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Original Research Article

ANTIMICROBIAL POTENTIAL OF ETHANOLIC LEAF EXTRACTS OF *PARKINSONIA ACULEATE* USING RESPONSE SURFACE METHODOLOGY

YOUNAS NS[1], ALI I[2]*, ASHRAF H[1], BUKHARI NT[3]

[1]*Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Pakistan*
[2]*Department of Life Sciences, School of Sciences, University of Management and Technology (UMT), Lahore, Pakistan*
[3]*Microbiology and Clinical Laboratory Sciences, Women University Swabi, KPK, Pakistan*
**Correspondence author email address: iftikhar.ali@umt.edu.pk*

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Abstract: *Plant extracts are commonly used to inhibit bacterial activities. In the current analysis, we aimed to study the antimicrobial activity of Parkinsonia aculeata. The plant powder was used to extract the phytocompounds present in the plant by standard method. Then this extract was used to inhibit the growth of different bacteria at different pH, temperate and concentration. Antimicrobial activity was noted under different conditions. These results showed that Parkinsonia aculeata has very important antibacterial compounds such as phenols, flavonoids, tannins, and alkaloids depicting promising antibacterial activity for various bacterial ailments but at a specific temperature, pH and concentration. Escherichia coli showed growth restriction at the optimal pH 5.0, temperature 35°C and concentration 5-10mg/dl. Salmonella typhi growth was inhibited at the optimal pH 5.0, temperature 35°C and concentration 9.4-10 mg/dl. Similarly, the Klebsiella pneumoniae offered the inhibition rate at the optimal pH 5-7, temperature 35.2°C and concentration 5.0-7.0mg/dl. All of these results were confirmed by contour plots by Response Surface Methodology. Hence, it is suggested from this research work that Parkinsonia aculeata, when used at proper pH, temperature and concentration, can be employed to treat various bacterial inflammations in the biological system.*

Keywords: *Parkinsonia aculeata*, phytocompounds, *Escherichia coli*, flavonoids, alkaloids, tannins

Introduction

Parkinsonia aculeata is a spiny, shrub small tree. It is famous for its drought-tolerance property. Its flower is a rich source of honey (Schuch and Kelly, 2008). It grows up to 5-10 m in height and has a trunk of 40 cm in 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 (Mohammad et al., 2017). Moreover, rheumatism can be treated its flower and leaf alcoholic extract. However, the salutary effects of these *Parkinsonia aculeata* extracts have not been investigated and are generally not noted at the biochemical and biological levels (Sonia and Adarsh, 2014). Its leaves appear in an alternate order and are designed for the special activity. Its flower shape resembles a pea to a small degree. They are golden yellow and have a pleasant smell. The plant is also an important source of various compounds with diverse chemical structures. Phytochemistry: It has been reported that the leaves of this plant contain C-glycosyl flavones like orientin in L form, vitexin and isovitexin. Other than these, iso-orientin, lucenin-II, vicenin-II, diosmetin 6-C-beta-glucoside, apigenin, luteolin, kaempferol and chrysoeriol were reported by (El-Sayed et al., 1991). Further studies on the leaves reported a new flavanone having an epoxy-isopentyl moiety known as Parkintin. The seed oil of *Parkinsonia aculeata* contains polyunsaturated fatty acids in large amounts. Rotenoids such as rotenone, elliptone and deguelin are known to be found in roots. Calcium, Magnesium, Sodium, Potassium, Phosphorus, Iron, Copper, Zinc, and Manganese have been isolated from the leaves of this plant. Pharmacological investigations revealed antibacterial, antidiabetic, antioxidant, antirabies, amoebicidal, antipyretic, antimalarial, hepatoprotective, as well as antispermatogenic activities of this plant. In other studies, it was observed that Parkinsonia aculeata withstand heavy metal effects such as zinc, chromium, lead, cobalt, and cadmium on germination and seedling growth. Since it was reported that the plant could grow in the presence of

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heavy metals, it can be cultivated in polluted areas where it can resist heavy metal toxicities (Divya et al., 2011). The scientific name of Parkinsonia aculeata is Parkinsonia aculeata L. Common names include Palo Verde, Horse bean and Jerusalem thorn. In the Pakistani language, it is known as Kabuli kikar and Vilayatikikar. It is from the Spermatophyta phylum. It belongs to the Fabaceae (Leguminosae) family and Genus *Parkinsonia*. It is found in Southern USA, Northern Mexico, Southern Africa, Pakistan and Central America. For several years research has been conducted on plants over the globe for their potential in medicine and other traditional uses. There is tremendous evidence of plants regarding human health. Infectious diseases have threatened the lives of many people across the world. Besides, microorganisms causing these diseases are now resistant to many antibiotics. Infection caused by it is very difficult to treat (Saljoughian, 2014). Therefore, medicinal plants are being explored to overcome such issues as a new drug source (Abubakar and Usman, 2016). Although new antimicrobial agents are being synthesized due to the increasingly widespread resistance, they will have a relatively short therapeutic life span.

It has been an age-long practice to combat infectious diseases using medicinal plants. The medicinal value of such plants is because they contain secondary metabolites which affect the physiology of an organism (Mohammad et al., 2017). As stated by World Health Organization (WHO), plants are meant to be the right source of antibacterial drugs. Therapy with plant extracts having antimicrobial properties is worthy of attention (Gislene et al., 2000). WHO has also indicated that about 20,000 plant species have been known to possess a therapeutic potential that could be attributed to the bioactive compound synthesized for the duration of the secondary metabolism of plants (Muhammad et al., 2016).

The objective of this study was to evaluate the antibacterial activity and phytochemical analysis and check the optimized antibacterial activity of Parkinsonia aculeata.

Methodology

Plant sample collection

Parkinsonia aculeata were arranged from the market in Lahore, Pakistan. After which, the plant leaves were dried and ground to powder form. The solvent employed for the extraction of phyto-compounds was ethanol. Culture media and the chemicals used during the study were from Oxoid, Ltd and were of analytical grade.

Bacterial species tested against the plant extract.

Escherichia coli, *Klebsiella pneumonia* and *Salmonella typhi*, were obtained from the Microbiology lab in the department of IMBB of The University of Lahore.

Extraction of phytocompounds from plant leaf powder

Two g of a Plant sample in its powdered form was dissolved in 40 ml of 100% ethanol in a flask for extraction and placed overnight on a bench top at room temperature. During 24 hours, 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 into a small petri dish and left for 48 hours for evaporation to concentrate the filtrate. The filtered extract was fully dried and dissolved in 2 ml DMSO solution. The latter was then centrifuged for 10 minutes at 14000 rpm. The supernatant was poured into a new Eppendorf tube and saved. The extract was stored in a refrigerator at 4°C until further use.

Preparation of Inoculum

A sterile platinum loop picked each bacterial sample under sterile conditions and inoculated it into 5ml, 0.85% NaCl tubes. These bacterial cultures were adjusted according to 0.5 McFarland standard solution.

Preparation of McFarland solution and culture media

1.175 g of BaCl2 was dissolved in 100ml of distilled water to make 1.175% of $BaCl_2$ solution. 1ml of 100% concentration of H_2SO_4 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 at room temperature at 25°C. Culture media was prepared based on the instruction given by the manufacturer by adding 2.8 g of Muller Hinton Broth, and 1.5 g of technical agar dissolved in 100 ml of distilled water in a flask. The media was sterilized in an autoclaved at 121°C and 15 Pa for 15 minutes. The pH of the media was adjusted according to the requirement of the experiment.

Determination of antibacterial activity

The filter paper was punched several times to make many discs. The antibacterial assay was performed using agar disc diffusion assay. Muller Hinton media was poured into sterilized glass Petri plates after cooling to 45°C. After pouring under an aseptic environment, the Petri plates were labelled according to the required organism, pH, temperature and concentration. 5-10 ųl of each prepared bacterial inoculum was added to every labelled Petri plate using a pipette and spread using a sterile glass spreader. Sterile filter paper discs were carefully placed onto the agar plates using sterile forceps. A pipette added plant extracts of the required concentration (5, 7.5, and 10) onto the discs Amikacin (30µg) discs were used as a positive control. 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.

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Phytochemical analysis:

Quantitative phytochemical analyses for the determination of tannins, flavonoids and Phenols were employed to determine the estimation of these compounds by colourimetric methods as described previously (Ahmad et al., 2022)

Statistical Analysis

Temperature, pH and concentration were selected as the main variables to optimize the antibacterial activity of the plant extracts. These factors have an important role in affecting the growth of all bacteria. Temperature, pH and concentration were the independent variables studied in different combinations designed by the response surface method. Sixty runs in three blocks were performed to check the extracts' optimal antibacterial activity level. Three repetitions of the same combination variables were run simultaneously to ensure the accuracy of the results. Antibacterial activity is measured in mm and was taken as a dependent variable. Minitab 17, statistical software, was used for data analysis using Response Surface Graphs. The model equation is represented on the graphs showing Response Surface Plots that represent test variables' individual and interactive effects on the response. After conducting the experiments and measuring the activity levels, a full second-order model was fitted to antimicrobial activity data, including interactions.

Results

The antibacterial activity of *Parkinsonia aculeata* ethanolic extracts was tested against three bacteria according to the design of experiments made by the response surface method of Minitab 17 software. The different combinations set were concentration: 5.0, 7.5, 10.0 pH: 5.0, 6.5, 8.0 and temperature: 35°C, 37.5°C, 40°C (table 1). The results indicated that the three concentrations were effective against tested bacteria. Moreover, the effects of pH caused a significant variation in the zone of inhibition. The response surface method is an effective method of analyzing data compared to other methods that would otherwise be costly and result in numerical noise. Using RSM, maximum antibacterial activity has been checked for ethanolic extract of *Parkinsonia aculeata* against three bacterial strains.

The optimization or maximum antibacterial activity has been checked for ethanolic extracts against three bacterial species using RSM. After the analysis, the final model's least square estimate of parameters was determined, and it was concluded that temperature and pH were significant among the three interaction terms extracted.

The three-dimensional response surface and counter presentations were plotted to study the interaction among various factors and determine the optimal level of each factor of the maximum zone of inhibition from antimicrobial activity. To understand the relationships among parameters, the Response Surface was investigated for each couple variable keeping the other variable constant. P value and the Response Surface Graphs show that temperature and pH are the effective parameters for giving the highest antibacterial activity against all bacteria, as clarified in a surface plot with significant interactions between the concentration, pH and temperature. Among all surface plots, there is a maximum interaction and antibacterial activity.

Table 1: Ranges and term of variables used in the experiment

Variables	Terms	Ranges
Temperature	X_1	35 to 40
pH	X_2	5.0 to 8.0
Concentration	X_3	5.0 to 10.0

Antimicrobial activity of ethanolic leaf extracts against E. coli

A contour plot is used to study the relationships among the test variable and to check the maximum level of each variable that can give a higher zone of inhibition due to the antibacterial activity of *Parkinsonia aculeata* extract. Out of three independent variables, one variable is kept constant, and the other two are kept on the x-axis and y-axis in the contour plot graph. Then the relationship between the two variables is examined using the graph for E. coli. The contour plot between the three variables is given below in figures 1 to 3. The graph indicates that plant extract's highest zone of inhibition has been achieved at concentrations of 5.0-10.0, pH 5.0 and temperature 35.0°C. It shows that by keeping the pH low, antibacterial activity will increase.

Antimicrobial activity of ethanolic leaf extracts against *S. typhi*. The relationship between the two variables is examined using the graph for *S. typhi*. A counterplot between the three variables is given below in figures 4 to 6. The graph indicates that plant extract's highest zone of inhibition has been achieved at a concentration of 9.4-10.0, pH 5.0 and temperature 35.0°C. It shows that by lowering the pH, antibacterial activity will increase.

Antimicrobial activity of ethanolic leaf extracts against *K. pneumoniae*. The relationship between the two variables is examined using the graph for *K. pneumoniae*. The counterplot between the three variables is given below in figures 7 to 9. The graph indicates that plant extract's highest zone of inhibition has been achieved at concentrations of 5.0-7.0, pH 5.0-5.7 and temperature 35.2°C. It shows by lowering the pH increases antibacterial activity.

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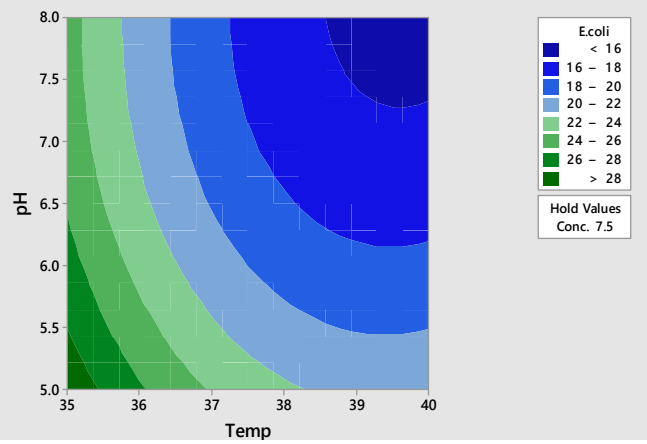
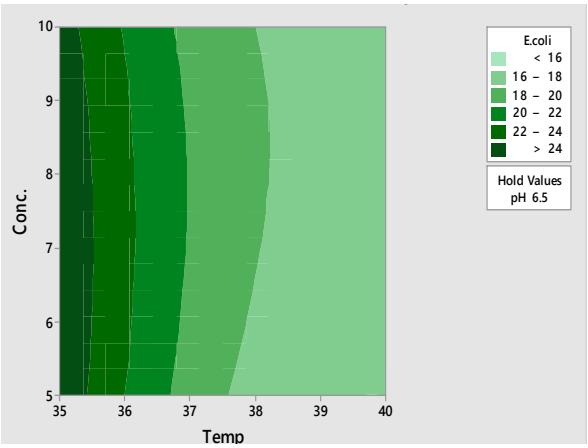


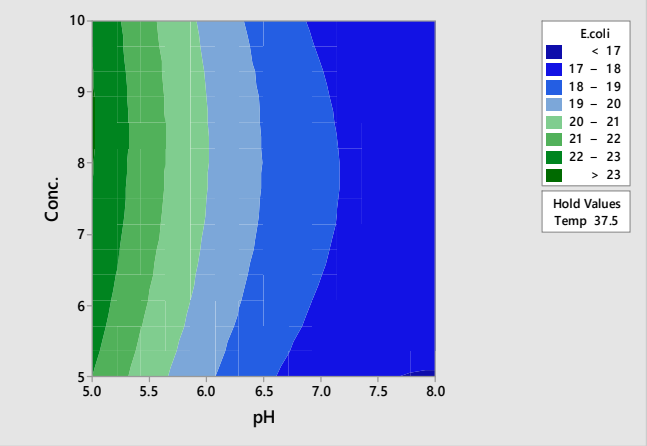
The highest zone of inhibition is at the pH of 5.0-5.5 and temperature 35-35.4°C at a fixed concentration of 7.5.

Figure 1: Contour Plot of *E. coli* vs pH, Temp



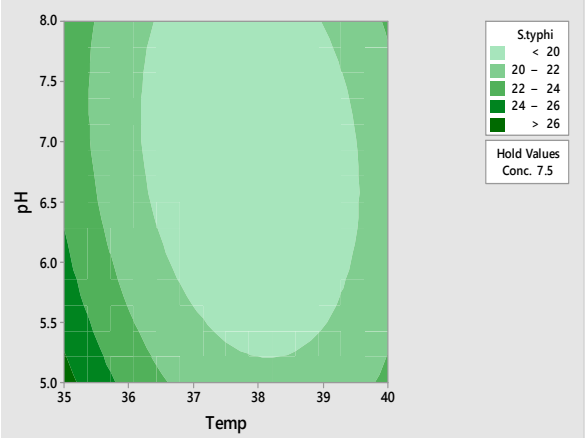
The highest zone of inhibition is at the concentration of 5.0-10.0 and temperature of 35.0- 35.4°C at a fixed pH of 6.5

Figure 2: Contour Plot of *E. coli* vs Conc., Temp



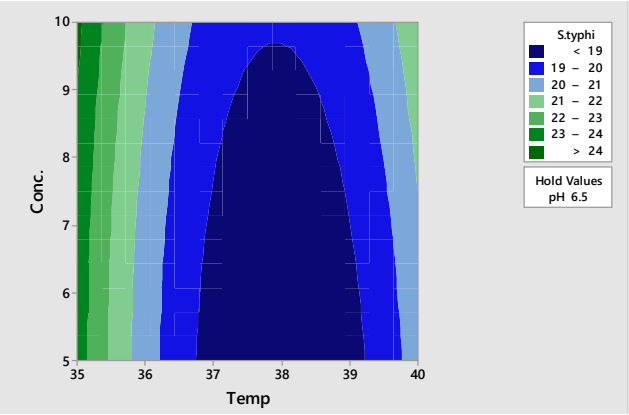
The highest zone of inhibition is at the concentration between 5.1-10.0 and pH 5.2 at a fixed temperature of 37.5°C

Figure 3: Contour Plot of *E. coli* vs Conc., pH



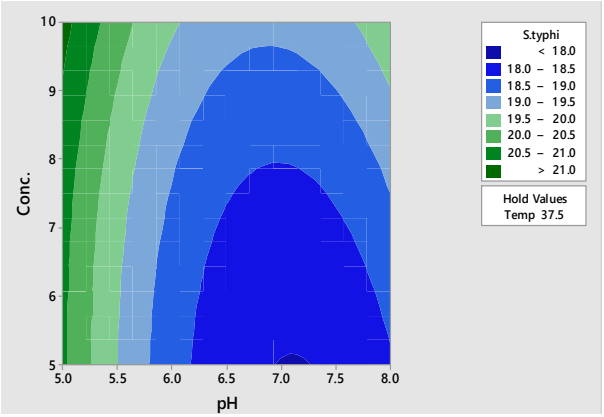
The highest zone of inhibition is at the pH 5.0-5.3 and temperature 35-35.1°C at a fixed concentration of 7.5

Figure 4: Contour Plot of *S. typhi* vs pH, Temp



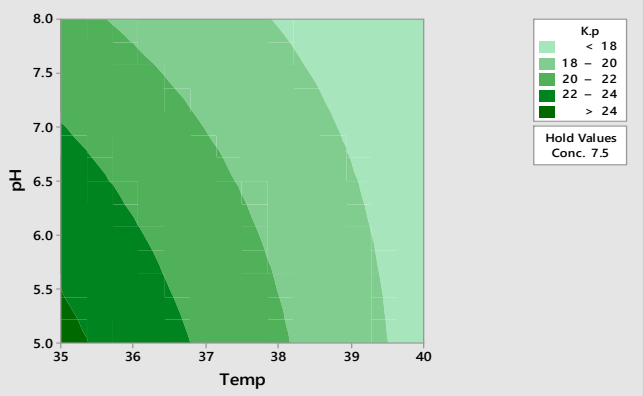
The highest zone of inhibition is at the concentration 9.4-10.0 and temperature 35.0°C at a fixed pH of 6.5

Figure 5: Contour Plot of *S. typhi* vs Conc., Temp

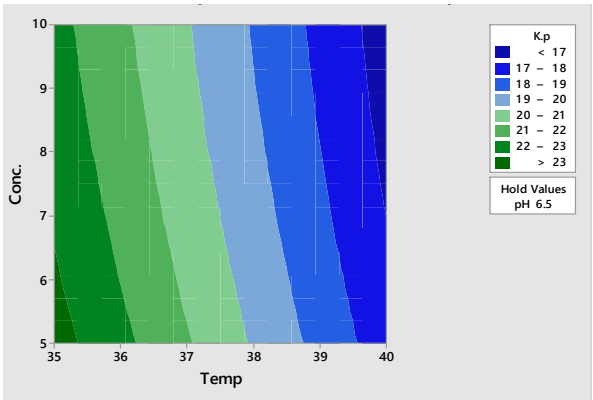


The highest zone of inhibition is at the concentration 9.4-10 and pH 5.0-5.1 at a fixed temperature of 37.5°C

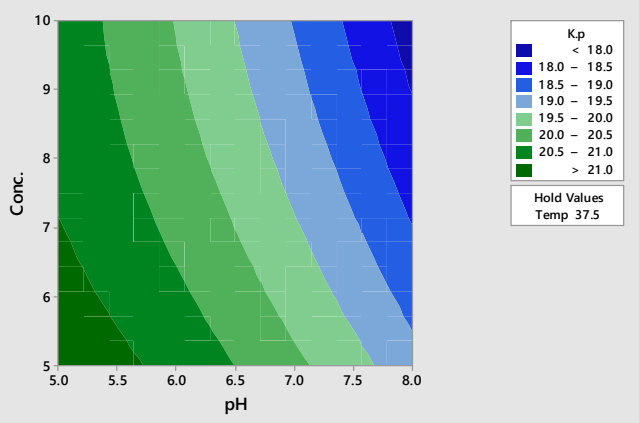
Figure 6: Contour Plot of *S. typhi* vs Conc., pH



The highest zone of inhibition is at the pH 5.0-5.4 and temperature 35.0-35.3°C at a fixed concentration of 7.5.
Figure 7: Contour Plot of *K. pneumoniae* vs pH, Temp



The highest zone of inhibition is at the concentration 5.0-6.3 and temperature at 35.2°C at a fixed pH of 6.5
Figure 8: Contour Plot of *K. pneumoniae* vs Conc., Temp



Thehighestzoneofinhibitionisattheconcentrationof5.0-7.0andpHat5.0-5.7atafixed temperature of37.5°C
Figure 9: Contour Plot of *K. pneumoniae* vs Conc., pH

TABLE 2: Total phenolic, tannin, alkaloid and flavonoid contents in the plant extract

Selected medicinalplant	Phenols mg of GAE/g of extract	Tannins mg of GAE/g of extract	Alkaloids mg of AE/g of extract	Flavonoids mg of QE/g of extract	Carotenoids mg of GAE/g of extract
Parkinsonia aculeata	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

Table 3: Optimized values giving maximum zone of inhibition

Bacteria	Optimized values giving maximum zone of inhibition Temperature /°C	pH	Concentration /ụl
E. coli	35.0	5.0	5.0-10.0
S.typhi	35.0	5.0	9.4-10.0
K.pneumoniae	35.2	5.0-5.7	5.0-7.0

Discussion

Infectious diseases continue to become the major cause of mortality and morbidity worldwide. Microorganisms occupy approximately 60% of the total earth's biomass. In addition, they have a wide variety in their genetic, metabolic and physiological makeup which makes them more complex for scientists to come up with the cure for the disease they cause as microorganisms become resistant to antibiotics in many ways making the situation worse.

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In our research, the antibacterial activity of Parkinsonia aculeata was observed using three factors. The antibacterial activity is indicated on the graphs by a different pattern of colours, through which the lowest to highest antibacterial activity can be judged, where the darkest colour indicates the maximum antibacterial activity. By looking at these ranges, interactions among three variables, the maximum antibacterial activity, and the variable affecting most of the results can be concluded. Temperature and pH have affected the results more than the concentration has affected, as clearly indicated by the contour plot graphs.

E.coli graph indicates that the combined effect of temperature and pH gives the maximum antibacterial activity. This shows that concentration plays less contribution in affecting the growth of E.coli as any concentration between 5 to 10 can inhibit the growth of E.coli. The lines of the contour graph of *S. typhi* are curved, indicating that the maximum antibacterial activity is due to the interaction among three variables. It also indicates that concentration is less significant in giving maximum antibacterial effects than pH and temperature. *K. pneumoniae* graph also shows less concentration contribution in getting maximum antibacterial activity. All the graphs of the three bacteria also indicated the maximum antibacterial activity could be achieved at the pH of 5.0-5.5.

The table below shows the optimized values that give the maximum zone of inhibition for each bacteria: Phenols are plant-derived that are further categorised according to their structure: simple phenolics, phenolic acids, quinones, flavonoids, flavones, Flavonols, coumarins and tannins. Phenolics are hydroxylated compounds with an aromatic ring. Phenolics are reported to cause membrane disruption in both gram-positive and gram-negative bacteria. Chlorogenic acid is reported to cause membrane disruption in several bacteria. Quercetin also leads to membrane disruption, DNA intercalation, DNA gyrase inhibition, inactivation of type III secretion and protein kinase inhibition. Apigenin belongs to the flavone family and is reported to cause dehydratase inhibition as well as protein kinase inhibition. It has been reported that 11 phenolic compounds inhibit DNA gyrase, and 9 compounds inhibit the type III secretion system. Phenolic compounds reportedly inhibit the helicase activity, and about 3 phenolic compounds inhibit multi-drug efflux pumps. Succinate dehydrogenase and malate dehydrogenase are also inhibited by phenolic compounds (Rempe et al., 2017).

Flavonoids are plant-derived, a major group of secondary metabolites. They are further classified according to their structures, flavones, Flavonols, flavone, isoflavones and anthocyanins (Xiao et al., 2014). They are heterocyclic organic compounds that can be used as antibacterial alone or in combination with antibiotics to give synergism effects. They are reported to suppress bacterial virulence activity. Scientists are approaching developing drugs that target virulence-causing agents only so that the host cellular components remain unnecessary for host cell survival. Flavonoids prevent infections such as sores, wounds, acne, respiratory infections, gastrointestinal diseases, and urinary tract infections. Therefore, flavonoids have become important in much antibacterial research. For decades, the antibacterial properties of flavonoids have been reported in the scientific literature. Due to this, their mode of action is now being pondered upon. Several mechanisms of action have been reported, such as they can cause perforation in the cytoplasmic membrane of the bacterial cell, resulting in leakage of various intracellular materials. Disruption of the cytoplasmic membrane also disrupts the proton motive force that affects ATP generation in the cell. When cellular energy generation becomes affected, the cell cannot synthesize many important macromolecules necessary for life, such as DNA and peptidoglycan synthesis. Other reports stated that flavonoids interrupt quorum sensing and communication used by bacterial cells in many processes. They interact with the receptors to which signalling molecules released by other bacteria bind. It disrupts the interaction between the signal molecule acyl-homoserine and its receptor. Baicalein is a flavone that inhibits TraR receptor in a cytoplasmic membrane. This study was proved via the docking scores from computer modelling. Also Bioassay data showed degradation of the receptor. Another study confirmed by RhlR-based biosensor showed catechin affects RhlR resulting in its inhibition. Sortases are the enzymes embedded in the cytoplasmic membrane of bacterial cells which synthesize adhesions and internalins, therefore important in bacterial infections. The report shows Morin, a flavonol that inhibits Sortases A and B. Isoflavone inactivates ureases. This shows that flavonol can synergise with antibiotics during the gastrointestinal tract caused by H. pylori. Flavonoids also neutralize toxins secreted by bacteria. Genistein is an isoflavone that inhibits endotoxin and catechin, epicatechin neutralizes lipopolysaccharides, an endotoxin (Cushnie and Lamb, 2011). Flavonoid also activates endonucleases, resulting in DNA fragmentation in bacteria (Anandhi et al, 2014).

Indoloquinoline and crytolepine induce cell lysis and cause morphological cell changes (Vinoth, 2013). Berberine and piperine intercalate with the cell wall and DNA (Silva and Fernandes, 2010). Berberine is also reported to disrupt membrane structure leading to a loss in cell membrane permeability (Chandra et al., 2017). Pergularinine and tylophorinidine are alkaloids from the indolizidine class that inhibit

nucleic acid synthesis and dihydrofolate reductase. Benzophenanthridine and Protoberberine belong to the isoquinoline class of alkaloids. They are known to inhibit cell division. Phenanthridine also inhibits nucleic acid synthesis. Squalamine is a Polyamine alkaloid which compromises the integrity of the cytoplasmic membrane and the bacterial cell's outer membrane, leading to leakage of cell contents (Cushnie et al, 2014). Tannins are a class of polyphenols that differ from other phenolic compounds in their properties. They can form complexes with proteins and polysaccharides. Therefore, they can inhibit enzymes. They are also known to form complexes with metal ions that affect metalloenzymes' activity. Tannins are also reported to inhibit oxidative phosphorylation and extracellular microbial enzymes (Scalbert, 1991).

Conclusion

It has been concluded that *Parkinsonia aculeata* consists of important antibacterial compounds such as phenols, flavonoids, tannins and alkaloids depicted by the phytochemical analysis, which are promising antibacterial agents for various bacterial ailments but at a specific temperature, pH and concentration. *E. coli* was effective in combating bacterial growth at the optimal pH 5.0, temperature 35 0 C and concentration 5-10mg/dl. *S. typhi* growth was inhibited at the optimal pH 5.0, temperature 35 0 C and concentration 9.4-10 mg/dl. Similarly, the *K. pneumoniae* offered the inhibition rate at the optimal pH 5-7, temperature 35.2 0 C and concentration 5.0-7.0mg/dl. All of these results were confirmed by contour plots by Response Surface Methodology. Hence, it is suggested from this research work that *parkinsonia aculaeta* when used in proper pH, temperature and concentration, can be employed to treat various bacterial inflammations in the biological system.

Conflict of interest

The authors declare no conflict of interest.

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