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Full Length Research Paper

Antioxidant and antimicrobial activity of propolis from Tamil Nadu zone

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Propolis, a natural product honeybee, has been used for thousands of years in folk medicine for several purposes. In this work, we have investigated the antimicrobial and antioxidant activity of propolis collected from west zone of India that is, Gujarat. The antimicrobial activity was done by agar diffusion method against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Candida albicans* and *Asparagus niger.* Ethanolic extracts of sample showed high antibacterial activity against Gram-positive (*B. subtilis*) but least activity against Gram-negative bacteria (*P. aeruginosa and E. coli*). The yeast *C. albicans* showed the moderate zone of inhibition where as *A. niger* did not show any activity. Pet. ether and chloroform extracts did not show any activity. The maximum zone of inhibition of the ethanolic extracts of propolis (EEP) was found against the *B. subtilis* at the conc. 200 mg/ml where as the least was in the 40% methanolic extracts. The free radical scavenging effect of propolis as well as of vitamin C in 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical system was determined. The free radical scavenging activity of EEP was 70.96% and 72.97% respectively in the concentration range of 100 mcg at the difference of 30 min and 1hr respectively. The result of free radical scavenging effect of vitamin C was 94.7% at 100 mcg and 93.4% at 10 mcg. The methanolic extracts showed the least free radical scavenging when compared to the 40% methanolic and ethanolic extract of the propolis.

Keywords: Propolis, ethanolic extracts of propolis, 1,1-diphenyl-2-picrylhydrazyl.

INTRODUCTION

Propolis is a sticky resinous hive product. It is used by bees as glue in general-purpose. Propolis is natural brownish-green resinous product collected by honey bees. The word is derived from the Greek pro (before) and polis (city). Propolis was being used to make the protective shield at the entrance of beehive. Also it used to fill the cracks in the hive, to attach the corners of frames to the grooves in the hive, and also to polish the cells of the honeycomb. The bodies of dead lizards, snakes and mice that die in hives are sealed into the walls with bee glue, thereby protecting the colonies against the unpleasant and bacterial flora of the putre-fying corpses.

Propolis was used specially in antiquity, in Egypt. Propolis was very well known to the priests who had monopolized medicine, chemistry and art of mummifying corpses.

The Holy Qur'an has a long Sorat with the name of bees (Al Nahl). The Ayahs number : In the name of God Most Gracious, Most Merciful "And thy Lord taught the Bee to build cells in hills, On trees and in (men's) habitations; Then to eat of all The produce (of the earth), And find with skill the spacious Paths of its Lord: there issues From within their bodies A drink of varying colors, Wherein is healing for men: Verily in this is Sign For those who give thought".

The extract contains amino acids, phenolic acids, phinolic acid esters, flavonoids, cinnimic acid, terpenes and caffeic acid. It purposes several biological activities such as antimicrobial, antifungal, antiviral (Kujumgiev et al., 1999), immunostimulatory, anti-inflammatory. It has been reported that propolis lowers blood pressure and cholesterol levels, the latter of which may persist for some weeks after drug withdrawal. These unexpected activities make propolis prospectively a very interesting compound for use in the prevention and treatment of atherosclerosis. Atherosclerosis is viewed as a multi-factorial disease whose pathogenesis cannot be exhaustively explained by recognized classic risk factors (hyper-

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tension, hypercholesterolemia, diet, smoking, etc.). Today there is growing evidence supporting the inflame-matory, immunologic pathogenesis of atherosclerosis. On the other hand, some data suggest that monocyte acti-vation could play a role in atherosclerosis pro-gression.

The precise composition of raw propolis varies with the source. In general, it is composed of 50% resin and vegetable balsam, 30%, wax, 10% essential and aromatic oils, 5% pollen and 5% various other substances, including organic debris (Criasino et al., 1987). The wax and organic debris are removed during processing, creating propolis tincture.

MATERIAL AND METHODS

Extraction of propolis

Propolis sample was obtained from Horticulture Department of Javadihills, Tamilnadu. Extraction was done by cold extraction method. Hand collected propolis was kept in a dry place and stored at 4^{o}C until its processing. The sample (100 g) was cut into small pieces grounded and successive solvent extraction was done using different solvents (pet. ether, chloroform, ethanol, methanol and 40% methanol) and kept for 5 days shaking occasionally. Then it was filtered through a Whatman # 41 filter paper and then dried.

Antimicrobial assay

Four bacterial strains *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa and Escherichia coli* and two fungal strains *Candida albicans* and *Asparagus niger* was obtained from the Department of microbiology, Al-Ameen college Bangalore. The bacterial suspension was prepared and adjusted by comparison against 0.5 Mc-Farland turbidity standard (5×10^{7} cells/ml) tubes. It was further diluted to obtain a final 5×10^{6} cells/ml. Both bacteria and fungus were subcultured on nutrient broth for further bacterial propagation (Cruickshank 1979). The broth was inoculated by the 0.2 µg /ml by all the bacteria and the fungus, and then added 40 µ of propolis. Dimethyl sulfoxide was used as the control and the propolis was dissolved in the same. The plates were then incubated at 37 ± 1 ^{o}C for 24 h and observed for colony growth. The lowest concentration that does not permit any colony growth was regarded as Minimum bactericidal concentration.

Antioxidant assay to determine DPPH scavenging activity

A simple method that has been developed to determine the antioxidant activity of foods utilizes the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The structure of DPPH and its reduction by an antioxidant are shown above. The odd electron in the DPPH free radical gives a strong absorption maximum at 520 nm and is purple in color. The color turns from purple to yellow as the molar absorptivity of the DPPH radical at 520 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to number of electrons captured.

Antioxidant compounds may be water-soluble lipid-soluble, insoluble, or bound to cell walls. Hence, extraction efficiency is an important factor in quantification of antioxidant activity of foods. Ascorbic acid (as the reference standard) and the sample are reacted with DPPH solution in ethanol/water for four hours at 35°C in a vessel mounted on a rotary shaker and the absorbance changes are measured at 520 nm. The quantity of sample necessary to react with one half of the DPPH is expressed in terms of the relative amount of Ascorbic acid reacted. Antioxidant activity of a sample is expressed in terms of micromole equivalents of ascorbic acid (AA) per 100 g of sample, or simply Ascorbic acid units per 100 g or AA/100 g.

DDPH preparations

2.366 mg of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was dissolved in 100 ml of absolute ethanol to obtain 60 µM DPPH.

Sample preparations

25 mg of the extract was dissolved in 25 ml of absolute ethanol and then it was further diluted to obtain 10 to 140 µg.

Procedure

The scavenging effect of propolis sample as well as vitamin C corresponding to the quenching intensity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) as carried out (Matsushige 1996). The sample solution of each tested (500 µl) material was mixed with the same volume of DPPH solution and allowed to stand for 30 min at room temperature. The absorbance was then measured at 520 nm. The sample and DPPH were dissolved in ethanol. The percentage scavenging effect was determined by comparing the absorbance of solution containing the test sample to that of control solution without the test sample taking the corresponding blanks. Then this was again measured after 1 hour. The result is the mean of the 3 measurements for each sample. The vitamin C was used as positive control.

Statistical analysis

The experimental results were repeated thrice and zone of inhibition were determined in mm. All the results were

Table 1. Antimicrobial activity of propolis.

Sl. No.	Extracts used	Zone of inhibition (mm)						
		Conc. (mg/ml)	*S. aureus*	*B. subitilis*	*P. aerugenosa*	*E. coli*	*C. albicans*	*A. niger*
1	Ethanolic Extracts	200 mg/ml	12.9± 0.1764**	14.5± 0.1453	11.7± 0.1155**	13.6± 0.0882	13.2± 0.1528***	Nil
2	Methanolic extracts	200 mg/ml	10.5± 0.1764	11.5± 0.1155***	11.1± 0.1155	12.1± 0.1155	11.6± 0.0577	Nil
3	40% methanolic extracts	200 mg/ml	9.86± 0.1202***	10.6± 0.1528	10.23± 0.230**	10.1± 0.1528*	10.0± 0.1453	Nil
4	Ampicilin	20 mg/100ml	23±0.00	23±0.00	24±0.00	22±0.00	Nil	Nil
5	Fluconazole	20 mg/100ml	Nil	Nil	Nil	Nil	23±0.00	24±0.00

Note: All values represent Mean ± SEM; n = 3 in each group. Values are significantly different from reference standard (Ampiciline) *p<0.05; **p<0.01; ***p<0.001.

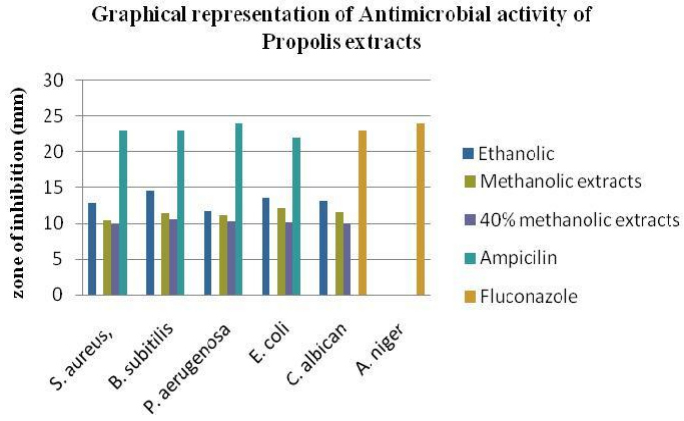

Figure 1. Antimicrobial activity of propolis extract. Results of the free radical scavenging effect of the propolis sample and positive control at the duration of 30 min in DPPH – free radical system were determined (Table 2 and Figure. 2).

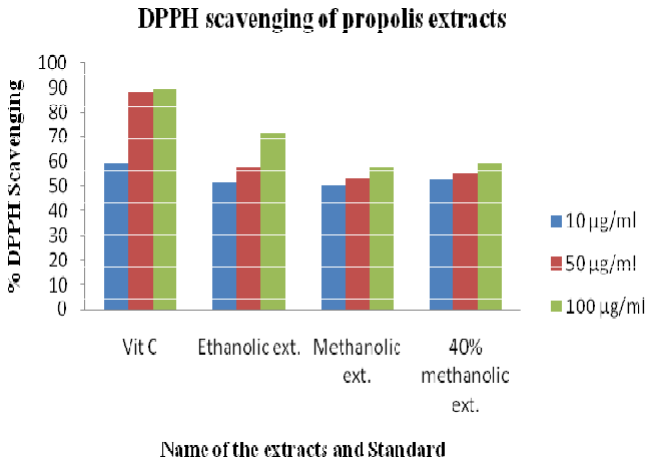

Figure 2. The DPPH free radical scavenging effect of propolis statistically expressed as the mean ± standard error of mean (SEM). Values of P < 0.05 were considered statistically significant.

RESULTS

The antimicrobial activity of all the five extracts was carried out by determining the zone of inhibition. Ethanolic extract showed higher zone of inhibition than methanolic extracts against all the microorganisms (Table-1). Interesting results were seen that ethanolic extract showed higher zone of inhibition against *B. subitilis* (14.5 mm) followed by *E. coil* (13.6 mm) and *C. albicans* (13.2 mm) at 200 mg/ml concentration. Among that against *C. albicans,* the ethanolic extract showed statistically higher activity (p<0.001) followed by *S. aureus* (p<0.01). Where as methanolic extract has showed high significant activity against *B. subitilis* (11.5 mm) (p<0.001) and against *S. aureus* (9.86 mm) with 40% methanolic extract (p<0.001). All the activities against microorganisms were resulted less than that of standard (Ampicilin and Fluconazole, 20 mg/ml) but has not showed any response against *A. niger*. (Figure 1)

The results of the free radical scavenging effect of propolis showed a concentration-dependent activity (Table 2) (Figure 2). The free radical scavenging activity of the EEP was 51.30, 57.62 and 70.96% respectively at a concentration of 10, 50 and 100 µg. Similarly for the methanolic extract it was found to have the maximum activity of 57.97 µ% at concentration of 100 µg. the results of the free radical scavenging effect of vitamin C was 94.7% at a concentration of 100µg but the activity at a concentration of 50 µg was 93.4% respectively.

DISCUSSIONS

Antimicrobial activities of various plant extracts were reported earlier in several journals. In this present study micro- biocides were evaluated against few pathogens which all are mentioned earlier in this text. Some of the extracts were found to the active against all the microbes while two extract was inactive that is, Petroleum ether and chloroform extracts. Similar results were also reported by Hegazi et al. (2002) . The extracts were found highly active in a concentration of 200 mg/ml but the acti-

Table 2. The DPPH free radical scavenging activity of ethanolic and methanolic extracts of propolis.

Sl.No.	Treatment	Concentration (µg) x 10^{-3} M		
		100 µg/ml	50 µg/ml	10 µg/ml
1	Control	0.00%	0.00%	0.00%
2	Vitamin C	89.47%	88.15%	59.21%
3	Ethanolic ext.	70.96%	57.62%	51.30%
4	Methanolic ext.	57.97%	53.14%	50.66%
5	40% methanolic ext.	59.26%	55.27%	52.49%

Note: The DPPH free radical scavenging effect was measured by the absorbance of DPPH radical at 520 nm in the reaction containing the test sample and 6 x 10^{-5} M DPPH

vities were lower than standards. The ethanolic extract was found to be most active against all the organisms except *A. niger* and 40% methanolic extract showed the least active against the entire organism. In our present study, *propolis* collected from Tamil Nadu zone shown high significant antimicrobial activities, determined by zone of inhibition.

The same results are also followed in case of antioxidant activity when the same was determined by DPPH method using ascorbic acid as standard at 520 nm. The results followed concentration dependent where ethanolic extract showed higher (70.96%) at 100 µg/ml followed by 40% methanolic extract (59.26%) at same concentration. But the same were lesser than that of standard ascorbic acid (89.47%). The results were also correlated with the earlier study carried out by the researchers for the ethanolic extracts of Egyptian propolis which was reported by Hegazi et al., (2002) and Russo et al. (2002).

Conclusions

Thus it was concluded that the EEP was the most active of all the five extracts showing the maximum zone of inhibition at the concentration of 200 mg/ml. Even in case of the free radical scavenging activity EEP showed the good activity. Further studies can be done for the identification of the chemical compounds responsible for the antimicrobial activity and its isolation along with its characterization. The exact mode of physiological or biochemical mechanisms responsible for the antibacterial effect is yet to be studied.

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