Research Article

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

M. Punnam Chander[1], P. Srivani[2], K. Suresh Babu[2] and P. Vijayachari[1*]

[1]Regional Medical Research Centre (ICMR), WHO Collaborating Centre for Diagnosis, Reference, research and training in leptospirosis, Port Blair -744101, Andaman and Nicobar Islands, India.
[2]Division of Natural Product Chemistry, CSIR-IICT, Uppal road, Hyderabad-500007, Telangana, India.

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 was 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[1]. In recent years development of drug resistance in human pathogens against commonly used antibiotics has necessitude a search for new antimicrobial substances from other sources including plants[2]. 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[3].

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[4].

Glyptopetalum calocarpum (Kurz.) Prain known as Miroonlő (Nicobarese language) which is endemic to Andaman and Nicobar Islands[5]. It is also commonly known as Andaman Spindle bush, belongs to *Celastraceae* family. It is endemic to Andaman and Nicobar 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[6]. Capsule is the size of a cherry, round, obscurely 4-lobed. There is one seed in each compartment[7]. 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[8].

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. ^{1}H-NMR, ^{13}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 (60-120mesh, Acm's make, Mumbai, India). Thin layer chromatography (TLC) was performed on Merck silica gel $60F_{254}$ plates (E. Merck, Darmstadt, Germany). Visualization was performed by spraying the TLC plates with 5% H_2SO_4 solution followed by heating.

Plant Material

The leaves of *G. calocarpum* were collected from forest area of Topoiming village (N $09^0$12.419 E $092^0$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 weak. 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[9]. 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[10].

Relative percentage inhibition of the test extract=

$$\frac{(X-Y)\quad x100}{(Z-Y)}$$

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**)[11], Lupeol (**2**)[12], Lupenone (**3**) [13], Stigmasterol (**4**) [12], β-Amyrin (**5**) [14], and β-Amyrin acetate (6) [15] 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 ^{0}C
^{1}H NMR ($CDCl_3$, 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). ^{13}C ($CDCl_3$, 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 $[M +]^+$.

Lupeol (2)
mp 172-174 ^{0}C; IR (KBr; ϑ_{max}, cm^{-1}) 3311, 2,946, 2870, 1638, 1464, 1189, 1035, 996. 680;
^{1}H NMR ($CDCl_3$, 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}C ($CDCl_3$, 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 $[M + H]^+$.

Lupenone (3)
mp 168-170 ^{0}C; IR (KBr; ϑ_{max}, cm^{-1}) 2,940, 1,700, 1,580, 1,454, 1,290, 1,140, 975, 767;
^{1}H NMR ($CDCl_3$, 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 × 6). ^{13}C NMR ($CDCl_3$): δ 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 $C_{30}H_{48}O$ *m/z*: 425 $[M+ H]^+$;

Stigmasterol (4)
White powder; mp: 174-176 ^{0}C;
IR (KBr; ϑ_{max}, cm^{-1}): 3435, 3019, 2940, 1636, 1422, 1380, 1215, 1020, 756, 669.
^{1}H NMR (CDCl3, 300 MHz): 5.29 (t, *J* = 6.1Hz, 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}C NMR ($CDCl_3$, 75MHz): 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.

β - Amyrin (5)
mp: 189-191 ^{0}C
IR (KBr; ϑ_{max}, cm^{-1}): 3360 and 1650
^{1}H NMR (CDCl3, 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 x3H), 0.95 (s, 3H), 0.85 (s, 3H), 0.77 (s, 3H), 0.74 (s, 3H). ^{13}C NMR ($CDCl_3$, 75MHz): 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.

β -Amyrin acetate (6)
Colourless needles, mp 241-242.5 ^{0}C
IR (KBr; ϑ_{max}, cm^{-1}): 1722, 1635, 1240, and 812. ^{1}H NMR (CDCl3, 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 X 3H), 0.87 (s, 3H), 0.86 (s, 3H), 0.83 (s, 3H). ^{13}C NMR ($CDCl_3$, 75MHz): 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 $[M +]^+$.

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. aeurognosa* 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 Lupenone 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.

ACKNOWLEDGEMENTS

The authors are thankful to the Indian Council of Medical Research (ICMR), New Delhi, India for providing financial support to the work.

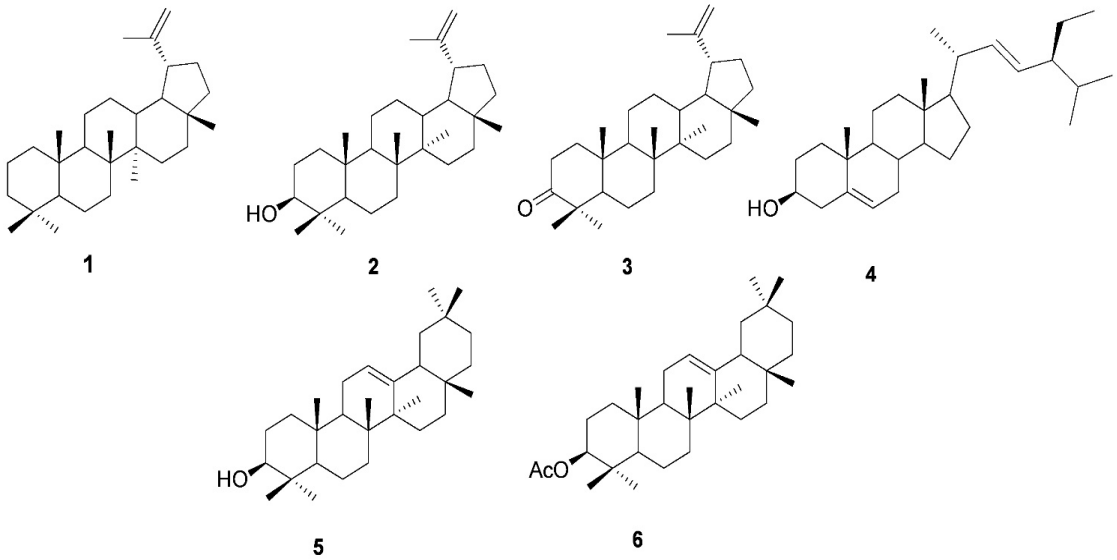

Fig. 1: Structures of isolated terpenes from leaves of *Glyptopetalum calocarpum*

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

Test compounds	Inhibition zone diameter (mm)			
	E.coli	*P. aeruginosa*	*S. epidermidis*	*S. aureus*
Lupenone	-	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 (%)		
	Lupenone	Stigmasterol	Crude extract
S. aureus	78.00	68.00	70.00
S. epidermidis	104.62	89.23	55.38
P. aeruginosa	81.58	84.21	97.37

REFERENCES

1. Tabuti J, Dhillion SS and Lye K. Traditional medicine in Bulamogi County, Uganda: its practitioner, uses and viability. J Ethnopharmacol. 2003;85:119-129.
2. Erdogrul OT. Antibacterial activities of some plant extracts used in folk medicine. Pharmaceutical Biology. 2002;40:269-273.
3. Diallo D, Hveem B, Mahmoud MA, Betge G, Paulsen BS and Maiga A. An ethnobotanical survey of herbal drugs of Gourma district, Mali. Pharmaceutical Biology. 1999;37:80-91.
4. Kaushal Kumar, Kumar B and Thiru Selvun. Ethnobotanical heritage of Nicobarese tribe. J Econ Taxon Bot. 2006;30(2);331-348.
5. Pandey RP and Diwakar PG. An Integrated check-List flora of Andaman and Nicobar Islands, India. J Econ Taxon Bot. 2008;32(2)403-500.
6. Dagar JC and Sing NT. Plant resources of the Andaman and Nicobar Islands. (Enumeration and utilisation of vascular plants) Bishen singh mahendra pal singh 23-A, New Connaught place, Dehra Dun, India. 1999;2:410.
7. Sclater WL and Walsh JH. Two additional species of Glyptopetalum – by Prain D. Journal of the Asiatic Society of Bengal. 1892;LX(II):206-210.
8. Peter KV. Handbook of herbs and spices. Boca Raton, CRC press. 2004.
9. Bauer AW, Kirby WMM, Sheriss JC and Turck M. Antibiotic susceptibility testing by standardised single method. Am J Clin Pathol. 1966;45:493-496.
10. Punnam chander M, Sachithanandam Veerargagavam and Vijayachari P. Antimicrobial and Hemolytic activity of seaweed Padina gymnospora from South Andaman, Andaman and Nicobar Islands of India. Int J Curr Microbio App Sci. 2014;3(6):364-369.
11. Dharmassree BT, Wijeratne, Vijaya Kumar, Uvais M and Sultanbawa S. JCS Perkin I. 1981;2724-2726.
12. Jain PS and Bari BS. Isolation of lupeol, stigmasterol and campesterol from petroleum ether extract of woody stem of Wrightia tictoria. Asian Journal of Plant Sciences. 2010;9:163-167.
13. Prakash CVVS and Prakash I. Isolation and Structural Characterization of Lupane Triterpenes from Polypodium Vulgare Res. J Pharmaceutical Sci. 2012;1:23-27.
14. Shan LY, Thing TC and Ping TS. Journal of Chemical and Pharmaceutical Research. 2014;6: 815-822.
15. Matsunaga S, Tanaka R and Akagi M. Phytochemistry. 1988;27:535-537.