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GC-MS Profiling and Antibacterial Activity of *Wrightia tinctoria* and *Lantana camara* Leaves Extract

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Abstract: *Wrightia tinctoria* and *Lantana camara* are distributed in the southern part of India particularly in Tamil Nadu and it has potential medicinal properties and used as antioxidant, antimicrobial, anticancer, diuretic, antitumor and for cardiovascular problems. The present work was designed to investigate the GCMS analysis of ethyl acetate and diethyl ether extracts of *Wrightia tinctoria* and *Lantana camara*. Both gram-positive and gram-negative strains of ATCC bacterial cultures and Clinical strains were used in this study. The GC-MS analysis showed the presence of 12 compounds in ethyl acetate extract of *Wrightia tinctoria*, 25 compounds in diethyl ether extract of *Wrightia tinctoria* whereas, *Lantana camara* showed 27 compounds in ethyl acetate extract and 19 compounds in diethyl ether extract. From the above study, it can be concluded that the *Wrightia tinctoria* ethyl acetate extract have great potential as antibacterial agents against ATCC culture when compared to *Lantana camara* solvent extracts. Hence, this study would lead to the development of some stable, biologically active compounds which can be employed in the formulation of antibacterial agents. Findings from current study support the use of *Wrightia tinctoria* in traditional medicine for treatment of various bacterial infections and can be used as multi-resistant drug in future.

Keywords: *Wrightia tinctoria*, *Lantana camara*, ATCC culture, Clinical strains, GC-MS analysis

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Introduction

More knowledge of different ethnic groups helped to the research in natural products, the creation and comprehension of the close link between the chemical structure and biological qualities of a given compound and the inter-relationships between animal and plant (Raven *et al.*, 1990; Martin *et al.*, 1999; Hensley *et al.*, 2013; Noujaim *et al.*, 2015; Correa *et al.*, 2016; Ching *et al.*, 2018;

Conde *et al.*, 2019). Medicinal plants therefore constitute valuable substances for the research of their traditional usage through pharmacological effect and may be natural composite sources acting as novel anti-infectious medicines. The purpose of the present study was to assess the *in vitro* antibacterial activity of *Wrightia tinctoria* and *Lantana camara* extracts against gram-

positive and gram-negative bacterial strains isolated from human infections (clinical strain) and ATCC strains of gram-positive and gram-negative bacterial cultures.

Wrightia tinctoria a well-known medicinal plant widely used in Ayurveda, Siddha and Unani. It is commonly known as Vetpalai or Venpalai in Tamil Nadu. It has the ability to produce blue dye when soaked in water hence called Pala indigo or dyer' oleander. *W. tinctoria* belongs to Apocynaceae family. It is named after a Scottish physician and Botanist William Wright (1740 – 1827) as reported by Beena Jose and Jesy (2014). This medicinal plant is used in healing in Ayurveda, Siddha, Unani and Naturopathy to treat diseases with no side effects (Bigoniya *et al.*, 2011). *W. tinctoria* is used in treating abdominal pain, skin diseases and the crushed leaves are used to relieve pain in tooth cavity (Khyade and Vaikos, 2009). *W. tinctoria* has a medicinal value for jaundice, tooth ache, stomach ache, anti-dysenteric and in treatment of piles. Anti-Pyretic activity of *W. tinctoria* was reported by Devi and Divakar (2014). *W. tinctoria* is rich in antibacterial, anticancer, anti HIV and antidiabetic properties and it is also more active against gram-positive bacteria and fungi (Nagalakshmi *et al.*, 2012). In the past, for more than three decades, several studies have been carried out on this medicinal plant species to facilitate evidences in favour of its conventional uses (Sakthivadivel *et al.*, 2014). Oil emulsion of leaves and pods are used to treat psoriasis. During headache, leaf and stem bark pastes are applied on the forehead or administered orally. Stem bark paste mixed with half cup of water were given twice a day for 2 days for relief from abdominal pain (Nagalakshmi and Murthy, 2015; Antony *et al.*, 2015; Chung *et al.*, 2017).

Lantana camara also known as Spanish flag or West Indian *Lantana*, is a species of flowering plant in the Verbenaceae family and Verbenaceae is native to American tropics (Singh *et al.*, 2015). In India it is considered as an ornamental plant. The

plant leaves are aromatic. The flowers are orange, blue, yellow, and bright red and usually change colours as they age (Ajitha *et al.*, 2015; Dash *et al.*, 2015). Flowers are having a yellow throat, in axillary head almost throughout the year. In Tamil it is called as Unnichedi, which is widely seen in pathway, deserted field, mountains etc. The fruit is consumed by birds and human. *Lantana camara* is a well-known medicinal plant in traditional medical system and recent scientific studies have emphasized the possible uses of *L. camara* in modern uses (Lee *et al.*, 2020). *L. camara* has several therapeutic values used mainly as herbal medicine and the leaf extracts exhibits antibacterial, fungicidal, insecticidal, nematocidal, biocidal activities, anticancer, antidiabetic and anti-inflammatory (Kumar *et al.*, 2016). Not only in India, in Philippines also, *L. camara* is used in folkloric treatment to cure various ailment like rheumatism, wound fever, asthma, antitumor, analgesic, antifungal and hepatotoxic activities (Jo-Ann *et al.*, 2015). For a long period of time people of India have been using many plant species as traditional medicines for a variety of ailments, including treatment of infectious diseases (Ram Kumar *et al.*, 2017). Discovery of novel drugs can be accomplished with the use of plant extracts, which is a reservoir of broad-spectrum secondary metabolites (Ved *et al.*, 2018; Negi *et al.*, 2019). Plants have proven themselves to be effective in preventing and treating the toxicity induced by other toxins or drugs. The efficacy of plant extracts and their derivatives in terms of their antibacterial activities have paved the way for the exploration of new and effective treatments against bacterial infections. The present study targets the use of such diverse traditional medicinal plants, which are capable of being used effectively as antibacterial drugs.

Materials and Methods

Collection and Preparation of Wrightia tinctoria and Lantana camara leaf extracts using solvents:

The plant leaves were collected from Tiruvanamalai District and were identified and

authenticated by Prof. P. Jayaraman, Plant Anatomy Research Centre (PARC) West Tambaram Chennai, Tamil Nadu, India.

Fresh leaves of *W. tinctoria* and *L. camara* were collected and washed thoroughly using running tap water and then finally with double distilled water, shade dried at room temperature to remove moisture. Then the dried leaves were ground into fine powder using hand blender. The powdered leaves were dissolved in two different solvents (ethyl acetate and diethyl ether) separately. The extraction was done according to the method of Tadesse *et al.* (2017)

GC-MS profiling of Wrightia tinctoria and Lantana camara leaf extracts:

The ethyl acetate and diethyl ether extract of leaves of *W. tinctoria* and *L. camara* is used for the GC-MS analysis. 2 µl of the ethyl acetate and diethyl ether extracts of *W. tinctoria* and *Lantana camara* were dissolved in HPLC grade methanol and subjected to GC and MS. JEOL GCMATE II GC-MS (Agilent Technologies 6890 N Network GC system for gas chromatography). The column (HP5) was fused silica 50 m x 0.25 mm I.D. Analysis conditions were 20 min, at 100 C, 3 min at 235 C for column temperature, 240 C for injector temperature, helium was the carrier gas and split ratio was 5:4. The sample (1 µl) was evaporated in a split less injector at 300 C. Run time was 22 min. The compounds were identified by gas chromatography coupled with mass spectrometry. The molecular weight and structure of the compounds of test materials were ascertained by the interpretation of mass spectrum of GC-MS using the database of National Institute Standard and Technology (NIST).

Antibacterial activities:

To determine the antibacterial potential of *Wrightia tinctoria* and *Lantana camara* leaf extracts of ethyl acetate and diethyl ether against human pathogenic bacteria such as – (i) Clinical strains –*Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter*, *Pseudomonas aeruginosa*,

Enterococcus faecalis and *Staphylococcus aureus*, and (ii) ATCC- *E. coli*(25922), *K. pneumonia* (13883), *P. aeruginosa* (25873), *S. aureus*(25923), *E. fecalis* (29212) and *Enterobacter* (200321) culture were used in this study according to the method of Vijilvania *et al.* (2020)

Results and Discussion

Mass spectrometry becomes a vital tool in the hands of the organic chemists and biochemists because of its potential to supply the definitive, qualitative and quantitative information on molecules based on their structural compositions. Gas chromatography attached to a Mass Spectrometer (GC-MS) enables mixture of small molecules mainly organic compounds of low molecular weight (<600) which can be analysed. The GC-MS analysis showed the presence of 12 compounds in ethyl acetate extract of *Wrightia tinctoria*, 25 compounds in diethyl ether extract of *Wrightia tinctoria* whereas, *Lantana camara* showed 27 compounds in ethyl acetate extract and 19 compounds in diethyl ether extract, Their activities are antimicrobial, antioxidant, anticancer, antitumor, anti-inflammatory, phototoxic, insecticidal, platelet-inhibitory, anti-diabetic, skin diseases, sexual disorders and gynaecological problems, neurological disorders, osteoporosis, neuroprotection, preservatives, plasticizers, dentistry and cardiovascular shown in the Tables 1-4 and Figures 1-4. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethyl acetate extract and diethyl ether extract of *W. tinctoria* and *L. camara* are presented in Tables 1-4.

The dl- α -Tocopherol had the major peak area of 4.02%. 7Benzene-1,3-dicarboxylic acid, 5-(2-methoxy-4-methylthiobenzoylamino)-dimethyl ester was found to have the lowest peak area of 0.13% in ethyl acetate extract of *Wrightia tinctoria* as shown in Table 1. *Wrightia tinctoria* leaves diethyl ether extract also showed high peak area at 31.45% in 12-Oleanen-3-ylacetate, (3 α .) and low peak area was observed in Ethyl1-(1-

Fig. 1: GC chromatogram of *Wrightia tinctoria* leaves ethyl acetate extract.

Fig. 2: GC chromatogram of *Wrightia tinctoria* leaves diethyl ether extract.

Fig. 3: GC chromatogram of *Lantana camara* leaves ethyl acetate extract.

Fig. 4: GC chromatogram of *Lantana camara* leaves diethyl ether extract.

adamantylcarbonyl)piperidine – 4 - carboxylate at 0.10% (Table 2). Similarly, *Lantana camera* extract showed increased peak in gamma-himachalene at 52.74% and low peak in Bicyclo [5.2.0] nonane, 2- methylene - 4, 8, 8- trimethyl-4-vinyl at 0.36% (Table 3). Table 4 shows increased peak at 51.27% in Dimethylsulfoxonium formylmethylide and low peak area in

Naphthalene, 1, 2, 4a, 5, 8, 8a – hexahydro - 4, 7-dimethyl-1-(1-methylethyl)-,[1S-(1.alpha.,4a.beta., 8a.alpha.)] at 0.20%. Jayamathi *et al.* (2009) reported that ethanolic leaf extract of *Wrightia tinctoria* exhibited twenty two compounds whereas the present study revealed 12 compounds in ethyl acetate extract of *Wrightia tinctoria*, 25 compounds in diethyl ether extract of

Table 1: Mass spectra of *Wrightia tinctoria* leaves ethyl acetate extract

RT	Phytocompounds	Area	Formula	Mass	Area%	Biological activity
14.21	Phenol,2,4-bis(1,1-dimethylethyl)-	1680794	C ₁₄ H ₂₂ O	206.2	0.48	Antioxidant, anti-inflammatory, cytotoxicity, antimicrobial, phototoxicity, insecticidal, and nematocidal (Zhao <i>et al.</i> , 2020)
17.11	DiethylPhthalate	2719250	C ₁₂ H ₁₄ O ₄	222.1	0.78	Plasticizers (Romeh <i>et al.</i> , 2013)
20.98	Phytol	3282638	C ₂₀ H ₄₀ O	296.3	0.95	Antinociceptive and antioxidant (Santos <i>et al.</i> , 2013)
28.26	Squalene	28186131	C ₃₀ H ₅₀	410.4	8.13	Antioxidant (Gunes, 2013)
28.30	Cortisone	439502	C ₂₁ H ₂₈ O ₅	360.2	0.13	Dentistry (Singh <i>et al.</i> , 2015 a)
31.10	l-Alanine,n-pentadecafluorooctanoyl-,isohexylester	644122	C ₁₇ H ₁₈ F ₁₅ NO ₃	569.1	0.19	Antibacterial, pharmaceutical and insecticidal (Li <i>et al.</i> , 2020)
31.49	Benzene-1,3-dicarboxylic acid, 5-(2-methoxy-4-methylthiobenzoylamino)dimethylester	436729	C ₁₉ H ₁₉ NO ₆ S	389.1	0.13	Antimicrobial (Paz <i>et al.</i> , 2012)
31.66	3,19:7,8-Diepoxystropane, 17-acetoxy-3-methoxy-4,4,7-trimethyl-	786556	C ₂₅ H ₃₈ O ₅	418.3	0.23	Food additives and flavoring agent (Yanni <i>et al.</i> , 2012)
31.68	4-(4-Fluoro- α -phenylstyryl)-N,N-dimethylaniline, isomer b	660565	C ₂₂ H ₂₀ FN	317.2	0.19	Alkylating agent, and a stabilizer (Mizoguchi and Adam, 1962)
31.93	dl- α -Tocopherol	13948512	C ₂₉ H ₅₀ O ₂	430.4	4.02	Antioxidant, anticancer, anti-inflammatory, platelet-inhibitory effect, down regulation of mutant p53 proteins, activation of heat shock proteins, anti-angiogenic, immune-enhancing activity, atherosclerosis, oxidative stress, cystic fibrosis, short bowel syndrome or bile duct obstruction (Rizvi <i>et al.</i> , 2014)
34.66	9,19-Cyclolanost-24-en-3-ol,acetate,(3. β .)-	50851938	C ₃₂ H ₅₂ O ₂	468.4	14.66	antifungal, antitumor, anti-diabetic, anti-cancer, antioxidant, anti-inflammatory and antimicrobial (Prayitno <i>et al.</i> , 2021)
34.92	12-Oleanen-3-ylacetate,(3. α .)-	129831861	C ₃₂ H ₅₂ O ₂	468.4	37.44	Antioxidant (Babatunde , 2017)
35.65	Lup-20(29)-en-3-ol,acetate,(3. β .)-	113348620	C ₃₂ H ₅₂ O ₂	468.4	32.68	Energy coupling inhibitor((Menezes-de-Oliveira <i>et al.</i> , 2011)

Table 2: Mass spectra of *Wrightia tinctoria* leaves diethyl ether extract

RT	Phytocompound	Area	Formula	Mass	Area%	Biological activity
11.84	Bicyclo[5.2.0]nonane,2-methylene-4,8,8-trimethyl-4-vinyl-	5814822	C ₁₅ H ₂₄	204.2	0.43	Cytotoxic (Pathak <i>et al.</i> , 2018)
12.22	Resorcinol	59126511	C ₆ H ₆ O ₂	110	4.37	Treatment of osteoporosis and symptoms of postmenopausal women (Durairaj <i>et al.</i> , 2005)
12.57	1,4,7,-Cycloundecatriene,1,5,9,9-tetramethyl-,Z,Z,Z-	6527892	C ₁₅ H ₂₄	204.2	0.48	Neurological disorders (Choudhary and Singh, 2018)
13.08	1HCyclopenta[1,3]cyclopropa[1,2]benzene,octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a.alpha.,3b.beta.,4.beta.,7.alpha.,7aS*)]-	4078750	C ₁₅ H ₂₄	204.2	0.30	Free radical and inhibit lipid peroxidation (Widyawati <i>et al.</i> , 2013)
16.75	Phytol, acetate	4366584	C ₂₂ H ₄₂ O ₂	338.3	0.32	Anti-inflammatory and metabolic properties (Olofsson <i>et al.</i> , 2014)
17.11	Diethyl Phthalate	8871704	C ₁₂ H ₁₄ O ₄	222.1	0.66	Antimicrobial (Premjanu and Jaynth, 2014)
19.50	Mandelicacid,4-hydroxy-3-methoxy-	4248728	C ₉ H ₁₀ O ₅	198.1	0.31	Treatment of acne (Debowska <i>et al.</i> , 2015)
19.58	Hexadecanoic acid, ethylester	4752599	C ₁₈ H ₃₆ O ₂	284.3	0.35	Antimicrobial (Abubakar and Majinda, 2016)
19.76	Ethanone,1,1'-(5-hydroxy-2,2-dimethylbicyclo[4.1.0]heptane-1,7-diyl)bis-,(1.alpha.,5.beta.,6.alpha.,7.alpha.)-	2625740	C ₁₃ H ₂₀ O ₃	224.1	0.19	Antimicrobial and antioxidant (Yang <i>et al.</i> , 2016)
20.33	2,3,3-Trimethyl-2-(4-methylpentanoyl)-cyclopentanone	10307768	C ₁₄ H ₂₄ O ₂	224.2	0.76	Fragrances, especially those related to jasmine and jasmine (Hua <i>et al.</i> , 2016)
20.39	1H-Indole-2,3-dione	13043170	C ₈ H ₅ NO ₂	147	0.97	Alkylation reaction on the nitrogen atom N, using a brominated alkylating agent in DMF under phase transfer catalysis conditions (Tribak <i>et al.</i> , 2016)
20.60	Acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-propenylester	9639847	C ₁₄ H ₂₂ O ₃	238.2	0.71	Natural flavor complexes (Cohen <i>et al.</i> , 2018)
21.83	Ethylolate	2590748	C ₂₀ H ₃₈ O ₂	310.3	0.19	Food, aromatics, cosmetics, detergents, flavors and pharmaceuticals (Neta <i>et al.</i> , 2012)
22.01	Linoleicacidethylester	3097337	C ₂₀ H ₃₆ O ₂	308.3	0.23	Functional food ingredient (Kim <i>et al.</i> , 2016)
22.32	9,12,15-Octadecatrienoicacid,ethylester,(Z,Z,Z)-	9823010	C ₂₀ H ₃₄ O ₂	306.3	0.73	Antioxidant, antihyperglacimic,

						antimicrobial and anti-inflammatory (Lan <i>et al.</i> , 2017)
28.26	Squalene	42762734	C ₃₀ H ₅₀	410.4	3.16	Antioxidant (Gunes, 2013)
31.93	dl- α -Tocopherol	24036233	C ₂₉ H ₅₀ O ₂	430.4	1.78	neuroprotection and cardiovascular, skin and bone health (Zaffarin, <i>et al.</i> , 2020)
31.98	Ethyl1-(1-adamantylcarbonyl)piperidine-4-carboxylate	1383051	C ₁₉ H ₂₉ NO ₃	319.2	0.10	Antimicrobial and anti-inflammatory (Al-Omar <i>et al.</i> , 2010)
33.67	Silane,[[[(3.β.,5.α.)-ergosta-8,24(28)-dien-3-yl]oxy]trimethyl-	18551567	C ₃₁ H ₅₄ OSi	470.4	1.37	Prosthetic and restorative dentistry (Metinlinna <i>et al.</i> , 2004)
34.67	9,19-Cyclolanost-24-en-3-ol,acetate,(3.β.)-	262711711	C ₃₂ H ₅₂ O ₂	468.4	19.44	Gastrointestinal problems, skin diseases, sexual disorders and gynaecological problems (Kalam <i>et al.</i> , 2019)
34.92	12-Oleanen-3-ylacetate,(3.α.)-	177875460	C ₃₂ H ₅₂ O ₂	468.4	13.16	Antibacterial, anthelmintic, anti-inflammatory, hepatoprotective, immunopotential, anti-pyretic, antioxidant, antifungal, anticancer and anti-HIV activity (Farooqui and Tyagi, 2018)
34.98	O,O-DiethylO-(6-methyl-2-(4-(trifluoromethyl)phenyl)-4-pyrimidinyl) thiophosphate	4670941	C ₁₆ H ₁₈ F ₃ N ₂ O ₃ PS	406.1	0.35	Pesticide (Villaverde <i>et al.</i> , 2014)
35.20	Urs-12-en-3-ol,acetate,(3.β.)-	96590623	C ₃₂ H ₅₂ O ₂	468.4	7.15	Anticancer, antifungal and antimicrobial (Hasan <i>et al.</i> , 2014)
35.57	12-Oleanen-3-ylacetate,(3.α.)-	425035837	C ₃₂ H ₅₂ O ₂	468.4	31.45	Antioxidant and cytotoxicity (Fabiyl <i>et al.</i> , 2012)
35.65	Lup-20(29)-en-3-ol,acetate,(3.β.)-	148995856	C ₃₂ H ₅₂ O ₂	468.4	11.02	

Table 3: Mass spectra of *Lantana camara* leaves ethyl acetate extract

RT	Phytocompounds	Area	Formula	Mass	Area%	Biological activity
10.07	Cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-,(3R-trans)-	9503477	C ₁₅ H ₂₄	204.2	0.41	Neurobiological effects of this volatile compound, including the cellular and molecular mechanisms of neurotoxicity, and to minimize the human health risk posed by the intentional or accidental inhalation of this potential drug of abuse (Campos-Ordóñez and Gonzalez-Perez, 2016)

10.76	.alpha.-Cubebene	20222896	C ₁₅ H ₂₄	204.2	0.86	Antioxidant activity and antibacterial effect (Sepahvand <i>et al.</i> , 2014)
11.24	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	20604372	C ₁₅ H ₂₄	204.2	0.88	Neurobiological effects of this volatile compound, including the cellular and molecular mechanisms of neurotoxicity, and to minimize the human health risk posed by the intentional or accidental inhalation of this potential drug of abuse (Campos-Ordóñez and González-Pérez, 2016)
11.84	Bicyclo[5.2.0]nonane,2-methylene-4,8,8-trimethyl-4-vinyl-	62114712	C ₁₅ H ₂₄	204.2	2.65	Sulfur fumigation (Ma <i>et al.</i> ,2016)
11.96	.alpha.-Guaiene	14364538	C ₁₅ H ₂₄	204.2	0.61	Flavoring agent (Nurjanah <i>et al.</i> , 2020)
12.57	1,4,7,-Cycloundecatriene,1,5,9,9-tetramethyl-,Z,Z,Z-	10014491	C ₁₅ H ₂₄	204.2	0.43	Antioxidant (El-Maati <i>et al.</i> , 2012)
12.65	Bicyclo[5.2.0]nonane,2-methylene-4,8,8-trimethyl-4-vinyl-	8527226	C ₁₅ H ₂₄	204.2	0.36	Sulfur fumigation (Ma, <i>et al.</i> ,2016)
13.09	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [S-(E,E)]-	201926090	C ₁₅ H ₂₄	204.2	8.62	Antioxidant (El-Maati <i>et al.</i> , 2012)
13.39	Azulene,1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-,[1S-(1.alpha.,7.alpha.,8a.beta.)]-	9570538	C ₁₅ H ₂₄	204.2	0.41	Anti-inflammatory with peptic ulcers, antineoplastic with leukemia, anti-diabetes, antiretroviral with HIV-1, antimicrobial, including antimicrobial photodynamic therapy, and antifungal (Bakun <i>et al.</i> , 2021)
13.45	Cyclohexane, 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-	18031357	C ₁₅ H ₂₄	204.2	0.77	Neurobiological effects of this volatile compound, including the cellular and molecular mechanisms of neurotoxicity, and to minimize the human health risk posed by the intentional or accidental inhalation of this potential drug of abuse(Campos-Ordóñez and González-Pérez, 2016)
13.80	Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,[1S-(1.alpha.,4a.beta.,8a.alpha.)]-	16837637	C ₁₅ H ₂₄	204.2	0.72	Antimicrobial (Rokade and Sayyed, 2009)
14.57	Azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-,[1R(1.alpha.,3a.beta.,4.alpha.,7.beta.)]-	30664390	C ₁₅ H ₂₄	204.2	1.31	Anti-inflammatory with peptic ulcers, antineoplastic with leukemia, anti-diabetes, antiretroviral with HIV-1, antimicrobial, including antimicrobial photodynamic therapy, and antifungal (Bakun, <i>et al.</i> , 2021)
16.74	7-Octadecyne,2-methyl-	61874958	C ₁₉ H ₃₆	264.3	2.64	Antimicrobial (Muthulakshmi and Mohan, 2012)
17.12	6-epi-shyobunol	48033051	C ₁₅ H ₂₆ O	222.2	2.05	Tranquilizing properties (St-Gelais <i>et al.</i> , 2016)
17.17	9,12-Octadecadienoicacid(Z,Z)-	13931393	C ₁₈ H ₃₂ O ₂	280.2	0.59	Used against ringworm infection of scalp, boils and syphilitic eruptions (Tyagi and Agarwal, 2017)
17.39	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	16759602	C ₂₀ H ₄₀ O	296.3	0.72	antibacterial, and antioxidant (Nithya <i>et al.</i> , 2018)
20.99	Cyclohexanol, 5-methyl-2-(1-methylethyl)(1.alpha.,2.beta.,5.alpha.)-(+/-)-	154064069	C ₁₀ H ₂₀ O	156.2	6.57	Neurobiological effects of this volatile compound, including the cellular and molecular mechanisms of neurotoxicity, and to minimize the human health risk

						posed by the intentional or accidental inhalation of this potential drug of abuse (Campos-Ordóñez and González-Pérez, 2016)
25.83	Octadecane,1-[2-(hexadecyloxy)ethoxy]-	11703054	C ₃₆ H ₇₄ O ₂	538.6	0.50	Anti-leaking ability in roof top (Nguyen <i>et al.</i> , 2021)
27.62	Sulfurousacid,butylheptadecylester	25188629	C ₂₁ H ₄₄ O ₃ S	376.3	1.07	Fruits and vegetable preservatives (Voegelé <i>et al.</i> , 2002)
28.47	Tetrapentacontane,1,54-dibromo-	8702462	C ₅₄ H ₁₀₈ Br ₂	914.7	0.37	Antitumor (Kamberovic <i>et al.</i> , 2019)
29.30	1-Decanol,2-hexyl-	37701011	C ₁₆ H ₃₄ O	242.3	1.61	Plasticizers, lubricants, surfactants and solvents (Kanikkannan and Singh, 2002)
30.68	3-(2-Ethoxycarbonylethyl)-4-methyl-1H-pyrrole-2,5-dicarboxylicacid,diethylester	9515066	C ₁₆ H ₂₃ NO ₆	325.2	0.41	Insecticide [Chattopadhyay and Veer, 2016]
30.87	Sulfurousacid,butylheptadecylester	55405887	C ₂₁ H ₄₄ O ₃ S	376.3	2.36	Fruits and vegetable preservatives (Voegelé <i>et al.</i> , 2002)
33.23	Stigmasterol	13999831	C ₂₉ H ₄₈ O	412.4	0.60	It is involved in the synthesis of many hormones like progesterone, androgens, estrogens and corticoids (Kaur <i>et al.</i> , 2011)
33.67	.beta.-Sitosterol	135420608	C ₂₉ H ₅₀ O	414.4	5.78	It is involved in the synthesis of many hormones like progesterone, androgens, estrogens and corticoids (Kaur <i>et al.</i> , 2011)
34.50	.gamma.-HIMACHALENE	1235925634	C ₁₅ H ₂₄	204.2	52.74	Antimicrobial and antioxidant (Chowdhury <i>et al.</i> , 2008)
34.91	6S-2,3,8,8-Tetramethyltricyclo[5.2.2.0(1,6)]undec-2-ene	92834255	C ₁₅ H ₂₄	204.2	3.96	Thermotherapy and aromatherapy (Silalahi <i>et al.</i> , 2019)

Table 4: Mass spectra of *Lantana camara* leaves diethyl ether extract

RT	Phytocompound	Area	Formula	Mass	Area%	Biological activity
5.01	Dimethylsulfoxonium formylmethylide	2286535349	C ₄ H ₈ O ₂ S	120	51.27	Antioxidant and cytotoxic (Paudel <i>et al.</i> , 2019)
10.76	.alfa.-Copaene	14754241	C ₁₅ H ₂₄	204.2	0.33	Cytotoxic and cytogenetic effects (Turkez <i>et al.</i> , 2014)
11.24	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	10890454	C ₁₅ H ₂₄	204.2	0.24	Neurobiological effects of this volatile compound, including the cellular and molecular mechanisms of neurotoxicity, and to minimize the human health risk posed by the intentional or accidental inhalation of this potential drug of abuse (Campos-Ordóñez and González-Pérez, 2016)
11.85	Bicyclo[5.2.0]nonane,2-methylene-4,8,8-trimethyl-4-vinyl-	39502354	C ₁₅ H ₂₄	204.2	0.89	Sulfur fumigation (Ma <i>et al.</i> , 2016)
11.96	.alpha.-Guaiene	9438619	C ₁₅ H ₂₄	204.2	0.21	Flavoring agent (Nurjanah <i>et al.</i> , 2020)
13.09	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [S-(E,E)]-	118496076	C ₁₅ H ₂₄	204.2	2.66	Antioxidant (El-Maati <i>et al.</i> , 2012)
13.80	Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,[1S-(1.alpha.,4a.beta.,8a.alpha.)]-	8869558	C ₁₅ H ₂₄	204.2	0.20	Antimicrobial (Rokade and Sayyed , 2009)

14.57	Azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-,[1R-(1.alpha.,3a.beta.,4.alpha.,7.beta.)]-	18145486	C ₁₅ H ₂₄	204.2	0.41	Anti-inflammatory with peptic ulcers, antineoplastic with leukemia, anti-diabetes, antiretroviral with HIV-1, antimicrobial, including antimicrobial photodynamic therapy, and antifungal (Bakun <i>et al.</i> , 2021)
16.74	7-Octadecyne,2-methyl-	25457465	C ₁₉ H ₃₆	264.3	0.57	Antimicrobial (Muthulakshmi and Mohan, 2012)
17.12	6-epi-shyobunol	26252848	C ₁₅ H ₂₆ O	222.2	0.59	Tranquilizing properties (St-Gelais <i>et al.</i> , 2016)
20.99	Cyclohexanol,5methyl-2-(1-methylethyl)(1.alpha.,2.beta.,5.alpha.)-(./-)-	69581962	C ₁₀ H ₂₀ O	156.2	1.56	Neurobiological effects of this volatile compound, including the cellular and molecular mechanisms of neurotoxicity, and to minimize the human health risk posed by the intentional or accidental inhalation of this potential drug of abuse (Campos-Ordóñez and Gonzalez-Perez, 2016)
25.83	Tetrapentacontane,1,54-dibromo-	15479016	C ₅₄ H ₁₀₈ Br ₂	914.7	0.35	Antitumor (Kamberovic <i>et al.</i> , 2019)
27.62	Sulfurousacid, butylheptadecylester	22663277	C ₂₁ H ₄₄ O ₃ S	376.3	0.51	Fruits and vegetable preservatives (Voegelé <i>et al.</i> , 2002)
28.26	Squalene	115760552	C ₃₀ H ₅₀	410.4	2.60	Antioxidant (Gunes, 2013)
29.30	1-Decanol,2-hexyl-	52873015	C ₁₆ H ₃₄ O	242.3	1.19	Plasticizers, lubricants, surfactants and solvents (Kanikkannan and Singh, 2002)
30.87	2-methylhexacosane	85392665	C ₂₇ H ₅₆	380.4	1.91	Oxidizing agent (Dhar <i>et al.</i> , 2020)
33.67	.beta.-Sitosterol	86640443	C ₂₉ H ₅₀ O	414.4	1.94	It is involved in the synthesis of many hormones like progesterone, androgens, estrogens and corticoids (Kaur <i>et al.</i> , 2011)
34.50	.gamma.-HIMACHALENE	552315939	C ₁₅ H ₂₄	204.2	12.38	Antimicrobial and antioxidant (Chowdhury <i>et al.</i> , 2008)
34.75	6S-2,3,8,8 Tetramethyltricyclo[5.2.2.0(1,6)]	900975294	C ₁₅ H ₂₄	204.2	20.20	Thermotherapy and aromatherapy (Silalahi <i>et al.</i> , 2019)

Table 5: Antibacterial activity of *Wrightia tinctoria* ethyl acetate extract against ATCC culture

S. No.	Name of the organisms	Zone of Inhibition in mm				
		AK	10 mg	20 mg	30 mg	40 mg
1	<i>Escherichia coli</i> - 25922	11	-	-	-	-
2	<i>Klebsiella pneumoniae</i> - 13883	13	12	12	13	14
3	<i>Pseudomonas aeruginosa</i> -25873	14	-	-	-	-
4	<i>Enterobacter</i> - 200321	14	09	12	12	15
5	<i>Staphylococcus aureus</i> -25923	15	13	15	16	16
6	<i>Enterococcus faecalis</i> - 29212	09	-	-	-	-

Wrightia tinctoria with different peak area and RT. Thus, the identification of a good number of compounds from various extracts of *Wrightia tinctoria* and *Lantana camara* by GC-MS might have some ecological significance. All the compounds have previously been reported from a number of other plant species. During extraction a number of polyphenol compounds are released, and these compound might serve to modulate the active compounds in the extract of plant materials in this study as shown in Tables 1-4.

Ethyl acetate extract of *Wrightia tinctoria* contained active and non-active constituents that may result in higher concentration of samples of the potential content of ethyl acetate extract, so its activity appear less than Amikacin due to the pure form so no interferences from other compounds of the standards were observed in this study as shown in Table 5. The procedure yielded inconsistent results and high resolution of bands. This may be due to the assertion that polyphenolic compounds present in *Wrightia tinctoria* ethyl acetate extract are thermally stable and may not decompose at high temperature in GC-MS analysis.

The present study is considered to be a new report on the identification and characterization of bioactive compounds of *W. tinctoria* and *L. camara* as natural and important herbal sources, analysis allowed us to validate the experimental results and to distinguish the *W. tinctoria* and *L. camara* based on their antibacterial activity and chemical composition. *W. tinctoria* ethyl acetate extract showed a powerful inhibitory effect against ATCC pathogens compared with standard; the bacteriostatic activity of Amikacin dramatically enhanced by addition of solvent extract that mean it exhibited a good synergism against pathogens. The factorial analysis of Amikacin and *W. tinctoria* ethyl acetate indicated intense positive interaction effects. This suggests that the main content polyphenolic substances and bioflavonoid could enhance the activity of Amikacin against pathogens in *in vitro* tests. Therefore, the use of commonly available plants such as *W. tinctoria*

will reduce the side effects and cost associated with the applications of synthetic antibiotics and will be an eco-friendly measure.

This study expands the knowledge of *W. tinctoria* and *L. camara* extracts chemical composition and provide an evidence of ethno-medicinal use of the plant by exploring antibacterial activity. The substantial antibacterial activity of *W. tinctoria* and *L. camara* extracts could be due to presence of polyphenol compounds and other reported compounds.

In the present study, initial screening was done to evaluate the antibacterial activity of selected antibiotics against available bacterial strains. A zone of inhibition measuring more than 8 mm signifies that bacteria were susceptible to the tested antibiotics. Table 5 to 10 summarizes the result of antibiotic sensitivity of experimented bacteria against different solvent extract i.e. ethyl acetate and diethyl ether. All the tested bacteria during experimental period showed resistance antibiotics which was active against all the bacteria when compared to solvent treated extracts.

The study aimed at evaluating the antibacterial activity synthesised in medicinal plant of *Wrightia tinctoria* ethyl acetate extract tested against ATTC strains by agar well diffusion method. *Staphylococcus aureus* had a greater zone inhibition (16 mm) in 40 mg, while *Klebsiella pneumoniae* had lower zone of inhibition (14 mm) in 40 mg (Table 5).

Staphylococcus aureus alone had a greater zone of inhibition (19 mm) in 40 mg when compared to other bacterial species when treated with *Wrightia tinctoria* diethyl ether extract (Table 6). Interestingly, ethyl acetate extract of *Wrightia tinctoria* showed high antibacterial activity than the diethyl ether extract when tested with ATCC culture. In this study Antibacterial activity of ethyl acetate may disintegrates the phospholipids present in the cell membrane of bacteria. Whereas, standard (Amikacin) showed

Table 6: Antibacterial activity of *Wrightia tinctoria* diethyl ether extract against ATCC culture

S. No.	Name of the organisms	Zone of Inhibition in mm				
		AK	10 mg	20 mg	30 mg	40 mg
1	<i>Escherichia coli</i> - 25922	09	-	-	-	-
2	<i>Klebsiella pneumoniae</i> - 13883	15	-	-	-	-
3	<i>Pseudomonas aeruginosa</i> -25873	09	-	-	-	-
4	<i>Enterobacter</i> - 200321	12	-	-	-	-
5	<i>Staphylococcus aureus</i> -25923	11	15	17	18	19
6	<i>Enterococcus faecalis</i> - 29212	12	-	-	-	-

Table 7: Antibacterial activity of *Wrightia tinctoria* ethyl acetate extract against clinical strains

S. No.	Name of the organisms	Zone of Inhibition in mm				
		AK	10 mg	20 mg	30 mg	40 mg
1	<i>Escherichia coli</i>	15	-	-	-	-
2	<i>Klebsiella pneumoniae</i>	12	-	-	-	-
3	<i>Pseudomonas aeruginosa</i>	14	-	-	-	-
4	<i>Enterobacter</i>	13	-	-	-	-
5	<i>Staphylococcus aureus</i>	15	17	18	18	19
6	<i>Enterococcus faecalis</i>	09	-	-	-	-

moderate activity when compared to 40 mg/ml of ethyl acetate tested bacteria strain of *Staphylococcus aureus*, this may be due to antibiotic nature of the drug.

Staphylococcus aureus alone had a greater zone inhibition (19 mm) in 40 mg/ml compared to other strains of bacteria as shown in Table 7.

Staphylococcus aureus had a greater zone of inhibition (18 mm) in 40 mg, while *Klebsiella pneumoniae* had lower zone of inhibition (11 mm) in 40 mg and remaining species of bacteria such as *Escherichia coli*, *Enterobacter*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* showed no activity when treated with different concentration of *Lantana camara* ethyl acetate extract as shown in Table 8. Table 9 shows antibacterial activity of the standard and no activity was noticed in the tested species when the concentration was

increased. The lack of visual zone does not mean that the antimicrobial agent (induced by *Lantana camara* ethyl acetate extract) is ineffective. The zone of inhibition test requires the antimicrobial agent to migrate into the nutrient agar. If the antimicrobial is not compatible with the nutrient agar, it will not migrate to create a visual zone of inhibition in the present study.

Staphylococcus aureus had a greater zone of inhibition (18 mm) in 40 mg, while *Enterobacter* had lower zone of inhibition (14 mm) in 40 mg when compared to the rest of the species of bacteria as shown in Table 10. No zone of inhibition was observed in *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* when tested with *Lantana camara* diethyl ether extract. Minimal antibacterial activity in diethyl ether extract could be due to a low concentration of antibacterial compounds in the diethyl ether

Table 8: Antibacterial activity of *Lantana camara* ethyl acetate extract against ATCC culture

S. No.	Name of the organisms	Zone of Inhibition in mm				
		AK	10 mg	20 mg	30 mg	40 mg
1	<i>Escherichia coli</i> - 25922	11	-	-	-	-
2	<i>Klebsiella pneumoniae</i> - 13883	15	9	10	11	11
3	<i>Pseudomonas aeruginosa</i> -25873	14	-	-	-	-
4	<i>Enterobacter</i> - 200321	17	-	-	-	-
5	<i>Staphylococcus aureus</i> -25923	10	11	12	16	18
6	<i>Enterococcus faecalis</i> - 29212	15	-	-	-	-

Table 9: Antibacterial activity of *Lantana camera* ethyl acetate extract against clinical strains

S. No.	Name of the organisms	Zone of Inhibition in mm				
		AK	10 mg	20 mg	30 mg	40 mg
1	<i>Escherichia coli</i>	14	-	-	-	-
2	<i>Klebsiella pneumoniae</i>	19	-	-	-	-
3	<i>Pseudomonas aeruginosa</i>	12	-	-	-	-
4	<i>Enterobacter</i>	13	-	-	-	-
5	<i>Staphylococcus aureus</i>	15	-	-	-	-
6	<i>Enterococcus faecalis</i>	10	-	-	-	-

Table 10: Antibacterial activity of *Lantana camera* diethyl ether extract against clinical strains

S. No.	Name of the organisms	Zone of Inhibition in mm				
		AK	10 mg	20 mg	30 mg	40 mg
1	<i>Escherichia coli</i>	09	-	-	-	-
2	<i>Klebsiella pneumoniae</i>	23	08	10	14	15
3	<i>Pseudomonas aeruginosa</i>	08	-	-	-	-
4	<i>Enterobacter</i>	15	-	-	13	14
5	<i>Staphylococcus aureus</i>	13	12	15	16	18
6	<i>Enterococcus faecalis</i>	15	-	-	-	-

extracts (Table 10). From the above study, it can be concluded that *Wrightia tinctoria* ethyl acetate extract have great potential as antibacterial agents against ATCC culture when compared to *Lantana camara* tested solvent extracts. Hence, this study would lead to the development of some stable, biologically active compounds which can be

employed in the formulation of antibacterial agents. In contrast to this, the zone of inhibitory concentration was evaluated for ethyl acetate and diethyl ether treated crude extracts of *Lantana camara* which showed no inhibition against pathogenic growth of *Pseudomonas aeruginosa*, *Enterobacter*, *Enterococcus faecalis*, *Escherichia*

coli, *Klebsiella pneumonia* and *Staphylococcus aureus* (Tables 9, 10). Generally, biological molecules can be affected as the temperature of treatment is increased with respect to various times of exposures. Accordingly, the community who need to consume medicinal plants either as nutrients or treatment of different ailments should not subject the plant material to temperature, especially above 50 °C even to make the plants texture decent for use and also should have thorough literature survey about the primary and secondary metabolites present in the plant.

Conclusion

The present study concludes that *Wrightia tinctoria* and *Lantana camara* are important medicinal plant with varied pharmacological spectrum. Current study on the evaluation of antibacterial property exhibited by the medicinal plant *Wrightia tinctoria* and *Lantana camara* 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 *Wrightia tinctoria* and *Lantana camara* for application in drug delivery, nutritional or pharmaceutical studies.

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