Research Article

Antimicrobial activity and identification of Essential oils constituents of leaves of *Wrightia tinctoria* (Apocynaeceae)

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Abstract

Wrightia tinctoria R.Br. is belonging to the family of Apocynaceae, it is distributed in the worldwide and throughout in the India. It is a commonly known as Tamil name is veppalai. The extract of the leaves of *W. tinctoria* was curing the various diseases such as jaundice, psoriasis and other skin diseases. In the present study was to evaluation of an antimicrobial activity of essential oils and identification constituents of essential oils of *W. tinctoria* leaves.

Methods: 250 gms of fresh leaves materials were added with 1lr water and essential oils were hydro- distilled for the Clevenger apparatus for 1 hr. Yielded of the essential oil compositions of *W. tinctoria* leaves were identified by GC, GC-MS method and literatures and to investigation of essential oils of *W. tinctoria* leaves were studied by antibacterial and antifungal activities studied by disc diffusion methods.

Results: The results of the present study observed that fifteen active essential oil compositions of *W. tinctoria* leaves were identified and major oil compounds were urs-12-en-24-oic acid-3-oxo-methyl ester (29.74%), 1-methyl-5-methylene-8-(1-methylethyl) (18.23%), and hydroquinone (16.32%) and minor level of active compounds in 4-methyl phenol (0.15%), caryophyllene (0.56%), and, indole (0.58%). antimicrobial activity was observed in essential oils of *Wrightia tinctoria.*

Keywords: Medicinal Plants, Apocynaceae; *Wrightia tinctoria*; veppalai; leaves, essential oils, GC-MS methods, antimicrobial activity.

Cite this Article

Maridass,M.(2024). Antimicrobial activity and identification of Essential oils constituents of leaves of Wrightia tinctoria (Apocynaeceae). International Journal of Biological Technology, Vol.No.15(2) :1-5. (August-November)

Article History

Article received dated on : 21-04-2024

Corrected and Resubmission dated on 12– 08-2024

Published Online : 15-08-2024

Journal Information

Journal : International Journal of Biological Technology (ISSN : 976-4313 Print)

Publisher :Gayathri Global Academic & Scientific Publisher (GGASP), Tirunelveli, Tamilnadu,India

Weblink:https://gtrpbiojournal.com/ijbt%20volume%20no%2015(1)/1508202401.pdf

1. Introduction

Wrightia tinctoria R. Br. belonging to the family of Apocynaceae, it is widely distributed in the Burma and India. It is a commonly known as "Veppalai". The plant parts are used to several medicinal purposes such as skin diseases, jaundice and relieve the toothache. Literature review of this plant is previously phytochemical analysis of several active compounds identified as falvonoids constitutes are glycoflavones-iso-orientin, and phenolic acids from the leaves. Steroids compounds are wrightial, cycloartenone, cycloeucalenol, β-amyrin, and β-sitosterol were identified from the immature seeds. 3,4-Seco-lup-20 (29)-en-3-oic acid, lupeol, stigmasterol and campetosterol, Indigotin, indirubin, tryptanthrin, isatin, anthranillate and rutin Triacontanol, Wrightial, cycloartenone, cycloeucalenol, β-amyrin, α -amyrin, and β-sitosterol, 14α-methylzymosterol and four uncommon sterols, desmosterol, clerosterol, 24-methylene-25-methylcholesterol, and 24-dehydropollinastanol, were identified in the leaves and pods (Srivastava,2014). The medicinal uses of leaves were cure of skin-healing properties of psoriasis, snake bites, and various inflammations. The present study is carried out the identification of essential oils compositions and its antibacterial and fungal activity of essential oils of *Wrightia tinctoria* leaves.

2. Materials and Methods

2.1. Plant Materials

The leaves materials of *W. tinctoria* were harvested at the flowering stage during the month of March 2022 in the region of Naranammalpuram, Tirunelveli District, Tamilnadu. The herbarium of this plants was submitted to Research Institute of Conservation Ecology (RICE), Tirunelveli, Tamilnadu-627011. The voucher number of this plant is RICEM2022201.

2.2. Hydro- distillation of essential oils

The extraction and collection of essential oils of leaves were carried out hydro -distillation method of Clevenger-type apparatus for 3hrs. The yields of essential oil of leaves were expressed in g relative to 100g of dry matter; it was calculated according to Equation (Majda Elyemni et al.,2019). The essential oils were collected and dried over anhydrous sodium sulphate, and stored in the dark at 4 ºC prior to GC-MS analysis.

$$\text{Yield of the essential oils (\%)} : \frac{\text{Amount of the extracted Oils (gm)}}{\text{Amount of dry vegetal matter mass}} \times 100$$

2.3 GC -MS Analyses and Identification of Essential Oils constituents

The chemical composition of the essential oils extracted by hydro distillation methods and its essential oils is analysis by gas chromatography and coupled with mass spectrometry (GC/MS). The GC analysis was performed using a chromatography equipped with a flame ionization detector (FID) and two capillary columns of different polarities OV type: 101 (25 m x 0.22 mm x 0.25 mm) and Carbowax 20 M (25 m x 0.22 mm x 0.25µm). The carrier gas was used as helium. The flow rate of 0.8 ml/min and the oven programming temperature was between 50 and 200°C with a gradient of 5°C/min. CPG/MS coupling was performed on a DB1-type fused silica capillary column (25 m x 0.23 mm x 0.25 µm). The peaks were identified by the based on the search NIST and Wiley library. Retention indices of essential oil compounds were carried out the according to standard method of Kováts Indices to support the identification of the compounds (Wagner et al.,2003; Shellie et al. 2002; Cai et al.,2006).

2.4 Antimicrobial activity

The investigation of essential oils of antimicrobial activities were studied against *S. aureus, E. coli, S. typhimurium, Candida albicans* and *Aspergillus niger*.

2.5 Screening for antibacterial activity

The effect of antibacterial activity of essential oils of leaves were studied by According to Puškárová, et al., (2017) method followed by a disc-diffusion assay method. To determine the essential oils of *Wrightia tinctoria* active against growth inhibition of bacteria and fungi. Essential Oils was tested concentration at 100% strength, and at various dilutions (5, 10, 25, 50, and 75%) in DMSO. Control as a pure DMSO was included with each test to ensure that microbial growth was not inhibited by DMSO itself. Chloramphenicol (30 µg/disc) was used as a positive control. Plates were then inverted and incubated for approximately 24 h at 37 °C and the diameter of the inhibition zones was measured in mm, including the diameter of the disc. The results were measured the sensitivity was classified in the not sensitive for a diameter less than 8 mm, sensitive for a diameter of 9-14 mm, very sensitive for a diameter of 15-19 mm, and extremely sensitive for a diameter larger than 20 mm (Ponce et al.2003). Each test was performed in three replicates.

2.6 Screening for antifungal activity

The antifungal activity of essential oils of *W. tinctoria* leaves were studied by De Lira Mota et al.(2012) method. Essential Oils of *Wrightia tinctoria* leaves were dissolved in DMSO at different concentrations of percentage of 5,10, 25, 50 and 75 was added. For each dilution, the same volume as the full-strength sample was placed on the sterile disc. Discs were impregnated with 10 µL of DMSO, nystatin (50 µg/mL) and cycloheximide (50 µg/mL) used as controls. Petri dishes were incubated at 26 ±° C for 5 days. Results were observed by diameters of inhibition zone were measured in mm and an inhibition zone larger than 1mm was taken to indicate a positive effect (Puškárová, et al.,2017).

3. Results and Discussion

The result of isolation of essential oils of *W. tinctoria* leaves were hydro-distillation method and yielded in 0.87% (v/w). The essential oils composition of leaves of *W. tinctoria* leaves were analyzed and identified by GC-MS method (Table-1). A total of fifteen active essential oil compositions were identified in leaves and which is represented a total of 94.99% (Table-1). The major oil compounds of leaves were urs-12-en-24-oic acid-3-oxo-methyl ester (29.74%), 1-methyl-5-methylene-8-(1-methylethyl) (18.23%), and hydroquinone (16.32%) and minor level of active compounds in 4-methyl phenol (0.15%), caryophyllene (0.56%), and, indole (0.58%).

Table - 1: Composition of the leaf essential oil of *Wrightia tinctoria* obtained by hydro-distillation

Sl.no	Identified compounds	Retention time (min)	Percentage (%)	Identification Method
1	Benzyl alcohol	2.43	4.21	GC-MS
2	Hydroquinone	3.41	16.32	GC-MS
3	1-methyl-4-(1-methylethyl)-Benzene	4.58	1.02	GC-MS
4	Indole	5.01	0.58	GC-MS
5	α-cubebene	5.59	3.29	GC-MS
6	3-methyl-2-(2-Pentenyl)-2-cyclopenten-1-one	8.21	8.12	GC-MS
7	1-methyl-5-methylene-8-(1-methylethyl) -1,6-cyclodecadiene	8.36	18.23	GC-MS
8	τ-cardinol	9.24	1.45	GC-MS
9	1-pentylheptyl-Benzene	11.17	0.89	GC-MS
10	9,12,15-Octadecatrienoic acid	15.38	6.42	GC-MS
11	4-methyl phenol	16.01	0.15	GC-MS
12	2-methoxy phenol	16.26	1.87	GC-MS
13	Caryophyllene	17.01	0.56	GC-MS
14	Urs-12-en-24-oic acid 3-oxo-methyl ester	22.35	29.74	GC-MS
15	Dodecanoic acid	22.41	2.14	GC-MS
Total percentage			94.99	

Table-2: Antimicrobial activity essential oils of *Wrightia tinctoria* leaves

Sl No.	**Tested organisms**	**Zone of Inhibition (mm)**				
		Tested Concentrations				
		5%	10%	25%	50%	75%
1	*S. aureus*	14±1.2	16±1.3	17±0.5	19±0.9	20 ±0.9
2	*E. coli*	16±1.2	18±0.5	19±0.9	21±0.9	22±0.9
3	*S. typhimurium*	11±1.3	12±1.9	14±1.5	15±0.9	15±0.9
4	*Candida albicans*	12±1.5	14±0.8	18±0.8	19±1.5	22±1.5
5	*Aspergillus niger*	13±1.5	15±1.5	16±1.5	17±0.89	19±0.8

"Values are triplicates"

3.2 Antibacterial activity

The hydro distillation of essential oils was screened for their antibacterial activity using paper disc diffusion method. The results of present study of the essential oils of *W. tinctoria* leaves showed a very good antibacterial activity active against *S. aureus, E. coli* and *S. typhimurium.* The maximum concentration of essential oils was active against the higher zone of inhibition of *E.coli* (22±0.9mm) observed in the present study (Table-1)**.** Wang et al. (2020) showed that ginger essential oil was a good antibacterial activity against *E. coli* and *S. aureus*. Previous studies, Abers, et al.,(2021) reported that 19 essential oils tested against *Staphylococcus* species range from 14 to 29 mm using a 6 mm disc and essential oils of *Salvia officinalis* were very low activity and active composition of 1,8-cineole was good active against both bacterial species of *S. aureus* and *E. coli*. Al-**Nabulsi et al., (2020)** demonstrated that the EOs of cinnamon and thyme showed the strongest inhibition against *E. coli* . Silva et al. (2020) in a meta-analysis study highlighted that lemon balm, sage, shallot, and anise EOs had the best inhibitory results against the pathogen *E. coli*.

Antifungal activity

The essential oils of *W. tinctoria* leaves were observed by good antifungal activity against both fungus species of *Aspergillus niger* and *Candida albicans*. The higher percentage of essential oils were producing the higher zone of inhibition active against *Candida albicans* (22±1.5mm). Thymol, one of the most famous components of thyme, exhibited efficient fungicidal activity against *Aspergillus flavus* (Shen et al.,2016). Many studies reported that the constituents of essential oils could act synergistic or antagonistic [Stevic et al.,2014; **(Adams** 2001]). These compounds are believed to be the active components in the oil as previous study indicated that both compounds were active against *C. albicans* (**Ibrahim & Sadikun, 1990**). Essential oils containing high levels of camphor exhibited strong antifungal activity against *Candida albicans* (Alvarez-Castellanos et al., 2001; Hammerschmidt et al., 1993).

Natural products have historically been used to discover new chemical entities to treat infectious diseases (Rossiter et al.,2017). The present study was good antimicrobial activity was observed in essential oils of *Wrightia tinctoria* leaves and 15 essential oils compounds were identified. Further studies have been going on control of skin diseases in our laboratory.

4. Conflicts of Interest

The author declare that they have no conflicts of interest.

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