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Evaluation of the Antimicrobial Activity of Extracts from *Acorus calamus* Rhizome against Multidrug-resistant Nosocomial Pathogens

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A B S T R A C T

Antibiotic drugs continue the leading therapeutics for healing microbial infections. The multidrug resistance has a global health crisis, and seek other substitutes. The present research work aimed to explore the in-vitro antibacterial property of *A. calamus* acetone extract against nosocomial pathogens. A total of 24 wound and skin specimens were collected and 28 significant nosocomial pathogens were identified based on their phenotypic and biochemical profiling viz., *E. coli* (5), *Staphylococcus aureus* (6), *Pseudomonas aeruginosa* (6), *Klebsiella pneumonia* (4), *K. oxytoca* (3), and *Acinetobacter baumanii* (4). The sensitivity and resistance pattern was observed on all the isolates. *K. pneumoniae* was 100% resistant against all three antibiotics. All six isolates were resistant to an antibiotic. They showed a multidrug-resistance (MDR) pattern. *E. coli, P. aeruginosa* and *K. pneumonia* were resistant to Tetracycline (30µg), *K. pneumonia*, and *K. oxytoca* were resistant to Gentamycin (10µg), *S. aureus, K. pneumonia*, and *A. baumanii* were resistant to Chloramphenicol (30µg). Hence, considerable inhibition of *A. calamus* acetone extract was found in all the isolates. The maximum inhibition of 17mm on *S. aureus* and the minimum inhibition of 13 mm on *A. baumanii* at 60 µg concentration. This research work evidenced that this plant extract has antibacterial potential to persuade a quick, wound healing.

***Key words*:** Nosocomial pathogens, MDR, Plant extracts, Antibacterial property

The ideal antimicrobial therapy to effectively treat infections involves the use of antibiotics either singly or in combination. With years passing by, the ridiculous use of antibacterial drugs, self-drug medication, improper supervision, over-prescription of medicine, recurrent hospitalization, morbidity, mortality, [1], and also the agricultural use of antibiotics which affects the environmental microbiome, everything leading to the emergence of multiple Multi-Drug Resistant (MDR) pathogens [2]. Universal level health issues bacterial Multidrug-resistant infections are coherent as leading pressures, and MDR bacteria causes nosocomial infections [3]. This predisposes us towards a future with ineffective antibiotics. Pathogens causing nosocomial infections are responsible for 15.5% of total MDR. Thereby, increasing mortality ultimately also leading to high health care costs [4]. Antibiotic development is no longer considered to be an economically wise investment for the pharmaceutical industry as antibiotics are used for relatively short periods and are often curative; antibiotics are not as profitable as drugs that treat chronic conditions (diabetes, psychiatric disorders, asthma, etc.) [5]. Inappropriate empirical antibiotic schedules easily translate into alarming morbidity and hospital mortality. The complex clinical scenarios nowadays pose a great challenge to new empirical antibiotic strategies that may further spread resistance [6]. Thus, in the last few years, the research thirst for new strategies more resilient to multidrug resistance is urgently warranted for the near future. Since time immemorial nature has sustained to provide us with novel remedies to help in relief from illnesses. Global, regional and local preventive measures should therefore be implemented to combat antimicrobial resistance, which includes the restriction of antibiotic prophylaxis to high-risk populations, stewardship programs on adequate antibiotic prescription, and increasing awareness among health professionals. This also decreases the risks of treatment failure and poor patient management. Therefore, reduced antibiotic production, increased drug resistance, and adverse effects make it imperative to find alternative therapies, including traditional plant-based

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medicines to end this MDR crisis [7]. This highlights the need for the use of plant natural products and plant extracts, as having particular promise for rapidly developing new, effective treatment to combat pathogens resistant to conventional antibiotic therapies [8]. Plants are the key source of drugs and alternative medicine for fighting against diseases since ancient times [9]. Medicinal and herbal plants is substitutes for battling MDR directing on their phytobioactives with resistance drug modulatory actions [10]. In search of new antibiotics for the rapidly emerging multidrug-resistant nosocomial pathogens, a bestowed and prestigious position in this study was given to *Acorus calamus* rhizome. *Acorus calamus* is a natural plant belonging to the order Acorales and family *Acoraceae* [11]. *Acorus* is a genus name and calamus its species and commonly known as "sweet flag" or "calamus". *A. calamus* has a lengthened past history of therapeutic use in Indian and Chinese herbal folklore. *A. calamus* (rhizome) are broadly employed by India, America, and China, and Europe for the healing of diverse ailments. Many ethnomedicinal, ethnobotanical, and pharmacological studies have revealed that *Acorus calamus* rhizome and its constituents possess a wide range of pharmacological activities such as anti-inflammatory, antioxidant, antimicrobial, etc. [12-13]. Hence this research work will aid in paving the path to examine the antibacterial potential of *Acorus calamus* rhizome acetone extract against the MDR nosocomial pathogens.

MATERIALS AND METHODS

Rhizome collection

Fresh plant materials of *A. calamus* rhizome were collected from the bazaar of Rasipuram, district Namakkal, Tamil Nadu. They were identified as A. *calamus* rhizome by taxonomy existence [14].

Preparation of rhizome extract

Fresh plant materials were washed, air-dried for 2 weeks, and ground into a fine powder using a grinder, kept at 4°C in sealed bottle, and subjected for extraction. Phytoextract was primed by soaking rhizome powder (50 gm) in the acetone solvent (250 ml) using Microwave-assisted extraction. Obtained rhizome extract was put together, resolute, and stored at 4°C in a sealed container for antimicrobial assay [15-16].

Specimen collection

Wound and skin swabs were collected from in and around Namakkal District using sterile cotton swabs aseptically and gently to avoid contamination of Specimens from patients who had developed purulent materials. Specimens were stamped and moved instantly to the research laboratory for microbiological investigation [17].

Examination of cultures and identification of isolates

In the laboratory, collected samples were inoculated on MacConkey agar and Blood agar. The inoculums were streaked out for discrete colonies with a sterile inoculating loop on the plate. Then the culture plates were incubated at 37°C for 24 hours for growth and the formation of colonies. After 24 hours, all the bacteria were isolated and identified using morphologically, microscopically, Gram staining, and biochemical tests. The biochemical tests carried out included: motility, Indole, MR, VP, citrate utilization, and carbohydrate fermentation (Glucose, Lactose, Sucrose, and Mannose). The species of bacteria were identified according to the standard microbiological methods [18-19].

Antibiotic susceptibility tests

According to CLSI guidelines, a disc diffusion test was performed according to the Kirby-Bauer method. A lawn of test pathogen was prepared by spreading 100µl inoculums with a sterilized spreader onto the entire surface of the Muller Hinton agar plate. The plates were dried before applying the antibiotic disc. Then, antibiotic discs such as Tetracycline (30µg), Gentamycin (10µg), and Chloramphenicol (30µg) were placed firmly on the agar plates, and then the plates were incubated at 37°C for 18 hours. If the antimicrobial activity was present on the plates, it was indicated by a zone of inhibition. The diameter of the zone of inhibition was measured in millimeters using an electronic scale. An organism was considered highly susceptible if the diameter of the zone of inhibition was above 19 mm, intermediate if the diameter was 15-18 mm and resistant if less than 13 mm [18].

Antibacterial assay

The aqueous rhizome extracts were placed to attain a concentration in conflict to check pathogens and these inoculums were swabbed consistently onto the Muller-Hinton Agar plates. Wells were punched using a sterile cork-borer (6 mm) and loaded with rhizome extract of varying concentrations of 20 µg, 40 µg, and 60 µg using a micropipette in each well under aseptic conditions. The plates were allowed to stand for 1 hour for pre-diffusion of the extract into the medium. Then the plates were incubated aerobically at 37°C overnight. The anti-bacterial screening was evaluated by measuring the zone of inhibition (ZOI) [16], [20-21].

Table 1 Microscopic and biochemical characterization of the isolates

Microscopy and morphology	Carbohydrate fermentation				Indole	Methyl red	Voges roskauer	Citrate	Identified pathogens
	Glucose	Lactose	Sucrose	Mannose					
G –ve Rod	P	N	N	P	N	N	N	P	*P. aeroginosa*
G +ve Cocci in clusters	P	N	P	P	N	P	N	N	*S. aureus*
G –ve Rod	P	N	N	N	N	N	N	P	*A. baumanii*
G –ve Rod	P	P	P	P	N	P	N	N	*E. coli*
G –ve Rod	P	P	P	P	N	N	P	P	*K. pneumoniae*
G –ve Rod	P	P	P	P	P	N	P	P	*K. oxytoca*

RESULTS AND DISCUSSION

Microscopic and biochemical characterization

The nosocomial pathogens from wound and skin swabs samples collected from hospitals in and around Namakkal district are unique on basis of microscopic and biochemical entities. In the existing study, six morphologically distinct bacterial strains were identified (Table 1). Among the strains Pseudomonas *aeruginosa* and *E. coli* are motile and the rest are non-motile [2].

From a sum of 24 samples, 28 significant nosocomial pathogens were screened. The rate of nosocomial infection was found to be predominant over gram-negative strains *P. aeruginosa* (6 isolates), *E. coli* (5 isolates), *K. pneumonia* (4 isolates), *K. oxytoca* (3 isolates), *A. baumanii* (4 isolates) followed by gram-positive strains *Staphylococcus aureus* (6 isolates) in our study (Table 2, Fig 1) [18].

Table 2 Screening of nosocomial pathogens from clinical samples

Number of wound and skin swab specimen	24
Significant pathogens	28
Pseudomonas aeruginosa	6
Staphylococcus aureus	6
Acinetobacter baumanii	4
Escherichia coli	5
Klebsiella pneumoniae	4
Klebsiella oxytoca	3

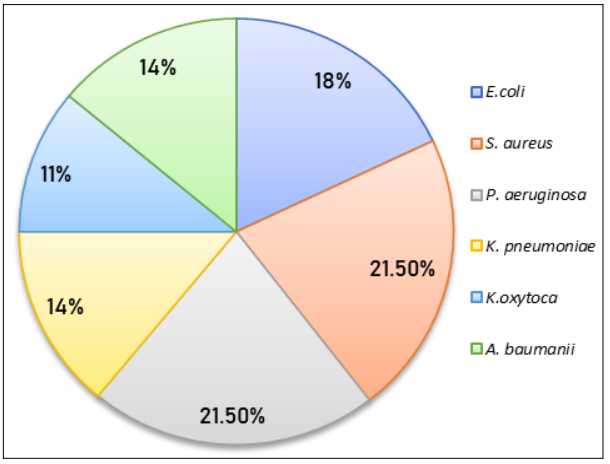

Fig 1 Isolates in the clinical specimens

Antibacterial assay

The study findings are based on the antibiotic susceptibility test performed on the identified pathogens against currently used antibiotics such as tetracycline (30 µg), Gentamycin (10 µg) and Chloramphenicol (30 µg) showed different zones of inhibition which were measured (mm) and analyzed (Table 3, Fig 2). It was alarming to discover that all the isolates showed multidrug-resistant patterns (MDR). In which high MDR pattern was shown by *Klebsiella pneumonia* and low by *S. aureus* against Chloramphenicol (30 µg) shown in (Fig 2) [2].

Table 3 Antibiotic sensitivity pattern against the pathogens (ZOI in mm)

Isolates	Chloramphenicol (30µg)		Tetracycline (30µg)		Gentamycin (10µg)	
	ZOI (mm)	Inf	ZOI (mm)	Inf	ZOI (mm)	Inf
Pseudomonas aeruginosa	18	S	16	R	19	S
Staphylococcus aureus	16	R	28	S	21	S
Acinetobacter baumanii	6	R	22	S	20	S
Escherichia coli	19	S	14	R	19	S
Klebsiella pneumoniae	11	R	12	R	12	R
Klebsiella oxytoca	23	S	21	S	14	R

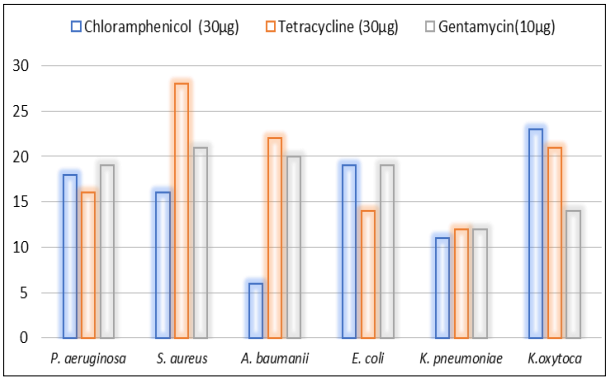

Fig 2 Multidrug resistance of sensitivity against the nosocomial pathogens (ZOI in mm)

In the current investigation, the antibacterial effects of *Acorus calamus* rhizome acetone extract at different concentrations (20–60 µg) were quantified and evaluated on the basis of the zone formation (Table 4, Fig 3). *Acorus calamus* rhizome acetone extract exhibited tremendous susceptibility action against *Staphylococcus aureus* even at the least concentrations. Antibacterial effects support the use of the medicinal plant in traditional therapeutics [22]. The plant extract reported the antibacterial activity towards *B. subtilis*, *E. coli*, *P. aeruginosa*, and *S. aureus* in MeOH and aqueous leaf extracts [23]. The *Acorus calamus* rhizome acetone extract was effective against all the identified pathogens with at least a minimum zone of inhibition of 11 mm and these results were compared with commercially available antibiotics [24]. Drug-resistant nosocomial infections are a pleasing severe crisis in healthiness and hospital alarm, escalating morbidity, lengthy stay, fatality, and elevation the healthcare-associated expenses [25]. The antibiotic drug resistance representation of nosocomial infections sightsaw luxations amongst hospitals on the earth. The mainstream of microbial infections are resilient to numerous antibiotic drugs [26].

Table 4 Antibacterial activity of *Acorus calamus* rhizome acetone extract

Name of the organism	20(µg)	40(µg)	60(µg)
Escherichia coli	12mm	14mm	15mm
Staphylococcus aureus	15mm	16mm	17mm
Pseudomonas aeruginosa	11mm	12mm	14mm
Klebsiella pneumoniae	12mm	13mm	15mm
Klebsiella oxytoca	12mm	14mm	16mm
Acinetobacter baumanii	11mm	12mm	13mm

CONCLUSION

The present study demonstrated the potential value of *Acorus calamus* rhizome extract in treating nosocomial skin and wound infections It can be concluded that it has antibacterial potential which can be utilized the pharmaceutical or biomedical fields in the future.

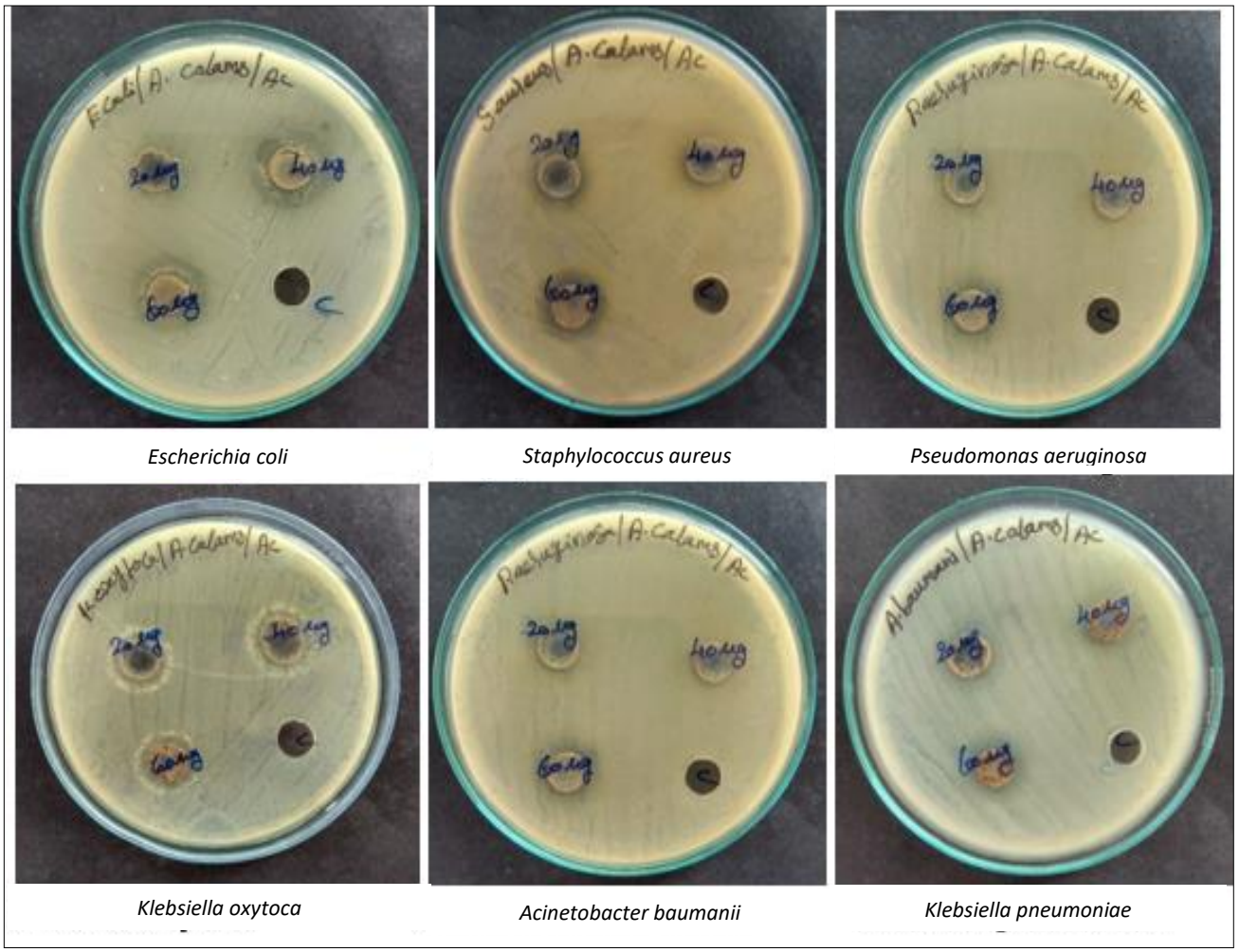

Fig 3 Antibacterial activity of *Acorus calamus* rhizome acetone extract

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LITERATURE CITED

1. WHO. 2018. World health organization. Antibiotic resistance.
2. Vakayil R, Krishnamoorthy S, Gnanendra S, Senthil Kumar N, Ramasamy S, Mathanmohun M. 2020. Screening and identification of multi-drug resistance nosocomial infection, isolates from clinical specimen: A cross-sectional study. *Plant Archives* 20(2): 7247-7251.
3. Motbainor H, Bereded F, Mulu W. 2020. Multi-drug resistance of bloodstream, urinary tract and surgical site nosocomial infections of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* among patients hospitalized at Felegehiwot referral hospital, Northwest Ethiopia: A cross-sectional study. *BMC Infectious Diseases* 20(1): 92.
4. Mulani MS, Kamble EE, Kumkar SN, Tawre MS, Pardesi KR. 2019. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: a review. *Frontiers in Microbiology* 10: 539.
5. Ventola CL. 2015. The antibiotic resistance crisis: part 1: Causes and threats. *Pharmacy and Therapeutics* 40(4): 277.
6. Huang H, Chen B, Liu G, Ran J, Lian X, Huang X, Wang N, Huang Z. 2018. A multi-center study on the risk factors of infection caused by multi-drug resistant *Acinetobacter baumannii*. *BMC Infectious Diseases* 18(1): 1-6.
7. Ansari S, Bari A, Ullah R, Mathanmohun M, Veeraraghavan VP, Sun Z. 2019. Gold nanoparticles synthesized with Smilax glabra rhizome modulates the anti-obesity parameters in high-fat diet and streptozotocin induced obese diabetes rat model. *Journal of Photochemistry and Photobiology B: Biology* 201: 111643.
8. Vakayil R, Anbazhagan M, Shanmugam G, Ramasamy S, Mathanmohun M. 2021. Molecular docking and in vitro analysis of phytoextracts from B. serrata for antibacterial activities. *Bioinformation* 17(7): 667-672.
9. Abirami K, Maghimaa M. 2019. Phytochemical screening and bioactivity of *Zingiber officinale* to combat the multidrug-resistant bacterial pathogens using foldscope. *Uttar Pradesh Journal of Zoology* 22: 67-74.

10. Anokwah D, Asante-Kwatia E, Mensah AY, Danquah CA, Harley BK, Amponsah IK, Oberer L. 2021. Bioactive constituents with antibacterial, resistance modulation, anti-biofilm formation and efflux pump inhibition properties from *Aidia genipiflora* stem bark. *Clinical Phytoscience* 7(1): 1-1.
11. Sudhakar C, Selvam K, Govarthanan M, Senthilkumar B, Sengottaiyan A, Stalin M, Selvankumar T. 2015. *Acorus calamus* rhizome extract mediated biosynthesis of silver nanoparticles and their bactericidal activity against human pathogens. *Journal of Genetic Engineering and Biotechnology* 13(2): 93-99.
12. Vakayil R, Muruganantham S, Kabeerdass N, Rajendran M, Ramasamy S, Alahmadi TA, Almoallim HS, Manikandan V, Mathanmohun M. 2021. Acorus calamus-zinc oxide nanoparticle coated cotton fabrics shows antimicrobial and cytotoxic activities against skin cancer cells. *Process Biochemistry*. Aug 25. https://doi.org/10.1016/j.procbio.2021.08.024
13. Essa MA, Younus RW, Salih MM. 2021. Synergetic effect of some plant essential oils with antibiotics against drug resistant pathogenic bacteria. *AMC* 6: 35.
14. Vakayil R, Kabeerdass N, Srinivasan R, Shanmugam G, Ramasamy S, Mathanmohun M. 2021. Invitro and insilico studies on antibacterial potentials of phytochemical extracts. *Materials Today: Proceedings*. 2021. https://doi.org/10.1016/j.matpr.2021.05.017.
15. Harsha K, Shreya KK. 2018. Topical nanoemmigel formulation of *Boswellia serrata*. *Indian Journal of Pharmaceutical Sciences* 80(2): 261-267.
16. Vakayil R, Krishnamoorthy S, Anbazhagan M, Kumar NS, Mathanmohun M. 2021. Antibacterial potential of *Acorus calamus* extracts against the multi-drug resistant nosocomial pathogens. *Uttar Pradesh Journal of Zoology* 24: 144-50.
17. Kabeerdass N, Krishnamoorthy S, Anbazhagan M, Srinivasan R, Nachimuthu S, Rajendran M, Mathanmohun M. 2021. Screening, detection and antimicrobial susceptibility of multi-drug resistant pathogens from the clinical specimens. *Materials Today: Proceedings*. https://doi.org/10.1016/j.matpr.2021.05.018.
18. Abedin MZ, Aktar MB, Zaman MSU, Jarin L, Miah MAS, Ahmed AA, Shilpi RY. 2020. Predominance of nosocomial pathogens among patients with post-operative wound infections and evaluation of the antibiotic susceptibility patterns in rural hospitals in Bangladesh. *Recent Adv. Biol. Med*. 6(4): 17990.
19. Vakayil R, Kabeerdass N, Kuppusamy A, Mathanmohun M. 2019. Phytochemical screening and antibacterial properties of *Punica granatum* extracts against gastrointestinal infection an in-vitro study. *Uttar Pradesh Journal of Zoology* 26: 25-32.
20. Atef NM, Shanab SM, Negm S I, Abbas Y A. 2019. Evaluation of antimicrobial activity of some plant extracts against antibiotic susceptible and resistant bacterial strains causing wound infection. *Bulletin of the National Research Centre* 43(1): 1-11.
21. Maghimaa M, Alharbi SA. 2020. Green synthesis of silver nanoparticles from Curcuma longa L. and coating on the cotton fabrics for antimicrobial applications and wound healing activity. *Journal of Photochemistry and Photobiology B: Biology* 204: 111806. https://doi.org/10.1016/j.jphotobiol.2020.111806.
22. Kabera JN, Semana E, Mussa AR, He X. 2014. Plant secondary metabolites: biosynthesis, classification, function and pharmacological properties. *Jr. Pharm. Pharmacology* 2(7): 377-392.
23. Awang-Jamil Z, Basri AM, Ahmad N, Taha H. 2019. Phytochemical analysis, antimicrobial and antioxidant activities of *Aidia borneensis* leaf extracts. *Jr. Appl. Biol. Biotechnology* 7(5): 92-97.
24. Devagi P, Suresh TC, Sandhiya RV, Sairandhry M, Bharathi S, Velmurugan P, Suresh G. 2020. Actinobacterial-mediated fabrication of silver nanoparticles and their broad-spectrum antibacterial activity against clinical pathogens. *Journal of Nanoscience and Nanotechnology* 20(5): 2902-2910.
25. Exner M, Bhattacharya S, Christiansen B, Gebel J, Goroncy-Bermes P, Hartemann P, Heeg P, Ilschner C, Kramer A, Larson E, Merkens W. 2017. Antibiotic resistance: What is so special about multidrug-resistant Gramnegative bacteria? *GMS Hygiene and Infection Control*. pp 12.
26. Asres G, Legese M, Woldearegay G. 2017. Prevalence of multidrug resistant bacteria in postoperative wound infections at Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia. *Arch Med*. 9(4): 12.