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THERANOSTIC AND ANTIBACTERIAL CHARACTERIZATION OF PYRUS PASHIA AGAINST BACTERIAL STRAINS OF MEDICAL IMPORTANCE

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ABSTRACT

Infectious diseases have taken the lives of many people across the globe. Antibiotics can tackle with such disease causing bacteria but unfortunately antibiotic resistance is being increasing to such an extent that cases of multi drug strains of bacteria have been reported. To overcome such issues plant derived antibacterial are under research, therefore the aim of the present study was not only to evaluate the antibacterial activity of *Pyrus pashia* but also to potentiate the antibacterial effect of the plant extract using RSM. Therefore the optimized antibacterial activity of extract was tested against *Escherichia coli* and *Bacillus subtilis* through the measurement of zone of inhibition. All the bacteria were inhibited by the ethanolic plant extract. Quantitative phytochemical analysis was also done using *in vitro* assays. The phenolic content of plant extract was 14.26 mg of GAE/g; tannins came out to be 41.26 mg of GAE/g, alkaloids 45.26 mg of GAE/g, flavonoids 86.26 mg of GAE/g and carotenoids mg of GAE/g. The plant extract was known to possess antibacterial activity may be due to the presence of these phytochemicals as their antibacterial mode of action have been researched previously by the scientists. An overview of mode of action of these phytochemicals is also provided. This study concludes that *Pyrus pashia* possess antibacterial activity which can also be enhanced using specific values for temperature, pH and concentration.

Keyword: Antibacterial activity, *Pyrus pashia, Escherichia coli, Staphylococcus aureus*

INTRODUCTION

Medicinal plants have always been a unique source in curing several illnesses as well as in the development of health. Many people seem to be interested in solving medical issues using plants and herbs, as synthetic medicines contains many side effects. It is known that about 1.42 billion people around the world depend on herbs to combat health problems especially in Asian countries where people choose nature over chemicals for their basic health necessities. In 2002 WHO launched its traditional medicine strategy emphasizing on safe and effective use of herbal medicine. Due to the tremendous efforts of WHO in describing the benefits of traditional medicines will lead to their utilization [1]. By keeping the importance of medicinal plants in view, this review represents the information about the antibacterial activity of Pyrus pashia.

Pyrus pashia is found mostly in tropical America, Southern US. It is cultivated in Florida, Jamaica, South Africa, Uganda and India. It grows quickly into a tree up to a height of 4 to 6m [1]. It has a smooth yellow-green bark. After 5 years of planting, flowers and fruits begin to grow on it. Its flower is yellow and gives off fragrance. It belongs to a Fabaceae family [2]. It is also called commonly as Palo Verde, Jerusalem thorn. *Pyrus pashia* is a spiny, shrub small tree. It is famous for its drought tolerance property. Its flower is the rich source of honey [3]. It grows up to 5-10 m of height and has a trunk of 40 cm diameter. It remains green all the time. This plant possesses many traditional uses. Its leaves, fruits and stems are used to treat malaria and fever. It is also used as an abortifacient drug. Its leaves have been reported to treat diarrhea [2]. Moreover rheumatism can be treated by its flower and leaf alcoholic extract. However the salutary effects of these *Pyrus pashia* extracts have not been investigated and are generally not noted at the biochemical and biological levels [4]. Its leaves appear in an alternate order and are designed for special activity. Its flower's shape resembles pea to a small degree. They are golden yellow in colour and have a pleasant smell. The plant is also an important source of various types of compounds with diverse chemical structures.

Pharmacological investigations revealed antibacterial, antidiabetic, antioxidant, antirabies, ameobicidal, antipyretic, antimalarial, hepatoprotective, as well as antispermatogenic activities of this plant. In other studies it was observed that *Pyrus pashia* withstand heavy metal

effects such as zinc, chromium, lead, cobalt, cadmium on germination and seedling growth. Since it was reported that the plant can grow in presence of heavy metals, it can be cultivated in polluted areas where it can resist heavy metal toxicities [1]. Plants hold much value regarding drugs. Even synthetic drugs are sometimes plant based. Therefore, it has become necessary to conduct plant based researches for the extraction of new drugs [5]. Antibiotics were produced to treat bacterial, fungal and protozoal infections. No doubt the discovery of antibiotics in 1950's has ease mankind but scientists have become aware of the microorganisms that continue to fight against drugs for survival. They are able to adapt themselves due to their diverse biochemical mechanisms which they use to resist antibiotic drugs. Other than this, inappropriate prescribing and the misuse of antibiotics has spread the resistance among microorganisms [6]. This challenge will remain until and unless scientists come up with alternative to kill drug resistant microbes. Microbes have the quality to adapt when they monitor the change in their environment. This is due to their flexible metabolic power [7]. In order for bacteria to cause infection they must remain persistence in the environment as well as able to interact with the humans. There are various virulence factors that facilitate disease development. These include adhesins and membrane bound proteins that helps bacteria to adhere itself on host cell membrane. This is followed by invasion and colonization. Moreover microbial toxins damages host tissues resulting in an inflammation response.

Bacteria are also able to resist host cell immune system due to its capsule and other cell wall components. Plant has been playing its role in the development of the well-being of humans in every aspect. Large number of plant products has been used in enhancing flavors, preservation of food as well as in sustaining human health. Plant derived compounds have also been reported previously to possess antibacterial activity. Out of which secondary metabolites which are only produced in limited set of species of plants as defensive molecules to protect plants against predators such as animals, insects. Secondary metabolites are derived from primary metabolites and are known to have complex chemical structures. They have many categories common one including alkaloids, terpenoids and phenols. Secondary metabolites possess several uses for humans. The major advantage of using plant based drug is that they donot possess any side effects whereas synthetic medicines

does. Moreover no report on the antimicrobial resistance came into knowledge against phytochemicals; this is probably due to their ability to affect bacteria in variety of ways. The effectiveness of antimicrobial activity, their low cost and non-toxicity increase their usage not only in medicines but in livestock and poultry industry, as disinfectants in food industry, veterinary and pharmaceutical industries [8]. The characteristics of plant compounds targeting bacterial cell have been discussed previously in some reviews. In this review study have also focused on how secondary metabolites modulate the virulence factors of bacteria. Secondary metabolites are classified according to their mode of actions, including multidrug resistance inhibitors and targeting virulence factors. Secondary metabolites are also classified based on their chemical structures, which also affects their antimicrobial properties. The major groups of phytochemical includes Phenolics and polyphenols such as flavonoids, quinones, tannins and coumarins. Flavonoids are pigments which are further categorized into flavone, flavanones, flavanols and anthocyanidins. Alkaloids and terpenoids. Sixteen phenolic compounds have been extracted from *Anacardium occidentale* which have shown a positive antimicrobial activity against two bacteria and two yeasts [9].

There are many modes of actions of plant derived antimicrobials suggested by researchers but the proper one remains uncertain. Some phytochemicals retard microbial growth. Some try to interfere with the metabolic pathways of bacterial cells. Signal transduction and regulation of gene expression is also modulated by phytochemicals. Other than this interaction with phospholipoidal cell membranes, damaging those enzymes involved in producing energy as well as synthesizing structural components has also been reported. DNA and RNA become nonfunctional. Proton motive force is disrupted as well as electron flow and active transport processes. Cell coagulation of intracellular components also occurs. Therefore by studying the mechanisms of phytochemicals, their chemotherapeutic and chemoprophylactic properties can be obtained to treat many infectious diseases [10]. After evaluating and confirming the antibacterial activity of Pyrus pashia, its antibacterial activity was then potentiate using Response surface Methodology which is a collection of statistical techniques to form empirical models. RSM have been

used in many ways to enhance the potency of metabolites, enzymes and drugs [11].

MATERIALS AND METHODS

3.1 Plant sample collection

Samples were arranged from the market in Lahore. After which the plant was distinguish by Prof. Dr. Ijaz Rasood from Department of Botany at Agriculture University of Faisalabad.

3.2 Solvents used for extraction

The solvent employed for extraction was Ethanol.

3.3 Bacterial species tested against the plant extract

Escherichia coli, Pseudomonas aeruginosa (ATCC-27853), *Staphylococcus aureus* (ATCC-25922), *Bacillus subtilis*, *Klebsiella pneumonia* and *Salmonella typhi* obtained from Microbiology lab in department of IMBB of The University of Lahore.

3.4 Culture media and the chemicals used during the study

Culture media and chemicals	Brand/Company
Muller Hinton Broth	Oxoid
Agar technical	Oxoid
NaCl	Riedel-de Haen
$BaCl_2$	BDH Laboratory, England
H_2SO_4	AnalaR

3.5 Antibiotic tested against the bacterial strains

Amikacin (30ug)

Extraction procedure

2g of a Plant sample in its powdered form were dissolved in 4 ml of 100% concentrated ethanol in a test tube for extraction and placed overnight on bench top at room temperature. During 24 hour time period 10 minutes at 14000 rpm. The supernatant was poured in a new ependroff tube and saved. The extract was stored in a refrigerator at 4°C until further use.

3.6 Determination of antibacterial activity

3.6.1 Preparation of McFarland solution

target compounds were extracted from the plant by ethanol. The extract was then subjected to filtration using Whatman filter paper. The extract was then poured in a small petri dish and left for 48 hr. for evaporation to concentrate the filtrate. When the filtered extract was fully dried it was then dissolved in 2 ml of a DMSO solution. The latter was then centrifuge for

1.175 g of $BaCl_2$ was dissolved in 100ml of distilled water to make 1.175% of $BaCl_2$ solution. 1ml of 100% concentration of H2SO4 was dissolved in 99ml of distilled water to prepare 1% H_2SO_4. Then 0.5ml of 1.175% of $BaCl_2$ was mixed with 99ml of

H_2SO_4 to form a turbid solution up to 100ml. It was stored in room temperature at 25°C.

3.6.2 Preparation of culture media

Culture media was prepared based on the instruction given by manufacturer by adding 28 g of Muller Hinton Broth, 15 g of technical agar dissolved in 500 ml of distilled water in a flask. The media was sterilized in an autoclaved at 121°C and 15 Pa for 1 hour.

Adjusting pH of media

The pH of media was adjusted according to the requirement of the experiment by using a pH meter in the CRiMM.

3.6.3: Antibacterial susceptibility test:

3.6.4: Preparation of discs

Filter paper was punched several times to make many discs.

3.6.5: Preparation of inoculum

Each bacterial samples were picked under sterile conditions by a sterile platinum loop and inoculated into each 5ml, 0.85 NaCl tubes. These bacterial cultures were adjusted according to 0.5 McFarland standard solution.

Disc diffusion method

The antibacterial assay was performed using agar disc diffusion assay. Muller Hinton media was poured in sterilized glass petri plates after it was cool down to 45°C. The sterilization of glass plates done before hand in hot air oven for 30 mins at 180°C. After pouring under aseptic environment the plates were kept on a bench to solidify and then incubated at 37°C for 24 hr. to check for any contamination. Petri plates were then labeled according to the required organism, pH, temperature and concentration.

5-10 µl of each of the prepared bacterial inoculum was added according to every labeled petri plates using pipette. Swabbing was done using a sterile cotton bud which was sterile beforehand in hot air oven. Sterile filter paper discs were placed very carefully onto the agar plates using a sterile forceps. Plant extracts of required concentration (5, 7.5, and 10) were added onto the discs using a pipette. Plates were kept upside down in incubators according to their required temperatures. After 24 hours of incubation the results were noted using a ruler of mm calibration.

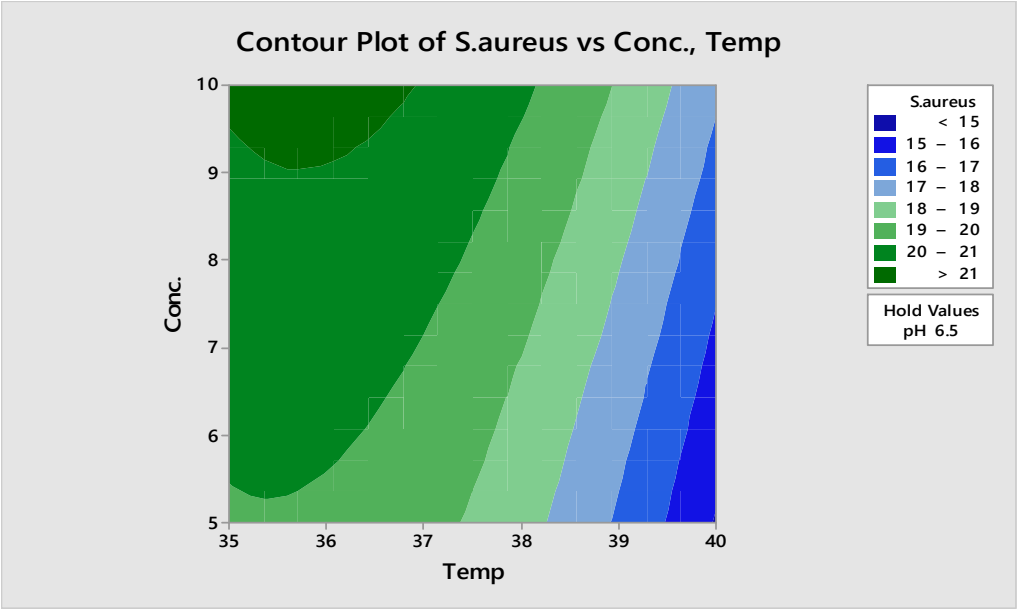
Quantitative Phytochemical Analysis

Tannins, Phenols and Flavonoids were determined by their respective methods.

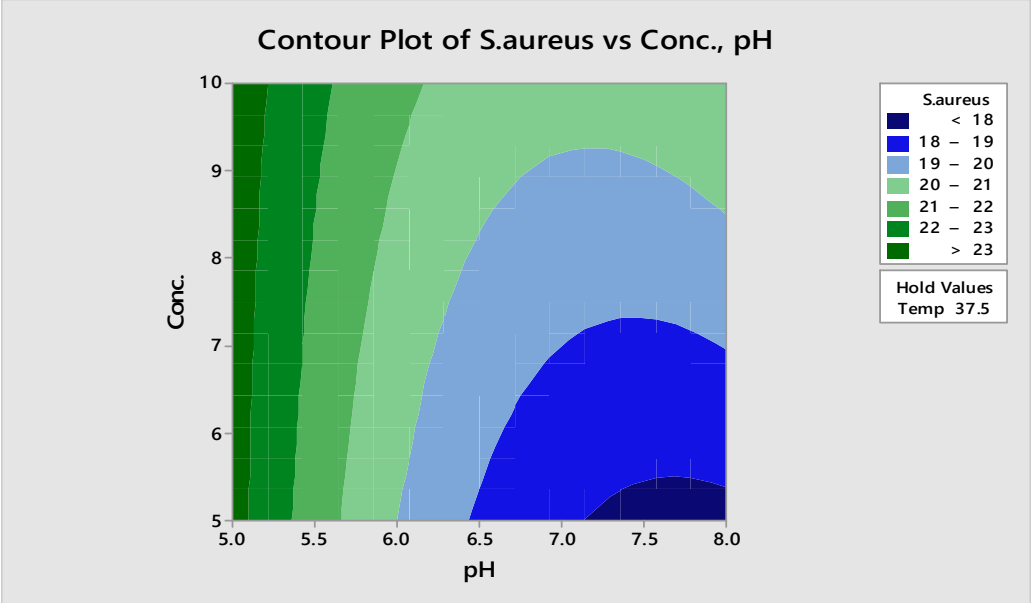
RESULTS

The maximum antibacterial activity that plant extract can give was checked using RSM. For maximum antibacterial activity the uncoded and actual level of three independent variables were chosen, temperature, pH, and concentration. A set of 60 experiments that were all factorials and

none of the experiment were zero-point test, performed to approximate their error. The treatment with the uncoded levels and the zone of inhibition gained by the test variable is in the table 1.1. Counter plot is used to study the relationships among the test variable and to check the maximum level of each variable that can give higher zone of inhibition due to antibacterial activity of *Pyrus pashia* extract.



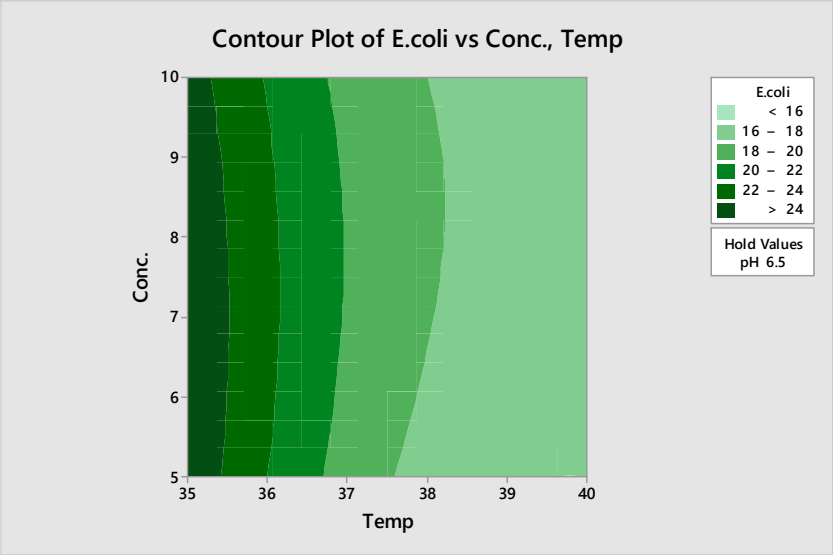
The highest zone of inhibition is at concentration 9.5-10.0 and temperature 35-36.9°C at a fixed pH of 6.5



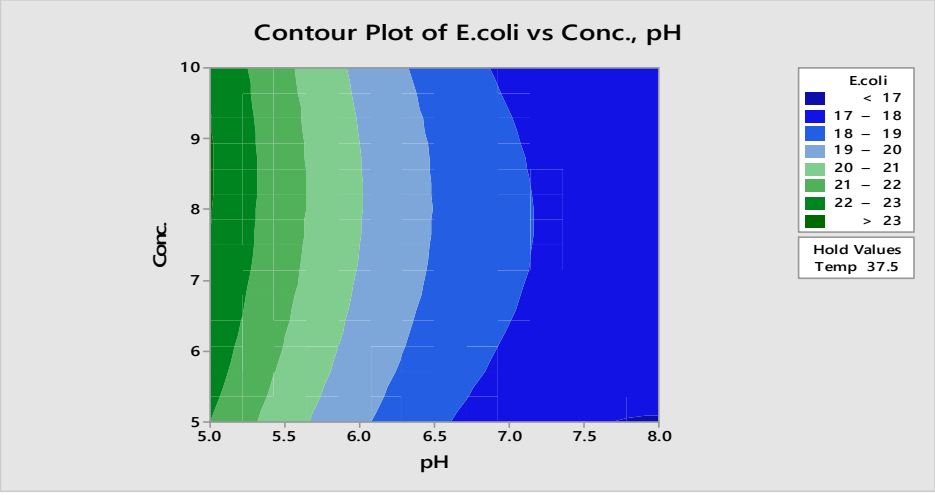
The highest zone of inhibition is at the concentration of 5-10 and pH 5.0-5.1 at a fixed Temperature of 37.5°C

The maximum antibacterial activity that a plant extract can give was checked using RSM. For maximum antibacterial activity the uncoded and actual level of three

independent variables were chosen, temperature, pH, and concentration. A set of 60 experiments that were all factorials and none of the experiment were zero-point test, performed to approximate their error. The treatment with the uncoded levels and the zone of inhibition gained by the test variable is in the table 1.1. Counter plot is used to study the relationships among the test variable and to check the maximum level of each variable that can give higher zone of inhibition due to antibacterial activity of *Pyrus pashia* extract.



The highest zone of inhibition is at the concentration of 5.0-10.0 and temperature of 35.0-35.4°C at a fixed pH of 6.5



The highest zone of inhibition is at the concentration between 5.1-10.0 and pH 5.2 at a fixed temperature of 37.5°C

Phytochemical analysis of *Pyrus pashia* shows the levels of alkaloids, tannins, flavonoids and total phenol contents in the ethanolic extract of plant extract. Analysis

shows 14.26 mg phenols of GAE/g, 41.26 mg tannins of GAE/g, 45.26mg Alkaloids of GAE/g, 86.26mg of flavonoids of GAE/g and 33.29mg of carotenoids of GAE/g of the ethanolic extract respectively.

TABLE 01: Total Phenololic, Tannin, Alkaloid And Flavonoid Contents In The Plant Extract

SELECTED MEDICINAL PLANT	Phenols mg of GAE/g of extract	Tannins mg of GAE/g of extract	Alkaloids mg of GAE/g of extract	Flavonoids mg of GAE/g of extract	Carotenoids mg of GAE/g of extract
Plant name*: Pyrus pashia*	14.26±2.19	41.26±3.29	45.26±4.29	86.26±7.16	33.29±4.16

Note: Each value is the average of three analyses (Mean) ± standard deviation (SD), Where GAE is gallic acid equivalents

DISCUSSION

Plants are immobile organisms, when they are attacked by herbivores or have to face environmental challenges they possess many mechanical defensives tools like spines and thorns [12]. In addition to these, they synthesize many classes of secondary metabolites, also known as phytochemicals. Plants have also evolved to rearrange the structures of secondary metabolites to ease in the interaction with DNA, RNA, proteins and cell membrane in microorganisms. The following table shows structural types of secondary metabolites [7, 12]. Polyphenols usually interfere with proteins. Saponins and terpenoids targets membranes of microorganisms. DNA is also targeted to cause alkylation or intercalating mutagenic effects. Secondary metabolites that targets DNA and membranes also possess cytotoxicity, which is they induce apoptosis. Several secondary metabolites are lipophilic which allow them to easily diffuse across cell membrane. These secondary metabolites may harm plant's oil cells and cuticles therefore they are present in dead tissue. Polar secondary metabolites are usually transported via transporters used for transporting sugars and amino acids. This is because polar secondary metabolites absorption is slower or negligible. More over secondary metabolites occur in combination, in which one metabolite such as saponins facilitates the uptake of polar one [8, 13]. Glycosyltransferase is present in *Streptococcus mutans* that produce glucans, an extracellular polysaccharide and a virulence factor causing dental caries. Glucan promotes adherence of bacteria resulting in biofilm dependent diseases. Plants have been known to contain glycosyltransferase inhibitors [6].

Phenols also known as phenolics, are chemical compounds that contain hydroxyl group bound to the aromatic ring. When there are multiple units of phenol together it is called as polyphenols. Plants usually synthesize phenolics by the action of phenylalanine ammonia lyase from phenylalanine [10]. It is one of the natural

product found in vegetable, seed and fruits. Phenolic compounds are found in many herbs and spices that have also been the basis of medical treatment [14]. Caffeic acid contains phenolic groups and are found in tarragon and thyme herbs. They show antimicrobial activity against viruses, fungi and bacteria. Catechol and pyrogallol are the class of phenols with two and three hydroxyl groups respectively. They are known to act against microorganisms. Flavonoids, quinones, tannins and coumarins are the subclass of phenols [8]. Gallic acids, type of polyphenols binds to bacterial enzyme called dihdrofolate reductase. They also inhibit bacterial gyrase and induce topoisomerases IV that cleaves DNA [10]. *Thymelaea hirsute* extracts contain phenolic compounds showed anti-bacterial activity by effecting cellular metabolism. Cranberry also known to have phenolic compounds showed antimicrobial effects against several bacteria. The phenolic compounds of Cranberry caused destabilizing and permeabilization of the bacterial membrane. They also affect energy metabolic pathways and extracellular microbial enzyme function. Phenols are also found to cause protein denature at higher concentration [5].

Quinones are coloured compound. They cause browning reactions in damaged fruits. They are intermediate metabolite in melanin synthesis pathway that occurs in the skin. They're found everywhere and are reactive compounds. Quinones are organic compounds derived from aromatic compound, which is they contain aromatic ring in their structure with two substituted ketone group. Ubiquinone accepts electron in electron transport chain. Quinone can also be made by hydroxylated amino acids by polyphenyoloxidase. Quinone causes protein function to lose by combining itself irreversibly with nucleophilic amino acids. This makes Quinone suitable for antimicrobial effects. As microbial cells contain protein on their surface known as adhesions, Quinone can interfere with these adhesion molecules and inactivate them. They also contain cell wall polypeptides and membrane bound enzymes.

Flavones are yellow colour compounds, class of flavonoids containg one carbonyl group found in herbs fruits and flowers. They function as chemo attractants for insects in pollination. They send signals to microbiota in rhizosphere. They protect plants from predators [8]. When 3-hydroxyl group is added in flavone, flavonol is formed. Bee glue (propolis) is reported to contain flavonol galangin which prevent honey from getting infected by

microorganisms. Galangin inactivate beta-lactamases activity, enzymes found in bacteria which breakdown beta lactam ring found in several antibiotics such as penicillin and cephalosporin therefore making them useless [1, 15]. Flavonoids also known as bioflavonoids. They are hydroxylated phenolic substances and are a group of heterocyclic organic compound that are numerously produced by plant species. They are plant secondary metabolites that protect them against microorganisms. They are also known to have antimicrobial effects against many microorganisms. They are able to interact with extracellular and soluble proteins as well as with cell wall of bacteria. If flavonoids are lipophilic, they damage microbial cell membrane. Catechins are part of flavonoids family found in oolong green tree. It was believed that green tea possessed antimicrobial activity due to the presence of many catechin compounds. Some authors believe that the more hydroxyl groups flavonoid contain, the greater will be its antibacterial activity. Some suggests flavonoids with any hydroxyl group are more toxic towards microorganisms. Flavonoids also prevent cytoplasmic membrane to function. They also work by inhibiting DNA gyrase and beta-hydroxyacyl-acyl carrier protein dehydratase activities. They have been reported to inhibit DNA and RNA synthesis in *Vibrio harveyi* [8].

CONCLUSION

It has been concluded that Pyrus pashia can be very effective in curing diseases caused by bacteria. Pyrus pashia is an effective plant to synthesize a very high potency antibacterial drug by using the optimize values of temperature and pH analyzed in this experiment. According to the literature review that supports the idea of plant derived antimicrobial compounds, further novel researches are in process to dig more about their characteristics. They can either be used alone in thereoutivcs or in combination with antibiotics. Although the antimicrobial activity of plants could be milder than the antibiotics but they proved promising antimicrobial due to their lower side effects. By studying the mode of action of secondary metabolites, new drugs from plants can be manufactured that will combat multidrug resistance and cure infectious diseases.

REFERENCES

[1] Divya B, K Mruthunjaya, and SN Manjula (2011). Parkinsonia aculeate: A

pharmacological Review. Asian journal of plant sciences, 10: 175-181.

[2] Mohammad SY, H Suleiman, M Abubakar, MR Sagir, SA Sule, BM Balarabe and K Mohammed (2017). Preliminary phytochemical screening of Parkinsonia aculeata leaf extracts. International Journal of Chemical Studies. 5(2):86-88

[3] Schuch UK and JJ Kelly (2008). Palo Verde trees for the Urban Landscape. Aridus. 20:1

[4] Sonia S and PV Adarsh (2014). Preliminary phytochemical screening and in vitro antioxidant activities of Parkinsonia aculeate Linn. BioMed Research International 756184, 8.

[5] Kamba AS and LG Hassan (2010). Phytochemical and Microbial Screening of Parkinsonia Aculeata L. leaves. International Journal of drug Development and Research. 2(1): 1-7

[6] Thelma O-A, M Rumana, R Taufiq and H Venter (2014). Phytochemicals increase the antibacterial activity of antibiotics by acting on a drug efflux pump. Microbiology Open. 3(6):885-896

[7] János Bérdy (2012). Thoughts and facts about antibiotics: where we are now and where we are heading. The Journal of Antibiotics. 65:385-395

[8] Abhinav U, U Indu, KJ Anup and V Kumar (2014). Combacting pathogenic microorganisms using plant-derived antimicrobials: A minireview of the Mechanistic basis. BioMed Research International. 18

[9] Ammer MR, S Zaman, M Khalid, M Bilal, S Erum, D Huang and S Che (2016). Optimization of antibacterial activity of Eucalyptus tereticornis leaf extracts against Escherichia coli through response surface methodology. Journal of radiation research and applied sciences. 9(4):376-385

[10] Omojate GC, FO Enwa, AO Jewo, O Eze Chritopher (2014). A Review; Mechanism of antimicrobial action of phytochemicals against enteric pathogens. Journal of Pharmaceutical, Chemical and Biological Sciences 2(2):77-85.

[11] Jacob J, RU Rajendran, SH Priya, J Purushothaman, D Kumar, N Bhaskaran and A Saraswathy (2017). Enhanced Antibacterial metabolite production through the application of statistical methodologies by a Streptomyces nogalater NIIST A30 isolated from Western Ghats forest soil. PLOS ONE, 12(4): e0175919.

[12] Michael W, LA Mohamed and Z Mahmoud (2012). Secondary metabolites

from plants inhibiting ABC transporters and reversing resistance of cancer cells and microbes to cytotoxic and antimicrobial agents. Frontiers in Microbiology. 3:130.

[13] Anandhi D, PT Srinivasan, G Praveen Kumar and Jagatheesh (2014). Influence of flavonoids and glycosides from Caesalpinia coriaria (Jacq) wild as bactericidal compound. International Journal of Current Microbiology and Applied Sciences. 3(4):1043-1051.

[14] Loers G, DV Yashunsky, NE Nifantiev and M Schachner (2014). Neural Cell Activation by Phenolic Compounds from the Siberian Larch (Larixsibirica). Journal of Natural Products, 77(7):15554-15561.

[15] Nadeem T, Khan MA, Ijaz B, Ahmed N, ur Rahman Z, Latif MS, Ali Q, Rana MA. (2018). Glycosylation of recombinant anticancer therapeutics in different expression systems with emerging technologies. Cancer research. 78(11):2787-98.