Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 6, Issue, 03, pp.5351-5354, March, 2014

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

EVALUATION OF THERAPEUTIC PROPERTIES OF PROPOLIS OBTAINED FROM *APIS MELLIFERA* (L) IN INDIA

***[1]Rajesh Kumar, [2]Gajendra Singh Rajpoot and [2]Reenu Chaudhary**

[1]School of Basic Sciences, Department of Bio-Sciences, Arni University, Kangra – 176401 (H.P.), India
[2]Entomology Research Unit, School of Studies in Zoology, Jiwaji University, Gwalior-474011 (M.P.) India

ARTICLE INFO

Article History:
Received 14th December, 2013
Received in revised form
20th January, 2014
Accepted 17th February, 2014
Published online 25th March, 2014

Key words:

Propolis, *Apis mellifera*, India, Antioxidant, Quercetin, Flavnoids.

ABSTRACT

Propolis is a natural composite balsam, collected by honey bees from the buds of various plants. Propolis collected from beekeepers was tested for it's antioxidant & free radical scavenging activities. Propolis was found to have polyphenol, flavnoids and quercetin substance in abundance, which possess properties like anti-inflammatory, anti-cancer, anti-allergic, anti-toxic and antioxidant properties. The aim of present study is to study therapeutic activities in Indian propolis obtained from *Apis mellifera* L.

INTRODUCTION

Honey bees are one of the most beneficial organisms for human beings. Bee hive products are interesting for their nutritional value as well as their healing, regenerating and cosmetic properties and attributes. Propolis, an important hive product, is a natural resinous substance collected by bees from parts of plants, buds and exudates (Ghisalberti, 1979). Bees use this material to seal hive (Garcia-Viguera *et al*., 1992), to strengthen the border of the combs, and embalm dead invaders (Marcucci, 1995) and more importantly, to prevent decomposition of creatures which have been killed by bees after an invasion of the hive (Brumfitt *et al*., 1990). It is believed that propolis not only hardens the cell walls but also contributes to the attainment of an internal aseptic environment (Ghisalberti, 1979). Propolis is well known for their antioxidative, antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant, immunostimulating, antitumor, anti-cancer, anti-allergic etc. Propolis exerts its beneficial effects as a natural antioxidant due to the presence of polyphenols and flavonoids found in it. It contains 150 polyphenolic compounds, which contribute to its therapeutic efficacy. It is found effective in the treatment of various diseases related to respiratory infection, viral infection, ear infection, dental treatment, inflammation, gynecological diseases, cardiovascular diseases, skin diseases etc.

Corresponding author: Rajesh Kumar
School of Basic Sciences, Department of Bio-Sciences, Arni University, Kangra – 176401 (H.P.), India.

Since ancient times (300 BC) propolis has been used in traditional medicine and it is still used as a remedy in folk medicine and as a constituent of cosmetics in many parts of the world. Due to various properties of propolis, it has been realized that it is a biologically active natural product that can be used for a variety of therapeutic and health promoting purposes. Chemical composition of propolis may vary due to different factors such as the geographical region, collecting time, methods of collection and plant source (Bankova *et al*., 2002; Sforcin *et al*., 2000). In general, the Propolis incorporates resins and balsams (40-50%), beewax (25-30%), volatile essential oils (10%) and pollen, mineral and other substances. (Ghisalberti *et al*.,1978). Several investigations have been made on therapeutic properties of propolis such as antibacterial (Kartal *et al*., 2003) anti-inflammatory, healing, anesthetic (Ghisalberti, 1979), anticariogenic (Park *et al*., 1999), antifungal (Prytzyk *et al*., 2003), antiprotozoan and antiviral (Guler *et al*., 2003) activities. There are only few reports on the composition of sample of Indian Propolis. However, a few Indian workers have studied biological and therapeutic properties of Indian samples of Propolis (Bhadauria *et al*., 2007a, b).

MATERIALS AND METHODS

Samples of propolis were procured from beekeepers of Morena, Gwalior (M. P.). First of all, wax was separated from the crude propolis. For this, prpoplis was heated in water bath at 60ºC for a period of 7-8 hours per day for 15 days. De-waxed propolis was extracted in ethanol according to the method of Alrarez *et al*., (1989) (Fig. 1).

Propolis sample

Propolis sample

Residue

Figure 1. Showing the propolis samples (Crude, Extracted & Residue)

Analysis of Propolis extract with the help of the TLC method with the help of: TLC aluminium sheets 20 × 20 cm, Silica gel 60 F_{254} with concentrating zone 20 × 2.5 cm, (Merck KGaA, Darmstadt, Germany). Samples were prepared by mixing propolis extract and quercetin (standard) with methanol. A mixture of petroleum ether and ethyl acetate in the ratio of 7:3 was also prepared for the mobile phase of the chromatography. After loading the sample and standard on the chromatographic plate the samples was run up to the top of the chromatographic plate and get dried under the room temperature for the 5 minutes. Visualisation of the TLC plate takes place in the U.V. chamber at 365nm. Total polyphenol content in extract of Propolis was estimated by Folin-Ciocalteau colorimetric method. Extract solution 0.5 ml (1mg/1ml ethanol) were mixed with 2.5 ml of the Folin-Ciocalteau reagent (1:10) and 2.0 ml of 4% Na_2CO_3 (3 replicates). Absorbance was measured at 740 nm after 2 hour incubation at room temperature, in the dark. Total flavonoid content in the Propolis extract was analyzed by the method proposed by Park *et al.*, 1999, with minor modifications. For this, 0.5 ml of EEP, 4.3 ml of 80% ethanol, 0.1 ml of 10% Aluminium nitrate and 0.1 ml of 1M aqueous potassium acetate was mixed in a test tube, then the absorbance was determined spectrophotometrically at 415 nm after 40 min. incubation at room temperature, in the dark. Free radical-scavenging activity of Propolis was measured by DPPH assay (Blios, 1958). 1 ml of various concentrations of the sample (10-50 µg/ml) was added to 2ml distilled water and 1 ml of DPPH solution (0.1 m mol). Absorbance at 517 nm was determined after 30 min and the inhibition was calculated by the formula given below

DPPH scavenging effect (%) = Ao – A_1 / Ao X 100

Where Ao was the absorbance of the control and A_1 was the absorbance in the presence of the sample.

RESULTS

Results obtained during the study have been described as follows. Thin Layer Chromatography was done on the chromatographic plates with the propolis extract and it was observed that the ethanolic extract of propolis contains quercetin substance. On the basis of results of this experiment it was concluded that Propolis sample contains the quercetin which is a flavonoid and is responsible for the antioxidant & anti-inflammatory activity of the Propolis (Fig. 2).

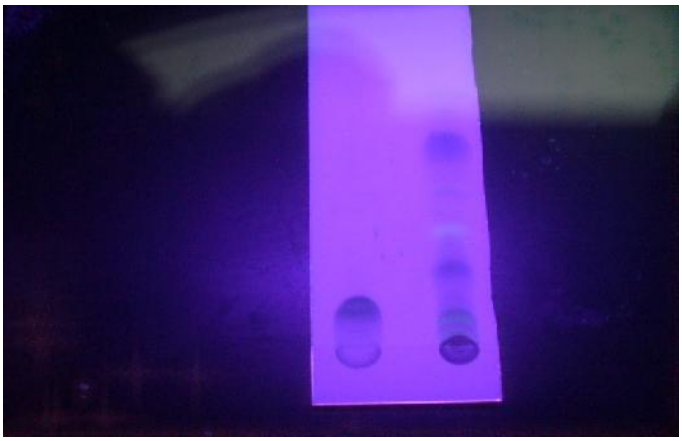

Figure 2. Chromatographic view of quercetin (left) and Propolis sample (right)

Results revealed the presence of 55mg of flavonoids in 1g of Propolis extract as the results being compared with the quercetin (Standard). Absorbance was recorded to be 0.727, 0.686 and 0.696 in test tube 1, 2 & 3 respectively at 415 nm. It has strong antioxidant properties and useful in the treatment of various cardiovascular, inflammatory and other diseases (Table 1). It was observed that the propolis sample contains 450 mg of polyphenols in 1g of propolis extract after comparing it with tannic acid (Standard). Absorbance was recorded as 0.542, 0.332 and 0.432 in test tube 1, 2 & 3 respectively at 740 nm. It was concluded that the propolis sample contains the abundant amount of polyphenol, which has strong antioxidant properties (Table 2). Propolis was tested for antioxidant activity using DPPH dye at different concentrations (10-50 µg/ml). The antioxidant capacity of propolis showed significant inhibition of DPPH free radical in dose dependent manner and found maximum 97% DPPH inhibition (10 µg/ml) followed by 96% (20 µg/ml), 96% (30 µg/ml), 94% (40 µg/ml) and 93% (50 µg/ml) respectively. Per cent DPPH inhibition of Vitamin C (standard) was found to be 71% (Table 3, Fig. 3).

Table 1. Absorbance in the propolis extract (triplicates) for flavonoid estimation

Sr. No.	Wavelength	Absorbance
Test Tube 1	415 nm	0.727
Test Tube 2	415 nm	0.686
Test Tube 3	415 nm	0.696

Table 2. Absorbance in the propolis extract (triplicates) for polyphenol estimation

Sr. No.	Wavelength	Absorbance
Test Tube 1	740 nm	0.542
Test Tube 2	740 nm	0.332
Test Tube 3	740 nm	0.423

Table 3. DPPH scavenging effect at different concentration of Propolis and Vitamin C

Sr. No.	Concentration	DPPH Scavenging Effect (%)
Propolis	10 µg	97%
Propolis	20 µg	96%
Propolis	30 µg	96%
Propolis	40 µg	94%
Propolis	50 µg	93%
Vitamin C (Standard)	10 µg	71%

DISCUSSION

Present observations made on the TLC of the ethanolic extract of propolis. propolis sample shows the presence of flavonoid in it like quercetin. Flavonoids are the most common anti-oxidant due to the presence of caffeic acid phenethyl ester (CAPE) thus preventing the integrity of liver cell membrane and thereby protecting the hepatic drug metabolizing enzymes and can act at the initiation stage of lipid peroxidation as scavengers, which may react with peroxy radicals of polyunsaturated fatty acids (PUFAs) breaking the chain reaction. Flavonoids is also found effective against different micro-organisms i.e. it has antimicrobial, antifungal and antibacterial properties. In addition to this, it is also found effective as an anti-inflammatory drug, it is a preventive drug against cancer, allergy, heart diseases and diabetic patients. It is observed that the flavonoids concentrated in propolis are powerful antioxidants, and have been shown to be capable of scavenging free radicals and thereby protecting lipids and other compounds such as Vitamin C from being oxidised or destroyed (Popeskovic *et al*., 1980). Kumazawa *et al*. (2004) also reported that the polyphenol content in Brazilian propolis is different from that of Europe and China. Moreira *et al*., 2008 investigated that Bornes propolis of Portugal showed the high amount of polyphenolic compounds, with 329.00 mg/g of gallic acid equivalents, twice the value found in the Fundão propolis (151.00 mg/g of gallic acid equivalents). According to (Alyane *et al*., 2008) study showed that total flavonoid concentration was 370 mg in the raw propolis sample. The flavonoid content of Brazilian propolis was 51.9 at 210 specific absorbance (Bankova *et al*., 2000). Korean propolis have flavonoid content 48.2, 56.5, 73.5, 77.9 at specific absorbance 297, 362, 346, 553 respectively. Earlier reports indicated that the flavonoids are phenolic compounds that exert multiple biological effects, including antioxidant properties and free radical scavenging abilities.

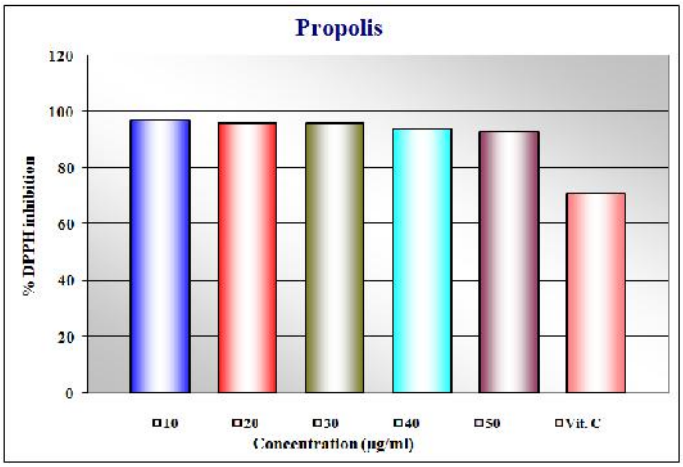

Figure 3. % DPPH inhibition as compared with positive control *i.e.* Vit. C (10 µg/ml)

Conclusion

It can be concluded at the end of study that propolis contains several therapeutic/pharmaceutical properties and can be used in the preparation of pharmaceutical drugs.

Acknowledgement

Authors are highly thankful to Dr. O. P. Agrawal for guidance during research work.

REFERENCES

Alyane, M., Benguedouar, L., Kebsa, W., Boussenane, H. N., Rouibah, H and Lahouel, M. 2008. Cardioprotective effects and mechanism of action of polyphenols extracted from propolis against doxorubicin toxicity. *Pakistan Journal of Pharmaceutical Science.*, 21**:** 201-209.

Bankova, V., Castro, S. L., Marcucci and M. C. 2000. Propolis: Recent advances in chemistry and plant origin. Apidologie., 31: 3-15.

Bhadauria, M., Nirala, S. K. and Shukla, S. 2007a. Propolis protects CYP2E1 enzymatic activities and oxidative stress induced by carbon tetrachloride. *Molecular and Cellular Biochemistry*., 302**:** 215–24.

Bhadauria, M., Nirala, S. K. and Shukla, S. 2007b. Duration dependent hepatoprotective effect of propolis extract against carbon tetrachloride induced acute damage in rats. Advances in Therapy., 24: 1134–43.

Blois, M. S. 1958. Antioxidant determinations by the use of a stable free radical. Nature., 181: 1199–1200.

Brumfitt, W., Hamllton-Miller, J. T. M. and Franklin, L. 1990. Antibiotic activity of natural products: 1, Propolis. Microbios., 62: 19-22.

Garcia-Viguera, C., Greenaway, W. and Whatley, F. R. 1992. Composition of propolis from two different Spanish regions. ZNaturforsch., 47(C): 634-637.

Ghisalberti, E. L. 1990. The pharmacological activity of propolis (Romanian), in *Romania* apicola, p.29 (transalation by Marlen Petrica from Bee World, 1979, pp.59-84).

Ghisalberti, E. L., Jefferies, P. R., Lanteri, R. and Matisons, J. 1978. Constituents of propolis. Experientia. 34**:** 157-158.

Guler, P., Sorkun, K. and Salih, B. 2003. Effect of some Turkish propolis on the product quantity of *Agaricus bisporus* (Lange.) Syng. *Pak. J. Botany*., 35(3): 439-447.

Kartal, M., Yildiz, S., Kaya, S., Kurucu, S. and Topcu, G. 2003. Antimicrobial activity of pro- polis samples from two different regions of Anatolia. *Journal of Ethanopharmacology*. 86: 69-73.

Kumazawa, S., Hamasaka, T. and Nakayama, T. 2004. Antioxidant activity of propolis of various geographic origins. Food Chemistry. 84: 329–339.

Marcucci, M. C. 1995. Propolis: chemical composition, biological properties and therapeutic activity. Apidologie. 26: 83-99.

Moreira, L., Luís, G., Dias, Pereira, J. A. and Estevinho, L. 2008. Antioxidant properties, total phenols and pollen analysis of propolis samples from Portugal. *Food and Chemical Toxicology*., 46: 3482–3485.

Park, E. H. and Kahng, J. H. 1999. Suppressive effects of propolis in rat adjuvants arthritis. *Archives of Pharmacological Research*. 22(6): 554-558.

Popeskovic, D., Kepcija, D., Dimitijevic, D. and Stojanovic, N. 1980. The antioxidative properties of propolis and some of its components. Acta veterinaria (Beograd)., 30:133–136.

Prytzyk, E., Dantas, A. P., Salomao, K., Pereira, A. S., Bankova, V. S., De Castro, S. L. and Aquino Neto, F. R. 2003. Flavonoids and trypanocidal activity of Bulgarian propolis. *Journal of Ethanopharmacology*. 88: 189-193.

Sforcin, J. M., Fernandes, Jr., Lopes, C. A. M., Bankova, V. and Funari, S. R. C. 2000. Seasonal effect on Brazilian propolis antibacterial activity. *Journal of Ethanopharmacology*. 73: 243-249.

****