1.1 Introduction

Antioxidants, scavenger for free radicals are a very special group of nutritional supplements. The free radicals have a strong tendency to impair the proper functioning of the immune system leads to infection and a hoard of degenerative diseases. The therapeutic efficacies of most of the synthesized medicine/ drugs are limited due to development of various side effects in the host [5]. To abate these side effects, better remedy has been used with varying success. Reports have shown that commonly used medicinal plants and herbal preparations are good sources of radioprotection in experimental models and in patients receiving radiotherapy [4,8]. A variety of plants extracts have been reported to possess high antioxidant properties and their components exhibit activities against oxidative damage mediated diseases.

Crude extracts of herbs and other plant materials rich in phenolics are of increasing interest in the food industries because they retard oxidative degradation of lipids and thereby improved the quality and nutritional value of food. Therefore the assessment of such properties from plants remain new area for finding sources of natural antioxidants, functional foods and nutraceutical [18]. In the present investigation, aimed to assess the free radical activities of four herbs used as food supplement by local people of Manipur and having medicinal values [19] by DPPH, hydroxyl radical and superoxide radical scavenging assays. Polygonum chinense Linn. of Polygonaceae is used in dyspepsia and cooked eaten in stomach trouble. Polygonum perfoliatum Linn. of Polygonaceae, the plant extract is used as oral contraceptive plant infusion is given in uterine disorder. Eclipta prostata Linn. of Compositeae, decoction of the plant is given in liver enlargement. Extract of leaves with honey is prescribed in fever and cough. Plant is tonic. Cardamine hirsuta Linn. of Brassicaceae, is a small herb and cooked eaten for better urination and used as culinary item in local dishes.

1.2 Materials and Methods

1.2.1 Chemicals

DPPH (2,2 diphenyl-1-pycryl hydrazyl) was purchased from Sigma Aldrich chemical company (USA), pyragallol from Merck (Mumbai, India), methanol from Qualigens (Mumbai, India), ascorbic acid (Merck), 2 thiobarbituric acid, hypoxanthine, 2-deoxy-D-ribose, nitroetrazolium blue, xanthine oxidase (Sigma).

1.2.2 Preparation of plant extracts

The plants parts which are used as the herbal food supplements are oven dried at 60°C. The dried plant parts were crushed into powder form. The powdered plant parts were weighed and crushed with the help of mortar and pestle, with absolute methanol (1gm/10ml). The crude extracts obtained were centrifuged twice and filtered using Whatman No. 1 filter paper, till a clear supernatant was obtained. The supernatant was vacuum evaporated till...
The percent inhibition of superoxide radicals was calculated from the optical density of the treated and control samples.

Inhibitory effect (%) = \[(A_{500 \text{ control}} - A_{500 \text{ sample}})/A_{500 \text{ control}}\] x 100

IC50 (half of the inhibitory concentration) was calculated for all the methods by using Linear Regression Analysis.

1.3 Results
The change in colorization from violet to yellow and subsequent fall in absorbance of the stable radical DPPH shows the scavenging activities of the four plant extracts. Among the plant extracts P. perfoliatum shows highest antioxidant activity at 500μg/ml with 88.4% IC50 values of 288.08. The order of antioxidant activity of these plant extracts are P. perfoliatum > C. hirsuta > E. prostata > P. chinense (Table 1).

When methanol extract of different plants were incubated with the reaction mixture used in the deoxyribose degradation assay, they removed hydroxyl radicals from the sugar and prevented its degradation. At the concentration of 10μg/ml, P. chinense shows 18.4%, P. perfoliatum 45.6%, E. prostata with 22.5%, C. hirsuta 25.5%. The hierarchy of these plant extract in scavenging hydroxyl radical is C. hirsuta > E. prostata > P. chinense > P. perfoliatum (Table 2).

Methanol extract of all experimental plants were found to scavenge the superoxide radicals generated from the hypoxanthine/xanthine oxidase method. All the plant extract shows dose dependent response of the superoxide radicals. The results show point towards an increased trend in the response with small increases in the concentration of the extract. The trend of antioxidant activity in scavenging superoxide radical is P. chinense > P. perfoliatum > E. prostata > C. hirsuta (Table 3).

The IC50 values of each plant extract for different antioxidant methods were calculated from the values of degree of decoloration. IC50 is the concentration of plant extract causing 50% inhibition of absorbance, a lower IC50 value would reflect greater antioxidant activity of the sample. The IC50 values of different plant extracts for different methods are shown in Tables.

1.4 Discussion
Free radicals generated either by endogenous metabolism or external environment agents are harmful to cellular constituents, such as proteins, lipids, DNA and carbohydrates, and result in possible alternation of cell function [20]. ROS are known to be carcinogens and act at several stages in malignant transformation [15], including permanent DNA sequence changes in the form of point mutations, deletions, gene amplifications, and rearrangements which may result in the activation of tumor-suppressor genes [16]. The role of these ROS in oxidative damage to the membranes and mechanism of apoptotic death of thymocytes have been well elaborated [3,2,21].

The ranking of the antioxidant activity of the sample may vary with the analysis methods. It is common to evaluate the antioxidant activity of plants using several
methods to measure various oxidation products. Many authors strongly suggested that when analyzing the antioxidant activity, it is better to use at least two methods due to differences between the test systems [10]. Recently it has been appreciated that there is no simple universal method by which antioxidant activity can be measured accurately and quantitatively [13]. In our experiment we used three common methods (DPPH, Hydroxyl radical and Superoxide radical) to analyze the antioxidant activity. The mechanism of each analysis is different. Despite various mechanisms of the methods, combined results of these in vitro assays have given an idea of relative antioxidant activity of different herbal vegetables.

The present studies made by using the DPPH, hydroxyl and superoxide radical scavenging assays have confirmed the antioxidant properties of the investigated 4 plants in elaborate manner and are hence quite relevant. Although the crude extracts of these various plants have numerous medicinal potentials, clinical applications can be made only after extensive research on the bioactivity, mechanism of action, pharmaco-therapeutics and toxicity studies of the different compounds present in these plants. Recent years have been seen an increased enthusiasm in treating various diseases with natural products. Many phytonutrients or phytochemicals having very high antioxidant profile need to be investigated for their application as anti-tumour or radioprotective agents to inhibit acute and chronic effects and even mortality after irradiation.

1.5 Conclusion

From the results of the investigation, all plants show moderate scavenging effect although one plant is more efficient than the other in scavenging different free radicals. In DPPH scavenging activity, P. perfoliatum shows highest activity; C. hirsuta shows the highest activity in hydroxyl radical and in superoxide radical scavenging assays whereas C. chinense shows the highest activity. All these plants show capability of using as nutraceuticals.

1.6 Acknowledgment

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1.7 References

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