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Review

Biological activity of propolis: an update

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Abstract: Antibiotic resistance was described soon discovery of penicillin by Fleming. In recent years, there has been increasing research interest in the development of alternatives to address this problem. Natural products, mainly, have long been considered as sources of drugs, and a great part of pharmaceuticals available in modern medicine are directly or indirectly derived from natural sources.

propolis been used to treat human diseases since ancient times, propolis, is a natural resinous mixture produced by honeybees (*Apis mellifera*) from collected parts of plants, he is increasingly recognized by their wide range of biological and pharmacological properties, As anti- infective agent, combination synergy with standard antibiotics could be very promising alternative strategy.

Keywords: bee propolis; biological activity; antibacterial

1. Introduction

Antimicrobial resistance has been a growing concern worldwide [1]. It has been defined as a global pandemic for the twenty-first century in [2], Recently, World Health Organization (WHO) has released a list (*12 bacteria names from bacterial families*) of the drug-resistant bacteria that pose a big sanitary challenges to human health [4,5], such problem is due to the lack of effective surveillance measures and widespread overuse,

In the 1990s a sharp reduction in development of new drug classes coupled with emergence of strains of human pathogens resistant with a great concern are multi-drug-resistant strains., now antimibrobial resistance causes 700,000 or more deaths each year which number could grow to 10 million by 2050 [4,6],with a loss of up to USD100 trillion to the global economy[7].

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In the USA, the prevalence of antimicrobial resistance has increased markedly in recent years, For example, methicillin-resistant *Staphylococcus aureus* (MRSA), In 1999, MRSA accounted for more than 50% of *S. aureus* isolates from patients in Intensive Care Units (ICUs) in the National Nosocomial Infection Surveillance (NNIS) system; in 2003, 59.5% of *S. aureus* isolates in NNIS ICUs were MRSA. Moreover, Antibiotic resistant *Mycobacterium tuberculosis* strains was reported, WHO estimates that, in 2018, there were about half a million new cases of rifampicin-resistant tuberculosis (TB) identified globally, of which the vast majority have multi-drug resistant TB (MDR-TB), a form of tuberculosis that is resistant to the two most powerful anti-TB drugs(https://www.who. int/news-room/fact-sheets/detail /anti microbial resistance).

A similar increase in prevalence has occurred in vancomycin-resistant enterococci (VRE). VRE causes nosocomial infections, and can also be transmitted from animals through ingestion or direct contact[3]. Emergence of antimicrobial resistance bacteria has human health consequences. Such consequences include increased frequency of treatment failures and increased severity of infection. The latter includes prolonged duration of illness, increased frequency of bloodstream infections, increased hospitalisation and increased mortality [8]..

It is clear that the need for new antibiotics is greater than ever before, because of the emergence of multi-drug-resistant and pan-resistant pathogenic microorganisms, paradoxically, despite the clinical need for new antimicrobial agents, the development of these agents is declining. Since the 1970s, few new antibiotics have been discovered, and [3,6]. It has been reported that, costs for pharmaceutical research and development, are estimated about $800 to $900 million and 10 to 15 years per approved agent (Monnet, 2005) in [3]. This may explain why the pharmaceutical industry currently concentrates on the development of analogs. However, the numbers of analogues which can be made from a single chemical core are limited and bacteria can develop resistance to new analogs and consequently more loss of antibiotic class efficacy [9]. In other hand, overuse, inappropriate use of antimicrobial or other factor like certain environmental conditions (for example; body compartments where antibiotic has a difficult access) can exacerbate resistance problem [10].

More recently, concerns have arisen over the extensive nontherapeutic antimicrobial (NTA) use. In the United States it was estimated to be equal to (WHO,1997) or eight times greater than the quantity administered for therapeutic use. NTA uses are also linked to the propagation of multidrug resistance (MDR), including resistance against drugs that were never used on the farm [11].

In other hand, It has been demostrated that bactericidal antibiotics induce the formation of toxic reactive oxygen species (ROS) both in bacteria and mammalian cells. antibiotics such as, quinolones, aminoglycosides, and β-lactams cause mitochondrial dysfunction and ROS overproduction in cells thus, can lead to oxidative tissue damage [12].Such imbalance increases the

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damage to the biomolecules, such as proteins, lipids, DNA, and sugars [12, 13, 14].

Finally, antimicrobial resistance represent multifactorial problems that must be addressed from different disciplines, including Medicine, Genetics, Microbiology, Epidemiology and Sociology [10].

In recent years, there has been increasing research interest in the development of alternatives to address this problem. Propolis or bee glue, as it is commonly named, is a natural resinous mixture produced by honeybees (*Apis mellifera*) from substances collected from parts of plants, buds and exudates [17].This resin is masticated, salivary enzymes are added, and then it is mixed with beeswax and probably with other compounds of bee metabolism [18]. Once collected by honey bees, this material is modified and mixed with wax and enzymatic secretions. The resulting substance is used by bees to seal holes in their hives and strengthen the thin borders of the comb. Propolis in the hive also acts as a biocide, and may have activity against invasive bacteria, fungi and even invading larvae [19,20, 21]. Propolis is increasingly recognized by their wide range of biological and pharmacological properties, with many useful application in medicines and cosmetics , food industries, packaging and animal husbandry[22],The activity of propolis, including antibacterial [17,23,24, 25 ,26,27,28], Antiviral [29], Antifungal [30], antioxidant [31, 32], immunostimulatory, anti-inflammatory [28, 33] and cytostatic effects [18].

2. Propolis :

The word "propolis" derives from Hellenistic Ancient Greek (suburb, bee glue) and describes the role of propolis to cement openings of the bee hive. Propolis is also «pro» = in front, «polis» = city, which means hive defensive substance [34] The meaning in front of the city suits well the protecting role of propolis for the bee colony [35,36]. Propolis has been used by humans as a traditional in folk medicine to maintain good health since ancient times by many civilizations; Egyptians, Arabs, Greek, Roman and many other civilizations [37]. Egyptians knew very well its anti-putrefactive properties and used bee glue to embalm their cadavers. Incas employed propolis as an anti-pyretic agent. Greek and Roman physicians used it as mouth disinfectant and as an antiseptic and healing product in wound treatment, skin abscesses, prescribed for topical therapy of cutaneous and mucosal wounds [38]. These therapeutic applications were perpetuated in the Middle Age and among Arab physicians. They described medicinal preparations containing propolis which was used for treatment of dental caries, oral and pharyngeal infections [36].

In the end of 19th century, propolis was widely used due to its healing properties, in the Second Global War it was employed in several Soviet clinics for tuberculosis treatment, due to the observed decline of lung problems and appetite recovery, it has been called "Russian penicillin" [39]. In the Balkan states it was one of the most frequently used remedies, applied to treat wounds and burns, sore throat and stomach ulcer [40].

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2.1. Plant Sources of propolis

Propolis is an important product of the hive, collected by the bees from living plants, particularly from flowers and leaf buds.which is mixed with various amounts of beeswax and resins, used as building and protective material against insects and pathogenic organisms [21].

according to the botanical source, its color varies from yellow-green, to red and to dark brown depending on its source and age. But, even transparent propolis has been reported by [19, 20].

Many studies on the properties and composition of propolis have been made without knowing the plant(s) from which the sample was obtained, or the sites where bees collected the material. However, phytochemical composition is complex and highly variable, depending on the season and available flora at the site of collection [41,42].

The source species can vary with geographical regions and chemical compositions of propolis, as shown in (**Figure.1)** different types of propolis are available worldwide **[**38**,**43,44,45]:

i) European propolis (Poplar type (*Populus spp*. which is originate mainly from Europe, non-tropic regions of Asia, New Zealand and North America), ii) Birch type or Russian propolis (*Betula verrucosa* which is derived from Russia), iii) Green type or green Brazilian propolis (South-eastern and western-central Brazil; derived from *Baccharis spp*.) iv) Red type or red Brazilian propolis (derived from *Dalbergia spp*. which is located in north-eastern part of Brazil,, Mexico and Cuba), v) Clusia type (from Clusia spp. From Cuba and Venezuela) and vi) Pacific type (*Macaranga tanarius* which is originate from Indonesia, Taiwan and Okinawa Prefecture) and vii) the most recent, Mediterranean type (plants mainly from *Cupressaceae* family which is located in Greece, Sicily and Malta. "Canarian" propolis (plant origin unknown), etc..

Many other works, reported that in temperate zones the bud exudates of *Populus* species and their hybrids are the main source of bee glue. In China, besides the main source poplar **[**46]: bees also use pines, cypress, willow and *sumacs* [34,47,48]. In Turkey, poplar seems to be the main origin, however other plant sources of bee glue is collected from pine trees, *eucalyptus* and *castanea* [49, 50,51,52, 53,54].

In tropical regions, other plant source of bee glue is collected. The most popular propolis type, the green Brazilian propolis(GBP), originates from the leaves of wild rosemary; *Baccharis dracunculifolia* (Asteraceae) [55, 56,57].

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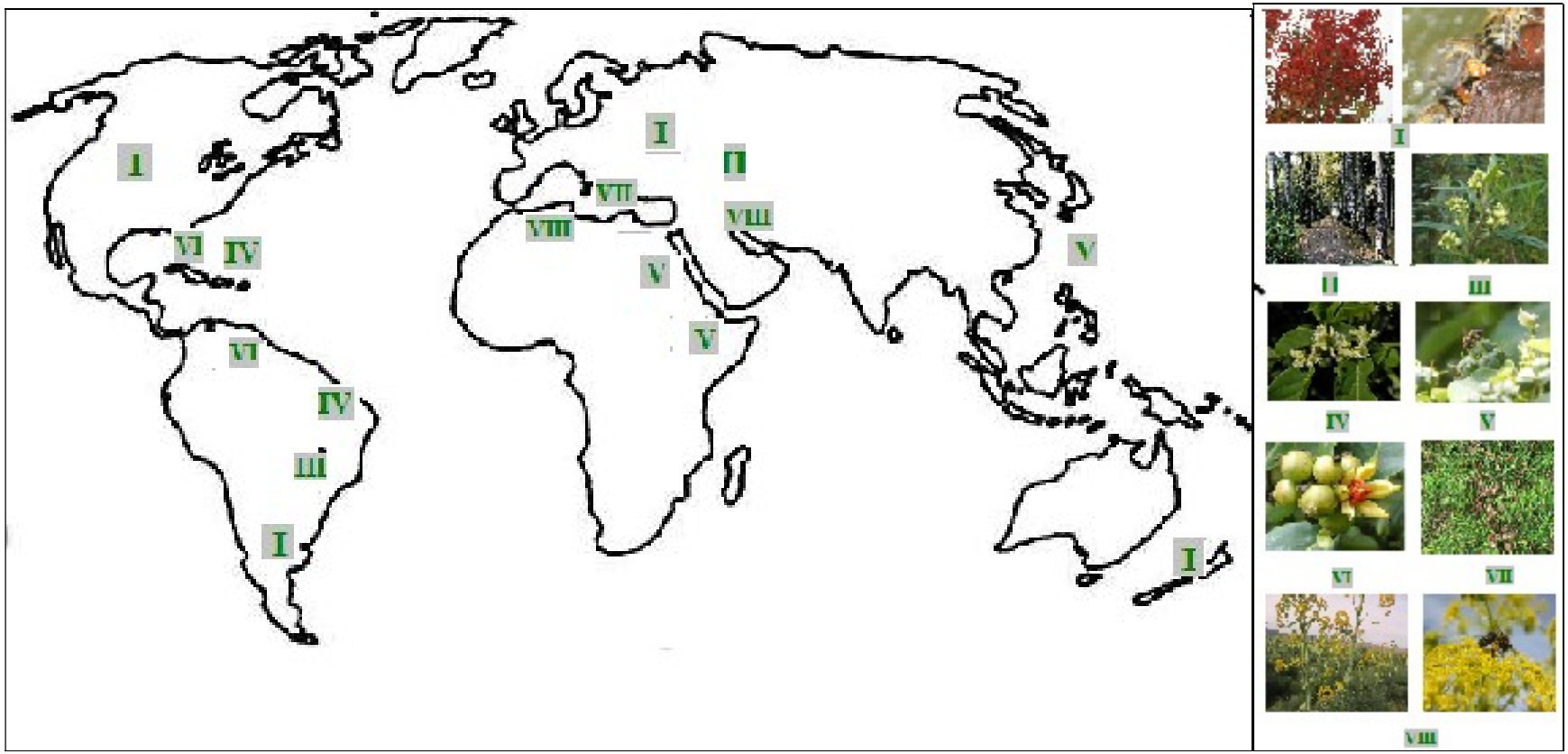

Figure. 1. Distribution of some propolis types.**I-** temperate poplar propolis,:http://beespoke.info/2015/10/20/bee-trees-poplar-populus-spp/.**II-** Birch type or Russian propolis.https://fr.wikipedia.or g/wiki /Bouleau verruqueux #/media/Fichier:Birch_walk_Weilerbach_Luxembg_01.jpg **III**-Brazilian green propolis,: derived from the buds of *Baccharis dracunculifolia* DC . **IV-** Red type or red Brazilian propolis(derived from *Dalbergia spp*. https://eol.org/pages/639535. **V**- Pacific type (derived from *Macaranga tanarius* [58]. **VI-**Clusia type (from *Clusia spp*.https://www.pronativascr.org/plantas/clusia-spp/) **VII-**Mediterranean type (plants from *Cupressaceae* family. https://fr.wikipedia.org/wiki/Cupressaceae). .**VIII-**Iranian propolis, Algerian propolis (derived from *Ferula spp* https:// fr. wikipedia.org wiki/Fichier: erula_communis03.jpg).

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Other type of propolis found Brazilian, Cuban and Mexican regions called red propolis, which bees collect vegetable material (**Figure. 2)** (red resinous exudates from plants; *Dalbergia* species (Fabaceae)) (**Figure. 4)** [57,59, 60].
In tropical islands in the Pacific Ocean (Taiwan, Okinawa, Indonesia), there is a specific propolis type, designated sometimes as "Pacific propolis". It contains prenylated flavanones (propolins) as major constituents [58,61,62,63] and its plant source is the resin on the fruits of the tropical tree *Macaranga tanarius*, (as shown in **Figure. 3)**[58]. Jeju Island, Korea (*Angelica keiskei Ito*) and Kangaroo Island, Australia (*Lepidosperma spp*., *Acacia paradoxa* D.C. and *Myoporum insulare* R. Br(family of *Scrophulariaceae Juss*) (**Figure. 5**)[46,64].Another tropical propolis type is the one originating from resin exuded by the flowers of different *Clusia* species found in Cuba and Venezuela [65,66,67]

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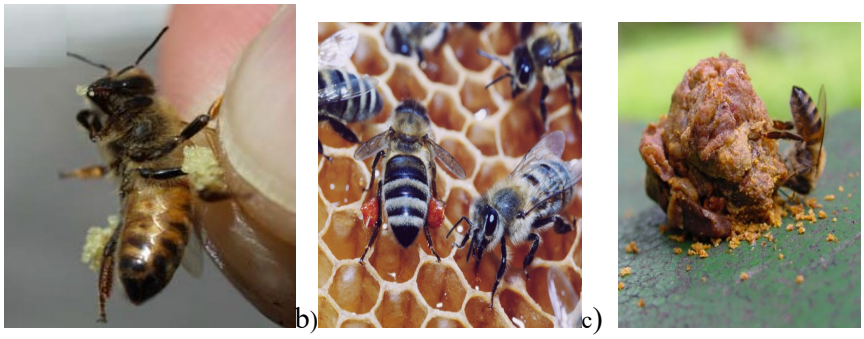

Figure 2. propolis collection by honeybees (role in beehive) and aspect of propolis after ollection by beekeeper.(a) honeybee with a piece of propolis on its leg. (b) propolis collector bee with propolis load on the corbicula ;(c) Propolis after collection from the hive by beekeeper [58,68].

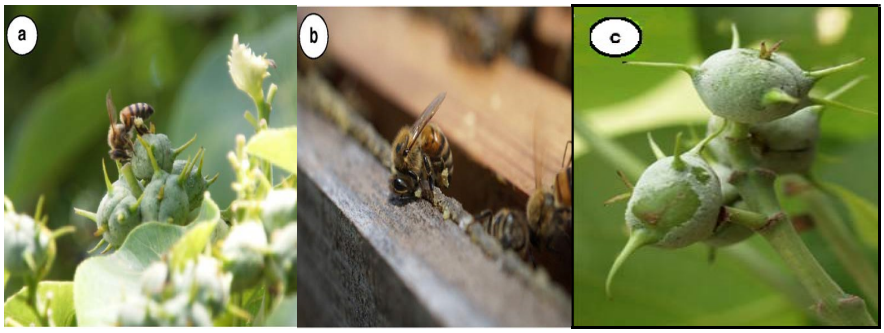

Figure 3. Photographs of a honeybee collecting the surface materials of M. tanarius fruit to bring them back to its nest as propolis. a) a honeybee collecting the surface material of M. tanarius fruit; b) a honeybee attaching the propolis to the nest; c) surface material of M. tanarius fruit remaining after collection by honeybees [58].

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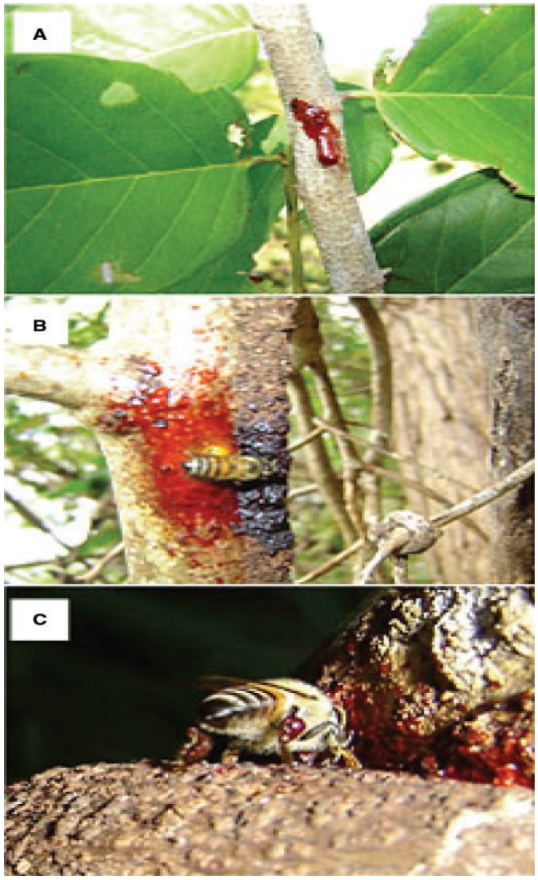

Figure 4.. Collection of propolis from reddish resinous exudates of D. ecastophyllum by africanized Apis mellifera. (A) Secrete reddish exudates from a hole in a branch of the tree. (B) Bee is collecting the reddish exudates. (C) The collected exudates passed to the hind leg to make propolis [59].

Figure. 5. (d) Propolis collection by bees from resinous leaves of Myoporum insulare; (e) Laboratory analysis of the bee legs with propolis collected from Myoporum insulare leaves surface [64]

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Table .1. propolis types: Geographic origin and plant source.

Propolis type	Geographic origin	Plant source	References
Poplar	Europe, North America, non-tropic regions of Asia, China, New Zealand	*Populus spp.*	[21,34,47,48 ,49,50,51,52, 53,54] 69 ,70,71]
Green	Brazil	*Baccharis* spp. (*B. dracunculifolia)*	[55, 56,57, 64,72]
		Hyptis divaricate	[73]
		Dalbergia ecastaphyllum	[55,72,74]
Red propolis	Cuba, Brazil, Mexico	*Dalbergia spp.*	[57,59, 60].
Mediterranean	Sicily, Greece, Crete, Gozo, Malta,Croatia Algeria	Cupressaceae *Populus* spp. and *Ferula* spp.	[75, 76,77,78]
		Populus spp.	[79]
		Cistus spp.	[80]
Clusia	Cuba, Venezuela	*Clusia spp.*	[19,65,66,67 ,81].
Pacific	Pacific region,Okinawa, Taiwan, Indonesia)	*Macaranga tanarius*	[58,61,62,63]
		Angelica keskei (Miq.) Koidz	
	Korea, Jeju Island	*Plumeria acuminata*	[82]
	Hawaii,	*W. T. Aiton , P. acutifolia* Poir	[83]
Australia Kangaroo Island		*Xanthorrhoea Lepidosperma spp.*	[17] [46,64].
"Canarian" Propolis	Canary Islands	Unknown	[23, 84]
"Chilean" Propolis	Chile	*Quillaja saponaria, Salix humboldtiana, S babylonica, Eucalyptus globules*	[85,86]
Birch propolis	Russia	*Betula verrucosa* Ehrh	[20,87]

One of the most important Subtropical propolis types turned out to be the so called Mediterranean propolis, which is characterized by the high concentration of diterpenics [75,76,77,78,79]. Its source belongs to the *Cupressaceae* family (*Cupressus sepmervirens*). In addition propolis from the Mediterranean region contains flavonoids, phenolic acid and its esters [88] .

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In Tunisia, where poplars are not always available, leaf exudates of some *Cistus* spp. act as propolis source [89], while in the Sonoran desert *Ambrosia deltoidea* and *Encelia farinosa* played this role [90,91]. In Algeria [92]., Iran, *Ferula spp, Juniperus polycarpus* [93], have been found to contribute to propolis as plant source, next to *Populus* spp [92,94], Thus support idea of direct impact of vegetation and environment on the compositions and biological activity of propolis[93].

Othewise, The composition of propolis differs greatly depending on its botanical origin.The measured compounds should be important from biological points of view [46].

2.2. Biological activities of propolis

The biological activities of propolis have been confirmed by numerous scientific studies, as shown in **Table 3**. Antimicrobial properties of all types of propolis are well documented by many researchers [95].against bacteria [18,19,96], fungus [97,98,99], virus [23,100,101] and parasites [102].

2.1.1 Antibacterial activity

The most popular, well studied and documented activity of propolis is the antibacterial one [45,81,101,103, 104]. As shown in Table N *mechanism of anti-bacterial activity of some active compound from propolis.*

It was evidenced that the mechanism of action of propolis on bacterial cell is complex and a simple analogy cannot be made to the mode of action of any classic antibiotics. Direct antibacterial activity of propolis was attributed to differents mechanisms; Inhibition of cytoplasmic membrane function, disruption of membrane potential and energy production, inhibition of protein synthesis, inhibition of nucleic acid synthesis [19,105,106,107,108].

Propolis affects the permeability and structure of the cellular membrane of microorganism, thus mechanism is due to electrostatic-based intermolecular interaction positively charged groups of propolis compounds (positively charged groups:cationic moieties) and the bacterial surface (negatively charged: anionic sites). Thus causing damage of the cell walls and bacteria form [107], alteration of the physico-chemical properties of surface layer cells [108], disruption of membrane potential and bioenergetic status (inhibition of adenosine triphosphate (ATP) production).

In addition, propolis extract inhibit biofilm formation [109]), and posses anti-quorum sensing activity [110], Recently, Wang and co workers [111] demonstrated that Australian propolis ethanol extract (APEE) possesse anti-planktonic, anti-biofilm and anti-adhesive activities against MRSA strains.

Otherwise, Vadillo-Rodríguez and collaborators 2021 suggested that initial mechanism of action of propolis resulting from interaction between different propolis components and bacterial cell wall structures. Presence of phenolic compounds and flavonoides are recognized for antimicrobial activity of propolis [112], Furthermore, phenylates flavonoids, is responsible for cell membrane damage, caused by lipophilic prenyl group. Synergistic activity between phenolic and other compounds was reported previously, by Castaldo [113] and recently, by Grecka [114]. Substances identified in propolis, such as pinocembrin, galangin, caffeic acid, ferulic acid, pinobanksin, benzyl ester, may act on the microbial membrane or cell wall site, causing functional and structural damages [19,105], constituents of propolis can interact with bacterial stucture by forming intramolecular hydrogen bonds that create

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hydrophobic interactions between the cell wall or membrane and propolis antimicrobial compounds [108].

It was reported by de Oliveira Dembogurski *et al*. 2018, that Artepillin C (3,5-diprenyl-*p*-coumaric acid, a prenylated derivative of *p*coumaric acid (*p*CA)),obtained from Brazilian green propolis posses a strong antimicrobial activity against planktonic and biofilm cells. Also, Artepillin C in synergy with other *p*CA derivatives is responsible for the antimicrobial effects [115]. Moreover, CAPE, an active component of propolis, is a competitive inhibitor of peptide deformylase, essential enzyme of *H. pylori.* CAPE can block interaction of substrate–active site with enzyme [116].

Table 2. mechanism of anti-bacterial activity *of some* active compound from propolis. Antibacterial potentials of propolis

Active Compound	Mechanism of action	References
pinocembrin, galangin, caffeic acid, ferulic acid, pinobanksin, benzyl ester	Functional and structural damages of cytoplasmic membrane or cell wall bacteria	[19,105].
flavonone pinocembrin flavonol galangin, caffeic acid phenethyl ester,	Inhibition of bacterial RNA polymerase	[18]
Flavonoids	Formation of strong ligand complex with heavy atoms of bacteria's mettaloenzymes; metabolic perturbation in ion channels and phosphorylation/dephosphorylation reactions. loss	[105,117]
Cinnamic acid and its derivatives	Damaging the cell membrane, inhibiting ATPases, cell division and biofilm formation. anti-quorum sensing activity .	[110].
Quercetin, naringenin.	Increase the permeability of inner bacterial membrane; inhibition of potential, decreasing the ATP production, membrane transport and mobility of bacteria	[28].
Apigenin,	Prevention of formation of dental plaque. inhibition of GTF's (glucosyltransferase) (enzymes considered as an important factor in the formation of dental plaque)	[118].
flavanone pinocembrin (5,7-dihydroxyflavanone) and its 3-OH analogue flavonol galangin (3,5,7-trihydroxyflavon) Caffeic acid (3,4-dihydroxycinnamic acid) and its esters, volatile fractions with phenols	Degradation of cytoplasm membrane of bacteria; loss of potassium ions from cell membrane and autolysis bacterial cell.	[18,119,120121,122]

and/or terpenoids and chrysin (5,7-dihydroxyflavone)		
ethanolic propolis extract i	Inhibition of protein synthesis	[123].
Polyphenols of propolis	Formation of hydrogen and ionic bonds in microbial proteins; Alteration of function and structure of proteins.	[106].
phenylates flavonoids	lipophilic prenyl group cause cell membrane damage	[124].
Australian propolis ethanol extract (APEE)	Disrupting cell structure; Inhibitory activity against planktonic bacterial cells and biofilm.	[125].
Cinnamoyloxy-mammeisin	Inhibition of bacteria adherence (MRSA) to host cells , disruption of biofilm development	[126].
Flavonoides	Modification of physical properties of membrane bilayers (*by changing phospholipids' gel-to-liquid crystal transition temperature*).	[127].
Galangin	Potassium loss from bacterial cells wall (*damage of cytoplasmic membrane of bacteria*)	[128].
flavone-based analogues	Inhibition of activity of DNA gyrase of *E.coli* (*by intercalating into DNA*).	[129].
guttiferone E, xanthochymol and oblongifolin B from ethanolic extracts of red (ERP) propolis,	antimicrobial activity against multidrug-resistant bacteria (MDRB) inhibited the biofilm formation of *S. aureus S. epidermidis*	[130].

Wink and co-workers 2008, 2015, demonstrated that polyphenols of propolis alter function and structure (three-dimensional structure 3D) of bacteria proteins. Such mechanism is due to formation of hydrogen and ionic bonds in microbial proteins by their interaction with polyphenols [106].

Flavonoids can form strong ligand complex with heavy atoms of bacteria's mettaloenzymes , such interaction probably are responsible of impairment in phosphorylation /dephosphorylation reactions that can lead to metabolic perturbation in ion channels and consequently, loss of viability of bacteria cells (bactericidial effect), [105,117]. It has been reported that propolis exhibits bacteriostatic activity against different bacterial strains and can be bactericidal in a high concentration [131],Vadillo-Rodríguez, [108] found that sub-bactericidal concentrations of propolis disturb and damge wall membrane and biofilm. Santos [132] , demonstrated that propolis extracts were effective in interfering with the formation of the *C. pseudotuberculosis* biofilm. Propolis also can affects cell division [27].

Tsuchiya and Iinuma, 2000, reported that the antimicrobial activity is potentially due to rutin, quercetin, naringenin. These compounds increase the permeability of the inner bacterial membrane and potential, decrease ATP production, membrane transport and mobility [28, 105]. Moreover, flavonoides modifiy physical properties of membrane bilayers by changing the phospholipids' gel-to-liquid crystal transition temperature to a more rigid, gel-like state [127]. In other hand,, galangin increase potassium loss

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rom bacterial cells wall, such mechanism is due to a direct damage to the cytoplasmic membrane of *S. aureus* [128].

Kim and Chung[28], reported that electron microscopic investigation of propolis- treated *Bacillus cereus* caused a structural damage by disintegration of the cell envelope, leakage of cytoplasmic and nuclear material from the cells. Other mechanism was described by Mirzoeva [105], EEP and some of its flavonoid components had negative effect on *B. subtilis* motility by inhibition of the membrane potential that can increased permeability of the membrane to ions.

It has been observed that the global synthesis of DNA and RNA cells was inhibited after addition of genistein. In a Study conducted by Verghese and co-workers [129],, demonstrated that, flavone-based analogues, inhibit activity of DNA gyrase of *E.coli* through intercalating into DNA. Flavonone pinocembrin and the flavonol galangin, and caffeic acid phenethyl ester, whose action mechanism is based on the inhibition of bacterial RNA polymerase. The inhibition of bacterial RNA-polymerase by the components of propolis was probably due to the loss of their ability to bind to DNA [133].

Lin *et al.,* [134]., investigated the effects of the flavonols myricetin, quercetin, kaempferol, and galangin on the inhibition of the binding activity of SSB (*Single-stranded DNA* (ssDNA)-*binding protein*) an important protein implicated in DNA replication, repair and recombination. They found that myricetin, was capable of inhibiting *P. aeruginosa* (PaSSB) activity. In addition, presence of aromatic acids and esters in propolis interferes with the division of *Streptococcus agalactie* through the formation of pseudo-multicellular forms, cytoplasm disorganization, protein synthesis inhibition and cell lysis Takaisi-Kikuni and Schilcher [123] [110]. In recent study conducted by de Souza [130],guttiferone E, xanthochymol and oblongifolin B obtained from ethanolic extracts of red (ERP) propolis, possess antimicrobial activity against multidrug-resistant bacteria (MDRB) They also inhibited the biofilm formation of *S. aureus* (ATCC 43300 and clinical isolate) and *S. epidermidis* (ATCC 14990 and clinical isolate) [130].

All investigation demonstrated that Gram positive microorganisms were sensitive to propolis, whereas, Gram negative ones were often resistant [19], Low sensitivity of Gram negative bacteria is that their outer membrane structure inhibits and/or retards the penetration of propolis. Also, propolis components could be destroyed in the bacterial suspension probably by hydrolytic enzymes released by the bacteria [135].

The antibacterial activities **(Table 3.)** of propolis were observed on aerobic and anaerobic Gram-positive (*Staphylococcus aureus, S. aureus* ATCC 25293, ATCC 29213,43300 [86,136,137,138],*Bacillus cereus,* Vancomycin resistant *Enterococcus* [137],*Enterococcus faecalis, Micrococcus luteus, Nocardia asteroides, Staphylococcus epidermidis, S. epidermidis* ATCC 14990 [138].*Staphylococcus haemolyticus, Streptococcus faecalis, Streptococcus pneumioniae, Streptococcus pyogenes*[139,140],*Streptococcus haemolyticus, Streptococcus mutans, Actinomyces naeslundii, Lactobacillus acidophilus, and Peptostreptococcus micros*) and Gram-negative (*Aeromonas hydrophila, Brucella abortus, , Klebsiella pneumoniae, Pseudomonas aeruginosa, P. aeruginosa* ATCC 27853, *Escherichia*

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coli, E.coli ATCC 35218[86,140,141] *Neisseria gonorrhoeae* [142], *Enterobacter cloacae .Salmonella enteritidis*[143], *Salmonella typhi, Salmonella Typhimurium, Proteus mirabilis, Proteus vulgaris, Shigella dysenteriae, Porphyromonas gingivalis, Fusobacterium nucleatum, and Prevotella oralis*), *Lactobacillus acidophilus, Prevotella oralis, P. gingivalis, Fusobacterium nucleatum, Peptostreptococcus anaerobius, Actinomyces naeslundii* and *Veillonella parvula* [144]. *Porphyromona,Fusobacterium, Propionibacterium, Clostridium, Prevotella, Actinomyces* and *Bacteroides* species [144,145,146].

Koo *et al.* in Brazil, found the antibacterial effect of propolis on *Streptococcus mutans, Streptococcus sanguis*. Such activity was due to inhibition of microbial glycosultransferase; enzyme implicated in pathogenicity of *Streptococcus spp* [147].

Otherwise, Dimov tested the efficacy of the water-soluble derivative (WSD) of propolis against experimental infections caused by Gram-negative pathogens (*Klebsiella pneumoniae, Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa*), [148].

In other study, Grochowski et al, (1985) in mice, study the effect of propolis ointment on experimental burns infection with *P. aeruginosa*. He found that the healing process in treated group was shorter than the control group (7- 13 days treated groups vs 14-18 days control group) [147]. Gregory et al (2002), found that the usage of skin cream based on propolis preparation, have a beneficial effects on the healing burn wounds [148]. Also, Santos and co-workers, 2008, evaluated the clinical efficacy of Brazilian propolis gel formulation in patients diagnosed with denture stomatitis [149].

In food application, numerous studies showed potential of propolis as a food preservative against pathogenic microorganisms such as *Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes, Enterococcus faecalis, Escherichia coli* and *Clostridium perfringenes* [150,151, 152].

Extracts of propolis have been shown to enhance the efficacy of certain antibiotics (streptomycin, ampicillin, gentamycin, cloxacillin…) [105,153-156]. Additionally, Orsi *et al.*, had studied the effect of combination of propolis and antibiotics (amoxicillin, ampicillin and cefalexin) against *Salmonella Typhi*. He reported that propolis extract diminished the resistance of the bacteria wall to antibiotics [157].

Therefore, Combination of propolis with antimicrobial drugs could potentiate the activities of known antibiotics, to reduced their marked side effects, and/or enhance the host immune system against microbial infection and to prevent emergence of resistant or multi-resistants strains [103].

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Table 3. Anti-microbial activities of propolis against different bacteria

Pathogens	***References***
Gram-positive bacteria	
Staphylococcus aureus , S.aureus Amme ATCC 29213, *S.aureus* ATCC 25923, *S. aureus* BAA ATCC 977 , *MRSA/NCTC* ATCC 10442, *Staphylococcus* spp.; *S.saprophyticus* ATCC 15305,*S. auricularis, S. capitis, S. epidermidis, S. haemolyticus, S. hominis, S. mutans, S. warnerii.*	[107,113,114,125,126,128,130, 136,137,138]
Streptococcus spp.; *S. cricetus, S. faecalis, S.epidermis* ATCC 14990, *S.epidermis , S.pneumoniae* ATCC *49619, S.pneumioniae, S. pyogenes* ATCC 12344, *S. pyogenes, S. β-haemolyticus, S. thermophilus* ATCC 19258, *S. mutans, S.sobrinus, S. agalactia* ATCC 27956, *S. viridians, S. oralis* ATCC 35037.	[1,2,17,23,24,25,26,27,28,50,51 .60,127] [3,4,5] [101]
Bacillus cereus, B. subtilis, B. subtilis ATCC 6051, *B. mesentericus, Listeria. monocytogenes L. monocytogenes* (ATCC, 19111)	[96,113,105,117,120,] [107, 114-121]
Enterococcus spp.; Enterococcus faecalis, E. faecalis (ATCC 29212) *E casseliflavus* (ATCC *70032) , VRE VanB ATCC 51299* *Micrococcus luteus, Diplococcus pneumonae, Mycobacteria sp., Mycobacterium tuberculosis.* *Actinomyces naeslundii, A. viscosus Lactobacillus acidophilus, Peptostreptococcus micros*	[139,140,144,145,146, [107,116,117,120,48,152, 153]
Gram negative bacteria	
Escherichia coli, E.coli (ATCC 25922), *E.coli O157:H7* ATCC 35150, *Pseudomonas aeruginosa, P. aeruginosa,* (ATCC 27853), *Helicobacter pylori, Proteus vulgaris, Shigella dysenteriae , S. flexneri* ATCC 29903, *Brucella abortus, Haemophilus influenzae* ATCC 49747,*Klebsiella pneumoniae , K. pneumoniae* (ATCC *700603)* ,(ATCC 13883), *K. ozaemae, K. oxytoca* ATCC 700324, *Acinetobacter baumannii* ATCC BAAm 747, *Enterobacter cloacae* ATCC700323, *Yersinia enterocolitis* ATCC 9610.	
Salmonella spp (S. enteritidis, S.dublin,S. typhi, S. typhimurium: choleraesuis, S. choleraesuis ATCC 554,*exneri, , paratyphi-A, paratyphi-B, typhi, gallinarum, pullorum, sonnei)*	[132,144,145,146,154,155]
Corynebacterium spp; Corynebacterium diphtheria, C. pseudotuberculosis. *Capnocytophaga gingivalis, Porphyromonas anaerobius , Porphyromonas gingivalis.* *Prevotella intermedia , Prevotella melaninogenica , Aeromonas hydrophila, Burkholderia cepacia* ATCC 25416. *Fusobacterium nucleatum, Branhamella catarrhalis.*	

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3. The target pathogen and Synergy in antimicrobial activity

The development of multidrug-resistant pathogens has been related to the occurrence of over- and under-dosage of antimicrobials [203,204].

In order to minimise the potential development of further antimicrobial resistance ''The Copenhagen Recommendations: Report from the Invitational EU Conference on The Microbial Threat'' were published (http://www.im.dk/publikationer/micro98/index.htm), which outlined the need for the development of ''Novel principles for treating or preventing Infections in humans and animals''. One strategy employed to overcome resistance mechanisms is the use of combinations of drugs and several plant extracts, which had exhibited synergistic activity against microorganisms [205]. . Among the natural sources of antimicrobial agents utilized to treat infectious diseases, propolis was exhaustively studied [27].

3.Combination effect of propolis and antibiotic

The synergistic effects of propolis and antibiotics are a new target for clinical research, in order to explore the benefits of this bee product [156].

Oksuz *et al*.,,2005, studied synergistic activity between ciprofloxacin and propolis in the treatment of experimental *Staphylococcus aureus* keratitis [157]. Other combination therapy in BALB/c mice salmonella infection (with *Salmonella typhimurium*), propolis and standard antibiotic efficacy of therapy was observed after 5 days of treatment [158].

Lin and colleagues. 2008 showed that the bactericidal action of ciprofloxacin was greatly enhanced by the sub-MIC addition of two polyphenols (kaempferol and quercetin) in [276]. Furthermore, propolis had synergistic effects with antibiotics acting on the bacterial wall and ribosome *in vitro* (chloramphenicol, tetracycline and neomycin) [160], in addition, EEP are most effective when combined with antibiotics that interfere with bacterial protein biosynthesis such as tetracycline, linezolid, chloramphenicol, gentamicin, tobramycin and netilmicin against MRSA and MSSA [160,161].

Recently, AL-Ani and co-workes[106], observed synergism between EEP and antibiotics that inhibit bacterial cell wall synthesis (vancomycin and oxacillin) against *Streptococcus pyogenes*, MRSA NCTC 10442, and VRE ATCC 51299. In addition, no synergistic activity of propolis and antibiotics acting on the DNA and folic acid (ciprofloxacin, norfloxacin and cotrimoxazole) was obtained [156]. In this same study, the synergism was investigated by using of sub minimal inhibitory concentration (½ and ¼ of the MIC) for propolis (Brazilian, Bulgarian) and antibiotics, the authors suggested that such interactions can lead to bacteriostatic action towards *Salmonella Typhi* [162]. Also, synergistic or additive activity was observed after combinations of propolis extract with clarithromycin against *Helicobacter pylori* [164].

In other hand, Hamoud and co-workers, studies combination effects of phenolic monoterpenes (thymol) and vancomycin, such mixture of two compounds have synergistic effects, thymol preferentially affect the outer cell membrane of Gram-negative bacteria helping the traffic of vancomycin to peptidoglycan (site of action) [164].

The *in vitro* synergistic effect of kaempferol and quercetin, in combination with rifampicin , was demonstrated against clinical rifampicin-resistant methicillin-resistant S. aureus (MRSA) isolates (Lin et al., 2008). Quercetin and kaempferol alone showed slight β-lactamase inhibition, but when combined with rifampicin, the complex exhibited good β-lactamase inhibitory effect. These compounds (quercetin/kaempferol) inhibit the catalytic activity of different bacterial topoisomerases (Bernard et al., 2008) and this might

explain some of the synergistic activities between ciprofloxacin and quercetin/kaempferol (Lin et al., 2008) in [160].
Wang and his colleagues [111], demonstrated that Australian propolis ethanol extract (APEE) decrease β-lactamase activity and inhibit expression of specific drug-resistant protein; *PBP2a* (penicillin binding protein 2a) in MRSA strains. Same authors suggested that combination of APEE with β-lactam antibiotics (ampicillin, methicillin…..),consequently, increasing therapeutic effect of antibiotic.
Kaempferol and its glycosides can also act synergistically with antibiotics (e.g. rifampicin, vancomycin, methicillin, erythromycin and clindamycin) against antibiotic-resistant bacteria (Lim et al., 2007; Xu et al., 2001) in [296] , therefore suggesting that kaempferol could be used in combination with these drugs in cases of resistance [296].

Conclusion

Propolis, known in folk medicine since ancient times and possesses various biological properties including antimicrobial, antioxidant and anti-inflammatory.
Recently, the propolis has regained interest however, he chemical composition and biological activities of propolis depend mainly upon the local flora, the geographic region, and the climate. hence, further investigations and comprehensive qualitative and quantitative analyses of the specific propolis constituents and mechanism of action are needed for its usage.
Sforcin and Bankova [38] discussed the potential of propolis for the development of new drugs. They suggested that:

- More investigations will be needed in order to isolate and identify the main bioactive(s) compound(s).
- Development of clinical studies, to evaluate propolis samples activity in patients and/or in healthy individuals.
- Studies of interactions between propolis and other drugs.

Finally, as anti-infective agent propolis will constitutes an interesting natural alternative and complementatry products that merit more deeply investigation in academic, pharmaceutical research industry and medical practice to overcome problem of resistance to antibiotics.

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