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Article

Potential Application of Modified α-Mangostin Xanthones from *Garcinia mangostana* as Antibacterial Agents in Food Packaging

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Abstract: The microbial contamination in food packaging have been a major concern that paved the way for the search for natural based new anti-microbial agents, such as modified α-mangostin. In the present work, twelve synthetic analogs were obtained via semi-synthetic modification of α-mangostin by Ritter reaction, reduction by palladium-carbon (Pd-C), alkylation, and acetylation. The evaluation of the anti-microbial potential of the synthetic analogs showed higher therapeutic value than the parent molecule. The anti-microbial studies proved that **I E** showed higher antibacterial activity whereas **I I** showed most significant antifungal activity. Due to their microbial properties, modified α-mangostin can be utilized as active anti-microbial agents in food packaging.

Keywords: α-mangostin; antibacterial; antifungal; food packaging; semi-synthetic modification

1. Introduction

The perishable foods market is under continuous search for novel anti-microbial materials due to the great economic losses caused by bacterial and fungal growth on perishable food throughout the entire food supply chain. Such anti-microbial materials should exert some effectiveness with regard to extending the shelf-life of the produce on the market shelves up to the consumer table. One challenge is to find methods for improved treatment (i.e. modified atmosphere, type of film, packages composed by various active materials) and application of effective, safe anti-bacterial and anti-fungal compounds. This will ensure food safety, protect human health, and alleviate the economic losses at retailer shops and during the food supply chain processes. It is envisaged that new anti-microbial compounds could be incorporated in food packaging and films to improve the shelf-life of ready-to-eat foods and packaged fresh products (e.g. salads, sliced fruits, etc).

The fruit of *Garcinia mangostana* Linn. (mangosteen), of the family *Guttiferae*, has been used in Asian traditional medicines for the treatment of skin infections, wounds, diarrhea, dysentery, suppuration, leucorrhea, chronic ulcer and gonorrhea [1,2]. In addition, mangosteen with essential minerals is commercially used as dietary supplement for cancer patients [3]. The pericarp of the fruit

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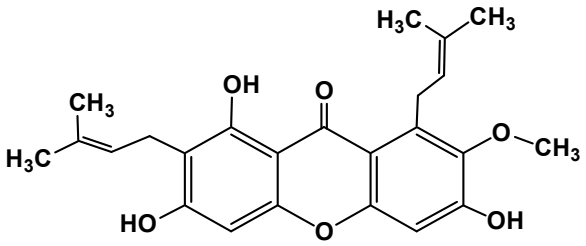
contains high amount of xanthones, such as α-mangostin (Figure 1), β-mangostin, γ-mangostin, etc. and considerable amounts of other bioactive compounds such as terpenes, anthocyanins, tannins, flavonoids and polyphenols [4].

Xanthones are naturally occurring compounds; have distinct chemical structure known as tricyclic aromatic system. Mangostin (3,6,8-trihydroxy-2-methoxy-1,7-bis(3-methyl but-2-enyl)xanthen-9-one) is a yellow crystalline solid of molecular mass 410.45 g/mol with a xanthone core structure. It is prepared by heating of phenyl salicylate (salol). It is used in preparation of xanthydrol, which is used for the determination of urea levels in the blood. Recent reports have revealed that α-mangostin from *G. mangostana* fruit possesses several medicinal properties, such as high anti-microbial activity against bacteria [5], anti-oxidant [6-9], anti-inflammatory [10], anti-cancer activities [3] as well as its action as a central nervous system depressant [11].

Figure 1. Structure of α-mangostin



Biologically active molecules from medicinal plants are utilized as therapeutic agents, but most of the secondary metabolites do not exhibit optimum efficacy. This is due to the lack of specificity and the absence of biologically active functional group. Thus, by elucidating the structure of the active compound and the pharmacophores, the functional groups are considered as essential for the bioactivity of a compound. In order to increase the bioavailability of the α-mangostin, the semi-synthetic modification of the compound were to lead to more active compounds, with no excessive toxicity.

In the present study, the search for new anti-bacterial agents paved the way to the semi-synthetic modification of α-mangostin using Ritter reaction, reduction by palladium-carbon (Pd-C), alkylation and acetylation to improvise the bioactivity of the compound. We herein studied the microbial activity of α-mangostin and their synthetic analogs and confirmed the development of new anti-microbial xanthones drugs with higher therapeutic activity. Alongside their anti-microbial properties, the synthetic analogs possess wound healing and anti-inflammatory activity, and hence, they could be exploited in the treatment of skin infections. Following the discovery of the medicinal properties of the synthetic analogs, it is suggested that these analogs possess better therapeutic value than the parent molecule and are potential drug candidates for preventive and therapeutic applications. In addition,

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many pathogens are able to survive on steel surfaces and in pipelines where food are processed, establishing biofilms. Therefore, it is of utmost importance to find new treatments of surfaces and processing line in food industry in order to eliminate bacterial contaminations. Several approaches have been proposed to release of active ingredients to the surface and kill the micro-organisms. For example, Poverenov and colleagues prepared numerous active anti-microbial surfaces on the basis of polymers, cellulose and glass, with potent inhibition against *Bacillus cereus* (*B. cereus*), *Alicyclobacillus acidoterrestris* (*A. acidoterrestris*), *Escherichia coli* (*E. coli*), and *Pseudomonas aeruginosa* (*P. aeruginosa*) [12-16]. Similar research on anti-microbial food-contact materials were developed based on natural phenolic compounds using nanotechnological approaches [17-24], essential oils to control pest pathogens [25,26], active-passive modified atmosphere for microbial control [27], and various polymeric based anti-microbial films [28-31].

2. Results and Discussion

2.1 α-Mangostin isolation

α-Mangostin was isolated from the dried fruits of *Garcinia mangostana* using ethyl acetate and dried to obtain ethyl acetate crude extract. The obtained ethyl acetate crude extract was subjected to a series of extractions and the obtained solid was repeatedly recrystallized using benzene until the purity reached 95% purity by HPLC analysis. The yield of the pure α-mangostin obtained was 5-6 g.

2.2 Synthetic Modifications

Following the isolation, α-mangostin was subjected to a series of chemical reactions to alter the core structure. The basic core structure xanthone (anthraquinone) was conserved intact while the functional groups iso-prenyl, phenolic hydroxy and ketone were subjected to semi-synthetic modification. Twelve different semi-synthetic derivatives were obtained (Table 1), each containing new key moieties that were evaluated to detect the increase in the microbial activity during minimal inhibitory concentration (MIC) test against various bacterial and fungal cultures.

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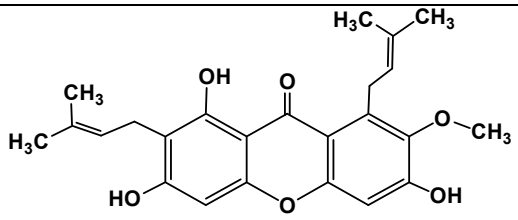
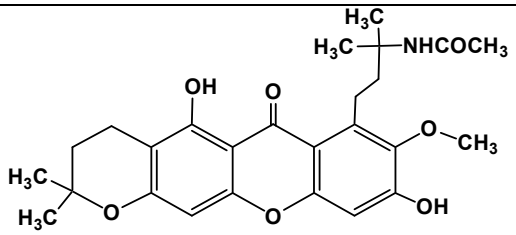
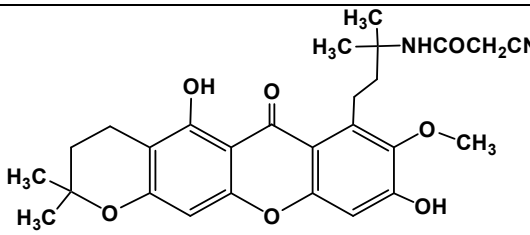
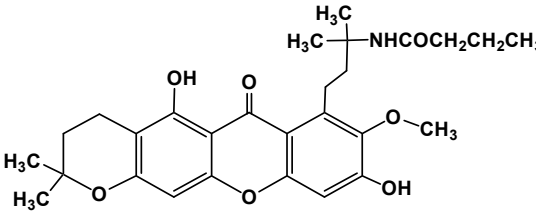
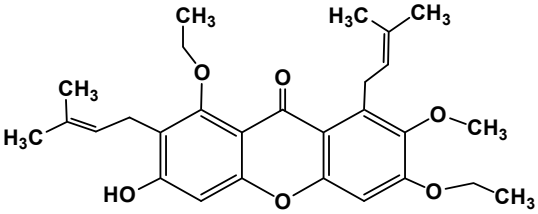
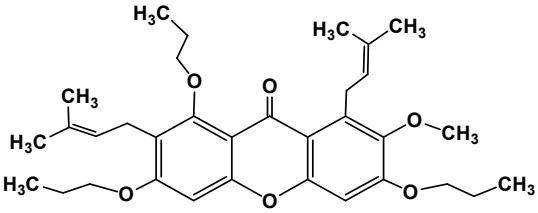
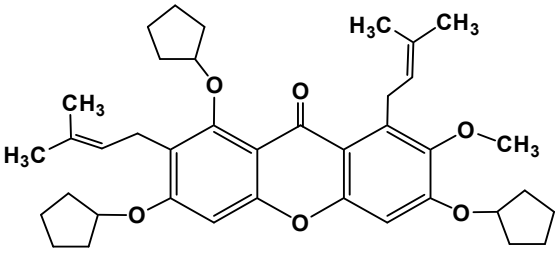
Table 1: α-Mangostin and its synthetic derivatives.

S. No.	Compound Information	Structure	Reaction of α-mangostin with	Molecular Formula	Mass
1.	α-Mangostin **(I)**		-	-	-
2.	Ritter product of α-Mangostin **(I A)**		Acetonitrile in Silica supported sulphuric acid	$C_{26}H_{31}NO_7$	470 $(M+1)^+$
3.	Ritter product of α-Mangostin **(I B)**		Malononitrile in Silica supported sulphuric acid	$C_{27}H_{30}N_2O_7$	514 $(M+2+NH_3)^+$
4.	Ritter product of α-Mangostin **(I C)**		Butyronitrile in Silica supported sulphuric acid	$C_{28}H_{25}NO_7$	498 $(M+1)^+$
5.	Alkylated product of α-Mangostin **(I D)**		Ethyl Iodide K_2CO_3/ACN M.W. 10 min	$C_{28}H_{34}O_6$	467 $(M+1)^+$
6.	Alkylated product of α-Mangostin **(I E)**		Bromopropane K_2CO_3/ACN M.W. 10 min	$C_{33}H_{44}O_6$	537 $(M)^+$
7.	Alkylated product of α-Mangostin **(I F)**		Cyclopentyl bromide K_2CO_3/ACN M.W. 10 min	$C_{39}H_{50}O_6$	615 $(M)^+$

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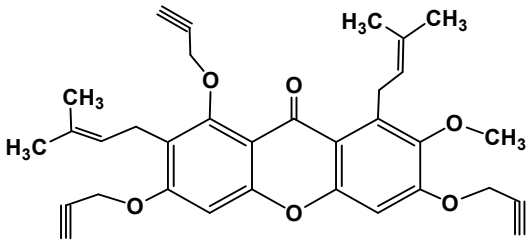
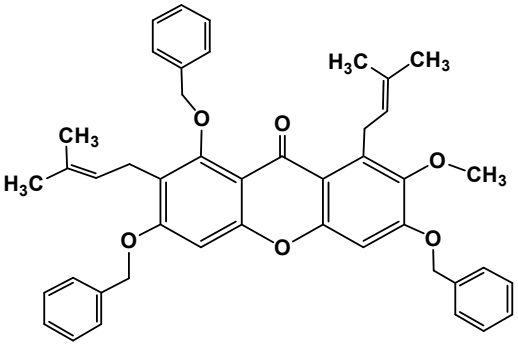
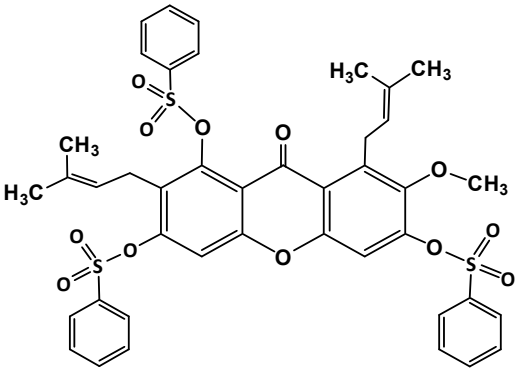
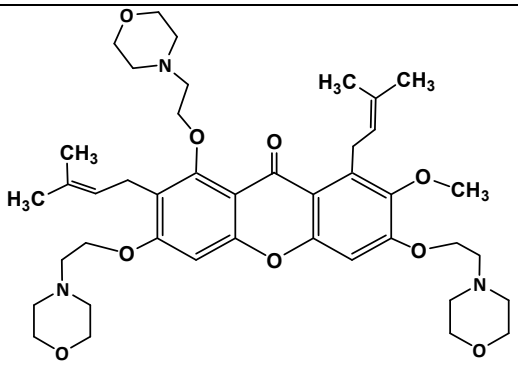
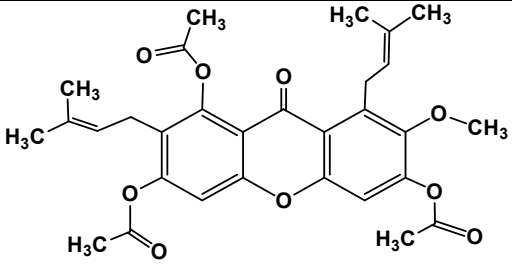
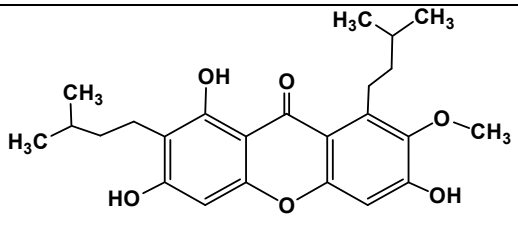
8.	Alkylated product of α-Mangostin **(I G)**		Propargyl bromide K_2CO_3/ACN M.W. 10 min	$C_{33}H_{32}O_6$	525 $(M)^+$
9.	Alkylated product of α-Mangostin **(I H)**		Benzyl bromide K_2CO_3/ACN M.W. 10 min	$C_{45}H_{44}O_6$	681 $(M)^+$
10.	Alkylated product of α-Mangostin **(I I)**		Benzene sulphonyl chloride K_2CO_3/ACN M.W. 10 min	$C_{42}H_{38}O_{12}S_3$	831 $(M)^+$
11.	Alkylated product of α-Mangostin **(I J)**		Chloroethyl morpholine hydrochloride K_2CO_3/ACN M.W. 10 min	$C_{42}H_{59}N_3O_9$	750 $(M)^+$
12.	Alkylated product of α-Mangostin **(I K)**		Acetic anhydride K_2CO_3/ACN M.W. 10 min	$C_{26}H_{28}O_7$	536 $(M)^+$
13.	Reduced product of α-Mangostin **(I L)**		Palladium / Carbon	$C_{25}H_{30}O_6$	415 $(M+1)^+$

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2.3 Biological Assays

2.3.1 Anti-bacterial Assay

The evaluation of the anti-bacterial potential for α-mangostin-based synthetic analogs was examined against *Escherichia coli* (*E. coli*) **(Fig. 2)**, *Bacillus subtilis* (*B. subtilis*) **(Fig. 3)**, *Staphylococcus aureus* (*S. aureus*), (**Fig. 4)** and *Pseudomonas aeruginosa* (*P. aeruginosa*) **(Fig. 5)**, in accordance with an experimental procedure. Two different concentrations (50 µg and 100 µg) of the α-mangostin and their synthetic analogs along with standard drug Ciprofloxacin were tested against the pathogens and the results are given in Table 2.

By measuring the zone of inhibition (in mm), it is observed that all the derivatives of α-mangostin exert moderate to high anti-bacterial activity. Compound (**I E**) showed maximum anti-bacterial activity (up to 12 mm) at 100 µg concentration against all bacterial stains tested in comparison to the other synthesized compounds. At low concentration of 50 µg, the acetyl derivative (**I G**) showed maximum inhibition against *E. coli*. The butyl derivative (**I C**) showed maximum inhibition against *B. subtilis*. The propenyl derivative (**I G**) showed maximum inhibition against *S. aureus*. The ethyl (**I D**) and benzene sulphonyl (**I I**) derivatives of α-mangostin showed maximum inhibition against *P. aeruginosa*. Among these compounds, the acetyl (**I K**) and benzene sulphonyl (**I I**) derivatives of α-mangostin showed maximum anti-bacterial activity against the four bacterial strains tested.

2.3.2 Anti-fungal Assay

The first compound evaluated was the natural product α-mangostin, which was compared against the synthetic analogs to prove that the analogs had better efficacy than the parent molecule. Compound (**I H** and **I J**) showed maximum anti-fungal activity (up to 13 mm) at 100 µg concentration against *Candida albicans* (*C. albicans*) tested in comparison to the other synthesized compounds. In addition, among all derivatives of α-mangostin, the acetyl (**I K**) and benzene sulphonyl (**I I**) derivatives displayed maximum inhibition of 13 mm in 100 µg/ml against *Aspergillus niger* (*A. niger*), the most potent anti-fungal activity. The alkylated product of α-mangostin **(I I)** showed maximum inhibition of 12 mm and 13 mm against *C. albicans* (**Fig. 6)** and *A. niger* **(Fig. 7)**, the most significant activity against fungal strains. The xanthonoid skeleton with benzene sulphonyl moiety showed enhanced anti-fungal activity for α-mangostin based derivatives.

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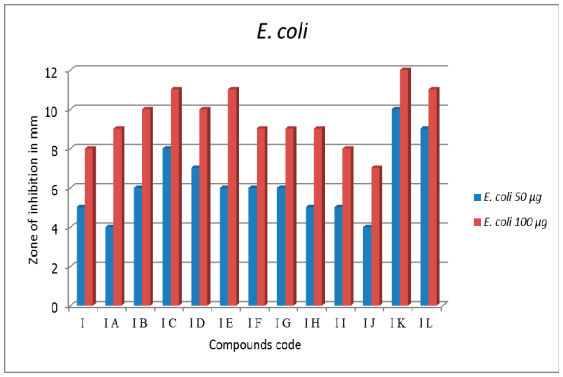
Table 2: Anti-microbial activity of the α-mangostin and their synthetic analogs

S. No.	Zone of inhibition in mm											
	E. coli		*B. subtilis*		*S. aureus*		*P. aeruginosa*		*C. albicans*		*A. niger*	
Conc. (μg/ml)	50	100	50	100	50	100	50	100	50	100	50	100
I	5	8	7	9	5	9	6	10	8	10	4	7
I A	4	9	6	10	5	11	5	8	7	10	9	12
I B	6	10	6	9	5	8	4	8	4	6	3	5
I C	8	11	10	12	6	9	5	10	2	6	2	5
I D	7	10	4	10	5	7	9	9	4	6	7	9
I E	6	11	9	12	7	11	7	12	4	7	3	5
I F	6	9	5	11	7	11	5	9	3	5	4	7
I G	6	9	7	10	10	11	6	9	4	6	3	5
I H	5	9	6	9	8	10	8	11	9	13	7	10
I I	5	8	6	10	7	9	9	12	10	12	9	13
I J	4	7	5	8	4	7	3	6	11	13	6	8
I K	10	12	4	7	5	9	6	9	8	11	10	13
I L	9	11	5	8	5	8	6	8	7	10	9	12



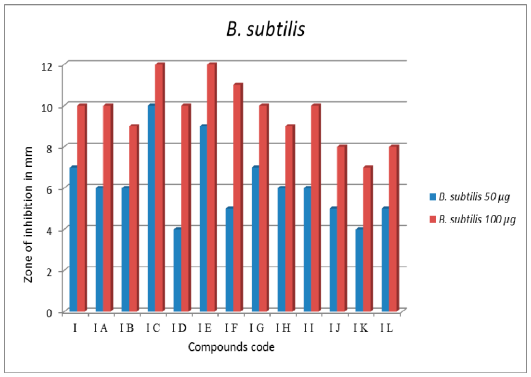


Fig. 2 –Fig. 3: Inhibitory effect of α-mangostin and their synthesized derivatives against *E. coli and B. subtilis*

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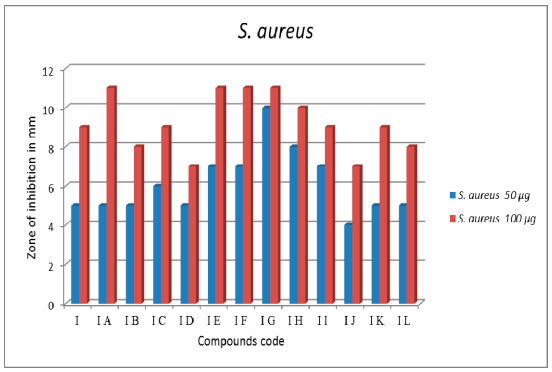


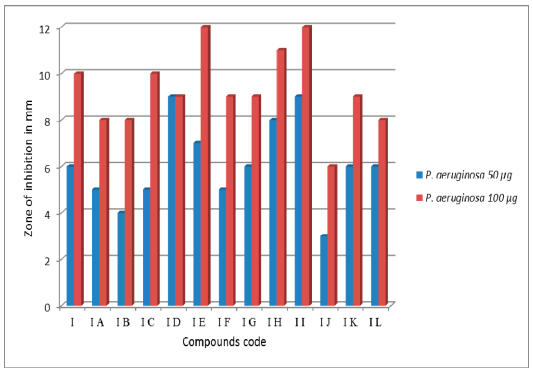
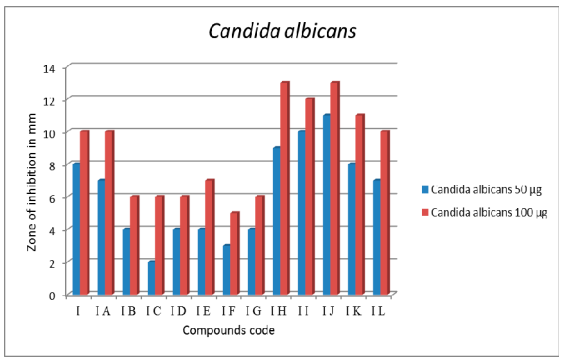


Fig. 4 – Fig. 5: Inhibitory effect of α-mangostin and their synthesized derivatives against *S. aureus* and *P. aeruginosa*



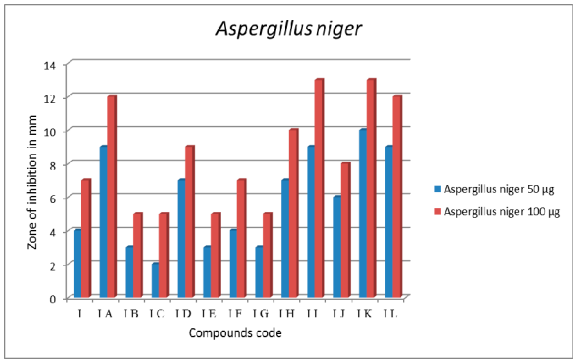


Fig. 6 – Fig. 7: Inhibitory effect of α-mangostin and their synthesized derivatives against *C. albicans* and *A. niger*

3. Experimental Section

3.1 General Methods

All the chemicals and reagents were purchased from either Sigma-Aldrich or Merck chemicals. The dried fruits of *Garcinia mangostana* were extracted twice with required amount of ethyl acetate and was dried using rota-vacuum to obtain the ethyl acetate crude extract. Then 20 g of ethyl acetate crude extract was washed with n-hexane repeatedly until the hexane became colorless. The insoluble portion was dissolved in benzene by heating under water bath; the soluble portion was filtered immediately under suction then cooled very slowly under room temperature. The solid was thrown out very slowly and filtered. The obtained solid is repeatedly recrystallized using benzene until the purity reaches 95% purity by HPLC analysis.

3.2 General Methods for Compound Analysis

The purity of the isolated α-mangostin and the progress of the reaction was monitored by HPLC on analytical reversed phase develosil ODS column C18 (150mm×4.6 mm, 0.5µm) using 0.02 M potassium dihydrogen phosphate in water and acetonitrile as a mobile phase for 30 min with a flow rate of 1.0 ml/min and UV detector wavelength of 254 nm. The main product was analyzed by nuclear magnetic resonance (NMR) data (^{1}H: 500 MHz, ^{13}C: 100 MHz) was recorded on a Bruker instrument

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and the chemical shifts was expressed in δ ppm. NMR spectra are obtained in MeOD with tetramethylsilane (TMS) as a reference compound. Mass were recorded in Shimadzu.

3.3 Anti-Microbial Activity Assay

The study of anti-microbial activity of the synthetic analogs was determined by the zone of inhibition (mm). The zone of inhibition of the α-mangostin was compared with synthetic analogs to determine the rate of inhibition. The zone of inhibition was determined in triplicates using the diffusion techniques and the values represent average zone of inhibition.

The nutrient broth medium was prepared for 50 ml and sterilized in an autoclave at pressure 15 lbs, 121 °C for 15 min. The Gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*) and Gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*) were inoculated into tubes of nutrient broth whereas the fungal culture (*Candida albicans, Aspergillus niger*) were inoculated into tubes of potato dextrose agar separately and incubated at 37 °C for 24 hr, then the suspension was centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A_{610} nm). All the microbial cultures were adjusted to 0.5 McFarland standards, which is visually comparable to a microbial suspension of approximately 1.5 $\times 10^8$ cfu/ml.

3.3.1 Anti-bacterial Assay

The following bacterial cultures are among the most important pathogenic bacteria of human diseases and it was chosen to evaluate the anti-bacterial activities of the synthetic compounds. The bacteria were maintained on Muller Hilton broth media at 37 °C. Then 20 ml of Muller Hilton agar media was poured into each Petri plate and the plates were swabbed with 100 µl inocula of the test microorganisms and kept for 15 min for absorption. Disc made of Whatmann No.1., diameter 6 mm was pre-sterilized and each concentrations (50, 100 and 200 µg/ml) of the test compounds diluted with DMSO were applied to the sterile disc papers. Standard drug Ciprofloxacin (10 µg) was used as a positive reference standard to determine the sensitivity of each bacterial species. Then the plates were inoculated at 37 °C for 24 hr. The diameter of the clear zone around the well was measured and expressed in millimeters as its anti-bacterial activity.

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3.3.2 Anti-fungal Assay

All the synthesized compounds were screened for anti-fungal activity by agar well diffusion method. PDA medium was autoclaved at 121 °C for 15 min and poured into each Petri plate and the solidified media plates were swabbed with 0.2 ml of fungal cultures. Using a sterile cork borer of 8mm diameter, wells were bored into the seeded agar plates and loaded with concentration of 50 and 100 µg/ml of the test compounds diluted with DMSO. Then the plates were inoculated at 28 °C for 72 hr. The diameter of the clear zone around the well was measured and expressed in millimeters as its anti-fungal activity. Standard drug Ketoconazole (10 µg) was used as a positive reference standard to determine the sensitivity of each fungal species.

4. Conclusion

In this study the semi-synthetically modified α-mangostin derived compounds were shown to exhibit bioactive properties and antimicrobial activity greater than the parent natural molecule when tested against various microbial and fungal cultures. Due to their anti-microbial properties, they can be utilized as wound healers and in the treatment of skin infections, and in packaging materials for the extension of food shelf life. We have shown that the structural modification of α-mangostin was attempted using Ritter reaction reduction by palladium-carbon (Pd-C), alkylation, and acetylation, in which I E showed higher anti-bacterial activity whereas I I showed most significant anti-fungal activity. These findings pave the way to the synthesis of non-toxic compounds with a better efficacy compared to the natural product for anti-microbial treatment. It is envisaged that these new anti-microbial compounds could be incorporated in packaging materials and films, to improve the food safety and shelf-life of ready-to-eat foods and packaged fresh products, such as salads and sliced fruits. The perishable foods represent a loss due to fungal and bacterial growth, requiring short times from the shelves to the table. Thus, improvement in treatments and use of anti-fungal films will ensure higher safety standard for human health in addition to decrease of economic losses at retailer shops and during food supply chain.

Supplementary Materials

Supplementary materials are available and include 1H-NMR spectra, 13C-NMR spectra and high resolution mass spectra for compounds **I A - I L**. Supplementary materials can be accessed from PhD thesis “Studies on isolation and the semi-synthetic modification of bio active molecules from *Garcinia mangostana* and *Embelia ribes* submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy by S. Maheshwaran, under the guidance of Dr. S. Narasimhan at the University of Madras (April 2013).

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Author Contributions

N.S. designed the experiments and supervised their execution. S.M. performed the chemical modifications. I.A.A.Y., A.F.M. and J.R. performed the structure analyses. P.E.D. provided the data analyses. P.P. designed and supervised the microbiological assays done by S.M..

Conflict of interest.

The authors declare no conflicts of interest.

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