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Isolation of *Garcinia mangostana* Extract to Evaluate Antioxidant and Anti-Diabetic Activity in Animals



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ABSTRACT

The aim of the current research was to isolate and evaluate the antioxidant action of *Garcinia mangostana* L. (Clusiaceae) is commonly known as mangosteen and its fruits are referred as the “queen of fruits”, as a result of its delicious taste in an experimental animal model. Isolation of extract having major bioactive substances alpha and gamma-mangostins, good amount of polyphenols with anti-oxidant and good anti-diabetic activities. Further, the present study was to provide in-vitro evidence for the potential inhibition activity of the α -mangostin rich fraction on the α -glucosidase enzyme, DPPH free radical scavenging and α -amylase. In the present research work, α -mangostin rich fraction was evaluated for the anti-diabetic activity, Antioxidant and anti-inflammatory activity. In conclusion, these two compounds assumed to be responsible for the very good antioxidant activity related to the health protecting benefits against nascent oxygen radical damage. Present study also gave us strong insight that, purified fraction containing rich content of α -mangostin can be a potent phytochemical entity for the development drug products; particular for the disease related to free radical damage such as Cardiovascular, Atherosclerosis and inflammation etc. Results on the Anti-diabetic activity were also showing good inhibition of Alpha-glucosidase and Alpha-amylase.

INTRODUCTION

Herbal drugs constitute a major part in all the traditional systems of medicine. Herbal medicine is a triumph of popular therapeutic diversity. Plants above all other agents have been used for the medicine from time immemorial because they have fitted the immediate personal need are easily accessible and inexpensive. Indian Meteria Medica includes more than 2500 natural products of therapeutic importance of which more than 800 are minerals and animal origin and rest are of vegetable origin¹. There are approximately 1500 Indian medicinal plants, which are used in formulation therapeutic preparations according to ayurvedic and other traditional systems of medicine.

In olden days traditional practitioners used to collect the materials directly from the wild. Presently everything is available in markets through proper raw material collection network which makes easy accessibility to the practitioners as well as researchers. This commercialization has lead to the problem of adulteration. Adulteration is a practice of substituting original crude drug partially or wholly with other similar looking substances or exhausted materials. Much controversy exists in herbal drugs regarding the correct identity of plants. Due to the high demand of herbal drugs, depletion of natural resources occurs and is alone one of the main cause for adulterations.

Before 18th century only slow progress was made in the field of phytochemistry. A few compounds such as glucose, starch, camphor and benzoic acid had long been known as their preparation was extremely simple and complex mixtures such as fats, fixed oils, volatile oils, tars and resins had been prepared and used although virtually nothing was known of their composition. The early scientific workers in the phytochemical field failed to appreciate the extreme complexity of the materials. They were trying to investigate and almost entirely lacked techniques necessary for real progress. Many hundreds of plants were burnt to yield ashes and early investigators were disappointed to find significant differences between the ashes of poisonous and those of non-poisonous plants. Percolation, extraction and distillation process had long been used for the preparation of many drugs from the plants.

India is one among the twelve mega biodiversity centres of the world with the presence of over forty five thousand diversified species. Of these, about fifteen to twenty thousand plants have good medicinal properties and out of which, only about nine to ten thousand are being used by the traditional practitioners. The Siddha system of medicine uses around 600 to 750,

Ayurvedic 900 to 1000, Unani 800 and modern medicine about 50 plant species. Projection is being made that after information technology, herbal technology will be India's biggest revenue earner².

Garcinia mangostana L. (Clusiaceae) is commonly known as mangosteen and its fruits are referred to in Thailand as the "queen of fruits", as a result of its delicious taste^{3,4}. The mangosteen plant grows slowly to 7-12 m high and has a straight trunk and dark brown bark. It is cultivated principally in Indonesia, Malaysia, the Philippines, and Thailand. The purple ripe fruits consist of 6-8 seeds and have a white and juicy pulp³. The edible fruit aril is white, soft, and juicy with a sweet, slightly acid taste and a pleasant aroma. The fruits of *G. mangostana* have been used as a traditional medicine in southeastern Asia for the treatment of diarrhea, dysentery, inflammation, and ulcers, as well as for wound healing^{3,4}. In the United States, mangosteen products are now widely available and are highly popular because of their perceived role in promoting human health⁵. Mangosteen fruit juice was ranked as one of the top three-selling "single botanicals" on the U.S. market in 2007⁶. Mangosteen extracts and purified constituents have been subjected to a wide array of biological tests germane particularly to infectious diseases, cancer chemotherapy and cancer chemoprevention, diabetes, and neurological conditions^{7,8,9}. This contribution summarizes studies reported on the structural characterization of the chemical constituents of *G. mangostana*, and the biological activity of the main secondary metabolites of this species, followed by the chemical synthesis of several mangosteen xanthenes. Recently, products manufactured from *G. mangostana* have begun to be used as a botanical dietary supplement in the United States, because of their potent antioxidant potential. The major secondary metabolites of mangosteen have been found to be prenylated xanthone derivatives; some members of this compound class isolated from this plant possess antifungal, antimicrobial, antioxidant, and cytotoxic activities.

Botanical Description

The tree grows from 7 to 25 meters tall. The edible fruit is deep reddish purple when ripe. Fruit is sweet and creamy, citrusy with a touch of peach flavour. The outer shell of the fruit is rather hard, typically 4 to 6 cm in diameter. Cutting through the shell, one finds an edible fruit shaped like a peeled tangerine but bright white, about 3-5 cm in diameter, nested in a deep red outer pod.

The shell of the mangosteens looked tough and hard, but they are soft and easy to open. To open a mangosteen, the shell is usually broken apart, one hold the fruit in both the hands and process it gently (thumbs on one side, the other fingers on the other) until the shell cracks. The endocarp is the white part of the fruit containing mild flavour that makes the fruit popular for eating. When analysed specially for its content of essential nutrients, it contains bundle of minerals and vitamins.

Origin and Distribution:

The plant is widely distributed in Southern India, Indonesia, Malaysia, Australia, Brazil, Central America, Hawaii, Thailand and other tropical countries.

Medicinal Uses:

Medicinal part of the plants is fruits and bark. Fruits had numerous uses in traditional medicine. The fruit hull of mangosteen has been used for hundreds of years in Southeast Asia as a medicine for skin infection, wound, dysentery and diarrhoea. Apart from the above stated, it also processes some very useful activities as antilipidemic, anti-hypertensive, prevents atherosclerosis, cardioprotective, hypoglycaemic and anti-obsessive.

Phytochemical Constituents:

The secret of its power appears to lie in its remarkable profile of anti-oxidant phytonutrients including polyphenols and xanthenes like Alpha-mangostin and Gamma-mangostin. Following are the list of some xanthenes ¹⁰ rich in mangosteen fruit shells;

Alpha-Mangostin	Beta-Mangostin	3-Isomangostin	Mangostanol
Gertanin	Garcinone A,B,C,D and E	Maclurin	Trapazifolixanthone
Tovophyllin B	Mangostenone A	Mangostenone B	Flavonoid- Catechin

Pharmacology:

Antimicrobial activity: scientific studies showed that xanthenes from mangostana fruit extract showing very good antimicrobial activity against methicillin resistant *Staphylococcus aureus* ¹¹.

In Arthritis: Arthritis is a very painful, debilitating condition resulting from inflammation of the joints in the body. It is linked directly to the effects of the COX-2 enzymes. Scientific research studies have shown mangostana extract as a strong COX-2 inhibitor.

Inflammation and pain: Mangostana more than any other fruit, has the ability to change the course of inflammation by effectively blocking the inflammatory process and the pain associated with it ¹².

Cancer: scientific studies showed that xanthonenes, the natural plant compounds found in the mangosteen fruit are powerful healers of the body and were shown to have ever greater antioxidant potency than vitamin C and E, two most powerful antioxidants known to science¹³.

Diabetes: it also improves blood sugar control by reducing blood sugar fluctuations and decreases the frequency of infection in people with diabetes. In some cases, blood sugar may drop significantly after mangosteen intake because cells resistance to glucose ceases. This lowers blood sugar levels.

Cholesterol (LDL) and Heart Disease: Independent scientific research studies have showed that potent, natural healing compounds found in the mangosteen prevents the oxidation of LDL (Low density lipoproteins); the cholesterol that causes clogged arteries which lead to heart disease.

Powerful Antioxidant: As part of ongoing research on cancer chemopreventive agents from botanical dietary supplements, *Garcinia mangostana* and its isolated compounds are proved to be very strong and powerful antioxidants ¹⁴.

2. MATERIALS AND METHODOLOGY

2.1. Experimental animals- Laboratory bred *Wistar albino rats* (110-160 g) of either sex were housed at $25^{\circ} \pm 5^{\circ}\text{C}$ in a well-ventilated environmental condition. The animals had free access to standard food pellets and water *ad libitum*.

2.2. Procurement of plant material and extraction- *Garcinia mangostana* raw material (Dried Fruit hulls) is collected from the local market in Bangalore. The plant were identified and authenticated. Dried and Powdered mangosteen raw material (Fruits were cleaned thoroughly and the edible part was removed. The fruit hull were cut into small pieces and shade dried for 15 days. Shade dried Mangosteen fruit hulls were made into powder) was taken for the extraction of different solvents extraction.

2.3. *Chemicals*- Anisaldehyde reagent, Sulphuric acid, Folin-ciocalteu reagent, Sodium carbonate, Gallic acid, 2,2-diphenyl- 1-picrylhydrazyl (DPPH), Ascorbic acid, p-nitrophenyl-Alpha-D glucopyranoside (DNPG), Alpha-Glucosidase, Acarbose, Carrageenan and sulindac, Methanol, Acetone, Benzene, chloroform, Toluene, TLC plates

Extraction: Fresh fruits obtained from the local market, Bangalore were washed with water and hand processed to remove hulls and inner layer of white colored flesh and seeds. The fruits were cleaned thoroughly and the edible part was removed. The fruit hull was cut into small pieces and shade dried for 15-20 days. Shade dried (2.1 kgs) mangosteen fruit were made into powder for the extraction. Dried powder was checked for the Alhamangostin content by HPLC before taking for the extraction.

2.0 Kg of the coarsely grinded RM was extracted with fresh 8.0 Liters of methanol for about 2 hrs under reflux condition, filtered the extract and two more similar extractions were done with 6.0 liters of fresh methanol. Combined all the extracts, concentrated and dried the combined liquid extract in rotaevaporator at temperature $< 70^{\circ}\text{C}$ under Vacuum (500 mm Hg) to afford 78.0 Gms of Crude extract. Washed the crude extract obtained, with 1:1 volume of DM (Demineralized) water (i.e., 78.0 ml) by constant stirring and allowed for the settling for 2 to 3 hrs. Filtered the mixture through Whatman No.1 filter paper to separate water and settled crude extract. Discard the filtrate and dried the extract obtained on the filter paper under vacuum (500 mm Hg) to afford 72.0 Gms of Water washed crude extract.

Purification: Purification of the Alpha-mangostin was performed as shown below and the starting material used for the purification was Water washed crude extract. 70.0 Gms of E2 was dissolved in 70.0 ml of Benzene (1:1 volume), heated at 50°C to dissolve and filtered into a separate beaker. Filtrate collected was concentrated in a buchii vacuum rotaevaporator at temperature $<70^{\circ}\text{C}$ under vacuum (500 mmHg) to get dark brown colored thick paste. Transferred the hot thick paste obtained immediately into separate beaker and left for 2-3 hrs for cooling to get hard cake like extract. Dissolved this hard thick cake like substance in 50 ml Toluene at 55°C with constant stirring and filtered into a separate beaker. Filtrate collected was concentrated in a buchii vacuum rotaevaporator at temperature $<70^{\circ}\text{C}$ under vacuum (500 mmHg) to get dark brown colored thick paste. Transferred the hot thick paste obtained immediately into another separate beaker and left for 2-3 hrs at room temperature to get hard cake like material. Obtained hard extract material was next dissolved in mixture of

Benzene, toluene and water (30ml, 30 ml and 10 ml respectively) at 50°C under stirring for 30 min.

Cooled the mixture for 5 to 6 hrs at room temperature resulted in the yellow colored shiny amorphous powder which is separated clearly from the rest of solvent mix. Separate the yellow colored shiny amorphous powder from the solvent mix by filtration through Whatman No.1 filter paper. Powder obtained by filtration is treated with hot water followed by separation by filtration by Whatman filter paper again. Now the separated yellow colored amorphous powder was dried very carefully under vacuum (500 mm Hg) at < 50°C to get dried, light yellow colored shiny powder. This purified material was again dried at low temperature (40-45°C) to lower the moisture content, grinded in mortar and pestle to get uniform size powder (Alpha-mangostin Rich Fraction) (34.0 Gms). Presence of purified Alpha-mangostin in the above fraction was confirmed by TLC. Purity of the alpha-mangostin was checked by HPLC with the Working standard.

TLC Separation of the Extracts

Tried many solvent systems for the optimization of the TLC system to get better separation of the phytocompounds in the extracts. After examining the suitability of the different solvent system for the optimum separation of each compound in the extract, one best system is selected. Solvent system Chloroform: Acetone: benzene with visualisation agent ANS was finalized for the TLC separation.

TLC conditions		
Solvent System	:	Chloroform/Acetone/Benzene
Ratio	:	4/3/3
Rf Value of the standard	:	0.59
Spraying reagent	:	Anisaldehyde Sulphuric Acid
Observation	:	Necked eyes
Colour of the spot	:	Dark green colour

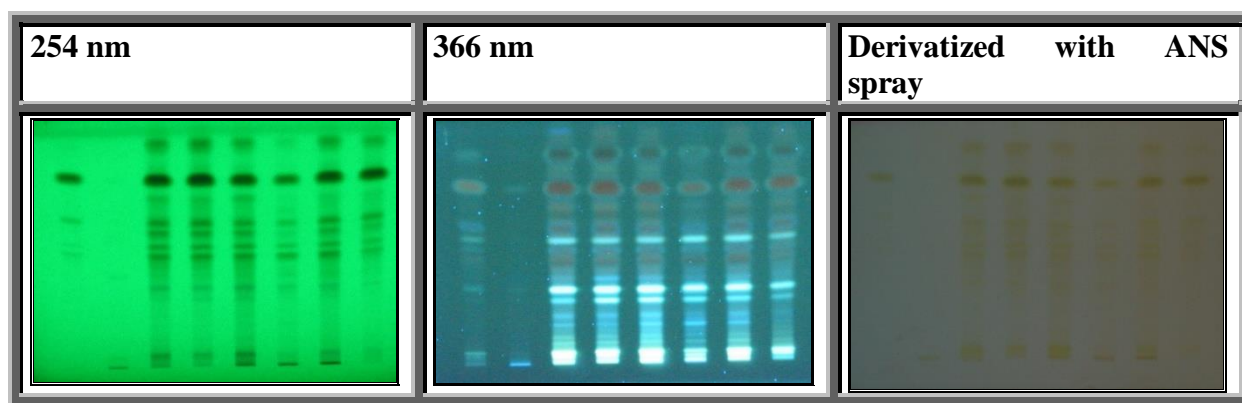


Fig 1: TLC plates of Separated Phytochemicals

Alpha-mangostin (E1) and Gamma-mangostin (E2) purified are run the TLC for the identification with working standards. Purified fractions show Alpha and gamma mangostins spots at the same Rf values as that of standards i.e., 0.58 and 0.52 respectively. Different solvent extracts of *G.mangostana* fruit were also run to understand the presence or absence of Alpha and Gamma-mangostin.

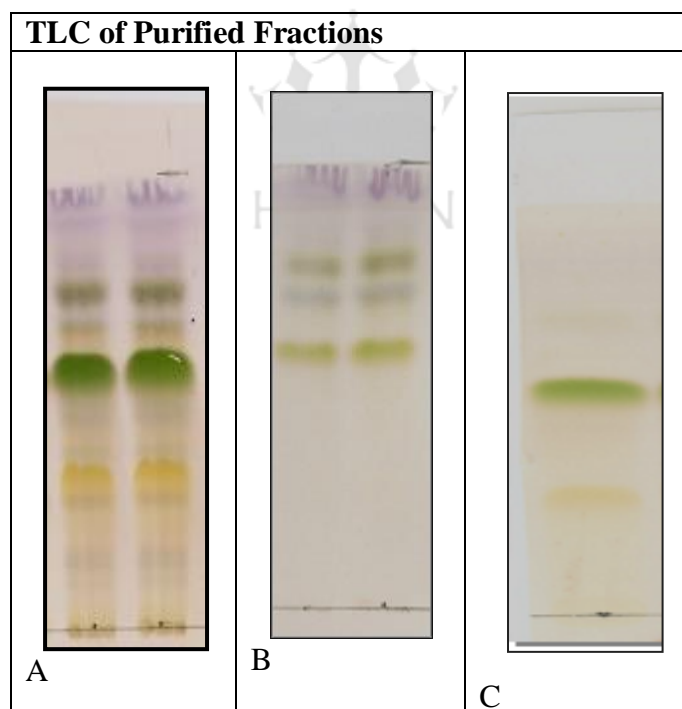


Fig 2: TLC plates of Separated Phytochemicals in (E1) and (E2)

A= Purified fraction.

B= Purified fraction.

C= Alpha-Mangostin Working standard

Standard used: Standard Alpha-mangostin used for this work was working standard confirms 80% of Alpha-mangostins. This 80% Alpha-mangostin extract was interned standardized against reference standard from chromadex 98% pure Alpha-mangostin.

Experimentation- The drug solutions were prepared in distilled water for oral administration. Evaluation of antioxidant antidiabetic effect was carried out by the following models.

RESULTS

IN-VITRO PHARMACOLOGICAL ACTIVITIES

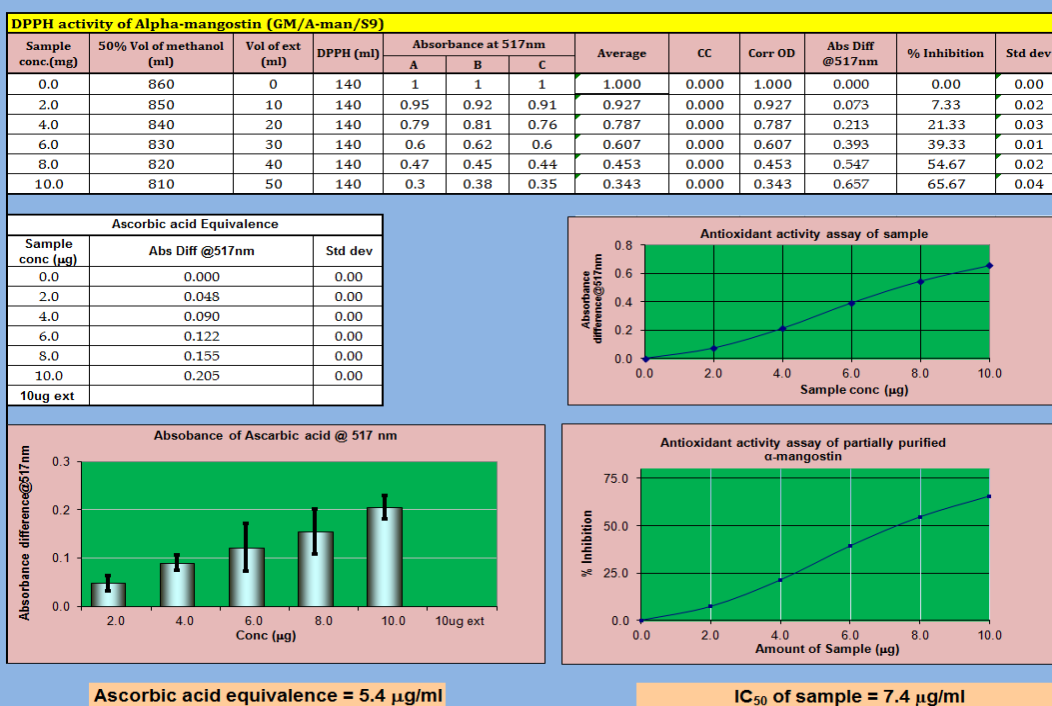
Antioxidant Activity:

Oxidative damage is involved in many chronic diseases including some of the prominent causes of death in Western societies such as cardiovascular disorders and cancer. Antioxidants may prevent these degenerative processes by various mechanisms including scavenging of free radicals. There are numerous reports about the reduction of the incidence of degenerative diseases due to the consumption of fruits and vegetables. These positive bioactivities are considered mainly to be due to the presence of various antioxidants in fruits and vegetables. Most of the antioxidant activity in foods is thought to be due to vitamins C and E, polyphenols, and carotenoids^{15,16}.

DPPH Activity:

DPPH radical is a commonly used substrate for fast evaluation of antioxidant activity because of its Stability in the radical form and simplicity of the assay. The principle behind this assay in the colour change of DPPH solution from purple to yellow as the radical is quenched by the antioxidant colour changes can be measured quantitatively by spectrophotometer absorbance at 517 nm. Partially purified Alpha-mangostin was screened for the antioxidant activity according to the method described and results were showed comparable with known antioxidant standard Ascorbic acid. Partially purified extract is showing reducing power activity almost equal to the Ascorbic acid. Purified Alpha-mangostin (E1) and gamma-mangostin (E2) exhibit a significant dose dependent inhibition of DPPH activity with 50% of inhibition (IC₅₀) at concentration of IC₅₀ value of Ascorbic acid was 5.4 ug/ml (Figure No.03).

DPPH Antioxidant Activity of Alpha-mangostin (E1)



DPPH Antioxidant Activity of Gamma-mangostin (E2)

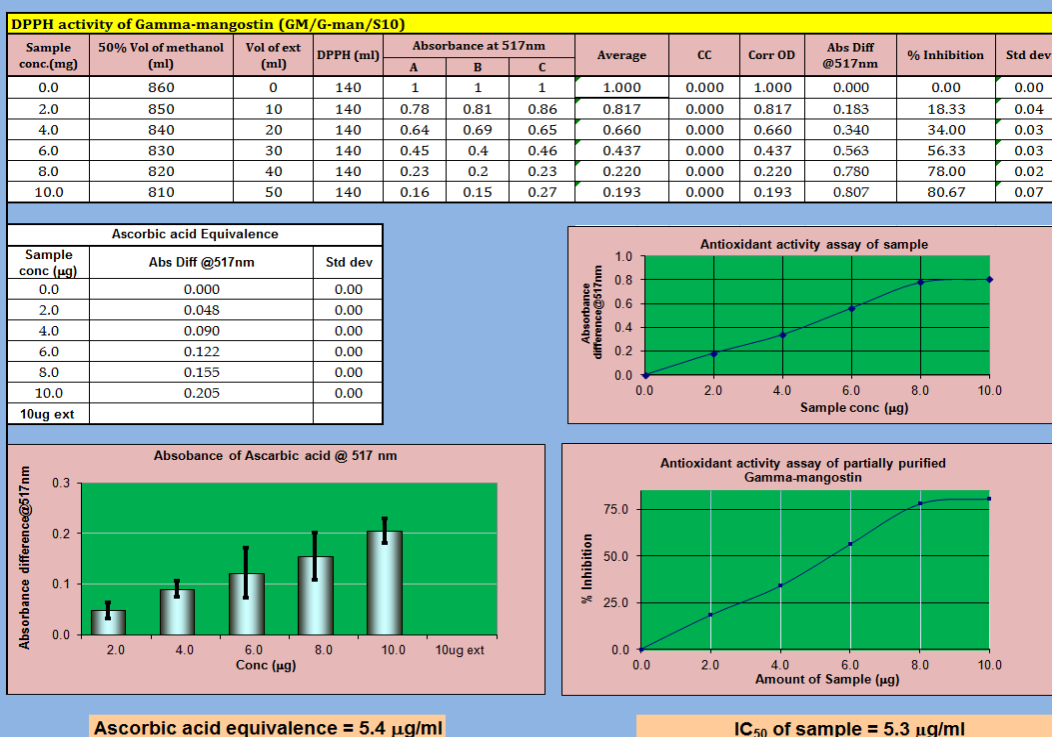


Fig 03: DPPH free radical scavenging activity at different concentrations of GM/A-man/S9 (E1) and GM/G-man/S10 (E2)

Antidiabetic Activity:

One of the most critical control points is the control of plasma postprandial glucose levels in the early treatment of diabetes mellitus (Oritz et al., 2007). Controlling the absorption of glucose produced from the breakdown of starch by hydrolysis by inhibiting the pancreatic Alpha-amylase and by limiting the absorption of glucose by inhibition of intestinal Alpha-glucosidase enzymes (Krentz and Bailey, 2005) are the two available therapeutic approaches for the Type II diabetes management.

Alpha-Glucosidase Inhibition activity:

Both purified Alpha-mangostin (E1) and Gamma-mangostin (E2) fractions exhibit a significant dose dependent inhibition of Alpha-Glucosidase and with 50% of inhibition (IC_{50}) at concentration of 45.2 ug/ml and 70.2 ug/ml respectively (Standard Acarbose was 11.6 ug/ml for the Inhibition of Alpha-glucosidase inhibition).



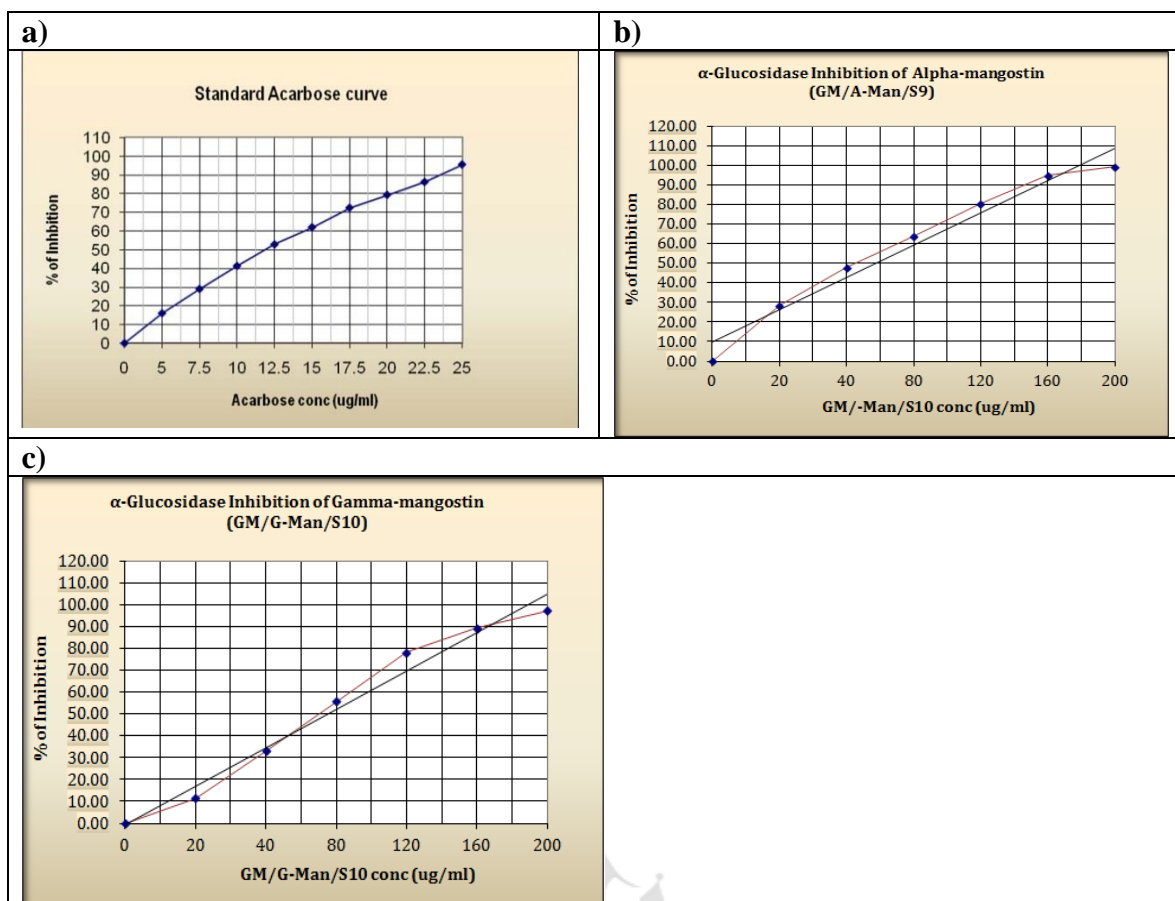


Fig. 04: Standard curve for determination of IC₅₀ value of (a) standard Acarbose and (b) purified Alpha-mangostin fraction and (c) Gamma-mangostin on Alpha-Glucosidase Inhibition

Alpha-Amylase Inhibition activity:

Alpha-mangostin (GM/A-man/S9-[E1]) and Gamma-mangostin (GM/G-man/S10 [E2]) fractions are also exhibits significant dose dependent inhibition of Alpha-amylase activity with 50% of inhibition (IC₅₀) at concentration of 30.6 ug/ml and 80.1 ug/ml respectively (Standard Acarbose was 6.3 ug/ml for the Alpha-amylase inhibition).

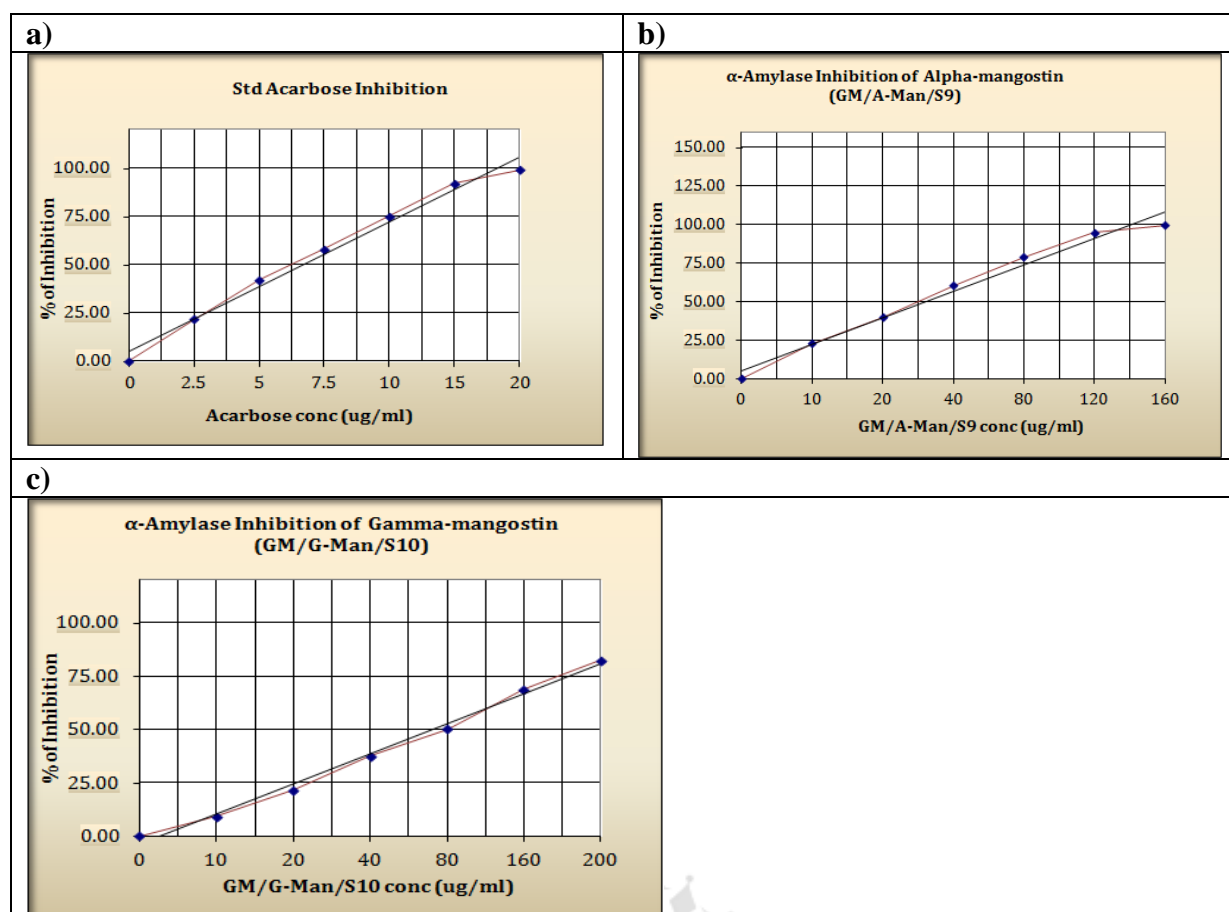


Fig. No 05: Standard curve for determination of IC₅₀ value of (a) standard Acarbose and (b) purified Alpha-mangostin fraction and c) Gamma-mangostin on Alpha-Amylase Inhibition

Anti Obesity Activity:

Aldolase Inhibition:

Both purified fractions; GM/A-man/S9 (Alpha-mangostin) and GM/G-man/S10 (Gamma-mangostin) were subjected for the aldolase inhibition assay. Results show both fractions are active inhibitors of Aldolase enzyme at conc. of IC₅₀ of 64.0 µg/ml and 86.5 µg/ml respectively. CuSO₄ is used as Standard and shows inhibition at IC₅₀ of 8.64 µg/ml (Ref table No.1)

Table No.1: IC₅₀ Values for the Inhibition of Aldolase enzyme by Alpha- and Gamma-mangostins

Sample	IC ₅₀ (MI %)
Reference Standard (CuSO ₄)	8.64 µg/ml (7.63-9.70)
S1 (Standard)	67.95 µg/ml (66.53-69.4)
GM/A-Man/S9 (Alpha-mangostin purified)	64.00 µg/ml (62.5-65.7)
GM/G-Man/S10 (Gamma-mangostin purified)	86.50 µg/ml (84.9-88.3)

Cell based assay to determine Antimicrobial activity by Minimal Inhibitory Concentration (MIC) and Time killing Curve

Minimal Inhibitory Concentration (MIC):

Antimicrobial activity of Garcinia Mangostins is shown in Table No.03 and Gamma Mangostin has good antimicrobial activity against all four strains of pathogenic micro-organisms. That means there was Growth pressure on all the microorganisms due to which they dint show any growth. Very less turbidity was seen with Gamma mangostin at a concentration of 100 to 400µg. Whereas Alpha mangostins showed less turbidity and No growth only at 200 and 400µg concentration.

Table 02: Shows MIC of Pathogenic strains for Alpha Mangostin.

Strain	Concentration of Alpha Mangostin				
	0.1 µg	10 µg	100 µg	200 µg	400 µg
<i>B.cereus</i>	Turbidity	Turbidity	Turbidity	Less Turbidity	Less Turbidity
<i>B.subtilis</i>	Turbidity	Turbidity	Turbidity	Less Turbidity	Less Turbidity
<i>P.aeruginosa</i>	Turbidity	Turbidity	Turbidity	No Growth	No Growth
<i>S.aureus</i>	Turbidity	Turbidity	Turbidity	No Growth	No Growth

Table 03: Shows MIC of Pathogenic strains for Gamma Mangostin

Strain	Concentration of Gamma Mangostin				
	0.1 µg	10 µg	100 µg	200 µg	400 µg
<i>B.cereus</i>	Turbidity	Turbidity	Less Turbidity	Less Turbidity	Less Turbidity
<i>B.subtilis</i>	Turbidity	Turbidity	Less Turbidity	Less Turbidity	Less Turbidity
<i>P.aeruginosa</i>	Turbidity	Less Turbidity	Less Turbidity	No Growth	No Growth
<i>S.aureus</i>	Turbidity	Less Turbidity	Less Turbidity	No Growth	No Growth

Inhibition or Time killing Curve:

Antimicrobial activity of Alpha and Gamma Mangos tins are shown in below Figures. *B.cereus* and *B.subtilis* was showed a minimal level of resistance as the O.D reached 3–4 at 10 hours of incubation with both Alpha and Gamma Mangostins. But *P.aeruginosa* and *S.aureus* showed very less resistance to both Alpha and Gamma Mangostins. With increase in incubation time and concentration the OD drop to 0.

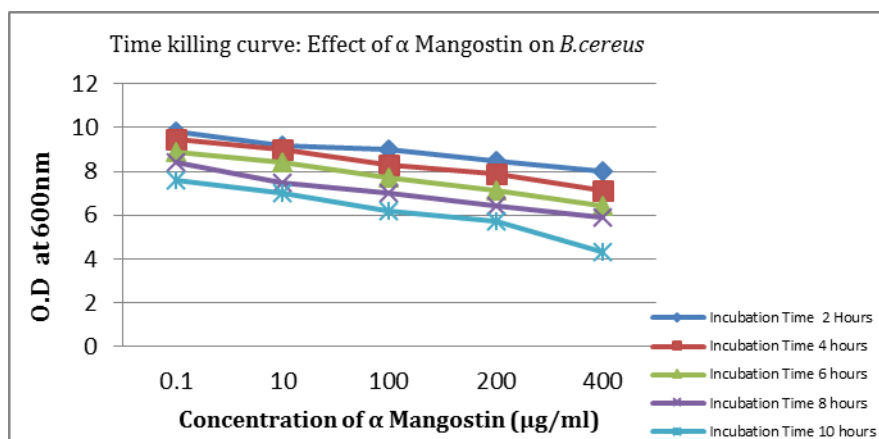


Fig 6a: Inhibition Curve of α -Mangostin (GM/A-man/S9) on *B. cereus*

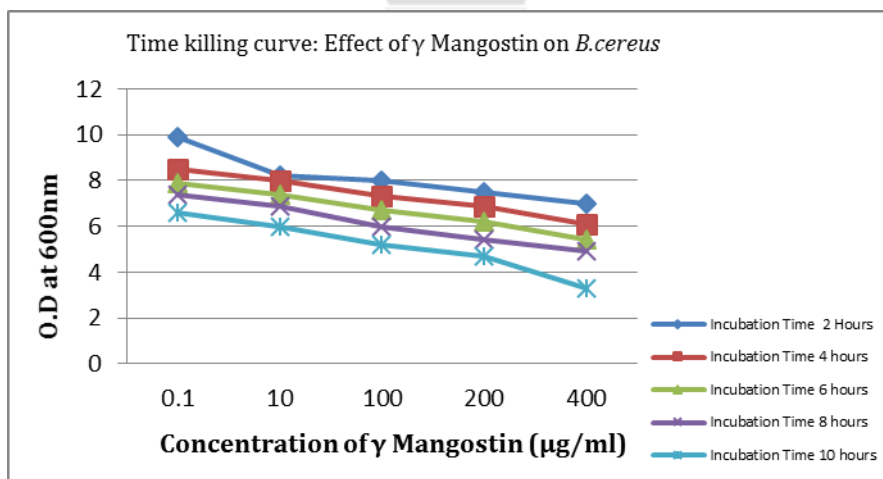


Fig 6b: Inhibition Curve of γ -Mangostin (GM/G-man/S10) on *B. cereus*

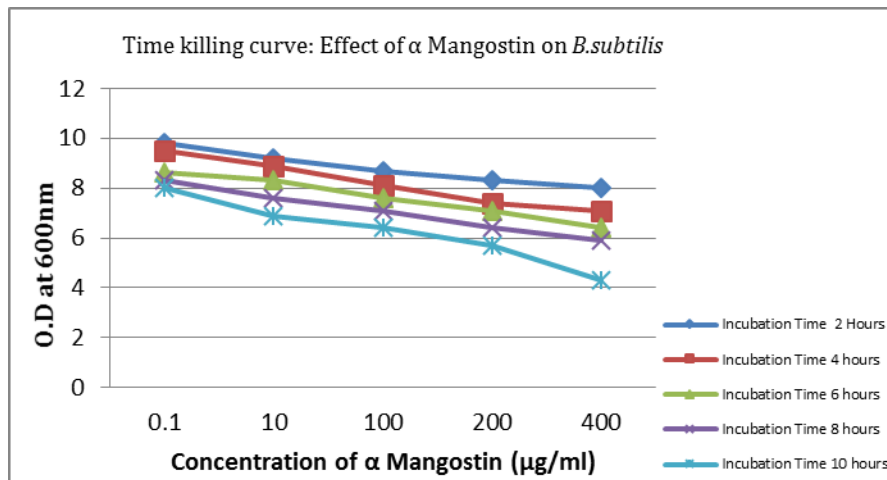


Fig 6c: Inhibition Curve of α - Mangostin (GM/A-man/S9) on *B. subtilis*

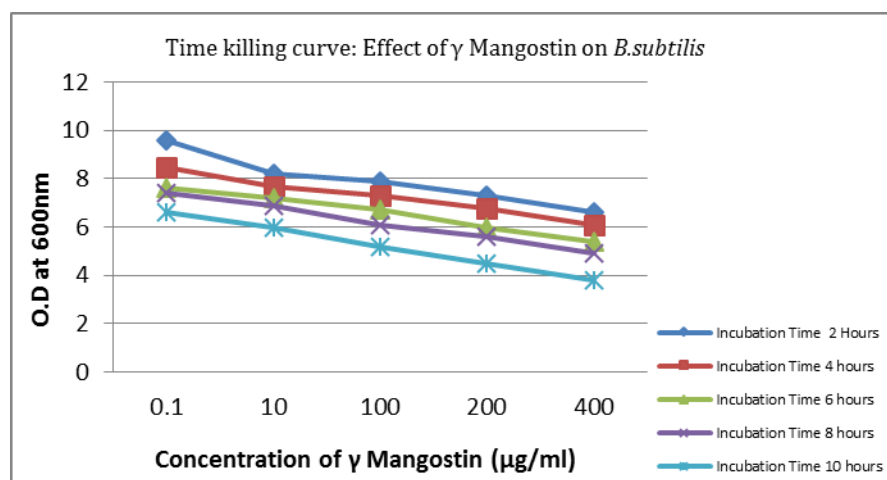


Fig 6d: Inhibition Curve of γ - Mangostin (GM/G-man/S10) on *B. subtilis*

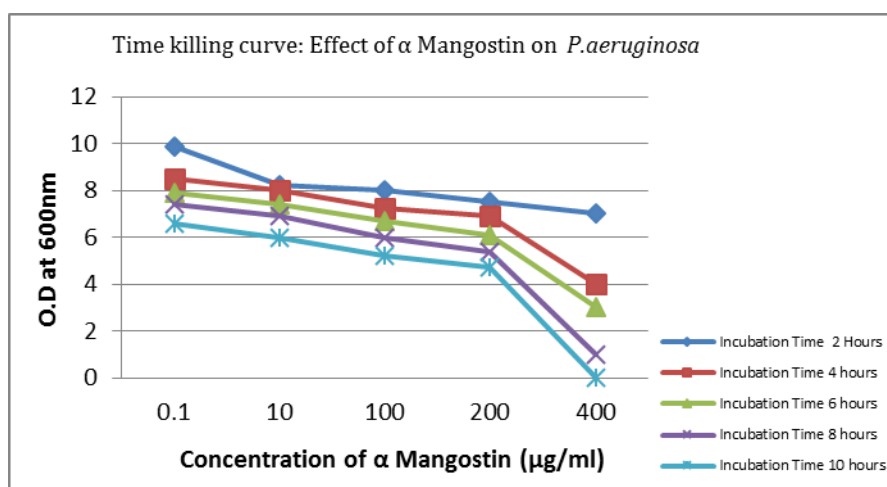


Fig 6e: Inhibition Curve of α - Mangostin (GM/A-man/S9) on *P.aeruginosa*

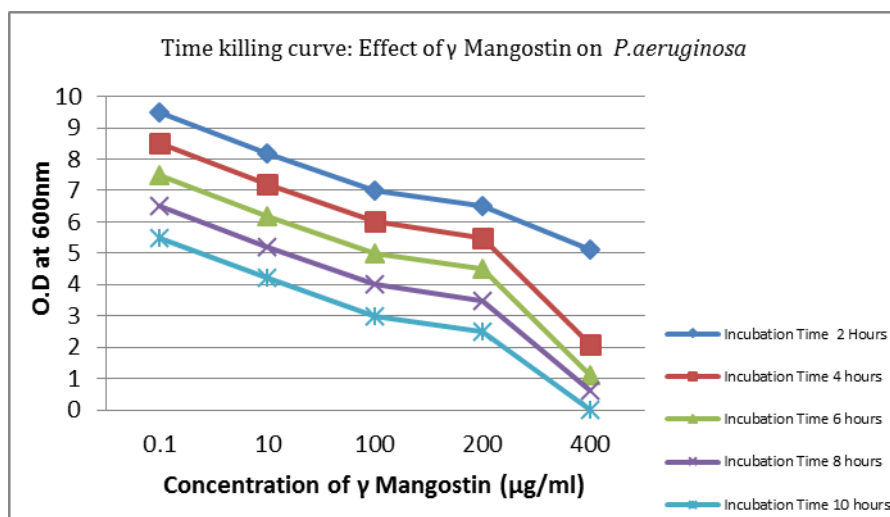


Fig 6f: Inhibition Curve of γ - Mangostin (GM/G-man/S10) on *P.aeruginosa*

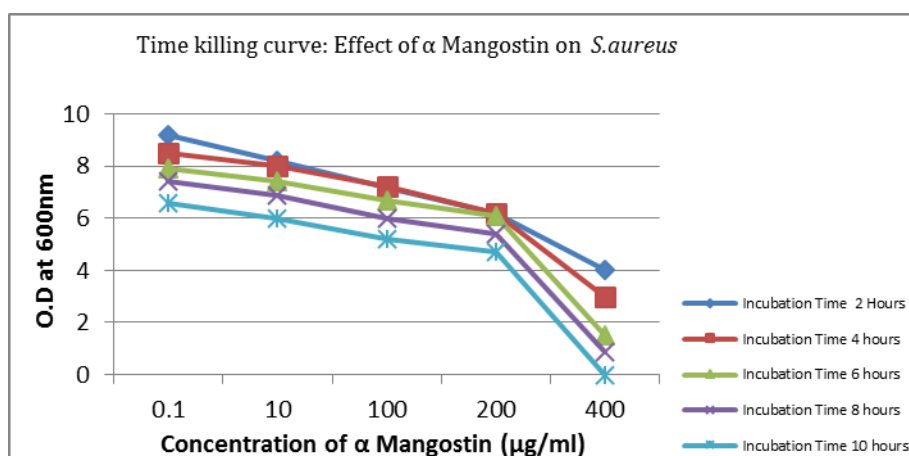


Fig 6g: Inhibition Curve of α - Mangostin (GM/A-man/S9) on *S.aureus*

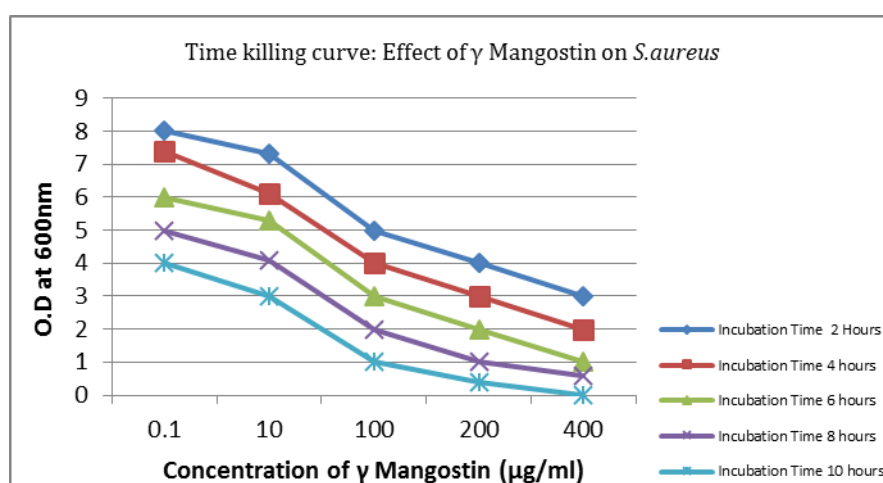


Fig 6h: Inhibition Curve of γ -Mangostin (GM/G-man/S10) on *S.aureus*

Based on the results obtained in our present study (Both MIC and Inhibition curve) revealed antibacterial activity of Mangostins against *B.cereus*, *B.subtilis*, *P.aeruginosa* and *S.aureus*. Microbial susceptibility assays using the disc diffusion method and the Minimal Inhibitory Concentration (MIC) and Inhibition curve was carried out. The Minimum Inhibitory Concentration and killing effect on *P.aeruginosa* and *S. aureus* was seen at 400 µg / ml. Mangostins has been known for its broad-spectrum antibacterial activity against several Gram-positive and Gram negative bacteria, especially those associated with skin infection, diarrhea, tuberculosis or acne. The active chemical components that are present in medicinal plants like *Garcinia mangostana* are responsible for its antimicrobial activity. Among xanthone derivatives from mangosteen extract, α-mangostin has been known to exert the most potent antimicrobial activity. But in the strains which we have used for the study γ-mangostin has showed better antimicrobial activity.

In conclusion, these two compounds assumed to be responsible for the very good antioxidant activity related to the health protecting benefits against nascent oxygen radical damage. Present study also gave us strong insight that, purified fraction containing rich content of Alpha-mangostin can be a potent phytochemical entity for the development drug products; particular for the disease related to free radical damage such as Cardiovascular, Atherosclerosis and inflammation etc. Results on the Anti-diabetic activity were also showing good inhibition of Alpha-glucosidase and Alphaamylase.

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