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Screening and antibacterial efficacy of selected Indian medicinal plants

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ABSTRACT

Objective: To evaluate the antibacterial efficacy of five Indian medicinal plants such as *Acalypha indica* L. (*A. indica*), *Aerva lanata* (L.) Juss. ex Schult. (*A. lanata*), *Clerodendrum inerme* (L.) Gaertn., *Pergularia daemia* (Forsk.) Chiov. and *Solanum surattense* Burm. f. against opportunistic bacterial pathogens isolated from HIV infected patients for the potential phytoconstituents in plant extracts.

Methods: The opportunistic bacterial pathogens such as *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa*, *Salmonella typhi* and *Serratia marcescens* from Gram-negative group and *Staphylococcus aureus* from Gram-positive group were isolated from HIV infected patients. The antibacterial efficacy of ethanolic extracts of selected medicinal plants was carried out by disc diffusion method. The potential phytoconstituents of medicinal plant extracts were identified by gas chromatography and mass spectrometry (GC–MS) analysis.

Results: Among the five medicinal plants tested, *A. indica* and *A. lanata* showed the significant antibacterial activity. *A. indica* showed potential activity against *Staphylococcus aureus* and *E. coli*. *A. lanata* significantly exhibited antibacterial activity against *E. coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*. A total of 19 phytoconstituents were identified in the ethanolic extract of *A. indica* and *A. lanata* by GC–MS analysis respectively.

Conclusions: The results of the present investigation revealed that *A. indica* and *A. lanata*, possessed significant antibacterial activity when compared with the other plant extracts tested. The presence of 3-*O*-methyl-D-glucose by GC–MS analysis in both *A. indica* and *A. lanata* extracts has not been reported elsewhere in the literature and the findings in this study could be the first one to report.

1. Introduction

Medicinal plants have been identified as a part of the evolution of human healthcare for thousands of years. Medicinal components from plants play an important role in traditional as well as modern medicine. Antimicrobial resistance is an increasingly serious threat to global public health. According to World Health Organization (WHO) report on antimicrobial resistance in 2014, overcoming the antibiotic resistance is the major issue to the WHO for the next millennium. Screening of plants for antimicrobial agents has gained much importance because WHO is encouraging and promoting in the development and utilization of medicinal plant resources in the traditional system of medicine. Accordingly, the last decade witnessed an increase in the investigation of plants as a source of human infectious disease management.

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Acalypha indica L. (*A. indica*) (family: Euphorbiaceae) is an annual herbaceous weed and widely distributed throughout the tropical region of India. According to ethno-medicinal uses, it is used for treating pneumonia, jaundice, piles, asthma, rheumatism, bedsores, wounds and skin disorders. It has been reported to have wound healing activity, snake venom neutralizing properties, antibacterial activity and anti-urolithiatic activity [1–4].

Aerva lanata (L.) Juss. ex Schult. (*A. lanata*) (family: Amaranthaceae) is a herbaceous perennial weed. It is commonly distributed throughout India. It is used as diuretic and demulcent. Its diuretic action is very effective in the treatment of urethral discharges and gonorrhea. It is also used for treating headache, cough, liver congestion, jaundice, biliousness, dyspepsia, pneumonia, typhoid, urinary and gall stones, skin diseases, scorpion stings and snake bites [5]. It is extensively studied for various pharmacological activities such as antimicrobial, anti-diabetic, antitumor, nephron-protective, hepatoprotective, immunomodulatory, anti-urolithiatic, antifertility, anti-metastatic and anti-HIV activity [6–19].

Clerodendrum inerme (L.) Gaertn. (*C. inerme*) (family: Verbenaceae) is a perennial shrub. It is used to treat fever, cough, scrofulous infection, venereal infection and skin diseases. It is also used to treat umbilical cord infection and for cleaning the uterus [20,21]. It has been accounted for antimalarial, anticancer, relieve hyperlocomotion and antimicrobial activities [22–25].

Pergularia daemia (Forsk.) Chiov. (*P. daemia*) (family: Asclepiadaceae) is a perennial climber throughout hot parts of India. It is used as stomachic, laxative and diuretic. It is also used to treat diarrhea, fever, cough, asthma, biliousness and sore eyes. The methanolic extract of *P. daemia* plant was evaluated for its antiurolithiatic and antidiabetic activities [26,27].

Solanum surattense Burm. f. (*S. surattense*) (family: Solanaceae) is a perennial herbaceous weed. It is distributed throughout the tropical regions of India. It is used for treating asthma, cough, leprosy, dropsy, sore throat and constipation. It has been reported for antimalarial and antioxidant activities [28–31].

As per the currently available literature, only few studies were evaluated the antibacterial properties of the selected medicinal plants. Consequently, the present research was planned to evaluate the antibacterial activity of ethanolic extracts of the selected medicinal plants against the opportunistic bacterial pathogens isolated from HIV infected patients and its potential phytoconstituents.

2. Materials and methods

2.1. Collection of plant leaves

The fresh aerial parts of medicinal plants including *A. indica, A. lanata, C. inerme, P. daemia* and *S. surattense* were collected from Tiruchirappalli District. The taxonomic identification of the plants was done and the voucher herbarium specimens were deposited in the Post Graduate & Research Department of Botany & Microbiology, A. Veeriya Vandayar Memorial Sri Pushpam College (Autonomous), Poondi, Thanjavur District, Tamil Nadu, India. All the plant leaves were cleaned and shade dried (Figure 1).

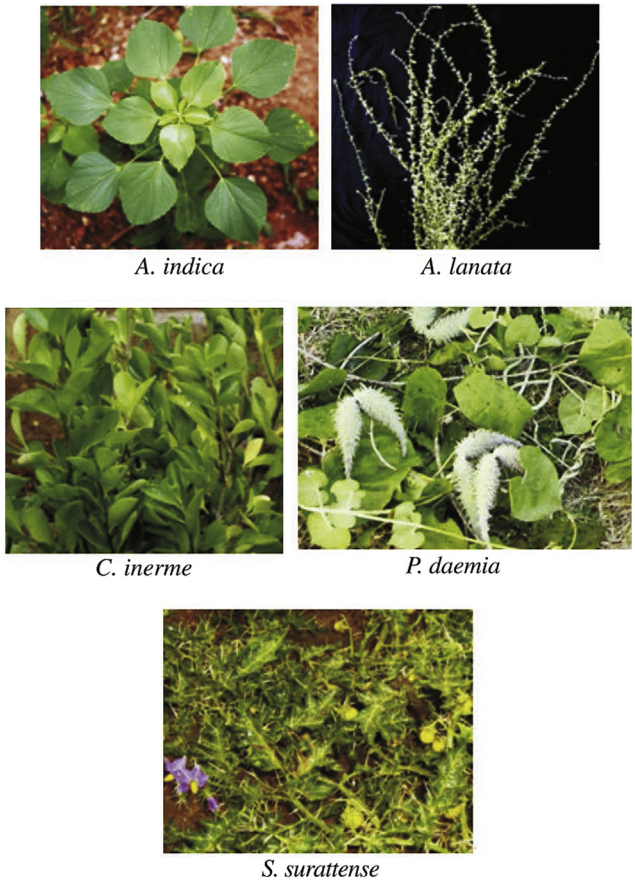

Figure 1. Selected Indian medicinal plants.

2.2. Preparation of ethanolic extracts

The shade dried leaves of selected plants were pulverized. About 50 g of powdered sample was extracted with the 100 mL of ethanol by using a Soxhlet extractor for 24 h. The obtained extracts were concentrated by using a rotary flash evaporator. The extracts were well preserved in airtight containers for further analysis.

2.3. Preparation of disc

The sterile discs (6 mm, Himedia) were impregnated with different concentrations of 5, 10, 20 and 30 μg of the plant extracts. The discs were identified with labeling and stored in airtight containers with silica gel desiccant.

2.4. Inoculum preparation

The opportunistic bacterial pathogens such as *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Salmonella typhi* (*S. typhi*), *Serratia marcescens* (*S. marcescens*) and *Staphylococcus aureus* (*S. aureus*) were isolated from HIV infected patients, who were referred to Doctors' Diagnostic Center, Tiruchirappalli. All these pathogens were suspended in 0.85% saline corresponding to No. 0.5 McFarland turbidity standard. All cultures were incubated on a shaker at 37 °C for 18 h and then diluted to 1/10 concentration to yield a culture density of approximately 1.5×10^8 CFU/mL.

2.5. Antibacterial assay

The antibacterial activity of ethanolic extracts was assessed by disc diffusion method [32]. Mueller-Hinton agar (0.2 g beef extract, 1.75 g peptone, 0.15 g starch, 2.0 g agar, 100 mL distilled water, pH 7.5) was prepared with lawn culture using desired test organisms. The inoculated plates were kept aside for a few minutes. The plant extract impregnated discs were placed over the medium. After diffusion, the plates were incubated at 37 °C for 24 h. After incubation, the diameter (mm) of the zone of inhibition was measured and compared with the standard antibiotics such as ofloxacin (5 μg/disc) for Gram-negative bacteria and vancomycin (30 μg/disc) for the Gram-positive bacteria (*S. aureus*). All the tests were performed in three replicates and the activity was expressed as the mean of inhibition diameters (mm) produced by the plant extracts.

2.6. Gas chromatography-mass spectrometry (GC–MS) analysis

About 20 g of powdered plant material was soaked in 50 mL of absolute alcohol overnight and then filtered through Whatman Grade No. 41 quantitative filter paper along with 2 g sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulfate was wetted with absolute alcohol. The filtrate was concentrated by bubbling nitrogen gas into the solution and reduced the volume to 1 mL. The extract contained both polar and non-polar phytocomponents of the plant material used.

The GC–MS analysis was carried out using a Clarus 500 PerkinElmer (AutoSystem XL) gas chromatograph equipped and coupled to a mass detector, turbo mass gold-PerkinElmer turbo mass 5.1 spectrometer with an elite-1 (100% dimethyl poly siloxane), 30 m × 0.25 mm (inner diameter) × 1 μm of capillary column. The instrument was set to an initial temperature of 110 °C and maintained at this temperature for 2 min. At the end of the period, the oven temperature was rose up to 280 °C at the rate of an increase of 5 °C/min and maintained for 9 min. Injection port temperature was ensured as 250 °C and helium flow rate was ensured as 1 mL/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45–450 (*m/z*). Using computer searches on a National Institute of Standards and Technology ver.2.1 MS data library and comparing the spectrum obtained through GC–MS compounds present in the plants sample were identified.

3. Results

3.1. Antibacterial activity of selected Indian medicinal plants

The antibacterial activity of ethanolic plant extracts was evaluated against the five opportunistic bacterial pathogens isolated from HIV infected patients. The results of antibacterial activity of different concentrations of *A. indica* extracts were summarized in Table 1. The results revealed that *A. indica* ethanolic extracts showed the maximum activity against *S. aureus* and *E. coli* and the minimum activity against *S. typhi*, *P. aeruginosa* and *S. marcescens* at 30 μg concentration. The antibacterial activity was distinctly varied against the bacterial test pathogens.

Table 1
Antibacterial activity of ethanolic extract of *A. indica* against the clinical pathogens. mm.

Name of the bacterial pathogens	Zone of inhibition			
	30 μg	20 μg	10 μg	5 μg
E. coli	17.90 ± 0.71	16.30 ± 1.12	10.20 ± 0.57	–
P. aeruginosa	10.20 ± 1.36	8.30 ± 0.93	–	–
S. typhi	11.70 ± 0.83	10.90 ± 0.75	–	–
S. marcescens	9.40 ± 1.10	10.50 ± 1.40	–	–
S. aureus	22.80 ± 1.21	20.00 ± 1.00	15.70 ± 0.79	10.20 ± 1.17

Results were expressed as mean ± SD, $n = 3$. –: Indicated no activity.

Table 2 demonstrates the results of antibacterial activity of *A. lanata* ethanolic extracts. The maximum antibacterial activity of *A. lanata* plant extract was exhibited against *E. coli*, *P. aeruginosa* and *S. aureus*. A minimal antibacterial activity was identified against *S. typhi* and *S. marcescens* at 30 μg concentration. The activity was concentration-dependent.

Table 2
Antibacterial activity of ethanolic extract of *A. lanata* against the clinical pathogens. mm.

Name of the bacterial pathogens	Zone of inhibition			
	30 μg	20 μg	10 μg	5 μg
E. coli	23.20 ± 1.21	20.40 ± 0.96	15.10 ± 0.66	11.00 ± 0.75
P. aeruginosa	20.90 ± 0.40	16.20 ± 0.86	9.20 ± 0.78	–
S. typhi	18.20 ± 0.67	15.30 ± 0.93	10.00 ± 1.04	–
S. marcescens	18.10 ± 0.61	16.00 ± 1.15	–	–
S. aureus	20.53 ± 1.17	18.20 ± 0.99	–	–

Results were expressed as mean ± SD, $n = 3$. –: Indicated no activity.

The antibacterial activity of *C. inerme* plant extract showed the activity against only *S. typhi* with the zone of inhibition (10.60 ± 0.85) mm at 30 μg concentration and didn't show any inhibitory activity against the other test pathogens such as *E. coli*, *S. aureus*, *P. aeruginosa* and *S. marcescens*. The results of antibacterial activity of *P. daemia* plant extract revealed the activity against only *E. coli* [(14.10 ± 0.75) mm and (12.10 ± 0.80) mm] at 30 μg and 20 μg concentrations and didn't show any inhibitory activity against the other tested pathogens. The antibacterial activity of *S. surattense* plant extract showed the activity against only *E. coli* [(10.10 ± 0.91) mm] at 30 μg concentration and did not show any inhibitory activity against the other test pathogens such as *S. aureus*, *S. typhi*, *P. aeruginosa* and *S. marcescens*.

The results of standard antibiotics sensitivity test on clinical pathogens were given in Table 3. Among the five plant extracts tested, the ethanolic extracts of *A. lanata* exhibited significant antibacterial activity when compared with the standard antibiotics tested.

Table 3
Standard antibiotics sensitivity test on clinical pathogens.

Name of the bacterial pathogens	Standard antibiotics tested (μg/disc)	Zone of inhibition (mm)
E. coli	Ofloxacin (5)	25.40 ± 1.72
P. aeruginosa	Ofloxacin (5)	26.20 ± 0.86
S. typhi	Ofloxacin (5)	25.30 ± 0.70
S. marcescens	Ofloxacin (5)	26.10 ± 0.71
S. aureus	Vancomycin (30)	17.90 ± 0.55

Results were expressed as mean ± SD, $n = 3$.

3.2. Assessment of phytocomponents in selected medicinal plants

Among the five plants, *A. indica* and *A. lanata* showed the significant antibacterial activity. Consequently, it was subjected to GC–MS analysis. The mass spectrum of ethanol extracts of *A. indica* showed 10 prominent peaks (Figure 2). The major phytoconstituents were 3-*O*-methyl-D-glucose with 50.18% peak area and 9,12,15-octadecatrienoic acid, (Z,Z,Z)- with 17.50% peak area. The identified phytoconstituents of *A. indica* extracts and its biological activity were given in Table 4. The biological activities listed were based on Dr. Duke's phytochemical and ethnobotanical databases created by Dr. Jim Duke of the Agricultural Research Service/United States Department of Agriculture.

The mass spectrum of ethanolic extracts of *A. lanata* showed 9 prominent peaks (Figure 3) and the identified phytoconstituents were given in Table 5. The major phytoconstituents were 3-*O*-methyl-D-glucose with 25.56% peak area, 9,12-octadecadienoic acid (Z,Z)- with 18.90% peak area, *n*-hexadecanoic acid with 16.02% peak area and squalene with 14.04% peak area.

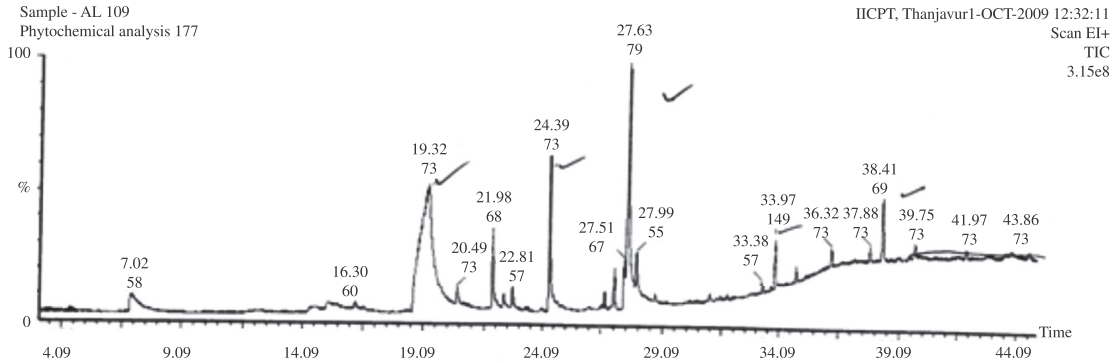

Figure 2. GC–MS chromatogram formed by ethanolic extract of *A. indica*.

Table 4
Phytochemicals identified in the ethanolic extract of *A. indica* by GC–MS.

Name of the compound	Molecular formula	RT	MW	Peak area (%)	Activity[a]
2,5-Pyrrolidinedione, 1-methyl-	$C_5H_7NO_2$	7.02	113	5.48	No activity reported
3-*O*-methyl-D-glucose	$C_7H_{14}O_6$	19.32	194	50.18	Antitumor activity [33]
Tetradecanoic acid	$C_{14}H_{28}O_2$	20.49	228	1.09	Antioxidant, cosmetic, cancer preventive, hypercholesterolemic, lubricant, nematicide
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	21.98	296	4.21	Cancer preventives
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	24.39	256	8.53	5-Alpha-reductase-inhibitor, antialopecic, antiandrogenic, antifibrinolytic, antioxidant, flavor, hemolytic hypercholesterolemic, lubricant, nematicide, pesticide, propecic, soap
Phytol	$C_{20}H_{40}O$	27.12	296	2.04	Cancer preventive
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	27.63	278	17.50	5-Alpha-reductase-inhibitor, antimicrobial, antiacne, antialopecic, antianaphylactic, antiandrogenic, antiarteriosclerotic, antiarthritic, anticoronary, antigranular, antihistaminic, antiinflammatory, antileukotriene, antimenorrhagic, antiprostatic, cancer preventive, carcinogenic, hepatoprotective, hypocholesterolemic, immunomodulator, insectifuge, metastatic
Oleic acid	$C_{18}H_{34}O_2$	27.99	282	5.56	5-Alpha-reductase-inhibitor, anemiagenic, antialopecic, antiandrogenic, antiinflammatory, antileukotriene-cancer preventive, choleretic, dermatitigenic, flavor, hypocholesterolemic, percutaneostimulant, perfumery
1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	33.97	390	2.41	No activity reported
Squalene	$C_{30}H_{50}$	38.41	410	3.00	Antibacterial, antioxidant, antitumor, cancer-preventive, chemopreventive, immunostimulant, lipoxygenase inhibitor, perfumery, pesticide, sunscreen

[a]: Dr. Dukes ethnobotanical database; RT: Retention time; MW: Molecular weight.

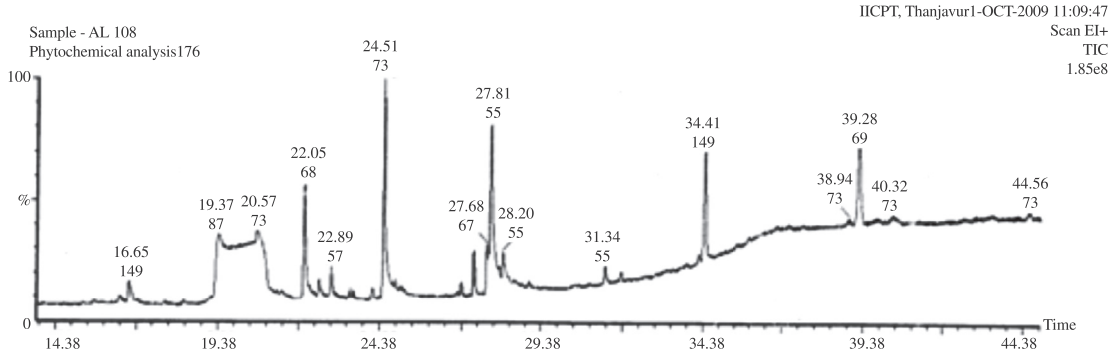

Figure 3. GC–MS chromatogram formed by ethanolic extract of *A. lanata*.

Table 5

Phytochemicals identified in the ethanolic extract of *A. lanata* by GC–MS.

Name of the compound	Molecular formula	RT	MW	Peak area (%)	Activity[a]
Diethyl phthalate	$C_{12}H_{14}O_4$	16.65	222	2.23	Antimicrobial and anti-fouling property
3-*O*-methyl-D-glucose	$C_7H_{14}O_6$	19.37	194	25.56	Antitumor activity [33]
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	22.05	296	8.57	Cancer preventive
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	24.51	256	16.02	5-Alpha-reductase-inhibitor, antialopecic, antiandrogenic, antifibrinolytic, antioxidant, flavor, hemolytic hypercholesterolemic, lubricant, nematicide, pesticide, propecic, soap
Phytol	$C_{20}H_{40}O$	27.28	296	2.66	Cancer preventive
9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	27.81	280	18.90	Antimicrobial, antiacne, anti-inflammatory, cancer preventive, nematicide
Oleic acid	$C_{18}H_{34}O_2$	28.20	282	4.65	5-Alpha-reductase-inhibitor, anemiagenic, antialopecic, antiandrogenic, anti-inflammatory, antileukotriene, cancer preventive, choleretic, dermatitigenic, flavor, hypocholesterolemic, percutaneostimulant, perfumery
1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	34.41	390	7.37	No activity reported
Squalene	$C_{30}H_{50}$	39.28	410	14.04	Antibacterial, antioxidant, antitumor, cancer-preventive, chemopreventive, immunostimulant, lipoxygenase inhibitor, perfumery, pesticide, sunscreen

[a]: Dr. Dukes ethnobotanical database; RT: Retention time; MW: Molecular weight.

4. Discussion

The discovery of novel antimicrobial metabolites from medicinal plants is an important alternative to overcome the increasing levels of drug resistance by human pathogens. Due to the world's urgent need for new antibiotics and chemotherapeutic agents, growing interest is taken into the research on the chemistry of medicinal plants. Antimicrobial activity of medicinal plants and their phytoconstituents have been deliberated in the late 19th century [34]. In the present study, five ethnobotanical herbs including *A. indica*, *A. lanata*, *C. inerme*, *P. daemia* and *S. surattense* were evaluated for antibacterial activity against opportunistic bacterial pathogens isolated from HIV infected patients.

Among the five plants, the significant antibacterial property was observed in *A. indica*. The present results were in concurrence with the findings of Mohan *et al*. [35]. Their results revealed that various extracts of *A. indica* showed considerable antibacterial activity against *S. aureus* and *E. coli*. The antibacterial potential of *A. lanata* plant extract showed significant activity against *E. coli* in all concentrations and potential activity against *S. aureus*, *S. typhi*, *P. aeruginosa* and *S. marcescens*. Results of antibacterial activity were comparable with the findings of Vidhya and Udayakumar, who found that *A. lanata* possessed significant antibacterial activity [36].

The antibacterial property of *C. inerme*, *P. daemia* and *S. surattense* plant extracts showed very minimal antibacterial activity. The plant extract of these plants could have lost their antibacterial potentials in drying due to heat sensitive nature of the phytochemical constituents in the plants.

GC–MS analysis plays a key role in the analysis of components of plant origin. Generally, the plant materials are highly complexes, which make GC–MS well suited for their analysis because of its high sensitivity and selectivity. It is considered to be the gold standard in scientific analysis [37,38]. Several phytochemical screening studies have been carried out in different parts of the world by using GC–MS [39,40]. Among the five plants, only two plants were justifiably chosen for bioactive compounds investigation using the GC–MS analysis.

In this study, we reported for the first time a high resolution GC–MS method for the evaluation of the chemical constituents of *A. indica* and *A. lanata* plant extracts. This accurate and sensitive analysis of *A. indica* and *A. lanata* revealed the various phytoconstituents present in the extracts.

The major bioactive compounds in *A. indica* and *A. lanata* are 3-*O*-methyl-D-glucose. The presence of 3-*O*-methyl-D-

glucose in *A. indica* and *A. lanata* extracts has not been reported in any previous research work and the findings in this study could be the first one to report. 3-*O*-methyl-D-glucose is a nontoxic nonmetabo lizable derivative of glucose and it has been reported for antitumor activity [33]. The antibacterial activity of *A. indica* and *A. lanata* may be due to presence of 3-*O*-methyl-D-glucose.

9,12,15-Octadecatrienoic acid, (Z,Z,Z)-, 9,12-octadecadienoic acid (Z,Z)- and *n*-hexadecanoic acid are another major compounds in the studied plant extracts and have been reported to have antimicrobial, antioxidant and anti-inflammatory activities [41–44]. The antibacterial activity of *A. indica* and *A. lanata* could be attributed to the presence of these phytoconstituents.

The *n*-hexadecanoic acid (synonym: palmitic acid) and 9,12-octadecadienoic acid (linoleic acid) were also reported in *Benincasa hispida* and *Carissa congesta* plant extracts [40]. Similarly, these phytocompounds were identified in various plants such as *Allium nigrum*, *Kielmeyera coriacea*, *Cyrtocarpa procera*, *Labisia pumila* and *Rosa indica* [42–46]. The identified squalene (14.04%) in *A. lanata* extract has been reported for antioxidant, antibacterial and anticancer activities [47].

The results of the present investigation also complement the ethnobotanical usage of the studied plants which possess several phytoconstituents with biological activity. Based on the present investigation, it is concluded that *A. indica* and *A. lanata* have potential source of bioactive compounds with great pharmaceutical value. The study can be extended for separation and *in vitro*, *in vivo* evaluation of bioactive compounds in novel drug discovery.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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