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RESEARCH ARTICLE

Profiling of Phytoconstituents and Antimicrobial Evaluation of *Wrightia tinctoria* Leaf Extracts

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ABSTRACT

The Indian traditional system of medicine recognizes *Wrightia tinctoria* R.Br. (Apocyanaceae) as a biologically effective therapy for jaundice. They are of interest to the pharmaceutical industry because they are a wild medicinal plant with a wide range of function and biological effects. Different organic solvents, including Petroleum ether, N-butanol, Carbinol, and Acetone, were employed for extracting phytocompounds. The extracted substances underwent phytochemical analysis, antibacterial testing, and compound characterization through techniques like GC-MS and FT-IR analysis. Among the four extracts, carbinol and N Butanol extracted the highest number of phytoconstituents, The extracts were tested for antibacterial activity against *E. coli*, *S. aureus*, *Pseudomonas*, *Klebsiella*, and *Enterobacter*. Among these, *Enterobacter* sp exhibited the greatest sensitivity in Carbinol extract (15.33±0.58), while *Klebsiella sp* showed similar sensitivity levels in both carbinol and N- butanol extract. (14.67±0). FT-IR analysis identified several functional groups, including hydroxyl (O-H), aliphatic (C-H), alkene (C=C), and carbonyl (C-O) groups. The GC-MS analysis of the carbinol extract from *Wrightia tinctori* identified 28 active compounds, with Butanoic acid (5.1419), Butanoic acid, 2-methylpropyl ester (6.3145), and n-Hexadecenoic acid (18.2658) possessing antibacterial, anti-inflammatory, and anticancer properties. This study depends the comprehension of the phytocompounds found in *Wrightia tinctori* leaf extracts and supports the plant's traditional medicinal applications by examining its antibacterial properties. The significant antibacterial properties of *Wrightia tinctori* leaf extracts are likely due to the presence of terpenes, flavonoids, vitamin E, and other well-documented compounds. This research involves the identification of phytochemicals and the antibacterial properties of the carbinol extract from *Wrightia tinctoria*, supporting therapeutic claims about this species in traditional medicine.

Keywords: *Wrightia tinctoria*, FTIR, GC-MS, phytocompounds, Antibacterial activity.

INTRODUCTION

Medicinal plants are known to contain innumerable biologically active substances which possess antibacterial properties (Brantner *et.al*. 1994). Medicinal plants are an important therapeutic aid for various ailments.(Mohan *et.al*, 2010) Around 15% of angiosperms have been investigated chemically and from that nearly 74% of pharmacologically active bioactive substance were identified (Perumalsamy *et al*.1999). In the recent years, there has been a renewed interest on medicinal plants and plant based drugs as it gives a holistic and harmonious balance of physical, biological and mental process through systems of medicine like Ayurvedha, Unani, Siddha and Homeopathy, due the emergence of multi-drug resistance microorganisms(Ravindra Sharma *et al*, 2004).

Wrightia tinctoria belongs to family Apocynaceae commonly called as Sweet Indrajao, Pala Indigo Plant, Dyer's Oleander. "Jaundice curative tree" in south India (Rajani Srinivatsa *et al*, 2014). The leaves of this tree yield a blue dye called "Pala Indigo". The five flavonoid compounds indigotin, indirubin, tryptanthrin, isatin and rutin were isolated and identified from the leaves. (Muruganandam *et al*, 2000). The juice of the tender leaves is used efficaciously in jaundice (Moorthy *et .al* 2012).Crushed fresh leaves when filled in the cavity of the decayed tooth relieve tooth ache (*Sridhar et al, 2011*). biologically effective plant for the treatment of jaundice in the Indian traditional system of medicine. *Wrightia tinctoria* is a wild medicinal tree possessing anti-inflammatory, antidiabetic, antinociceptive, hepatoprotective, antibacterial, antifungal, antiviral, antipsoriatic, anticancerous, anthelmintic, aphrodisiac, analgesic, and antipyretic activities. (Nausheen Khan *et al.*, 2021).

The leaves are applied as a poultice for mumps and herpes. In folk medicine, the dried and powdered roots of Wrightia along with *Phyllanthus amarus* (Keezhanelli) and Vitex negundo (Nochi) is mixed with milk and orally administered to women for improving fertility (Beena Jose *et al*, 2014).Notably, the leaves are particularly effective in treating skin ailments and are integrated into Siddha medicine for psoriasis management. Key compounds such as indigotin, indirubin, tryptanthrin, isatin, anthranilate, and rutin are extracted from *W. tinctoria* leaves, alongside β-amyrin, lupeol, β- sitosterol, and ursolic acid. The leaves possess an acrid taste, whereas the bark and seeds taste bitter. Additionally, the leaves exhibit thermogenic and hypotensive properties, while the bark and seeds are noted for their *thermogenic, carminative, anthelmintic, depurative, and aphrodisiac attributes* (Meenu *et.al*, 2022). The present study has been extensively investigated antimicrobial activity of *Wrightia tinctoria* leaves petroleum ether, carbinol, N-butanol and acetone extracts on selected organisms. Phytochemical analysis. And its characterized by FTIR and GC-MS analysis.

METHODOLOGY

Collection of plant materials
Fresh healthy leaves of *Wrightia tinctoria*, were collected from the locality near Coimbatore, Tamil Nadu, India.

Extraction of plant materials

Healthy leaf samples were washed thoroughly with sterile water, shade-dried and pulverized into fine powder. About 50gms of powdered material was extracted by cold maceration method with 200 ml of each of the following organic solvents such as Petroleum ether, n -butanol, Acetone , carbinol and aqueous and kept undisturbed for 24 hr. The extracts obtained with each solvent were filtered through Whatmann filter paper No.1 and the respected solvents were evaporated (at room temperature). The sticky light greenish-brown substances were obtained and suspended in dimethyl sulphoxide (DMSO) for further analysis.(Dhale *et,al* 2011)

Phytochemical analysis of the extracts:

Preliminary qualitative phytochemical analysis of the crude extracts collected were determined as follows (Horyomba Siaka Ouandaogo *et al*,2023)

Test for Alkaloids: Wagner's Test

Each extract were put into a test tube with a volume of around 2 mL. Wagner's reagent was applied, followed by 2 mL of diluted 1% HCL, and incubated for 15 min.

Test for Flavonoids

A test tube containing 5 mL of each extract was Test for Flavonoids: Alkaline Reagent Test Two milliliters (2 mL) of weak NaOH was added to 3 mL of each extract in test tubes.

Test for Phenolics

3 mL of each extract were placed in a test tube , along with a mixture of 10% ferric chloride and 10% ferrocyanide.

Test for saponins: froth test

A test tube containing 1 ml of extract were taken and mixed with 1% lead acetate.

Test for Tannis

A test tube containing 5 ml of solvent extracts were taken and mixed with 10% lead acetate.

Antimicrobial Screening

Source of microbial strains

Microbial strains used in this study are as follows: *Escherichia coli, Staphylococcus aureus, Pseudomonas sp, Klebsiella sp* and *Enterobacter sp.* and two fungal strains are *Aspergillus sp* and *Fusarium sp.* Cultures were maintained in nutrient agar slants and stored at 4°C. All cultures were checked for viability and purity by regular plating.

Anti bacterial activity

Antibacterial activity was tested by using well diffusion method. Use 24 hours old bacterial culture in nutrient broth. Muller Hinton Agar plates were prepared. Log phase test culture *Escherichia coli, Staphylococcus aureus, Pseudomonas sp, Klebsiella sp* and *Enterobacter sp.* were swabbed on the Mueller Hinton Agar plate using the sterile cotton swab. Make a well using well cutter. And the extract is loaded in the wells. The plates were incubated at 37°C for 24hrs and the zone of inhibition around the well was measured in millimeter (mm).(Kiran *et al.*, 2021)

Anti fungal activity

Aspergillus sp and *Fusarium sp* were used for antifungal activity. Prepare Sabouraud Dextrose broth and inoculate the fungal culture at 30°C for 3 days in shaker incubator. Then the culture was swabbed on MHA plate using sterile swab. Make a well using well cutter. And the extract is loaded in the wells. The plates were incubated at 30°C for 2-3days.

Characterization of Extract

FTIR Analysis

FTIR is a tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plants extract. *Wrightia tinctoria* extract with each solvent were given for FTIR analysis (Revathi *et al*, 2019)

GC-MS Analysis

The GC-MS [Gas Chromatography-Mass Spectrometry] analysis of *Wrightia tinctoria* extract provided critical insights into its chemical profile, revealing the presence of various bioactive compounds. The separation and identification of individual components through retention times and mass spectral data underscore the extract's complex phytochemical composition.

RESULT

Phytochemical Screening

The preliminary phytochemical screening of *Wrightia tinctoria* with different solvent showed the presence of bioactive constituents such as alkaloids, Tannis, saponins, flavanoids, saponin and phenolic compounds (Table 1). These substances are said to be responsible for the antimicrobial activity of this plant.

Antibacterial Activity

The antibacterial activity of the extracts (Petroleum ether, N- butanol, Carbinol, and Acetone) were screened by well diffusion method and the zone of inhibition was assessed in millimetre diameter. The maximum inhibitory activity was observed in carbinol extract against *Enterobacter sp* of 15.33mm, carbinol and N- butanol extract shows similar activity towards *Klebsiella sp* of 14.67mm followed by Acetone extract against Pseudomonas of 12.33 mm. Petroleum ether extract against *Klebsiella sp 12.67 mm*. The result were given in the table 2 and fig:1,2&3.

Antifungal Activity

Antifungal activity of W. tincoria on two species *Aspergillussp* and *Fusarium sp* using well diffusion method. The result showed in the table 3 and fig: 4&5.

Characterization

FTIR Analysis

FTIR analysis of *Wrightia tinctori* extract has been used to identify functional groups. FTIR spectra confirm the presence of Alcohol, Aliphatic hydrocarbons, Alkyne and Carboxylic acid. And the spectrum of betacyanins. FT-IR results from *Wrightia tinctoria* flower extract can be seen in table: 4 figure 6

GC-MS Analysis oF *Wrightia tinctoria*

Chromatographic fingerprint analysis of Indian variety *Wrightia tinctoria* carbinol extract using GC-MS has shown 28 peaks indicating the presence of 28 phytochemical compounds. The peak observed at GC-MS Analysis presented in *Fig 7*. The identification of the phytochemical compounds was based on the peak , Retention time and molecular formula [MW] and Molecular concentration % (MC%) . The molecular activity of some of the identified compounds and structures of some of the identified compound's in table 5.

DISCUSSION

Infectious diseases had a major role in increasing morbidity and mortality and those disease causing agents are resist towards drugs . Therefore an alternative drug sources are needed such as plants, microbes and chemicals. The study includes the antimicrobial effect of *Wrightia tinctoria* and its phytochemical analysis, FTIR Analysis and its GC-MS content. From all the above analysis *Wrightia tinctoria* had effectiveness against selective microbes. Similar studies with *Wrightia tinctoria* along with different solvents like acetone, methanol, petroleum ether and ethanol where more studies show higher activity in methanol extract and elucidating its bioactive components. However this study shows, high sensitivity towards *Enterobacter sp*, *Klebsiella sp* and *Pseudomonas sp.* Especially Its sensitive towards *Enterobacter sp* in carbinol extract. The antimicrobial activity of *W. tinctoria* may be associated with the presence of alkaloids, tannis, saponins and flavonoids. And some functional groups such as alcohol, aliphatic hydrocarbon alkyne and carboxylic acid. In GC-MS it provides 28 components. This suggests, these components may also provide antimicrobial activity against certain microorganisms. Results of this study may further helpful in development of drug formulation. On the other hand, a few unknown minor components present have not been elucidated in terms of their activity. Further studies are required to validate the potential of bioactive compounds detected in this study. In the nearest future, thorough investigation is needed to better ascertain the antimicrobial effect of these plant extracts.

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Table 1: Qualitative Phytochemical Analysis of *Wrightia tinctoria*

Phytochemical compound	Petroleum ether	N-butanol	Carbinol	Acetone	Aqueous
Alkaloids	-	+++	+++	++	+
Tannis	-	-	-	-	++
Saponins	+	+++	-	-	-
Flavonoids	-	+	+++	-	-
Phenolic	-	-	-	-	-

Table 2 : Antibacterial Activity of *Wrightia tinctoria* Extract

Name of the test organisms	Zone of inhibition in mm								
	Petroleum ether		N-Butanol		Acetone		Carbinol		Chloromphenicol
	Concentration of the extract in µg/ml								
	50	20	50	20	50	20	50	20	1
E.coli	6.67±0.58	0	10.33±0.58	6.67±0.58	5.33±0.58	0	7.33±0.58	0	12.33±0.58
S.aureus	10.33±0.58	0	5.67±0.58	0	6.33±0.58	0	10.33±0.58	0	14.33±0.58
Pesudomonas	6.33±0.58	0	10.33±0.58	0	12.33±0.58	0	10.33±0.58	0	14.33±0.58
Klebsiella sp	12.67±0.58	0	14.67±0.58	0	5.33±0.58	0	14.67±0.58	0	12.33±0.58
Enterobacter sp	10.33±0.58	0	5.67±0.58	0	8.33±0.58	0	15.33±0.58	0	10.33±0.58

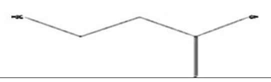
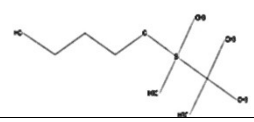
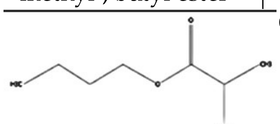
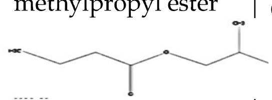
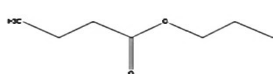
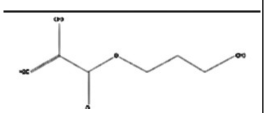
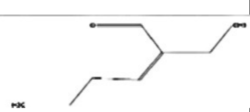
Table 3: Antifungal Activity of *Wrightia tinctoria* Extract

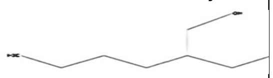
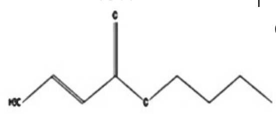
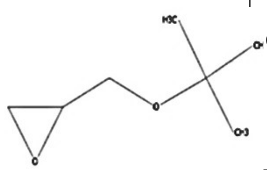
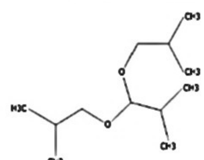
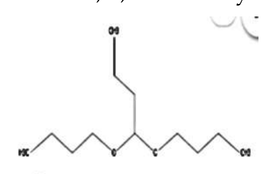
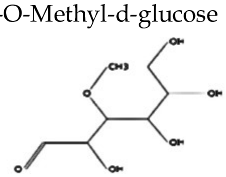
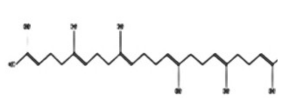
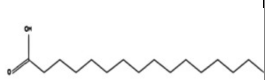
Zone of inhibition in mm						
Test Organism	n- Butanol					
	Concentration of the extract in µg/ml					
Aspergillus sp	25	50	75	100	125	200
	2±0.5	0	0	0	1.5±0.5	0
Fusarium sp	1.6±0.5	1.9 ±0.5	1.5±0.5	0	1.9±0.5	0.9 ±0.5

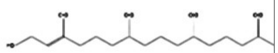
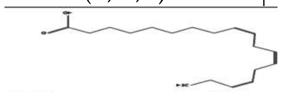
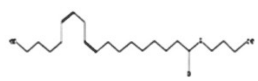
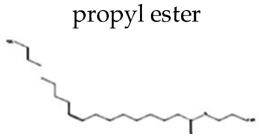
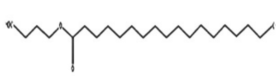
Tabe 4: Functional Groups of *Wrightia tinctori* Flower Observed By FT-IR

Peak Position [cm]	Functional Group	Functional Group Names
3848.26	O-H	hydroxyl group
3680.48	O-H	hydroxyl group
2851.24	C-H	Aliphatic hydrocarbons
2357.55	C=C	Alkene
1541.81	N-H	amine group
1255.43	C-O	carbonyl group
1034.62	C-O	carbonyl group

Table 5 Phytochemical Profiling and Gc -Ms Analysis of *Wrightia tinctori*

s.no	R Time	compound name	molecular formula	molecular weight [g\mol]	molecular%	Class	Activity
1	5.1419	Butanoic acid 	$C_4H_8O_2$	88.11	96.1	Fatty acid	Butanoic acid is used as a food additive, disinfections and pharmaceutical
2	5.8738	1,3-Propanediol, TBDMS derivative 	$C_9H_{22}O$ Si	190.356	81.9	Alcohols	-
3	6.2890	Propanoic acid, 2-methyl-, butyl ester 	$C_8H_{16}O_2$	144.21	95.9	Propionic acid	Used in food and beverage Flavors
4	6.3145	Butanoic acid, 2-methylpropyl ester 	$C_8H_{16}O_2$	144.21	97.5	Carboxylic acid esters, fatty acid esters, organic oxides	Antimicrobial, Pheromone component
5	6.4565	Butanoic acid, butyl ester 	$C_8H_{16}O_2$	144.2114	97.0	Carboxylic acid, Fatty acid esters, organic oxides	Antimicrobial, antioxidant, Anti-inflammatory
6	6.5694	n-Butyl methacrylate 	$C_8H_{14}O_2$	142.2	88.8	Methacrylic acid	-
7	6.8352	2-Hexenal, 2-ethyl 	$C_8H_{14}O$	126.192	97.2	Alkenal	Antifungal,antimcrobial

8	7.1120	1-Hexanol, 2-ethyl 	$C_8H_{18}O$	130.23	98.0	Alcohol	
9	7.2795	2-Butenoic acid, butyl ester 	$C_8H_{14}O_2$	142.192	98.2	Cratonic acid	-
10	8.2627	tert-Butyl glycidyl ether 	$C_7H_{14}O_2$	130.18	83.8	-	-
11	8.8198	1,1-Diisobutoxy-isobutane 	$C_{12}H_{26}O_2$	202.33	97.4	-	-
12	9.4571	Butane, 1,1- dibutoxy 	$C_{12}H_{26}O_2$	170.328	89.1	-	-
13	15.3672	3-O-Methyl-d-glucose 	$C_7H_{14}O_6$	194.182	81.9	Fattyalcohol, D-aldohexoses	Anti-inflammatory, Anti-cancer
14	17.0022	Neophytadiene 	$C_{20}H_{38}$	278.52	91.3	Diterpenoid, terpenoid	Antimicrobial, antioxidant, antioxidant
15	18.2658	n-Hexadecenoic acid 	$C_{16}H_{32}O_2$	256.43	97.5	Iong-chain fatty acids	Antimicrobial, antioxidant, Anti-inflammatory, cancer prevention,hypocholesterolemic

16	19.6933	Phytol 	$C_{20}H_{40}O$	296.53	97.8	Acyclic diterpenoids	Antimicrobial, antioxidant, Anti-inflammatory, Anti-cancer
17	19.9628	9,12,15-Octadecatrienoic acid, (Z, Z,Z) 	$C_{18}H_{30}O_2$	278.42	94.3	Polyunsaturated fatty acid	Anti-inflammatory, Anti-arthritic,hypocholesterolemic
18	20.3233	Hexadecanoic acid, butyl ester 	$C_{20}H_{40}O_2$	312.538	97.7	Fatty acid ester	Plant metabolite, animal metabolite
19	21.7981	Butyl 9,12-octadecadienoate 	$C_{22}H_{40}O_2$	336.54	92.8	Chemical compound	Antileishmanial activity
20	21.8345	Cis-9-Octadecenoic acid, propyl ester 	$C_{21}H_{40}O_2$	282.46	86.4	Oleic acid	Antimicrobial,antioxidant,inflammatory properties
21	21.8673	Butyl 9,12,15-octadecatrienoate 	$C_{22}H_{38}O_2$	334.524	85.6	Chemical compound	Anticancer, antioxidant, antileishmanial
22	22.0311	Octadecanoic acid, butyl ester 	$C_{22}H_{44}O_2$	340.59	93.7	Fatty acid ester	Emollient,solvent,lubricant

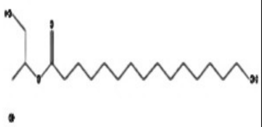
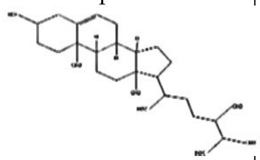
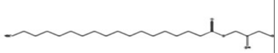
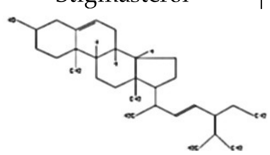
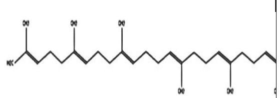
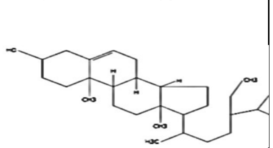
23	23.0653	Hexadecenoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester 	$C_{19}H^{38}O_4$	330.50	96.5	monoglyceride	Antioxidant,antibacterial,pesticidal properties
24	23.2014	Campesterol 	$C^{28}H^{48}O$	400.68	95.4	phytosterols	Anti-inflammatory, Anti-cancer
25	24.6239	Octadecanoic acid, 2,3-dihydroxypropyl ester 	$C_{21}H_{42}O_4$	358.56	96.3	Monoacylglycerols	Antimicrobial and antioxidant
26	24.6239	Stigmasterol 	$C_{29}H_{48}O$	412.69	97.5	stigmastanes	Antimicrobial, antioxidant, Anti-inflammatory, Anti-cancer,anti-allergy, neuroprotective
27	25.4068	Supraene 	$C_{30}H_5O$	410.7180	91.6	General anethetic, Inhalation	-
28	25.7309	Gamma-Sitosterol 	$C_{29}H_{50}O$	414.71	97.4	Stigmastanes , derivatives	Antihyperlycemic

Fig: 1 Petroleum ether

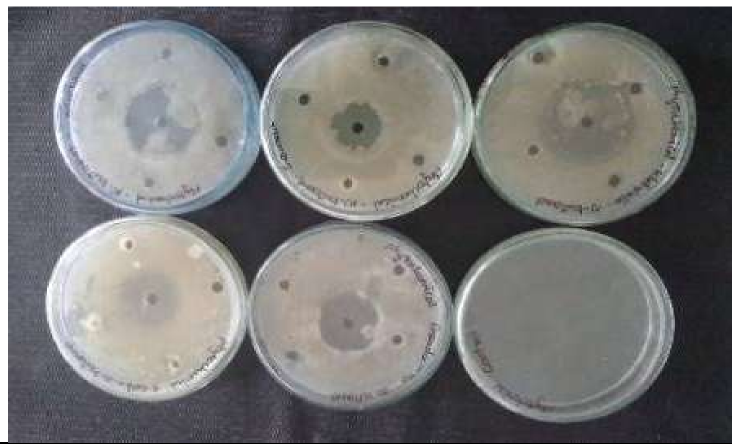


Fig:2 N- Butanol

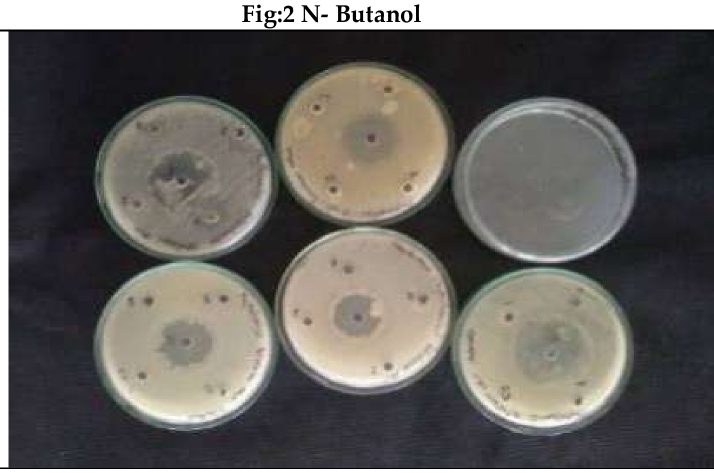


Fig:3 Carbinol

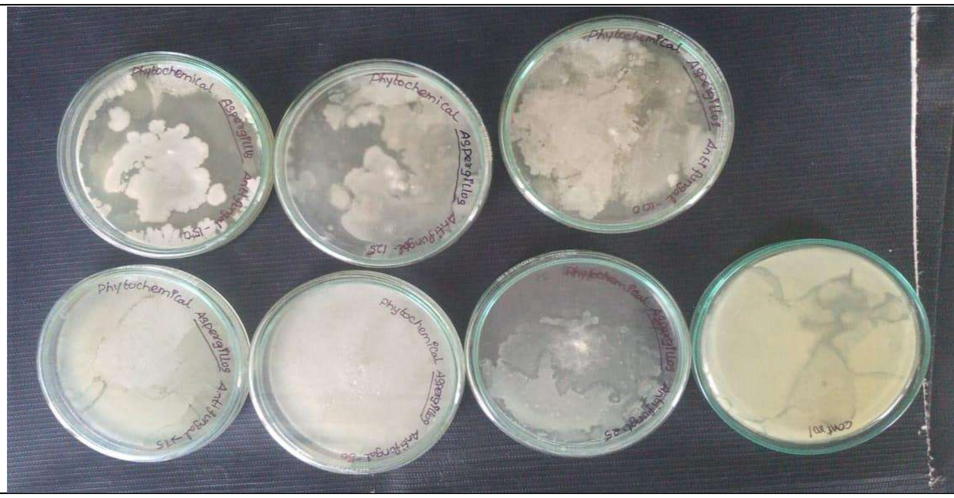


Fig:4 *Aspergillus sp*

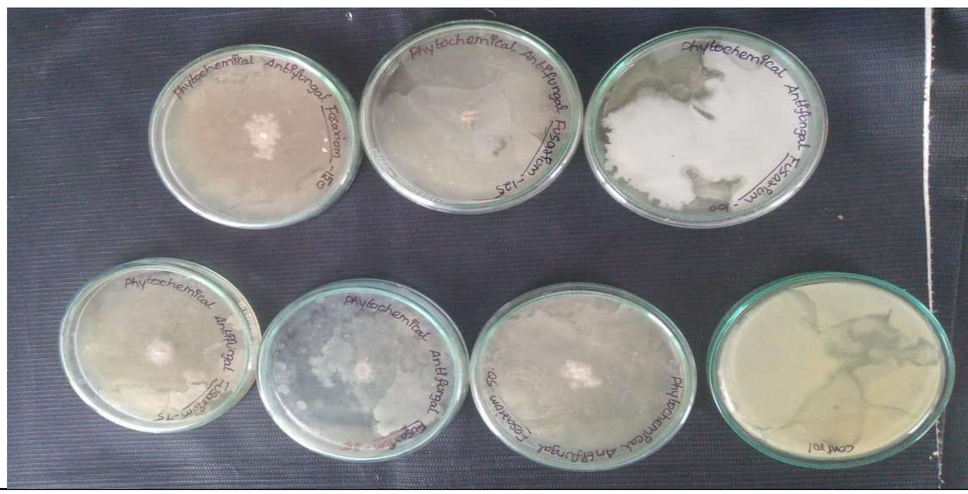


Fig: 5 Fusarium sp

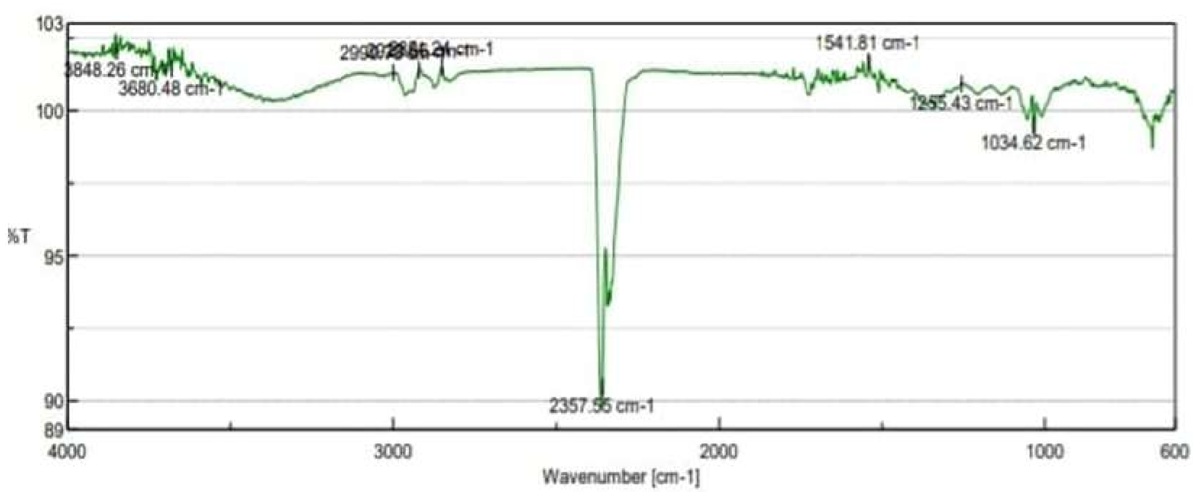


Fig 6: FT-IR Analysis of A *Wrightia tinctori*

Vignesh *et al.,*

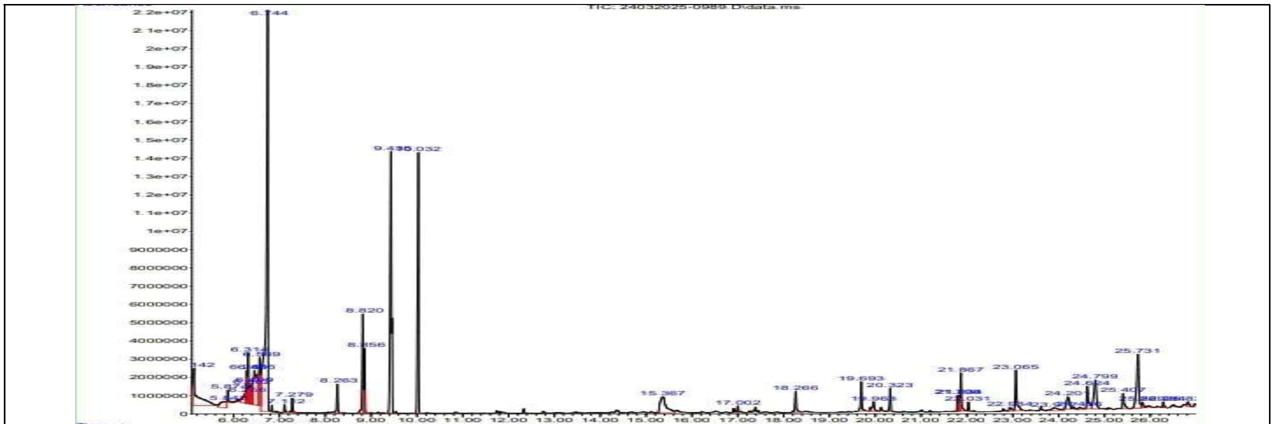


Fig. 7. The Peaks Observed GC-MS Chromatogram of *Wrighti tinctoria*