

# Isolation of novel phytoconstituents from the stem part of *Cleome gynandra* Linn and their antimicrobial activity

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## Abstract

Dried and powdered stem of *Cleome gynandra* Linn were extracted with methanol and chloroform solvents. From the extract, four individual components were identified using UV-Visible Spectrophotometer, FT-IR, GC-Mass and NMR technique. The extracted components were isolated as pure form using Silicagel-G column chromatography. Identity of these four novel compounds such as  $\beta$ -amyirin,  $\beta$ -Amyrin-3-O- $\beta$ -Glucopyranoside, Stigmasetrol and Sitosterol was confirmed by spectral interpretation. These compounds were tested for their antimicrobial activity and showed inhibition against all the tested bacterial and fungal cultures.

**Keywords:** *Cleome gynandra* Linn, Antibacterial and antifungal activities, phytoconstituents, Spectral interpretation.

## Introduction

Medicinal plants possess various pharmacological activities which has wide application in the field of pharmaceuticals [1]. *Cleome gynandra* (*Capparidaceae*), a common weed grows throughout India, West Africa, Tanzania, Uganda, and Nigeria. It is an erect herbaceous annual herb which has long been used as houses hold remedy for a variety of ailments including inflammation [2]. High amounts of proteins, vitamins (A and C) and minerals are found in the leaves of this plant. Leaves and seeds of *Cleome gynandra* are used for earache, epileptic fits, stomachache, constipation and inflammation in most of the countries [3-6], fresh leaves of *Cleome gynandra* are used in ayurveda and siddha medicine for a variety of disease conditions [7]. It is used as an anthelmintic in ayurveda for ear diseases, pruritis and several other diseases like gastro intestinal disorders and gastrointestinal infections etc [8]. The phytochemical studies revealed that it contains several constituents like Carotenoids, Cardiac glycosides, Cyanogenic Glycosides, Flavonoids, Saponins, Triterpenes, sugars, Tannins etc [9].

The *Cleome gynandra* seed contains the presence of oleic acid, linolic acid, palmitic acid, stearic acid, arachidic acid and a phytosterul have been reported. Amino acid content of *Cleome gynandra* was analyzed in an experimental study [10] and reports the presence of glutamic acid, arginine, aspartic acid, leucine, valine, glycine, proline, phenylalanine, isoleucine lysine, tyrosine and histidine. In the rural areas of Kenya, *Cleome gynandra* had been used to cure scurvy [11]. In other countries, the leaves will be boiled and taken as nutritious meal and known to improve eyesight, provide energy and cure marasmus, which is highly recommended for pregnant and lactating women. In Taiwan, *Cleome gynandra* is used as a medicine for dysentery, gonorrhoea, malaria, rheumatoid arthritis. In India, the plant has been used as anthelmintic and

rubefacient. Free radical scavenging activities [9] of this plant was reported previously.

Anticancer activity of *Cleome gynandra* was tested in Swiss albino mice and found that it has dose dependent activity. Based on the pharmacological analysis, it has known to possess immunosuppressant activity in the ethanolic and aqueous extract. Even though, the hypoglycemic properties had not studied scientifically, it is believed to have Antidiabetic activity with the efficacy to lower the blood sugar level. Ancient times, the whole plant was used to treat tumor, inflammation and lysosomal stability actions. A report [12] showed it has dose dependent anticancer activity comparable to 5-fluorouracil.

However the antimicrobial property has not reported so far hence the present work deals with the isolation of novel compounds, characterization, structural analysis and its antimicrobial activity.

## Materials and Methods

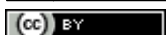
### Reagents

Chemicals used in the experiments were purchased from Sigma Aldrich chemicals Co (St. Louis, USA). Muller Hinton and Sabouraud dextrose medium were procured from Himedia (Mumbai).

### Plant material

The plant *Cleome gynandra* was collected near the rural region of Vellore district, Tamilnadu, India in the year 2012. The voucher specimen was deposited in the Herbal garden, VIT University for future reference.

### Fractionation and isolation



The stem of *Cleome gynandra* was separated and dried under the shade [13]. It was powdered (250g) and extracted with methanol and chloroform in a soxhlet extractor for 12-15hr at room temperature. The extracts were concentrated under reduced pressure and controlled temperature (40-50°C) in a rotavapor. About 2.98g of crude plant extract with methanol and chloroform was obtained, partitioned and chromatographed over silica column, eluted with chloroform and methanol (8:2) with increasing polarities. The column is packed with silica gel 60 mesh in n-hexane by wet method. The crude extract in chloroform-methanol extract is fractionated using solvents of different polarity viz., Hexane:Ethyl acetate (9.5:0.5), Hexane: Ethyl acetate (9:1), Hexane: Ethyl acetate (8:2). The crude n-hexane extract is separated into various compounds by column chromatography. About 10 fractions are collected and they are reduced into four fractions based on their  $R_f$  values.

### UV-Visible

Ultraviolet Spectra were recorded on a Hitachi 2800 Double Beam UV-Visible Spectrophotometer with a range of 190-1100 nm.

### FTIR

FTIR spectra were registered in a spectral range of 4000-400  $\text{cm}^{-1}$  using Thermo Nicolet Avatar FTIR Spectrum 330 Spectrometer. The samples were added with KBr and pressed into thin pellets (1:100).

### Gas Chromatography–Mass Spectrometry (GC-MS)

GC-MS analysis was done using MSD/DS spectrometer (Agilent, India), which was equipped with mass selective detector, a diffusion pump and a capillary PH Innowax column (30m 250 $\mu\text{m}$  0.50  $\mu\text{m}$ ). Gradient temperature was 150-250 C at heating rate equal to 2 C/min. Helium with chromatography grade was used as a carrier gas at a flow rate of 1ml/min.

### NMR Spectroscopy

VXR 500S NMR spectrometer (Varian, India) was used to record  $^1\text{H}$  NMR spectra at an operating condition of 500 MHz. 1% DMSO was used as a solvent to record the spectra. Chemical shifts are relative to TMS.

### Antimicrobial Activity

The extracted compounds were screened for Antibacterial activity [14] against 2 bacterial strains and 2 fungal strains. The test organisms were *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922); *Candida albicans* (MTCC 227) and *Candida glabrata* (MTCC-3019) respectively. The compounds (10mg) were dissolved in DMSO (Dimethyl Sulfoxide) to prepare a stock solution of 1000  $\mu\text{g/ml}$  from which concentrations of 25, 50, and 100 $\mu\text{g/ml}$  were prepared for the determination of minimum inhibitory concentration (MIC). The standard control comprises of

the medium, organism culture and dilution of similar order of standard drug.

### Preparation of inoculums

The stock cultures were stored at 4 C on nutrient agar slopes. The active cultures, before the experiments, were made by taking a loopful of individual colony from the stock cultures to conical flask containing mueller-hinton broth (MHB) for bacteria and sabouraud dextrose broth (SDB) for fungi. After incubation, the flasks were incubated for 24 hrs at 37 C for bacteria and 25 C for fungi. After the cells grown, the cultures were diluted to obtain an optical density of  $2.0 \times 10^6$  colony forming units (CFU/ml) for bacteria and  $2.0 \times 10^5$  spore/ml for fungal strains.

### Antimicrobial Susceptibility Test

For antimicrobial screening, disc diffusion method [15] has been used to screen the antimicrobial activity. Mueller Hinton agar (MHA) was sterilized and poured in sterile petriplates. After that the plates were allowed to solidify, bacterial and fungal cultures of 0.1 ml was swabbed uniformly on the molten medium and allowed to dry for 5 min. sterile disc was loaded with different concentrations of plant extracts (100, 50 and 25 mg/disc). Then the disc was placed on the medium and kept for 5 min for the compounds to diffuse. After that, the plates were incubated at 37 C for 24 hrs. After incubation, zone of inhibition around the disc was measured and expressed in millimeter. Zone of inhibition against fungus was also tested by the same procedure. Studies were performed in triplicate for conformation. For Comparison, Trimethoprim (MIC -1mg/ml) was used as a standard drug. Antifungal testing was done in sabouraud dextrose agar broth (pH-6.0); the sterile medium was dispensed in a series of tubes. Test solution was added to attain a final concentration of 100, 50 and 25  $\mu\text{g/ml}$  and 100  $\mu\text{l}$  of test fungus was added and incubated at 28-30°C in dark. Visual examination was carried out to determine the presence/absence of growth. For Comparison, Miconazole (MIC-6.25mg/ml) was used as a standard drug.

### Results and Discussion

*Cleome gynandra* methanol and chloroform extracts of stem were partitioned with methanol/chloroform. The crude plant extracts underwent purification in Silica gel-G column chromatographic technique. Twenty fractions were collected and reduced into four groups. The four fractions were subjected to spectral analysis. The isolated bioactive compounds were identified using UV-Visible, FTIR, GC-Mass and NMR spectral analysis. The spectral data shows that the presence of  $\beta$ -amyrin,  $\beta$ -Amyrin-3-O- $\beta$ -Glucopyranoside, Stigmasetrol and Sitosterol in the extracts of *Cleome gynandra*. Identification of the structures of isolated compounds was presented in Fig. 1 and 2. To the best of our knowledge, this is the first report on the presence of these compounds in *Cleome gynandra*.

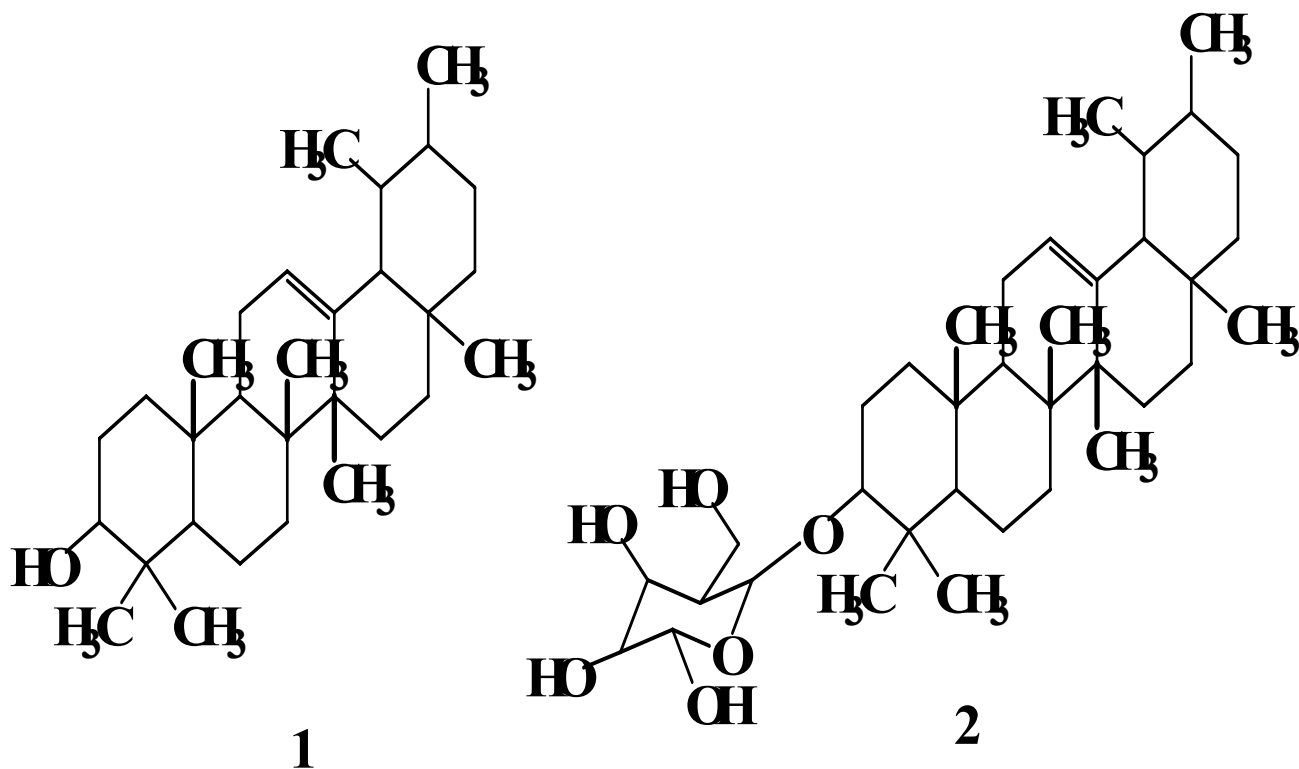


Figure. 1: Bioactive compounds extracted from *Cleome gynandra*. Compound (1)  $\beta$ -Amyrin Compound (2)  $\beta$ -Amyrin-3-O- $\beta$ -D-Glucopyranoside. The compound characterization was analyzed with UV, FTIR, GC-MS and NMR.

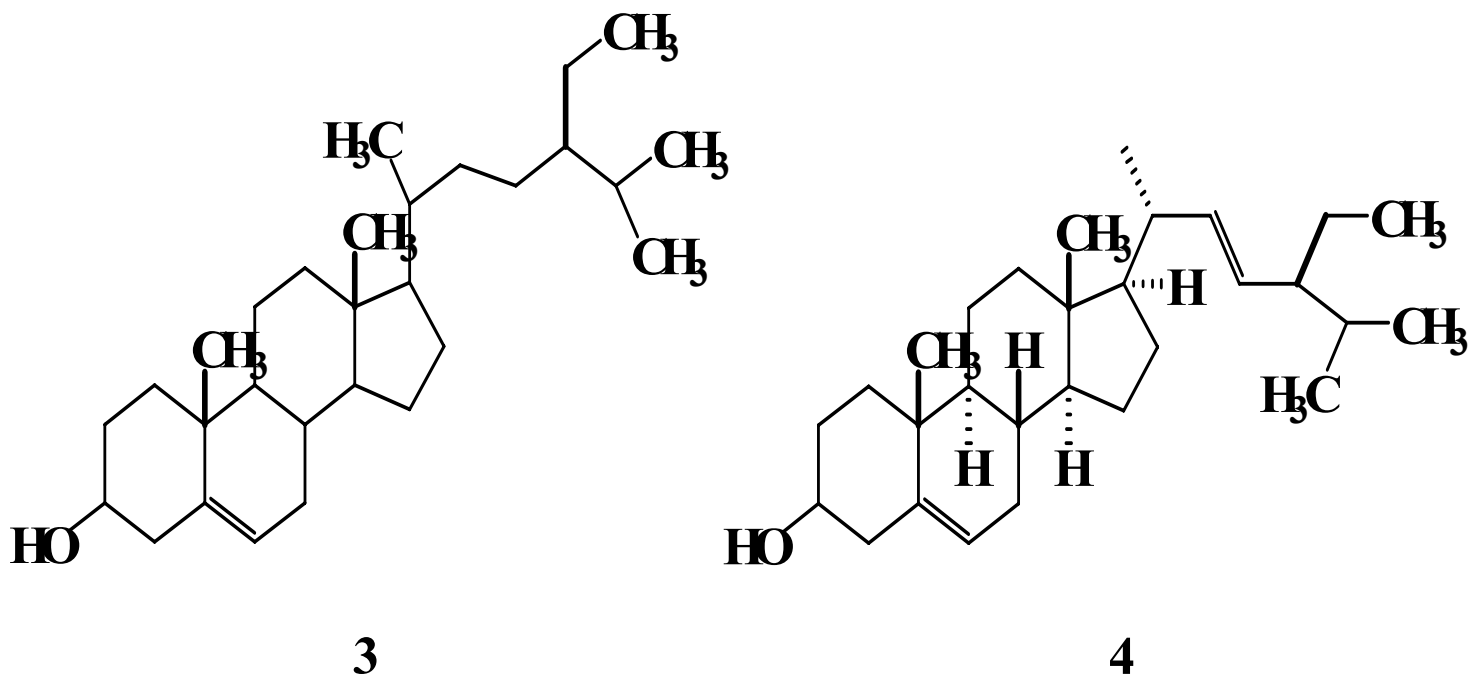


Figure. 2: Bioactive compounds extracted from *Cleome gynandra*. Compound (3) Sitosterol Compound (4) Stigmasterol. The compound characterization was analyzed with UV, FTIR, GC-MS and NMR.

Compound (1):  $\beta$ -Amyrin - Molecular Formula;  $C_{30}H_{50}O$ , Molecular Weight; 426. UV-Visible ( $CHCl_3$ )  $\lambda$  nm (A); 421(0.849), 444 (0.867), 473 (1.016). FTIR ( $CHCl_3$ , KBr,  $\nu_{max}$ ,  $cm^{-1}$ ); 3511, 3056, 1636, 822. EIMS m/z (Re. int); 426 [ $M^+$ ]  $^1H$ NMR (DMSO, 500MHz):  $\delta$  5.11 (m, H-12), 1.08 (s, H-27), 1.02 (s, H-26), 1.01 (s, H-23), 0.96 (s, H-25), 0.88 (s, H-29 and H-30), 0.93 (s, H-28) and 0.80 (s, H-24).

Compound (2):  $\beta$ -Amyrin-3-O- $\beta$ -Glucopyranoside - Molecular Formula;  $C_{36}H_{60}O_6$ , Molecular Weight; 588. UV-Visible ( $CHCl_3$ )  $\lambda$  nm (A); 424(0.879), 446 (0.888), 475 (1.010). FTIR ( $CHCl_3$ , KBr,  $\nu_{max}$ ,  $cm^{-1}$ ); 3517, 3059, 1632, 828. EIMS m/z (Re. int); 588 [ $M^+$ ]  $^1H$  NMR (DMSO, 500MHz):  $\delta$  0.73, 0.78 (s, 3H), 0.89 (s, 6H), 0.97, 1.01, 1.26 (3H, s), 3.02 (s, H-3), 5.17 (br-s, 1H, H-12), 2.75 (br-s, 1H).

Compound (3): Sitosterol - Molecular Formula;  $C_{29}H_{50}O$ ; Molecular Weight; 414; UV-Visible ( $CHCl_3$ )  $\lambda$  nm (A); 234 (0.510), 408 (0.990), 670 (2.693). FTIR ( $CHCl_3$ , KBr,  $\nu_{max}$ ,  $cm^{-1}$ ); 3424, 2935, 2867, 2363, 1654, 1382, 1241, 1192, 1133, 1107, 1062. EIMS m/z (Re.int); 414 [ $M^+$ ].  $^1H$ NMR (DMSO, 500MHz): 3.53 (dd, 1H, C-3); 5.36(t, 1H, C-5); 0.93 (d, 3H, C-19); 0.84 (t, 3H, C-24); 0.83 (d, 3H, C-26); 0.81 (d, 3H, C-27); 0.68 (s, 3H, C-28); 1.01 (s, 3H, C-29).

Compound (4): Stigmasterol - Molecular Formula;  $C_{29}H_{48}O$ ; Molecular Weight; 412. UV-Visible ( $CHCl_3$ )  $\lambda$  nm (A); 235 (0.515),

410 (0.995), 672 (2.697). FTIR ( $CHCl_3$ , KBr,  $\nu_{max}$ ,  $cm^{-1}$ ); 3400, 3025, 1410, 1250, 820. EIMS m/z (Re.int); 412 [ $M^+$ ].  $^1H$ NMR (DMSO, 500MHz): 3.51 (dd, 1H, C-3); 5.31 (t, 1H, C-5); 0.91 (d, 3H, C-19); 4.98 (m, 1H, C-20); 5.14 (m, 1H, C-21); 0.83 (t, 3H, C-24); 0.82 (d, 3H, C-26); 0.80 (d, 3H, C-27); 0.71 (s, 3H, C-28); 1.03 (s, 3H, C-29).

The results were shown in the Table. 1. Trimethoprim and miconazole were used as standard for antimicrobial activities. The results showed that, the isolated compounds  $\beta$ -Amyrin,  $\beta$ -Amyrin-3-O- $\beta$ -Glucopyranoside, Stigmasterol and Sitosterol could able to inhibit the tested microbes to some extent. Promising activity was seen against *S.aureus* and *C.albicans*, moderate activity was against *E.coli* and *C.glabrata*. Similar to previous reports, methanol extracts of the plant possess good antimicrobial activity [15-21]. There were no reports on *Cleome gynandra* antimicrobial activity. Yet, other *Cleome* species had been tested for antimicrobial activity [22] and found that the methanol extract of the plant showed better activity against *B.subtilis* and *E.coli*. Another study [23] reported good activity against *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa* by methanolic extracts of *Cleome viscosa*. According to our extensive literature survey, this is the first report on *Cleome gynandra* antimicrobial activity.

**Table 1: Antimicrobial activity of the novel compounds isolated from *Cleome gynandra***

S.No	C	Microorganisms											
		Zone of Inhibition (mm)											
		Concentration (mg/ml)											
		<i>S.aureus</i>			<i>E.coli</i>			<i>C.albicans</i>			<i>C.glabrata</i>		
25	50	100	25	50	100	25	50	100	25	50	100		
1.	C <sub>1</sub>	16	19	29	13	16	27	13	15	19	11	19	26
2.	C <sub>2</sub>	11	14	27	9	11	24	16	18	22	6	14	23
3.	C <sub>3</sub>	15	17	25	18	21	33	12	17	34	10	13	21
4.	C <sub>4</sub>	13	16	21	14	17	27	15	24	36	9	17	29

\* C-Isolated Compounds: C1-  $\beta$ -amyrin; C2-  $\beta$ -Amyrin-3-O- $\beta$ -Glucopyranoside; C3-Stigmasterol; C4-Sitosterol; Standards used for antimicrobial activity: Trimethoprim (antibacterial); Miconazole (Antifungal).

## Conclusion

The present investigation reports the isolation of four compounds from the stem part of the *Cleome gynandra* (An Indian Medicinal Plant). To ascertain the therapeutic value, the application of the isolated compounds was tested upon some selected microorganisms and the findings towards inhibition of microorganisms were correlated with a standard drug. The observed result allows us to conclude that the compounds exhibited good antimicrobial activities and can be further developed for application as effective antimicrobial agent. Apart from this, the

present study also scientifically supports the therapeutic use of plant materials by indigenous people against a number of infections since generations. These plants could serve as useful sources for new antimicrobial agents.

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