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Antioxidant and antibacterial activity of lichen extracts, honey and their combination

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ABSTRACT

The present study was carried to evaluate antioxidant and antibacterial activity of two macrolichens *Parmotrema pseudotinctorum* (des. Abb.) Hale and *Ramalina hossei* H. Magn & G. Awasthi individually and in combination with honey. The powdered lichen materials were extracted with methanol solvent. Antioxidant activity was assessed using DPPH free radical scavenging assay and antibacterial activity was determined by performing Agar well diffusion method. Antioxidant activity of combination of honey and lichen extracts was not as effective as scavenging potential of individual lichen extracts. Extracts of *P. pseudotinctorum* and *R. hossei* exhibited marked antibacterial activity individually than in combination with honey. Among all, honey showed less antioxidant and antibacterial activity. Thus, combination of lichen extracts and honey was not found to exert any synergistic action.

Keywords: *Parmotrema pseudotinctorum* (des. Abb.) Hale, *Ramalina hossei* H. Magn & G. Awasthi, Honey, Antioxidant activity, Antibacterial activity

INTRODUCTION

India is a rich center of lichen diversity, contributing nearly 15% of the 13,500 species of lichens so far recorded in the world [1]. Lichens and lichen products have been used in traditional medicines for centuries and still hold considerable interest as alternative treatments in various parts of the world. They produce characteristic secondary metabolites that are unique with respect to those of higher plants [2]. A wide range of secondary metabolites of lichens were characterized. According to their chemical structure, most lichen substances are phenolic compounds, dibenzofuranes, Usnic acids, depsidones, depsones, lactones, quinines and pulvunic acid derivatives [3]. In various systems of traditional medicine worldwide, including the Indian system of medicine, these lichen species are said to effectively cure dyspepsia, bleeding piles, bronchitis, scabies, stomach disorders, and many disorders of blood and heart [4,5,6]. Honey is a product from the hive prepared by honeybees from the nectar and other sugary substance derived from many plants and is considered a part of traditional medicine all over the world [7,8,9]. Honey has been reported to be effective in the healing of wounds and burns [10,11] and as an antimicrobial agent [12,13] and in providing gastric protection against acute and chronic gastric lesions [14,15]. In this study, we describe the antioxidant and antibacterial activity efficacy of lichen extracts, honey and their combination.

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MATERIALS AND METHODS

Collection and Identification of lichens

Three lichen species namely *P. pseudotinctorum* (des. Abb.) Hale (Voucher no. KSV/KU01130) and *R. hossei* H. Magn & G. Awasthi (Voucher no. KSV/KU00905) growing on barks of trees, were collected from the forest area of Bhadra wildlife sanctuary. The voucher specimens of the selected lichens were deposited in the Dept. of Applied Botany, Shankaraghatta for future reference. The collected lichen specimens were dried and identified by using standard manual [16] and also by morphological, anatomical, chemical tests. Thin layer chromatography (TLC) in solvent A (180 ml toluene: 60 ml 1,4, dioxine: 8 ml acetic acid) was performed to detect secondary metabolites [17,18].

Extraction of lichen material

For extraction, air-dried lichen samples were first ground and then 20 g portions were taken and added to 100 ml of methanol separately. The mixtures were sonicated for 30 min, and then left at room temperature overnight. The extracts were filtered over Whatman No 1 filter paper, and the filtrates were concentrated under reduced pres-

sure to pasty mass [19]. The condensed extract was used for antioxidant and antibacterial assay alone and in combination with honey.

Screening for Antioxidant activity of lichen extracts, honey and their combination by DPPH free radical scavenging assay

DPPH free radical scavenging assay was performed to determine the antioxidant activity of different concentrations (0.062, 0.25, 0.50 and 1.00mg/ml) of lichen extracts, honey and the standard Ascorbic acid prepared in methanol [20,21]. 0.002% of DPPH was prepared in methanol. In clean and labeled test tubes, 2ml of DPPH solution was mixed with 2ml of different concentrations of lichen extract and standard separately. For combination trial, equal volumes of lichen extract and honey were mixed and then added to DPPH solution. The tubes were incubated at room temperature in dark for 30 minutes and the optical density was measured at 517nm using UV-Vis Spectrophotometer. The degree of stable DPPH* decolorization to DPPHH (reduced form of DPPH) yellow indicated the scavenging efficiency of the extract. The scavenging activity of the extract against the stable DPPH* was calculated using the following equation.

$$\text{Scavenging activity in \%} = A - B / A \times 100$$

Where A is absorbance of DPPH and B is absorbance of DPPH and extract combination.

Screening of lichen extracts, honey and their combination for Antibacterial activity

The bacteria namely *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were screened for their sensitivity towards the lichen extracts, honey and their combination by Agar well diffusion method [22]. In this method, 24 hours old Muller-Hinton broth cultures of test bacteria were swabbed uniformly on solidified sterile Muller-Hinton agar plates using sterile cotton swab. Then, aseptically wells of 6 mm diameter were bored in the inoculated plates with the help of gel puncher and the extract (10mg/ml of DMSO), Standard (Chloramphenicol, 1mg/ml) and Control (DMSO) were added into the respectively labeled wells. The plates were incubated at 37°C for 24 hours in upright position and the zone of inhibition was recorded. The experiment was carried in triplicates to get average reading.

RESULTS AND DISCUSSION

The TLC in solvent A showed the presence of various secondary metabolites in lichens selected. *P. pseudotinctorum* showed the presence of Atranorin and Lecanoric acid. Usnic acid and Sekikaic acid were detected in *R. hossei* (Table-1).

Table.1: Secondary metabolites detected in TLC of selected lichens

Metabolite	*P. pseudotinctorum*	*R. hossei*
Atranorin	+	-
Lecanoric acid	+	-
Usnic acid	-	+
Sekikaic acid	-	+
Salanizic acid	-	-

'+' present; '-' absent

Radical scavenging activity of honey, lichen extracts and their combination is presented in Table-2. Extracts of *P. pseudotinctorum* and *R. hossei* exhibited marked radical scavenging activity individually. Antioxidant activity of combination of honey and lichen extracts was not as effective as scavenging potential of individual lichen extracts. Among all, honey showed less antioxidant activity. A dose dependent radical scavenging activity was observed in all the trials. It is evident from the result that honey with lichen extracts has no synergistic radical scavenging activity as revealed by decreased scavenging activity when compared to lichen extracts singly. Further it may be concluded that the lichen extracts possess active principle which demonstrate a marked antioxidant potential in a concentration dependent manner.

Table-2: Antioxidant activity of Lichen extracts, Honey and their combination

Treatment	Radical scavenging activity (in %) of different concentrations (mg/ml)			
	0.0625	0.25	0.50	1.00
Honey	62.03	64.81	66.66	71.29
Parmotrema	75.92	79.62	90.74	93.51
Honey+Parmotrema	68.51	72.22	81.48	89.81
Ramalina	78.70	88.88	89.81	91.48
Honey+Ramalina	69.44	75.01	86.58	90.74
Standard (Ascorbic acid)	85.66	91.02	93.66	97.06

Table-3 presents antibacterial activity of Lichen extracts, Honey and their combination against tested bacteria. Among trials, honey exhibited least antibacterial activity. Extract of *P. pseudotinctorum* exhibited marked antibacterial activity against *E. coli* with zone of inhibition of 32mm followed by *P. aeruginosa* (28mm) and *S. aureus* (24mm) whereas methanol extract of *R. hossei* showed more inhibition of *E. coli* and *S. aureus* with zone of inhibition of 24mm followed by *P. aeruginosa* (21mm). Combining *P. pseudotinctorum* extract and honey revealed lesser activity in case of *P. aeruginosa* and *E. coli* when compared to *P. pseudotinctorum* extract alone. Same observation was made in case of *R. hossei* extract and honey combination where combination revealed less activity in case of *S. aureus*. Thus, combination of lichen extracts and honey did not revealed synergistic action.

Table-3: Antibacterial activity of Lichen extracts, Honey and their combination

Treatment	Zone of inhibition mm		
	S. aureus	*P. aeruginosa*	*E. coli*
Control	-	-	-
Standard	41	38	38
Honey	11	08	10
Parmotrema	24	28	32
Parmotrema + Honey	24	26	24
Ramalina	24	21	24
Ramalina + Honey	22	21	24

DISCUSSION

Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS [23, 24]. The synthetic antioxidants like BHA,

BHT, gallic acid esters etc., have been suspected to cause or prompt negative health effects. Strong restrictions have been placed on their application [25, 26]. In recent years much attention has been devoted to natural antioxidant and their association with health benefits [27]. Phenolics are the largest group of phytochemicals and have been said to account for most of the antioxidant activity of plant extracts [28]. There are several methods available to assess antioxidant activity of compounds. An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1,1, diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. In presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases [29]. The antioxidant activity of lichen extracts and honey might be due to the presence of various groups of phytochemicals in them.

Antioxidant capacity of several types of honey from different countries has been demonstrated and was found to be dependent on the concentration of phenolic groups [30]. The antioxidant capacity of a genuine honey produced in Mérida, Venezuela was studied using the ferrous iron oxidation with xylenol orange method, the thiobarbituric acid method, and the determination of antioxidant activity. It was found that this honey has the capacity to decrease significantly the concentration of lipid hydroperoxides and malondialdehyde, produced during the lipid peroxidation process, in a comparable way with other widely studied antioxidants such as melatonin and vitamin E [31]. The antibacterial activity of honey varies not only between floral sources but even within one floral source. The antimicrobial activity in most honeys is due to the enzymic production of hydrogen peroxide, but honey from some *Leptospermum* species, such as manuka, can also have a high antimicrobial activity due to an unidentified phytochemical component [32]. The susceptibility to the antibacterial activity of honey of pathogens *in vitro* has been established [33,34]. The antibacterial activity of honey against coagulase-negative Staphylococci was determined [35].

Numerous lichens were screened for antibacterial activity in the beginning of the antibiotic era in the 1950s [36]. Several lichen metabolites were found to be active against Gram-positive organisms [37]. The antimycobacterial activity of lichen compounds was reported against nontubercular species of *Mycobacterium* [38]. A wide range of secondary metabolites of lichens were characterized. According to their chemical structure, most lichen substances are phenolic compounds, dibenzofuranes, Usnic acids, depsidones, depsones, lactones, quinines and pulvunic acid derivatives [3]. The antibacterial activity of lichen extracts and honey could be attributed to the presence of chemical constituents in them. In this study, the antioxidant and antibacterial activity of lichen extracts, honey and their combination have been described. From the results, it is evident that the combination has not shown enhanced radical scavenging and antibacterial potential. The possible reason for this observation may be the presence of more inhibitory principles in lichen extracts than the honey. Further, *in vivo* experiments in animal models have to be carried to find out whether combination has more efficacy.

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