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BIOCHEMICAL AND ANTIOXIDANT ANALYSIS OF MADHUCA INDICA J.F GMEL.

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Abstract

In recent time focus on plant research has increased all over the world and a large no ofevidence has collected to show immense potential of plants and their uses in various aspects. Madhuca indica J.F.Gmel. is commonly known as Mahua is an economically important plant belongs to family Sapotaceae. The goal of research work is comparative estimation of primary compounds such as total carbohydrate, reducing sugar, total lipid, total amino acids, protein, moisture and carotenoids from both dry and fresh corolla and assay of catalase, Peroxidase, and SOD activity from leaves. Highest amount of carbohydrate (602mg/g), reducing sugar (298mg/g) and protein (53.86mg/g) was observed in dry corolla, as compared to carbohydrate (213mg/g), reducing sugar (99.28mg/g), and protein (15.54mg/g) content of fresh corolla. Moisture content was high in fresh corolla (728mg/g) in contrast to dry corolla (110.62mg/g).Comparative account of total amino acid (16.4mg/g),carotenoids (33.39µg/g) content of dry corolla showed higher level than fresh corolla total amino acid (5.6mg/g), carotenoids (2.868µg/g).Dry corolla content lower amount of total lipid (22.6mg/g) as compared to fresh corolla (113.6mg/g).The antioxidant activity of the enzymes Catalase, peroxidase and superoxide dismutase was 270unit-1g, 7×103unit-1g and 1.02unit-1 in leaves.

Key words: Madhuca indica, total carbohydrates, reducing sugar, total lipid, total amino acid, protein, catalase, peroxidase, SOD.

Introduction: Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine for the treatment of various kinds of diseases (Chah et al., 2006). The widespread use of herbal remedies from medicinal plants plays an important role

in tribal culture. The Herbalism is a traditional medicine or folk medicine practice based on the use of plants and plant extracts (Acharya and Srivastava, 2008).The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed due to effectiveness, easy availability, low cost and devoid of side effects (UNESCO, 1996,Tambe et al.,2010). Furthermore, an increasing reliance on the use of medicinal plants in the industrialised societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants (UNESCO, 1996).

Medicinal plants-therapy is based on the empirical finding of hundred and thousand years (Gurib-fakim, 2006).Plants are like natural laboratories where a great number of chemicals are biosynthesized and in fact they may be considered the most important source of chemical compounds.

Madhuca indica J.F.Gmel is one amongst those multipurpose forest medicinal plants having great socio-economic importance as most of its parts are used for various purposes (Banerji and Mitra, 1996). The plant, popularly called 'Mahua' or 'butter tree' produces edible flowers and fruits. Mahua is a common tropical tree grows naturally in deciduous forest, all over the world, especially Asian and Australian countries (Mishra and Pradhan, 2013). In India this plant is found in the state Dehradun, Saharanpur, Chota Nagpur, Siwaliks, Uttar Pradesh, Madhya Pradesh, Odisha, Chhattisgarh, Jharkhand, Gujarat, Andhra Pradesh, Maharashtra, Bihar, West Bengal, North circars, Deccan and Karnataka (Patel et al., 2012). Mahua is a medium sized tree, usually of 12 to 15 meter height, having thick dark colour cracked bark, inner bark dark red, short trunk, and numerous branches. Leaves are 10-30 centimetre long, thick and leathery, having pointed tips. Flowers are small and fleshy, dull orpale white in colour and in define fascicles near the end of branches. Corolla are tubular, freshly, pale yellow, aromatic and caduceus. Fruits are 2-6 cm long, fleshy and greenish (Patel et al., 2012). This plant is economically important because of its role, in yielding county liquor from edible succulent corolla and oil from the seeds (Boral et al., 1999). Fleshy

corolla is greenish white in colour and contains high reducing sugar, vitamin, calcium and other nutrient contents (Sastry and kavathekar 1994, Bhanja 2000, and Jayasree et al., 1998).Every part of Mahua plant possesses some medicinal properties due to presence of some bio-active compounds, either in small of large proportion. Different parts of a plant often contain a quit different active ingredients, so that one part may be toxic and another one quite harmless (Erik and Michael, 2004). *Madhuca indica* J.F.Gmel., a plant of Indian origin

having tremendous therapeutic value. It has several pharmacological activity and potential toprovide health to the society. Flowers are used as stimulant, removal of catarrhal matter and phelgem from the bronchial tubes. Leaves are used for wounds and burn dressing, eczema and orchitis treatment. Vapours of boiling Madhuca leaves are useful in relieving the pain of orchitis or the inflammation of testicles (Thambe, 2013). The bark is used traditionally in the treatment of rheumatism, ulcers, tonsillitis, and diabetes mellitus. It is also useful in the treatment of helminths, acute and chronic tonsillitis, pharyngitis (Nadkarni, 1954).

Materials and methods

Plant materials

Madhuca indica dry flowers and fresh flowers were collected from Mayurbhanja district and Keonjhar district of Odisha respectively in the month of March-April. Leaves of *Madhuca indica* were collected from the same area of Odisha. Only fleshy corolla were used for biochemical analysis and rest of the parts like anther, filament, and scaly-corolla were removed carefully .Both young and matured healthy leaves were selected for enzymatic assay. Only healthy portion of leaves without any infection were thoroughly washed with water and used. Ribs were removed from all the leaves.

Biochemical analysis

Carbohydrate

Carbohydrate was estimated and extracted from the petals by Hedge method, 1962 (Das etal., 2010) using 80% alcohol and Anthrone reagent. Optical density was taken at 660nm against blank with glucose as standard.

Reducing sugar

Reducing sugar was quantitatively estimated by Miller, 1959 method (Sadasivam and Manickam, 2008)using 80% ethanol, DNS reagent and 40% Rochelle salt solution. Optical density was taken at 510nm using glucose as standard.

Lipids

The extraction and estimation of total lipid was done by the method given by Vijayvargia and Kumar (2007) adapting Jayaraman's method (1981) using distilled water and chloroform-methanol and chloroform-DH2O. Lower chloroform layer containing all lipids was collected in a pre-weight beaker. After evaporation of solvent completely, weight of the beaker was taken which was referred as the weight of total lipids of the sample.

Total amino acid

Total amino acid content of petal was extracted and estimated by using the Moore and Stein method (1948) (Das et al., 2010) using 80% ethanol, Ninhydrin solution and diluents solvent. Absorbency was taken at 570 nm, against a blank. The total amino acid content was estimated comparing with the standard curve of a Glycin.

Carotenoids

500mg of both dry and fresh petals were homogenated with 80% acetone and the volume was made up to 50ml. The content was centrifuged at 4000rpm for 25min and absorbency of supernatant was taken at 480nm, 663nm, 645nm. The amount of carotenoids was calculated (Wellburn, 1994).

Protein

Protein was quantified by using the method of Lowry et al., 1951, (Tambe et al., 2010) using extraction buffer, alkaline copper reagent and Folin phenol reagent After appearance of blue colour, optical density was taken at 750nm against blank and using BSA as standard.

Moisture

Moisture amount was determined by (Berwal et al., 2009). 5g of both dry and fresh sample was taken and placed it in a tries. These tries are kept shady place where no sunlight fall on them. Covered it with fine white cloth and weight this sample after 72 hour.

Antioxidant activity

1g of fresh and healthy leaves was homogenised with phosphate buffer and extract was centrifuged at 10000 rpm at 40C for 10min. The supernatant was decanted and used for various enzymatic assays within 24 hour of extraction. (Sadasivam and Manickam, 2008).

Catalase

Catalase activity was estimated by methodology opted by Sinha. (Kumar et al., 2011) using reaction mixture (1ml of 0.01M phosphate buffer pH7, 0.2M of 0.5ml hydrogen peroxide to 0.4 ml of DH2O) acid reagent (5% potassium dichromate in glacial acetic acid (1:3, v/v). The optical density was taken at 610nm after cooling the mixture against blank. Catalase activity was expressed in units/g.

Superoxide dismutase

Superoxide dismutase activity was measured by Beauchamp and Fridovich (1971), Sadasivum and Manickum 2008 using 200µl with 50mM phosphate buffer and 3ml of reaction mixture. Read the absorbency at 560nm against blank. Superoxide dismutase activity was expressed in units/g.

Peroxidase

Enzyme activity was measured in zero and full time of action. Different volume of buffer was taken (3.8-3ml) for both zero time and full time and made the volume up to 4ml with Pyrogallol. Then 0.5 ml of DH2O2was added to all the tubes and 0.5 ml of sulphuric was added to zero time action.1ml of enzyme extract was added to full time and incubated at room temperature for 10min.Then 1ml of enzyme extract and 0.5 ml of sulphuric acid was corresponding ones. OD was taken at 420 nm and Peroxidase activity was calculated using an extinction coefficient of oxidized Pyrogallol.

Results and discussion

Biochemical analysis

From the beginning of the present investigation attempt was made to maintain the quality of both fresh and dry flowers. Dry flowers were taken out intermittently and spread out in bright sun light using traditional practiced methods (Jerkin, 1982; Dejene, et al.,2006) and fresh flowers were stored at a low temperature to avoid any microbial spoilage (Lee and Resurrection, 2006).

In recent times, focus on plant research has increased over the entire world and evidence has collected to show immense potential of medicinal plants used in various traditional systems. Plants are rich sources of high value metabolites like proteins, phenols, sugars, starch and lipids, useful in flavouring, fragrances, insecticides, sweeteners and natural dyes.

Carbohydrates are one such group of carbon compounds which are essential for life and almost all organisms use carbohydrates as building blocks of cells and as a matter of fact, exploit their rich supply of potential energy to maintain life. They are of special importance because this is the direct product of photosynthesis therefore the primary substance from which most other the primary energy storage compound and the basic organic compound found in plant synthesized. The present investigation confirmed the high percentage of carbohydrate contents in the flowers of *M. indica,* i.e. 60.2%. It was found that the total carbohydrate content of the dry flowers was 602mg/g and it was higher than that of fresh flowers which was 213mg/g.

Mahua flowers are rich in total sugars out of which maximum proportion is of reducing sugars. Sugars identified are sucrose, maltose, glucose, fructose, arabinose and rhamnose, Levulose. When flowers are mature and ready to fall, there is maximum total sugar content in the flowers. Fructose is present in a greater proportion than glucose and in the ripened stage the quantities of both are almost equal. Sucrose increases in amount up to shedding of corollas and is later converted into invert sugars (Singh et al. 2013). Present investigation

showed the presence 50% of carbohydrates in form of reducing sugar in both fresh and dry flowers. Reducing sugar was 99.28 mg/g in fresh flower which was lower than 298mg/g of reducing sugars in dry flower.

The quantitative analysis of different biochemical compounds, in the present investigation showed 2.26% lipid content in dry flowers. The Fresh flowers exhibited higher lipid (113.6mg/g) as compared to dry flowers (22.6mg/g). The dry flower accumulates more protein (53.876mg/g) as compared fresh one (15.54mg/g) and the amount of protein present in dry flowers was 5%. Higher percentage of amino acid content (16.4mg/g) was found to be present in dry flowers than fresh lowers (5.6mg/g).

Moisture is the major constituent for flowers. Present investigation established higher amount of moisture to be present in fresh flowers (728.28mg/g) as compared to dry flowers (110.62mg/g). The amount of moisture content in the fresh flowers was found to be 72% as compared to 11% of moisture in dry flowers. The amount of carotenoids was also high in dry flowers (33.39mg/g) as compared to fresh flowers (2.868mg/g).

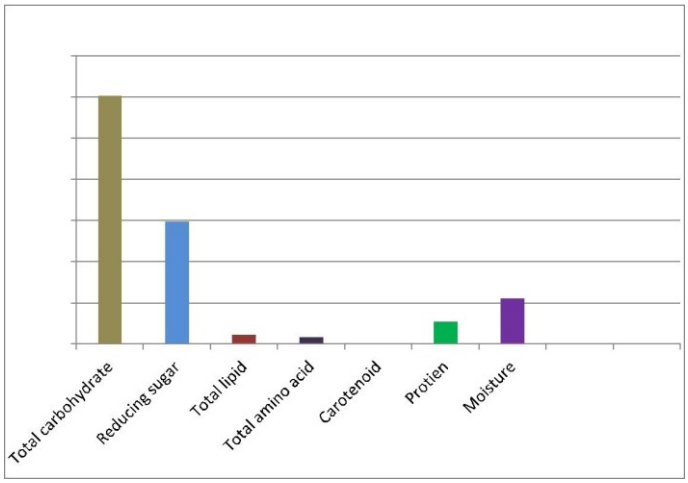
TABLE 1 Biochemical constituent of dry and fresh corolla of Madhuca indica J.FGmel.in terms of mg/g, µg/g and %.

constituent	**dry corolla in mg/g, µg/g**	**dry corolla in %**	**fresh corolla in mg/g, µg/g**	**fresh corolla in %**
total carbohydrate	602**	60.2	213**	21.3
reducing sugar	298**	29.8	99.28**	9.92
total lipid	22.6**	2.26	113.6**	113.3
total amino acid	16.4**	1.64	5.6**	56
carotenoids	33.39***		2.868***	
protein	53.867**	5.38	15.54**	1.55
moisture	110.62**	11.06	728.28**	72.28

***-µg/g, **-mg/g.

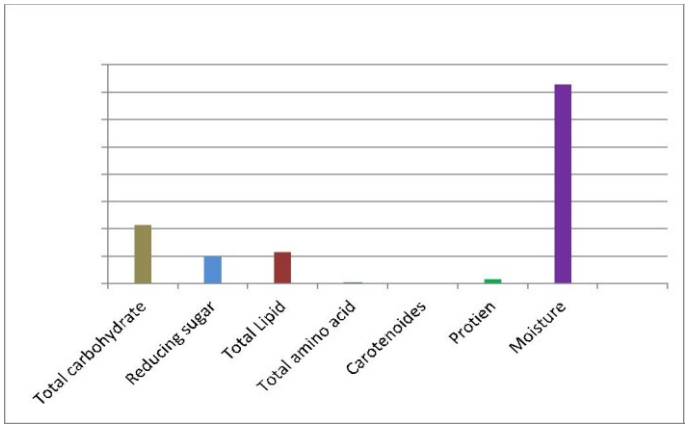
GRAPH 1

Graph showing the different biochemical content in dry Corolla



GRAPH 2

Graph showing the different biochemical content of fleshy Corolla



Enzymatic analysis

Fresh and clean mature leaves were used for analysis of enzymatic activity of catalase, peroxidise and superoxide dismutase. Present investigation showed 270units/g of catalase activity, 1.02units/g of superoxide dismutase activity and 7×10^3units/g of peroxidase activity.

TABLE 2

Table showing enzymatic activities of Madhuca *indica* leaves in terms of units/g

Enzyme	Amount
catalase	270units/g
superoxidase dismutase	1.02units/g

peroxidase	7×10^3units/g

Conclusion

M. indica has great importance due to the presence of various active ingredients in different parts of this tree. It produces edible flowers and fruits. The leaves of Mahua tree contain saponins, an alkaloid glycoside; sapogenin and other basic acids in the seeds. Due to the high reducing sugar and nutrient content Mahua flowers are used as sweetener in preparation of many local dishes (Patel and Naik, 2008) in the Mahua-producing belt of India. Proper scientific investigation can open up new possibilities to use it as an alternative of sugar.

However, due to the lack of proper scientific investigation and post harvest processing technologies, they are collected and subjected to open yard sun drying till about 80% moisture is lost, before storage (Patel and Naik, 2010). This process results in heavy microbial load and degrades their food value, finally making them suitable only for the liquor distillation units and as cattle feed. Thus, a precious, organic and easily available source of natural sugar is being under-utilized.

Sound scientific knowledge and a series of unit operations on the basis of that knowledge can convert the high nutritional value and effective medicinal properties of various parts of *Madhuca indica* into authenticated value added products. The potentialities and the therapeutic values of different chemical ingredients found in *M. indica* posses immeasurable value to cure different diseases. Proper analysis of these biochemical ingredients in a scientific manner will definitely give these, the Government approval and authentication for its consumption and medicinal uses. *Madhuca Indica* has found to have several pharmacological activities, yet several other activities have to be found out.

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