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Evaluation of Antibacterial and Structural Elucidation of Biological Compounds from *Gymnema kollimalayanum* A. Ramachandran and M.B. Viswan: A New Record Plant

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Abstract: The prime aim of present investigation was focused on the *in vitro* antibacterial and GC-MS analysis of *Gymnema kollimalayanum*, a new record plant from India. The aqueous and 5 different organic solvents (chloroform, acetone, methanol, ethanol and dichloromethane) extracts were tested against both gram negative and gram positive bacterial strains by agar well diffusion assay (*Staphylococcus aureus*, *Streptococcus pneumoniae*, *Bacillus substilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsilla pneumonia* and *Salmonella typhi*). The results highlights that the chloroform, acetone and aqueous extracts exhibited significant antibacterial activity than other solvents. The results of GC-MS analysis shows ten biologically active compounds were isolated. Out of these, two compounds are reflecting the highest peak values.

Key words: *Gymnema kollimalayanum,* antibacterial tests, human pathogenic bacteria, GC-MS, India

INTRODUCTION

Plants have been utilized as medicines for thousands of years (Samuelsson and Bohlin, 2004). These medicines initially took in the form of crude drugs such as tinctures, teas, poultices, powders and other herbal formulations (Samuelsson and Bohlin, 2004). The acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led to investigate the antimicrobial activity of medicinal plants. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has lead to the screening of several medicinal plants for their potential antimicrobial activity (Elizabeth, 2005).

Antimicrobials of plant origin are not associated with side effects and have an enormous therapeutic potential to heal many infectious diseases. For example, vincristine (antitumor drug), digitalis (a heart regulator) and ephedrine (a bronchodilator used to decrease respiratory congestion) were all originally discovered through research on plants. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes (Iwu, 1999). Concern has been expressed about the rising prevalence of pathogenic microorganisms which are resistant to the newer or modern antibiotics that have been produced in the last three decades worldwide (Cohen, 1992; Nascimento *et al*., 2000). The identification of organic compounds from the extracts of plants is of great importance, mainly because they can be used as an excellent source of pharmaceutical products for phytotherapy (Melecchi *et al*., 2002; Lang and Wai, 2001). GC-MS technology appears to be the requirement for chemical derivatization prior to quantitative analysis (Mueller *et al*., 2002; Birkemeyer *et al*., 2003; Schmelz *et al*., 2004). Gymnema is an antidiabetic plant and well known herbal medicine due to the therapeutic efficacy of its different species.

Among them, the biological activity of *G. kollimalayanum* is not explored so far except the taxonomic sketch (Ramachandran and Viswanathan, 2009). Based on the above cited information's, the present and first-time report focused on the screening of antibacterial activity and isolation of biological compounds from the pulverized leaf extracts of *G. kollimalayanum*, a new record plant from peninsular India.

MATERIALS AND METHODS

Plant collection: The fresh and healthy leaves of *Gymnema kollimalayanum* A. Ramachandran and M.B. Viswan were collected from the higher altitudes (>1100 in MSL) of Kolli hills at Namakkal district, Tamil Nadu, India. The plant leaves were washed, air dried and powdered.

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Extraction procedures: Both polar (water, methanol, ethanol and acetone) and non polar (Di-chloromethane and chloroform) solvents were mixed with the known amount of plant powder (each 10 g in 100 mL). This mixture was kept in a shaker upto 72 h in room temperature and the mixture was filtered through whatmann no. 1 filter study. The filtered solvent was kept in 100 mL of glass beaker. The crude extraction was kept in hot air oven for allow to evaporation until it reach the dried condition. The required amount of extraction could be taken for the antibacterial test.

Screening for antibacterial activity

Preparation of bacterial inoculums: The eight bacterial cultures (*E. coli*, *P. aeruginosa*, *P. vulgaris*, *S. aureus*, *S. pneumoniae*, *K. pneumoniae*, *S. typhi* and *B. subtilis)* were collected from the clinical laboratory, Govt. Hospital, Salem, Tamil Nadu. The stock culture of each bacterium was subculture on nutrient broth at 37°C for 12-14 h prior to carry out the antibacterial tests.

Agar well diffusion method: The antibacterial activity was performed by the agar-well diffusion method as described by Natarujan *et al.* (2005) with few modifications. A volume of 15 mL of agar medium was inoculated with 0.1 mL of fresh overnight culture. Three wells of each plate (5 mm in diameter) were punched in the agar and filled with 70 µL of the each extract. After holding the plates at room temperature for 2 h to allow the diffusion of the extract into the agar, the plates were incubated at 37°C for 24 h and the diameter of the inhibition zones of each well was measured (13) (Gupta, 1977). The standard antibiotic (streptomycin) and DMSO are served as positive and negative control.

GC-MS study: Gas chromatography and mass spectroscopy analysis was performed by GC clarus 500 Perkin Elmer using Elite- 5MS column (5% diphenyl/95% dimethyl poly siloxane) 30×0.25 mn×0.25 µm of thickness. Helium was used as carrier gas at a flow of 1 mL min^{-1}. The injection port was maintained at 250°C and the split ratio was 10:1. Oven temperature programming was done from 5-280°C at 10°C min^{-1} and it was kept at 280°C for 9 min.

Interface temperature was kept at 250°C. The ionization mode was electron impact ionization and the scanning range was from 45-450 (m/z). Mass spectra were obtained at 0-2 min interval. The spectra of the compounds were matched with NIST Version year 2005 library. The structure of selected biologically active compounds were drawn by Chemdraw, Version 8.0.0 Cambridge Soft Corporation, UK.

RESULTS AND DISCUSSION

The results of GC-MS study from the ethanol leaves extracts of *G. kollimalayanum* showed a total of 10 compounds were identified with 99.99%. The major compounds were identified as 2-penten-1-ol (E) and 2, 6, 10-dodecatrien-1-ol, 3, 7, 11-trimethyl-acetate (E, E) which accounted for 43.74 and 26.33%, respectively and rest of them are minor compounds (Table 1 and Fig. 1) and their structures are shown in Fig. 2.

This study was supported by Sathya *et al.* (2010) investigated on the identification of phytochemicals (terpenoids, glycosides and alkaloids) from the successive leaf extracts of *G. sylvestre* (Asclepiadaceae) using similar technique.

The results of antibacterial properties of aqueous and different organic solvents extracts of *G. kollimalayanum* were tested against several bacterial strains (Table 2) by agar well diffusion method. The methanolic extracts showed moderate to least activity in all the bacteria except *P. aeruginosa*. The ethanol extracts of the plant highlighted better antibacterial potentiality against the organisms tested. Similarly, the chloroform extracts

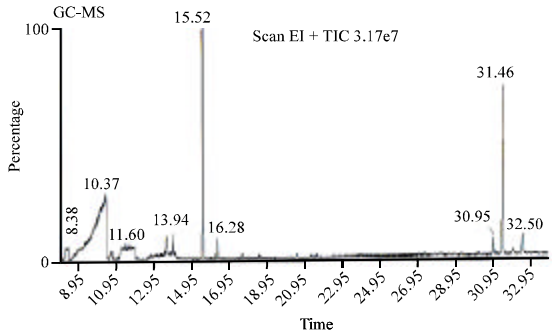

Fig. 1: GC-MS study of ethanolic extraction of *G. kollimalayanum*

Table 1: GC-MS analysis of leaves of *G. kollimalayanum*

RT	Name of the compounds	Molecular formula	Mw	Peak area (%)
8.38	dl-arabinose	$C_5H_{10}O_5$	150	2.01
10.37	2-penten-1-ol (E)-	$C_5H_{10}O$	86	43.74
11.60	L-galactose, 6-deoxy	$C_6H_{12}O_5$	164	3.74
13.60	n-decanoic acid	$C_{10}H_{20}O_2$	172	2.16
13.94	Pentadecanoic acid, 2, 6, 10, 14-tetramethyl-methyl ester	$C_{20}H_{40}O_2$	312	1.29
15.52	Phytol	$C_{20}H_{40}O$	296	14.39
16.28	9,12,15-octadecatrienoic acid (Z, Z, Z)-	$C_{18}H_{30}O_2$	278	2.01
30.95	1, 6, 10, 14-hexadecatetraen -3-0l, 3, 7, 11, 15-tetramethyl- (E, E)	$C_{20}H_{34}O$	290	1.15
31.46	2, 6, 10-dodecatrien-1-ol, 3, 7, 11-trimethyl-acetate (E, E)	$C_{17}H_{28}O_2$	264	26.33
32.50	Diazoprogesterone	$C_{21}H_{30}N_4$	338	3.17

Table 2: Antibacterial activity of leaf extracts of *G. kollimalayanum*: a new record plant

	Zone of Inhibition (diameter in mm)							
Pathogens extracts	*B. subtilis*	*P. aeruginosa*	*S. aureus*	*K. pneumoniae*	*S. typhi*	*S. pneumoniae*	*E. coli*	*P. vulgaris*
Methanol	10±1.41	18.6±1.63	6.3±0.81	7±0	6.3±0.81	7.6 ±0.82	6.3±0.81	7±1.41
Ethanol	12.3±0.81	11±1.41	11.3±0.81	7±1.41	11.6±1.63	12±1.41	11.6±2.16	10±1.41
Chloroform	10.3±0.81	9.3±2.5	15±1.41	9±1.41	13.6±3.56	10.3±3.26	10.6±2.94	12±1.41
Dichloromethane	11.6±2.94	7±0	7.3±1.6	8.3±0.81	8±2.4	7±0	8±1.41	7.6±0.82
Acetone	11±0	9.33±0.82	8±1.41	7.6±0.81	12.3±3.26	14.6±2.16	12±3.74	8.3± 2.16
Water	9±1.41	19.6±0.82	9±1.411	8.3±0.81	8±1.41	7.6±0.82	10±0	11±1.41
Streptomycin	21	18	22	12	15	18	-	13
DMSO	-	-	-	-	-	-	-	-

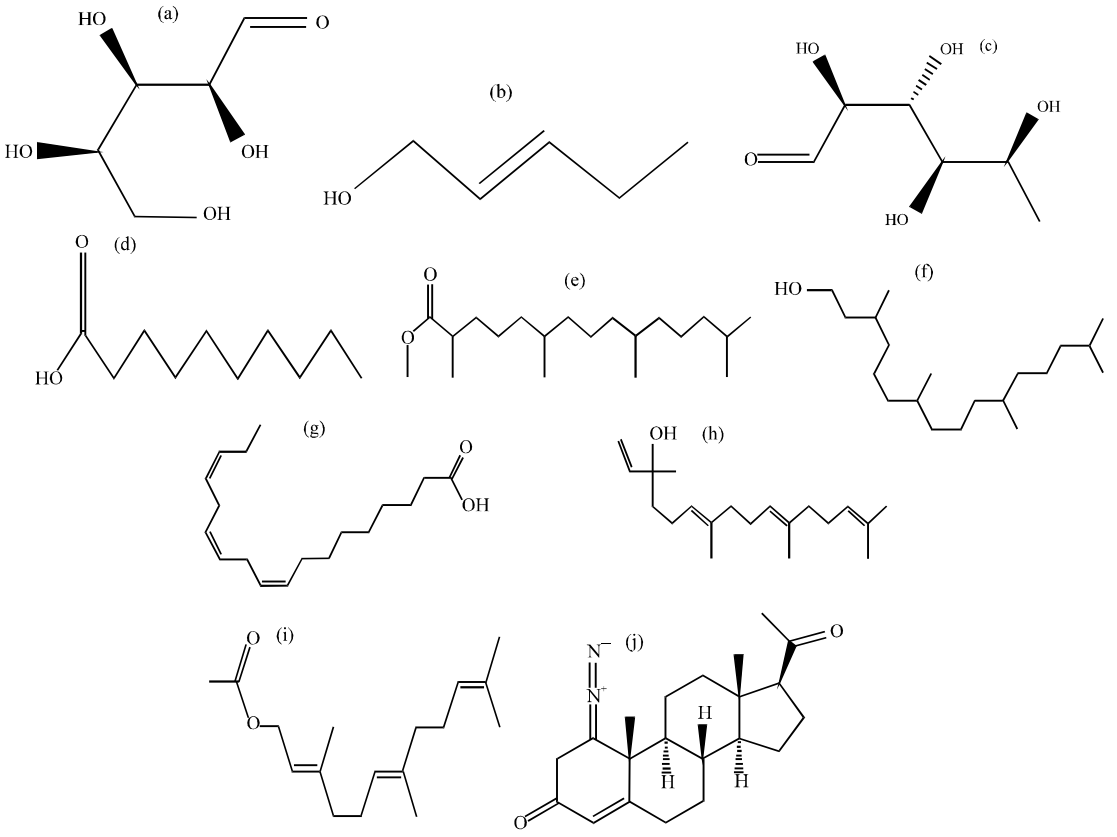

Fig. 2: Structural elucidation of some biologically active compounds: a) dl-Arabinose; b) 2-penten-1-ol (E); c) L-Galactose, 6-deoxy; d) n-Decanoic acid; e) pentadecanoic acid, 2, 6, 10, 14-tetramethyl-, methyl ester; f) phytol; g) 9, 12, 15-Octadecatrienoic acid (Z, Z, Z); h) 1, 6, 10, 14-Hexadecatetraen-3-ol, 3, 7, 11, 15-tetramethyl-(E, E); i) 2, 6, 10-Dodecatrien-1-ol, 3, 7, 11-trimethyl-, acetate (E, E) and j) Diazoprogesterone

expressed significant activity against most of the bacteria except *P. aeruginosa* and *K. pnuemoniae*. The dichloromethane extracts contribute least activity against tested bacterial strains excluding *B. subtilis* showed moderate inhibitory effect. Whereas, the acetone extract showed broad spectrum of antibacterial potentiality against all the pathogens. The aqueous extract was reported remarkable antibacterial activity against *P. aeruginosa* over standard antibiotic and the same extracts were found to be least to moderate activity in remaining tested organisms. The overall results indicate that the aqueous and organic solvents extracts of *G.kollimalayanum* showed moderate to better antibacterial activity against most of the tested bacterial strains. Similarly, the alcoholic (Raja and Devi, 2010), ethanolic (Satdive *et al*., 2003), aqueous-methanol (Pasha *et al*., 2009), chloroform and ethyl acetate extracts of *G. sylvestre* and *G. montanum* (Ramkumar *et al*., 2007) showed broad spectrum of antimicrobial activity against several bacterial strains include the tested

bacteria's. The present results were also comparable to other genera of Asclepiadaceae, i.e., *Cryptostegia grandiflora* (Mukherjee *et al*., 1999), *Oxystelma esculentum* (Khan *et al*., 2008), *Tylophora hirsuta* (Bashir *et al*., 2009), *T. indica* (Reddy *et al*., 2009), *Pergularia daemia* (Ignacimuthu *et al*., 2009), *Calotropis gigantea* (Kumar *et al*., 2010) and *Pentatropis microphylla* (Prabha and Vasantha, 2010) have been proved significant antimicrobial activity. The present study paves the way for further attention to test clinical validation of the crude drugs.

CONCLUSION

The results revealed that plant extracts were effective both for controlling gram positive and gram negative human pathogens. This study encourage the use of herbal extracts as therapeutic agents for treat several diseases caused by the pathogenic bacteria.

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