Activity of Various Fractions of *Saussurea lappa* Herb against MDR Gram Negative Rods

Iqra Tabassum[1*], Sidrah Saleem[1], Irfan Ahmad[2,3] and Usman Arshad[1]

1. Department of Microbiology, University of Health Sciences, Lahore, Pakistan
2. Department of Biomedical and Allied Health Sciences, University of Health Sciences, Lahore, Pakistan
3. Department of Molecular Biology, Faculty of Medicine, Umea University, Umea, Sweden

*Correspondence: iqra.tabassum@cemb.edu.pk

Keywords: *Saussurea lappa* herb, MDR gram negative rods, Carbapenemase producing organisms, Methanol, n-hexane, chloroform

doi: 10.37978/tijfs.v6i1.407

Submitted: February 03, 2022
Accepted: April 12, 2022
Published Online: June 22, 2022

How to cite this:
Tabassum *et al*., 2022. Activity of Various Fractions of *Saussurea lappa* Herb against MDR Gram Negative Rods. Int J Front Sci, 6, 1.

Significance:
The worldwide emergence of multidrug resistance is increasing, limiting the efficacy of drugs which are currently use and ultimately treatment failure occurs. As the pipeline of antibiotic drugs are nearly to be empty due to excessive use of effective drugs and resistance against them. This study may benefit in making a bridge between traditional medicine and modern medicine for making new and effective drug from its components.

Abstract

Background: Microorganisms are turning out to be greatly resistant to existing antibiotics, specifically gram-negative rods which shows resistance to currently accessible antibiotics. Beta-lactam antibiotics are the main therapeutic option to treat infections of gram-negative microorganisms i.e., ESBL and Carbapenemase producers. *Saussurea lappa* herb is a medicinal herb use since many times. Basically, the roots of *S. lappa* herb were used as medicines. Current study was conducted to find out activity of various fractions of *S. lappa* herb against multidrug resistant gram-negative rods.

Methods: Crude extracts of ethanol, methanol and water and fractions of n-hexane, chloroform, and methanol from *S. lappa* herb were used. Total of sixty multidrug resistant organisms were included in which thirty were ESBL and thirty were carbapenemase producing organisms. Mean MIC and comparative analysis of various extracts and fractions have been evaluated.

Results: The mean MIC value of crude extracts of ethanol, methanol, n-hexane, chloroform and methanol fractions and water extract from *S. lappa* against ESBL producing organisms were 109.33± 6.915 mg/ml, 154.67± 5.164 mg/ml, 150.00± 5.345 mg/ml, 55.33±5.164 mg/ml, 178.00±6.103 mg/ml, 64.00± 7.701 mg/ml respectively. Similarly, the mean MIC value against carbapenemase producing organisms were 100.67± 8.683 mg/ml, 158.67± 3.519 mg/ml, 150.67± 5.936 mg/ml, 54.67±5.164 mg/ml, 176.67±14.223 mg/ml, 64.33± 9.353 mg/ml respectively.

Conclusion: This study suggests that extracts and fractions of *S. lappa* herb can form the basis to develop novel broad-spectrum formulation for antimicrobial drugs as it contains compounds that has novelty to perform its action against multi-resistant mechanisms.

Introduction

Among gram negative bacterial infections, gram negative rods are threatening cause of infections. A rapid emergence of antibiotic resistance and changes in their mechanism compromised the options of treatments and these mechanisms are more prevalent in gram negative organisms so that chemotherapeutics agents to clinicians are narrow for treatment of invasive infections (1). Most important microorganisms that are included in normal flora of human and animal intestine have been on the rise most of which are belonging to *Enterobacteriaceae* family notably *Escherichia coli*, *Klebsiella Pneumoniae*, *Proteus Providencia*, and *Enterobacter* species. The resistance is against the most important group of antibiotics, the β-lactams (mainly cephalosporins and carbapenem group) that are mostly used for gram negative bacterial infections. Clinicians, scientists and public health authorities pay attention to their alarming spread and fear of spreading infections during the recent years (2). These infections are mainly treated by β-lactam agents including broad spectrum penicillins, cephalosporins, monobactams (aztreonam) and carbapenems. Because of their speed of action, potency and safety, these antimicrobials are widely prescribed antibiotics worldwide. Over the last two decades, however the number of pathogens which are resistant to this type of antibiotics is increased, usually life-threatening infections were due to therapeutic failures. This abundant resistance to this class of drugs has paralleled to the introduction, administration, and overuse of β-lactams drugs in clinical settings (3). The main cause of evolution of antibiotic resistance is overuse of antibiotics. Over-prescription of antibiotics results in leaving resistant pathogens in the patient. Inappropriate prescription is the promotion of resistant bacteria. Moreover, 30% to 60% of antibiotic prescriptions in intensive care units are unnecessary, inappropriate, or suboptimal. Wrong prescribed antibiotics have exposed patients to complications and have questionable therapeutic outcomes (4). Hospital settings have been said as the birthplace of antimicrobial resistant bacteria. In view of the least therapeutic options available to the health care professionals for treating such infections, it is feared

that the medical profession may face a therapeutic dead end against these highly resistant organisms soon. Bacteria are intrinsically resistant to more than one class of antibiotics. Antimicrobial drug resistance of bacteria may exhibit through various mechanisms. One reported mechanism is destruction of antibiotics by bacterial β-lactamases. In another mechanism, antibiotics are expelled out of cell using efflux pump and no interaction with target site occurs. Moreover, bacteria also mutate their genome for modification of cell wall structure and antibiotic target sites. These genomic mutations are favored by survival of fittest model of natural selection. The event of transferring genetic element by transformation, conjugation, or transduction towards antibiotic resistance also occurs in some species (5).

Increasing incidence of antimicrobial resistant infections raise concerns on the selection of effective antimicrobial drug therapy (6-10). Consequently, counter-steps are needed to be taken to eradicate this issue. An alternate path may be the use of plant extracts as antimicrobial agents. That's among other reasons why plant extracts have been popular among researchers to be screened against bacterial pathogens (11). Therapeutic plants are rich source of antimicrobial compounds. Before the advent of Western medicine, therapeutic plants were utilized as remedies for cure of different illnesses including resistant ailments. Bodies are suggesting screening of dietary plants for potential application as antibiotics (12). *Saussurea lappa* (*S. lappa*) is one of such plants commonly known as Costus roots. It is famous for its medicinal properties and widely used in the treatment of many inflammatory diseases indigenously (13).

S lappa Clarke belongs to the family Compositae and is indigenous to China, India, and Pakistan. It grows in the Himalayas at 2500-3500m altitude. Dried roots of this plant that are basically known as Costus roots and have well reputed for their usage in traditional remedies of China, India, and Japan (14, 15). In this study we examined antibacterial function of various fractions of *S. lappa* herb against multidrug resistant gram-negative bacteria. We specifically tested *S lappa* against extended spectrum β-lactamases (ESBLs) and carbapenemase producing gram negative rods.

Materials and Methods

The study was conducted at Microbiology Department of University of Health Sciences Lahore Pakistan. The study was approved by Advanced Study and Research Board of University of Health Sciences (UHS) Lahore Pakistan.

Test Organisms: Multidrug resistant gram-negative rod isolates were collected from participating hospitals and transported to the Microbiology Laboratory of UHS. The organisms were re-confirmed for ESBL and carbapenemase production in UHS.

Plant Material: Roots of *S lappa* herb were collected in august 2016 from the local market of Lahore. The roots were identified phenotypically by the botany department of Government College University of Lahore.

Minimal inhibitory concentration (MIC) defines as susceptibility or resistance at invitro level of specific bacterial strains to applied antibiotic. Reliable evaluation of MIC can be implemented on the choice of a therapeutic strategy, which affects efficiency of a treatment strategy (16)

To determine MIC values, all quantitative methods use Mueller–Hinton (MH) medium either in the form of agar (MHA) or broth (MHB), in some cases additionally supplemented with, for example, 5% lysed horse blood or other compounds depending on bacteria or antibiotic type. Only for anaerobic bacteria Brucella agar with Hemin (5 µg/mL), Vitamin K (1 µg/mL) and 5% lysed horse blood is used. To determine MIC by dilution methods, for most antibiotics, water is used both as a solvent and a diluter, including for most beta-lactams, fluoroquinolones and aminoglycosides. Some require alcohol as a solvent, especially macrolides, chloramphenicol and rifampicin, while others require a phosphate buffer or dimethyl sulfoxide DMSO. Dissolved and diluted antibiotics are used to make working solutions in Mueller-Hinton broth or agar (17, 18).

Working solutions should contain double dilutions of antibiotics. The procedure involves preparing two-fold dilutions of the antimicrobial agent (e.g., 1, 2, 4, 8, 16 and 32 µg/mL) in a liquid growth medium dispensed in tubes containing a minimum volume of 2 mL (macrodilution) or with smaller volumes using 96-well microtitration plate (microdilution) with the range of concentrations used for testing depending on the medication concerned and should take into account the MIC breakpoints for reference strains (19).

In the agar dilution method, each of the obtained antibiotic concentrations at a volume of 1 mL is added to 19 mL of liquid MHA medium at a temperature of 45–50 °C and is poured on petri dishes with a diameter of 9 cm. In the agar dilution method, interpretation is done by the evaluation of the growth of 1–2 colonies or faint haze colonies are contempted. In the broth microdilution method, for certain antibiotics, separate rules for reading the MIC value are used (20, 21)

Identification of ESBLs by double disc diffusion method: A panel of five sensitivity discs were placed

on the Muller-Hinton agar plate inoculated with test organism. They were arranged as: amoxicillin + clavulanic acid (20+10µg) disc in center while aztreonam (30µg), cefotaxime (30µg), ceftazidime (30µg) and ceftriaxone (30µg) discs around at distance of 15mm (edge to edge). After an incubation period of 18-24 hours at 37ºC, zones of inhibition around the discs were examined. ESBL production of the organism is indicated by the enhancement of the zone of inhibition around one or more third generation cephalosporin discs or aztreonam on the side which is nearest to the amoxicillin+clavulanic acid disc by 5mm or more (CLSI Guidelines 2015) (22).

Identification of carbapenemase producers using modified Hodge test:

The modified Hodge test (MHT) was performed according to Anderson *et al*. In brief, Mueller-Hinton agar plates were inoculated with a 1:10 dilution of 0.5 McFarland suspension of E. coli ATCC 25922 and streaked for confluent growth with a swab. A 10µg-meropenem disk was placed in the center, and each test isolate was streaked from the disk to the edge of the plate. The presence of a distorted inhibition zone after overnight incubation was interpreted as a positive result (CLSI Guidelines 2015) (22).

Processing of *S lappa* roots for extraction and fractionation: The extract and fraction were outsourced to Pakistan Council for Scientific and Industrial Research Lahore. Following procedure was used:

Crude ethanolic extract: The material was crushed using a grinder. Ten kilogram of crushed material was soaked in 5 liters of ethanol for one week and filtered on Whatmann filter paper grade 1. The material was again soaked in 4 liters of ethanol and filtered after one week. Two percolates were mixed and dried under vacuum (reduced pressure at the temperature below 45°C) in a rotary evaporator to obtain ethanol extract (23, 24). Yield was calculated as approximately 90 grams of crude ethanolic extract of *S.lappa.*

N-hexane and Chloroform fraction: 45 grams of the crude extract of *S. lappa* was partitioned with n-hexane and chloroform. Crude ethanolic extract were undertaken for solvent-solvent partitioning. The crude extract (45g) was triturated by dissolving in ethanol with few drops of water to make the homogenous solution. It was further partitioned by two solvents n-hexane and chloroform respectively in order to increase polarity using separating funnel. Separate layers were formed in flask. Fractions of *S.lappa* extract were dried by evaporating respective solvent using rotary evaporator. All extracts were stored at 4°C in airtight containers till further analysis (25, 26).

Crude Methanolic extract: Similarly, two kilogram of ground material was soaked in 1 liter of methanol for one week and filtered and dried under vacuum in a rotary evaporator to obtain crude methanol extract of *S. lappa***.** Yield was calculated as approximately 98 grams of crude methanolic extract of *S.lappa*.

Methanol fraction: 49 grams of crude methanolic extract was partitioned with n-hexane and chloroform. Yield was calculated as 44 grams of chloroform fraction of *S.lappa*.

Water extract: One kilogram of ground material was soaked in 1liter of distilled water for one week and filtered and dried under vacuum in a rotary evaporator to obtain crude water extract of *S. lappa.* Yield was calculated as approximately 44 grams of water extract of *S.lappa* (27).

Determination of minimum inhibitory concentration: Minimum inhibitory concentration of *S. lappa* herb was determined by agar dilution method. It is a quantitative method for determination of minimum inhibitory concentration of various extracts and fractions against given organisms (28).

Incorporation of extract and fraction in Muller Hinton Medium: The minimum inhibitory concentration (MIC) of the crude extract and fractions was determined for each of the test organisms in duplicates using following method (29)

1. Muller Hinton medium was prepared by dissolving 38 grams of Muller Hinton agar per liter of distilled water by intermittent heating and mixing.

2. 40 ml of the Muller Hinton medium was then poured in volumetric flask of 100 ml capacity. The flasks were then autoclaved at 121ºC for 15 minutes.

3. Autoclaved sterile Muller Hinton agar medium cooled and maintained at 45-55ºC in a water bath.

4. Each tested concentration was labeled in duplicate on the base of the plates (90mm diameter) and flasks containing the medium.

5. The volume of extract/fraction for each concentration was calculated by standard formula ($M_1V_1 = M_2V_2$) and was then incorporated into the medium at 50ºC and mixed well for even distribution of the extract/fraction.

6. The medium was then poured in pre-labeled plates for each concentration and allowed to solidify at room temperature for 10-15 min.

7. Plates were kept at 4ºC.

N-hexane fraction and chloroform fraction contain hydrophobic compounds. They were not dissolved in water-based Muller Hinton medium. For dissolving of these fractions, we added 2% v/v of TWEEN-80 which

is also called polysorbate 80 in Muller Hinton medium (Difco company).

Preparation of Inoculums: The organism was streaked on Nutrient Agar medium or Muller-Hinton agar medium for obtaining isolated colonies. After incubation at 37°C for 18- 24 hours, 4 or 5 pure colonies were selected for making bacterial suspension and were transferred to a sterile normal saline tube and vortex thoroughly. The bacterial suspension was considered equivalent to 0.5 McFarland standard. The suspension was then diluted in 1:10 sterile normal saline. This dilution had made an inoculum concentration of 10^7CFU/ml. The suspension was used within 15 minutes of preparation.

Inoculation procedure: The plates were dried before performing the test. Multipoint inoculator was used for the inoculation of multiple organisms on the prepared plates of multiple dilution. The 35 wells were present in the sterile grid and each well was filled with 600 µl of each bacterial suspension corresponding to their respective grid number. Multipoint Inoculator has 35 pins each with a diameter of approximately 3mm, delivering approximately 3 µl of the suspension per spot. The pointer pin was used for investigating the starting point. The instrument inoculated our samples simultaneously on a single plate. Then we left plates at room temperature for proper absorption of the inoculums into the medium. Then the plates were incubated at 35 to 37ºC for 18-24 hours without inverting.

Interpretation: After incubation of 18-24 hours, the MIC was examined by reading the values against dark background as concentration that inhibited the growth of the organisms completely. The presence of faint hazy growth of the organism by the inoculum was considered as organism in inhibition state. Confirmed ESBL and carbapenemase positive samples were used positive control. Sterile Muller Hinton agar incorporated with DMSO was used as negative control.

Results:

Sixty gram-negative rod isolates including 30 ESBL and 30 carbapenemase positive were incorporated in this study (Table 1). Among ESBL positive isolates, 21 were Escherichia *coli* and nine *Klebsiella pneumoniae*. All of them showed resistance or reduced susceptibility among third generation cephalosporins and/or aztreonam. Among carbapenemase positive isolates, six were *Pseudomonas aeruginosa*, seven *Klebsiella pneumoniae*, four *Escherichia coli*, five *Enterobacter* and eight *Acinetobacter baumanii*. All of them were meropenem resistant and MHT positive organisms.

Out of 30 ESBLs and 30 carbapenemase positive isolates, 15 isolates from each group were selected to perform MIC for n-hexane fraction, chloroform fraction and crude methanolic extract. The mean MIC of each fraction against ESBLs carbapenemase positive isolates is given in tables 2 and 3. ANOVA followed by Post-hoc Tukey test was applied for comparison of MIC of various groups of extract and fraction. The result showed that there were statistically significant differences between and within these groups. A significant difference was observed in MIC values of extract and fraction against ESBL and carbapenemase positive isolates except crude methanolic extract and n-hexane fraction.

In correspondence to the figure 1 and figure 2 we summarized the mean MIC of ESBL and Carbapenemase producing organisms against multiple fractions and extract to check the efficacy of the medicinal herb.

The mean MIC of Chloroform fraction against ESBLs gram negative rods was 55.33 ± 5.164 mg/ml and against carbapenemase producing organisms was 54.67±5.164 mg/ml. The mean MIC of water extract against ESBLs gram negative rods was 64.00 ± 7.701 mg/ml and against carbapenemase producing organisms was 64.33±9.353 mg/ml. The mean MIC of crude ethanolic extract against ESBLs gram negative rods was 109.33 ± 6.915 mg/ml and against carbapenemase producing organisms was 100.67±8.683 mg/ml. The mean MIC of n-hexane against ESBLs gram negative rods was 150 ± 5.345 mg/ml and against carbapenemase producing organisms was 150.67±5.936 mg/ml. The mean MIC of crude methanolic extract against ESBLs gram negative rods was 154.67 ± 5.164 mg/ml and against carbapenemase producing organisms was 158.67±3.519 mg/ml. The mean MIC of methanol fraction against ESBLs gram negative rods was 178.00 ± 6.103 mg/ml and against carbapenemase producing organisms was 176.67±14.223 mg/ml.

Table 1: Organisms included in our study

Organisms	Isolates (60)	ESBL producers	Carbapenemase producers
Escherichia coli	25	21	4
Klebsiella pneumonia	16	9	7
Acinetobacter baumanii	8	-	8
Enterobacter species	5	-	5
Pseudomonas aeruginosa	6	-	6

Table 2: Mean MIC of various extract and fractions against ESBL

Extract/Fraction 2	Mean MIC Against ESBL (mg/ml)
Ethanolic Extract	109.33± 6.915
Methanolic Extract	154.67± 5.164
N-Hexane Fraction	150.00± 5.345
Chloroform Fraction	55.33± 5.164
Methanol Fraction	178.00± 6.103
Water Extract	64.00± 7.701
P-Value	< 0.005

Table 3: Mean MIC of various extract and fractions against Carbapenemase producing organisms

Extract/Fraction 3	Mean MIC Against Carbapenemase producing organisms (mg/ml)
Ethanolic Extract	100.67± 8.683
Methanolic Extract	158.67± 3.519
N-Hexane Fraction	150.67± 5.936
Chloroform Fraction	54.67± 5.164
Methanol Fraction	176.67± 14.223
Water Extract	64.33± 9.353
P-Value	< 0.005

Figures

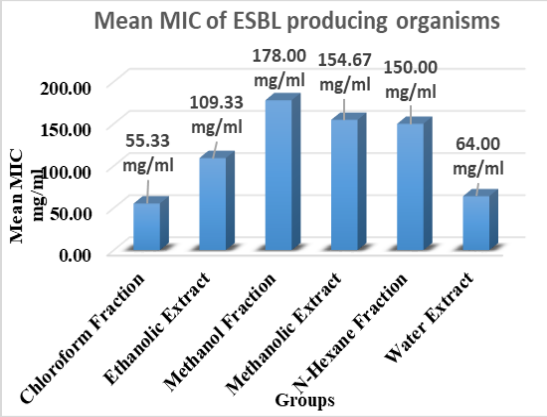


Figure 1: Comparative Analysis of means of MIC against ESBL producing organisms. This figure shows that Chloroform fraction has least MIC value and shows best antimicrobial activity against ESBL producing organisms followed by water extract, Crude Ethanolic Extract, n-Hexane fraction, Crude Methanolic Extract and Methanol fraction respectively.

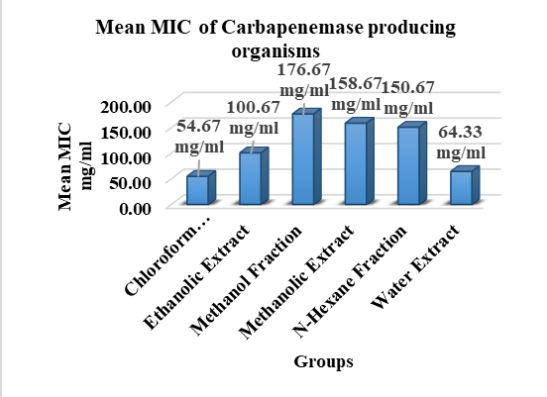


Figure 2: Comparison of means of MIC of extract and fraction against Carbapenemase producing organisms. This figure shows that Chloroform fraction has least MIC value and shows best antimicrobial activity against Carbapenemase producing organisms followed by water extract, Crude Ethanolic Extract, n-Hexane fraction, Crude Methanolic Extract and Methanol fraction respectively.

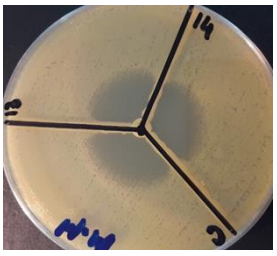
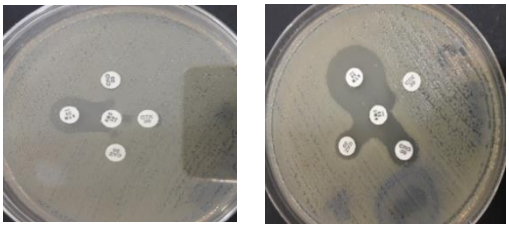


Figure 3: Modified Hodge Test: Clover leaf Pattern



A: ESBL Pattern 1 **B: ESBL Pattern 2**

Figure 4: ESBL Detection: Double Disc Diffusion Method for ESBL detection

Discussion

Data achieved in our study shows that all the extracts and fractions displayed different level of activity against multidrug resistant gram-negative rods. Low sensitivity of some of the test strains to extracts and fractions may be because of variable antibacterial effect of extracts and fractions and emergence of resistant strains. In the present investigation, different extracts and fractions of *S.lappa* were evaluated to explore their antimicrobial activity against multidrug resistant gram-negative rods.

In this analysis, three extracts were used i.e., water extract, crude ethanolic extract and crude methanolic extract. Water extract proved more effective than

crude ethanolic extract and crude methanolic extract. Similarly, three fractions were used for antibacterial analysis i.e., chloroform fraction, n-hexane fraction and methanol fraction. From these fractions, chloroform fraction showed best antibacterial activity against multidrug resistant gram-negative rods. N-hexane fraction and methanol fraction showed least activity as compared to chloroform fraction.

In our study, the chloroform fraction showed good antibacterial activity against tested ESBL and carbapenemase producing organisms. However, its antibacterial activity varies species to species. The chloroform fraction showed the greatest action against almost all the multidrug resistant species tested. Chloroform extract of *S.lappa* contains alkaloids, coumarins, flavonoids, saponins, sterols and triterpenes. HPLC analysis of chloroform extract of *S.lappa*, isolated several compounds. These included umbelliferon glucoside, costunolide, luteolin-7-O-β D-glucoside, rutin and apigenin-7-O-β-D-glucoside. These compounds seem responsible to exhibit strong antibacterial activity (30). Chloroform fraction has 68.56±0.08 grams equivalent total flavonoids contents and 33.79±0.12 grams equivalent total phenolic contents (31). It can be assumed that highest activity of chloroform fraction is due to the presence of flavonoid compounds.

Water extract has the second-best antibacterial activity among the examined extracts and fractions. It inhibited the tested multidrug resistant gram-negative organisms at MIC value of 64.00 ± 7.701 mg/ml and 64.33±9.353 mg/ml against ESBL and carbapenemase producing organisms respectively. Water extract of *S.lappa* contains alkaloids, coumarins, flavonoids, saponins, tannins and triterpenes (*32*). Slight variation in MIC values of chloroform fraction and water extract can be due to the presence of sterol compounds in chloroform fraction while tannin compound in water. Our investigation further showed that both water extract and chloroform fraction were active against multidrug resistant gram-negative rods.

N-hexane fraction obtained from crude ethanolic extract of *S.lappa* herb did not exhibit good antibacterial activity. Chemical constituent of n-hexane has been documented to contain completely non-polar compounds and lower yield of phenolic and flavonoid compounds (31, 32). N-hexane extract contains two active compounds costunolide and erementhin having potent antifungal activity than antibacterial activity as per previous reports (33).

Several authors have reported the antimicrobial activity of crude methanolic extract (34). In our study, MIC value of crude methanolic extract is higher than chloroform fraction, water extract, crude methanolic extract and n-hexane fraction. Methanol extract contains alkaloids, coumarins, flavonoids, saponins, sterols and triterpenes, tannins and cardiac glycosides (*30*). So, it can be supposed that cardiac glycoside phytochemicals interfere to express good antimicrobial activity of crude methanolic extract. Methanol is responsible to extract sesquiterpene, a dehydrocostus lactone from *S.lappa* (35). The variation in antibacterial activity of crude extracts might be due to distribution of antimicrobial compounds of crude extracts (36).

The methanol fraction has highest MIC value examined in our study. These values were too high to be considered active against the multidrug resistant pathogens than other extracts and fractions. If crude methanolic extract is compared with the methanol fraction of *S.lappa* in order to reveal the antimicrobial action against MDR gram negative rods, then we can conclude that crude methanolic extract showed good synergistic interaction of Phyto-compounds (36).

Based on the results obtained in this study it might be concluded that *S. lappa* root extracts and fractions have broad-spectrum antimicrobial activity against ESBL and carbapenemase producing organisms. ESBL and carbapenemase producing organisms are resistant to old regimen of antibiotics so biologically active compounds in *S.lappa* can be a substitute. Extracts and fractions of *S.lappa* may be used to isolate biologically active compounds with the development of new and effective antimicrobial drugs. This study suggests development of novel broad-spectrum formulation containing extracts and fractions of *S. lappa* herb against antibiotic resistant gram-negative rods.

Conclusion

S.lappa extracts and fractions are effective against ESBL and carbapenemase producing gram-negative rods. Biological active compounds in these extracts and fractions have potential to be used in antibiotic resistant gram-negative infections.

Conflict of interest: Authors do not have any conflict of interest to declare.

Disclosure: None

Human/Animal Rights: No human or animal rights are violated during this study.

References

1. Berezin EN, Solórzano F. Gram-negative infections in pediatric and neonatal intensive care units of Latin America. The Journal of Infection in Developing Countries. 2014 Aug 13;8(08):942-53.

2. Östholm Balkhed Å. Extended-Spectrum ß-Lactamase-Producing Enterobacteriaceae: Antibiotic consumption, Detection and Resistance Epidemiology (Doctoral dissertation, Linköping University Electronic Press).

3. Ventola CL. The antibiotic resistance crisis: part 2: management strategies and new agents. Pharmacy and Therapeutics. 2015 May;40(5):344.
4. Tenover FC. Mechanisms of antimicrobial resistance in bacteria. The American journal of medicine. 2006 Jun 1;119(6):S3-10.
5. Bush K. Alarming β-lactamase-mediated resistance in multidrug-resistant Enterobacteriaceae. Current opinion in microbiology. 2010 Oct 1;13(5):558-64.
6. Paterson DL. Resistance in gram-negative bacteria: Enterobacteriaceae. American journal of infection control. 2006 Jun 1;34(5):S20-8.
7. Paterson DL, Ko WC, Von Gottberg A, Mohapatra S, Casellas JM, Goossens H, Mulazimoglu L, Trenholme G, Klugman KP, Bonomo RA, Rice LB. Antibiotic therapy for Klebsiella pneumoniae bacteremia: implications of production of extended-spectrum β-lactamases. Clinical infectious diseases. 2004 Jul 1;39(1):31-7.
8. Rossi F, Baquero F, Hsueh PR, Paterson DL, Bochicchio GV, Snyder TA, Satishchandran V, McCarroll K, DiNubile MJ, Chow JW. In vitro susceptibilities of aerobic and facultatively anaerobic Gram-negative bacilli isolated from patients with intra-abdominal infections worldwide: 2004 results from SMART (Study for Monitoring Antimicrobial Resistance Trends). Journal of Antimicrobial Chemotherapy. 2006 Jul 1;58(1):205-10.
9. National Nosocomial Infections Surveillance (NNIS) System R. National Nosocomial Infections Surveillance (NNIS) system report, data summary from January 1992 through June 2004, issued October 2004. Am J infect control. 2004;32:470-85.
10. Kumar VP, Chauhan NS, Padh H, Rajani M. Search for antibacterial and antifungal agents from selected Indian medicinal plants. Journal of ethnopharmacology. 2006 Sep 19;107(2):182-8.
11. Sarwat SZ, Ahmad N. Screening of potential medicinal plants from district sawat specific for controlling women diseases. Pak J Bot. 2012 Aug 15;44(4):1193-8.
12. Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. International journal of food microbiology. 2004 Aug 1;94(3):223-53.
13. Rao RN, Raju SS, Babu KS, Vadaparthi PR. HPLC determination of costunolide as a marker of Saussurea lappa and its herbal formulations. Int J Biochem. 2013;3(1):99-107.
14. Wang F, Xie ZH, Gao Y, Xu Y, Cheng XL, Liu JK. Sulfonated guaianolides from Saussurea lappa. Chemical and Pharmaceutical Bulletin. 2008 Jun 1;56(6):864-5.
15. Hancock RE. Mechanisms of action of newer antibiotics for Gram-positive pathogens. The Lancet infectious diseases. 2005 Apr 1;5(4):209-18.
16. Kowalska-Krochmal B, Dudek-Wicher R. The minimum inhibitory concentration of antibiotics: Methods, interpretation, clinical relevance. Pathogens. 2021 Feb 4;10(2):165.
17. Satlin MJ, Lewis JS, Weinstein MP, Patel J, Humphries RM, Kahlmeter G, Giske CG, Turnidge J. Clinical and Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing position statements on polymyxin B and colistin clinical breakpoints. Clinical Infectious Diseases. 2020 Nov 1;71(9):e523-9.
18. Nagayama A, Yamaguchi K, Watanabe K, Tanaka M, Kobayashi I, Nagasawa Z. Final report from the Committee on Antimicrobial Susceptibility Testing, Japanese Society of Chemotherapy, on the agar dilution method (2007). Journal of infection and chemotherapy. 2008 Jan 1;14(5):383-92.
19. Andrews J. BSAC Working Party Report on Susceptibility Testing. 2001. Determination of inhibitory concentrations. J. Antimicrob. Chemother.;48:48-71.
20. EUCAST. European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID): terminology relating to methods for the determination of susceptibility of bacteria to antimicrobial agents. Clin Microbiol Infec. 2003;9:1-7.
21. Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature protocols. 2008 Feb;3(2):163-75.
22. Patel JB. Performance standards for antimicrobial susceptibility testing; twenty-fifth informational supplement.
23. Hasson SS, Al-Balushi MS, Al-Busaidi J, Othman MS, Said EA, Habal O, Sallam TA, Aljabri AA, AhmedIdris M. Evaluation of anti–resistant activity of Auklandia (Saussurea lappa) root against some human pathogens. Asian Pacific Journal of Tropical Biomedicine. 2013 Jul 1;3(7):557-62.
24. Rahman MA, Sultana R, Bin Emran T, Islam MS, Rahman MA, Chakma JS, Rashid HU, Hasan CM. Effects of organic extracts of six Bangladeshi plants on in vitro thrombolysis and cytotoxicity. BMC complementary and alternative medicine. 2013 Dec;13:1-7.
25. Rashid U, Khan MR, Jan S, Bokhari J, Shah NA. Assessment of phytochemicals, antimicrobial and cytotoxic activities of extract and fractions from Fagonia olivieri (Zygophyllaceae). BMC complementary and alternative medicine. 2013 Dec;13:1-7.
26. Rojas JJ, Ochoa VJ, Ocampo SA, Muñoz JF. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections. BMC complementary and alternative medicine. 2006 Dec;6(1):1-6.
27. Adebiyi AO, Koekemoer T, Adebiyi AP, Smith N, Baxter E, Naude RJ, Van de Venter M. Antimicrobial and antioxidant activities of crude extracts of two Nigerian chewing sticks. Pharmaceutical Biology. 2009 Apr 1;47(4):320-7.
28. Kang CG, Hah DS, Kim CH, Kim YH, Kim E, Kim JS. Evaluation of antimicrobial activity of the methanol extracts from 8 traditional medicinal plants. Toxicological research. 2011 Mar;27:31-6.
29. Panda SK. Screening methods in the study of antimicrobial properties of medicinal plants. International Journal of Biotechnology and Research. 2012;2(1):1-35.
30. Alaagib RM, Ayoub SM. On the chemical composition and antibacterial activity of Saussurea lappa (Asteraceae). The Pharma Innovation. 2015 Apr 1;4(2, Part C):73.
31. Chang KM, Choi SI, Chung SJ, Kim GH. Anti-microbial activity of Saussurea lappa CB Clarke roots. Journal of Food Science and Nutrition. 2011 Dec;16(4):376-80.
32. Mukherjee S, Dey A, Das T. In vitro antibacterial activity of n-hexane fraction of methanolic extract of Alstonia scholaris LR Br. stem bark against some multidrug resistant human pathogenic bacteria. European Journal of Medicinal Plants. 2012 Jan 1;2(1):1.
33. Duraipandiyan V, Abdullah Al-Harbi N, Ignacimuthu S, Muthukumar C. Antimicrobial activity of sesquiterpene lactones isolated from traditional medicinal plant, Costus speciosus (Koen ex. Retz.) Sm. BMC complementary and alternative medicine. 2012 Dec;12(1):1-6.
34. Rathi SG, Patel KR, Bhaskar VH. Isolation of herbal plants: antifungal and antibacterial activities. Journal of Pharmaceutical Science and Bioscientific Research. 2012;2(1):25-9.
35. Sarwar A, Enbergs H. Effects of Saussurea lappa roots extract in ethanol on leukocyte phagocytic activity, lymphocyte proliferation and interferon-gamma (IFN-gamma). Pak J Pharm Sci. 2007 Jul 1;20(3):175-9.
36. Alam MA, Sarder M, Awal MA, Sikder MM, Daulla KA. Antibacterial activity of the crude ethanolic extract of Xylocarpus granatum stem barks. Bangladesh Journal of Veterinary Medicine. 2006;4(1):69-7