COMPARATIVE EVALUATION OF *IN VITRO* ANTIOXIDANT ACTIVITY OF PLANT *NICOTIANA TABACUM & PLUMBAGO ZEYLANICA*

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Abstract- *Plumbago zeylanica* Linn. is a useful medicinal plant. The various part of the plant contains Plumbagin, napthoquinone and other active chemical constituents, which exhibits various medicinal properties. The leaves of *Nicotiana tabacum* contain high amounts of the key flavor component cembranoids. Tobacco α and ß-2,7,11-cembratriene-4,6-diols display potent anticancer activity. The aim of present study is to compare in vitro antioxidant activity in both the plant. Determinations of their *in vitro* antioxidant activity were carried out by using DPPH radical scavenging activity method and reducing power assay method. Results of the present study indicated that the methanolic extract of *Plumbago zeylanica* Linn. is better antioxidant in comparison to *Nicotiana tabacum* Linn.

Key words: *Plumbago zeylanica* Linn, *Nicotiana tabacum* Linn, Antioxidants, DPPH, Reducing power assay.

Introduction-

Plants have been utilised as a form of drug since ancient time. People have been used natural product derivatives likes plants minerals & animals to treat their diseases.

Plumbago zeylanica L. is a multipurpose medicinal herb of family Plumbaginaceae. *P. zeylanica* is the most common plant used in Indian traditional system of Ayurveda. Presence of phytoconstituent plumbagin have been show many pharmacological properties. ***Nicotiana tabacum***, or cultivated tobacco, is from solanaceae family. This plant grows once in a year. It is the most widely cultivated because *Nicotiana*, and its leaves are produced to be transformed into tobacco commercially in many countries. In India it is named as Tambaku in Hindi and Hogesoppu in Kannada (Vaidyaratnam 1990).The crop conventionally has been used to cure bronchitis, skin diseases, inflammation regional allergies and asthma. (Kirtikar *et. al.*,1991). A lotion is prepared by boiling its leaves with cooking oil and applied it to cure painful tumors and ulcer (Nadkarni *et. al.,* 1996). Flovonoids, terpenoids polyphenols etc many bioactive components are present in the leaves of Tobacco. Nicotine is also present in this plant which is very useful to synthesize nicotinamide and nicotinic acid (CSIR 2001).

Substances which neutralize free radicals & its action is known as Antioxidants. Oxidation process is important in approx. all living beings to produce energy for biological process. Free radicals forms during the process of oxidation. On the other hand uncontrolled production of free radicals causes of many diseases like aging, rheumatoid arthritis, cancer and arteriosclerosis etc (Valko *et. al.,* 2004). Antioxidants control

production of free radicals in living organism and widely present in various parts of the plant (Flora 2007). Here is the comparison of antioxidant activity of plant *P. zeylanica* and *N. tabacum*

Plant- *Plumbago zeylanica*

Plant – *Nicotiana tabacum*

Material and methods

Collection of Plant Material & authentication

Leaves of plant *Nicotiana tabacum* were bought from the local market of Bhopal. While the leaves of plant *Plumbago zeylanica* was collected from the Sanjivani Bhopal and authenticated by Dr. Zia Ul Hasan Professor, Department of Botany, Safia College of Art & Science, Bhopal.

Preparation of Plant Extracts

The leaves of both plants were collected, cleaned to remove adulterants and dried it at 25-27 ^{o}C temperature. The leaves were crushed to make powdered. These powders of leaves were subjected to successive extraction by maceration process. Different solvents like petroleum benzine, methanol and water were used to prepare extracts. The filtrates were concentrated and dried at 40^{o}C. Weighed these crude extracts and kept in refrigerator for further antioxidant activity investigation.

Chemical requirement:

DPPH, methanol, phosphate buffer (0.2 M, pH 6.6), potassium ferricyanide (0.5 ml, 1%W/V), tri-chloroacetic acid solution (10% W/V), ferric chloride (0.1% W/V).

DPPH radical scavenging activity

The DPPH free radical scavenging assay of extract was measured by the process illustrated by (Gulçin *et al.*2006). Prepared 0.1mM solution of DPPH in methanol. Prepared samples of concentration of 1mg/ml in methanol, diluted every sample by adding 2 ml methanol now added to this 1 ml of DPPH solution and incubated at room temperature for 10 min. Measured the absorbance at 515 nm against blank. The free radical scavenging ability was calculated using following equation.

Radical Scavenging effect (%) = [($Abs_{Control}$ – Abs_{sample} / $Abs_{Control}$) x 100]

Where $Abs_{Control}$ is the initial absorbance of stable DPPH radical without extract (sample) and Abs_{Sample} is the absorbance of DPPH radical in presence of sample. DPPH radical scavenging activity of extract was expressed as IC50 value and compared with Ascorbic acid.

Measurement of reducing power

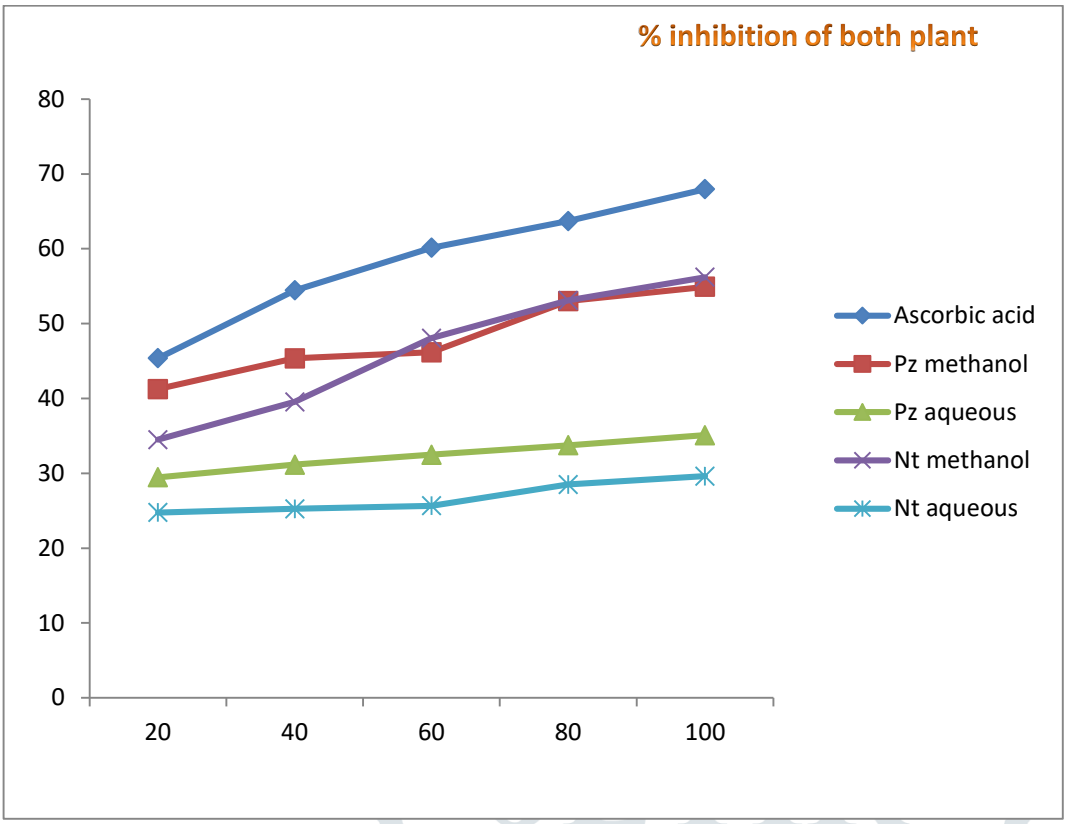
The reducing power of the samples was determined by the process explained by Elmastas et al. & Jain 2011 with some small changes. 0.5 ml of different concentrations of samples was taken then 0.5 ml of phosphate buffer (0.2 M, pH 6.6) and 0.5 ml of potassium ferricyanide (0.5 ml, 1%W/V) was added. Reaction mixture was incubated at 500 ^{o}C for 20 min. After cooling, 1.5 ml of trichloroacetic acid solution (10% W/V) was added to terminate the reaction. 0.5 ml ferric chloride (0.1% W/V) was added and absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increase in reducing power.

Result and discussion-

Table no. 1

Comparisons of Antioxidant activity of *P. zeylanica* & *N. tabacum* by DPPH radical scavenging activity

S. No.	Concentra-tion in µg/ml	Values of % inhibition in various extracts of both plants & Ascorbic acid (A.A.)				
		Ascorbic acid % inhibition	*P. zeylanica* % inhibition		*N. tabacum* % inhibition	
			Methanol	Aqueous	Methanol	Aqueous
1	20	45.37	41.26	29.47	34.50	24.75
2	40	54.45	45.36	31.15	39.53	25.25
3	60	60.14	46.17	32.49	48.06	25.66
4	80	63.70	53.01	33.75	53.10	28.50
5	100	67.97	54.92	35.10	56.20	29.61
Value of IC50		**29.448**	**70.9**	**315**	**73.309**	**424.06**

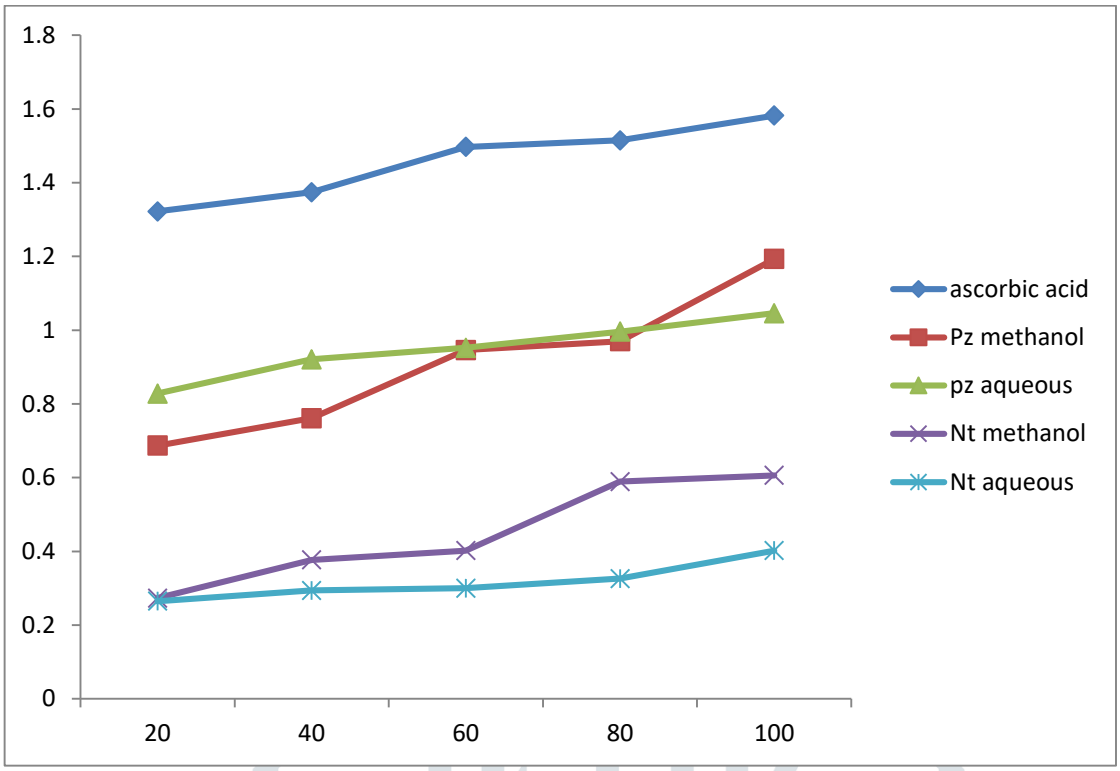


Graph 1 % inhibition in *P.zeylanica* and *N. tabacum* by DPPH assay

Table no. 2

Comparisons of Antioxidant activity of *P. zeylanica* & *N. tabacum* by reducing power Assay

S. No.	Concentra-ion in µg/ml	Absorbance at 700 nm at various extracts of both plants & Ascorbic acid (A.A.)				
		Abs. in Ascorbic acid	Abs. *in P. zeylanica*		Abs. in *N. tabacum*	
			Methanol	Aqueous	Methanol	Aqueous
1	20	1.322	0.687	0.828	0.273	0.265
2	40	1.374	0.761	0.921	0.377	0.294
3	60	1.497	0.946	0.952	0.402	0. 300
4	80	1.515	0.970	0.996	0.589	0.326
5	100	1.582	1.193	1.046	0.606	0.402



Graph 2 comparisons by reducing power assay

Discussion-

Antioxidants may stop formation of free radicals or disrupt oxidizing chain reaction to reduce damage caused by free radicals. DPPH is a stable free radical species which shows strong absorption at 515 nm. Methanolic extract of leaf of *P. zeylanica* show % inhibition in both extracts, in methanol the value of IC50% was 70.9 and aqueous it was 315 while the values IC50% of *N. tabacum* for methanolic extract was 73.309 and 424.06 for aqueous extract. Since the value of methanolic extract of both plant were very similar so we can say that *N. tabacum* also contains high antioxidant property in DPPH assay. On the other hand in reducing power assay plotted graph between concentration and absorbance depict that *N. tabacum* don't have similarity with *P. zeylanica.* High value of absorbance shows increasing reducing power of extracts. In *P. zeylanica* the values of absorbance were increasing significantly at 80 & 100 µg/ml concentration in both extract while in *N.tabacum* these values of absorbance were far away from ascorbic acid as shown in table no. 2.

The reducing power of both extract of *P.zeylanica* was parallel to their increasing concentrations. Increasing concentration also obtains in *N. tabacum* but its values are not considered to be good when we compare to Ascorbic acid. The antioxidant activity of the *P. zeylanica* extract was found to be considerable when compared with the standard ascorbic acid as we can see in graph 2.

Conclusion

Plumbago zeylanica and *Nicotiana tabacum* both contains antioxidant activity. *P. zeylanica* showed high anti oxidant activity in both methanolic and aqueous extract.

Many biological tests have been suggested that *Nicotiana tabacum* and cigarette smoke toxicity and its ill effects. Here in this work, we tried to interpret Antioxidant activity of leaf extracts of *Nicotiana tabacum* and

compare with *P. zeylanica* plant and found that methanolic extract of *Nicotiana tabacum* shows similar antioxidant activity as *P. zeylanica* in DPPH assay. Results shows that antioxidant also present in tobacco (Nicotiana tabacum) and this can be use a form of medicine in future.

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