Antibacterial Activity and Phytochemical Analyses of Propolis (Bee Glue) Extract against *Escherichia coli* and *Staphylococcus aureus*

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Abstract: Propolis (bee glue) is a sticky dark-colored material that honeybees collect from plants and use in the hive, which contains higher amounts of bioactive components. The study was aimed at evaluating phytochemical constituents and antibacterial activity of propolis extract against *Staphylococcus aureus* and *Escherichia coli.* Phytochemical analyses of the extract were carried out using qualitative and quantitative procedures. *Staphylococcus aureus* and *Escherichia coli* were isolated and identified using Bergy's manual of determinative bacteria. Evaluation of the antibacterial activity of extracts was carried out using the agar well diffusion method. The result of antibacterial activity showed that *Escherichia coli* had the higher zone of inhibition in ethanolic extract than in aqueous at the concentration of 50mg/ml and 25mg/ml (18.5±0.17 and 10.0±0.17), (4.0±2.67 and 3.3±0.89) respectively, the higher the concentration the higher the zone of inhibition. The result of phytochemical screening revealed the presence of saponins, alkaloids, tannins, phenolics, flavonoids, steroids, and cardiac glycosides in ethanolic extract while anthraquinone was not detected. While quantitative phytochemical screening revealed that phenolic compounds had the highest absorbance followed by flavonoids and tannins. The ethanolic extract of propolis can be an alternative material for treating skin and wound infection

Keywords: Anthraquinone, Phytochemicals, Propolis, and *Staphylococcus aureus*

INTRODUCTION

In traditional medicine, propolis has been an important source of natural products for treating aliments for humans. It is a resinous substance produced by bees through the mixture of jaw secretions and the exudate collected from plant materials (Bankova *et al.,*2000; Teixeira *et al.,* 2005). This resin is used in the building, maintenance, and asepsis of the nest whereby are referred to as "blue glue" (Simone-Finstrom and Spivak, 2010). Raw propolis consists of 50% –60% of resins and balms (including phenolic compounds), 30% –40% of waxes and fatty acids, 5% –10% of essential oils, 5% of pollen, and about 5% of other substances including amino acids, micronutrients, and vitamins (thiamin, riboflavin, pyridoxine, C, and E (Boisard *et al.,* 2014; Bonamigo *et al.,* 2017).

There are records that ancient civilizations, such as the Incas, Greeks, Egyptians, and Romans, used propolis for its therapeutic properties, being described as antiseptic, healing, antipyretic, and used to embalm cadavers (Sforcin and Bankova, 2011). Propolis has also been used in the preparation of drinks and foods for human nutrition, aiming at the improvement in health and disease prevention (Umthong *et al.,* 2009). Propolis has many antimicrobial properties as secondary metabolites such as alkaloids, phenolic compounds, etc.

The phytochemicals and antimicrobial properties of propolis have been investigated by several researchers worldwide and have been reported to possess antibacterial, antifungal, antiviral, antiparasitic, antioxidative, anticancer, anti-inflammatory, antiulcer, and antidiabetic effects (Pasupuleti *et al.,* 2017; Al-Ani *et al*., 2018). Propolis and bee pollen extracts are used instead of the raw substance since they contain higher amounts of bioactive components (Denisow and Denisow, 2016). The present occurrence of antibiotic resistance is among the

significant problems in the 21st century and has necessitated the need for continuous research for more and safe therapeutic agents (Chikezie *et al.,* 2015). The objective of the study was to determine the antibacterial activity and phytochemical analyses of ethanolic and aqueous propolis extract against some clinical isolates.

MATERIALS AND METHODS

Sample Collection and Sampling

The study sample was collected from an apiary containing modern beehives located in More Area, Kware Local Government Area of Sokoto State, Nigeria. Hives were chosen randomly and from each selected hive sample of propolis was taken. The samples were then transported to microbiology laboratory in an air-tight paper envelope, mixed and a representative sample was collected using the method reported by Alan (1996). Before the analysis, the sample was kept in an air-tight container in a refrigerator, this is because raw propolis can remain frozen in an air-tight container for several years (Bankova *et al.,* 2016).

Preparation of the Extracts

Aqueous Extraction

Thirty grams (30g) of the sample were dissolved in 100ml of distilled water for 24 hours. The mixture was filtered using Whatman's filter paper No 1 to obtain a solution free of solids. The filtrate collected was evaporated to dryness using a water bath. The extract was collected in fresh sterile universal bottles and stored at 4^{o}C until required for further use (Akinnibosun, 2009)

Ethanolic Extraction

This was carried out as described by Ozkok and Ozcan (2010), Thirty grams (30g) of propolis was dispensed in 100ml ethanol (70%) and kept at room temperature. The extracts were filtered using Whatman's No1 filter paper after a week and evaporated under vacuum at 50^{o}C.

Isolation and Identification of Bacterial Isolate

Staphylococcus aureus and *Escherichia coli* were obtained from patients with wounds infection and burns at Usmanu Danfodiyo University Teaching Hospital, Sokoto. Gram's staining and biochemical test such as Triple sugar iron, methyl red, Voges Proskauer, indole test, oxidase, coagulase urease, motility, catalase were carried out as described by Oyeleke and Manga (2008) for identification.

Phytochemical Screening of the Extracts

Phytochemical analysis of the extract for qualitative and quantitative detection of alkaloids, balsams, flavonoids, tannins, saponins, glycosides, saponin glycosides, steroids, anthraquinones, and volatile oil was performed as described by Sofowora (1994).

Determination of Antibacterial Activity of the Crude Extract

The susceptibilities of the bacterial isolates to the propolis extracts were assayed as described by Aliyu *et al*. (2009). Bacterial isolates grown on nutrient agar incubated at 37^{o}C for 18 hours were suspended in saline solution (0.85% NaCl) and adjusted to match turbidity of 0.5 McFarland standard (10^{8} cell/ml). The standardized suspension was used to inoculate the surfaces of Mueller Hinton agar plate's sterile using a bent glass rod. A standard cork borer of 6mm in diameter was used to cut a well and filled with different concentrations (50, 25, 12.50, and 6.25mg/ml) of the aqueous and ethanol extracts and a commercial antibiotic (Tetracycline 500mg/ml) as standard drug control. The plates were allowed to stand for 5 hours at room temperature for the extract to diffuse into the agar and incubated at 37^{o}C for 24hrsthen observed for the zone of inhibition of the growth. The zones were measured with a transparent ruler and the result was recorded in millimeters. The screening was done in triplicates.

Determination of Minimum Inhibitory Concentration (MIC) of the Crude Extract

Minimum inhibitory concentration (MIC) was determined according to Cheesebrough (2006) for the crude extracts of Propolis against test organisms. To each 5ml of the various extracts in different tubes were added 5ml of nutrient broth each and serially diluted out to various concentrations ranging from 50 to 6.25mg/ml. A loopful of each test organism was inoculated at 37^{o}C for 24hours. The MIC was the lowest concentration of the extracts that inhibited growth.

Determination of the Minimum Bactericidal Concentration (MBC) of the Crude Extract

The minimum bactericidal concentration (MBC) was determined by the method described by Ibekwe *et al.,* (2001). The contents of negative tubes from MIC above were sub-cultured on freshly prepared Mueller Hinton agar plates and incubated for 37^{o}C for 24hrs. The tubes with the lowest concentration of the extract that show no growth at sub-culturing were recorded as minimum bactericidal concentration.

RESULTS

The result of the antibacterial activities of aqueous and ethanolic extracts of Propolis is presented in Table 1. At concentrations of 50mg/ml, 25mg/ml, 12.5 mg/ml, and 6.25mg/ml both *Staphylococcus aureus* and *Escherichia coli* were susceptible with the zone of inhibition of 18.5 ± 0.17, 15.5± 0.17, 12.5± 0.17 and 6.67±0.87 respectively.

The result of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the aqueous and ethanolic extract shown in Table 2. The MIC of *Staphylococcus aureus* is susceptible or sensitive at a concentration of 12.5mg/ml and 6.25mg/ml and *Escherichia coli* at a concentration of 12.5mg/ml for both aqueous and ethanolic extract. While MBC of *Staphylococcus aureus* showed a minimum bactericidal concentration of 50mg/ml for both aqueous and ethanolic solution and *Escherichia coli* showed the minimum bactericidal concentration of 50mg/ml and 12.5mg/ml for aqueous and ethanol extracts respectively.

The qualitative phytochemical screening of the extract is presented in Table 4. Alkaloids, tannins, saponin, phenolics, flavonoids, steroids, and cardiac glycosides were detected, while anthraquinone was found absent in the ethanolic extract. The quantitative phytochemical screening of ethanolic and aqueous extract of Propolis is shown in Table 5, the absorbance of phenolic compounds was the highest followed by flavonoids and tannins.

Table 1: Antibacterial activities of ethanolic and aqueous extracts of propolis in different concentrations.

Test Organisms	**Zone of inhibition (Diameter in mm)**									
	Aqueous Extract (Conc. = mg/ml)					**Ethanolic Extract (Conc. = mg/ml)**				
	50	25	12.5	6.25	Control	50	25	12.5	6.25	Control
S. aureus	1.30±0.89	1.30±0.49	3.3±0.89	-	-	10.0±0.17	8.0±017	8.0±017	5.5±017	6.33±1.57
E. coli	4.0±2.67	4.0±2.67	4.0±0.67	2.0±1.0	-	18.5±0.17	15.5±0.17	12.5±0.17	10.5±0.17	6.67±0.87

Keys: Control= *Tetracycline;* Values are Mean ± S.D in triplicate, 6mm^2 Size of the well.

Table 2: Minimum Inhibitory and Bactericidal concentration (MIC and MBC) of Propolis extract on *S. aureus* and *E. coli*

	Concentration of Extracts (mg/ml)			
Isolates	**MIC_E**	**MIC_A**	**MBC_E**	**MBC_A**
S. aureus	12.5	6.25	50	50
E. coli	12.5	12.5	50	12.5

Keys: MIC_E = Minimum Inhibition Concentration of Ethanol Extract
MIC_A = Minimum Inhibition Concentration of Aqueous Extract; MBC_E = Minimum Bactericidal Concentration of Ethanol Extract
MBC_A = Minimum Bactericidal Concentration of Aqueous Extract;

Table 3: Qualitative Phytochemical Screening of Ethanolic Extract of Propolis

Phytochemicals	Inference
Alkaloids	**+**
Tannins	**+**
Saponins	**+**
Phenolics	**+**
Flavonoids	**+**
Steroids	**+**
Cardiac, Glycosides	**+**
Anthraquinone	**-**

Key: + =Detected; - = Not detected

Table 4: Quantitative Phytochemical Screening of Ethanolic and Aqueous Extract of Propolis

Phytochemicals	**Absorbance**
Tannin	0.512
Flavonoid	0.738
Phenolic compounds	1.117

The antibacterial activities shown in Table 1 of ethanolic and aqueous extract of propolis were determined using two bacteria: gram-positive and gram-negative bacteria, *Escherichia coli,* and *Staphylococcus aureus* respectively. The ethanolic extract is highly active against *Escherichia coli* and *Staphylococcus aureus* with the 50mg/ml concentration showing the highest zone of inhibition at 18.5±0.17 and 10.0±0.17mm respectively. This shows that the higher the concentration the higher the zone of inhibition, and the lower the concentration, the lower the zone of inhibition. In this study, ethanol revealed the most effective extract than aqueous, which might be due to the polarity of the compound. Our findings confirm the report on the antibacterial activity of propolis extract from the previous study of Siddhuraju *et al.,* (2012) and Milena, *et al*., (2013) reported that gram-negative bacteria are more sensitive to the action of propolis than gram-positive bacteria which may be due to the structural differences of the cell wall. Silivia *et al.,* (2013) and Manimaram *et al.,* (2015) supported the findings concerning the antibacterial properties of the various extract varied with the solvents used.

The result of Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of ethanolic and aqueous extract of propolis as shown in Table 2 that ethanolic extract shows highest Minimum inhibitory concentration (MIC) than aqueous, *Escherichia coli* shows highest (MIC) than *Staphylococcus aureus* in an aqueous while, the value was the same in ethanolic extract. This result is in agreement with the previous works of Teixera *et al.,* (2005) who reported that resistance exerted by Gram-negative bacteria might be due to cell wall structure which is more complex than that of Gram-positive bacteria. While minimum bactericidal concentration (MBC) of ethanolic and aqueous extract of propolis showed in Table 2 that ethanolic extract of *Staphylococcus aureus* has the highest minimum bactericidal concentration (MBC). In this study, it is observed that the MIC value obtained were lower than MBC values. This shows that Propolis extracts were bacteriostatic at lower concentrations but bactericidal at higher concentrations. This result agrees with the work of Ahn *et al.,* (2004) who reported that ethanolic extract of propolis showed high MBC against Gram-positive cocci but had weak MBC against Gram-negative bacteria.

The results of qualitative phytochemical screening of the ethanolic extract as shown in Table 3 revealed the presence of various phytochemicals such as; alkaloids, tannins, saponins, phenolics, flavonoids, steroids, and cardiac glycoside, while anthraquinone was absent. This is consistent with the findings of Sharma *et al.,* (2012). Oda *et al.,* (2000), which stated that phytochemicals are chemical compounds formed during the plants' normal metabolic processes; these chemicals are often referred to as secondary metabolites of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, polysaccharides, phenols, tannins, terpenes and terpenoids. In this study, flavonoid was detected in the ethanolic extract of propolis, which is in agreement with the previous findings of Daniel *et al*., (2012). Saponin functions as an antioxidant because it possesses a special moiety (2,3-dihydric-2,5-dihyroxy6-methyl-4-pyran-4-one), which acts by forming hydroperoxide intermediates removing free radicals and possess hemolytic action on human erythrocytes (Hu *et al*., 2002). Milena *et al*., (2013), Silvia *et al*., (2013), and Manimaran *et al.,* (2015) as supported by the findings concerning the antibacterial properties of flavonoids, tannins, and steroids.

The result of quantitative phytochemical screening of ethanolic and aqueous extract of propolis shown in Table 4 revealed the presence of tannins, flavonoids, and phenolic compounds. The absorbance of the phenolic compound was the highest followed by flavonoids and tannins

respectively, These results are in agreement with the findings of Hu *et al.,* (2002) which reported that the highest antibacterial activity might be due to the presence of phytochemical constituents present in propolis and their ability to penetrate through the cell wall and cytoplasmic membranes of the microbes.

CONCLUSION

The research revealed that the ethanolic extract of propolis is active against *Escherichia coli* and *Staphylococcus aureus*. The MIC of ethanolic extract for *E. coli* was recorded at 12.5mg/ml and aqueous extract for *S. aureus* was recorded at 6.25mg/ml. While MBC was 50mg/ml for *S. aureus* and 12mg/ml for *E. coli.* The phytochemical screening revealed the presence of many phytochemicals such as alkaloids, flavonoids, tannins, saponins which could be attributed to its antibacterial activity. The results obtained suggest the potential use of propolis as an alternative material for treating ailments such as skin infections and wounds.

Recommendations

Further research work should be carried out to determine the antifungal activities of propolis and also to isolate secondary metabolites from the extract to test for specific antimicrobial activities. Evaluation of propolis extract through *in vivo* based research is highly recommended to achieve low cost, less side effect treatment, and prevent recurrent infections.

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