DOI: 10.7860/JCDR/2026/77379.23287

Original Article

Microbiology Section

Antibacterial, Antioxidant Activities and GC-MS Analysis of Methanol Extracts of *Andrographis paniculata, Indigofera tinctoria, Moringa oleifera*, and *Justicia adhatoda*: An In-vitro Study

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ABSTRACT

Introduction: Medicinal plants possess different groups of phytocompounds such as flavonoids, alkaloids, phenols, and tannins and saponins and offer protection against various infectious diseases. It has been proved that medicinal plants have antimicrobial, antispasmodic, emetic, and anticancer properties. The conventional synthetic drugs often lead to undesirable side effects and contribute to resistance, highlighting the need for alternative therapeutic agents. Medicinal plants have traditionally been used as effective remedies for a wide range of ailments in traditional medicine systems.

Aim: To evaluate the antioxidant and antibacterial properties of *Andrographis paniculata*, *Indigofera tinctoria*, *Moringa oliefera*, and *Justicia adhatoda* methanol extracts, in in-vitro.

Materials and Methods: The present in-vitro study was conducted at the Department of Microbiology, Dr ALM PGIBMS, University of Madras (January-December 2017). Methanol extracts of powdered *A paniculata, I tinctoria, M oleifera, and J adhatoda* were prepared via 48-hour maceration with methanol, followed by Seitz filtration and lyophilisation. Antibacterial activity was evaluated using the agar-well diffusion method against six gram-negative and two gram-positive bacterial pathogens. Antioxidant potential was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Major phytochemical constituents were identified through Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Data were analysed using Excel and two-way Analysis of Variance (ANOVA); graphs were made in GraphPad Prism 5. and $p<0.05$ was considered significant.

Results: *A. paniculata* showed higher zone of inhibition against *Shigella, S. aureus* and *Enterococcus. I. tinctoria* showed higher zone of inhibition against *P. aeruginosa. S. aureus and Shigella*. and showed moderate inhibition against *K. pneumoniae. M. oleifera* had higher zone of inhibition against *P. aeruginosa* and moderate activity against *Enterococcus and P. vulgaris. J. adhatoda* had moderate inhibition against *Enterococcus* and K. *pneumoniae. A. paniculata* and *I. tinctoria* had shown the highest antioxidant activity. The 1-Hexene, 1-butoxy-2-ethyl, cyclopentaneundecanoic acid methyl ester, 17 Alpha-ethyl-6beta-methooxy-3alpha, 5cyclo-5alpha androstane-17 beta diol and Methyl Z-tetradecnoate were the major phytochemicals identified in *A. paniculata, I. tinctoria*, *M.* oleifera and *J. adhatoda*, respectively.

Conclusion: Combinations of these four extracts might have broad-spectrum antibacterial properties. The extracts of *A. paniculata*, *I. tinctoria*, and *J. adhatoda* have significant antioxidant properties. Using these medicinal plants could support recovery from bacterial infections and oxidative stress of chronic diseases, along with additional healthcare benefits.

Keywords: Bacterial pathogens, Free radicals, Gas chromatography-mass spectrometry, Medicinal plants, Oxidative stress

INTRODUCTION

The use of medicinal plants offer defence against range of communicable and non-communicable disease. Medicinal plants have numerous phytochemicals that have been identified that have antibacterial, antispasmodic, emetics, and anticancer properties [1]. Nowadays, more than 30% of pharmaceutical medications (quinine, berberine, allicin, paclitaxel (taxol), codeine, digoxin, topotecan, galantamine etc.,) on the market are derived from medicinal plants, and the use of phytochemicals for therapeutic purposes has steadily expanded [2]. According to the World Health Organisation (WHO), nearly 80% of people in developed nations use medications made of plants [3,4]. Important phytocompounds with antibacterial activities found in medicinal plants are secondary metabolites, such as phenolics, terpenoids, and flavonoids [5]. Studies have shown that phytocompounds have anti-inflammatory, wound-healing, anti-anxiety, anti-mutagenic, and spasmolytic properties. They are used to treat cutaneous infections, respiratory diseases, gastrointestinal disorders, and urinary tract infections [6,7]. The world population was seriously threatened and treatment became more challenging due to the common antibiotics decreased susceptibility to bacterial pathogens [8]. Using medicinal plants to treat bacterial infections could be an alternative approach [9].

Free radicals can cause oxidative damage by reacting with biological molecules, which is responsible for many chronic illnesses [10]. Phytochemicals from medicinal plants has free radical scavenging molecules called antioxidants [11,12]. Plant-derived antioxidants function as singlet and triplet oxygen quenchers, peroxide decomposers, enzyme inhibitors, and synergists [13]. New antimicrobial and antioxidant molecules are highly needed to support the treatment of various emerging infectious and non-infectious diseases. Based on the pharmacological properties reported and the merits of *Andrographis paniculata* (*A. paniculata*) [14], *Indigofera tinctoria* (*I. tinctoria*) [15], *Justicia adhatoda* (*J. adhatoda*) [16] and *Moringa oleifera* (*M. oleifera*) [17], the present

study aimed to evaluate the in-vitro antioxidant and antibacterial activities of methanol extracts of plant extracts and to identify their major phytocompounds through GC-MS analysis. Exploring and validating the medicinal values of plants and discovering new bioactive compounds could help in validating its traditional uses and promotes its biodiversity conservation.

The primary objective of the present study was to assess the antibacterial and antioxidant activities of methanol extracts from *A. paniculata, I. tinctoria, M. oleifera*, and *J. adhatoda* in vitro and the secondary objectives is to further compare the effectiveness of each extract against various bacterial strains and to identify key phytochemicals using GC-MS analysis.

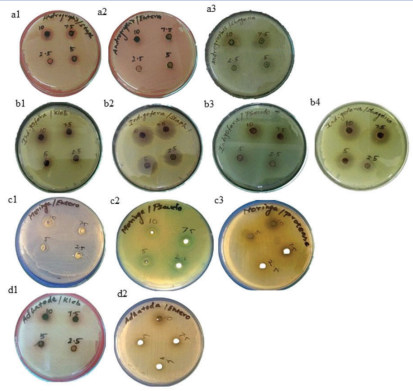
MATERIALS AND METHODS

The present in-vitro study was done at Department of Microbiology, Dr ALMPG Institute of Basic Medical Sciences, University of Madras, Chennai, Tamil Nadu during the period of January 2017 to December 2017. The powdered plant materials of *A. paniculata* (whole plant), I. *tinctoria* (leaves), *M. oliefera* (seed) and *J. adhatoda* (leaves) were procured from local country drug store.

Study Procedure

Preparation of methanolic extract: Powdered material (50 g) was extracted with 500 mL of methanol by maceration for 48 hr at room temperature. After extraction, the mixture was filtered under sterile conditions using a Seitz filter combined with Whatman filter paper. The filtrate was subsequently concentrated by lyophilisation at -80°C to obtain a dry extract. The lyophilised powder was stored at 4°C in airtight containers until further analysis.

Screening of antibacterial activity: The antibacterial activity of extracts was studied against 8 bacterial pathogens belonging to the Gram-negative bacteria such as *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Shigella flexneri, Proteus vulgaris, Salmonella typhi* and Gram-positive bacteria such as *Staphylococcus aureus* and *Enterococcus faecalis* by agar-well diffusion method as described by Dahiya P and Purkayastha S 2012 [18]. Briefly the cultures of bacterial strains were prepared using Brain Heart Infusion (BHI) broth and antibacterial susceptibility testing was performed using Mueller-Hinton Agar medium (MHA). The bacterial strains were inoculated into sterile BHI broth and compared with 0.5 McFarland standard to give an approximate bacterial concentration of $1.5X10^8$ CFU/mL culture suspension of bacterial strains was spread on the MHA plates using sterile cotton swab. Then, well with diameter of 6 mm has punched aseptically with sterile cork borer. Increasing concentrations of (2.5 mg, 5 mg, 7.5 mg, and 10 mg) of extract were added to the respective wells [Table/Fig-1]. Agar plates were incubated at 37°C for 24 hours. The antibacterial activity of the extracts was measured in mm using a standard measuring scale.



[Table/Fig-1]: Agar-well diffusion antibacterial activity of methanol extracts of *A. paniculata* with (a1) *S. aureus*, (a2) *Enterococci*, (a3) *Shigella. I. tinctoria* with (b1) *K. pneumoniae*, (b2) *S. aureus*, (b3) *P. aeruginosa*, (b4) *Shigella. M. oleifera* with (c1) *Enterococci*, (c2) *P. aeruginosa*, (c3) *P. vulgaris. J. adhatoda* with (d1) *K. pneumoniae* and (d2) *Enterococci*

Screening of antioxidant activity: The antioxidant properties of methanol extracts were estimated as described by StankovićN et al., 2016 using DPPH (1, 1, diphenyl 2, picrilehyde) method. Briefly, 800 µL of Tris (100 mM, pH 7.4) was mixed with 200 µL of extract with increasing concentration 2.5 mg, 5 mg, 7.5 mg, and 10 mg (test), ascorbic acid (positive control), distilled water (negative control). To this mixture, an equal volume of DPPH (500 µM in ethanol) was added and incubated in the dark at room temperature for 20 minutes with intermittent shaking. After incubation, the absorbance was read in UV-spectrophotometer at 517 nm. The percentage of scavenging activity was calculated using the following formula. DPPH. RSC (%) = 100 (A0 - A1/A0) where A0 was the absorbance of the control reaction (without extract) and A1 was the absorbance in the presence of extract [19].

GC-MS Analysis: It was performed using the instrument JEOL GCMATE II GC-MS at the sophisticated analytical instrument facility centre, Indian Institute of Technology - Madras, Chennai, India. This analysis was performed by injecting 1µL of each extract into GC-MS instrument followed by 40 min run. The major compounds were identified by comparing the compounds found in the extracts with the stranded reference library.

STATISTICAL ANALYSIS

Microsoft Excel was used to perform descriptive statistics. The significance between different extracts and concentrations was analysed by two-way ANOVA. All the graphs were generated by GraphPad Prism 5. The p-value<0.05 was considered as statistically significant.

RESULTS

In the present study, the methanol extract of *A. paniculata* showed zone of inhibition of 14 mm, 17 mm, 20 mm, and 23 mm against *Shigella* sp., 10 mm, 13 mm, 15 mm and 18 mm against *Staphylococcus aureus*, 0 mm, 10 mm, 13 mm, and 15 mm against *Enterococcus* sp. at the concentrations of 2.5 mg/mL, 5 mg/mL, 7.5 mg/mL and 10mg/mL, respectively and which had no antibacterial activity against *E. coli, K. pneumoniae, P. aeruginosa, S. typhi* and *P. vulgaris* [Table/Fig-2]. There was significant difference in zone of inhibition between the organisms (p=0.0350) and concentrations (p=0.0001) were observed.

Organism	10 mg	7.5 mg	5 mg	2.5 mg	p-value	F value
	Zone of Inhibition (mm)					
E. coli	–	–	–	–	0.0350* <0.0001***	3.451* 34.48***
K. pneumoniae	–	–	–	–		
S. aureus	18±1.2	15±1.0	13±1.0	10±0.8		
Enterococci sp.	15±1.1	13±1.0	10 ±0.9	–		
P. aeruginosa	–	–	–	–		
S. typhi	–	–	–	–		
Shigella sp.	23±1.3	20±1.2	17±1.0	14±0.9		
P. vulgaris	–	–	–	–		

[Table/Fig-2]: Antibacterial activity of methanol extracts of *A. paniculata*. The significance between the different concentrations and organisms was analysed by two-way ANOVA.
*Column Factor, ***Row Factor

The methanol extract of *I. tinctoria* exhibited the zone of inhibition of 23 mm, 24 mm, 25 mm, and 26 mm against *P. aeruginosa*, 20 mm, 22 mm, 23 mm and 25 mm against *S. aureus*, 15 mm, 18 mm, 20 mm and 22 mm against *Shigella* and 0 mm, 0 mm, 11 mm and 13 mm against *K. pneumoniae* at the concentrations of 2.5 mg/mL, 5 mg/mL, 7.5 mg/mL and 10 mg/mL respectively and this plant does not shown zone of inhibition against *E.coli, Enterococcus, S. typhi* and *P. vulgaris* [Table/Fig-3], *I. tinctoria* showed significance difference in zone of inhibition between the organisms (p=0.0347) and concentrations (p=0.0001).

Organism	10 mg	7.5 mg	5 mg	2.5 mg	p-value	F value
	Zone of Inhibition (mm)					
E. coli	0	0	0	0	0.0347* <0.0001***	3.460* 79.95***
K. pneumoniae	13±1.2	11±1.1	0	0		
S. aureus	25±0.8	23±0.9	22±0.7	20±0.8		
Enterococci sp.	0	0	0			
P. aeruginosa	26±1.5	25±1.4	24±1.2	23±1		
S. typhi	0	0	0	0		
Shigella sp.	22±1.3	20±1.1	18±1.4	15±1.0		
P. vulgaris	0	0	0	0		

[Table/Fig-3]: Antibacterial activity of methanol extracts of *I. tinctoria*. The significance between the different concentrations and organisms was analysed by two-way ANOVA.
*Column Factor, ***Row Factor

The methanol extract of *M. oleifera* exhibited antibacterial activity with the zone of inhibition of 15 mm, 17 mm, 19 mm, and 24 mm against *P. aeruginosa*, 0 mm, 0 mm, 12 mm and 15 mm against *Enterococcus*. and 0 mm, 0 mm, 11 mm and 13 mm against *P.vulgaris* at the concentrations of 2.5 mg/mL, 5 mg/mL, 7.5 mg/mL and 10 mg/mL, respectively and zone of inhibition was not observed against *E. coli, K. pneumoniae, S. aureus, S. typhi* and *Shigella* [Table/Fig-4]. *M. oleifera* showed significance difference in zone of inhibition between the organisms (p=0.0324) and concentrations (p=0.0001).

Organism	10 mg	7.5 mg	5 mg	2.5 mg	p-value	F value
	Zone of Inhibition (mm)					
E. coli	0	0	0	0	0.0324* <0.0001***	3.533* 14.78***
K. pneumoniae	0	0	0	0		
S. aureus	0	0	0	0		
Enterococci sp.	15±1.2	12±1.0	0	0		
P. aeruginosa	24±1.5	19±1.2	17±1.1	15±0.8		
S. typhi	0	0	0	0		
Shigella sp.	0	0	0	0		
P. vulgaris	13±1.2	11±1.3	0	0		

[Table/Fig-4]: Antibacterial activity of methanol extracts of *M. oleifera*. The significance between the different concentrations and organisms was analysed by two-way ANOVA.
*Column Factor, ***Row Factor

The methanol extract of *J. adhatoda* showed the zone of inhibition of 0 mm, 10 mm, 12 mm, and 14 mm against *Enterococcus* sp. and 0 mm, 0 mm, 8 mm and 10 mm against *K. pneumoniae* at the concentrations of 2.5 mg/mL, 5 mg/mL, 7.5 mg/mL and 10mg/mL respectively and not showed the zone of inhibition against *E. coli, S. aureus, P. aeruginosa, S. typhi, Shigella* sp. and *P. vulgaris* [Table/Fig-5]. *J. adhatoda* showed significance difference in zone of inhibition among the concentrations (p=0.0006) but not had difference between the organisms (p=0.152).

Organism	10 mg	7.5 mg	5 mg	2.5 mg	p-value	F value
	Zone of Inhibition (mm)					
E. coli	0	0	0	0	0.1523 <0.0006***	1.951 6.051***
K. pneumoniae	10±1.5	8 ±1.1	0	0		
S. aureus	0	0	0	0		
Enterococci sp.	14 ±0.9	12±1.2	10±1.5	0		
P. aeruginosa	0	0	0	0		
S. typhi	0	0	0	0		
Shigella sp.	0	0	0	0		
P. vulgaris	0	0	0	0		

[Table/Fig-5]: Antibacterial activity of methanol extracts of *J. adhatoda*. The significance between the different concentrations and organisms was analysed by two-way ANOVA.
Column Factor; No significance, ***Row Factor

Among the four extracts *A. paniculata* showed highest antioxidant activity with percentage free radical scavenging activity of 91.21%, 95.6%, 97.4% and 98.7% at the concentration of 2.5 mg/mL, 5 mg/mL, 7.5 mg and 10 mg, respectively. *I. tinctoria* extract exhibited 83.73%, 95.3%, 97.1%, and 98.21%, *J. adhatoda* exhibited 87. 97%, 90.8%, 91%, and 94.2% and *M. oleifera* exhibited 9.81%, 31.06, 53.3, and 56.71% of inhibition of free radicals at the concentration of 1.5 mg/mL, 2.5 mg/mL, 5 mg and 10 mg, respectively [Table/Fig-6]. Antioxidant result showed significant difference between the extracts (p=0.0001) and not had difference between the concentrations (p=0.069).

The dose dependent Zone of inhibition measured against bacterial pathogens using methanol extracts of the four medicinal plants, indicating their potential antimicrobial efficacy [Table/Fig-7].

Antioxidant activity of methanol extract of *A. paniculata*, *I. tinctoria*, *M. oleifera*, and *J. adhatoda* by the DPPH free radical scavenging method [Table/Fig-8]. Methanol extracts of *A. Paniculata, I. tinctoria, M. oleifera*, and *J. adhatoda* were subjected to the GC-MS analysis [Table/Fig-9]. The results showed that the following compounds such as 1-Hexene,1-butoxy-2-ethyl, Pyrrolidine,1-(7-oxo-2,4,6 trimethylheptanoyl), 3-Heptadecanone, Benzo(e) (1,2)-thiazine-2-one,8a-cyano-4,4-pentamethylene-perhydro, 7H-Indeno (2,2-a) anthracen-one was found in the methanol extract of *A. paniculata* [Table/Fig-10].

In the *I. tinctoria*, the phytocompounds found were Cyclopentaneundecanoic acid methyl ester, E,E,Z1 3,12-nonadecatrinen-5,14 diol, Spiro-(isoquinolone-1,2-indene) 1-2-1,2,3,4,2,3 tera hydro- 6- hydroxy-6,7,3,7-tetramethoxy-2-methyl-1-oxa, 5-(2- Morpholino-1- triophen-2-yl-vinyl)-1,2,4-thiadiazole, Spiro(isoquinoline-1,2-indene)1,2,3,4,2,3-tetrahydro-6-hydroxy-6,7,3,7-tetramethoxy-2methyl-1-oxa [Table/Fig-11].

From *M. oleifera* the compounds identified were 17 Alpha-ethyl-6beta-methooxy-3alpha, 5cyclo-5alpha androstane-17 beta diol, Strychane, 1-actyle-20a –hydroxy 16 methylene, 18,19-seco-15ayohimban19-oicacid 20,21didehydro-16a (hydroxymethyle): methyle ester and beclomethasone [Table/Fig-12].

The major phytocompounds from the methanol extract of *J. adhatoda* were Methyl Z-tetradecnoate, 13-Heptadecyn-1-ol, 1-Benzazirene-1-Carboxylic acid, 2,25a trimethyl-1-a (3-oxo 1-butenyl) perhydro; methyl ester, Spiro (bicycle (3.3.0) oct-6-ene-3-cyclopropane) 6-(1-trimethylsilyloxy) cyclopropyl and carnegine [Table/Fig-13].

DISCUSSION

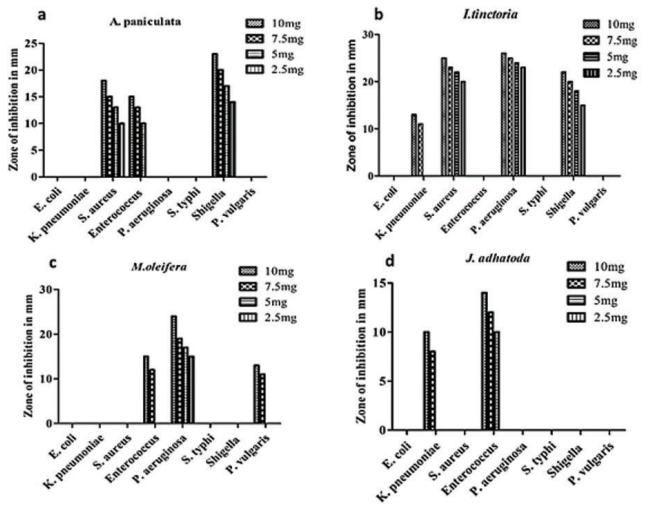
Finding new, promising medications is urgently needed given the rise of infectious diseases. Medicinal plants' active biological components aid in the search for new lead molecules [20,21]. Nearly half of the compounds from *A. paniculata* were not studied for their pharmacological properties [22]. The methanol extracts of *A. paniculata*, *I. tinctoria*, *M. oliefera*, and *J. adhatoda* were examined in this study for their antioxidant and antibacterial activities.

Khan AV et al., reported that the dichloromethane extract of leaves of *A. paniculata* showed a better antibacterial activity against the gram-positive organisms [23]. Dhwani NV et al., reported that methanol leaf extracts showed significant antibacterial activity against *S. aureus* and *B. subtilis* and moderate activity against *E. coli*, K. *pneumoniae* and *P. vulgaris* [24]. *A. paniculata* demonstrated a greater zone of inhibition against *Shigella, Staphylococcus aureus*, and *Enterococcus*, reflecting significant antibacterial activity against both Gram-negative and Gram-positive pathogens.

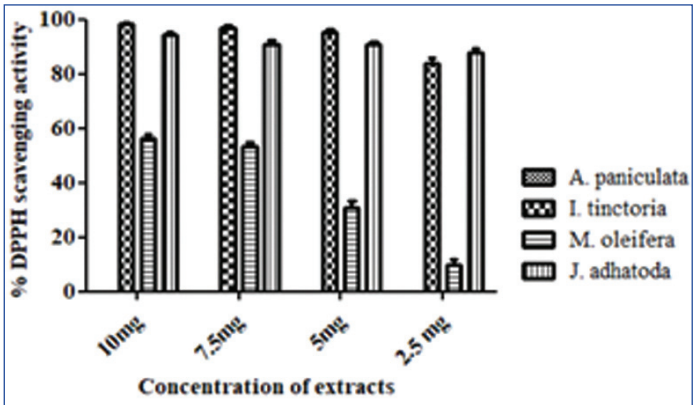
Vijayan M et al., [25] reported that the methanol extract of *I. tinctoria* exhibited antibacterial activity, which was found to be more active than the standard antibiotic linezolid. *I. tinctoria* exhibited a higher zone of inhibition against *P. aeruginosa*, *S. aureus*, and *Shigella*, while showing moderate inhibition against *K. pneumoniae*.

Concentration	*A. paniculata*	*I. tinctoria*	*M. oleifera*	*J. adhatoda*	p-value	F value
10 mg	98.69±0.85	98.21±0.72	56.51±1.25	94.18±0.95	0.0693 <0.0001***	3.348 37.09***
5 mg	97.39±0.92	97.07±0.88	53.30±1.45	90.98±1.15		
2.5 mg	95.60±1.08	95.28±1.13	31.06±2.10	90.78±1.25		
1.25 mg	91.21±1.35	83.73±1.95	9.81±1.90	87.97±1.50		

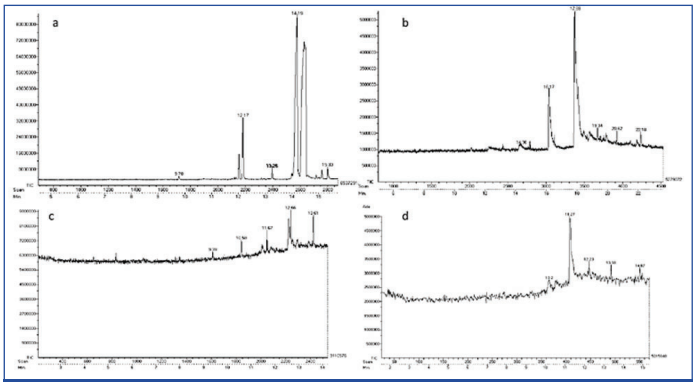
[Table/Fig-6]: Antioxidant activity of methanol extract of *A. paniculata*, I. *tinctoria*, *M. oleifera*, and *J. adhatoda*. The significance between different extracts and concentrations was analysed by two-way ANOVA.
Column Factor; No significance, ***Row Factor



[Table/Fig-7]: Antibacterial activity of methanol extracts from selected medicinal plants: (a) *A. paniculata*; (b) *I. tinctoria*; (c) *M. oleifera*; and (d) *J. adhatoda*.



[Table/Fig-8]: Antioxidant activity of methanol extract of *A. paniculata*, *I. tinctoria*, *M. oleifera*, and *J. adhatoda*.



[Table/Fig-9]: GC-MS Chromatogram of methanol extracts of: (a) *A. paniculata*; (b) *I. tinctoria*; (c) *M. oleifera*; and (d) *J. adhatoda*.

Name of the compound	RT	% Area	M.W	Molecular formula	Structure
1-Hexene,1-butoxy-2-ethyl	9.78	51.3	184.31	$C_{12}H_{24}O$	

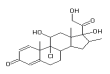
Pyrrolidine,1-(7-oxo-2,4,6 trimethylheptanoyl)	12.17	100	239.18	$C_{14}H_{25}NO_2$	
3-Heptadecanone	13.26	100	254.45	$C_{17}H_{34}O$	
Benzo(e)(1,2)-thiazine-2-one,8a-cyano-4,4-pentamethylene-perhydro	14.19	100	264.38	$C_{14}H_{20}N_2OS$	
7H-Indeno(2,2-a) anthracen-one	15.33	100	280.32	$C_{21}H_{12}O$	

[Table/Fig-10]: Major active compounds identified in methanol extracts of *A. paniculata*.

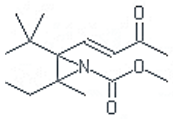
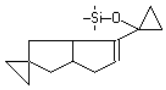
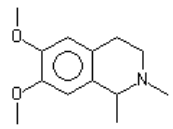
Name of the compound	RT	% Area	M.W	Molecular formula	Structure
Cyclopentaneundecanoic acid methyl ester	16.17	43.6	268.435	$C_{17}H_{32}O_2$	
E,E,Z1 3,12-nonadecatrinen-5,14 diol	17.88	69.5	294.479	$C_{19}H_{34}O_2$	
5-(2-Morpholino-1-triophen-2-yl-vinyl)-1,2,4-thiadiazole	22.18	8	279.376	$C_{12}H_{13}N_3OS_2$	
Spiro(isoquinoline-1,2-indene)1,2,3,4,2,3-tetrahydro-6-hydroxy-6,7,3,7-tetramethoxy-2methyl-1-oxa	20.62	9.3	403.511	$C_{22}H_{31}NO_6$	

[Table/Fig-11]: Major active compounds identified in methanol extracts of *I. tinctoria*.

Name of the compound	RT	% Area	M.W	Molecular formula	Structure
17 Alpha-ethyl-6beta-methooxy-3alpha,5cyclo-5alpha androstane-17 beta diol	9.39	7.9	344.48	$C_{22}H_{32}O_3$	
Strychane,1-actyle-20a -hydroxy 16 methylene	11.67	28.00	338.44	$C_{21}H_{26}N_2O_2$	
18,19-seco-15ayohimban19-oicacid 20,21didehydro-16a(hydroxymethyle): methyle ester	12.66	39.10	360.49	$C_{21}H_{32}N_2O_3$	

Beclomethasone	13.61	36.5	408.91	$C_{22}H_{29}ClO_5$	

[Table/Fig-12]: Major active compounds identified in methanol extracts of *M. oleifera*.

Name of the compound	RT	% Area	M.W	Molecular formula	Structure
Methyl Z-11-tetradecnoate	10.2	6.6	240.38	$C_{15}H_{28}O_2$	
13-Heptadecyn-1-ol	11.27	18.7	252.44	$C_{17}H_{32}O$	
1-Benzazirene-1-Carboxylic acid,2,25a trimethyl-1-a (3-oxo 1-butenyl) perhydro; methyl ester	12.23	12.23	265.35	$C_{15}H_{23}NO_3$	
Spiro [bicycle (3.3.0) oct-6-ene-3-cyclopropane) 6-(1-trimethylsilyloxy) cyclopropyl	13.38	18.6	262.47	$C_{16}H_{26}OSi$	
Carnegine	14.87	14.87	221.30	$C_{13}H_{19}NO_2$	

[Table/Fig-13]: Major active compounds identified in methanol extracts of *J. adhatoda*.

Mujeeb F et al., [26] reported that seed extract of *M. oleifera* showed a broad-spectrum antibacterial activity. The highest zone of inhibition was found against *P. aeruginosa*. Methanol extract possessed moderate antibacterial activity against bacterial strains such as *S. aureus*, *B. subtilis*, *V. cholerae*, *B. cereus*, *S. typhi*, *S. dysenteriae*, *P. aeruginosa, Klebsiella* species and *Proteus* species. *M. oleifera* exhibited a higher zone of inhibition against *P. aeruginosa* and showed moderate activity against *Enterococcus* and *P. vulgaris*.

Pa R and Mathew L concluded that *J. adhatoda* had a broad spectrum of antimicrobial activity and a potential source of antimicrobial agents that could be useful for chemotherapy and control of infectious diseases [16]. *J. adhatoda* exhibited moderate inhibition against *Enterococcus* and *Klebsiella pneumoniae*. Overall, the results of the antibacterial activity indicated that all four plant extracts possessed specific antibacterial effects against particular bacterial pathogens.

Antioxidant potential is one of the most significant biological properties of medicinal plants, as it lowers the risk of oxidative stress, which is linked to a number of chronic diseases [27,28]. The free radicals scavenging property of methanol extracts revealed that A. *paniculata* and *I. tinctoria* had shown the highest antioxidant activity. The antibacterial and antioxidant property of these plants might be due the compounds identified in the GC-MS analysis. Products containing these herbs provide improved patient compliance, less side effects, a wider therapeutic activity, and synergistic results.

Limitation(s)

The study was limited to in-vitro experiments and did not investigate the mechanisms of action or evaluate potential synergistic effects between the plant extracts.

CONCLUSION(S)

The present study concludes that *I. tinctoria* had more antibacterial potential than other extracts. It was also found that each plant extract had antibacterial activity against specific bacterial pathogens. Hence, developing combinations of these four extracts might have broad-spectrum antibacterial properties. The extracts of *A. paniculata, I. tinctoria*, and *J. adhatoda* have significant antioxidant properties. Using these medicinal plants could support recovery from bacterial infections and oxidative stress of chronic diseases, along with additional healthcare benefits. Further studies are warranted to elucidate the mechanism of action.

Authors' contribution: All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Dec 17, 2024
- Manual Googling: Aug 07, 2025
- iThenticate Software: Aug 09, 2025 (9%)

ETYMOLOGY: Author Origin

EMENDATIONS: 8

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? NA (No human or animal subjects involved in the present study)
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Dec 16, 2024**
Date of Peer Review: **Apr 03, 2025**
Date of Acceptance: **Aug 12, 2025**
Date of Publishing: **May 01, 2026**