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A STUDY OF IN-VITRO ANTIOXIDANT ACTIVITY OF PARMOTREMA GRAYANUM AND IT'S BIOACTIVE COMPOUNDS

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ABSTRACT

The medicinal property of Parmotremagrayanum tends to add value in drug industry. The most sensible organism of P.grayanumhas its own regal and exclusive biological property among other plant kingdoms. Antioxidant activity of P.grayanumhas been evaluated using four different choice of solvents such aspetroleum ether, ethanol, ethyl acetate and water. To attain and strengthen the respective results, the following assay has been carried out. DPPH, FRAP and H2O2 assay are achieved in order to ascertain the consistency of all the results. The present assessment was instigated from antibacterial activity of lichen extract against Salmonella enterica, Bacillus anthrax, Escherichia coli, Enterobacteraerogenes, Streptococcusaureus. The hypothesis on the four extracts were build up on the basis of deserving maximum biological activity at various concentration. It was revealed by the investigated ethanolic extract which has maximum ability to inhibit the growth of pathogenic bacteria and the production of free radicals at the concentration of 100µg/ml. It lucidly shows the extracts are concentration dependent. Lichens are the tenets of ethnic group who are still used as "medicinal tea" in Nepal, India. The fact behind is the contemporary action of lichen substances which are consist of active compounds. They were summarized here which may cause the potential efficacy of lichen in biomedical application.

KEYWORDS: Parmotremagrayanum, Duality, Scavenging activity, Lichen substances, Biomedical application.

INTRODUCTION

One of the most sensitive and liable member of plant kingdom is lichen. The symbiosis between an alga and fungus reveals the dual nature of lichen as well as "Purely imaginary" and "The baseless fabric of a vision" [1]. India, China, Malaysia, South Korea, Australia are the countries who constantly show their interest in taxonomic study of lichen since they are rich in abundance of lichen thallus. Whilst considering India about lichen divergence, Western Himalayas, Western Ghats, Uttaranchal are highly conserved region of lichen spp. As per Awasthi, 2450 Indian lichen species were reported until 2000 [2]. The ethnicity of lichen belongs to India which has unswervingly shows the traditional and household values. The aromatic plant of lichen is being used as a doctrinal herb in improving the flavor of food, in preparation of perfume to raise an economic impact across global market and the distinct formulation of drug or medicine to defend the human population [3]. The present work deals with the customaries effect on Parmotremagrayanum.Since the connotation of lichen is known worldwide, which may also use as crude drug in a particular ethnic group on geographical area. Some of the lichen species pose "Doctrine of signatures" because of having medicinal values against lung disease. The 30% of dry weight of lichen products or metabolites are used as food to serve animal and human, dyes and in the production of litmus paper [4]. Extracellular secondary metabolites of lichen are further subjected into quantification. In recent scenario, researchers put their attention to inquire the active compounds retrieved from lichen spp. More than 1050 compounds are being reported as per the choice of interest taken [5] which influence the pathways such as Polyketide pathway, Mevalonate Pathway, Shikimic acid pathway [6]. It triggers the research scarve on lichen and their metabolism depends on their own physiological characteristic. The conceivable function of lichen substance [7] proved as well they do have antimicrobial, antitumor [8], antioxidant, anti-inflammatory [9], antidiabetic, allelopathic [10] and an enzymatic inhibitory activities [11], anti-adhesive activity [12].

Since there is no much regal reports on P.grayanum, the current study has been dealt with the evaluation of antibacterial and free radical scavenging activity of P.grayanum growing in Yercaud hill, Tamilnadu, India. The identification of lichen substances is addressed here with GC-MS analysis.

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MATERIALS AND METHODS

Lichen sample

P.grayanum was collected from extremely preserved locality of Yerkaud Hills, Tamilnadu, India. The basic biochemical test was performed. Then the collected lichen thallus was allowed to dry at room temperature.

Preparation of extract

The air dried lichen sample was homogenized to make them into powder form. Five grams of lichen powder was weighed and extracted separately using different solvents such as petroleum ether, ethyl acetate, ethanol and water. 250 ml of each solvent was used and diluted with the Soxhelt extractor system for 24 hours. The extracts were filtered and then concentrated at 40^0C in a rotary evaporator. The dry extracts were kept at -80^0C for further study.

Determination of antimicrobial activity

The inhibitory effects of lichen extracts were studied by the following bacterial strain Salmonella enterica, Bacillus anthrax, Escherichia coli, Enterobacteraerogenes, Staphylococcusaureus through agar well diffusion method. The zone of inhibition was obtained and measured in mm . Bacterial cultures were maintained with Muller-Hinton Agar medium at 4^0C.

Determination of antioxidant activity

Evaluation of free radical scavenging effects on P.grayanum extracts were studied against DPPH, FRAP, Hydrogen peroxide assay.

DPPH Assay : (1,1 – Diphenyl-2-picryl-hydrazyl radical scavenging assay)

The estimation of free radical scavenging activity of each lichen extract was performed using DPPH (Mensor*et al* 2002). During the reduction of free radical, the colour will be turned into yellow.

The different concentration of each lichen extract was taken in the range of 20, 50, 100 and 200µg/ml and then added with 500µl of 100mMmethanolic solution of DPPH. The reaction mixture was incubated for 30 min at room temperature in dark. The colour change was observed from deep violet to pale yellow then it was subjected to measure the absorbance at 517nm using UV-vis spectrophotometer. To calculate the antioxidant activity, the absorbance was further converted into percentage by following formula.

% of inhibition = (A0-**A1**)/A0 * 100

A0 = Absorbance of the test control

A1 = Absorbance of the test sample

FRAP assay

This assay is based on the reduction of ferrin (Szollosi*et al* 2002). 10mM of 2, 4, 6 tripyridyltriazine was added to 20mM ferric chloride in 0.25M acetic buffer. The pH was maintained at 3.6. The various concentration of lichen sample recommended above was prepared and added to 3ml of FRAP reagent. The reaction mixture was kept for 5 min incubation at 37^0C. The absorbance was measured at 593nm using UV-vis Spectrophotometer. Percentage of scavenging power was calculated.

Hydrogen peroxide scavenging activity

The reduction of hydrogen peroxide by the extract was assessed here (Ruch*et al* 1989). The reaction mixture consists of 0.6ml of 40mM hydrogen peroxide, 0.1M of phosphate buffer (pH 7.4) and extract at different concentration mentioned above. The total volume was made upto 3ml. Then the absorbance was measured at 230nm using UV-vis spectrophotometer. The scavenging activity of extract was converted into percentage by the given formula.

Identification of potential compound

The investigation of active compounds present in the *P.grayanum* was carried out by Hewlett Packard 6890N gas chromatograph-mass spectrometry equipment. The chemical composition of ethanol extract was used here to identify the organic materials.

RESULTS AND DISCUSSION

Determination of antibacterial activity

The iatric usage of P. grayanum emerges in recent days. The antibacterial activity of aqueous, petroleum ether, ethyl acetate and ethanol extract of lichen sample was tested against the given bacteria using well diffusion method. The assessment was performed on the basis of inhibitory zone formation. The result of antibacterial activity was summarized in table. 1

Table:

Table 1. Antibacterial activity of different extracts of P.grayanum

Name of the sp.	Petroleum ether	Ethyl acetate	Ethanol	Distilled water
S.enterica	–	–	–	–
B.anthrox	+	+	+	+
E.coli	–	–	–	–
E.aerogenes	–	–	–	–

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S.aureus	+	+	+	+

(+) – Presence of inhibitory zone,
(–) – Absence of inhibitory zone

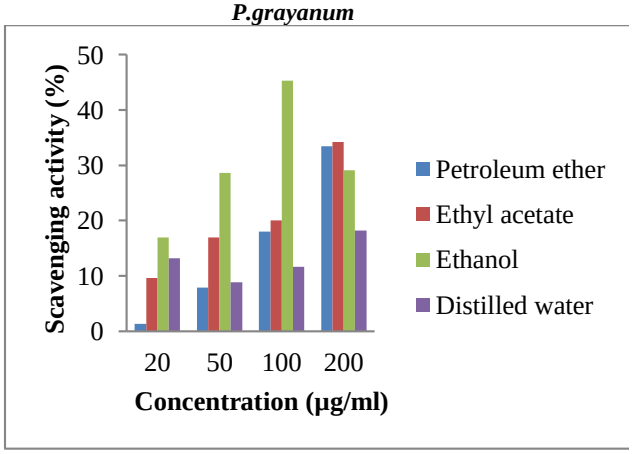
Among five different species, B.anthrox and S.aureus showed the maximum zone of inhibition on each solvent extract. The remaining species such as S.enterica, E.coliandE.auroginase did not show inhibitory zone or less than B.anthroxand S.aureus produced. It indicates that the recommended lichen spp. has efficient inhibition or to the extent of inactivation of gram positive bacteria. There is no expected results on gram negative bacteria such as S.enterica, E.coliandE.auroginase. However, the obtained results demonstrate maximum inhibitory action against tested human pathogenic bacteria, it can be further used to cure bacterial infection. In previous studies, most of the researchers reported the antimicrobial activity of other kind of lichen thallus. The modern work divulges medicinal society about the significance of P.grayanum in new medical evolution since this is a first attempt made in P.grayanum.

Determination of antioxidant activity

DPPH

All the four extracts were subjected to DPPH assay. DPPH was converted into diphenyl-picryl hydrazine when it reacts with an antioxidant compound. The conversion is due to the donation of hydrogen by antioxidant compound. It can be clearly identified by the colour change from purple to pale yellow. The free radical reduction ability was investigated and summarized in fig. 1.

Fig 1. DPPH assay to evaluate the reducing power of P.grayanum

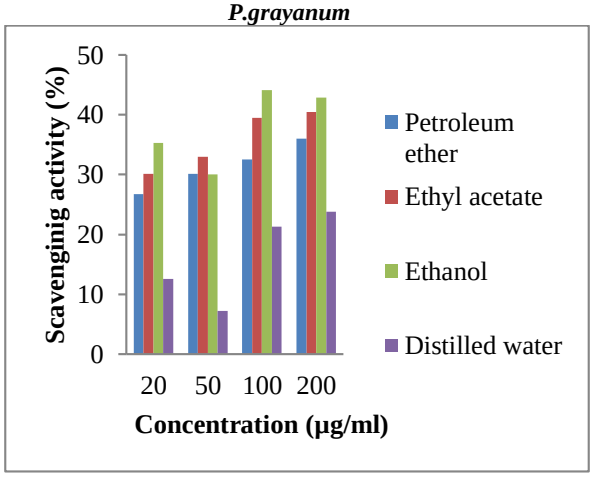


Amongst various solvent, ethanolic extract showed the best activity in scavenging and the values were depicted in a given graph.In such case, ethanol extract might be concentration-dependent since it carries significant in-vitro antioxidant potential when concentration increases. All other extracts might demonstrate the variance in significant level at each concentration.

FRAP assay

The inhibition of the production of ferrin by lichen extract is illustrated in fig. 2. The difference in absorbance indicates the individual potential ability of scavenging by lichen extract. The result clearly represents that the ethanol extract gives maximum percentage of reducing capacity. Petroleum ether, ethyl acetate shows minimal reduction when compare to ethanolic extract. Water extract shows less activity at the concentration of 20, 50µg/ml.

Fig 2. FRAP assay to evaluate the reducing power of P.grayanum



Petroleum ether extract also caused the maximum reduction of ferrin at 100µg/ml. Water extract shows lesser activity compare to other extracts since it has no significant level of reducing free radicals.

Hydrogen Peroxide Scavenging Assay

The reduction of hydrogen peroxide occurred at the maximum concentration of all three extracts except water which is depicted in fig 3. It indicates all the extracts have inhibitory efficacy against hydrogen peroxide. When the concentration increases the efficiency also gets increased. The lowest concentration of all the extracts have shown lesser activity which tells the reduction is concentration dependent.

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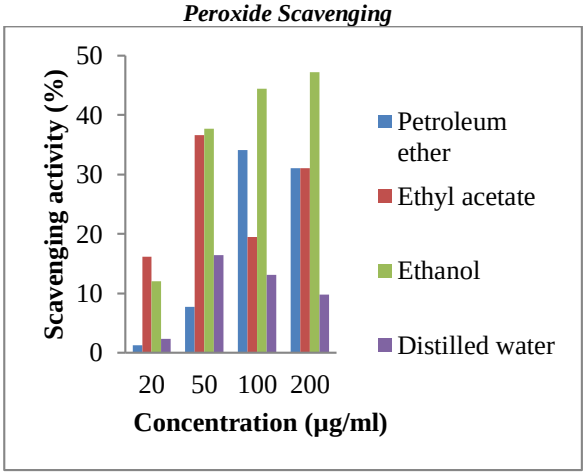
Fig 3. Antioxidant activity of P.grayanum by Hydrogen Peroxide Scavenging



Compound Analysis

The beneficial compounds fromP.grayanum is listed in table 2. They would be the major candidates of empirical biological activity of mentioned lichen extract which also enhances drug likeliness property against various disease.

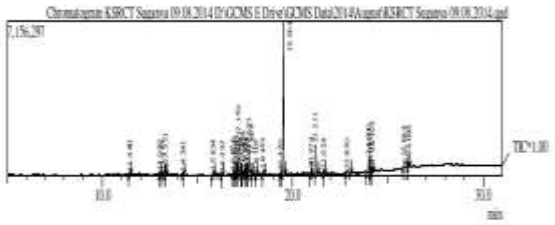


Table 2: Analysis of bioactive compounds of P.grayanum

S. No	R.Time	I.Time	F.Time	Area	Name of the Compounds
1	11.440	11.375	11.517	0.82	Azulene
2	13.020	12.975	13.092	0.61	Undecane
3	13.173	13.117	13.267	0.94	Tetradecane
4	13.331	13.267	13.392	2.16	Nonadecane
5	14.241	14.200	14.308	0.67	Nonane
6	15.834	15.767	15.892	0.94	Heptadecane
7	16.297	16.225	16.358	1.34	Decane
8	16.876	16.833	16.942	0.66	Docosane
9	16.990	16.942	17.017	0.54	Tetracontane
10	17.159	17.092	17.250	10.78	1,2-Benzene dicarboxylic acid
11	17.294	17.250	17.333	1.39	Octadecane
12	17.531	17.483	17.558	1.73	Eicosane
13	17.575	17.558	17.642	1.89	Tricosane
14	17.691	17.642	17.808	6.93	Pentadecane
15	17.864	17.808	17.925	0.85	1-Undecene
16	18.105	18.042	18.142	0.94	2,4-Ditert butyl phenol
17	18.452	18.383	18.533	1.31	Nonacosane
18	19.370	19.325	19.425	0.57	Pentadecane
19	20.979	20.925	21.025	1.00	Dotriacontane
20	21.211	21.150	21.342	7.69	Benzoic Acid
21	21.624	21.583	21.708	0.82	MYRISTIC acid
22	22.850	22.775	23.083	1.37	9-Octadecenoic acid
23	24.000	23.883	24.058	4.63	Pentadeconoic acid
24	24.075	24.058	24.150	1.19	4-Chromone
25	25.884	25.792	26.017	4.57	9-Octadecenal
26	26.086	26.017	26.167	0.99	Eicasanoic acid

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CONCLUSION

Antibacterial activity is an instigation to reveal the efficacy of lichen extract in attacking virulent pathogen such as B.anthroxand S.aureusand become commercial remedy against them. It fosters the human population free from microbial attack. Free radicals are uncharged molecule which are having unbalanced valency electron. When they start reacting with cellular components, there is an opportunity to inactivate the cellular function or cause damage to the cells. To prevent this, defense mechanism of antioxidants are being used by body as natural substance. Lichens "folk medicine" appear as a natural antioxidant agents. The study on flourish species of P.grayanum stated that the investigated lichen extract may have strong in-vitro antibacterial and antioxidant activity. Ethanolic extract ofP.grayanum sounds great in the assessment of maximum inhibitory action at 100µg/ml. The recommended active compounds from P.grayanum are the potential aspirants which can be applicable in drug development. The functional property of those compounds will be studied in future.

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