Research Article

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Preliminary phytochemical screening and *in vitro* antibacterial activity of *Plumbago indica* (Laal chitrak) root extracts against drug-resistant *Escherichia coli* and *Klebsiella pneumoniae*

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Abstract: Drug resistance is one of the problems affecting the world where drug-resistant *Escherichia coli* and *Klebsiella pneumoniae* have been shown to be ubiquitous, frequently isolated from foods and commonly associated with surgical site infection in hospitals worldwide. The aims of this work were to analyze the antibacterial activity of root extracts of the plant obtained by serial extraction (using petroleum ether, chloroform, methanol, and water) and by *in vitro* techniques and preliminary screen phytochemicals present in the extract by qualitative means. Fresh roots of *Plumbago indica* were collected, oven-dried, and extracted using Soxhlet apparatus; antibacterial activity, minimum inhibitory concentrations (MICs), and minimum bactericidal concentrations (MBCs) of the active extract were evaluated by standard methods against clinically isolated drug-resistant *E. coli* and *K. pneumoniae;* preliminary phytochemical screening was taken to detect the presence of alkaloids, saponins, flavonoids, steroids, tannins, reducing sugars, phenolics, protein, and oil and fat; and bioactive compounds were detected by GCMS analysis of the active extracts. Determination of antibacterial activity showed that the test organisms were susceptible to methanol and aqueous extracts only. MIC of methanolic extract was found to be 20 µg/mL on both *E. coli* and *K. pneumoniae,* while aqueous extract had MIC of 10 and 20 µg/mL on *E. coli* and *K. pneumoniae,* respectively. Preliminary phytochemical screening showed the presence of all the above-mentioned phytochemicals except oil and fat. The significance of this work is to find a lasting solution to the current problem of emerging drug-resistant bacteria (*E. coli* and *K. pneumoniae*) through the use of extracts obtained from *P. indica* which have long history of use as traditional medicine. The methanolic and aqueous extract can be recommended as an alternative and candidates for drug development against drug-resistant *E. coli* and *K. pneumoniae*.

Keywords: *Plumbago indica*, drug-resistant, *Escherichia coli*, *Klebsiella pneumoniae*, phytochemicals, antibacterial

1 Introduction

Infectious diseases are one of the leading causes of death worldwide with an estimated death of about 50,000 every year; this is further aggravated nowadays with the emergence of new strains of multidrug-resistant strains of microorganisms with bacteria found at forefront [1]. Majority of such diseases affect all people irrespective of age, race, gender, or social and economic status with increased mortality and morbidity [2]. A lot of researchers nowadays are required to be in a race to discover new drugs, antibiotics, or preparations from different sources that can be used to solve the problem of resistance so as to save humanity from the threat of drug-resistant infectious diseases [3].

Bacteria are at the forefront of most infectious diseases where members of the family Enterobacteriaceae currently dominate the scene with *Escherichia coli* and *Klebsiella pneumoniae* being the most active members [4]. Most resistant strains are known to emerge initially from hospital setting and spread to the general population as nosocomial infections; the first known multidrug-resistant strains were detected among the members of the Enterobacteriaceae [5]. *E. coli* and *K. pneumoniae* are

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widely known to cause many infections such as those affecting urinary tract, kidney, and lungs with increased mortality and morbidity, which is coupled with excess financial burden [6]. Multidrug-resistant Enterobacteriaceae are regarded as the greatest threat to the entire world which requires immediate attention to researchers [7].

Drug-resistant *E. coli* and *K. pneumoniae* have recently been showed to be ubiquitous and frequently isolated from foods including meat, poultry, milk, and cheese [8], in addition to the most commonly isolated bacteria associated with surgical site infection in hospitals [9]. In European countries, the carbapenem resistance in *E. coli* and *K. pneumoniae* was showed to be higher in Greece, Italy, and Romania with respect to other countries and its distribution was shown to be endemic in USA, Brazil, China, Argentina, Colombia, and Taiwan [10].

Many efforts are put in place worldwide for effective control of spread of multidrug resistance such as through cohorting both patients and healthcare workers, which was, however, showed to be expensive and challenging to implement [11]. Another approach was cefoxitin-based antibiotic therapy, which was showed to be effective, but with prolonged therapy, financial burden, and long hospital stay [12].

In recent years, an increase in awareness on the use of medicinal plants is gaining popularity due to their promising nature against many commonly known and newly emerging diseases [13]. Plants were showed to contain large amount of phytochemicals which were believed to play key roles in their medicinal properties due to their valuable resources [14]. Plants have so many compounds embedded within their leaves [15,16], stems and fruits [17], nuts, and seeds [18] with good and safe medicinal properties [19].

Medicinal plants proved to be of both medicinal value as well as great economic importance as botanical wealth from nature; in fact many people rely solely on plant as source of medicine [20,21]. Crude extracts of some medicinal plants have been showed to possess good antimicrobial properties against many organisms such as *Staphylococcus aureus, Candida albicans, E. coli,* and *K. pneumoniae* with root extracts showing more promising result than latex from the plants [22]. Plants extracts exert their effect on many microbes based on the fact that they contain innumerable biologically active chemical constituents that act in combination to exert damage to the microorganisms [23]. Many civilizations have been using various medicinal plants in the past and present to cater for their health needs; the Chinese, Indians, Africans, Egyptians, and Arabians have for long acquired knowledge in medicinal plants and use it from ancient time, middle age, and modern era in curing many diseases [24].

Plumbago indica (also known as *P. rosea* or Indian leadwort) belonging to the family Plumbaginaceae is an evergreen perennial herb plant that can reach up to the height of 1.5 m and characterized with stems that sometimes undergo drooping and rooting. The plant is widely found or scattered around the world, but mostly in tropical Africa, Asia, and Pacific region; it is also commonly found in south-eastern Asia and cultivated in other tropical and subtropical regions where it can grow at elevation up to 1,000 m. In India, the plant is commonly called Laal Chitrak and usually cultivated for medicinal and ornamental uses [25]. It is the most preferred plant in West Bengal and Kerala as compared to its close relation (*P. zeylanica*); the root of the plant is used in traditional medicine to normalize intestinal flora, treatment of skin diseases, opening of abscesses as well as influenza and black water fever treatment [26].

The aims of this work were to analyze the antibacterial activity of root extracts of the plant obtained by serial extraction (using petroleum ether, chloroform, methanol, and water) and by *in vitro* techniques and screen phytochemicals present in the extract by qualitative means. The significance of this work thus lies in finding a lasting solution to the current problem of emerging drug-resistant bacteria (*E. coli* and *K. pneumoniae*) through the use of extracts obtained from medicinal plant (*P. indica*) which have long history of use as traditional medicine, which is further supported by *in vitro* technique as well as gas chromatography mass spectroscopy.

2 Materials and methods

2.1 Sample collection

Fresh roots of *Plumbago indica* were collected from Jalandhar, Punjab, India, and transported to research laboratory, Lovely Professional University, Phagwara. Authentication of the plant was carried out by expert botanists and voucher specimen kept in laboratory for future reference as per method reported earlier [27].

2.2 Sample processing

The plant sample was processed as per standard processing methods reported earlier by other researchers [27,28]. The authenticated roots were carefully sorted out, thoroughly rinsed with distilled water, and then oven-dried at a temperature of 40°C. The roots were then ground

into fine particles using mechanical grinder. 30 g of the powder was extracted by serial extraction method first with petroleum ether, chloroform, methanol, and then water with the aid of Soxhlet apparatus. Extracts were concentrated using rotary evaporator and dried at low temperature (40°C) in a hot air oven to ensure removal of the remaining solvent.

2.3 Collection of test isolates

Drug-resistant isolates of *Escherichia coli* and *Klebsiella pneumoniae* were collected from Johal Hospital Rama mandi and Punjab Institute of Medical Sciences (PIMS), Jalandhar. These were confirmed by testing with standard antibiotics (imipenem and meropenem) using standard method described earlier [29].

2.4 Standardization of inocula and antibacterial activity test

The petroleum ether, chloroform, methanol, and aqueous extracts were dissolved in dimethylsulfoxide and extract concentration of 5 mg/mL was prepared as per standard method [30]. Inoculum was prepared using protocol reported earlier [31].

The antibacterial activity test of different extracts was carried out using well diffusion method as per standard protocol [29,32]. With the aid of sterile cotton swab, an inoculum was taken from the standardized culture and inoculated on a freshly prepared solidified Mueller–Hinton agar and allowed to stand for about 15 min; this was then followed by making wells into the inoculated plates with the aid of 8 mm cork borer and extract concentration was dispensed into the well. The plates were allowed for a pre-diffusion time of 15 min and then incubated upright for 24 h at 37°C. Later, the zone of inhibition formed around the well was measured and plates were photographed.

2.5 Determination of minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs)

The MIC was carried out by tube dilution assay as per the method reported earlier [30,33,34] where extracts concentrations were made between 100 and 4 µg/mL through mixing the extract with an inoculated double strength Mueller–Hinton broth followed by incubation at 37°C for 16–18 h. Evidence of growth in the broth was indicated by presence of turbidity when compared with the control tube. MIC was recorded as minimum concentration that shows no evidence of growth in the tubes, while MBC was determined by subculturing the growth-free tubes on to extract–free Mueller-Hinton agar and incubated for 18–20 h at 37°C.

2.6 Phytochemical analysis

Solvent extracts of *P. indica* (methanolic and aqueous extracts) were subjected to phytochemical analysis by standard method described earlier. Testing was carried out for the presence of phytochemicals such as alkaloids, saponins, flavonoids, steroids, tannins, reducing sugar, phenolics, proteins, and oil and fats [20,35,36].

2.7 GCMS analysis

The methanolic and aqueous extracts of *P. indica* were subjected to GCMS analysis as per standard protocols reported earlier with some modifications [37,38]. The analysis was performed using SHIMADZU GCMS-TQ8040NX with auto sampler AOC-20i plus interfaced to mass spectrometer and equipped with SH-RXi-5SilMS (cross bond similar to 5% diphenyl/95% dimethyl polysiloxane) GC capillary column (30 mts × 0.25 mm ID × 0.25 µm df). The analysis was taken in electron impact mode with ionization energy of 70 eV. Helium (99.99%) was used as carrier gas with a flow rate of 0.97 mL/min, purge flow of 3.0 mL/min, pressure of 62.7 kPa, equilibrium time of 0.5 min, and linear velocity of 36.3 cm/s with a split injection mode (at a ratio of 5:1). The injection temperature and ion-source temperatures were maintained at 250°C; the oven temperature was programmed from 80°C (holding time of 2 min) to 250°C (with 3 min holding time at 250°C) at a ramp rate of 7°C/min with a solvent cut time of 1 min. Samples were dissolved in methanol and the mass spectra were taken at 70 eV at a scan range of 50–650 *m/z*. The GCMS running time was 29 min and relative percentage of each compound was obtained by comparing the average peak area to the total area of detected compounds. The detected compounds were compared with the database of National Institute of Standards and Technology (NIST) and name, molecular weight, and structure of each compound were obtained.

Table 1: Antibacterial activity of root extracts of *P. indica* against drug-resistant *E. coli* and *K. pneumoniae* at 5 mg/mL

Isolates	Zone of inhibition (mm) ± SD				
	Petroleum ether	Chloroform	Methanol	Water	Positive control
E. coli	NZI	NZI	14.33 ± 0.58	15.67 ± 0.58	18.67 ± 1.53
K. pneumoniae	NZI	NZI	14.33 ± 0.58	15.33 ± 0.58	19.67 ± 1.15

Positive control: Imipenem (10 µg/disk); NZI: No zone of inhibition.

2.8 Statistical analysis

All the experiments were performed in triplicates to minimize experimental error while data were reported as mean ± SD (n = 3).

3 Results

3.1 Extract yield

The extraction process gave a yield of 0.17 g (0.57%), 0.18 g (0.60%), 1.32 g (4.40%), and 3.96 g (13.2%) of petroleum ether, chloroform, methanol, and aqueous extracts, respectively, from the original 30 g of the dried root sample used. This showed that different solvents play significant role in the extraction process due to variation in their polarity levels.

3.2 Antibacterial activity test

At the end of the incubation period, the methanolic and aqueous extracts were found to show antibacterial activity on the tested isolates, while the extracts from petroleum ether and chloroform were found to show no activity against the drug-resistant *E. coli* and *K. pneumoniae* as presented in Table 1. This different effect of extracts could be the result of ability of the different solvents to extract the bioactive constituents from the root of *P. indica*; most of this ability is associated with the polarity of the solvent as well as that of the phytoconstituents embedded within the plant roots. As only methanolic and aqueous extracts showed antibacterial activity against the test isolates, they were subjected for further studies.

Table 2: Minimum inhibitory concentration and minimum bactericidal concentration of methanolic and aqueous root extracts of *P. indica* against drug-resistant *E. coli* and *K. pneumoniae*

Isolates	MIC (µg/mL)		MBC (µg/mL)	
	Methanol	Water	Methanol	Water
E. coli	20	10	30	10
K. pneumoniae	20	20	20	20

3.3 Determination of MIC and MBC

The MIC of the methanolic and aqueous root extracts of *P. indica* was found to be 20 and 10 µg/mL, respectively, on *E. coli*, while it was 20 µg/mL against *K. pneumoniae* on both the extracts. On the other hand, the MBC for methanolic and aqueous extracts against *E. coli* was found to be 30 and 10 µg/mL, respectively, while towards *K. pneumoniae*, it was recorded at 20 µg/mL for both extracts (Table 2).

3.4 Phytochemical screening

The phytochemical screening that was undertaken on *P. indica* methanolic and aqueous extracts (qualitatively)

Table 3: Phytochemical composition of methanolic and aqueous root extracts of *P. indica*

Test	Methods	Methanolic extract	Aqueous extract
Alkaloids	Wagner's test	+++	+++
	Mayer's test	++	++
Saponin	Foam test	++	+++
Flavonoids	Shinoda test	++	++
Steroids	Sulfuric acid test	++	+++
Tannins	5% ferric chloride test	++	++
Carbohydrates	Benedict's test	+++	+++
	Fehling test	+++	+++
Phenolics	Ferric chloride test	+	+
Protein	Biuret test	++	++
Oil and fats	Spot test	—	—

Key: +: presence, —: absence of tested phytochemical.

revealed the presence of various plant secondary metabolites in different quantities such as alkaloids, saponins, flavonoids, steroids, tannins, reducing sugars, phenolics, and protein, while oil and fat were found to be missing from both extracts which could be the result of defattening or removal of it by the initial two solvents used during the extraction process (petroleum ether and chloroform) as presented in Table 3. The relative amounts of the phytochemicals in the extracts were found to vary with respect to the type of the test taken (as in the case of alkaloids).

3.5 GCMS analysis

The result of GCMS analysis of methanolic and aqueous extracts of *P. indica* showed the presence of a variety of compounds as mapped from NIST library. The gas chromatogram of the methanolic and aqueous root extracts of *P. indica* showed various peaks with different retention time (Figure 1). The methanolic extract showed the presence of many compounds with the prominent ones including 2-pentadecyn-1-ol; 5-hydroxymethylfurfural; benzoic acid, 4-hydroxy-; plumbagin; 2*H*-1-benzopyran-2-one, 3,4-dihydro-6-hydroxy-5,7-dimethyl-; 2-pentanone, 4-methyl-, oxime; benzoic acid, 4-hydroxy-3,5-dimethoxy-; 3-methyl-4-(2,3-dihydroxyphenyl)-4-oxo-butanoic acid; 2-quinoxalineacetic acid, 3-hydroxy-, hydrazide; *n*-hexadecanoic acid; 6-octadecenoic acid, methyl ester, (*Z*)-; 9-octadecenoic acid, (*E*)- and methylchromone present in the methanolic root extract of *P. indica*, which correspond to 63.34% of the total peak area of the chromatogram (Table 4).

The aqueous extract, on the other hand, showed many compounds with the prominent ones including ethane, 1-chloro-2-nitro-; acetic acid; trimethylsilyl fluoride; dimethylsulfoxonium formylmethylide; 4*H*-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; benzoic acid; benzoic acid, 4-hydroxy-; vanillic acid; 2*H*-1-benzopyran-2-one, 3,4-dihydro-6-hydroxy-5,7-dimethyl-; L-(+)-ascorbic acid 2,6-dihexadecanoate; (*E*)-3,3′-dimethoxy-4,4′-dihydroxystilbene; 1-*cis*-vaccenoylglycerol; *cis*-11-eicosenamide and corresponding to 82.67% of the total peak area of the chromatogram (Table 5).

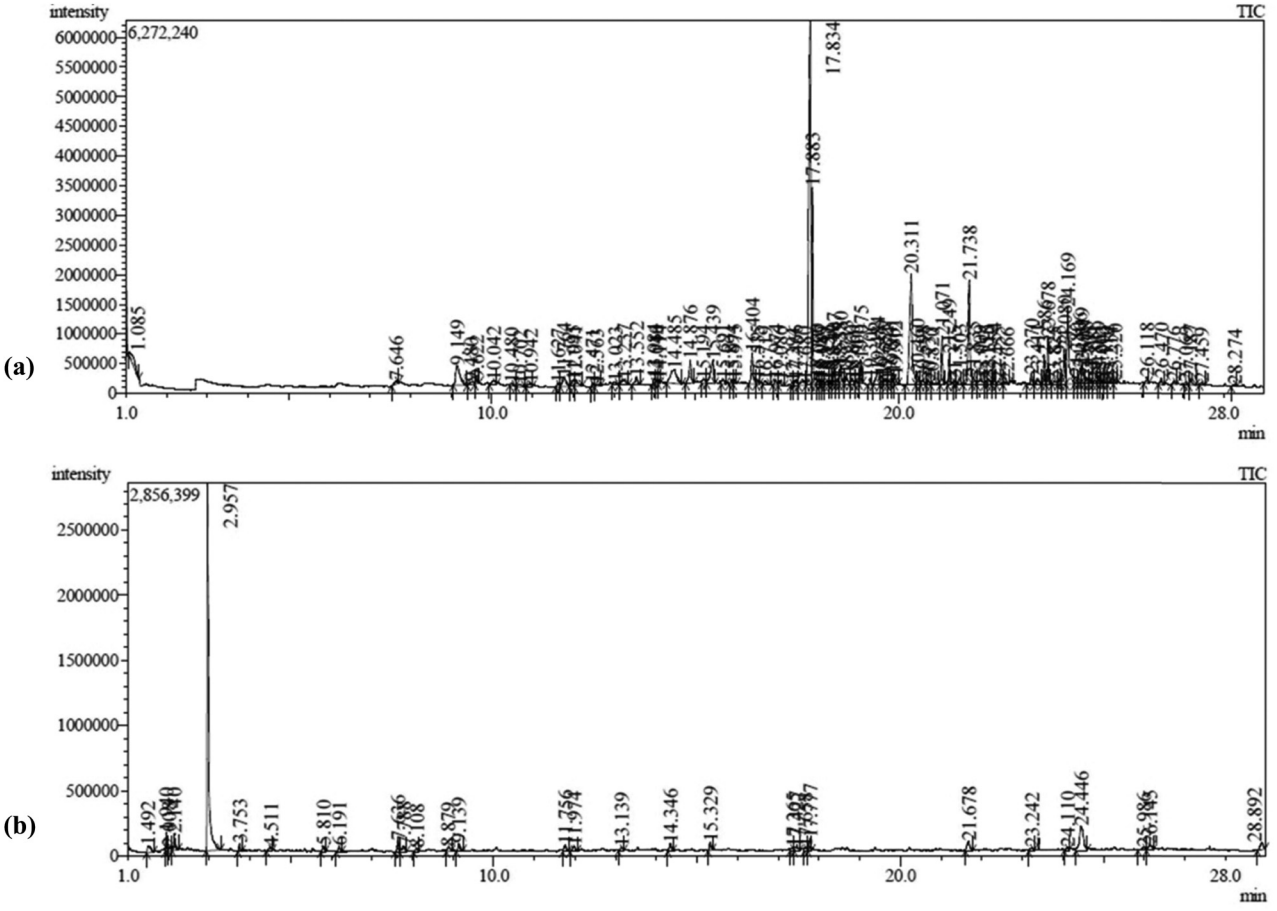

Figure 1: Gas chromatography analysis of *P. indica* root extracts of methanolic (a) and aqueous (b) extracts.

Table 4: Compounds identified in methanolic extract of *P. indica* by GCMS

Retention time	Name of compound	Peak area %	Molecular formula	Molecular weight
1.085	2-Pentadecyn-1-ol	1.24	$C_{15}H_{28}O$	224
9.149	5-Hydroxymethylfurfural	2.36	$C_6H_6O_3$	126
14.485	Benzoic acid, 4-hydroxy-	2.35	$C_7H_6O_3$	138
16.404	Plumbagin	1.91	$C_{11}H_8O_3$	188
17.834	2*H*-1-Benzopyran-2-one, 3,4-dihydro-6-hydroxy-5,7-dimethyl-	22.91	$C_{11}H_{12}O_3$	192
17.883	2-Pentanone, 4-methyl-, oxime	7.45	$C_6H_{13}NO$	115
19.484	Benzoic acid, 4-hydroxy-3,5-dimethoxy-	1.15	$C_9H_{10}O_5$	198
20.311	3-Methyl-4-(2,3-dihydroxyphenyl)-4-oxo-butanoic acid	8.60	$C_{11}H_{12}O_5$	224
21.071	2-Quinoxalineacetic acid, 3-hydroxy-, hydrazide	2.02	$C_{10}H_{10}N_4O_2$	218
21.738	*n*-Hexadecanoic acid	5.74	$C_{16}H_{32}O_2$	256
23.678	6-Octadecenoic acid, methyl ester, (*Z*)-	1.68	$C_{19}H_{36}O_2$	296
24.169	9-Octadecenoic acid, (*E*)-	4.61	$C_{18}H_{34}O_2$	282
24.519	Methylchromone	1.32	$C_{10}H_8O_2$	160

Table 5: Compounds identified in aqueous extract of *P. indica* by GCMS

Retention time	Name of compound	Peak area %	Molecular formula	Molecular weight
1.492	Ethane, 1-chloro-2-nitro-	2.16	$C_2H_4ClNO_2$	109
1.940	Acetic acid	1.55	$C_2H_4O_2$	60
2.140	Trimethylsilyl fluoride	1.90	C_3H_9FSi	92
2.957	Dimethylsulfoxonium formylmethylide	53.36	$C_4H_8O_2S$	120
7.636	4*H*-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	1.62	$C_6H_8O_4$	144
7.788	Benzoic acid	1.55	$C_7H_6O_2$	122
14.346	Benzoic acid, 4-hydroxy-	1.65	$C_7H_6O_3$	138
15.329	Vanillic acid	1.36	$C_8H_8O_4$	168
17.777	2*H*-1-Benzopyran-2-one, 3,4-dihydro-6-hydroxy-5,7-dimethyl-	1.66	$C_{11}H_{12}O_3$	192
21.678	L-(+)-Ascorbic acid 2,6-dihexadecanoate	2.72	$C_{38}H_{68}O_8$	652
24.446	(*E*)-3,3′-Dimethoxy-4,4′-dihydroxystilbene	9.32	$C_{16}H_{16}O_4$	272
26.145	1-*Cis*-vaccenoylglycerol	1.87	$C_{21}H_{40}O_4$	356
28.892	*Cis*-11-eicosenamide	1.95	$C_{20}H_{39}NO$	309

Further, some of the identified molecules with reported medicinal properties have been short-listed and the structure of these eluted compounds at different time and their peaks at different *m*/*z* ratio by mass spectrometer which serve as a fingerprint of each identified compounds are shown in Figure 2.

4 Discussion

Over centuries, humans are in close proximity with plants and their products to cater to their health needs in a variety of forms. Almost all civilizations have used some parts of plants to meet their health need which were proved to be reliable, readily available, and safe for human consumption [37]. Since ancient time to current date, many civilizations are known for their extensive knowledge on herbal medicine with India, China, Africa, and Arabian Peninsula at the forefront in such knowledge [20,24]. Many modern researches have also demonstrated a valuable and promising future in plants research and advocate their use in modern drug development. Studies have shown many plants to possess high antibacterial and antifungal properties and emphasize their use in drug development [39,40]. Previous study has showed that different parts of the same plant such as root and latex have different inhibitory effect on the same strain of microorganisms [22].

P. indica is a plant that has been used traditionally for the cure of various ailments; its roots are used for normalizing intestinal flora, treatment of skin diseases, influenza, and black water fever, and prevent accumulation of triglyceride in liver and aorta [26]. However,

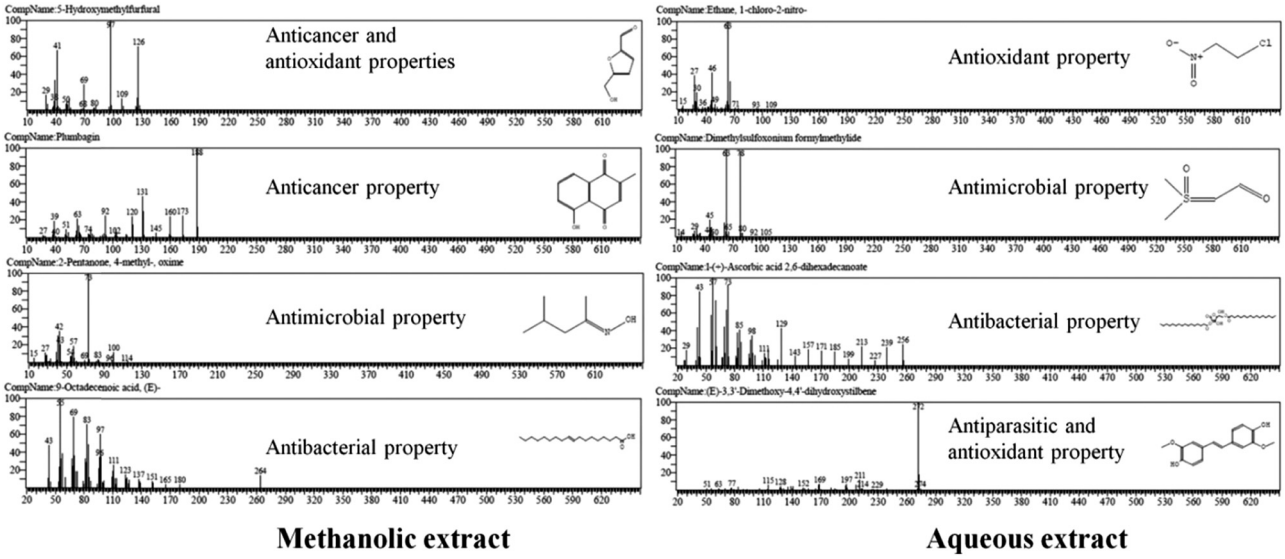

Figure 2: Structures and mass spectra of compounds identified by mass spectrometer in methanolic and aqueous root extracts of *P. indica*.

unlike its close counterpart *Plumbago zeylanica* which was widely investigated by modern research [41], *P. indica* has been relatively less explored.

During this study, the antibacterial activity shown by this plant against drug-resistant *E. coli* and *K. pneumoniae* is worth of note due to the fact that it supports activity reported in the other member of Plumbaginaceae family [42]. The present result of *P. indica* on the drug-resistant *E. coli* and *K. pneumoniae* is found to be better than that reported with *P. zeylanica* being a member of its family [52]. The previous report on the use of leaves and stem of *P. indica* as antibacterial agent against bacterial isolates showed a promising result [43]. The present study can be a breakthrough based on the fact that the tested root extracts are active against drug-resistant clinical isolates which are widely known problem of the healthcare industry [44,45]. In addition, this plant has also been reported to show other activities such as antiviral and haemagglutination inhibition effect [46], antimicrobial [47] and antifertility [48] activity indicating the medicinal potential of this plant and these studies also emphasize that the medicinal properties shown by this plant are because of various phytocomponents present in it.

The preliminary phytochemical screening carried out on this plant and the compounds detected (qualitatively) showed that some of the results are in agreement with earlier study, although such study employed a different extraction method [49]; however, in the present study, oil and fats were found to be missing in the methanolic and aqueous extract mostly due to the fact that a serial extraction technique was employed which make the oil and fat to be removed by the initial two solvents used (petroleum ether and chloroform). Some of the phytochemical compounds identified by GCMS from both extracts were earlier reported to have biological activities; 9-octadecenoic acid, (*E*)- and L-(+)-ascorbic acid 2,6-dihexadecanoate were showed to have good antibacterial properties [50,51], 2-pentanone, 4-methyl-, oxime and dimethylsulfoxonium formylmethylide have antimicrobial activity [52,53], while 5-hydroxymethylfurfural, plumbagin, and (*E*)-3,3′-dimethoxy-4,4′-dihydroxystilbene were showed to possess anticancer, antioxidant, and antiparasitic activities [54–57]. Plants belonging to Plumbaginaceae family are usually rich in plumbagin which is a compound known for medicinal properties [26]. The compound derived from *P. indica* was showed to possess good antibacterial activity which is almost similar to that of naphathoquinones and anthraquinones [25]. During this study also, methanolic extract exhibited the presence of plumbagin along with other important compounds with medicinal properties. Many different stilbene compounds (3-bromo-3′, 5′-dimethoxystilbene-2-nitrogen; 3-bromo-3′, 4′, 5′-trimethoxystilbene-2-nitrogen; 3-bromo-3′, 4′-dimemethoxystilbene-2-nitrogen; 3-bromo-4′-hydroxy stilbene-2-nitrogen; 3-bromo-3′, 4′-dihydroxy stilbene-2-nitrogen) were previously showed to be very active compounds in most plant extracts as antimicrobials, some of which were active at very low concentrations [58]. Presence of the above-mentioned compounds could be the reason for the high antibacterial property with low MIC as well as MBC [59]. A previous study conducted with different plants also showed low MIC and MBC towards resistant bacteria, which is consistent with the present study [60].

The versatility of plant in curing many diseases, easy accessibility, and their low cost led to amplification of usage in herbal medicine against many pathological diseases [51]. Many of the biological activities of the *P. indica* root extracts can thus be attributed to the known compound or as a result of synergistic effect with other minor compounds.

5 Conclusion

In this study, methanolic and aqueous extracts of *P. indica* exhibited good antibacterial activity, with low MIC and MBC towards drug-resistant *E. coli* and *K. pneumoniae*. These findings justify the use of these extracts as herbal remedy for the treatment of drug-resistant *E. coli* and *K. pneumoniae*. It also emphasizes on the potential use of methanolic and aqueous extracts of *P. indica* to be used for modern drug development against the tested isolates. However, it is recommended to conduct detailed studies such as *in vivo* confirmation of antimicrobial activity, toxicity, and pharmacokinetic before its use in medicine. In addition, the isolation of active molecules can also be performed which will be very helpful in modern drug designing.

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