ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 10, Issue 9, 2017

Online - 2455-3891
Print - 0974-2441

Research Article

BIOLOGICAL ACTIVITIES OF *GARCINIA MANGOSTANA*

JEBALSY LALITHA L, PRINCE CLARANCE P, JERIN SALES T, MARY ARCHANA A, AGASTIAN P*

Research Department of Plant Biology and Biotechnology, Loyola College, Chennai - 600 034, Tamil Nadu, India.
Email: agastian@loyolacollege.edu

Received: 20 March 2017, Revised and Accepted: 31 May 2017

ABSTRACT

Objective: The aim of the present study was to investigate *in vitro* antibacterial, antifungal, and antioxidant activities of different solvent extracts of *Garcinia mangostana* leaves.

Methods: The powdered leaf was subjected to sequential extraction using hexane, ethyl acetate (EA), and methanol. The extracts were subjected to quantitative and qualitative phytochemical analysis, antimicrobial, and antioxidant activities. The best solvent extract was subjected to gas chromatography–mass spectrometry (GC-MS) analysis.

Results: The highest activity was recorded in EA extract which was subjected to GC-MS analysis revealing the presence of squalene (17.09%).

Conclusion: From this present study, we conclude that EA is the best solvent for extracting antimicrobial and antioxidant compounds from the leaves of *G. mangostana.*

Keywords: Antibacterial activity, Antifungal activity, Phytochemical assay, Antioxidant activity, Gas chromatography–mass spectrometry analysis, *Garcinia mangostana.*

 DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i9.18585

INTRODUCTION

According to the World Health Organization, approximately 85% of the world's inhabitants currently rely on indigenous or traditional medicines for their primary health needs, and most of this therapy involves the use of plant extracts, often in aqueous solutions [1]. Many traditional cultures remain mostly dependent on plants for their food and medicine and often consider them both in the same context of the plant-based foods used as medicines; none have received more attention as a group than herbal remedies [2]. They have stood the test of time for their safety, potency, cultural acceptability, and low side effects. The chemical constituents present in them are a part of the physical functions of living flora and hence they are believed to have better compatibility with the human body. Antique literature also mentions herbal medicines for age-related diseases, namely, memory loss, diabetic wounds, osteoporosis, immune, and liver disorders. Traditional medicine is the synthesis of therapeutic experience of generations of practicing physicians of indigenous systems of medicine [3]. *Garcinia mangostana* (Mangosteen) is a highly recognized equatorial fruit and has universal appeal because of its quality in color, shape, and flavor. The white, moist, soft, and juicy flesh is sweet and has high sugar content [4]. The pulp has an excellent flavor and, though slightly acidic, it is sweet and delicious. Mangosteen "queen of fruits" (*G. mangostana*) (Family: *Clusiaceae*) is a tropical evergreen tree originated in Southeast Asia and used for centuries as a folk medicine [5]. *G. mangostana* is a seasonal medicinal plant occurs only during the month of November, not much explored for its bioactivities. Very scanty work has been carried out in the test plant *G. mangostana*. Hence, the present study was taken up to investigate the solvent extracts for antibacterial, antifungal, phytochemical, and antioxidants assay followed by the identification of active compounds using gas chromatography–mass spectrometry (GC-MS) [6].

METHODS

Collection and identification of plant

The plant *G. mangostana* was collected from the Kerala, India and was identified by the taxonomist Dr. G. Jeya Jothi and the voucher specimen (LCH 313) was deposited in the Department of Plant Biology and Biotechnology, Loyola College, Chennai.

Solvent extraction from plant material

The leaves of the plant *G. mangostana* were collected from Kerala, India. Dried in shade and crushed to fine powder, equally soaked in hexane and ethyl acetate (EA), and methanol for 3 days and shaken the flask intermittently [7]. After 3 days, the solution was filtered using filter paper. The solvent was evaporated using rotary vacuum evaporator. The crude extract was collected and stored at 4°C for further uses.

Test organisms

Staphylococcus aureus (Microbial Type Culture Collection [MTCC] 96), *Staphylococcus epidermidis* (MTCC 3615), *Enterobacter aerogenes, Vibrio parahaemolyticus, Proteus vulgaris*, *Escherichia coli* (American Type Culture Collection [ATCC] 25922), *Klebsiella pneumoniae*, *Yersinia enterocolitica* (MTCC 840), *Salmonella typhimurium,* and *Micrococcus luteus.* All the bacterial cultures were obtained from IMTECH-Chandigarh, India, and the clinical isolates were obtained from the Department of Microbiology, Christian Medical College, Vellore, Tamil Nadu, India.

Disc diffusion method

Antibacterial activity was carried out using disc diffusion method [8]. Petri plates were prepared with 20 ml of sterile Muller Hinder agar (Hi-media, Mumbai). At a temperature between 40 and 50°C and poured immediately into Petri dishes to occupy a depth of 3-4 mm. The prepared dishes must be stored such that no significant growth or death of the test bacteria occur before use and the surface of the agar layer was dry at the time of use. The test culture (100 µl of suspension containing 10^8 CFU/ml bacteria) was swabbed on the top of the solidified media and allowed to dry for 10 minutes. Five different dilutions (62.5, 125, 250, 500, and 1000 µg/ml) of the EA, hexane, and methanol extracts were placed in appropriate positions on the plate with quadrants marked at the back of the each petri dishes. Sterile discs of 6 mm diameter were impregnated with 25 µl (2.5 mg) each

hexane, EA, and methanol extract of the test plants. These discs were then dried at 37°C before use. Streptomycin (10 µg/disc) was used as a positive control. These discs were placed on the microbe-inoculated plate as a control. These plates were incubated for 24 hrs at 37°C. Zone of inhibition was recorded in millimeters (mm), and the experiments were repeated thrice.

Antifungal activity

Test fungal strains

The following test fungal strains were used for the experiment: *Aspergillus flavus, Scopulariopsis* species*, Curvularia lunata* 46/01, *Aspergillus niger* MTCC 1344, *Trichophyton rubrum* 57/01 and *Trichophyton mentagrophytes* 66/01.

Antifungal assay using broth micro dilution method

The antifungal activity was done according to the standard reference method [9,10]. The extracts were dissolved in water with 2% dimethyl sulfoxide (DMSO). The initial concentration of the extract was 2 mg/ml. The first test concentration was serially diluted two-fold in 96 well plates. Each well was inoculated with 5 µl of suspension containing approximately 10^4 spore/ml of fungi. The positive control for fungi was fluconazole, and minimum inhibitory concentration (MIC) was determined as the lowest extract concentration showing no visible fungal growth after incubation time.

Qualitative analysis phytochemical screening

Preparation of plant extract

The leaves of the selected medicinal plants were removed from the plants and then washed under running tap water to remove dust. The plant samples were then shade dried for few days, and the leaves were crushed into powder and stored in room temperature. The plant powder was taken in a test tube, and distilled water was added to it such that plant powder soaked in it and shaken well. The solution was then filtered with the help of a filter paper and filtered extract of the selected plant samples were taken and used for further phytochemical analysis. Phytochemical examinations were carried out for plant powder as per the standard methods as follows [11].

Test for phlobatannins

Distilled water was added to the plant powder in a test tube, then shaken it well, and filtered to take plant extract. Then, to each plant extract, 1% aqueous hydrochloric acid was added and each plant sample was then heated with the help of hot plate stirrer. Red precipitation confirmed a positive result.

Test for reducing sugar

An amount of 0.50 g of selected plant sample was added in 5 ml of distilled water. Then, 1 ml of ethanol was mixed in plant extract. After that 1 ml of Fehling solution A and 1 ml of Fehling solution B was added in a test tube, boiled it and then poured it in the aqueous ethanol extract. Change in color indicates the presence of reducing sugar.

Test for terpenoids

0.8 g of selected plant sample was taken in tube, then poured 10 ml of methanol in it, shaken well and filtered to take 5 ml extract of plant sample. Then, 2 ml of chloroform were mixed in extract of selected plant sample and 3 ml of sulphuric acid were added in selected sample extract. Reddish brown color indicates the presence of terpenoids in the selected plants.

Test for flavonoids

For the confirmation of flavonoid in the selected plants, 0.5 g of each selected plant extract were added in a test tube and 10 ml of distilled water, 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by the addition of 1 ml concentrated H_2SO_4. Appearance of yellow color indicates the presence of flavonoid in each extract.

Test for alkaloids

For the purpose of phytochemical analysis of the selected plants, 0.2 g of the selected plant samples were added in each test tube and 3 ml of hexane were mixed in it, shaken well, and filtered. Then, 5 ml of 2% HCl was taken and poured in a test tube having the mixture of plant extract and hexane. The test tube having the mixture was heated, filtered it and poured few drops of picric acid in a mixture. Formation of yellow color precipitate indicates the presence of alkaloids.

Test for phytosterols Salkowski's test

Extracts were treated with 2 ml chloroform and filtered. The filtrates were treated with few drops of conc. sulfuric acid, shaken, and allowed to stand. Golden yellow color appears indicating the presence of triterpenes.

Test for phenols ferric chloride test

Extracts were treated with 4-5 drops of ferric chloride solution leading to the formation of bluish black indicating the presence of phenols.

Test for tannins

0.5 g of powdered sample of each plant is heated in 20 ml of distilled water in a test tube and filtered 0.1% $FeCl_3$ is added to the filtered samples and observed for brownish green or a blue-black coloration which shows the presence of tannins.

Quantitative analysis

Determination of total phenolic content

Total phenolic content of *G. mangostana* hexane, EA, and methanol extracts were assessed according to the Folin-Ciocalteau method [12]. 0.1ml of extracts (200-1000 µg/ml), 1.9 ml distilled water, and 1 ml of Folin-Ciocalteu's reagent were seeded in a tube, and then, 1 ml of 100 g/l Na_2CO_3 was added. The reaction mixture was incubated at 25°C for 2 hrs and the absorbance was read at 765 nm. The experiment was repeated thrice in and a calibration curve with six data points for catechol was obtained. The results were compared to a catechol calibration curve and the total phenolic content of *G. mangostana* was expressed as mg of catechol equivalents per gram of extract.

Total flavonoid assay

Flavonoid content was measured by the aluminum chloride colorimetric assay [13]. Sample and standard were incubated at room temperature for 5 minutes. Then, 0.3 ml of 5% sodium nitrite and add 0.3 ml of 10% of aluminum chloride was added and incubated at room temperature for 6 minutes. 2 ml of 1 M sodium hydroxide was added and immediately made up to 10 ml with distilled water. The absorbance was read at 510 nm.

Determination of *in vitro* α-glucosidase inhibition and antioxidant assays

α-glucosidase inhibition of G. mangostana leaf extract

To investigate the inhibition activity of *G. mangostana* leaves hexane, EA, and methanol extracts, an *in vitro* a-glucosidase inhibition test was performed. α-glucosidase from yeast is used extensively as a screening material for a-glucosidase inhibitors, but the results do not always agree with those obtained in mammals. Therefore, we used the mouse small-intestine homogenate as an α-glucosidase solution because we speculated that it would better reflect the *in vivo* state. The inhibitory effect was measured using the method slightly modified from Pfaller *et al*. [14]. After fasting for 20 hrs, the small intestine between the part immediately below duodenum and the part immediately above the cecum was cut, rinsed with ice-cold saline, and homogenized with 12 ml of maleate buffer (100 mM, pH 6.0). The homogenate was used as the α-glucosidase solution. The assay mixture consisted of 100 mM maleate buffer (pH 6.0), 2% (w/v) each sugar substrate solution (100 µl), and the sample extract (200-1000 µg/ml). It was preincubated for 5 minutes at 37°C, and the reaction was initiated by adding the crude α-glucosidase solution (50 µl) to it, followed by incubation for 10 minutes at 37°C. The glucose released in the reaction mixture was

determined with the kit described above. The rate of carbohydrate decomposition was calculated as the percentage ratio to the amount of glucose obtained when the carbohydrate was completely digested. The inhibition percentage was calculated by the following formula:

Inhibition (%) = [(amount of glucose produced by the positive control) − (amount of glucose produced by the addition of sample)/(amount of glucose produced by the positive control)] ×100

Reducing power activity

The reducing power of *G. mangostana* hexane, EA, and methanol leaf extracts was evaluated according to the method Wadood *et al*. [15]. Different amounts of the extracts (200-1000 µg/ml) were suspended in distilled water and mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6), and 2.5 ml of 1% $K_3Fe(CN)_6$. The mixture was incubated at 50°C for 20 minutes; 2.5 ml of 10% trichloroacetic acid (TCA) was added to the mixture and centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increase in absorbance of the reaction mixture indicated the ability of reducing power. Butylated hydroxytoluene (BHT) was used as standard.

1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

DPPH quenching ability of solvent extracts of the test plant was measured according to a methanol DPPH solution (0.15%) was mixed. 3.7 ml of absolute methanol was taken in all test tubes along with blank. Then, 100 µl of absolute was added to 3.7 ml of absolute methanol in all test tubes along with blank. 100 µl of absolute methanol in blank samples to all other tubes marked as tests were added. Finally, 200 µl of DPPH reagent was added to all the test tubes including blank and incubated all test tubes at room temperature and dark condition for minimum 30 minutes. Absorbance of all samples was read at 517 nm. DPPH radical scavenging assay was calculated by the following formula [16]:

% Antioxidant activity = {(absorbance at blank) − (absorbance at test)/(absorbance at blank)}×100

Hydroxyl radical scavenging activity

The hydroxyl radical assay was described by the method of Slinkard and Singleton [17]. Various concentrations (200, 400, 600, 800, 1000, and 100 ug/ml in methanol) of extract were taken in different test tubes and evaporated to dryness. 1 ml of iron-ethylenediaminetetraacetic acid (EDTA) solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of EDTA (0.018%), and 1 ml of DMSO (0.85% v/v in 0.1 M phosphate buffer pH 7.4) were added to these tubes, and the reaction was initiated by adding 0.5 ml of 0.22% ascorbic acid. Test tubes were capped tightly and heated on a water bath at 80-90°C for 15 minutes. The reaction was terminated by the addition of 1 ml of ice cold TCA (17.5% w/v). 3 ml of Nash reagent (75.0 g of ammonium acetate, 3 ml of glacial acetic acid, and 2 ml of acetyl acetone were mixed and made up to 1 L with distilled water) was added to all of the tubes and left at room temperature for 15 minutes for color development. To different concentrations of sample and standard add 100 µl 2-deoxyribose (28 mm), 200 µl of EDTA, 200 µl of $FeCl_3$, and 100 µl of H_2O_2 100 µl of ascorbic acid incubate for 1 h. Then, add 1 ml of TBA and 1 ml of TCA. Boil at 100°C for 20 minutes. Measure absorbance at 532 nm.

Calculation:

%Inhibition = Control−test/control × 100.

RESULTS

Antibacterial activity

The effect of the different extracts from *G. mangostana* leaves on growth of different bacteria is presented in Table 1. Different extracts inhibited growth to variable extents, among these extracts, EA extract was found to be the most effective against each bacteria with a larger zone of inhibition values (13.4-19.8 mm) followed by methanol and the lowest for hexane extract. However, the MIC and minimum bactericidal concentration values of the hexane extract for tested Gram-negative bacteria strains have not yet been gained at the highest concentration of extract. In this study, *S. aureus*, *P. aeruginosa*, *S. typhimurium,* and *B. subtilis* were highly susceptible to *Mangostana* whereas *Proteus* sp., *Klebsiella* sp. and *E. coli* were only moderately susceptible to them. Some studies reported that extracts of *G. mangostana* inhibited the growth of *S. aureus* and *S. epidermidis*. Three different solvent extracts of the leaves of *G. mangostana* were screened against bacterial cultures. Zones of inhibition of the crude extracts were noted. Among the three different solvent (hexane, EA and methanol) extracts tested for antimicrobial activity against the ATCC and MTCC reference cultures, most of the Gram-positive and Gram-negative bacteria were inhibited at different levels (Table 1). With reference to Gram-positive bacteria tested, *S. aureus*, *S. epidermidis* (10-17 mm), and *M. luteus* (10-19 mm) were affected by all the solvent extracts. Higher activity found in EA extracts followed by methanol and least activity found with hexane extracts. With reference to Gram-negative bacteria, all the tested bacteria were affected significantly by all the solvent systems used for the test plant. *E. aerogenes* (MTCC 111), *P. vulgaris* (MTCC 1771), and *K. pneumoniae* affected significantly by all the solvent extracts of all the three tested solvents. Moderate activity was found in *S. typhimurium, V. parahaemolyticus, Y. enterocolitica,* and *E. coli* in all the extracts. Standard streptomycin was used as positive control (10 µg/disc).

From the data given in the tabular column, we can conclude that EA extract showed the best antibacterial activity followed by methanol and hexane extracts of *G. mangostana* leaves.

Antifungal activity

Antifungal activities of different solvent (EA, methanol, and hexane) extracts of the plant (*G. mangostana*) were carried out using micro

Table 1: Antibacterial activity of hexane, ethylacetate, and methanol extracts of *Garcinia mangostana*

Bacteria	Zone of inhibition in mm			
	Standard (streptomycin)	Hexane	EA	Methanol
Gram-positive bacteria				
Staphylococcus epidermidis	19	11	15	13
Staphylococcus aureus	18	10	14	12
Micrococcus luteus	17	10	14	12
Gram-negative bacteria				
Enterobacter aerogenes	17	11	13	11
Escherichia coli	16	10	14	13
Vibrio parahaemolyticus	21	10	18	14
Proteus vulgaris	19	11	15	14
Klebsiella pneumoniae	18	12	14	12
Yersinia enterocolitica	18	13	16	15
Salmonella typhimurium	19	12	17	14

Table 2: Antifungal activity of hexane, EA and methanol extracts of *Garcinia mangostana* MIC test

Fungi	Hexane µg/ml	EA µg/ml	Methanol µg/ml	F1 µg/ml
Aspergillus flavus	125	162.5	125	50
Trichophyton mentagrophytes 66/01	62.5	31.25	62.25	25
Trichophyton rubrum 57/01	125	31.25	125	25
Scopulariopsis species	250	62.5	125	100
Aspergillus niger MTCC 1344	250	125	250	100
Curvularia lunata 46/01	250	62.5	125	<12.5

MIC: Minimum inhibitory concentration, EA: Ethyl acetate, MTCC: Microbial type culture collection, F1: Fluconazole (antifungal agent)

broth dilution method (Table 2). About six fungal pathogens which could cause disease in both animals and plants were considered for the present study. Fluconazole was used as a positive control. MIC value was significantly found high in hexane extract that affects *T. rubrum* and *Scopulariopsis* sp. (31.25 µg/ml) while for methonal extract for *T. rubrum, T. mentagrophytes,* and *A. niger* found to be 62.5 µg/ml. EA extracts of this plant found to be moderate in antifungal activity with medium MIC value *C. lunata* (31.25 µg/ml). *A. niger, Scopulariopsis* species required maximum MIC values (250 µg/ml) for EA extract of *G. mangostana.* With reference to *C. lunata* 46/01, *T. mentagrophytes* 66/01, least MIC value was found with EA extract.

Qualitative phytochemical screening

Phytochemical screening

Phytochemical examinations were carried out using plant extracts as per the standard methods. Many compounds have been isolated from the plants flavonoids and xanthones were found to be present in this plant. The presence of catechins, saponins, steroids, and triterpenoids were reported by earlier workers [18].

A preliminary phytochemical screening experiment reveals the presence of different compounds from the hexane, EA, and methanol extracts of the test plant (Table 3). Most of the compounds were extracted using EA and methanol. Hexane was found to be moderate in extracting compounds. From the perusal of the antibacterial, antifungal activities, EA extract was found to be comparatively the best. Hence, the EA extracts of the test plant were subjected to further studies.

Quantitative analysis

Total phenolic content

The total phenolic content of hexane EA and methanol extracts of *G. mangostana* was found to be 370.24±0.57, 270.46±0.46, and 330.37±1.11 mg catechol equivalent/g extract, respectively.

Total flavonoids content

The total flavonoids content of hexane EA and methanol extracts of *G. mangostana* was found to be 320.99±0.46, 220.94±0.57 and 300.49±1.11 mg catechol equivalent/g extract, respectively.

GC-MS analysis on EA extract of G. mangostana leaves

GC-MS analysis on EA extract of *G. mangostana* leaf clearly showed the presence of diverse molecules. Fig. 1 shows molecules chemical nature, retention time, concentration (peak are %). Around, 45 compounds were detected, out of which the maximum area was found to be squalene (17.09%). From the perusal of above study, the antibacterial, antifungal, and antioxidant activity of the EA extract would be due the presence of the above listed various chemical constituents.

***In vitro* antioxidant activity of *G. mangostana* leaf extracts**

In vitro α-glucosidase inhibition and antioxidant assays of G. mangostana

α-glucosidase inhibition

The results for α-glucosidase inhibition assay of EA extract and the standard acarbose are shown in Table 4. The concentration for 50% inhibition of methanol extracts and acarbose was found to be

Table 3: Phytochemicals in the leaves of *Garcinia mangostana*

S.No.	Phytochemical	Result
1	Alkaloids	+++
2	Terpenoids	++
3	Flavonoids	+++
4	Reducing sugar	++
5	Phlobatannins	-
6	Phytosterols	+
7	Phenols	++
8	Tannins	+++

Table 4: *In vitro* α-glucosidase inhibition and antioxidant assays of *Garcinia mangostana*

Sample	Concentration (µg/ml)	Percentage of inhibition	IC_{50} (µg/ml)
Hexane	200	2.77±2.54	886.16±2.03
	400	27.21±2.53	
	600	31.20±2.71	
	800	48.96±2.77	
	1000	56.31±1.90	
EA	200	31.41±2.34	659.89±2.63
	400	37.64±2.37	
	600	39.80±2.04	
	800	53.31±2.28	
	1000	73.27±2.54	
Methanol	200	24.73±0.87	774.02±2.37
	400	31.72±1.94	
	600	37.74±1.55	
	800	53.21±1.21	
	1000	61.38±1.67	
Acarbose	200	32.04±1.74	606.99±1.43
	400	39.46±0.39	
	600	47.98±0.29	
	800	54.23±0.29	
	1000	74.56±0.40	

IC_{50}: 50% inhibition concentration, EA: Ethyl acetate

380.40±2.37 and 338.32±1.23 µg/ml, respectively. The hexane and methanol extracts showed less inhibition compared to EA extract.

Reducing power activity

Fig. 2 shows the reductive capabilities of hexane, EA, and methanol extracts of *G. mangostana* compared to BHT. The reducing power of EA extract was very potent than hexane and methanol extracts, and the power of the extract was increased with quantity of sample. The plant extract could reduce the most Fe^{3+} ions, which had a lesser reductive activity than the standard of BHT.

DPPH radical scavenging activity

EA extract exhibited a significant dose-dependent inhibition of DPPH activity compared to hexane and methonal extracts, with a 50% inhibition concentration (IC_{50}) at a concentration of 343.77±1.02 µg/ml. The results are presented in the IC_{50} value of ascorbic acid was 314.54±2.25 µg/ml (Fig. 3).

Hydroxyl radical scavenging assay

To neutralize the substrate deoxyribose hydroxyl radicals were generated by reaction of ferric-EDTA together with H_2O_2 and ascorbic

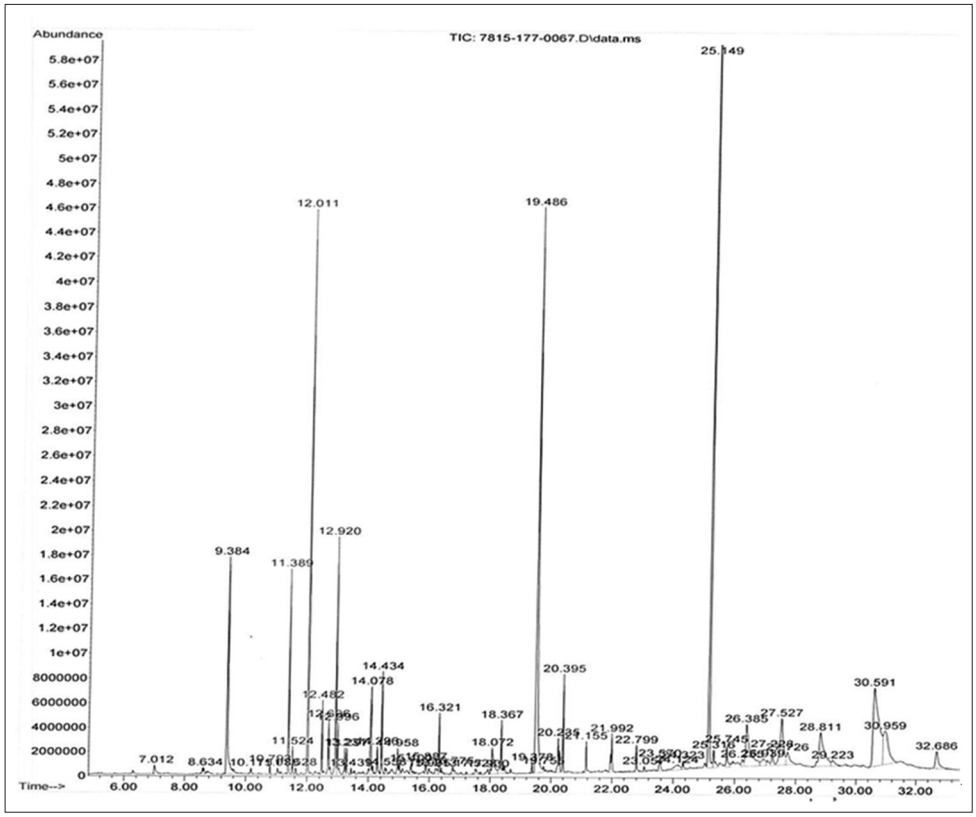

Fig. 1: Gas chromatography–mass spectrometry analysis on ethyl acetate extract of *Garcinia mangostana* leaves

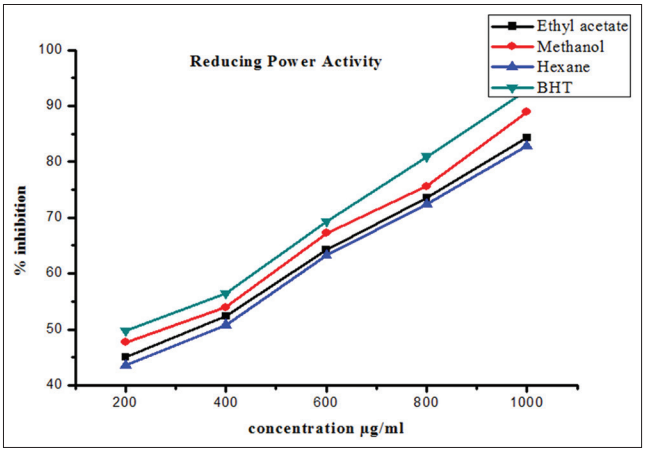

Fig. 2: Reducing power activity

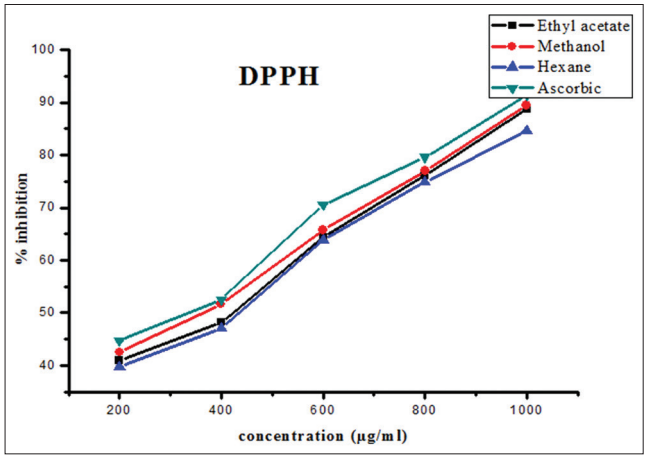

Fig. 3: 1, 1-diphenyl-2- picrylhydrazyl radical scavenging activity

acid. To prevent the damage against sugar, the plant extracts were incubated with the above reaction mixture. The results for hydroxyl scavenging assay are shown in Fig. 4. The concentrations for 50% inhibition were found to be 407.19±1.51 and 361.27±1.71 µg/ml for the EA extract and vitamin C, respectively. Hexane and methanol extracts showed less effect.

Statistical analysis

The data for biochemical and physiological parameters were analyzed in triplicate and expressed as mean±standard deviation. The IC_{50} values were calculated from linear regression analysis. Results were processed by computer program, Microsoft Excel (2010).

DISCUSSION

In the traditional systems of medicine, there is enough variation in the identity of the various plants of the individual drug selected for use. Based on the tribal information, leaves are the important and common parts used for external diseases in the form of powder/paste. This study can serve as a valuable source of information and provide suitable diagnostic tool for identification of adulterant in powdered drugs for standardization in future. It will also be of immense use in carrying out further research and revalidation of its use. The preliminary phytochemical analysis was done on the crude extracts obtained from different polar solvents such as EA, methanol, and hexane. The presence of various secondary metabolites such as alkaloids, flavonoids, saponins, steroids, terpenoids, tannins, and volatile oils may confirm the different medicinal properties of plants. Alkaloids, which are reported to have dramatic physiological activities and act mainly on controlling nervous system, were observed in *G. mangostana*. Flavonoids having anti-inflammatory, antiviral and cytotoxic activities and used in the treatment of capillary fragility, retinal hemorrhage, diabetic retinopathy, hypertension, rheumatic fever, and arthritis were observed. Saponins,

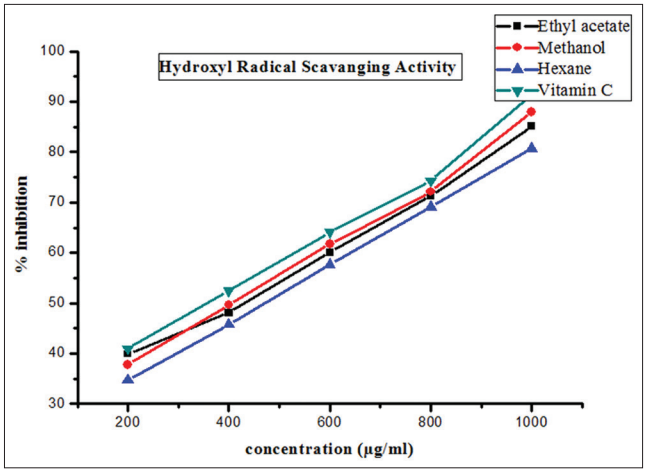

Fig. 4: Hydroxyl radical scavenging assay

well known for their expectorant, spasmolytic and antitissue activities (Raghavaiah) were observed. Steroids and triterpenoids, which are known for anti-inflammatory, lipolytic and anticholesteremic activities were recorded [19]. Gallic tannins, which are well documented for the astringent, cytotoxic, and antineoplastic activities and used in diarrhea, hemorrhage, wounds healing, and deep burns were observed [20]. During study, *G. mangostana* crude drug was screened for antimicrobial activity. Three different solvents, namely, hexane, EA, and methanol were used for the preparation of crude extracts. The alcoholic extracts exhibited inhibitory activity against most of the organisms. A majority of the plant extracts of *G. mangostana* (leaves) have shown significant inhibitory activity against tested pathogenic microorganisms.

The observation made in the studies of phytochemical coupled with medicinal uses mentioned by traditional herbal healers provides valuable information for further biological screening and antioxidant property of each plant extract. The screening for antimicrobial and antifungal activity of crude extracts of the plant leaves was carried out for their inhibitory effects individually on active cultures of ten different pathogenic microorganisms. Secondary metabolities such as alkaloids, anthraquinones, dihydrochalcones, aucubins, coumarins, emodins, gallic-tannins, reducing compounds, and polyoses are known to affect physiological activities. Anthraquinones, coumarins, gallic-tannins, and volatile oils are known to have antimicrobial activity [21]. Alkaloids, coumarins, flavonoids, phenols, tannins, phlobatannins, and terpenoids were present in the sample screened for the study. Individual (or) combination of several compounds may be one of the reasons for antibacterial activity. It is realized that many diseases at present are due to the "oxidative stress" that results from an imbalance between neutralization and formation of pro-oxidants. The free radicals initiate oxidative stress, which seek stability through electron pairing with biological macromolecules such as lipids, proteins, and DNA in healthy human cells and cause it also DNA and protein damage along with lipid peroxidation. These changes contribute to atherosclerosis, cardiovascular diseases, aging, and inflammatory diseases [22]. Many synthetic drugs protect against oxidative damage but they also have adverse side effects. A different solution to the problem is to consume natural antioxidants from food supplements and traditional medicine. Plants are rich sources of natural antioxidants, which play a vital role in the prevention or progression of the degenerative diseases. Plant compounds, mainly derived from natural sources that showed potential antioxidant activity includes carotenoids, flavonoids, tannins, etc. Plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators [23]. Phenolics are aromatic secondary metabolites, widespread throughout the plant kingdom. The phenols contain hydroxyls that are responsible for the radical scavenging redox properties [24]. Tannins are antioxidants often characterized by reducing power [25]. Plant-derived antioxidants especially polyphenols and flavonoids have ascribed been to various properties like antidiabetic, anticancer, antiaging, and prevention of cardiovascular diseases [26]. Polyphenolic compounds such as flavonoids have been labeled as high-level natural antioxidants based on their abilities to scavenge free radicals and active oxygen species [27].

The present study revealed the comprehensive data on ethnobotany, preliminary phytochemical screening followed by potential fractions of various compounds present in the test species, and biological screening with reference to crude extracts used. This helps to promote the future investigations on locally available medicinal plants and capture the biological and cultural data of local people. Further, the data can be used for intensive studies to evaluate active principle, which is biologically active against the causative pathogenic microorganisms. It is apparent through this review that the pace of natural product research and level of global interest in this particular area has raised dramatically in the past few years. This trend is projected to continue for the interface between chemistry and biology which becomes more blurred, and the public demand rises for cost-effective medications and biological agents from sustainable natural resources.

The present study provides evidence that *G. mangostana* extract in spite of having comparatively less amount of flavonoid and phenolic contents, process potential antioxidant activity. These *in vitro* assays demonstrate that plant extracts are important sources of natural antioxidants, which might be useful as preventive agents against oxidative stress and hence currently, the evaluation of *in vivo* antioxidant activity of these extracts are in progress. To elucidate the prime source of antioxidant properties, further studies should be carried out with isolate active principles.

CONCLUSION

This study highlights phytochemical and some pharmacological information on the use of this plant as a food supplement, there are no clinical data available that would provide evidence of efficacy of mangosteen xanthones or extracts on humans. Extracts and constituents of *G. mangostana* may have considerable clinical potential in humans and need to be studied further by *in vivo* models and ultimately in clinical studies. However, making unsubstantiated therapeutic claims will result in consumer expectations which cannot be met. Therefore, based on antioxidant property mangosteen may best be considered as (certainly healthy) food supplements. The ethnobotanical survey reveals that there are several therapeutic uses, which is been practiced for a long time, and they do not use modern methods of medication unnecessarily. At this stage, it is too early to assess the advantage of this fruit and any other products derived from *G. mangostana.* This study supports the development of evidence-based nutraceuticals and to globalize the local knowledge systems which would increase the benefits obtained to a wider population. In the present study leaves of the plants with different solvent extracts of were subjected for *in vitro* antioxidant activity and free radical scavenging activity. All the extracts (hexane, EA, methanol) in the present study exhibited different extent of antioxidant activity and free radical scavenging activity. Very little or undocumented antioxidant activities were observed in the stem extracts of *G. mangostana*. Results suggest that the above-mentioned crude extract samples serve as good candidates for further evaluation of their bio efficacies, active constituents, and biological mechanisms by *in vitro* as well as *in vivo* on antioxidation or chemoprevention effects.

REFERENCES

1. Attele AS, Zhou YP, Xie JT, Wu JA, Zhang L, Dey L, *et al*. Antidiabetic effects of *Panax ginseng* berry extract and the identification of an effective component. Diabetes 2002;51(6):1851-8.
2. Gryglewski RJ, Korbut R, Robak J, Swies J. On the mechanism of antithrombotic action of flavonoids. Biochem Pharmacol 1987;36(3):317-22.
3. Akao Y, Nakagawa Y, Iinuma M, Nozawa Y. Anti-cancer effects of xanthones from pericarps of mangosteen. Int J Mol Sci 2008;9(3):355-70.
4. Kanchanapoom K, Kanchanapoom M. Mangosteen. In: Shaw PE, Chan HT Jr, Nagy S, editors. Tropical and Subtropical Fruits. Vol. 15.

Auburndale, Florida: AG Science, Inc.; 1998. p. 68-74.
5. Saito M, Ogino S, Kubo K, Nagata J, Takeuchi M. High dose of *Garcinia cambogia* is effective in suppressing fat accumulation in developing male Zucker obese rats, but highly toxic to the testis. Food Chem Toxicol 2005;43(3):411-9.
6. Gutierrez-Orozco F, Failla ML. Biological activities and bioavailability of mangosteen xanthones: A critical review of the current evidence. Nutrients 2013;5(8):3163-83.
7. Akao Y, Nakagawa Y, Iinuma M, Nozawa Y. Anti-cancer effects of xanthones from pericarps of mangosteen. Int J Mol Sci 2008;9(3):355-70.
8. Bindu AR, Rosemary J, Akhila S. Antimicrobial activity screening of *M. minuta* extracts. Int J Pharm Pharm Sci 2014;6:581-3.
9. Sunil C, Ignacimuthu S, Agastian P. Antidiabetic effect of *Symplocos cochinchinensis* (Lour.) S. Moore. In Type 2 diabetic rats. J Ethnopharmacol 2011;134:298-304.
10. Glorybal L, Kannana KB, Arasu MV, AL-Dhabi NA, Agastian P. Some biological activities of *Epaltes divarcata L.*-an *in vitro* study annals os clinical microbiology and antimicrobials. Ann Clin Microbiol Antimicrob 2015;14:1-14.
11. Nimal Christhudas IV, Praveen Kumar P, Sunil C, Vajravijayan S, Lakshmi Sundaram R, Jenifer Siril S, *et al. In vitro* studies on a-glucosidase inhibition, antioxidant and free radical scavenging activities of *Hedyotis biflora* L. Food Chem 2013;138(2-3):1689-95.
12. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolke RH. Manual of Clinical Microbiology. 6th ed. Washington, D.C: American Society for Microbiology; 1995.
13. Pinto E, Pina-Vaz C, Salgueiro L, José Gonçalves M, Costa-de-Oliveira S, Cavaleiro C, *et al*. Antifungal activity of the essential oil of *Thymus pulegioides* on Candida, *Aspergillus* and dermatophyte species. J Med Microbiol 2006;55:1367-73.
14. Pfaller MA, Messer SA, Coffmann S. Comparison of visual and spectrophotometric methods of MIC endpoint determinations by using broth microdilution methods to test five antifungal agents, including the new triazole D0870. J Clin Microbiol 1995;33(5):1094-7.
15. Wadood A, Ghufran M, Jamal SB, Naeem M, Khan A, Ghaffar R, *et al*. Phytochemical analysis of medicinal plants occurring in local area of Mardan. Biochem Anal Biochem 2013;2:1-4.
16. Hepsibah AH, Jothi GJ. A comparative study on the effect of solvents on the phytochemical profile and biological potential of *Ormocarpum cochinchinense* Auct. Non (Lour.) Merrill. Int J Pharm Pharm Sci 2017;9:67-72.
17. Slinkard K, Singleton VL. Total phenol analyses: Automation and comparison with manual methods. Am J Enol Viticult 1977;8:4955.
18. Hanato T, Kagawa H, Yasuhara T, Okuda T. Two new flavonoids and other constituents in licorice root: Their relative astringency and radical scavenging effects. Chem Pharm Bull (Tokyo) 1988;36:2090-7.
19. Elizabeth K, Rao MN. Oxygen scavenging activity of curcumin. Int J Pharm 1990;58:237-40.
20. Smirnoff N, Cumbes QJ. Hydroxyl radial scavenging activity of compatible solutes. Phytochemistry 1989;25:1057-60.
21. Krishnaiah D, Sarbatly R, Bono A. Phytochemical antioxidants for health and medicine: A move towards nature. Biotechnol Mol Biol Rev 2007;1:97-104.
22. Marxen K, Vanselow KH, Lippemeier S, Hintze R, Ruser A, Hansen UP. Determination of DPPH radical oxidation on caused by metabolic extracts of some microalgal species by linear regression analysis of spectrophotometric measurements. Sensors 2007;7:2080-95.
23. Aruoma OI. Free radicals, oxidative stress and antioxidants in human health and disease. J Am Oil Chem Soc 1998;75:199-212.
24. Steer P, Millgård J, Sarabi DM, Basu S, Vessby B, Kahan T, *et al.* Cardiac and vascular structure and function are related to lipid peroxidation and metabolism. Lipids 2002;37(3):231-6.
25. Arora S, Brits E, Kaur S, Kaur K, Sohi RS, Kumar S, *et al.* Evaluation of genotoxicity of medicinal plant extracts by the comet and VITOTOX tests. J Environ Pathol Toxicol Oncol 2005;24(3):193-200.
26. Inglis TJ. Microbiology and infection. A Clinically Oriented Core Text with Self-Assessment. New York: Churchill Livingstone, Long Man Singapore Publishers (Pte)Ltd; 1996.
27. Bannister BA, Begg NT, Gillespie SH. Infectious Disease. 2nd ed. Osney Mead, Oxford: Blackwell Science Ltd.; 2000.