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Research Article

REVIEW ARTICLE ON DIOSPYROS MELANOXYLON FIND OUT VERIOUS TYPE OF THERAPEUTICAL IMPORTANCE OF ALL PART OF DIOPYROS MELANOXYLON PLANT

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The aim to present this study to highlight the therapeutic activity of diospyros melanoxylon plant D. melanoxylon have [1]antimicrobial activity, [2]anticandidal activity, [3]wound healing activity, [4]analgesic activity, [5]anti-diabetic activity, [6]drug effect on cholesterol, [7]anti- ulcer activity, [8]anti- inflammatory activity

INTRODUCTION

The generic name is derived from the Greek word "Dios" means divine and "Pyros" means fruits. It's also known as Kendu, darkwood, temburini, tendu, abnus, nallatumki, timburni, karundumbi etc [chintala at al][1]. The plant Diospyros melanoxylon (Roxb.) belonging to family Ebenaceae. This plant is widely distributed in the northern part of India (Bihar, Madhya Pradesh, Chhattisgarh, Himachal Pradesh, west Bengal, Mumbai etc) and Tamil Nadu (Coimbatore, Dharmapuri, saltem, etc). It's also used an Indian cigarette product known as beedi making leave through the word. The genus Diospyros consist of 240 species out of this 59 distributed in India. Diospyros melanoxylon is a medium size tree or shrub up to 25 m and 1.9 girths. The primary root is long, thick and fleshy at first, afterword woody, greyish, often swollen in upper part near ground level. The root from vertical loops in sucker-generated plants. Leave opposite or alternate and coriaceous, up to 35 cm long tomentose on both side when fully grown [gupta v.1* and maitili v[2] visvkarma pk *et al*][2].

Traditional/folk medicines

According to previous studies, different parts of the *D m* plant are beneficial in many diseases.

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The parts of plant have individual therapeutic index *i.e*. bark are used in the treatment of diarrhoea, dyspepsia, astringent dysentery and ulceration of cornea [Gupta v.1* maitili visvkarma pk *et al*][3], leaves are used in the treatment of scabies, old wound, laxative and carminative [Gupta v.1* maitili visvkarma pk *et al*][4], fruits are used in the stomach disorders have cooling effect, mental disorders, palpitation of heart also used in nervous breakdown [Rath *et al*][5], and flowers are used in the treatment of skin, blood diseases, urinary discharge, anaemia and is also used as a diuretic [Gupta v.1* maitili visvkarma pk *et al*][6].

Antimicrobial activity

[Ruth *et al*. 2009] collected *D. Melanoxylon* Roxb. (Ebenaceae) barks from the forests of Similipal Biosphere Reserve, Orissa, India and the dried barks have been homogenised to powder followed by extraction. Crude extracts were obtained by successive extraction with petroleum ether, chloroform, ethanol, methanol and aqueous extraction (yield 1.07, 0.8, 5.6, 4.8 and 2.06%, respectively) and were evaporated to complete dryness by vacuum distillation and stored at 4°C in airtight containers. The inhibitory potential of the extracts against standard human pathogenic microorganism has been evaluated using three Gram positive (*Staphylococcus aureus* MTCC1144, *Staphylococcus epidermidis* MTCC3615, *Bacillus lichenoformis* MTCC7425), five Gram negative bacteria (*Escherichia coli* MTCC1089, *Pseudomonas*

aeruginosa MTCC1034, *Pseudomonas fluorescens* MTCC1748, *Salmonella typhi* MTCC3216, *Vibrio cholerae* MTCC3904) and three fungal strains (*Aspergillus niger* MTCC 871, *Trichosporon rubrum*, *Aspergillus fumigatus*). In the agar well diffusion method (Khalid *et al*., 2007), the activity has been evaluated at 50 mg/ml concentration and minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) has been determined. For the different solvent extracts, phytochemical screening has been performed (Mukherjee, 2002; Parekh and Chanda, 2007) and ethanol extract has been found to contain most of the phytochemicals such as alkaloids, steroids, tannins, saponins, ascorbic acid, fixed oil, fats, carbohydrate, gums and mucilage. This might be the reason behind maximum activity of ethanol extract. Presence of the active constituents interferes with the growth and metabolism of microorganisms as phenolic compounds (tannins) and saponins possess antimicrobial and antifungal properties, respectively (Aboaba and Efuwape, 2001; Aboaba *et al*., 2006). The plant extracts were found to be most active against Gram positive microorganisms as compared to the Gram-negative microorganisms. *Staphylococcus aureus* and *Escherichia coli* were most susceptible and only petroleum ether extract has shown activity against fungal strain with *Trichosporon rubrum* showing zone of inhibition (< 15mm).

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Anticandidal activity

[Panda *et al* 2010] collected the bark and leaves of diospyros melanoxylon from similipal biosphere reserve, mayurbhajn, Orissa. And perform phytochemical screening the extract was screened and chemical present in extract of diospyros bark and leave such as – Alkaloid, Flavonoid, Carbohydrate, Glycoside, Protein, Amino acid, Tannin, Triterpenoid, Phenolic compound, Gum, Mucilage, Steroid and sterols. The extract of diospyros melanoxylon bark and leave have anticandidal activity against particularly species of Candida viz. C.albicans, C krusei, Cparapsilosis and C.tropicalis (Evan WC)[1] assay perform by (Eloff JN.)[2] for anticandidal activities by cup plate method, the plate of sabouraud dextrose agar media incubate with 100 micro litre (1.0 multiply 10 the power five CFU/ml) of suspension incubate for growing culture yeast over night and bottoms plate using a sterile standard cork borer and in this plate pouring 50-100 micro litre of molten sabouraud dextrose agar into scooped out wells (10mg/ml in DSMO) after that extract was poured into the walls and allow to evaporate water. After that the yeast seeded plate was incubated at 31 digree temperature for 48 hours**.** And measure zone of inhibition for evaluating the anti candidal activities. TLC bio- autography was tested with ethanol extract against C. tropcalis, C. Parapsilosis the zone of inhibition was found 22mm for C. Tropicalis and 21 mm for C. Parapsilsis with RF value 0.72. The result of MIC showed that 20% extract were active concentration in a concentration of 0.375 mg/ml and 50 % of active in concentration 1.6 mg/ml and 75% extract were active in a concentration of 3.0 mg/ml however 100% concentration give 6.0 mg/ml. The result of MFC was find concentration of 6.0 mg/ml 45% of candida straion was inhibited and remaing 55% killed same concentration

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Wound healing activity

The powdered material of bark of *Diospyros melanoxylon (Roxb.)* was refluxed successively with the 250ml of hydro alcohol in a Soxhlet extractor for 48 hrs, and removed *chlorophyll* by treated Ethyl acetate than dried by using desiccators in order to remove moisture content. The extract obtained from hydro alcohol was labelled, weighed and used for various studies. This alcohol-free extract of *Diospyros melanoxylon* bark was used for following preparation i.e., ointment 0.5 % (W/W) using soft paraffin base for topical use, Povidone iodine 0.2 %(w/w) ointment applied as a standard and gum tragacant suspension (1%) for oral use. (Mukherjee *et al* 2000, Charde *et al* 2005, and Majumdar *et al* 2005) performed wound healing activity by using Albino rats (Wistar) and followed excision wound method. (Myers *et al* 1980) The animals were anesthetized by open mask method with *aesthetic ether*. The rats were depilated on back. One excision wound was inflicted by cutting away 500 mm2 full thickness of skin on ethanol sterilized dorsal thoracic region of rats. The wound was left undressed to the open environment. Group of *albino rat* was used individually for wound healing activity and apply the dose according to rout of administration. The parameters studied were wound closure and time of epithelialisation. The wounds was traced on mm2 graph paper on the days of (Povidone iodine ointment reference standard,0.2 %w/w) 4th day 37.98±0, 8th day 83.93 ±0, 12th day 90.01, 16th day 98.65 ± average value of this was found 13.57 ±0 and (Hydroalcoholic + extract ointment,0.5%w/w) 4th day 45.41 ±0,8th day 52.78 ±0, 12th day 81.75 ±0, 16th day 92.51 ±0, average value of this was found14.63 ± according to the present data the diospyros melanoxylon bark was found more potent in comparision to providon ointment (Mortan *et al* 1972)The percentage of wound closure was calculated. The number of days required for falling of the scar without any residual of the raw wound gave the period of epithelialization the progressive changes in wound area were measured planimetrically by tracing the wound margin on a graph paper every alternate day. The changes in healing of wound i.e.,

measurement of wound on graph paper were expressed as unit (mm2) (Mustafa *et al* 2005)

Analgesic activity (stem bark and root bark)

Previous phytochemical studies revealed that stem bark contains tannins (19%), ceryl alcohol, lupeol, betulin, β-sitosterol, sequoyitol, carboxylic acid, diospyric acid and naphthoquinones [Rastogi *et al* 1970]. The powder of diospyros melanoxylon root and stem bark was sieved (sieve no. 60) to get the coarse powder. This powder was subjected to continuous hot extraction with ethanol. The yield was found to be 15.04% w/w for stem barks and 16.24%w/w for root barks. [Gupta *et al* 2013]. The extract of diospyros melanoxylone has analgesic properties in the stem bark and root bark by ethanolic extracts in mice. The analgesic effect of ethanolic extracts was evaluated by 'hot plate' and 'acetic acid-induced writhing test' in mice. The present analgesic drug was used as a reference drug. Pentazocine (5 mg/kg) and Aspirin (50 mg/kg). *Diospyros melanoxylon* (Roxb.) stem bark and root bark both have analgesic activity. Evaluation of Analgesics Activity by using Albino Swiss mice. Both the extracts (prepared as fine suspension in 1%CMC) were evaluated for their analgesic activity by acetic acid induced writhing method and hot plate method. In acetic acid-induced writhing method, albino mice were grouped into four different groups (six animals each). Group I served as control, where normal saline (2 ml/kg) was given. Group II served as standard, where Aspirin (50 mg/kg) was given. Groups III and IV received ethanolic extract (each 200 mg/kg) orally. The writhing movements were observed and counted after acetic acid administration. [Jackson *et al* 2011 and Bachhav 2009]. Before and after drug administration, the basal reaction time to the heat stimulus was taken by placing the rats on Eddy's hot plate maintained at 55}1°C at 30 min, 60 min, 120 min and 180 min was noted. When the animal licked the fore or hind paws or jumped, it was taken as the end point and was noted [Snedeer 1967] and the results showed that ethanolic extracts of stem bark and root bark both possess analgesic activity the ethanolic extract of stem bark (200 mg/kg) possessed significant analgesic activity in response to acetic acid-induced writhing test, where the writhing count was significantly reduced comparatively. In hot plate method, ethanolic extract of stem bark (200 mg/kg) showed the basal reaction time 10.02 s (p<0.01) and percentage increase in threshold to pain was 148.78% as compared to the standard pentazocine, which showed basal reaction time 10.6 s (p<0.01) and percentage increase in threshold to pain 152.38%. Whereas the basal reaction time for root bark's ethanolic extract (200 mg/kg) was 9.8 s and percentage increase in threshold to pain was 133.3%, which was comparatively less than stem bark extract. Finally, it was concluded that ethanolic extract of stem bark showed higher analgesic activity against acute inflammatory pain as compared to the potent inhibitory [Gupta *et al* 2013]

Antidiabetic activity (D. melanoxylon leaves)

Alloxan induced hyperglycaemic effect the animals were allowed to fast for 24hrs and rendered Diabetic by injection a single dose of alloxan at 150mg/kg body weight administered as a 5% w/v in distilled water by i.p. route. It produces diabetes by selected necrosis of beta –cells (produce insulin and amylin about 70 %) in blood for degradation of sugar (islets of Langerhans) of pancreas. After 48 hrs of injecting Alloxan, Diabetes was confirmed by testing blood sugar with *Erba CHEM 5 Plus* Auto analyzer. The animals with sugar level more than 250mg/dl were selected. Animals were maintained for four days in diabetic condition for well establishment of diabetes. Glipalamides at the dose of 2.5mg/kg was used as a standard drug [Mohan M et.al 2009]. Body weight of animal increase because the insulin level low and sugar level increase in body shows an increase in the mean body weight. *Normal rat* 0 day 230.33±1.47 gm, 7^{th} day 240.00±1.06gm, 14^{th} day 249.2±0.94gm and 21th day 253.3±2.29 gm. This shows that the group of normal rats gained body weight during the treatment period of 21 days. *Diabetic rat* was show change in body weight from a mean (± SEM) value of 0day 190.5±1.2gm.7^{th} day 175.6±1.28gm, 14^{th} day 160.8±1.07gm and 21th day 128.8±1.22gm. These changes in the body weight that the diabetic rats show a progressive loss of body weight, which was found to be significant ($p \leq 0.05$) during the 21 days of treatment period as against the gain in body weight seen in normal group of rats.The *Glibenclamide* (2.5 mg/kg) treated group of diabetic rats shows a mean (±SEM) body weight of 0 day 189.83±1.4gm, 7^{th} day 204.6±1.08gm, 14^{th}day 212.5±1.6gm and day 217.6±1.17 gm on day 21.The body weight gain in this group of rats from day 0 day,7^{th} day,14^{th}day and 21th day was founded relatively less when compared with the normal group. This shows that glibenclamide treatment has protected the diabetic rats from losing the body weight in a significant ($p<0.01$) manner when compared with the diabetic control group of rats. The *ethanolic extract of diospyros melnoxylone* (EEDM 200 mg/ kg) treated group of diabetic rats was found to have mean body weight (± SEM) of 185.60±1.30 gm on day 0, 198.00±1.4 gm on day 7, 207.3±0.9 gm on day 14 and 219.5±1.05 gm on day 21. These values show that the body weight was maintained in a significant manner ($p \leq 0.05$) throughout the study period in this group when compared with diabetic animals. The *Aqueous ethanolic extract of diospyros melanoxylone* (AEDM 200 mg/kg) treated group of diabetic rats show mean (± SEM) body weight of 189±1.15 gm on day 0, 191.80±0.9 gm on day 7, 201.80±1.13 gm on day 14 and 208.16±0.76 g on day 21. These values show that the body weight was maintained in a significant manner ($p \leq 0.05$) throughout the study period in this group when compared with diabetic animals. *[Srikanth et al. 2014] D. melanoxylon extracts* (ethanolic and aqueous) was reduces the fasting serum glucose of normal rats at the dose of 200 mg/kg, body weight of EEDM and AEDM i.e., ethanolic and aqueous extract of *Diospyros melanoxylon* was able to reduce fasting serum glucose which was comparable with the reduction caused by glibenclamide. The ethanolic and aqueous extract of *Diospyros melanoxylon* was able to reduce fasting serum glucose which was comparable with the reduction caused by glibenclamide.

Drug effect on cholesterol

The serum cholesterol levels of the different groups of animals during of study shows that the mean (±SEM) serum cholesterol of the normal animal's group of rats was 141±1.9 mg/dl on day 21. The mean serum cholesterol (±SEM) in the diabetic control group of rats was found to be 255±1.12 mg/dl on day 21. Which was found higher when compared with the normal rats on the respective days. These elevated serum cholesterol levels were found to be increasing throughout the 21 days of study period. The glibenclamide (2.5 mg/kg) treated diabetic rats show a mean (±SEM) serum cholesterol of 132±0.93 mg/dl on day 21, which was found to be

significantly reduced as against the serum cholesterol of diabetic was rats however these values also found to be having no significant difference when compared with the normal rats which means that the values are comparable with those of normal rats and infect lower than the normal rats after 21 day of treatment. This shows that the cholesterol levels have reduced in glibenclamide treated diabetic rats. The ethanolic extract of diospyros melanoxylone [EEDM 200mg/kg] treated diabetic rats show mean (±SEM) serum cholesterol of 154.16±1.35 mg/dl on day 21, which was found to be significantly reduced as against the serum cholesterol of diabetic was rats during the entire study period. The *Aqueous extract of diospyros melanoxylone* [AEDM 200 mg/kg] treated diabetic rats show means (±SEM) serum *cholesterol* of 169.16±1.07 mg/dl on day 21. This change in serum cholesterol values illustrate that the diabetic rats treated with the 200 mg/kg of EEDM show a significant reduction in serum cholesterol after the 21 days of treatment period in comparison to diabetic group of rats. Among these groups i.e., EEDM, AEDM (200 mg/kg) the *serum cholesterol* was not only reduced significantly when compared with diabetic rats but the values were also comparable with those of normal rats on day 21 and glibenclamide treated diabetic rats. The above observations indicates that the treatment of diabetic rats with the EEDM,AEDM reduces the serum cholesterol of diabetic rats at all the tested dose, was able to reduce the serum cholesterol significantly compared to diabetic rat after the 21 days of treatment.[Srikanth *et al*. 2014, Mohan M *et al* 2009 and Tiet *et al*.1991].The effect of Diospyros melanoxylon leaves petroleum ether extract on blood glucose level, lipid level, insulin level, body weight, water and food intake in Streptozotocin (STZ) induced diabetic rats. The extract exhibited hypocholesterolemic and hypotriglyceridemic effects while increased level of HDL in diabetes induced rats. In-vitro activity showed more than 75% viability of cells and significant inhibition in differentiated cells as compared to non-differentiate cells in 3T3-L1 cell line. The extract exhibited the concentration-dependent inhibitory effect with an IC50 value of 689.22 µg/ml.**:** The extract exhibited significant results for antiadipogenic, antidiabetic and hypolipidemic activity both in-vivo and in-vitro and it may prove to be effective for the treatment of both types of diabetes, i.e., Insulin Dependent Diabetes Mellitus (IDDM) and Noninsulin Dependent Diabetes Mellitus (NIDDM).[Rathore *et al* 2014]

Anti-Ulcer Activity

Anti - Ulcer Activity Pyloric Ligation Method In this study the animals (Rats) divided in to four groups (n=6) Group I: Served as control group received normal saline (10ml/kg) (p.o). Group II: Served as standard group received Ranitidine (50mg/kg) i.p. route. Group III & IV: Served as test groups received 100, 200 mg /kg. Body weight. Diospyros melanoxylon leaves methanolic extract in sodium CMC (1%), per oral. Procedure Wister rats weighing between 150 – 250 g were selected for pyloric ligation ulcer model as described by Njar *et al*., 1995). Rats were divided into 4 groups, each group consisting of five animals. The animals were fasted for 24 hrs before the operation procedure. However they given free access to water. Prevent the cannabolism and coprophagy. After 24 hrs fasting, all the groups were treated with standard and test drugs as per the study design. At 25th hr under ether anaesthesia pyloric ligation carried out by a one-inch middle abdominal incision was given below the xiphoid process. The pylorus is carefully lifted out with a minimal handling and traction and ligated without damaging its blood supply. Animals were sacrificed 4-6 h later by cervical dislocation. The stomach was opened to collect the gastric contents. Scoring of gastric ulceration was done as described by [kunchandy 1985]. Ulceration in the stomach was accessed by means of a scoring technique whereby macroscopic examination of the stomach was made using a hand (10 xs) and ulcers were scored using the method and criteria.3, Analgesic Activity Acetic acid-induce writhing in mice In this study the animals (mice) divided in to four groups (n=6). Group I: Served as toxic control group received acetic acid (1%) of 0.1ml/10g, (i.p). Group II: Served as standard group received Aspirin (100mg/kg) in sodium CMC (1%) per oral. Group III: Served as test groups received 100, 200 mg /kg. Body weight. Diospyros melanoxylon methanolic leaf extract in sodium CMC (1%), (p.o) Group IV: Served as test group received 200 mg /kg. Body weight. Diospyros melanoxylon methanolic leaf extract (DMMLE) in sodium CMC (1%) (p.o) [Ramakrishna, Chukka *et al*, 2014]

Anti-inflammatory

The pentacyclic triterpenes are reported to exhibit excellent surface-active properties [Khadzhieva *et al*., 1987]. In fact, ursolic acid is being used as an emulsifier in the pharmaceutical, cosmetics and food industries (Mezzetti *et al*., 1971). The pentacyclic triterpenes have long been thought of pharmacologically inactive but gaining importance in view of their recently attributed interesting activities like anti-cancer (Ishida *et al*., 1990), antiHIV (Xu *et al*., 1996) and anti-inflammatory (Recio *et al*., 1995). Interestingly, ursolic acid and oleanolic acid have been recommended for skin cancer therapy in Japan (Muto *et al*., 1990). Cosmetic preparations containing ursolic acid/oleanolic acid are patented in Japan for the prevention of skin cancer for topical use (Ishida *et al*., 1990). Recently the dihydroxy triterpenic acid, corsolic acid was found to be active as protein kinase inhibitor and cytotoxic agent. In the biocidal front, ursolic acid was found to exhibit potent antifeedant activity against Spilosoma obliqua and Spodoptera litura insects (Shukla *et al*., 1996). In the case of amyrins, a-amyrin palmitate was reported to exhibit excel

CONCLUSIONS

Diospyros melanoxylon plant found very important in aspect of medicinal use its part individually have various type or therapeutic activity like anti-microbial activity, anti-candidal activity, analgesic activity, anti-diabetic activity, effective on cholesterol management, anti-ulcer activity, wound healing properties, anti-oxidant, anti-HiV, skin cancer, anti-feedent properties looking this benefit I thought to aware about these properties of specific plant.

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