ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 14, Issue 7, 2021

Online - 2455-3891
Print - 0974-2441

Research Article

PHYTOCHEMICAL AND ANTIBACTERIAL ACTIVITY OF HEXANE AND ETHYL ACETATE EXTRACT OF *KNOXIA SUMATRENSIS* (RETZ.) DC.

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Received: 20 September 2021, Revised and Accepted: 05 May 2021

ABSTRACT

Objective: The study was carried out the phytochemical and antibacterial activity of *Knoxia sumatrensis* (Retz.) Dc. using hexane and ethyl acetate extracts.

Methods: The phytochemical screening was extracted from hexane and ethyl acetate solvent and its screening was analyzed by standard procedure. GC-MS technique was analyzed in ethyl acetate extract to identify the components present in the extract. The hexane and ethyl acetate extract of the plant was tested for antibacterial activity against human pathogenic bacteria using disk diffusion method.

Results: The phytochemical screening was revealed the presence of phenols, saponins, flavonoids, cardiac glycosides, steroids, and tannins. The GC-MS results showed that the presence of seven bioactive compounds in ethyl acetate extract. The major compounds were identified such as N-Hexadecanoic Acid (9.336), 2-Piperidinone, N-[4-Bromo-N-Butyl] - (37. 883). The ethyl acetate extract showed good antibacterial activity. The maximum zone of inhibition was noticed in *S. aureus* (8.25 mm) using ethyl acetate extracts at 50 µg.

Conclusion: Thus, this study the information regarding the phytochemical constituents present in the both extract. Hence, it can be used for further therapeutic applications in the near future study.

Keywords: Phytochemical, Ethyl acetate, Disk diffusion method, *Staphylococcus aureus*.

 DOI: http://dx.doi.org/10.22159/ajpcr.2021v14i1.39750. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Plants are producing large amount of secondary metabolites which have been used for a therapeutic applications. The role of plants in medicinal production is two-fold and served as a natural drawing for the development of new drugs. Medicinal plants represent a rich source of antimicrobial agents [1]. Their capabilities serve as a possible source for the application of wet finishes on the textiles to treat skin, wounds, antifungal, and antimicrobial [2]. Medicinal plants would be the greatest source to obtain a variety of medicines, according to the World Health Organization (WHO), and 80% of the world's population depends on traditional medicine and a large part of traditional therapies requires the use of plant extracts or their active constituents. [3].

In bacterial diseases stay leading causes of death worldwide [4]. Bacterial standard medication can diminish the degree of death among irresistible patients. However, several cases the growing prevalence of strains from resistance to easily obtainable and inexpensive antimicrobials from bacterial pathogens is harmful in eroding the impact [5]. The defeating of issues thusly required the creation of the new antibacterial specialist. Therapeutic plants are the best way to deal with the bacterial disease.

Knoxia sumatrensis (Retz). DC. belongs to the family Rubiaceae. Around seven species are occur in the Indo-Malayan area in addition two species in Africa [6]. It is profound in deep forest, hills and moist places among the circar Mountains *K. sumatrensis* leaf paste to apply for affected places of wounds healing [7]. The objective the study, phytochemical, GC-MS, and antibacterial activities of hexane and ethyl acetate extract using *K. sumatrensis* leaves.

METHODS

Plant collection

Knoxia sumatrensis (Retz). DC. Plant was collected in September 2018 from vathalmalai hills (12.0464 N, 78.2220 E) which is located in Dharmapuri district, Tamil Nadu, India (Fig. 1). The collected plant was authenticated by Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India, and the specimen voucher number (BSI/SRC/4/23/2018/Tech/620).

Extraction

Plant leaves were shade dried for 2 weeks at room temperature. The dried leaves were grounded using steel blender. The powdered sample was extracting using Soxhlet extraction. Further, the concentrated extracts were evaporated using rotary evaporator. Final extracts were well-preserved in airtight bottle and used for further analysis.

Phytochemical screening

The qualitative phytochemical screening is described earlier [8]. Dragendorff's tests used for alkaloids, lead acetate used for flavonoids, foam test used for saponins, Ferric chloride test used for tannins, Salkowski test used for terpenoids and steroids, Keller–Kiliani test used for cardiac glycosides, Borntrager's test used for anthraquinones, Ferric chloride test used for tannin and phenol as well as Biuret test used for protein.

Gas chromatography mass spectrophotometry (GC-MS)

GC-MS analysis of the ethyl acetate of *Knoxia sumatrensis* was performed using Perkin Elmer GC-MS model. The Clarus 680 GC used in analysis of a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250 µmdf) and the components were separated using Helium as transporter gas

at a constant flow of 1 mL/min. The temperature of injector was set 260°C in the chromatographic run. Sample 1 µL was injected in to the instrument at the oven temperature 60°C (2 min); after that 300°C at the rate of 10°C min^{-1} and 300°C were its hold for 6 min. The mass detector temperature 240°C, ion source temperature 240°C, ionization mode electron impact 70 eV, a scan time 0.2 s, and scan interval 0.1 s. The characterizations of compounds were compared with the database of spectrum of known components stored in the GC-MS NIST (National Institute of Standard Technology) library using Turbo mass software (5.4.0).

Fig. 1: *Knoxia sumatrensis* (Retz.) DC

Antibacterial activity

Collection of microbes

The bacterial strains were used throughout the investigation namely; *Staphylococcus aureus* and *Escherichia coli* were obtained from Department of Microbiology, Periyar University, Salem.

Disk diffusion method

A total of two microbial pathogens, namely., *Staphylococcus aureus* and *Escherichia coli* were tested using of *Knoxia sumatrensis* leaves extract. In this study, we performed by Bauer *et al*., [9] disk diffusion method. Nutrient broth was used to culture bacteria as well as fresh overnight culture of inoculums. The test cultures were swabbed on nutrient agar (NA) Plates and permissible to dry for 10 min. A total of two concentrations used like as (25 µg and 50 µg). DMSO and chloraphenicol were used as negative as well as positive controls (30 µg/disc). The culture plates were incubated for 12 h in an incubator at 37°C. The diameters of the inhibition zones were measured in millimeters (mm).

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screenings is represented in Table 1. The presence of phytoconstituents includes flavonoids, saponins, tannins, steroids,

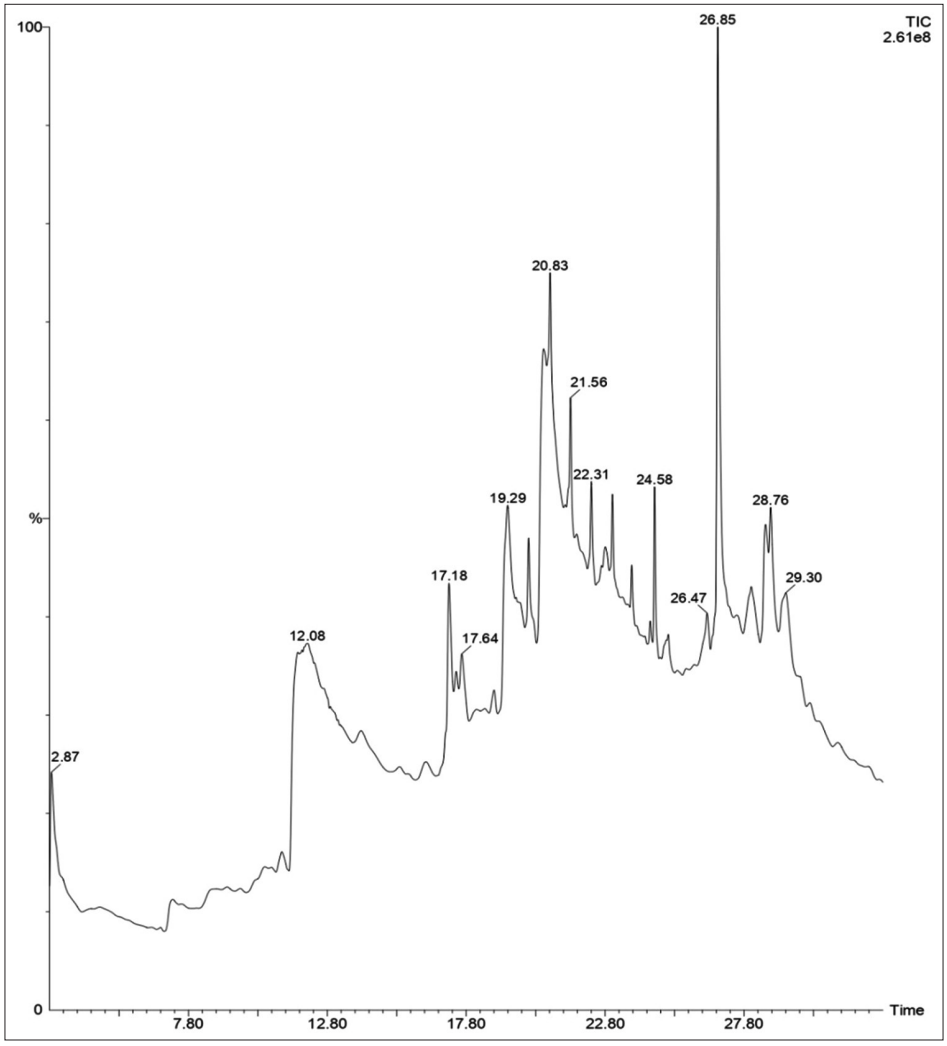


Fig. 2: GC-MS chromatogram of *K. sumatrensis* ethyl acetate leaf extract

cardiac glycosides, and phenol. Alkaloids, anthraquinones, terpenoids, and protein were absent in both extracts. In, Similar ethyl acetate extract were finding in *Myristica dactyloids* fruit were presence of the steroids, proteins, carbohydrates, cardio glycosides, and saponins [10]. In earlier findings, *Tiliacora racemosa* different solvent extract showed that the presence of various secondary metabolites [11].

GC-MS analysis

Chromatogram is shown in Fig. 2. A total of seven bioactive compounds were present in the ethyl acetate extract of *K. sumatrensis* (Table 2). Nair and Gangprasad [12] were reported that the *Gynochthodes ridsdalei* stem extract revealed the presence of 52 bioactive components. In the previous study on *Lactuca runcinata* ethanolic leaf extract showed that 20 bioactive compounds were present using in GC-MS [13]. In this study, the major compounds are present in the ethyl acetate extract and are showed that the N-Hexadecanoic Acid (9.336%), 2-Piperidinone, N-[4-Bromo-N-Butyl]- (37. 883%), 7-Methyl-Z-Tetradecen-1-Ol Acetate (7.012%), and Vitamin E (15. 211%). The compound of N-Hexadecanoic acid and Vitamin E has been noticed in plant extract exhibited in antioxidant and antibacterial activity [14,15]. Al-Salman *et al*. [16] were reported that the compound of 2-Piperidinone, N-[4-Bromo-N-Butyl]-showed the strong antimicrobial activity. The pharmacological activities of other compounds of ethyl acetate of *Knoxia sumatrensis* are yet to be determined. Therefore, we assume that the strong biological activities exhibited by *Knoxia sumatrensis*. In this study are correlated to the occurrence of these bioactive compounds in the ethyl acetate extract. Further, the study, on the isolation of these compounds is needed to confirm their potential benefits.

Table 1: Phytochemical screening of *K. sumatrensis* leaves extracts

S. No.	Phytochemical constituents	Hexane extract	Ethyl acetate extract
1.	Alkaloids	-	-
2.	Flavonoids	+	+
3.	Saponins	-	+
4.	Tannins	-	+
5.	Steroids	+	+
6.	Cardiac glycosides	+	+
7.	Anthraquinones	-	-
8.	Phenol	-	+
9.	Terpenoids	-	-
10.	Protein	-	-

+: Present, -: Absent

Antibacterial activity

The result of the antibacterial activity of *K. sumatrensis* hexane and ethyl acetate extracts is represented in Tables 3 and 4. The hexane and ethyl acetate extract of *K. sumatrensis* was confirmed the antibacterial activity against *S. aureus* and *E. coli* (Figs. 3 and 4). The result of the agar disk diffusion method showed the high efficient antibacterial activity against *S. aureus* (8.25 mm), followed by *E. coli* (7.25 mm). The *Carica pubescens* ethyl acetate fraction showed significant activity against *S. aureus*, *E. coli,* and *S. flexnery* [17]. In this study, ethyl acetate extracts showed maximum zone inhibition when compare to hexane

Table 2: Bioactive compounds identified from GC–MS analysis of *K. sumatrensis* ethyl acetate leaf extract

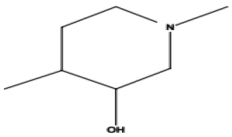
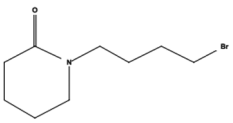
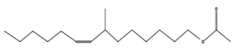
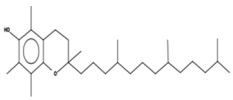
S. No.	R/T	Peak area %	Name of the compound	Molecular formula	Molecular weight (M/W)	Compound structure	Biological activity
1.	17.184	4.101	3,7,11,15-TETRAMETHYL-2-HEXADECEN-1-OL	296	$C_{20}H_{40}O$		
2.	17.639	2.470	3-PIPERIDINOL, 1,4-DIMETHYL-, TRANS	129	$C_7H_{15}ON$		
3.	19.290	9.336	N-HEXADECANOIC ACID	256	$C_{16}H_{32}O_2$		Antibacterial and properties (Tyagi and Agarwal, 2017)
4.	20.831	37. 883	2-PIPERIDINONE, N-[4-BROMO-N-BUTYL]-	233	$C_9H_{16}ONBr$		Antimicrobial activity (Al-Salman *et al*., 2019)
5.	21.551	7.012	7-METHYL-Z-TETRADECEN-1-OL ACETATE	268	$C_{17}H_{32}O_2$		
6.	22.301	3.385	HEXATRIACONTAN	506	$C_{36}H7_4$		Rsdical scavenger (Arora and Meena, 2017)
7.	26.848	15. 211	VITAMIN E	430	$C_{29}H_{50}O_2$		Antibacterial, Antioxidant and Anticancer (Arora and Meena, 2017).

Table 3: Antibacterial activity of hexane leaf extracts of *K. sumatrensis.*

Microorganisms	Zone of inhibition (mm)			
	Positive control (chloramphenicol)	Negative control (DMSO)	Hexane extracts	
			25 (µg/ml)	50 (µg/ml)
S. aureus	23.5±0.7	00.0±0.00	00.0±0.00	7.5±0.2
E. coli	23.25±0.4	00.0±0.00	00.0±0.00	7.25±0.5

Value are given as Data (Mean±SD) (n=3) represent the zone of bacterial growth inhibition (mm)

Table 4: Antibacterial activity of ethyl acetate leaf extracts of *K. sumatrensis.*

Microorganisms	Zone of inhibition (mm)			
	Positive control (chloramphenicol)	Negative control (DMSO)	Ethyl acetate extracts	
			25 (µg/ml)	50 (µg/ml)
S. aureus	26.5±0.5	00.0±0.00	7.5±0.1	8.25±0.3
E. coli	22.5±0.6	00.0±0.00	00.0±0.00	7.00±0.02

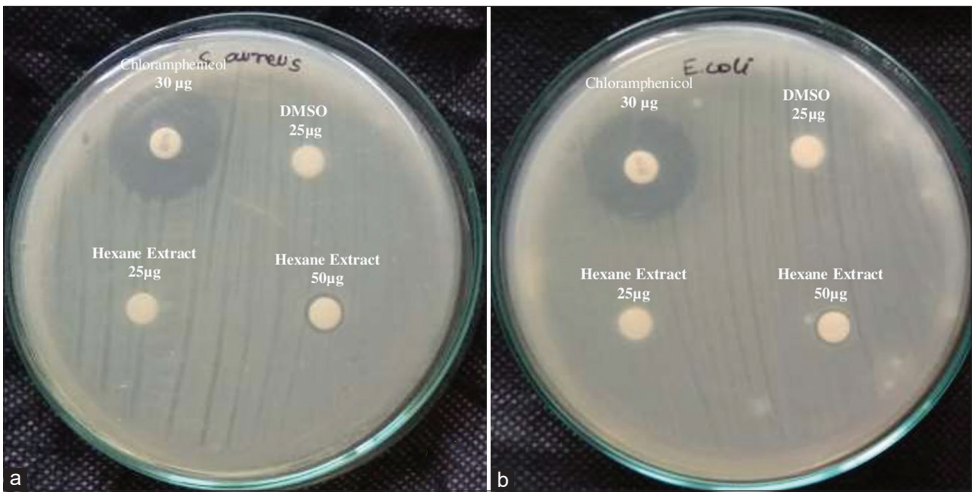


Fig. 3: Antibacterial activity of *K. sumatrensis* hexane leaf extracts against (a) *S. aureus* and (b) *E. coli*

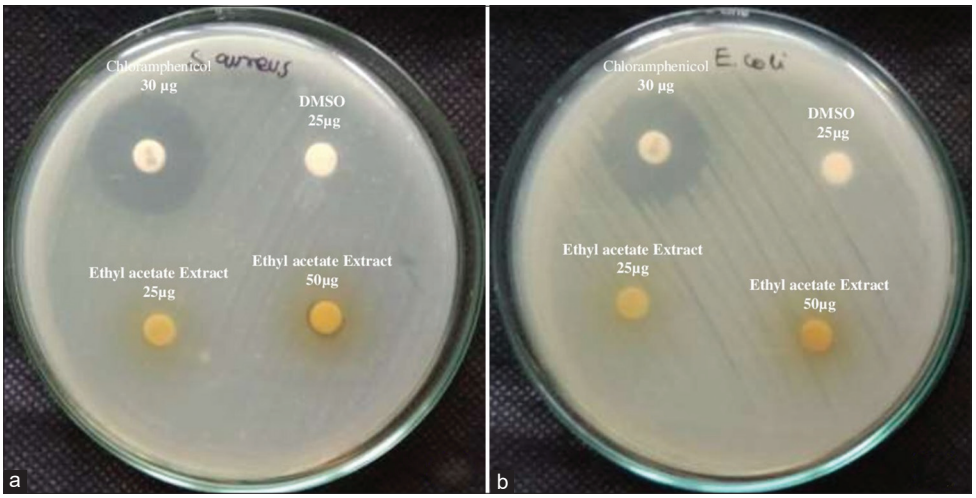


Fig. 4: Antibacterial activity of *K. sumatrensis* ethyl acetate extracts against (a) *S. aureus* and (b) *E. coli*

extract. Anita *et al*. [18] were studied the ethanolic tissue extract of *Purpura bufo* exhibited the maximum zone inhibition against *S. aureus*. Chimahali *et al*. [19] were reported that the maximum zone inhibition when observed by *S. aureus* (19mm) using ethyl acetate tuber extract of *Gloriosa superba*. In earlier findings, the ethyl acetate extract of *Marselia minuta* was showed the more active toward *S. aureus* and *P. aeruginosa* [20]. In ealier study, Soni and Dahiya [21] were reported that the maximum zone of inhibition was noticed in *S. aureus* 1 using *Vetiveria zizanoides* ethanol extract. The present study, the zone of inhibition was increase when increasing the concentrations of both extracts of *K. sumatrensis*.

CONCLUSION

The plant *K. sumatrensis* exhibited the presence of large amount of phytoconstituents. The GC-MS study showed that seven bioactive compounds and antibacterial study revealed that the significant activity against *S. aureus* using hexane and ethyl acetate extract at 50 µg. These investigations potentially recommend the possibility of the plant as an essential source of antibacterial drug may be development pharmaceutical industry.

ACKNOWLEDGMENT

The authors acknowledge to Periyar University, Salem to providing University Research Fellowship (Ref. No. PU/AD-3/URF/2016). The authors also thank to the Department of Botany, School of Life Sciences, Periyar University, Salem - 636 011, Tamil Nadu, India.

AUTHOR'S CONTRIBUTIONS

SL Lab related work to bioassay test, preparation of the plant extract. SL and KS data analysis. The experimental work designed and manuscript corrected KS. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The author's declared that they have no conflicts of interest in publishing this research article.

AUTHOR'S FUNDING

This work is completely self-funded.

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