

## Comparative Study on the Antibacterial Activities of Bee Product (Propolis, Pollen, Bee Wax and Honey)

<sup>1</sup>ogbu, K. I., <sup>2</sup>ochai, S.O., <sup>3</sup>olabode, M.P., <sup>4</sup>olaolu, O.S., <sup>1</sup>maimadu, A.A.,  
<sup>1</sup>gurama,

<sup>1</sup>Department Of Animal Health, Federal College Of Animal Health And Production Technology,

<sup>2</sup>Faculty Of Veterinary Medicine, University Of Maiduguri, Maiduguri, Borno State Nigeria

<sup>3</sup>National Veterinary Research Institute, Vom, Plateau State, Nigeria

<sup>4</sup>Ahmadu Bello University Zaria, Kaduna State, Nigeria

Corresponding Author: ogbu, K. I

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**Abstract:** Comparative Study On The Antibacterial Activities Of Bee Products (Propolis, Pollen, Bee Wax And Honey) Was Studied To Determine The Antibacterial Efficacy Of These Products Against Pathogenic Gram-Positive Bacteria. (*B. Subtilis*, *S. Aureus*, *S. Faecalis*) And Gram Negative Bacteria (*P. Multocida*, *S Typhi*, And *E.Coli*). Ditch Diffusion Method, Minimum Inhibitory Concentration (Mic) And Minimum Bactericidal Concentration (Mbc) Were Employed To Evaluate The Antibacterial Activities Of These Products. The Result Obtained Were Analyzed Using One Way Analysis Of Variance (Anova). Among The Bee Products, Pollen Was Discovered To Be More Active Against All The Pathogenic Gram-Positive And Gram-Negative Bacteria Used. The Result For The Minimum Inhibitory Concentration (Mic) Of The Bee Products Against All The Pathogenic Gram-Positive And Gram-Negative Bacteria Ranges Between 125 And 250mg/ml. The Result Of Minimum Bactericidal Concentration (Mbc) Of The Bee Products (Propolis, Pollen Wax And Honey) Shows At Varying Concentrations Used, The Effect Of The Products Were Static On The Growth Of The Test Organism And Recorded Highest Mic Against *S. Faecalis* (Gram-Positive) And *P. Multocida* (Gram-Negative). It Is Therefore Recommended That Higher Concentration Of These Products Against Other/Same Test Organism Should Be Done To Assess Their Activities.

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### I. Introduction

Natural Medicinal Products Have Been Used For Millennia For The Treatment Of Multiple Ailments. Although Many Have Been Superseded By Conventional Pharmaceutical Approaches, There Is Currently Resurgence In Interest In The Use Of Natural Products By The General Public. In Addition, The Pharmaceutical Industry Continues To Examine Their Potential As Sources Of Novel Medicinal Compounds To Identify Novel Growth Factor, Immunomodulatory And Potential Anti-Microbial Activity<sup>1</sup>. Honey And Propolis Are Bee Products That Have Been Used For Centuries In Folk Medicine<sup>2</sup>. These Products Have Received Increased Attention From The General Population Because Of Their Health Claims<sup>3</sup>.

The Use Of Honey As A Traditional Remedy For Microbial Infections Dates Back To Ancient Times. Honey Exhibits Potent Antimicrobial Activities Against Pathogenic And Non-Pathogenic Bacteria, Yeast And Fungi<sup>4</sup> (Zagholoul *Et Al.*, 2001). Laboratory Studies Have Demonstrated That Honey Is Effective Against Several Human Pathogens, Including *Escherichia Coli*, *Enterobacter Aerogenes*, *Salmonella Typhimurium*, *Staphylococcus Aureus*, Methicillin-Resistant *S. Aureus* (Mrsa), Haemolytic Streptococci And Vancomycin Resistant Enterococci (Vre)<sup>5</sup>.

Propolis Is A Resinous Substance Collected From Various Plants By Bee It Is Used In The Construction Of, And To Seal The Cracks In The Bee Hive. For This Reason Propolis Is Often Referred To As "Bee Glue" It Is A Mixture Of Resin, Essential Oils And Waxes And Also Contains Amino Acids, Minerals, Ethanol, Vitamin A, B Complex, E, And Flavonoids<sup>6</sup>. Propolis Displays Strong Antimicrobial Activity And Has Been Used As A Chemotherapeutic Agent Since Ancient Times. It Was Used In Folk Medicine As Early As 300 Bc For Medical And Cosmetic Purposes, And As An Anti-Inflammatory Drug And Wound Healing Agent. Propolis Is One Of The Most Potent Natural Antibiotics Characterized By A Very Wide Spectrum Of Effect. Its Therapeutic Application Does Not Induce Germ Resistance And Does Not Destroy Useful Micro Flora<sup>7</sup>.

Bee Wax Is Also A Product Of Bees, Secreted From The Wax Gland Of Bee Workers. It Is A Mixture Of Esters, Fatty Acids, Higher Alcohols And Saturated Hydrocarbons In Addition To Aromatic Substances And Pigments<sup>8</sup>. The Antimicrobial Character Of Beeswax Has Been Documented In European And Asian Holistic Remedies For Centuries. It Was Found To Be Particularly Active Against *Bacillus Alvei*, *Proteus Vulgaris*,

*Salmonella Gallinarum* And *Bacillus Subtilis*. In A Study Conducted By Ghanem Et AL., (2011)<sup>8</sup> It Was Found That The Bee Wax Sample Was Effective Against Gram Positive And Gram Negative Bacteria And Showed Pronounced Inhibitory Effect Against *Candida Albicans*. In Another Study, It Was Found That The Extracts Of Beeswax Were Effective Against Pathogenic Bacteria (*Listeria Monocytogenes*, *Pseudomonas Aeruginosa*; *Staphylococcus Aureus*; *Salmonella Enteric*, *Escherichia Coli*) And Microscopic Fungi (*Aspergillus Fumigatus*, *Aspergillus flavus*, *Aspergillus Niger*, And Different Strains Of Yeasts *Candida Krusei*, *Candida Albicans*, *Candida Glabrata*, *Candidaparapsilosis*, *Candida Tropicalis*, *Geotrichum Candidum*, *Rhodotorula Mucilaginosa*)<sup>9</sup>.

The Bee's Wax Has An Extremely Wide Spectrum Of Useful Applications And Occupies A Very Special Position Among Waxes Of Plant And Animal Origin. The Major Part Of Bees Wax Produced Is Used For Technical Purposes, Candles, Modeling, Polishes Etc. It Is Also Utilized In Cosmetics, Food Processing, Food Packaging And Preservation, Natural Food Additive And Medicine<sup>10</sup>. The Aim Of This Study Is To Investigate The Antibacterial Activities Of Bee Product On Some Gram Positive And Gram Negative Bacteria.

Bee Pollen Is A Fine Powder-Like Material Produced By Flowering Plants And Gathered By Bees. A Pollen Grain Contains The Male Gametophyte. Pollen Is A Bee's Primary Food Source Being Rich In Nutrients And Phytochemicals Such As Carotenoids, Flavonoids And Phytosterols<sup>11</sup>. Bee Collected

Pollen And Pollen Products Have Been Successfully Used For The Treatment Of Benign Prostatitis And For Oral Desensitization Of Children Who Have An Allergy<sup>12</sup>. In Addition, Bee Pollen Has Antimicrobial Effects<sup>13</sup>.

Thus The Aim Of This Study Which Was To Determine The Antibacterial Activities Of Bee Products (Propolis (Bee Glue), Bee Pollen, Honey And Be Wax) Against Some Gram Positive And Gram Negative Bacteria

## II. Materials And Method

**Study Location:** This Study Was Carried Out At Federal College Of Animal Health And Production Technology, National Veterinary Research Institute Vom Plateau State. Vom Is Quite A Rocky Village In Jos South Local Government Area Of Plateau State And Situated 1285 Meters Above Sea Level. The Nearest Towns Are Bukuru, And Jos, 12.8 And 24km To The North East Respectively.

**Study Design:** Bee Product Was Obtained From The College Orchard (Bee Farm), Bacterial Isolate Were Also Obtained From The Microbiology Laboratory Central Diagnostic Division, National Veterinary Research Institute Vom.

An Ethanolic Extracts Of The Bee Product Was Obtained From The Biochemistry Laboratory, Three Gram Positive And Three Gram Negative Bacteria Were Used To Determine The Efficacy Of These Products, Ditch Diffusion Methods Was Used To Evaluate The Antibacterial Activities Of These Products, Broth Micro-Dilution Method Established Bynational Committee For Clinical Laboratory Standard (Nccls 1996)<sup>14</sup> Was Used To Determine The Minimal Inhibitory Concentration (Mic) And Minimal Bacteria Concentration (Mbc).

**Procedure For The Ethanolic Extraction:** According To The Method Adopted By Sofowora (1989)<sup>15</sup>, 200g Of Each Of The Sample (Bee Pollen, Bee Wax, Honey And Propolis (Bee Glue) Was Weighed Into Two Liter Conical Flask Using Weigh Balance, 300mls Of Ethanol Was Added To Each And Was Shaken Vigorously To Dissolve, After Shaking, It Was Then Sealed, Labeled, And Was Allowed To Stand For 48 Hours, After Which It Was Sieved Using A Laboratory Sieve Of 150 Microns, It Was Then Filtered Using Whatman Number One Filter Paper In To A Conical Flask And Dried In An Oven At 45<sup>o</sup>c To Obtain The Ethanol Extracts.

**Preparation Of Culture Media (Nutrient Agar):** Six Gram Of The Nutrient Agar Was Dissolve In 300mls Of Distilled Water And Was Shacked Until It's Dissolves Completely, It Was Then Put Into An Autoclave To Heat For 45 Minutes And Allow To Stand, It Is Then Removed From The Autoclave And Allow To Cool More, After Cooling, It Was Poured Into The Sterile Petri-Dishes And Allow To Solidify, It Was Incubated At 37<sup>o</sup>c For 24 Hours<sup>16</sup>.

**Preparation Of The Extracts:** 250mg/ml Concentration Of Each Extract Was Prepared By Weighing 0.5g And Was Dissolve Into 2ml Of Tween-80. (Extract Dissolver)

**Preparation Of The Bacteria Isolate (Broth Culture):** Six Sterile Flavour Bottle Were Arranged And Labeled According To The Number Of Organism To Be Used, 10ml Of Normal Saline Was Dispense Into Each Bottle, A Colony Of The Each Organism Was Picked From The Media Using A Wire Loop And Was Put Into The Normal Saline In Each Of The Bottle<sup>17</sup>.

### Determination Of Antibacterial Activity:

Antimicrobial Activity Of The Ethanolic Extract Of The Bee Products Sample Was Determined Using Ditch Diffusion Method. The Standardized Bacterial Inoculum Was Flooded On Nutrient Agar. They Were Done In Triplicates. Excess Of The Inoculum Was Discarded And Allowed To Stay On The Bench For 15 Minutes. Ditches Were Bored Using A Cork Borer Equidistant From Each Other. Using A Micropipette, 100ul

Of The Extracts Was Introduced Into The Ditches At 250mg/MI Concentrations. This Was Done Alongside The Controls. The Plates Were Incubated At 37° C For 24 Hours. Antimicrobial Activity Was Determined By Measuring The Clearance Of The Zones Of Inhibition Using A Meter Rule<sup>16</sup>.

**Determination Of Minimum Inhibitory Concentration (Mic)**

The Minimum Inhibitory Concentration (Mic) Of The Extract Was Determined For Each Of The Test Organisms In Duplicates. 0.5ml Of Varying Concentrations Of The Extracts (250, 125, 62.5, 31.25, 15.625 Mg/MI), Was Added To 4.5 MI Of Nutrient Broth, 100ul Of The Test Organism Previously Diluted To 0.5 Mcfarland Turbidity Standard (For Bacterial Isolate) Were Introduced Into The Tubes. The Procedure Was Repeated On The Test Organisms Using The Standard Antibiotics (Ciprofloxacin, 20mg/MI). A Tube Containing Nutrient Broth Only Was Seeded With The Test Organisms As Described Above To Serve As Control. Tubes Containing Bacterial Cultures Were Then Incubated At 37° C For 24 Hours. After Incubation, The Tubes Were Then Examined For Microbial Growth By Observing For Visible Turbidity

**. Determination Of Minimum Bactericidal Concentration (Mbc)**

To Determine The Mbc, For Each Set Of Test Tubes Used In The Mic Determination, A Loopful Of Broth Culture Was Collected And Inoculated On Sterile Nutrient Agar By Streaking. Nutrient Agar Only Were Streaked With The Test Organisms Respectively To Serve As Control. Plates Inoculated With Bacteria Were Then Incubated At 37° C For 24 Hours. After Incubation, The Concentration At Which No Growth Was Seen Or With Least Growth Was Noted As The Mbc.

**Statistical Analysis**

The Data Obtained Were Analyzed Using Descriptive Statistical Method. One Way Anova P < 0.05 Were Considered Significant

**III. Result**

Table No 1 Shows That Propolis And Honey Has An Antibacterial Activity Against *Pasteurella Multocida* With 8.8±0.20 Mm In Diameter Clearance Of Zone Of Inhibition, Pollen Shows 11.7±0.30 Mm And Bee Wax Shows 10.7±0.30 Mm And Honey Is 8.9±0.10 Mm Against The Above Mentioned Organism. All The Bee Products (Propolis, Pollen, Wax And Honey) Show The Same Activity Against *E. Coli* And *Salmonella Typhi*. Propolis, Wax, And Honey Have The Same Activity Against *B. Subtilis* Which Is 8.8±0.20 Mm While Pollen Has 7.7±0.30 Mm. Propolis Is Active Against *S. Faecalis* With 8.9±0.10 Mm, Pollen Is 11.7±0.30 Mm, Wax Is 9.9±0.10 Mm And Honey Show The Least Activity With 8.8±0.20 Mm. Propolis And Honey Are Active Against *S. Aureus* With 7.7±0.30 Mm While Pollen And Wax Has 8.8±0.20 Mm. The Overall Antibacterial Activities Of Bee Products Against The Pathogenic Gram Positive And Gram Negative Is Statically Significant. P < 0.05.

**Table No 1:**Antibacterial Activities Of Bee Products Against Gram-Positive And Gram-Negative Bacteria.

Organism	Concentration Of Extracts (250 Mg/MI)/Diameter Of Zone Of Inhibition (Mm)				Positive Control (Mg/MI)
	Propolis 250	Pollen 250	Bee Wax 250	Honey 250	
Pm	8.8±0.20 <sup>a</sup>	11.7±0.30 <sup>c</sup>	10.7±0.30 <sup>b</sup>	8.9±0.10 <sup>a</sup>	13.8±0.20 <sup>d</sup>
Ec	6.7±0.30 <sup>a</sup>	10.7±0.30 <sup>c</sup>	9.7±0.30 <sup>b</sup>	9.9±0.10 <sup>b</sup>	13.8±0.20 <sup>d</sup>
St	6.7±0.30 <sup>a</sup>	10.8±0.20 <sup>c</sup>	9.8±0.20 <sup>b</sup>	9.9±0.10 <sup>b</sup>	13.8±0.20 <sup>d</sup>
Bs	8.8±0.20 <sup>a</sup>	7.7±0.30 <sup>a</sup>	8.8±0.20 <sup>b</sup>	8.7±0.30 <sup>b</sup>	12.8±0.20 <sup>d</sup>
Sf	8.9±0.10 <sup>a</sup>	11.7±0.30 <sup>c</sup>	9.9±0.10 <sup>b</sup>	8.8±0.20 <sup>a</sup>	13.8±0.20 <sup>d</sup>
Sa	7.7±0.30 <sup>a</sup>	8.8±0.20 <sup>b</sup>	8.8±0.20 <sup>b</sup>	7.8±0.20 <sup>a</sup>	13.8±0.20 <sup>d</sup>

**Key:**Pmpasteurella *Multocida*, **E**cescherichia *Coli*, **S**tsalmonella *Typhi*, **B**sbacillus *Subtilis*, **S**fstreptococcus *Faecalis*, **S**a *Staphylococcus Aureus*.**Different Superscript In A Row (A,B,C And D) Indicate Significance Difference Between The Group Mean At (P<0.05)**

The Antibacterial Activity Of Propolis Against Different Gram Positive Pathogenic Bacteria (*B. Subtili*, *S. Aureus* And *S. Faecalis*) Are Shown In (Table No 2.). According To The Analysis Among The Tested Gram Positive Bacteria, *B. Subtilis* And *S. Faecalis* Were More Sensitive During The 24 Hours Incubation. With 8.8±0.20 And 8.9±0.10 The Sensitivity Decreased On *S. Aureus* With 7.7±0.30 the Antibacterial Activities Of Pollen Extracts Against Different Gram Positive Pathogenic Bacteria (*B. Subtilis*, *S. Aureus* And *S. Faecalis*) With 11.7±0.3, The Sensitivity Decreased As Follows *S. Aureus* 8.8±0.02 And 7.7±0.30 The Activities Of Bee Wax Extract Against These Gram Positive Bacteria (*B. Subtilis*, *S. Aureus* And *S. Faecalis*) Were As Follows *S. Faecalis* 9.9±0.10, *B. Subtilis* And *S. Aureus* 8.8±0.20. The Activities Of Honey Extract Against These Gram

Negative Bacteria Are: *S. Faecalis* 8.8±0.20, *B. Subtilis* 8.7±0.30 And *S. Aureus* 7.8±0.30. The Antibacterial Activity Of These Products Against Gram Positive Bacteria Is Not Statistically Significant. P > 0.05.

**Table No 2:**Antibacterial Activity Of Bee Products Against Gram-Positive Bacteria

Organism	Concentration Of Extracts (250 Mg/MI)/Diameter Of Zone Of Inhibition (Mm)				Positive Control (Mg/MI)
	Propolis 250	Pollen 250	Bee Wax 250	Honey 250	
Bs	8.8±0.20 <sup>a</sup>	7.7±0.30 <sup>a</sup>	8.8±0.20 <sup>b</sup>	8.7±0.30 <sup>b</sup>	12.8±0.20 <sup>d</sup>
Sa	7.7±0.30 <sup>a</sup>	8.8±0.20 <sup>c</sup>	8.8±0.20 <sup>b</sup>	7.8±0.20 <sup>a</sup>	13.8±0.20 <sup>d</sup>
Sf	8.9±0.10 <sup>a</sup>	11.7±0.30 <sup>b</sup>	9.9±0.10 <sup>b</sup>	7.8±0.20 <sup>a</sup>	13.8±0.20 <sup>d</sup>

The Antibacterial Activities Of Propolis Extract Against Different Garm Negative Pathogenic Bacteria Are Shown In (Table No 3.) The Inhibition Zones Vary Among The Bacteria Used. *P. Multocida* Was Discovered To Be More Sensitive To Pollen Extract With 8.8±0.20 The Sensitivity Decreased On *E. Coli* And *S. Typhi* With 6.7±0.30 *P. Multocida* Is More Sensitive To Pollen Extract Than That Of Propolis. The Sensitivity Decreased On *S. Aureus* With 10.8±0.20 And *E. Coli* 10.7±0.30 *P. Multivcida* Is More Sensiyive To Pollen Extract Than That Of Propolis With 10.7±0.30. The Sensitivity Of *E. Coli* And *S. Typhi* On Honey Is 9.9±0.10 And *P. Multocida* Is 8.9±0.10. The Antibacterial Activities Of These Products Against Gram Positive Bacteria Are Statistically Significant. P < 0.05.

**Table No 3:**Antibacterial Activity Of Bee Products On Gram-Negative Bacteria

Organism	Concentration Of Extracts (250 Mg/MI)/Diameter Of Zone Of Inhibition (Mm)				Positive Control (Mg/MI)
	Propolis 250	Pollen 250	Bee Wax 250	Honey 250	
Pm	8.8±0.20 <sup>a</sup>	11.7±0.430 <sup>c</sup>	10.7±0.30 <sup>b</sup>	8.9±0.10 <sup>a</sup>	13.8±0.20 <sup>d</sup>
St	6.7±0.30 <sup>a</sup>	10.8±0.20 <sup>c</sup>	9.8±0.20 <sup>b</sup>	9.9±0.10 <sup>b</sup>	13.8±0.20 <sup>d</sup>
Ec	6.7±0.30 <sup>a</sup>	10.7±0.30 <sup>c</sup>	9.7±0.30 <sup>b</sup>	9.9±0.10 <sup>b</sup>	13.8±0.20 <sup>d</sup>

P < 0.05

The Result Of The Minimum Inhibitory Concentration Of Propolis Shows That *Pasteurella Molticida*, *E. Coli*, *S. Aureus* And *S. Typhi* Was Recorded At 125 Mg/MI Concentrations, *B. Subtilis* And *S. Faecalis* Was Recorded At 250 Mg/MI Concentration.

The Result Of The Minimum Inhibitory Concentration Of Pollen Against *P. Molticida* And *S. Faecalis* Was Recorded At 125 Mg/MI Concentrations While That Of *E. Coli*, *S. Typhi*, *B. Subtilis* And *S. Aureus* Was Recorded At 250 Mg/MI Concentration.

Minimum Inhibitory Concentration Result Of Bee Wax Against *P. Molticida* Was Recorded At 125 Mg/MI Concentration, While *E. Coli*, *S. Typhi*, *B. Subtilis*, *S. Faecalis* And *S. Aureus* Were Recorded At 250 Mg/MI Concentration.

125 Was Recorded As The Minimum Inhibitory Concentration Of Honey Against *E. Coli* And *S. Typhi*, 250 Was Recorded Against *P. Molticida*, *B. Subtilis*, *S. Faecalis* And *S. Aureus* Respectively.

The Entire Table For The Minimum Bactericidal Concentration (Mbc) On All The Bee Products (Propolis, Pollen Wax And Honey) Shows Positive (+) Which Means There Was Growth Of All The Organism Used On The Nutrient Agar On Subsequence Transfer To A New Environment This Is Because The Activity Of These Bee Products At Varying Concentration Of 250, 125, 62.5, 31.25, And 15.625 Mg/MI During The Mic Was Static Not Cidal

**Table No 4:** Minimum Inhibitory Concentrations (Mic) Of Propolis On The Gram-Positive And Gram-Negative Bacteria

Organism	Concentration Of Propolis Extract (Mg/MI):					Mic (Mg/MI)
	250	125	62.5	31.25	15.625	
Pm	-	-	+	+	+	125
Ec	-	-	+	+	+	125
St	-	-	+	+	+	125
Bs	-	+	+	+	+	250
Sf	-	+	+	+	+	250
Sa	-	-	+	+	+	125
	20	10	5	2.5	1.25	Positive Control
Pm	-	-	-	-	+	2.5
Ec	-	-	-	-	-	1.25
St	-	-	-	-	-	1.25
Bs	-	-	-	+	+	5

Sf	-	-	-	+	+	5
Sa	-	-	-	-	-	1.25

**Key:** - No Visual Turbidity, + Visual Turbidity

**Table No 5:** Minimum Inhibitory Concentrations (Mic) Of Pollen On The Gram-Positive And Gram-Negative Bacteria

Organism	Concentration Of Pollen Extract (Mg/MI):					Mic (Mg/MI)	
	250	125	62.5	31.25	15.625		
Pm	-	-	+	+	+	125	
Ec	-	+	+	+	+	250	
St	-	+	+	+	+	250	
Bs	-	+	+	+	+	250	
Sf	-	-	+	+	+	125	
Sa	-	+	+	+	+	250	
	20	10	5	2.5	1.25	Positive Control	Mic (Mg/MI)
Pm	-	-	-	-	+		2.5
Ec	-	-	-	-	-		1.25
St	-	-	-	-	-		1.25
Bs	-	-	-	+	+		5
Sf	-	-	-	+	+		5
Sa	-	-	-	-	-		1.25

**Table No 6:** Minimum Inhibitory Concentrations (Mic) Of Bee Wax On The Gram-Positive And Gram-Negative Bacteria

Organism	Concentration Of Bee Wax Extract (Mg/MI):					Mic (Mg/MI)	
	250	125	62.5	31.25	15.625		
Pm	-	-	+	+	+	125	
Ec	-	+	+	+	+	250	
St	-	+	+	+	+	250	
Bs	-	+	+	+	+	250	
Sf	-	+	+	+	+	250	
Sa	-	+	+	+	+	250	
	20	10	5	2.5	1.25	Positive Control	Mic (Mg/MI)
Pm	-	-	-	-	+		2.5
Ec	-	-	-	-	-		1.25
St	-	-	-	-	-		1.25
Bs	-	-	-	+	+		5
Sf	-	-	-	-	+		2.5
Sa	-	-	-	-	-		1.25

**Table No 7:** Minimum Inhibitory Concentrations (Mic) Of Honey On The Gram-Positive And Gram-Negative Bacteria

Organism	Concentration Of Honey Extract (Mg/MI):					Mic (Mg/MI)	
	250	125	62.5	31.25	15.625		
Pm	-	+	+	+	+	250	
Ec	-	-	+	+	+	125	
St	-	-	+	+	+	125	
Bs	-	+	+	+	+	250	
Sf	-	+	+	+	+	250	
Sa	-	+	+	+	+	250	
	20	10	5	2.5	1.25	Positive Control	Mic (Mg/MI)
Pm	-	-	-	-	+		2.5
Ec	-	-	-	-	-		1.25
St	-	-	-	-	-		1.25
Bs	-	-	-	+	+		5
Sf	-	-	-	+	+		5
Sa	-	-	-	-	+		2.5

**Table No 8:** Minimum Bactericidal Concentration (Mbc) Of Propolis On Gram-Positive And Gram-Negative Bacteria

Organism	Concentration Of Propolis	Mbc
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	Extract (Mg/MI):					(Mg/MI)	
	250	125	62.5	31.25	15.625		
Pm	+	+	+	+	+	0	
Ec	+	+	+	+	+	0	
St	+	+	+	+	+	0	
Bs	+	+	+	+	+	0	
Sf	+	+	+	+	+	0	
Sa	+	+	+	+	+	0	
	20	10	5	2.5	1.25	Positive Control	Mbc (Mg/MI)
Pm	-	-	-	-	+		2.5
Ec	-	-	-	-	-		1.25
St	-	-	-	-	-		1.25
Bs	-	-	-	+	+		5
Sf	-	-	-	+	+		5
Sa	-	-	-	-	-		1.25

Key: - No Growth, + Growth

**Table No 9:** Minimum Bactericidal Concentration (Mbc) Ofpollen On Gram-Positive And Gram-Negative Bacteria

Organism	Concentration Of Pollen Extract (Mg/MI):					Mbc (Mg/MI)	
	250	125	62.5	31.25	15.625		
Pm	+	+	+	+	+	0	
Ec	+	+	+	+	+	0	
St	+	+	+	+	+	0	
Bs	+	+	+	+	+	0	
Sf	+	+	+	+	+	0	
Sa	+	+	+	+	+	0	
	20	10	5	2.5	1.25	Positive Control	Mbc (Mg/MI)
Pm	-	-	-	-	+		2.5
Ec	-	-	-	-	-		1.25
St	-	-	-	-	+		2.5
Bs	-	-	-	+	+		5
Sf	-	-	-	+	+		5
Sa	-	-	-	-	+		2.5

**Table No 10:** Minimum Bactericidal Concentration (Mbc) Of Bee Wax On Gram-Positive And Gram-Negative Bacteria

Organism	Concentration Of Bee Wax Extract (Mg/MI):					Mbc (Mg/MI)	
	250	125	62.5	31.25	15.625		
Pm	+	+	+	+	+	0	
Ec	+	+	+	+	+	0	
St	+	+	+	+	+	0	
Bs	+	+	+	+	+	0	
Sf	+	+	+	+	+	0	
Sa	+	+	+	+	+	0	
	20	10	5	2.5	1.25	Positive Control	Mbc (Mg/MI)
Pm	-	-	-	+	+		5
Ec	-	-	-	-	-		1.25
St	-	-	-	-	-		1.25
Bs	-	-	+	+	+		10
Sf	-	-	-	-	+		2.5
Sa	-	-	-	-	-		1.25

**Table No 11:** Minimum Bactericidal Concentration (Mbc) Of Honey On Gram-Positive And Gram-Negative Bacteria

Organism	Concentration Of Honey Extract (Mg/MI):					Mbc (Mg/MI)	
	250	125	62.5	31.25	15.625		
Pm	+	+	+	+	+	0	
Ec	+	+	+	+	+	0	

<b>St</b>	+	+	+	+	+	<b>0</b>	
<b>Bs</b>	+	+	+	+	+	<b>0</b>	
<b>Sf</b>	+	+	+	+	+	<b>0</b>	
<b>Sa</b>	+	+	+	+	+	<b>0</b>	
	<b>20</b>	<b>10</b>	<b>5</b>	<b>2.5</b>	<b>1.25</b>	<b>Positive Control</b>	<b>Mbc (Mg/MI)</b>
<b>Pm</b>	-	-	-	-	+		<b>2.5</b>
<b>Ec</b>	-	-	-	-	-		<b>1.25</b>
<b>St</b>	-	-	-	-	-		<b>1.25</b>
<b>Bs</b>	-	-	-	+	+		<b>5</b>
<b>Sf</b>	-	-	-	+	+		<b>5</b>
<b>Sa</b>	-	-	-	-	+		<b>2.5</b>

#### IV. Discussion

The Result Obtained From This Research Showed That Propolis, Pollen, Bee Wax And Honey Have Antibacterial Activity Against Both The Gram-Positive And Gram-Negative Bacteria. (Table 1) This Is In Relation With The Work Conducted By Kujumgiev *Et AL.*, (1999)<sup>18</sup> Who Reported That All Bee Products Sample Used In Their Experiment Were Active Against Gram-Positive Bacteria<sup>19</sup> castaldo And Capasso (2002) Also Reported That Bee Products Sample Showed In-Vitro Antimicrobial Activity Mainly Against Gram-Positive Bacteria (*Staphylococcus Spp And Streptococcus Spp*) And Gram-Negative Bacteria (*E. Coli, Klebsiella Pneumonia, Proteus Vulgaris And Pseudomonas Aeuginosa*).

In This Study, Pollen Showed The Highest Activity Against The Entire Organism Used Than That Of The Other Bee Products (Table 1.) The Variation In The Antibacterial Activity May Be Due To The Different Plant Material Used By The Honey Bees, Phenolic And Flavonoid Constituent Of The Samples. According To Cihangir *Et AL.*, (2005)<sup>20</sup> who Reported That One The Determining Factor For The Antibacterial Activities Of Bee Products Is The Botanical Origin Of The Honey Bees And Geographical Location/ The Region. Based On The Result Obtained In This Study, Bee Wax Is Active Against The Tested Organism But Showed High Inhibitory Activity Against *P. Multocida* The Sensitivity Decreases On *E. Coli, S. Typhi, And S. Feacalis*. *S. Aueus* And *B. Subtilis* Showed The Same Zones Of Inhibition. This Is Accordance With The Work Done By Bogdanov (2004)<sup>21</sup> on The Antibacterial Properties Of Bee Wax It Was Found To Be Particularly Active Against *B. Alvei, Proteus Vulgaris, Salmonella Gallinarum And B. Subtilis*.

The Result Also Showed That Honey Is Active Against The Tested Organism Used But *E. Coli* And *S. Typhi* Are More Sensitive (Table 1.) This Is In Agreement With The Research Conducted By Miorin *Et AL.*, (2003)<sup>22</sup> On The Antibacterial Activity Of Honey Against *E. Coli* 0157:H7 and *S. Typhi*. The Variation In The Activity May Be Due To The Method Used In Testing The Antibacterial Activity, The Solvent Used For The Extraction, The Botanical Origin, Phenol Or Flavonoid Content Of The Products.

The Antibacterial Activity Of Propolis Against Different Gram Positive Pathogenic Bacteria (*B. Subtilis S. Aureus And S. Faecalis*) Are Shown In (Table 2.). According To The Analysis Among The Tested Gram Positive Bacteria, *B. Subtilis* And *S. Faecalis* Were More Sensitive After 24 Hours Of Incubation. The Sensitivity Decreased On *S. Aureus*, There Is Antibacterial Activities Of Pollen Extracts Against These Gram Positive Pathogenic Bacteria (*B. Subtilis, S. Aureus And S. Faecalis*) The Sensitivity Decreased On *S. Aureus* These Gram Positive Bacteria (*B. Subtilis, S. Aureus And S. Faecalis*) Were Sensitive On Bee Wax Extract. Honey Extract Was Active Against These Gram Negative Bacteria Used But The Activity Vary Among The Organism The Antibacterial Activity Of These Products Against Gram Positive Bacteria Is Not Statistically Significant.  $P > 0.05$ .

This Is Not Related With The Work Conducted By Kujumgiev *Et AL.*, (1999)<sup>18</sup>, Who Reported That All Bee Products Sample Used In Their Experiment Were Active Against Gram-Positive Bacteria. The Antibacterial Activities Of Propolis Extract Against Different Gram Negative Pathogenic Bacteria Are Shown In (Table 3.) The Inhibition Zones Vary Among The Bacteria Used. *P. Multocida* Was Discovered To Be More Sensitive To Pollen Extract The Sensitivity Decreased Among The Organism *E. Coli* And *S. Typhi* *P. Multocida* Is More Sensitive To Pollen Extract Than That Of Propolis. The Sensitivity Decreased On *S. Aureus, E. Coli. P. Multocida* Is More Sensiyive To Pollen Extract Than That Of Propolis. The Sensitivity Of *E. Coli And S. Typhi* On Honey Is The Same. *P. Multocida* Vary Abit Among Them. The Antibacterial Activities Of These Products Against Gram Positive Bacteria Are Statistically Significant.  $P < 0.05$ . This Is In Relation With The Work Conducted By Castaldo And Capasso (2002)<sup>19</sup>, Also Reported That Bee Products Sample Showed In-Vitro Antimicrobial Activity Mainly Against Gram-Negative Bacteria (*E. Coli, Klebsiella Pneumonia, Proteus Vulgaris And Pseudomonas Aeuginosa*).

All The Result For The Minimum Inhibitory Concentration (Mic) Of The Bee Products Against The Entire Pathogenic Gram Positive And Gram Negative Bacteria Ranges Between 125 And 250mg/MI

Respectively (Table 4-7.) This Is Not In Agreement With The Work Conducted By Fernandes (1995)<sup>23</sup>,The Variation May Be Due To Method In Determining The (Mbc).

The Entire Table For The Minimum Bactericidal Concentration (Mbc) On All The Bee Products (Propolis, Pollen Wax And Honey) Shows Positive (+) Which Means There Was Growth Of All The Organism Used On The Nutrient Agar On Subsequence Transfer To A New Environment This Is Because The Activity Of These Bee Products At Varying Concentration Of 250, 125, 62.5, 31.25, And 15.625 Mg/ML During The Mic Was Static Not Cidal (Table 8-11.) These Does Not Relate With The Research Carried Out By<sup>24</sup>.Among All Pollen Have The Highest Activity Against All The Bacteria's Used.

## V. Conclusion

Among All The Pathogenic Gram Positive And Gram Negative Bacteria Used In This Study, It Is Discovered That All The Tested Organism Are Sensitive To All The Bee Products But It Is Statically Significant On Gram Negative Bacteria. In A Nut Shell These Bee Products Are More Active Against Gram Negative Bacteria.

## Recommendation

We Recommend That Further Work Should Be Carried Out On The Antibacterial Activity Of Bee Products From Different Geographical Location.

We Recommend That Further Research Should Be Carried Out To Increase The Concentration Above 250mg/ML.

We Also Recommend That Any Of These Bee Products Can Be Used To Treat Any Disease Caused By Gram Negative Bacteria At A Higher Concentration.

## References

- [1] Ghosh S And Playford R. Bioactive Natural Compounds For The Treatment Of Gastrointestinal Disorders. *Clinical Science*. 2003; (104): 547–556
- [2] Ghisalberti El. Propolis: (1979). A Review *Bee World* (60): 59-84.
- [3] Dobrowolski Jw, Vohora Sb, Sharma K, Shah Sa, Naqvi Sah, Dandlya Pc (1991) Antibacterial, Antifungal, Antiamoebic, Anti-Inflammatory And Antipyretic Studies On Propolis Bee Products. *Jurnal Of Ethnopharmacology* 35: 77-82.
- [4] Zaghoul Aa, El-Shattawy Hh, Kassem Aa, Ibrahim Ea, Reddy Ik And Khan Ma. (2001) Honey, A Prospective Antibiotic Extraction, Formulation, Stability. *Pharmazie*. (56): 643-647.
- [5] Kingsley A. (2001). The Use Of Honey In The Treatment Of Infected Wound. *British Journal Of Nursing*. 10 (22): S13-S20.
- [6] Gene B. (2005). Bee Pollen, Propolis , Royal Jelly. Huntington College Of Health Sciences.
- [7] Ramanauskieni K, Inkeniene Am, Savickas A, Masteikova R And Brusokas V. (2009) Analysis Of The Antimicrobial Activity Of Propolis And Lysozyme In Semisolid Emulsion Systems. *Acta Poloniaepharmaceutica - Drug Research*. 66(6): 681 - 688.
- [8] Ghanem Nevine B. (2011). Study On The Antimicrobial Activity Of Honey Products And Some Saudi Folkloric Substances. *Research Journal Of Biotechnology*. 6 (4): 38 – 43.
- [9] Kacaniova, M; Vukovic, N; Chlebo, R; Hascik, P; Rovna, K; Cubon, J; Dzugan, M; Pasternakiewicz, A (2012). The Antimicrobial Activity Of Honey, Bee Pollen Loads And Beeswax From Slovakia. *Archives Of Biological Sciences* 64 (3): 927-934.
- [10] Krell, R. (1996) Value-Added Products From Beekeeping. *Rome: Fao*, 409. Isbn 92-5-1038198.
- [11] Broadhurst, C. L. (1999) Bee Products: Medicine From The Hive. *Nutritional. Science. News*, 4, 366-368.
- [12] Campos, M. G., Cunha, Markham R. A., And K. (1997) Bee-Products Properties, Applications And Apitherapy In *Bee-Pollen Composition, Properties And Applications*, A. Mizrahi & Y. Lensky, Eds.; London, Uk, Plenum Publishers, 93-100.
- [13] Haas, E. M.(1992) *Staying Healthy With Nutrition*. Newyork: Celestial Arts Publish, P. 297–298.
- [14] National Committee For Clinical Laboratory Standards (Nccls) (2000). Approved Standard M7-A5: Methods For Dilution Antimicrobial Susceptibility Test For Bacteria That Grow Aerobically. 5th Edition. Nccls, Wayne, Pa.
- [15] Sofowara, E.A (1989) Medicinal Plant And Traditional Medicina In Africa. 2<sup>nd</sup> Edition. Spectrum Books Limited No. (2): 16
- [16] Collins C. H. And Patricia M. Lyne, (1967). Microbiological Methods Fourth Edition Isbn: 007600781110.
- [17] Cowan St. M.D., D. Sc. F. C. Path And Steel K. J. B. Phrm., Phd., Fps. (1965). Manual For Identification Of Medical Bacteria First Edition. Isbn: 0521047366.
- [18] Kujungiev A., Tsvetkova I., Serkedjieva Y., Bankova V.S., Christov R., Popov S. (1999). Antibacterial, Antifungal And Antiviral Activity Of Propolis Of Different Geographic Origin, *Journal Of Ethnopharmacology* (64): 235-240.
- [19] Castaldo, S And Capass, S. (2002). Propolis, An Old Remedy Used In Modern Medicine. *Fitoterapia*, V.73, N.1, P. S1-S6.
- [20] Cihangir, N., Sorkun., And Salih, B. (2005). Chemical Composition And Antibacterial Activities Of Propolis Collected From Different Regions Of Turkey. *Hacettepe Journal Of Biology And Chemistry* 34:59-67.
- [21] Bogdanov S. (2011) Functional And Biological Properties Of The Bee Products: A Review Bee Products Science [www.Bee-Hexagon.Net](http://www.Bee-Hexagon.Net).
- [22] Miorin, P.L., Levy, N.C.J., Custodio, A.R., Bretz, W.A, Amd Marcucci, M.C. (2003). Antibacterial Activity Of Honey And Propolis From *Apis Mellifera And Tetragonisca Angustula* Against *staphylococcus Aureus*. *J. Appl. Microb*. 95:913-920.
- [23] Fernandes, A. Jr., Sugizaki M.F., Fogo, M.L. Funari, S.R.C. And Lopes, C.A.M. (1995). In Nitro Activity Of Propolis Against Bacterial And Yeast Pathogens Isolated From Human Infections. *J. Venom. Anim. Toxins.*, 1:63-69
- [24] Somal, N., Coley, K.E., Molan, P.C. And Hancock, B.M. (1994). Susceptibility Of *Helicobacter Pylori* To The Antibacterial Activity Of Manuka Honey. *J. R. Soc. Med*. 87:9-12

ogbu, K. I"Comparative Study on the Antibacterial Activities of Bee Product (Propolis, Pollen, Bee Wax and Honey)"IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 13.2 (2018): 20-27.