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**Research Article**

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# Antibacterial and Antifungal Activity of the Propolis Growing in the Basin of Mediterranean City Misurata, Libya

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**Abstract** Poroplis, a sticky material collected by bees and used in their hives as a general purpose sealer, is well known for its diverse and useful biological activities: antimicrobial, antioxidant, anti-inflammatory, immuno-stimulating, and many others. The aim of the present research was focused on investigating the preliminary phytochemical screening, antibacterial and antifungal activity of Propolisvia *in vitro* approach. The ethanolic extract of Propolis was tested against *E. coli, Shegella sonnei, Pseudomonas aeruginosa* and *Klebsiella pneumonia* by agar well diffusion method and broth dilution method. The antifungal activity was tested with ten different fungal strain *A. oryza, A. niger, A. altera, A. flavus, P. chrogin, P. notatum, B. fubae, P. digitatum, F. solanai* and *F. moliniforme.* Results showed promising antibacterial and antifungal activity against the bacterial and fungal strain tested. The ethanolic extract was found to have a more potent inhibitory effect comparing with the standard antibiotics which prove the potentiality of the plant extracts for the treatment of various skin and gastrointestinal infections in humans.

**Keywords** Propolis, Phytochemical, Antibacterial, Antifungal activity and Pathogens.

## Introduction

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value [1]. Human infections particularly those involving microorganisms i.e., bacteria, fungi, viruses, nematodes cause serious damages in tropical and subtropical countries of the world. Medicinal plants are of great value in the field of treatment and cure of disease. Over the years, scientific research has expanded our knowledge of the chemical effect and composition of the active constituents, which determine the medicinal properties of plant. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country [2, 3]. Herbal medicine is still the mainstay of about 75-80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents [4, 5]. Following the advent of modern medicine, herbal medicine suffered a setback, but during last two or three decades, advances in phytochemistry and in identification of plant compounds, effective against certain diseases have renewed the interest in natural medicines [6]. Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions [7]. This situation forced to search for new antimicrobial substances from natural origins. Therefore, there is a need to develop alternative antimicrobial drugs

for the treatment of infectious diseases from medicinal plants. Antimicrobials of plant origin have enormous therapeutic potential [8]. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [9]. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, flavonoids, steroids, resins fatty acids gums which are capable of producing definite physiological action on body [10]. Propolis is one of the most fascinating honey bee (*Apis mellifera* L.) products. It is a plant derived product that beesproduce from resins that they collect from different plant organs and with which they mix beeswax [11]. Propolis is a building material and a protective agent in the bee hive. It also plays an important role in honey bee social immunity, and is widely used by humans as an ingredient of nutraceuticals, over-the-counter preparations and cosmetics. Its chemical composition varies by geographic location, climatic zone and local flora [12, 13]. Many vegetables protect their leaves, flowers, fruits and buds by producing aresinous compound with potent antimicrobial, anti-putrefaction, waterproofing and heat-insulating properties. These resins are gathered from the gum of various plant sources by honey bees (*Apis mellifera* L.) that form pellets with their mandibles, probably mixing it with products of their salivary glands and with bee wax. So, propolis (beeglue) is an amalgamation of plant resins collected and transformed by bees, and is a strongly adhesive and resinous substance whose color varies from yellow-green to dark brown depending on its source and age [14, 15]. The biological activity of propolis is associated mainly with phenolic compounds such as flavonoids and derivatives of hydroxycinnamic acids. The understanding of the chemical diversity of Propolis is very important in Propolis research [16]. The main objective of the research is to screen and evaluate antibacterial and antifungal activity of crude extracts of Propolis and to find out minimum inhibitory concentration (MIC) of the extract against both gram positive as well as gram negative bacteria.

## Materials and Methods

### Collection and Authentication

Propolis samples were collected from palm tree of the Misurata, Libya in the month of November- December 2016 by scrapping the propolis sample off the top of the hiveusing a spatula and collected in a clean dry tray.

### Extraction

The 300gm of raw propoils were grounded and placed in a flask with 500 ml of 70% ethanol, which was placed on agitator for 72 hrs. Then the macerate was filtered with muslin cloth and filtered was evaporated under reduced pressure and vacuum dried. The yielded a brownish residue of 11% w/w extract of propolis with reference to dry starting material.

#### *Preliminary Phytochemical Screening*

Preliminary phytochemical screening for the detection of various phytoconstitunts such as alkaloids, carbohydrates, steroids, terpenes, flavonoids, phenolic compounds, tannins, saponins, glycosides, protein, and mucilage was carried out by using standard procedures described by Ali et al., 1997 [17– 19].

### Thin Layer Chromatography (TLC)

Thin layer chromatography studies of the ethanol and chloroform extracts carried out in various solvents at 30°C using Silica gel G as adsorbent and the $R_f$ values were determined [20 -23].

### Antibacterial Activity

#### *Pathogenic Bacteria Used for the Present Study*

About three human pathogenic bacterial strains were used. The gram-negative *E. coli, Shegella sonnei, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were included. Axenic cultures of bacterial strains were obtained from the Department of Microbiology, College of Science, Misurata University, Misurata, Libya.

#### *Agar well diffusion method*

One loopful of bacterial stock culture were incubated in 10 ml nutrient broth and incubated at 37°C for overnight. Cell count of the log phase cultures were adjusted to 106-107 cells per ml. 20 ml of 2% nutrient agar was poured in each petridish and allowed to solidify. 25 ml of 0.8% Nutrient agar with bacterial inoculants was poured as second

layer over basal nutrient agar. The well was made in the agar after it solidified each plate by using sterilized cork borer. Different amount of each extract were filled in the well. The plates were incubated at 37°C for 18-24 hrs. Diameter of zone of inhibition on bacterial growth around the well were measured and noted. Another set of petridishes were prepared in the same way in which four different commercially available antibiotics disc i.e. Gentamycin (10µg), ampicillin (10µg), penicillin (10µg/disc) and tetracycline (10µg) [24].

***Broth Dilution Test***

Overnight cultures of test bacteria grown in nutrient broth cultures were diluted 100 folds in Nutrient broth (NB). Gradually increasing volumes of the extracts were added to the test tubes containing the bacterial cultures to know the inhibitory concentration in a particular tube inhibiting the bacterial growth. The tubes were incubated at 37°C for 18-24 hrs. The tubes were examined for visible turbidity and optical density of cultures was determined at 620 nm using NB as control [25].

**Antifungal Activity**

The same procedure was followed as that for antibacterial activity. Nystatin (50 µg/disc) was used as reference and from the stock culture solution (5 µg/µl). The incubation period was 48-72 h. The fungi (*A. oryza, A. niger, A. altera, A. flavus, P. chrogin, P. notatum, B. fubae, P. digitatum,F. Solanai* and *F. moliniforme*) were collected from Department of Microbiology, Faculty of Science, Misurata University, Misurata, Libya.

## Results

### Preliminary Phytochemical Screening

The preliminary phytochemical investigation of the ethanol extract of propolis showed the presence of phytosterols, flavonoids, terpenoid saponins, carbohydrates, tannins, glycosides alkaloids proteins organic acids (Table 1).

**Table 1:** Preliminary phytochemical screening of ethanolic extract of Propolis

| S. No. | Constituents | Ethanolic extract of Propolis |
|---|---|---|
| 1 | Alkaloids | - |
| 2 | Carbohydrates | + |
| 3 | Glycosides | - |
| 4 | Phenolic compounds | + |
| 5 | Tannins | + |
| 6 | Flavonoids | + |
| 7 | Terpenoids | + |
| 8 | Saponins | + |
| 9 | Sterols | + |
| 10 | Proteins | + |
| 11. | Resins | + |
| 12. | Mucilage | - |

+ = Present, - = Absent

### Thin Layer Chromatography (TLC)

Thin layer chromatography of the ethanol and chloroform extracts was carried out using Toluene: Ethyl acetate (8.5: 1.5) as mobile phase respectively and the Rf values were recorded (Table 2). The visualizing reagent employed was anisaldehyde-sulphuric acid reagent to effect visualization of the resolved spots (Fig.1).

**Table 2:** TLC profile of the ethanolic extract of Propolis

| Test extract | Solvent system | Number of spots | $R_f$ value |
|---|---|---|---|
| Ethanol extract | Toluene : Ethylacetate (8.5 : 1.5) | 7 | 0.17, 0.19, 0.31, 0.49, 0.56, 0.65, 0.72 |

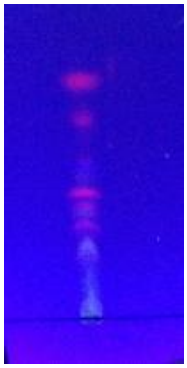

*Figure 1: TLC image of ethanolic extract of Propolis*

**Antibacterial and Antifungal Activity**

The results of antibacterial activities by agar well diffusion method were presented in table-3 and minimum inhibitory concentration (MIC) values were tabulated in table-4.The antibacterial activity was tested on the basis of the magnitude of zones of inhibition (in mm) and minimum inhibitory concentration (in mg/ml). The activity of ethanolic extract of Propolis has also been compared with the broad spectrum commercially available antibiotics. Some bacteria were found to be resistant towards commercially used antibiotics while others were found to be sensitive (Table-5). The detailed analysis of the antibacterial activity of the ethanolic extract showed dose dependent activity and the activity was shown at an amount of 150 mg/ml. While less activity was shown when 50 mg/well amount of extract was used. The MIC of extract for *E. coli* was 1.75mg/ml, for *S. sonnei* was 2.0 mg/ml, for *K. Pneumonia* was 1.25 mg/ml and for *P. Aeruginosa* was 1.5 mg/ml. When compared to the standard antibiotics, it was seen that ethanolic extract of Propolis was effective than ampicillin, and penicillin against *E. coli, S. sonnei, K. pneumoniae* and *P. aeruginosa.* By analyzing the overall data it was observed that the gram positive pathogens were more susceptible towards the different leaf extracts tested. While gram negative bacteria showed little resistance towards some extracts. It is not known exactly why gram negative bacteria should be less susceptible, but it may be related to its outer membrane which endows the bacterial surface with strong hydrophilicity and acts as a strong permeability barrier [26]. Some of the phytochemical compounds e.g. glycoside, tannin, flavonoids, alkaloids, have variously been reported to have antimicrobial activity [27]. It might be possible that the antibacterial activity of *Propoils* extracts was due to the inhibition of bacterial cell wall synthesis or because of leakage from cell membranes of bacteria or it might be possible that the effect of extract was shown due to the inhibition of protein synthesis of bacterial cell or due to the possibility to interfere with DNA function of the bacteria.

Antifungal activity of ethanolic extract of Propolis among the ten tested fungi *A. oryza, A. niger, A. altera, A. flavus, P. chrogin, P. notatum, B. fubae, P. digitatum, F. Solanai* and *F. moliniforme* were responsive to the ethanolic extract with the zones of inhibition given (Table 6) below in comparison to the inhibition by the standard Nystatin. There was no activity in case of *P. Notatum* and *P. digitatum* the tested compounds. However, Nystatin showed the prominent zone of inhibition at 50 µg/disc in all tested fungi.

**Table 3:** Antibacterial activity of ethanolic extract of Propoils against different pathogenic bacterial strains by agar cup diffusion method

| **Pathogenic bacteria** | **Diameter of inhibition zone (mm)** | | |
|---|---|---|---|
| | **Ethanolic extract** | | |
| | **50** | **100** | **150** |
| *E. coli* | 13 | 25 | 29 |
| *Shegella sonnei* | 17 | 26 | 28 |
| *Klebsiella pneumoniae* | 16 | 21 | 23 |
| *Pseudomonas aeruginosa* | 10 | 15 | 21 |

**Table 4:** Minimum inhibitory concentration of ethanolic extract of Propoils against different pathogenic bacterial strains

| Pathogenic bacteria | Minimum inhibitory Conc. (mg/ml) | |
|---|---|---|
| | Ethanolic extract | 10% DMSO |
| *E. coli* | 1.75 | - |
| *Shegella sonnei* | 2 | - |
| *Klebsiella pneumoniae* | 1.25 | - |
| *Pseudomonas aeruginosa* | 1.50 | - |

Data is a mean of three replications
"-" No inhibition observed
10% DMSO Negative control

**Table 5:** Diameter of inhibition of zone of antibiotics against bacterial strains

| Pathogenic bacteria | Diameter of inhibition zone (mm) | | | |
|---|---|---|---|---|
| | Gentamycin | Ampicillin | Penicillin | Tetracycline |
| *E. coli* | 14 | - | - | 16 |
| *Shegella sonnei* | 16 | 10 | - | 18 |
| *Klebsiella pneumoniae* | 10 | 16 | - | 22 |
| *Pseudomonas aeruginosa* | 18 | - | 21 | 20 |

Data is a mean of three replicates
"-" No inhibition observed
Antibiotics Positive control

**Table 6:** *In vitro* antifungal activity of ethanolic extract of Propolis and the standard Nystatin

| Diameter of Zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|
| Test fungus | µg/disc | | | | Nystatin |
| | 50 | 100 | 150 | 200 | 50 µg/disc |
| *A. oryza,* | - | 5 | 8 | 11 | 15 |
| *A. niger* | - | 9 | 4 | 10 | 19 |
| *A. altera* | 3 | 7 | 5 | 11 | 10 |
| *A. flavus* | 7 | 12 | 14 | 17 | 19 |
| *B. fubae* | - | 9 | 11 | 15 | 18 |
| *F. solanai* | - | 3 | 7 | 12 | 15 |
| *F. moliniforme* | 4 | 7 | 9 | 11 | 14 |
| *P. chrogin,* | - | 5 | 10 | 13 | 20 |
| *P. notatum,* | - | - | - | - | 04 |
| *P. digitatum* | - | - | - | - | 18 |

**Discussion**

Propolis is a heterogeneous material consisting of resin collected by honey bees from the leaf, buds and bark of certain tree species, which is then admixed with beeswax produced from the hypo-pharyngeal glands of the worker bees [28]. Propolis is used by bees to defend the hive against invaders, and to reduce air flow into the hive to retain heat. In modernbee-keeping practice, the beekeeper places mats into the hives with grid slots ideally sized for Propolis to be deposited. These mats are then removed from the hives to recover the Propolis for commercialuse [29]. *In vitro* studies in the present work concluded that the plant extract inhibited bacterial growth but their effectiveness varied. The antimicrobial activity has been attributed to the presence of some active constituents in the extracts. This antibacterial study of the plant extracts demonstrated that folk medicine can be as effective as modern

medicine to combat pathogenic microorganisms [30]. The present work also revealed that the Propolis extract inhibited fungal growth but their effectiveness varied. The antifungal activity has been attributed to the presence of some active constituents in the extracts. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases [31].

## Conclusions

These findings support the traditional knowledge of local users and it is a preliminary, scientific, validation for the use of these plants for antibacterial activity to promote proper conservation and sustainable use of such plant resources. Hence it would be interesting to investigate the potentiality of this plant for possible application in foods to increase shelf life or promote safety. It can also be suggested to promote the use of this plant against various gastrointestinal disorders as well as in skin infections.

This study is a substantial step and it further requires a long term study to evaluate therapeutic efficacy and toxicity of propolis. This result may provide a basis for the isolation of compounds of from Propolis extract. Further studies are needed to identify the pure component and establish the mechanism of action for antibacterial action of the plant extract.

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## Ethical statement

The experimental protocols were approved by Ethical Committee of Faculty of Science, Misurata University, Misurata, Libya and their guidelines were followed for the studies.

## Conflicts of Interest

The authors report no declaration of interest.

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