Research Article

ANTIBACTERIAL ACTIVITIES OF PLANT EXTRACTS AND HONEY ON LOCAL BACTERIAL ISOLATES

Humaira Abdul Wahid

Assistant Professor, Govt. Girls Degree College Jinnah Town Quetta, Pakistan

humairawahid@yahoo.com

Abstract

Since ancient times, plants have been a valuable source of natural products for maintaining human health. The use of plant extracts with known antibacterial properties can be of great significance in therapeutic treatments. In the present study nine plants extracts, honey and three antibiotics were tested against six bacterial species isolated from different sources. The isolated organisms were *staplylococcus saprophyticus*, *Streptococcus faecum*, *Lactobacillus bulgaricus*, *Escherichia coli*, *Enterobacter cloacae* and *Obesium proteus*. The antibacterial activity was performed by disc diffusion method and Standard antibiotic discs of Fosfomycin, Gentamycin and Ampicillin were used as standard comparison purpose. The inhibitory effect of the treatment against each test bacterium was determined by measuring the diameter of zones of inhibition in millimeters. Of the extracts tested the *Swertia chirata*, *Butea monosperma*, *Syzygium cumini*, *Carum copticum* and *Melia azadirachta* has shown good inhibiting results against most of the tested organisms, while all the tested organisms were resistant to the *Peanum harmala*, *Artemisia meritima*, *Zingiber officinale* and *Calotropis procera*. Honey used in this study has shown no inhibiting effect on any organism.

Key words: Antibacterial activity, Aqueous & ethanol extracts, Disc diffusion method, Medicinal plants

Introduction

From the ancient times to date, people healed themselves with traditional herbal medicines [1]. Medicinal plants have important contributions in the healthcare system of local communities as the main source of medicine for the majority of the rural population [2]. Plants have provided a source of inspiration for novel drug compounds as plant-derived medicines have made significant contribution towards human health [3]. Out of about 258,650 species of higher plants reported from the world, more than 10% are used to cure ailing communities [4].

Pakistan has a variety of climatic zones and quite rich in medicinal herbs which are scattered over a large area. Majority of the people in Pakistan rely on medicinal plants to find treatment for their minor, even in some cases major diseases [4].

Today, thousands of plants, traditionally used as medicines, are being explored for their antimicrobial activities and chemical components [5]. Even there are thousands of species of plants which have to be explored in the future [6]. New and effective antimicrobial agents from natural sources are now becoming the demand of the day because of the development of resistance of pathogens against antibiotics caused by the indiscriminate use of modern antibiotics. The American Society of

Microbiology has recommended the development of novel antimicrobial agents because of the emergence of multi drug resistant bacterial pathogens [7]. Antibacterial activity of different extracts is being sorted out throughout the world [8, 9, 10, 11, 12, 13, 14, 15].

Medicinal plants play a key role in the development and advancement of modern studies by serving as a starting point for the development of novelties in drugs [16]. This study was aimed to see the antibacterial effects of medicinal plants which are commonly available against the bacterial species isolated from different food and water sources.

Materials and methods:

In this study, nine plants extracts were screened for potential antibacterial activity (Table.1).

Plant materials:

Different parts of the plants were collected, air dried, and powdered in a homogenizer, and 10 g of each plant was used for aqueous and ethanol extraction.

Extract preparation:

Aqueous extraction

The aqueous extraction was done in distilled water for 6 hours at slow heat. The extract was concentrated to 1/5th of the original volume for antibacterial assay.

Ethanolic extract

For ethanol extraction, the sample was extracted in ethanol kept on a rotary shaker overnight. The filtrate was collected and centrifuged at 5000 rpm. The extract was concentrated to 1/5th of the original volume and used for antibacterial assay [17].

Test microorganism:

The following test microorganisms (bacteria) were isolated from different sources. These organisms were identified by colonial and cellular morphology, staining and biochemical tests (Tab.2.1 and Table.2.2).

Gram positive bacteria: *staphylococcus saprophyticus*, *Lactobacillus bulgaricus*, *Streptococcus faecum*,

Gram negative bacteria: *Escherichia coli*, *Enterobacter cloacae*, *Obesumbacterium proteus*.

Antibacterial assay:

Disc susceptibility method was adopted as proposed by Bauer et al., [18]. Mueller Hinton Agar was used for this purpose and for the approximate turbidity of the inoculum the McFarland standard 0.5 was used.

Preparation of 0.5 McFarland standard: The required standard was prepared by adding 0.5 ml. of 0.048 M BaCl₂ (1.17% w/v BaCl₂·2H₂O) to 99.5 ml. of 0.18 M H₂SO₄ (1% w/v) while stirring constantly [19].

Disc diffusion method:

Sterile nutrient agar plates were prepared for bacterial strains and inoculated by a spread plate method under aseptic conditions. The filter paper disc of 6 mm diameter (Whatman's No. 1 filter paper) was prepared and sterilized. The sterile impregnated disc with plant extracts were placed on the agar surface with framed forceps and gently pressed down to ensure complete contact of the disc with the agar surface. Fosfomycin, Gentamycin and Ampicillin were used as control. The inhibitory effect of the treatment against each test bacterium was determined by measuring the diameter of zones of inhibition in millimeters excluding paper disc incubated at 37°C for 24 h. The zone of inhibition above 6 mm in diameter was taken as positive result. Each extract was used in triplicate.

Results and Discussion:

Plants Extracts used in the disc diffusion assay varied in their antibacterial activity (Table.3). Among the extracts tested *Swertia chirata* extract has significant antibacterial activity against *Staph. Saprophyticus* (16mm) *Strep.* effective against both gram positive and gram negative strains. All the organism tested were

Faecum (14mm) and *E. coli*(14mm). Similar result was observed by Alam et al., [20] against *Staphylococcus aureus* and Anwar et al., [21] against *Bacillus subtilis*. Ahirwal et al., [22] also observed significant antibacterial activity of *Swertia chirata* methanol extract against *Bacillus . subtilis* , *E. coli* and *Staph. typhi*. *Butea monosperma* extract was found to be effective against *Staph. Saprophyticus* (12mm) and *Lact. Bulgaricus* (13mm). Dhale et al, [23] reported ethanol extract of *Butea monosperma* effective against *Bacillus subtilis*, and *Staphylococcus aureus*. *Syzygium cumini* and *Carum copticum* have shown good inhibitory results against the Gram positive bacterial isolates. Guilherme et al, [24] have tested *Syzygium cumini* against *E. coli* and observed that *E. coli* was resistant to the said plant, same results were observed against *E. coli* in this study. *Syzygium cumini* has shown the inhibition zone of 20 mm against *Staph saprophyticus* as comparable to the antibiotics fosfomycin (26mm) gentamycin (19mm) and Ampicillin (22mm), 16mm inhibition zone against *Strep. faecum* as comparable to ampicillin (16mm) and 16mm inhibition zone against *Lact.bulgaricus* as comparable to gentamycin (21mm) and ampicillin (23 mm).

The antibacterial activity noticed by the *Carum copticum* was 15 mm against *Staph. saprophyticus*, 15mm against *Strep. faecum* and 13mm against *lactobacillus bulgaricus*. *E. coli*, *Enterobacter cloacae* and *Obesium proteus* were resistant to the *Carum copticum*. Antibiotics used in this study i.e. Gentamycin, Fosfomycin and Ampicillin had shown promising results. Gentamycin tested in this study has shown the inhibiting zone of 19 mm against *Staph saprophyticus*, 21 mm against *Lactobacillus bulgaricus*, 14 mm against *E. coli*, 17 mm against *Enterobacter Cloacae* and 19 mm against *Obesium proteus*. *Strep faecum* was resistant to Gentamycin.

From the results it is clear that leaves of *M. azedarach* are effective against both gram positive and gram negative strains. Ethanol extract of *M. azedarach* inhibited the growth of *Staph. Saprophyticus*(14mm), *Strep. Faecum*,(13mm)

Lact. Bulgaricus,(15mm) and *E. coli* (14mm) however, maximum inhibition zone was recorded against the gram positive bacteria *Lact. Bulgaricus* (15mm). Ramya et al, [25] also reported that leaves of *M. azedarach* are resistant to the *Peganum harmala*, *Artemisia meritima*, *Zingiber officinale*, *Calotropis procera*

and honey. Same results observed by Mintesnot and Mogessis,[26] in *Artemisia* crude preparations against *E.coli*. Mangena and Muyima,[27] have tested *Artemisia afra* against *Enterobacter cloacae* and observed inhibitory zone of 10 mm, where as in our study we used *Artemisia merittima* which shown no antibacterial activity against same bacterial species. Onyeagba et al., [28] and Nelson et al.,[29] has tested *Zingiber officinale* against *E.coli* but not found any antibacterial activity, same results observed in this study, they have also tested *Zingiber officinale* against *Staph. aureus* and not observed any inhibitory effect although the species different but same observed in this study with *Staph. saprophyticus*.

Honey used in this study has shown no antibacterial effect against any organism tested. Vilma, [30] used honey samples to check the antibacterial effects against *Staph. aureus* and *Staph. epidermidis* but he could not find any results in all the concentrations used, same in this study honey did not show any inhibitory effect on any isolate used.

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Table.1: Plants extracts used for antibacterial activity

Plant name	Part of Plant used	Extract
<i>Swertia chirata</i>	leaves and stem	Ethanollic extract
<i>Butea monosperma</i>	leaves	Ethanollic extract
<i>Peganum harmala</i>	Seeds	Ethanollic extract
<i>Syzygium cumini</i>	leaves	Ethanollic extract
<i>Artemisia merittima</i>	leaves	Ethanollic extract
<i>Carum copticum</i>	Seeds	Ethanollic extract
<i>Calotropis procera</i>	leaves and stem	Ethanollic extract
<i>Melia azadirachta</i>	leaves	Aqueous extract
<i>Zingiber officinale</i>	Rhizome	Aqueous extract

Table 2.1: staining colonial and cellular morphology of organisms

S. No:	Organism	Gram Staining	Cellular Morphology	Colony Morphology on BHIA / NA	Catalase	Oxidasei	Hemolysis	Diameter >5 mm 48 h	Carotenoid pigment	Urea Hydrolysis	Aerobic growth	Anaerobic growth	Nitrate Reduction	ONPG	D-lactic acid productn	Growth on 10 % NaCl	Growth on 15 % NaCl	Growth at 15 C	Growth at 45 C	Arginine Dihydro-lase	Growth on Am.Sulph	Coagulase	Clumping factor	Fibrinolysin	DNase agar	Heatstable Nuclease
1	<i>Strep Faecium</i>	+	cocci		-	-	-	-	-	-	+	+	-	+		-	-	-	+	+		-			-	
2	<i>Staphylococcus saprophyticus</i>	+	cocci		+	-	-	-	-	+	+	+	-	+		+	+	-	+	+		-			-	
3	<i>Lactobacillus bulgaricus</i>	+	Rods		+	-	-	-	-	+	+	+	-	-		-	-	-	+	-		-			-	

S. No:	Organism	Gram Staining	Cellular Morphology	Pigment BHIA / NA	MacConkey Agar	MacConkey Agar	EMBA	EMBA	Motility	Catalase	Oxidase	Indole	Citrate Utilization	Hydrogen sulphide	Urea Hydrolysis	Voges-Proskauer (VP)	Methyl Red (MR)	Phenilalanine	Gelatinase	Nitrate Reduction	ONPG	Arginine Dihydro-lase	Decarboxylase test	Gas from Glucose	Tartrate (Jordans)	DNase
					Gtt	Clr	Grt	Clr															Ly	Or		
1	<i>Enterobacter Cloacae</i>	-	S. Rods		+	R	+	MS	+	-	-	-	+	-	-	-	-	-	-	+	+	+	-	+	+	
2	<i>Obesumbacterium Proteus</i>	-	S. Rods		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	-	
3	<i>E. Coli (2a)</i>	-	S. Rods		+	R	+	MS	+	-	-	+	-	-	-	-	+	-	-	+	+	+	+	+	-	

Table 2.2: Biochemical tests of organisms

S. No:	Organism	CARBOHYDRATE (SUGAR) UTILIZATION														
		Lactose	Glucose	D-Mannitol	D-Sorbitol	L-Rhamnose	Sucrose	Melibiose	L-Arabinose	Cellobiose	Dulcitol	Maltose	D-Mannose	Raffinose	Salicin	Trehalose
1	<i>E. coli</i>	+	+	+	+	+	+		+	-		+	+	+	-	+
2	<i>Enterobacter Cloacae</i>	+	+	+	+	+	+		+	+		+	+	+	-	+
3	<i>Obesumbacterium Proteus</i>	-	-	-	+	-	-		-	-		-	-	-	-	-

S.No	Organism	Glucose	Fructose	Galactose	Mannitol	Lactose	Sucrose	Arabinose	Cellobiose	Maltose	Mannose	Raffinose	Salicin	Trehalose	Xylose
1	<i>Strep Faecalis</i>	+	+	+	+	+	+	+	-	+	+	+	-	+	+
2	<i>Lactobacillus bulgaricus</i>	+	+	-	-	-	-	-	-	-	-	-	-	-	-
3	<i>Staph saprophyticus</i>	+	+	+	+	+	-	+	-	+	+	-	-	+	+

TABLE 3: Diameters (mm) of zones of inhibitions produced by plants extracts, honey and antibiotics

S#	Organisms													
		Swertia chirata	Butea monosperma	Peaunum harmala	Syzygium cumini	Artemisia meritima	Carum copticum	Calotropis procera	Melia azadirachta	Zingiber officinale	Honey	Fosfomycin	Gentamycin	Ampicillin
1	Staph. Saprophyticus	16	12	R	20	R	15	R	14	R	R	26	19	22
2	Strep. Faecum	14	R	R	16	R	15	R	13	R	R	R	R	16
3	Lact. Bulgaricus	R	13	R	16	R	13	R	15	R	R	R	21	23
4	E coli	14	R	R	R	R	R	R	14	R	R	21	14	R
5	Enterobacter cloacae	R	R	R	R	R	R	R	R	R	R	17	17	R
6	Obesium proteus	R	R	R	15	R	R	R	R	R	R	R	19	23

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