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Identification of Bioactive Compounds from *Alphonsea madraspatana* Leaves against Multi Drug Resistance Bacteria

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In this study, the antimicrobial compounds present in leaves of *Alphonsea madraspatana* was identified by using RP-HPLC, LC/MS and GC/MS and found to be active against multiple drug resistant (MDR) bacteria *e.g*. *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Extraction of dried plant leaves was performed by solvent gradient technique. Antimicrobial study was performed with each extract. *n*-Hexane and methanol extracts were further subjected to analysis for identification of potent antimicrobial compounds due to their better antimicrobial activity as compared to remaining extracts. The methanolic extract was more active than *n*-hexane extract. As *n*-hexane extract contains non-polar compounds, it was subjected to GC-MS analysis and methanol extract was subjected to RP-HPLC followed by LC/MS due to presence of polar components. Among the isolated compounds, four compounds were identified as potent antimicrobial. RP-HPLC and LC/MS analysis of methanol extract also ensured the presence of four potent antimicrobial compounds as follows: (i) kaempferol-3-O-rotinoside-orhamnoside (*m/z* 748), (ii) 3-hydroxypropylglucosinolate (*m/z* 377), (iii) luteolin-7-O-glucoside (*m/z* 448) and (iv) genistein-7-O-glycoside (*m/z* 432). Leaves of this plant may be used as potent antibacterial agents due to presence of antimicrobial compounds.

Keywords: *Alphonsea madraspatana*, Antimicrobial activity, MDR bacteria, Phytochemical screening.

INTRODUCTION

Bacterial infection is being a serious health concern across the world and the situation is gradually being complicated by the appearance of multidrug-resistant pathogens [1]. Now a days, multidrug resistant bacteria are becoming massive threat to public health and are associated with nosocomial and community acquired infections resulting morbidity, mortality and antibiotics dependency [2].

The exposure and growth of multiple drug resistant (MDR) pathogens are significantly alarming the contemporaneous antimicrobial therapy [3]. Around the globe, the emergence of multidrug-resistant Gram-negative bacteria is a solemn. Over the past two decades, infections originating by these pathogens have become a challenge [4]. High resistance of the pathogens to commonly used antibiotics, lead to decline the efficiency of treatment for common infections [5,6]. These resistant pathogens are prime cause of hospital acquired infections as well as community infections. Infections of post-surgical (wound), pneumonia, respiratory tract, bloodstream (septic) and urinary tract are the most attributable common infections [7]. *Klebsiella pneumonia*, *Escherichia coli* and *Pseudomonas aeruginosa* have been identified as major MDR pathogens [8,9].

As a result of enormous fight against infections and continual usage of antimicrobial agents, bacteria have developed its immeasurable protection against antimicrobial agents [10]. Initiation of novel approaches for the search of antimicrobial substitutes should be encouraged [11]. Flavonoids in medicinal plants can play a prominent role in detecting antibacterial agents against such MDR bacteria. *Alphonsea madraspatana* (Annonaceae) being an important medicinal plant is vastly available in Malaysia, north-east India, southwards to Ceylon and South China. The *Alphonsea arborea* fruits (boiled) have been reported to have antidiarrheal, antipyretic and emmenagogue properties and crude extracts of this genus have proved many pharmacological activities like antibacterial, antioxidant, anti-cancer, antifungal and anti-inflammatory [12].

The primary objective of the present work is to investigate the antimicrobial potency of both polar and non-polar fractions

of *A. madraspatana* leaves. As *n*-hexane extract are highly non-polar and volatile, hence, gas chromatography coupled with mass spectroscopy (GC-MS) was selected for the identification of the active compounds present in this extract. Due to presence of polar (ionic and non-ionic) molecules as a result of solvent gradient extraction technique, the methanolic extract is preferred to be analyzed by liquid chromatography (HPLC) and liquid chromatography coupled with mass spectroscopy (LC-MS). Considering the chemical diversity of phytochemicals and their role in mitigating challenges of antimicrobial resistance, in the present investigation, efforts have been made to authenticate the traditional value of *A. madraspatana* against selective MDR bacteria followed by antibiotic susceptibility test to identify the potential bioactivity against *E. coli* for the development of new and potent antimicrobial drugs.

EXPERIMENTAL

The leaves of *Alphonsea madraspatana* were collected from the forest region of Khandagiri in Bhubaneswar, India and authenticated by a taxonomist, Dr. P.C. Panda, Principal scientist of Regional Plant Resource Center (RPRC). A voucher specimen (Voucher No. As-1) was also deposited at taxonomy department of RPRC. The collected leaves were cleaned with double distilled water and shade dried for 3 weeks. The dried leaves were crushed and sieved with mesh size 20. The accumulated leaf powder was kept in a borosilicate glass jar and stored in a cool and dry place with proper label.

Mueller Hinton agar and antibiotic disc were purchased from HI Media Laboratories), HPLC-grade solvents were purchased from Sigma-Aldrich. Double-distilled water was used for preparation of plates. All other chemicals used were of analytical grade.

Preparation of plant extract: Cold maceration with solvent gradient technique (successive solvent extraction) were adopted for the extraction of the air-dried powdered leaf material with a series of solvents such as *n*-hexane, dichloromethane, ethyl acetate and methanol.

Isolation and identification of pathogenic bacteria: Samples were collected from ICU of IMS, SUM Hospital and cultured on blood agar, cystine lactose electrolyte deficient agar and MacConky agar plates. Based on lactose fermenting and non-lactose fermenting growth on MacConky agar plate, bacterial isolates were subjected to biochemical tests and different species were identified.

Antibiotic susceptibility test: Three isolated strains such as *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were subjected to antibiotic sensitivity test by the Kirby-Bauer disc diffusion method [13,14]. A bacterial suspension was swabbed uniformly on the surface of a Muller-Hinton agar plate using sterile cotton. The plates were then incubated at 37 ºC for 30 min. Discs of eight types of antibiotics like cefpodoxime, oxacillin, norfloxacin, amikacin, ciprofloxacin, gentamicin, nitrofurantoin and vancomycin (controls) were applied on the inoculated agar plate. The plate was inverted and further incubated at 37 °C. The inhibition zones were recorded after 24 h of incubation. The sensitivity of the bacterial strains were studied according to NCCLS criteria [12].

Antimicrobial activity

Agar diffusion well assay: The antibacterial activity of different solvent extracts of *A. madraspatana* leaves was determined by agar-well diffusion method. The protocol was slightly modified according to the current context of experimental conditions. The antibacterial potentials of four different solvent extracts were compared with a standard drug, ciprofloxacin (10 µg/mL). In this method, a 6 mm thickness of bacterial lawn was prepared with agar media. After 30 min of preparation, agar lawn was punched to make wells and 50 µL of molten MHA medium was transferred into each well. 20 µL of solution of different solvent extracts in 10% DMSO were transferred into respective wells. Plates were incubated at 37 ºC for 24 h and subjected for determination of zone of inhibition. The inhibitory effect of DMSO (10%) was found no activity and ciprofloxacin (10 µg/mL) was taken as the reference control [15].

Statistical analysis: All the activities were carried out in triplicate. The antibacterial activity of the test samples and standard were evaluated by following ANOVA.

GC-MS analysis of *n*-hexane extract: Thermo Trace 1300GC coupled with Thermo TSQ 8000 Triple Quadrupole MS detector guided by XCalibur 2.2SP1 and Foundation 2.0SP1 software were used for separation and mass analysis of elements in sample. For separation TG 5MS (30 m × 0.25 mm, 0.25 µm) column made up with 5% diphenyl and 95% dimethyl polysiloxane was used as stationary phase and helium carrier gas was used as mobile phase with a flow of 1.5 mL/min. Split injector with split ratio of 13:1 and split flow of 20 mL/min was programmed. 1.0 µL injection was done with injector temperature at 250 ºC. Oven was programmed with initial temperature at 50 ºC, hold for 4 min and temperature gradient of 3 ºC/min to final temperature of 260 ºC, holded for 3 min. Ion source temperature was settled to be 230 ºC with MS transfer line temperature at 280 ºC with mass range programmed for 50-700. NIST 2.0 library was used to identify the elements.

RP-HPLC analysis of methanol extract: Methanolic extract of *A. madraspatana* leaves was subjected to analysis in reversed phase mode using HPLC system (Make: Waters, Model Alliance-e2695) coupled with PDA detector (Make: Waters, Model-2998). LiChroCART C18 column (250 mm length, 4.6 mm internal diameter and 5 µm particle size) was used to separate the phytoconstituents. Gradient flow method was adopted with solvent-A (water) and solvent-B (acetonitrile) at a flow rate of 1 mL/min with column oven temperature maintained at 45 ºC (Table-1).

LC/MS analysis of methanol extract: The compounds, those peaks were observed in methanolic extract by RP-HPLC

TABLE-1
GRADIENT FLOW FOR WATER AND ACETONITRILE AT DIFFERENT TIME INTERVAL PROGRAMMED FOR HPLC

Time (min)	Solvent-A (%)	Solvent-B (%)
0	90	10
6	40	60
20	40	60
24	90	10
30	90	10

were further identified by LC-MS analysis by using Waters UPLC-MS/MS system consists of an Acquity H-class UPLC pump, Acquity H-class FTN auto-sampler and a triple quadrupole Xevo TQD mass spectrometer (Waters India Pvt. Ltd.). Acquity UPLC BEH C18 column (150 mm length, 2.1 mm internal diameter and 1.7 µm particle size) was used in this study. Gradient flow method was adopted with solvent-A (0.1% v/v formic acid in aqueous) and solvent-B (0.1% v/v formic acid in acetonitrile) at a flow rate of 0.15 mL/min with column oven temperature at 45 ºC. The gradient flow was followed as the conditions given in Table-1 to mimic with the HPLC method.

The tandem MS system equipped with an ESI source was used in this analysis (Waters India Pvt. Ltd). The mass spectrometric conditions were optimized in order to achieve maximum sensitivity by following spray voltage of -3 kV, capillary temperature of 200 ºC and API gas (N_2) pressure at 25 mm/Hg. Argon gas was used as collision gas at a pressure of 1.5 m torr and collision energy was 20 V for GA and 25 V for IS.

RESULTS AND DISCUSSION

Isolation and identification of pathogenic bacteria: All three isolated bacteria, which undergoes biochemical tests like catalase, oxidase, indole, methyl red, Voges-Prausker citrate, urease, triple sugar iron, nitrate reduction, bile esculine have been carried out. Identification of bacteria was done by the methods as given for lactose forming test isolates IMVIC. The TSI tests were carried out for antibiotic susceptibility for all bacteria (Table-2).

Antibiotic susceptibility test: Among eight antibiotics, only ciprofloxacin was found to be effective against all the three bacterial strains (Table-3). Henceforth, ciprofloxacin was considered as standard to evaluate the antibacterial activity of the leaf extract in further studies.

Antimicrobial activity

Agar well diffusion assay: In this study, the antibacterial potentials of various extracts of *A. madraspatana* leaves were evaluated by using agar-well diffusion method against selected MDR bacteria. The antibacterial activity of four different solvent extracts of *A. madraspatana* leaves was evaluated against one Gram-positive and two Gram-negative bacterial strains (Table-4). The methanolic extract of *A. madraspatana* leaves recorded the maximum zone of inhibition against *E. coli* (24 ± 1 mm) followed by *n*-hexane extract (21 ± 1 mm), dichloromethane (18 ± 2 mm) ethyl acetate extract (14 ± 2 mm), respectively.

Many studies have reported antibacterial activity of genus Alphonsea. Joshi *et al.* [16] evaluated the antibacterial activity of methanolic extract of leaf of *A. sclerocarpa* and found the zone of inhibition to be 18 against *E. coli*. In this study, better activity is observed as compared to Table-4. The alcoholic extracts of *A. arborea* and *A. sclerocarpa* showed similar observation against *E. coli.* The antimicrobial activity of methanol extract is probably associated to the presence of phenolic compounds and flavonoids [12,17,18]. It was previously reported that some species of Annonaceae family contain important bioactive compounds, exhibiting various activities like antimicrobial, insecticidal and antiparasitic activities [19,20].

GC/MS analysis: GC/MS analysis of *n*-hexane extract of *A. madraspatana* leaves enabled to identify 30 compounds (Fig. 1) belonging to different chemical families. Among these 30 compounds only 16 prominent and major constituents (Table-5) are reported based upon there abundance in the extract considering their response and percentage in the chromatogram. The

TABLE-2
SUMMARY RESULT OF BIOCHEMICAL TEST OF BACTERIA

Bacterium	Catalase	Oxidase	Indole	MR	VP	Citrate	Urease	TSI	NT	BE
E. coli	+	–	+	+	–	–	–	A/G	+	–
S. aureus	+	–	–	+	+	+	+	–	+	+
P. aeruginosa	+	+	–	–	–	+	–	+	+	–

VP: Voges-Prausker; MR: methyl red; NT: nitrate reduction; TSI: triple sugar iron BE: bile esculin; A/G: acid and gas production; K/A: alkaline and acid production; +: positive; -: negative A/GH2S: acid gas and hydrogen sulfide production.

TABLE-3
SELECTED CLINICALLY ISOLATED PATHOGENIC STRAINS ANTIBIOGRAM

Name of organism	Cefpodoxime	Oxacillin	Norfloxacin	Amikacin	Ciprofloxacin	Gentamicin	Nitrofurantoin	Vancomycin
E. coli	RE	RE	RE	RE	SE	RE	RE	RE
S. aureus	SE	SE	SE	SE	SE	SE	RE	RE
P. aeruginosa	SE	SE	SE	SE	SE	SE	RE	SE

RE: Resistant; SE: Sensitive, Antibiotics (µg/disc)

TABLE-4
ANTIBACTERIAL ASSAY BY AGAR-WELL DIFFUSION METHOD OF FOUR COLD LEAF-EXTRACTS OF *A. madraspatana* OF AGAINST MDR BACTERIAL STRAINS (ZONE OF INHIBITION IN mm)

Strain	*n*-Hexane	Dichloromethane	Ethyl acetate	Methanol	Ciprofloxacin (10 µg/mL)
E. coli	21 ± 1	18 ± 2	14 ± 2	24 ± 1	29 ± 1
S. aureus	15 ± 3	20 ± 4	13 ± 3	14 ± 5	28 ± 2
P. aeruginosa	10 ± 2	19 ± 4	14 ± 3	17 ± 3	28 ± 2

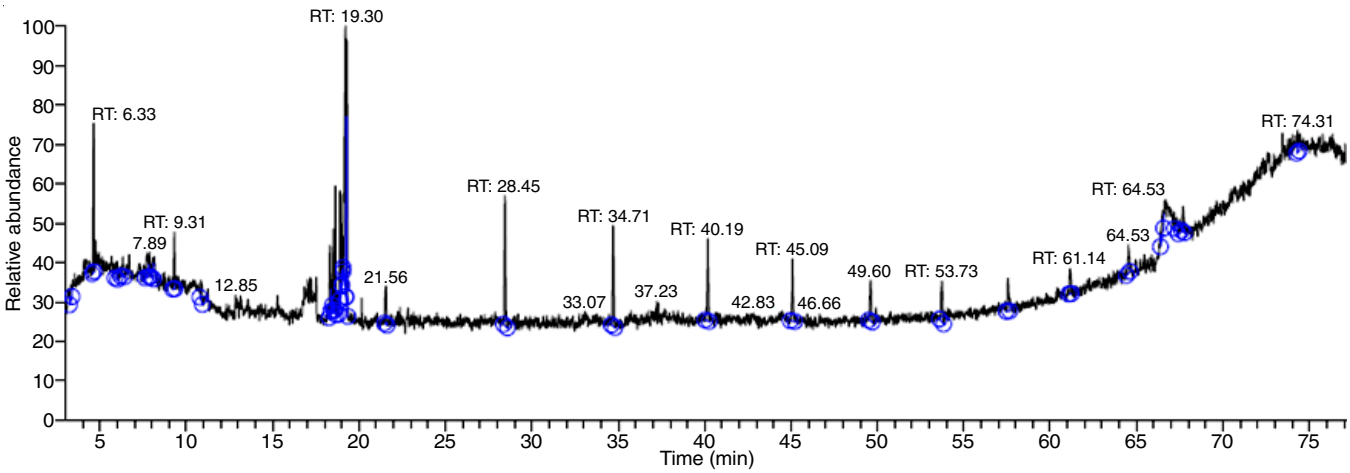

Fig. 1. GC chromatogram of *n*-hexane leaves extracts of *Alphonsea madraspatana*

TABLE-5
CHEMICAL COMPOSITION OF *n*-HEXANE EXTRACT FROM *Alphonsea madraspatna* LEAVES ANALYZED BY GC-MS

Retention time (min)	Compounds	m.f.	Area (%)	Peak height	Peak area
4.64	Ditungsten, *tris*(cyclooctatetraene)	$C_{24}H_{24}W_2$	4.64	891329.05	2489483.70
9.31	Octadecane, 3-ethyl-5-(2-ethylbutyl)	$C_{26}H_{54}$	2.48	342205.29	1331120.13
18.32	2,2-*Bis*[4-[(4,6-dichloro-1,3,5-triazin-2-yl) oxy]phenyl]-1,1,1,3,3,3-hexafluoropropane)	$C_{21}H_8Cl_4F_6N6_2$	2.82	415725.48	1514843.38
18.50	Pregn-4-ene-3,11,20-trione,6,17,21 *tris*[(trimethylsilyl)oxy]-, 3,20-*bis*(O-methyloxime), (6α)	$C_{32}H_{58}N_{2O}6Si_3$	2.61	468527.61	1401765.59
18.62	L-Proline, 1-[O-(1-oxohexyl)-*N*-[*N*-[*N*6-(1-oxohexyl)-*N*2-[*N*-(1-oxohexyl)-L-valyl]-L-lysyl]-L-valyl]-L-tyrosy l]-, methyl ester	$C_{49}H_{80}N_6O_{10}$	2.98	758603.22	1599348.08
18.89	Pregn-5-en-20-one, 3,16,17,21-*tetrakis* [(trimethylsilyl)oxy]-, O-(phenylmethyl) oxime, (3α,16β)	$C_{40}H_{71}NO_5Si_4$	2.60	686454.07	1394363.36
18.95	Anodendroside E 2, monoacetate	$C_{32}H_{40}O_{12}$	3.36	594144.32	1802842.31
19.02	Fucoxanthin	$C_{42}H_{58}O_6$	1.25	270595.57	672606.49
19.22	Silane, [[(3α,5β,11α,20S)-pregnane-3,11,17,20,21-pentayl] *pentakis* (oxy)] *pentakis* [trimethyl	$C_{36}H_{76}O_5Si_5$	15.84	1581813.32	8501561.78
19.30	Lycoxanthin	$C_{40}H_{56}O$	12.37	1584971.79	6639262.10
21.56	Cyclohexasiloxane, dodecamethyl	$C_{12}H_{36}O_6Si_6$	2.02	227596.30	1083819.38
28.45	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	$C_{16}H_{50}O_7Si_8$	6.76	777783.49	3625481.74
34.71	1-Monolinoleoyl glycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	5.28	601497.94	2834851.32
40.19	Cyclodecasiloxane, eicosamethyl	$C_{20}H_{60}O_{10}Si_{10}$	4.74	491430.60	2542006.73
45.09	2,4-Imidazolidinedione, 5-[3,4-*bis*[(trimethylsilyl)oxy] phenyl]-3-methyl-5phenyl-1-(trimethylsilyl)	$C_{25}H_{40}N_{2O}4Si_3$	3.42	371709.01	1836689.94
49.60	Bufa-20,22-dienolide,3,14-dihydroxy-, (3α,5α)-	$C_{24}H_{34}O_4$	2.55	242599.37	1370008.82

major constituents are (1) silane, [[(3α,5β,11α,20S)pregnane-3,11,17,20,21-pentayl]*pentakis*(oxy)]*pentakis*[trimethyl] (15.84%); (2) lycoxanthin (12.37%); (3) octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15 hexadecamethyl (6.76%); (4) 1-monolinoleoyl glycerol trimethyl silyl ether (5.28%); (5) cyclodecasiloxane, eicosamethyl (4.74%); (6) ditungsten, *tris*(cyclooctatetraene) (4.64%); (7) 2,4-imidazolidinedione, 5-[3,4-*bis*-[(trimethylsilyl)oxy]phenyl]-3-methyl-5phenyl-1-(trimethylsilyl) (3.42%); (8) anodendroside E 2, monoacetate (3.36%); (9) L-proline, 1-[O-(1-oxohexyl)-*N*-[*N*-[*N*6-(1-oxohexyl)-*N*2-[*N*-(1-oxohexyl)-L-valyl]-L-lysyl]-L-valyl]-L-tyrosyl]-, methyl ester (2.98 %); (10) 2,2-*bis*[4-[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]phenyl]-1,1,1,3,3,3 hexafluoropropane) (2.82%); (11) pregn-4-ene-3,11,20-trione, 6,17,21-*tris*[(trimethylsilyl)-oxy]-, 3,20-*bis*(O-methyloxime), (6α) (2.61%); (12) pregn-5-en-20-one, 3,16,17,21-*tetrakis*[(trimethylsilyl)oxy]-, O-(phenylmethyl)oxime, (3α,16β) (2.60%); (13) bufa-20,22-dienolide, 3,14-dihydroxy-, (3α,5α)- (2.55%); (14) octadecane, 3-ethyl-5-(2-ethylbutyl) (2.48%); (15) cyclohexasiloxane, dodecamethyl (2.02%) and (16) fucoxanthin (1.25%).

Among the above mentioned 16 compounds, only five compounds *viz*. (i) ditungsten, *tris*(cyclooctatetraene) [21], (ii) octadecane-3-ethyl-5-(2-ethylbutyl) [22], (iii) octasiloxane-1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl [23], (iv)1-monolinoleoylglycerol trimethylsilyl ether [24] and (v) cyclodecasiloxane, eicosamethyl [25] have been reported to have antimicrobial activity.

As per our knowledge, GC/MS is performed for the first time to identify the chemical composition of *n*-hexane extract of *Alphonsea madraspatna* leaves. Thang *et al*. [26,27] reported chemical constituents of essential oils identified by GC/MS from *A. tonkinensis*, *A. philastreana* and *A. gaudichaudiana*.

It was reported that the secondary plant metabolites and bioactive compounds identified by GC/MS in *n*-hexane extract possess antimicrobial, anticancer, anti-inflammatory, antioxidant and antidiabetic activities [28,29].

RP-HPLC analysis of methanol extract: RP-HPLC chromatogram (Fig. 2) illustrates the presence of five compounds with significant abundance in methanolic extract of *A. madraspatna* leaves. Individual UV absorption spectrums were acquired by PDA detector and respective absorption maxima were compared with available literature data to support [30] and confirm the molecules identified by LC-MS. The details of the identified peaks are mentioned in Table-6.

Liquid chromatography-mass spectrometry (LC/MS) analysis: The molecular weights of five compounds observed in RP-HPLC were confirmed by LC-MS on the basis of *m/z* ratio (Table-6) and identified as (i) kaempferol-3-O-rotinoside-7-O-rhamnoside (*m/z* 748), (ii) digitoxoigenin-3-O-α-L-thevetopyranoside (*m/z* 534), (iii) 3-hydroxypropylglucosinolate (*m/z* 377), (iv) luteolin-7-O-glucoside (*m/z* 448), (v) genestein-7-O-glucoside (*m/z* 432). Out of five compounds, four compounds have been reported to have antibacterial potency excluding digitoxoigenin-3-O-α-L-thevetopyranoside. Kaempferol-3-O-rotinoside-7-O-orhamnoside, luteolin-7-O-glucoside and genistein-7-O-glycoside are the flavonoidal glycosides. Luteolin contains one extra hydroxyl belongs at the 2-phenyl substituent compared to kaempferol, which makes luteolin more hydrophilic than kaempferol [31]. Genistein is structurally close to luteolin, which belongs to flavanones. These are active against antibiotic-resistant bacteria as well as potent inhibitors of DNA topoisomerase I by binding to the enzyme. Furthermore, Tadera *et al.* [32] reported that luteolin is a potent α-glucosidase inhibitor due to presence of –OH group and the hydroxyl sub-stitution on its ring.

Xu & Lee [33] found that the presence of at least one hydroxyl group in rings A or B of luteolin at C-3,5,7 is associated for its antibacterial activity. However, the antibacterial potential of this flavonoid against these MDR bacteria is due to its hydroxyl groups in ring B. It was also found that luteolin has antibacterial activity against certain Gram-negative like *E. coli* and *P. aeruginosa* and no activity against the Gram-positive *S. aureus* [34,35].

Farhadi *et al.* [36] reported that the kaempferol with the higher C log P and the positive charge on C_3 has most potent activity against *E. coli*. It also interacts with some crucial

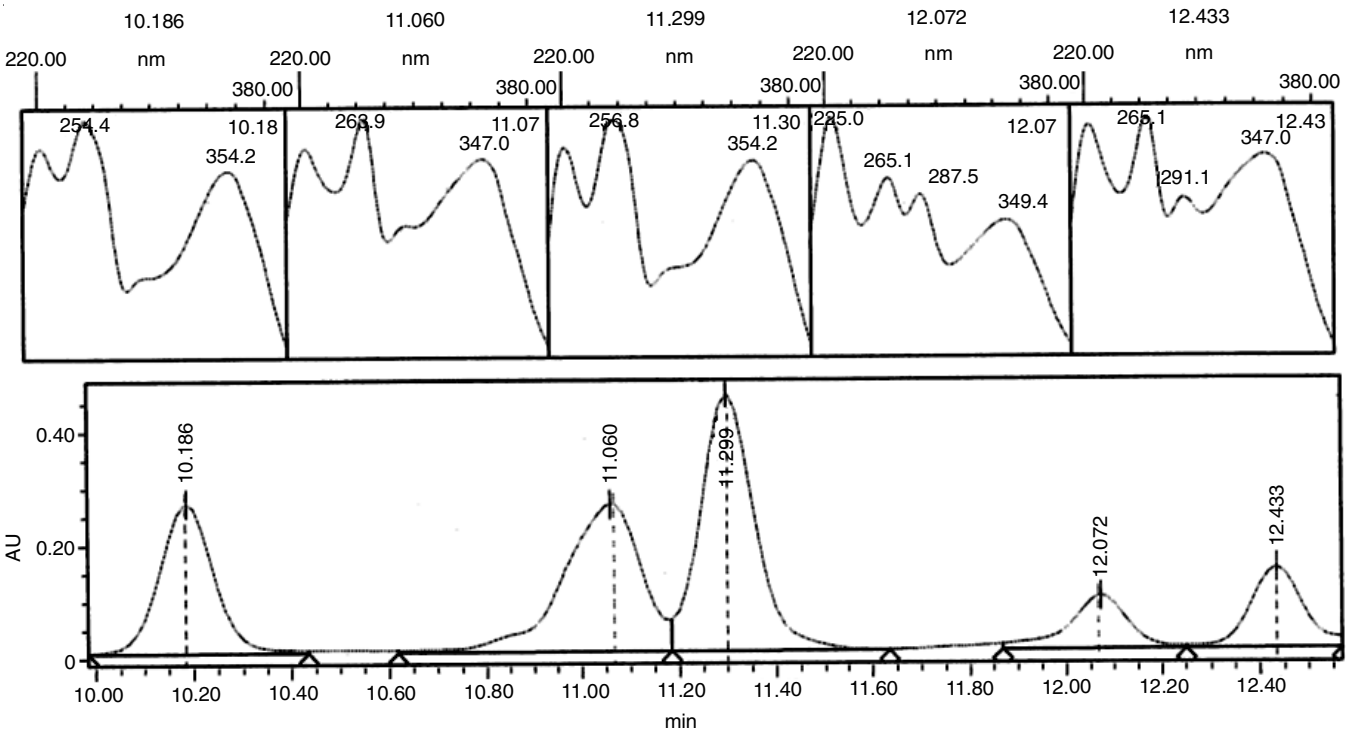

Fig. 2. HPLC chromatogram and spectrum of individual peaks of methanol extract

TABLE-6
CHEMICAL COMPOSITION OF METHANOL EXTRACT FROM *Alphonsea madraspatna* LEAVES ANALYZED BY RP-HPLC AND LC-MS

HPLC RT	λ_{max}	Area (%)	Molecular weight (g/mol)	Compound name
10.186	254.4, 354.2	19.79	748	Kaempferol-3-O rutinoside-7-O-rhamnoside
11.060	263.9, 347.0	28.23	534	Digitoxoigenin-3-O-α-L-thevetopyranoside
11.299	256.8, 354.2	33.72	377	3-Hydroxypropylglucosinolate
12.072	225.0, 265.1, 287.5, 349.4	7.95	448	Lutiolin-7-O-glucoside
12.433	265.1, 291.1,347.0	10.32	432	Genestein-7-O glucoside

HPLC RT: Retention time as in HPLC chromatogram, λ_{ax}: Absorbance maxima as obtained by PDA detector, % Area: Percentage by Area in HPLC chromatogram

enzymes like β-ketoacyl acyl carrier protein synthase (KAS) II and III, which are responsible for the production of precursors of bacterial cell membrane. These enzymes result in fatty acid biosynthesis, such as FabG, FabI and FabZ. Similarly, the present study supports that the plant extract rich in flavonoids are more active against *E. coli* as the flavonoids bind potently to KAS enzymes.

The glucosinolates of *Cleomehave* and their hydrolytic products have been associated with antibacterial potency [37]. Surprisingly it is also found that genistein is an inhibitor of DNA topoisomerases type I [38] and type II [39]. It showed significant antimicrobial activity on the strain *E. coli* isolated from the compound *Flemingia paniculate* [40]. Similarly, kaempferol-3-O-rutinoside-orhamnoside was isolated from Korean mulberry leaves and also exhibit antibacterial activity [41,42].

Conclusion

An attempt was made to identify the antimicrobial compounds present in *Alphonsea madraspatana* leaves. This study was designed to establish the experimental evidence to ensure antimicrobial activity of leaves and to establish a qualitative approach by identifying the compounds responsible for the activity. Although all extracts showed positive antimicrobial response against MDR bacteria, however, a significant antimicrobial effects were observed with *n*-hexane and methanol extracts. The antibacterial activity of this plant could be accredited due to its high content of flavonoids and identified essential components.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. J.A. Seukep, L.P. Sandjo, B.T. Ngadjui and V. Kuete, *S. Afr. J. Bot.*, **103**, 1 (2016); https://doi.org/10.1016/j.sajb.2015.08.014
2. D. van Duin and D.L. Paterson, *Infect. Dis. Clin. North Am.*, **30**, 377 (2016); https://doi.org/10.1016/j.idc.2016.02.004
3. S. Basak, P. Singh and M. Rajurkar, *J. Pathogens*, **2016**, 1 (2016); https://doi.org/10.1155/2016/4065603
4. N. Singh and V. Manchanda, *Clin. Microbiol. Infect.*, **23**, 216 (2017); https://doi.org/10.1016/j.cmi.2017.02.034
5. K. Le Doare, J. Bielicki, P.T. Heath and M. Sharland, *J. Pediatric Infect. Dis. Soc.*, **4**, 11 (2015); https://doi.org/10.1093/jpids/piu014
6. H.H. Kumburu, T. Sonda, B.T. Mmbaga, M. Alifrangis, O. Lund, G. Kibiki and F.M. Aarestrup, *Trop. Med. Int. Health*, **22**, 454 (2017); https://doi.org/10.1111/tmi.12836
7. B. Allegranzi, S.B. Nejad, C. Combescure, W. Graafmans, H. Attar, L. Donaldson and D. Pittet, *Lancet*, **377**, 228 (2011); https://doi.org/10.1016/S0140-6736(10)61458-4
8. G. Sganga, *J. Med. Microbiol. Diagn.*, **03**, (2014); https://doi.org/10.4172/2161-0703.1000132
9. D. Oduro-Mensah, N. Obeng-Nkrumah, E.Y. Bonney, E. Oduro-Mensah, K. Twum-Danso, Y.D. Osei and S.T. Sackey, *Ann. Clin. Microbiol. Antimicrob.*, **15**, 29 (2016); https://doi.org/10.1186/s12941-016-0144-2
10. S. Fournier, C. Brun-Buisson and V. Jarlier, *Antimicrob. Resist. Infect. Control*, **1**, 9 (2012); https://doi.org/10.1186/2047-2994-1-9
11. W.A. Abdel-Naime, J.R. Fahim, M.A. Fouad and M.S. Kamel, *S. Afr. J. Bot.*, **124**, 228 (2019); https://doi.org/10.1016/j.sajb.2019.05.011
12. A. Sahu, G. Ghosh and G. Rath, *Curr. Pharm. Biotechnol.*, **21**, 613 (2020); https://doi.org/10.2174/1389201021666200107114846
13. C.A. Slaughter, *J. Biomol. Technol.*, **30**, S25 (2019); https://doi.org/10.7171/jbt.19-3002-002
14. K. Gupta, *JAMA*, **281**, 736 (1999); https://doi.org/10.1001/jama.281.8.736
15. R.H. Rashid, A.A. Shah, A. Shakoor and S. Noordin, *BioMed Res. Int.*, **2013**, 252356 (2013); https://doi.org/10.1155/2013/252356
16. S.D Joshi, R.G. Venkata, P.M. Satya, B.M. Kishore, N.S. Surya and S. Krishna, *J. Pharmacogn. Phytochem.*, **6**, 1280 (2017).
17. S.-Y. Pan, S.-F. Zhou, S.-H. Gao, Z.-L. Yu, S.-F. Zhang, M.-K. Tang, J.-N. Sun, D.-L. Ma, Y.-F. Han, W.-F. Fong and K.-M. Ko, *Evid. Based Complement. Alternat. Med.*, **2013**, 627375 (2013); https://doi.org/10.1155/2013/627375
18. K.C. Ndukwe, I.N. Okeke, A. Lamikanra, S.K. Adesina and O. Aboderin, *J. Contemp. Dent. Pract.*, **6**, 86 (2005); https://doi.org/10.5005/jcdp-6-3-86
19. A. Plazonic, F. Bucar, Z. Males, A. Mornar, B. Nigovic and N. Kujundzic, *Molecules*, **14**, 2466 (2009); https://doi.org/10.3390/molecules14072466
20. M.M. Rahman, S.S. Lopa, G. Sadik, Harun-or-Rashid, R. Islam, P. Khondkar, A.H.M.K. Alam and M.A. Rashid, *Fitoterapia*, **76**, 758 (2005); https://doi.org/10.1016/j.fitote.2005.08.011
21. M.A. Syed, U. Manzoor, I. Shah and S.H.A. Bukhari, *New Microbiol.*, **33**, 329 (2010).
22. R. Baskaran, P.M. Mohan, K. Sivakumar and A. Kumar, *Acta Microbiol. Immunol. Hung.*, **63**, 27 (2016); https://doi.org/10.1556/030.63.2016.1.2
23. H.N.B. Marzoug, M. Romdhane, A. Lebrihi, F. Mathieu, F. Couderc, M. Abderraba, M.L. Khouja and J. Bouajila, *Molecules*, **16**, 1695 (2011); https://doi.org/10.3390/molecules16021695
24. O. Oluwasegun Victor, *J. Plant Sci.*, **3**, 133 (2015); https://doi.org/10.11648/j.jps.20150303.14
25. G. Camino, S.M. Lomakin and M. Lageard, *Polymer*, **43**, 2011 (2002); https://doi.org/10.1016/S0032-3861(01)00785-6
26. T.D. Thang, L.T. Huong, D.N. Dai and I.A. Ogunwande, *Nat. Prod. Res.*, **27**, 2022 (2013); https://doi.org/10.1080/14786419.2013.821121
27. N.V. Hung, D.N. Dai, T.H. Thai, T.D. Thang, I.A. Ogunwande and A.L. Ogundajo, *Chem. Nat. Compd.*, **54**, 1170 (2018); https://doi.org/10.1007/s10600-018-2584-8
28. M.J. Abad, L.M. Bedoya, L. Apaza and P. Bermejo, *Molecules*, **17**, 2542 (2012); https://doi.org/10.3390/molecules17032542
29. P.A. Biacs and H.G. Daood, *Biochem. Soc. Trans.*, **28**, 839 (2000); https://doi.org/10.1042/bst0280839
30. L.-Z. Lin and J.M. Harnly, *J. Agric. Food Chem.*, **55**, 1084 (2007); https://doi.org/10.1021/jf062431s
31. N.F. Al-Tannak, N.A. Al-Hasawi and L. Novotny, *Curr. Pharm. Anal.*, **15**, 295 (2019); https://doi.org/10.2174/1573412914666171220154224
32. K. Tadera, Y. Minami, K. Takamatsu and T. Matsuoka, *J. Nutr. Sci. Vitaminol. (Tokyo)*, **52**, 149 (2006); https://doi.org/10.3177/jnsv.52.149
33. H.X. Xu and S.F. Lee, *Phytother. Res.*, **15**, 39 (2001); https://doi.org/10.1002/1099-1573(200102)15:1<39::AID-PTR684>3.0.CO;2-R
34. A. Basile, S. Giordano, J.A. López-Sáez and R.C. Cobianchi, *Phytochemistry*, **52**, 1479 (1999); https://doi.org/10.1016/S0031-9422(99)00286-1

35. A. Mori, C. Nishino, N. Enoki and S. Tawata, *Phytochemistry*, **26**, 2231 (1987); https://doi.org/10.1016/S0031-9422(00)84689-0
36. F. Farhadi, B. Khameneh, M. Iranshahi and M. Iranshahy, *Phytother. Res.*, **33**, 13 (2019); https://doi.org/10.1002/ptr.6208
37. M. Sudhakar, Ch.V. Rao, P.M. Rao and D.B. Raju, *Fitoterapia*, **77**, 47 (2006); https://doi.org/10.1016/j.fitote.2005.08.003
38. Y.-C. Chang, M.G. Nair and J.L. Nitiss, *J. Nat. Prod.*, **58**, 1901 (1995); https://doi.org/10.1021/np50126a016
39. Y. Azuma, Y. Onishi, Y. Sato and H. Kizaki, *Cell. Immunol.*, **152**, 271 (1993); https://doi.org/10.1006/cimm.1993.1287
40. K. Ulanowska, A. Tkaczyk, G. Konopa and G. Wêgrzyn, *Arch. Microbiol.*, **184**, 271 (2006); https://doi.org/10.1007/s00203-005-0063-7
41. R.A. Escandón, M. del Campo, R. López-Solis, E. Obreque-Slier and H. Toledo, *Eur. Food Res. Technol.*, **242**, 1495 (2016); https://doi.org/10.1007/s00217-016-2650-z
42. W.-T. Ju, O.-C. Kwon, H.-B. Kim, G.-B. Sung, H.-W. Kim and Y.-S. Kim, *J. Food Sci. Technol.*, **55**, 1789 (2018); https://doi.org/10.1007/s13197-018-3093-2