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Phytochemical screening and anti-oxidant property of *Diospyros melanoxylon* used by Gothi Koya and Konda Reddi Tribes of Kinnerasani Wildlife Sanctury, Paloncha, Bhadradri Kothagudem district, Telangana

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Abstract
The Present study emphasizes that the preliminary phytochemical screening and the anti-oxidant activity of an important medicinal plant which was often used by primitive tribal groups of Gothi Koya and Konda Reddi located at Kinnerasani wildlife Sanctuary, Paloncha, Bhadradri Kothagudem district, Telangana state, India. Based on the uses of whole plant, phytochemical constituents, anti-oxidant property *Diospyros melanoxylon* Roxb was considering an important medicinal plant.
Diospyros melanoxylon Roxb leaf extracts were screened for phytochemical components and for anti-oxidant activity. DPPH, Nitric Oxide, Superoxide, Hydrogen peroxide scavenging activity of methanol leaf extracts at different concentrations were studied (ascorbic acid used as standard). The present study revealed the presence of phytochemical compounds and anti-oxidant activity of the extracts of *Diospyros melanoxylon* Roxb at all concentrations tested.

Keywords: *Diospyros melanoxylon* Roxb, anti-oxidant activity, DPPH, Nitric Oxide, superoxide, hydrogen peroxide, gothi koya, konda reddi, palonacha, kinnerasani wildlife sanctuary, khammam

Introduction
The tropical zones sharing 2/3 of the biodiversity of the globe. The Largest tribal population across the globe after Africa was recorded in India [1]. India represents one of the great emporia of ethno botanical wealth. Since time immemorial, plants and their products, animals and minerals have been exploited for the welfare of mankind. It is inevitable to trace out the therapeutic compounds from the nature itself to treat various ailments of man and his domesticated biota. Plants are the basis of every resource like commerce, food, medicine, drugs, furniture, shelter etc. Ayurveda is most acclaimed for its incredible nature of healing. Ayurveda is unique in its treatment, it is individual centric; it treats on the basis of individuals' genetic composition. Ayurveda and nature co-evolved in such a way that it meets the demands of every living being including wild biota.

Ayurveda is a way of life and it has percolated even every ritualistic event of Hindus and can be envisaged in every walk of life. It had imbibed the habitat, diet, lifestyle, culture, civilization and every footprint of Indian mode of life and it represents a holistic approach towards sustainable life in a dynamic way in the changing environmental conditions. As per W.H.O. almost 80% of the mankind relied on conventional methods by using bioactive compounds [2]. Therefore, exploration of plants has become inevitable. W.H.O. defines traditional medicine as the "diverse health practices, approaches, knowledge and beliefs incorporating plant, animal and/or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose, or prevent illness". There is a need to validate the information scientifically for the successful healing, either in combination with currently being used methods, or as a new method for new drug discovery.

Diospyros melanoxylon Roxb is a member of Ebenaceae, it is known as Tuniki or Beedi aaku in Telugu, Tendu in Hindi, Malabar ebony in English and Tinduka in Sanskrit is such tribal fruit. It is famous among tribal communities of the states Telangana, Andhra Pradesh, Madhya Pradesh, Chhattisgarh, Orissa and other states of southern and central parts of India. Solapur-city which is located in Maharashtra state has huge count of small beedi making units. Huge quantity debris from these industries in the form of trimmed leaf residues is available as feed material for preparation of vermi compost [3] Beedi rolling is a popular small-scale industry in Telangana which provides employment to over a million people [4]. It is a well-known tree among the people and ethnic tribes of India. In folklore practice its fruit is employed as a cooling and astringent agent and as an agent of rejuvenation and also used as anti-flatulence agent. It is employed in treatment of rheumatoid arthritis and abdominal pain [5, 6]. Wine prepared from tendu fruit very popular among the tribal community. Pelican colour is seen on bark, exfoliating in rectangular scales. The bark is burnt by local tribe to "heal" disease small-pox. Fruit which is dried and powdered has carminative and astringent property; its tannin - content is 15% and that of half ripe fruit is 23%. (https://abcofagri.com/)

Antioxidants counter the oxidative stress. The free radicals are part of defensive machinery of the body and antioxidants scavenge the free radicals and an imbalance of this results in tissue damage and would result in numerous disorders like neuro degenerative diseases like Alzheimer's, cancer, atherosclerosis, diabetes mellitus, hypertension and ageing [7, 8].

The ever changing modern lifestyle has resulted in a tremendous stress on the lives and the outcome is many disorders. Plants are the sources of several bio active compounds that are being used as components in the medical formulations in treating diseases. In recent past an increased demand for plant derived products has gain momentum due to the minimal side effects, availability and low cast. Several studies revealed the antioxidant nature of medicinal plants, foods and beverages that are rich in phenolic compounds. They reduce the hydrogen donors, free radical scavengers.

Many subtropical plants with potential medicinal value are distributed in Kinnerasani wild life sanctuary, Telangana. Gothikoya and Kondareddi tribes of Kinnerasani wild life sanctuary use bark preparation of *D. melanoxylon* to treat various ailments. In addition to the medicinal value, it is also used in boxes, combs, ploughs and beams make up [9].

The antioxidant property of some selected medicinal plants has been reported from Telangana [10]. Phytochemical and pharmacological properties of *D. melanoxylon* have been reviewed [11]. The antioxidant and antibacterial activities of *Diospyros* have been studied [12] phenolic and antioxidant activities were evaluated [13]. Antioxidant and anti-fungal properties of *Diospyros melanoxylon* were documented [14, 15]. Antimicrobial and antioxidant and antibacterial activity nature of *Diospyros melanoxylon* were studied [16, 17].

Diospyros melanoxylon Tree

Diospyros melanoxylon-Leaf

Materials and Methods

Study site

The present investigation is an attempt to document the utilisation of plants and their products in treatment by Gothikoya and Kondareddi inhabiting in and around Kinnerasani Wild life sanctuary. The area under study was located in between 17°.46′ 30″ N, 80°.33′ 32.4″ E and it was established in 1973, and covers an area of 635.40 km^2 with lush and dark forest. It is a southern tropical dry deciduous forest. The perennial Kinnerasani, is a tributary of Godavari and is the life supporting in the sanctuary.

Phytochemical Analysis

Phytochemical screening was carried out by following standard procedure to assess the qualitative chemical composition of crude extracts and to ascertain major natural chemical groups such as alkaloids, Terpenoids, steroids, flavonoids, tannins, carbohydrates and amino acids. Qualitative phytochemical analyses of the extracts were performed [18].

DPPH radical scavenging activity

The total anti-oxidant potential was determined [19]. Various concentrations of test sample were prepared by serial dilution and 0.1mL of each dilution was added to 3.9 mL of a 6.0×10^{-5} µM methanol solution of DPPH, followed by vortexing. The reaction was allowed to take place in the dark at room temperature to reach a plateau.

The decrease in the absorbance was measured at 517 nm was determined by using a Shimadzu spectrophotometer. The concentration of remaining DPPH in the reaction medium was calculated from the calibration curve as follows:

$$\text{Scavenging effect (\%)} = \frac{(1 - \text{A Sample (517 nm)}}{\text{A Control (517 nm)}} \text{X } 100$$

Super oxide free radical scavenging activity

Different concentrations of 50, 100, and150 µg/mL (10, 20, 30 µL) of plant extracts were taken and the volume was made up to150 µL with methanol, to each of this, 100 µL of riboflavin, 200 µL EDTA, 200 µL methanol and 100 µL NBT was mixed in test tubes and further diluted up to 3 mL with phosphate buffer and absorbance was measured after illumination for 5 min, at 590 nm on UV visible spectrophotometer (Shimadzu, UV-1601), Japan and results were compared with ascorbic acid (10 µg/mL as standard).

Scavenging of nitric oxide

Sodium nitroprusside (5 µM) in standard phosphate buffer solution was incubated with different concentration of the test extracts dissolved in standard phosphate buffer (0.025 M, pH 7.4) and the tubes were incubated at 25 °C for 5 h. After 5 h, 0.5 mL of incubation solution was removed and diluted with 0.5 mL Griess reagent (prepared by mixing equal volume of 1% sulphanilamide in 2% phosphoric acid and 0.1% naphthyl ethylene di amine dihydro chloride in water). The absorbance of chromophore formed was recorded at 546 nm.

The control experiment was also carried out in similar manner, using distilled water in the place of extracts. The activity was compared with ascorbic acid.

Scavenging of hydrogen peroxide

A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffered saline (PBS, pH 7.4). Various concentrations of 1mL of the extracts or standards in methanol were added to 2 mL of hydrogen peroxide solutions in PBS. The absorbance was measured at 230 nm, after 10 min against a blank solution that contained extracts in PBS without hydrogen peroxide. IC_{50} value is the concentration of the sample required to scavenge 50% free radical.

The percentage inhibition was calculated by using the following formula

$$\text{Scavenging activity (\%)} = \frac{\text{OD of control - OD sample}}{\text{OD of control}} \text{ x } 100$$

Results and Discussion

Phytochemical screening of *Diospyros melanoxylon* extracts revealed the presence of different secondary metabolites of pharmacological significance and the results were presented in table no. 1.

Table 1: Phytochemical screening of *Diospyros melanoxylon*

1	Flavonoids	+
2	Alkaloids	+
3	Terpenoids	+
4	Tannins	+
5	Carbohydrates	+
6	Glycosides	+
7	Amino acids and proteins	+
8	Phenols	+
9	Steroids	+
10	Quinones	+
11	Anthraquinones	+
12	Saponins	+

In the present study, DPPH scavenging activity revealed the leaf extracts of *Diospyros melanoxylon* possesses scavenging activity at the studied concentrations.

Pairing of unpaired electrons results in neutralization and converts it into 1-1 di phenyl-2- picryl hydrazine and becomes colorless from purple color. The DPPH radical was measured at 517 nm. DPPH, Superoxide, Nitric oxide, Hydrogen Peroxide scavenging activity of *Diospyros melanoxylon* methanol leaf extracts at different concentrations measured [ascorbic acid (10 µg/ml) was used as standard].

The present study revealed that the *Diospyros melanoxylon* methanol leaf extracts have shown significant reduction of the super oxide anions and they inhibited the formation of formazan.

It interacts with O_2 and results in nitrites and peroxy nitrites formation. Sodium nitroprusside at an optimum pH results in nitric oxide formation and it reacts with O_2 and forms nitrate ions, they are quantitatively measured by use of Greiss reagent. The extracts of plant are potent scavengers of nitric oxide and subsequently compete with oxygen (to inhibit the production of nitric oxide). H_2O_2 oxidizes thiol (-SH) groups and inactivates some enzymes. The cytotoxicity of H_2O_2 is due to hydroxyl radical generated by H_2O_2 interaction with $Fe^{+2}$and Cu^{+2} in the cell. Methanol leaf extracts were noticed for significant inhibitory activity of H_2O_2 due to the antioxidant and free scavenging of radical activity (Table no-2 and Figure no. 1).

Table 2: Anti-oxidant activity of *Diospyros melanoxylon* [All values in this table represent the mean ± SD (n = 6)]

Diospyros melanoxylon	**Extract 50µg/ml**	**Extract 100µg/ml**	**Extract 150µg/ml**	**Standard 10µg/ml**
DPPH	61±0.14	63±0.12	69±0.43	71±0.13
Superoxide	24±0.17	38±0.21	48±0.67	59±0.47
Nitric Oxide	54±0.18	61±0.34	65±0.21	72±0.17
Hydrogen Peroxide	26±0.41	39±0.24	41±0.40	59±0.11

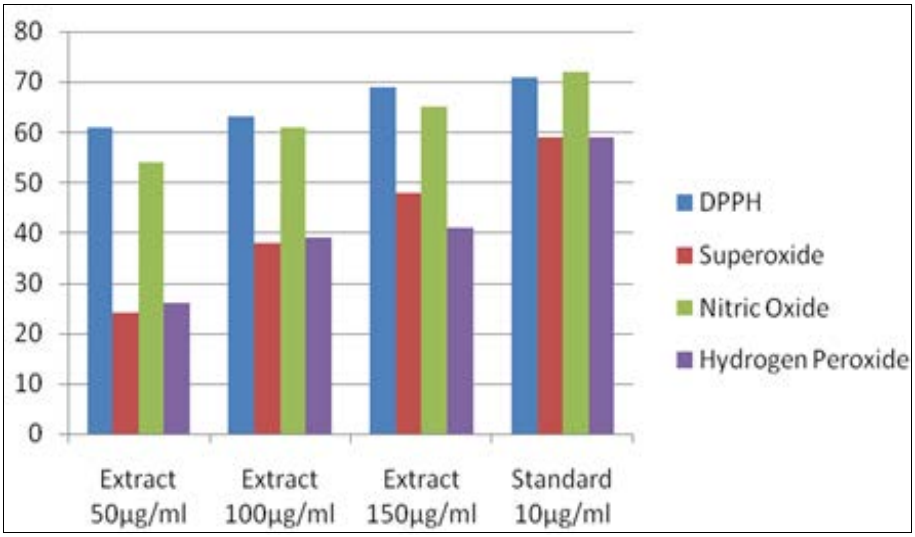

Fig 1: Anti-oxidant activity of *Diospyros melanoxylon*

Antioxidant property of *Diospyros melanoxylon* might be due to phenolic compounds, flavonoids, tocopherols and carotenoids.

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