Journal of Food Processing & Technology
ISSN: 2157-7110

Garai et al., J Food Process Technol 2018, 9:2
DOI: 10.4172/2157-7110.1000717

Research Article Open Access

Anti-microbial and Anti-cancer Properties of Echinocystic Acid Extracted from *Luffa cylindrica*

Garai S[1]*, Ghosh R[2], Bandopadhyay PP[3], Mandal NC[2] and Chattopadhyay A[3]

[1]*Organic and Medicinal Chemistry Division, CSIR-Indian Institute of Chemical Biology, Kolkata-700032, India*
[2]*Department of Botany, Visva-Bharati, Santiniketan-731235, West Bengal, India*
[3]*Department of Zoology, Visva-Bharati, Santiniketan-731235, West Bengal, India*

Abstract

Triterpenoids are the most ubiquitous non-steroidal secondary metabolites in terrestrial and marine flora and fauna occurring in the free form as well as in the forms of ether, ester and glycosides. The diverse structural types of triterpenoids with useful biological activities, identification, biosynthesis for feed industry increases interest in triterpenoids. The widely occurring plant *Luffa cylindrica* was investigated for active triterpenoid sapogenins. The triterpenoid sapogenins were isolated by solvent extraction followed by chromatography and were defined by newer spectroscopic methods. These triterpenoid sapogenins from *Luffa cylindrica* were isolated and characterized. The anti-microbial and anti-cancer properties of echinocystic acid will be discussed.

Keywords: *Luffa cylindrica*; Anti-microbial activity; Anti-cancer activity; Disc Diffusion assay; MTT assay

Introduction

Triterpene of diverse structural types are widely distributed in terrestrial and marine sediments, prokaryotes as well as eukaryotes. The triterpenoids have a range of unique and potentially usable biological effects. The most important triterpenoid structures are oleanane, ursane, lupane and dammarane-euphane triterpenoids on biological point of view. *Luffa cylindrica* (Cucurbitaceae) is a traditionally important medicinal plant. It is widely distributed in tropical and subtropical areas throughout India. It is used in clinical problems relating to child birth. The chemical constituents such as sapogenins, saponins and proteins of *Luffa cylindrica* used as potentially effective clinical agent in health care in many communities reported [1-4]. The saponins and sapogenins isolated from the plant have the same oleanane type skeleton. The two major sapogenins of the plants are mainly oleanolic acid **(1)** and echinocystic acid **(2)** [5]. Its seed and fruits are used as a vegetable either prepared like squash or eaten like cucumbers. Khajuria et. al [6] reported the immunomodulatory effects of two oleanane sapogenins, oleanolic acid **(1)** and echinocystic acid **(2)** in mice isolated from *Luffa cylindrica*. In this study, the anti-microbial and anti-cancer properties of oleanane triterpenoid sapogenin, echinocystic acid **(2)** will be discussed.

Materials and Methods

Materials

Seeds of *Luffa cylindrica* were collected from Jammu, India and identified in the Botany Department of Regional Research Laboratory (Formerly RRL, CSIR-IIIM), Jammu. The voucher specimen is deposited in the herbarium of the institute. Dulbecco's Modified Eagle's Medium (DMEM), Roswell Park Memorial Institute (RPMI)-1640 culture media, fetal bovine serum (FBS), penicillin–streptomycin antibiotic solution, phytohemagglutinin (PHA-M) and 3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide (MTT) were purchased from HiMedia (Mumbai, India). Heparin and trypan blue were purchased from SRL (India); Histopaque, were procured from Sigma-Aldrich (St Louis, MO, USA).

Sample preparation

The methanolic extract (60 g) of the defatted powdered seeds of *L. cylindrica* was hydrolysed with aqueous MeOH-HCl under reflux for 4 h. The acid hydrolysate was separated into acidic and neutral fractions by treatment with a saturated solution of $NaHCO_3$. The acidic fractions were separately subjected to column chromatography on silica gel using n-Hexane-$CHCl_3$ in various ratios 9:1, 3:1, 1:1 and $CHCl_3$-MeOH (24:1) and similar fractions so obtained were further purified by preparative TLC ($CHCl_3$-Pyridine-H_2O 9:0.5:0.5) followed by crystallization. Thus, two sapogenins, 1 (950 mg) and 2 (200 mg) were obtained. The two sapogenols were identified by IR, NMR and ESIMS. The two polar sapogenins are oleanolic acid **(1)** mp. 308°C-310°C and echinocystic acid **(2)**, mp. 300°C-305°C. The echinocystic acid **(2)** was dissolved in non-toxic organic solvent dimethyl sulphoxide (DMSO) and stored at 4°C until use.

Microorganisms

The standard bacterial strains used as test organisms were obtained from Microbial Type Culture Collection (MTCC) from Institute of Microbial Technology, Chandigarh, India. They are belonged to the Gram Positive and Gram-negative category. The Gram-positive food borne pathogens are *Bacillus subtilis* (MTCC121), *Staphylococcus aureus* (MTCC96) and *Listeria monocytogenes* (MTCC657). *Escherichia coli* (MTCC1667) and *Pseudomonas aeruginosa* (MTCC741) are Gram negative bacteria. *Salmonella typhimurium* (MTCC98) is most predominating food spoilage human pathogenic Gram-negative bacteria.

Anti-bacterial activity screening

The Anti-bacterial screening of echinocystic acid **(2)** was determined by Disc Diffusion method of Bauer et al. [7] against some Gram positive and Gram negative pathogenic bacterial strains. This study was performed using 2500 µg/ml, 250 µg/ml, 25 µg/ml and 2.5

***Corresponding author:** Garai S, Organic and Medicinal Chemistry Division, CSIR-Indian Institute of Chemical Biology, 4 Raja S.C. Mullick Road, Jadavpur, Kolkata-700032 India, Tel: 009474767565, 91-33-2499-5947, Fax: 91-33-2473-5197/2414-9475; E-mail: saraswatigarai@iicb.res.in

Received January 18, 2018; **Accepted** February 05, 2018; **Published** February 12, 2018

Citation: Garai S, Ghosh R, Bandopadhyay PP, Mandal NC, Chattopadhyay A (2018) Anti-microbial and Anti-cancer Properties of Echinocystic Acid Extracted from *Luffa cylindrica*. J Food Process Technol 9: 717. doi: 10.4172/2157-7110.1000717

μg/ml of echinocystic acid **(2)**. The inhibition zones were obtained at 250 μg/ml and values were found to be at one fourth of 250 μg/ml of oleanolic acid **(1)**. Ciprofloxacin was used as positive controls.

Anti-microbial mode of action of Echinocystic acid (2) on Gram positive and Gram negative bacterial cells by counting colony forming units (CFUs)

Echinocystic acid **(2)** once applied to actively growing liquid culture (exponential phase of growth) of *Listeria monocytogenes* and *Salmonella typhimurium* and their colony forming units (CFU) were counted for next 24 hours along with untreated control.

The echinocystic acid **(2)** at its MIC was treated on actively growing culture (8 hrs grown) and allowed to grow further till 24 hrs. In every two hours interval aliquot of culture (both treated and untreated) was withdrawn aseptically diluted serially with sterilized distilled water and plated into nutrient agar medium, incubated at 37°C for another 24 hrs. The colonies appeared were counted per ml of culture broth [7-11].

Antifungal activity screening

The antifungal screening of echinocystic acid **(2)** was determined by same Disc Diffusion method of Bauer et al. [7] against *Candida albicans* MTCC3018. Fluconazole was used as standard.

Cell culture

MCF7 and MDA-MB-231 breast cancer cell lines were procured from the National Centre for Cell Science (Pune, India). Cell culture was done according to the description of Banerjee et al. [12]. Briefly, cells were grown in DMEM, supplemented with 1% penicillin–streptomycin and 10% (v/v) FBS solution at 37°C in a humidified CO_2 incubator (5% CO_2) (Thermo Fisher Scientific, Waltham, MA, USA).

MTT assay

Cells (1×10^4) were seeded per well of a 96-well plate and incubated in standard culture condition for 24 h. After that, complete DMEM media was removed and cells were replenished with incomplete media (without FBS) and then treated with different concentrations of echinocystic acid **(2)**. Both negative and positive controls were used along with the treated groups. Treated cells were incubated at 37°C in the presence of 5% humidified CO_2 for 48 h, and the proliferation rates were estimated by MTT assay at 595 nm using an ELISA reader (Bio Rad, USA). The percentage of viable cells was calculated taking viability of untreated cells as 100%.

Isolation of human peripheral blood mononuclear cells; culture and MTT assay

Fresh blood was collected from five healthy non-smokers, non-alcoholic male donors (21–25 years of age) with written informed consents by venipuncture into heparinised falcon tubes. Blood was collected by pathologists employed at the university hospital, under the supervision of medical doctors. All studies were performed complying fully with the approved "Ethical Guidelines for Biomedical Research on Human Subjects" formulated by the Indian Council of Medical Research, India. The work was reviewed and approved by the Institutional Ethics Committee for Human Research of Visva-Bharati University. Human peripheral blood mono nuclear cells (HPBMC) were isolated according to the method of Bøyum [13] with minor modification. Blood was layered over equal amount of Histopaque and centrifuged at 1000 × g for 30 min. The buffy coat was aspirated into 3-5 ml PBS and centrifuged at 1000 × g for 10 min, and the washing process was repeated thrice. The pellet was resuspended in RPMI-1640 media (1×10^6 cells/ml) and viability was checked using the trypan blue dye exclusion method.

Cell viability was found to be >95%, which indicated successful isolation. Isolated HPBLs (0.5 ml) were stimulated by PHA and cultured in 5 ml of RPMI-1640 media with 10% FBS along with antibiotics. After 24 h, cells were treated for 48 h with respective IC_{50} doses of echinocystic acid **(2)** in MCF7 and MDA MB 231 cell lines and proliferation rates were estimated by MTT assay at 595 nm using a ELISA reader (Bio Rad, USA). Percentage of viable cells was calculated taking viability of untreated cells as 100%.

Statistical analysis

Results were expressed as mean ± standard error (SE). Statistical analysis of the data was performed by paired *t*-test, and $p<0.05$ were considered significant.

Results and Discussion

In the Disc Diffusion study, the anti-microbial activity of echinocystic acid **(2)** was evaluated against all the Gram-positive *Bacillus subtilis*, *Listeria monocytogenes*, *Staphylococcus aureus* and Gram-negative *Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa* bacteria (Table 1) [8-11]. It showed significant anti-microbial potential against the Gram-positive *Bacillus subtilis*, *Listeria monocytogenes*, *Staphylococcus aureus* and Gram-negative *Salmonella typhimurium* bacteria (Figure 1). The echinocystic acid **(2)** could kill all the tested bacteria at 250 μg/ml and maximum zones of inhibition were obtained using highest concentration (2500 μg/ml) suggests that Echinocystic acid **(2)** killed the Gram-positive food borne pathogens *Bacillus subtilis*, *Listeria monocytogenes*, human pathogenic *Staphylococcus aureus* and one Gram-negative *Salmonella typhimurium* bacterium. MIC of echinocystic acid **(2)** obtained against all the tested bacteria was 62.2 μg/ml. A clear cut bacteriostatic pattern of growth for both the strains, the Gram-positive *Listeria monocytogenes* and Gram-negative *Salmonella typhimurium* was found in the anti-microbial mode of action study (Table 2). Figures 2 and 3 clearly indicated static type of mode of action upon treatment with echinocystic acid **(2)** at its MIC. Echinocystic acid **(2)** has moderate anticandidal activity against *Candida albicans* (Table 3). With regard to the structure-activity relationship it was suggested that both the hydroxyl and carboxyl groups are important

Bacterial strains	Echinocystic acid (2)
Bacillus subtilis MTCC 121 Gram (+) ve	+++
Listeria monocytogenes MTCC 657 Gram (+) ve	+++
Staphylococcus aureus MTCC 96 Gram (+) ve	+++
Escherichia coli MTCC 1667 Gram (-) ve	-
Salmonella typhimurium MTCC 98 Gram (-) ve	+++
Pseudomonas aeruginosa MTCC 741 Gram (-) ve	-
+++, inhibition zone diameter more than 20 mm; -, no inhibition	

Table 1: Anti-bacterial screening of Echinocystic acid **(2)** against six Gram-positive and Gram-negative bacteria.

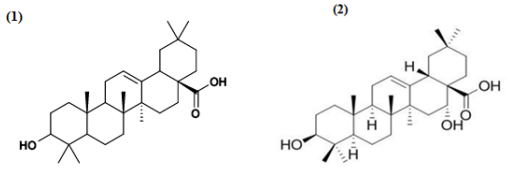

Figure 1: Structures of Oleanolic acid **(1)** and Echinocystic acid **(2)**.

for their Anti-bacterial activity of echinocystic acid **(2)**. MTT assay results showed that echinocystic acid **(2)** inhibited the cell viability of human breast cancer cell lines (Figure 4). Both MCF7 and MDA-MB 231 cell lines showed dose dependent inhibition of cell growth after treatment with echinocystic acid **(2)**. Significant inhibitory effect was observed at the dose of 60 µM. Calculated IC_{50} value for MCF7 cell line was 41.72 µM and for MDA-MB 231 cell line was 48.17 µM. Isolated HPBMCs were treated with both these IC_{50} for 48 h. 96.29% of cell growth inhibition was observed at 41.72 µM which was 84.34% at the dose of 48.17 µM. This shows that echinocystic acid **(2)** is not cytotoxic to normal cells.

Log CFU count at different hours	Control Gram positive *Listeria monocytogenes*	Treated on actively growing culture (8 hrs culture)	Control Gram negative *Salmonella typhimurium*	Treated on actively growing culture (8 hrs culture)
0	0.8	--	0.7	--
2	1.0	--	0.9	--
4	1.2	--	1.3	--
6	1.9	--	2.0	--
8	3.0	3.0	2.9	2.9
10	5.2	5.2	5.0	5.0
12	7.6	5.2	7.4	4.8
14	9.4	5.1	9.3	4.8
16	10.7	5.0	10.5	4.6
18	11.0	4.8	11.2	4.5
20	11.3	4.5	11.6	4.3
22	11.4	4.3	11.7	4.2
24	11.4	4.2	11.8	4.1

Table 2: Mode of action of Echinocystic acid **(2)** on Gram positive and Gram negative bacterial cells by counting colony forming units (CFUs).

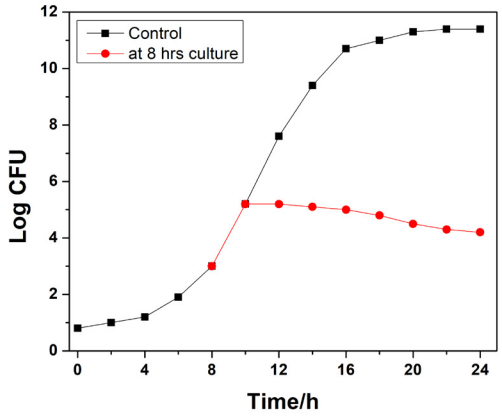

Figure 2: Time-kill curve of Echinocystic acid **(2)** against *Listeria monocytogenes*.

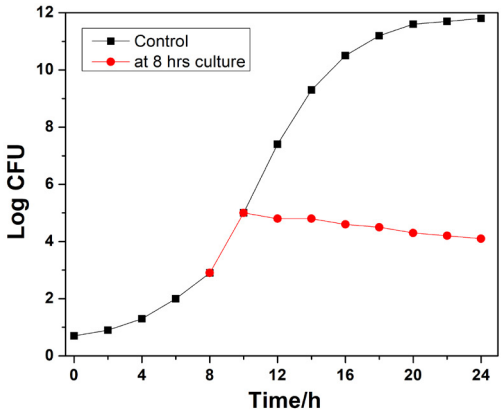

Figure 3: Time-kill curve of Echinocystic acid **(2)** against *Salmonella typhimurium*.

Fungal species	Echinocystic acid (2)	Echinocystic acid (2)	Fluconazole
	MIC_{100}	MIC_{80}	MIC_{100}
Candida albicans MTCC3018	300	250	300

MIC_{100} and MIC_{80}: Concentration of echinocystic acid **(2)** that caused 100% and 80% reduction of the growth control respectively.

Table 3: Minimum inhibitory concentration (MIC) in µg/ml of Echinocystic acid **(2)**.

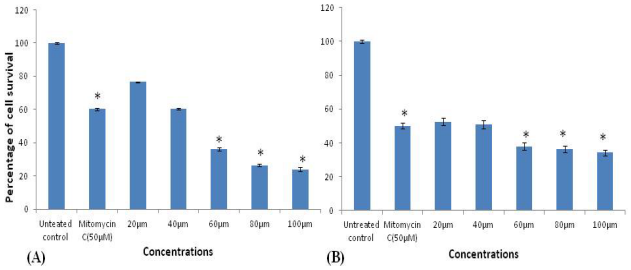

Figure 4: Histograms showing cell survival (%) of (A) MDA-MB-231and (B) MCF7 cells after treatment with different concentrations of echinocystic acid **(2)** for 48 h (*significantly different from control. p<0.05). Mitomycin C (50 µM) was taken as positive control.

Conclusion

Due to its potential anti-microbial properties echinocystic acid **(2)** can be considered as natural preservatives in food processing industry to reduce the microbial growth in processed food products. Echinocystic acid has potential anti-cancer activity on human breast cancer cell lines and it can be used in human health care. We are pursuing further studies of anti-microbial and anti-cancer activities of the two major oleanane sapogenins, oleanolic acid **(1)** and echinocystic acid **(2)** of the plant to provide a basis for discussion of their activity in relation to their chemical structures. This is the first report of anti-microbial and anti-cancer properties of echinocystic acid of *Luffa cylindrica*.

Acknowledgement

Financial support from CSIR, New Delhi is gratefully acknowledged.

References

1. Azeez MA, Bello OS, Adediji AO (2013) Traditional and medicinal uses of *Luffa cylindrica*: A Review. J Med Plants Studies 1: 102-111.
2. Nair DU, Saraswathy MP, Kishore N, Reddy Pully N (2010) Phytochemical and antimicrobial evaluation of *Luffa cylindrica* Linn. leaf and flower extracts: An *in-vitro* study. Res J Pharm Tech 3: 438-441.
3. Velmurugan V, George S, Surekha PS (2011) Phytochemical and biological screening of *Luffa cylindrica* Linn. fruit. Inter J Pharm Tech Res 3: 1582-1585.
4. Muthumani P, Meera R, Mary S, Devi PJ, Kameswari B, et al. (2010) Phytochemical and antiinflammatory, bronchodilator and antimicrobial activities of the seeds of *Luffa cylindrica*. Res J Pharm Bio Chem Sci 1: 11-21.
5. Yoshikawa K, Arihara S, Wang JD, Narui T, Okuyama T, et al. (1991) Structures of two new fibrinolytic saponins from the seed of *Luffa cylindrica* ROEM1). Chem Pharm Bull 39: 1185-1188.
6. Khajuria A, Gupta A, Garai S, Wakhloo BP (2007) Immunomodulatory effects

of two sapogenins 1 & 2 isolated from *Luffa cylindrica* in Bulb/C mice. Bioorg Med Chem Lett 17: 1608-1612.

7. Bauer AW, Kirby WMM, Sherries T (1966) Antibiotic susceptibility testing by a standard single disc method. Am J Clin Pathol 45: 493-496.
8. Mondal V, Sen SK, Mondal NC (2011) Isolation and characterization of pediocin NV 5 from vacuum-packed fermented meat product. Ind J Microbiol 51: 22-29.
9. Huang G, Mason SL, Hudson JA, Clerens S, Plowman JE, et al. (2014) Proteomic differences between *Listeria Monocytogenes* isolates from food and clinical environments. Pathogen 3: 920-933.
10. Colagiooprgi A, Bruini H, Aldo Di-Ciccio P, Zanardi E, Ghidini S, et al. (2017) Listeria monocytogenus Biofilms in the wonderland of Food Industry. Pathogen 6: 41.
11. Boukhira S, Balour M, Monsouri LE, Youbi AEHE, Bouarfa M, et al. (2017) Development of natural preservative from *Silene vulgaris* extract in topical formulation under a challenge test and its stability study. J Appl Pharm Sci 7: 142-148.
12. Banerjee PP, Bandyopadhyay A, Nagesh HS, Policegoudra RS, Bhattacharya S, et al. (2017) *Mentha arvensis* (Linn.) mediated green silver nanoparticles trigger caspase 9 dependent cell death in MCF7 and MDA-MB-231 cells. Breast Cancer: Target Therapy 9: 265-273.
13. Bøyum A (1976) Isolation of lymphocytes, granulocytes and macrophages. Scand J Immunol 5: 9-15.