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Phytochemical Investigation of *n*-Hexane Extract of Leaves of *Cleome gynandra*

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Dried and powdered leaves of *Cleome gynandra* were extracted using *n*-hexane solvent. From this extract, four individual components were identified using GC-Mass technique. The identified components were isolated in their pure form using column chromatography. The identity of these 4 isolated components was confirmed by spectral interpretation. These isolated components showed inhibitory activity against various bacteria and fungi.

Key Words: *Cleome gynandra*, Phytochemical investigation, GC-Mass, Column chromatography, Inhibitory activity.

INTRODUCTION

Cleome gynandra (Capparidaceae), a common weed that grows throughout India, West Africa, Tanzania, Uganda and Nigeria is an erect herbaceous annual herb which has long been used as a house hold remedy for a variety of aliments including inflammation[1]. The leaves contain excessive amounts of proteins, vitamins (A & C) and minerals. The leaves and seeds of *Cleome gynandra* are used in many countries for ear ache, epileptic fits, stomach ache, constipation and inflammation[2-5], fresh leaves of *Cleome gynandra* are used in Ayurveda and Siddha medicine for a variety of disease conditions[6]. The claimed medicinal effects of *Cleome gynandra* have not been investigated using controlled experiments in detail. Hence an attempt has been made to identify the presence of bioactive compounds and its antimicrobial activity in the plant material of *Cleome gynandra*.

EXPERIMENTAL

The leaves of the plant *Cleome gynandra* are well know and were obtained from a rural region of vellore district, Tamil Nadu, India and the plant was identified by one of the authors, Dr. P.N. Sudha, Department of Chemistry, D.K.M. College for Women's, Vellore and the voucher specimen of the plant is being retained in the department herbarium (No. 1228).

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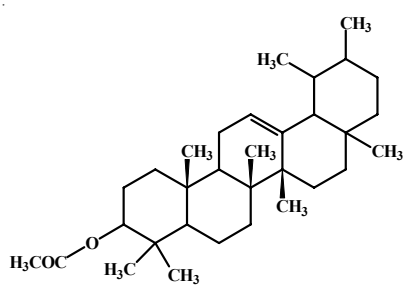
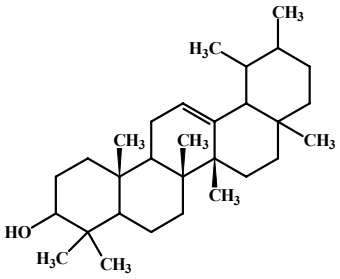
Infrared spectra were measured in a KBr pellet on a Thermo Nicolet Avatar FTIR spectrum 330 spectrometer. EIMS were recorded on a Shimadzu GC-MS QP2010 spectrometer. Ultra-violet spectra were recorded on a Hitachi 2800 double beam UV-Visible spectrophotometer.

Extraction and isolation of compounds: Air-shade dried powdered material of *Cleome gynandra* (250 g) was extracted in a soxhlet extractor for 15 h using *n*-hexane solvent (40-60). Solvent was removed under reduced pressure. About 3.08 g of crude *n*-hexane extract was fractionated by column chromatography over silica gel to yield α-amyrin acetate (**1**), α-amyrin (**2**), sitosterol (**3**) and stigmasterol (**4**).



(**1**)



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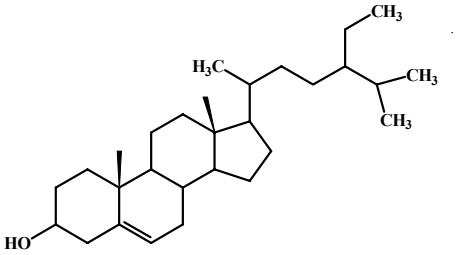
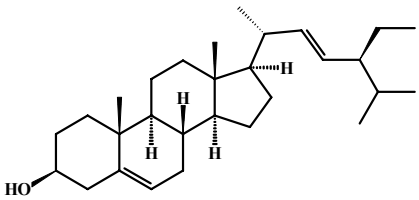


(**3**)



(**4**)

α-Amyrin acetate (1): m.f. $C_{32}H_{52}O_2$, m.w. 468, UV-Visible ($CHCl_3$) λ nm (A); 216 (0.969), 412 (1.040), 671 (3.082). FTIR ($CHCl_3$, KBr, ν_{max}, cm^{-1}); 3058, 1712, 1660, 1462, 1384, 1182, 812. EIMS m/z (Re. int); 468 $[M^+]$ (27), 426 $[M\text{-}Ac]^+$ (35), 411 $[M\text{-}Ac\text{-}Me]^+$ (11), 408 $[M\text{-}Ac\text{-}H_2O]^+$ (12), 257 $[M\text{-}C_{11}H_{21}O]^+$ (26), 218 $[M\text{-}C_{14}H_{24}O]^+$ (100), 207 $[M\text{-}C_{16}H_{27}O]^+$ (8), 203 $[M\text{-}C_{15}H_{27}O]^+$ (55), 189 $[M\text{-}C_{16}H_{29}O]^+$ (75).

α-Amyrin (2): m.f. $C_{30}H_{50}O$, m.w. 426, UV-Visible ($CHCl_3$) λ nm (A); 421 (0.849), 444 (0.867), 473 (1.016). FTIR ($CHCl_3$, KBr, ν_{max}, cm^{-1}); 3511, 3056, 1636, 822. EIMS m/z (Re. int); 426 $[M^+]$ (15), 411 $[M\text{-}Me]^+$ (18), 408 $[M\text{-}H_2O]^+$ (16), 393 $[M\text{-}Me\text{-}H_2O]^+$ (32), 257 $[M\text{-}C_{11}H_{21}O]^+$ (20), 218 $[M\text{-}C_{14}H_{24}O]^+$ (100), 207 $[M\text{-}C_{16}H_{27}O]^+$ (10), 203 $[M\text{-}C_{15}H_{27}O]^+$ (40), 189 $[M\text{-}C_{16}H_{29}O]^+$ (55).

Sitosterol (3): m.f. $C_{29}H_{50}O$; m.w. 414, UV-Visible ($CHCl_3$) λ nm (A); 234 (0.510), 408 (0.990), 670 (2.693). FTIR ($CHCl_3$, KBr, ν_{max}, cm^{-1}): 3424, 2935, 2867, 2363, 1654, 1465, 1382, 1241, 1192, 1133, 1107, 1062. EIMS m/z (Re. int); 414

$[M^+]$ (75), 396 $[M\text{-}H_2O]^+$ (20), 274 $[M\text{-}C_{19}H_{30}O]^+$ (30), 258 $M\text{-}Me\text{-}C_{19}H_{30}]^+$ (25), 122 $[M\text{-}C_9H_{14}]^+$ (10), 82 $[M\text{-}C_6H_{10}]^+$ (15).

Stigmasterol (4): m.f. $C_{29}H_{48}O$; m.w. 412, UV-Visible ($CHCl_3$) λ nm (A); 235 (0.515), 410 (0.995), 672 (2.697). FTIR ($CHCl_3$, KBr, ν_{max}, cm^{-1}); 3400, 3025, 1410, 1250, 820, 690. EIMS m/z (Re. int); 412 $[M^+]$ (75), 397 $[M\text{-}Me]^+$ (10), 394 $[M\text{-}H_2O]^+$ (20), 379 $[M\text{-}Me\text{-}H_2O]^+$ (28), 369 $[M\text{-}C_3H_7]^+$ (35), 351 $[M\text{-}C_3H_7\text{-}H_2O]^+$ (70), 300 $[M\text{-}C_8H_{16}]^+$ (67), 327 $[M\text{-}H_2O\text{-}C_5H_7]^+$ (60), 301 $[M\text{-}H_2O\text{-}C_7H_9]^+$ (18), 273 $[M\text{-}H_2O\text{-}C_9H_{17}]^+$ (30), 270 $[M\text{-}C_{10}H_{21}]^+$ (25).

Antimicrobial activity: The isolated components were screened for their antimicrobial activity[7], *Staphylococcus aureus, E. coli, Candida albicans* and *Candida glabrata* were used for this antimicrobial activity determination. The test material (10 mg) was dissolved in dimethyl sulfoxide to prepare a stock solution of 1000 mcg/mL from which concentrations of 25, 50, 100 and 200 mcg/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.

Antibacterial testing: For antibacterial testing the tube dilution technique was used. Muller Hinton Broth (pH-7.4) was used as a culture medium. This was sterilized and suspended in series of borosilicate test tubes. The test solution was then added, so as to attain a final concentration of 200, 100, 50 and 25 mcg/mL. Then 0.1mL of test organism strain 10^6 cfu/mL was added. These tubes were incubated at 37 ºC for 48 h and then examined, for the presence (or) absence of growth of microorganisms. For comparison, trimethoprim (MIC- 1 mcg/mL) was used as a standard drug.

Antifungal testing: For antifungal testing, Saboraud Dextrose Agar medium (pH 6.0) was employed for growth. The sterile medium was dispensed in a series of borosilicates test tubes. The test solution was then added, so as to attain a final concentration of 200, 100, 50 and 25 mcg/mL. Then 0.1 mL of test organism strain was added. The tubes were incubated at 28-30 ºC in a dark place. Visual examination was carried out for determining the presence (or) absence of growth of microorganisms. For comparison, miconazole (MIC-6.25 mcg/mL) was used as a standard drug. The results of this antimicrobial activity have been listed in the Table-1.

TABLE-1
ANTIMICROBIAL ACTIVITY

Compound	Microorganisms zone of inhibition (mm)			
	S. aureus	*E. coli*	*C. albicans*	*C. glabrata*
α-Amyrin acetate	14	10	12	8
α-Amyrin	13	8	14	7
Sitosterol	14	15	13	10
Stigmasetrol	15	13	14	9
Trimethoprim (Standard)	24	22	–	–
Miconazole (Standard)	–	–	24	13

RESULTS AND DISCUSSION

The column is packed with silica gel 60 mesh in *n*-hexane by wet method. The crude extract in *n*-hexane 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 20 fractions are collected and they are reduced into four fractions based on their R_f values.

All the four fractions collected from the column chromatographic technique were subjected to spectral analysis. The isolated four bioactive compounds are identified by using UV-Visible, FTIR and GC-Mass spectral data's. The spectral datas shows that the presence of α-amyrin, α-amyrin acetate, stigmasetrol and sitosterol in the *n*-hexane extract of the leaves of *Cleome gynandra*. Comparison was made with available literatures data[8,9].

The concentrated *n*-hexane extract of the leaves of *Cleome gynandra* exhibited in general activity against certain strains of bacteria and fungi[10]. Trimethoprim and miconazole were used as the standard for reference. The isolated compounds α-amyrin, α-amyrin acetate, stigmasetrol and sitosterol exhibited moderate activity against *S. aureus* and *C. albicans*, but less activity against *E. coli* and *C. glabrata*.

Conclusion: The present investigation reports the isolation of four compounds from the leaf part of the *Cleome gynandra*. 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 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.

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