Research article

Phytochemical analysis and antibacterial activity of traditional plants for the inhibition of DNA gyrase

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ABSTRACT

Introduction and Aim: DNA gyrase is a class of Type II Topoisomerases that plays an important role in bacterial viability. It is found in all bacteria and is involved in replication, repair, recombination, and DNA transcription. Negative supercoiling of bacterial DNA by DNA gyr B is essential in replication which further influences all the metabolic activities. *Staphylococcus aureus* (ATCC 25923) is one of the pathogens that can modify its genome easily under multidrug resistance. This study explores the activity of medicinal compounds to inhibit DNA gyrB. Plant species *Solanum nigrum, Vitex negundo, and Euphorbia hirta* were studied for the potential plant-based molecules. The compounds alkaloids, glycosides, flavonoids, and terpenoids were considered to have high-potential targets. The study focuses on DNA gyrase as a target and shows insights into future drug development. The research focuses on the discovery of novel plant-based therapeutic compounds to target DNA gyrase B activity.

Methods and Materials: Phytochemical screening was performed to study the medication options that could inhibit DNA gyrB. Phytochemicals were determined using GC-MS.

Results: Utilizing GC MS and FT-IR analysis, the phytochemical constituents of S*olanum nigrum, Vitex negundo,* and *Euphorbia hirta* were discovered. It will be simpler to do a follow-up study on discovering bioactive compounds and evaluating their effectiveness in inhibiting DNA gyrB with the help of this preliminary data from the analytical procedures.

Conclusion: There are countless applications for the phytochemicals that medicinal plants produce. *Staphylococcus aureus* will be stopped by DNA gyrB inhibition. The study employs DNA gyrase as its target and provides information on potential therapeutic targets. The study aims to identify innovative plant-based medicinal molecules that specifically target DNA gyrase B activity.

Keywords: Topoisomerases; *Staphylococcus aureus; Solanum nigrum; Vitex negundo; Euphorbia hirta*; alkaloids; terpenoids.

INTRODUCTION

The search for drugs to combat diseases is never-ending. The developing resistance against the compounds seeks for new improved drugs with desirable properties (1). DNA Topoisomerases are proven to have therapeutic qualities against effective targets (2). DNA topoisomerase enzymes were first discovered by James Wang in 1971 within *Escherichia coli* (3). Supercoiling by Gyrase is involved in all DNA-related metabolic processes and is required for replication (4). Gyrase uses a method known as sign inversion to supercoil DNA, in which a positive supercoil is inverted to a negative one by passing a DNA segment through a temporary double-strand break. The ability of gyrase to catenate and un-catenate DNA rings is due to the reversal of this strategy, which relaxes DNA (5). Adenosine triphosphate (ATP) binding causes a conformational shift that drives each round of supercoiling; ATP hydrolysis allows for new cycles (6). The inhibition of gyrase by two antimicrobial classes reflects the fact that it is made up of two reversibly linked subunits. The A subunit is linked to coordinated DNA breakage and rejoining, while the B subunit is linked to DNA replication. The GyrA and GyrB subunits of DNA gyrase contain three gates that play an important role in the enzyme's activity. The GyrA subunit of DNA gyrase contains an active-site tyrosine residue that is important for double-stranded DNA breakage and reunion (dsDNA). The GyrB subunit, on the other hand, contains the ATPase active site, which provides the energy required for DNA supercoiling (7). The GyrA subunit contains 4 domains whereas the GyrB consists of 3 domains. The winged-helix domain (WHD), long domain, tower domain, and variable C-terminus are all domains of GyrA whereas the GyrB subunit is comprised of only three domains i.e., GGHKL, ATP transducer, and TOPRIM (8).

Staphylococcus aureus as a causative agent

Staphylococcus aureus was thought to be a normal flora inhabiting the human population overall, but it has emerged as the causative agent of many severe infections in immunocompromised patients and healthy people in the community (9,10). *S. aureus* infections have recently become a major cause of

human morbidity and mortality in both community and hospital settings (11). Furthermore, *S. aureus* strains combining resistant and virulent genes have become a major treatment issue in Europe, the United States, and developing countries such as India. Because of antibiotic resistance, enzyme and toxin production, biofilm formation capacity, and immune evasion capability, the available therapies are no longer fully effective in treating *Staphylococcal* infections (12). *S. aureus* (ATCC 25923) is one of the pathogens that can modify its genome easily under multidrug resistance (13). In this study, one of the regions of *S. aureus* and its potential platform for drug resistance will be explored. Plant-based molecules such as alkaloids, phenols, flavonoids, and saponins, are considered to have high potential (14). These consist of high biological values that can be studied.

MATERIALS AND METHODS

Preparation of plant extract

The plant species *Solanum nigrum, Vitex negundo, and Euphorbia hirta* are herbs and are seen throughout the places in India. These are very common household plants that can be seen to be growing in moist soil conditions. The plants *Solanum nigrum, Vitex negundo, and Euphorbia hirta* were collected from the college premises of Vels Institute of Technology and Advanced Sciences (VISTAS), Pallavaram, Chennai, Tamil Nadu. Healthy and mature leaves were selected then the leaves were thoroughly washed under water to remove the dirt and kept for drying. After cleaning the leaves were kept for drying. They were left to dry in newspapers for a week under a shady place to remove the present moisture content.

Ultrasonication

The dried leaves were pulverized and ready for extraction once the moisture content was eliminated. Using polar (ethanol and ethyl acetate) and non-polar (petroleum ether and hexane) solvents, extraction was done using an ultrasonic method. Ultrasonication is the application of ultrasound waves for the disintegration of sludge. The intense sonication produces compression and refraction which agitates the particles by breaking the droplets and thus disrupting the cells causing homogenization and dispersion effects. The solvents were kept for 20 min and extracts were purified using Whatman filter paper.

Phytochemical analysis

Examination of the medicinal plants' phytochemical qualities was utilized to identify and separate the medication, lead compounds, and component parts from the plant's parts. The qualities of the phytochemicals in plants can be used to pinpoint their distinct biological activity. Most of the plant parts used for the investigation of the phytochemical qualities were the leaves, roots, stems, bark, and fruits. A variety of phytochemicals were extracted from medicinal plants using various solvents, including ethanol, methanol, chloroform, acetone, hexane, petroleum ether, ethyl acetate, and water (15, 16).

Thin layer chromatography

To separate, detect, and quantify various types of bioactive components, thin layer chromatography (TLC) is a crucial analytical technique. Each solvent extract was subjected to thin-layer chromatography. The silica gel was prepared on the TLC plates. It was performed to analyze the bioactive variation of compounds present. The glass slides were coated with silica gel and kept in the hot air oven for 20 minutes. The powdered sample was extracted with solvents such as ethanol, petroleum ether, hexane, and ethyl acetate. The mobile phase of the solvents was ethyl acetate: petroleum ether, Ethyl acetate: hexane; ethanol: petroleum ether; ethanol: hexane. These mobile solvents were used for the detection of active compounds such as carbohydrates, Alkaloids, Saponins, Terpenoids, Glycosides, Phenol, Steroids, and Flavonoids. The developed chromatograms were studied under UV light. Retention factor values were calculated with the following with the formula given below.

Rf = Distance traveled by solute / Distance traveled by the solvent

Column chromatography

The highest retention factor of the TLC results of the plant extract is proceeded for column chromatography. A liquid solvent (mobile phase), which gently descends the glass cylinder-shaped column with the aid of gravity or applied external pressure, is in contact with the solid phase at the top. The removal of chemicals from a mixture is accomplished using this method. The sample is put into the column's top portion once it is ready. The mobile solvent is then permitted to pass through the column as it descends. The compounds in the mixture interact differently with the solid phase and the mobile phase, which causes them to migrate all along the mobile phase at varying rates or intensities. Compounds are successfully separated from the mixture in this manner (17). After the mobile phase, the solvents were chosen in different concentrations as ethyl acetate: hexane. The solvents were mixed in the ratio such as 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0:10 respectively. The solvent mixture is poured in the column and the samples are collected in the test tubes.

FTIR (Fourier transform infrared) analysis

FTIR analysis is a technique for identifying inorganic, organic, and polymeric substances by scanning materials with infrared light. FTIR has proven to be a successful method for characterizing and validating the chemicals or chemical links present in an undetermined blend of plant extract. Three of the main IR spectroscopic sampling techniques are attenuated

total reflection (ATR), attenuated reflection, and transmission. For some examples, each model is effective, while others present difficulties. The infrared spectrum, which depicts the intensity of infrared spectra, is represented by the x-axis, or horizontal axis. When exposed to the infrared portion of the electromagnetic spectrum, the sample's distinct atomic vibrations correlate to the peaks frequently referred to as absorption bands (18,19).

GC-MS analysis

Compounds present in a plant sample can be determined and identified using the combined analytical approach known as gas chromatography-mass spectroscopy (GC-MS). The phytochemical screening and chemotaxonomic investigations of medicinal plants with physiologically active components depend heavily on GC-MS (20-22). Peak regions, on the other hand, are correlated with the concentration of the relevant chemical. Complex samples segregated by GC-MS will produce many different peaks, every one of which produces a unique mass spectrum used for determining the compound. Huge, commercialized collections of mass spectra could be used to find and analyze unknown compounds and target analytes.

Antibacterial activity

Anti-bacterial activity was determined by an agar well diffusion test. The glassware was washed and dried in the hot air oven. The agar medium was transferred to the Petri dishes and kept for solidifying at room temperature. The test organism used is *E. coli* and it was spread across the agar with a cotton swab. A sterile borer was used for making wells of 8mm diameter. After the well is made the sample is loaded with a control. Ampicillin is used as the control and the plant sample was added to the well and kept in incubation at 37^0C for 24 hours in the incubator. After 24 hours the zone of inhibition was observed, and diameter was measured.

Rate of kill assay

Rate of kill assay is the study of the activity of any antimicrobial agent against a bacterial strain to determine the bactericidal activity over time. The E. coli culture was prepared in LB broth and incubated for 24 hours. The plant sample is mixed with DMSO solution and serially diluted. To each of the Eppendorf 2 ml of the sample and 1 ml of the bacterial culture are added. The reading is observed in the 0th, $2^{nd,}$ and 24^{th} hour. The reading is measured in 595 nm.

RESULTS

Phytochemical analysis

The phytochemical analysis of *Solanum nigrum, Vitex negundo,* and *Euphorbia hirta* confirmed the existence of bioactive chemicals, and tests were conducted using four main solvents: ethanol, ethyl acetate, petroleum ether, and hexane. The testing of phytochemical components and the accompanying observations are shown in Tables 1 and 2 below.

Table1: Phytochemical screening

Identification of bioactive constituents	**Result analysis**
Test for Carbohydrates Equal volumes of Fehling A and Fehling B reagents were mixed, and 2 ml of the mixed reagents were added to the 2 ml leaves extract and gently boiled.	A brick-red precipitate appeared at the bottom of the test tube indicating the presence of reducing sugar.
Test for Alkaloids 2 ml of the Mayer's reagent was added to the 2 ml of the crude extract	A cream precipitate was formed which showed the presence of Alkaloids.
Test for Saponins 2 ml of the sample was dissolved in the distilled water and the sample was shaken vigorously	A forth formation indicates the presence of Saponins.
Test for Terpenoids 2 ml of the sample, 2 ml of Chloroform, and 2 ml of conc. Sulfuric acid was added to the test tube. The solution was shaken well	The appearance of the reddish-brown color of the interference indicated the presence of terpenoids.
Test for Glycosides 2 ml of Sample, 1 ml of glacial acetic acid, 1 ml of ferric chloride, and 1 ml of conc. Sulfuric acid was added. The solution is shaken gently.	The appearance of greenish brown-blue color indicated the presence of glycosides.
Test for Phenol 2 ml of Sample and 1% of 2 ml ferric chloride were added to a test tube and shaken gently.	The appearance of dark green color indicates the presence of phenol.
Test for Steroid 2 ml of sample, conc. Sulphuric acid was added and gently shaken.	The appearance of the lower layer yellow indicates the presence of steroids.
Test for Flavonoid 2 ml of the sample with 1 % of 2 ml Sodium hydroxide flakes were added. Drops of Hydrochloric acid were added to the sides of the test tube.	The appearance of yellow color indicates the presence of flavonoids.

Table 2: Phytochemical analysis of *Solanum nigrum, Vitex negundo, Euphorbia hirta*

Phyto chemical tested	***Solanum nigrum***				***Vitex negundo***				***Euphorbia hirta***			
	Ethanol	Ethyl acetate	Petroleum ether	hexane	Ethanol	Ethyl acetate	etroleum ether	Hexane	Ethanol	Ethyl acetate	Petroleum ether	Hexane
Carbohydrate	+	+	+	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	-	+	-	+	+	+
Terpenoids	+	+	+	+	+	+	-	-	-	+	+	-
Saponins	-	-	-	+	-	+	-	-	-	-	-	-
Glycosides	+	+	+	-	+	+	+	+	+	+	+	+
Phenol	+	-	+	-	+	-	+	-	-	-	-	-
Steroids	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoid	-	-	+	-	-	-	+	-	-	-	+	-

Table 3: Retention factor values of crude extracts of *Solanum nigrum* and *Vitex negundo*

No.	**Solvent system**	**Rf value**		
		Solanum nigrum	***Vitex negundo***	***Euphorbia hirta***
1	Ethyl acetate	0.824	0.271	0.434
2	Ethanol	0.472	0.236	0.236
3	Petroleum ether	0.7636	0.903	0.602
4	Hexane	0.763	0.682	0.809

TLC (Thin layer chromatography)

Ethyl acetate, ethanol, petroleum ether, and hexane were the solvent systems employed. For the mobile the combination of ethyl acetate: petroleum ether, ethyl acetate: hexane, ethanol: petroleum ether, and ethanol: hexane was used. The Retention factor for the crude extracts of *Solanum nigrum* and *Vitex negundo* are shown in Table 3.

Column chromatography

Hexane crude extract of *Euphorbia hirta* showed the highest retention factor of 0.909 and thus the stationary phase silica gel is mixed with the solvent ethyl acetate and hexane and filled inside the tube to get settled. After that for the mobile phase, the solvents were chosen in different concentrations as ethyl acetate: hexane. The solvents were mixed in the ratio such as 10:0, 9:1, 8:2, 7:3, 6:4, 5:5 , 4:6 , 3:7, 2:8, 1:9, 0:10 respectively. The solvent mixture is poured in the column and the samples are collected in the test tubes. The collected solutions are shown in the figure below. From the 11 concentrations, the ratios 7:3 and 6:6 showed good results and it proceeded to the GCMS analysis.

FTIR (Fourier transform infrared) analysis

The infrared spectrum showed a wave number for a mid-range IR ranging from 4,000 and 400 cm^{-1}. Three medicinal plants—*Solanum nigrum, Vitex negundo,* and *Euphorbia hirta*—had their ethyl acetate and hexane extracts studied using FT-IR spectroscopy. The various functional groups present in extracts are often seen in the FT-IR tests with various peak characteristics. The highest values in the infrared (IR) band were used to characterize the function groups of the active elements found in the extract using the FT-IR spectrum. Based on the peak ratio of the extract after FT-IR processing, the components were divided into functional categories. According to the findings of the FT-IR study, the functional groups O-H, C-C, C=C, C-H, O-H, R-COO, and CH3 were confirmed. There is proof that FTIR analysis is a precise and trustworthy technique for determining the composition of biomolecular systems.

GC-MS analysis

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250 μm df), and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The extract sample (1μL) was injected into the instrument The oven temperature was as follows: 60°C (2 min); followed by 300 °C at the rate of 10 °C min^{-1}; and 300 °C, where it was held for 6 min. The mass detector conditions were transferring line temperature of 240°C; ion source temperature of 240°C; and ionization mode electron impact at 70 eV, a scan time of 0.2 sec, and a scan interval of 0.1 sec. The fragments range from 40 to 600 Da. The mass conditions were solvent Delay=2.00 min, Transfer Temp=24^0C, Source Temp=24^0C, Scan: 50 to 600Da.

Antibacterial activity

The agar well diffusion method showed good results *Escherichia coli* was used as the pathogen and a 10μg/mL concentration of plant extracts of *Euphorbia hirta* was used in the well. The solvents ethyl acetate, petroleum ether, ethanol, and hexane showed zones of inhibition of 2 mm 2.2 mm 2.9 mm, and 3.2 mm respectively, which is shown in Fig. 1. The crude extract hexane of *E. hirta* showed the highest zone of inhibition of 3.2 mm (Fig. 2).

Table 4: GC-MS analysis of hexane extracts of *Vitex negundo* and *Euphorbia hirta*

Compound ID/Name	Extract	Molecular weight (g/mol)	Formula
Heptacosane	*Vitex negundo*	380.7	$C_{27}H_{56}$
Heptadecane, 2,6,10,15-tetramethyl-	*Vitex negundo*	296.6	$C_{21}H_{44}$
1-iodo-2-methylundecane	*Vitex negundo*	296.23	$C_{12}H_{25}I$
4-(2,2,6-trimethyl-bicyclo[4.1.0]hept-1-yl)-butan-2-one	*Vitex negundo*	208.34	$C_{14}H_{24}O$
1-hexyl-2-nitrocyclohexane	*Vitex negundo*	213.32	$C_{12}H_{23}NO_2$
1h-benzocyclohepten-7-ol,2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-, cis-	*Vitex negundo*	222.37	$C_{15}H_{26}O$
Bicyclo [7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1r-(1r*,4z,9s*)]-	*Vitex negundo*	204.35	$C_{15}H_{24}$
m-dioxan-4-ol, 2,6-dimethyl-	*Euphorbia hirta*	132.26	$C_6H_{12}O_3$
Propanamide, 2-hydroxy-	*Euphorbia hirta*	89.09	$C_3H_7NO_2$
1-heptadecanamine	*Euphorbia hirta*	255.5	$C_{17}H_{37}N$
Hexadecanal	*Euphorbia hirta*	240.42	$C_{16}H_{32}O$

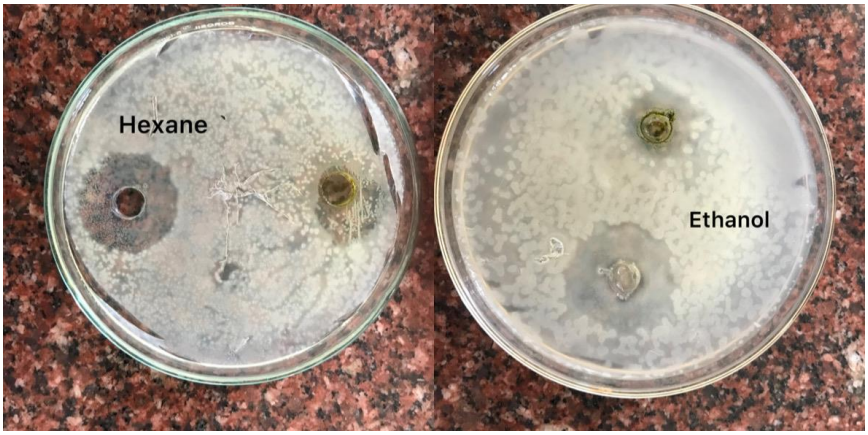

Fig. 1: Antibacterial activity of hexane and ethanol crude extract against *E. coli*

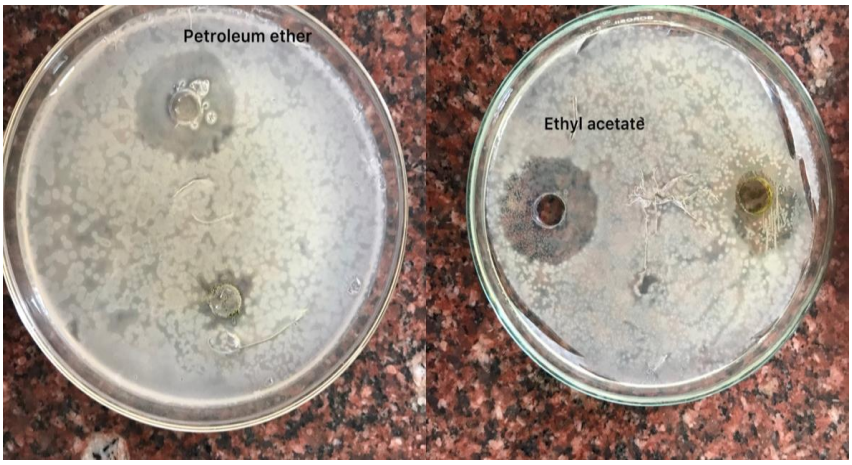

Fig. 2: Antibacterial activity of hexane and ethanol crude extract against E. coli

Rate of kill assay

The rate kill assay was performed at 595 nm and the absorbance was observed for the crude extract hexane of *Euphorbia hirta* and the absorbance reading was taken against the bacteria *E. coli*. The percentage inhibition was found to be 45%.

Table 5: Rate of kill assay

Concentration (µg/ml)	0^{th} hour (nm)	4^{th} hour (nm)	24^{th} hour (nm)	Inhibition (%)
1000	0	0.443	1.470	45%
500	0	0.301	1.070	40%
250	0	0.156	1.292	37%

DISCUSSION

Plant discipline is one of the vast fields that has been showing vast research potential in intense pharmacological studies for the last few decades, the value of medicinal plants as potential sources of new compounds of therapeutic value and as sources of lead compounds in drug development. Supercoiling by gyrase is involved in all DNA-related metabolic processes and is required for replication and other cellular activities. With the developing resistance to the available antibiotics, there is a need for new drugs that can prevent infections. Bioactive compounds alkaloids, flavonoids, glycosides, and terpenoids

reported high potential for the target of DNA gyrase B inhibition.

CONCLUSION

The targets chosen in this research, *Staphylococcus aureus* (ATCC 25923) DNA Gyrase B, are appealing because they are relatively undiscovered targets in the field of drug discovery and thus hold enormous potential for the development of novel agents. The designed primer shows potential insights into the cloning of DNA gyr B enzyme from the *S.aureus* (ATCC 25923) in the future**.** The thin layer chromatography performed on the hexane crude extract of *Euphorbia hirta* shows the highest retention factor of 0.909 cm followed by the ethyl acetate crude extract of v*itex negundo* and petroleum ether crude extract of *Solanum nigrum* as 0.907cm and 0.781cm respectively. *E. hirta was* reported to have the highest alkaloid content of 17.29% followed by *Vitex negundo.* Thus, the research concludes that *Euphorbia hirta* can be considered as a potential therapeutic compound against the *S. aureus* (ATCC 25923) DNA Gyr B inhibition.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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