



Phytochemical Analysis of Leaf Extracts from *Wrightia tinctoria* R.Br. and its Antidermatophytic Activity

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Accepted on: 17-07-2016; Finalized on: 30-09-2016.

ABSTRACT

Medicinal plants are the most important source of life saving drugs for the majority of the world population. Dermatophytes is common contagious fungal diseases, discovery of new drugs are essential against dermatophytes. The aim of the present study is to screen the phytochemical compounds in the Chloroform leaf extracts of *Wrightia tinctoria* by GC-MS analysis and carry out in-vitro antifungal activity against seven dermatophytes fungi. The chemical composition of *W.tinctoria* Chloroform leaf extract using Perkin-Elmer Gas Chromatography - Mass Spectroscopy analysis revealed the presence of eight major compounds. The antifungal activity was carried out by agar well diffusion method against seven fungi and the tested sample showed remarkable activity in comparison to standard antibiotic drug Clotrimazole. The obtained result interprets that *W. tinctoria* possess potential anti-dermatophytic activity.

Keywords: *Wrightia tinctoria*, Dermatophyte, GC -MS analysis, Agar well diffusion, Clotrimazole.

INTRODUCTION

Medicinal plants are a source of great economic value all over the world. The World Health Organization (WHO) had estimated that up to eighty percent of people still rely mainly on traditional remedies such as herbs for their medicines. Plants are considered as an important source of nutrition and as a result of this; plants are recommended for their therapeutic values¹. The use of plants as medicines has involved the isolation of active compounds, beginning with the isolation of morphine from opium in the early nineteenth century^{2,3}. Plants continue to serve as possible sources for new drugs and chemicals derived from various parts of plants⁴. *Wrightia tinctoria* is belong to Apocynaceae is a small to medium size deciduous tree, which is widely distributed throughout India^{5,6}. The plant is commonly known as Paalai and generally called "Sweet Indrajao". The plant is useful as stomachic, in the treatment of abdominal pain, anti- diarrhoeal and anti-haemorrhagic⁷. Leaves indicated the presence of flavonoids, glycoflavones, iso-orientin and phenolic acids, the leaves of this tree yield a blue dye called pala indigo and its leaves were soaked in coconut oil for few hours and applied for eczema psoriasis and other skin diseases^{8,9}. The bark and seeds are effective against psoriasis and non-specific dermatitis. It has anti-inflammatory and anti-dandruff properties and is used in hair oil preparations¹⁰. One of the most important groups of fungi, which causes worldwide human infections are the dermatophytes. They have the ability to invade keratinized tissues, such as hair, skin and nails to produce an infection, commonly referred to as ringworm¹¹. Some dermatophytes are spread directly from one person to another (anthropophilic organisms), others live in and are

transmitted to humans from soil (geophilic organisms), and still others spread to humans from animal hosts (zoophilic organisms). There are limited number of antifungal drugs are available but some of them are shown resistant to fungal strains¹². Herbal medicines have been important sources of products for the developing countries in treating common infections including fungal diseases. Hence, the aim of study is to characterization leaf compounds with the aid of GC-MS technique, from the leaves of *Wrightia tinctoria* and to carry out its antidermatophytic activity.

MATERIALS AND METHODS

Collection of Plant Material

The leaves of *Wrightia tinctoria* R.Br. (Apocyanaceae) were collected during the month of December from the natural habitats of Villupuram district, Tamil nadu, India. The plant material was identified and authenticated by Department of Botany, Ramakrishna Mission Vivekananda College, Chennai, Tamil Nadu, India.

Preparation of Leaf Extracts

The leaves were shade dried and pulverized to powder in a mechanical grinder, 50 gm powder was extracted in a soxhlet apparatus using chloroform. The Chloroform extracts were dried under reduced pressure using rotary evaporator to get the extract and were stored at 4°C until further used. 2 µl chloroform extract was employed in GC-MS analysis.

GC-MS Analysis

GC-MS analysis of the chloroform extract of *Wrightia tinctoria* was performed in a Perkin-Elmer GC Clarus 500 system comprising an AOC-20i auto-sampler and a Gas



Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with an Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 µm ID × 0.25 µm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2µl was employed (a split ratio of 10:1). The injector temperature was maintained at 250°C, the ion-source temperature was 200°C, the oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min to 200°C, then 5 °C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min, and the total GC-MS running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass-detector used in this analysis was Turbo-Mass Gold-Perkin-Elmer, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.2.

Identification of Compounds

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Antifungal activity by agar well diffusion method

Antifungal screening was performed by agar well diffusion method using Sabouraud dextrose agar (SDA) medium. In the culture plates, wells were prepared with the help of sterile cork borer (6 mm in diameter) standard inoculum of fungal cultures were streaked on the medium, and 1

mg of the plant extracts in 1ml of DMSO (1:1), with 50µg concentration dispensed into each well. The extracts were allowed to diffuse into the medium for 1 hour at room temperature and then plates were incubated at 37°C for 72 hours under aerobic conditions. The Clotrimazole of 10µg/ml concentration was taken as standard reference. The zone of inhibition was measured around each well and recorded 13.

RESULTS AND DISCUSSION

Phytochemical Screening of *Wrightia tinctoria* leaf

The results pertaining to GC-MS analysis leads to the identification of eight compounds. The prevailing eight different compounds in the chloroform extract were Pentadecanoic acid, 13-methyl-, methyl esters (retention time 17.07), 8-Octadecanoic acid, methylester,(E)- (retention time 19.00), Heptadecanoic acid, 14-methyl-, methyl ester(ñ) (retention time 19.15), 10,13-Eicosadienoic acid, methyl ester (retention time 20.68), 9,12-Hexadecadienoic acid, methyl ester (retention time 22.37), 7,10-Octadecadienoic acid, methyl ester (retention time 24.63), E,Z-1,3,12-Nonadecatriene (retention time 25.67), 2,2,4a,6a,8a,9,12b,14a Octamethyl-1,2,3,4,4a,5,6, 6a,6b,7,8,8a,9,12,12a, 12b,13,14, 14a,14b- eicosahydricene (retention time 28.07) The compounds present in the chloroform extract of *W.tinctoria* identified by GC-MS analysis as shown in Fig. 1. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the chloroform extract of *W.tinctoria* are presented in Table 1.

The 8-Octadecanoic acid, methylester,(E) and Heptadecanoic acid, 14-methyl-,methyl ester(ñ) had the major peak area of 39.47. 7,10-Octadecadienoic acid, methyl ester was found to be having the lowest peak area of 1.75.

Table 1: Phytocomponents identified in the Chloroform leaf extract of *Wrightia tinctoria* by GC-MS

Retention Time	Name of the compound	Molecular Formula	Peak area (%)	Molecular Weight
17.07	Pentadecanoic acid, 13-methyl-,methyl esters	C ₁₇ H ₃₄ O ₂	6.87	270.45
19.00	8-Octadecanoic acid, methylester,(E)-	C ₁₉ H ₃₆ O ₂	39.47	296.48
19.15	Heptadecanoic acid, 14-methyl-,methyl ester(ñ),	C ₁₉ H ₃₈ O ₂	39.47	298.50
20.68	10,13-Eicosadienoic acid, methyl ester	C ₂₁ H ₃₈ O ₂	3.05	322.52
22.37	9,12-Hexadecadienoic acid, methyl ester	C ₁₇ H ₃₀ O ₂	1.75	266.41
24.63	7,10-Octadecadienoic acid, methyl ester	C ₁₉ H ₃₄ O ₂	1.66	294.47
25.67	E,Z-1,3,12-Nonadecatriene	C ₁₉ H ₃₄	2.95	262.47
28.07	2,2,4a,6a,8a,9,12b,14a-Octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,12,12a,12b,13,14,14a,14b-eicosahydricene	C ₃₀ H ₅₀	4.75	410.00

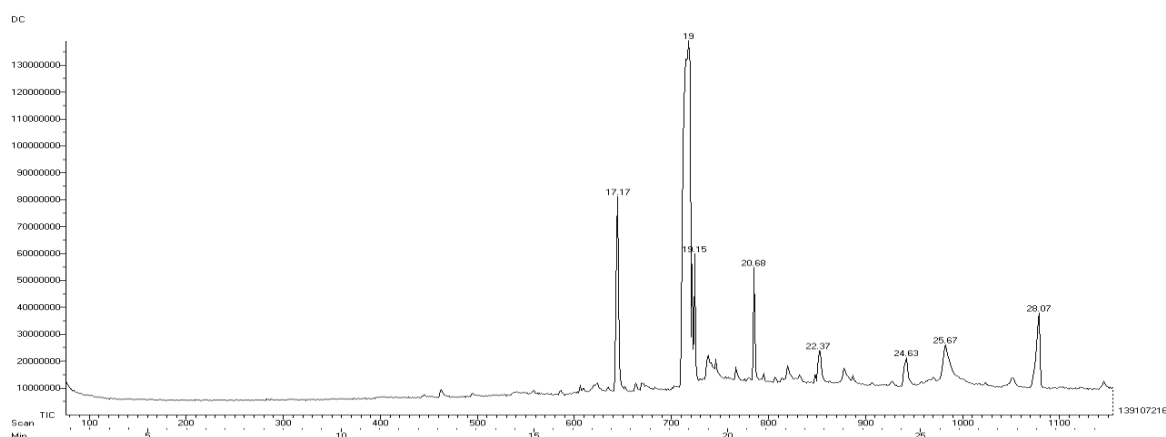


Figure 1: GC-MS Chromatogram of chloroform leaf extract of *Wrightia tinctoria*

Antifungal activity

Leaf extract of *W.tinctoria* showed zone of inhibition against all test the organisms at the concentration of 50µg/ml. Chloroform extract showed intermediate activity against *Candida tropicalis*, *Candida albicans*, *Trichophyton mentagrophytes*, *Microsporium nanum*, *Aspergillus flavus*, *Epidermophyton floccosum*, *Penicillium sp.* The extract showed remarkable antifungal activity against *Microsporium nanum*, *Penicillium sp.*, with compared to that of positive control. The results are interpreted in table 2.

Table 2: Antifungal activity of leaf extracts of *Wrightia tinctoria*

Name of the fungus	<i>Wrightia tinctoria</i> (mm)	Clotrimazole (mm)
<i>Candida tropicalis</i>	11.00	15.00
<i>Candida albicans</i>	12.00	14.00
<i>Trichophyton mentagrophytes</i>	11.00	14.00
<i>Microsporium nanum</i>	11.00	12.00
<i>Aspergillus flavus</i>	12.00	15.00
<i>Epidermophyton floccosum</i>	10.00	14.00
<i>Penicillium sp.</i>	14.00	17.00

Wrightia tinctoria is an important medicinal plant used in the Indian system of medicine for the treatment of variety of diseases and it was reported to possess analgesic and antifungal activities^{14,15}. The more precise information in qualitative analysis can be obtained by gas-chromatography coupled with mass spectrometry (GC-MS)¹⁶. Jayamathi et al (2009), reported that leaf extracts of *W. tinctoria* using ethanol exhibited twenty two compounds whereas the present study revealed eight dissimilar compounds with different peak area and retention time¹⁷. Heptadecanoic acid, 14-methyl-, methyl ester was reported to be having therapeutic properties and 9,12-Hexadecadeciseteroic acid, methyl ester was having used for anti-inflammatory, cancer preventive,

hepatoprotective, hypocholesterolemic, insectifuge, antiacne, alpha reductase, anticoronary, antihistaminic, nematocidal and anti-arthritic activity¹⁸. E,Z-1,3,12-Nonadecatriene and 7,10-Octadecadienoic acid, methyl ester had been reported to be having anti-inflammatory and antioxidant activity¹⁹.

The leaf extract exhibited antifungal activity against the *Microsporium nanum*, *Aspergillus flavus* at the concentration of 50µg/ml whereas, Manoharan Sharanya et al (2013), reported that leaf extract from *W.tinctoria* represents the effective antimicrobial activity against the fungal species of *Microsporium nanum*, *Aspergillus flavus* and *Aspergillus niger* at the concentration of 150µg/ml²⁰.

Moorthy et al (2012) had reported in-vitro antifungal activity using methanol extract against *Candida albicans* at the concentration of 512µg/ml whereas; in the present study chloroform extract showed antifungal activity at 50µg/ml concentration against *Candida albicans*²¹. Chadha et al (1976) reported least activity on fungal species of methanol extract, he also reported that fungal cultures to be susceptible to chloroform extract, whereas in the present study chloroform extract exhibited antifungal activity against *Candida albicans* and *Candida tropicalis*²². In addition, further research is necessary to purify the active compounds responsible for various pharmaceuticals activity.

CONCLUSION

The present research work concludes that *Wrightia tinctoria* is important medicinal plant with varied pharmacological spectrum. Current study on the evaluation of antifungal property exhibited by the medicinal plant *W. tinctoria* would in near future greatly assist the mankind in the identification of novel compounds against the ailing diseases. Gas chromatography and mass spectroscopy analysis showed the existence of various compounds with variable chemical structures. Further studies are needed to explore the potential compounds responsible for the biological activity from *W. tinctoria* for application in drug delivery, nutritional or pharmaceutical studies.

Acknowledgment

The authors are thankful to Secretary and Principal, Ramakrishna Mission Vivekananda College (Autonomous), Mylapore, Chennai, India for providing all facilities and we specially thank Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology, Chennai, India for carried out GC-MS studies and validation of the results. Sincere thanks to Dr.S.Sasikala, Head, Department of Microbiology & Biotechnology, Presidency College (Autonomous), Chennai, for helping the antifungal studies.

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Source of Support: Nil, Conflict of Interest: None.

