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Isolation, Characterization and Biological Activities of Stigmasterol from Leaf Part of *Crescentia alata* Kunth (*Bignoniaceae*)

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

The present investigation deals with the extraction, separation, isolation, identification, characterization and biological evaluation of the stigmasterol from methanolic extract on leaves of *C. alata* Kunth using bio-guided fractionation and spectral analytical methods. The biological activities investigated were antimicrobial and cytotoxicity. Leaf crude extract of *C. alata* obtained from 80% methanol was successively extracted with hexane, chloroform, ethyl acetate and n-butanol. Ethyl acetate fraction afforded a bioactive compound by bioassay-guided fractionation. The characterization of isolates was done by biochemical and spectral methods. The active fraction obtained and isolated compound were tested for their antimicrobial activities. The cytotoxicity of the isolated compound on Hela cell line, estimated with the MTT assay. The ethyl acetate fraction has exhibited highest effective antimicrobial activities and the fraction afforded a compound stigmasterol. The compound isolated stigmasterol from leaf of *C. alata* showed strongest antimicrobial effect against all microbial strains were tested with minimum inhibitory concentration values ranging from 1.95 to 125 µg/mL. The cytotoxicity studies indicated that the isolated stigmasterol possesses much potential against Hela (mammalian cancer) cell line. Overall, the stigmasterol compound was the most dynamic as far as the antibacterial and antifungal potential of

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the leaves of *C. alata* confirm the conventional utilization of this plant in treating different respiratory sufferings and its related manifestations. The properties of the bioactive phytochemical compound stigmasteol recommend that the powerful and wide range of antimicrobial and anticancer operators and may fill in as the lead compound in the advancement of novel restorative medications.

Keywords: Crescentia alata; Bio-guided isolation; Spectral analysis; Stigmasterol; Antimicrobial activity; Cytotoxicity studies; Pure compound; Medicinal plant; Extraction; Traditional medicines.

1. INTRODUCTION

Each plant in the nature is special not only to be a piece of biodiversity but also for certain medicinal value. Therapeutic properties of the different plant species is because of the presence of a particular unique type of medicinally important chemical compounds. There are variable in various plant species. The chemical compound concentrations of the inside of the plant body in each individual of the plant species are found to be differing. Plants are utilized as an essential source of medicines for the treatment of various diseases since the ancient time for humans due to the presence of certain chemicals/active ingredients inside of their body. Presence, the concentration and types of the biochemical performing to be usefulness of the usefulness of the various plant parts are utilized as safe medicine for the treatment of various disorders [1].

Now a days, antimicrobial resistance is on the increase globally because of greater access to antibiotic drugs in developing countries. One out of two people is dying prematurely from infectious diseases. When compared to developed countries, the premature mortality is more frequent in developing countries [2-4]. The plant-based drugs used in the traditional systems continue to play a significant role in health care and it has been projected by the World Health Organization. 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care; remaining 20% of the populations, mostly residing in developed countries the plant products also play an important role in the health care systems. Extraction, isolation, identification and biological properties of chemical substances from the various medicinal plants have now framed the significant field of study in biochemical and pharmaceutical sciences. This may be due to the presence of wide range phytoconstituents present in it. Thus, keeping the importance of plants is being screened for newer and effective chemotherapeutic agents. Higher plants can serve as potential anti-infective and source of new antimicrobial agents.

Crescentia alata belonging to the family *Bignoniaceae* and it is a blooming plant that is native to Southern Mexico and Central America and it is naturalized in India [5-6]. In India, it is found in Delhi, Uttar Pradesh, Bihar, West Bengal, Sikkim, Assam, Tripura, Gujarat, Maharashtra and Tamil Nadu. It is an evergreen tree reaching up to 6 to 10m in height with a broad, irregular crown composed of long, spreading branches clothed in 5 to 15cm long bright green leaves. The tree is most important ornamental in the landscape for its year-round production of flowers and fruit, both of which are unordinary. They have 5 cm wide flowers, which bloom at night, are yellow/green with red or purple veins, cup-shaped, and appear to emerge directly from the branches. These are followed by the emergence of the large, round and cannonball-like fruit 7-10 cm diameter that are difficult to break into, with a smooth, hard shell, which hang directly beneath the branches. The calabash tree grows in clayey soils with inadequate drainage subject to frequent flooding. The fruits of *C. alata* are developed only after pollination by bats and the ovoid fruit has a hard green woody shell. Inside fruit there is a pulp that has medicinal applications [7]. The important traditional use of this plant is a decoction is prepared from the leaves has been employed as an astringent and anti-hemorrhagic. The fruit of *C. alata* is pectoral. The pulp of the fruit is mainly used in the treatment of colds, haemoptysis, dysentery and for diseases of the kidney. In traditional medicine the plant is used to decrease various respiratory illnesses and diseases, including chronic bronchitis, asthma, cold, flus and cough, and also is used to treat skin disorders and internal inflammations [8-9].

The *Bignoniaceae* family has previously demonstrated a number of pharmacological activities such as immunostimulant, antiulcer activity, molluscicidal, anti-inflammatory, trypanocidal, antidiabetic, mosquito larvicidal,

anti-snake venom, antiplasmodial, neurotrophic antimicrobial, antidepressant, anticancer, antinociceptive, and antioxidant activities [10-15].

Phytosterols are a group of steroidal alcohol, naturally occurring compounds found in plant cell membranes. It has been recognized as an important component of healthy diets and has been demonstrated to reduce blood cholesterol levels in hyper and normocholesterolemic subjects. Phytosterols have anticarcinogenic properties and it has been experimentally proved to inhibit colon cancer development [15]. The compound stigamasterol has been reported that a valuable human growth hormone that plays an important physiological functions including in the tissue repair, regrowth, regeneration, mechanisms of lost tissues and regulatory mechanisms related to estrogen effects of the human body, it has also been identified as a suitable precursor in the manufacture of semisynthetic progesterone, as well as acting as an intermediate in the biosynthesis of steroid hormones such as corticoids, estrogens, and androgens which is responsible for the regulation and development of the human body reproductive system and secondary sex characteristics. Stigmasterol is a diet high in phytosterols may inhibit the absorption of cholesterol and lower serum cholesterol levels by competing for intestinal absorption. Similarly, the main pharmacological activities reported for stigmasterol are Antioxidant, Anti-cancer, Anti-arthritic, Diuretic, Anti feeding, insecticide, Anticomplementary, Hypoglycemic Thyroid Inhibiting Properties, Precursor of Progesterone, Female activating hormone, Anti-microbial, Anti-asthama, Anti-inflammatory, Antimutagen, Neurotoxic, and Cytotoxic activities [10-16].

Therefore, the current study aims to identify and characterize the bioactive chemical compound responsible for some of the biological activities exhibited by leaf extract of *C.alata* using bio-guided fractionation and spectral analytical methods. The biological activities investigated were antimicrobial and cytotoxicity. To our best knowledge, this is the first study to isolate and characterize this compound from the leaves of *C. alata* belonging to the family *Bignoniaceae.*

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Chemicals, thin-layer chromatography (TLC) silica gel 60 F_{254}, and all other solvents analytical grade were procured from Merck, HiMedia, and Fisher Scientific, Mumbai, India.

2.2 Procurement, Authentication, and Process of *C. Alata*

The leaf materials of *C. alata* were collected from Pudukkottai, Tamilnadu, India. It was collected during the onset of monsoon season. The identification and authentication of plant material was by the authorities of the Botanical Survey of India (BSI), Tamil Nadu Agricultural University Campus, Southern Regional Centre, Coimbatore and the specimen samples are deposited in the BSI (Voucher Number: BSI/SRC/5/23/2017/Tech/3525). The leaves were separated from the stems and dried under the shade for 7 days. Finally it was powered by using an electric grinder, sieved and used to isolate phytochemical component.

2.3 Extraction and Solvent Partitioning of the Crude Extracts

The powdered leaf material of *C. alata* (1.5 Kg) was extracted with 10 L of 20% aqueous methanol (MeOH) at room temperature for 24 hours with occasional shaking and the crude extract filtered through Whatman No.1 filter paper. Then the filtrate afforded from the extraction process was concentrated in vacuo at 40°C to about of its original volume. This afforded the crude extracts (158.6g) of the leaves of *C. alata.*

The 80% Methanolic concentrated crude extract was suspended in 500mL water:methanol (9:1) and in turn sequentially extracted with *n*-hexane C_6H_{14} (3× 800 ml), chloroform $CHCl_3$ (3× 800 ml), ethyl acetate $C_4H_8O_2$ (3× 800 ml) and n-butanol $C_4H_{10}O$ (1×500 ml). The solvent fractions were concentrated to dryness using a rotary evaporator and these yielded four solvent fractions. The mass of each fraction was 15.2g (*n*-hexane), 18.15g (chloroform), 21.13g (ethyl acetate) and 36.4 g (n-butanol) respectively. All the four fractions from the methanolic crude extracts were subsequently assayed for preliminary antimicrobial activities. As a result of the favorable antimicrobial potential of ethyl acetate fraction during the preliminary tests, further it was selected for isolation process. The bio-guided isolation assay performed on the leaf part of *C. alata* and the fractionation process is illustrated in Fig. 1.

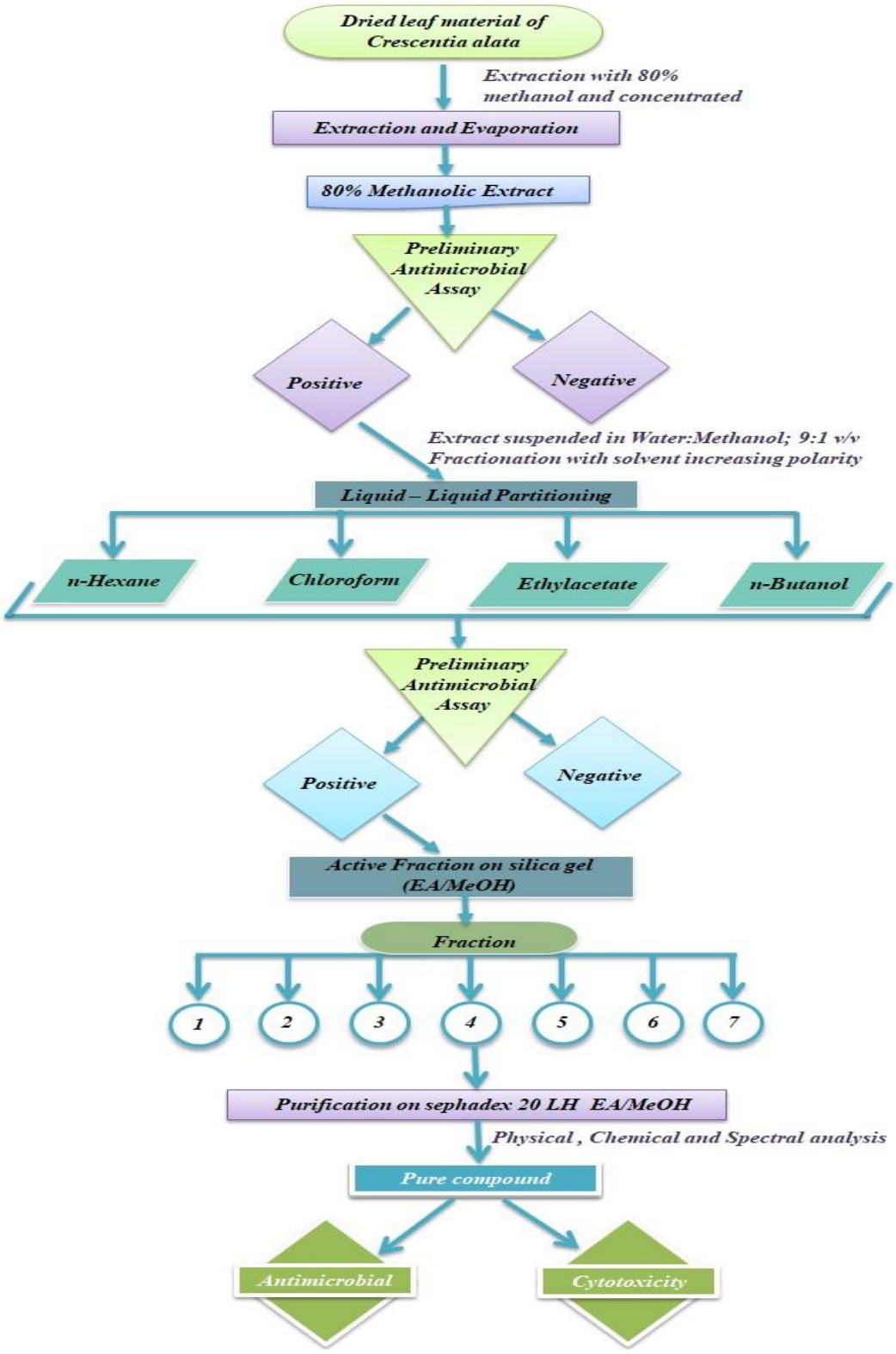

Fig.1. Flowchart of the bio-guided isolation assay performed on the leaf part of *C. alata*

2.4 Isolation of Bioactive Compound from the Portioned Fractions

The most bioactive ethyl acetate fraction, (21.13g) was fractionated on a silica gel column (230-400 mesh) starting by using 100% chloroform followed by an increasing gradient of an acetate fraction of 30% up to 100% as mobile phase and then it was followed in turn by an increasing gradient of ME up to 60%. Based on TLC analyses seven fractions were obtained (E1-E7). Then, the collected seven fractions were performed for antimicrobial assay by following the same procedure described in Section 2.6.2 to determine the minimum inhibitory concentration (MIC) values. From that, the fraction 4 exhibited good antimicrobial activities against all the tested bacterial and fungal strains. Further, the fraction 4 was subjected to column chromatographic fractionation on Sephadex LH 20 using ethyl acetate/methanol (8:2) as a mobile phase followed by an increasing gradient of methanol up to 30% and resulted in the isolation of a bioactive compound (42.4 mg) after analysis on TLC plate using ethyl acetate/methanol (9:1).

2.5 Characterization and Structural Elucidation of Isolated Compound

The isolated pure compound was characterized using various spectroscopic analytic techniques. The UV–visible spectrum of the isolated compound in methanol was recorded using a Shimadzu 1700 double beam UV-visible spectrophotometer in the range of 200-800 nm at the scanning rate 100nm min^{-1} and chart speed of 5 cm min^{-1}. The λ_{max} of the compound was determined using DMSO. The Fourier transform infrared spectrum was recorded using a Perkin-Elmer instrument with spectrum RZX software version 5.0.1 at room temperature. The FT-IR spectra were recorded with a nominal resolution of 4 cm^{-1} and a wave number range from 400 to 4000 cm^{-1} using the KBr pellet technique. FT-IR spectrophotometer to identify the linkages and functional groups. ^{1}H NMR spectra were recorded at 400 MHz and ^{13}C NMR was recorded at 100 MHz in FT-NMR Cryomagnet Spectrometer (BRUKER-AMX) with 5mg of purified compound in *DMSO-d_6*. The ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO solvent and Tetramethylsilane (TMS) is used as internal standard (chemical shift values were given in δ, ppm) using topshim software. The NMR spectral analyses were performed to elucidate the chemical structure of the isolated compound and these methods are used to confirm and explore the molecular connectivity of the atoms in the molecule of a corresponding compound.

2.6 *In Vitro* Biological Evaluation of Crude Extract, Fractions, and Isolated Compound

2.6.1 Antimicrobial assay test microorganisms

The methanolic leaf crude extract, fractions and isolated compound from the leaf part of *C. alata* were screened for antimicrobial activity against a panel of Gram-positive (*Staphylococcus aureus* MTCC 3160 and *Bacillus subtilis* MTCC 2423) and Gram-negative (*Escherichia coli* MTCC 732) bacterial strains as well as fungal (*Candida albicans* MTCC 183, *Aspergillus flavus* MTCC 1783 and *Aspergillus niger* MTCC 10180) strains. The bacterial strains were selected for this study because of their respiratory pathogenesis [17]. The microbial strains that were used in this study were obtained from the Microbial type culture collection and Gene bank (MTCC), Institute of Microbial Technology, Chandigarh, India. Re-suspension was done at a concentration of 50 mg/ml (extracts and fractions) and 1 mg/ml (compound).

2.6.2 Minimum inhibitory concentrations (MIC)

In terms of the antibacterial activity, the minimum inhibitory concentrations (MIC) were determined using the micro-dilution assay in 96- well micro-plates [18]. Chloramphenicol was used as the positive control and details of the procedure for the antibacterial assay was as described by Aremu et al. [19]. Antifungal activity of the extracts, fractions and isolated compounds were evaluated using the micro-dilution assay modified for the fungi Fluconazole was included as the positive control. The MH broth, 80% methanol, 10% dimethyl- sulfoxide (DMSO) and water were used as negative and solvent controls respectively. Both antibacterial and antifungal assays were conducted triplicates.

2.7 *In-Vitro* Cytotoxicity on Cancer cells (MTT test)

In Vitro anticancer activity of the isolated Stigmasterol compound was analyzed using HeLa cell line obtained from National Centre for

Cell Sciences Repository (NCCS- Pune, India) by the MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] with slight modification of the procedure reported by Vasanthakumar et al., [20]. The minimum Essential Medium (MEM) supplemented with 100 units of penicillin, streptomycin and 10% (v/v) of fetal bovine serum (FBS) in a humidified atmosphere of 5% CO_2 at 37ºC until confluent. The cells were seeded at 1×10^4 cells/well in 96-well culture plates for 24 h. A miniaturized viability assay using MTT was performed as per ISO 10993:5 method. The control and test sample in triplicates were added to the cell. After incubation at 37±1 ºC for 24 h. Fluorescence Microscopy Fluorescence microscopy was used to visualize and examine the cell morphological features which can be exploited to understand therapeutic outcome. As per the Table 1, the reactivity were graded as 0, 1, 2, 3 and 4 based on zone of lysis, vacuolization, detachment and disintegration of membrane.

3. RESULTS AND DISCUSSION

3.1 Physical and Chemical Analysis of Isolated Compound

The appearance of the isolated compound was white in color (colorless); crystalline. Libermann-burchard test and Salkowski tests were found to be positive and the reactions confirms and indicate it to be as sterol. The observed melting point of the isolated compound was 167°C to 169°C and the mp was similar as the reported in literature [21-22]. On the basis of comparison of melting point with those reported in literature, the isolated compound was identified as stigmasterol a well known phytosterol.

3.2 Structural Elucidation of the Isolated Compound

White needle shaped crystals (colourless); m.p. 167-169 °C; UV λ_{max}: 210 nm in DMSO Fig. 2; IR: (KBr) ν_{max}: 3415, 2927, 2853, 1631, 1383, 1116, 1023, 617 cm^{-1} Fig. 3; ^{1}H NMR (δ ppm, 400 MHz, *DMSO-d_6*): δ 5.36 (H-6), 5.18 (H-22), 4.62 (H-23), 3.58 (OH, H-3), 1.29 (H-21), 1.68 (H-19), 0.89 (H-26), 0.81 (H-27), 0.7 (H-29), 0.58 (H-18) Fig. 4 ^{13}C NMR (100 MHz, *DMSO-d_6*): δ (δ 141.14, 138.78, 129.63, 121.86, 72.15, 56.88, 56.22, 52.25, 50.10, 42.48, 42.45, 39.90, 37.62, 36.88, 31.88, 31.85, 25.42, 24.43, 21.53, 20.21, 19.88, 12.27, 12.18 Fig. 5.

Based on the results of melting point, chemical test, and spectral analysis of the isolated phytoconstituent, it was identified as stigmasterol Fig. 6 Structure of bioactive compound stigmasterol has been elucidated from obtaining results which were compared with reported values of Stigmasterol and ^{1}H and ^{13}C NMR spectral data were in good agreement with the published data and confirmed [23-24]. So it was observed that the isolated compound from the leaves of *C. alata*, was found to be stigmasterol and structure is shown in Fig. 6. The compound stigmasterol was isolated by several authors from different plant species such as *Parkia speciosa* [25], *Rubus suavissimus* [24], *Heliotropium ellipticum* [26], *Butea monosperma* [27], *Calotropis gigantea* [28]. However, the stigmasterol compound was isolated from the *C. alata* plant species for the first time in our laboratory and the chemical structure of the isolated stigmasterol compound shown in Fig. **6.**

Stigmasterol is reported to exhibit a spectrum of pharmacological activities against various disease conditions. These include conditions such as arthritis, cardiovascular ailments, renal disorder, inflammation, diabetes, microbial infections, hepatic toxicity, and cancer. Earlier reports by many investigators have proven that the biological effects of stigmasterol to have anti-inflammatory, inhibiting tumor promotion, anti HIV reverse transcriptase. Furthermore, literature has been reported that the stigmasterol possess the potent antioxidant, hypoglycemic, thyroid inhibiting properties, anti-tumor effect against ovarian skin and breast cancer cells [25,27].

Table 1. Cytotoxic activity grade and their description

Grade	Activity	Description of reactivity zone
0	None	No detectable zone around or under specimen
1	Slight	Some malformed or degenerated cell under specimen
2	Mild	Zone limited to area under specimen
3	Moderate	Zone extending specimen size upto 1cm
4	Severe	Zone extending farther than 1 cm beyond specimen

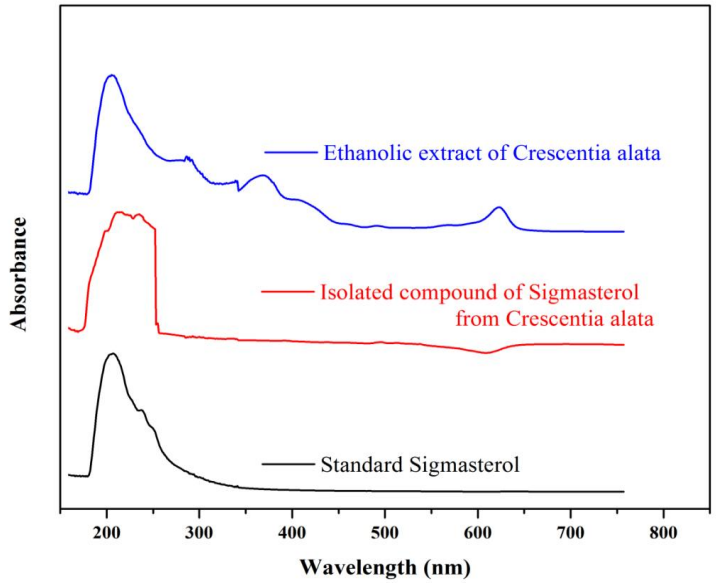

Fig. 2. UV spectra comparison of standard, isolated compound and ethanolic extract of *C. alata* (200-800 nm)

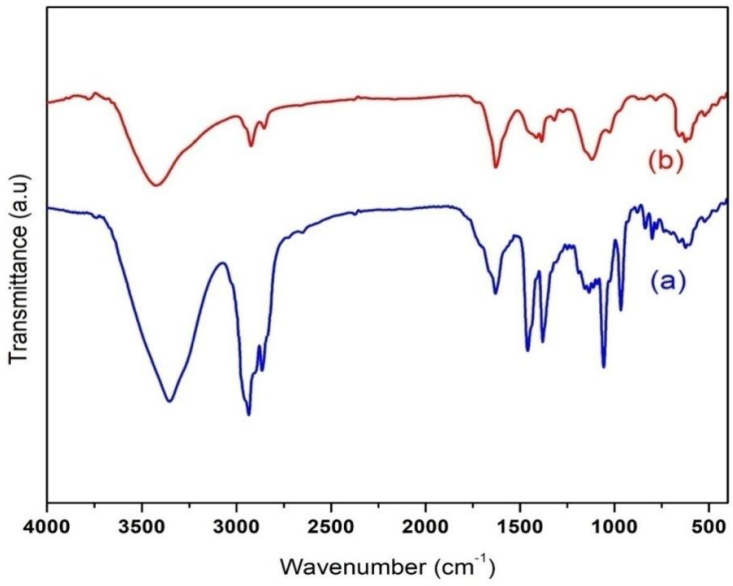

Fig. 3. FT-IR spectra of standard stigamasterol (a) and compound isolated (b)

3.3 *Invitro* Biological Studies

3.3.1 Biological potential of the crude extract, partitioned fractions and isolated compound

The results of the MIC, MBC and MFC values are demonstrated by the methanolic crude extract, fractions and isolated compound from the leaf part of *C. alata* against bacterial and fungal strains are presented in Table 2. The results showed MIC values less than 1000 µg/mL had good antimicrobial activity [29-30]. The antibacterial activity against Gram-positive and Gram-negative strains of crude extract and fractions exhibited a broad spectrum of activities with MIC values ranging from 98 to 3250 mg/mL with the exception of hexane fraction that was only active against *Bacillus subtilis* with (390

µg/mL). Overall, the most active with significant antibacterial activity in this study was demonstrated by the ethyl acetate fraction at a MIC of 98 to 390 µg/mL against *Bacillus subtilis*. This led to the selection of the EA fraction of *C. alata* for isolation of compound responsible for good antimicrobial activity observed. The isolated compound stigmasterol also demonstrated a strong antibacterial effect with MIC ranging from 1.95 to 125 µg/mL against all the bacterial strains were tested. Particularly, the compound was the most active and it was highly inhibitory with a MIC value of 1.95µg/ mL against *Bacillus subtilis* of the three bacterial strains tested.

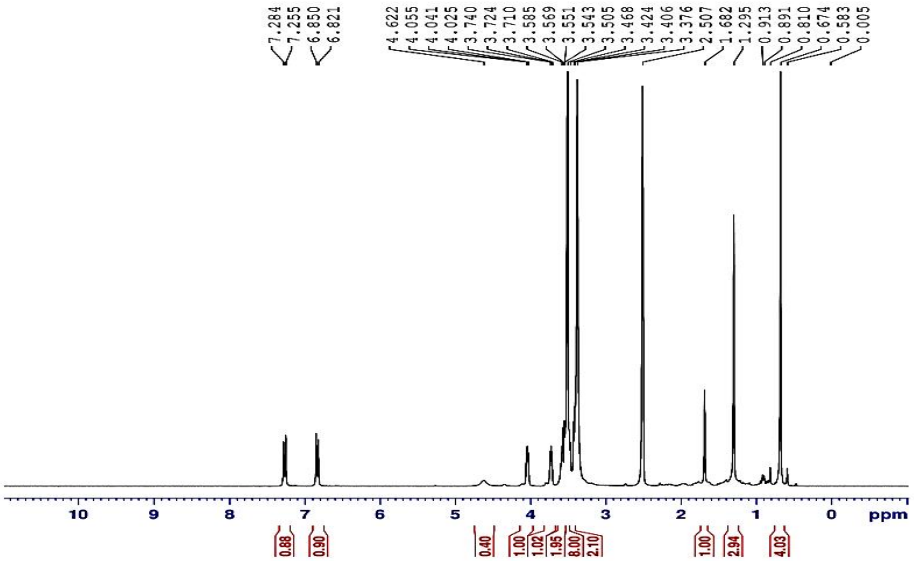

Fig. 4. ^{1}H-NMR spectrum (400 MHz, *DMSO-d_6*) of compound isolated from leaf of *C. alata*

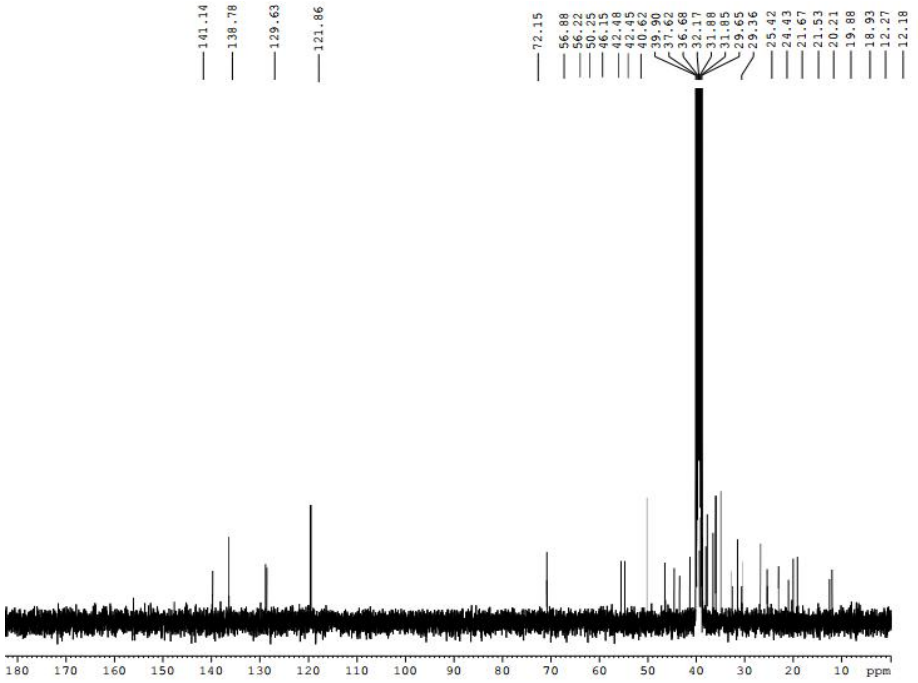

Fig.5. ^{13}C-NMR spectrum (100 MHz, *DMSO-d_6*) of compound isolated from leaf of *C. alata*

Table 2. Antimicrobial activity of crude extract, fractions and isolated compound from the leaves of *C. alata.* The MIC values less than 1000 and 100 µg/mL for crude extract, fractions and isolated compound respectively

Microorganisms	Activity	LCE	Fractions				ICS	Positive control Chloramphenicol (Bacteria) and Fluconazole (Fungal)	
			CL	EA	HE	BU			
Bacterial strains									
Staphylococcus aureus	MIC	780	780	390	1560	390	62.5	1.56	
	MBC	1560	1560	780	3250	780	125	4.9	
Bacillus	MIC	195	98	98	390	195	1.9	1.56	
subtilis	MBC	390	195	195	780	390	3.9	0.39	
Escherichia coli	MIC	195	780	390	1560	780	3.9	0.39	
	MBC	390	1530	780	3250	1560	7.81		2.45
Fungal strains									
Candida albicans	MIC	780	390	390	1560	390	3.9	0.15	
	MFC	1560	390	780	3250	780	3.9	12.4	
Aspergillus flavus	MIC	390	195	98	195	195	3.9	1.56	
	MFC	780	390	195	780	390	7.81	6.24	
Aspergillus niger	MIC	390	1560	195	1560	1560	125	3.12	
	MFC	780	3250	390	3250	3250	250	6.24	

MIC, minimum inhibitory concentration; MBC minimal bactericidal concentration; MFC, minimum fungicidal concentration; LCE, leaf crude extract; CL, chloroform; EA, ethylacetate; HE, hexane; BU, butanol; ICS, isolated compound stigmasterol

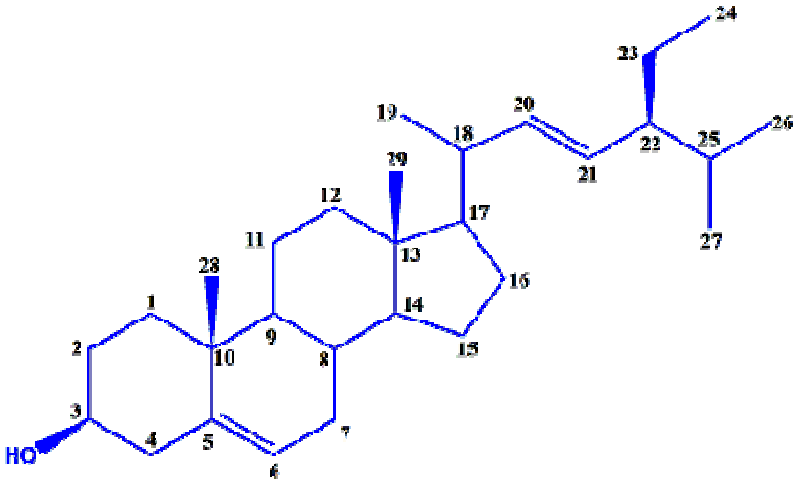

Fig. 6. Chemical structure of the isolated compound stigmasterol (Molecular Formula: $C_{29}H_{48}O$) from the leaf of *C. alata*

The leaf crude extract and fractions of *C. alata* demonstrated some degree of MIC with the value of 98-3250 µg/mL) of antifungal activity Table 2. Relative to the crude extract, the partition fractions had better inhibition against *Aspergillus flavus* among various fungal strains tested with MIC value ranging from 98 to 390 µg/mL. In addition, as shown in Table 2, the ethyl acetate fraction was the most effective noteworthy MIC values and active in terms of the fungicidal effect with the MFC value of 98 µg/ mL against *Aspergillus flavus*. Apart from the hexane fraction with MIC of 125 µg/mL, the isolated compound stigmasterol had effective with MIC value of 3.9-7.8 µg/mL fungistatic (MIC) and fungicidal (MFC) effects against *Candida albicans* and *Aspergillus flavus.* Overall, the stigmasterol compound was the most active in terms of the antibacterial and antifungal potential.

3.3.2 Cytotoxicity studies (*in-vitro*) of isolated bioactive compound Stigmasterol from *C. alata*

The cytotoxicity of the stigmasterol isolate compound assessed against HeLa (mammalian cancer) cell line using MTT assay. We examined the isolated stigmasterol compound at four final concentrations such as 25, 50, 75 and 100 µg at 37 ± 1 ºC for 24 h. Suitable positive control was run and this experiment was repeated thrice. Fluorescence morphology examination of cell viability of control cells and treated HeLa cancer cells are represented in Fig. 7. From the results a significant change in cell viability is observed to change in concentration of the stigmasterol compound. Fig. 7(c-d). shows that at higher concentrations (75 and 100 µg) the most of cells zone were expanding farther 1 cm beyond than specimen, which shows the severe cytotoxic activity of the stigmasterol. Fig. 7(a-b) shows that for concentration of 25 and 50 µg, the cells are zone round-shaped and loosely attaching degenerated cells under the specimen are observed, which shows slight cytotoxic action against the cancer cells. The control gave none cytotoxic activity as expected. Based on the response around the test sample, the cytotoxic activity was graded, as appeared Table 3. The achievements of numerical grade greater than 2 are considered as cytotoxic effect. Since the test sample achieved a numerical grade greater than 2 in 75 and 100 µg, it reveals that the isolated stigmasterol compound is considered as a best cytotoxic agent at higher concentration.

Table 3. Cytotoxic reactivity grade for the isolated stigmasterol from leaves of *C. alata*

Sample	Grade	Activity
Control	0	None
Isolated Stigmasterol 25 µg	1	None
Isolated Stigmasterol 50 µg	1	Slight
Isolated Stigmasterol 75 µg	4	Severe
Isolated Stigmasterol 100 µg	4	Severe

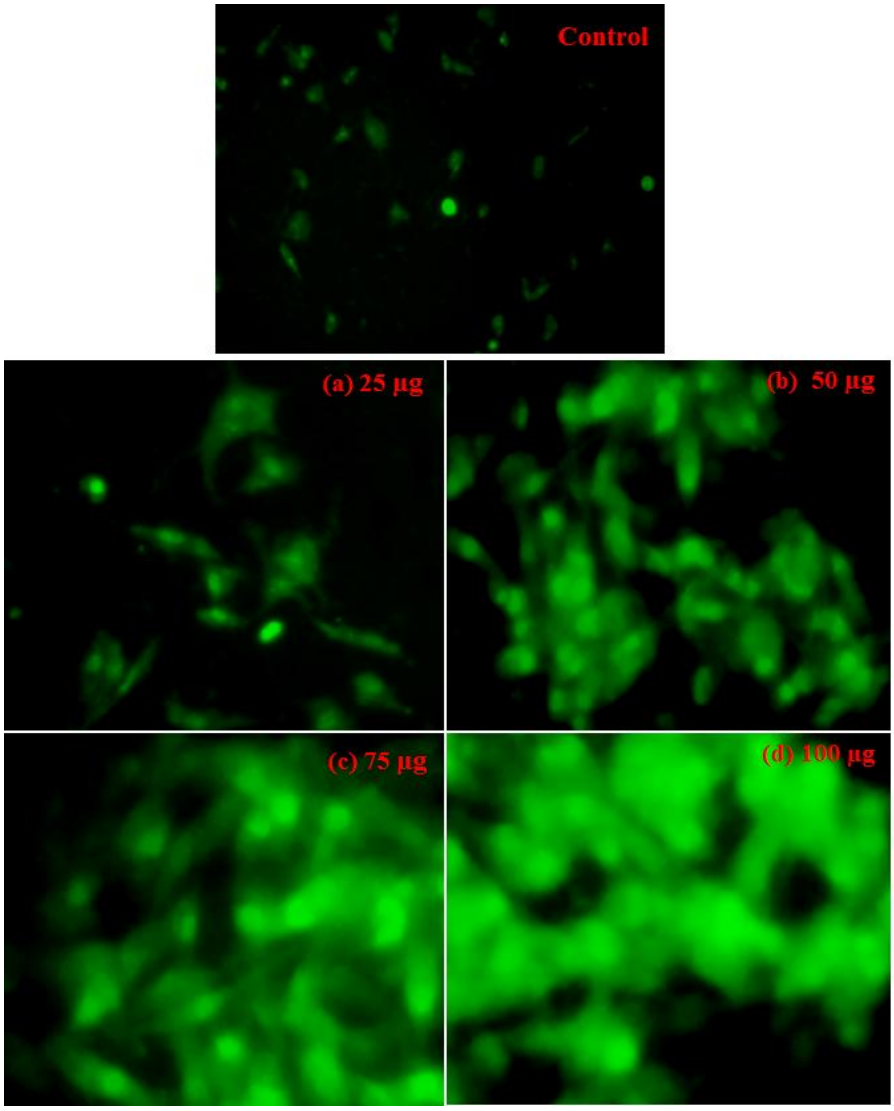

Fig. 7. Fluorescence images of control and different concentration of treated HeLa cancer cells line on isolated Stigmasterol compound from the leaves of *C. alata*.

4. CONCLUSION

In the present study, we have isolated the bioactive compound stigmasterol, a well known phytosterol from the leaves of *C. alata* is belonging to the family *Bignoniaceae*. From these physical, chemical and spectral analytic evidences the isolated phytoconstituent, was affirmed as stigmasterol. To the best of our knowledge, this is the first ever report of steroidal compound from the leaf of this plant. Further, the biological activities of the isolated compound flash more light in the pharmacological efficacy. Pharmacological investigations, such as antimicrobial activity and anticancer activity were undertaken on the of isolated stigmasterol bioactive compound from *C. alata* Kunth established their efficacy and to support its medicinal significance. The antimicrobial investigations demonstrated that the stigmasterol possessed effective antibacterial and antifungal properties against both bacterial and fungal strains. The cytotoxicity studies revealed that the stigmasterol showed strong cytotoxic activity against Hela (mammalian cancer) cell line. Our findings suggest that the bioactive stigmasterol from the plant *C. alata* possesses antimicrobial and anticancer potential against pathogenic microorganisms and cancer cell lines respectively. Thus the present bioactive natural

compound recommended for various therapeutic uses to formulation of drugs. This study can be useful to discover bioactive natural products that may serve as a lead compound for the development of new pharmaceutical drugs. The results could justify the use of the leaf parts of *C. alata* in traditional herbal medicine for the treatment of various diseases. So, the present scientific investigation work will enhance the scientific communities to do more work on this important medicinal plant in near future. However, Besides the findings, it would be more appropriate to enhance further researches are required for clinical applications to explore the exact mechanism of action and its pharmacological evaluation by using suitable animal models for improving the plant based drug and the development of new medications of natural source.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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