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REVIEW ARTICLE

REVIEW ON DIFFERENT METHODS TO ASSESS THE ANTIOXIDANT ACTIVITY OF SOME COMMON PLANTS OF INDIAN TRADITIONAL MEDICINE

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ABSTRACT
A lot of medicinal plants, traditionally used for thousands of years, are present in a group of herbal preparations of the Indian traditional health care system (Ayurveda) named Rasayana proposed for their interesting antioxidant activities. Among the medicinal plants used in ayurvedic Rasayana for their therapeutic action, some of these have been throughly investigated. In the present paper seven plants (Emblica officinalis L., Curcuma longa L., Mangifera indica L., Momordica charantia L., Santalum album L., Swertia chirata Buch-Ham, Withania somnifera (L.) Dunal) are viewed for their historical, etymological, morphological, phytochemical and pharmacological aspects. The plants described contain antioxidant principles that can explain and justify their use in traditional medicine in the past as well as the present. In order to identify the plants with antioxidant activity in Ayurveda, a formulation of some rasayanas with well defined antioxidant properties has been examinated. For this purpose, we have considered Sharma's work on the preparation MAK4, MAK5, MA631, MA 471, MA Raja's Cup, MA Student Rasayana, MA Ladies Rasayana. The different methods to assess the antioxidant total peroxyl radical-trapping parameter (TRAP), oxygen radical absorbance capacity (ORAC) and hydroxyl radical averting capacity (HORAC) methods. ORAC, TRAP and HORAC values well correlated with polyphenol content. A good correlation was found also between the methods for measuring antioxidant capacity. Nevertheless, ORAC has been found to be the most sensitive method to measure chain-breaking antioxidant activity. Although we have found a good correlation between TRAP, ORAC and HORAC, using more than one antioxidant assay is recommended for more detailed understanding the principles of antioxidant properties of samples. Some other assays are also there are discussed in the article
Keywords- antioxidant, TRAP, ORAC, HORAC

INTRODUCTION

INDIA has a rich history of using plants for medicinal purposes. We all are aware that India is one of the richest sources of medicinal plants. Nature always stands as a golden mark to exemplify the outstanding phenomenon of symbiosis. It has provided a complete storehouse of remedies to cure all ailments of mankind. The history of herbal medicine is as old as human civilization.

Plants have been used in the treatment of various diseases from the time of immemorial the use of plant as a source of medicine lies deep in the history of mankind. Thousands of plant species growing throughout the world have medicinal uses containing active constituents that have direct action in the body. They are used both in herbal and conventional medicine. Hence herbal drugs are valuable as well as precious gift from nature to mankind.

A herbal drug consists of a definite part or parts of single plant or mixture of plants which may be further processed through crushing, drying, powdering, etc., or extracting the juice either through pressure or by means of water at room temperature or by the application of heat.

In local health tradition system, spices, raw drugs, fruits, vegetables and weeds are also used. All parts of plant have some use; it can be the root, stem, leaf, stamen and pistils. Un-doubtfully, the plant kingdom still holds many species of plants containing substances of medicinal value, which have yet to be discovered; large number of plants is constantly being screened for their possible pharmacological values.

The aim of the herbal pharmacopoeia is to study the most extensively used herbal plants and carrying out standardization and formulating them. [1]

Antioxidants, scavenge for free radicals, and consequently are a very special group of nutritional supplements. The free radicals have a strong tendency to impair the proper functioning of the immune system which leads to infection and a hoard of degenerative diseases. Due to biochemical processes occurring in the body, it is normal for free radicals to be present in the body at all times, however when the free radicals increase to an abnormal level the danger begins. Antioxidants are substances with free radical chain reaction breaking properties. Among the numerous antioxidants available, flavonoids are naturally occurring phenolic compounds in plants. The antioxidative effect of flavonoids had long been recognized. They are known to inhibit lipid peroxidation, to scavenge free radicals and active oxygen, to chelate iron ions and to inactivate lipoxygenase. We have been interested in Zingiberaceae family species as these could be new source of natural antioxidants because most of these wild rhizomes are used as traditional medicines and spices. It is known that several species from Zingiberaceae[2-3] displayed antioxidant properties, some of the abundantly available

wild species that grow in Manipur in two different solvents such as dichloromethane (less polar) and methanol (polar) solvents for crude extraction. The species of Zingerberacea family chosen for our study are *Zingiber cassumunar, Alpinia galanga, Alpinia allughas, Hydychium corancium, Hydychium coccinum and Kaempferia galaugalim* which grow particularly in Manipur, although this family is widely distributed in all tropical forests. However, little is known about their antioxidant properties and compounds responsible for antioxidant activity in these species. This prompted us to carry out this study of assessment of antioxidant properties of these rhizomes. Most members of the family are easily recognized by the characteristic aromatic fleshy rhizomes.[2]

Plants from Zingiberaceae family having high antioxidant activity

1. *Zingiber Cassiumunar* (fresh rhizome)
2. *Alpinia allughas*
3. *Alpinia galanga*
4. *Hydychium Cornacium*
5. *Hydychium Coceineum*
6. *Zingiber cassumunar* (Dry Ginger powder)
7. *Curcuma longa* (Dry turmeric powder)[3]

Antioxidant Activity of Some Selected Medicinal Plants in Western Region of India

1. *Calotropis procera* (stem and leaf parts)
2. *Hibiscus cannabinus*
3. *Parthenium hysterophorus*
4. *Gmelina arborea Roxb.*
5. *Kigelia pinnata*[4]

Some medicinal plants such as Ginkgo (Ginkgo biloba L., Ginkgoaceae) and Green tea (Camellia sinensis L. (Kuntze), Theaceae) are very important regarding the content of natural antioxidant substances and are widely used in folk medicine and pharmacy. In many studies of plant antioxidant substances, these plants serve as natural standards for evaluation of antioxidant activity and other pharmaceutical purposes of medicinal plants . Whole plant extracts were investigated in numerous studies of biological activity of medicinal plants.[5,6]

DIFFERENT METHODS FOR ASSESSING THE ANTIOXIDANT ACTIVITY

TRAP assay

The luminol-enhanced chemiluminescence (CL) was used to follow up the peroxyl radical reaction and the principle was described previously in. The CL signal is driven by the production of luminol derived radicals from thermal decomposition of AAPH. The TRAP value is determined from the duration of the time period (T sample) during which the sample quenched the CL signal due to the present antioxidants. A known quantity (8.0 nM) of trolox, a water-soluble analogue of tocopherol was used as a reference inhibitor (Ttrolox) instead of the sample. The calculation of the TRAP value is represented by the equation:

$$\mathbf{TRAP = 2.0[Trolox]T_{Sample}/\mathit{f}\text{-}T_{Trolox}}$$

Where 2.0 is the stoichiometric factor of trolox (the number of peroxyl radicals trapped per one molecule of trolox) and f is the dilution of the sample.[9]

ORAC assay

The ORAC assay measures the antioxidant scavenging function against peroxyl radical induced by AAPH at 37 ˚C. Fluorescein is used as a fluorescent probe. The loss of fluorescence of fluorescein is an indication of the extent of damage from its reaction with the peroxyl radical Working solution of fluorescein (70 nM) was prepared by dissolving fluorescein disodium salt in phosphate buffer (75 mM, pH = 7.4).The total reaction mixture volume was 200 µl and all solutions were prepared in a phosphate buffer (75 mM, pH = 7.4). One-hundred and seventy micro-litres of fluorescein solution (60 nM final concentrations) and 10 µl of the sample were placed in the well of the microplate and incubated at 37˚C directly in the FLUOstar plate reader for 10 min. After the incubation 20 µl of AAPH (51.5 mM final concentration) was added rapidly using a multichannel pipette to start the reaction. The fluorescence was recorded every minute and the microplate was automatically shaken prior to each reading. A blank using phosphate buffer instead of the antioxidant and calibration solutions of Trolox (12.5, 20, 50, and 100 lM) as antioxidant were also carried out in each assay. The final ORAC values were calculated using a regression equation between the Trolox concentration and the net area under the curve (AUC). The net AUC corresponding to the sample was calculated by subtracting the AUC corresponding to the blank. ORAC values were expressed as l mol Trolox equivalents per gram of fresh weight (FW) of the samples. [10, 11]

HORAC assay

The HORAC assay developed by Ou et al. (2002) measures themetal-chelating activity of antioxidants in the conditions of Fenton-like reactions employing a Co(II) complex and hence the protecting ability against formation of hydroxyl radical.Hydrogen peroxide solution of 0.55 M was prepared in distilled water. 4.6 mM Co (II) was prepared as follows: 15.7 mg of CoF2.4H2O and 20 mg of picolinic acid were dissolved in 20 ml of distilled water.

Fluorescein – 170 µl (60 nM, final concentration) and 10 µl of sample were incubated in 37˚C for 10 min. directly in the FLUOstar plate reader. After incubation 10 µl H2O2 (27.5 mM, final concentration) and 10 µl of Co(II) (230 l M final concentration) solutions were added subsequently. The initial fluorescence was measured polyphenols.

Using the ORAC assay we obtained a hierarchy of antioxidant capacity ranging from 113.0 to 1.2 l mol TE/g of fresh weight. Interestingly,The TRAP values ranged from 68.1 to 0.0 l mol TE/g of fresh weight with hierarchy similar to ORAC on the first five positions and the last three positions. The range of HORAC values was from 82.9 to 0.0 GAE/g fresh weight. The total polyphenol content in the vegetable extracts analyzed was in the range of 605.6–20.0 mg GAE/100 g fresh weight.The lowest total polyphenol content was recorded in tomato, carrot, cucumber and vegetable marrow corresponding to their low antioxidant function. There was a direct relationship

between the total polyphenol content and antioxidant function in the extracts of various vegetables.

ORAC, TRAP and HORAC values were significantly linearly correlated to the total polyphenol content: r = 0.95, r = 0.97 and r = 0.94, respectively.In addition, there was a good correlation between the methods for measuring peroxyl radical-trapping antioxidant function of vegetables – TRAP vs. ORAC, r = 0.96. A good correlation was also found between methods for measuring the peroxyl radical-trapping capacity and hydroxyl radical averting capacity – TRAP vs.HORAC, r = 0.94 and ORAC vs. HORAC, r = 0.94. [12]

DPPH method

The antioxidant properties were assessed by DPPH radical scavenging method. The different extracts were measured in terms of hydrogen donating or radical scavenging ability using a stable radical DPPH. 2.8 ml of DPPH solution (45 µg/ ml) were rapidly mixed with 200 µl and 400 µl of methanolic solution of plant extract one at a time in cuvette placed in the spectrophotometer. The absorbance at 515 nm was measured after 5 min.The initial absorbance of the DPPH was 1.2 -1.3. The decline in radical concentration indicated the radical scavenging activity of the sample.Pyragallol solution (125 µg/ ml) was used as a reference corresponding to 100% radical scavenging activity. Radical scavenging activity or antioxidant properties was evaluated as percentage was calculate

$$\frac{(A_0 - A_{test})}{(A_0 - A_{ref})} \times 100$$

Where as A_0 is the initial absorbance (DPPH + sample absorbance) and A_{ref} and A_{test} are absorbance after 5 min with pyragallol solution and sample solution.[13]

B-carotene-linoleic acid method

β- carotene-linoleate method (by Miller 1971)6 A solution of β- carotene is prepared by dissolving 2 mg of β-carotene in 10 ml chloroform. 2 ml of this solution is pippetted into 100 ml RB flask after chloroform was removed under vacuum 40 mg 0f purified linoleic acid , 400 mg of tween 40 emulsifier and 100 ml of aerated distilled water are added to shake vigorously. Aliquots (4.8 ml) of this emulsion are added to test tubes containing different concentrations of the extracts. BHT was used for comparison purpose. As soon as the emulsion was added to each tube, zero time absorbance was measured on UV-VIS spectrophotometer at 470nm. The tubes were then placed in water bath at 50°C and the measurement of absorbance was continued until the color of β- carotene disappeared. A blank devoid of β- carotene was prepared for background correction. [14]

$$AA = \frac{\beta - carotene\ content\ after\ 2\ hours\ of\ assay}{initial\ \beta - carotene\ content}$$

Determination of Total antioxidant capacity

The determination of total antioxidant activity was done as per the phosphomolybdenum method with some modifications. The basic principle of the assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate Mo (V) complex at acidic pH. 0.3 ml extract was combined with a mixture of 3ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were then capped and incubated at 95 °C for 90 minutes. After the samples had cooled to room temperature, the absorbance of the solution was then measured at 695 nm against blank. Methanol (0.3 ml) in the place of extract is used as the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid. [15]

ABTS or TEAC assay.

This method is based on the inhibition of the absorbance of the radical cation of 2,2´-azinobis (3-ethylbenzothiazoline 6-sulfonate) ABTS•+, which has a characteristic long-wavelength absorption spectrum showing a maximum at 734 nm (Re et al., 1999). Results were compared with a standard curve prepared with different concentrations of Trolox, a water-soluble analogue of vitamin E, and were expressed as milimolar Trolox equivalents. [16]

CONCLUSION

The review describes the different methods to assess the antioxidant activity of various medicinal plants. Approximately 80% of the world population depends exclusively on plants for their health and healing. Whereas in the developed world realize on surgery and pharmaceutical medicine is more usual but in recent years, more and more people are complementing their treatment with natural supplements. Plant materials containing phenolic constituents are increasingly of interest as they retard oxidative degradation of lipids and thereby improving quality and nutritional value of food. The various plants present in the world having antioxidant activity and different parts of the plant show the antioxidant activity. Different methods are discussed in the review as TRAP assay, ORAC assay, HORAC assay, DPPH method, β-carotene-linoleic acid method, Determination of Total antioxidant capacity, ABTS or TEAC assay.

Antioxidants are component, which inhibits oxidation, or free radicals induced oxidative damage and therefore are potential quenchers of oxidative stress induced lipid peroxidiation. Antioxidants effectively prevent free radical induced cellular and tissue damage which are solely responsible for several pathogenic condition including cancer. Antioxidants are naturally present in biological systems such as plant and plant derived products. Such naturally occurring antioxidants provide a protection against oxidation. Antioxidants are the compounds that when added to food products, especially to lipids and lipid-containing foods, can increase the shelf life by retarding the process of lipid peroxidation, which is one of the major reasons for deterioration of food products during processing and storage. Synthetic antioxidants, such as

butylated hydroxyanisole (BHA) and butylated hydroxytolune (BHT), have restricted use in foods as these synthetic antioxidants are suspected to be carcinogenic (Madhavi and Salunkhe, 1995). Therefore, the importance of the search and exploitation of natural antioxidants, especially of plant origin, has greatly increased in recent years (Gulcin, 2010; Sarikurkcu, 2011).

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