Acta Pharm. Sci. Vol 62:(2), 2024
Research article
DOI: 10.23893/1307-2080.APS6225

Chemical composition and biological activities of seed butter extracted from *Garcinia gummi-gutta* (L.) Roxb.

Neenthamadathil Mohandas KRISHNAKUMAR[1*], George SHINEY[2]

1 Department of Biosciences, Rajagiri College of Social Sciences (Autonomous), Rajagiri P. O., Kalamassery, Cochin, 683104, Kerala, India

2 Department of Biotechnology, Presentation College of Applied Sciences, Puthenvelikkara, Ernakulam, 683594, Kerala, India

ABSTRACT

Garcinia gummi-gutta (L.) Roxb. is a commercially viable fruit crop and it is a rich source of valuable edible fat known as seed butter. In the present study, the cytotoxic, antiproliferative and antimicrobial properties of *G. gummi-gutta* seed butter (GGSB) were investigated. The cytotoxic effects of the seed butter were assessed with Dalton's Lymphoma Ascites (DLA) and Ehrlich's Ascites Carcinoma (EAC) cell lines, and also on normal splenocytes. The seed butter was found to be cytotoxic for DLA an EAC and it exhibited a dose dependent effect and IC_{50} value of 289.04 μg/mL and 299.18 μg/mL for DLA and EAC respectively while less toxic to normal spleen cells. Further, human triple negative breast cancer (MDA-MB-231) and hepatocellular carcinoma (HepG2) cell lines in culture exhibited reduction in cell viability revealing its antiproliferative effect. The IC_{50} value of GGSB against MDA-MB-321 and HepG2 were determined as 66.70±2.60 μg/mL and 110.98 μg/mL respectively. The antimicrobial effect of the seed butter was determined by broth dilution method. The gas chromatography mass spectrometry (GC-MS) and gas chromatography with flame ionization detection (GC-FID) analysis revealed the presence fatty acids as the major phytochemical compounds responsible its bioactivities.

Keywords: *garcinia gummi-gutta*, cytotoxicity, antiproliferative effect, antimicrobial activity

*Corresponding author: Neenthamadathil Mohandas KRISHNAKUMAR
E-mail: krishnakumarnmohandas@gmail.com
ORCIDs:
Neenthamadathil Mohandas KRISHNAKUMAR: 0000-0002-8510-5391
George SHINEY: 0009-0001-2575-4760
(Received 11 May 2023, Accepted 29 Sep 2023)

INTRODUCTION

The regular consumption of dairy butter has increased risks of weight gain and coronary diseases and therefore search for alternate plant based butter viz., seed butter is important. The seeds and nuts are consumed in various ways as ingredients recipe, spreads, snacks and as a delicacy[1]. Plant based butters are made from plant nuts like cashew butter, peanut butter, almond butter, sunflower butter, soy butter, sesame butter and pumpkin seed butter[2]. The previous reports suggested that nut or seed butter consumption protects against lifestyle diseases like diabetes, coronary heart diseases, metabolic syndrome and gall stone disease[3]. The plant based seed butters contain higher percentage of unsaturated fats, essential fatty acids and minerals like magnesium, calcium, zinc etc. The phytocompounds present in seed butters exhibited protection against breast, prostate and colon cancer[4]. The efficacy of anticancer drugs improves when combined with natural compounds like curcumin, resveratrol, apigenin, cyclopamine, quercetin, tetrandrine etc. The anticancer effect is achieved through perturbing various cellular signaling pathways in cancer development. The phytocompounds prevent tumor cell proliferation, development and metastasis by various mechanisms such as induced cell cycle arrest, reactive oxygen species generation, activating intrinsic and extrinsic apoptotic pathways and down regulating the activated signaling pathways[5]. It has been reported that dietary polyphenols exhibited cancer chemoprevention effect through the epigenetic regulation of cancer associated genes via noncoding RNAs (ncRNAs) and long noncoding RNA (lncRNA)[6].

Only some limited literature is available on the therapeutic effects of plant based seed butters. *Cucurbita pepo* (pumpkin) seeds alleviated the signs of benign prostatic hyperplasia by decreasing protein binding prostate (PBP) levels and weight of ventral prostate[7]. In another study, Ortiz et al. (2019) reported the cytotoxic and antiproliferative activities of grape seed and extracts of grape pomace on colon cancer cells (Caco-2) and the effect was achieved through the down- regulation of Mfc gene expression levels[8]. In addition, the roasted almond butter significantly lowered total cholesterol and low density lipoprotein levels in healthy volunteers suggesting cardiac protective effect of the butter[9].

Garcinia gummi-gutta (L.) Roxb. (Family: Clusiaceae) commonly called 'Malabar Tamarind', is a small tree up to 25 m height. It is an under exploited, economically important tree species, tropically distributed and found in the evergreen forests of Western Ghats from Konkan to Kerala of the Indian peninsula. It is also common in the home gardens and fields of Kerala. The fruit rind of *G. gummi-gutta* is acidic and used in fish and meat curries as a souring

condiment[10]. *G. gummi-gutta* is traditionally used against constipation, delayed menstruation, edema, fever, hemorrhoids, dysentery and diarrhea. It is an established plant for reducing body weight[11]. The dried seeds yield edible fat commonly known as 'seed butter', due to its solid state in room temperature, and it is a rich source of protein and fat. The physicochemical characteristics such as acid value, saponification value and peroxide value of the seed butter are within the range of domestic oils used for cooking purposes and therefore it forms an important source for edible oil[12].

A closely related species of the family *Garcinia indica* is a good source of edible fat known as 'Kokum butter'. It is used by the people of coastal Konkan region of the states of Goa and Maharashtra, India for cooking purposes. The butter is also used in cosmetic industries and a better substitute for cocoa butter in chocolate industries[13]. Earlier, *G. gummi-gutta* seeds were considered as waste product of post-harvest operations. The seed oil *G. gummi-gutta* is traditionally used against skin diseases[14] and recently it has been explored as a source of biodiesel[15]. Rani et al. (2021) evaluated the toxicity by acute oral toxicity and acute dermal toxicity studies *in vivo* and the results showed that there was no significant change in the bodyweight, no mortality, morbidity or clinical signs of dermal responses[16]. The importance of the present study is to find out the chemical composition of GGSB from *G. gummi-gutta* seeds. The therapeutic efficacy of the seed butter has not been scientifically validated and the present study was undertaken to investigate the cytotoxic, anti-proliferative and antimicrobial properties of *G. gummi-gutta* seed butter. Furthermore, GC-MS and GC-FID analyses were performed to understand the chemical composition of GGSB.

METHODOLOGY

Chemical reagents

RPMI-1640, Trypan blue, fetal bovine serum, penicillin and streptomycin were purchased from Sigma Aldrich, USA. Commercial kit for MTT assay was purchased from Merck, Germany. All the other chemicals used were of analytical reagent grade.

Cell cultures

DLA (Dalton's Lymphoma Ascites) and EAC (Ehrlich's Ascites Carcinoma) cell lines were obtained from Amala Cancer Research Centre, Thrissur, India. Human triple negative breast cancer cell line (MDA-MB-231) (RRID: CVCL_0062) and hepatocellular carcinoma (HepG2) (RRID: CVCL_0027) cell lines were procured from National Centre for Cell Sciences, Pune, India.

Animals

Sprague-Dawley rats, males (200–250g) and Swiss albino mice, males (25-30 g) were obtained from the Animal House of Amala Cancer Research Centre, Thrissur, Kerala, India. They were housed in polyacrylic cages under standard conditions of temperature (24–28◦C), relative humidity (60–70%) and 12h dark–light cycles), fed commercial rat feed (Lipton India Ltd., Mumbai, India) and boiled water *ad libitum*. Animals were acclimatized for 1 week before starting the experiments. All experiments involving animals were carried out according to guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, after getting the approval of the Institute's Animal Ethics Committee.

Extraction of the seed butter

The seeds of *G. gummi-gutta* were collected from the homesteads of Mala locality, Thrissur District, Kerala, India and it was authenticated by the plant taxonomist. A voucher specimen (PCASH-01 dated 15/05/2017) was deposited in the herbarium of Biotechnology Department of the College for future reference. The seed butter was extracted according to the standardized method[12]. The seeds were separated from the succulent aril of fresh ripen fruits, washed thoroughly and dried in oven at 60°C. The seed kernels were separated from the seed coat and second level of drying was carried out. 100 g of the kernels were washed in hot water to remove the impurities and they were slightly roasted in gentle heat in a pan and grinded, boiled with 1 L distilled water in an open container for 2 to 3 h. The oily upper layer was separated and decanted into a clean vessel and allowed to evaporate water content. The yield (%) was noted and the *G. gummi-gutta* seed butter (GGSB) was stored in dry and air tight containers.

Short term *in vitro* cytotoxicity studies on normal rat spleen cells

The seed butter was evaluated for short term cytotoxicity effect *in vitro* in rat spleen tissues collected from male Sprague-Dawley rats according to the standard protocol. It was then smashed to single cell suspension in RPMI complete medium containing antibiotics and filtered using mesh cloth. The collected cells were washed thrice and suspended in known volume of RPMI complete medium containing antibiotics and counted. Viable cell suspension ($1x10^6$ cells in 0.1 mL) was added to tubes containing various concentrations of GGSB and the volume was made up to 1 mL using RPMI media. Control tubes contained only cell suspension (without additives). These tubes were incubated for 3h at 37°C. At the end of incubation cell suspension in the tubes were mixed with 0.1 mL of 1% trypan blue and kept for 2-3 minutes and loaded on a haemocy-

tometer. Dead cells take up the blue colour of trypan blue while live cells did not take up the dye. The number of stained cells were counted separately and percentage cytotoxicity was determined[17,19].

$$\% \text{ Cytotoxicity} = \frac{\text{No. of dead cells} \times 100}{\text{No. of live cells} + \text{No. of dead cells}}$$

Evaluation of cytotoxicity effect

The cytotoxic effect of the seed butter GGSB was evaluated by determining the percentage viability of DLA and EAC cell lines *in vitro* using Trypan blue exclusion method[18]. The tumor cells were aspirated aseptically from the peritoneal cavity of tumor bearing male Swiss albino mice after 15 days of inoculation. The aspirated tumour cells were washed thrice with phosphate buffered saline (PBS) and centrifuged at 1500 rpm for 3 min. Cell viability was determined by trypan blue exclusion method. The pellets of cells were re-suspended and the count was adjusted to a concentration of 1×10^7 cells/mL. The different concentrations (10, 20, 50, 100 and 200 µg/mL) of GGSB in 1 mL PBS were added to test tubes containing approximately 1×10^6 cells. Control tube contained only cell suspension. These assay mixtures were incubated at 37°C for 3 h. Further cell suspension was mixed with 0.1 mL of 1% trypan blue and kept for 2-3 minutes and then loaded on haemocytometer to evaluate the cell viability. The percentage viability and IC_{50} of GGSB against tumour cells were calculated[18].

Antiproliferative activity *in vitro* using MTT assay

Antiproliferative activity of GGSB dissolved in dimethyl sulphoxide (DMSO) was evaluated in human triple negative breast cancer cell line (MDA-MB-231) and human hepatocellular carcinoma (HepG2) cell lines, using 3-(4, 5-dimethylthiazol-2-yl) - 2, 5-diphenyltetrazolium bromide (MTT) assay[20]. All the cell lines were maintained in DMEM media, supplemented with 10% fetal bovine serum, 100 µg /mL penicillin and 100 µg/mL streptomycin and kept at 37°C in an incubator with 5% CO_2. The cells were passaged at 80-90% confluency and medium was changed every third day. Cytotoxicity of the test materials was performed by MTT assay. Approximately $1x10^5$ cells/mL were seeded in a 24 well plate, with complete growth medium (DMEM) and allowed to attach and grow. At 80% confluency, the medium was replaced with fresh medium containing different concentrations of GGSB (0-120 µg/mL) and incubated for 48 hrs. At the end of incubation period, the medium was again replaced with

fresh medium containing 40 µL of 5mg/ mL MTT and incubated for 4 hrs. The formazan crystals formed were dissolved in dimethyl sulfoxide and the absorbance was measured at 570 nm in ELISA microplate reader (BioTek, USA). All the experiments were performed in triplicate and the average of the percentage absorbance was plotted against concentration and IC_{50} was calculated[21]. The percentage viability was calculated using the formula:

$$\% \text{ Viability} = \frac{\text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

Evaluation of antimicrobial activity

The seed butter GGSB was dissolved in 1% DMSO to get a final concentration of 1 mg/mL and evaluated for antimicrobial effects. A total of seven microbial strains were tested for their susceptibility to GGSB. These strains include one yeast: *Candida albicans* (ATCC 90028); one filamentous fungus: *Aspergillus niger* (ATCC 9029); three Gram-negative bacteria: *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25292) and *Salmonella typhimurium* (ATCC 14028); and two Gram-positive bacteria: *Staphylococcus aureus* (ATCC 29213) and *Bacillus subtilis* (ATCC 6051). These strains were maintained on agar slant at 4°C and sub-cultured on fresh and appropriate agar plates 24 h prior to the experiment. The broth dilution method was carried out to evaluate the antimicrobial effect of the sample[22].

Fatty acid methyl esters (FAMEs) preparation from GGSB

Lipids extracted from the seed butter were methylated and converted to their respective fatty acid methyl esters (FAMEs), according to the modified method of Moss et al. (1974)[23]. Further, 2 mL of 0.5 M NaOH in 2% (w/v) methanol was added to a 100 mL flask containing the lipid with stirring and heating to 100°C for 5 min under reflux. Boron trifluoride reagent (BF_3) (3 mL) was added, followed by stirring for 2 min for acid catalysis to occur, after which 3 mL of NaCl solution was added at 20% (w/v). The sample was transferred to a separating funnel with containing 20 mL hexane. The organic phase was separated and dried using anhydrous sodium sulfate (2 g). The solvent was evaporated under stream of N_2 on steam bath and the samples were weighed for further analysis.

GC-MS and GC-FID analysis

The seed butter of *G. gummi-gutta* (GGSB) 1 µL (mg/mL) was employed in GC-MS for various phytoconstituents. The analysis was performed on a capillary system, GC Varian CP-3800, containing Combi PAL auto-sampler and a gas chromatograph interfaced to a mass spectrometer (Saturn 2200 MS) provided with a VF-5ms, a Factor Four column, (0.25mm × 30m ID × 0.25µm df). The average peak area to the total areas of each component was compared and relative percentage amount of compound was calculated. Ion trap is the mass-detector used in the analysis. The software used for the analysis of mass spectra and chromatograms was MS work station with National Institute Standard and Technology (NIST) library, Gaithersburg, Maryland, USA. The NIST database, having more than 62,000 patterns was used for the identification of each component by comparing the spectrum of the unknown compounds with the spectrum of known compounds. GC-FID analysis was employed for quantitation of the constituent fatty acids. A gas chromatograph GC-FID with GC6890N equipped with a fused-silica capillary column (SP 2560, dimensions of 100 m x 0.2 mm x 0.25 µm film thickness) was used for quantitative analysis. An analytical curve from FAME MIX-37 standard and internal standardization was used to analyze them qualitatively and quantitatively. An internal standard solution containing non adecanoate (Sigma Aldrich) was prepared at 2 mg/mL concentration.

Statistical analysis

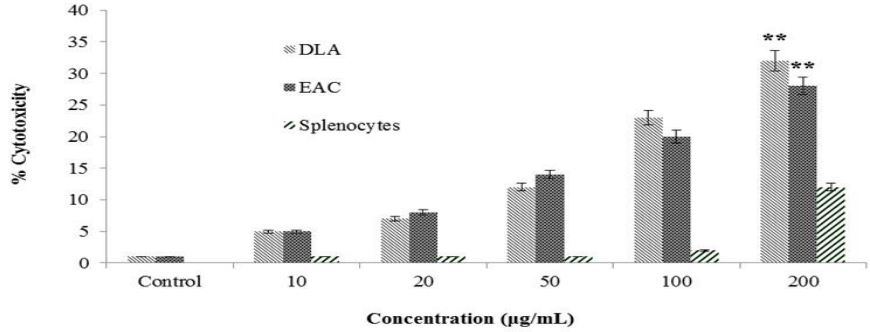
All the analyses were performed in triplicate. Analysis of Variance (ANOVA) was used to find out the statistical significance between the samples. The data were indicated as mean ± standard deviation (SD), and $p \leq 0.05$ was considered to be statistically significant. Dunnett's multiple comparison test was used to determine the significant differences between means. The IBM SPSS Statistics, version 20 (USA) was the computer software used for the analysis.

RESULTS and DISCUSSION

The yield of *G. gummi-gutta* seed butter (GGSB) was estimated as 15%. The isolated butter was yellowish brown in color without any characteristic odor and comparatively high yield of the fixed oil indicated presence of fatty acids in the seeds.

Cytotoxic and antiproliferative effects of *G. gummi-gutta* seed butter (GGSB)

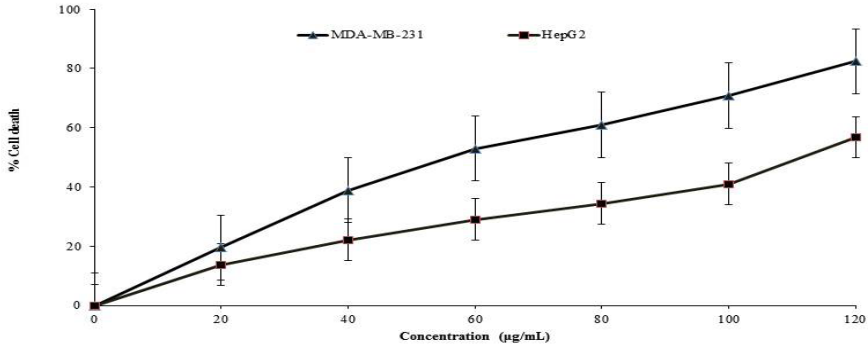
In the present study, the cytotoxicity of GGSB towards DLA and EAC were evaluated in a short-term *in vitro* assay. It was carried out by Trypan blue dye exclusion method and the viable cells remained unstained by the dye and they were counted by using a haemocytometer. The intact membranes of live cells exclude the dye, whereas the dead cells do not. *In vitro* cytotoxic study showed that GGSB exhibited dose dependent cytotoxic effect towards DLA and EAC (Figure 1). The IC_{50} values obtained are 289.04 µg/mL and 299.18 µg/mL for DLA and EAC respectively. The oil GGSB was found non-cytotoxic to rat splenocytes *in vitro* with IC_{50} value above 200 µg/mL concentrations (Figure 1).



**Significance $p \leq 0.05$ compared to the control

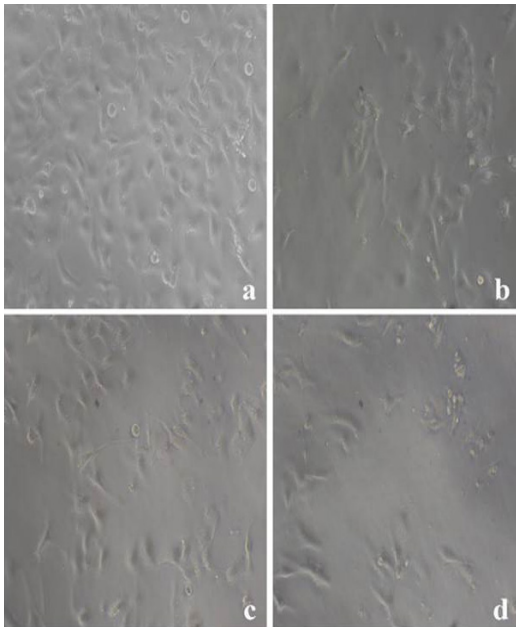
Figure 1. Cytotoxicity effect *in vitro* of *Garcinia gummi-gutta* seed butter (GGSB) on Dalton's Lymphoma Ascites (DLA), Ehrlich Ascites Carcinoma cells (EAC) and normal spleen cells. Values are expressed as mean ± SD of at least three consecutive experiments, Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test.

Anti-proliferative effect *in vitro* of GGSB was estimated by MTT assay and the relative cell proliferative activities of human triple negative breast cancer cell line (MDA-MB-231) and human hepatocellular carcinoma (HepG2) cell lines following exposure with GGSB are summarized in Figure 2. The seed butter exhibited a dose dependent anti-proliferative effect on MDA-MB-231 and HepG2 cell lines (Figure 2). The IC_{50} value of GGSB against MDA-MB-321 was determined as 66.70±2.60 µg/mL. Significant cytotoxicity was observed towards HepG2 cell line with an IC_{50} of 110.98 µg/mL. The cells in cultures have a proliferating population which is shown to be inhibited by GGSB. In MTT assay GGSB exhibited cytotoxicity towards MDA-MB-231 and HepG2. Photomicrographs of cell at 20, 40 and 100 µg/ mL concentration are shown in Figure 3 and the images were taken under 20 x magnification (total 200 x) of phase contrast microscope (Magnus INVI, Chennai, India).



**Significance p≤0.05 compared to the control

Figure 2. Antiproliferative effect *in vitro* of *Garcinia gummi-gutta* seed butter (GGSB) on human triple negative breast cancer (MDA-MB-231) and human hepatocellular carcinoma (HepG2) cell lines following exposure with GGSB. Values represent mean ± SD of at least 3 replica cultures. Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test.



**Significance p≤0.05 compared to the control

Figure 3. Antiproliferative effect *in vitro* of GGSB on Human triple negative breast cancer cell line (MDA-MB-231) (a) Control (b) 20 µg/mL (c) 40 µg/mL and (d) 100 µg/mL concentration treatments. The images were taken under (magnification 200 x) Phase contrast microscope (Magnus INVI, Chennai, India)

Cytotoxic and antiproliferative compounds of plant origin, that are known to target specific oncogenes or malignant cells and interrupt carcinogenesis process and prevent tumor growth. Ursolic acid, corosolic acid, conophylline, kaempferide and campesterol are some of the plant-based chemopreventive agents[24]. The chemopreventive effects of natural compounds is achieved by mechanisms such as antioxidant effect, anti-inflammatory pathways, ability to induce phase II enzymes, cell cycle arrest and apoptosis[25]. The plant species coming under *Garcinia* genus are very important due to its therapeutic effects and it contains phytocompounds having antitumor effects. Prenylxanthones isolated from leaves of *Garcinia bracteata*[26] and garcinol, a polyisoprenylated chalcone from *Garcinia indica*[27] showed cytotoxic effects against different cancer cell lines and exhibited potent antitumor activity. The cytotoxic effect of the seed butter may be achieved by making pores on the cell membrane, through which Trypan blue dye enters and the dead cells stained blue and viable cells were excluded from the staining[28]. Most of the currently using allopathic chemotherapeutic agents have significant immune-suppressing effect. The result is significant that the seed butter is non-cytotoxic to rat splenocytes and as spleen cell population represent the immune cell population. In view of this, it is assumed that the differential toxic potential of GGSB may be an advantage and it can be considered as a good candidate for anticancer drug development.

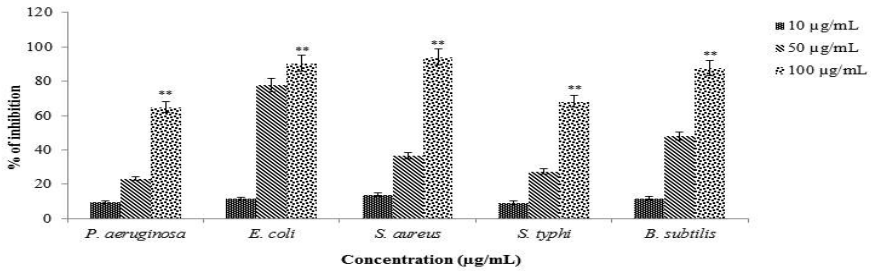
MTT assay is a colorimetric assay based on the principle of the ability of nicotinamide adenine dinucleotide phosphate (NADPH) dependent cellular oxidoreductase to reduce the tetrazolium to its insoluble purple colored formazan. It measures cell viability in terms of reductive activity as enzymatic conversion of tetrazolium to water insoluble formazan by dehydrogenases present in mitochondria and endoplasmic reticulum. A solubilizing agent was added to dissolve the formazan product and absorbance of the solution was noted[29]. MDA-MB-231 cells are aggressive and invasive triple negative breast cancer cells that are resistant to several anticancer agents[30]. HepG2 cells have high proliferation rates, adherent properties and grow as monolayers in small aggregates in cultures[31]. The cytotoxicity and antiproliferative effects of GGSB may be achieved by inducing apoptosis, cell cycle arrest at sub G_0/G_1 phase and anti-angiogenesis[32]. Several mechanisms have been proposed by the researchers for explaining the antiproliferative effect of unsaturated fatty acids.

One of the major compounds detected in GGSB is oleic acid, which is a monounsaturated omega-9 fatty acid. Oleic acid has been shown in numerous reports to inhibit cellular proliferation in several cancer cell lines. It plays crucial role in intracellular calcium signaling pathways related to apoptosis and growth

induction. The mechanisms related to apoptosis are linked to the rise in intracellular caspase-3 activity and development of reactive oxygen species[33]. It can suppress the over expression of human epidermal growth factor receptor-2 (HER-2/erbB-2), an oncogene which is involved in the development and metastasis of numerous human cancers[34]. Autophagy dependent anticancer effect of oleic acid has been reported in human hepatocellular carcinoma cell lines[35].

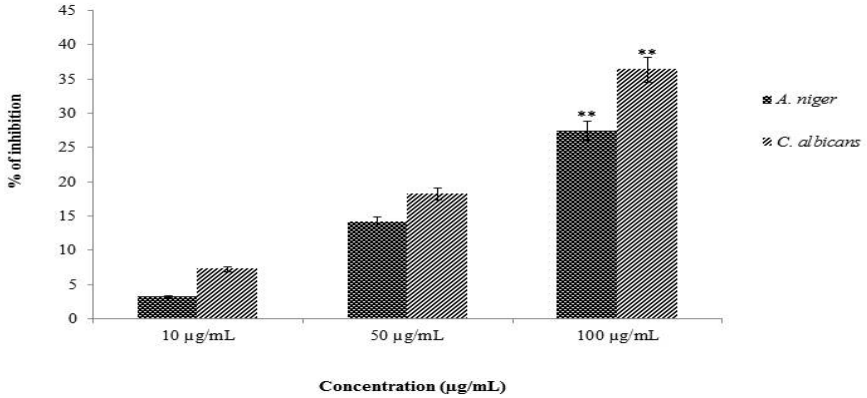
Antimicrobial activity of *G. gummi-gutta* seed butter (GGSB)

The antimicrobial property of GGSB was evaluated with concentration range 10-100 µg/mL and the results showed that GGSB inhibited the microbial growth after 24 h of incubation. It significantly ($p \leq 0.05$) inhibited the growth of pathogens such as *E. coli*, *S. aureus* and *B. subtilis* (Figure 4). The maximum microbial growth inhibition percentage (94.19%) was observed in *S. aureus* by GGSB (100 µg/mL) treatment. The minimum inhibition percentage was observed in the case of *A. niger*. The seed butter exhibited a dose dependent growth-inhibitory effect on the microorganisms selected for the study. Amongst the pathogens screened, *E. coli*, *S. aureus* and *B. subtilis* were found to be more sensitive to GGSB, which exhibited the percentage of inhibition of microbial growth ranged between 94.19% to 87.75%. Moderate level of activity was showed by GGSB against *P. aeruginosa* and *S. typhi* compared to the control. The seed butter (100 µg/mL) showed maximum growth inhibition percentage of 27.40% and 36.36% against *A. niger* and *C. albicans* respectively compared to the control (Figure 5). The IC_{50} values of 78.03 µg/mL, 25.43 µg/mL, 58.75 µg/mL, 73.40 µg/mL and 51.78 µg/mL were obtained with GGSB against *P. aeruginosa*, *E. coli*, *S. aureus*, *S. typhimurium* and *B. subtilis* respectively.



**Significance $p \leq 0.05$ compared to the control.

Figure 4. Antibacterial effect *in vitro* of GGSB (10-100 µg/mL) by broth dilution method. Values are the mean ± SD, n=6 in each group, Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test.



**Significance p≤0.05 compared to the control.

Figure 5. In vitro antifungal effect of GGSB (10-100 µg/mL) by broth dilution method. Values are the mean ± SD, n=6 in each group, Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test.

Chemical composition of *G. gummi-gutta* seed butter (GGSB)

In the present study, the chemical composition of the seed butter GGSB was investigated using both GC-FID and GC-MS. GC-MS analysis was used for the chemical characterization of GGSB (Figure 6) and the main compound classes identified were fatty acids. The identified compounds are listed in Table 1.

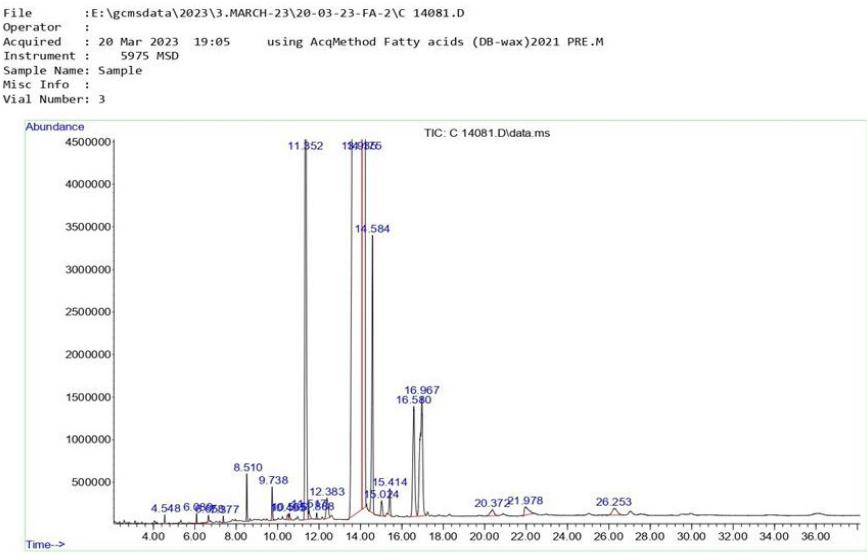


Figure 6. Phytocompounds detected in GC-MS Total Ion Chromatogram of *Garcinia gummi-gutta* seed butter (GGSB)

Table 1. Phytocompounds detected in GC-MS spectrum of *Garcinia gummi-gutta* seed butter (GGSB)

Compound Name	Peak Number	Retention Time RT (min)	Molecular Formula	Molecular Weight	Area (%)
Caprylic acid	1	15.108	$C_8H_{16}O_2$	144.21	0.02
Capric acid	2	18.143	$C_{10}H_{20}O_2$	172.26	0.02
Lauric acid	3	21.926	$C_{12}H_{24}O_2$	200.32	0.14
Myristic acid	4	25.963	$C_{14}H_{28}O_2$	228.37	0.13
Pentadecanoic acid	5	27.948	$C_{15}H_{30}O_2$	242.40	0.03
Palmitic acid	6	29.891	$C_{16}H_{32}O_2$	256.42	4.34
Heptadecanoic acid	7	31.758	$C_{17}H_{34}O_2$	270.50	0.20
Stearic acid	8	33.689	$C_{18}H_{36}O_2$	284.50	63.81
Elaidic acid	9	34.379	$C_{18}H_{34}O_2$	282.50	43.40
Oleic acid	10	34.839	$C_{18}H_{34}O_2$	282.50	26.31
Linoleic acid	11	36.503	$C_{18}H_{32}O_2$	280.40	1.51
Arachidic acid	12	37.068	$C_{20}H_{40}O_2$	312.50	1.06
Cis-11-Eicosenoic acid	13	38.282	$C_{20}H_{38}O_2$	310.50	0.30
Gamma-Linolenic acid-	14	38.593	$C_{18}H_{30}O_2$	278.40	0.06
Cis-11, 14-Eicosadienoic acid-	15	40.105	$C_{20}H_{36}O_2$	308.50	0.28
Behenic acid	16	40.723	$C_{22}H_{44}O_2$	340.60	0.11
Lignoceric acid	17	44.928	$C_{24}H_{48}O_2$	368.60	0.16
Cis-4, 7, 10, 13, 16, 19-Docosahexaenoic acid-	18	53.522	$C_{22}H_{32}O_2$	328.50	0.19

The gas chromatography analysis of GGSB fatty acid composition (Figure 7) showed that the main compounds with high proportions were stearic acid, C18:0 (47.74%), oleic acid, C18:1n9c (43.29%) and palmitic acid, C16:0 (2.53%) as the major compounds (Table 2).

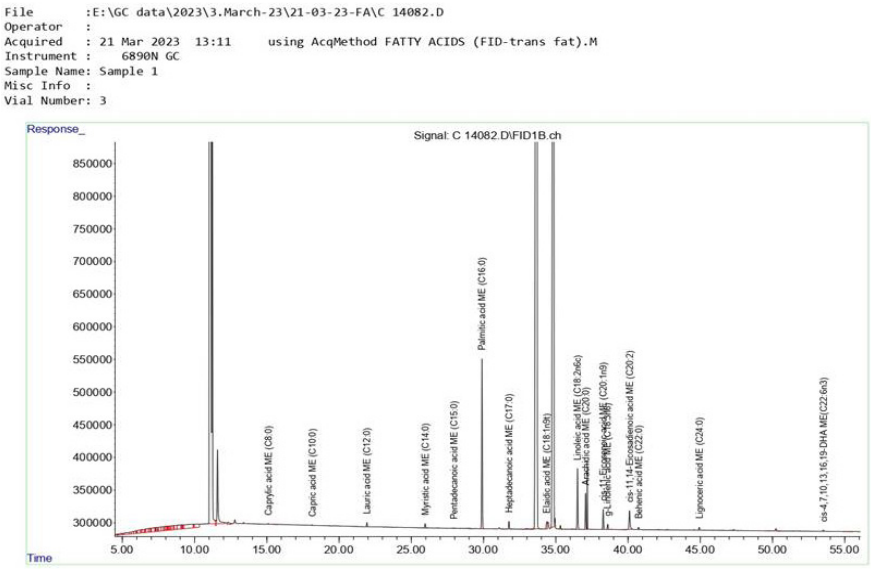


Figure 7. GC-FID chromatogram of *Garcinia gummi-gutta* seed butter (GGSB)

Table 2. The fatty acid composition of *G. gummi-gutta* seed butter (GGSB) determined by GC-FID

Fatty Acid Composition	Concentration (%)	Molecular Formula	Molecular Weight
C16:0 (Palmitic acid)	2.530	$C_{16}H_{32}O_2$	256.42
C17:0 (Heptadecanoic acid)	0.103	$C_{17}H_{34}O_2$	270.50
C18:0 (Stearic acid)	47.740	$C_{18}H_{36}O_2$	284.50
C20:0 (Arachidic acid)	0.438	$C_{20}H_{40}O_2$	312.50
C18:1n9c (Oleic acid)	43.290	$C_{18}H_{34}O_2$	282.50
C18:1n9t (Elaidic acid)	0.144	$C_{18}H_{34}O_2$	282.50
C18:2n6c (Linoleic acid)	0.825	$C_{18}H_{32}O_2$	280.40
C20:2 (cis-11, 14-Eicosadienoic acid)	0.393	$C_{20}H_{36}O_2$	308.50

The presence of phytocompounds such as heptadecanoic acid, arachidic acid, elaidic acid, linoleic acid and cis-11, 14-eicosadienoic acid was also detected.

The nuclear magnetic resonance (NMR) spectroscopy analysis of *G. gummi-gutta* seed oil helps to evaluate the quality of the edible oils by identifying the fatty acids compounds present in it. Rani et al. (2021) carried out the NMR spectroscopy analysis of the seed oil extracted from *G. gummi-gutta*[16] and the study revealed the tentative presence of compounds like stearic acid, 9-octadecenoic acid and the saturated fatty acid such as oleic acid was detected in the oil.

Stearic acid ($C_{18}H_{36}O_2$) is saturated long chain fatty acid with 18 carbon backbone and 9-octadecenoic acid ($C_{18}H_{34}O_2$) is monounsaturated fatty acid. The composition of GGSB is similar to the seed oil from a closely related species, *G. indica*[36]. The previous phytochemical studies conducted in the petroleum ether extract of *G. gummi-gutta* seeds reported to contain margaric acid and oleic acid as the major fatty acid compounds[37]. However, in the present study, it was not detected in the seed butter. The combination of monounsaturated fatty acids and long chain saturated fatty acids provides higher levels of melting temperature, heat of combustion and oxidation stability[38]. Due to the stable and solid nature at room temperature, stearic acid is widely used in food industries and cosmetic products. It is used to increase the hardness and heat resistance property of the chocolates by using along with cocoa butter. In hot climatic conditions the butter helps to prevent softening and loss of consistency of chocolates caused by heat[39]. GGSB is in the solidified form at room temperature, suggesting it as a better component for chocolates.

Garcinia seed kernel consists of fixed oil (30-40%) compared to other seed fats like coconut (60%), palm kernel (36%), sunflower (32%), castor seed (50%), mustard (35%), sesame (50%) and ground nut kernel (42%). *Garcinia* butter is rich in stearic acid and it is considered as demulcent, nutritive, emollient and astringent[40]. Stearic acid reduces blood pressure, improves cardiac function and reduces the risk for cancer[41]. It had been reported that the compounds stearic acid and oleic acid exhibited anticancer and antimicrobial effects[42]. According to Li et al. (2011), stearic acid can induce apoptosis of breast cancer cells[43] by arresting the cell cycle and dietary stearic acid has the potential to decrease the incidence of mammary tumor in carcinogenesis models[44]. Cell culture studies indicated that stearic acid showed anti-cancer activities including inhibition of the invasion and proliferation of cancer cells, changes in cell morphology and inducing apoptosis[45]. Dietary stearate delayed tumor development and decreased tumor incidence in experimental animals by inhibited metastasis to the lungs through a mechanism independent of primary tumor size[46]. It has been reported that oleic acid promoted antitumor effect against prostate, breast and colorectal cancers through inducing apoptosis and increased production of reactive oxygen species[47]. Stearic acid showed antibacterial activity against both Gram positive and Gram negative bacteria. The antibacterial effect of stearic acid derivative isolated from the hexane-soluble fraction of the leaf and stem extracts of *Stemodia foliosa* has been reported[48]. Oleic acid is a component of omega-9-unsaturated fatty acid and it reported to exhibit antifungal and antibacterial effects[49]. Liposomal oleic acid loaded (LipoOA) antibiotics were reported to exhibit enhanced antimicrobial activity against multidrug-resistant *Pseudomonas aeruginosa*. Thus, LipoOA could be utilized to encapsulate antibiotics for the development of novel and more effec-

tive methods to tackle emerging multidrug resistance in pathogenic microbes[50]. The phytochemical compounds present in GGSB are responsible for its therapeutic effects. In the present study, the *in vitro* cytotoxic and antiproliferative effects are reported and pharmacological studies using *in vivo* models should be carried out to understand the effects of GGSB on biological system.

In conclusion, the present study deals with the evaluation of cytotoxic, antiproliferative and antimicrobial effects of *G. gummi-gutta* seed butter. The seed butter exhibited dose dependent cytotoxic and antiproliferative effects *in vitro*. But at the same time, it was found non-cytotoxic to normal splenocytes and GGSB showed significant antimicrobial effect also. The GC-MS and GC-FID analysis revealed the presence of fatty acid compounds in the seed butter and these compounds are responsible for its therapeutic effects. Detailed *in vivo* studies, molecular gene expression analysis and detailed phytochemical studies are warranted for the isolation of bioactive compounds from GGSB. Future research in this field should be carried out to understand the exact mechanism of action of the compounds isolated from the seed butter.

STATEMENTS OF ETHICS

All experiments involving animals were carried out according to guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, after getting the approval of the Institute's Animal Ethics Committee.

CONFLICT OF INTEREST STATEMENT

Authors declare to have no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept: GS, NMK; Design: GS, NMK; Data Collection or Processing: NMK; Analysis or Interpretation: GS, NMK; Literature Search: GS, NMK; Writing: NMK; Revision and Proofreading: GS, NMK.

FUNDING SOURCES

This study obtained financial support from the State Science and Technology programme of the Department of Science and Technology (DST), Ministry of Science and Technology, Govt. of India, New Delhi (DST/SSTP/Kerala/453).

ACKNOWLEDGMENTS

The authors thank Rajagiri College of Social Sciences (Autonomous), Kalamassery and Presentation College of Applied Sciences, Puthenvelikkara for the research support.

REFERENCES

1. King JC, Blumberg J, Ingwersen L, Jenab M, Tucker KL. Tree nuts and peanuts as components of a healthy diet. J Nutr, 2008;138:1736-1740. Doi: 10.1093/jn/138.9.1736S

2. Gorrepati K, Balasubramanian S, Chandra P. Plant based butters. J Food Sci Technol, 2015;52:3965-3976. Doi: 10.1007/s13197-014-1572-7

3. Jenkins DJA, Hu FB, Tapsell LC. Possible benefit of nuts in type-2 diabetes. J Nutr, 2008;138:1752-1756. Doi: 10.1093/jn/138.9.1752S

4. Mangels R. Guide to nut and nut butters. Vegetarian J, 2001;21:20-23.

5. Rahman MM, Sarker MT, Tumpa MAA, Yamin M, Islam T, Park MN, et al. Exploring the recent trends in perturbing the cellular signaling pathways in cancer by natural products. Front Pharmacol, 2022;13. Doi: 10.3389/fphar.2022.950109

6. Shah D, Gandhi M, Kumar A, Martins NC, Sharma R, Nair S. Current insights into epigenetics, noncoding RNA interactome and clinical pharmacokinetics of dietary polyphenols in cancer chemoprevention. Crit Rev Food Sci Nutr, 2021;63(12):1755-1791. Doi: 10.1080/10408398.2021.1968786

7. Rahman KMA. Effect of Pumpkin seed (*Cucurbita pepo* L.) diets on benign prostatic hyperplasia (BPH): Chemical and morphometric evaluation in rats. World J Chem, 2006;1:33-40.

8. Ortiz JMP, Alguacil LF, Salas E, Gutiérrez IH, Alonso SG, Martin CG. Antiproliferative and cytotoxic effects of grape pomace and grape seed extracts on colorectal cancer cell lines. Food Sci Nutr, 2019;7:2948-2957. Doi: 10.1002/fsn3.1150

9. Spiller GA, Miller A, Olivera K, Reynolds J, Miller B, Morse SJ, et al. Effects of plant-based diets high in raw or roasted almonds or roasted almond butter on serum lipoproteins in humans. J Am Coll Nut, 2003;22:195-200. Doi: 10.1080/07315724.2003.10719293

10. Abraham Z, Malik SK, Rao GE, Narayanan SL, Biju S. Collection and characterization of Malabar tamarind [*Garcinia cambogia* (Gaertn.) Desr.]. Genet Resour Crop Evol, 2006;53:401-406. Doi: 10.1007/s10722-004-0584-y

11. Madappa MB, Bopaiah AK. Preliminary phytochemical analysis of the leaf of *Garcinia gummi-gutta* from Western Ghats. IOSR J Pharm Biol Sci, 2012;4:17-27.

12. George S, Krishnakumar NM, Vincent L, Rini KT, Athira S, Agnesjini PJ, Ramesh B. Physicochemical characterization of *Garcinia gummi-gutta* (L.) Robs. seed oil. Int J Applied Biol Pharmaceut Technol, 2018;9:5-12. Doi: 10.21276/Ijabpt

13. Maheshwari B, Reddy YS. Application of Kokum (*Garcinia indica*) fat as cocoa butter improver in chocolate. J Science Food Agri, 2005;85:135-140. Doi: 10.1002/jsfa.1967

14. Sharmila KP, Vijaya R, Pushpalatha KC, Kumari NS. Phytochemical profile and *in vitro* antioxidant activity of *Garcinia gummi*-gutta (L.) peel extracts. J Biol Scientific Opinion, 2015;3:7-12.

15. Janakiraman S, Lakshmanan T, Chandran V, Subramani L. Comparative behavior of various nano additives in a diesel engine powered by novel *Garcinia gummi-gutta* biodiesel. J Cleaner Prod, 2020; 245:118940. Doi: 10.1016/j.jclepro.2019.118940

16. Rani MP, Raj MRG, Rameshkumar KB. *Garcinia gummi-gutta* seeds: a novel source of edible oils. J Sci Food Agri, 2021;102:3475-3479. Doi: 10.1002/jsfa.11671

17. Moldeus P, Hogberg J, Orrenius S. Isolation and use of liver cells. Methods Enzymol, 1978;52:60-71. Doi: 10.1016/s0076-6879(78)52006-5

18. Talwar GP. Hand Book of Practical Immunology. New Delhi: National Book Trust;1974.

19. Ramavarma SK, Unnikrishnan AP, Babu TD, Achuthan CR. *In vitro* cytotoxic and antiproliferative activities of *Uvaria narum* seed oil (UNSO). Int J Advanced Res, 2018;6:912-916. Doi: 10.21474/IJAR01/7751

20. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods, 1983:65:55-63. Doi: 10.1016/0022-1759(83)90303-4

21. Cree IA. Cancer Cell Culture-Methods and Protocols. 2nd ed., New Jersey: Humana Press; 2011.

22. Nagalakshmi S, Saranraj P, and Sivasakthivelan P. Determination of minimum inhibitory concentration (MIC) and percentage bacterial growth inhibition of essential oils against gram positive bacterial pathogens. J Drug Deliv Ther, 2019;9:33-35. Doi: 10.22270/jddt.v9i3.2596

23. Moss CW, Lambert MA, Merwin WH. Comparison of rapid methods for analysis of bacterial fatty acids. Applied Microbiol, 1974;28:80-85. Doi: 10.1128/am.28.1.80-85.1974

24. Mazumder K, Biswas B, Raja IM, Fukase K. A review of cytotoxic plants of the Indian subcontinent and a broad-spectrum analysis of their bioactive compounds. Molecules, 2020;25:1904. Doi: 10.3390/molecules25081904

25. Wu SJ, Lin YX, Ye H. Prognostic value of alkaline phosphatase, gamma-glutamyl transpeptidase and lactate dehydrogenase in hepatocellular carcinoma patients treated with liver resection. Int J Surg, 2016;36:143-151. Doi: 10.1016/j.ijsu.2016.10.033

26. Thoison O, Fahy J, Dumontet V, Chiaroni A, Riche C, Tri MV, Sevenet T. Cytotoxic phenylxanthones from *Garcinia bracteata*. J Nat Prod, 2000; 63: 441-446. Doi: 10.1021/np9903088.

27. Saadat N, Gupta SV. Potential role of garcinol as an anticancer agent. J Oncol, 2012;2012:647206. Doi: 10.1155/2012/647206

28. Latha PG, Panikkar KR. Cytotoxic and antitumor principles from *Ixora coccinea* flowers. Cancer Lett, 1998;130:197-202. Doi: 10.1016/S0304-3835(98)00140-2

29. Kuete V, Karaosmanoglu O, Sivas H. Medicinal spices and vegetables from Africa. In: Kuete, V. ed. Medicinal Species and Vegetables from Africa: Therapeutic Potential against Metabolic, Inflammatory, Infectious and Systemic Diseases. 1st ed. USA: *Academic Press*, 2017.

30. Gest C, Joimel U, Huang L, Pritchard LL, Petit A, Dulong C, et al. Rac3 induces a molecular pathway triggering breast cancer cell aggressiveness: Differences in MDA-MB-231 and MCF-7 breast cancer cell lines. *BMC Cancer*, 2013;13:63. Kuete V, Karaosmanoglu O, and Sivas, H. Medicinal spices and vegetables from Africa. In: Kuete, V. ed. Medicinal Species and Vegetables from Africa: Therapeutic Potential against Metabolic, Inflammatory, Infectious and Systemic Diseases. 1st ed. USA: *Academic Press*, 2017. Doi: 10.1186/1471-2407-13-63

31. Chang TT, Fulford MH. Monolayer and spheroid culture of human liver hepatocellular carcinoma cell line cells demonstrate distinct global gene expression patterns and functional phenotypes. Tissue Engineering, 2009;15:559-567. Doi: 10.1089/ten.tea.2007.0434

32. Razak NA, Abu N, Ho WY, Zamberi NR, Tan AW, Alitheen NB, et al. Cytotoxicity of eupatorin in MCF-7 and MDA-MB-231 human breast cancer cells via cell cycle arrest, antiangiogenesis and induction of apoptosis. Sci Rep, 2019;9:1514. Doi: 10.1038/s41598-018-37796-w

33. Farag MA, Gad MZ. Omega-9 fatty acids: Potential roles in inflammation and cancer management. J Genet Engg Biotechnol, 2022;20:48. Doi: 10.1186/s43141-022-00329-0

34. Nelson R. Oleic acid suppresses over expression of ERBB2 oncogene. Lancet Oncol, 2005;6:69. Doi: 10.1016/s1470-2045(05)01722-5

35. Giulitti F, Petrungaro S, Mandatori S, Tomaipitinca L, de Franchis V, D'Amore A, et al. Anti-tumor effect of oleic acid in hepatocellular carcinoma cell lines via autophagy reduction. Front Cell Develop Biol, 2021;9. Doi: 10.3389/fcell.2021.629182

36. Nagavekar N, Kumar A, Dubey K, Singhal RS. Supercritical carbon dioxide extraction of kokum fat from *Garcinia indica* kernels and its application as a gelator in oleogels with oils. Ind Crops Prod, 2019;138:111459. Doi: 10.1016/j.indcrop.2019.06.022

37. Choppa T, Selvaraj CI, Zachariah A. Evaluation and characterization of Malabar tamarind [*Garcinia cambogia* (Gaertn.) Desr.] seed oil. J Food Sci Technol, 2015;52:5906-5913. Doi: 10.1007/s13197-014-1674-2

38. Nikolskaya E, Hiltunen Y. Determination of carbon chain lengths of fatty acid mixtures by time domain NMR. Appl Magnetic Resonance, 2018;49:185-193. Doi: 10.1007/s00723-017-0953-2

39. Utpala P, Nandakishore OP, Senthil KR, Nirmal BK. Chromatographic fingerprinting and estimation of organic acids in selected *Garcinia* species. Int J Innov Hortic, 2012;1:68-73.

40. Rameshkumar KB. Diversity of *Garcinia* Species in the Western Ghats: *Phytochem. Persp*. 1st ed. Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Thiruvananthapuram, India. 2016.

41. Senyilmaz TD, Pfaff DH, Virtue S, Schwarz KV, Fleming T, Altamura S, et al. 9-dietary stearic acid regulates mitochondria *in vivo* in humans. Nat Commun, 2018;9:3129. Doi: 10.1038/s41467-018-05614-6

42. Gheda SF, Ismail GA. Natural products from some soil cyanobacterial extracts with potent antimicrobial, antioxidant and cytotoxic activities. Bras Academic Sci, 2020;92: e20190934 Doi: 10.1590/0001-3765202020190934

43. Li C, Zhao X, Toline EC, Seigal GP, Evans LM, Hashim AI, et al. Prevention of carcinogenesis and inhibition of breast cancer tumor burden by dietary stearate. Carcinogenesis, 2011;32:1251-1258. Doi: 10.1093/carcin/bgr092

44. Bennett AS. Effect of dietary stearic acid on the genesis of spontaneous mammary adenocarcinomas in strain A/ST mice. Int J Cancer, 1984;34:529-533. Doi: 10.1002/ijc.2910340416

45. Evans LM, Cowey SL, Siegal GP, Hardy RW. Stearate preferentially induces apoptosis in human breast cancer cells. Nutrition and Cancer, 2009; 61: 746-753. Doi: 10.1080/01635580902825597

46. Habib NA, Wood CB, Apostolov K, Barker W, Hershman MJ, et al. Stearic acid and carcinogenesis. British J Cancer, 1987;56:455-458. Doi: 10.1038/bjc.1987.223

47. Carrillo C, Cavia MM, Alonso TSR. Antitumor effect of oleic acid; mechanisms of action: a review. Nutrici Hospi, 2012;27:1860-1865. Doi: 10.3305/nh.2012.27.6.6010

48. da Silva LD, Nascimento M, Silva DHS, Furlan M, Bolzani VS. Antibacterial activity of a stearic acid derivative from *Stemodia foliosa*. Planta Med, 2002; 68:1137-1139. Doi: 10.1055/3-2002-36346

49. Shobier AH, Ghan, SAA, Barakat KM. GC/MS spectroscopic approach and antifungal potential of bioactive extracts produced by marine macroalgae. Egyptian J Aqu Res, 2016;42: 289-299. Doi: 10.1016/j.ejar.2016.07.003

50. Selvadoss PP, Nellore J, Ravindrran MB, Sekar U, Tippabathani J. Enhancement of antimicrobial activity by liposomal oleic acid-loaded antibiotics for the treatment of multidrug-resistant *Pseudomonas aeruginosa*. Artificial Cells, Nanomed Biotechnol, 2017; 46:268-273. Doi: 10.1080/21691401.2017.130720