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Determination of Presence of Various Antioxidants in Aqueous Extract of Various Plants - A preliminary study

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Abstract

Plants are thought to be one of the most potential sources of various antioxidants. These antioxidants play a significant role in scavenging superoxide molecules produced during various biochemical reactions. Each plant has different concentrations of these compounds. In the present study five common plants namely Cassia auriculata, Ficus bengalensis, Ficus religiosa, Tecoma stans and Calliandra haematocephala were selected. Their phytochemicals were extracted using cold water. Each of these plants has shown presence of higher concentration of tannins, quinones and terpenoids, whereas flavanoids was found least in concentration among all the phytochemicals.

Keywords: Antioxidants, phyochemical analysis, aqueous extraction, qualitative determination.

Introduction

Antioxidants have been classified into groups such as natural antioxidants and synthetic antioxidants. Phenolic compounds such as flavonoids and phenolic acids, other than this nitrogen based compounds such as alkaloids, amino acids, amines and chlorophyll, ascorbic acid and caretonoids are natural antioxidants[1-3]. Some of the natural antioxidants are synthesized in the body whereas maximum number of antioxidants is taken up from plants as the dietary supplement. The reason behind the increased usage of natural antioxidants is its minimal side effect as well as it has shown potential results against many of the diseases. Two alkaloids (secondary metabolite) namely vinblastine and vincristein which are isolated from the plant *Catharanthus roseus*, as the therapeutic agent against cancer is widely used in chemotherapy. Other than these they influence broad range of effect as antibacterial, antiallergic, anti-inflammatory, and vasodilatory and antithrombic functions[1,4]. The other class of the antioxidant is synthetic antioxidant which covers mainly two types of antioxidants such as BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoulene)[3,5]. But the use of synthetic antioxidants for human health is prohibited due to their carcinogenic effect and other long term generative diseases. On the basis of enzymology, antioxidants are further divided into two major classes: enzymatic and non-enzymatic antioxidants. Superoxide dismutase, catalase and glutathione peroxidase are enzymatic antioxidants which are synthesized endogenously[6,7]. Tocopherol, caretonoids, flavonoids, tannins and ascorbic acid are obtained from natural resources such as plants are non-enzymatic antioxidants[3,8].

Any naturally occurring chemical derived from plants with antioxidant activity is known as phytochemical. The term is originated from the Greek word phyto, which means plants. They contribute to both micro- as well as macro nutrients required for human health. Other than this they also provide defense mechanism to the plants from external as well as internal destruction and gives colour, fragrance and flavor to it. Approximately 4000 phytochemicals have been identified and are defined according to their function such as protection, physical attribute and chemical function. Out of these 4000, only 150 phytochemicals have been studied in great detailed so far. Phytochemicals are found in each and every part of plant including fruits, vegetables, whole grains, nuts, seeds, herbs, fungi. The most common sources are broccoli, cabbage, carrots, onions garlic, cherries, grapes, beans and soy foods. They get stored in different parts of the plants such as leaves, fruits, flowers, bark, stem etc. These compounds are also known as secondary metabolites and have biological characteristics such as antioxidant activity, antimicrobial activity, stimulation of immune system, anticancer property etc.

First phytochemical was isolated in 19th century, as the active principle alkaloids such as morphine, strychnine, quinine etc., which was marked as the beginning of new modern era of medicinal research. After that these phytochemicals were classified as phenolic, flavanoids and carotenoids, tannins, saponins, steroid etc. Keeping in mind the current value and demand of antioxidant, a study was designed to find out the presence of such compounds in the commonly available plants. Hence five plants were selected. These plants were *Cassia auriculata, Ficus bengalensis, Ficus religiosa, Tecoma stans* and *Calliandra haematocephala.* Phytochemicals from these plants were extraction using three different methods. Qualitative analysis of all the extracted were done to determine the presence of various components.

Materials and Methods

Preparation of Extract: Before starting the extraction, leaves from each plant were shade air dried until they completely loose the water content and become coarse. These shade air dried leaves are then crushed finely by either mortar-pestle or in mixer-grinder. Larger particles were removed by using mesh sieve and fine powder was kept into tight poly-bags until further use.

Extraction of phytochemicals by Cold Aqueous Method: 10 gram of air dried leaf powder was soaked in 75ml of cold water in a conical flask and left for 24 hours at room temperature. The extract is filtered via sterile filter paper. The filtrate is concentrated by evaporating ¾ volume of total solvent at low temperature. The extract was then stored at 4˚C in air tight bottles for further used.

Determination of phytochemicals: Terpenoids (Salkowski Test-with slight modification): To perform the test for terpenoid 5ml extract was mixed with 4ml of chloroform, to that mixture 6ml of conc. sulphuric acid was carefully from the side of tube. Reddish brown colouration at the interface indicates the presence of terpenoid.

Flavonoids: For the presence of flavonoid, 5ml of extract was mixed with 5 ml (1%) dilute ammonia, to that solution 1ml conc. sulphuric acid was added. The disappearance of yellow colour on standing indicates presence of flavonoids.

Saponins: For saponins, 5ml of extract was mixed with equal amount of distilled water; this solution was shaken vigoursly and observed for stable persistent froth. Froth was then mixed with 3 drops of olive or castor oil and mixed again. Emulsion formation in the solution indicates presence of saponins.

Tannins: Test for tannin was performed by adding few drops of 0.1%$FeCl_3$ into 5ml of boiled and filtered extract. The formation of brownish green or blue-black colouration shows presence of tannins.

Alkaloids: For alkaloid testing, 1 drop of 0.1%$FeCl_3$ was added in 1ml of extract. Formation of yellow precipitation indicates positive test for alkaloids.

Phenols (Ellagic Acid test): Phenolic were determined by dissolving 1 ml of 5% glacial acetic acid and 1 ml of 5% of sodium nitrite into 1ml of extract. Muddy niger brown colour appears, which gives positive test results for phenols

Quinones (Alcoholic KOH Test): Quinones were determined by adding 1ml of 1% alcoholic KOH to 1ml of extract. Appearance of blue colour shows positive test results for quinones.

Results and Discussion

Results of various qualitative assays were noted in the Table-1. Based on the overall results it was found that the selected five plants contain various phytochemicals within them in various concentrations. Tannins, quinones and terpenoids were found present in all the plants. Among all the phytochemical, tannin was the most prominent one, followed by terpenoids and quinines. Flavonoids were present in least concentration in all the plants as compare to other components. Similar kinds of observation were made by the other previous study[4,9–11]. Choice of method for extraction of these components also has significant effect on their concentration in the extract[12,13]. It is believed that extraction carried out using solvent has more potential than the aqueous extract. Not only this, previous studies have also suggests that if the extraction carried out at higher temperature than phytochemicals could be extracted more efficiently[12]. However, the only problem with hot extraction is loss of certain volatile and heat labile compounds which may have antioxidant activity. Here only cold extraction with water was performed to determine the quality of extract produced under such condition. Qualitative analysis can only help in determination of presence or absence of compounds and may also give tentative idea about the concentration. For accurate concentration determination of these components, quantative assay must be carried out for each compound.

Conclusion

Based on the overall study it was concluded that, cold aqueous method for phytochemical extraction could be a potential methods extraction of antioxidants from the plants available at nearby area.

Table-1
Results of various qualitative assays

Name of Plant	Terpenoids	Saponins	Alkaloids	Quinones	Phenols	Flavonoids	Tannins
F.bengalensis	+ +	+ + +	-ve	+ +	-ve	+	+ + +
F.religiosa	+ + +	-ve	+	+ + +	+ + +	-ve	+ + +
C.haematocephala	+	+	+ + +	+	+	+	+ + +
C.auriculata	+ +	+	+ +	+ + +	+ +	-ve	+ + +
T.stans	+ + +	+ +	-ve	+	+ +	-ve	+ + +

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