

## FREE RADICAL SCAVENGING AND ANTI-INFLAMMATORY ACTIVITY OF INDIAN PROPOLIS

R. Ambardekar<sup>a</sup>, S. Gilda<sup>a</sup>, K. Mahadik<sup>a</sup>, A. Harsulkar<sup>a</sup> and A. Paradkar<sup>a,b\*</sup>

<sup>a</sup> Department of Pharmaceutics, Bharati Vidyapeeth University, Poona College of Pharmacy, Erandwane, Pune - 411 038, Maharashtra, India.

<sup>b</sup> Institute of Pharmaceutical Innovation, University of Bradford, Bradford, BD7 1DP, UK.

\*Corresponding author

Phone: +91-20-25437237

Fax: +91-20-25439383

Email: arparadkar@rediffmail.com

### Summary

Propolis, a natural product produced by the honeybee, has been used for thousands of years in folk medicine for several purposes. Chemical composition of propolis is known to alter with geographical origin leading to variation in pharmacological activities. Propolis from various geographical origins have been evaluated and screened for antioxidant, anti-microbial, anticancer, anti-inflammatory, Hepatoprotective, etc activities. Despite of these reports no attempt has been ever made in screening the effectiveness of propolis from Indian origin. Therefore, in this work, we have investigated the antioxidant and anti-inflammatory activity of Indian propolis. Ethanolic, aqueous and hydro-alcoholic extract of propolis were screened and compared for anti-oxidant potential by various *in-vitro* anti-oxidant models such as, DPPH, Nitric oxide and Hydrogen peroxide scavenging assay alongwith Anti Lipid Peroxidation potential. Further the total polyphenol content using Folin ciocalteau method and Flavanoid contents by aluminum chloride colorimetric method, were estimated for Indian propolis extract. Anti-inflammatory activity of ethanolic extract of Indian propolis was checked in rat paw edema model. All the three studied extracts of Indian propolis were found to be capable of *in vitro* scavenging following free radicals DPPH, nitric oxide and hydrogen peroxide. These extracts showed strong anti-lipid peroxidation potential. IC<sub>50</sub> values calculated for each type of extract for above mentioned assays showed that ethanolic extract has highest potency followed by Hydro-alcoholic extract and aqueous extract sequentially. Ethanolic extract showed positive results for anti-inflammatory activity in rat paw edema model. It can be concluded from above results that Indian propolis extract possess good anti-oxidant and anti-inflammatory potential.

**Key words:** Indian propolis, polyphenol, flavonoid, anti-oxidant, rat paw edema.

## 1. Introduction

Propolis is a sticky resin that seeps from the buds of some trees and oozes from the bark of trees, mainly conifers. The bees gather propolis, sometimes called bee glue, and carry it home in their pollen baskets. They blend it with wax flakes secreted from special glands on their abdomens. With its antiseptic properties, this Propolis lining ensures a hospital-clean environment for the rearing of brood. Propolis is not only a building material; it is the most important anti-microbial agent of bees against pathogenic microorganisms and has been used as a remedy by humans since ancient times. Because of its potent medicinal applications, propolis has attracted many researchers for screening of pharmacological as well as chemical studies. Many studies have proven its significant pharmacological activities: antibacterial, antifungal, antiviral, anti-inflammatory, hepatoprotective, antioxidant, antitumor, etc. [1].

Propolis contains a variety of chemical compounds such as polyphenols (flavonoid aglycones, phenolic acids and their esters, phenolic aldehydes, alcohols and ketones), sesquiterpene quinones, coumarins, steroids, amino acids and inorganic compounds. Propolis samples contain more than 160 constituents, and differs greatly due to variation in its geographical and botanical origin [2-3].

It has been suggested that the biological activities of propolis mainly depend on the presence of a large number of flavonoids [4]. These are reported to have antioxidant [5], anti-inflammatory, anticancer and antiviral activities, as well as antimicrobial effects [6].

Major of these activities have been attributed to caffeic acid phenethyl ester (CAPE). CAPE inhibits the lipoxygenase pathway of arachidonic acid, resulting in anti-inflammatory activity [7]. CAPE is also known to have anticarcinogenic, antimutagenic and immunomodulatory properties. CAPE has been found to inhibit the activation of the nuclear transcription factor NF-Kappa B by tumor necrosis factor (TNF), as well as by other pro-inflammatory agents. The inhibition of NF-Kappa B activation may provide the molecular basis for its immunomodulatory and anti-inflammatory activities [8]. Although CAPE has been considered as vital component, propolis as whole has shown to be more active than isolated CAPE due to synergistic activity of various other minor components [9].

The biological activities of propolis have been studied extensively in Europe, mainly in eastern countries but available literature indicates that no previous studies have been done on Indian propolis and there are no reports on their chemical constituents. The objective of the present report is to investigate and compare the antioxidant properties of ethanolic, hydro-alcoholic and aqueous extracts of Indian propolis. In this study, total flavonoid content was determined by aluminum chloride colorimetric method. Further Folin ciocalteau assay was used for determination of total polyphenol content. We have used scavenging of diphenyl picryl-hydrazyl, a colorimetric method for free radical-scavenging activity determination, nitric oxide scavenging activity which involves generation and detection of nitrite ions in accordance with Griess Illosvoy reaction. We have also checked *in-vitro* Anti-lipid Peroxidation potential. Further ethanolic extract which has been proven best anti-oxidant agent was tested for its *in-vivo* anti-inflammatory activity using carragenan induced rat paw edema model.

## 2. Materials:

Propolis samples were received from Central Bee Research and Training Institute, Ganeshkhind Road, Pune, India. Folin-phenol Reagent was purchased from Qualigens. Quercetin was from Sigma USA.1, 1-Diphenyl-2-picrylhydrazal (DPPH) and Gallic Acid were obtained from Fluka. Sodium nitroprusside, Butylated hydroxy anisole (BHA), ascorbic acid, Iron (III) chloride

(FeCl<sub>3</sub>), Sodium nitroprusside, Trichloroacetic acid (TCA), Hydrogen peroxide was purchased from Merck; Phosphoric acid, potassium chloride was purchased from SD Fine Chem. Ltd. N-1-Naphthylethylenediamine dihydrochloride was from Unichem, Sulphanilamide from SISCO research Laboratories Pvt. Ltd., and Sodium nitrite, Potassium Ferricyanide was from Thomas Baker & Co. All reagents used were of analytical grade.

### **3. Methods:**

#### **3.1 Preparation of ethanolic extract of propolis (EEP)**

20 g of finely ground propolis was extracted with 400 ml of absolute ethanol in a Soxhlet extractor for 24 hours at a maximum temperature of 60 °C. The extract thus obtained was vacuum filtered and the filtrate was refrigerated at 4°C overnight in order to crystallize waxes. The filtrate thus obtained was vacuum filtered again and the solvent was evaporated in rotary evaporator. The dry mass obtained was collected and was further assessed for antioxidant activities [10].

#### **3.2 Preparation of aqueous extract of propolis (WEP)**

Aqueous extract of propolis was obtained as described by Nagai et al [11], with slight modification. In brief, 100.0 g of propolis was suspended and extracted with 10 volumes of distilled water with shaking at 80 °C for 1 day. The extracts were vacuum filtered using buchner assembly and filtrates were pooled. The residue was re-extracted under the same conditions. Finally all the extracts were pooled together and solvent was evaporated using rotary evaporator. The dry mass obtained was collected and 100 ml of 10 mg/ml stock solution was prepared. This solution was utilized to prepare different concentration of extract for further assessments.

#### **3.3 Preparation of hydroalcoholic extract of propolis (HAP)**

Hydro-alcoholic extract of propolis was obtained by similar method as described for aqueous extract but with modification in extracting solvent composition as 50% ethanol was used as solvent instead of water and the procedure was carried out at 40 °C.

#### **3.4 Total polyphenol content**

The Folin–Ciocalteu colorimetric method was used to determine total polyphenol contents in extracts of propolis. 0.5 ml of extract solution was mixed with 0.5 ml of the Folin–Ciocalteu reagent and 0.5 ml of 10% Na<sub>2</sub>CO<sub>3</sub>, and the absorbance was measured at 760 nm after 1 h incubation at room temperature. Total polyphenol contents were expressed as mg/g gallic acid equivalents.

#### **3.5 Total Flavonoid content**

Total flavonoid contents were determined using the aluminium chloride colorimetric method. To 0.5 ml of extract solution, 0.5 ml of 2% AlCl<sub>3</sub> ethanol solution was added. After 1 h at room temperature, the absorbance was measured at 420 nm. Total flavonoid contents were calculated as quercetin (mg/g) from an analytical curve. The amount of 2% aluminium chloride was substituted by the same amount of distilled water in blank [12].

#### **3.6 Free radical scavenging activity by DPPH method:**

One ml Different concentrations of extract solution and standard were taken in different vials. To this 5 ml of methanolic solution of DPPH was added, shaken well and mixture was incubated at 37 °C for 20 min. Absorbance was measured against methanol as blank at 517 nm.

Take absorbance of DPPH as control. Percent antiradical activity can be calculated by using following formula [13];

$$\% \text{ Anti-radical activity} = (\text{Control Abs} - \text{Sample Abs.}) \times 100 / \text{Control Abs}$$

### 3.7 Nitric Oxide Scavenging Activity:

Nitric oxide radical scavenging activity was determined according to following method [14]. 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations and the mixture incubated at 25 °C for 150 min. From the incubated mixture 0.5 ml was taken out and added into 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally, 1.0 ml naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min. The absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radicals scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = (A_0 - A_1) / A_0 \times 100$$

Where  $A_0$  was the absorbance of the control (blank, without extract) and  $A_1$  was the absorbance in the presence of the extract.

### 3.8 Hydrogen peroxide scavenging assay

Hydroxyl radical generated from hydrogen peroxide ( $H_2O_2$ ) within body has major role in inflammation. Hydrogen peroxide is generated as a result of various enzymatic reactions in biological system, due to unstable nature it dissociates into hydroxyl radical and nascent oxygen atom. Hydroxyl radicals as well as nascent oxygen has important role in oxidative damage to cellular machinery. Hence removal of hydrogen peroxide is vital in antioxidant defense of biological cell. The ability of propolis extracts to scavenge  $H_2O_2$  was determined based on the method of Ruch et al [15]. 2 ml of various concentrations of propolis extracts were mixed with 1 ml of  $H_2O_2$  solution (10 mM) and incubated at 37 °C for 10 minutes. Absorbance of reaction mixture was determined at 260 nm against a blank solution containing same extract solution without  $H_2O_2$ , while control absorbance of  $H_2O_2$  (10 mM) was taken against distilled water as blank. Here Ascorbic acid was taken as standard reference. The percentage of  $H_2O_2$  scavenging of both the extract and standard compound were calculated as follows

$$H_2O_2 \text{ Scavenged (\%)} = \frac{(A_{\text{cont.}} - A_{\text{test}})}{(A_{\text{cont.}})} \times 100$$

Where,  $A_{\text{cont.}}$  = Absorbance of control reaction

$A_{\text{test}}$  = Absorbance of test reaction

As most of compounds having hydroxyl group, and hence most phyto-extracts absorbs at 260nm, same extract solution (or ascorbic acid solution in case of reference) without  $H_2O_2$  was utilized as blank.

### 3.9 *In vitro* anti – lipid Peroxidation assay.

A modified thiobarbituric acid reactive species (TBARS) assay was used to measure the lipid peroxide formed using liver tissue homogenate as lipid media. Lipid peroxidation was induced by ferric chloride. Percentage inhibition of lipid peroxidation by different concentrations (100-500µg/ml) of propolis extracts were calculated. The liver 10 percent (w/v) homogenate was prepared using a tissue homogenizer under ice-cold conditions. The mixtures containing 0.5 ml of homogenate, 1 ml of 0.15 M KCl, and 0.5 ml of different concentrations (100-500µg/ml) of propolis extracts were prepared. Along with this ascorbic acid were taken in different vials as standard. Lipid peroxidation was initiated by adding 100 µl of 1mM ferric chloride. The reaction mixtures were incubated for 30 min at 37°C. After incubation, the reaction was stopped by adding 2 ml of ice-cold 0.25 N HCl containing 15percent trichloroacetic acid (TCA) and 0.38percent thiobarbituric acid (TBA) and 0.2 ml of 0.05 percent butylated hydroxyl toluene (BHT). These reaction mixtures were heated for 60 min at 80°C, cooled and centrifuged at 5000 g (≈6900 rpm) for 15 min. The absorbance of the supernatant was measured at 532 nm against a blank, which contained all reagents except liver homogenate and propolis extracts. Identical experiments were performed to determine the normal (without drug and FeCl<sub>3</sub>) and induced (without drug) lipid peroxidation level in the tissue. The percentage of anti-lipid peroxidation effect (percent ALP) was calculated by the following formula [16].

$$\text{Percent ALP} = [A_{\text{FeCl}_3} - A_{\text{test}} / A_{\text{FeCl}_3} - A_{\text{Normal}}] \times 100$$

Where, A<sub>FeCl<sub>3</sub></sub>: Absorbance of FeCl<sub>3</sub>,

A<sub>Normal</sub>: Absorbance of control reaction, A<sub>test</sub>: Absorbance of test reaction.

### 3.10 Anti-inflammatory study:

**Animals:** Wistar rats of either sex weighing 200 g, respectively, were purchased from National Toxicological Centre, Pune. They were housed in polypropylene cages at 25± 2°C with relative humidity of 45-55 % under 12 hours light and 12 hours dark cycles and had unlimited access to food and water. Experimental procedures were conducted in accordance with the regulations of Animal Ethical committee (CPCSEA/ 41/ PCP/2008-09).

**Toxicity study:** Toxicity testing was carried out according to OECD guidelines. For acute toxicity studies the mice were divided into four groups each containing 10 mice and they were treated with graded doses. After treatment the animals were observed for behavior changes and their mortality. From the study it was revealed that the ethanolic extract was found to be safe up to 5 g/kg orally since there was no mortality. Further work was carried out by selecting doses as 0.5 g/kg and 1 g/kg b. w. orally.

**Rat Paw edema Model:** The animals were maintained under standard environmental conditions and had free access to standard diet and water. Anti-inflammatory activity was measured using carrageenan induced rat paw edema model [17-18]. Groups of 6 rats were given a dose of the extract (EEP was suspended in sterile distilled water using clinically approved quantity of SLS (1mg/100ml), and administered orally at different dose levels namely 1g/kg and 0.5g/kg). After 1h, 0.1 ml, 1% carrageenan suspension in 0.9% NaCl solution was injected into the sub-plantar tissue of the hind paw. The linear paw circumference was measured at time interval (minutes) of 0, 30, 60, 120, 180, 240, 360 [19]. Anti-inflammatory activity was measured as the percentage reduction in edema level. Dichlofenac was used as a standard at 10 mg/kg [20].

### 3.11 Statistical analysis

All data were expressed as mean  $\pm$  SD and one-way ANOVA was applied to determine the significance of the difference between the control groups and rat treated with the test compounds. GRAPHPAD (Instat USA Ver. 3) Statistical software was used for analysis of data obtained.

## 4. Results and discussion

### 4.1 Total Polyphenol and Flavanoid contents

A series of experiments were performed in an attempt to evaluate the antioxidant activity of aqueous, hydroalcoholic and ethanolic extracts of Indian propolis in order to show the ability of these extracts in scavenging different radicals in different systems. The antioxidant activity may be related to polyphenol and flavonoid content since it has been reported that these phenolic compounds can act breaking the chain reaction of lipid peroxidation, inhibiting chemiluminescence reactions, scavenging several ROS, etc. The Folin–Ciocalteu and the  $AlCl_3$  coloration method were used to determine the total polyphenol and flavonoid contents, respectively. The activity of these propolis extracts was compared against that of gallic acid, which was used as standard. The results were expressed in terms of mg/g of gallic acid equivalent (GAE) units, which is defined as amount of standard (in mg) which gives same number of polyphenols present in 1 g of extract. In the present study, these methods were applied to determine total polyphenol and flavonoid contents of these extracts of Indian propolis. Extracts of propolis, collected from different geographical regions may get differ in respective composition and concentration.

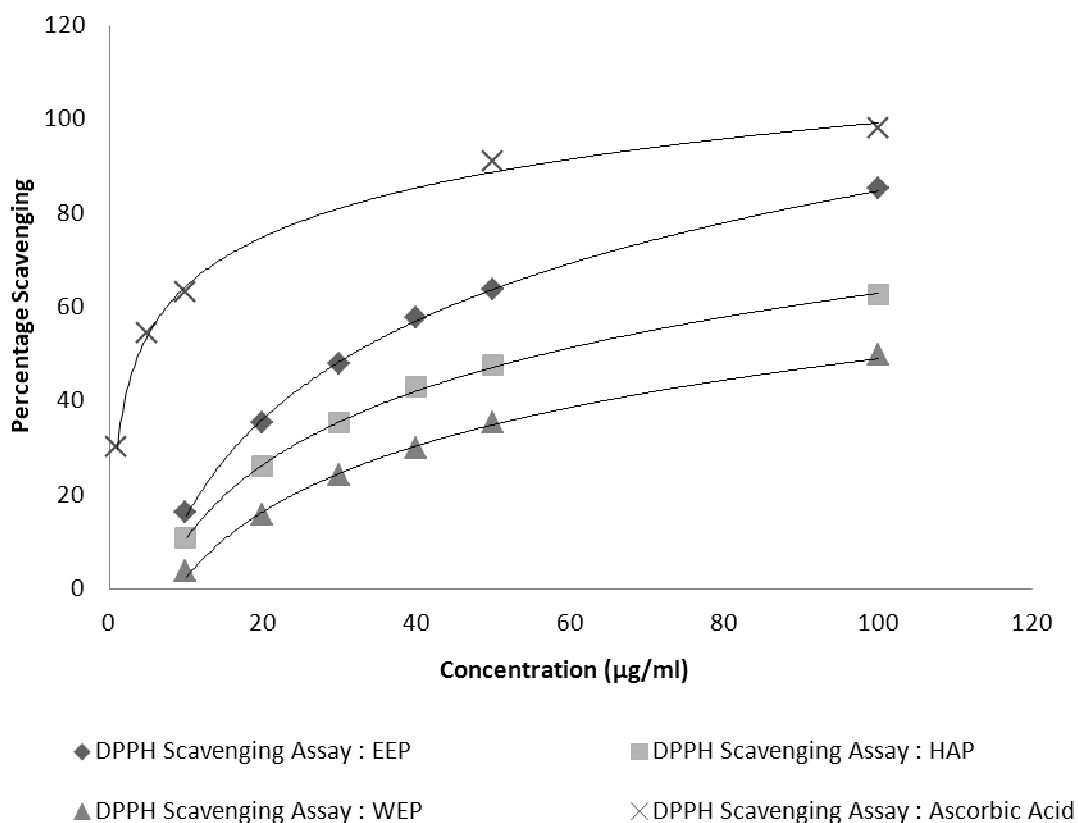
The flavonoid content in these extracts was determined using  $AlCl_3$  coloration method. Quercetin was used as standard and the activities of these extracts were compared with that of quercetin. The results were expressed as mg/g Quercetin Equivalent Unit (QAE), which is defined as amount of standard (in mg), which gives same number of flavonoids present in 1 g of extract.

Total Polyphenol contents of EEP, HAP, WEP were  $48.59 \pm 1.8357$  mg/g,  $29.10 \pm 0.3462$  mg/g,  $23.88 \pm 0.9951$  mg/g of Gallic acid respectively while total Flavanoid contents of the same were  $31.23 \pm 1.9275$  mg/g,  $17.99 \pm 0.1839$  mg/g,  $13.86 \pm 0.5880$  mg/g of quercetin respectively. Total phenolics and Flavanoid contents of ethanolic extract of Indian propolis were higher than found in ethanolic extract of Iranian propolis [21], but fewer than that of Brazilian red propolis [22].

### 4.2 Free Radical Scavenging Activity by DPPH

The model of scavenging stable DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radicals can be used to evaluate the antioxidative activities in a relatively short time. The absorbance decreases as a result of a color change from purple to yellow as the radical is scavenged by antioxidants through donation of hydrogen to form stable DPPH-H molecule. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. Radicals formed in the presence of oxygen ( $ROO^\cdot$ ,  $HO^\cdot$ ,  $RO^\cdot$ ,  $O^{2-\cdot}$ ) are highly reactive species greatly differing in their lifetimes and chemical properties, making their direct detection and evaluation difficult, hence Stable radical species (DPPH, ABTS etc) are often used for evaluation of the general radical scavenging abilities of antioxidants.

Antioxidants on interaction with DPPH, either transfer electron, or hydrogen atom to DPPH thus neutralizing its free radical character and converting it to 1,1-diphenyl-2-picrylhydrazine. This reaction is accompanied by change of color from violet to yellow and the degree of discoloration indicates the scavenging activity of the drug. The decrease in the absorbance measured at 517nm has been used as a measure of antioxidant activity.



**Fig. 1:** Percentage Inhibition – DPPH scavenging assay on propolis extracts.

Ascorbic acid which was used as standard and various propolis extracts showed logarithmic progression in DPPH free radical scavenging activity (Fig. 1).  $IC_{50}$  Values obtained from equations of graphs were  $3.82\mu\text{g/ml}$ ,  $31.58\mu\text{g/ml}$ ,  $56.50\mu\text{g/ml}$  and  $105.17\mu\text{g/ml}$  for Ascorbic acid, EEP, HAP and WEP respectively. Hence Percent DPPH scavenging activity was remarkably higher for ascorbic acid than propolis extracts wherein it decreased in order  $EEP > HAP > WEP$ .

The remarkable higher scavenging activity of ethanolic extract may be attributed to the reason that these extracts are rich in polyphenol and flavonoid contents as evident from the tests of polyphenols and flavonoids carried out earlier.

#### 4.4. Nitric Oxide Scavenging Activity

Nitric oxide (NO) is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilation and antimicrobial and antitumor activities. NO, which is a free radical generated by a group of enzymes named nitric oxide synthase (iNOS), is produced copiously after injury. The excess production of NO can cause the oxidative damage of tissue by forming peroxynitrite with super oxide anion. Peroxynitrite is responsible for most severe toxic effects of NO. In our study we estimated NO radical scavenging activity of various propolis extracts based on Griess Illosvoy reaction. In this nitric oxide generated by sodium nitroprusside reacts with oxygen to form nitrite ions. The absorbance of chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthalene diamine can be measured as 540 nm. Antioxidant compounds may act by various mechanisms like scavenging of nitric oxide or oxygen but finally leading to fewer diazotization

reaction and hence lesser absorbance of the reaction mixture in presence of it. This can be easily determined spectrophotometrically. Curcuminoids were used as the reference standard [14].

Concentration Of extract µg/ml	Percentage Scavenging Activity (Mean ± SD)			
	Ascorbic Acid	EEP	HAP	WEP
100	70.19 ± 0.39	35.95 ± 0.91	6.566 ± 0.44	2.209 ± 0.92
200	79.53 ± 0.22	44.74 ± 0.41	12.71 ± 3.43	7.972 ± 0.64
300	88.32 ± 0.35	51.01 ± 0.51	18.58 ± 0.92	13.61 ± 0.62
400	94.29 ± 0.65	58.06 ± 0.83	25.92 ± 0.34	20.44 ± 0.45
500	96.8 ± 0.67	61.19 ± 0.72	33.15 ± 0.52	28.82 ± 0.55
IC <sub>50</sub> (µg/ml)	71.43	138.88	762.19	1785.71

**Table 1:** Nitric Oxide scavenging assay on various extracts of propolis, using Curcumin as standard (% Inhibition and IC<sub>50</sub> Values).

Nitric Oxide Scavenging activity of curcuminoids and various propolis extracts (Table 1) showed logarithmic increase in activity with concentration. IC<sub>50</sub> value for curcuminoids was 15.72 µg/ml while for EEP, HAP and WEP it was 405.55 µg/ml, 798.82 µg/ml and 7549.33 µg/ml respectively. Hence Curcuminoids were found to have far greater activity than propolis but in case of various propolis extracts ethanolic extract was found to have more potent activity than Hydro-alcoholic extract which in turn was better than activity of water extract of propolis.

#### 4.5 Hydrogen peroxide scavenging assay.

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is generated under physiological conditions by peroxisomes, by several oxidative enzymes including glucose oxidase and d-amino acid oxidase, and by dismutation of superoxide radical, catalysed by superoxide dismutase. Hydroxyl radicals generated from hydrogen peroxide via fenton reaction plays vital role in worsening inflammation, while nascent oxygen is involved in oxidative damage to DNA and other cellular substrates. Hydrogen peroxide can inactivate a few enzymes directly, usually by oxidation of essential thiol groups. Hydrogen peroxide can cross biological membranes rapidly, once inside the cell, it can react with Fe<sup>2+</sup> and Cu<sup>2+</sup> ions to form cytotoxic hydroxyl radical. Hence hydrogen peroxide scavenging activity of a compound is indicative of its antioxidant as well as anti-inflammatory activity. One of the most common methods for assessing the scavenging capacity against this molecule is based on the intrinsic absorption of H<sub>2</sub>O<sub>2</sub> in the UV region. As the H<sub>2</sub>O<sub>2</sub> concentration is decreased by scavenger compounds, the absorbance value at 230 nm is also decreased.

Hydrogen peroxide scavenging activity of ascorbic acid as positive standard was found to be higher than propolis. Ethanolic extract of propolis has highest H<sub>2</sub>O<sub>2</sub> scavenging activity followed by hydro-alcoholic extract and then aqueous extract of propolis. All the extracts including reference standard showed linear rise in scavenging activity with concentration. IC<sub>50</sub> values for Ascorbic acid, EEP, HAP and WEP were found to be 60.05 µg/ml, 291.56 µg/ml, 603.30 µg/ml and 853.66 µg/ml respectively (Table 2).



Concentration Of extract $\mu\text{g/ml}$	Percentage Scavenging Activity (Mean $\pm$ SD)			
	Ascorbic Acid	EEP	HAP	WEP
100	47.38 $\pm$ 0.56	27.37 $\pm$ 0.25	15.32 $\pm$ 0.79	10.79 $\pm$ 0.36
200	70.16 $\pm$ 0.4	45.56 $\pm$ 0.49	26.02 $\pm$ 0.29	19.92 $\pm$ 0.21
300	80.47 $\pm$ 0.98	53.14 $\pm$ 0.75	33.43 $\pm$ 0.37	24.91 $\pm$ 1.15
400	86.61 $\pm$ 0.46	60.55 $\pm$ 0.32	36.98 $\pm$ 0.59	28.21 $\pm$ 3.64
500	93.02 $\pm$ 0.55	67.44 $\pm$ 0.44	41.71 $\pm$ 0.51	31.03 $\pm$ 0.68
IC <sub>50</sub> ( $\mu\text{g/ml}$ )	60.05	291.56	603.30	853.66

**Table 2:** H<sub>2</sub>O<sub>2</sub> scavenging assay on various extracts of propolis, using Ascorbic acid as standard (% Inhibition and IC<sub>50</sub> Values).

#### 4.6 *In vitro* anti-lipid peroxidation assay

Lipid Peroxidation involves series of free radical mediated chain reaction processes, which is associated with several biological damages. Lipid Peroxidation mainly affects biological membranes of especially liver, brain, spinal cord, containing highly oxidizable PUFA (polyunsaturated fatty acids). Malondialdehyde (MDA) is volatile  $\beta$ -scission product formed as major product of lipid peroxidation along with other aldehydes and ketones. These substances are mainly measured by (TBA) thiobarbituric acid, so called as thiobarbituric acid reactive substances (TBARS).

Concentration Of extract $\mu\text{g/ml}$	Percentage Scavenging Activity (Mean $\pm$ SD)			
	Ascorbic Acid	EEP	HAP	WEP
100	70.19 $\pm$ 0.39	35.95 $\pm$ 0.91	6.566 $\pm$ 0.44	2.209 $\pm$ 0.92
200	79.53 $\pm$ 0.22	44.74 $\pm$ 0.41	12.71 $\pm$ 3.43	7.972 $\pm$ 0.64
300	88.32 $\pm$ 0.35	51.01 $\pm$ 0.51	18.58 $\pm$ 0.92	13.61 $\pm$ 0.62
400	94.29 $\pm$ 0.65	58.06 $\pm$ 0.83	25.92 $\pm$ 0.34	20.44 $\pm$ 0.45
500	96.8 $\pm$ 0.67	61.19 $\pm$ 0.72	33.15 $\pm$ 0.52	28.82 $\pm$ 0.55
IC <sub>50</sub> ( $\mu\text{g/ml}$ )	71.43	138.88	762.19	1785.71

**Table 3:** Anti-Lipid Peroxidation assay on various extracts of propolis, using Ascorbic acid as standard (% Inhibition and IC<sub>50</sub> Values).

We found that the positive standard, Ascorbic acid showed prominently high anti-lipid peroxidation activity, while ethanolic extract of propolis has highest activity amongst the studied extracts; followed by hydroalcoholic extract and then aqueous extract (Table 3). IC<sub>50</sub> values were calculated and were found to be as 71.43 µg/ml for ascorbic acid and it was 291.56 µg/ml, 603.30 µg/ml, 853.66 µg/ml for EEP, HAP and WEP respectively. Higher anti-lipid peroxidation inhibitory activity in ethanolic extract can be attributed to higher flavanoid content.

Various monocytes, macrophages, polymorphonuclear leucocytes (PMNs) and endothelial cells as well as cellular markers, enzymes such as, Catalase, Superoxide dismutase and Glutathione are mainly involved in inflammatory response, where altered levels of these enzymes in human body mainly provokes inflammatory conditions. However, anti-oxidant defense system in human body is not capable of scavenging increased free radical level and oxidative stress which ultimately resulting in severe inflammation even cell death [23]. Propolis extracts that have strong anti-oxidant activity are therefore having potential beneficial role in inflammatory treatment. Hence, above all antioxidant properties of ethanolic extract of propolis that it can potentially cure inflammation, can modulate antioxidant defense system and can be a logical choice in relieving symptoms in inflammatory disorders.

#### Rat Paw Edema Model:

Any extract having potent antioxidant activity is likely to show anti-inflammatory activity as well due to its ability to scavenge various free radicals. Also COX-2 mediated prostaglandin production in the central nervous system contributes to the severity of inflammation and pain responses. Any extract that show effective reduction of edema in this model has potential to effectively and selectively inhibit Cox-2 or PGE<sub>2</sub> and should prove to be effective against EP<sub>3</sub> receptor.

Time (hrs.)	Diclofenac (cm)	Propolis ethanolic extract 1 g/kg (cm)	Propolis ethanolic extract 0.5 g/kg (cm)
0	3.68	3.47	3.54
30	3.65	3.48	3.53
60	3.51	3.45	3.55
120	3.37	3.40	3.48
180	3.09	3.34	3.37
240	2.93	3.16	3.31
360	2.81	3.01	3.25
<b>% Inhibition</b>	<b>77 %</b>	<b>26%</b>	<b>22%</b>

**Table 4:** Percent reduction in paw volume, as a marker of anti-inflammatory activity.

The result of anti-inflammatory activity of the, ethanolic extract of Indian propolis has shown in Table 4. The extracts were tested at two different dose levels. It was observed from the results that the ethanolic extract with a dose of 0.5 g/kg b.w showed 22 % of inhibition on carrageenan induced rat paw edema after 6 hours. Whereas reference drug diclofenac showed 77 % inhibition. Ethanolic extract with a dose 1 g/kg b.w produced 26 % of inhibition. Anti-inflammatory activity of ethanolic extract (0.5 g/kg b.wt) of Propolis may be due to inhibition of

the mediators of inflammation such as histamine, serotonin and prostaglandin. The present result indicates the efficacy of ethanolic extract (0.5 g/kg b. wt) of Indian Propolis as an efficient therapeutic agent in acute anti-inflammatory conditions.

### 5. Conclusion

It can be concluded that Indian Propolis contains ample amount of polyphenols and flavonoids which confers various biological activities such as anti-oxidant to it. Various anti-oxidant assays have evidenced that Indian propolis has free radical scavenging activity, as it was successfully able to scavenge DPPH, H<sub>2</sub>O<sub>2</sub>, NO radicals. Also we were able to prove that Indian propolis has anti lipid Peroxidation activity *in-vitro*. Hence antioxidant, free radical scavenging activity of Indian propolis was proven for first time. Amongst various extract of propolis, ethanolic extract showed more potent activity followed by hydro-alcoholic extract and then aqueous extract. This indicated presence of more alcohol soluble components in Indian propolis which are bestowing antioxidant activities on it. When anti-inflammatory potential of ethanolic extract was screened using carrageenan induced edema model, it evidenced good anti-inflammatory agent. Hence various mechanistic as well as analytical studies are required to explore Indian propolis medicinally as well as commercially.

### 6. Acknowledgement

We would like to acknowledge Mrs. L. Rao, Central bee research and training Institute (CBRTI) for generous gift of Indian propolis.

### 7. References

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