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In-silico and *in-vitro* Antibacterial Activity and GC-MS Analysis of *Anogeissus acuminata* Leaf Extract with Host Toxicity Testing

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Abstract

Background: *Anogeissus acuminata* leaf extract is utilized in the different purposes. **Objective:** Antibacterial activity of organic solvent fractions of crude leaf extract of *Anogeissus acuminata* was used against multidrug-resistant strains of 9 pathogenic bacteria isolated from urine samples of patients with urinary tract infections. **Methodology:** The n-butanol fraction revealed effective control over pathogens, and the zone size was 21 mm as a measure of antibacterial activity against *Proteus mirabilis*. The gas chromatography-mass spectroscopy analysis indicated the presence of 15 phyto-compounds with docking score values -5 to -7 kcal/mol in a molecular docking attempt against β-lactamase of *Pseudomonas aeruginosa*. **Results**: The isolated chemicals, conocarpan and dihydrodehydrodiconiferyl alcohol, had comparatively effective docking scores, -10.908 and -10.081 kcal/mol, respectively. In toxicity testing, the experimental minimum inhibitory concentration value was 600 mg/L, and the computed lethal concentration$_{25}$ value was 1698.24 mg/L during cytotoxicity assay; it was observed that comet was not found in the cells treated leaf extract. **Conclusion**: The isolated compounds conocarpan and dihydrodehydrodiconiferyl alcohol achieved successful docking scores against -lactamase in the molecular docking attempt. When the leaf extract was seen using lymphocytes grown from UCB, it was determined that it had no host toxicity. [*Bangladesh Journal of Infectious Diseases, December 2024;11(2):93-101*]

Keywords: *Anogeissus acuminata*; GC-MS analysis; molecular docking; cytotoxicity

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Introduction

Anogeissus species (fam. Combretaceae) is widely used as ethnomedicines by tribes and communities in Asia and Africa. Mainly, *Anogeissus acuminata*, a timber-yielding plant native to South Asia, is used as ethnomedicines mainly in gastric disorders, diarrhea, dysentery, skin diseases, wounds, cough, burns as well as diabetes[1-2]. The presence of several bioactive secondary metabolites such as alkaloids, anthraquinones, essential oils, flavonoids, glycosides, saponins, steroids, tannins, terpenoids, and xanthones was elucidated, which are *a priory*, responsible for diverse ethnomedicinal uses[3]. Previously, the screening of methanolic leaf extracts of 21 timber-yielding plants, including *A. acuminata*, had been elucidated in vitro control of (MDR) strains of 9 UTI-causing Gram-positive

(GP) and Gram-negative (GN) bacteria isolated from urine samples in hospitalized patients[4].

Furthermore, it has often been reported that pathogenic bacteria resist the applied antibiotics[5,6]. In the odyssey of the development of drugs, there had been several chemical manipulations of existing drugs/antibiotics along with the addition of newer antibiotics[7]. Progressively, bacteria resistant to several antibiotics emerge, and multidrug-resistant (MDR) strains predominate; consequently, the spectrum of antibiotics in present use becomes selectively effective[5,8]. Specifically in empiric therapy, the failure of the employed antibiotic(s) causes an infection to proceed to BSI, ending up intractable bacteremia and fatality, and a physician prescribes an antibiotic of a higher generation in anticipation of failure of administered antibiotic. In this perspective, searching for newer antibiotics/ antimicrobials/ drugs from conventional and non-conventional sources would be prudent.

This paper describes the antibacterial activities of crude leaf extracts of *A. acuminata* and fractions were extracted using five non-polar and polar solvents. The best solvent fraction was used against MDR strains of nine pathogenic bacteria isolated from urine samples of in-house patients and outdoor patients reporting urinary tract infection (UTI). The best solvent fraction was used for gas chromatography-mass spectroscopy (GC-MS) analysis to locate lead compounds that could be the coveted antimicrobial agents. Moreover, the advanced bioinformatics tool, molecular docking of characterized phyto-constitutes by GC-MS analysis along with reported phytochemical, conocarpan, and dihydrodehydrodiconiferyl alcohol was attempted against β-lactamase enzyme of the pathogenic bacterium, *Pseudomonas aeruginosa* for individual activity. The crude plant extract was tested for possible host toxicity by monitoring its activity against human lymphocytes cultured from umbilical cord blood (UCB). This study is anticipated to be useful in the drug development module with two lead plant chemicals.

Methodology

Study Design and Settings: This study investigates the antibacterial potential of a plant leaf extract using *in vitro* and *in silico* methods, GC-MS analysis, and cytotoxicity testing. Fresh leaves were evaluated, and the extract's antibacterial activity was estimated using the agar well diffusion method. Major bioactive components were identified through GC-MS analysis, and molecular docking experiments were conducted to determine their binding affinity with bacterial target proteins. An *In-vitro* cytotoxicity study of crude leaf extract was done with UCB using acridine orange/ethidium bromide (AO/EB) staining.

Bioassay-guided fractionation of leaf extract: A methanolic extract of the dried leaf powders of *A. acuminata* was obtained by hot extraction method using a Soxhlet apparatus, and the crude methanol extract was subjected to a bioassay-guided fractionation by solubilizing in water followed by a sequential partition with an additional four solvents, n-hexane, chloroform, ethyl acetate, and n-butanol. The extract was filtered and concentrated, as detailed[9].

Antibiotic sensitivity and antibacterial activity: Two GP bacteria, *Staphylococcus aureus* and *Enterococcus faecalis*, and 7 GN bacteria, *Acinetobacter baumannii*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*, were isolated from clinical samples[5]. Bacterial strains were subjected to antibiotic sensitivity tests by Kirby-Bauer's disc diffusion method, following the standard antibiotic susceptibility test, as described[5]. The antibacterial activities of the seven solvent fractions (Merck, Hi-Media) were determined using the agar-well diffusion method[10].

GC-MS analysis: The GC-MS analysis of the n-butanol fraction was carried out using an instrument equipped with a VF-5ms fused-silica capillary column of 30 m length, 0.25 mm diameter, and 0.25 mm film thickness. An electron ionization system with an ionization energy of 70 eV was used as the detector. Helium gas (99.99 %) was used as the carrier gas at the constant flow rate of 1.51 mL/min. The injector and mass transfer line temperatures were 200 and 240ºC, respectively. The oven temperature was programmed from 70 to 220ºC at 10ºC/min, held constant for 1 minute, and finally increased to 300ºC at 10ºC/min. Diluted fraction samples in aliquots of 2 mL were manually injected in the split-less mode with the split ratio 1:40 and a mass scan range of 50-600 AMU, with a running time of 60 minutes.

Identification of Compounds: Phytochemicals of the biologically active fraction (the n-butanol fraction) were identified by comparing their mass spectra fragmentations and retention indices with those stored in the following databases: NIST14.LIB (Stein SE National Institute of Standards and Technology, Mass Spectral Database and Software version 2.2, NIST, Gaithersburg,

USA, 2014) and WILEY11.LIB[11]. For the molecular docking study, individual chemical structures were designed by Chem-Draw software, and the other two chemicals were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The target enzyme, β-lactamase enzyme of *Pseudomonas aeruginosa* with PDB ID: 4NK3, was retrieved from Protein Data Bank (http://www.rcsb.org/pdb/home/home.do), and a docking study was carried out using software, Auto Dock Vina[12]. An *In-vitro* cytotoxicity study of crude leaf extract was done with UCB using acridine orange/ethidium bromide (AO/EB) staining[13].

Statistical Analysis: The data was stored in Microsoft Excel and analyzed using IBM-Statistical Package for the Social Sciences (SPSS, Version 23) software to convert the percentage responses to probits automatically.

Ethical Clearance: Approval for the study was obtained from the Institutional Ethics Committee (IEC), Centurion University of Technology and Management, Bhubaneswar.

Results

Antibacterial activities of 5 solvent fractions were monitored by the agar–well diffusion method on separate lawn cultures of 9 bacterial isolates, 2 GP and 7 GN bacteria.

The n-butanol leaf fraction registered the maximum zone diameter of inhibition against S. aureus as 28 mm, and the minimum zone size against P. mirabilis was 21 mm. The n-hexane and chloroform fractions individually registered low antibacterial activity compared to the other three solvent fractions (Table 1).

Table 1: Antibacterial assay by agar-well diffusion method of hot solvent leaf extract fractions of *Anogeissus acuminata* against MDR UTI pathogenic bacteria

Bacteria	n-Hexane	Chlor-oform	Ethyl acetate	n-Butanol	Methanol	Gentamicin (30µg/ml)
Enterococcus faecalis	13	18	23	26	24	25
Staphylococcus aureus	16	21	25	28	27	28
Acinetobacter baumannii	12	17	19	23	22	20
Citrobacter freundii	11	19	17	24	24	21
Enterobacter aerogenes	10	15	15	22	21	23
Escherichia coli	14	20	16	24	19	27
Klebsiella pneumoniae	12	16	18	23	21	20
Proteus mirabilis	15	15	17	21	20	23
Pseudomonas aeruginosa	15	17	21	27	24	26

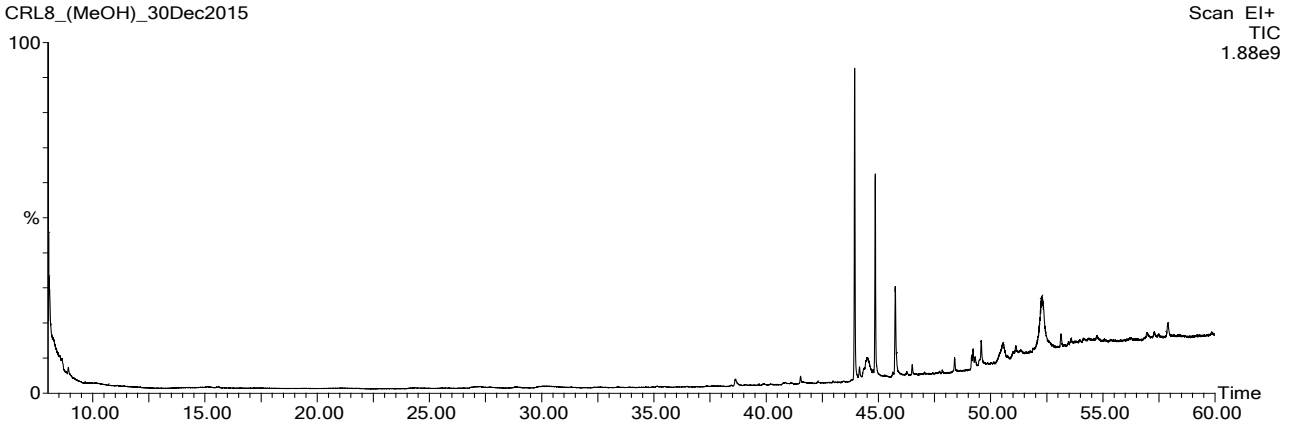
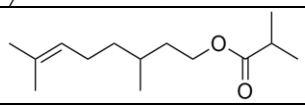
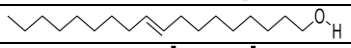
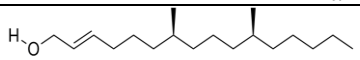
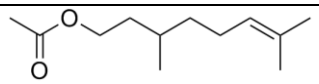
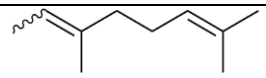
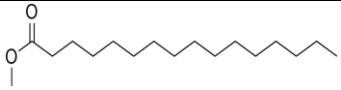
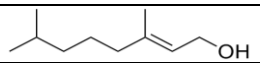
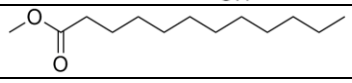


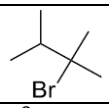
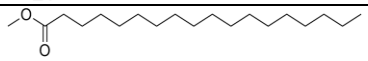
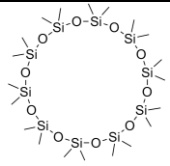
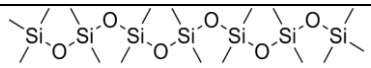
Figure I: Gas chromatography-mass spectrometry chromatogram of the n-butanol fraction of *Anogeissus acuminata*

Table 2: Phyto-components Identified in n-butanol Fraction of Leaf Extract of *Anogeissus acuminata*

Peak	RT (min)	Area	Area (%)	M.W.	M.F.	Name and class of compounds
1	38.63	5,952,871.0	0.974	278	$C_{20}H_{38}$	3-eicosyne (alkyne)
2	39.89	1,616,438.9	0.264	306	$C_{20}H_{40}O$	1,21-docosadiene (alkadine)
3	41.09	1,306,406.9	0.214	226	$C_{14}H_{26}O_2$	Citronellyl isobutyrate (ester)
4	41.54	3,122,980.2	0.511	268	$C_{18}H_{36}O$	9-octadecen-1-ol, (E)- (alcohol)
5	43.94	67,972,776.0	11.119	296	$C_{22}H_{42}$	Phytol (steroidol)
6	44.16	2,800,062.2	0.458	198	$C_{12}H_{22}O_2$	6-octen-1-ol, 3,7-dimethyl-, acetate(ester)
7	44.38	3,503,395.5	0.573	138	$C_{10}H_{18}$	2,6-octadiene, 2,6-dimethyl- (alkadiene)
8	44.50	23,105,936.0	3.780	222	$C_{16}H_{30}$	8-hexadecyne(alkyne)
9	44.86	49,055,816.0	8.025	270	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl Ester(ester)
10	45.74	1,468,473.1	0.240	156	$C_{10}H_{20}O$	2-octen-1-ol, 3,7-dimethyl-(alkene)
11	49.58	10,877,252.0	1.779	214	$C_{13}H_{26}O_2$	Dodecanoic acid, methyl ester(ester)
12	50.56	22,220,580.0	3.635	164	$C_6H_{13}Br$	Butane, 2-bromo-2,3-dimethyl-(alkane)
13	51.12	7,156,221.5	1.171	298	$C_{19}H_{38}O_2$	Octadecanoic acid, methyl ester(ester)
14	52.28	83,221,960.0	13.614	740	$C_{20}H_{60}O_{10}Si_{10}$	Cyclodecasiloxane, eicosamethyl-(silane ether)
15	53.13	4,478,469.0	0.733	532	$C_{16}H_{48}O_6Si_7$	Heptasiloxane, hexadecamethyl- (silane)

Table 3: Chemical Structure and Biological Activity of the Identified Compounds

Peak	Chemical structure	Biological activity	References
1		antimicrobial, antioxidant	Swamy et al.,2015; Verma et al., 2016.
2		antimicrobial, insecticidal	Eid, 2015; Halawa et al., 2007
3		antimicrobial, antiplasmodial	Wangchuk et al., 2013; Kuljanabhagavad et al., 2010
4		No activity reported	
5		antimicrobial, antinociceptive, antioxidant	Santos et al., 2013; Ghaneian et al., 2015
6		No activity reported	
7		antimicrobial	Wei and Wee, 2012
8		antifungal	Hossain and Rahman, 2011
9		antimicrobial, anticancer, antioxidant, anti-inflammatory	Ajoku et al., 2015; Belakhdar et al., 2015
10		antioxidant	Sasikala and Chandra Mohan, 2014
11		antibacterial, antiviral, antifungal	Belakhdar et al., 2015

Peak	Chemical structure	Biological activity	References
12		No activity reported	
13		antimicrobial	Gehan et al., 2009; Belakhdar et al., 2015
14		Antimicrobial, antioxidant	Nehal et al, 2016; Boominathan and Bakiyalakshmi, 2016
15		Antibacterial, antioxidant	Mohy El-Din and El-Ahwany, 2015

The phytochemical screening of the n-butanol leaf fraction of *A. acuminata* revealed the presence of alkaloids, glycosides, terpenoids, steroids, saponins, and tannins, the GC-MS analysis (Figure I). The chemical profiles of the identified compounds, their retention time, percentage peak area, molecular formula, molecular weight, structure, nature of the compound, and reported activity (Tables 2 and 3).

The GC-MS analysis clearly showed the presence of 15 biologically active compounds in *A. acuminata* (area %): (1) 3-eicosyne (0.97 %); (2) 1, 21-docosadiene (0.26 %); (3) citronelly is butyrate (0.21 %); (4) 9-octadecen-1-ol, (E)- (0.51 %); (5) phytol (11.12 %); (6) 6-octen-1-ol, 3,7-dimethyl-, acetate (0.45%); (7) 2,6-octadiene, 2,6-dimethyl- (0.57 %); (8) 8-hexadecyne (3.78 %); (9) hexadecanoic acid, methyl ester (8.02 %); (10) 2-octen-1-ol, 3,7-dimethyl- (0.24 %); (11) Dodecanoic acid, methyl ester (1.77 %); (12) Butane, 2-bromo-2,3-dimethyl- (3.63 %); (13) Octadecanoic acid, methyl ester (1.17 %); (14) cyclodecasiloxane, eicosamethyl- (13.61 %); and (15) heptasiloxane, hexadecamethyl- (0.73 %). The GC-MS spectrum confirmed the presence of 15 peaks of different compounds with retention times of 38.63, 39.89, 41.09, 41.54, 43.94, 44.16, 44.38, 44.50, 44.86, 45.63, 48.39, 49.58, 50.56, 51.12, 52.28 and 53.13 minutes, respectively. The mass spectrometer characterized the compounds at different times to identify the chemical nature and structure of the eluted compounds. The large compounds fragment into small compounds giving rise to peaks at different m/z ratios (Supplementary Figure 1A-O).

Table 4: Docking scores and drug-likeness values of phytochemicals of *Anogeissus acuminata* from GC-MS analysis of two reported phytochemicals against β-lactamase of *Pseudomonas aeruginosa*

Sl. No.	Phytochemicals/ antibiotic	Docking score (kcal/mol)	Drug-likeness score
1	3-eicosyne	-5.241	-1.18
2	1,21-docosadiene	-6.080	-1.03
3	Citronellylisobutyrate, (E)-	-7.842	-1.07
4	9-octadecen-1-ol	-5.672	-1.11
5	Phytol	-7.763	-0.87
6	6-octen-1-ol, 3,7-dimethyl-, acetate	-6.431	-0.92
7	2,6-octadiene, 2,6-dimethyl-	-5.815	-1.05
8	8-hexadecyne	-6.643	-1.18
9	Hexadecanoic acid, methyl Ester	-5.897	-1.21
10	2-octen-1-ol, 3,7-dimethyl-	-6.093	-1.14
11	Dodecanoic acid, methyl ester (ester)	-7.756	-1.28
12	Butane, 2-bromo-2,3-dimethyl- (alkane)	-7.041	-1.24
13	Octadecanoic acid, methyl ester	-5.698	-1.28
14	Cyclodecasiloxane, eicosamethyl-	-6.513	-1.37

Sl. No.	Phytochemicals/ antibiotic	Docking score (kcal/mol)	Drug-likeness score
15	Heptasiloxane, hexadecamethyl- (silane)	-7.890	-1.16
16	Conocarpan*	-10.908	0.13
17	Dihydrodehydrodiconiferyl alcohol*	-10.081	0.13
18	Ampicillin**	-10.515	0.20

* Isolated phytochemicals; **Used standard antibiotic in docking study.

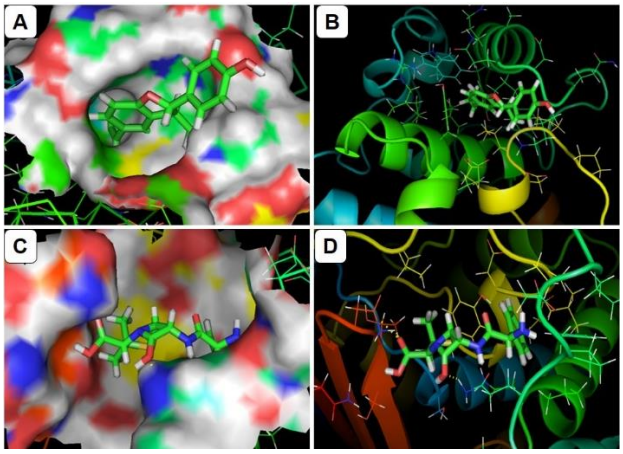


Figure II: Protein-ligand interaction during docking study; A) and B) views two different interaction of the phytochemical, conocarpan; C) and D) views of two different interactions of antibiotic, ampicillin against β-lactamase of *Pseudomonas aeruginosa* through PyMOL software.

In the molecular docking attempt, phytochemicals identified by GC-MS had docking score values (Table 4) within -5 and -7 kcal/mol. Still, the isolated chemicals, conocarpan and dihydrodehydrodiconiferyl alcohol had effective docking scores, -10.908 and -10.081kcal/mol, respectively (Figure II).

This preliminary GC-MS analysis confirmed that the active fraction of *A. acuminata* contains several classes of phytochemicals with lower molecular weight and are synergistically active against MDR pathogens, *in vitro.* Furthermore, the leaf extract might be containing more active constituents with high molecular weight including, conocarpan and dihydrodehydrodiconiferyl and those would be isolated in the future using column chromatography. Percent lethality (PL) values recorded from AO/EB staining and MTT assay as cytotoxicity and corresponding probit values were used to construct the probit plot for individual LC values (Table 5).

Treatment of cells with graded concentrations of leaf extract for 24 hr resulted in a slow decreasing pattern of living cell counts. Single-cell gel electrophoresis was carried out to study DNA damage of the cells treated with graded concentrations of leaf extract. It was observed that the comet was not found in the cells treated with leaf extract (Figure III).

Table 5: Lethality Values During Leaf Extract Toxicity to Human Lymphocytes Growing in DMEM, assessed by AO/EB staining with probits.

Concentration of leaf extract (mg/L)	Log_{10} concentrations	Lethality of cells by AO/EB staining (%)	Probits of the lethality of cells by AO/EB staining
0	-	-	-
300	2.47	-	-
600	2.77	6.8	3.51
900	2.95	8.2	3.61
1200	3.07	13.4	3.89
1500	3.17	17.6	4.06
1800	3.25	21.4	4.21
2100	3.32	24.8	4.32
2400	3.38	31.2	4.51
2700	3.43	32.4	4.54
3000	3.47	33.1	4.56

*AO/EB: Acridine orange/ethidium bromide; DMEM: Dulbecco's modified Eagle's medium

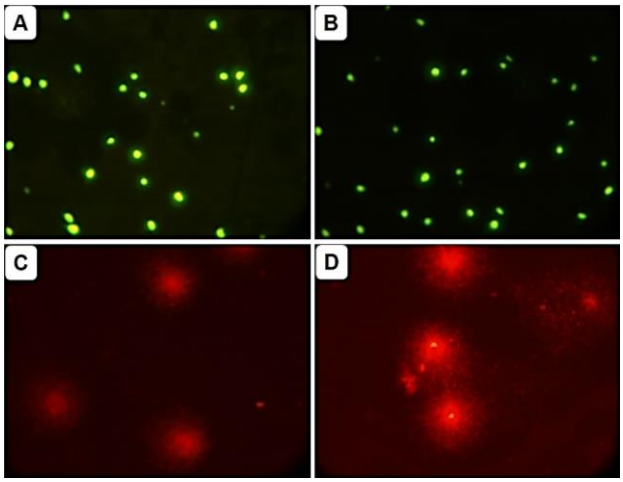


Figure III: AO/EB staining: A) Control lymphocytes; B) Lymphocytes after growing with 1800 mg/L leaf extract of *A. acuminata*; Comet assay: C) Control lymphocytes; D) Lymphocytes after growing with 1800 mg/L leaf extract of *A. acuminata*.

The Experimental minimum inhibitory concentration (MIC) value was 600 mg/L and the computed LC_{25} value was 1698.24 mg/L during cytotoxicity (Figure IV). As the plant is non-toxic, LC_{100} values could not be obtained.

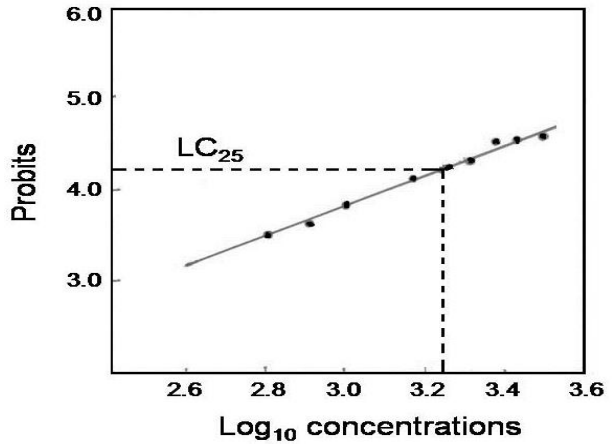


Figure IV: Probits of percentage lethality values plotted against log_{10} concentrations of leaf extract of *A. acuminata* in the toxicity study of lymphocytes; log_{10} concentration values were determined, taking probit points by AO/EB staining.

Discussion

Indeed, phyto-drugs, when scaled up clinically, could control resistant bacteria, which can never win over natural phytochemicals of non-microbial origin. The roles of crude Phyto extracts in traditional medicine used by ethnic and elite masses in developed and developing countries for hundreds of age-tested plants are enormous. Without testing host toxicity, commercial formulations of plant drugs are produced by several pharmaceutical companies now, generating millions of US dollars. Well-known companies market alternative phytomedicines, and those have been popular worldwide; in the US mainly, phyto-medicines have been used for lowering blood pressure, coagulation effects, cardiac effects, sedative effects, controlling infective ailments and cancer, etc[14,15] and integrative medicine system has been followed for an eclectic effect, wherein the regular allopathic medicines are mixed up with phyto-drugs as conjugate druggable agents for treating acute diseases, including cancer[16,17]. The use of crude plant extracts in infusions has been growing unusually popular worldwide, mainly consisting of a 63% use of plant products for acute diseases, cancer, and heart problems; this is a new concept, neo-herbalism[18,19]. Furthermore, even for the treatment of osteoarthritis, herbal medicines have been popular in the US[20-22]. Herbal products are widely held today for health-boosting preventives by the WHO. They would be deeply held for more specific needs as complementary and alternate medicines (CAMs) if those could be scaled as remedial measures without any dyslogistic prejudice, often seen with phyto-drugs[23,24]. As it is, host toxicity testing of non-edible plant products remains an essential pharmacological work in CAM before being recommended as a drug.

Three novel bio-active complex tannins of the group flavano-ellagitannins, viz., anogeissinin, anogeissusins A, and B, along with 8 known C-glycosidic hydrolysable tannins, acutissimins A, acutissimins C, eugenigrandin A, castalin, castalagin, vescalagin carboxylic acid, castamollinin and grandinin were isolated from a 60 % aqueous acetone extract of stem bark, through size exclusion method with Sephadex LH-20 column chromatography[25]. Furthermore, the methanolic bark extract was partitioned between chloroform and water and the former fraction was subjected to separation by silica gel column chromatography yielding the following compounds, astilbene, pterostilbene, conocarpan, dihydrodehydrodiconiferyl alcohol, anolignan A, anolignan B, anolignan C, (-)-secoisolariciresinol[26,27]. High-performance liquid chromatography (HPLC) analysis of methanol sub-fraction crude methanol extract of stem bark revealed 2.30mg/g of xanthones[2,3]. Conocarpan, dihydrodehydrodiconiferyl alcohol, and pterostilbene isolated from stem showed *in-vitro* cytotoxicity in various cancer cell lines.

The GC-MS analysis of the n-butanol fraction of *A. acuminata* revealed several secondary metabolites with therapeutic properties, such as antibacterial, antifungal, anti-inflammatory, anticancer, antioxidant, and wound-healing activities. The compounds with higher percentages in peak areas, namely, phytol (11.1%), 8-hexadecyne (3.8%), hexadecanoic acid, methyl ester (8.02), Dodecanoic acid, methyl ester (1.8%), Octadecanoic acid, methyl ester (1.2%), cyclodecasiloxane, eicosamethyl- (13.6%) are present in the n-butanol fraction. These six compounds have previously been reported to have medicinal properties. Acyclic diterpene alcohol and phytol have antimicrobial, antinociceptive, and antioxidant activities, and 8-hexadecyne, an alkyne, has antifungal activity[28-30]. The saturated fatty acids, hexadecanoic acid methyl ester, dodecanoic acid methyl ester, and octadecanoic acid methyl ester have antimicrobial, anticancer, antioxidant, and anti-inflammatory activities[31,33]. Cyclodecasiloxane, eicosamethyl-, a silane ether, has antimicrobial and antioxidant activities[34-35].

Conclusion

This preliminary GC-MS analysis confirmed that the active fraction of *A. acuminata* contains several classes of phytochemicals with lower molecular weight and is synergistically active against 9 MDR pathogens, *in vitro.* In the molecular docking attempt, the isolated chemicals, conocarpan and dihydrodehydrodiconiferyl alcohol had effective docking scores, against β-lactamase. The leaf extract had been seen as not having host toxicity when monitored with lymphocytes cultured from UCB.

Supplementary Material
Supplementary material relating to this paper is available online and includes Fig. 1 A-O.

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Conflict of Interest
The authors have no conflicts of interest to declare.

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None

Contribution to authors:
Prusty JBK, Mishra MP: Conception and design, or design of the research; Mishra MP, Padhy RN: the acquisition, analysis, or interpretation of data; conceptualized and designed the overall study; Mishra MP: involved in data collection; Prusty JBK: Drafting the manuscript or revising it critically for important intellectual content; Prusty JBK: conducted data analysis; Mishra MP: drafted the manuscript. All authors reviewed and approved the final manuscript.

Data Availability
Any questions regarding the availability of the study's supporti ng data should be addressed to the corresponding author, who can provide it upon justifiable request.

Ethics Approval and Consent to Participate
The Institutional Review Board granted the study ethical approval. Since this was a prospective study, every study participant provided formal informed consent. Each method followed the appropriate rules and regulations.

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References

1. Afifi FU, Wazaify M, Jabr M, Treish E. The use of herbal preparations as complementary and alternative medicine (CAM) in a sample of patients with cancer in Jordan. Complementary Therapies in Clinical Practice. 2010;16:208-212.
2. Ajoku GA, Okwute SK, Okogun JI. Isolation of hexadecanoic acid methyl ester and 1,1,2-ethanetricarboxylic acid- 1-hydroxy-1, 1-dimethyl ester from the calyx of green Hibiscus Sabdariffa (Linn). Natural Products Chemistry and Research. 2015;3:1-5.
3. Al-Disi SS, Anwar MA, Eid AH. Anti-hypertensive Herbs and their Mechanisms of Action: PartI. Frontiers in Pharmacology. 2016;6:323.
4. Altman RD. The future of herbal medicine in the treatment of osteoarthritis. Osteoarthritis and Cartilage. 2008;16:10-11.
5. Belakhdar G, Benjouad A, Abdennebi EH. Determination of some bioactive chemical constituents from Thesium humile Vahl. J Materials and Environmental Sciences. 2015;6: 2778-2783.
6. Boominathan M, Bakiyalakshmi SV. Analysis of bioactive compounds in navara (njavara) rice by GCMS. International Journal of Recent Scientific Research. 2016;7: 14307-14311.
7. Dubey D, Patnaik R, Ghosh G, Padhy RN. In Vitro antibacterial activity, Gas Chromatography-Mass Spectrometry analysis of Woodfordia fruticosa Kurz. leaf extract and host toxicity testing with in Vitro cultured lymphocytes from human

umbilical cord blood. Osong journal of Public Health Research Perspectives. 2014;5:298-312.
8. Gehan MA, Hanan AE, Hassan AHI, Okbah MA. Marine natural products and their potential applications as anti-infective agents. World Science Journal. 2009;7:872-880.
9. Ghaneian MT, Ehrampoush MH, Jebali A, Hekmatimoghaddam S, Mahmoudi M. Antimicrobial activity, toxicity and stability of phytol as a novel surface disinfectant. Environmental Health Engineering and Management Journal. 2015;2:13–16.
10. Hossain MA, Rahman A. Chemical composition of bioactive compounds by GC-MS screening and antifungal properties of the crude extracts of cabbage samples. Asian Journal of Biotechnology. 2011;DOI:10.3923/ajbkr.
11. Lin TC, Tanaka T, Nonaka G, Nishioka I, Young TJ. Tannins and related compounds. CVIII. Isolation and characterization of noyel complex tannins (flavano-ellagitannins), anogeissin in and anogeissusins A and B from Anogeissus acuminata (ROXB exDC.) Guill. Et Perr.yar. lanceolata Wall. ExClarke. Chemical and Pharmaceutical Bulletin. 1991;39:1144–1147.
12. McLafferly FW. Registry of mass spectral data, New York, USA, John Wiley & Sons, 5th edition. 1989
13. Mishra MP, Debata NK, Padhy RN. Surveillance of multidrug resistant uropathogenic bacteria in hospitalized patients – an Indian study. Asian Pacific Journal of Tropical Biomedicine. 2013;3:315-324.
14. Mishra MP, Padhy RN. In vitro antibacterial efficacy of 21 Indian timber-yielding plants against multidrug resistant bacteria causing urinary tract infection. Osong Journal of Public Health Research Perspectives. 2013;4:347-57.
15. Mishra MP, Rath S, Swain SS, Ghosh G, Das D, Padhy RN. In vitro antibacterial activity of crude extracts of 9 selected medicinal plants against UTI causing MDR bacteria. Journal of King Saud University-Science. 2017;29:84-95.
16. Mishra MP, Sarangi R, Padhy RN. Prevalence of multidrug resistant uropathogenic bacteria in pediatric patients of a tertiary care hospital in eastern India. Journal of Infection Public Health. 2016;9;308-14.
17. Murthy EN, Madhav NV. Enumeration of medicinal plants of Ramagiri-Khilla forests of Karim nagar district, Telangana, India. International Journal of Pharmacy and Life Sciences. 2015;6:4405–4416.
18. Nehal N, Mann S, Gupta RK.Nutritional and phytochemical evaluation of A. lividus L. syn. Amaranthus blitum subsp. Oleraceus (L.) Costea leaves. Indian Journal of Traditional Knowledge. 2016;15:669-674.
19. Patnaik R, Padhy RN. Human umbilical cord blood-derived neural stem cell line as a screening model for toxicity. Neurotoxicity Research. 2017;31:319–326.
20. Patra JK, Das G, Fraceto LF, Campos EVR, Rodriguez-Torres MP, et al. Nano based drug delivery systems: recent developments and future prospects. Journal of Nanobiotechnology. 2018;16:1-33.
21. Rath SN, Padhy RN. Surveillance of multidrug resistance in community acquired urinary tract bacterial infections in an Indian hospital. Journal of Acute Disease. 2015;4:186-195
22. Rimando AM, Pemto JM, Farnsworth NR, Santisuk T, Reutraku V. Revision of the NMR assignments of pterostilbene and of dihydrodehy- drodiconieferyl alcohol: cytotoxic constituents from Anogeissus acuminata. Natural Product Letter. 1994a;4:261–212.
23. Rimando AM, Pezzuto JM, Farnsworth NR, Santisuk T, Reutraku V, Kawanishi K. New lignans from Anogeissus acuminata with HIV-1 reverse transcriptase inhibitory activity. Journal of Natural Product. 1994b;57:896–904.
24. Samojlik I, Mijatović V, Gavarić N, Krstin S, Božin B. Consumers' attitude towards the use and safety of herbal medicines and herbal dietary supplements in Serbia. International Journal of Clinical Pharmacy. 2013;35:835–840.
25. Santos CCMP, Salvadori MS, Mota VG, Costa LM, de Almeida AAC, et al. Antinociceptive and Antioxidant Activities of Phytol In Vivo and In Vitro Models. Neurosciience Journal. 2013
26. Sen S, Chakraborty R, De B. Challenges and opportunities in the advancement of herbal medicine: India's position and role in a global context. Journal of Herbal Medicine. 2011;1: 67-75.
27. Shakeel M, Bruce J, Jehan S, McAdam TK, Bruce DM. Use of complementary and alternative medicine by patients admitted to a surgical unit in Scotland. Annals of Royal College of Surgeons of England. 2008;90:571-576.
28. Sharf BF, Martin GP, Cosgriff-Hernandez KK, Moore J. Trailblazing healthcare: institutionalizing and integrating complementary medicine. Patient Education and Counselling. 2012;89:434-438.
29. Singh D, Baghel US, Gautam A, Baghel DS, Yadav D, Malik J, Yadav R. The genus Anogeissus: A review on ethnopharmacology, phytochemistry and pharmacology. Journal of Ethnopharmacology. 2016;194:30–56.
30. Swain SS, Paidesetty SK, Padhy RN. Antibacterial activity, computational analysis and host toxicity study of thymol-sulfonamide conjugates. Biomedicine and Pharmacotherapy. 2017;88:181–193.
31. Tabassum N, Ahmad F. Role of natural herbs in the treatment of hypertension. Pharmacognosy Reviews. 2011;5:30-40.
32. Wright GD. Antibiotics: A new hope. Chemical Biology. 2012;19:3-10.
33. Zaruwa MZ, Manosroi J, Akihisa T, Manosroi W, Manosroi A. Anti-diabetic activity of Anogeissus acuminata a medicinal plant selected from the Thai medicinal plant recipe database MANOSROI II. Wudpecker Journal of Medicinal Plants. 2012;1:8–15.
34. Zeddou M. Osteoarthritis Is a Low-Grade Inflammatory Disease: Obesity's Involvement and Herbal Treatment, Evidence-Based Complementary Alternative Medicine. 2019;2037484:1-11
35. Zhang W, Robertson WB, Zhao J, Chen W, Xu J. Emerging Trend in the Pharmacotherapy of Osteoarthritis. Frontiers in Endocrinology. 2019;