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Full Length Research Paper

Estimation of phytoconstituents from *Cryptostegia grandiflora (Roxb.) R. Br. in vivo* and *in vitro*. II. Antimicrobial screening

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The hexane and ethyl acetate extracts of *Cryptostegia grandiflora* yielded a mixture of phytosterols and triterpenoids namely lanosterol, β-sitosterol, stigmasterol, campesterol, friedelin, lupeol, ursolic acid and β-amyrin. The quantification of these phytosterols and triterpenoids was carried out and found that β-sitosterol (0.43±0.000241) was major phytosterol in 6 weeks old callus. In recent years, elicitors are being used for increasing the quantity of medicinally important products so the effect of salicylic acid (abiotic elicitor) and ergosterol (biotic elicitor) in cell cultures was also investigated and observed that the quantity of lupeol was raised (0.11±0.0081) at 0.01 mM concentration of ergosterol as biotic elicitor. The isolated compounds and reference antibiotics were tested against pathogenic bacteria and fungi. The inhibition zones (IZ) of isolated compounds were also recorded and the activity index (AI) was calculated. It has been reported that lanosterol was most active (IZ-19.2; AI-1.032) against *Escherichia coli* and campesterol had greater activity against *Candida albicans* (IZ-15.6; AI-1.006).

Key words: *Cryptostegia grandiflora,* phytosterols, triterpenoids, *in vivo* and *in vitro*, antimicrobial activity.

INTRODUCTION

Cryptostegia grandiflora (Roxb.) R. Br. (Asclepiadaceae) is a perennial climber widely distributed in Madagascar, Southern Florida and throughout India. The juice of aerial parts of *C. grandiflora,* when exposed to sunshine, produces caoutchoue (Bailey, 1942). It is considered to be purgative (Satyavati et al., 1976), plant decoction is consumed to treat nervous disorders (Britto and Mahesh, 2007; Augustus et al., 2000), leaves are toxic and contain rubber (Chopra et al., 1956; Kirtikar and Basu, 1987; Aronson, 2009; Nunes et al., 2010). The aqueous solution of ethanolic extract of aerial parts exerted significant hypoglycemic action in normal rabbits (Sharma et al., 1967; Sharma and Shukla, 1977), wound healing remedies (Steenkemp et al., 2004). The latex of *C. grandiflora* used in blood coagulation, source of vegetable rubber (Vishwanath et al., 1943). The plant species also possesses various biological activities like as antioxidant (Abdelsalam and Bagdadi, 2009; Mukherjee et al., 1999), antitumour (Doskotch et al., 1972), antiviral (Vijayan et al., 2004) and also control the schistosomiasis (Adewunmi, 1984).

The plant species has been reported to contain wax, which on treatment with alcoholic potash gave ursolic and oleic acids (Sastri, 1950), phytoecdysteroids (Dinan et al., 2001), anthocynins (Forsyth and Simmonds, 1954), alkaloids (Daniel, 2006; Hadi and Bremner, 2001), cardenolides (Kreis and Müller, 2010; Doskotch et al., 1972; A Abd El-Mawla, 2010; Sanduja et al., 1984; Radford et al., 1994; Krider et al., 1957), flavonoids (El-Zalabani et al., 2003). The present study deals with the estimation of phytosterols and triterpenoids in cell cultures, effect of salicylic acid and ergosterol on quantity of isolated compounds and testing for their antimicrobial

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activity.

MATERIALS AND METHODS

Plant material

C. grandiflora (Roxb.) R. Br. (Asclepiadaceae) was collected (July, 2007) from the Moti Dungari Hills, Jaipur and authenticated by Professor R. S. Mishra, from the Herbarium, Department of Botany, University of Rajasthan, Jaipur, India (specimen sheet no. 27754).

General experimental conditions

The melting points were recorded on a capillary Toshniwal melting point apparatus and are uncorrected. The spectral data were obtained on the following instruments: ir, Perkin-Elmer, Model 283; uv, Perkin-Elmer, Model 200; ms, Hewlett Packard HP 5930A; hplc, Millipore Waters, Model 501; adsorbents for TLC (silica gel 60, 230 to 400 mesh for column chromatography and silica gel G for TLC, E-Merck). The standard compounds (lanosterol, β-sitosterol, stigmasterol, campesterol, friedelin, lupeol, ursolic acid and β-amyrin) were obtained from the laboratory of Professor Pahup Singh, Department of Chemistry, University of Rajasthan, Jaipur, India.

Extraction and characterization

Shade-dried powdered aerial parts (leaves and bark) of plant material (10.0 kg) were defatted with petroleum ether (60 to 80°C, E-Merck, 13.0 l) for 24 h, filtered and resultant residue was further Soxhlet extracted (pressure -0.2 atm) with 95% ethanol (9.0 l) for 48 h, filtered and dried *in vacuo* (yield 198.43 g). Phytosterol I to IV. The crude ethanolic extract (85.632 g) was refluxed with 15% ethanolic HCl for 4 h (Tomita et al., 1970), filtered and fractionated with EtOAc. The EtOAc fraction was brought to neutrality by repeated washing with distilled water and passed over sodium sulphate (Na_2SO_4) to remove moisture. Later, it was dried *in vacuo,* weighed (yield 08.812 g) and investigated for phytosterols.

A portion of EtOAc fraction was chromatographed on silica gel and purified by preparative TLC with development by C_6H_6 - EtOAc (85:15, v/v), detection on TLC by 50% H_2SO_4, yielded **I**, lanosterol (147 mg), mp 138-140°C, $C_{30}H_{50}O$, R_f ~ 0.96, white crystals, pink-brown colour spot appeared on TLC after spray, positive to LB test (Heble et al., 1968; Corey et al., 1966; Abe et al., 1993). Phytosterol II, β-sitosterol (269 mg), mp 164 to 166°C, $C_{29}H_{48}O$, $[\alpha]_D$ - 37° ($CHCl_3$), R_f ~ 0.90, pink colour spot appeared on TLC after spray, positive to LB test (Conolly and Hill, 1994). Phytosterol III, stigmasterol, mp 170 to 170.5°C, $C_{29}H_{48}O$, $[\alpha]_D$ - 50° ($CHCl_3$), water insoluble, R_f ~ 0.64, purple colour spot appeared on TLC plate after spray, yield–349 mg, positive to LB test (Kowshanul Habib et al., 2007). Phytosterol IV, campesterol, $C_{28}H_{48}O$, $[\alpha]_D$ - 33°, yield - 332 mg, mp 158°C, white crystals, R_f ~ 0.23, blue colour spot appeared on TLC after spread, positive to LB test (Kallianos et al., 1963). These isolated and purified compounds were subjected to various physical and spectral studies and were identical with standard samples.

Triterpenes V to VIII- The crude ethanolic extract (70.831 g) was subjected to partition among hexane (Fr. **I,** 38.713 g, 1.0 l), C_6H_6 - hexane (2:1, v/v, Fr. II, 18.613 g, 800 ml), which were used in subsequent work. The column chromatography of Fr. I, with elution by petroleum ether - C_6H_6, 4 fractions, A-D; Fr. **II**, with elution by hexane - C_6H_6, 4 fractions, E-H, were collected (Singh et al., 2006). A portion of fraction A to B were combined (08.413 g) and re-chromatographed on silica gel and purified by preparative TLC with development by heptane - C_6H_6 - EtOH (100:100:1, v/v; Heftmann, 1975), R_f ~ 0.24, triterpene V, friedelin, $C_{30}H_{50}O$, yield-237 mg, mp 198 to 200°C, $[\alpha]_D$ – 27.8° ($CHCl_3$), brown colour spot appeared on TLC plate sprayed with 20% $SbCl_3$ in $CHCl_3$, positive to LB test (Corey and Ursprung, 1956). Fr. C to D (09.316 g), were further separated and purified by preparative TLC, solvent system: n-heptane - C_6H_6 - EtOH (100:100:1, v/v), yielded triterpene VI, lupeol, $C_{30}H_{50}O$, $[\alpha]_D$ + 20° ($CHCl_3$), detected by 20% $SbCl_3$ ($CHCl_3$), R_f ~ 0.37, violet-orange colour spot, positive to LB test, yield 620 mg (Nigam and Mitra, 1966). Fr. E-F, pooled together (8.316 g) and purified by means of preparative TLC, yielded triterpene VII, ursolic acid (223 mg), $C_{30}H_{48}O_3$, mp 291°C, detected by 20% $SbCl_3$ ($CHCl_3$), R_f ~ 0.75, brown colour spot, appeared on TLC after spray, positive to LB test (Ramadan et al., 2009). Fr. G-H, further combined together (7.516 g) and purified by preparative TLC, yielded triterpene VIII, β-amyrin, $C_{30}H_{50}O$, mp. 197 to 197°C, $[\alpha]_D$ + 88°, solvent system: n-heptane - C_6H_6 - EtOH (100:100:1, v/v), R_f ~ 0.24, detected by 20% $SbCl_3$ ($CHCl_3$), brown-grey colour spot appeared on TLC plate, yield-217 mg (Burrel and Honston, 1948). The isolated compounds were subjected to various physical and spectral studies and were identical with their respective standards (Heilbron and Bunbury, 1957; Yamaguchi, 1970).

Tissue culture

The unorganized callus tissue of *C. grandiflora* was induced by nodal segments (1.0 to 2.0 cm). The nodal segments were sterilized with 0.1% (w/v) $HgCl_2$ solution for 2 to 3 min and then rinsed three times with sterilized distilled water. These sterilized nodal segments were then aseptically inoculated on to MS (Murashige and Skoog, 1962) medium supplemented with 1.0 mg/l indole-3-acetic acid (IAA), 5.0 mg/l benzyl amino purine (BAP), 1.0 mg/l kinetin and 3.0% sugar. The nodal segments differentiated to callus tissue formation 23 to 27 days of inoculation. These cultures were incubated at 25±1°C with 60% relative humidity under room light conditions (300 lux).

The callus tissue sample was transferred onto the fresh MS medium after 5 to 6 weeks intervals. The callus tissue was harvested at the transfer age of 2, 4, 6, 8 weeks and the growth index was calculated. The fresh tissue samples (1.0 kg) were first dried at 100°C for 15 min to inactivate the enzymatic activity and then at 40°C until the weight of each sample became constant. Each of the dried tissue samples were powdered, refluxed in Soxhlet apparatus with hexane for 24 h, filtered and dried (yield : 10.491 g, 1.37%; 16.461 g, 2.74%; 21.201 g, 3.46%; 20.562 g, 3.19%) *in vacuo* and taken up separately for quantification and characterization studies.

Elicitation

Liquid MS medium (50 ml) containing 5.0 g of suspension cells were grown on reciprocal shaker for 6 days and on 7th day, the medium was supplemented with different concentrations (0.01, 0.025, 0.05, 0.1 mM) of each salicylic acid and ergosterol as elicitors and the cultures were allow to grow for 24 h. Later, these cultures were harvested, dried at 100°C for 15 min to inactivate the enzymatic activity and later at 40°C until constant weight were achieved. Resultant calli were powdered and subjected for the quantification of phytosterol and triterpenoid contents (Eilert, 1987).

HPLC analysis

The HPLC determination of phytosterols and triterpenoids was achieved on Millipore Waters Model 501, fitted with pump-solvent delivery system, injector (Model 6 UK) by using µ Bondapak C_{18}

column (30 cm × 3.9 mm, temperature 24±1°C). The mobile phase used for the separation was MeOH (HPLC grade: E-Merck) and the flow rate was adjusted to 0.7 ml/min; effluent was monitored by UV absorption at 254 nm with a detector adjusted at an attenuation of 0.5 AUFS (Lambda Max Model 481 LC spectrophotometer, detector-Waters). Before use, the columns were washed with MeOH. The ethanolic extract was filtered, dissolved in mobile phase and a 10 µl volume of *in vivo* and *in vitro* samples injected (chart speed 0.50 cm/min). The calibration graphs were constructed by plotting the ratio of the peak area for determination (Foulsen et al., 1991).

Sources of microorganisms

The pure cultures of bacteria, *Escherichia coli* (ATCC 5922), *Staphylococcus aureus* (ATCC 25923), *Enterobacter cloacae* (ATCC 19523), *Klebsiella pneumoniae* (ATCC 10031), *Pseudomonas aeruginosa* and *Proteus vulgaris* were obtained from S.M.S. Medical College, Jaipur, and maintained on a nutrient broth agar medium by incubating at 37°C for 48 h. Fungi, *Aspergillus fumigatus, Penicillium chrysogenum, Rhizoctonia phaseoli* and *R. stolonifer* (from Seed Pathology Laboratory, Department of Botany, University of Rajasthan, Jaipur) were grown on potato dextrose agar medium and incubated at 27°C for 48 h. Similarly, *Candida albicans,* obtained from S.M.S. Medical College, Jaipur and maintained at Sabouraud dextorse agar (SDA) medium and incubated at 30°C for 5 days (Chang and Cury, 1998).

Antimicrobial assay

Antimicrobial activity was screened by using the disc diffusion method (Gould and Bowie, 1952). The filter paper discs (Whatman No. 1, 6.3 mm in diameter) impregnated with hexane, was used as negative control, isolated and purified compounds (concentration at 1.0 mg/disc eluted with hexane), while as reference antibiotics (gentamycin -1.0 mg/disc for bacteria and nystatin -1.0 mg/disc eluted with water used for fungi) used as positive control drugs. For this experiment three sets of discs were performed for each microorganism. The plates were incubated at 30°C for 24 h and inhibition zone diameter (including the 6.3 mm disc) were recorded. The sterile conditions were maintained throughout the experiment. The activity index (AI) was calculated by comparison with respective compounds (AI = Inhibition zone of test sample / Inhibition zone of standard).

RESULTS AND DISCUSSION

The aerial parts of plant material defatted with petroleum ether for 24 h, filtered and residue was Soxhlet extracted with 95% EtOH for 48 h, filtered and dried *in vacuo.* The crude ethanolic extract of *C. grandiflora* refluxed with 15% ethanolic HCl, filtered and fractionated with EtOAc. The EtOAc fraction was neutralized with sodium sulphate for moisture removal. Later, dried *in vacuo* and gave 4 phytosterols: I, lanosterol (147 mg); II, β-sitosterol (269 mg); III, stigmasterol (349 mg); IV, campesterol (332 mg). For triterpenes, the EtOH extract partitioned among hexane (Fr. I) and C_6H_6 - hexane (2:1, v/v, Fr. II) and gave 4 triterpenes - V, friedelin (237 mg); VI, lupeol (620 mg); VII, ursolic acid (223 mg) and VIII, β-amyrin (217 mg; Figure 1).

The callus cultures of *C. grandiflora* were also maintained and the growth indices showed an increase up to a period of 6 weeks but later on the growth was declined up to 8 weeks. The growth index was maximum (5.83±0.00256 w/w) in 6 weeks old callus while at minimum of (1.25±0.00112 w/w) 2 weeks old callus (Table 1). On quantification, the β-sitosterol was found to be maximum in quantity (0.43±0.000241w/w) in 6 weeks old callus (Table 2). Microscopical observations suggest that much of the early increase in cell dry weight is due to accumulation of starch grains while subsequently disappear during growth cycle. Growth was therefore balanced, since rates of dry weight, proteins, and carbohydrates were in proportion to cell division rate (Wilson, 1976; Wilson and Marron, 1978). The cells are therefore continuously undergoing physiological changes and there is no point in time at which cultured cells in balanced growth (Wilson, 1971).

Accumulation of secondary metabolites in cell culture by the use of elicitors has attributed the attention of workers in the recent years (Wolters and Eilert, 1982; Dicosmo and Misawa, 1985; van der Luit et al., 2000; Madrid and Corchete, 2010). In the present study, the effect of salicylic acid (abiotic elicitor) on the phytosterols and triterpenoids biosynthesis has been evaluated using suspension cultures treated at various concentrations (0.01, 0.025, 0.05 and 0.1 mM). From reported results, it is evidenced that quantity of campesterol (0.095±0.0021 w/w) was higher at 0.025 mM dose. Likewise, ergosterol (biotic elicitor) also demonstrated strong effect on the yield of phytosterols and triterpenoids in the cell suspension cultures (Muffler et al., 2010). The quantity of lupeol was also maximum (0.11±0.0081 w/w) at 0.01 mM dose (Table 3). The above finding shows that the elicitor concentration is a factor that strongly affects the biosynthetic yield of individual compounds.

Intensive research work has been devoted to establishing the mechanism of elicitation in plants. Research was focused mainly on the biotic and particularly carbohydrate elicitors, and the effect of abiotic elicitors on the overproduction of secondary metabolites in plants poorly understood, elicitation is hypothesized to involve the very messenger Ca^{2+}, factors affecting cell membrane integrity, inhibition/activation of intracellular pathways and changes in osmotic pressure acting as stress agents (Pitta-Alvarez et al., 2000; Angelova et al., 2006). Veersham et al. (1995) reported the addition of biotic elicitors, cell extracts of *Gliocladium deliquescens* improved the production of taxol and related taxanes in *Taxus* species cell cultures. Enhanced production of saponin content by *Panax ginseng* cell cultures with biotic elicitors are known (Jeong et al., 2005). Lipid domain formation is dependent on sterol component having a structure that allow tight packing with lipids having saturated acyl chain. The domain promoting activities of various natural sterols were compared with that of cholesterol, both plant and fungal sterols promote the

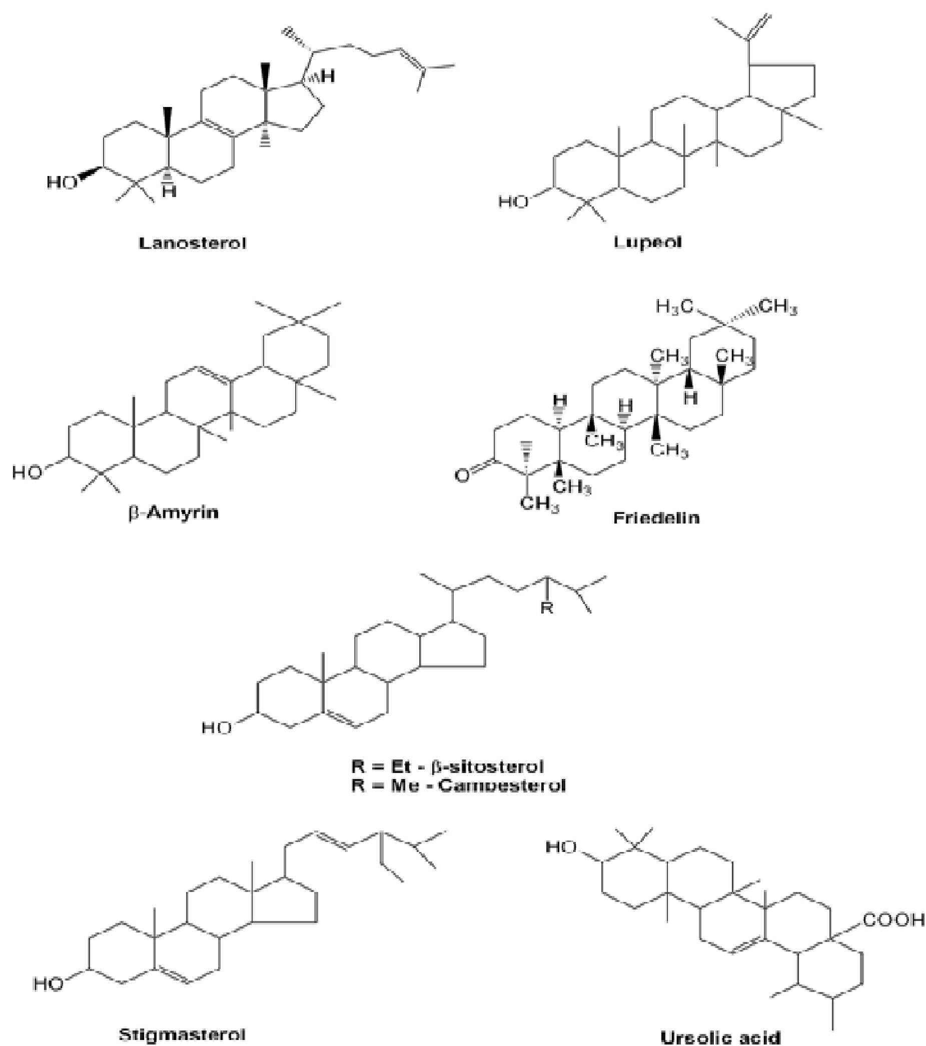

Figure 1. Structures of isolated compounds.

Table1. Growth indices of *C. grandiflora* cell cultures.

Callus age (in weeks)	*Growth index (GI) ± S.D.
2	1.25±0.00112
4	3.64±0.00232
6	5.83±0.00256
8	5.61±0.00189

*Growth index = Final dry weight of callus – Initial dry weight of callus/Initial dry weight of callus. Values are mean of triplicate readings.

formation of tightly packed, ordered lipid domains by lipids with saturated acyl chains (Xu et al., 2001). The role of fungal sterol, ergosterol as a general elicitor in the triggering of plant innate immunity in sugar beet. Evidence for this specific function of ergosterol is provided by careful comparison with the cholesterol and three plant sterols (stigmasterol, campesterol and sitosterol) which enable the integrity of responses leading to elicitation.

Our results demonstrate the modification of H^+ influx by ergosterol due to the direct inhibition of the H^+ ATPase activity on plasma membrane vesicles purified from

Table 2. Estimation of phytosterols and triterpenoids from *C. grandiflora* cell cultures.

Isolated compounds	*Quantity of isolated compounds (w/w) ± S.D. *In vitro* (age of callus in weeks)				
	0	2	4	6	8
I	0.011±0.00212	0.029±0.000712	0.056±0.000665	0.092±0.000232	0.089±0.000987
II	0.023±0.00319	0.061±0.000619	0.086±0.000339	0.43±0.000241	0.41±0.000448
III	0.022±0.00268	0.042±0.000319	0.071±0.000819	0.12±0.000161	0.11±0.000312
IV	0.013±0.00239	0.031±0.000118	0.062±0.000223	0.089±0.000119	0.083±0.000111
V	0.019±0.00369	0.029±0.000216	0.039±0.000333	0.082±0.000667	0.077±0.000286
VI	0.027±0.00819	0.049±0.000116	0.082±0.000441	0.31±0.000717	0.22±0.000770
VII	0.015±0.00619	0.028±0.000119	0.056±0.000569	0.098±0.000561	0.089±0.000505
VIII	0.012±0.00559	0.026±0.000816	0.062±0.000619	0.096±0.000534	0.091±0.000339

*Values are mean of triplicate readings.

Table 3. Effect of salicylic acid (abiotic elicitor) and ergosterol (biotic elicitor) on level of isolated compounds from *C. grandiflora*.

Isolated compounds	Saliycylic acid					Ergosterol				
	Elicitor concentration (mM)					Elicitor concentration (mM)				
	Control	0.01	0.025	0.05	0. 1	Control	0.01	0.025	0.05	0. 1
I	*0.031±0.0083*	0.059±0.045	0.066±0.0022	0.45±0.0058	0.039±0.0036	0.031±0.0052	0.056±0.0089	0.041±0.0087	0.036±0.0064	0.035±0.016
II	0.043±0.0075	0.064±0.0062	0.075±0.0029	0.059±0.0070	0.048±0.0041	0.043±0.0061	0.086±0.0081	0.044±0.0069	0.044±0.0048	0.046±0.0028
III	0.058±0.0071	0.071±0.0041	0.089±0.0019	0.077±0.0067	0.072±0.0071	0.058±0.0065	0.098±0.0078	0.062±0.0063	0.056±0.0054	0.054±0.0026
IV	0.061±0.0065	0.085±0.0035	0.095±0.0021	0.087±0.0079	0.069±0.0065	0.061±0.0075	0.089±0.0061	0.071±0.0069	0.065±0.0059	0.060±0.0018
V	0.049±0.0051	0.067±0.0041	0.058±0.0044	0.055±0.0056	0.052±0.0078	0.049±0.0061	0.067±0.0065	0.059±0.0056	0.054±0.0064	0.052±0.0062
VI	0.069±0.0062	0.091±0.0054	0.084±0.0035	0.077±0.0065	0.075±0.0092	0.069±0.0045	0.11±0.0081	0.098±0.0096	0.089±0.0076	0.081±0.0056
VII	0.045±0.0054	0.064±0.0056	0.048±0.0086	0.046±0.0046	0.052±0.0061	0.045±0.0056	0.061±0.0039	0.056±0.0086	0.049±0.0071	0.046±0.0052
VIII	0.039±0.0068	0.045±0.0071	0.042±0.0075	0.049±0.0052	0.050±0.0065	0.039±0.0044	0.042±0.0059	0.040±0.0077	0.044±0.0066	0.040±0.0043

*Quantity of estimated compounds. Values are mean of triplicate readings

Table 4. Bioefficacy of phytosterols and triterpenoids from *C. grandiflora.*

Microorganisms		Isolated compounds ± S.D.							
		I	II	III	IV	V	VI	VII	VIII
Bacteria									
E. coli	*IZ	16.4±0.186	12.3±0.189	14.3±0.182	13.6±0.119	11.4±0.888	12.8±0.0868	19.2±0.113	13.5±0.316
	AI	0.937	0.702	0.817	0.777	0.612	0.688	1.032	0.725
S. aureus	IZ	16.3±0.55	15.5±0.265	16.6±0.441	13.4±0.540	15.4±0.716	11.5±0.662	16.7±0.189	12.9±0.114
	AI	0.900	0.856	0.917	0.740	0.802	0.598	0.854	0.671
E. cloacae	IZ	15.6±0.667	14.1±0.656	18.2±0.339	12.9±0.448	10.6±0.789	11.6±0.710	15.6±0.0216	15.9±0.365
	AI	0.776	0.701	0.905	0.641	0.642	0.703	0.945	0.963
K. pneumoniae	IZ	14.3±0.678	14.0±0.467	17.6±0.565	11.6±0.419	12.2±0.668	13.5±0.691	14.8±0.335	19.5±0.415
	AI	0.777	0.760	0.956	0.630	0.635	0.703	0.770	1.015
P. aeruginosa	IZ	12.5±0.719	13.4±0.415	17.0±0.619	11.2±0.550	15.2±0. 568	14.1±0.654	18.4±0.410	18.8±0.484
	AI	0.631	0.676	0.858	0.565	0.741	0.687	0.897	0.917
P. vulgaris	IZ	13.5±0.834	12.6±0.590	16.4±0.589	12.8±0.610	12.4±0.330	15.1±0.543	16.6±0.868	17.6±0.435
	AI	0.678	0.633	0.824	0.643	0.659	0.803	0.882	0.936
Fungi									
A. fumigatus	IZ	11.5±0.911	10.8±0.556	09.5±0.519	13.3±0.561	10.6±0.434	10.8±0.639	12.4±0.569	13.4±0.568
	AI	0.804	0.755	0.664	0.930	0.670	0.683	0.784	0.854
C. albicans	IZ	13.4±0.232	11.5±0.661	10.2±0.439	15.6±0.689	11.5±0.543	11.8±0.384	10.5±0.667	13.9±0.479
	AI	0.864	0.741	0.658	1.006	0.777	0.797	0.709	0.939
P. chrysogenum	IZ	15.2±0.436	11.8±0.735	11.2±0.441	14.3±0.614	13.5±0.445	12.6±0.541	11.4±0.369	12.9±0.395
	AI	0.962	0.746	0.708	0.905	0.789	0.736	0.666	0.754
R. phaseoli	IZ	14.1±0.557	12.2±0.192	10.6±0.436	13.8±0.556	12.6±0.435	10.5±0.549	15.5±0.871	11.8±0.529
	AI	0.774	0.670	0.582	0.758	0.677	0.564	0.833	0.634
R. stolonifer	IZ	13.8±0.666	10.5±0.239	10.4±0.337	12.8±0.437	11.6±0.562	13.5±0.465	14.8±0.736	10.9±0.561
	AI	0.841	0.640	0.634	0.780	0.748	0.870	0.954	0.703

*IZ = Inhibition zone (in mm) includes the diameter of disc (6.3 mm); AI = IZ of test sample / IZ of standard. Values are the mean of triplicate readings.

leaves. The ergosterol-induced oxidative burst is related to enhanced NADPH-oxidase and superoxide dismutase acivity (Rossard et al., 2010). In the present investigation, the isolated phytosterols and triterpenoids were screened for their antimicrobial activity and found that ursolic was the most active against *E. coli* (IZ= 19.2; AI = 1.032) and campesterol showed potent activity against *Candida albicans* (IZ = 15.6; AI = 1.006). The observed results indicate that the isolated phytosterols and triterpenes demonstrated greater efficacy against bacteria rather than fungi (Table 4). Development of chemical evaluation for the drug has been hampered by previous lack of knowledge for the precise active principles (Trease and Evans, 1973).

The interest of pharmaceutical industries to screen natural product extracts for new biological active compounds is increasing due to high through-put screening methods. For this, our huge biodiversity can be explored effectively to find out lead consisting of a large number of chemical structures with potent therapeutic activity for the treatment of deadly ailments. The number of compounds have been isolated and identified for their specific characteristics which will not only help to screen the compounds but also proper validation for the formations of all traditional medicine can be made more effectively (Mukerjee et al., 1999).

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