REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 1S (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

Indian Propolis: Chemical Composition, Antioxidant and Antimicrobial Properties

Charushila papal[1*], Dr Rohini patil[2]

[1*,2]R.K.T College, Ulhasnagar ,(University of Mumbai)

Abstract: -
Propolis, a resinous substance collected by honeybees from various plant sources, has been utilized for centuries in traditional medicine due to its diverse biological activities. This research focuses on exploring the chemical composition, antioxidant potential and antimicrobial properties of Indian propolis.
The chemical constituents of Indian propolis were elucidated, revealing a complex mixture of phenolic compounds, flavonoids, terpenoids, and other bioactive substances. These constituents contribute significantly to the therapeutic properties exhibited by propolis.
Moreover, the antioxidant activity of Indian propolis revealed its capability to scavenge free radicals and protect against oxidative stress-induced damage. The findings underscore the potential application of Indian propolis as a natural antioxidant supplement for mitigating oxidative stress-related diseases.
Furthermore, the antimicrobial properties of Indian propolis were investigated against different standard pathogens. Results revealed its effectiveness in inhibiting bacterial growth, suggesting its potential as an alternative or adjunctive therapy for bacterial and fungal infections.
In conclusion, Indian propolis exhibits promising chemical composition, antioxidant potential and antimicrobial activity, highlighting its therapeutic significance and potential applications in pharmaceuticals, nutraceuticals, and cosmeceuticals. Further research is warranted to elucidate its mechanisms of action and explore its clinical efficacy in various health conditions.

Keywords: antimicrobial activity, chemical constituent, antioxidant activity.

Introduction: -
Many natural compounds have found widespread application as therapeutic agents in pharmaceuticals. The medical community has always placed a strong emphasis on treating illnesses using natural remedies rather than allopathic ones. Many natural items are currently employed in biomedical applications to cure illnesses. These consist of miswak (**Niazi, *et al*. 2016)**, chitosan **(Husain, *et al*.2017; Qasim, *et al*.2017)**, herbal tea **(Khurshid, *et al*.2016; Niazi, *et al*. 2016)** and natural silk **(Zafar, *et al*.2015; Zafar, *et al.* 2014).**

All prehistoric societies were aware of the benefits of using goods made from bees as a source of medicine. Bees gather propolis, a resinous material, from plant buds and exudates in many parts of the world. The Greek word propolis, which means "at the entrance to" and "polis," means "community" or "city." This indicates that propolis is a natural product utilised for bee defence. Blue glue is another name for propolis. One of the natural materials used by bees to construct and preserve their hives is propolis **(Sforcin, *et al.*2011; Freires, *et al.* 2016)**. The main component of propolis is resin. In addition, it includes pollen, essential oils, waxes and fatty acids, as well as other organic materials **(Burdock, G. A., 1998; Marcucci, *et al* 1995)**.

In temperate climates, propolis' chemical components include chrysin, galangin, pinocembrin, and pinobanksin **(Huang, Shuai, *et al*. 2014)**. Galangin and chrysin were found to be the two polyphenols in a brown propolis extract with the highest concentrations **(Curti V. *et al*, 2019)**.Galangin, 3, 5, 7-trihydroxyflavone, is known for its great biological benefits, including its antioxidant properties **(Russo A. *et al*, 2002).**

Propolis has exceptional antimicrobial qualities despite its chemical makeup and origins from various geographical areas. Natural resin has limited efficacy against Gram negative bacilli but is efficient against both Gram-positive rods and *Mycobacterium tuberculosis* **(Kalogeropoulos, Nick, *et al*.2009**). Propolis has shown *in vitro* activity against *Staphylococcus* and *Streptococcus spp*. and certain Gram negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* etc. Propolis improves wound healing in diabetes (**McLennan, *et al*.2008**). Propolis's beneficial effects have been applied most frequently in dermatology. It is applied to tissue repair acceleration, thermal damage, injury healing, and healing time reduction **(Ramos, A. F. N, *et al*.2007)**.It proved to have the ability to enhance the effects of traditional antifungal medications. The development of propolis-based antibiotic and antifungal combinations is gaining attraction (**Stepanović, S, *et al*.2003).**

Present research work is based on chemical composition, antioxidant and antimicrobial activity of Indian Propolis.

Materials and Methods
A) Collection of Propolis sample
Propolis sample was collected from Indian apiary at Pune, Maharashtra and stored at 4^0 C until further processing.

B) Characterisation of Propolis
Basic physical parameters like appearance, color, odour, melting point, solubility etc. was studied. Chemical characterization of extracts of propolis was done using GC/MS analysis.

C) Preparation of various extracts of propolis
1) Ethanolic extract of propolis (EEP)
The ethanolic extract of propolis was prepared as per the methodology given by *Sawaya et al*. 2004.
2) Water extract of Propolis (WEP)
The liquid or water extract of propolis was prepared according to the methodology given by *Miguel et al.*2014.
3) Methanolic extract of propolis (MEP)
Methanolic extract of propolis prepared according to the methodology given by Midorikawa *et al.2001*.
All the extracts prepared were kept at 4^0 C in dark until testing.

D) Antioxidant property of propolis
Propolis extracts were tested for activity using the DPPH technique. Gallic acid was used as an antioxidant standard to compare the antioxidant activity. In this method, the capacity of extract to scavenge free radicals was assessed using the stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH). Equal volumes of various extract concentrations of different extracts of propolis were added to a methanolic DPPH solution (100 µM). At room temperature, the absorbance was measured at 515 nm after 15 minutes. **(Ebrahimzadeh *et al*., 2008)**

E) Study of *In vitro* antimicrobial activity of different extracts of propolis
Agar well diffusion method
Various concentrations of different propolis extracts were prepared in the range of 100 ppm to 10,000 ppm using 2% DMSO as a solvent. For this, 20 ml sterile molten Mueller and Hinton agar was bulk seeded with young cell suspension of standard Gram positive (*S. aureus* ATCC 29213), Gram negative (*E.coli* ATCC 35218, *Pseudomonas aeruginosa* 27853 and *Klebsiella pneumoniae* 700603) with cell suspension having cell density $1X10^8$cells/ml and for yeast (*C. albicans* ATCC10231) sterile molten Saboroud's agar was used. Wells of 6mm were made on the medium using a sterile borer and 65 microliters of diluted extracts were added to respective agar cups. The plates were then kept in refrigerator for pre-diffusion and then incubated at 37^0C for 24 hours. The zone of inhibition was measured in millimetre. Appropriate positive control and solvent controls were maintained (**Magaldi, S. *et al.*, 2004*;* Valgas. *et al.*, 2007).**

Results and Discussions
Physio-chemical characterisation of Indian propolis
The propolis was subjected to its characterization and the physical characteristics were as given in Table 1.

Sr. number	Characteristics	Interpretation
1	Appearance	Irregular shiny Mass or fragment
2	Colour	Dark brown
3	odour	Sweet aromatic
4	Melting point	$60\text{-}70^0$ C
5	Solubility	DMSO, Ethanol, Methanol
6	consistency	Hard
7	Visible impurity	Wood shavings, Waxes, bee part, plant remains

Table 1: Physical characteristics of Indian propolis

Indian propolis under study appeared as irregular masses or fragments with a shiny surface and dark brown in colour. An appearance of irregular masses may be due to its resinous nature. Its appearance can vary depending on the specific botanical sources from which it was collected. The dark brown color of Indian propolis is typical but it varies depending on plant source, age and its origin (**Wagh, V. D. 2013).** It varies from yellow, red, green, light or dark brown and is attributed to the presence of various plant resins, flavonoids, and other organic compounds (**Ahmed, R., *et al.*2017**). A sweet aromatic odour of Indian propolis is due to its composition of volatile organic compounds, including essential oils and resins collected from plants. This characteristic aroma is often described as pleasant and herbal. **(Alfarrayeh, *et al.* (2021).**The melting point of Indian propolis range ($60\text{-}70^0$ C) aligns with typical melting points reported for propolis by **Martinotti, *et al.*2015.**

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 1S (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

Indian propolis exhibited solubility in different organic solvents like ethanol, methanol and DMSO. Many researchers have also reported best solubility of propolis in ethanol (**Galeotti, F.*et al* 2018; Paviani, L. C., *et al*. 2013; Kubiliene, L., *et al*. 2015)**. The presence of visible impurities such as wood shavings, waxes, bee parts, and plant remains indicates that the propolis sample may contain contaminants or residues from the natural environment.
GC/MS analysis of different propolis extract was done for chemical composition of Indian propolis.

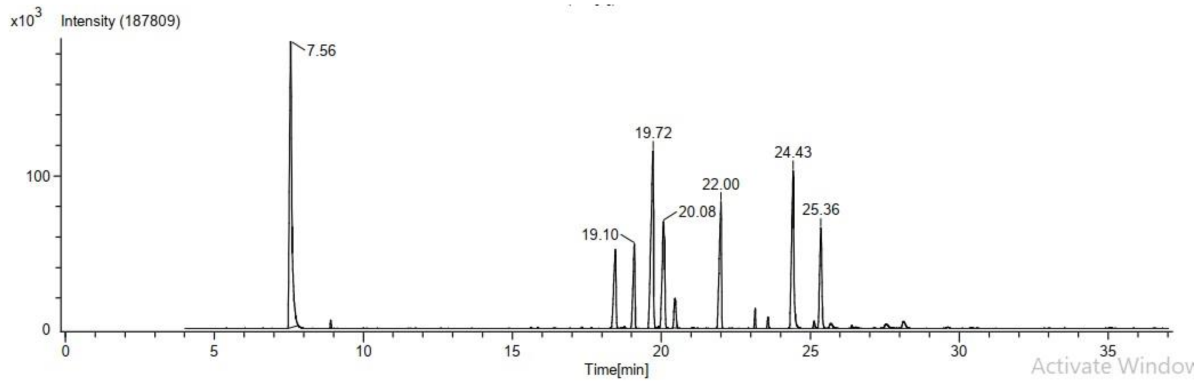

Figure 1.GCMS-W water extract

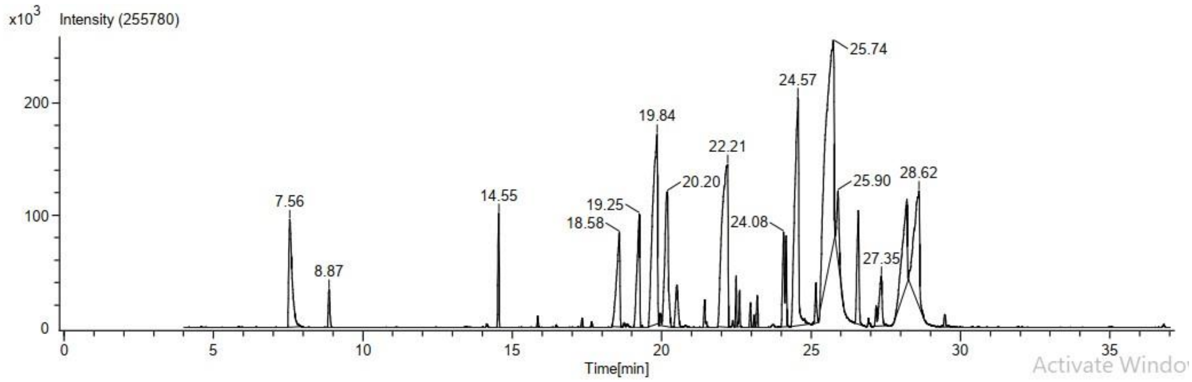

Figure 2. GCMS-E Ethanol extract

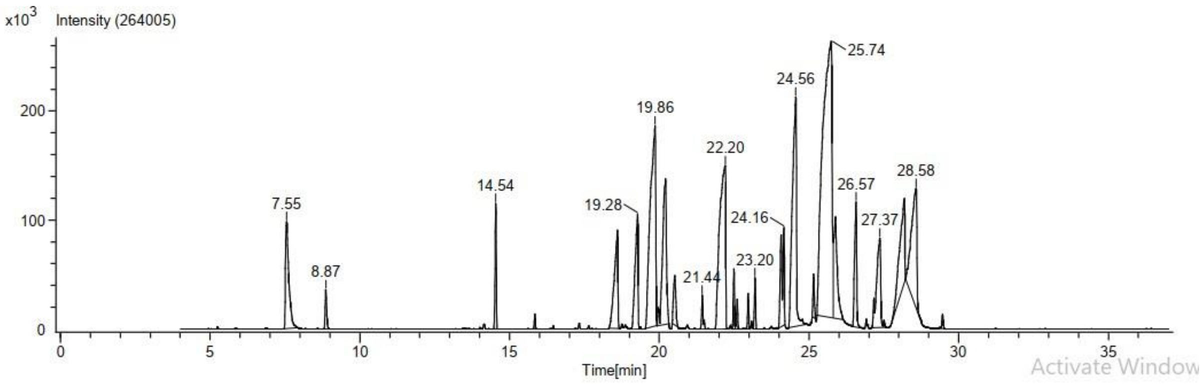

Figure 3. GCMS-M Methanol extract

Some of the components present were as depicted in Table 2. They were mainly flavonoids, phenolic compounds, organic acids, sugars, alcohols, esters etc. These compounds were extracted by using various solvents including ethanol, methanol and water. The degree of extracts of these components was different in 3 different solvents namely ethanol, methanol and water. More than 80% components were extracted by ethanol followed by methanol (70-80%) and less than 30% by water. The best solvent for extraction of bioactive component was ethanol. Similarly many researchers have also reported best extraction of bioactive components by ethanol from propolis (**Sun, C., *et al.*2015; Kara, Y, *et al*.2022).**

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 1S (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

No	Name of the compound	Mol. Weight	EEP	MEP	WEP
	Flavonoid				
1	Chrysin (5,7-dihydroxy-2-phenylchromen-4-one)	254	+	+	+
2	Galangin (3,5,7-trihydroxy-2-phenylchromen-4-one)	270	+	+	-
3	Genistein (5,7-dihydroxy-3-(4-hydroxyphenyl)chromen-4-one)	270	+	+	-
4	Vestitol (3-(2-hydroxy-4-methoxyphenyl)-3,4-dihydro-2H-chromen-7-ol)	274	+	+	-
5	Kaempferol	288	-	+	-
6	Pinocembrin	256	+	+	+
7	Pinobanksin/naringenin	272	+	+	-
8	Daidzein	254	+	+	-
9	Liquiritigenin	256	+	+	-
10	Quercetin methyl ether	314	+	-	+
	Phenols				
11	Pterostilbene	256	+	+	-
12	Artepillin C ((E)-3-[4-hydroxy-3,5-bis(3-methylbut-2-enyl)phenyl]prop-2-enoic acid)	300	-	+	-
	Acids				
13	3-isopropyl-p-caumeric acid	230	+	-	-
14	Anacardic acid	344	+	-	-
15	Benzoic acid	120	+	+	+
	Carbohydrates & sugar				
16	Cellobiose	342	-	-	+
17	Maltose	342	+	+	+
18	Sucrose	342	+	+	+
	Others				
19	Bisabolol (Non cyclic alcohol)	222	+	+	-
20	1-monoacetin (Ester)	134	+	-	-
21	Ethyl trans caffeate (Ester)	210	+	+	-
22	Thiazole (Heterocyclic compounds)	354	+	-	-
23	Palmitic acid (Fatty acids)	256	+	+	-
24	Quercetin (Pigments)	304	+	+	-

Table 2: Chemical components from Indian propolis

Key: 1) + = presence of compound 2) - = absence of compound

All three extracts of propolis under study exhibited antioxidant activities (Table 3). The ethanolic and methanolic extract had same activity whereas water extract had the least activity. Since ethanol and methanol has very good extraction ability for flavonoids and phenolic compounds, both EEP and MEP exhibit very high antioxidant ability than WEP. The pronounced antioxidant properties of propolis, may be due to its rich polyphenolic content. Thus propolis extracts may offer significant protection against oxidative stress, damage and inflammation. Similar activity of propolis was also reported by **Nilesh, Kumar, *et al*. 2008 ;(Sowmya, S., *et al*.2019)**. Propolis contains a high level of antioxidants due to the presence of phenolic, alkaloids, vitamins, flavonoids, coumarins, tannins, terpenoids, etc.(**Mulyati, A. H., *et al*.2020)**.

Sr.No.	Sample	Absorbance at 515 nm	Percentage inhibition of DPPH radical
1	WEP	0.69	6.66%
2	EEP	0.36	51.35%
3	MEP	0.36	51.35%

Table 3: Antioxidant activity of different extracts of propolis

In vitro evaluation of antimicrobial property of various extracts of Indian propolis was done and the average zone of inhibition in mm was as depicted in Table 4 and Fig 4. All three extracts demonstrated antimicrobial activity but it was very high in both the alcoholic extracts where as poor in water extract. Both bacteria and fungi were inhibited by all 3 extracts, indicating antibacterial and antifungal property of all 3 extracts. Propolis exhibited better antibacterial activity than antifungal activity. Among the bacteria Gram positive were more sensitive towards extract of propolis than Gram negative. Since bioactive components were better extracted in ethanol and methanol than water, antimicrobial activity is higher in EEP and MEP. Among Gram negative *E. coli* were comparatively less sensitive to extracts of propolis.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 1S (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

The observed activity may be attributable to the synergistic interactions of its bioactive compounds, which disrupt microbial cellular functions and integrity. Notably, the diverse array of chemical constituents within propolis, enhances its effectiveness against pathogenic microorganisms, thereby supporting its traditional use in folk medicine.

no	**Name of Standard culture↓**	**Average diameter of zone of inhibition (mm) At different concentrations of propolis extract (ppm)**								
	Extracts→	**EEP**			**MEP**			**WEP**		
	Concentration(ppm)→	**100**	**1000**	**10000**	**100**	**1000**	**10000**	**100**	**1000**	**10000**
1	***S. aureus* 29213**	10	13	15	10	07	15	-	07	10
2	***P. aeruginosa 27853***	07	09	13	07	09	13	-	-	08
3	***K. pneumoniae700603***	08	10	13	8	10	13	-	-	08
4	***E. coli 35218***	-	08	10	-	8	13	-	-	-
5	***C. albicans 90028***	08	10	13	8	9	11	-	09	10

Table 4: Average diameter of zone of inhibition by different extracts of propolis
Key: - EEP=Ethanolic extract of propolis, MEP=Methanolic extract of propolis
WEP=Water extract of propolis

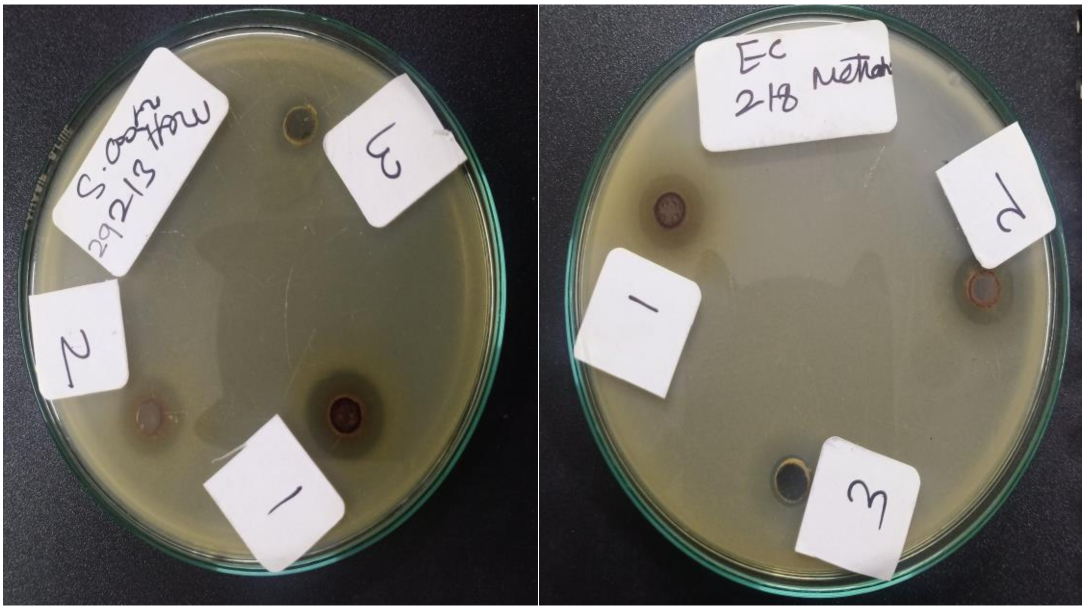

Fig.4 *In vitro* antibacterial activity of propolis against standard *S. aureus* ATCC 29213 and *E.coli* ATCC 35218
Key:-1=10,000 ppm, 2=1000 ppm, 3=100 ppm

Ethanolic and methanolic extracts of propolis were more effective than water extract of propolis. Similar activity was also reported by **Dönmez, S,*et al*.2020** and **Park, Y. K .*et al* 1998**.Also, propolis extracts were effective against fungal pathogens associated with humans **(Sokolonski, A. R., *et al*. 2021).**

Conclusion

The multifaceted pharmacological profile of propolis, encompassing its antimicrobial, chemical and antioxidant properties, underscores its considerable potential as a therapeutic agent. Through a detailed examination of its chemical composition, it is evident that propolis is a complex amalgam of resins, flavonoids, phenolic acids, essential oils, and waxes. Each of these constituents contributes uniquely to its biological activity mainly flavonoids and phenolic acids. Propolis extracts have broad spectrum antimicrobial activity that highlights its utility as a natural alternative to conventional antimicrobial agents. Similarly high antioxidant capacity confirms its role in preventing oxidative stress-related diseases. Thus propolis represents a promising candidate for further investigation and potential clinical application which may lead to development of novel and effective applications in medicine and health care.

References

1. Zafar, Muhammad Sohail, Zohaib Khurshid, and Khalid Almas. "Oral tissue engineering progress and challenges." *Tissue Engineering and Regenerative Medicine* 12.6 (2015): 387-397.
2. Zafar, Muhammad Sohail, and Khalid H. Al-Samadani. "Potential use of natural silk for bio-dental applications." *Journal of Taibah University Medical Sciences* 9.3 (2014): 171-177.

3. Husain, Shehriar, *et al*. "Chitosan biomaterials for current and potential dental applications." *Materials* 10.6 (2017): 602.
4. Qasim, Saad B., *et al*. "Potential of electrospun chitosan fibers as a surface layer in functionally graded GTR membrane for periodontal regeneration." *dental materials* 33.1 (2017): 71-83.
5. Khurshid, Zohaib, *et al*. "Suppl-1, M3: Green Tea (Camellia Sinensis): Chemistry and Oral Health." *The open dentistry journal* 10 (2016): 166.
6. Niazi, Fayez, *et al*. "Role of Salvadora persica chewing stick (miswak): A natural toothbrush for holistic oral health." *European journal of dentistry* 10.2 (2016): 301.
7. Sforcin, José Maurício, and Vassya Bankova. "Propolis: is there a potential for the development of new drugs?." *Journal of ethnopharmacology* 133.2 (2011): 253-260.
8. Freires, Irlan Almeida, Severino Matias de Alencar, and Pedro Luiz Rosalen. "A pharmacological perspective on the use of Brazilian Red Propolis and its isolated compounds against human diseases." *European journal of medicinal chemistry*110 (2016): 267-279.
9. Burdock, G. A. "Review of the biological properties and toxicity of bee propolis (propolis)." *Food and Chemical toxicology* 36.4 (1998): 347-363.
10. Marcucci, Maria Cristina. "Propolis: chemical composition, biological properties and therapeutic activity." *Apidologie* 26.2 (1995): 83-99.
11. Huang, Shuai, *et al*. "Recent advances in the chemical composition of propolis." *Molecules* 19.12 (2014): 19610-19632.
12. Kalogeropoulos, Nick, *et al*. "Chemical composition, antioxidant activity and antimicrobial properties of propolis extracts from Greece and Cyprus." *Food chemistry* 116.2 (2009): 452-461.
13. Stepanović, Srdjan, *et al*. "In vitro antimicrobial activity of propolis and synergism between propolis and antimicrobial drugs." *Microbiological Research* 158.4 (2003): 353-357.
14. McLennan, Susan V., *et al*. "The anti-inflammatory agent Propolis improves wound healing in a rodent model of experimental diabetes." *Wound Repair and Regeneration* 16.5 (2008): 706-713.
15. Ramos, A. F. N., and JL de Miranda. "Propolis: a review of its anti-inflammatory and healing actions." *Journal of Venomous Animals and Toxins Including Tropical Diseases* 13.4 (2007): 697-710.
16. Curti, V., Zaccaria, V., Tsetegho Sokeng, A. J., Dacrema, M., Masiello, I., Mascaro, A., ... & Daglia, M. (2019). Bioavailability and in vivo antioxidant activity of a standardized polyphenol mixture extracted from brown propolis. *International journal of molecular sciences*, *20*(5), 1250.
17. Russo, A., Longo, R., & Vanella, A. (2002). Antioxidant activity of propolis: role of caffeic acid phenethyl ester and galangin. *Fitoterapia*, *73*, S21-S29..
18. Sawaya, Alexandra CHF, *et al*. "Electrospray ionization mass spectrometry fingerprinting of propolis." *Analyst* 129.8 (2004): 739-744.
19. Miguel, Maria Graça, *et al.* "Phenols, flavonoids and antioxidant activity of aqueous and methanolic extracts of propolis (Apis mellifera L.) from Algarve, South Portugal." *Food Science and Technology* 34.1 (2014): 16-23.
20. Midorikawa, K., Banskota, A. H., Tezuka, Y., Nagaoka, T., Matsushige, K., Message, D., & Kadota, S. (2001). Liquid chromatography–mass spectrometry analysis of propolis. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques*, *12*(6), 366-373.
21. .Ebrahimzadeh, M. A., Pourmorad, F., & Bekhradnia, A. R. (2008). Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran. *African journal of Biotechnology*, *7*(18).
22. Magaldi, S., Mata-Essayag, S., De Capriles, C. H., Pérez, C., Colella, M. T., Olaizola, C., & Ontiveros, Y. (2004). Well diffusion for antifungal susceptibility testing. *International journal of infectious diseases*, *8*(1), 39-45.
23. Valgas, C., Souza, S. M. D., Smânia, E. F., & Smânia Jr, A. (2007). Screening methods to determine antibacterial activity of natural products. *Brazilian journal of microbiology*, *38*, 369-380.
24. Wagh, V. D. (2013). Propolis: a wonder bees product and its pharmacological potentials. *Advances in Pharmacological and Pharmaceutical Sciences*, *2013*(1), 308249.
25. Ahmed, R., Tanvir, E. M., Hossen, M. S., Afroz, R., Ahmmed, I., Rumpa, N. E. N., ... & Khalil, M. I. (2017). Antioxidant properties and cardioprotective mechanism of Malaysian propolis in rats. *Evidence-Based Complementary and Alternative Medicine*, *2017*(1), 5370545.
26. Alfarrayeh, I. I. S. (2021). Bioactivities and potential beneficial properties of propolis ethanolic extract, caffeic acid phenethyl ester, and Arabic coffee beans extract. *University of Pécs Pécs, Hungary*.
27. Martinotti, S., & Ranzato, E. (2015). Propolis: a new frontier for wound healing?. *Burns & trauma*, *3*, 1-7.
28. Galeotti, F., Maccari, F., Fachini, A., & Volpi, N. (2018). Chemical composition and antioxidant activity of propolis prepared in different forms and in different solvents useful for finished products. *Foods*, *7*(3), 41.
29. Paviani, L. C., Fiorito, G., Sacoda, P., & Cabral, F. A. (2013, April). Different solvents for extraction of Brazilian green propolis: Composition and extraction yield of phenolic compounds. In *III Iberoamerican Conference on Supercritical Fluid* (pp. 1-5).

30. Kubiliene, L., Laugaliene, V., Pavilonis, A., Maruska, A., Majiene, D., Barcauskaite, K., ... & Savickas, A. (2015). Alternative preparation of propolis extracts: comparison of their composition and biological activities. *BMC complementary and alternative medicine*, *15*, 1-7.
31. Sun, C., Wu, Z., Wang, Z., & Zhang, H. (2015). Effect of ethanol/water solvents on phenolic profiles and antioxidant properties of Beijing propolis extracts. *Evidence-Based Complementary and Alternative Medicine*, *2015*(1), 595393.
32. Kara, Y., Can, Z., & Kolaylı, S. (2022). What should be the ideal solvent percentage and solvent-propolis ratio in the preparation of ethanolic propolis extract?. *Food Analytical Methods*, *15*(6), 1707-1719.
33. Mulyati, A. H., Sulaeman, A., Marliyati, S. A., Rafi, M., & Fikri, A. M. (2020, June). Phytochemical analysis and antioxidant activities of ethanol extract of stingless bee propolis from Indonesia. In *AIP Conference Proceedings* (Vol. 2243, No. 1). AIP Publishing.
34. Kumar, N., Ahmad, M. K. K., Dang, R., & Husain, A. (2008). Antioxidant and antimicrobial activity of propolis from Tamil Nadu zone. *Journal of Medicinal Plants Research*, *2*(12), 361-364.
35. Sowmya, S., Gujjari, A. K., Mruthunjaya, K., Padmanabhan, T. V., Anupama, C., Sushma, R., & Gaekwad, S. S. (2019). Antioxidant and antimicrobial activity of propolis.
36. Park, Y. K., & Ikegaki, M. (1998). Preparation of water and ethanolic extracts of propolis and evaluation of the preparations. *Bioscience, biotechnology, and biochemistry*, *62*(11), 2230-2232.
37. Sokolonski, A. R., Fonseca, M. S., Machado, B. A. S., Deegan, K. R., Araújo, R. P. C., Umsza-Guez, M. A., & Portela, R. W. (2021). Activity of antifungal drugs and Brazilian red and green propolis extracted with different methodologies against oral isolates of Candida spp. *BMC complementary medicine and therapies*, *21*, 1-14.
38. Dönmez, M., Karadeniz, Ş., Yoldas, T., Aydın, G., Karagül, P., Aksu, O., & Rasgele, P. G. (2020). Comparison of chemical contents of extracts in different solvents of propolis samples produced in Duzce province. *International Journal of Traditional and Complementary Medicine Research*, *1*(3), 137-146.
39. https://www.iitb.ac.in