichia coli* (gram negative)

Fungal strains

1. *Fusarium solani*
2. *Aspergillus niger*
3. *Aspergillus parasiticus*
4. *Microsporum ferrugineum*

The bacterial strains were cultured during the night at 37 °C and the fungal strains were cultured in sabourad dextrose agar during night at 28 °C.

Preparation of Reagents:

Nutrient agar (2.8%)

2.8% Nutrient agar was autoclaved at 121 °C for 15 minutes and used for bacterial medium.

Sabouraud Dextrose Agar (6.5%)

Sabouraud Dextrose Agar was used as assay for fungal medium and was prepared in distilled water by autoclaving at 121⁰c for 15 minutes having a concentration of (6.5%).

Nutrient broth (1.3%)

1.3% Nutrient broth was used for fungal medium and prepared in distilled water by autoclaving at 121⁰c for 15 minutes.

Potato Dextrose Agar (3.9%)

Potato dextrose agar having concentration (3.9%) was was utilized as assay for bacterial medium and prepared in distilled water by autoclaving at 121⁰c for 15 minutes.

Antibacterial assay:

Petri dishes were used to keep the pure bacterial culture. Nutrient broth (13g/ml) was poured in distilled water, shaken well and was autoclaved. Bacterial colonies were constantly stir in incubator shaker at 37 °C for 24 hours. The inoculants were prepared and store at 4 °C.

Antibacterial assay by disc diffusion method:

Disc diffusion method was used to analyze the antibacterial activity of *Punica granatum*. Nutrient agar, (14g/500 ml) was shaking well and discrete homogenously after poring it in the distilled water. The nutrient medium was sterilized and autoclaved for 15 minutes at 121 °C. Inoculums (50µl/50ml) were added to the medium and poured in Petri plates. After this small paper discs were laid smooth on growth medium containing the solution of (100µg/ml) of trial compounds. For the development and amplification of bacteria the Petri

plates having culture medium were then incubated for 24 hours at 37°C. The extracts of *Punica granatum* repressed and hindered the bacterial growth free zones were around the discs where the solution of the extracts of *Punica granatum* was located.

Antifungal assay:

Disc diffusion technique was utilized to assess the antifungal action of *Punica granatum*. Sabourad Dextrose agar medium was utilized to keep up the uncontaminated and unadulterated culture of fungal microorganism that was cleaned in hot air boiler at 180°C for 5 hours. For the fine development and advancement of fungal strains the cultures were incubated for 2-3 days.

Antifungal assay by disc diffusion method:

When growth medium was shifted to sterilized Petri plates, a paper discs were laid smooth on growth medium containing (20 mg/ml) of the solution of concentrates of pomegranate and loquat, was applied on nutrient medium and incubated at 28°C for 2 days for culturing fungus. Growth free zones were found around the plates because of the antifungal action uncovered by the extract of pomegranate.

Minimum inhibitory concentration (MIC) of extracts:

Micro well plate reader was used to estimate the minimum inhibitory concentration of plant extracts. Concentrations of the extracts of *Punica granatum* were prepared ranging from 50mg/ml to 5 mg/ml and dissolved in 1ml of solvent.

ENZYME INHIBITION STUDIES:

Acetyl-cholinesterase assay:

The AChE inhibition activity was performed according to the slightly modified method. Total volume of the reaction mixture was 100µL. it contained 60µL Na₂HPO₄ 50mM and pH 7.7. 10µL test compound (0.5 mm/well) was added, followed by the addition of 10µL (0.005 unit/well) enzyme. The content were mixed and pre-read at 405 nm. Then, content were pre-incubated for 10 min at 37°C. The reaction was initiated by the addition of 10µL of 0.5 mm/well substrate (acetylthiocholine iodide), followed by the addition of 10µL DNTB (0.5 mm/well). After 30 min of incubation at 37°C, absorbance was measured at 405 nm using 96-well plate reader Synergy HT, Biotek, USA. All experiments were carried out with their respective controls in triplicate. Galatamine (0.5 mm/well) was used as a positive control. The percent inhibition was calculated by the help of following equation:

$\% \text{ Inhibition} = 100 - (\text{Absorbance of Test} / \text{Absorbance of Control}) \times 100$ IC₅₀ values

Where, Control= total enzyme activity without inhibitor; test = activity in the presence of test compound.

IC₅₀ values were calculated using EZ-Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA).

Butyrylcholinesterase assay:

The BChE inhibition activity was performed according to the slightly modified method. Total volume of the reaction mixture was 100µL containing 60µL, Na₂HPO₄ buffer, 50 mm and pH 7.7. 10µL test compound 0.5 mm/well was added followed by the addition of 10µL (0.5 unit/well) BChE (Sigma Inc.). The content were mixed and pre-read at 405 nm and then pre-incubated for 10 min at 37°C. The reaction was initiated by the addition of 10 µL of 0.5 mm/well. After 30 min of incubation at 37°C, absorbance was measured at 405 nm using 96-weplate reader Synergy HT, Biotek, USA. All experiments were carried out

with their respective controls in triplicate. Galatamine (0.5mM/well) was used as positive control. The percent inhibition was calculated by the help of following equation.

$\% \text{ Inhibition} = 100 - (\text{Absorbance of Test} / \text{Absorbance of Control}) \times 100$ IC 50 values

Urease Assay:

The enzyme assay is the modified form of the commonly known Berthelot reaction (Creno et al., 1970). A total volume of 85L assay mixture contained 10 μ L of phosphate buffer of pH 7.0 in each well in the 96-well plate followed by the addition of 10...L of sample solution and 25 ...L of enzyme solution (0.1347 units). Contents were pre-incubated at 37°C for 5 minutes. Then, 40..L of urea stock solution (20mM) was added to each well and incubation continued at 37°C for further 10 min. After given time, 115...L phenol hypochlorite reagents were added in each well (freshly prepared by mixing 45...L phenol reagents with 70L of alkali reagents).

For color development, incubation was done at 37°C for another 10 mint. Absorbance was measured at 625 nm using the 96-well plate reader Synergy HT. The percentage enzyme inhibition was calculated by the following formula:

$\text{Inhibition (\%)} = 100 - (\text{Absorbance of test sample} / \text{Absorbance of control}) \times 100$ IC 50 values (concentration at which 50% enzyme catalyzed reaction occurs) of compound were calculated using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA).

RESULTS

ETHNOBOTANY SURVEY

Traditional health care system and remedies have a prime importance in society. Medicinally important flora has been used to cure different ailments since ancient times. Folkloric and common people used commonly grown plants to cure certain diseases. Local people and specially the herbal specialists have great knowledge regarding the medicinally uses of these plant keeping in view these points. The ethnobotanical survey of medicinal plants was conducted in Kot Momin (District Sargodha) to evaluate the properties of selected plants belonging to two different families such as Punicaceae by visiting the study area and interviewing the local inhabitants as well as medical practitioners. A self-structured questionnaire was filled to get information related to medicinal uses of selected plant during the visit of studied area.

Responses of Practitioners

Data collected from people of age ranging from (20-35)

For collection of ethnobotanical information, different areas were selected randomly. All respondent gave the positive response about the medicinal uses of selected plants. Most of people were villagers. It was noticed that information related to use of plants for medicinal purposes were mostly same.

The data given in the Table 01 depicts different parameters that were used to collect ethnobotanical data. In this survey plant name, common name, plant part used and their medicinal uses were interviewed.

According to data presented in Table 01 shows that common name of *Punica granatum* is pomegranate. *Punica granatum* is commonly used to cure influenza, digestive disorders and cancer. It is also involve in regulation of blood insulin level, so can be used to cure diabetes.

In the second phase data related to medicinal uses of plant was collected from medicinal plant of age ranging from 35-55. It is observed after survey that this group of peoples has extended knowledge of medicinally important plants.

Data collected from people of age ranging from (35-50)

After the survey it was revealed that all the parts of plant can be used to cure different diseases.

Punica granatum has many anti-inflammatory and anti-septic properties. It was traditionally used to cure digestive disorders such as for the treatment of pinworm, hook worms and round worms as well as for the treatment of dysentery and diarrhea. *Punica granatum* extract had also many anti cancerous properties. It was also used as a coagulant. Pomegranate seeds have a potential to ameliorate the throat and cardiac diseases. Fruit of pomegranate decoction are used to treat stomach disorder. Data collected from specialists revealed that all component of *Punica granatum* shows strong astringent effects. The data mentioned in Table 01 represents that Pomegranate fruit control the insulin level in blood, so it plays a role in handling diabetes. Many herbal specialists referred that paste of leaves is useful for curing scabies, eczema, itchiness and ringworm. It was cropped up after survey report that fruit rind powder is very effective in gum bleeding and teeth whiteness.

Data collected from people of age ranging from (50-65)

According to the data mentioned in Table 01, it is evaluated that local people used *Punica granatum* for the treatment of various health issues. Traditionally it was used for the treatment of cancer. The whole plant of Pomegranate is being considered more beneficial for the cure of number of health issues like diabetes, diarrhea, stomachic and blood pressure.

Traditional Uses of Pomegranate in Pregnancy:

Pomegranate Seed Tea: Local healers might recommend boiling pomegranate seeds in water to create a tea-like infusion. This concoction is believed to be beneficial for pregnant women, as it is thought to alleviate nausea and provide essential nutrients. Some variations might include adding other herbs or spices for flavor or additional medicinal properties.

Pomegranate Peel Decoction: The dried peel of pomegranate fruit can be boiled in water to create a decoction. This decoction is often consumed as a traditional remedy for digestive issues such as diarrhea or dysentery. Pregnant women experiencing gastrointestinal discomfort might be advised to drink this decoction for relief.

Pomegranate Juice with Honey: Pomegranate juice mixed with honey is a common traditional remedy believed to boost immunity and provide energy. Pregnant women may be encouraged to consume this mixture to maintain their health and vitality during pregnancy.

Pomegranate and Milk Drink: Some traditional recipes involve blending pomegranate seeds or juice with milk to create a nutritious beverage. This drink is thought to be particularly beneficial for pregnant women, as it provides a combination of essential nutrients like calcium, vitamins, and antioxidants.

Pomegranate Seed Paste: Ground pomegranate seeds can be mixed with other ingredients like honey or yogurt to create a paste. This paste may be applied topically to the skin as a traditional remedy for various skin ailments such as acne, eczema, or wounds. Pregnant women experiencing skin issues might seek relief through this natural remedy.

Pomegranate Seed Infusion for Anemia: Pomegranate seeds or juice may be infused in water overnight, and the resulting liquid is believed to be effective in treating anemia due to its iron content. Pregnant women, who are often at risk of iron deficiency anemia, may be advised to consume this infusion regularly to boost their iron levels naturally.

Traditional Uses of Pomegranate after Pregnancy:

Cultural Significance of Pomegranate Post-Pregnancy:

Pomegranate holds significant cultural importance in Pakistan and India as a symbol of fertility, prosperity, and good health. After childbirth, it is commonly believed that consuming pomegranate aids in postpartum recovery and helps mothers regain strength and vitality.

Remedial Practices and Folklore:

Local traditions and folklore attribute various healing properties to pomegranate after pregnancy. It is believed that eating pomegranate seeds or drinking pomegranate juice can help replenish blood loss during childbirth and alleviate postpartum weakness. Additionally, some folklore suggests that hanging pomegranates in the home can ward off evil spirits and bring blessings to the newborn.

Nutritional Benefits and Healing Properties:

Pomegranate is rich in essential nutrients such as vitamin C, vitamin K, folate, and antioxidants. These nutrients are beneficial for new mothers as they support immune function, aid in tissue repair, and promote overall health and well-being. Pomegranate is also believed to stimulate lactation and provide energy to combat postpartum fatigue.

Traditional Recipes and Preparations:

Traditional recipes often include pomegranate as a key ingredient in dishes designed to nourish and revitalize new mothers. For example, "Anar ka Sharbat" (pomegranate juice) is a popular beverage consumed for its refreshing taste and purported health benefits. Additionally, pomegranate seeds are commonly added to salads, desserts, and savory dishes to enhance flavor and nutritional value.

Modern Perspectives and Integration:

While traditional beliefs regarding the use of pomegranate after pregnancy persist, modern healthcare practices also recognize the nutritional benefits of this fruit. Healthcare providers may recommend including pomegranate in postpartum diets to support recovery and promote optimal maternal and infant health. This integration of traditional wisdom with modern healthcare practices highlights the holistic approach to postnatal care in Pakistan.

Table 01: Data collected from people of different age group

Age	Scientific name	Local name	Part used	Form used	Diseases treated
Age (20-35)	<i>Punica granatum</i>	Pomegranate/ Anaar	Whole plant part	Decoction/ Juice	Anti-diabetic, diarrhea, treating cancer, pregnancy
(35-50)	<i>Punica</i>	Pomegranate/	Whole plant part	Decoction/	Anti-diabetic, heart problems, blood thinner,

	<i>granatum</i>	Anaar		Juice/ Raw fruit	intestinal parasites, dysentery, diarrhea, treating pregnancy, cancer,
(50-65)	<i>Punica granatum</i>	Pomegranate/ Anaar	Whole plant parts	Decoction/ Juice/ Raw fruit	Anti-diabetic, heart problems, blood thinner, diarrhea, stomach disorder, treating cancer, pregnancy

Percentage of age of respondents

Indigenous people rely on the herbal medicine to cure diseases. Data represented in Table 02 depicts that according to 31% respondents, *Punica granatum* is beneficial for diabetics. 15% respondent used for digestive disorder while according to 25% people heart problems can be cured by using *Punica granatum*. High %age of respondents (37%) prefer it to cure cancer, only 9% suggest it to cure high blood pressure while 39% recommended it for Pregnancy/Post Pregnancy cures.

Table: 02 Percentage of responses for the use of selected plants as traditional medicine:

Diseases	%age of <i>Punica granatum</i> Usage by people
Cancer	37%
Diabetes	30%
Digestive disorder	15%
Heart problems	25%
Blood pressure	9%
During pregnancy	37%
Post Pregnancy	39%

ANTIMICROBIAL

In the present research work, one objective was to check the antimicrobial activity of selected plants against different bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Pasteurella multocida* and *Bacillus substillus*) and fungal strain (*Aspergillus paracistic*,

Fusarium solani, *Aspergillus niger*, *Sportium ferrugenium*). Minimum inhibitory concentration (MIC) was noticed to check the antimicrobial potential of different part of selected plants (*Punica granatum*) by using ethanol, methanol and water as a solvent.

Antibacterial activity of *Punica granatum* against Bacterial strains

Antibacterial activity against *E.coli*

The control value of leaf extract against *E. coli* was (43±0.33µg/ml).when ethanol, methanol and water were used as solvent for leaf extract ,MIC values were -51%,-45% and -26% low as compared to control (Rifampicine 44±0.19µg/ml). i.e (21±0.32µg/ml) in ethanol, (22±0.20µg/ml) in methanol and (31±0.12µg/ml) in water.

When stem extract in selected solvent methanol, ethanol and water was checked for antibacterial activity almost same results were obtained like leaf extract i.e (32±0.30µg/ml), (25±0.23µg/ml) and (31±0.20µg/ml) which were again -25%,-45% and -27% lower as compared to control (44±0.19µg/ml).

Data presented in Table 06 depicts that the antibacterial activity of peel extract was much better as compared to leaf and stem extract. When ethanol were used as solvent MIC value was (45±0.34µg/ml) a little higher than control (43±0.33µg/ml).but in case of methanol and water, MIC was (34±0.20µg/ml) and (22±0.23µg/ml) i.e -0% & -25%.

When seed extracts was used to check the antibacterial activity, it was noticed that maximum MIC value was recorded in methanol (42±0.05µg/ml) against *E.coli* as compared to control. When ethanol and water were used as solvents, MIC value were (35±0.05µg/ml) and (39±0.04µg/ml), quite lower as compared to Rifampicine (27±0.23µg/ml) as given in Table 06.

Antibacterial activity against *S.aureus*

When *S.aureus* was used against different parts of plants increased antibacterial activity was recorded as compared to control (26±0.27µg/ml).

Maximum antibacterial activity (33±0.21µg/ml), (46±0.04µg/ml) and (58±0.13µg/ml) was observed when leaf extracts in different solvent ethanol, methanol and water was used against *S. aureus*. A slightly decrease (32±0.14µg/ml), (40±0.12µg/ml) and (51±0.13µg/ml) in antibacterial activity was recorded when stem extract was used in different solvent against *S. aureus*. But antibacterial activity was 20%, 59% & 101% higher than control.

Almost same result of antibacterial activity were recorded when peel extract was used in different solvent against *S.aureus*. Antibacterial activity were (33±0.04µg/ml), (40±0.13µg/ml) and (53±0.21µg/ml). MIC was recorded which were 31%, 56% & 106% higher than control.

The result of antibacterial activity was recorded (23±0.06µg/ml) -5%, (33±0.74µg/ml) 24% and (51±0.05µg/ml) 101%, when root extract was used in different solvent .In this case only the antibacterial activity of root extract in ethanol was lower as compared to control (26±0.27µg/ml).

Antibacterial activity against *P. multocida*:

When *P. multocida* was used strain against different part of plants, higher antibacterial activity was noticed as compared to control (35±0.58µg/ml).

When leaf extract of *Punica granatum* was used to ensure the antibacterial activity the highest susceptibility was measured in case of methanol (67±0.14µg/ml), but less

antibacterial susceptibility was recorded when ethanol and water were used as solvent i.e (56±0.03µg/ml), and (40±0.05µg/ml).

The stem extract of *Punica granatum* were found to be more active in case of water (73±0.04µg/ml) 105% as compared to control (35±0.58µg/ml).when ethanol was used as solvent, MIC values were (50±0.33µg/ml) and (61±0.14µg/ml) 41% & 75% higher than control.

The results of antibacterial activity were also recorded when peel extract was used, it exhibited an extensive range of antibacterial activity as compared to control (35±0.58µg/ml).MIC values in ethanol, methanol and water were (42±0.13µg/ml), (53±0.34µg/ml) and (64±0.24µg/ml) against *P.multocida*.

According to the data given in the Table 03, it was revealed that antibacterial activity of root extract was much closer to control (35±0.58µg/ml) in case of all solvents. When ethanol was used as solvent, the MIC value was (35±0.14µg/ml) and in case of water it was equal it was equal (36±0.06µg/ml) to MIC value of control. A slightly increase in MIC value was observed when methanol (43±0.05µg/ml) was used as solvent.

Antibacterial activity against *B. substilis*:

When *B. substilis* was used strain against different part of plants, higher antibacterial activity was noticed as compared to control (26±0.24µg/ml).

When leaf extract was checked for antibacterial activity in different solvent i.e ethanol, methanol and water given in Table 03. MIC values were (34±0.32µg/ml), (45±0.05µg/ml) and (32±0.33µg/ml) which were 38%, 81% & 39 % higher than control (26±0.24µg/ml).

Data presented in Table 03 shows that, when stem extract was used to check the antibacterial activity, it was recorded that MIC value were again 7%,49% & 82%higher as compared to control i.e (42±0.03µg/ml) in water, (34±0.11µg/ml) in methanol and (27±0.34µg/ml) in ethanol.

When peel extract was used to check the antibacterial activity against *B.substilis*, it was noticed that MIC values were slightly higher than control (26±0.24µg/ml). Antibacterial activity was (32±0.11µg/ml), (25±0.57µg/ml) and (40±0.44µg/ml) which were 43%, 15% & 56% higher than control.

Maximum antibacterial activity was observed, when root extract in different solvent was used MIC value were (50±0.25µg/ml), (38±0.05µg/ml), (46±0.36µg/ml), which were 102%, 117% & 85% higher than control against *B. substilis*.

Table: 03 Antibacterial activity of *Punica granatum* against Bacterial strain

Plant Parts	Bacterial strains	<i>E. coli</i>	% increase/ Decrease over control	<i>S. aureus</i>	% increase/ Decrease over control	<i>P. multocida</i>	% increase/ Decrease over control	<i>B. substilis</i>	% increase/ Decrease over control
Leaf	Ethanol	21 ± 0.32	-51	33± 0.21	31	56 ± 0.03	59	34± 0.32	38
	Methanol	22 ± 0.20	-45	46 ± 0.04	75	67 ± 0.14	85	45 ± 0.05	81
	Water	31 ± 0.12	-26	58 ± 0.13	120	40 ± 0.05	17	32 ± 0.33	39
Stem	Ethanol	32 ± 0.30	-25	32 ± 0.14	20	50 ± 0.33	41	27 ± 0.34	7
	Methanol	25 ± 0.23	-45	40 ± 0.12	59	61 ± 0.14	75	34 ± 0.11	49
	Water	31 ± 0.20	-27	51 ± 0.13	101	73± 0.04	105	42 ± 0.03	82
Peel	Ethanol	45 ± 0.34	0	33 ± 0.04	31	42 ± 0.13	21	32 ± 0.11	43
	Methanol	34 ± 0.20	-25	40 ± 0.13	56	53 ± 0.34	45	25 ± 0.57	15
	Water	22 ± 0.23	-49	53 ± 0.21	106	64 ± 0.24	76	40 ± 0.44	56
Seed	Ethanol	35 ± 0.05	-21	23 ± 0.06	-5	35 ± 0.14	-3	50 ± 0.25	102
	Methanol	39± 0.04	-6	32 ± 0.74	24	43 ± 0.05	19	38 ± 0.05	117
	Water	27 ± 0.23	-42	51 ± 0.05	101	36 ± 0.06	0	46 ± 0.36	85
	Rifampicine	43 ± 0.33		26 ± 0.27		35± 0.58		26± 0.24	

Antifungal activity of *Punica granatum* against fungal strains

Antifungal activity against A. paracistic

Data presented in Table 04 indicates that when leaf extract was used against different solvent, increased antifungal activity was recorded as compared to control ($22\pm 0.57\mu\text{g/ml}$). The MIC was recorded as ($26\pm 0.33\mu\text{g/ml}$), ($28\pm 0.27\mu\text{g/ml}$) and ($33\pm 0.12\mu\text{g/ml}$) in ethanol, methanol and water against *A. paracistic*.

The minimum potential was measured in stem extract against *A. paracistic* in methanol ($30\pm 0.34\mu\text{g/ml}$) i.e -36% MIC value was observed as compared to control ($22\pm 0.57\mu\text{g/ml}$). Antifungal activity was ($35\pm 0.15\mu\text{g/ml}$) and ($37\pm 0.25\mu\text{g/ml}$) which was methanol and water i.e -15% higher than control.

The antifungal activity of peel extract was much better as compared to leaf and stem extract. The result of antifungal was recorded ($34\pm 0.33\mu\text{g/ml}$) in ethanol ($49\pm 0.15\mu\text{g/ml}$) in methanol and ($27\pm 0.22\mu\text{g/ml}$) in water (74%), (141%) and (32%) higher than control.

According to data given in Table 04, it was revealed that antifungal activity of root extract of pomegranate was much closer to control in case of water ($32\pm 0.12\mu\text{g/ml}$) i.e 56% higher than control. While maximum antifungal activity was observed in ethanol and methanol ($40\pm 0.33\mu\text{g/ml}$) and ($24\pm 0.47\mu\text{g/ml}$) which is 56% and 109% higher than control against *A. paracistic*.

Antifungal activity against Fusarium solani

Data given in Table 04 presented that when *F. solani* was used against different part *Punicagranatum* of increased antifungal activity was recorded as compared to control ($20\pm 0.17\mu\text{g/ml}$). When leaf extract was checked for antifungal activity in different solvent i.e ethanol, methanol and water. MIC value were ($32\pm 0.12\mu\text{g/ml}$), ($37\pm 0.32\mu\text{g/ml}$) and ($44\pm 0.43\mu\text{g/ml}$) i.e -5%, 18% and 29% higher than control.

Result in Table 04 revealed that stem extract of *Punicagranatum* in water ($30\pm 0.17\mu\text{g/ml}$) and 17% more active against *Fusarium solani*. While in case of ethanol and methanol, MIC values were ($38\pm 0.15\mu\text{g/ml}$) and ($46\pm 0.57\mu\text{g/ml}$) i.e 13% & 42% higher than control. But all were higher as compared to control ($20\pm 0.17\mu\text{g/ml}$).

From the given data in Table 04 it is quite clear that peel extract of Pomegranate showed high results ($33\pm 0.61\mu\text{g/ml}$), ($40\pm 0.13\mu\text{g/ml}$), ($55\pm 0.16\mu\text{g/ml}$). Maximum susceptibility was noticed in case of water as compared to control ($19\pm 0.11\mu\text{g/ml}$) against *F. solani*.

When root extract was checked for antifungal activity in different solvent i.e ethanol, methanol and water MIC value were ($25\pm 0.24\mu\text{g/ml}$), ($34\pm 0.74\mu\text{g/ml}$) and ($51\pm 0.22\mu\text{g/ml}$) i.e 135%, 23% & 132% higher than control.

Antifungal activity Against A. niger:

When *A. niger* was used as strain in leaf extract of Pomegranate, higher antifungal activity was noticed as compared to control ($19\pm 0.41\mu\text{g/ml}$). MIC values in ethanol, methanol and water were ($42\pm 0.13\mu\text{g/ml}$), ($33\pm 0.34\mu\text{g/ml}$) and ($47\pm 0.43\mu\text{g/ml}$).

Data presented in Table 04 depicts the highest susceptibility was measured in case of methanol ($42\pm 0.33\mu\text{g/ml}$) in stem extract of Pomegranate. But less antifungal activity was recorded, when ethanol and water were used as solvent. MIC value were ($31\pm 0.34\mu\text{g/ml}$) and ($50\pm 0.22\mu\text{g/ml}$) -9% & 32% less than control.

According to data given in Table 04, it was revealed that antifungal activity of peel extract in methanol was 107% higher than control. When ethanol and water was used as solvents MIC value were $(36 \pm 0.67 \mu\text{g/ml})$ and $(23 \pm 0.79 \mu\text{g/ml})$ i.e 107% & 48% higher than control.

As far as antifungal activity of root extract is concerned, it was observed that methanol $(19 \pm 0.41 \mu\text{g/ml})$ was much closer to control $(19 \pm 0.41 \mu\text{g/ml})$. It is quite clear from data that maximum antifungal activity was noticed in ethanol $(35 \pm 0.57 \mu\text{g/ml})$ and water $(24 \pm 0.33 \mu\text{g/ml})$ against *A.niger*.

Antifungal activity against S. ferrugenum:

When leaf extract of Pomegranate was used it exhibited an extensive range of antifungal activity as compared to control $(13 \pm 0.76 \mu\text{g/ml})$. MIC value in ethanol, methanol and water were $(26 \pm 0.57 \mu\text{g/ml})$, $(39 \pm 0.43 \mu\text{g/ml})$ and $(28 \pm 0.44 \mu\text{g/ml})$ i.e -26%, -7% & -26% lesser than control.

Data given in Table 04 depicts that when stem extract in selected solvents ethanol, methanol and water was used to check the antifungal activity, it was recorded that MIC value were again higher as compared to control. MIC values were $(21 \pm 0.22 \mu\text{g/ml})$ -45% , $(32 \pm 0.28 \mu\text{g/ml})$ -18% and $(39 \pm 0.69 \mu\text{g/ml})$ 0%.

When peel extract was used to check the antifungal activity, it was noticed that maximum MIC value was recorded in methanol $(13 \pm 0.76 \mu\text{g/ml})$, which was much closer to control $(13 \pm 0.76 \mu\text{g/ml})$ in case of all solvents. On the other hand of ethanol and water MIC values were $(43 \pm 0.35 \mu\text{g/ml})$ and $(34 \pm 0.32 \mu\text{g/ml})$ i.e 257% and 176% higher than control.

According to data given in Table 04, it was revealed that antifungal activity of root extract in ethanol $(16 \pm 0.43 \mu\text{g/ml})$ and water $(44 \pm 0.27 \mu\text{g/ml})$, and in methanol it was much closer to control $(13 \pm 0.76 \mu\text{g/ml})$. But maximum antifungal activity was noticed in water $(44 \pm 0.27 \mu\text{g/ml})$ against *S.ferrugenum*.

Table 04: Antifungal activity of *Punica granatum* against fungal strains

Plant Parts	Fungal strains	<i>A. paracistic</i>	% increase/ Decrease over control	<i>Fusarium solani</i>	% increase/ Decrease over control	<i>Aspergillus niger</i>	% increase/ Decrease over control	<i>Sportium ferrugenum</i>	% increase/ Decrease over control
Leaf	Ethanol	26 ± 0.33	-41	32 ± 0.12	-5	42 ± 0.33	16	26± 0.57	-26
	Methanol	28 ± 0.27	-40	37 ± 0.32	18	31 ± 0.34	-9	39 ± 0.43	-7
	Water	33 ± 0.12	72	44 ± 0.43	29	50 ± 0.22	32	28 ± 0.44	-26
Stem	Ethanol	35± 0.15	-15	30 ± 0.17	-17	35 ± 0.11	-6	21 ± 0.22	-45
	Methanol	30 ± 0.34	-36	38 ± 0.15	13	45 ± 0.45	25	32 ± 0.28	-18
	Water	37 ± 0.25	-15	46 ± 0.57	42	36 ± 0.37	0	39 ± 0.69	0
Peel	Ethanol	34 ± 0.33	74	33 ± 0.61	40	36 ± 0.67	107	43 ± 0.35	257
	Methanol	49 ± 0.15	141	40 ± 0.13	102	15± 0.74	-5	13 ± 0.76	0
	Water	27 ± 0.22	32	55 ± 0.16	30	23 ± 0.79	48	34 ± 0.32	176
Root	Ethanol	32 ± 0.12	56	25 ± 0.24	135	35± 0.57	101	16 ± 0.43	24
	Methanol	40± 0.33	109	34 ± 0.74	23	19 ± 0.41	0	13± 0.76	17
	Water	24± 0.47	16	51 ± 0.22	132	24 ± 0.33	34	44 ± 0.27	282
	Rifampicine	22 ± 0.57		20± 0.17		19 ± 0.41		13 ± 0.76	

ENZYME INHIBITION ACTIVITIES OF SELECTED PLANTS:

Enzyme inhibition activity in (*Punica granatum*) against urease:

Table 05 depicts the antiurease activity of selected parts in different solvent i.e ethanol, methanol and water. The plants extract that shows the enzyme inhibition activity are given in Table 05. By comparing all the values with control Thiourea ($IC_{50}=21.25\pm 0.17\mu M$), It was concluded that almost all of extract exhibited minimum antiurease activity, as compared to control. Only the root extract of (*Punica granatum*) and stem extract of (*Punica granatum*) in ethanol resulted in maximum antiurease inhibitory activity.

Root extract of pomegranate showed the strongest antiurease inhibition activity ($IC_{50}=49.04\pm 0.04\mu M$) in methanol followed by the ethanol ($IC_{50}=34.13\pm 0.03\mu M$) and water ($IC_{50}=33.03\pm 0.14\mu M$) as compared to control i.e ($IC_{50}=21.25\pm 0.17\mu M$).

As far as the antiurease activity of leaves extract of Pomegranate is concerned the strongest antiurease inhibitory activity was measured in ethanol ($IC_{50}=15.04\pm 0.05\mu M$) as compared to control ($IC_{50}=21.25\pm 0.17\mu M$). When methanol and water were used as solvent, the inhibition activity were ($IC_{50}=46.05\pm 0.06\mu M$) and ($IC_{50}=22.04\pm 0.15\mu M$) minimum as compared to control.

Data presented in the Table 05 indicates that Pomegranate stem also exhibit strongest antiurease inhibition activity in ethanol ($IC_{50}=19.15\pm 0.04\mu M$) as compared to control. While in case of methanol and water, lower activity was measured as compared to control i.e ($IC_{50}=48.06\pm 0.13\mu M$) and ($IC_{50}=32.16\pm 0.13\mu M$).

Table 05 depicts that when ethanol extract was used as solvent for Pomegranate seed, it was noticed that all these values were minimum as compared to control ($IC_{50}=21.25\pm 0.17\mu M$). The antiurease activity were exhibited by ethanol, methanol and water were ($IC_{50}=35.07\pm 0.14\mu M$), ($IC_{50}=30.07\pm 0.35\mu M$) and ($c=36.67\pm 0.06\mu M$).

Table: 05 Anti-urease activities in *Punica granatum*

Sr. No	Plant part	Anti-urease activity		
		Compound	Inhibition (%) at 0.5 Mm	IC ₅₀ μM
1.	<i>Punica granatum</i> Root	Ethanol	33.06±0.17	34.13±0.03
		Methanol	36.02± 0.13	49.04± 0.04
		Water	43.03±0.24	33.03±0.14
2.	<i>Punica granatum</i> Leaves	Ethanol	35.04±0.35	15.04±0.05
		Methanol	42.05±0.26	46.05±0.06
		Water	22.03±0.17	22.04±0.15
3.	<i>Punica granatum</i> Stem	Ethanol	98.14±0.74	19.15±0.04
		Methanol	31.25±0.95	48.06± 0.13
		Water	28.36±0.73	32.16±0.13
4.	<i>Punica granatum</i> Seed	Ethanol	35.05±0.04	35.07±0.14
		Methanol	27.64±0.45	30.07± 0.35
		Water	36.53±0.26	36.67±0.06
	A*	Standard-Thiourea	99.15±0.13	21.25±0.17

Anti-cholinesterase activity in *Punica granatum*

Table 4.8 describes the studies on plant extracts i.e *Punica granatum* and *Eriyobotrya japonica* that have been found to be a good acetyl –cholinesterase (AChE) and butyryl- cholinesterase inhibitors (BChE).

When **root** extract of Pomegranate was used to check (AChE) and inhibitors (BChE) activity, it was observed that water ($IC_{50}=2.34\pm 0.64\mu M$) showed strongest AChE inhibitory activities than control ($IC_{50}=4.0\pm 0.10\mu M$). Whereas ethanol ($IC_{50}=37.3\pm 17\mu M$) and methanol showed minimum (AChE) inhibitory activity as compared to control. As far as the root extract of *Punica granatum* is concerned it was observed that the BChE inhibitory activity were again maximum in water ($IC_{50}=12.5\pm 3.74\mu M$) as compared to control. But in ethanol ($IC_{50}=27.3\pm 4.12\mu M$) and methanol ($IC_{50}=18.4\pm 0.53\mu M$) quite low AChE activity were measured as compared to control. Table 4.8 depicts that when *Punica granatum* **leaves** Extract was used in different solvents minimum AChE inhibition activity were recorded as compared to control ($IC_{50}=4.0\pm 0.10\mu M$). But in case of BChE, it was noticed that in water ($IC_{50}=12.6\pm 0.24\mu M$) highest inhibition result was noticed while little difference was showed in methanol ($IC_{50}=18.6\pm 0.33\mu M$) and ethanol ($IC_{50}=26\pm 0.35\mu M$).

Stem extract of *Punica granatum* showed the strongest inhibitory activity ($IC_{50}=3.2\pm 0.46\mu M$) in Methanol as compared to control ($IC_{50}=4.0\pm 0.10\mu M$). While ethanol ($IC_{50}=15.4\pm 0.75\mu M$) and water shows lower activity as compared to control. In case of BChE the ethanol ($IC_{50}=17.6\pm 0.36\mu M$) and water ($IC_{50}=22.4\pm 0.66\mu M$) exhibited minimum inhibitory activity as compared to control, only the stem extract of *Punica granatum* in methanol ($IC_{50}=14.4\pm 0.25\mu M$) resulted maximum inhibitory activity.

According to Table it is clear that when seed extract of Pomegranate was used to check the AChE activity, it was noticed that minimum inhibitory activity was recorded i.e ($IC_{50}=23.5\pm 0.67\mu M$), ($IC_{50}=16\pm 0.45\mu M$), ($IC_{50}=43\pm 0.74\mu M$) as compared to control. Almost same result were obtained in BChE. Minimum inhibitory activity was observed in different solvent. BChE inhibitory activity were exhibited by ethanol, methanol and water were ($IC_{50}=28.3\pm 0.87\mu M$), ($IC_{50}=19.3\pm 0.48\mu M$) and ($IC_{50}=27.5\pm 0.59\mu M$) less than control.

By comparing all solvent i.e water, ethanol and methanol of extract in Pomegranate plant part (root, stem, peel, seed), it was clear that Pomegranate root and stem showed the strongest activity against AChE and BChE in all solvents. Among all these values root extract of Pomegranate in water ($IC_{50}=2.34\pm 0.64\mu M$ and $IC_{50}=12.5\pm 3.74\mu M$) shows the least activation value against AChE and BChE with selective index (SI) **i.e 2.2** and same result exhibited in Pomegranate stem in methanol. The ($IC_{50}=3.2\pm 0.46\mu M$ and $IC_{50}=14.4\pm 0.25\mu M$) is the least activation value against AChE and BChE with selective index (SI) **i.e 0.4** was noticed.

Table 06: Anti-cholinesterase activity of *Punica granatum*

Sample Code	Compounds	(µM ±SEM) ^a		Selectivity Index (SI) ^b
		EeAChE	eqBChE	
<i>Punica granatum</i> root	ETHANOL	37.3±17	27.3±4.12	1.3
	METHANOL	33±0.13	18.4±0.53	3.5
	WATER	2.34±0.64	12.5±3.74	2.2
<i>Punica granatum</i> leaves	ETHANOL	23±0.35	26.06±0.35	0.5
	METHANOL	13.2±0.16	18.6±0.33	1.7
	WATER	24.3±0.33	12.6±0.24	0.8
<i>Punica granatum</i> stem	ETHANOL	15.4±0.75	17.6±0.36	0.3
	METHANOL	3.2±0.46	14.4±0.25	0.4
	WATER	13±0.46	22.4±0.66	1.4
<i>Punica granatum</i> seed	ETHANOL	235±0.67	28.3±0.87	0.8
	METHANOL	16±0.45	19.3±0.48	1.9
	WATER	43±0.74	27.5±0.59	2.7
A*	Galatamine	4.0±0.10	15.0±0.67	3.7

DISCUSSION

ETHNO MEDICINAL

Medicinal plants play an important role in primary health care system. So, the ethnobotanical survey in Kot Momin (District Sargodha) was held to reconnoiter the ethnobotanical importance of *Punica granatum* belonging to Puniceae to check out that how people use these plants for ailments of different diseases. The ethnobotanical survey of medicinal plants was conducted by visiting the study area and interviewing the herbal experts of age ranging from (as 25-40 and 40-55, 55-70 years of age) well as local people (25-40 and 40-55, 55-70 years of age).

According to data composed from the survey the common and local names of the selected plant are asked by the herbal expert and local people. The common name, local name, scientific name and family name all were collected and listed in Table 01. The respondent gave the positive response about the medicinal uses of plants against various diseases because the herbal product had no noticeable side effects (Sonibare *et al.*, 2009). The method used in survey were similar to other that old people are well aware of the use of the plants to cure the different disease and concluded that old peoples are more liable to the use of plants in primary health care with comparison to young generation (Qureshi & Bhatti, 2009; Sardar & Khan 2009).

From the present survey the information was collected from the respondent (Hakim and Local people) about the medicinal use of *Punica granatum*. The local name of *Punica granatum* (pomegranate) is anar, recently described as nature's power fruit used on folk medicine for curing disease (Abdel *et al.*, 2011) commonly cultivated in mediterranean region. It is noted that native use Pomegranate to cure certain diseases like skin disorder (Pacheco-Palencia *et al.*, 2008), cough, urinary infection, digestive disorder, most effective during pregnancy and post pregnancy and dysentery. This studies match with traditional medicines prepared from *Punica granatum* to cure different diseases. It is used to stabilize the blood insulin level (Das. S & Barman, 2012), reduce blood pressure (Disilvestro *et al.*, 2009) and reduce the risk of cardiac disorders (Aviram *et al.*, 2000). The information collected through the survey is correlated to literature.

Debjit *et al.*, (2017) also concluded that Pomegranate have best cultural and civilization values for boosting of circulatory, digestive, respiratory, immune, skeleton system as well as it can also retard the production of cancer causing oncogenes.

5.2: ANTIMICROBIAL ACTIVITY:

The major aim of present study was to established a comprehensive protocol to check the antimicrobial activity by using disc diffusion method of the extract of plants of *Punica granatum* through different solvents such as ethanol, methanol and water against the growth of selected microbes like antibacterial such as (*Escherichia coli*, *Staphylococcus aureus*, *Pasteurella multocida* and *Bacillus subtilis*) and fungal strain (*Aspergillus paracetic*, *Fusarium solani*, *Aspergillus niger* and *Sportium ferrugineum*). The presences of active compounds in plants shows that they inhibit the microbial growth. In our experiments disc diffusion method was used to check the antimicrobial activity in selected plants.

Grover & Moore, (1962) used disc diffusion method for the study of antimicrobial activity. Melendez & Capriles (2006) also using this method for the study of antimicrobial activity against *E.coli* and *S. aureus*.

It is determined from results that all extract of Pomegranate prepared by using different solvent methanol, ethanol, acetone, and water showed considerable antimicrobial activity. Mathabe *et al.*, (2005) used different solvent ethanol, methanol and water against microorganisms included (*S. aureus*, *E. coli*, *Salmonella typhi*, *Vibrio cholera*, *S. dysenteriae*, *S. sonnei*, *S. flexneri*, *S. boydii*) by using *Punica granatum* extracts and concluded that pomegranate were active and effective against the tested microorganisms.

It is clearly indicated by present study that the methanol extract showed more effectiveness as compared to the other extract. The fruit rind of *Punica granatum* in methanol solvent to be active against all microorganisms tested in their study and also supported by literature (Prashanth *et al.*., 2001).

Nuamsetti *et al.*, (2012) reported that *Punica granatum* fruit peels and arils showed antibacterial activity of against bacteria and showed that Gram positive (*Bacillus subtilis* & *Staphylococcus aureus*) were more susceptible to the plant extracts. The peels pomegranate extracts was the most persuasive antibacterial agent as compared to the arils in hot-water solvent.

Shaygannia *et al.*, 2015 explained the process of inhibitory mechanisms due to bacterial and microbial activity. For the purpose *Punica granatum* was tested due to its ancient medicinal value after experiment and chemical investigation. It was concluded that *punica granatum* contain phenolic compounds rich in concentration as well as considered as the best source of Ellagic acid with reference to anti inflammation, antimicrobial and antioxidant activity.

Drinking *Punica granatum* juice has been shown to have antimicrobial properties against harmful bacteria that can exist in the stomach, such as *Eschericia Coli* and *Bacillus subtilis*, both of which can cause painful infections and serious stomach conditions (Debjct *et al.*, 2013).

Tiancha iNuamsitt *et al.*, (2012) examined various part of *Punica granatum* for observation of antibacterial activities. Dilution method with ethanol acetate and hot water was done and various part of *Punica granatum* were grinded and concluded that peel extract containing more resistance to bacteria especially to Gram-negative and Gram-positive bacteria. This extract can permanently retard and ceased the bacterial growth.

From the current research work shows that leaves of *P.granatum* have high concentration of phenolic compounds and shows a good antibacterial activity (Duh, 1994).Pomegranate are also used for different medicinal purpose like Stomachic, inflammation, fever, bronchitis, diarrhea, dysentery, vaginitis, urinary tract infection and show antimicrobial activity (M. Reddy *et al.*, 2007).

It is clearly indicated by research work that different solvents (ethanol, methanol and water) were used to study the antimicrobial activity of extracts of *Punica granatum* pericarp and concluded that these extract were persuasive against different bacterial than fungal strains (Moorthy *et al.*, 2013).

Pavan *et al.*, confirmed that *Punicagranatum* has antimicrobial and antifungal properties. They examined four different local variety of Pomegranate against different bacterial and fungal strains. The present work about antimicrobial activities of plant extracts was supported by literature. The peels of *Punica granatum* are used to study the antibacterial activity (Braga *et al.*, 2005) & (Oliveira *et al.*, 2009).

Shimizu *et al.*, (1986) concluded that extract of whole part of *Eriobotrya japonica* is considered having best antimicrobial activity. The ethanol solvent of leaves extract of Loquat is best for curing diseases.

Enzyme Inhibition Activities

The data on enzyme inhibition activity of *Punica granatum* against urease and cholinesterase presents intriguing insights into the potential pharmacological properties of this plant. The findings suggest that different parts of the pomegranate plant exhibit varying degrees of enzyme inhibitory activity, with the root and stem extracts showing particularly promising results.

In the case of urease inhibition, it's noteworthy that while most extracts exhibited lower activity compared to the control, the root extract in methanol demonstrated the strongest inhibition, followed by ethanol and water extracts. Similarly, the stem extract displayed potent urease inhibitory activity, especially in ethanol. These results underscore the potential of *Punica granatum* as a source of natural urease inhibitors, which could have implications for conditions associated with urease activity dysregulation.

Furthermore, the data on anti-cholinesterase activity highlights the diverse bioactive potential of *Punica granatum*. The root extract, particularly in water, exhibited significant inhibitory activity against both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), indicating its potential in the management of neurological disorders such as Alzheimer's disease. Similarly, the stem extract showed notable inhibition, especially in methanol, suggesting its relevance in combating cholinergic dysfunction.

Overall, these findings contribute to the growing body of research supporting the medicinal properties of *Punica granatum*. Further investigations into the underlying mechanisms and bioactive compounds responsible for the observed enzyme inhibitory activities could pave the way for the development of novel therapeutic agents derived from this versatile plant.

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