International Standard Serial Number (ISSN): 2249-6807

*International Journal of Institutional Pharmacy and Life Sciences 4(5): September-October 2014*

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

**Pharmaceutical Sciences**

**Research Article……!!!**

Received: 10-10-2014; Revised: 20-10-2014; Accepted: 21-10-2014

# ANTIMICROBIAL STUDIES ON PLANT BASED *DIOSPYROS MELANOXYLON* EXTRACTS

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**Keywords:**

*Diospyros melanoxylon*, Antibacterial activity, cup-plate method

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## ABSTRACT

Antibacterial activity of *Diospyros melanoxylon* stem extracts was determined using cup-plate method. In cup-plate method, inhibition zone sizes were used to determine the susceptibility of gram positive, negative bacteria and fungi to the extracts. The results showed that the *Diospyros melanoxylon* stem extracts have significant antimicrobial activity. Chloroform extract shown promising antibacterial and antifungal activity extracts with highest inhibition zone followed by ethyl acetate, methanol and pet ether extracts. The control (streptomycin and gentamycin) was however, more effective than plant extracts. The chloroform extract was shown highest activity against gram positive bacterial *S. aureus* and fungi *C. albicans*. Phytochemical screening of the extracts revealed the presence of phyto-compounds such as triterpenoids, flavonoids and tannins which are known to inhibit bacterial growth by different mechanisms from those of synthetic drugs. These phyto-constituents may be responsible for the *D. melanoxylon* antimicrobial activity.

## INTRODUCTION

Traditional medicine usage is a common practice in developed and developing countries at the primary healthcare level (Essawi and Srour, 2000). According to World Health Organization, greater than 80% of world population depends on traditional medicine for their primary healthcare needs (Duraipandiyan et al., 2006). Due to increased and indiscriminate use of antibiotics for treatment of humans and animals, develops the antibiotic resistance and multidrug resistance in microorganisms. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated. Last from few years, the research in antimicrobial field have been intensively increased in search of safe drugs which has increased due to the misuse of antibiotics (Grayer and Harborne, 1994).

*Diospyros melanoxylon* Roxb. (Ebeaceae), commonly known as '*Tendu patta',* is a medium sized tree native to India, Pakistan, Sri Lanka. The leaves are important raw materials of the "Bidi" (Indian cheap smoke) industry. The seeds can be intoxicating; they have been prescribed in India as a cure for mental disorders, nervous breakdowns, and palpitations of the heart. The fruits have a cooling and an astringent effect. Dried flowers are reportedly useful in urinary, skin and blood diseases. The bark is astringent; its decoction is used in diarrhea (Barve et al., 2013). Earlier studies on bark showed to posses anticandidal and antihyperglycemic activity (Ande et al., 2012) ; while leaves and bark posses antimicrobial activities (Barve et al., 2013).

Except these studies, so far no other pharmacological investigations have been reported on this plant. This plant is commonly available in Marathwada region of Maharshtra. This motivates us to investigate this plant on scientific line. The survey was carried out for its ethnopharmacological use, it was observed that along with the bark the tender stem of plant being utilized for in early healing of wounds, cuts and other skin problems amongst the local tribes. So, in present study attempt was made to investigate the antimicrobial property of *D. Melanoxylon* stem extracts.

## MATERIALS AND METHODS

### Plant material collection

Fresh stems of *D. Melanoxylon,* free from disease were collected during October - November 2011 from different Nanded districts, Maharashtra, India. The identification of collected plant specimen was carried out with the help of taxonomist Prof. Vishal R. Marathe, Science College, Nanded. The specimen of herbarium was preserved at institute for future identification.

**Preparation of extracts**

Fresh stem twigs of the plants were sun and shade dried. The extracts of the plants were prepared by successive extraction as described by Kalaskar and Surana (2011) with slight modification. Dried coarsely powdered stems were extracted with petroleum ether (60-80°), chloroform, ethyl acetate and ethanol successively by hot continuous percolation technique. The successive extracts were collected, evaporated, and stored in desiccators for further use.

**Test organisms**

The test organism includes the following gram positive bacteria - *Staphylococcus aureus*, *Bacillus subtilis* and Gram negative bacteria - *Pseudomonas aeruginosa*, *Escherichia coli.* The fungal strains - *Aspergillus niger, Candida albicans* were used.

**Preliminary phytochemical investigation**

The extracts were qualitatively evaluated for presence of different secondary metabolites using standard qualitative chemical tests (Kalaskar et al., 2012).

**Anti-microbial Activity**

The antibacterial activity of successive extracts was performed using agar cup-plate method (Mallemula et al; 2013). 20 ml of sterile nutrient agar medium was poured into sterile petri-dishes and allowed to solidify. The petri dishes were incubated at 37°C for 24 hrs and 28°C for 48 hrs to check for sterility. The medium was seeded with the 0.1 ml of gram positive/negative and fungi test micro-organisms by spread plant method using. The 5 mm bores were made on the medium using sterile borer. Dried extracts of *D. Melanoxylon* stems was dissolved in Dimethyl sulfoxide (DMSO) to obtained a concentration (500 µg/ml) and sterilized by filtration through a Whatman filter paper no. 1, and 0.1 ml of the different concentrations of extract were added to the respective bores. 0.1 ml of streptomycin amd gentamycin at a concentration of (50 µg/ml) was taken as standard reference. The petri-dishes were kept in refrigerator at 4°C for 1 hr for diffusion. After diffusion the petri-dishes were incubated at 37°C for 24 hours for antibacterial study while, 28°C for 48 hrs for antifungal evaluation. The zone of inhibition were observed and measured. DMSO was used as the control.

**Table 1. Antibacterial activity of different plant extract by cup-plate method**

| Micro-organism / Extracts | Zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | **PE-DM** | **CH-DM** | **EA-DM** | **ME-DM** | **SMC** | **GMC** |
| ***S. aureus*** | 7.27 ±0.15 | 15.23 ± 0.35 | 14.37 ± 0.38 | 10.10 ± 0.26 | 17.97 ± 0.40 | - |
| ***B. subtilis*** | 5.70 ± 0.26 | 14.30 ± 0.40 | 12.17 ± 0.31 | 11.30 ± 0.20 | 17.20 ± 0.39 | - |
| ***E. coli*** | 5.97 ± 0.32 | 14.10 ± 0.26 | 13.77 ± 0.15 | 12.40 ± 0.20 | 19.97 ± 0.51 | - |
| ***P. aeroginosa*** | 5.33 ± 0.51 | 12.20 ± 0.26 | 13.03 ± 0.32 | 8.57 ± 0.35 | 15.20 ±0.41 | - |
| ***C. albicans*** | 5.93 ± 0.15 | 15.40 ± 0.20 | 14.67 ±0.15 | 13.70 ± 0.20 | - | 16.43 ± 0.25 |
| ***A. Niger*** | 5.23 ± 0.35 | 7.20 ± 0.30 | 9.97 ± 0.31 | 6.26 ± 0.21 | - | 12.15 ± 0.21 |

Mean ± SD (n=3) PE-DM- Petroleum ether (60-80°) *D. melanoxylon stem extract*

CH-DM- Chloroform *D. melanoxylon stem* extract

EA-DM- Ethyl acetate *D. melanoxylon stem* extract

ME-DM- Methanol *D. melanoxylon stem* extract

SMC- Streptomycin GMC- Gentamycin

**RESULTS AND DISCUSSION**

In present study, the *Diospyros melanoxylon* stem were extracted successively with increasing polarity of solvents to separate the phytoconstituents based on solubility. The preliminary phytochemical investigation showed the presence of bioactive secondary metabolite in moderate polar extracts. The chloroform extracts shown the presence of flavonoids, steroids and triterpenoids; while ethyl acetate extracts showed the presence of tannins, traces of flavonoids and steroids while methanol extracts shown strongly presence of tannins and phenols.

The antimicrobial activity of successive *Diospyros melanoxylon* stem extract of was determined against pathogenic gram positive, negative bacteria strain and fungal strain. The results were compared with standard streptomycin and gentamycin. The two extracts such as chloroform and ethyl acetate extracts shown promising antibacterial and antifungal activity may be due presence of secondary metabolites such as flavonoids, steroids and triterpenoids. According to Cowan (1999); Bhalodia and Shukla (2014), the flavonoids, steroids and triterpenoids chemical classes have been shown to have inhibitory effects on all types of microorganisms *in vitro*. Flavonoids in plant shown to accumulate in response to microbial infection as antimicrobial agent. Additionally, they have been found as effective antimicrobial substances against a wide array of microorganisms *in vitro*. The activity is probably due to the ability of extracellular and soluble proteins to form complex with bacterial cell walls (Moghadam et al., 2010). Additionally,

methanol showed significant activity. Among the extracts, maximum activity observed in chloroform extract, where as petroleum ether showed the least activity against all bacterial and fungal strain (table 1). Results of present study demonstrates that both gram strains and fungal strain were found susceptible to *D. melanoxylon* stem extract, comparatively were most active against gram positive bacteria than gram negative and *C. albicans* than *A. niger* fungi.

## CONCLUSION

Our findings suggest that there are still many plants which are used traditionally with medicinal values but not documented. So, scientific studies also need to be focused toward undocumented tribal medicinal plants. In present study, *Diospyros melanoxylon* were screened for antimicrobial study based on undocumented tribal use of the plant which are being utilized by tribes in Marathwada region. As *D. melanoxylon* showed the good antimicrobial activity, a detailed biological and phytochemical study is needed to find out the chemical constituent responsible for their activities.

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