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Free Radical Scavenging Activity of Chloroform and Ethyl Acetate Extracts of Leaves of *Piper betle* Linn

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Abstract: The free radical scavenging activities of chloroform and ethyl acetate extracts of leaves of *Piper betle* were evaluated in vitro with the spectrophotometric method based on the reduction of the stable DPPH free radical. The reducing power ability of these extracts was also determined. Both extracts of leaves of the plant has shown significant antioxidant activity in all assay techniques. In DPPH method, IC_{50} values of these chloroform and ethyl acetate extracts were found to be 9.187 µg/ml and 4.56 µg/ml. The results obtained in the present study indicate that the leaves of *Piper betle* are a potential source of natural antioxidants.

Key words: *Piper betle* % Antioxidant % DPPH % Free radical scavenging activity % Reducing power

INTRODUCTION

By definition, the free radicals are chemical agents (0_2, OH., RO., ROO., H_20_2,) [1] produced in the living body during the chemical reaction that contribute to the development and the maintenance of the cellular life. Production of free radicals and other reactive species in cells and body tissues has been linked to aging and more than one hundred diseases states [2]. Antioxidants may be defined as radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation, neurodegeneration, Parkinson's diseases, mongolism, ageing process and perhaps dementias. Flavonoids and flavones are widely distributed secondary metabolites with antioxidant and antiradical properties [3-6].

Plants are potent biochemical factories and have been components of phytomedicine since times immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. Plants based natural constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, seeds, etc i.e. any part of the plant may contain active components [3]. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. Antioxidant based dugs/formulations for the prevention and treatment of complex diseases like atherosclerosis; stroke, diabetes, Alzheimer's disease and cancer have appeared during the last 3 decades [7, 8, 4, 9, 10].

This has attracted a great deal of research interesting natural antioxidants. Subsequently, a worldwide trend towards the use of natural phytochemicals present in berry crops, tea, herbs, oilseeds, beans, fruits and vegetables have increased. Several herbs and spices have been reported to exhibit antioxidant activity, including rosemary, sage, thyme, nutmeg, turmeric, white pepper, chili pepper, ginger and several Chinese medicinal plants extracts. The majority of the active antioxidant compounds are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins,and isocatechins. In addition to the above compoundsfound in natural foods, vitamins C and E, beta-carotene and tocopherol are known to possess antioxidant potential.

The Plant *Piper betle* , locally named as pan (Fam. Piperaceae) is a stout twining climber with broadly ovate-oblong or ovate-cordate leaves, tiny yellow-green flowers and small spherical fruits, extensively cultivated as a cash crop throughout Bangladesh, India, Srilanka [11,12]. Leaves contain up to 1% of an essential oil with burning tastes, composed of cadinene, chavicol, chavibetol and cineole. They also contain an alkaloid, arakene, tannins, starch, sugars and diastases. In addition, they contain beta-carotene and alpha-tocopherol [13,14]. Roots contain diosgenin [15]. Leaves are

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popularly used in carminative, astringent, stimulant and antiseptic drugs. They also used in headache and coughs of children. Roots induce permanent sterility in woman [16]. Extract of leaves exerts anti-tumor activity in carcinogenesis and suppresses mutagenic and carcinogenic actions of tobacco-specific nitrosamines [17, 13].

Literature survey revealed that no detailed phytopharmacological work has been done on this plant and chloroform and ethyl acetate leaves extracts has yet not been investigated for its evaluation of free radical scavenging activity. So, the present report gives an account of antioxidant activities of chloroform and ethyl acetate extract of leaves of *Piper betle* Linn.

MATERIALS AND METHODS

Preparation Of Crude Plant Extracts: The fresh leaves of *Piper betl* were collected from the local market of Natore city of Bangladesh and was identified by a taxonomist. Leaves were dried in the sun for five days and ground into powder. About 200 gram of dried, ground leaves powder were macerated in chloroform and another 200 gm in ethyl acetate at room temperature for 2-3 days. The final extracts were (chloroform and ethyl acetate extract) passed through Whatman filter paper No. 1. The filtrates were concentrated and dried at room temperature to get chloroform and ethyl acetate extracts. This crude extracts were used to investigate potential antioxidant activity.

Antioxidant Assay: The antioxidant activities of the plant extracts were determined by two different in vitro methods such as DPPH free radical scavenging assay and reducing ability assay.

DPPH Free Radical Scavenging Assay: The antioxidant activities of the chloroform and ethyl acetate extracts of leaves of the plant *Piper betle* were assessed on the basis of the stable 1,1-diphenyl-2-picryl-hydrazyl(DPPH)-free radical scavenging activity by modified method [18]. DPPH solution was prepared in 95% methanol. The stock solutions of two crude extracts were prepared by dissolving separately in a known amount of dry extract in 95% methanol. The working solutions (500µg/ml, 250µg/ml, 125µg/ml, 62.5µg/ml, 31.25µg/ml, 15.625 µg/ml, 7.81µg/ml, 3.91µg/ml, 1.95µg/ml and 0.98µg/ml) of these extracts were prepared from the stock solution using suitable dilution in each case. *tert*-butyl-1-hydroxytoluene (BHT) was used as standard using the above same concentrated solution as used in case of extracts. 0.002% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution separately. These solution mixtures were kept in dark for 20 minutes and the absorbance was measured at 517 nm using a spectrophotometer (UV mini 1240 Shimadzu UV-Visible spectrophotometer). Methanol (1ml) with DPPH solution (0.002%, 1ml) was used as blank. The absorbance was recorded and percentage scavenging inhibition (I%) was determined using the following equation and was compared with that of BHT, which was used as the standard.

$$\text{Percentage of inhibition (I\%)} = (A - B)/B \times 100$$

Where, A was the Absorbance of the sample, B was the absorbance of the control.

The IC_{50} value for each sample, defined as the concentration of the test sample leading to 50% reduction of the initial DPPH concentration, was measured from linear regression curve of concentration of the test extract (µg/ml) against the percentage of the radical scavenging inhibition.

Reducing Power Assay: The reducing power of the present extracts was determined as described previously [5]. Different concentrations of chloroform and ethyl acetate leave extract solution using methanol were prepared and 1 ml of each solution was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was then incubated at 50°C for 20 minutes. After that 2.5 ml of Tricholoacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 1.25 ml from the supernatant was mixed with 1.25 of distilled water and 0.25 ml $FeCl_3$ solution (0.1%, w/v). The absorbance was measured at 700 nm. in a spectrophotometer (UV mini 1240 Shimadzu UV-Visible spectrophotometer). Ascorbic acid was used as standard and phosphate buffer used as blank solution. Increases absorbance of the solution proves higher reducing power.

RESULTS AND DISCUSSION

The antioxidant activity of chloroform and ethyl acetate leave extracts and standard BHT, determined using DPPH method is showing in Figure 1. Results showed that the chloroform and ethyl acetate extracts exhibited antioxidant activity with IC_{50} values of 9.187µg/ml and 4.56 µg/ml, respectively. Between these two extracts tested, chloroform extract was more antioxidant active than ethyl acetate extract and standard BHT, with IC_{50} value 6.09 µg/ml.

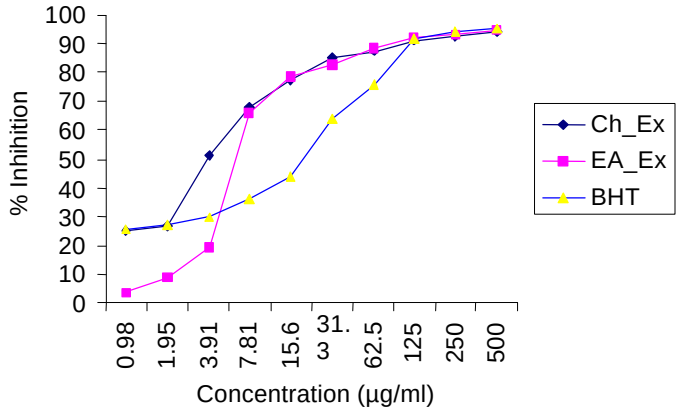

Fig. 1: The antioxidant activity of chloroform and ethyl acetate leave extracts of *Piper betle* and standard BHT, determined using DPPH method Ch_Ex = Chloroform Extract; EA_Ex = Ethyl Acetate Extract BHT = *tert*-butyl-1-hydroxytoluene

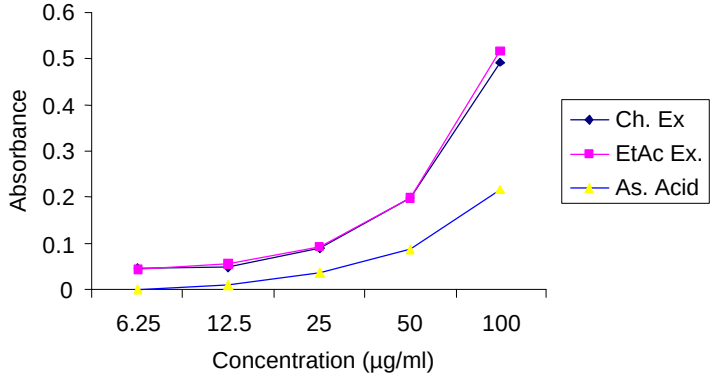

Fig. 2: Reducing power of methanolic leaves and flowers extracts of *Lippia alba* as compared to Ascorbic acid. Ch. Ex = Chloroform Extract; EtAc Ex = Ethyl Acetate Extract As.Acid = Ascorbic Acid

Natural antioxidants present in the medicinal plants are useful for inhibiting or preventing the deleterious consequence of oxidative stress. In the present study, the free radical scavenging activities of chloroform and ethyl acetate extracts of leaves of *Piper betle* was evaluated using DPPH free radical scavenging assay & reducing power assay.

Actually, the reducing power of any substance is dependent on the presence of reductants which show anti-oxidative potential by breaking the free radical chain, donating a hydrogen atom. The presence of reductants or antioxidants in chloroform and ethyl acetate leave extracts caused the reduction of the ferric (Fe^{3+})/ferricyanide complex, used in this method to the ferrous form (Fe^{2+}). Thus, the concentration of Fe^{2+} can be determined by measuring the formation of Perl's Prussian blue at 700 nm wavelength. An illustration of reducing power of the same extracts as a function of concentrations with comparison to standard ascorbic acid was given in Figure 2. Results obtained revealed that the reducing ability of both extracts went up with their increasing order of concentration. Between the two extracts, chloroform extract had higher reducing power than ethyl acetate extract. But both extracts have higher value of reducing ability than that of ascorbic acid.

Thus, the antioxidant activity of chloroform and ethyl acetate leave extracts of *Piper betle* plant was confirmed in the present investigations. However, it needs to be screened for chemical constituents that present in these extracts, which are responsible for these activities. Conclusively, the present investigations suggest that leaves of *Piper betle* might be used as a natural source of antioxidant.

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