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ANGIOTENSIN CONVERTING ENZYME INHIBITION, ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL EVALUATION OF *GARCINIA CAMBOGIA* SEEDS

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ABSTRACT

This study evaluated the antioxidant activity and angiotensin converting enzyme (ACE) inhibitory activities of seed extracts of *Garcinia cambogia*. Among the three extracts tested, methanol extract has shown significant DPPH radical scavenging activity with IC_{50} value of 15.99 µg mL^{-1}, total antioxidant activity (TAA) of 19.7 µg mL^{-1} and ferric reducing potential of 130.2 µg $mL^{-1.}$ Further, methanol extract has shown potential ACE inhibitory activity with IC_{50} value of 198.82 µg mL^{-1}. Gas chromatography-Mass spectrometry analysis was carried out to locate the possible active principles responsible for the ACE inhibition and antioxidant activity. GC-MS analysis revealed the presence of various phytochemicals such as phenolics, flavanoids, tannins and terpenoids. Results of *in vitro* studies of antioxidant and ACE-inhibitory activities suggest that *G. cambogia* seeds have strong antioxidant and ACE-inhibitory properties. Further, isolation and characterization of ACE-inhibitory molecules will be of greater importance.

Keywords: *G. cambogia*, Angiotensin converting enzyme, Antioxidant, Hypertension, GC-MS

INTRODUCTION

Hypertension is the the major public health concern worldwide because of its frequency and concurrent increase in the risk factor for atherosclerosis, myocardial infarction, stroke and renal diseases **[1, 2]**. In association with renin angiotensin system, angiotensin converting enzyme (ACE) plays a prominent role in regulation of blood pressure **[3]**. ACE is a dipeptidyl carboxypeptidase which releases the active angiotensin II enzyme, a potent vasoconstrictor by cleaving the C-terminal decapeptide from angiotensin I **[4]**. ACE also inactivates bradykinin, a vasodilator there by increasing blood pressure **[5]**. Therefore inhibition of ACE has been considered as a good rationale for treating hypertension **[6]**. However, inspite of their advantages commercially available ACE inhibitors have exhibited side effects includes skin rashes, cough, allergic reactions angioneurotic edema and taste disturbances **[7]**. Therefore there is an extremely large demand for ACE inhibitors from natural sources which having lesser side effects for regulating high blood pressure.

In addition, reactive oxygen species (ROS), free radicals as well as non free radical compounds are considered to play a crucial role in secondary complications of hypertension and many degenerative diseases including atherosclerosis, diabetes and cancer **[8]**. Antioxidants also prevents ROS mediated oxidative damage and lipid peroxidation. There are many synthetic antioxidants are available which are used mainly in food industry as food preservative and to increase shelf-life of food products. But these are associated with nutritional losses and quality deterioration. In addition they are also reported to exhibit toxic and mutagenic effects **[9]**.

Naturally the human system has the ability to overcome the consequences of ROS, based on the non-enzymatic antioxidant compounds and antioxidant enzymes. However these defence mechanisms are not enough to entirely prevent cellular damage. So there is need for dietary component containing antioxidants to reduce the oxidative stress in the human body **[10, 11]**. Medicinal plants provide an excellent source of phytoconstituents having potential ACE inhibitor activity and antioxidant activities. Phytochemical molecules from the medicinal plant extract can be a rich source for isolation of active molecule for drug discovery **[12]**.

Garcinia gummi-gutta (L) or Garcinia cambogia, a plant is commonly known as malabar tamarind mainly grows in Southeastern Asia, belongs to the family

Clusiaceae. This is a averaged sized tree grows up to 12 m tall with hanging branches. Different parts of *G. cambogia* known to contain different phytochemicals such as phenolics, alkaloids, flavanoids, saponins, tannins **[13]**. The epicarp of the fruit is usally used as a food-bulking agent, food preservative and traditionally it is used to treat rheumatism, oedema, constipation and intestinal parasites **[14, 15]**. Numerous scientific studies have also reported the hypolipidemic, anti-obesity, and anticancer activity **[16, 17]**. Hyroxy citric acid (HCA), which is a α-, β- dihydroxy tricarboxylic acid is the main compound responsible for the weight loss property present in the fruit rind **[18]**. The fruit epicarp is also used to cure bowel complaints and it is also used as hydragogue, anthelmintic, purgative and emetic. In addition to this, it is also employed in veterinary medicine to cure mouth infections in cattle **[19]**.

In this study, we have evaluated the antioxidant property and ACE inhibitory activity of the different solvent extracts of *G. cambogia* seeds.

MATERIALS AND METHODS

Chemicals

2, 2 diphenyl-1-picryl hydrazyl, Hippuryl histidyl leucine, Sepharose CL-4B, HEPES, Sephadex G 200, Nonidet P-40, 1,4-butanediol ether, dialysis membranes and lisinopril were procured from Sigma-Aldrich (St. Louis, MO, USA). Porcine kidney which is the source for angiotensin converting enzyme (ACE) was collected from the pork stall, Tumkur, India. All other chemicals and reagents were of analytical grade.

Plant seeds

G. cambogia seeds were procured from the city market and recognized by Dr. P. Sharanappa, Professor, Department of Studies & Research in Bioscience, Hemagangotri, University of Mysore, Hassan, Karnataka, India. Specific voucher specimens (TU16DOSRBC005) of the plant were deposited in the herbarium of the Department of Studies and Research in Botany, Tumkur University, Tumkur, Karnataka, India. The plant seeds were dried and powdered.

Extract preparation

The seeds extraction was carried out by extracting the powdered *G. cambogia* seeds using different solvents separately. The seeds powder (20 gm) was extracted using soxhlet apparatus with ethyl acetate (EA), acetone (AC) and methanol (ME) separately. The extracts were filtered and condensed under vaccum at 45°C. The extracts were stored in refrigerator until further use.

In vitro antioxidant activity

Total antioxidant activity

The assay was determined in accordance **[20]** with some modifications. Different concentrations of *G. cambogia* seed extracts (100-500 µg) were mixed with 1 mL reagent. The reaction mixture was incubated for 60 min at 95°C and after cooled, absorbance was recorded at 695 nm. Total antioxidant activity was expressed as Gallic acid equivalents (GAE).

FRAP (ferric reducing antioxidant power) Assay

The FRAP assay of *G. cambogia* seed extracts was determined according to **[21]** with slight modifications. Seed extracts of EA, AC, ME from varying concentrations 100-500 µg were added to 1 ml of reagent and incubated at for 30 min at 37° C. Absorbance monitored spectro-photometrically at 593 nm. The values were expressed as GAE.

2,2-Diphenyl-1-Picrylhydrazyl- (DPPH) assay

Radical scavenging ability of the *G. cambogia* seed extracts was performed according to **[22]** with slight modifications. In this method, varied concentrations of extracts (5-30 µg) were allowed to react with 1 mM DPPH solution. After 30 minutes of incubation at room temperature, absorbance was read at 517 nm against blank in UV-visible spectrophotometer. The percent of radical scavenging activity was calculated using the following formula:

$$\%\ \textbf{Radical scavenging activity} = \frac{\textbf{Absorbance of control} - \textbf{Absorbance of sample}}{\textbf{Absorbance of control}}$$

The gas chromatography-mass spectrometry (GC-MS) analysis

The (GC-MS) analysis of *G. cambogia* seed extracts (methanol, ethyl acetate and acetone) was performed using GC-MS (model; QP 2010 plus, Shimadzu, Japan). The temperature of the oven was computed from 75 to 312°C for 2°C min^{-1}. Ionisation of the sample was achieved through electron impact mode (EI, 70eV). Injector temperature was set to 280°C and detector to 220 °C. Helium was the carrier gas. The flow rate of the carrier gas was set to 1.21mL $min^{-1.}$ Compounds from mass range 40-60 m/z were scanned in the range of 3 scans/s. 2 µL of all the three plant seed extracts of *G. cambogia* were injected to GC-MS separately using Hamilton syringe in split injection technique. The phytoconstituents of the extracts were recognized by comparing their patterns of mass spectra and retention time with reference to chemstation software.

Purification of ACE

The porcine kidney was collected from a local slaughter house and the acetone

powder was prepared as described by **[23]**. The acetone powder was dissolved in phosphate buffer (pH 8.3), extracted on magnetic stirrer under ice cold condition, centrifuged at 12000 rpm for 45 min. Ammonium sulphate precipitation (20%) of the resulting supernatant was carried out on magnetic stirrer and centrifuged at 10000 rpm for 15 min. The pellets were dialyzed using 12 kDa cut off dialysis membrane and then lyophilized which serve as crude ACE. Then it is dissolved in 1 mL of HEPES buffer (pH 8.0), centrifuged for 5000 rpm for 10 minutes and the supernatant was loaded on to a column pre-equilibriated with Sephadex G-200 **[24]**. Protein elution was monitored at 280 nm using UV- visible spectrophotometer. The fractions were tested for ACE activity and then pooled and stored at -20°C. Further affinity chromatography was performed to purify the enzyme. Sephdex CL-4B resin was coupled with lisinopril and activated with epoxy was assembled and purification was implemented as described earlier **[24]**. Briefly, the peak II fraction having higher ACE activity was dissolved in equilibriation buffer, centrifuged and loaded onto the affinity column (1cm×22 cm). Protein was eluted by adding 10 µm/L lisinopril and 2mL fractions were collected, pooled and dialyzed.

SDS-PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed to visualize the protein bands in accordance with the method of **[25]**. Electrophoresis was performed for 2 h at 90 V at room temperature in a running buffer containing glycine (192 mM), Tris (25 mM) and SDS (0.1%). For detecting protein bands, the gel was stained with 0.25% Coomassie brilliant blue R-250.

ACE inhibition assay

The ACE activity was determined in accordance with the method of **[26]** with some modifications. Briefly, different concentrations of porcine kidney ACE were incubated in 350 µL reaction mixture of 50 mM HEPES buffer (pH 8.3) containing 300 mM Nacl and 8.3 mM of hippuryl histidyl leucine (HHL) for 30 minutes at 37 °C. 250 µL of 1 M HCl was added to terminate the reaction. To this, 1 mL of ethyl acetate was added to precipitate the hippuric acid. After centrifugation for 10 minutes at 3000 rpm, 0.5 mL of upper ethyl acetate layer was pippetted out and transferred to new series of test tubes and vacuum dried. Precipitated hippuric acid is dissolved in 1 mL of distilled water. Absorbance was monitored at 228 nm using UV-vis spectrophotometer. Specific activity is determined as nmoles of hippuric acid released/min/mg of protein at 37°C.

For inhibition studies, porcine kidney ACE (5mU) was incubated with varied concentrations of *G. cambogia* seed extracts (50-400 µg) in 350 µL reaction mixture of 50 mM HEPES buffer (pH 8.3) containing 300 mM Nacl for 30 minutes at 37 °C. Decrease in the concentration of hippuric acid released compared to test reaction is expressed as percentage of inhibition and is calculated using fallowing formula.

$$\% \textbf{ inhibition} = \frac{\textbf{Absorbance of control} - \textbf{Absorbance of sample}}{\textbf{Absorbance of control}}$$

RESULTS AND DISCUSSION

Medicinal plants contain many phytoconstituents which are responsible for the exhibited antioxidant property **[27]**. *G. cambogia* is used in the treatment of rheumatism, oedema, constipation, irregular menstruation and intestinal parasites. Solvent extracts of *G. cambogia* seeds (methanol, ethylacetate and acetone) were evaluated for antioxidant activities including DPPH, TAA, and FRAP, in order to understand the possible mechanisms responsible for antioxidant activities. The extracts showed antioxidant activities and the results have been tabulated in **Table 3, Figure 1**.

In TAA, Mo (VI) will reduced to Mo (V) by the antioxidants present in the sample and it leads to the formation of green colored complex at low pH. Among the three extracts tested, acetone extract has shown highest TAA of 21.2 µg mL^{-1} (expressed as µg of GAE mL^{-1} extract). TAA is increased gradually with the increasing concentration of extracts from 100-500 µg/ml. In the FRAP assay, antioxidants present in the seed extracts will donate the hydrogen atom there by breaking the free radical chain. Thus it acts as a important indicator of reduction potential **[28]**. The results conformed significant reduction potential of all the three extracts of *G. cambogia* seeds. Among them methanol extract has shown highest reducing potential of 92.63 µg mL^{-1}, which was gradually increased with the increasing concentration measured in terms of GAE µg mL^{-1} extract. As antioxidant activity is directly related to reducing potential, FRAP assay contribute a definitive method for determining the antioxidant activity of different extracts **[21]**. The results are tabulated in **Table 4**.

DPPH assay is based on the reduction of DPPH radical by the antioxidant by donating hydrogen atoms, is frequently used method for determining the antioxidant potential of plant seeds extracts

[29]. *G cambogia* seed extracts have significantly scavenge the DPPH radicals, as shown in **Figure 1** in a dose dependent manner. Methanol extract has shown significant radical scavenging activity and its percentage of scavenging reached 83.05% at 30 µg/mL of concentration (IC_{50} value 15.99 µg/mL) followed by acetone extract.

Purification of angiotensin converting enzyme was carried out for screening the ACE inhibitory activities of *G. cambogia* seeds. Acetone powder from the porcine kidney was prepared and the crude ACE was subjected to gradients of ammonium sulphate precipitation, and 20% precipitate fraction having higher ACE activity was subjected to fractionation on sephadex G-200 column chromatography, fractionation has shown two major peaks and the peak II showed maximum ACE activity. Eventually, ACE purification was accomplished by fractionating peak II on affinity column. The homogeneity of the protein elute from affinity column was established through SDS-PAGE **(Figure 2)**. All the three solvent extracts of *G. cambogia* seeds were assessed for their ACE-inhibitory activity. *G cambogia* seed extracts showed dose dependent ACE inhibition. Among the extracts evaluated, methanol extract showed highest inhibition followed by ethylacetate and acetone. ME extract has shown significant inhibition with IC_{50} value of 198.82 µg mL^{-1} followed by ethyl acetate extract (IC_{50} value of 268.60 µg mL^{-1}) and acetone extract (IC_{50} 357.55 µg mL^{-1}). From the clinical studies it is confirmed that ACE inhibitors could effectively act as antihypertensive compounds and hence play a crucial role in regulating high blood pressure. However, in spite of their beneficial effects, synthetic drugs have many side effects cough, skin rashes, taste disturbances, angioneurotic edema, and allergic reactions. Therefore across the globe, researchers and scientists have shown interest in developing new ACE inhibitors from natural sources **(Figure 3)**.

GC-MS analysis was determined to reveal the possible active molecules responsible for ACE-inhibitory activity and antioxidant activity in *G. cambogia* seed extracts. GC-MS analysis has shown varied distribution of phenolics, terpenoids, flavanoids, glycosides and steroids in methanol, acetone and ethyl acetate extracts *of G. cambogia* seeds. The retention time and peak area percentage of various bioactive principles was shown in **Table 1-3, Figure 4, 5**. In methanol, acetone, ethyl acetate extracts, twenty five, twenty nine, twenty two compounds respectively were detected from the GC-MS analysis of the *G. cambogia* seeds. The different

pharmacological properties associated with these molecules have been tabulated in the **Table 5**.

In these phytoconstituents, ACE inhibition was greatly correlated to phenolics and it is reported that phenolics and flavanoids are excellent radical scavengers **[30]**.

Preliminary studies reveled that, antihypertensive properties of the most of the plants is mainly because of ACE inhibition. Different types of active principles with ACE-inhibition properties have been extracted from plants including alkaloids, flavanoids, phenolics, glycosides, terpenes, xanthones, tannins, and peptides/proteins **[31, 32]**. Even though extensive work has done on medicinal plants, till date, there is no information available on ACE-inhibitory activities of *G. cambogia* seeds **(Table 5)**.

Table 1: Phytocomponents identified by GC-MS in methanol extract of *G. cambogia* seeds

S. No	R.T	Compound name	Molecular formula	MW	Peak area %	Activity
1.	5.28	n-Tetradecane	198	$C_{14}H_{30}$	0.06	Anaphylactic, antibacterial, antitumour, inhibit production of tumour necrosis factor, NADH oxidase inhibitor
2.	7.14	2,5,9-Trimethyldecane,	184	$C_{13}H_{28}$	3.00	
3.	7.79	Adipic acid,	370	$C_{22}H_{42}O_4$	0.81	Arachidonic acid inhibitor
4.	9.56	tert-Butyl 3á-acetoxy-12,21-dioxo-23E-phenylthio-5b,20E cholan-24-oate	610	$C_{36}H_{50}O_6S$	0.04	Anticancer, Cytochrome P450 inhibitor
5.	10.95	Imidazole-2-carboxaldehyde, 1-methyl-	110	$C_5H_6N_2O$	0.07	2-Lipoxygenase inhibitor, 5-Alpha-Reductase-Inhibitor,
6.	12.25	4',6'-Dihydroxy-2',3'-dimethylacetophenone	180	C10H12O3	4.45	17-beta hydroxysteroid dehydrogenase-inhibitor,5-HETE-Inhibitor,5-hydroxytryptamine inhibitor,
7.	12.9	3-Benzoyl-3-benzyl-2,4(1H,3H)-quinolinedione	355	$C_{23}H_{17}NO_3$	0.76	17- Beta hydroxysteroid dehydrogenase inhibitor, hematopoetic
8.	13.1	2,4,6-Tritert-butyl-4-methyl-2,5-cyclohexadien-1-one #	276	$C_{19}H_{32}O$	0.75	Catechol-O-Methyl-Transferase-Inhibitor, Methyl-Guanidine-Inhibitor
9.	15.57	2,4-Ditert-butylphenyl 5-hydroxypentanoate	306	$C_{19}H_{30}0_3$	5.60	
10.	16.06	5,8-Diethyldodecane	226	$C_{16}H_{34}$	0.30	Antioxidant, Antimicrobial
11.	16.57	Phthalic acid	372	$C_{23}H_{32}O_4$	4.55	Arachidonic acid inhibitor, acidifier
12.	18.48	Oxalic acid	272	$C_{15}H_{28}O_4$	2.48	Arachidonic acid inhibitor, acidifier
13.	20.66	Biphenyl-, 2,2',5,5'-tetramethyl-	210	$C_{16}H_{18}$	2.96	
14.	21.19	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	234	$C_{15}H_{22}O_2$	4.03	Anti-inflammatory, Analgesic
15.	21.87	Propionitrile, 3-(5-methyl-3-phenyl-1-pyrazolyl)-3-oxo-	225	$C_{13}H_{11}N_3O$	4.04	Catechol-O-Methyl-Transferase inhibitor.
16.	23.11	Heptadecane,	296	$C_{21}H_{44}$	14.49	Antimicrobial activity
17.	23.8	1,2-Benzenedicarboxylic acid, bis(2 methylpropyl) ester	278	$C_{16}H_{22}O_4$	0.90	Antibacterial, Arachidonic acid inhibitor
18.	24.11	Sulfurous acid,	320	$C_{17}H_{36}O_3S$	2.01	Arachidonic acid inhibitor
19.	25.13	(3E)-3-Icosene	280	$C_{20}H_{40}$	22.48	Anticancer, antitumour
20.	26.19	2,6,10-Trimethyltetradecane	240	$C_{17}H_{36}$	6.93	Allelopathic, Antibacterial
21.	26.92	(17E)-17-Pentatriacontene #	490	$C_{35}H_{70}$	3.17	Antioxidant, Anticancer
22.	27.79	Methyl (10E)-10-octadecenoate	296	$C_{19}H_{36}O_2$	1.60	Anticancer, edematogenic, elastase inhibitor, antidiaareal, antiprolifarative
23.	28.06	(10E)-10-Henicosene	294	$C_{21}H_{42,}$	3.08	Antiasthmatics ,urine acidifiers Antimicrobial
24.	30.69	Phthalic acid	362	$C_{22}H_{34}O_4$	8.12	Arachidonic acid inhibitor
25.	33.07	Tributyl acetylcitrate	402	$C_{20}H_{34}O_8$	3.21	

Table 2: Phytocomponents identified by GC-MS in ethyl acetate seed extract of *G. cambogia* seeds

S. No	R.T	Compound name	Molecular formula	MW	Peak area %	Activity
1.	19.36	1,4-Dimethyl-3-n-octadecylcyclohexane	$C_{26}H_{52}$	364	0.07	Anticancer, anaphylactic, NADH oxidase inhibitortable
2.	19.71	Spiro(cyclohexanol-5,6'-piperidine), 6-butyl-	$C_{14}H_{27}NO$,	225	0.24	Spirocheticide
3.	19.89	4-Ethylundecane	$C_{13}H_{28,}$	184	0.24	
4.	20.28	Squalene	$C_{30}H_{50}$		0.05	Monooxygenase inhibitor
5.	22.09	10,12-dihydroxy-1,3,7,8-tetramethyl-	$C_{18}H_{28}0_3$	292	5.00	
6.	23.78	Hexadecen-1-ol	$C_{16}H_{32}O$,	240	1.49	Oligo saccharide provider
7.	24.39	Phthalic acid	$C22H_{34}O_4$,	362	0.35	Arachidonic acid inhibitor, Increases aromatic aminoacid decarboxylase activity
8.	24.6	Lupeol	$C_{30}H_{50}O$	426	0.45	Antioxidant, antiinflammatory, antimicrobial, antitumour
9.	25.6	Cyclohexadecane	$C_{16}H_{32}o2$	224	1.77	Antioxidant, hypocholesterolemic, nematicide, anti-androgenic, hemolytic, Lubricant, 5-Alpha reductase inhibitor, antipsychotic
10.	27.8	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	5.37	Anticarcinogenic, alkaline phosphatase inhibitor
11.	28.08	Stearic acid	$C_{18}H_{36}O_2$	284	0.68	Antibacterial, antioxidant
12.	28.71	13-Octadecenoic acid,	$C_{19}H_{36}O2$	296	4.42	Anticancer,anti-inflammatory, hypocholesterolemic, 5-alpha reductase inhibitor, anemiagenic, insectifuge,
13.	29.16	Lup-20(30)-en-3-one	$C_{30}H_{48}O$,	424	21.02	Antimicrobial, antioxidant, antiprotozoal, anti-inflammatory, antitumour
14.	30.63	2-Ethyl-1',8-dimethyl-5,6,7,8-tetrahydro-spiro[1,3,2-benzodioxaborin-4, 1'-cyclohexane]	$C_{16}H_{27}BO_2$	262	22.35	
15.	35.25	Acetic acid, 4-acetoxy-3-(3,7,11-trimethyl-dodeca-2,6,10-trienyl)-phenyl ester	$C_{25}H_{34}0_4$	398	1.26	Arachdonic acid inhibitor
16.	35.52	4,9,13,17-Tetramethyl-4,8,12,16-octadecatetraenal	$C_{22}H_{36}O$,	316	1.01	
17.	35.86	Dihydrosqualene	$C_{30}H_{52}$,	412	2.24	Antibacterial
18.	36.9	9,19-Cyclolanostan-3-ol, 24,24-epoxymethano-, acetate	$C_{33}H_{54}O_3$,	498,	7.22	Provides oligosaccharides
19.	37.12	3-Thioacetoxycholest-4-one	$C_{29}H_{48}OS$,	444	1.10	
20.	37.19	Friedelin	$C_{30}H_{50}O$,	426	4.11	
21.	37.78	Cyclohexanone, 2,4-diacetyl-5-hydroxy-3-(4-hydroxy-3-methoxyphenyl)-5-methyl-	$C_{18}H_{22}O6$	334	1.31	Testosterone hydroxylase inhibitor, aryl hydrocarbon hydroxylase inhibitor, 17 beta hydroxyl steroid dehydrogenase inhibitor
22.	38.11	Acetic acid, 4-acetoxy-3-(3,7,11-trimethyl-dodeca-2,6,10-trienyl)-phenyl ester	$C_{25}H_{34}04$	398	5.36	Arachidonic acid inhibitor
23.	38.49	Baccharane	$C_{30}H_{54}$,	414	1.97	Antioxidant, anti-inflammatory
24.	38.78	5-Cholestene-3beta,7beta-diol 3-benzoate	$C_{34}H_{50}O_{3,}$	506	2.02	17 beta hydroxylsteroid dehydrogenase inhibitor, beta glucuronidase inhibitor.
25.	39.65	Propanoic acid,	$C_{27}H_{42}O_4$	430	0.95	Arachidonic acid inhibitor
26.	40.04	Unknown	$C_{36}H_{54}OS$	534	3.14	
27.	40.49	5-Chloro-6beta-nitro-5alpha-cholestan-3-one	$C_{27}H_{44}ClNO_3$,	465	1.08	alpha reductase inhibitor, Alpha glusidase inhibitor, testosterone 5 alpha reductase inhibitor,
28.		17á-Acetoxy-1',1'-dicarboethoxy-1á,2á-dihydrocycloprop[1,2]-5à-androst-1-en-3-one	$C_{28}H_{40}O_7$	488	0.39	
29.	41.65			364	3.82	

Table 3: Phytocomponents identified by GC MS analysis in acetone extract of *G. cambogia*

S. No	R.T	Compound name	Molecular formula	MW	Peak area %	Activity
1.	2.35	4"-Dehydroxy-2",3',3",4',5,6",7 hepta-O-methylisoorientin	$C_{28}H_{34}O_{11}$	546	26.98	Anticancer, antitumour, aldehyde oxidase inhibitor, NADH oxidase inhibitor, inhibit production of tumour necrosis factor
2.	4.96	2-(2-Furyl)-2,5-dimethyltetrahydrofuran	$C_{10}H_{14}O_2$	166	0.05	
3.	5.73	N,N-Diethyl-3-buten-1-amine	$C_8H_{17}N$	127	0.02	Anaphylactic, antitumour, increase natural killer cell activity
4.	6.77	Acetonyldimethylcarbinol	$C_6H_{12}O_2$	116	0.04	
5.	8.87	1,2,4,5-Tetrazine, 3,6-dimethyl	$C_4H_6N_4$	110	0.17	Allelopathic, Antibacterial
6.	10.66	1,2-Benzenediol, mono(methylcarbamate)	$C_8H_9NO_3$	167	0.26	Antioxidant activity, Anticancer
7.	16.08	1,2-Benzenediol, o-propoxycarbonyl	$C_{10}H_{12}O_4$	196	0.06	Anticancer, edematogenic, elastase inhibitor, Antidiaareal, Antiprolifarative
8.	17.91	5-Methyldodecane	$C_{13}H_{28}$	186	0.07	
9.	19.91	6-Methyl-7-oxo-1,7-dihydro-triazolo(4,3-b)triazine	$C_5H_5N_5O$	151	0.05	Anticancer, cytochrome P450 inhibitor
10	22.11	All-trans-Squalene	$C_{30}H_50$	410	2.86	Monooxygenase inhibitor
11	23.29	Phthalic acid, butyl nonyl ester	$C_{21}H_{32}O_4$	348	0.31	Arachidonic acid inhibitor, increases aromatic aminoacid decarboxylase activity
12	23.82	1-(4-Bromobutyl)-2-piperidinone	$C_9H_{16}BrNO$	233	0.38	Antimicrobial
13	25.27	1,4-Dimethyl-3-n-octadecylcyclohexane	$C_{26}H_{52}$,	364	1.42	Anaphylactic, antitumour
14	26.39	Phthalic acid	$C_{17}H_{24}O_4$,	292	0.10	Arachidonic acid inhibitor, increase aromatic aminoacid decarboxylase activity
15	27.8	(9E)-9-Hexadecen-1-ol	$C_{16}H_{32}O$	240	1.61	Anticancer, antitumour, cytochrome P450 inhibitor
16	28.73	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$,	282	10.261	Arachidonic acid inhibitor, increases aromatic aminoacid decarboxylase activity
17	29.18	á-Estradiol, 3-(tert-butyldimethylsilyl) ether	$C_{24}H_{38}O_2S$	386	10.62	
18	30.65	Octadecanoic acid	$C_{18}H_{36}O_2$	284	14.32	Arachidonic acid inhibitor, increases aromatic aminoacid decarboxylase activity
19	34.38	2-(3,7-Dimethyl-octa-2,6-dienyl)-4-methoxy-phenol	$C_{17}H_{24}O_2$	260	23.00	Lipoxygenase inhibitor, ACE inhibitor, Acetyl choline esterase inhibitor, alcohol dehydrogenase inhibitor.
20	36.9	Lup-20(29)-en-3-one	$C_{30}H_{48}O$,	424	3.10	Antimicrobial, antioxidant, antiprotozoal, anti-inflammatory, antitumour
21	38.11	Acetic acid, 4-acetoxy-3-(3,7,11-trimethyl-dodeca-2,6,10-trienyl)-phenyl ester	$C_{25}H_{34}O_4$	398	2.92	Arachidonic acid inhibitor
22	39.8	Dihydrosqualene	$C_{30}H_{52}$,	412	1.27	Antibacterial

Table 4: Total Antioxidant ctivities (TAA) and FRAP of *G. cambogia* seeds extracts. Gallic acid equivalents (GAE) mg/ml of extract

Concentration of extract (µg)	Total antioxidant activity (GAE)			Ferric reducing antioxidant power (GAE)		
	Methanol	Ethyl acetate	Acetone	Methanol	Ethyl acetate	Acetone
100	4.81	4.81	5.78	27.50	14.05	34.22
200	7.75	6.91	11.36	41.64	17.84	41.91
300	10.69	9.43	16.83	47.25	22.88	68.26
400	14.47	11.66	19.1	77.92	27.92	76.66
500	17	12.37	21.2	92.63	36.32	80

Table 5: ACE inhibition and antioxidant activity of *G. cambogia* seed extracts, IC_{50} µg/ml

Extracts	Antioxidant activity (DPPH)	ACE inhibition
Methanol	15.991	198.82
Ethyl acetate	20.136	268.60
Acetone	23.34	357.55

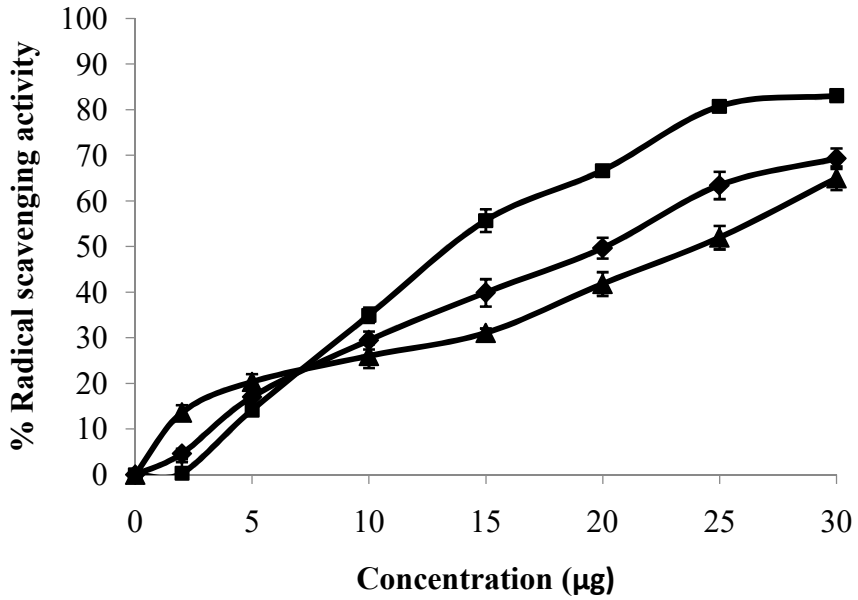
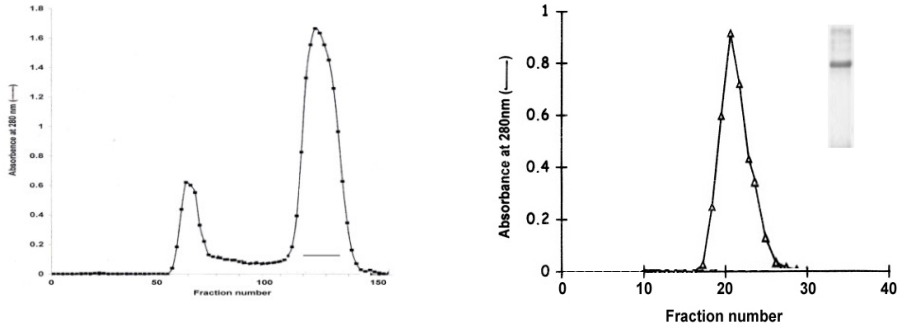


Figure 1: Antioxidant activity of *G. cambogia* seed extracts. DPPH was incubated with different concentrations of ME [♦], AC [●], EA [▲] *G. cambogia* seed extracts at room temperature for 30 min. Values are expressed as average of ± SD of three independent experiments



A. **B.**

Figure 2: ACE purification

A: Sephadex G-200 gel filtration of porcine kidney ACE. Crude ACE obtained after ammonium sulphate precipitation was loaded on to the column of Sephadex G 200. 2mL fractions were collected and tested for ACE activity. Active fractions were pooled and stored at -20°C.

B: Affinity column chromatography: The fraction having higher ACE activity (peak II fraction of gel filtration chromatography) was loaded onto affinity column. Elution of the enzyme was achieved by adding 10 µmol/L lisinopril and the flow rate was adjusted to10mL/h

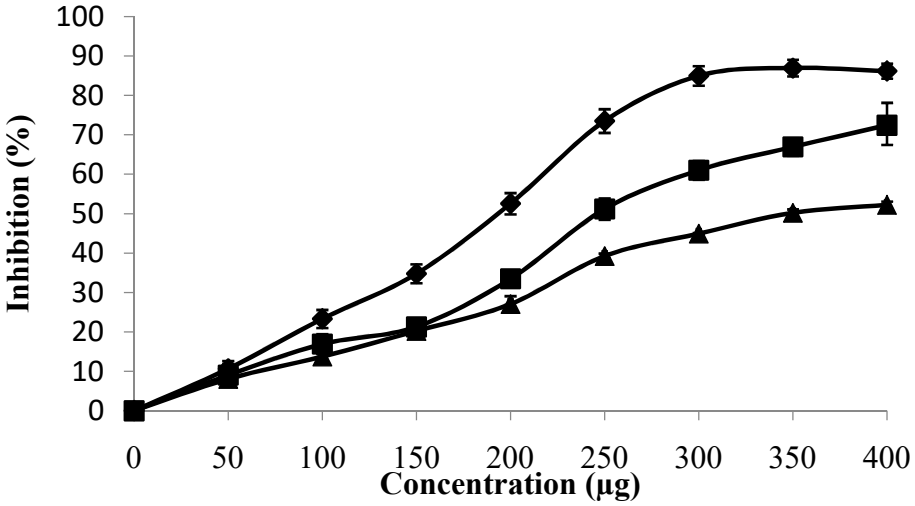


Figure 3: ACE inhibition of *G. cambogia* seed extracts
ACE from the porcine kidney (5 IU µL^{-1}) was pre-incubated with various concentrations of methanol [♦], acetone [●], ethyl acetate [▲], *G. cambogia* seed extracts. HHL was treated with the reaction mixture and incubated at 37°C for 40 min. Absorbance of hippuric acid precipitated was monitored spectrophotometrically at 228 nm. Values are mean ± SD of triplicate determinations

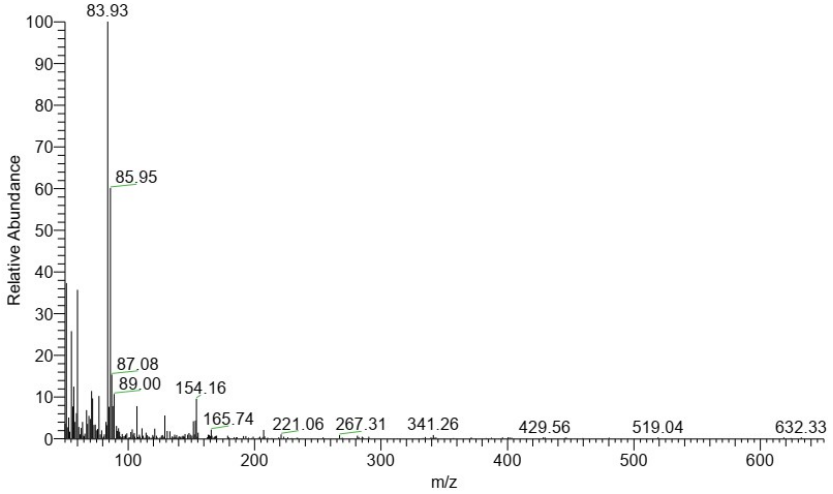


Figure 4: GC-MS analysis of methanolic extract of *G. cambogia* seed extracts

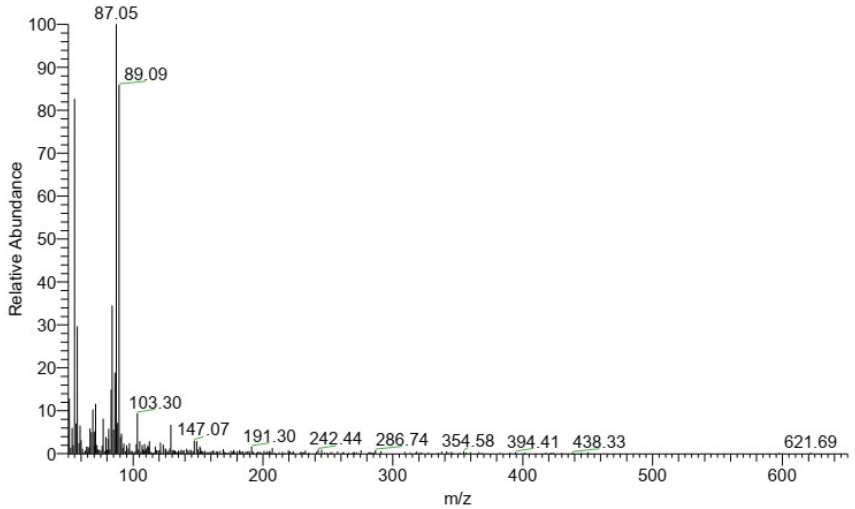


Figure 5: GC-MS analysis of ethyl acetate extracts of *G. cambogia* seeds

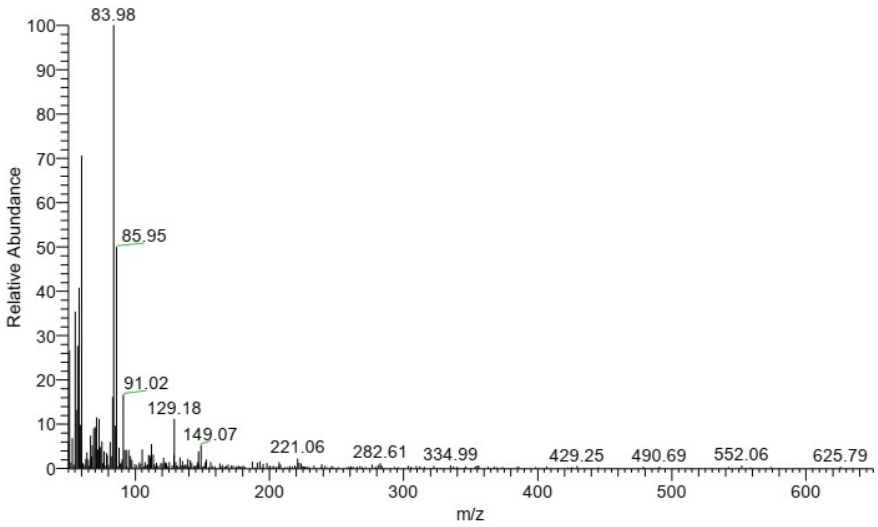


Figure 6: GC-MS analysis of acetone extracts of *G. cambogia* seeds

Conflicts of interest

The authors have no conflicts to disclose.

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