International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2023; 11(3): 46-49

Received: 01-02-2023
Accepted: 05-04-2023

Deepa Kumari
Assistant Professor, Department of Chemistry, Ram Krishna College, L.N.M.U., Darbhanga, Bihar, India

Farheen Jahan
Assistant Professor, Department of Chemistry, Ram Krishna College, L.N.M.U., Darbhanga, Bihar, India

Corresponding Author:
Deepa Kumari
Assistant Professor, Department of Chemistry, Ram Krishna College, L.N.M.U., Darbhanga, Bihar, India

Exploring the phytochemical profiles and antioxidant activity of propolis and their potential applications

Deepa Kumari and Farheen Jahan

Abstract
We have performed a detailed study on the phytochemical profile and antioxidant activity of the aqueous extracts and ethanol extracts (AEP and EEP respectively) of the Propolis, also known as Bee Glue. The antioxidant activity of the extracts was measured by *in-vitro* chemical analyses involving the assays of (1) 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (2) Ferric ion reducing power. In all the assays, AEP showed significantly greater activity over EEP. This work provides a scientific support for the high antioxidant activity of this Biogenic material and thus it may find potential applications in the treatment of the diseases caused by ROS.

Keywords: Antioxidant activity, biogenic agents, propolis, extraction, bioactive compounds, chemical constituents

1. Introduction
Nature with its unique biogenic sources is the biggest and the greatest combinational library of naturally occurring chemical compounds. Such natural products have been proven as excellent source of medicinal compounds. The biogenic sources and their active constituents have important roles in the traditional system of healing and they have also been an integral part of history and culture in many regions of the world. These materials are also the sources of wide spectrum of pharmaceutical activity. Therefore, these biogenic materials are becoming the part of integrative health care system known as "Complementary and alternative system of medicine". Propolis, also known as 'bee glue' is the most important and interesting 'Chemical Weapon' of honey bees. Bees collect resinous exudates from various plant parts and transport it to the hives where it is modified by their enzymes to the propolis. It is used by the bees to prevent the spread of microbial (bacterial and fungus) infections, embalm dead animals that had entered the hives, as construction material for the hives [1]. Propolis is the unique source of a wide range of bioactive natural products e.g. polyphenols, flavonoids, caffeic acid and its esters [2, 3]. In general, it is composed of 50% of resin and balm, 30% of wax, 10% of essential and aromatic oils, 5% of pollen and 5% several other substances [1]. Until the recent time, more than 200 chemical compositions have already been identified in propolis and among them, flavonoids, aromatic acids, terpenoids, alcohols, aliphatic acids and esters, amino acids, steroids, sugars are important [1, 4-6]. Analyzing the recent trends of research on propolis obtained from various geographical regions, it is evident that this unique biogenic material is the source of various potentially bioactive chemical constituents, mainly polyphenolic compounds which belong to various classes of natural products e.g. flavonoids, terpenes, various cinnamic acid derivatives such as caffeic acid, caffeic acid phenethyl esters (CAPE) and its various derivatives.
It has been observed that the biological activities of propolis which depend on its chemical composition also very much influenced by geographical diversity and the genetic variety of the queens [2-3]. However, comparative biological studies of propolis of different geographical regions and of different chemical compositions are the most interesting trends in the recent propolis research. Though, the number of this type of works is as yet limited on international level as well as on national level. The results of these type of studies unambiguously prove that in spite of the great differences in the chemical compositions of propolis from different geographical areas, all the samples exhibit significant antibacterial and antifungal (and most of them shows antiviral) activities [6, 12].

Here in the following table we have summarized the biologically different types of propolis. Active chemical constituents of propolis different regions and their biological activities are summed up in the following table:

Table 1: Different types of propolis and their biological activities

Propolis type	Active constituents in different types of propolis responsible for					
	Antibacterial activity	**Anti-inflammatory actively**	**Antitumor activity**	**Hepatoprotective activity**	**Antioxidant actively**	**Allergenic action**
European (Poplar type)	Flavanones Flavones Phenolic acids and their esters [13]	Flavanones, Flavones, Phenolic acids and their esters [14]	Caffeic acid phen-ethyl ester [15]	Caffeic acid, ferulic acid, caffeic acid Phenethyl ester [14]	Flavonoids, phenolic and their esters [14]	3,3-Dimethyl allyl -caffeate [27]
Brazilian (Baccharis type)	Prenylated p-coumaric acid, labdane diterpenes [14]	Unidentified [10]	Prenylated P-coumaric acid, clerodane diterpenes, Benzofuran [10]	Prenylated P-coumaric acids, flavonoids, lignans, caffeoylquinic acids [10]	Prenylated P-coumaric acids, flavonoids [10]	Not tested
Cuban	Prenylated benzophenone [16]	Not tested	Prenylated benzophenone [16]	Unidentified [10]	Prenylated benzophenones [16]	Not tested
Taiwanese	Not tested	Not tested	Prenylated flavonoids [17-18]	Not tested	Prenylated flavonoids [18]	Note tested
Chinese	Not reported		Pinobanksin [19]	Not reported		
Greek	7-Prenyl pinocembrin (flavanone derivative) [20]	Not reported				

So it is one of the most versatile sources of which have tremendous possibility to be explored by natural product chemists in search of potential bioactive compounds and throughout the world, numerous studies are carried out with the combined efforts of phytochemists and pharmacologists to evaluate the chemistry and biological activities of propolis [1, 4].

The chemical composition and biological activities of propolis of various countries have been studied extensively by various scientific research groups, but only a few reports can be found on Indian propolis though there are huge references of works related to the uses of honey in Indian traditional medicine.

This motivated us to explore the antioxidant activity of Indian propolis and its chemical constituents. During literature survey it is evident that chemical composition of propolis which in turn depends on the geographical diversity is very crucial for its biological activity. To get an idea about the chemical composition we have conducted a detailed in-vitro antioxidant activity assay of the aqueous and ethanol extracts of Indian Propolis (AEP and EEP respectively) followed by isolation of some of its individual compounds. We used following three assay systems: (1). 1, 1- diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity assay, (2). and Ferric ion reducing antioxidant power (FRAP) assay. Total flavonoid and polyphenol contents of EEP and AEP were determined by standard colorimetric methods [21-22]. Furthermore, DPPH radical scavenging activity guided isolation gave two flavonoid components from EEP; which were pinocembrin and galangin. These two compounds were also subjected to DPPH radical scavenging assay.

2. Materials and Methods

2.1 Collection of Indian Propolis and its processing:

Indian propolis was collected from UPES Campus, Dehradun, Uttarakhand, India during December, 2021. It was put through a cold water washing process to remove extrinsic wax. The remaining propolis was then dried on stainless steel screens. The propolis was then ground and stored at 4 °C for further use.

2.2 Preparation of EEP

For the preparation of EEP, 30 gm of propolis was dissolved in 80% 100 ml ethanol and stirred for 24 h at 35 °C. Then, it was filtered through Whatman No. 42 filter paper to eliminate the residual mass. The filtrate (EEP) was subjected to evaporation under reduced pressure whereby a gummy mass was obtained which was stored at 4 °C for further use.

2.3 Preparation of AEP

To get AEP, 30 gm of propolis was refluxed in 100 ml water for 3 h; followed by filtration (Whatman No. 42) under hot condition. The extract (AEP) was lyophilized and a gummy mass was obtained which was preserved at 4 °C for further use.

2.4 Isolation of pinocembrin (1) and galangin (2) from EEP

The gummy mass obtained from EEP (section 4.2.1) was extracted with petroleum ether (PE) (100 ml X 5 times) to remove the low polar compounds. PE fraction of EEP was subjected to DPPH radical scavenging activity assay. But it did not show any positive response. The residual part of EEP was also subjected to the same test and it showed high DPPH radical scavenging activity. To separate out the individual components responsible for this radical scavenging activity, we have carried out further isolation of this part using column chromatography over silica gel (60-120 mesh). Out of large number of fractions we have successfully isolated (1) using [3:1] mixture of PE and ethyl acetate (EA) eluting agent. Crystalline (1) was obtained by the repeated fractional crystallization in PE and dichloromethane (DCM) [1:2] medium. Finally it was recrystallised in DCM medium. Compound (2) was obtained from the PE and EA [1:4] fraction. It was purified by repeated fractional crystallization in PE and EA [1:4] medium and finally it was recrystallised in ethyl acetate medium.

3. Result and Discussion

3.1 Total polyphenol and flavonoid content

The total polyphenol and flavonoid contents of AEP are

269.10 mg GAE $g^{-1}$and 25.50 mg QE g^{-1} respectively. Whereas that for EEP are 159.10 mg GAE g^{-1} and 57.25 mg QE g^{-1} respectively. Interestingly, AEP has higher polyphenol content while EEP has higher flavonoid content. Kumazawa *et al*. [25] previously reported that the polyphenol content of EEP of Europe and China was in the range of 200-300 mg GAE g^{-1}. The polyphenol content of AEP of Indian propolis is comparable with that reported data but polyphenol content of EEP was lower than the reported value [25].

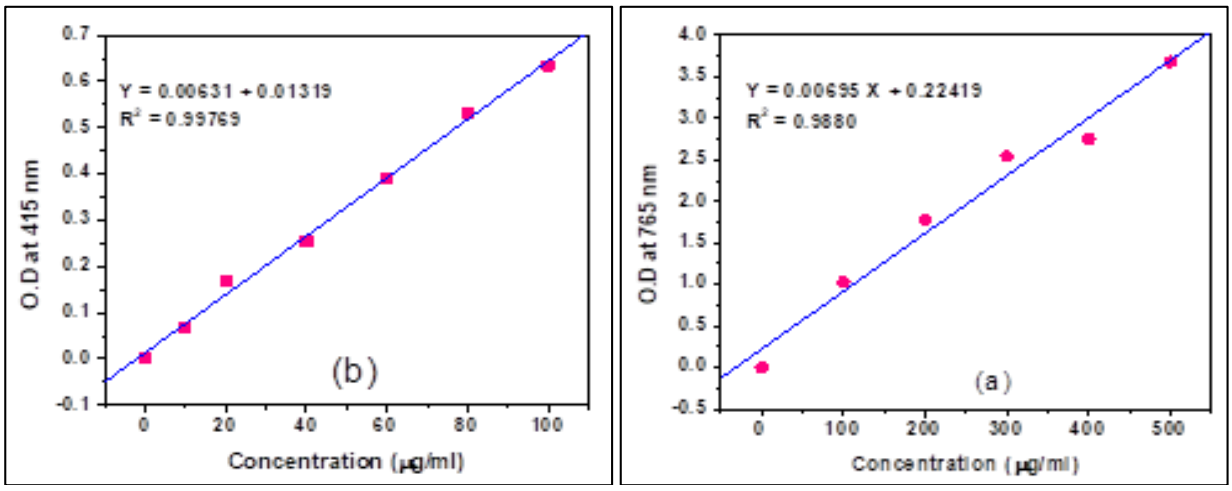

Fig 2: Calibration curve of (a) polyphenol content (Gallic Acid) and (b) flavonoid content (Quercetin)

Table 1: Polyphenol and Flavonoid content of AEP and EEP

Extract	Polyphenol content (mg GAE g^{-1})	Flavonoid content (mg QE g^{-1})
AEP	269.1 ± 0.17	25.5 ± 0.36
EEP	159.1 ± 0.26	57.2 ± 0.24

3.2 Ferric reducing antioxidant power assay

Reducing power of a compound is also a supporting feature for its antioxidant activity. Reducing power characteristics of AEP and EEP and ascorbic acid (standard compound) are given in Fig.2. The concentration dependent reducing power followed the order of: ascorbic acid > AEP > EEP. At lower concentration region, EEP showed slightly higher reducing power, but as a whole AEP had higher reducing activity. This may be due to the higher polyphenol content of this extract. Because being good electron donor, phenolic compounds have the ability to convert Fe^{3+} to Fe^{2+} and hence show higher reducing activity.

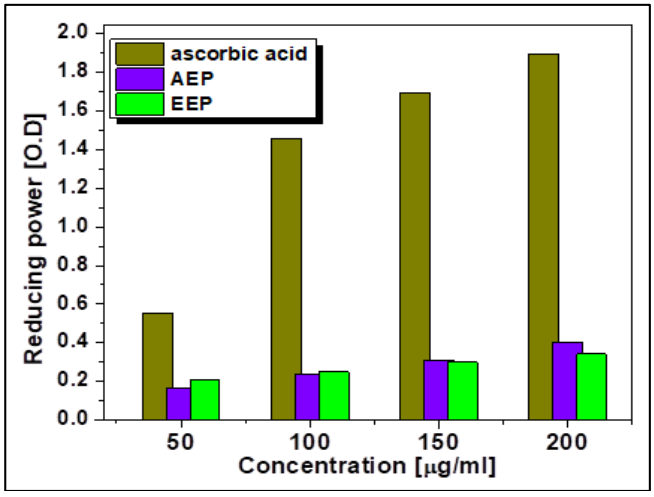

Fig 3: Reducing power of propolis extracts and ascorbic acid solutions at different concentrations (0 to 400 µg ml^{-1})

3.3 DPPH radical scavenging activity

The free radical scavenging activities of AEP, EEP and its isolated compounds (1) and (2) were evaluated through their ability to quench the synthetic DPPH radical. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The methodology involves reaction of specific compound or extract with DPPH in methanol solution. In the presence of hydrogen donors, DPPH is reduced and a free radical is formed from the scavenger. The reaction of DPPH is monitored by measuring the decrease of the absorbance of its radical at 517 nm. Upon reduction of this radical by an antioxidant, the absorbance at 517 nm disappears.

IC_{50} value of AEP was 0.05 mg ml^{-1} while that of EEP is 0.07 mg ml^{-1}. Both of these two values are comparable with the IC_{50} value of the standard compound, gallic acid; which was found to be 0.01 mg ml^{-1}. Thus AEP showed higher radical scavenging activity than that of EEP. Moreover, both of these two values are significantly lower than that of some Indian green leafy vegetables [26].

Table 2: IC_{50} value of propolis extracts, isolated compounds and standard compound

Sample	AEP	EEP	Pinocembrin	Galangin	Gallic Acid
IC_{50} (mg/ml)	0.05	0.07	8.42	0.05	0.01

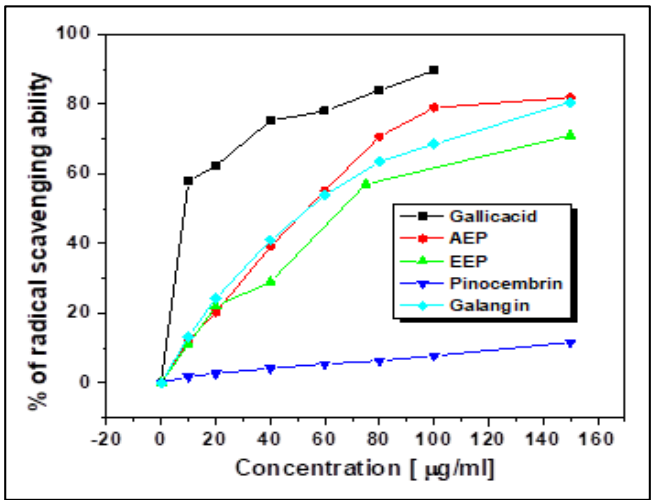

Fig 4: % of radical scavenging ability

The higher activity of AEP was probably due to its higher polyphenol content and also due to the better solubility of its polyphenol constituents in water. The IC_{50} value of pinocembrin is 8.42 mg ml^{-1} which is higher than that of galangin (0.05 mg ml^{-1}).

4. Conclusion

India is the country of biodiversity, has a number of varieties of propolis differing in chemical compositions and medicinal values. But, unfortunately it is still to be explored. We have collected propolis from Uttrakhand state simply because being in the Himalayan region; it is one of the most fascinating states of India with respect to its flowerings and medicinal plants. To our knowledge, this is the first report describing the antioxidant activity of Indian propolis extracts and its chemical constituents**.** In all the antioxidant assay systems which we have used here, AEP showed higher activity compared to the EEP. This may be due its very higher polyphenol content. So AEP can be a good substitute of ethanol extract. Moreover, it can be used in the prevention of various free radical related diseases.

5. References

1. Marucci MC. Apidologie; c1995. p. 26-83.
2. Nagaoka T, Banskota AH, Tezuka Y, Midorikawa K, Matsushige K, Kadota S. Biological and Pharmaceutical Bulletin**.** 2003;26:487.
3. Banskota AH, Teruka Y, Adnyana IK, Midosikawa M, Matsushige K, Kadota S. Phytomedicine. 2001;8:16.
4. Cuesta A, Rodriguez A, Esteban MA, Meseguer J. Fish and Immunology. 2005;18:71-80.
5. Teixeira EW, Negri G, Renata MSA Meira, Message D, Salatino A. eCAM. 2005;2(1):85-92.
6. Bankova V. eCAM. 2005;2(1):29-32.
7. Su Z, Lin J, Prewett M, Goldstein NI, Fisher PB. Anticancer Res. 1995;15:1841-1848.
8. Frankel K, Wei It, Bhimani R, Ye J, Zadunaisky JA, Huang MT *et al.* Cancer Research. 1993;53:1255-1261.
9. Marquez N, Sancho R, Calzado MA, Feibich BL, Munoz E. J. Pharmcol, Expt. Theapetics. 2004;308(3):993-1001.
10. Lin MW, Yang SR, Huang MH, Wu SN. J. Biol. Chem. 2004;279(26):26885-26892.
11. Ito J, Chang F, Wang H, Park YK, Ikegaki M, Kilgore N, *et al*, J. Nat. Prod., 2001;64:1278-1281.
12. Banskota AH, Nagaoka T, Sumioka LY *et al*., J. Ehtnopharm. 2000;80:67-73.
13. Burdock GA. Food Chem. Texicol. 1998;36:347-363.
14. Banskota AH, Tezuka Y, Kadota S. Phytother. Res. 2000;15:561-571.
15. Grunberger D, Banerjee R, Eisingu K *et al*., Experientia. 1988;44:230-232.
16. Hernandez NMR, Cuesta OR, Aviles A, Avelianede DLS. Rev. Cubana Farm., 2001;35(Suppl. Especial):197-199.
17. Cuesta OR, Uriba Frontana BA, Ramirez TA, Cardenas J. Z. Naturforsch., 2002;517C:372-378.
18. Chen CH, Weng M, Wu CH, Lin J. eCAM., 2004;1:175-185.
19. Usia T, Banskota AH, Teznka YA, Midorikawa K, Matsushige K, Kadota SH. J. Nat. Prod., 2002;65:673-676.
20. Milliou E, Chinou I. Planta Med., 2004;70:515-519.
21. Chang C, Yang M, Wen H. J. Chern, 2002;10:178.
22. Mcdonald S, Prenzler PD, Autolovich M, Robards K. Food Chem. 2001;73:73.
23. Jaipetch T, Kanghae S, Pancharoen O, Patrick VA, Reutrakul V, Tuntiwachwuttikul P, White AH. Australian Journal of Chemistry. 1982;35:351.
24. Li XC, Joshi AS, ElSohly HN, Khan SI, Jacob MR, Zhang Z *et al*. Cihlaro, Journal of Natural Products. 2002;65:1909.
25. Kumazawa S, Hamasaka T, Nakayama T. Food Chemistry. 2004;84:329.
26. Gupta S, Prakash J. Plant foods and Human Nutrition. 2009;64:39.
27. Zielinska D, Szawara-Nowak D, Ornatowska A, Wiczkowski W. J. Agric. Food Chem. 2007;55:9891.