Research Article

GC-MS Analysis and Antimicrobial Activities of Ethanol Alkaloid Leaf Extracts of *Delonix elata*.L

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Received: 18-03-2021; **Revised:** 22-05-2021; **Accepted:** 30-05-2021; **Published on:** 15-06-2021.

ABSTRACT

Delonix elata L., belongs to family Fabaceae used by the traditional various medicinal practices to cure jaundice, skin disease, heart disease, cancer cell formation, physiological abnormalities, heptoprotective, bronchial and rheumatic problems. The present study was screen the antimicrobial and phytochemical activity of alkaloid leaf extracts. This extracts was assessed on multidrug resistant clinical isolated from both gram positive, gram negative and antifungal strains including *Bacillus subtilis, Staphylococcus aureus, Escherchia coli, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger.* The zone of inhibition was determined by Agar well diffusion method with various concentration. GC- MS analysis was performed to identify major bioactive compounds present in the extracts. The GC – MS studies shown the present of 25 compound were identified in the leaf extract composition. The antimicrobial analysis revealed that *C. albicans* showed a highest zone of inhibition 25mm at 100 mg/ml of extracts. Present finding suggest that *D. elata* as plant pharmaceutical and pharmacological importance.

Keywords: Alkaloid leaf extracts, Antimicrobial, GC- MS analysis, pharmacological importance.

QUICK RESPONSE CODE →

DOI:
10.47583/ijpsrr.2021.v68i02.016

DOI link: http://dx.doi.org/10.47583/ijpsrr.2021.v68i02.016

INTRODUCTION

Antibiotics have saved millions of lives and contributed to significant increases in life expectancy over the last century. However, the emergence of multi-drug resistant pathogens is threatening the clinical efficacy of many existing antibiotics. [1, 13] The recent emergence of strains with decreased susceptibility, as well as the unfavorable side effects of certain antibiotics [2]. Infectious diseases caused by resistant microorganisms are associated with longer hospital stays, higher costs, and an increased risk of morbidity and mortality. Resistance is a particularly difficult issue for people with compromised immune systems, such as those suffering from AIDS or cancer patients. The Promiscuous antibiotic use accounts for a significant portion of the community burden of antibiotic use and significantly contributes to the rising prevalence of resistance among major human pathogens. The development of extended-spectrum antibiotics that target Gram-negative bacteria has resulted in infections that can be extremely difficult to treat, resulting in significantly increased illnesses and deaths. The resistance problem necessitates a renewed effort to screen various medicinal plants for potential antimicrobial traits caused by compounds synthesised in the plant's secondary metabolism. Alkaloids, flavonoids, tannins, phenolic compounds, steroids, resins, fatty acids, and gums are the most important bioactive compounds found in plants. Another motivator for scientists to look for new antimicrobial substances from various sources, including medicinal plants, has been the rapid extinction of plant species. Medicinal plants are used by 80 percent of the world's population, and India has a long history of using herbal medicine to treat a variety of infectious diseases, inflammations, injuries, and illnesses. Many plant materials used in traditional medicine have been shown to be more effective and less expensive than modern medicine [4, 15] against certain ailments while also mitigating many of the side effects commonly associated with synthetic antimicrobials [5-7]. The majority of the research is aimed at determining the activity of plant extracts against a variety of test bacteria, including both pathogenic and nonpathogenic strains.

Delonix elata is a belonging to the family Fabaceae and deciduous tree about 2.5-15 m tall, with a spreading, rather rounded crown, crooked poor stem form and drooping branches. Bark smooth, shining; sometimes flaking. Leaves 3-6 or more, bipinnate; pinnae usually 4-6 pairs; leaflets 10-14 pairs, oblong or oblanceolate-oblong, 0.6-1.2 cm long. Leaflets 1.25-4 mm wide, smaller than those of D. regia. Flowers in terminal corymbs; stalks pubescent, lowest flowers stalks longest. Flowers open one at a time. Sepals 1.8 cm long, with a broadly ovate or rotundate-cuneate lamina narrowing into a distinct claw. Petals rounded in outline and crisped on margins 1.6-3.8 cm long, 1.8-4.2 cm wide; upper one smaller than rest, pale yellow; the remainder white; later all turning apricot. Staminal filaments pale brown or reddish, hairy at the

base, 5-10 cm long; pedicels up to 3.75 cm. Ovary pubescent or tomentose all over. Pods red-brown or purple-brown, up to 20 cm long and smooth, compressed elliptic-oblong. *Delonix* is widely used by the traditional medical practitioners of Karnataka, India, to cure jaundice, diabetics, bronchial, Wound healing and rheumatic problems. Bark extracts have shown potential antioxidant and hepatoprotective activity.

Despite an abundance of literature on the antimicrobial properties of plant extracts, none of the plant-derived chemicals have been successfully used as antibiotics in clinical trials. A significant portion of plant chemical diversity is thought to protect plants from microbial pathogens. As a result, the purpose of this study was to investigate the antimicrobial potentiality of various fungal and bacterial strains and Phytochemical investigation of *D. elata.*

MATERIALS AND METHODS

Collection of Plant Materials

Delonix elata. L leaves were collected from Somarasam pettai, Tiruchriappalli district of Tamil Nadu, India. Department of Botany, Bishop Heber College, Tiruchirappalli, has authenticated the *Delonix elata*. L. I t was ensured that the plant was healthy and uninfected. Collected materials i.e. leaves were collect in bulk quantity. After collection the plant materials are washed properly by using running tap water to remove the adhering soil and dirt particles and then shade dried. Voucher specimen of the plant was deposited at The Rapinet Herbarium, St. Joseph's College, Tiruchirapppalli for further reference. The dried plant materials were coarsely powered and stored in airtight, non-toxic polyethylene bags until used. Leaves were separated and powdered by using blender. It was stored in a freezer for further use.

Preparation of Leaf Extracts

Fresh leaves (60-80 gm) of *D. elata* were shade dried at room temperature (32 – 35 °C) to constant weight over a period of 5 days. The dried seeds were ground into powdered using a mortar and pestle. 25 g of the powdered seeds were separately extracted in 250ml conical flasks seed powered was packed in soxhlet apparatus. The powdered leaves of *D. elata* was extracted with different solvents like ethanol (60 - 80°C) for 24 h to de-fat it and then the remaining plant materials were extracted by maceration process by using acetone as solvent. Filtered was occurred with the extractives where the solvents were incorporate and subsequently the marc is pressed to squeeze out residual extractives. This process was repeated thrice to achieve complete extraction. The extracts obtained during the three cycles were combined and reduced to 1/8th of its original volume in a rotary evaporator at 45°C and then lyophilized in a freeze dryer to obtain the yield. After obtaining the product the product is concentrated by drying method and then preserved for further study.

Extraction and Isolation of Alkaloids

Powdered plant material (10 g) was wetted with 15mL of NH_4OH (25%, m/m) and room temperature solvent extraction was performed with 300 mL of ethyl acetate for 72 h. The extract was filtered and the solvent was evaporated in a rotary evaporator under reduced pressure at 40 °C. The residue, dissolved in H2O and acidified with H_2SO_4 to pH 3-4, was extracted with petroleum ether and diethyl ether to remove lipophilic, acidic and neutral material. After basifying the aqueous solution to pH 9-10 with NH4 OH (25%, m/m), it was extracted with chloroform, the extract washed with distilled water to neutral pH, dried with Na2 SO4 and concentrated to dryness under reduced pressure to obtain crude alkaloids.

GC –MS

The GC-MS analysis for identification of compounds present in different fractions prepared from crude methanol extract of *D. elata* had been carried to identify the compounds. Gas chromatography study includes the important optimization process such as i) introduction of sample extract onto the GC column, ii) separation of its components on an analytical column and iii) detection of target analysis by using mass spectrometry (MS) detector. 5ml of ethanol extract was evaporated to dryness and reconstituted into 2ml ethanol. The extracts were then subjected to GC-MS analysis. Chromatographic separation was carried out with instrument GC-MS-QP 2 (SHIMADZU instrument) with Db 30.0 column (0.25 µm diameter × 0.25 µm thickness). The oven temperature was programmed from 70°C (isothermal for 5 minutes), with an increase of 10°C/min. up to 200°C, then 5°C/min. up to 280°C and ending with 35 minutes isothermal at 280°C. Mass spectra were taken at 70 eV; scan interval of 0.5 seconds and scan range from 40–1000 m/z. Helium was used as the carrier gas at 99.99 % pressure with flow rate of 1.0 ml/min. and electronic pressure control on Samples were dissolved in Acetone and injected automatically. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adapted to handle mass spectra and chromatograms was a Turbo Mass Version 5.2.0. Interpretation on mass-spectrum.

MS Programme

Library used NIST Version-Year 2015, Inlet line temperature 200°C, Source temperature 200°C Electron energy:70 eV, Mass scan (m/z): 45-450,Solvent Delay: 0-2 min, Total MS running time:36 min.

Identification of compounds

Interpretation on mass spectrum generated during GC-MS analysis was done by using National Institute Standard and Technology (NIST) database 2015 to identify the compounds present. To identify the compounds well established known compounds are considered as a standard whereas Unknown compounds of spectra were kept as test in the NIST library by considering different

parameters like retention time, molecular weight and structure. GC- MS having more 62,000 patterns. The Structure of the compound and the Molecular weight of the compound with name of the test materials were confirmed. The major compounds identified were searched in Dr. Duke's Phytochemical and Ethnobotanical Database.

Source of Microorganisms

The organisms used were Two gram-positive bacteria such as *Bacillus subtilis, Staphylococcus aureus,* two gram-negative bacteria *Escherchia coli, Pseudomonas aeruginosa,* and the two fungal strains *Candida albicans* and *Aspergillus niger.* The organisms were obtained from Kauvery Medical Hospital, Tiruchirappalli, Tamil Nadu and maintain according to specification. Sub culturing was done at the interval of 15 days. Bacterial strains were maintained on Nutrient agar slants (Hi media) at 4˚C. The fungal strains were maintained on Sabouraud dextrose agar slants at 4˚C.

Inoculum preparation

Stock cultures were maintained at 4°C on slopes of nutrient agar and sabouraud dextrose agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Muller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 h at 37°C respectively and Sabouraud dextrose broth (SDB) for fungi that were incubated for 72 hours at 27°C. To 5ml of MHB and SDB, 0.2 ml of culture was inoculated and incubated till it reached the turbidity equal to that of the standard 0.5 McFarland solution 8h at 600nm which is equivalent to 10^6– 10^8 CFU/ml. These suspensions were prepared immediately before the test was carried out.

Microbial inoculum preparation

The nutrient broth and Sabouraud dextrose broth were prepared, then identified bacterial and fungal colonies were inoculated into the broth culture were used for antimicrobial activity.

Determination of Antimicrobial Activity

The antibacterial and antifungal activity of the *D. elata* alkaloid leaf extracts was determined using agar well diffusion method by following the known procedure. Nutrient agar and Sabouraud dextrose agar were inoculated with the given microorganisms by spreading the bacterial and fungal inoculums on the media. Wells of 6mm were punched in the agar and filled with plant extracts. Control wells containing neat solvents (negative control) were also run parallel in the same plate. The plates were incubated at 37°C for 24 hours and 28°C for 72 hours the antimicrobial activity was assessed by measuring the diameter of the zone of inhibition. The antimicrobial activity of the different alkaloid extracts were evaluated by comparing their zones of inhibition with standard antibiotic streptomycin.

RESULTS AND DISCUSSION

The ethanol leaf extracts of *D.elata* were tested against the pathogenic bacteria and fungi and viz. *B. subtilis* which can cause Endocarditis, pneumonia and Septicemia; *S. aureus* which can cause of skin and soft tissue infections such as abscesses, furuncles and cellulitis; *P. aeruginosa* causes Urinary tract infections, respiratory infections, dermatitis, soft tissue infections, bacteremia bone and joint infections, gastrointestinal infections and variety of systematic infections; *E.coli* causes cholecystitis, cholangitis, urinary tract infection and traveler's diarrhea; *C. albicanse* is the most common cause of genital yeast infections and superficial infections; *A.niger* cause Aspergillosis, allergic reactions, lung infections. Antimicrobial activity of *D.elata* were seen against four bacteria and two fungus strains. The four different concentration of leaf extracts of *D.elata* were also used against six microbial pathogens. The ethanol alkaloid leaf extract showed maximum activity against *Candida albican* and minimum activity against *Bacillus subtilis* shown in the Table 1. The alkaloid leaf extracts higher concentration (100 µl) showed maximum inhibitory zone (25 mm) and lower concentration (25 µl) showed minimum inhibitory zone (12 mm) against to *B. subtilis* Table-1.The zone of inhibition of microbial strains was ranged from 12 mm to 25 mm. The antibacterial and antifungal activity demonstrated by *D.elata* is suggestive of the presence of compounds and/or possible synergistic interaction of compounds that can disrupt fungal and bacterial membranes. The current finding is an indication of the therapeutic relevance of the tested plants in managing dermatological conditions of bacterial and fungal origin.

Table 1: Antimicrobial activity of *D. elata*

SAMPLE	ETHANOL Extract 100 µl added and Zone of inhibition (mm/ml)				
	25 µl	50 µl	75 µl	100 µl	Control
Bacillus subtilis	12	14	15	16	20
Staphylococcus aureus	14	16	18	22	20
Escherchia coli	15	18	20	23	20
Pseudomonas aeruginosa	12	14	16	18	18
Candida albicanse	16	19	22	25	22
Aspergillus niger	14	18	20	23	20

The GC-MS studies revealed that the presence of twenty-five compounds in ethanol alkaloid leaf extract of *D. elata.* The compounds with their Retention Time (RT), Molecular Formula, Molecular Weight (MW) and Peak Area (%) have been presented in different tables. The major phyto compounds had been identified on basis of the percentage Peak Area in the Chromatograph. The compounds identified in the ethanol alkaloid leaf extract (Table 2 and figure 1) are Styrene, Benzaldenyde, 2,2,4,4,6,6,8,8-Octamethyl-1,3,5,7,2,4,6,8-Tetraoxyatertrasilocane, 1-Tetradecanol, n-Pentadecanol, Neophytadiene, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-Tetradecamethyl -, Dibutyl phthalate, Hexadecanoic acid, ethyl ester, trans,trans-9,12-Octadecadienoic acid, propyl ester, Ethyl (9Z,12Z)-9,12-Octadecadienoate, Octadecanoic acid, ethyl ester, Cyclooctasiloxane, Hexadecamethyl-,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15 Hexadecamethyl ocatasiloxane, Cyclonosiloxane, Octadecamethyl-,2,5,7,8-Tetramethyl -2-(4,8,12-Trimethyl tridecyl)-3,4-Dihydro-2H- Chromen- -YL Hexofuranoside, Cyclodecasiloxane, eicosamethyl-,Cyclododecasiloxane, Tetracosamethyl-,Cyclooctasiloxane, hexadecamethyl-,Z-11,13-Tetradecadien-1-ol acetate, Cyclononasiloxane, octadecamethyl-, Cyclononasiloxane, octadecamethyl-, Hexacontane, Cyclodecasiloxane and eicosamethyl- may be synergistically responsible for the antimicrobial activity. The GC-MS spectrum confirmed the presence of various components with different retention times as illustrated in [Figure 1, Table 2]. The mass spectrometer analyses the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. The present study helps to predict the formula and structure of 25 biomolecules. Further investigation may lead to isolation of bio-active compounds and their structural elucidation and screening of pharmacological activity will be helpful for further drug development [8-17].

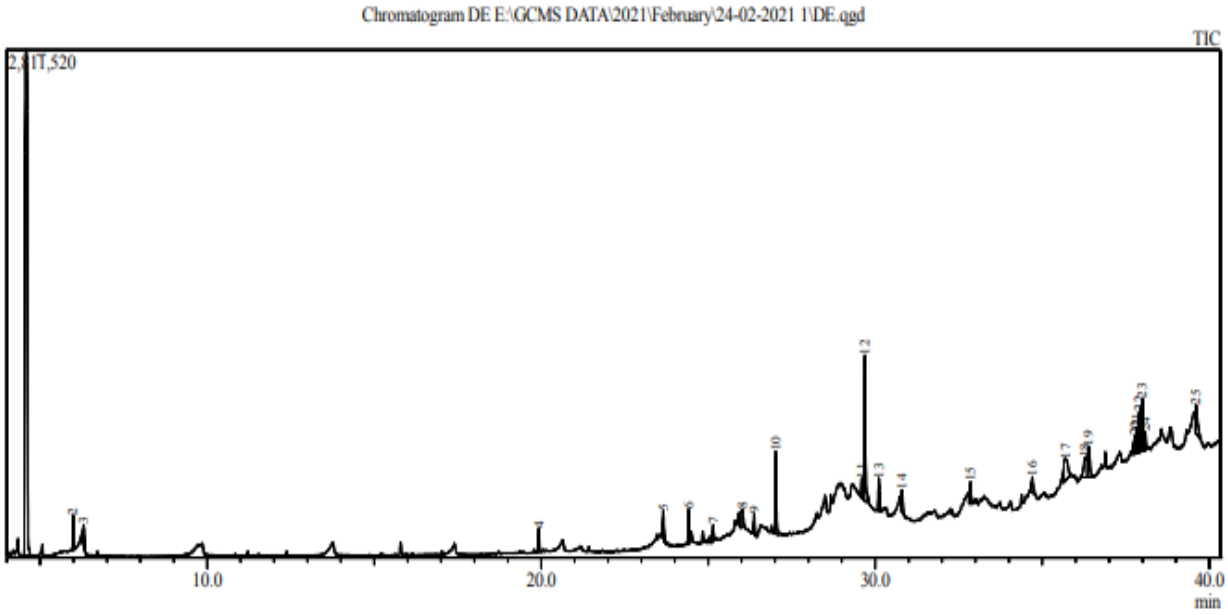
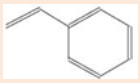
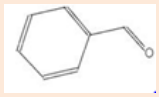
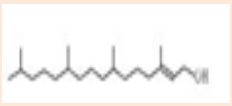
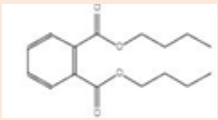
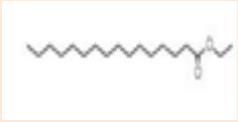
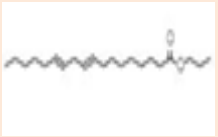
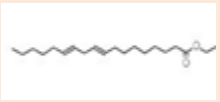
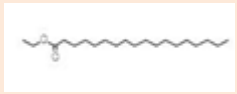
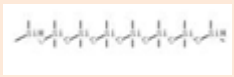
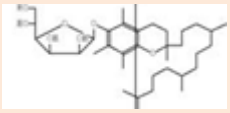
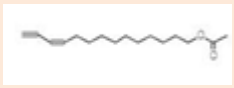
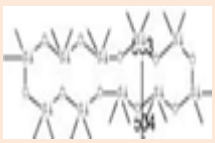


Figure 1: GC – MS analysis of D.elata

Secondary metabolites of plants such as alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds serve as a defence mechanism against prediction by many microorganisms, insects, and other herbivores. In very small doses, alkaloids had strong biological effects on animal and human organisms. Alkaloids are found not only in food and beverages, but also as stimulant drugs [18-26]. They demonstrated anti-inflammatory, anticancer, analgesic, local anaesthetic, and pain relief properties, as well as neuropharmacologic, antimicrobial, antifungal, and other activities. Alkaloids can be used as diet ingredients, supplements, pharmaceuticals, and in medicine, among other things. Alkaloids are also important compounds in organic synthesis for the discovery of new semisynthetic and synthetic compounds that may have greater biological activity than parent compounds [27-36]. Alkaloids are a large class of naturally occurring organic compounds that have a nitrogen atom or atoms (amino or amido in some cases) in their structure. These nitrogen atoms are responsible for the alkalinity of these compounds. These nitrogen atoms are typically found in a ring (cyclic) system. Indole alkaloids, for example, are those that have a nitrogen atom in the indole ring system. Alkaloids are classified into several classes based on their structures, including indoles, quinolines, isoquinolines, pyrrolidines, pyridines, pyrrolizidines, tropanes, terpenoids, and steroids.

Table 2: GC – MS analysis of *Delonix elata*.

S. No	R. Time	Mass Peak	Name of the Compound	MW	Molecular Formula	Molecular Structure	Action
1	4.579	1	Styrene	104	C8H8		Antimicrobial activity
2	5.994	2	BENZALDEHYDE	106	C7H6O		Great anti Oxidant, Antimicrobial Activity
3	6.305	3	2,2,4,4,6,6,8,8-OCTAMETHYL-1,3,5,7,2,4,6,8-TETRAOXATETRASILOCANE	296	C8H24O4Si4		Reactant for Synthesis of Ethylhydro Silicone fluids
4	19.931	4	1-Tetradecanol	214	C14H30O		Antimicrobial Activity
5	23.657	5	n-Pentadecanol	228	C15H32O		Lubricating oil, Consumer product such as loations andcreams
6	24.412	6	Neophytadiene	278	C20H38		Antioxident, Anti inflammatory, antimicrobial and algal metabolite
7	25.149	7	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296	C20H40O		Synthesis form of vitamin E and vitamin K1
8	26.027	8	HEPTASILOXANE, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-TETRADECAMETHYL-	504	C14H44O6Si7		Antimicrobial Activity
9	26.375	9	Dibutyl phthalate	278	C16H22O4		Bioactive Compound, Antifungal and Antibacterial Activity
10	27.017	10	Hexadecanoic acid, ethyl ester	284	C18H36O2		Anti oxidant, Anti inflammatory, Antibacterial activity
11	29.596	11	trans,trans-9,12-Octadecadienoic acid, propyl ester	322	C21H38O2		Biosynthesis of prostaglandins acid Cell membrane
12	29.688	12	ETHYL (9Z,12Z)-9,12-OCTADE CADIENOATE #	308	C20H36O2		Anti fungal and Antibacterial activity

13	30.107	13	Octadecanoic acid, ethyl ester	312	C20H40O2		Plant metabolite
14	30.799	14	CYCLOOCTASILOXANE, HEXADECAMETHYL-	592	C16H48O8Si8		Antimicrobial activity, Antibacterial activity
15	32.85	15	1,1,3,3,5,5,7,7,9,9,11,11,13 ,13,15,15HEXADECAMETHYLOCTASILOXANE #	578	C16H50O7Si8		Antimicrobial activity
16	34.701	16	CYCLONONASILOXANE, OCTADECAMETHYL-	666	C18H54O9Si9		Antibacterial, Antimicrobial activity
17	35.702	17	2,5,7,8-TETRAMETHYL-2-(4,8,12-TRIMETHYLTRIDECYL)-3,4-DIHYDRO-2H-CHROMEN-6-YL HEXOFURANOSIDE	592	C35H60O7		Antimicrobial, Antioxidant
18	36.27	18	Cyclodecasiloxane, eicosamethyl-	740	C20H60O10Si10		Antiparasitic, Pesticidal, antifungal Activity
19	36.393	19	CYCLODODECASILOXANE, TETRACOSAMETHYL-	888	C24H72O12Si12		Antimicrobial, Antifungal activity
20	37.77	20	Cyclooctasiloxane, hexadecamethyl-	592	C16H48O8Si8		Anticancer, Antibacterial, Antimicrobial activity
21	37.83	21	Z-11,13-Tetradecadien-1-ol acetate	252	C16H28O2		Antioxidant, Antibacterial activity
22	37.9	22	Cyclononasiloxane, octadecamethyl-	666	C18H54O9Si9		Antiparasitic, Pesticidal, Antimicrobial, Antifungal activity
23	37.994	23	Cyclononasiloxane, octadecamethyl-	666	C18H54O0Si9		Antiparasitic, Pesticidal, Antimicrobial, Antifungal activity
24	38.085	24	Hexacontane	842	C60H122		Antibacterial activity, Antioxidant activity
25	39.602	25	Cyclodecasiloxane, eicosamethyl-	740	C20H60O10Si10		Antimicrobial, Antioxidant, Anticancer activity and anti inflammatory

CONCLUSION

Gas Chromatogram Mass Spectrometry (GC-MS) analysis was used to identify twenty three chemical constituents from ethanolic alkaloid leaf extract in this study. The presence of various bioactive compounds justifies traditional practitioners' use of the plant in various ailments. The antimicrobial activities demonstrated by *D.elata*, despite being at high concentrations, continue to provide a clue to the local traditional system of medicine that employs it. To identify major biologically active phytoconstituents, phytochemical screening was performed. Furthermore, we looked into the biological activity of the powerful alkaloid leaf extracts against a variety of antibacterial and antifungals Agar overlay bioautography assay for strains. It is hoped that these active constituents will provide useful information for the development of new compounds with greater activity against multidrug resistant bacteria and fungi than currently available agents.

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Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.