زانکۆی سەلاحەددین- هەولیر

Salaheddin University- Erbil

The effect of natural product on human health

Research Project

Submitted to the department of (general science) in partial fulfillment of the requirements for the degree of B.A or BSc. in (biology)

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Confirm supervisor approval

I confirm that this research has been completed and written under my supervision, and I agree to submit it in its current form for discussion to obtain a bachelor's degree for each of the female students:

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the signature :

Name: ***Suhair Waleed***

the date:

Dedication

This research is dedicated to

- our family
- our supervisor
- General Science department
- All people and laboratory that helped us

Acknowledgment

First of all thanks for Allah to giving us the ability to do this research, a special thanks to our parent to supporting us all the time . We would like to express our deepest thanks and respect to our supervisor Asst Prof. Dr. Suhair waleed for his help supervision and guidance and support to accomplish this study. We would like to express a special thanks to all members who helped us to finish this research.

Abstract

Propolis is an extremely safe natural antifungal substance that has been reported to have powerful antifungal efficacy. The aim of this study was to evaluate the inhibitory effects of propolis against *Candida albicans*. Propolis was collected from the honey bee *Apis mellifera*. The strain of *C. albicans* was cultivated overnight in liquid media incubated at 37℃. The antifungal activity was investigated propolis (the results were (11-11.1-12.5-14mm) at (20-40-60-80µ/ml) respectively

Therefore, propolis can be a new antifungal therapy for fungal disease in traditional medicine.

CONTENTS

List of figures

Introduction:
Chapter 1

Introduction

1.1Natural product :

is anything that is produced by life, and includes biotic materials (e.g. wood, silk), bio-based materials (e.g. bioplastics, cornstarch), bodily fluids (e.g. milk, plant exudates), and other natural materials that were once found in living organisms (e.g. soil, coal) *natural product* is any organic compound that is synthesized by a living organism. The science of organic chemistry, in fact, has its origins in the study of natural products, and has given rise to the fields of *synthetic organic chemistry* where scientists create organic molecules in the laboratory, and *semi-synthetic organic chemistry* where scientists modify existing natural products to improve or alter their activities.

In fact, natural organic products find their way into almost every facet of our lives, from the clothes on our backs, to plastics and rubber products, health and beauty products. One of most important natural products it is the propolis has been successfully used in the treatment of infectious diseases for centuries.

Plants can be serving as source of bioactive compounds with new chemical entities of wide structural diversity, which can be used: directly as bioactive compounds, as drug precursors, as drug prototypes, as pharmacological tools and as marker compounds for standardization of extracts (Marcy,et al 2005).

Natural products may be extracted from the cells, tissues, and secretions of microorganisms, plants and animals. A crude (unfractionated) extract from any one of these sources will contain a range of structurally diverse and often novel chemical compounds. Chemical diversity in nature is based on biological diversity, so researchers travel around the world obtaining samples to analyze and evaluate in drug discovery screens or bioassays. This effort to search for natural products is known as *bioprospecting*. Soderberg (2016).

Plant Natural Products for Human Health" is intended to be a compilation of scientific reports to cover different aspects of biologically active plant natural products, such as chemical characterization, in vitro and in vivo activities, clinical effects, mechanism of action, structure-activity relationship, and pharmacokinetic/pharmacodynamic properties. With the global trend growing in popularity for botanical dietary supplements and plant-based drugs, it is our hope that this Special Issue would serve as a reference for researchers and scholars who are interested in the discovery of potentially useful molecules from plant sources for health-related applications Soderberg (2016).

1.2Propolis

is a resinous material produced by bees from plant buds and exudates, showing biological activities such as antibacterial. The chemical composition of propolis is very complex and is dependent upon the source plant. The main vegetal source of propolis in, Brazil, The brazilian propolis sample, collected in the Beekeeping Section of the University, was analysed by GC, GC-MS and TLC, revealing that its main components are phenolic compounds (flavonoids, aromatic acids, benzopyranes), di- and triterpenes, essential oils (Siqueira,et al.2015).

Thus, the aim of this study was to evaluate the in vitro fungicidal activities of red propolis alcoholic extract against the *Candida albicans* isolated from cases of periodontitis.

1.3 Natural Product division:

Natural products are often divided into two major classes: primary and secondary metabolites.

1.3.1Primary metabolites:

Are organic molecules that have an intrinsic function that is essential to the survival of the organism that produces them (i.e. the organism would die without these metabolites). Examples of primary metabolites include the core building block molecules (nucleic acids, amino acids, sugars, and fatty acids) required to make the major macromolecules (DNA, RNA, proteins, carbohydrates, and lipids) responsible for sustaining life. Soderberg (2016).

Primary metabolites are components of basic metabolic pathways that are required for life. Primary metabolites include the building blocks required to make the four major

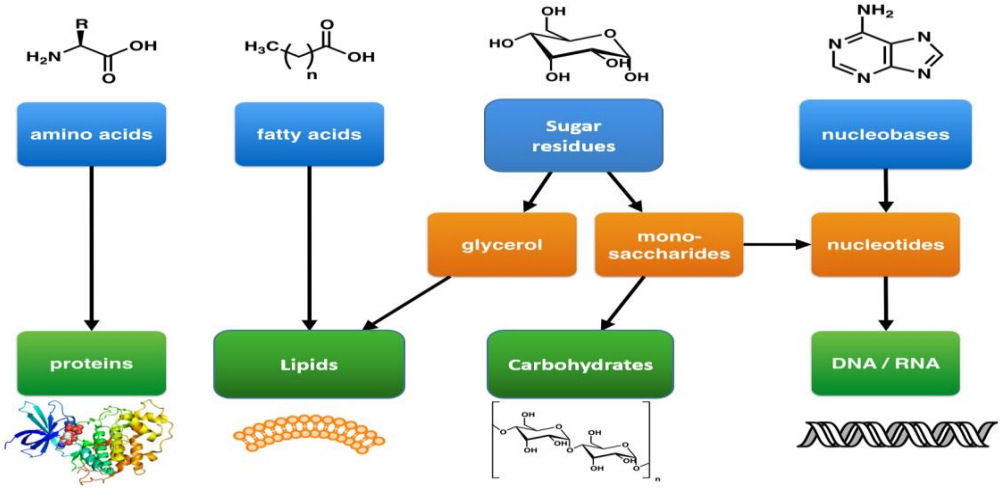

Fig.(1):macromolecules within the body: carbohydrates, lipids, proteins, and nucleic acids (DNA and RNA). Soderberg (2016).

1.3.1 Secondary metabolites:

In contrast are organic molecules that typically have an extrinsic function that mainly affects other organisms outside of the producer.

Natural products, especially within the field of organic chemistry, are often defined as primary and secondary metabolites. A more restrictive definition limiting natural products to secondary metabolites is commonly used within the fields of *medicinal chemistry* and *pharmacognosy*,

Secondary metabolites have a diversity of structures and include examples such as alkaloids, phenylpropanoids, polyketides and terpenoids (plant), *Alkaloids* are secondary metabolites that contain nitrogen as a component of their organic structure and can be divided into many subclasses of compounds. Nicotine, the addictive substance in tobacco is provided as an example alkaloid. The *Phenylpropanoids* are a diverse family of organic compounds that are synthesized from the amino acids phenylalanine and tyrosine (phenylalanine is). Cinnamic acid one of the volatile flavor molecules found in cinnamon is a phenylpropanoid. *Polyketides* are assembled from the building blocks of acetate and malonate to form large, complex structures. Alflatoxin B1, shown below, is a polyketide structure produced by fungi from the *Aspergillus* genus. These types of molds commonly grow of stored food crops, such as corn and peanuts and contaminate them with aflatoxins. Aflatoxins damage DNA molecules and act as a *carcinogen*, or cancer causing agent. Food crops contaminated with aflatoxins have been linked with cases of liver cancer. *Terpenoids* are another large class of natural products that are constructed from 5-carbon monomer units called isoprene . Natural rubber is a good example of a terpenoid-based structure. It is assembled from multiple reapeating isoprene units. As we explore organic structures in more detail in the next few chapters we will continue to evaluate examples from these diverse classes of metabolites and how they impact our lives. Soderberg (2016).

such as diabetes, inflammation, cancer, neurological disease, cardiovascular disease, liver damage, bacterial and fungus infection and malarial. They provide important insights into the current state of research on drug discovery and new techniques in the following areas of plant natural products.

In the diabetes area, Kang et al. reported that the Amadori rearrangement compounds obtained from heat-processed onion extract were able to suppress carbohydrate absorption through inhibition of intestinal sucrose, thereby reducing the postprandial increase of blood glucose.

In cancer studies, the potential of hinokitiol in lung cancer chemoprevention was described by Jayakumar et al,2009. The compound was shown to inhibit the migration of lung adenocarcinoma A549 cells through several pathways, such as activation of capases-3 and -9, induction of p53/Bax and the antioxidant enzymes CAT and SOD, as well as reduction of MMP-2 and -9 activities.

In another study, fucoidan, a sulphated polysaccharides found in seaweeds, was reported by Wang et al. to be able to protect against hepatotoxicity.

To better understand their medicinal properties and to establish stronger evidence of potentials for further development, preclinical and clinical investigations regarding their mechanisms of action, safety and efficacy are warranted. Currently, about 60% of drugs in the market worldwide are natural productderived new drugs against some common respiratory diseases, including asthma, tuberculosis, pneumonia, influenza.

1.4Candida

are almost universal in low numbers on healthy adult skin and *C. albicans* is part of the normal flora of the mucous membranes of the respiratory, gastrointestinal and female genital tracts. The dryness of skin compared to other tissues prevents the growth of the fungus, but damaged skin or skin in intertriginous regions is more amenable to rapid growth (Goehring, 2008).

This propolis variety is obtained from exudates collected by bees *(Apis mellifera)* from the bee hive /College of Science/ Mousel University on January 2023. The antifungal activity of this red propolis proved effective against *C. albicans.* The present study is the first to investigate the antifungal activity of red propolis against *Candida albicans* isolated from cases of periodontitis. The chemical composition of red propolis alcoholic extract indicates a predominance of flavonoids, such as rutin, liquiritigenin, daidzein, pinobanksin, quercetin, luteolin, dalbergin, isoliquiritigenin, pinocembrin, pinobanksin-3-acetate, biochanin A and formononetin, the last being the predominant component (Machado, et, al. 2015).

Thus, the aim of this study was to evaluate the in vitro fungicidal activities of red propolis alcoholic extract against the *Candida albicans* isolated from cases of periodontitis.

Material and Methods

(Chapter 2)

2.1Fungi samples

C. albicans were isolated from lesions and identified by sequencing /Lab. (Microbiology Rzgare Hospital) microdilution assays using commercial antifungal red propolis extracts.The diameter of the inhibition zone for C.albicans growth at four concentrations of redpropolis (20, 40, 60, 80 µg/ml) were measured.

Fig(2):***Candida albicans*** on Sabouraud agar.

2.2Propolis samples

The red propolis was collected the green propolis was collected from the honey bee hives from College of Science/Biology Department /Mousel University. Propolis Ethanolic extract was used in this experiment. The ethanolic propolis extract was obtained using conventional methods ((Park, *et,al.* 2002) briefly, frozen red propolis was crushed and sieved (60 mesh), samples of each propolis was extracted with ethanol (15 mL, 80%) by mixing the samples for 30 min under constant agitation in an incubation shaker (MA 420/MARCONI—Brazil) at 70 °C (Park, *et,al.* 2002).

2.3Evaluation of antifungal activity

The red propolis alcoholic extract, originally from was solubilized in 70% ethyl alcohol (v/v), according to to obtain concentrations from of4 to 2048 μg/mL.

All *Candida* isolates were plated on Sabouraud Dextrose Agar medium contained in test tubes and maintained at 28°C (± 1ºC), for 48 h, to standardize the inoculum.

Red propolis was deposited on separate Sterile petridishs , in rows from 1 to 5, and each concentration was deposited in a row as a disc in different concentration (20-80%) and control.

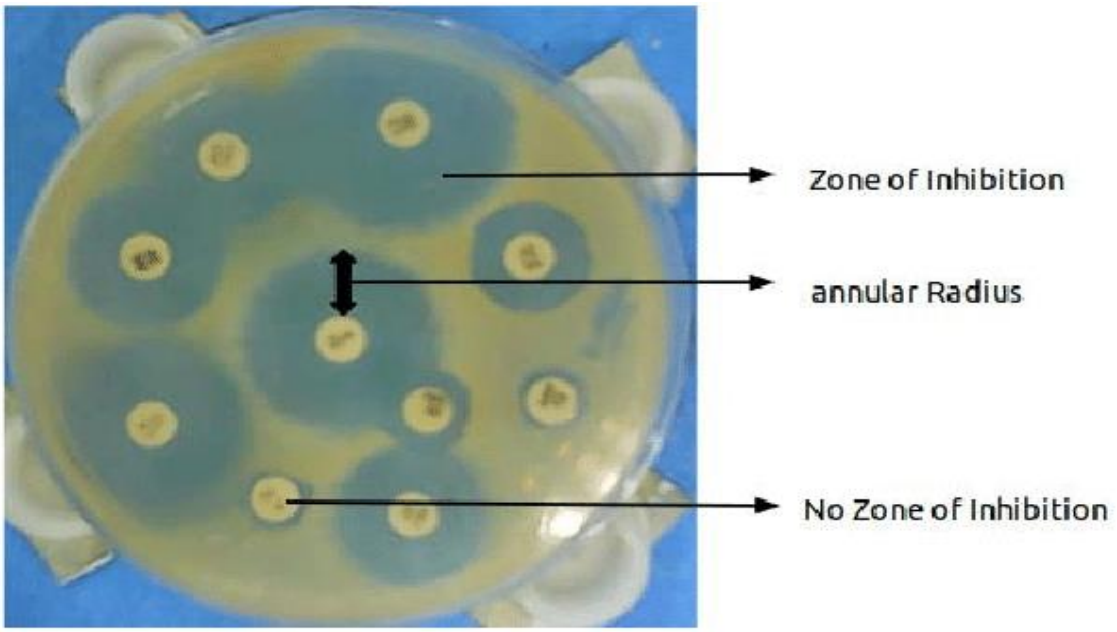

Fig.(3):show how we calculate the inhibition zone

Results
(Chapter 4)

Growth inhibition was observed in *C. albicans* isolates incubated with red propolis extracts. At all concentration of red propolis extracts (20-40-60-80 μg/ml) presented significant antifungal activity. The fungicidal activity of red propolis alcoholic extract was observed for *C. albicans(Fig.4)*..

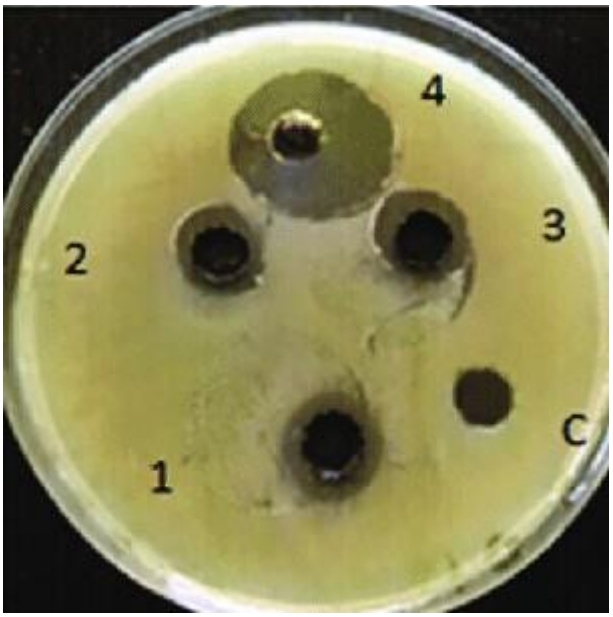

Fig. (4): The diameter of the inhibition zone for *C.albicans* growth at four concentrations of redpropolis (20, 40, 60, 80 μg/ml).

We could verify that *C. albicans* is susceptible to propolis With regards to ethanol 70% effects, used as a solvent for propolis in this assay; its inhibitory action was seen in all concentration v/v. the results were (11-11.1-12.5-14mm) at (20-40-60-80μ/ml) respectively

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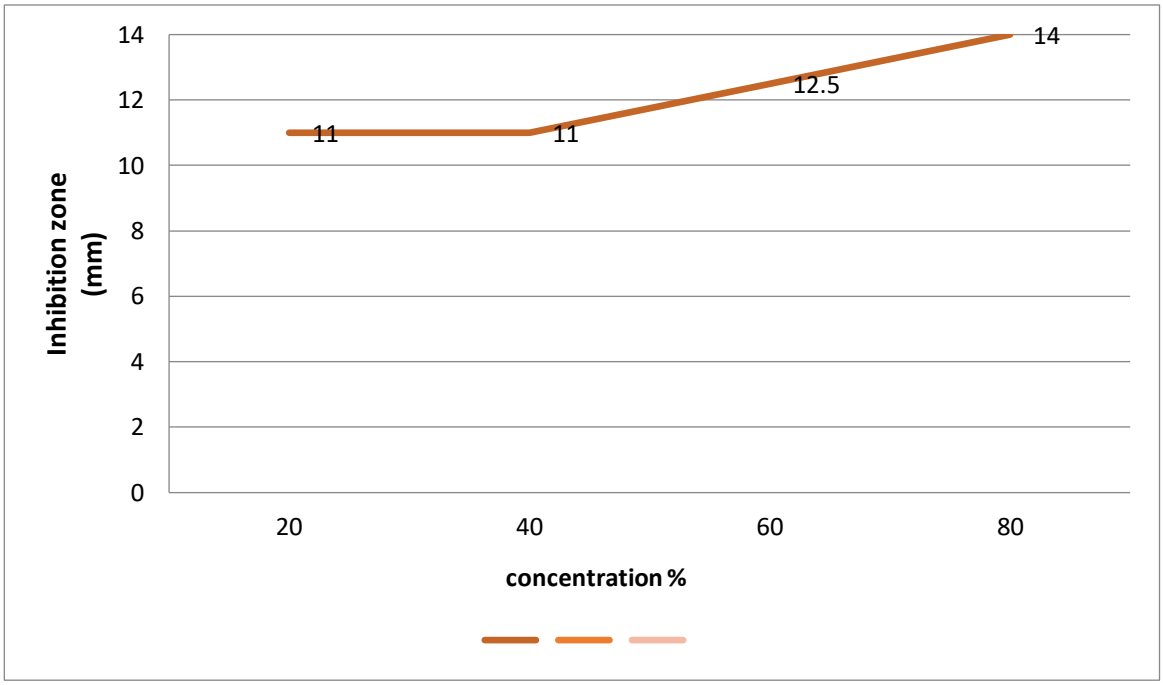

Figure (5) shows the diameter of the inhibition zones for *C.albicans* growth.

Discusion
(Chapter5)

To evaluation of the Brazilian green and red propolis extracts dose-response curves indicated that both red propolis extracts are potent agents against *Candida* spp. when compared to the green propolis extracts. Antimicrobial activity of the propolis extracts is attributed to the phenolic and favonoid content. The high formononetin content of the red propolis can be associated to the best Candida growth inhibition by red propolis when compared to green propolis, and this situation can be supported by the results presented by the treatment with the ultrasoundtreated extracts. Regarding total phenolic compounds and favonoids, both green and red propolis ethanolic extracts demonstrated to have high contents of these compounds. (Machado, et.al. 2016).

Our study accepted with (Hyeon,et.al. 2014)who reported the antibacterial activity of C.albicans of propolis (5, 10µL/ml) was evaluated in solid and liquid culture with zones of inhibition of 15, 14, 16and 17respectively. Also our study accepted with (Anne,et.al. 2015)

Who showed greater antifungal activity for C. tropicalis, with an average of 12 mm of inhibition zone diameter and especially the Palmas-CPI propolis (16 mm). It showed a slightly lower activity for C. albicans, average diameter of 11 mm, with the best result corresponding to propolis from Nova Olinda-CPI (15mm).

Conclusion

This antifungal activity may hold a promise for future applications as an alternative treatment for infections caused by these fungi. Further investigation into the use of red propolis for the prevention and treatment of periodontal diseases is required, including microbiological, randomized controlled trials and longitudinal studies.

Refrences

Refrences

1- Anne Milane Formiga Bezerra1, Maria do Carmo Andrade Duarte de Farias2, Kévia Katiúcia Santos Bezerra2, Francisco Geyson Fontenele Albuquerque3, Antônio Fernandes Filho2, Geofabio Sucupira Casimiro2, Elicarlos Marques Nunes4, Edivânia Maria Leite da Silva5, Wilma Kátia Trigueiro Bezerra1, Palloma Batista de Almeida1, Tereza Lourença Matias de Araújo1, Kennia Sibelly Marques de Abrantes2,6, Luiz Carlos de Abreu6, Patrício Borges Maracajá7, Alfredina dos Santos Araújo7, Rosilene Agra da Silva7.(2015). Red Propolis Antifungal Action on Species of Candida of the Oral Cavity. International Archives of Medicine Section: Microbiology ISSN: 1755-7682.

2- ANVISA - Agência Nacional de Vigilância Sanitária. Detecção e Identificação dos Fungos de Importância Médica 2004. https://www.anvisa.gov.br/servicosaude/microbiologia/mod_7_2004.pdf. Accessed 7 Aug 2021.

3- Gleiznys A, Zdanavičienė E, Žilinskas J, Eglė DDS. Juozas Žilinskas DDS (2015). Candida albicans importance to denture wearers. A literature review. Balt Dent Maxillofac J.;17:54–66. –

4- Goehring, Richard V. (2008). *Mims' medical microbiology* (4th Ed.). Philadelphia, PA: Mosby Elsevier. p.656.

5- G. Yang, Q. Jin, C. Xu, S. Fan, C. Wang, P. Xie Synthesis, characterization and antifungal activity of coumarin-functionalized chitosan derivatives Int. J. Biol. Macromol., 106 (2018), pp. 179-184, 10.1016/j.ijbiomac.2017.08.009

6- Hyeon-Sook Kwun1 , Seoul-Hee Nam1 , Min-Kyoung Park2 Mi SooK Cho3 , Sae-Hee Cheon(2014). Antimicrobial Activities of Propolis against Oral Candidiasis by Candida Albicans -Effect of Microbial Inhibition Using Propolis. Journal of the Korea Academia-Industrial cooperation Society Vol. 15, No. 9 pp. 5644-5651,

7- Machado BAS, Silva RPD, Barreto G de A, Costa SS, Silva DF da, Brandão HN, Rocha JLC, DEllagostin OA, Henriques JAP, Umsza-Guez MA, Padilha FF.(Chemical composition and biological activity of extracts obtained by supercritical extraction and Ethanolic extraction of Brown, green and red Propolis derived from diferent geographic regions in Brazi)l. PLoS One. 2016;11:e0145954.

8- Machado BAS, Barreto G de A, Costa AS, Costa SS, Silva RPD, da Silva DF, Brandão HN, Rocha JLC, Nunes SB, Umsza-Guez MA, Padilha FF (2015) Determination of parameters for the supercritical extraction of antioxidant compounds from green Propolis using carbon dioxide and ethanol as co-solvent. PLoS One 10:e0134489. https://doi.org/10.1371/journal.pone.0134489.

9- Ohl, M.E.; Miller, S.I.(2001). Salmonella: a model for bacterial pathogenisis. Ann. Rev. Med., 52, 259-274, 2001.

10- Pazin WM, Mônaco L da M, Egea Soares AE, Miguel FG, Berretta AA, Ito AS.(2017). Antioxidant activities of three stingless bee propolis and green propolis types. J Apic Res. ; 56:40–9.

11- Park YK, Alencar SM, Aguiar CL.(2002). Botanical origin and chemical composition of Brazilian Propolis. J Agric Food Chem. 2002;50:2502–6. https://doi.org/10.1021/jf011432b.

12- Regezi JA. Ciubba, James J. Jordan RCK(2017). Patologia Oral correlações clinicopatológicas. 7 th. Rio de Janeiro: Guanabara Koogan.

13- Siqueira ABS, Rodriguez LRN de A, Santos RKB, Marinho RRB, Abreu S, Peixoto RF, Gurgel BCV(2015). Antifungal activity of propolis against Candida species isolated from cases of chronic periodontitis. Braz Oral Res;29: 1–6. 10.1590/1807-

14- Soderberg,T (2016).(Organic chemistry with Biological Emphasis.Published under Creative Commons by-nc-sa 3.0.