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Determination of Antioxidants in the Barks of Common Roadside Fruit Trees of NCR

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Abstract: **Medicinal plants have been used for antioxidants in the traditional medicine in the treatment of several diseases in humans. Plants antioxidants are composed of broad variety of different substances like ascorbic acid polyphenols or terpenoids. They perform several important functions in plants and humans. The trees chosen for the research is the guava - *Psidium guajava*, bael – *Aegle marmelos* and Jamun- *Syzygium cumini* trees. Antioxidants are known to revert oxidation process. Tree bark is a major waste product from paper pulp industries hence it is worth while to determine them. Total phenolic content was expressed as gallic acid equivalent by Folin Ciocalteu reagent method. Total Flavonoids content was determined by Aluminium Chloride Colorimertric method and expressed as quercetin equivalent. Alkaloids were expressed as atropine equivalent by Bromo Cresol Green Colorimetric test. Alkaloids have been found highest of all the antioxidants with specially high in the barks of Bael- *Aegle marmelos* and guava- *Psidium sps.* The phenols were lesser and flavonoids the least. The phenols and flavonoids are still higher in the bark of guava- *Psidium sps* than the other tree barks.**

Keywords: **Alkaloids, Flavonoids, Phenols, Alternative Medicines, Free Radicals Scavengers.**

I. INTRODUCTION

Plant products have been part of phytomedicine since time immemorial. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are natural bioactive compounds found in plant food, leaves or other parts of plants that interplay with nutrients and dietary fiber to protect them. Recent research demonstrate that these antioxidants can protect humans against diseases as well as, reduce the risk of a variety of chronic or inflammatory disease.[Afolabi C et al 2007]

Antioxidants are compounds that protect cells against reactive oxygen cell or free radical. These free radicals are believed to play role in many health conditions [Food ChemToxicol 2008]. Our body is under constant free radical action and antioxidants prevent these free radical as scavengers. Free radicals are unstable, unpredictable structures that pose a potential threat to healthy molecules on a cellular level and can destroy a protein, enzyme. They can multiply through chain reactions. Diet alone cannot provide the kind of physiological defense the body requires. There is no way to escape the exposure. [Rita Elkins M.H. 1992]. Antioxidants act as free radical scavengers when added to the food products and prevent the radical chain reaction. The free radicals are related to degenerative diseases.[Dr Mark Percival 1998].

Several different types of such compounds have been extracted from barks of trees. These compounds have numerous defense functions in plants example predation by many microorganism, insects and herbivore. Thus several environmental factors such as light, temperature, humidity, internal factors, nutrients, hormones, etc. Contribute to their synthesis.

They can be derived from any part of the plant like barks, leaves flowers, fruits, roots, seeds etc. Such phytochemical screening of various medicinal plants and common fruits has been reported by many workers. The search for raw materials containing antioxidants continues to attract the attention of researches.

Compared with the leaves, bark is most economical and convenient resource for extraction of possible antioxidants. Herbal medicine have become more popular in treatment of many diseases they are called green medicines as they are safe, easily available with less side effects.

There are different kinds of antioxidants eg. Phenols, alkaloids, flavonoids, etc.

Alkaloids:

These are heterogeneous group of naturally occurring compounds found in the leaves, bark, roots or seeds of plants. Although they vary greatly in their chemical structures, alkaloids have several common characteristics: they possess nitrogen (most are derived from a few common amino acids), and are alkaline (basic), but have nonbasic forms such as quaternary compounds and N-oxides.

Some alkaloids stimulate the nervous systems; others can cause paralysis, elevate blood pressure or lower it. Certain alkaloids act as pain relievers, others act as tranquilizers. Others have also been noted to contain antimicrobial properties.

Phenols:

Phenols are most abundant antioxidant in the diet. This is a crystalline aromatic organic compound. It is a constituent of coal tar. Dilute solutions of phenol are useful antiseptics, but strong solutions of phenols are caustic and scarring to tissues. Phenols are widely used in the manufacture of resins, plastics, insecticides, explosives, dyes and detergents and as raw materials for the productions of medicinal drugs such as aspirin

Flavonoids:

Flavonoids are class of secondary metabolite [A. Edreva1 2008]. These are organic compounds that are not directly involved in normal growth, development, or reproduction of an organism. [Ammar Al-Temimi and Ruplal Choudhary 2013] They have protective effects including anti inflammatory, anti-oxidant, antiviral, and anticarcinogenic properties. They are generally found in a variety of foods, such as oranges, tangerines, etc

The present study has been planned to phytochemical analysis of the barks of three common trees of NCR like the Jamun, guava and bael. These trees are commonly seen in the NCR areas. They are common trees of the family Myrtaceae ie. Jamun tree is scientifically known as *Syzygium cumini.*. This tree is an evergreen tree with dense foliage. The wood is strong and water resistant. Another plant of this family is the common guava tree or *Psidium guajava*. It is a low evergreen tree with wide spreading and crooked branches and downy twigs. Lastly the bael tree called as *Aegle marmelos* of family Rutaceae is a medium sized deciduous tree with straight sharp axillary thorns and yellowish brown shallowly furrowed corky bark. Flowers are greenish white axillary panicle.

II. MATERIAL AND METHODS

Collection of Barks

The common trees given above namely Guava (*Psidium guajava)*, Bael (*Aegle marmelos)*and Jamun (*Eugenia cumini* or *Syzygium cumini*) were choosen, and there barks were removed using a scalpel from the green belt and parks of Sector 35 NOIDA Uttar Pradesh in the month of September 2011 and stored in plastic containers.

Drying and Grinding of the barks

The barks which were collected were later on washed with deionised water and then were dried in a hot air oven. When dried the excess tissue other than bark which had come out when removing the bark was scrapped and removed using blade and a forcep. The rest of the bark was broken into small pieces in a pestle and mortar and then finally was made into a powder using a electric grinder. The powdered barks were then stored in containers, which would be used for performing different tests as samples.

III. QUANTIFYING ANTIOXIDANTS

Test for Alkaloids

Took 1 gm of the powdered bark in 10ml of methanol, kept the same for 2 days at 50^0C in a covered bottle. After two days removed the supernatent and then centrifuged the supernatent for 10 minutes and kept the supernatent in a beaker and left it to evaporate, the residue after evaporation was used for testing alkaloids. For preparing a Standard curve we took atropin [1ml/10mg] and used concentration 0ml, 1ml and 2ml.To the above we added phosphate buffer [4.7 pH]

5ml- 7ml depending on the volume to make the total volume equal. Then we added 5ml of BCG [bromo cresol green] and 4 ml of chloroform and kept it for 15 minutes in a separating funnel. Made the total volume up to 16ml with the buffer. Alkaloid dissolves in lower layer that is the Chloroform layer as chloroform is heavier than water, this layer was removed from below and OD seen at 470nm. Similarly this was done with the samples when 7 ml of buffer was added to the residue and the above steps were repeated and the OD taken for each samples. Quantification of the samples was done using standard curve made using different concentration of atropin [Fazel Shamea et al 2008]

Test For Total Phenols – Folin-Ciocalteu reagent method

Here we took **gallic acid** for our standard curve we took 0.1gm of gallic acid to it we added 2ml of ethanol and made the volume up to 20ml with distilled water. This was the stock. Now took 0,1, 2 ,3, 5, 8 ml from stock and again made volume up to100ml with distilled water this concentration now was 50mg/ml. Took out 20ul with a pippete from each test tube and to each added 1.58ml of distilled water and 100ul of Folin-Ciocalteu reagent. Mix well then added 300ul of 20% Na_2C0_3 . Made the volume up to 2ml. Leave for 2 hours at 20^0 C. Then noted the absorbance OD at 710nm.

For the samples we took 0.5gm powder and added 5ml of 80% ethanol. Homogenized and then centrifuged the mixture. Removed the supernatent and add 2.5ml of 80% ethanol to the residue. Now again removed the supernatent in the same beaker. Now let it evaporate and dry. Now to it we added 3ml of distill water . Now pippette out 20ul of the each sample and do the same like above test and we noted OD at 710nm .

Quantified the samples using the standard curve prepared through gallic acid. [Iva Juranović Cindrić et al, 2011]

Test for Flavinoids-

Here we used again a known standard **qucertin** by dissolving 10mg of it in methanol 10ml diluted. We used different concentration like 6.25, 12.5,25, 50 ,80 and100 ug/ml were the dilution was 100ug/ml.

For samples we took 0.5gm powdered bark and added 5ml methanol kept it for 2 days at 40^0C then centrifuged and removed the filtrate in a beaker. To the residue added 2.5 ml of methanol and centrifuged it. Then we removed the filtrate in the same beaker. Filtrate was evaporated and to the residue we added 3ml of methanol. From this we took only 0.5ml and performed the test given below for the standard and the samples.

To 0.5ml samples added 1.5 ml methanol, 0.1ml aluminium chloride, 0.1ml potassium acetate, 2.8 ml of distills water. Kept the above for 30 minutes and then seen the OD using colorimeter at 430nm. For each sample of bark blank has to be prepared without aluminium chloride and the colorimeter was adjusted with the blank each time before taking the OD. [S. M. Hassan et al 2013]

IV. RESULTS

The results of the test for antioxidants in the different samples are as follows:

In mg/gm of tissue-

Samples/antioxidants	Bael	Jamun	Guava
Alkaloids	80	25.7	77.1
phenols	2.16	2.61	10.8
Flavonoids	0.69	0.42	0.9
Total Antioxidants	82.85	28.73	88.82

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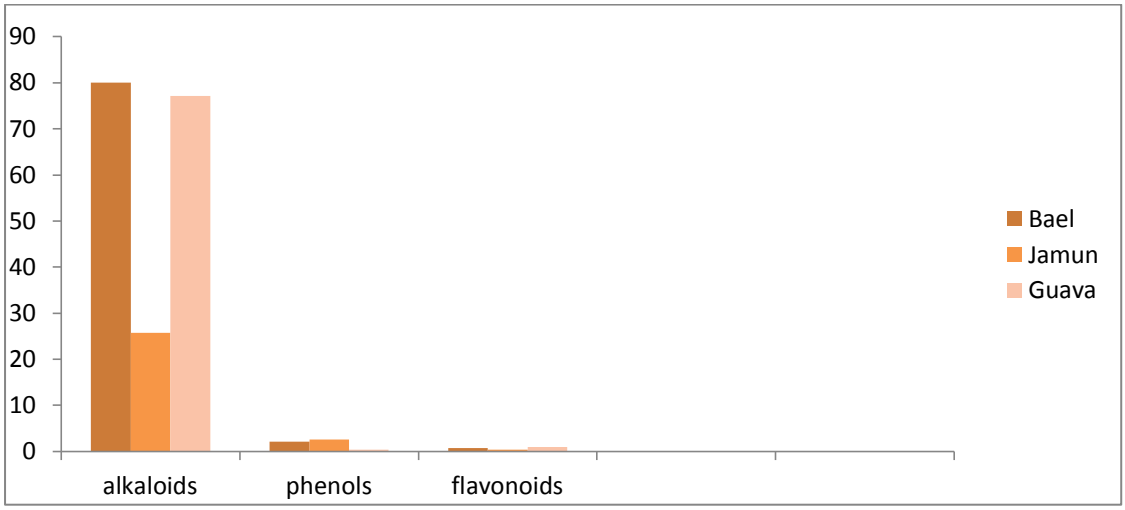

From the results we see that plant barks have high level of alkaloids specially in the bark of Bael or *Aegle marmelos* which has a maximum of 80 mg/gm of tissue followed by Guava or *Psidium sps*. with 77.1 mg/gm of tissue. The phenols are very less in the barks followed by flavonoids which are negligible.

V. CONCLUSION

The reason for high level of alkaloids in the bark may be as they are strong chemically and the bark requires protecting the inner tissue from various environmental stresses and predation. The phenols and flavonoids being mainly aromatic and give smell, flavor, etc which is not so much required by the barks. The bark is constantly being exposed to high temperature, humidity, etc. the phenols and flavonoids may be a little volatile and hence found less in the barks. But as seen guava barks has still little higher level of phenols and flavonoids which gives it a reason for the plant guava bark used in alternative medicines.

So now growing of trees goes beyond afforrestation purposes as to make them available and accessible of their natural chemical composition for management and cure of both human and animal diseases The tree bark is a major waste and voluminous product from the paper pulp industry which is only used as energy source through incernation, hence it is worthwhile to extract the nutrients.

According to P.C. Sharma et al (2007) in his research paper A Review On Bael Tree pointed out that each part of this tree is utilized in day to day life in various forms. The products obtained from bael being highly nutritive and therapeutic in Indian and now in International markets. In India where there is large area of wasteland which can be used for bael cultivation as this plant acts as **sink** for chemical pollutants as it absorbs poisonous gases from atmosphere and make them inert or neutral. It is a climate purifier which emits greater percentage of oxygen in sunlight as compared to other and also neutralizes bad smell and thus saves life from bacterial attack. So hence it is another important tree which is a savior.

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