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Exploration of antioxidant and antimicrobial potential of prunus dulcis and arachis hypogaea shells extracts

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***Abstract*---**Almond *(Prunus dulcis)* and Peanut *(Arachis hypogaea)* both are the edible dry fruits, belong to families *"Rosaceae"* and *"Fabaceae"* respectively. There are number of studies have reported

the biological activities of fruit but a very little data is available on shells. Shells extracts possess antiviral, antibacterial, anti-inflammatory, antifungal, and anticancer activities due to the presence of bioactive compounds, such as alkaloids, flavonoids, and polyphenolic compound. In the present study, green extraction technique was performed. The green extraction technique has many benefits over traditional extraction technique such as use of alternative solvents, reduction of energy consumption and unit operation and by-products in it. Firstly, shells were collected and crushed then their extracts were prepared through green extraction technique. The shells extract of Almond and Peanut were screened to investigate antioxidant, and antimicrobial potential. Antimicrobial activity was measured through well diffusion method while DPPH scavenging assay was performed to determine antioxidant potential. In our study, the antimicrobial activity of Almond shells extract was maximum against *Staphylococcus aureus* with zone of inhibition 19mm ±0.20 and minimum against the *Salmonella enterica* with zone of inhibition 7mm ±0.85. On the other-hand the antimicrobial activity of Peanut shells extract was maximum against *Pasteurella multocida* with zone of inhibition 21.5mm ±0.90 and minimum against *Bacillus cereus* with zone of inhibition 16.5mm ±0.20. The Almond shells extract possessed higher radical scavenging activity with IC_{50} 27.78 µg/ml than Peanut with IC_{50} 50.834 µg/ml. Our results revealed that the Almond shells extract has high antioxidant activity, and maximum potential against Staphylococcus aureus, while Peanut shells extract has moderate antioxidant activity, but high potential against *Pasteurella multocida*. Bioactive compounds of Almond and Peanut shells extracts should be identified and in-vivo model study against *Staphylococcus aureus* and *Pasteurella multocida* respectively.

***Keywords*---**Phytochemicals, Antioxidant, Antimicrobial, Potential, Shells extracts.

Introduction

The plants groups are not only the sources of the large number of the valuable bioactive natural compounds. Other living organisms such as fungi, animals and microorganisms including insects can also provide valuable organically active compounds [1].Natural products are those compounds that are manufactured by bacteria, animals, plants, and molds. Natural products are enhanced to bind with many biological macromolecules [2]. Natural compounds have the ability to induce immunogenic cell death and activate the adaptive immune systems. NPs have therapeutic potential against various diseases such as inflammation and cancer. This potential is due to the presence of molecular scaffolds in living organisms' extracts [3].

The metabolism and absorption of natural products in the human body are favorable with low toxicity in comparison with synthetic compounds. The selection of lead compounds can be achieved by natural compounds and novel drugs can

also be designed [4]. NPs have gained popularity because of their reliable therapeutic effects, cost-effectiveness, and availability to ordinary people [5].The metabolism and absorption of natural products in the human body are favorable with low toxicity in comparison with synthetic compounds. The selection of lead compounds can be achieved by natural compounds and novel drugs can be designed [6]. NPs have gained popularity because of their reliable therapeutic effects, cost-effectiveness, and availability to ordinary people [7].Medicines derived from natural sources have been broadly used against various diseases for many years in different parts of the globe. These parts of the world are China, Egypt, India, Sumer, and Greece. The worldwide medicine market is almost 1.1 trillion US dollars. And medicines derived from natural sources are about 35 percent [8].

The study of natural products to make drugs is not a new thing because mostly medical therapeutics are natural[9]. The history of natural products in medicine is as old as human history itself. The first record of the cultured therapeutic system from natural compounds in Mesopotamia, dates back to 2600 BC, comprising around 1000 drugs procured from botanic products and are plants derivatives [10].Natural compounds have the capability to induce immunogenic cell death and activate the adaptive immune systems. NPs have therapeutic potential against various diseases such as inflammation and cancer. This potential is due to the presence of molecular scaffolds in living organisms' extracts [11].

Antibiotics and most other medications on the market have caused undesired side effects, the rise of resistant pathogenic bacteria, toxic side effects, and withdrawal concerns in many countries, limiting their usage [12].Novel chemical entities account for 28% of all new chemical entities (NCEs) present in markets are organic products or their derivatives, according to the investigation of the origin of the medicated compounds that were aroused during the time of 1981 to 2002 [13]. An analysis of drugs from 1981 to 2007 indicates that half of the drugs were derived from natural products since 1994. 13 drugs from natural products were approved in 2005–2007 [14].

Almost 547, natural compounds along with their derivatives are approved by FDA by 2013. In the last 30 years, the percentage of natural compounds has increased to almost 50% total and in the anticancer area 74%. 25 natural products or drugs derived from natural products were approved to use as medicines in the period 2008–2013. The need for natural products is increasing in the whole world to cure chronic diseases in form of dietary supplements, botanicals, and herbal drugs [15].NPs are alluring sources to prepare new therapeutic & medicinal agents. NPs having anticancer efficacy is more demanding because of weaker side effects in comparison to chemically synthesized drugs to cure cancer [16]. Almost 50% of all antitumor drugs are consequent from natural resources [17].

NPs have bestowed abundant and remarkable innovation in the chasing of recent drug therapies, having numerous therapeutics persuaded by natural products. A significant proportion of present drugs (40%) in the market are natural compounds or derived from them [18]. Natural products with the potential to prevent microbial strains might be used as such substitutes [19]. At times, natural compounds have been utilized to prevent and cure microbial infections.

Technologists have discovered a extensive portion of natural compounds from plants, peculiarly secondary phytochemicals having antibacterial efficacy and are efficient against multidrug-resistant bacteria. The trouble of resistance arouses the demand for efficacious and environmentally friendly substitutes [20].

Antimicrobial resistance is growing at an alarming level due to significant public health issues according to the WHO report of 2014. The disclosure of multidrug-resistant bacteria causes antimicrobial treatments useless, increases the time of illness and hospitalization, and expands the mortality rate [21].The clinical efficacy of antibiotics is decreasing because of bacterial resistance as time passes that frighten human and animal health. There is a remarkable issue about the expansion of antibiotic-resistant superbugs that are resistant to many antibiotics. Infectious diseases caused by antibiotic resistance are great summons for medicine throughout the world. Deaths due to methicillin-resistant *S. aureus* (MRSA) can be compared with the deaths due to HIV, and estimation showed at least 10 million people will die annually by 2050 because of anti-microbial resistance [22].

Antibiotic-resistant bacteria cause infections that have increased now and cause a major reason of mortality and morbidity throughout the world in developed countries too. Multidrug-resistant organisms (MDRO) like vancomycin-resistant enterococci, 3rd generation cephalosporin-resistant Gram -ve bacteria and methicillin-resistant *S. aureus* are going to cause a more rapid rate of death as compared to cancer in the future [23].The rapid spread of multidrug-resistant micro-organisms causes failure of treatment. Antibacterial resistance in humans is influenced by excessive inappropriate antibiotic usage in human health but it is also influenced by antibiotic usage in animal husbandry. Some national action plans, such as antibiotic usage restrictions in human and animal health are already in place around the world but the antimicrobial resistance problem is far from solved. As a result, finding new antibacterial agents is critical. Many plant-derived natural chemicals, particularly phenolic compounds, are identified as antibacterial and resistance-modifying agents due to their structural variety (RMAs).

During the era of antibiotics, powerful therapeutic drugs are prepared from natural products against infectious microorganisms, in the mid-twentieth century. NPs have provided a major foundation for developing antibiotic drugs [24].Protective compounds which are natural in origin mainly derived from plants are used as substitutes for synthetic fungicides is a hot topic these days [25].

Natural antimicrobial compounds are less expensive and have efficacy to treat fungal infections [26].Free radicals such as superoxide anion, hydroxyl radicals, singlet oxygen, and ROS have been shown to have adverse effects upon cellular functioning in living systems. Free radicals can interact with biological components, resulting in DNA damage, lipid peroxidation, and protein degradation. Excessive generation of free radicals can disrupt the body's pro-oxidant and antioxidant systems, resulting in a variety of pathological illnesses including rheumatism, arterial hypertension, diabetes, inflammation, neurological diseases, genetic mutations, and cancer [27]. Various plant extracts have been declared by researchers as a natural and unlimited source of antioxidants.

Excessive formation of free radical master natural defensive mechanism, engendering liver injury, as scientists identified an extensive diversity of plant extracts having hepato-protective effects generally coupled with antioxidant activity. Antioxidants are chemicals that can counteract the adverse effects of free radicals. In biological systems, antioxidants operate as electron donors, free radical scavengers, and free catalytic metal chelating agents. Exogenous antioxidants consist of both natural and synthetic substances that have the capacity to forage free radicals [28].Natural products have provided powerful therapeutic drugs in opposition to infectious microorganisms during the era of antibiotics, mid-twentieth century. NP have given a major foundation for the production of antibiotic drugs. The dependence on natural products to bestow new molecular entities for effectively each disease is too well established [29].

Plants are utilized in ancient medication for Millennium of years, have also been continuously evaluated to design new drugs to cure various infectious diseases [30].

Almonds are introduced into South and North America, Australia and in more than 60 countries [31]. The almond is cultivated in the dry area of mountainous and desert like places such as Middle East Central Asia and, particularly in Iran.

The top six almond producer countries in the world are Spain, Iran, Morocco, the United States, Turkey, and Italy, in production rate order [32].The almond tree is infrequently up to 12 m long, it has branches in pale red color, and rectangular leaves in shape. Its flowers are of light pink to white color, which has an aromatic flavor and is 19-20 mm long. The tree of almond starts producing fruit after 3 to 4 years, fruit are 3.5–4.6 and 2.5–3 cm long and width respectively [33]. The kernel, seed coat, middle shell, and almond hull or shell are the most important parts of almond fruit. The bitter and sweet are the taste of almond fruits. The eatable part of the *Prunus dulcis* is the kernel, which is supplemented with nutraceutical food [34].

Arachis hypogaea is commonly called peanut which belongs to the family "*Fabaceae*". The peanut contains 80 species and is native to South America. The *Arachis* is economically most an important species which is mostly scattered in the tropical and subtropical areas [35]. Flavonoids, resveratrol, and sterols are abundant in peanuts. Peanut shells are a rich source of unsaturated that help to lower blood cholesterol level [36]. Peanut also maintain blood glucose levels, more effective in diabetic patients [37]. Peanut shell extract (PSE) maintains body abnormalities such inflammation prevention agents, a free radical scavenger, and modulation in immune system. Peanut shell contains numerous purposeful compounds among these most important anti-vascular inflammation agent luteolin is also present [38]. PSE also protects from oxidative stress to the retinal epithelial cells which induced cell death [39].

Objectives: The main objectives of the present study were:

- To investigate the antimicrobial potential of *Prunus dulcis* and *Arachis hypogaea*shells extracts.

- To explore the antioxidant activities of *Prunus dulcis* and *Arachis hypogaea*shells extracts.

Materials and Methods

The present study was done to determine the characterization of antioxidant and antimicrobial potential of *Prunus dulcis* and *Arachis hypogaea* shells extracts. The experiment was launched in the research and cell culture laboratory, Department of Zoology, Government College University Faisalabad.In the study following steps were performed:

- Collection of almond and peanut shells, preparation of extracts by green technology, antioxidant assay, antimicrobial assay, and statistical analysis.

Materials

The chemicals that were used for the experiment are given below:

Glucose, Nutrient agar, Wagner's reagent, Sulphuric acid, Methanol, Ethanol, Ferric chloride, Sodium Hydroxide, Gentamycin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) (MTT), Dimethyl sulfoxide (DMSO) and Distal water.

The apparatus and equipment that were used for the experiment are given below:

Microwave oven, rotary evaporator, incubator, weight balance, vortex, petri dishes, test tubes, cotton plugs, cotton swabs, vials, micropipette, aluminum foil, falcon tubes, para film or masking tape, forceps Eppendorf tubes, beakers, test tubes racks, discs.

Micro-organisms used in the experiment

Following bacterial and fungal strains were used

There are the following bacterial strains were used during the experiment in research lab.

Escherichia coli, Bacillus cereus, Aeromonas hydrophila, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and *Pasteurella multocida*

There are the following bacterial strains were used during the experiment in research lab.

Aspergillus niger, Fusarium avenaceum, and *Fusarium brachygibbosum*

Extracts preparation:

Almond and peanut shells were collected from Faisalabad then shells were grinded through grinder to obtain a fine powder, and then stored in air tight jars.

200 g of powdered material was subjected to flask and 600 ml (80%) ethanol then heated at 400 watts for 6 min then filter it and repeat this cycle for three time.

Then rotary that filtrate and crude extracts then lyophilized for 2 to 3 days then these extracts were stored at 4^{o}C for further analysis.

Anti-oxidant activity

For antioxidant activity we followed the protocol [40].

For this 4 mg of sample was taken in Eppendorf. 1 ml of methanol was added in 4 mg sample and vortex it. Then this solution was placed in falcon tube and in that tube 9 ml of methanol also added to make final concentration 400 µg/ml. Then from falcon tube took 62.5µl and put in one Eppendorf, 125 µl from falcon tube put into second Eppendorf, 250 µl in third Eppendorf, 500 µl in forth and 1 ml in fifth one. After this from falcon tube added the 937.5 µl in Eppendorf of 62.5 µl, 875 µl in 125 µl Eppendorf, 750 µl in Eppendorf of 250 µl and 500 µl in Eppendorf of 500 µl to make total concentrations 25 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml and 400 µg/ml. We removed 340 µl from each Eppendorf to get 660 µl volume and added 1ml DPPH solution in each Eppendorf. Similar concentrations for standard were also prepared from ascorbic acid. Then tubes were covered with aluminum foil and placed in dark place. Then absorbance of the solution was calculated at 517 nm using a spectrophotometer against reference solution DPPH and methanol only as control. In a reference solution all reagents were present except plant extract. Percentage (%) antioxidant activity was calculated by following equation.

Antioxidant activity %age = $(A^{o}-A^{1}) / A^{o}\times 100$

Anti-microbial activity

To check antimicrobial activity of Almond and Peanut shells extracts by green extraction (APGE), the protocol designed by [41] was followed. Following bacterial and fungal strains were used to evaluate antimicrobial activity of both extracts of almond and peanut shells.For determination of anti-bacterial activity of Almond and Peanut shells extracts media was prepared as:

- We took 2.8 g nutrient agar and 100ml of distilled water in a flask and autoclaved it.

- Media was prepared then poured it in 4 petri dishes in equal concentration and incubated overnight at 37 °C.

For determination of anti-fungal activity of Almond and Peanut shells extracts media was prepared as:

We Took 35 g potato, boiled it in 200 ml distilled water. When the boiled potato mixture remained 190 ml then lifted down it from the hot plate. Then we added 2 g glucose and 2 g of nutrient agar and mixed it well. After autoclaving we poured the media in sterilized petri dishes and after solidification incubated it overnight

at 37 °C. Next day by using well diffusion method we performed following methodology:

We Put 100 µl of strain in center of petri dish.Spread it evenly by the help of spreader.With the help of decontaminated borer, we made 6 wells at the equal distances.5 wells were for drug and 1 was for control. Marked concentrations at the bottom i.e., 20 mg/ml, 40 mg/ml, 60 mg/ml, 80mg/ml, 100 mg/ml and filled the wells accordingly pouring 50 µl of each concentration.Used anti-biotic for the positive control of both bacterial and fungal species and DMSO (according to drug dissolved) for negative control. After that we kept all these plates in incubator at 37°C for overnight. Very next day we checked the results in plates in the form of zones and noted the reading with the help of Vernier caliper.

Statistical analysis

One way ANOVA was applied by SPSS (Statistical Package for the Social Sciences) software on result data to compare mean values and standard deviation was calculated.

Results and Discussion

Antioxidant assay

Antioxidant potential of Peanut and Almond shells extract by green extraction technology were examined through DPPH (2,2-Diphenyl-1-Picrylhydrazyl) assay.

DPPH is a free radical and has the capability to give the hydrogen atom by reacting with the various compounds. This activity is used to measure the reducing capacity of various antioxidants by DPPH free radicals. Antioxidant activity was performed at different concentrations i.e. (25µg/ml, 50µg/ml, 100µg/ml, 200µg/ml and 400µg/ml). Both Almond and Peanut shells extracts displayed dose dependent DPPH free radical scavenging activity as compared with standard ascorbic acid. Among both IC_{50} observed by Peanut and Almond shell extracts against DPPH radical is 27.78µg/ml and 50.834 µg/ml respectively as compared to positive control ascorbic acid who's IC_{50} is 8.5µg/ml. The results shows that Peanut shells extracts have more scavenging properties as compared to the Almond shells.

Radical scavenging property of Almond and Peanut shell extracts were evaluated by DPPH assay. The qualitative estimation of antioxidants in the extracts which was estimated by the IC_{50} standards, low IC_{50} shows powerful antioxidant potentials.

Table 1: Percent Inhibition of DPPH free radical by extracts of Almond and Peanut shells at 517nm.

Name of Compounds	Conc. (µg/ml)	Percent inhibition			Mean ± SD	IC_{50}
Almond shells extract	25	31.9	32.2	32.6	34.23±0.35	27.78 µg/ml
	50	40.4	40.6	40.6	42.53±0.11	
	100	48.0	48.2	48.2	50.13±0.11	
	200	50.1	50.2	50.0	52.1±0.10	
	400	55.2	55.0	54.0	61.4±0.52	
Peanut shells extract	25	13.1	12.9	13.2	13.07±0.15	50.834 µg/ml
	50	19.3	19.5	19.6	19.46±0.15	
	100	32.0	31.7	31.8	31.84±0.15	
	200	43.5	43.3	43.3	43.36±0.11	
	400	50.5	51.0	51.1	50.86±0.32	
Ascorbic acid	25	85.77	84.50	86.05	85.44 ±0.82	8.5 µg/ml
	50	87.60	88.16	87.46	87.74 ±0.37	
	100	89.85	89.57	90.14	89.85 ±0.28	
	200	93.52	92.95	92.81	93.09 ±0.37	
	400	94.50	94.22	94.08	94.26 ±0.21	

Data values (n = 3) represent mean and Standard Deviation (SD)

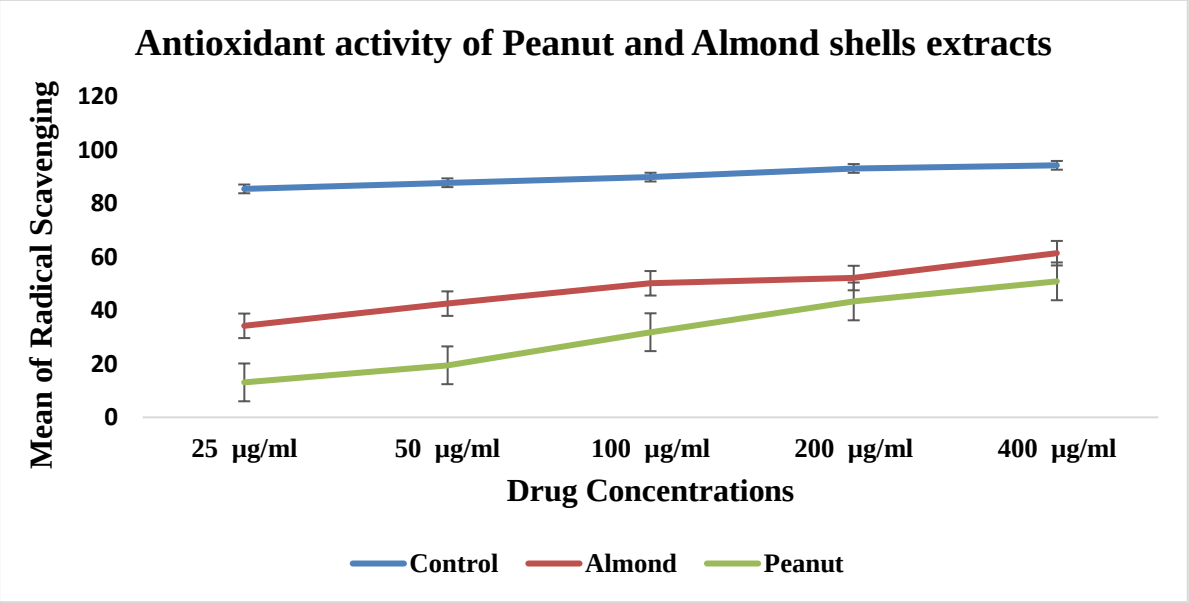

Figure 1:Antioxidant activity exhibited by Almond and Peanut shells with reference to standard ascorbic acid.

Antibacterial activity of *Arachis hypogaea* (Ah) shells extracts against various strains

Antimicrobial activity of the Peanut shells extracts was performed against the various strains of bacteria such as, *Pasteurella multocida, Staphylococcus aureus, Escherichia coli, Bacillus cereus, Aeromonas hydrophila, Salmonella enterica,* and *Pseudomonas aeruginosa.* The Peanut shells extract was checked against bacterial strains from 20 mg to 100 mg at five different concentrations. The Peanut shells extract showed minimum antibacterial activity against the *Bacillus cereus* which show the zone of inhibition 12 mm at the 20 mg and 16.5 mm at 100 mg of the shell extract as compared to positive control, which shown the 18 mm zone of inhibition. And Peanut shells extract show highest activity against the *Pasteurella multocida*which is 13 mm at 20 mg and 21.5 mm at 100 mg of the shells extract as compared to positive control, which shown the 10 mm zone of inhibition.

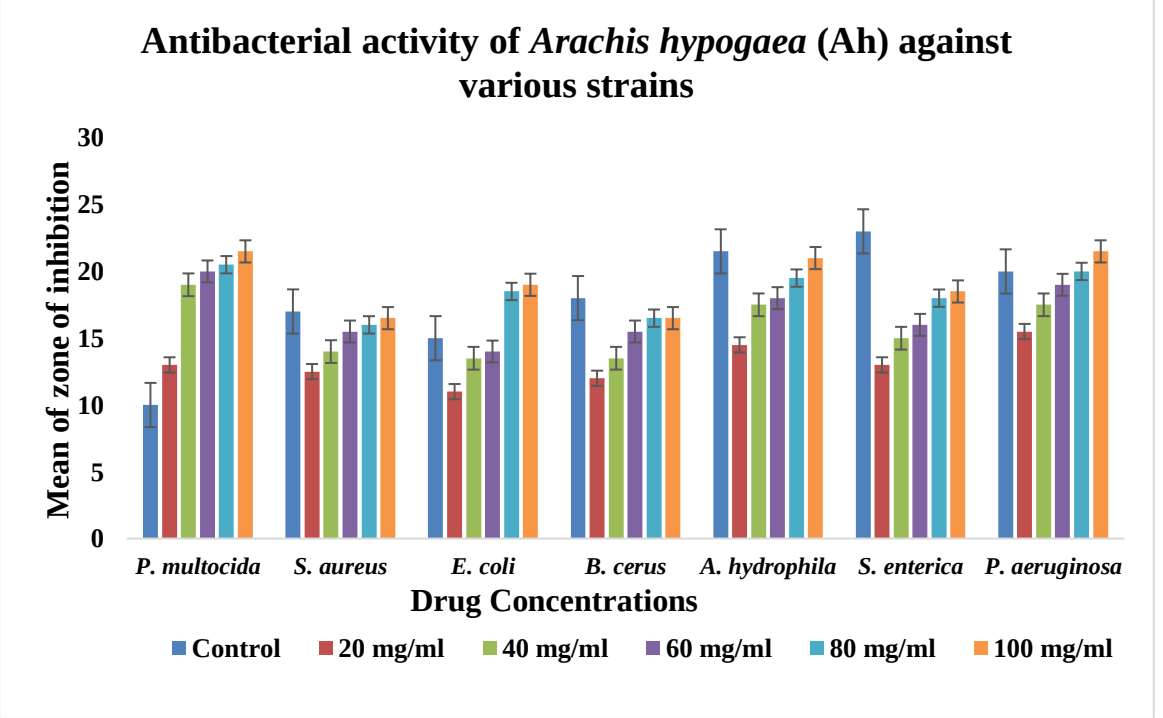

Figure 1:Comparative graph of antibacterial activity of*Arachis hypogaea* (Ah) shells extract against different bacterial strains.

Antibacterial activity of Almond (*Prunus dulcis)* shells extracts against various strains

Antimicrobial activity of the Almond shells extracts was performed against the various strains of bacteria such as, *Pasteurella multocida, Staphylococcus aureus, Escherichia coli, Bacillus cereus, Aeromonas hydrophila, Salmonella enterica,* and *Pseudomonas aeruginosa.* The Almond shells extract was checked against bacterial strains from 20 mg to 100 mg at five different concentrations. The Almond shells extract showed highest antibacterial activity against the *Staphylococcus aureus* which show the zone of inhibition 11 mm at the 20 mg and 19 mm at 100 mg of the shell extract as compared to positive control, which shown the 19 mm zone of inhibition. And Almond shells extract show lowest activity against the *Salmonella enterica* which is 0 mm at 20 mg and 7 mm at 100 mg of the shells extract as compared to positive control, which shown the 18.5 mm zone of inhibition.

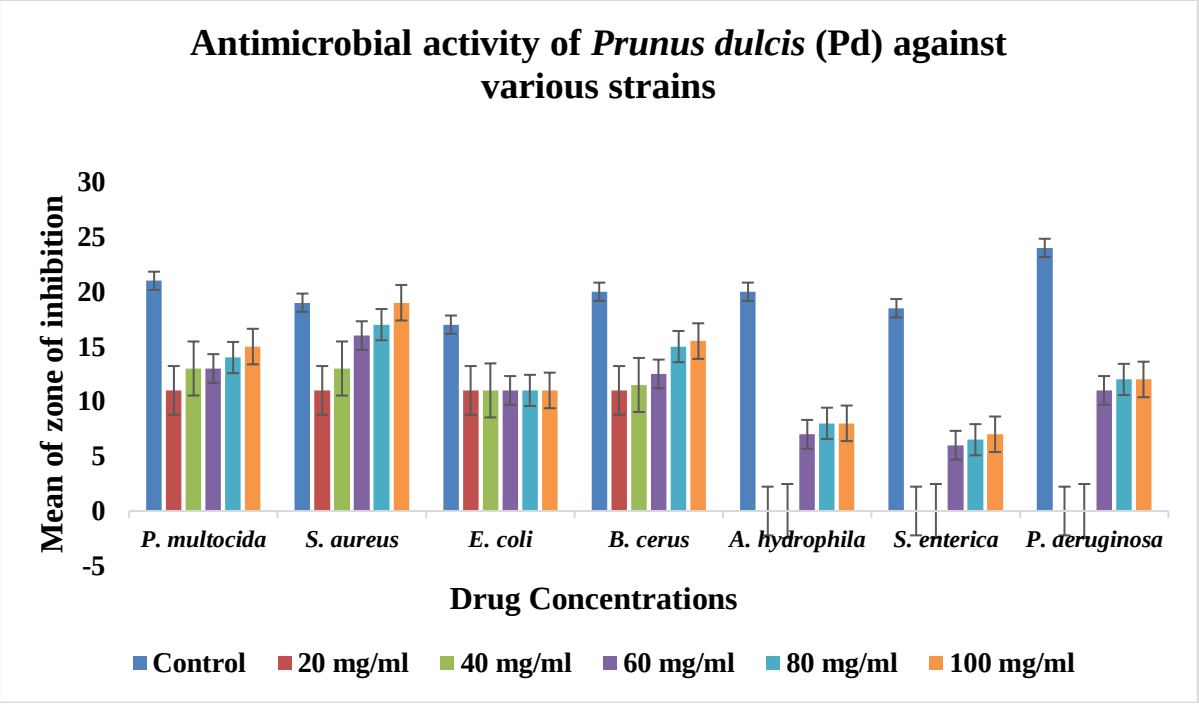

Figure 1: Comparative graph of antibacterial activity of *Prunus dulcis*(Pd)shells extract against different bacterial strains

Antifungal activity of *Arachis* hypogaea (Ah) shells extracts against various strains

Antifungal activity of the Peanut shells extracts was performed against the various strains of fungus such as *Aspergillus niger, Fusarium avenaceum,* and *Fusarium brachygibbosum*. The Peanut shells extract was checked against bacterial strains from 20 mg to 100 mg at five different concentrations. The Peanut shells extract showed highest antifungal activity against the *Fusarium barcchygibbosum* which show the zone of inhibition 13.5 mm at the 20 mg and 17.5 mm at 100 mg of the shell extract as compared to positive control, which shown the 10.5 mm zone of inhibition. And Peanut shells extract show lowest activity against the *Fusarium avenaceum* which is 0 mm at 20 mg and 14 mm at 100 mg of the shells extract as compared to positive control, which shown the 10 mm zone of inhibition.

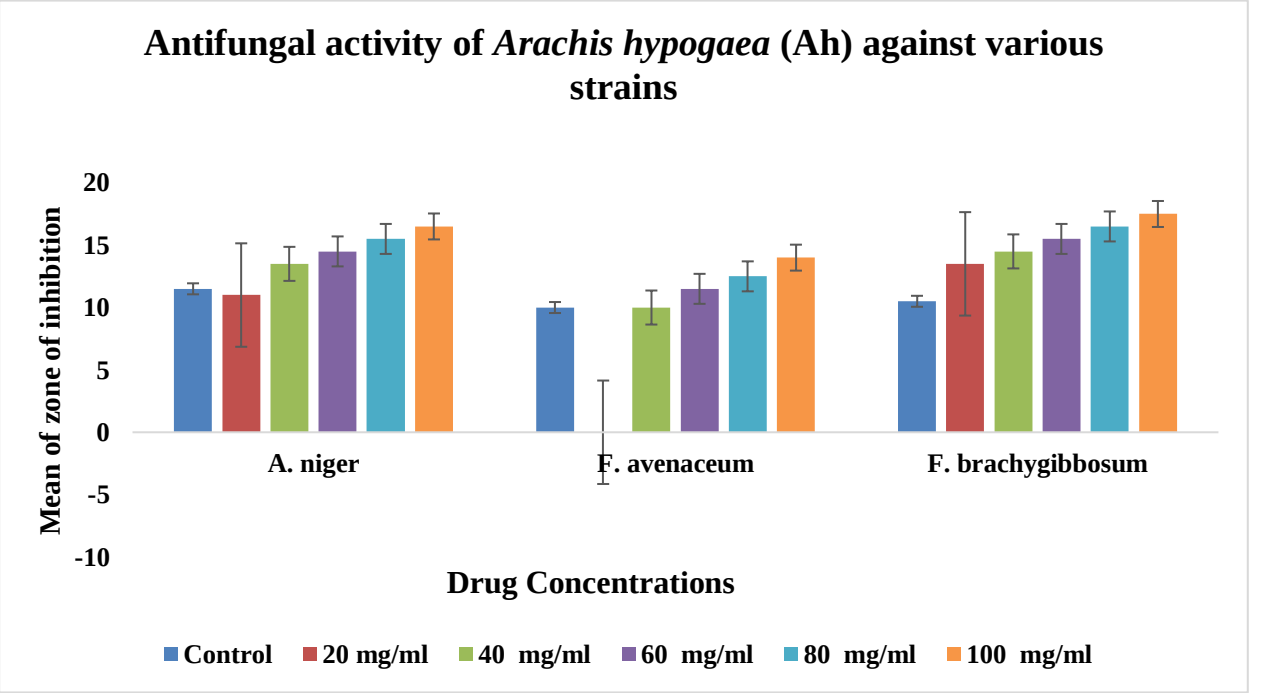

Figure 1:Comparative graph of antifungal activity of *Prunus* dulcis (Pd) shells extract against different fungal strains.

Antifungal activity of Almond (*Prunus dulcis)* shells extracts against various strains

Antifungal activity of the Almond shells extracts was performed against the various strains of fungus such as *Aspergillus niger, Fusarium avenaceum,* And *Fusarium brachygibbosum*. The Almond shells extract was checked against bacterial strains from 20 mg to 100 mg at five different concentrations. The Almond shells extract showed highest antifungal activity against the *Fusarium avenaceum* which show the zone of inhibition 18.5 mm at the 20 mg and 25 mm at 100 mg of the shell extract as compared to positive control, which shown the 15 mm zone of inhibition. And Almond shells extract show lowest activity against the *Aspergillus niger* which is 9 mm at 20 mg and 14 mm at 100 mg of the shells extract as compared to positive control, which shown the 11 mm zone of inhibition.

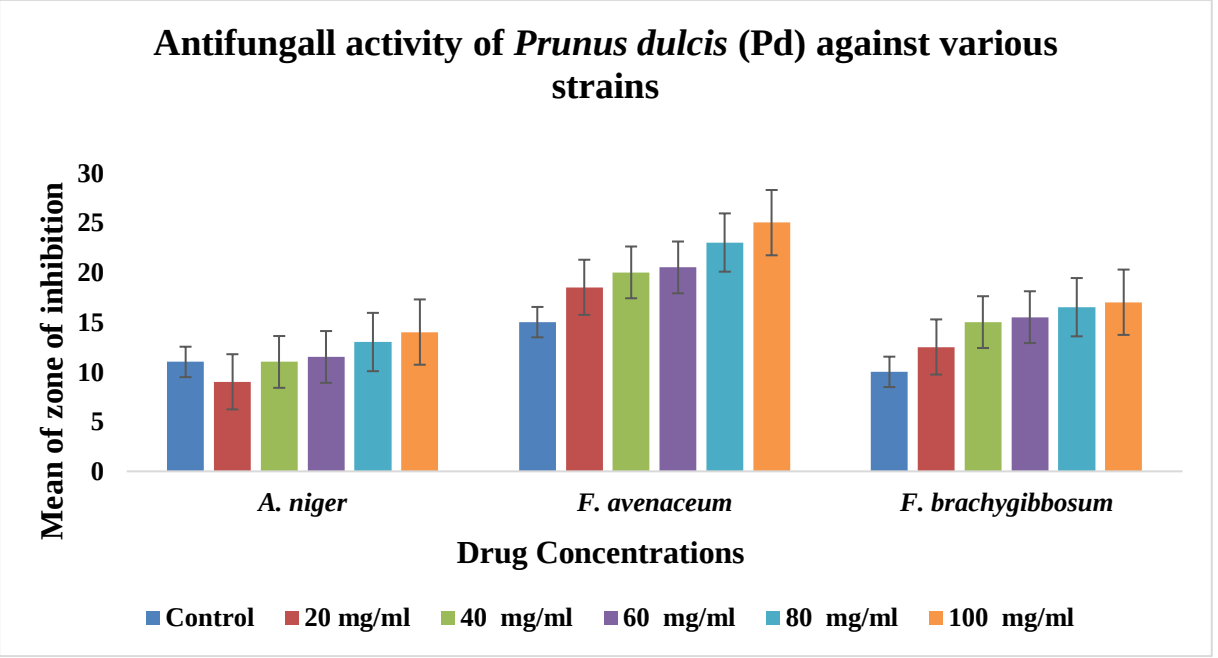

Figure 1: Comparative graph of antifungal activity of Almond (*Prunus dulcis)* shells extract against different fungal strains.

Overall data of antimicrobial study of Peanut and Almond shells extracts proved that both shells extracts have remarkable antimicrobial potential. Comparison of both extracts for each bacterial and fungal strain exhibited that Peanut shells extracts was the strongest antifungal and antibacterial. The inhibition zone of Peanut shells extracts was against *Pasteurella multocida>Pseudomonas aeruginosa>Aeromonas hydrophila>Escherichia coli>Salmonella enterica>Staphylococcus aureus>Bacillus cereus*.

And the trend of inhibition zone of Almond shells extracts *Staphylococcus aureus> Bacillus cereus>Pasteurella multocida >Pseudomonas aeruginosa >Escherichia coli> Salmonella enterica*.

The trend of inhibition zone (mm) of Peanut shells extracts was against *Aspergillus niger >Fusarium avenaceum >Fusarium brachygibbosum*. While the inhibition zone of Almond shells extract was against *Aspergillus niger>Fusarium avenaceum>Fusarium brachygibbosum.*

Conclusion

Our experimental aims to investigate the antioxidant, and antimicrobial potential of*Prunus dulcis* and *Arachis hypogaea* shells extracts.The presence of variety of bioactive compounds in these extracts have medicinal and pharmacological properties. Antimicrobial activity was performed against different strains of bacteria and fungi by well diffusion method. Gentamicin was used as a positive control. Peanut and Almond shells extract displayed dose-dependent DPPH free

radical scavenging activity. IC_{50} observed by DPPH radical was 50.834 µg/ml and 27.78 µg/ml respectively as compared to positive control ascorbic acid that's IC_{50} was 8.5µg/ml.

Future recommendations

The results of our study demonstratethat extracts of these shells also should be prepared by using other solvents than ethanol, all bioactive compounds should be identified by using different techniques such as HPLC, TLC and GC-MS. Bioactive compounds of Almond and Peanut shells extracts should be identified and study in vivo model against *Staphylococcus aureus* and *Pasteurella multocida* respectively. There is a need to investigate the bioactive compounds of shells other than Almond and Peanut that are discarded.

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