

Glycyrrhiza glabra and Azadirachta indica against Salmonella Typhi: Herbal Treatment as an Alternative Therapy for Typhoid Fever

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Abstract

Herbal medicine is a very ancient art of healing. Antibiotics developed resistivity against many pathogens due to misuse of these agent that's why antimicrobial potential is studied all over the world. The present study was conducted to analyze an *in-vitro* anti-bacterial activity of methanolic extract of *Glycyrrhiza glabra* and *Azadirachta indica* against two gram positive and three gram negative organism by using disk diffusion method at the concentrations of 20 ppm, 30 ppm and 40 ppm. The zones of inhibition were measured with mm scale and found in the range of 6 mm to 17 mm. The result reveals that methanolic extract of both plant posses anti-bacterial activity. These plant extracts may prove their promising role as antibiotics and may help to overcome the problem of resistivity against many pathogens. Moreover, anti-typhoid activity of these medicinal plants is promising to act as potential agent for treating typhoid fever from natural plant source.

Keywords: Antibacterial activity, *Glycyrrhiza glabra*, *Azadirachta indica*, disc diffusion method and typhoid fever

Highlights

- The present study was conducted to analyze in-vitro anti-bacterial activity of methanolic extract of *Glycyrrhiza glabra* and *Azadirachta indica*
- These plant extracts may prove their promising role as antibiotics and may help to overcome the problem of resistivity against many pathogens
- Anti-typhoid activity of these medicinal plants is promising to act as potential agent for treating typhoid fever from natural plant source.

Introduction

Antibiotics provide the main basis for the therapy of bacterial and fungal infection [1]. Bacterial resistance is due to misuse of antibiotics [2]. Bacterial infections are hazardous and responsible for many death in each year [3], and Currently used antimicrobials are failing to bring an end to many bacterial infections due to super

resistant strains for this reason research is ongoing for natural anti-microbial activity [4,5]. Herbal medicine was a very ancient art of healing one that use both perceptual and intellectual awareness as well as focus on outcome and anti bacterial activity also reported [6]. The antimicrobial potential of different medicinal plants are being extensively studied all over the world [7]. Chemical agent with general as well as specific activities [8]. Novel phytochemical substance of plant origin has been shown to inhibit the growth of bacteria [7,9]. Recent screening of bioactive compound from plants results in discovery of new medicinal substance [8,10]. The traditionally used medicine, *Glycyrrhiza glabra* and *Azadirachta indica* having antibacterial activity also reported [5,9]. *Glycyrrhiza glabra* also known as Licorice and sweet wood belonging to family Papilionaceae. It contains Saponin glycoside that is glycyrrhizin, Flavonoids and many other constituents [11]. The *Azadirachta indica* commonly known as Neem, belonging to family Meliaceae (mehogani family). Neem seeds contain azadirachtin, alkaloids, flavonoids, triperpenoids, phenolic compounds, carotenoids, steroids and ketones. [12]. In

2012, a phytochemical constituents, pharmacological action and medicinal use through the millennia of *Glycyrrhiza glabra* were conducted and it was accomplished that it is used as a traditional medicine, it contain glycyrrhizin and other chemical constituents extracted from root of licorice. It is used as a prophylactic as well as therapeutic drug for major body ailment sat any age group [13]. In 2013, the phytochemical screening and anti-oxidant potential of Glycyrrhiza root extract was conducted, it was concluded that it have significant anti-bacterial and hydroxyl radical scavenging activities, it is helpful in treating bacterial infection and scavenging hydroxyl radical which are generated during carcinogenesis. In 2012, the antimicrobial activity of leaf extract of neem was conducted against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi* and *Bacillus pumillas* and concluded that ethanol and methanol extract show maximum inhibition on *Bacillus pumillas*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* in ascending order [14].

The present study was carried out to evaluate plant extract of *Glycyrrhiza glabra* and *Melia azadirachta* of different dilution to screen their anti-bacterial activity. These plants are used as traditional medicine to cure common ailment. To give a scientific approach to the use of these plant, this study was carried out to analyze anti-bacterial activity against gram positive (*Pseudomonas aeruginosa*, *Staphylococcus aureus*) and gram negative organism (*Escherichia coli*, Klebsiella and salmonella).

Material and Methods

Material and reagent

Ampicillin, licorice stem, neem seeds, methanol, Nutrient broth and nutrient agar. Chemicals and media of analytical grade were purchased from Merck's and oxide chemical manufacturer. All the solutions and media were prepared fresh before experiment.

Plant extract

The plant extract were prepared by using maceration method [4]. The crude drug of licorice and neem seeds was grinded into powder and soak required amount of sample in methanol for 2 days and filter the extract with the help of watts man filter paper 1. The plant extract is concerntrated in rotary evaporator and with the help of this extract further dilution of 20 ppm, 30 ppm and 40 ppm were prepared by using methanol as a solvent.

Antibacterial activity

The plant extract liquorice and neem were screened for their antimicrobial activity against two gram positive (*Pseudomonas aeruginosa*, *Staphylococcus aureus*) and three gram negative (*Escherichia coli*, Klebsiella and *Salmonella typhi*) organism by the conventional cylinder plate method [4,15]. The solutions of plant extract for soaking disk were made of different dilution that is 20, 30, and 40 ppm using methanol as a solvent. To ensure that the solution had no effect on bacterial growth, a negative control test was performed with test medium supplemented with methanol and positive control ampicillin at the same dilutions as used in the experiment.

Nutrient agar was prepared, autoclaved at 124°C for 15 minutes cooled and then pour in Petri plates swabbing was done with the help of sterile cotton swab, soaked disks of solution were placed in them and the plates were incubated for 48 hours at 37°C.

Finally, the zones of inhibition were carefully measured with the help of scale in millimeter [16].

Result

The result of anti-bacterial activity is shown in **Table 1 and Table 2**. The diameter of zone of inhibition of extract from 6 mm to 17 mm, whereas standard Ampicillin disc produced zone of inhibition ranging from 7 to 31 mm in diameter. The result shown in **Table 1 and 2** and also represent in **Graph 1 and 2** shown below:

Discussion

Plants are renowned to have valuable therapeutic effects. Though bioactive products of Neem and licorice have been used in treatment of various diseases since various decades, role of phytochemical in inhibition of microorganisms growth has provide little evidence [17]. The anti-bacterial activity of plant extract, liquorice and neem were analyzed against a five pathogenic bacterial strains which include both gram positive and gram negative organisms that is *Pseudomonas Aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* and *Salmonellae*. They all are pathogenic organisms and cause serious body ailment and infect vital organs.

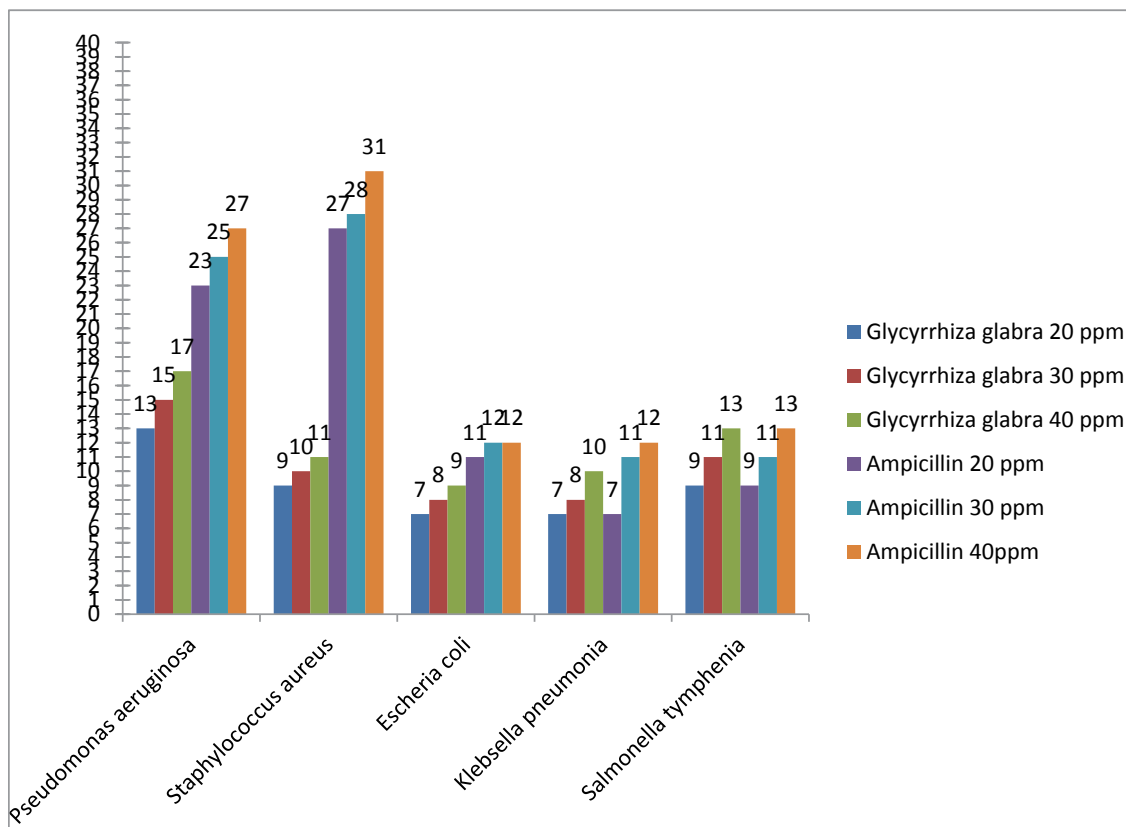
Both plant exhibits anti-bacterial activities against both Gram positive and Gram negative microorganism. The liquorice and neem both are used as a traditional medicine all over the world. The concept of alternative medicine increases day by day, especially herbal medicine because of no or least harmful effects. In our study, the control (methanol) showed no zone of inhibition. The diameters of zone of inhibition of both extract are of different concentrations that are 20 ppm, 30 ppm and 40 ppm and the results were compared with the activity of the well known antibiotic, Ampicillin. It exhibited strong anti bacterial activity against selected organism, shown in **Table -1 and Graph -1** represent the comparative analysis of methanolic liquorice extract with the reference drug of Ampicillin. The Azadirachta indica also used in cosmetic products as well as a home remedies. It has antiseptic effect and used to treat many skin diseases, also exhibit remarkable antibacterial activity. They contain bioactive components which are responsible for anti bacterial activity, shown in **Table 2 and Graph 2**. From the result it is evidence that methanolic extracts of these plants has remarkable zone of inhibition against salmonella typhi. The zone of inhibition is nearest to the controlled drug that is ampicillin. *Salmonella typhi* is causative agent of typhoid fever. Currently, typhoid fever is a major health problem in developing countries with limited success of treatment with antimicrobial agent [18,19]. The previous study evaluated the anti Salmonella typhi activity of ethanol, hot and cold crude water extracts of Vitex doniana (root), Cassia tora (Leaf), Alstonia boonei (bark), Stachytarpheta jamaicensis (leaf), and Carica papaya (leaf) used as traditionally medicine in Ebonyi state, Nigeria [9]. The neem seeds extract and glycyrrhiza extract shows comparable same result as compare to ampicillin. The plant extract as naturally synthesize mostly preferable because of having no destructive effects. The frequent use of antimicrobial agent is restricted because the frequent use may cause resistivity, G I upset and loss of appetite. The antimicrobial activity of *Glycyrrhiza glabra* and *Azadirachta indica* has shown in **Table 1 and 2**. The tested methanol

Table 1 Anti-bacterial activity of *Glycyrrhiza glabra*.

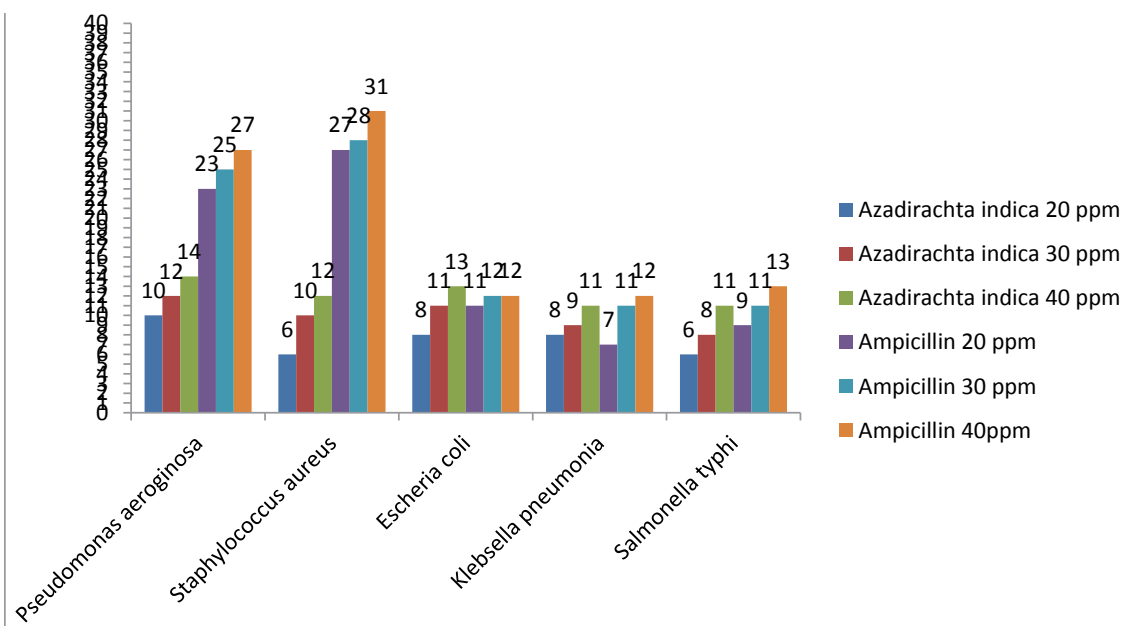
S.No.	Bacterial strain	Organism name	Zone of inhibition (mm)						
			Glycyrrhiza glabra			Positive control Ampicillin			Negative control methanol
			20 ppm	30 ppm	40 ppm	20 ppm	30 ppm	40 ppm	
1	Gram positive	<i>Pseudomonas aeruginosa</i>	13 mm	15 mm	17 mm	23 mm	25 mm	27 mm	NIL
		<i>Staphylococcus aureus</i>	9 mm	10 mm	11 mm	27 mm	28 mm	31 mm	NIL
2	Gram negative	<i>Escherichia coli</i>	7 mm	8 mm	9 mm	11mm	12 mm	12 mm	NIL
		<i>Klebsella pneumonia</i>	7 mm	8 mm	10 mm	7 mm	11 mm	12 mm	NIL
		<i>Salmonella tympheia</i>	9 mm	11 mm	13 mm	9 mm	11 mm	13 mm	NIL

Table 2 Anti-bacterial activity of *Azadirachta indica*.

S.No.	Bacterial strain	Organism name	Zone of inhibition			Positive control			Negative control
			Azadirachta indica			Ampicillin			
			20 ppm	30 ppm	40 ppm	20 ppm	30 ppm	40 ppm	
1	Gram positive	<i>Pseudomonas aeruginosa</i>	10 mm	12 mm	14 mm	23 mm	25 mm	27 mm	NIL
		<i>Staphylococcus aureus</i>	6mm	10 mm	12 mm	27 mm	28 mm	31 mm	NIL
2	Gram negative	<i>Escherichia coli</i>	8mm	11 mm	13 mm	11mm	12 mm	12 mm	NIL
		<i>Klebsella pneumonia</i>	8mm	9 mm	11 mm	7 mm	11 mm	12 mm	NIL
		<i>Salmonella typhi</i>	6mm	8 mm	11 mm	9 mm	11 mm	13 mm	NIL



Graph 1 Graphical representation of micro –organism VS zone of inhibition (mm).



Graph 2 Graphical representation of micro –organism VS zone of inhibition (mm).

extracts of *Glycyrrhiza glabra* and *Azadirachta indica* have profound antimicrobial activity against both gram positive and gram negative bacteria. The valuable zone of inhibition of both extract is especially against salmonella typhi. The result clearly shows that the herbal extract of both plant shows the same zone of inhibition as compare to controlled drug i.e. ampicillin. The quantitative and qualitative differences in inhibition variation are influenced by the method of extraction and concentration. In this study the anti-bacterial activity was tested by using the disc diffusion method, which is widely acceptable for preliminary screening of plant extracts. It is essentially an initial test indicating the sensitivity and resistivity of microorganisms to the test sample [20-22]. It became also clear in the current study that concentrations affect the degree of antimicrobial activity, as the concentration increases the antibacterial activity also increases. Various plant shows inhibitory effect on the growth of bacteria and also effective for systemic diseases, and our finding also prove that both plant extracts have great potential as antimicrobial activity against enteric bacterial pathogens and they can be used in the treatment of communicable diseases with no destructive

effects. The data obtained in this study justify the use of herbal preparations of neem and liquorice in typhoid. It is concluded from the result that both plant extracts have anti-bacterial activity against *Salmonella typhi* and they can be used in typhoid fever as alternate medicine in order to prevent antimicrobial resistivity. Currently, we reported experimental proof of the same. Further research should be proceeded to go into more detail studies to find out the bioactive constituents [23,24].

Conclusion

The present study showed the traditional roles of plants in curing typhoid. Methanolic extract of *Glycyrrhiza glabra* and *Azadirachta indica* have broad spectrum antibacterial activity against gram-positive and gram-negative bacteria. The study support the use of these herbal extracts as to prevent or control the typhoid, and anti-typhoid activity of these medicinal plants are promising to act as potential agent for treating typhoid fever from natural plant source.

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