"STUDY OF AN ANTIOXIDANT-MIRACLE FOR HUMAN LIFE".

Dr. Bharati M Pardeshi[1] Somnath A Raut[2]
[1]Associate Professor, Baburaoji Gholap College, Sangvi, Pune, Maharashtra, India
[2]Ph.D. Research Scholar, Baburaoji Gholap College, Sangvi, Pune, Maharashtra, India

Abstract - Oxidative stress plays an important role in various diseases such as atherosclerosis, alcoholic liver cirrhosis, cancer, diabetes, asthma, Alzheimer, bronchitis, HIV-AIDS, inflammation, ulcers, malaria, hypertension, male infertility viz. Oxidative stress is initiated by free radicals, especially reactive oxygen species (ROS). In the present study, we have screened the antioxidants and Phytochemicals from *Ficus religiosa* L. and *Azadirachta indica* L. using standard methods of extraction for Phytochemicals and instrumental methods like Spectroscopy.

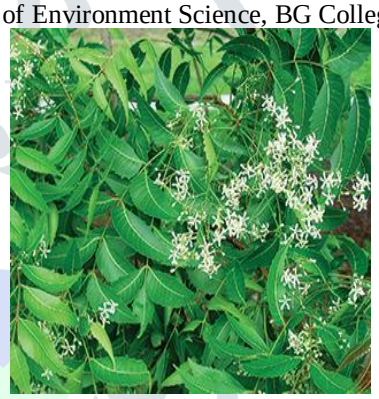
Introduction - Antioxidants are the substance that inhibit free radicals and improves the immunity Phytochemicals such as phenolics, tannins, flavonoids, carbohydrates, alkaloids etc. and Antioxidant like vit.-A, vit.-C, vit-E, beta –carotene are present in plants are able to reduce or prevent the oxidative damage to the human cells.

Methodology - Standard Chemical and spectrophotometric methods were used to determine the presence of different Antioxidants and Phytochemicals.

Collection of Sample:

Leaves of *Ficus religiosa* L. and *Azadirachta indica* L. was collected from the PMC and PCMC area of Pune City and thoroughly rinsed with distilled water and shade dried in the laboratory of Department of Environment Science, BG College, Sangvi, Pune.

Ficus religiosa L.



Azadirachta indica L.

Preparation of extracts

Dried leaves were then grinded into fine particles with the help of grinder, further stored into air tight packets.

Distilled water extract (aqueous extraction): 5gm of powdered leaves was taken in small conical flask. Then 50ml of distilled water added. Further flask was kept on the rotary shaker at 200 rpm for 24hrs.

Methanol extract (solvent extraction): 5gm of each powdered leaves sample in 2 different small conical flasks is taken. Then 50ml of ethanol is added into both of the conical flask. Both the conical flask was kept on soxhlet till the solvent is vaporized completely.

Test for Alkaloids: The extract of plant samples was evaporated to dryness and the residue was heated on a boiling water-bath with 2% Hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Meyer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation.

Detection of Carbohydrates: Extract was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate.

Test for Saponins: 5 ml of extract was shaken vigorously with 5 ml of distilled water in a test tube and heated. The formation of stable foam was accepted as an indication of the presence of saponins.

Detection of Phenols: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Test for Flavonoid: 4 mg of extract solution was treated with 1.5ml of 50% methanol solution. The solution was warmed and metal magnesium was integrated. To this solution, 5-6 drops of concentrated
hydrochloric acid was integrated and orange or red color was observed for presence of flavonoids.

Detection of Proteins: To the extract ninhydrin reagent (2,2-dihydroxyindene-1,3-dione) was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

Detection of Tannins: About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Methods of Antioxidant activity

Ferric reducing antioxidant power (FRAP) assay: The FRAP assay was used to estimate the reducing capacity of plant extracts, according to the method of Benzie and Strain. It was freshly prepared and warmed at 37ºC. 900 µl FRAP reagent was

mixed with 90 µl water and 30 µl of the extract. The reaction mixture was incubated at 37ºC for 30 minutes and the absorbance was measured at 593 nm.

Results and Discussion – *Ficus religiosa* L. and *Azadirachta indica* L. are screened for presence of Antioxidants and phytochemicals and explain what is present /absent in it. The FRAP assay suggesting that the reducing ability of polyphenols seemed to be an important factor dictating free radical scavenging capacity of these compounds. FRAP assay has many advantages over many radical scavenging assays such as excellent reproducibility, linearity over a wide range and high sensitivity. In contrast, the FRAP assay measures the reducing capability by increased sample absorbance and the assay may not complete even several hours after the reaction starts, such that a single end point of the reaction cannot be determined.

Table 1. Phytochemical screening of crude extracts of *Ficus religiosa L and Azadirachta indica L.*

Sr. No.	**Phytochemicals**	***Ficus religiosa L***		***Azadirachta indica L***	
		Aqueous extract	***Methanol extract***	***Aqueous extract***	***Methanol extract***
1	Alkaloids	-	-	+	++
2	Carbohydrates	+	+	+	+
3	Saponins	+	+	++	+
4	Phenols	+	+	+	+
5	Flavanoids	+	+	+	++
6	Protein	-	+	-	-
7	Tanins	+	+	++	++
8	Terpenoids	+	+	-	-

Conclusion – *Ficus religiosa* L. and *Azadirachta indica* L can be used as natural booster of Antioxidants and Phytochemicals. Natural antioxidants such as phenols, flavonoids, alkaloids and tannins are increasingly attracting attention because they are having qualities of disease-preventing, health-promoting and anti-ageing substances.

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