

## Research Article

## Anti-microbial Constituents From Leaves of *Glyptopetalum calocarpum* (Kurz.) Prain

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### ABSTRACT

Phytochemical investigation of MeOH extract of *Glyptopetalum calocarpum* (Kurz.) Prain leaves led to the isolation of six known triterpenes (1-6). Occurrences of these compounds are reported for the first time from the leaves of *Glyptopetalum calocarpum*. The compounds were identified and characterized on the basis of various spectral techniques including 1D, Mass, and IR spectral data. In addition, these compounds were tested against selected human pathogens, *E.coli*, *S. aureus*, *S. epidermidis* and *P. aeruginosa*.

**Keywords:** *Glyptopetalum calocarpum*, Celastraceae, triterpenes, antimicrobial activity.

### INTRODUCTION

Medicinal plants have been harvested from the wild since health care system in many rural communities because of its effectiveness, lack of modern medical alternatives and cultural preferences<sup>1</sup>. In recent years development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants<sup>2</sup>. A vast knowledge of how to use the plants against different illness may be expected to have accumulated in areas where the use of plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance<sup>3</sup>.

Andaman and Nicobar Islands tribal communities, in particular Nicobarese tribes, depend on plant resources mainly for herbal medicines, food, making household implements, sleeping mats and for fire<sup>4</sup>.

*Glyptopetalum calocarpum* (Kurz.) Prain known as Miroonió (Nicobarese language) which is endemic to Andaman and Nicobar Islands<sup>5</sup>. It is also commonly known as Andaman Spindle bush, belongs to *Celastraceae* family. It is endemic to Bay Islands, found along sea shores in Nancowry Islands, North and South Andaman Islands. A small tree bearing greenish-white flowers, globose capsules and red seeds<sup>6</sup>. Capsule is

the size of a cherry, round, obscurely 4-lobed. There is one seed in each compartment<sup>7</sup>. Nicobarese use its leaves as a remedy for fever, body ache, joint pains and body swelling. Isolation of pure, pharmacologically active constituents from plants remains a long and tedious process. It is necessary to have methods available for efficient separation from plant extracts, which are typically mixtures of thousands of different molecules<sup>8</sup>.

The present study was focused to isolate active metabolites from the methanolic extract of *Glyptopetalum calocarpum* (Kurz.) Prain leaves along with their antimicrobial biological activity. The compounds were identified and characterized on the basis of various spectral techniques including 1D, Mass, and IR spectral data. To the best of our knowledge it is the first report on isolation of chemical constituents of the plant *Glyptopetalum calocarpum*.

### EXPERIMENTAL

#### General Procedures

Melting points were recorded on a Fisher scientific melting point apparatus and were uncorrected. Mass spectra were recorded on an Agilent LC/MSD trap SL 1100 series with a 70 eV (ESI probe) and IR spectra were recorded on a Thermo Nicolet Nexus 670 FTIR spectrometer. <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, DEPT

experiments were recorded on a Bruker 300 MHz spectrometer using TMS as an internal standard. All the solvents used were of analytical grade. Column chromatography was performed on silica gel (60-120 mesh, Acme's make, Mumbai, India). Thin layer chromatography (TLC) was performed on Merck silica gel 60F<sub>254</sub> plates (E. Merck, Darmstadt, Germany). Visualization was performed by spraying the TLC plates with 5% H<sub>2</sub>SO<sub>4</sub> solution followed by heating.

#### Plant Material

The leaves of *G. calocarpum* were collected from forest area of Topoiming village (N 09°12.419 E 092°47.499) of Car Nicobar Island during March 2013. The plant material was brought to the research laboratory, Regional Medical Research Centre (ICMR), Port Blair. Plant specimen was identified in Botanical Survey of India, Port Blair and voucher specimen was maintained in Institute library (Voucher No.: ANH/CN/290).

#### Extraction and isolation

The air dried leaves (1kg) were grinded and made into powder, soaked with MeOH for one week. The resulting extract was then concentrated under vacuum to obtain a residue. This residue (90 g) was subjected to column chromatography over silica gel (60-120 mesh) using an eluent system of increasing polarity of n-hexane: ethyl acetate (100:0 to 0:100) to get nine major fractions (F1-F9). Fraction F1 on further chromatographic analysis (solvent system using n-hexane: ethyl acetate 97:03) yielded compound **1** (5 mg). Fraction F2 on further chromatographic analysis (solvent system using n-hexane: ethyl acetate 90:10) yielded compound **2** (5 mg). Compound **5** (4 mg) and **3** (6 mg) were isolated from fraction F3 when subjected to preparative TLC using n-hexane: ethyl acetate 95:05. Fraction F4 on repeated column chromatography yielded compound **4** with minor impurity, this was further purified (using n-hexane: ethyl acetate 94:06, isocratic solvent system) to get compound **4** (7mg) in pure form. Fraction F5 on subjecting repeated chromatography (solvent system n-hexane: ethyl acetate 85:15) eluted compound **6** (8 mg).

#### Disc diffusion assay

Antibacterial activity of *G. calocarpum* leaves extract and purified compounds was determined using disc diffusion method<sup>9</sup>. Briefly, 100µl of the test bacteria was spread onto the Muller Hinton agar and different test compounds and crude extract were loaded to

the sterilized sterile 6mm discs, allowed to dry and then the impregnated discs with 25µL (1000µl/mL) onto the inoculated plates. The plates were allowed to stand at 4°C for 2 hours before incubated at 37°C for 24 hours. The diameter of the inhibition zones were measured in mm. All the assays were done in triplicate and the results were given in mean ± SD. Standard antibiotic streptomycin served as positive control.

#### Determination of relative percentage inhibition

The relative percentage inhibition of the test extract with respect to positive control was calculated by using the following formula<sup>10</sup>.

#### Relative percentage inhibition of the test extract=

$$\frac{(X-Y) \times 100}{(Z-Y)}$$

Where,

X: total area of inhibition of the test extract.

Y: total area of inhibition of the solvent.

Z: total area of inhibition of the standard.

## RESULTS AND DISCUSSION

#### Extraction and isolation

The MeOH extract of *G. Calocarpum* leaves were subjected column chromatography on silica gel afforded compounds (**1-6**). These compounds were identified as Alpha-Lupene (**1**)<sup>11</sup>, Lupeol (**2**)<sup>12</sup>, Lupenone (**3**)<sup>13</sup>, Stigmasterol (**4**)<sup>12</sup>, β-Amyrin (**5**)<sup>14</sup>, and β-Amyrin acetate (**6**)<sup>15</sup> from 1H, 13C NMR data which were reported in literature. Structures of all the six identified compounds were given in Figure 1. All the isolated compounds are first time to report from this plant and these are the first phytochemicals to be reported.

#### Spectroscopic characterization of the isolated compounds

##### Alpha-Lupene (1)

White solid; mp: 181-182 °C

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): 4.69 (brs, 1H), 4.58 (brs, 1H), 2.52-2.31(m, 3H), 2.06-1.84 (m, 3H), 1.68 (s, 3H), 1.52- 1.34 (m, 19H, overlapped each other), 1.63-1.52 (m, 2H), 1.07 (s, 2 x 3H), 1.03 (s, 3H), 0.96 (s, 3H), 0.88 (s, 3H), 0.80 (s, 3H). <sup>13</sup>C (CDCl<sub>3</sub>, 75 MHz): 150.8, 109.5, 54.8, 49.7, 48.2, 47.9, 47.3, 42.9, 42.8, 40.7, 39.9, 39.6, 38.1, 36.8, 35.4, 34.1, 33.5, 31.9, 29.3, 27.4, 26.3, 25.1, 22.6, 21.4, 21.0, 19.6, 17.9, 15.9, 15.7, 14.1. EI-MS *m/z* 410 [M + ]<sup>+</sup>.

**Lupeol (2)**

mp 172-174 °C; IR (KBr;  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ) 3311, 2,946, 2870, 1638, 1464, 1189, 1035, 996, 680;

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz): 4.68 (1H, brs), 4.56 (1H, brs), 3.18 (dd,  $J = 5.0, 10.7$  Hz, 1H), 2.37 (1H, m), 1.20-0.90 (25H, all peaks merged with each other), 1.68 (3H, s), 1.03 (3H, s), 0.96 (3H, s), 0.94 (3H, s), 0.83 (3H, s), 0.79 (3H, s), 0.76 (3H, s).  $^{13}\text{C}$  ( $\text{CDCl}_3$ , 75 MHz): 203.5, 151.0, 109.3, 78.9, 55.2, 50.5, 48.3, 47.9, 43.0, 42.7, 40.8, 39.9, 38.8, 38.6, 38.0, 37.1, 35.5, 34.2, 29.8, 28.0, 27.3, 25.1, 20.9, 19.6, 18.3, 17.9, 16.0, 15.9, 15.3, 14.5; EI-MS  $m/z$  427 [ $\text{M} + \text{H}$ ] $^+$ .

**Lupenone (3)**

mp 168-170 °C; IR (KBr;  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ) 2,940, 1,700, 1,580, 1,454, 1,290, 1,140, 975, 767;

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz): 4.66 (d,  $J = 2.4$  Hz, 1H), 4.57 (d,  $J = 2.4$  Hz, 1H); 2.52-2.45 (m, 1H), 2.43-2.35 (m, 2H), 1.97-1.87 (m, 2H), 1.73-1.70 (m, 1H), 1.68 (s, 3H), 1.65-1.61 (m, 2H), 1.52-1.26 (16H, merged with each other), 1.23-1.18 (m, 1H), 1.07, 1.06, 1.03, 0.96, 0.93, 0.80 (each 3H, s, Me  $\times$  6).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  218.2, 150.8, 109.3, 54.9, 49.7, 48.2, 47.9, 47.3, 42.9, 42.8, 40.7, 39.9, 39.6, 38.1, 36.8, 35.4, 34.1, 33.5, 29.8, 27.4, 26.6, 25.1, 21.4, 21.1, 19.6, 19.3, 18.0, 15.9, 15.7, 14.4. EIMS for  $\text{C}_{30}\text{H}_{48}\text{O}$   $m/z$ : 425 [ $\text{M} + \text{H}$ ] $^+$ ;

**Stigmasterol (4)**

White powder; mp: 174-176 °C;

IR (KBr;  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 3435, 3019, 2940, 1636, 1422, 1380, 1215, 1020, 756, 669.

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz): 5.29 (t,  $J = 6.1$  Hz, 1H), 5.10 (m, 1H), 4.93 (m, 1H), 3.49 (m, 1H), 2.51- 2.07 (m, 5H), 1.97-1.91 (m, 3H), 1.69 (3H, s), 1.59 (m, 1H), 1.55 (m, 2H), 1.31-1.40 (m, 3H), 1.16 (m, 2H), 1.15 (m, 2H), 1.06 (s, 3H), 1.04 (s, 3H), 1.00 (m, 1H), 0.95 (m, 1H), 0.90 (d,  $J = 6.2$  Hz, 3H), 0.82 (t,  $J = 7.2$  Hz, 3H), 0.81 (d,  $J = 6.6$  Hz, 3H), 0.79 (d,  $J = 6.6$  Hz, 3H), 0.71 (s, 3H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz): 141.0, 138.5, 129.4, 121.6, 72.0, 56.6, 56.1, 50.4, 46.3, 42.6, 42.4, 40.4, 39.8, 37.5, 36.4, 32.3, 31.8 (2C), 29.4, 29.2, 25.2, 24.1, 21.6, 21.4, 20.1, 19.7, 18.7, 12.1, 12.0.

 **$\beta$  - Amyrin (5)**

mp: 189-191 °C

IR (KBr;  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 3360 and 1650

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz): 5.25 (d,  $J = 6.0$  Hz, 1H), 3.2 (dd,  $J = 4.9, 10.8$  Hz, 1H), 2.10-1.85 (m, 2H), 1.82-1.68 (m, 4H), 1.64-1.49 (m, 11H, overlapped with each other), 1.45-1.27 (m, 7H, overlapped), 1.04 (s, 3H), 1.0 (s, 3H), 0.97 (s, 2  $\times$  3H), 0.95 (s, 3H), 0.85 (s, 3H), 0.77 (s, 3H), 0.74 (s, 3H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ,

75 MHz): 139.8, 118.8, 79.0, 55.2, 47.6, 47.2, 42.3, 42.1, 41.0, 39.2, 38.8, 38.7, 37.1, 36.7, 36.2, 34.3, 34.1, 29.6, 27.9, 27.6, 27.3, 27.0, 22.5, 21.5, 18.3, 17.6, 16.2, 15.9, 15.3, 14.6.

 **$\beta$  -Amyrin acetate (6)**

Colourless needles, mp 241-242.5 °C

IR (KBr;  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 1722, 1635, 1240, and 812.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz): 5.21 (t,  $J = 3.5$  Hz, 1H), 4.54 (dd,  $J = 11.6$  Hz, 1H), 2.07 (s, 3H), 2.03-1.84 (m, 5H), 1.80-1.72 (m, 1H), 1.69-1.60 (m, 5H), 1.59- 1.45 (m, 4H), 1.43-1.38 (m, 4H), 1.36-1.30 (m, 3H), 1.09-1.03 (m, 1H), 1.14 (s, 3H), 0.98 (s, 3H), 0.96 (s, 3H), 0.88 (s, 2  $\times$  3H), 0.87 (s, 3H), 0.86 (s, 3H), 0.83 (s, 3H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz): 171.0, 145.1, 121.6, 80.9, 55.2, 47.5, 47.2, 46.7, 41.6, 39.7, 38.2, 37.7, 37.1, 36.8, 34.7, 33.3, 32.5, 32.4, 31.0, 28.3, 28.0, 26.9, 26.1, 25.9, 23.6, 23.5, 21.3, 18.2, 16.7, 16.6, 15.5. EIMS  $m/z$ : 468 [ $\text{M} + \text{H}$ ] $^+$ .

**Antimicrobial assay**

The antimicrobial activity of six purified compounds and methanol crude extract of the *Glyptopetalum calocarpum* was presented in the Table 1. The compounds Lupenone, Stigmasterol shows a strong inhibition in the growth of tested bacteria. The maximum zone of inhibition was observed against *S. epidermidis* and *S. aureus*. Moderate activity observed against *P. aeruginosa* and no activity found against *E. coli*.

**Relative percentage inhibition**

The result of antimicrobial activity of six compounds and crude extract was compared with the positive control for evaluating their relative percentage inhibition (Table 2). The compound Lepeone exhibits maximum relative percentage inhibition against *S. epidermidis* (104.62%) followed by *P. aeruginosa* (81.58%) and the compound stigmasterol exhibits maximum relative percentage inhibition against *S. epidermidis* (89.23%) followed by *P. aeruginosa* (84.21%). The crude extract showed maximum relative percentage inhibition against *P. aeruginosa* (97.37%) followed by *S. aureus* (70.00%).

**CONCLUSION**

The present study found that the methanol extract of *Glyptopetalum calocarpum* (Kurz.) Prain leaves holds great promise as a potential source of beneficial antimicrobial components which are active against both gram positive and gram negative human pathogens. It also resolve the problem of drug resistance and the harmful effects of synthetic compounds. However, further in vivo studies

are needed to investigate the pharmacological and toxicological properties of *Glyptopetalum calocarpum* (Kurz.) Prain extract before it can be considered as a new antimicrobial agent.

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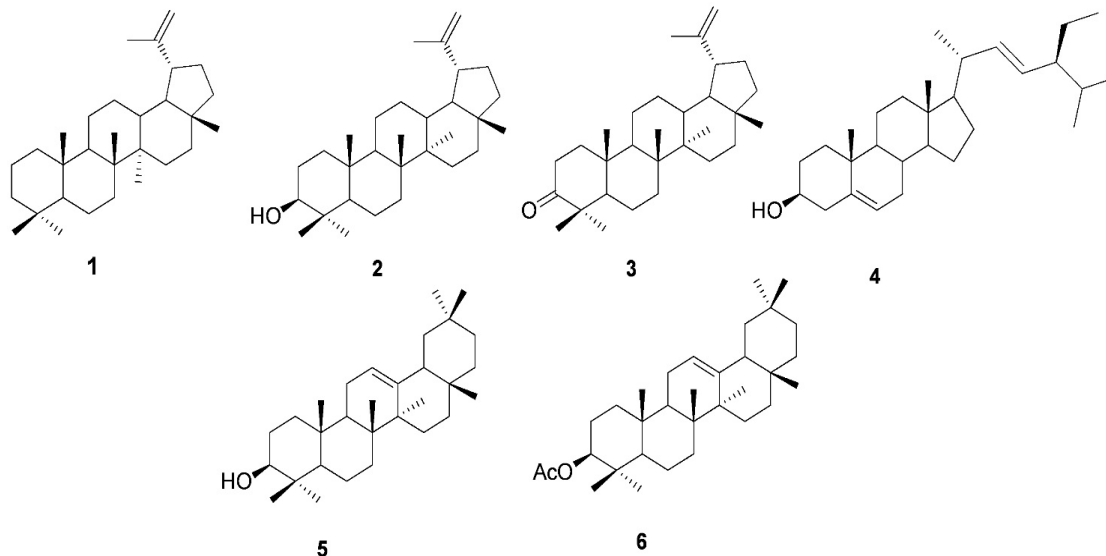


Fig. 1: Structures of isolated terpenes from leaves of *Glyptopetalum calocarpum*

Table 1: Antimicrobial activity of isolated compounds and crude extract of *Glyptopetalum calocarpum*

Test compounds	Inhibition zone diameter (mm)			
	<i>E.coli</i>	<i>P. aeruginosa</i>	<i>S. epidermidis</i>	<i>S. aureus</i>
Lupeone	-	10.33±0.58	22.67±0.58	13.00±1.00
Stigmasterol	-	10.67±0.58	19.33±1.53	11.67±1.15
α-Lupene	-	-	-	-
Lupeol	-	-	-	-
β-amyrin	-	-	-	-
β-amyrin acetate	-	-	-	10.33±0.58
Crude extract	-	13.67±0.58	12.00±0.00	11.33±1.53
Streptomycin	12.33±0.58	12.67±0.58	21.67±1.15	12.33±0.58

- indicates no activity

Table 2: Relative percentage inhibition of isolated compounds and crude extract of *Glyptopetalum calocarpum* compare to standard antibiotic

Test organisms	Relative percentage of inhibition (%)		
	Lupeone	Stigmasterol	Crude extract
<i>S. aureus</i>	78.00	68.00	70.00
<i>S. epidermidis</i>	104.62	89.23	55.38
<i>P. aeruginosa</i>	81.58	84.21	97.37

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