

Evaluation of Antioxidant Properties of Certain Traditional Medicinal Plants Used to Treat Diabetes mellitus in Manipur

Katingpou Panmei* and Jiten Singh Soram

Asufii Christian Institute, Mao, Manipur, India

*Corresponding Author E-mail: katingpoupanmei@gmail.com

ABSTRACT

Methanolic extracts of four medicinal plants used to treat diabetes mellitus grown in Manipur, India were evaluated for their radical scavenging activity using DPPH assay. Out of the four medicinal plants evaluated, two plants showed high efficacy, one plant extract gave moderate antioxidant activity and the other plant exhibited a remarkable lower antioxidant potential. Syzygium cumini, a popular and widely used plant to treat DM, gave the highest antioxidant activity in the present investigation.

KEY WORDS: Radical scavenging activity, DPPH, Ascorbic acid, methanolic extracts and antioxidant.

INTRODUCTION

Numerous physiological and biochemical processes in the human body produce oxygen-centered free radicals and other reactive oxygen species (ROS) as by-products. These free radicals are also generated through a variety of environmental agents- ultra violet rays, radiation and toxic chemicals etc. In the human body any imbalance between radical production and the defensive scavenging system based on the action of naturally- present antioxidant species such as superoxide dismutase can produce 'oxidative stress' capable of leading to biochemical alterations and even to cell death¹.

Plants (fruits, vegetables, medicinal herbs etc) contain a wide variety of free radical scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids, and some other endogenous metabolites that are rich in antioxidant activity^{4,5,6,7,8,9}.

The biological activity of antioxidant compounds is exerted on the reactive species of oxygen and on the free radicals produced via an aerobic metabolism, which represent the main causes of ageing and the onset of cardiovascular and degenerative diseases. The antioxidant compounds contained in some foods, especially vegetables, are important protective agents for human health^{2,3}.

Antioxidants are important in diabetes, with low levels of plasma antioxidants implicated as a risk factor for the development of the disease^{15,18} and circulating levels of radical scavengers impaired throughout the progression of diabetes¹⁶. Many of the complications of diabetes, including retinopathy and atherosclerotic vascular disease, have been linked to oxidative stress¹⁴ and antioxidants (ie. vitamin C or E) have been considered as treatments¹².

Hyperglycaemia results in the generation of free radicals which can exhaust antioxidant defenses thus leading to the disruption of cellular functions, oxidative damage to membranes and enhanced susceptibility to lipid peroxidation^{12,13}.

The seeds of *Oroxylum indicum* are known as the crude drug ' Mu Hu Die' in China and it has been used as an analgesic, antitussive, and anti- inflammatory agent for the treatment of cough, bronchitis, and other diseases. The flavonoids in the seeds of *O. indicum* are probably bioactive compounds. Flavonoids present in the seeds of *O. indicum* are Baicalein-7-O- glucoside, Baicalein and Chrysin¹⁰. Some of the flavonoids, due to their phenolic structure, are known to be involved in the healing process of free radical-mediated diseases including diabetes¹¹.

We have been particularly interested in these certain traditional medicinal plants viz., *Oroxylum indicum*, *Meyna spinosa*, *Syzygium cumini* and *Lagenaria siceraria* grown in Manipur as these could be new potent sources of antioxidants because these plant materials are widely used in treatment of diabetes mellitus and other ailments by the folks of Manipur for centuries and also of the fact that no proper scientific investigation is carried out on these plants. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay used for hydrogen-donating capacity is commonly employed for screening plant extracts^{20,21}. Present investigation is aimed at screening and evaluation of antioxidant property of these certain plant extracts using DPPH method.

MATERIALS AND METHODS

Plant material

All the plant materials (*Oroxylum indicum*, *Meyna spinosa*, *Lagenaria* and *Syzygium cumini*) were collected from their native habitats ie., forests and gardens in Manipur.

Chemical material

DPPH was purchased from Hi Media Company, Mumbai, India and Ascorbic acid was obtained from MERCK. All the other chemicals were of the highest analytical grade.

Extraction

1g of the dried-powdered sample was weighed and put into 100 ml volumetric flask. Each flask was added 50 ml of 80% methanol. After 1 week of storage at room temperature, the supernatant was filtered and the filtered extract was dried at room temperature (30⁰ C). The dried sample was dissolved in 10ml methanol.

Antioxidant capacity

The antioxidant capacity of the plant extracts was examined by comparing it to the activity of known antioxidants, such as ascorbic acid by the chemical assay- scavenging of DPPH radical.

DPPH free-radical scavenging activity

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging activity was measured by the method of Cuendat *et al*,¹⁹ with slight modification. The reaction mixture contained 6×10⁻⁵ methanolic solution of DPPH and various concentrations of the test substances and was kept in dark for 30 minutes. Ascorbic acid solution of 150(μg/ml) was used as a standard to compare with the fruit extracts corresponding to 100 % radical scavenging activity. Optical density (OD) of the samples was measured at 515 nm against the sample blank. The assay was carried out in triplicate. Percent inhibition was determined by comparison with a methanol treated group. The decline in the radical concentration indicated the radical scavenging activity of the sample.

The percentage of DPPH decolorization was calculated as

$$\% \text{ DPPH decolorization} = [1 - \text{OD sample} / \text{OD control}] \times 100$$

RESULTS AND DISCUSSION

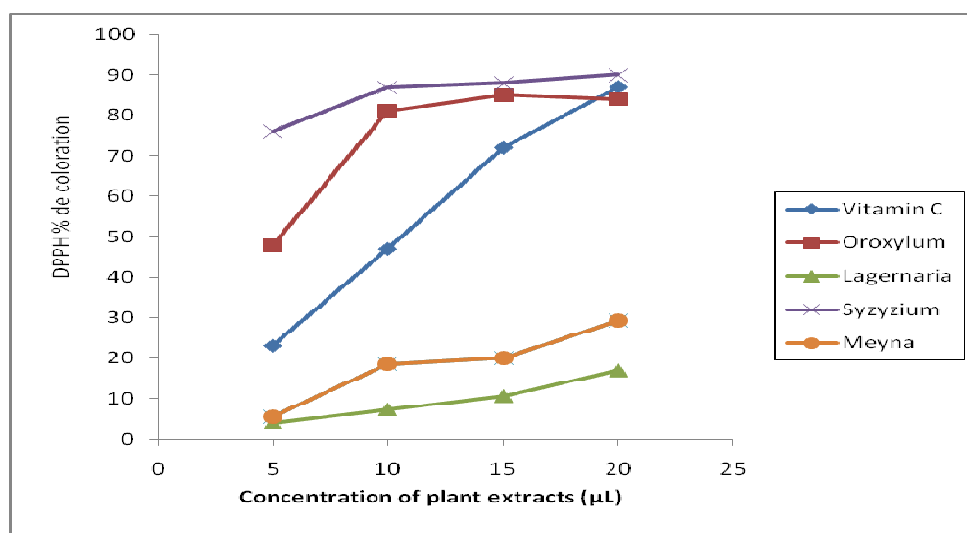
The change in the colourization from violet to yellow and subsequent fall in the absorbance of the stable DPPH was measured at 515 nm for various concentrations and the result is presented in Figure 1. In the present investigation, methanolic extracts of two plants were most effective DPPH radical scavengers and showed percent inhibition of DPPH activity- *Syzygium cumini* (88.9%) and *Oroxylum indicum* (84.4%). The extract of seed of *Meyna spinosa* (30%) showed somewhat moderate radical scavenging activity, whereas the extract of the fruit *Lagenaria siceraria* (17%) contained remarkable lower amount of radical scavenging compound. Extracts of these plants except that of *Oroxylum indicum* are dose-dependent and showed an increase in DPPH free radical scavenging activity, *in vitro*. The extract of *O. indicum* exhibited peak scavenging activity at the concentration of 15 μl, beyond which it declined. Noteworthy significance of the radical scavenging activity of the different plant extracts as compared with the standard is that the extracts of *Oroxylum* and *Syzygium* have higher radical scavenging potentials than the standard's whereas those of *Meyna* and *Lagenaria* have a lower value than that of the standard.

The total antioxidant activity of a sample is extracted in an organic medium. Therefore the extracts of all the plants were prepared in methanol. DPPH can generate stable free radicals in methanolic solution.

It is relatively easier to evaluate antioxidant properties in an *in vitro* than in *in vivo* system because of the fact that in an *in vivo* system a variety of the radical species with varying rates of reactivity is generated. The percentages of radical scavenging potentials of the plant extracts can be considered as a full absorption inhibition of DPPH.

The antioxidative properties of phenolics arise from their reactivity as hydrogen or electron donors and from the ability of polyphenol-derived radicals to stabilize and delocalize the unpaired electron or from their ability to chelate transition metal ions (ie. cause termination of the Fenton reaction)¹⁷.

Fig. 1. Graph showing radical scavenging potentials of the different plant extracts



CONCLUSION

Quite often it is difficult to decide in a screening for antioxidants from natural sources which of the plant species studied can be considered as the best one, as each of them exhibits different antioxidant and/or scavenging activities. The extracts of the four plants showed high to moderate to low antioxidant potentials. During the assaying of the four medicinal plants used to treat diabetes, *Syzygium cumini*, *Oroxylum indicum*, *Meyna spinosa* and *Lagenaria siceraria*, in this order, were found to be the most promising ones. But it requires further investigation by more methods with regards to its individual antioxidant components.

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