TOXICITY PROFILING OF CARICA PAPAYA LEAVES

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RESEARCH COMPLETION CERTIFICATE

It is certified that **Ms. Ayesha Shahzad** of **Bsc (HONS) (session 2019 – 2023)**, Department of Zoology has carried out research work entitled **"Toxicity profiling of carica papaya leaves"** under my supervision. It is assured that research work is original and has not yet been published anywhere else.

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AYESHA SHAHZAD

ABSTRACT

The common name of papaya plant is papita. The scientific name is *Carica. papaya*. It is soft stem long plant. The peel, pulp and seed of the papaya contains phytochemical and the carotenoids. The plant extract was done by drying the leaves of papaya plant in shade and stock solution was prepared (1mg/ml, 2mg/ml, 10mg/ml, 50mg/ml, 100mg/ml). three bacterial strains were used (Streptococcus pneumonia, Staphylococcus aureus, Pseudomonas aeruginosa) for the determination of antibacterial activity, and for the determination of antifungal activity aspergillus niger was used. For the determination of antioxidants DPPH and catalase assay was done. For cytotoxicity nalysis MTT assay was performed. The DPPH assay shows the dose P10 is show significant results, P50 and P100 show highly significant result. The catalase assay shows the dose P10(10mg/ml,5.5±0.19), P50(50mg/ml, 7.2±0.055) and P100 (100mg/ml, 11±0.14) show significant result as compared to control. The antibacterial activity of papaya leave extract against the S.pneumonia, the results shown in dilution 20 µl of dose P10 (10 mg /ml, 1.5333±0.120). and for the P. aeruginosa, the best results were shown in dilution 40 µl of dose P50 (50 mg/ml, 2.433±0.067). for the S.aereus, the best results were shown in 40 µl of dose P100(100mg/ml, 2.666±0.033). for the determination of antifungal activity The antifungal properties of papaya leave extract against the A.niger. The best result 40 µl show abundant zone of inhibition of dose P100(100mg/ml, 3.033±0.033). To check the toxicity profiling by MTT assay of the *C. papaya* leaves, shows no toxcicity. Our research concludes that the ethanolic papaya leave extract have ability to inhibit the growth of bacteria and fungus, and have antioxidant capacity.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION

Sr.no	Abbreviation	Full Form
1	C.papaya	Carica papaya
2	Ca, Fe, Zn	Calcium, iron, zinc
3	Gram (+) ve	Gram Positive Bacteria
4	Gram (-) ve	Gram Negative Bacteria
5	P. dulce	*Pithecellobium dulce*,
6	A. sapota	*Achras sapota*,
7	C. edulis	*Casimiroa edulis*
8	C. limon	*Citrus limon*
9	C. mexicana	*Crataegus mexicana*
10	P. guajava	*Psidium guajava*
11	S. purpurea	*Spondias purpurea*
12	E. coli	*Escherichia coli*
13	S.aureus	*Staphylococcus aureus*
14	P. aeruginosa	*Pseudomonas aeruginosa*
15	*S. mutans*	*Streptococcus mutans*
16	A.niger	*Aspergillus niger*
17	B.ciliata	*Bergenia ciliata*
18	B. subtilis	*Bacillus subtilis*
19	DPPH	α, α-diphenyl-β-picrylhydrazyl
20	MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
21	UV	Ultra voilet
22	DMSO	Dimethyl sulfoxide

CHAPTER 1
INTRODUCTION

The foremost causes of death in whole world is Infectious diseases. These diseases are causing death of approximately more than fifty thousand people every year (1). Microbiologists and phytochemists are becoming more conscious of this situation's limitations on the efficacy of most synthetic medicinal products to control infectious disorders(2). Traditional herbal remedies provide an exciting and mostly untapped source for the creation of potentially innovative drugs that could help combat the developing problem of antibiotic toxicity and resistance (3). The fact that man has long used plants for medicinal purposes and It is well known that this information of herbal therapy has developed into a good-sized collection of helpful knowledge that constitutes a old medical system (4).

Papaya is a common plant which we can find in Pakistan easily. Papaya plant belongs to family caricaceae. The scientific name of the papaya plant is *c. papaya.* The papita is common name of papaya. It is a dioecious plant with a very soft stem plant and it's up to 5 to 10-meter-tall, the leaves are very large (5, 6).The peel, pulp and seed of the papaya contains phytochemical and the carotenoids also including the polyphenols and also papaya leaves contain various minerals like Ca, Fe, Zn and including multivitamins. Usually in tropical climates Papaya trees grow in the world, it is identified by their scratch stem, and leave fruit and roots are also consumed for making medicine. papaya fruit is use traditionally to treat the diseases related to the gastric issues and the it also acts as the anti-bacterial (7). There are specialized tissues present in Papaya, these tissues have large amount of proteases and alkaloids (8).

Since ancient times, different human ailments have been prevented by using plants and plant-based products. Globally, almost 80% of the people be subject to on solely on plants used for their primary healthcare. (9). In terms of nutrition, papaya plant is a great source of the vitamins A, B, and C, as well as a reasonable supplier of Ca and Fe. (10). The papaya fruit's seed extract contains benzyl isothiocyanate, a fungicidal,

bactericidal, and bacteriostatic substance (11). Papaya has exceptional antioxidant properties that help to prevent the pathogenesis of diseases and neutralize the production of free radicals (12).

The nervous pains can also treated by the extract of the leaves of papaya plant. The papaya plant leaves smoked which benefit to relief for the asthma in many distant areas. The usage of papaya fruit in diet helps to reduce the acidity of urine on the other hand the flower of papaya leaf also for the healing of jaundice. In the preparation of different compounds, the ability of treatment with C. papaya is tested. In some regions, it is used in the feed of animal after childbirth. In various countries it is believed that hypertension and the scorpion bites can be treated by papaya leaves (13).

In many countries it is used to treat the various diseases like infection of eyes, joint pain and the diarrheal problem. The papaya leaves also help in the cure of the malaria and dengue fever. The roots of the papaya plant help to treat the respiratory disorders and cough (14).The enzyme presents in the papaya named papain which is efficient to remove the worms from the human body. The indication of the papain is milky substance is present in every part of papaya plant to tender the meat (15). Pharmacologist use the powder of the leaves in capsule to treat the related diseases. In this research we tested the ethanoic solution of the *C.papaya* leaves against the pathogenic bacteria and fungi (16).

The properties of antitumor and antipesticidal, which are naturally present in the tissues of leaves and barks of the C. papaya plant by leaf extract of papaya, we tested the Antimalarial and anti-plasmodial activities which are treated against the malaria. To treating the urinary tract infections, the c. papaya leaves are very effective (17). The Kaprain is present in the leaves of the papaya plant which helps to kill microorganisms.

The extract of papaya leaves in both ways dried and fresh act as antibacterial and antifungal papaya. disc diffusion method is used for isolation of fungi and bacteria and tested with the both dry and fresh papaya leave extract. The significant result

showed against the bacteria and the fungi. The aqueous extract is not efficient but the organic extract is more efficient. The outcome shows both bacteria gram (+) ve and (-)ve are effected by dry extract, on the other hand only gram (-)ve bacteria affected by the fresh extract of leaves. The antibacterial activity is more than the antifungal activity by the extract of leaves of papaya (18).

To assess cytotoxicity activity of the papaya plant tried on the cell line of various cancers like breast cancer, ovarian cancer, neuroblastoma, cervical cancers, leukaemia and many other cancers (19, 20).We assumed that C. papaya extracts from leaves were cytotoxic to human squamous cell carcinoma in vitro (6).

To evaluate the antifungal action of ethanolic extract leave of the papaya plants (21). The sodium bicarbonate shows the inhibitory effects and three species of fungi were tested by the extract of leaves of papaya plant. The results shows that highest occurrence of a artificial fungicide A. Niger (22).

Fungus are the microorganisms the effect the human plant and animal body. Fungus live on organic matter. Fungus grow on the moist place and the environment which is suitable for fungus. mostly fungus having a filamentous structures except yeast. The fungi structure consist of the tread like structure which is called hyphae. The fungal cells have cell wall which contain chitin. Papaya is high-quality international for its nutritional properties and flavor Papaya is a yield that exhibits excellent yields and precociousness as it starts scaling up productivity before the 1st year (23). Fruit crop generates a variety of byproducts because immature or poor-quality fruit is rejected as well as plants that have completed their production cycle (24).

Post-harvest infections, which cause losses of up to 40% during transference and storing, limit papaya production and value in Mexico (25). Fungi cause anthracnose, white rot, and fruit dry rot, primarily R stolonifer, C. gloeosporioides. To excellently manage fungus beatings on fruit, artificial fungicides are obligatory. Some of these fungicides are hazardous to wildlife, humans, and the environment who come into

touch with them, and owing to inappropriate implementation, their efficiency may be diminished when fungus develop sensitivity (26).

Fungal diseases harm more than just papaya, and now a days high demand for natural and decomposable fungicides. The potential for biological pathogen control using vegetal solutions containing secondary metabolites has drawn more attention as a result of this need. People have employed plant extracts having antifungal properties for thousands of years. For instance, eucalyptus, garlic, acacia, and mint powders or extracts all function as fungicides that can treat a variety of illnesses. There has been a lot of research on biological fungicides as a result of the present focus on managing phytopathogenic fungus naturally. Numerous biographers exist (27), have stressed the need of analysing the chemical summaries of inferior metabolites in plant extracts that have antifungal effects. Alternaria species are successfully controlled by aq. extracts of the leaves of P. dulce, A. sapota, C. edulis, C. limon, C. mexicana, C. papaya, P. guajava, and S. purpurea (28). It has been demonstrated that powdered seeds, leaves, and aq. and ethanolic extracts of P. dulce guard in contradiction of B. cinerea, P. digitatum, and R. stolonifer infection in strawberries throughout storage (29).

Proteolytic enzymes, alkaloids, sulphurous compounds, oils, organic acids, flavonoids and have all been found in Carica papaya leaves and seeds. Bioactivity has been demonstrated for extracts from various papaya tissues. Antifungal activity of aqueous leaves extracts of and aqueous seeds extract against C. gloeosporioides has been demonstrated.(30), Seed aqueous and organic extracts show anthelminthic action against C. elegans. Alcoholic extracts of the roots, epicarp and seeds of not fully prepared food and full prepared papaya fruit have ability antidysenteric, antidiarrheic, and antibacterial characteristics, and aqueous seeds extracts have effects in masculine albino rats (31).

While the fruitlet is infrequently consumed as food of animal, particularly during the dry season when food is short, the dispose of papaya waste are usually in undeveloped areas. High transportation costs severely limit uses, this waste is left for

decaying, allowing phytopathogens to proliferate and causing ecological concerns and a risk to human health (24). Extraction of physiologically active metabolites is one possible alternate use for these wastes. Similarly, minimal study was conducted to estimate taking out yields using readily accessible solvents in an effort to create straightforward, profitable strategies for extracting physiologically active metabolites from unconventional sources. Foodborne illnesses can be brought on by microbial infections, and as multidrug-resistant and disinfectant-resistant bacteria including S. aureus, E. coli, and P. aeruginosa have seemed more frequently, morbidity and mortality have increased (32).

This study base on the determine the antibacterial and antifungal and cytotoxity or toxicity profiling of the papaya leaves by using the well diffusion method, and other various methods.

RATIONALE

To evaluate the cytotoxic properties of papaya plant. Papaya has historically been utilized for its antibacterial, antifungal, antiviral, and antioxidant qualities. Investigations are currently being conducted to highlight the significance of plants with medicinal properties that have no negative side effects, are cheap to produce and for improving the health issues of individuals as well as animals. To check cell viability by DPPH assay and the Catalase assay, and observe antibacterial, antifungal activity by well diffusion method of the C. papaya leaves to inhibit bacterial growth.

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AIM & OBJECTIVES

The aim of our research to observe the efficiency of ethanolic extract of *C. papaya* leaves against the bacterial and fungal strains also attempt to identify the cytotoxicity of the leaves of *C. papaya* plant.

Objective of the research

- To acquire the skill of preparing an ethanolic extract from papaya plant leave powder.
- Antibacterial and antifungal activity of plant extract
- Evaluation of Cytotoxicity and antioxidant profiling of plant

CHAPTER II

LITERATURE REVIEW

They investigated garlic plant extract and calculated its antibacterial effectiveness against the bacterial S. aureus and E. coli using the agar diffusion method (33). Different polarity is given by the four different types of solvents to extract of garlic. Garlic extract had antibacterial activity which was highly efficient against the gram (+) ve and gram (-)ve bacteria. Allicin disturbed the growth of bacterial strain The extract had a greater effect on E. coli than on S. aureus (34).

They have studied antimicrobial activity of neem plant. They used leaves and bark of neem plant. They were used aqueous extract of neem plant's leaves and bark. This extract tried in contradiction of E. coli and S. aureus bacterial strains that were resilient to various antibiotics (35). To determine the efficiency of the extracts different concentration dropped on the cultured bacterial strains by using several one of the methods in which the method was disc diffusion methods. The fresh extract of leave and the bark of neem plant was more efficient then the dry extract (36).

To test antibacterial activity against bacteria, a solvent leaf extract of two plants, A. sessilis and A. philoxeroides, was used. The Ethanoic leaf extracts of plants tested against the bacteria by using well diffusion methods and four-gram negative bacterial species and also with the four grams' positive bacterial species (37). The result shows that plants *A. philoxeroides* is not efficient against the bacterial strain *A. sessilis* plants. The ethanoic extract of the plant was linked with the Gentamycin for antibacterial activity. They conclude that leaf extract of the both plants show indications antibacterial activity in contradiction of the bacteria (38).

In additional, studies they have studied growth inhibition of the *s. mutans* by the testing of the antibacterial effects of leaf and the stick extract of neem plant. which are dipped into the ethanol. For the inoculation of bacterial strain, they were used MHA ager and incubate 24 hours for the culture of bacteria. Analyze the statically data which shows that the ethanolic extract of the neem, S. mutans has a strong

antibacterial activity. The extract of the neem stick was more efficient than the leaf extract (39).

In additional studies they took ginger plant extract to evaluate the antibacterial activity against pathogenic bacteria. Disk diffusion method was used by them. Ginger extract was found to be more efficient against gramme positive bacteria than gramme negative bacteria. As a result, hot water extract outperformed cold water extract (40).

Furthermore, studies Tulsi plant leaves were taken by them. *S. mutans* was a bacterial strain that was treated by the tulsi plant Antimicrobial activity must be determined. Using the cold extraction procedure, they extracted the ethanoic. The ethanoic extract was then diluted with a passive solvent, C_3H_7NO (41). The result shows that the great zone of inhibition of bacterial strain by dropping the extract with 15 concentration Tulsi extract exhibited antibacterial activity against S. mutans (42).

They studied the efficiency of plant activity against infection of pathogenic bacteria. Plant extract were treated by the cold and hot water. They employed the agar-based well diffusion method. From three therapeutic herbs, B.ciliata preparations shown ensuring antibacterial pathogen action (43). The extract of plant with cold water was more efficient against the B. subtilis and also seen the zone of inhibition which showed more efficiency of specific plant against the specific bacteria (44).

Many herbal products were known to treat the bacteria. They took ten herbal medicinal plants. To evaluate the antibacterial activity of each plant against the gram (-) ve and (+)ve bacteria (45). This all equipped by the agar based well diffusion method and extract of crude with usage of methanol. In result that the zone of inhibition is maximum activity of plant *H. officinalis* in contradiction of *S. aureus.* Product of herbal plant shows the high antibacterial activity (46).

The antibacterial activity of the extract of the C. papaya plant's leaf will be determined more effectively by employing the broth dilution methods, with the agar well diffusion being more efficient against some bacteria and fungi such as *S. aureus, E. coli, S. pneumoniae, B. subtilis and C. albicans*. The ethanolic extract of leave of

the *C. papaya* plant revealed a significant wide spectrum antimicrobial activity against both gram (+)ve positive and gram (-)ve bacteria (14).

They investigated the antibacterial properties of C. papaya using various solvent extracts. These extracts were then evaluated against Gramme (+)ve and Gram(-)ive bacterial strains, as well as fungus. Extracts of ethanol, methanol, C3H6O, and CHCl3 were found to be antibacterial and antifungal. Carica papaya extract has been shown to have antibacterial and antifungal properties against microorganisms (47).

C. papaya's antitumor activity was determined. In general, papaya plant components including as roots, leaves, stems fruits, and bark are used for medicinal purposes. Jamaicans use the prepared fruit consumed healing of ulcer, and decrease in long-lasting skin ulcers (48). Traditional healers in Pakistan use the unripe fruit and many other nations, and it has also been used to treat a variety of human and veterinary problems in various countries (49). They study numerous cancer cell lines in vitro and in vivo, no specific data they take various cancer patients, including 5 patients with lung cancer, 3 with breast cancer, 3 with stomach cancer, 1 with pancreatic, 1 with liver, and 1 with blood cancer, and all the patients drink the C. papaya leaves extract observed all the patients and its very effective against the cancer. The breast cancer cell line was studied in vitro. Effects of papaya extracts on the survival of the MCF-7 breast cancer cell line were noted. There are 14 plant are commonly consumed in Mexico (black sapote, pineapple, avocado, guava, (nopal), tomato, and grapes), only papaya was found to significantly reduce the multiplication of breast cancer cells (6).

The study was carried out in order to create stringent criteria for male and female plant leaves. An FTIR profile of the samples was also created, which validated the unique peak values associated with the functional groups displayed by the male and female plants of C. papaya. This study compares the phyto-constituents of male and female plants. The data obtained from this study will be used for subsequent Instrumental and Pharmacological evaluation of the plant, which can be valuable not only in perceptual as well as disinfecting the kind numerous phytochemicals contained in C. papaya masculine and feminine leaves, but also to create excellence criteria for future study (50).

This study aims to examine different A. squamosa and C. papaya extracts of seeds for their capacity to scavenge free radicals, antioxidant properties, total flavonoid compounds and phenolic contents. Microwave helped extraction was used to obtain samples from each of the seeds. Following the determination of their antioxidant properties, The chloroform methanolic extract of C. papaya seeds had the greatest antioxidant and phenol concentration. Aquoeus extract of A. squamosa seeds had the highest radical scavenging properties, but acetonic extract of C. papaya had the all extracts with the highest flavonoid concentration. It was discovered that more polar extracts were more potent free radical scavengers than lesser-polar extracts. Hexane extracts had the least amount of DPPH radical scavenging action. Flavonoids were better extracted with acetone, while phenols were finest extracted with a mixture of methanol(CH3OH) and chloroform($CHCl_3$). Few studies have been conducted on the antioxidant activity of nonedible portions of regularly used fruits. According to the findings, seeds auspicious source of antioxidants, which may have medicinal benefits (51).

This study's objective was to look into the potential of C. papaya leaves extract in comparison to patients who were 45 years old and bitten by mosquitoes infected with dengue virus. For the cure of Dengue fever, 25 mL of extract were made. A patient with dengue fever took 25 mL of an aqueous extract of C. papaya plant leaves twice day for five days, in the morning and evening. Prior to administration, a blood sample was analyzed. Following the administration of the leaves extract, the blood samples were checked again. Platelets were found to be rapidly increasing. The patient is feeling better, and the blood sample report is improving day by day, indicating that the aq. extract of C. papaya leaves is effective ability against the dengue fever. Moreover, the several parts of this specie like leave, stem, root are very strong against the viral activity (52).

A global threat to human health is posed by infectious diseases brought on by germs and contamination of food brought on by decay bacteria (32). The effectiveness of different antimicrobial medications has decreased due to microbial resistance, which

are now employed to increase the shelf-life and raise the protection of food products in the foodstuff business and to suppress germs in medicine which causing disease (53). As a result, new antimicrobial agents that can incredulous resistance must be identified. There are many spices like clove, cumin, cinnamon, oregano and basil influenced important antimicrobial activities against spoilage of food bacteria comparable P. fluorescens and B. subtilis, pathogens such as V. parahaemolyticus and S. aureus, harmful fungi such as A. flavus, even antibiotic resistant microbes such as S. aureus is resistant to methicillin (54). As a result, spices have a high ability for development as advanced and harmless antibacterial agents. This examination precises research on the antimicrobial properties of various of these spices (55).

In current an age, there has been a flow in concentration in determining and manufacturing new antimicrobial compounds derived from numerous sources in order to overcome bacteriological confrontation (56). As a result, antimicrobial activity screening and evaluating approaches have received more attention. Several assays are well recognized and commonly used, such as disk-diffusion, well diffusion, and broth, However they can be responsible for immediate evidence of anti-microbial agent's special effects and a better considerate of the effect they have on the ability to survive and cell damage caused to cells, methods like flow cytofluorometric and bioluminescent are not commonly used because they need specialized apparatus and furthermore valuation for reproducibility and standardization (57). This article includes a complete list of in vitro antibacterial and anti fungal exposure testing methods, as well as complete information on their benefits and drawbacks (58).

Herbal medications provide a wide range of antibacterial activity and antifungal activity. Garlic is a herbal medication with numerous active ingredients such as diallyl trisulphate, Allicin, Alliin, Allylpropl, Ajoene, and vinyl dithiines. Ajoene is a active component which sulphur-containing oil-soluble with antifungal action and platelet combination inhibitory action (59). The antimicrobial activity of powder of garlic tested using the agar disc diffusion method, in which the garlic powder was combined with vegetable oil as a solvent (60).

Eleven ethanolic extracts of spices from C. annum, C. cyminum, L. nobilis, J. oxycedrus, D. coryophyllum, R. coriaria, M. officinalis, E. arborea, M. piperita, P. nigrum and C. arborescens together from numerous regions of Turkey were tested for in vitro antimicrobial activity against three (61). Furthermore, utilizing both agar dilution and disc-diffusion procedures, It is observed that their potential toxicity to C. albicans and A. niger . For all bacteria evaluated, the slightest inhibitory concentration of the M. piperita, L. nobilis, and J. oxycedrus ethanolic extracts was 5 mg/mL. The most sensitive bacterial strain to P. nigrum was P. aeruginosa. With a slightest inhibitory concentration of 5 mg/mL, E. arborea extracts were found in both Gram(-)and Gram(+) bacteria. Extracts of C. arborescens, D. coryophyllum, J. oxycedrus and L. nobilis were found to have greater inhibitory efficacy in contradiction of the fungus C. niger yeast and the C.albicans than the conventional antifungal nystatin (62).

Medicinal plants of African are a rich source of novel vigorous ingredients. Three plants were chosen for biological studies in this context based on their earliest applications. The antibacterial and also anti-proliferative properties of 3 medicinal herbs were studied. Materials and procedures Ficus bubu Warb's Methanolic extracts stem bark and leaves, P. Beauv S. campanulata flowers, and C. papaya were tested for antibacterial activity (63). Latex of papaya plant in contradiction of a variety of anti-microbial pathogens including fungus and bacteria, like, S. saprophyticus, S. aureus, K. pneumonia, S. typhimurium, S. epidermididis, E. coli, E. faecalis, T. rubrum, and C. albicans,, were evaluate using the broth dilution method (64). The extract concentrations tested ranged from 2500.0 to 2.4 mg/mL, and MIC values were determined during a 24-hour development period at 37 degrees Celsius. Following that, the MTT assay was utilized to evaluate the anti-proliferative effect of these methanolic extracts on 3 cancer cells of human, as well as F. bubu latex. The methanol-based extract of the stem bark of F. bubu shown significant anti-proliferative action against glioma and lung cancer cells. These results simply that the three plants under investigation could be used to create efficient treatments (65).

The leaves, fruit, and seed of the C. papaya plant were collected, dried out in a dark place, then ground in a handheld chopper. Using a soxhlet extractor, the powdered parts of the plant and were separately placed in the thimble and successively extracted with chloroform, acetone, ethanal and distilled water (66). To ascertain the existence of phytochemical elements, a phytochemical screening was performed on all extracts. All of the following are present: tannin, carbohydrates, vitamin C, protein, flavanoids, alkaloids, saponin, and steroids. All extracts' antibacterial potency was assessed using the well diffusion method (67). Ethanol extracts from the Carica papaya leaf showed the most inhibitory efficacy of any plant material in this investigation against all test pathogens. The fruit sample's FT-IR analysis turned up 18 functional groupings chemicals in the bands 400–4000 nm (1).

The quantity of the various chemical components included in the formulation determines the efficacy of C. papaya therapies. The total quantity of chemical substances in the fruit, vegetables, leaves, latex-based substances and roots vary based on the removal procedure, plant component age, cultivar, and tree sex (68). The fresh and dried leaves of C. papaya were tested for antimicrobial activity against bacteria and fungi of medical interest. Using the disc diffusion method acetone and aqueous extracts of garden-fresh and desiccated leaves were observed at 25, 50, and 100 mg/ml concentrations on bacterial and fungal isolates. Antimicrobial activity bacteria and fungus was found to be quite significant (1). Organic extracts outperformed aqueous extracts in terms of efficacy. Furthermore, the desiccated sample was efficient against both Gram(-) and Gram (+) bacteria, but the garden-fresh sample was more efficient against Gram(-) bacteria. The dried leaf extract proved effective against microorganisms that regular antibiotics were unable to inhibit (64). The antifungal activity of C. papaya leaves was higher than the antibacterial activity. The sample of antifungal and antibacterial activity against the examination of isolates suggests that this plant may be a basis of substitute antibiotic compounds for the development of innovative antibacterial medicines (65).

Carica papaya leaf utilized in ancient medicine for a variation of medicinal purposes, comprising skin ailments and cancer (69). The cytotoxicity of Carica papaya leaf extracts on human mouth squamous cell carcinoma cell line and non-cancerous human HaCaT cells in vitro, was examined in this work. 2 of the 4 extracts consumed a substantial selective effect on sarcoma cells and included high quantities of phenolic and flavonoid chemicals. Using comparative analysis, The main chemicals discovered were flavonoids glycosides or flavonoid, some of them earlier been originate to have anticancer properties. These findings confirm that papaya leaves is a possible basis of anticancer substances and call for more logical research to rationalize the old usage of papaya leave in treatment of cancer (19).

Millions of people in various traditional systems have used medicinal plants to treat their illnesses, and antioxidant-rich compounds have recently attracted extraordinary interest as possible medicinal and preventative agents (70). The current study concentrated on the antioxidant and cytotoxicity assessment of The leaves of C. papaya are an important medicinal herb. The MTS colorimetric test was used to assess cytotoxicity, meanwhile the antioxidant properties of C.papaya leaves crude extract from water and portions of solvent was calculated in terms of capability to scavenge (DPPH) 2,2-diphenyl-1-picrylhydrazyl free radicals (71). The antioxidant capacity of the crude extract and fractions was substantial, with fraction 3 being the most potent radical scavenger, followed by portion 2, portion 1, crude water, portion 4, and portion 5 (72). The outcomes of this study provide a solid foundation for medical applications of C.papaya plant (73).

C. papaya is a famous medicinal plant that is used to treat a diversity of diseases in Western and Asian countries. In Pakistan the dengue fever pandemic and stimulated patients to eat papaya fruit on a regular basis. The aim of study was to evaluate the potential profile, flavonoids, antioxidant and polyphenols of extracts of all important portions of the C. papaya using seven solvents: ethanol, water, , ethyl acetate, n-hexane, n-butanol, dichloromethane, and methanol (74).

.Antioxidant and antibacterial characteristics of C. papaya leaves, flesh, and skin were tested utilizing various aqueous and solvent-based compounds, as well as trace element detection. Total soluble phenolics as well as flavonoids were detected in high concentrations particularly in methanolic and ethanolic extracts (75). With the DPPH free radical scavenge assay, extracts of all part of papaya plant like leaves, bark, roots, and pulp demonstrated >75.0% foraging capability, The leaves and pulp, on the other hand, suppressed peroxidation by 84.9 and 80.9%, respectively (76). The reducing power experiment demonstrated that leaf, pulp, and root extracts were capable of converting Fe3+ ions to Fe2+ ions. According to antibacterial research, pulp extract is the best technique to combat bacteria's infectious behavior (77). Apparently Ethanol and methanol have been shown to be the best solvents for extracting natural compounds with the highest medical benefits, and they may be used in pharmaceutical compositions against a variety of infectious diseases (78).

CHAPTER III

MATERIAL AND METHODS

Ethanol , payaya leaves powder, nutrient Agar, Distilled water

3.1 COLLECTION AND PREPARATION OF SAMPLE

3.1.1 SAMPLE COLLECTION AND EXTRACT PREPARATION

The garden-fresh leaves of the *C. Papaya* was gathered from the Lahore in the end of august and start of September(47). The garden-fresh leaves of C. papaya were harvested from the tree, washed, dried in the shade, and utilizing a mortar and pestle to make a fine powder. (39). Add 10 gram of papaya leaves powder into the 90 ml ethanol. Leave it for 2-3 days. After that filter the extract into the flask with the help of funnel and what man filter paper, each empty petri dish was weigh, then filtered plant extract was added into the petridish and left for four days to air dry. After 4 days when the extract dry in the petridish weigh the petridishes was weight again.

3.1.2 PREPARATION OF DOSES:

Doses were prepared using the stock solution. In P1, place 1 mg of plant extract in 1 ml of distilled water using a micropipette in an eppendorf tube. Add 2 mg of plant extract to 1 ml of distilled water in an eppendorf tube with a micropipette for the second dosage P2. Add 10 mg of plant extract to 1 ml of distilled water in an eppendorf tube with a micropipette for the third dosage P10. In an eppendorf tube with a micropipette, mix 50 mg of plant extract with 1 ml of distilled water for the fourth dose P50. In an eppendorf tube with a micropipette, mix 100 mg of plant extract with 1 millilitre of distilled water for the fifth dose P100.

3.2 ANTIOXIDANT ACTIVITY:

3.2.1 DPPH ASSAY

Despite the fact that the antioxidant properties of NADES has not been extensively studied or understood, many research papers was written on the topic(79). The capacity of the stabilized free radicals (DPPH) 2, 2-diphenyl-1-picrylhydrazyl to be scavenged was examined. According to the DPPH Assay theory, the hydrogen donor

acts as an antioxidant. It is offered the crystalline form of a free radical that is stable of DPPH that transfers the hydrogen from its atom to molecules that are unsteady and prevents oxidation (80). The antioxidant capacity of the test specimen and the same amount of control distilled water was measured using spectrophotometry, and the chemical combination displayed good DPPH absorbance efficiency. The UV spectrophotometer detected the highest absorbance by DPPH radicals that were free at 517 nm. The existence of an abundance of free radicals causes enhanced absorption. Lower absorbance suggests that the solution contains fewer free radicals and has a higher inhibitory capacity. As a result, antioxidant capability and absorption are inversely related, but DPPH disappearance and antioxidant capacity are closely linked (81).

Table 3.1: Chemical and amount of chemicals use in DPPH assay

Sr.no	Group	Group details
1	N	Untreated cell
2	P1 (1mg/ml)	100 µl DPPH+80µl Tris HCL+ 20 µl P1(1mg/ml)
3	P2(2mgl/ml)	100µl DPPH+80µl Tris HCL+ 20 µl P2 (2mg/ml)
4	P10(10mg/ml)	100µl DPPH+80µl Tris HCL+ 20 µl P10 (10mg/ml)
5	P50(50mg/ml)	100µl DPPH+80µl Tris HCL+ 20 µl P50 (50mg/ml)
6	P100(100mg/ml)	100µl DPPH+80µl Tris HCL+ 20 µl P100 (100mg/ml)

3.2.2 CATALASE ASSAY

A 96-well plate was used for the catalase assay. In 45ml of falcon tube, prepare the **1Molar stock solution A** by adding 8.89ml of distilled water and 1.11ml of H_2O_2. After that made 100mM **stock solution B** by added 2ml of stock solution A and add 18ml of distilled water. One well was filled with 50mM KH2PO4, The PH must be 7 and a medium from one of the groups being examined served as a blank. While the other Wells were filled with 12.5mM hydrogen peroxide in 50mM, PH must be 7. KH_2PO_4 and medium from several experimental groups. The optical density at 240 nm was measured against a blank after 45 to 60 seconds in the dark (82)

Table 3.2: Chemical and amount of chemicals use in Catalase assay

Sr	Group	Group details
1	N	Untreated cell
2	P1 (1mg/ml)	31.25 µl Stock solution B+ 12.5 µl KH_2PO_4 + 206.26 µl P1 (1mg/ml)
3	P2(2mg/ml)	31.25 µl Stock solution B+ 12.5 µl KH_2PO_4 + 206.26 µl P2(2mgl/ml)
4	P10(10mg/ml)	31.25 µl Stock solution B+ 12.5 µl KH_2PO_4 + 206.26 µl P10(10mg/ml)
5	P50(50mg/ml)	31.25 µl Stock solution B+ 12.5 µl KH_2PO_4 + 206.26 µl P50(50mg/ml)
6	P100(100mg/ml)	31.25 µl Stock solution B+ 12.5 µl KH_2PO_4 + 206.26 µl P100(100mg/ml)

3.3 PREPARATION OF CULTURE MEDIA

3.3.1 APPARATUS

250 ml flask, inoculating loops, petridish, sprit lamp, match stick, 3 bacterial strains.

3.3.2 MODEL BACTERIAL STRAINS

These bacterial strains provided by the institute.

Kc1	Streptococcus pneumonia
Kc2	S. aureus
Kc3	P. aeruginosa

3.3.3 MEDIUM FORMATION FOR BACTERIA:

For the culture medium use Nutrient agar. Flask of 500ml was taken and then distilled water was added 250 ml. Then 15 grams of nutrient agar were added. Put the flask on the magnetic stirer for the 15 minutes. Autoclave the agar flask, inoculating loop and petridishes. Place everything in the laminar air flow cabinet after the autoclave. Fill the petridish in the laminar flow cabinet with nutritional agar. Allow it to leave for 10-15 minutes.

3.3.4 BACTERIAL STRAINS INOCULATION

The agar was solidify. For the disinfection we use the sprit lamp and heat the inoculate loop. After heat the inoculating loop though on the agar for the 1-2 sec for cool. The bacterial strains are inoculate with the help of inoculating loop. There were

3 bacterial strain; streptococcus pneumonia, S.aureus, P. aeruginosa inoculate by streak plate method. After streaking name the petridish and incubated at 37C for 72h (47).

3.3.5 SUB CULTURING OF BACTERIAL STRAIN

For the culture medium use Nutrient broth. Take 250 ml flask and add 100 ml distilled water. To make nutrient broth add the 2.5grams of nutrient broth, 1 gram of tryptan and 1 gram of NaCl, and yeast extract 0.5 grams. Put the flask on the magnetic stirrer for the 15 minutes. Autoclave the nutrient broth flask, inoculating loop and petridish. After the autoclave place all things in the laminar air flow cabinet. Pour the nutrient broth into the black caped test tube in the laminar flow cabinet. Leave for the 2-3 minutes for the cool down the broth and incubate for the 24 hours at the 28*C.

For the culture medium we use Nutrient agar. Take 500 ml flask and add 250 ml distilled water. Then add the 15 grams of nutrient agar. Put the flask on the magnetic stirrer for the 15 minutes. Autoclave the agar flask, petridish. After the autoclave place all things in the laminar air flow cabinet. Pour the nutrient agar into the petridish in the laminar flow cabinet. Leave for the 10-15 minutes. The agar was solidifying. Make the well on the agar plate with the help of sterile T. Sterile swabs were used for the streaks of bacterial growth on the solidified nutrient agar. Each sterile swab was used for each bacteria strain. After that incubate those petridish on 37C for 72 h.

3.4 ANTIBACTERIAL ACTIVITY BY WELL DIFFUSION METHOD:

For the evaluation of the antibacterial properties of the plant extracts of the *C. papaya* with ordinary antibiotic Ciprofloxacin by using well diffusion method(47).

Well diffusion method used for antimicrobial exposure testing was carried out to evaluate the presence of antibacterial activities of the papaya leaves extract. The culture of bacteria was used spread to nutrient agar plates consistently using a sterile swab. The wells containing a variety of plant extracts have been placed on the nutrient agar surface. Each test plate contains four well. Each well 6mm in diameter. There are 5 doses like P1, P2, P10, P50, P100 each plate having the 4 different concentration

like well A having 10µl, well B having 20µl, well C having 30 µl and well D having 40µl of each dose.

Ampicillin (10) was the usual antibiotic for S. aureus and S. pneumonia, while Amikacin (30) was for P. aeruginosa. The plate was then incubated for 24 hours at 37°C. The inhibitory zone was studied after the plates had been incubated. The inhibitory zone was then determined. To guarantee dependability, the test was repeated three times.

3.5 DETERMINATION OF ANTIFUNGAL ACTIVITY

For the evaluation of the antifungal activity of the plant extracts of the *C. papaya* by well diffusion method (47).

3.5.1 MEDIA PREPARATION

For the culture medium use potato dextrose agar. 500 ml flask was taken and add 250 ml distilled water. Then 15 grams of potato dextrose agar were added. Put the flask on the magnetic stirer for the 15 minutes. Autoclave the agar flask, inoculating loop and petridish. After the autoclave place all things in the laminar air flow cabinet. Pour potato dextrose agar into the petridish in the laminar flow cabinet. Leave it for the 10-15 minutes

3.5.2 ANTIFUNGAL ACTIVITY BY WELL DIFFUSION METHOD:

Well diffusion method for antifungal exposure testing was carried out to determination of the occurrence of antifungal properties of the papaya leaves extract. A fungal culture was used spread to potato dextrose agar plates consistently using a sterile swab. The wells containing a variety of plant extracts have been placed on the potato dextrose agar surface. Each test plate contains four wells. Each well 6mm in diameter. There were 5 doses like P1, P2, P10, P50, P100 each plate having the 4 different concentration like well A having 10µl, well B having 20µl, well C having 30 µl and well D having 40µl of each dose. Nystatin was used as control inhibitory zone and give 3.5 cm zone against A. niger.

3.6 DETERMINATION OF CYTOTOXICITY OF C. PAPAYA LEAVES EXTRACT.

We determine the cytotoxicity of extract of papaya leave by the MTT assay and cell proliferation to measure activity cellular metabolic of a cell.

On cultivated cells (NIH/3T3) in 96 well plates, the MTT test was performed. Five different concentrations, namely 50µg, 100 µg, 200 µg, 500 µg, and 1000 µg, were applied to cells in triplicate and the entire assay was done three times. After rinsing the cells with phosphate buffer saline (PBS), they were incubated for 2 hours in 500 L media comprising 60 L of MTT solution. In living cells, MTT generated insoluble purple formazan crystals. Before measuring absorbance at 570 nm, 10% sodium dodecyl sulphate (SDS) was employed to solubilize those intractable crystals of formazan. The % of live and dead cells were calculated.

3.7 STATISTICAL ANALYSIS

All the data of different experimental groups expressed as ± SEM in duplicate experiment. For statistical analysis, group mean were looked at by one way was ANOVA and Bonferroni test was utilized to recognize the contrasts between experimental group.

CHAPTER IV

RESULTS

4.1 COLLECTION OF SAMPLE AND EXTRACT FORMATION

Pour 90 ml ethanol over 10 gram powdered papaya leaves. Allow it to sit for 2-3 days. The extract was then filtered with the help of filter paper, extract was poured to the petridish and left to air dry. When the extract dried in the petridish weigh after 4 days, the petridish were weighed.

Table 4.1: Weighing of petridish before and after pouring of agar

Sr no	Weight of petri plates before pour plant extract	Weight of petri plates after pour plant extract	Amount of extract pour into the petri plates
1	41g	42g	23ml
2	45g	46g	22ml
3	44g	45g	22ml
4	46g	47g	24ml

From every 25ml of crude extract almost 1 gram of powder extract was collected.

We use this solution as the stock solution. From this solution make the 5 different doses like P1, P2, P10, P50, P100.

Figure 4.1 Collection, grinding of leaves and weigh of powder

Figure 4.2 Filter and drying of extract

4.2 PREPARATION OF DOSES:

Doses were prepared using the stock solution, with 1 mg of plant extract in 1 ml of distilled water, 2 mg of plant extract in 1 ml of distilled water, 10 mg of plant extract in 1 ml of distilled water, 50 mg of plant extract in 1 ml of distilled water, and 100 mg of plant extract in 1 ml of distilled water. There were 5 different doses like P1, P2, P10, P50, P100.

Figure 4.2.1 Stock Solution Formation and doses preparation

4.3 ANTIOXIDANT ASSAY

4.3.1 DPPH ASSAY

The capacity of stabilized free radicals (DPPH) 2, 2-diphenyl-1-picrylhydrazyl to be scavenged was examined. The UV spectrophotometer detected the highest absorbance by DPPH radicals that were free at 517 nm. The distilled water used as control group, P1 and P2 is not show the significant result. And P10(10mg/ml), P50 (50mg/ml),

P100(100mg/ml), show very significant result. All measurements were performed in triplicates.

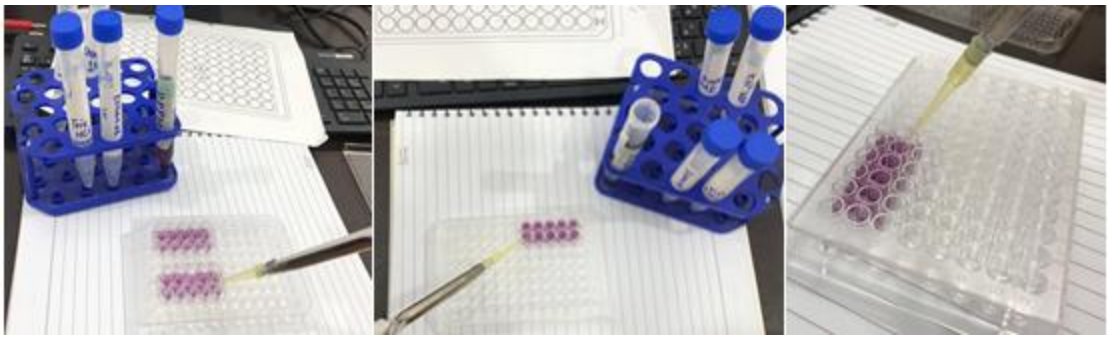


Figure 1.3.1 Performing DPPH assay of Papaya leaves Extract

Table 2.2: Show DPPH assay activity of different concentration of ethanolic extract of papaya leave

Sr no	Control	1mg	2mg	10mg	50mg	100mg
1	0.00±0.00	5.0±1.5	9.7±1.9	46±4.8	67±0.00	83±0.58

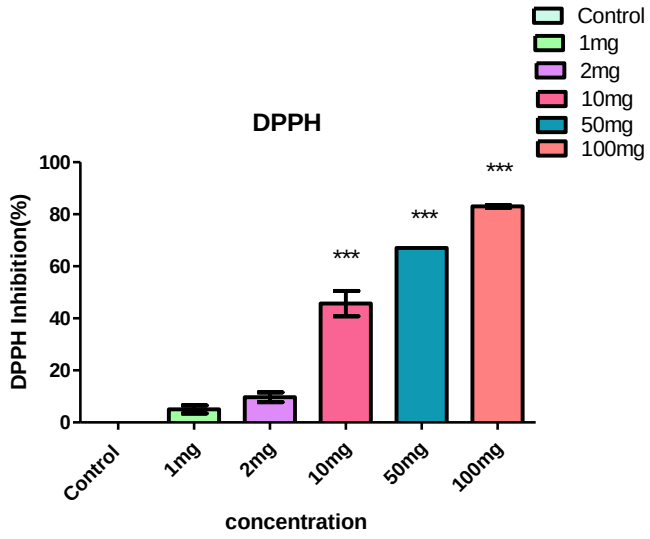


Figure 4.3.2: Show DPPH assay activity of different concentration of ethanolic extract of papaya leave. The values are expressed as ±SEM, where p>0.05is considered to be significant (*) show the level of signicance.

4.3.2 CATALASES

A 96-well plate was used for a catalase assay, with one well filled with 50mM KH2PO4, PH 7, and a blank from one group. The optical density was measured against a blank after 45-60 seconds in the dark. The catalase activity of different doses of papaya leave extract P1 (1mg/ml), P2 (2mg/ml) not showing significant result. But P10(10mg/ml,5.5±0.19), P50(50mg/ml, 7.2±0.055) and P100 (100mg/ml, 11±0.14) show significant result.

Figure 4.3.3 Performing catalase assay of Papaya leave extract

Table 4.3: Show Catalase activity of different concentration of ethanolic extract of papaya leave

Sr no	Control	1mg	2mg	10mg	50mg	100mg
1	2.0±0.003	3.0±0.058	3.8±0.017	5.5±0.19	7.2±0.055	11±0.14

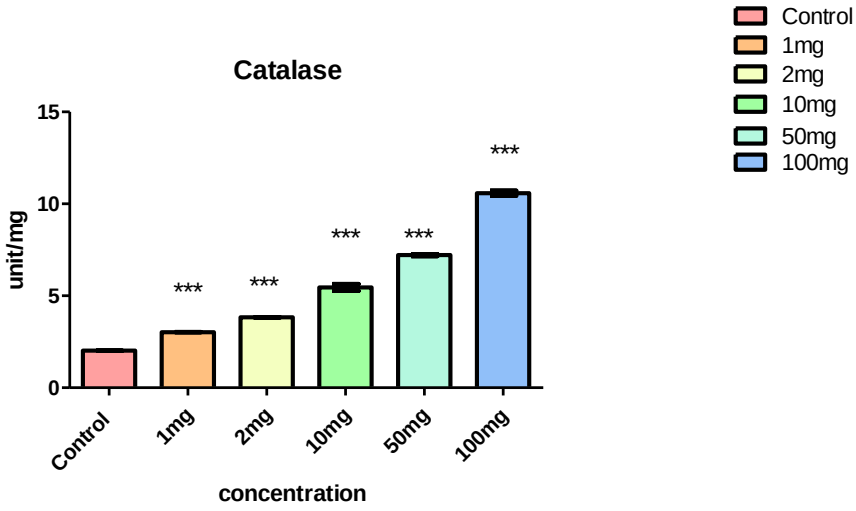


Figure 4.3.4: Show Catalase activity of different concentration of ethanolic extract of papaya leave. The values are expressed as ±SEM, where p>0.05is considered to be significant (*) show the level of significance.

4.4 MEDIUM FORMATION FOR BACTERIA CULTURE:

The culture medium used was Nutrient agar, 250ml of distilled water and 15 grams of nutrient agar. The flask was stirred for 15 minutes, then autoclaved and placed in a laminar air flow cabinet. The petridish was filled with nutritional agar and left for 10-15 minutes.

Figure 4.4.1: Medium preparation for Bacterial Culture

4.4.1 BACTERIAL STRAINS INOCULATION

The agar was solidified, disinfected innoculating loop with a sprit lamp and inoculated with 3 bacterial strains: S. pneumonia, S.aureus, and P. aeruginosa. After streaking, the petridish was named and incubated at 37C for 72h. After growth of bacteria perserved those petri plates for the further culturing.

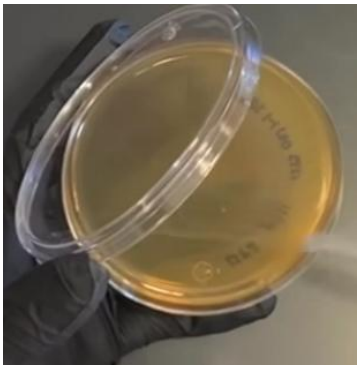


Figure 4.4.2: Inoculation of Bacterial Strain

4.4.2 Sub culturing of bacterial strain

Sub culturing a bacterial strain requires the use of Nutrient broth, tryptan, NaCl, and yeast extract. The culture medium is made with 250 ml flask, 100 ml distilled water, 2.5 grams of nutrient agar, 1 gram of tryptan, 1 gram of NaCl, and 0.5 grams of yeast extract. The nutrient broth was autoclaved, all the things was placed in a laminar air flow cabinet, and the nutrient broth was poured into the black caped test tube in the laminar air flow cabinet. The broth were cultured with the disinfected innoculating loop. The test tubes was incubated on 37C for 24 hours.

From broth we subculture the bacteria on the nutrient agar. Make well on the solidified agar and inoculate the culture the bacteria with the help of sterile swabs and make well with sterile T shaped tool and pour doses and incubate for 24 hours.

Figure 4.4.3 Sub culturing of Bacteria

4.5 ANTIBACTERIAL ACTIVITY BY WELL DIFFUSION METHOD:

The well diffusion method was used to assess the presence of antibacterial activities in papaya leaves extract. A sterile swab was used to transfer the bacterium culture to nutrient agar plates. Each test plate includes four wells with five doses: P1, P2, P10, P50, and P100. Each dose has four distinct concentrations: 10µl, 20µl, 30µl, and 40µl. different dillution of each dose of ethanolic extract of leaves of papaya plant show different behaviour against the different bacteria.

The antibacterial activity of papaya leave extract against the S.pneumonia. 20 µl show abundant zone of inhibition of dose P10 (10 mg /ml, 1.5333±0.120). The dose 40 µl P50 (50 mg /ml, 2.233±0.067) and the dose 40 µl of P100 (100 mg /ml, 1.367±0.088)not show the abundant zone of inhibition due to overdose toxicity.

Amphicin (am) 10 was served as control its zone of inhibition was 1.5 cm after 24 hours.

The antibacterial properties of papaya leave extract in contradiction of the P. aeruginosa, 40 µl show abundant zone of inhibition of dose P50(50 mg/ml, 2.433±0.067) as compare to the dose P100(100mg/ml, 2.133±0.033) not show the abundant zone of inhibition due to overdose toxicity. Amikacin (30) was served as control its zone of inhibition was 2.8 cm after 24 hours.

The antibacterial activity of papaya leaves extract against the S.aereus. 30µl show the minor difference with 40 µl. 40 µl show abundant zone of inhibition of dose P100(100mg/ml, 2.666±0.033). Papaya extract is highly effective against the S.aereus. The zone of inhibition seen in the picture the different cocentartion of dose P1 show less zone of inhition as compare to others doses. Amphicin (am) 10 was served as control its zone of inhibition was 3 cm after 24 hours.

Table 4.5.1: Inhibition zone of Papaya leaves ethanolic extract against the S. pneumonia

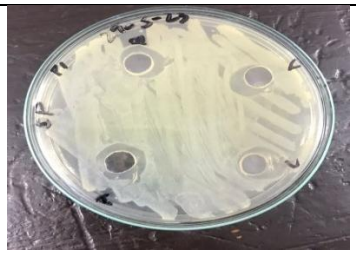
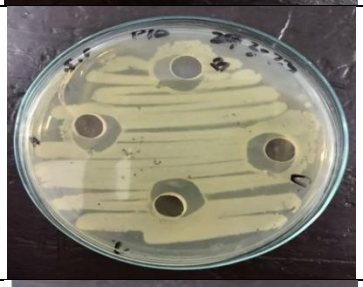
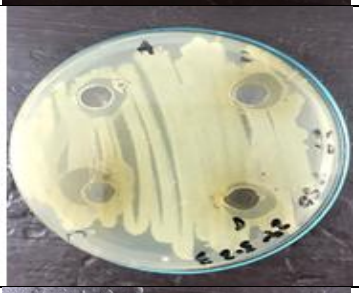
S.pneumonia		
Sr.no	Concentrations	Pictures
1	P1 (1mg /ml)	
2	P2(2mg/ml)	
3	P10(10mg/ml)	
4	P50(50mg/ml)	
5	P100(100mg/ml)	

Table 4.5.1: The different concentration and dilution of papaya plant extract show the zone of inhibition against the bacteria S. pneumonia.

Sr no	Concentration	A(10µl)	B(20µl)	C (30µl)	D (40µl)
1	P1 (1mg/ml)	1.166±0.033	1.333±0.033	1.300±0.058	1.400±0.058
2	P2(2mg/ml)	1.200±0.058	1.400±0.058	1.500±0.153	1.233±0.033
3	P10(10mg/ml)	1.300±0.058	1.5333±0.120	1.300±0.058	1.333±0.067
4	P50(50mg/ml)	1.400±0.058	1.433±0.033	1.333±0.088	1.500±0.058
5	P100(100mg/ml)	1.233±0.067	1.333±0.088	1.367±0.033	1.367±0.088

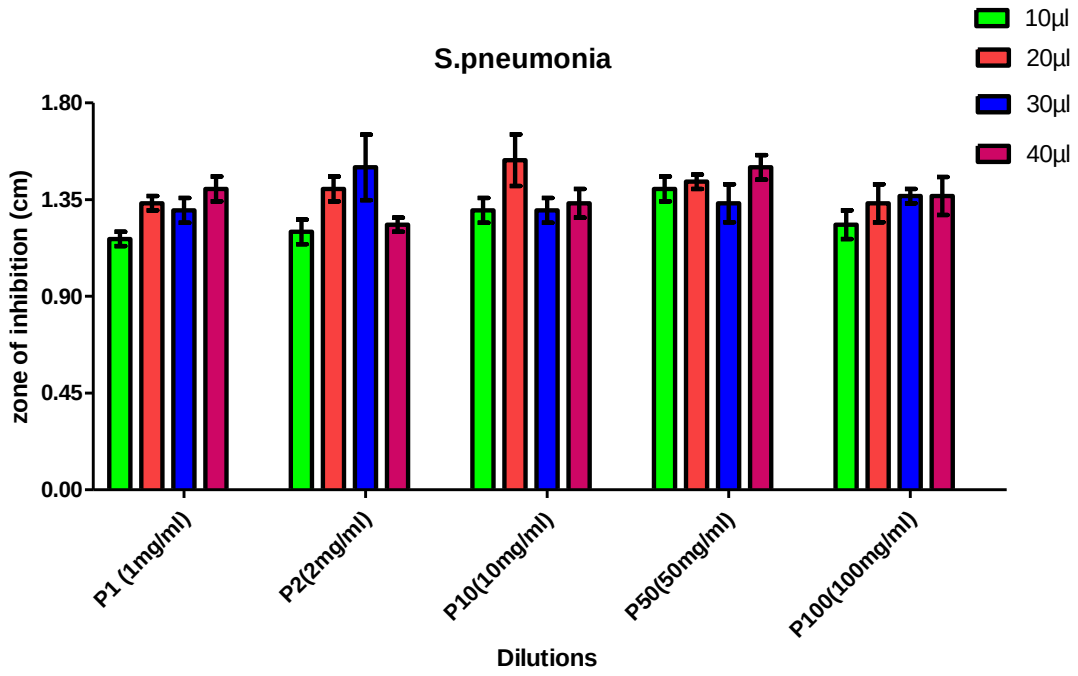


Figure 4.5.3: The different concentration and dilution of papaya plant extract show the zone of inhibition against the bacteria S. pneumonia

Table 4.5.2: Inhibition zone of Papaya leaves ethanolic extract against the P. aeruginosa

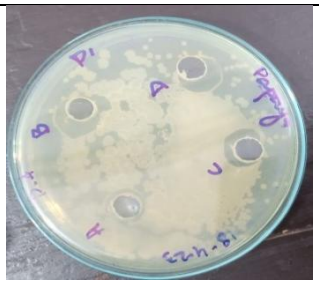
P.aeruginosa		
Sr.no	Concentrations	Pictures
1	P1 (1mg /ml)	
2	P2(2mg/ml)	
3	P10(10mg/ml)	
4	P50(50mg/ml)	
5	P100(100mg/ml)	

Table 4.5.4: The different concentration and dilution of papaya plant extract show the zone of inhibition against the bacteria P. aeruginosa

Sr no	Concentration	A(10µl)	B(20µl)	C (30µl)	D (40µl)
1	P1 (1mg/ml)	1.166±0.033	1.300±0.058	1.333±0.033	1.466±0.033
2	P2(2mg/ml)	1.400±0.058	1.500±0.058	1.500±0.058	1.500±0.058
3	P10(10mg/ml)	1.266±0.033	1.700±0.058	2.200±0.058	2.266±0.033
4	P50(50mg/ml)	1.400±0.058	2.200±0.058	2.333±0.067	2.433±0.067
5	P100(100mg/ml)	1.200±0.058	1.433±0.067	1.333±0.033	2.133±0.033

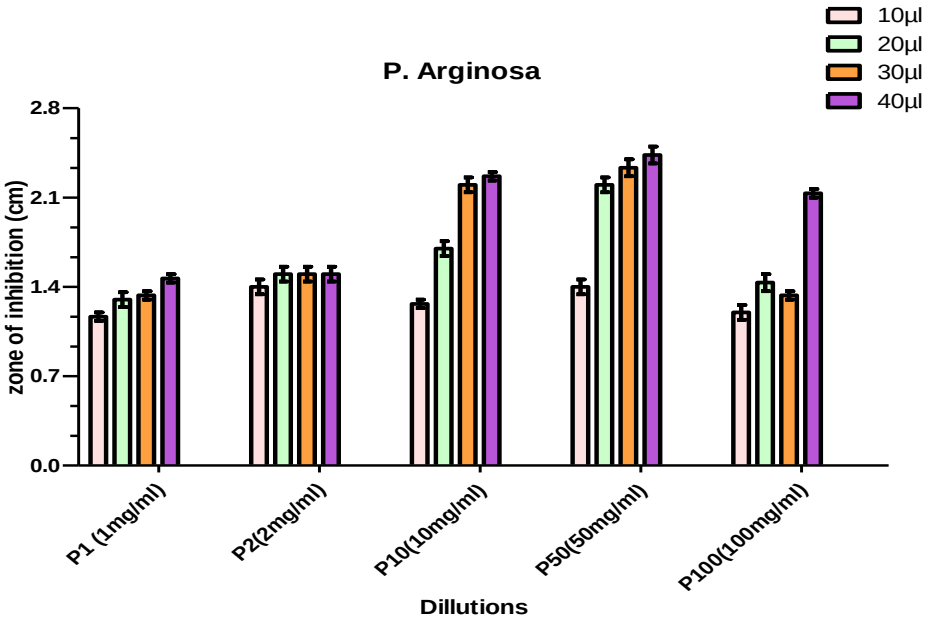


Figure4.5.5: The different concentration and dilution of papaya plant extract show the zone of inhibition against the bacteria P. aeruginosa

Table 4.5.5: Inhibition zone of Papaya leaves ethanolic extract against the S.aureus

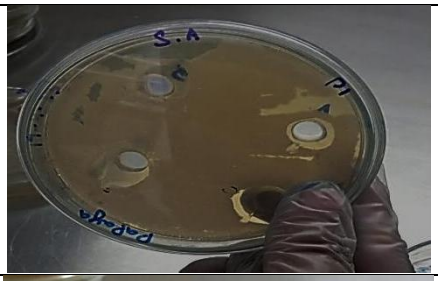
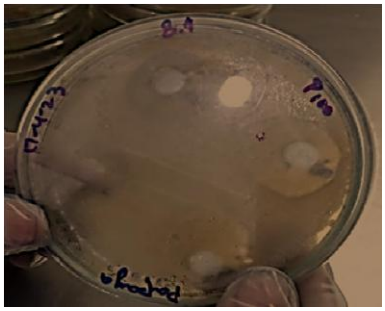
S.aureus		
Sr.no	Concentrations	Pictures
1	P1 (1mg /ml)	
2	P2(2mg/ml)	
3	P10(10mg/ml)	
4	P50(50mg/ml)	
5	P100(100mg/ml)	

Table 4.5.6 : The different concentration and dilution of papaya plant extract show the zone of inhibition against the bacteria S.aereus

Sr no	Concentration	A(10µl)	B(20µl)	C (30µl)	D (40µl)
1	P1 (1mg/ml)	1.200±0.058	1.266±0.033	1.366±0.033	1.500±0.058
2	P2(2mgl/ml)	1.566±0.033	1.466±0.033	1.466±0.120	2.033±0.033
3	P10(10mg/ml)	1.266±0.033	1.466±0.033	1.600±0.058	2.133±0.033
4	P50(50mg/ml)	1.266±0.033	1.366±0.033	1.866±0.033	2.233±0.067
5	P100(100mg/ml)	2.200±0.058	2.400±0.058	2.633±0.033	2.666±0.033

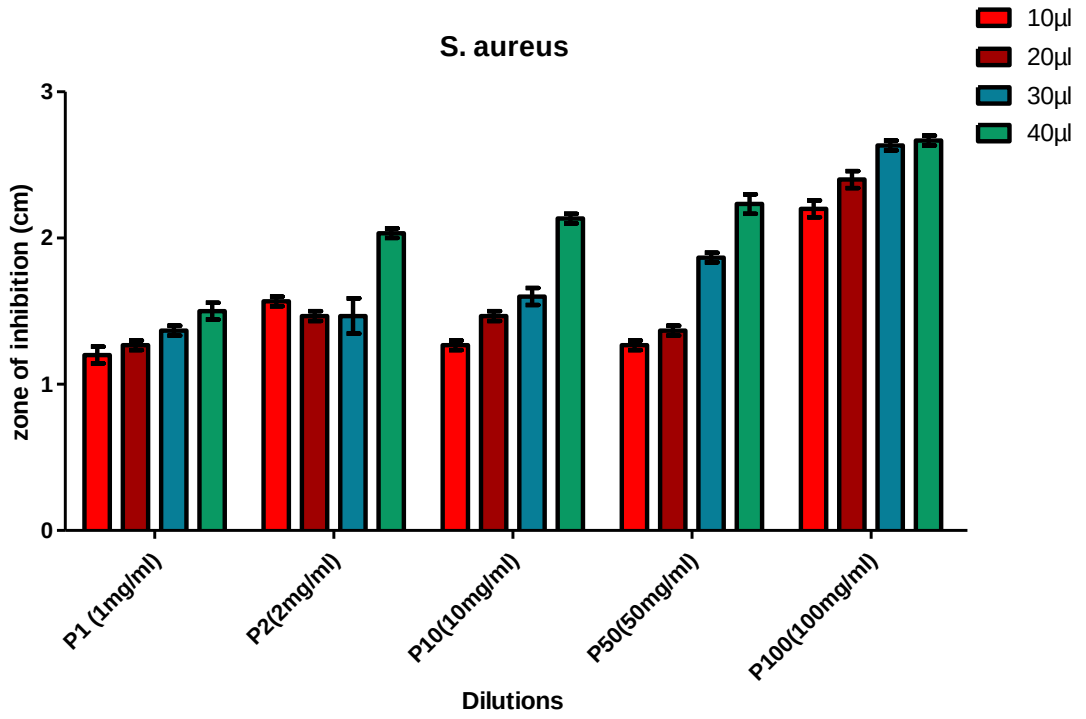


Figure 4.5.3: The different concentration and dilution of papaya plant extract show the zone of inhibition against the bacteria S.aereus.

4.6 DETERMINATION OF ANTIFUNGAL ACTIVITY:

Well diffusion method was used to determine the antifungal properties of papaya leaves extract. A fungal culture was spread to potato dextrose agar plates using a sterile swab. Each test plate contained four wells, each 6mm in diameter, with 5 doses of different concentrations. Each dose had 10µl, 20µl, 30µl and 40µl of each dose. The zone of inhibition seen in the picture the different cocentartion of dose P1 show

less zone of inhition as compare to others doses. As a control group we use 100 unit Nystatin was used as inhibitory zone and give 3.5 cm zone.

The antifungal properties of papaya leave extract against the A.niger.40 µl show abundant zone of inhibition of dose P100(100mg/ml, 3.033±0.033).

Table 4.6.1: Inhibition zone of Papaya leaves ethanolic extract against the A.niger

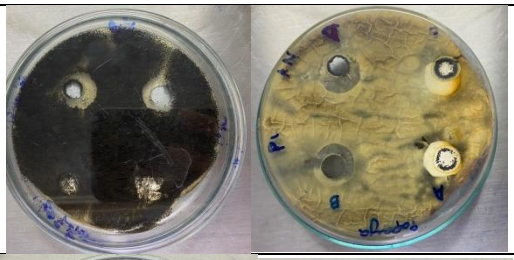
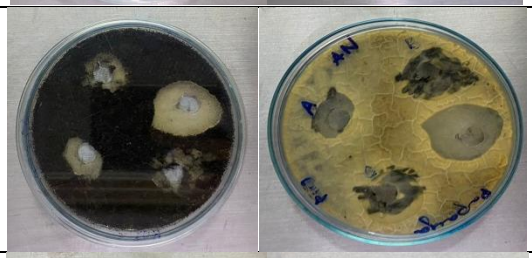
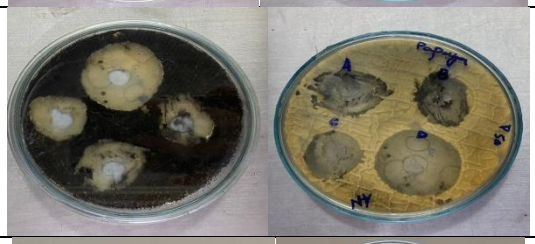
A.niger		
Sr.no	Concentrations	Pictures
1	P1 (1mg /ml)	
2	P2(2mg/ml)	
3	P10(10mg/ml)	
4	P50(50mg/ml)	
5	P100(100mg/ml)	

Table 4.6.2: The different concentration and dilution of papaya plant extract show the zone of inhibition against the fungus A.niger

Sr no	Concentration	A(10µl)	B(20µl)	C (30µl)	D (40µl)
1	P1 (1mg/ml)	1.266±0.033	1.466±0.033	1.466±0.033	1.666±0.033
2	P2(2mgl/ml)	1.033±0.033	1.233±0.033	1.466±0.033	1.566±0.033
3	P10(10mg/ml)	1.500±0.058	1.666±0.033	1.666±0.033	2.466±0.033
4	P50(50mg/ml)	1.533±0.033	1.666±0.033	1.833±0.033	2.766±0.033
5	P100(100mg/ml)	2.066±0.033	2.366±0.088	2.566±0.033	3.033±0.033

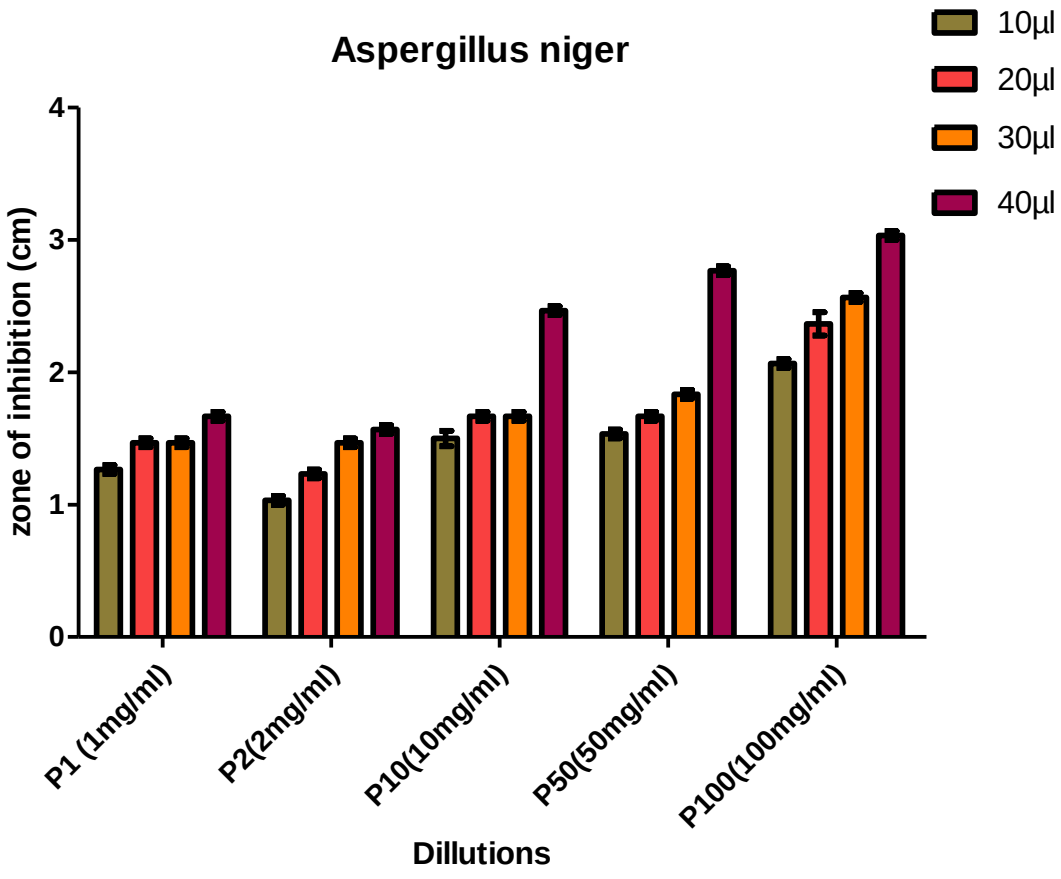


Figure 4.6.1The different concentration and dilution of papaya plant extract show the zone of inhibition against the fungus A.niger

4.7 DETERMINATION OF CYTOTOXICITY OF C. PAPAYA LEAVES EXTRACT.

We determine the cytotoxicity of extract of papaya leave by the MTT assay and cell proliferation to measure activity cellular metabolic of a cell.

On cultivated cells (NIH/3T3) in 96 well plates, the MTT test was performed. Five different concentrations, namely 50µg, 100 µg, 200 µg, 500 µg, and 1000 µg, were applied to cells in triplicate and the entire assay was done three times. After rinsing the cells with phosphate buffer saline (PBS), they were incubated for 2 hours in 500 L media comprising 60 L of MTT solution. In living cells, MTT generated insoluble purple formazan crystals. Before measuring absorbance at 570 nm, 10% sodium dodecyl sulphate (SDS) was employed to solubilize those intractable crystals of formazan. The % of live and dead cells were calculated using the formula:

$$\% \text{ viablity cell} = \frac{\text{Abs. of sample}}{\text{Abs of control}} \times 100$$

Table 4.7.1: Cytotoxicity of ethanolic papaya leaf extract

Sr. no	N	50 µg	100 µg	200 µg	500 µg	1000 µg
1	99.7±0.338	98.4±0.586	97.7±0.338	98.4±0.000	100±0.338	99.7±0.338

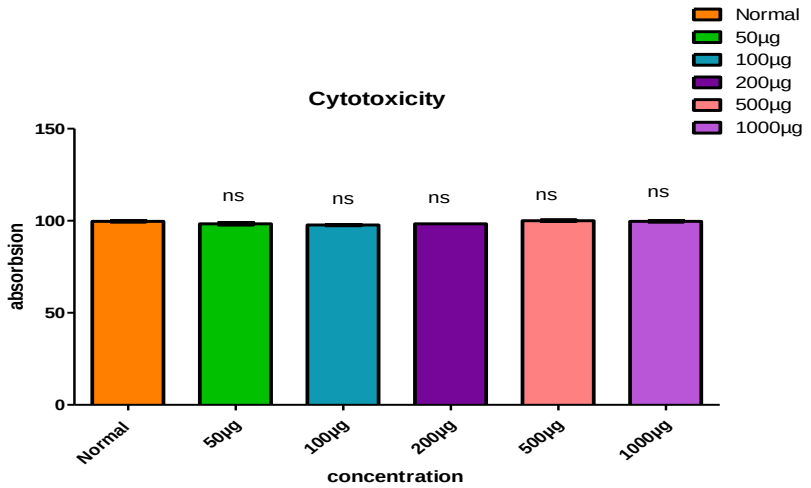


Figure 4.7.1: Cytotoxicity of ethanolic papaya leaves extract. The values are expressed as ±SEM, where p>0.05is considered to be significant (*) show the level of significance.

Chapter 5

DISCUSSION

Papaya is a dioecious plant that can grow up to 10 meters tall and has soft stems and huge leaves. It contains minerals, vitamins, phytochemicals, carotenoids, and polyphenols. It is found in tropical climates and is used to make medicine. Traditionally, papaya fruit has been used to treat gastrointestinal problems and is an anti-bacteria agents(5,6).

The agar well diffusion is more successful against some bacteria and fungi, such as S. aureus, S. pneumoniae, P. aeruginosa, and C. albicans, the cirica papaya leaf extract more effective in determining the antibacterial activity. Gramme (+)ve positive and gramme (-)ve bacteria were both significantly resistant to the ethanolic extract of the leaves from the C. papaya plant.

The sensitivity of various bacteria and fungi to varied amounts of extract from dried C. papaya leaves was evaluated in vitro. The result of the antibacterial properties of the papaya leaf is both the negative and the positive results. There were 3 bacterial strains against the dried leaf ethanolic extract of papaya. There were 5 different doses of papaya leaf extract and further five concentrations and observe the zone of inhibition according to the concentration added in the bacterial plates.

C. papaya leaf and root extracts were prepared and tested for antibacterial efficacy against specific human pathogenic microorganisms. The root extracts outperformed the gram-negative bacteria, with the greatest effectiveness against P. aeruginosa. The MIC and MBC were 50 to 200 mg/ml (13).

The antioxidant properties of NADES have not been extensively studied, but many research papers have been written on the topic (80). The presence of an abundance of free radicals causes enhanced absorption, while lower absorbance suggests that the solution contains fewer free radicals and has a higher inhibitory capacity. Antioxidant capability and absorption are inversely related, but DPPH disappearance and antioxidant capacity are closely linked (81).

The DPPH assay of ethanolic papaya leave extract shows the % inhibition. The absorbance was measured spectrophotometrically at 517 nm after a 30 minutes incubation at room temperature. The capability of the sample to scavenge the DPPH radical was calculated. The distilled water used as control group, P1 and P2 is not show the significant result. And P10 (10mg/ml, 46±4.8), P50 (50mg/ml, 67±0.00), P100(100mg/ml, 83±0.58), show very significant result. All measurements were performed in triplicates.

The DPPH free radical scavenging of sample was determined by measuring the decrease in absorbance of DPPH solution at 517 nm in the presence of the extract. Methanolic DPPH solution with initial concentration of 0.2 mM was prepared and incubated in dark for 2 hours at room temperature. Fresh DPPH solution was prepared for each assay. Then, the DPPH solution (100 µL) was mixed with the sample (50 µL). The absorbance of a sample was measured spectrophotometrically at 517 nm and its ability to scavenge the DPPH radical was calculated (21).

A 96-well plate was used for a catalase assay, with one well filled with 50mM KH2PO4, PH 7, and a blank from one group. The optical density was measured against a blank after 45-60 seconds in the dark. The catalase activity of different doses of papaya leave extract P1 (1mg/ml), P2 (2mg/ml) not showing significant result. But P10(10mg/ml,5.5±0.19), P50(50mg/ml, 7.2±0.055) and P100 (100mg/ml, 11±0.14) show significant result.

There are 5 different doses P1, P2, P10, P50, P100 against the each bacteria and each dose have 4 different dilution like in well A 10 µl, well B 20 µl, well C30 µl, well D 40 µl.

The antibacterial activity of papaya leave extract against the S.pneumonia. The different dilutions dose P1 (1mg/ml) show the minor zone of inhibition against S.pneumonia. The different dilutions dose P2 (2mg/ml) show the minor increase in zone of inhibtion as compared to the P1 (1mg/ml) dose against S.pneumonia. the dilution 20 µl show abundant zone of inhibition of dose P10 (10 mg /ml, 1.5333±0.120). The dose 40 µl P50 (50 mg /ml, 2.233±0.067) and the dose 40 µl of

P100 (100 mg /ml, 1.367±0.088)not show the abundant zone of inhibition due to overdose toxicity.

The antibacterial properties of papaya leave extract in contradiction of the P. aeruginosa the dilution of other doses show result significantly but some dilution doses like the dilution of P100(100mg/ml) show small zone of inhibition due to overtoxicity, The 40 µl show abundant zone of inhibition of dose P50 (50 mg/ml, 2.433±0.067) as compare to the dose P100(100mg/ml, 2.133±0.033) not show the abundant zone of inhibition due to overdose toxicity.

The antibacterial activity of papaya leaves extract against the S.aereus. 30µl show the minor difference with 40 µl. 40 µl show abundant zone of inhibition of dose P100(100mg/ml, 2.666±0.033). Papaya extract is highly effective against the S.aereus.

The antifungal properties of papaya leave extract against the A.niger the dose P2 (2mg/ml) show that when we increase the concentration the zone of inhibition is also increase. The best result 40 µl show abundant zone of inhibition of dose P100(100mg/ml, 3.033±0.033). The papaya leave extract shows the direct relationship between dose and zone of inhibition. When we increase the dose concentration it shows large zone of inhibition.

MTT assay was used to measure the cytotoxicity which is based upon on the metabolic reduction of soluble MTT salt. MTT assay on NIH/3T3. Our in-vitro of papaya plant extract shows that it does not cause any injury as show in MTT assay results.

CONCLUSION

Papaya is a common plant which we can find in Pakistan easily. Papaya plant belongs to family caricaceae. The scientific name of the papaya plant is *c. papaya.* The papita is common name of papaya. The antioxidants assay (DPPH assay, Catalase Assay) done to check the viability of the papaya leave extract this show the significant result. From this entire experiment, It can be concluded that the papaya leave have ability to kill antibacterial and antifungal activity. This experiment done by making ethanolic extract of the papaya leaves and check against the bacteria and fungus. It show the significant results. Tha ethanolic papaya leave extract also have ability to inhibit the growth of bacteria and spore of fungus. The MTT assay shows the no cytotoxicity against the mammalian cell. The evaluation of papaya leaf extract can be performed as preclinical trials, in order to examine the actual effect of this extract for preventing/curing other viral or bacterial infections.

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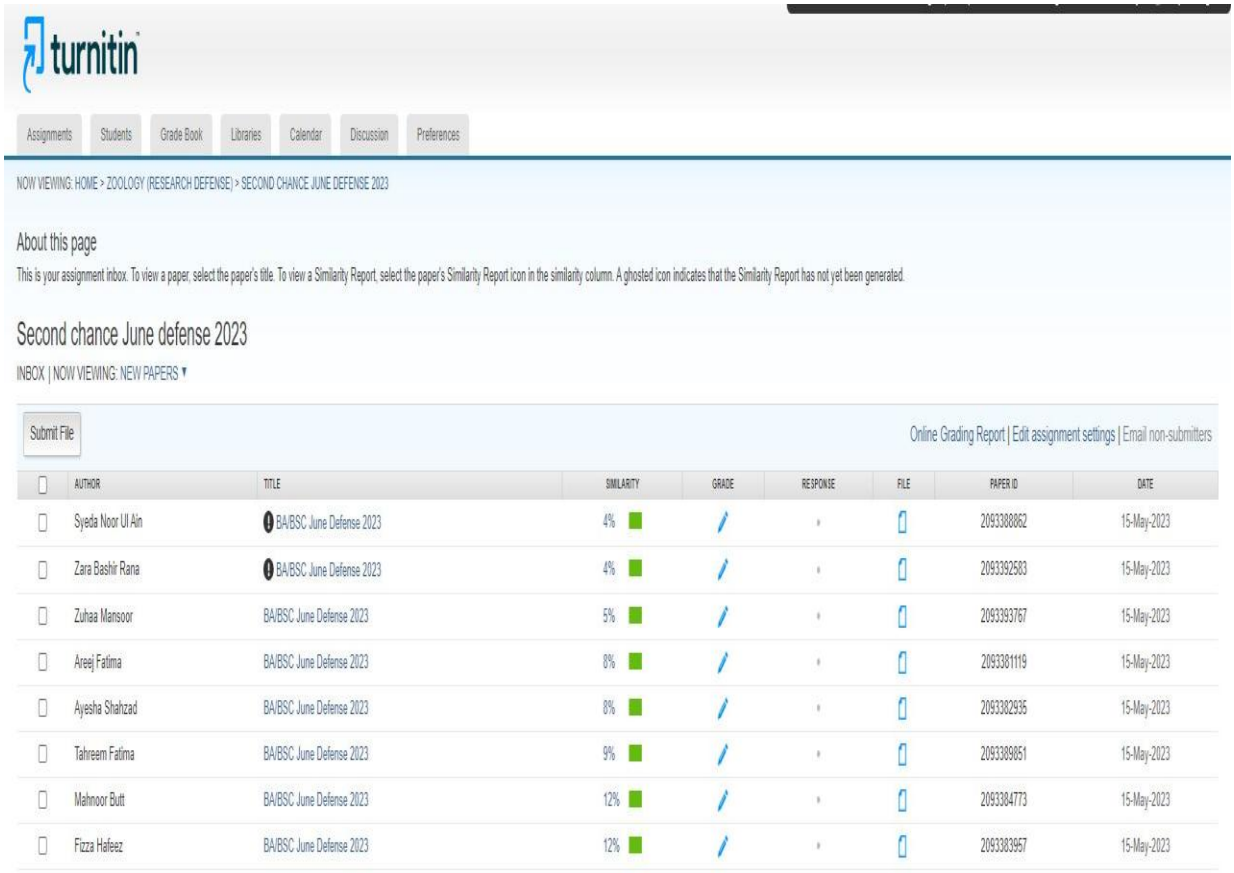

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