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Research Article

Biochemistry for better diagnosis and therapy

Insights into the *Invitro* Antioxidant, Anti-Inflammatory and Anticancer Activities of *Limonia acidissima* Fruits

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Abstract: Wood apple botanically identified as *Limonia acidissima* is an indigenous fruit with amazing nutritional and health benefits. Reports from traditional literature of Ayurveda and Siddha portrays the medicinal properties of this fruit. The scooped pulp of the ripe fruit is consumed as such or it can be made into different recipes. But inclusion of this fruit in our diet is not found as a regular practice. Many people are still unaware of the benefits of this fruit. Hence, this study was taken up to unravel the biological potencies of this fruit by conducting *in vitro* experiments. Phytochemicals such as alkaloids, flavonoids, phenols, saponins and ascorbic acid have been estimated. Anti-inflammatory activity of the aqueous extract of fruit pulp combined with outer rind has been evaluated through inhibition of albumin denaturation. Among the 5 different concentrations (200, 400, 600, 800, 1000 µg/ml), at 1000 µg/ml wood apple has shown 74.55% of protein denaturation inhibition which was compared with standard Diclofenac sodium. Antioxidant capacity of the extract was expressed as mg/100g ascorbic acid equivalent through phosphomolybdenum assay. Dose dependent increase in the antioxidant activity was observed. About 8 different concentrations of the aqueous extract of *L. acidissima* were evaluated for their cytotoxic activity on MCF 7 cell line. At a concentration of 1000 µg/ml, the extract has shown 93.43% of cytotoxicity and 6.57% of cell viability. Apoptotic induction was evaluated and confirmed by the formation of DNA ladders through DNA fragmentation assay. GCMS analysis of wood apple fruit pulp and rind revealed the presence of several phytochemicals among which many of them had therapeutic activity reported earlier.

Keywords: Anti-inflammatory, Antioxidant, *Limonia acidissima*, MCF 7 cell line, Anti-cancer, Wood apple

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1. INTRODUCTION

Edible fruits play a significant role in nutrient supplementary diet as well as in herbal medicine. They are the rich sources of vitamins, minerals, polyphenols, fibers and antioxidants which provide various health benefits. In Ayurveda and siddha medicines, several edible fruits are used as medicines for various health disorders. *Limonia acidissima* Linn, syn. *Feronia limonia* (Rutaceae) belongs to the monotypic genus *Limonia*, is a moderate-sized deciduous tree confined to India, Pakistan, Sri Lanka and Southeast Asia.[1] It is also known as wood apple, elephant-apple, monkey fruit, curd fruit, kath bel and kaitha. The fruit is berry, round to oval, globose, large, 2 to 5-inch-wide, with a hard, woody rind, which is greyish-white, scurfy rind about 6 mm thick. The pulp is sticky brown, aromatic odorous, resinous, astringent, acid or sweetish, white seeds scattered through it. [2] There are 2 forms, one with large, sweet fruits and the other with small, acid fruits. Wood apple fruit can be eaten plain or mixed into a variety of beverages and desserts, or preserved as jam. The scooped-out sticky pulp is eaten raw with or without jaggery, or is blended with coconut milk and palm-sugar syrup and frozen as an ice cream. The pulp is blended with curd and taken to treat ulcers. In Indonesia, wood apple is mixed with honey and eaten for breakfast. In Thailand, leaves are eaten in salads while in India the pulp is used in savoury chutneys. The wood serves as fuel.[2] In Tamil Nadu, people prepare an appetizing soupy Ras in their regular cuisine using either pulp or the outer rind of wood apple. About 100g of wood apple pulp contains 140kcal. The fruit contains carbohydrates and proteins. It is also rich in beta carotene, vitamin C, thiamine and riboflavin. The fruits are used as a substitute for *Aegle marmelos* in diarrhea and dysentery.[3-4] The bark and leaves of the plant are used for vitiated conditions of vata and pita while the fruits are used for treating tumours, asthma, wounds, cardiac debility and hepatitis. The fruit contains flavonoids, glycosides, saponins and tannins.[5] Some coumarins[5-6] and tyramine derivatives[3] have also been isolated from the fruits of *Limonia*. The leaves are reported to possess hepatoprotective activity[7] while the fruit shells contain antifungal compounds, namely, psoralene, xanthotoxin, 2, 6-dimethoxybenzoquinone and osthenol.[8] Studies carried out by researchers across the globe on different parts of this plant reveal antidiabetic,[9-11] antioxidant,[12-15] hepatoprotective,[3] wound healing,[3] diuretic,[16] anti-bacterial [17] and anti-cancer [18-19] activities. Chronic inflammation and oxidative stress are the major contributors to cause health implications. Hence, anti-inflammatory and antioxidant properties of medicinal plants make them suitable to treat several ailments through their inherent pharmacological potencies of accumulated phytochemicals. In this view the present study was designed to evaluate the anti-inflammatory, antioxidant and anti-cancer properties of aqueous extract of wood apple using in vitro experiments. Earlier studies on the anti-breast cancer activity of *L. acidissima* fruits was carried out using alcoholic extracts or using pulp alone.[19-21] This prompted us to carry out this study using aqueous extract of *L. acidissima* fruit pulp along with outer rind to evaluate the biological potentials of this traditional indigenous fruit.

2. MATERIALS AND METHODS

2.1 *Collection and Authentication of the Plant*

The Fruit of *Limonia acidissima*. L was collected from Raja Street Market, Coimbatore, Tamil Nadu. The selected plant *L. acidissima.* L and fruits were authenticated by Dr. V. Sampath Kumar, Scientist 'D'-in-charge, Botanical Survey of India, Southern Regional Centre, Coimbatore (BSI/SRC/5/23/2019/Tech/434).

2.2 *Preparation of aqueous extract*

The collected fruits were washed and air dried. Fruits are broken and the irregular pieces of outer hard rind, sticky brown pulp and small white seeds everything was dried under shade. These dried materials were mechanically powdered. The coarse powder was boiled with desired volume of distilled water in low flame and filtered. The filtrate was lyophilized and it was used for further analysis.

2.3 *Phytochemical analysis*

2.3.1 *Estimation of Crude Alkaloids*

Weighed 2.5 g of the sample in a 250 ml beaker and added 100 ml of 10 percent ethanol acetic acid. It was covered and allowed to stand for 4 hours. This was diluted, and the extract condensed to one-quarter of the original volume in a water bath. Concentrated ammonium hydroxide was added to the extract in a drop wise manner until the precipitation was complete. The whole solution was allowed to settle, and then the precipitate was collected, washed and filtered again with dilute ammonium hydroxide. The resultant alkaloid was dried and weighed. weighed and dried.[22]

2.3.2 *Estimation of Total Phenols*

Total phenols were estimated using Folin Ciocalteu method.[23] An extract aliquot (100 µl) was mixed with 250 µl of Folin Ciocalteu reagent and allowed to stand for 5 min at room temperature. Added to the mixture sodium bicarbonate (20 %, 1.5 ml) and incubated for 120 min at room temperature. Absorbance was measured spectrophotometrically at 765 nm. A standard curve was plotted using different concentrations of standard gallic acid and the total phenols were measured as gallic acid equivalents in µg/mg of dried extract.

2.3.3 *Estimation of Total Flavonoids*

Total flavonoid content was measured with the aluminium chloride colorimetric assay. About1ml of extract was taken in a test tube and added 4ml of distilled water and 0.3 ml of 5 % sodium nitrite solution. After 5 minutes, 0.3 ml of 10 % aluminium chloride was added. At the 6th minute, 2 ml of 1 M sodium hydroxide was added. Finally, volume was made up to 10 ml with distilled water and mixed well. Orange yellowish color was developed. The absorbance was measured at 510 nm. Distilled water was taken as blank. Quercetin was used as standard. The calibration curve was plotted using standard quercetin. The results were expressed in µg/ mg of the dried extract as Quercetin equivalents (QE). [24]

2.3.4 *Estimation of Saponins*

5 g of plant powder was taken in a conical flask with 50 ml of 20 % aqueous ethanol. The sample was heated in a hot water bath for 4 h with continuous stirring at about 55 °C. It was filtered and the residue obtained was re-extracted with another 50 ml of 20 % ethanol. The combined extracts were reduced to 10 ml over water bath at about 90 °C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was

recovered while the ether layer was discarded. The purification process was repeated. 15 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in an oven to a constant weight and the saponin content was calculated as percentage.[25]

2.3.5 *Estimation of Ascorbic acid*

Ascorbic acid was estimated using DNPH method.[26] Ascorbic acid is oxidized to dihydro ascorbic acid which then reacts with 2,4 dinitrophenyl hydrazine to form oxazole crystals and the solution is treated with sulphuric acid to give orange red color measured at 520 nm. The extract was taken in a test tube and added with 3ml distilled water. 0.5 ml of DNPH reagent was added. A blank was set. The contents were mixed and incubated for 3 h. After incubation the orange red color oxazole crystals were dissolved by adding 1.5 ml of ice-cold 65% sulphuric acid and were allowed to stand at room temperature for 30 min. The colored solution was measured colorimetrically at 520 nm. Graph was drawn with standard ascorbic acid. From the graph, the amount of ascorbic acid present in the extract was calculated.

2.4 *Determination of total antioxidant activity*

2.4.1 *Phosphomolybdenum assay*

This method is based on the reduction of phosphomolybdic acid to phosphomolybdenum blue complex by sodium sulfide. The obtained phosphomolybdenum blue complex is oxidized by the addition of nitrite and this causes a reduction in intensity of the blue color. Different aliquots of aqueous extract of wood apple and standard ascorbic acid were taken. 1ml of reagent solution was added to it and incubated in a boiling water bath at 95 °C for 90 min. After 90 min, the absorbance of the solution was read at 695 nm. The Phosphomolybdenum reduction potential (PRP) of the studied extracts were reported in percentage.[27]

2.5 *Determination of anti-inflammatory activity*

2.5.1 *Albumin Denaturation Assay*

The protein denaturation assay was conducted by taking 50 µl of different concentrations of water extract of wood apple with 450 µl of 5 % bovine serum albumin (BSA). The pH of the reaction mixture was adjusted to 6.3 using 1 N HCl and vortexed and then incubated at 37 ^{0}C for 20 min. The mixture was further incubated at 57 ^{0}C for 3 min and thereafter allowed to cool to room temperature. Then 2.5 ml of phosphate buffered saline (pH 6.3) was added to each tube and the turbidity was measured at 660 nm. Distilled water was added instead of extract in blank whereas for control water was used instead of BSA.[28] The percentage of inhibition of protein denaturation was calculated using the formula:

Inhibition of protein denaturation (%) = (100 – (Abs. Test - Abs. Control)/Abs.

2.6 *Determination of anticancer activity*

2.6.1 *MTT assay*

MCF 7 cell line was obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in Dulbecco's modified Eagle's medium supplemented (DMEM) supplemented with 10% Foetal bovine serum (FBS), penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37 °C. Initially the cells were washed with PBS for two times, centrifuged and the cell Pellet was collected and re-suspended in DMEM medium and incubated at 37° C for 2 days in a CO_2 incubator. The anticancer effect of compounds was analyzed by treating with human liver cancer cells for 24 h and the cell viability will be checked using MTT assay.[29] After arriving 60% of confluency, the cells were trypsinized and dispersed in 96 well plate with a cell count of 9000 cells per well and incubated for 24 h. Then the compounds were added at different concentrations and then again incubated for 24 h. At the end, the medium was discarded, cells were washed with PBS and 20 microliters of MTT reagent was added in each well and incubated for 6 h at 37°C in a water bath. Then 150 microliters of acidic isopropanol was added and shaken for 30 min on a plate shaker under dark. The absorbance was measured at 540 nm and the percentage of cell viability was calculated and from that the anticancer effect was derived.

2.7 *DNA Fragmentation assay*

MCF-7 cells were plated in 6 well plates and kept in a CO_2 incubator to attain confluence. Sample was added into the well and incubated for 24 hrs. After this, cells were harvested using TPVG and 1.5 ml of cell suspension was dispensed in Eppendorf. Cells were centrifuged at 200xg at 4 ^{0}C for 10 min. 0.5 ml of TTE solution was added to the pellet and vortexed vigorously. This procedure allows the release of fragmented chromatin from nuclei, after cell lysis (due to the presence of Triton X- 100 in the TTE solution) and disruption of the nuclear structure (following Mg2+ chelation by EDTA in the TTE Solution). To separate fragmented DNA from intact chromatin, tubes were centrifuged at 20,000xg for 10 min at 4 ^{0}C. The supernatant was carefully removed and added 500 µl of TTE solution into the pellet and 500 µl of Ice-cold NaCl and vortexed vigorously. The addition of the salt is to remove histones from DNA. Added 700 µl of ice-cold isopropanol and vortexed vigorously. Allowed to precipitate overnight at -20 ^{0}C. After precipitation, DNA was recovered by pelleting for 10 min at 20,000x g at 4 ^{0}C. Pellets were rinsed by adding 500-700µl of ice-cold 70% ethanol. Tubes were centrifuged at 20,000x g for 10 min at 4 ^{0}C. DNA was dissolved by adding to each tube 20-50 µl of TE solution and placed the tubes at 4 ^{0}C. The samples of DNA were mixed with a loading buffer by adding a 10x loading buffer to a final concentration of 1X. The addition of a loading buffer to samples allows us to load in wells more easily and to monitor the run of samples. The electrophoresis was run in a standard TE buffer after setting the voltage to the desired level. During electrophoresis it is possible to monitor the migration of samples by following the migration of bromophenol blue dye contained in the loading dye. The electrophoresis was stopped when the dye reached about 3

cm from the end of the gel. DNA was visualized by placing the gel on a UV Transilluminator.[30]

2.8 *Analytical Standardization*

2.8.1 *GC-MS analysis*

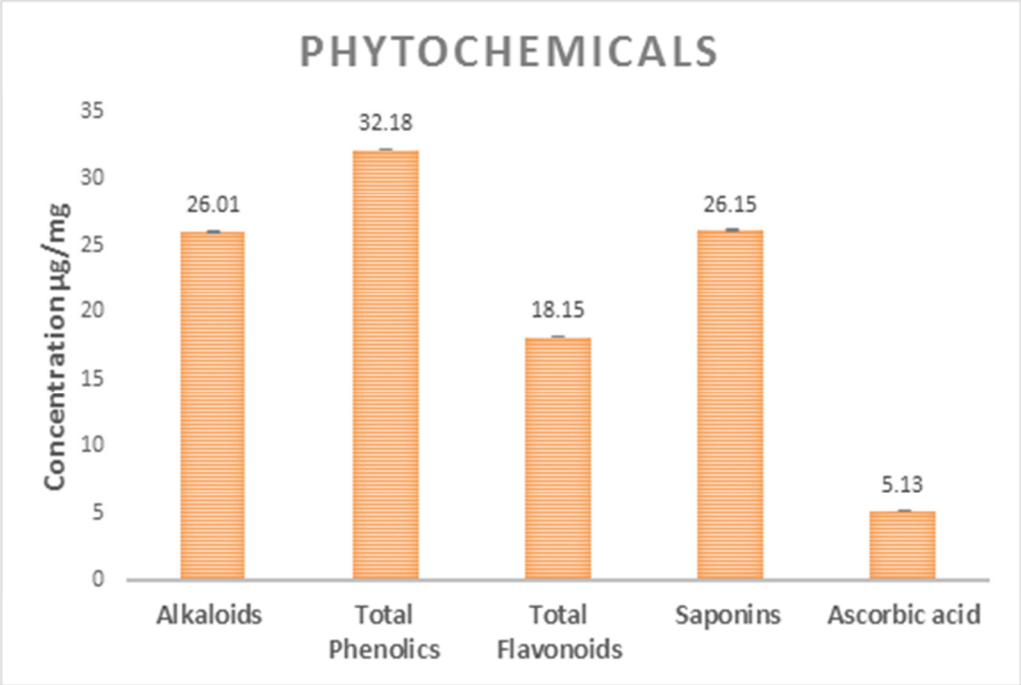
The test extract was subjected to GC-MS analysis using Gas Chromatographic system coupled with Mass Spectrometry (Perkin Elmer, Model: Clarus-500). Silica capillary column (30 m x 0.25 mm, 0.25 µm film thicknesses, Elite-5 MS non-polar fused 5% Phenyl 95% dimethyl-poly-siloxane) was used. Oven temperature was programmed with an increase of 6° C/min from room temperature to 150 °C and then an increase of 4°C/min from 150 °C to 280 °C was set. Injector temperature was 280 °C. Carrier gas was helium with the flow rate of 1 ml/min. Sample (1.0 µl) was injected with a split ratio of 1:10. Ionization energy 70 ev was used in the electron ionization mode; ion source temperature was set at 160-200 °C, mass was scanned in the range of 40-600 amu. The instrument was operated using Turbo mass software version 5.2.0. The resulting mass spectrum was compared with the inbuilt NIST library (2005) database and fragments of various compounds present in the extracts were identified.

3. STATISTICAL ANALYSIS

All values are expressed as mean ± standard deviation (n=3) wherever necessary. MS Excel is used for statistical analysis.

4. RESULTS AND DISCUSSION

Fruits continue to play an essential role in health care. In the present study *in vitro* experiments were carried out to evaluate the biological potentials of aqueous extract of *L. acidissima* fruits. The powdered plant material was subjected to the estimation of secondary metabolites such as alkaloids, flavonoids, phenols, saponins and ascorbic acid (Fig.1). The fruit extract was rich in phenolics, saponins, alkaloids and flavonoids.



Values are mean ± SD (n=3)

Fig 1. Quantitative analysis of phytochemicals

Chronic inflammation has become the major contributor of several ailments. Fruits represent a novel source of newer compounds with significant anti-inflammatory activity. In the present study, aqueous extract of wood apple was subjected to albumin denaturation inhibition assay, in order to ascertain its anti-inflammatory activity. Denaturation of tissue proteins is one of the underlying causes of inflammatory and arthritic disease. Production of auto antigens in certain arthritic disease may be due to denaturation of protein *in vivo*.[31-32] In the present study, 5 different concentrations (200, 400, 600, 800, 1000 µg/ml) of aqueous extract of wood apple were evaluated for inhibition of protein denaturation. At a concentration of 1000 µg/ml wood apple has shown 74.55% of protein denaturation inhibition. Increasing activity was found when the concentration of the extract increased. The anti-inflammatory potential of the extract was compared with standard Diclofenac sodium. The data of the results obtained are presented in Table 1.

Table.1: Effect of *L. acidissima* on inhibition of albumin denaturation

S. No.	Concentration µg/ml	Denaturation Inhibition Percentage ±SD	
		L. acidissima fruit extract	Diclofenac Sodium
1.	200	23.64±0.01	48.38±0.03

2.	400	36.36±0.03	58.06±0.01
3.	600	47.27±0.02	75.80±0.02
4.	800	63.64±0.02	80.65±0.03
5.	1000	74.55±0.01	93.55±0.03

Values are mean ± standard deviation (n=3)

Oxidative stress, arising as a result of an imbalance between free radical production and antioxidant defences contributes to the aetiology of many chronic health problems such as inflammatory disease, cataract and cancer. Antioxidants prevent free radicals induced tissue damage by preventing the formation of radicals, scavenging them or by promoting their decomposition.[33] There are many reports that support the use of antioxidant supplementation in reducing the level of oxidative stress and in slowing or preventing the development of complications associated with diseases. They exert their action either by scavenging the ROS or protecting the antioxidant defence mechanisms.[34] The accumulating evidence suggests the antioxidant potential of plant extracts as their therapeutic index. [35-38] Phosphomolybdate reduction assay is an important in vitro antioxidant assay to access the total antioxidant capacity of the plant extract. The assay principle follows the conversion of Mo (VI) to Mo (V) by extract or the compound which possesses antioxidant potential resulting in green phosphate Mo (V). The electron/hydrogen donating pattern of antioxidants depends upon its structure and series of redox reactions occurring in the activity.[39] Our findings showed that aqueous extract of wood apple has good antioxidant potential due to presence of flavonoid and phenolic contents. In the phosphomolybdenum assay, which is a quantitative method to evaluate the antioxidant capacity,[40] aqueous extracts of wood apple at different concentrations revealed significant activity as shown in Fig. 3. It has been found that antioxidant capacity increases with increasing concentration which was expressed as mg/100g ascorbic acid equivalents (Fig. 2). Presence of phenolic compounds and flavonoids might be correlated with the total antioxidant capacity of the extract.

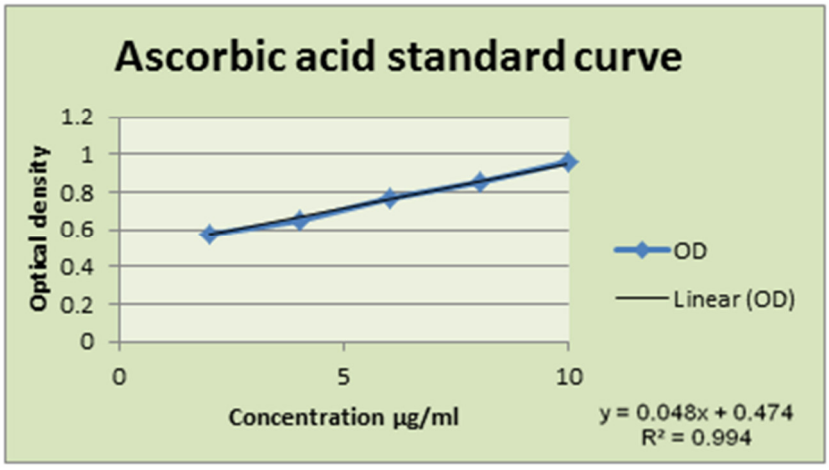


Fig 2. Ascorbic acid standard curve

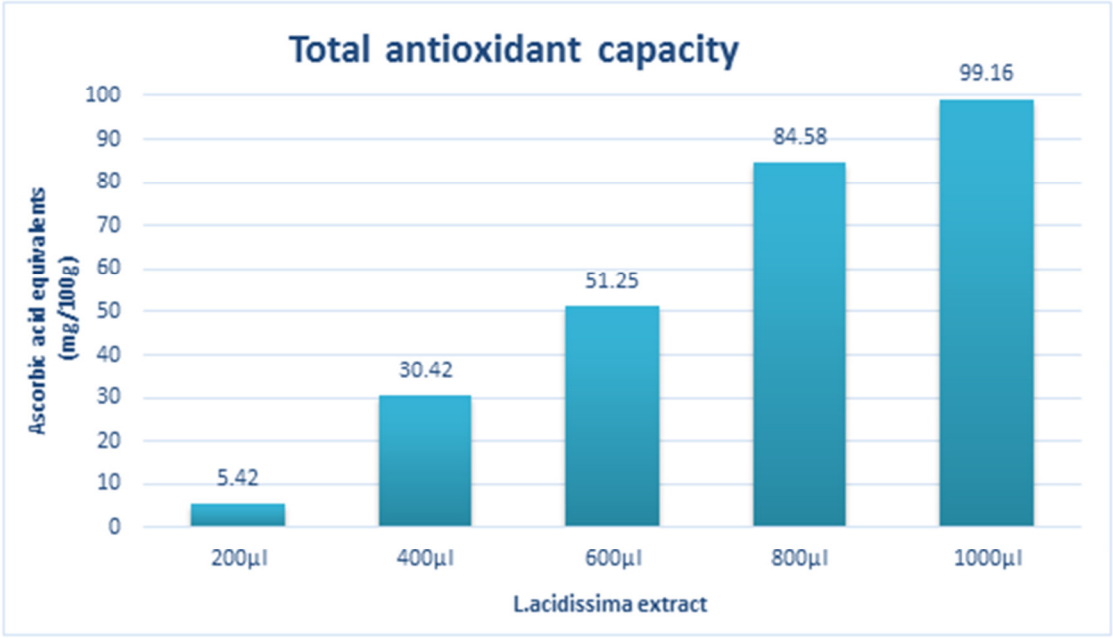


Fig 3. Total antioxidant capacity of aqueous extract of *L. acidissima*

Chemoprevention is a rapidly growing field of oncology which aims at preventing cancer growth using natural or synthetic interventions. [41] Chemotherapy using synthetic drugs can produce severe toxic side effects, which resulted in restricted usage of the same. In recent years, a considerable attention has been paid to identify naturally occurring chemo preventive substances capable of inhibiting, retarding or reversing the process of carcinogenesis.[42] Many chemical molecules isolated from plants and dietary sources have been reported to possess potentials to inhibit and delay the multistage process of tumour growth.[43] The important advantages of plant based medicines are their safety, efficacy and affordability. In this view, attempts were made in the present study to evaluate the anti-cancer activity of the aqueous extract of *L. acidissima* on MCF7 cell line through MTT assay and DNA fragmentation assay. MTT is the commonly applied method for evaluation of cell viability and cytotoxicity for screening the drugs. It significantly helps the researchers to determine whether any of the test compounds has cell toxicity or proliferative activity.[44] There are many advantages of MTT assay in particular its simplicity and effectiveness, which makes it more suitable to assess the anti-inflammatory and anti-cancer activities of any test samples at preliminary levels. In the present study, the systematic experimental steps were carried out in order to determine the potential cytotoxicity of the test substance at different concentrations against MCF7 cell line by MTT assay and the results are presented in Table 2, Fig.4 and 5. Decrease in absorbance at 540 nm in the cells treated with increasing concentration of the test substance was observed suggesting cytotoxicity in comparison to the control cells without any treatment. About 8 different concentrations of the aqueous extract of *L. acidissima* were evaluated for their cytotoxic activity on MCF 7 cell line. At a concentration of 1000 µg/ml, the extract has shown 93.43% of cytotoxicity and 6.57% of cell viability. At a concentration of 15.6 µg/ml, cytotoxicity was found to be 47.99% and cell viability was 52.01%. From the data of the results obtained, it could be ascertained that the aqueous extract of *L. acidissima* fruit pulp along with outer woody rind is having high degree of cytotoxic potential against breast cancer cell line MCF7.

Table 2: *In vitro* cytotoxic effect of *L. acidissima* on MCF 7 cell line

S. No.	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)	Cell cytotoxicity (%)
1	1000	-	0.057	6.57	93.43
2	500	1:1	0.122	14.07	85.93
3	250	1:2	0.187	21.56	78.44
4	125	1:4	0.253	29.18	70.82
5	62.5	1:8	0.326	37.60	62.40
6	31.2	1:16	0.391	45.09	54.91
7	15.6	1:32	0.451	52.01	47.99
8	7.8	1:64	0.523	60.32	39.68
9	Cell control	-	0.867	100	0

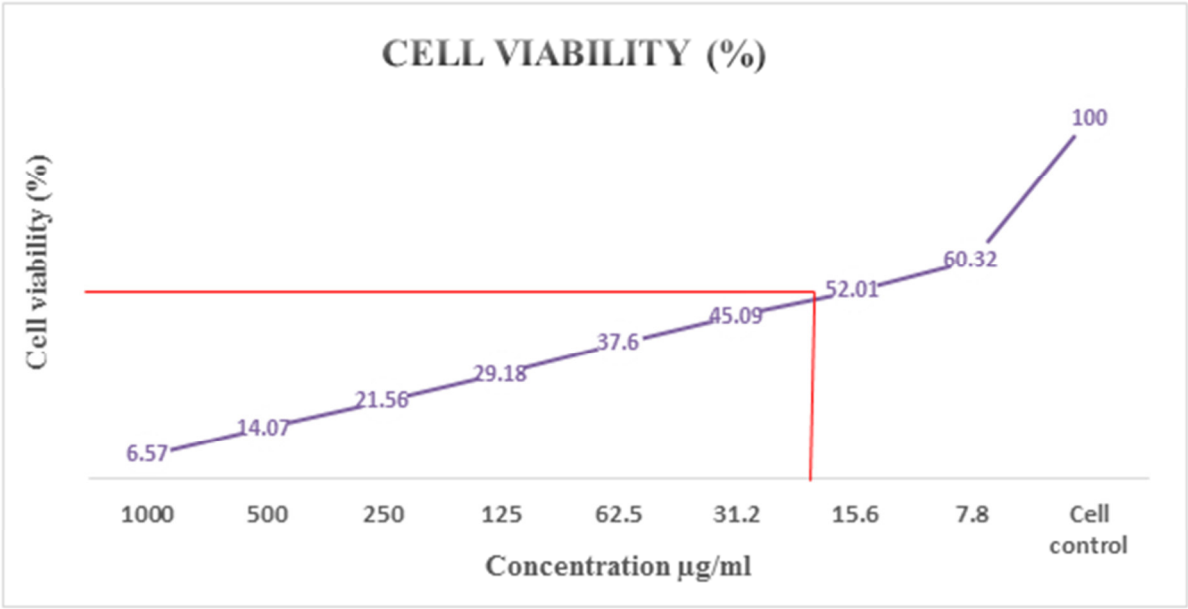
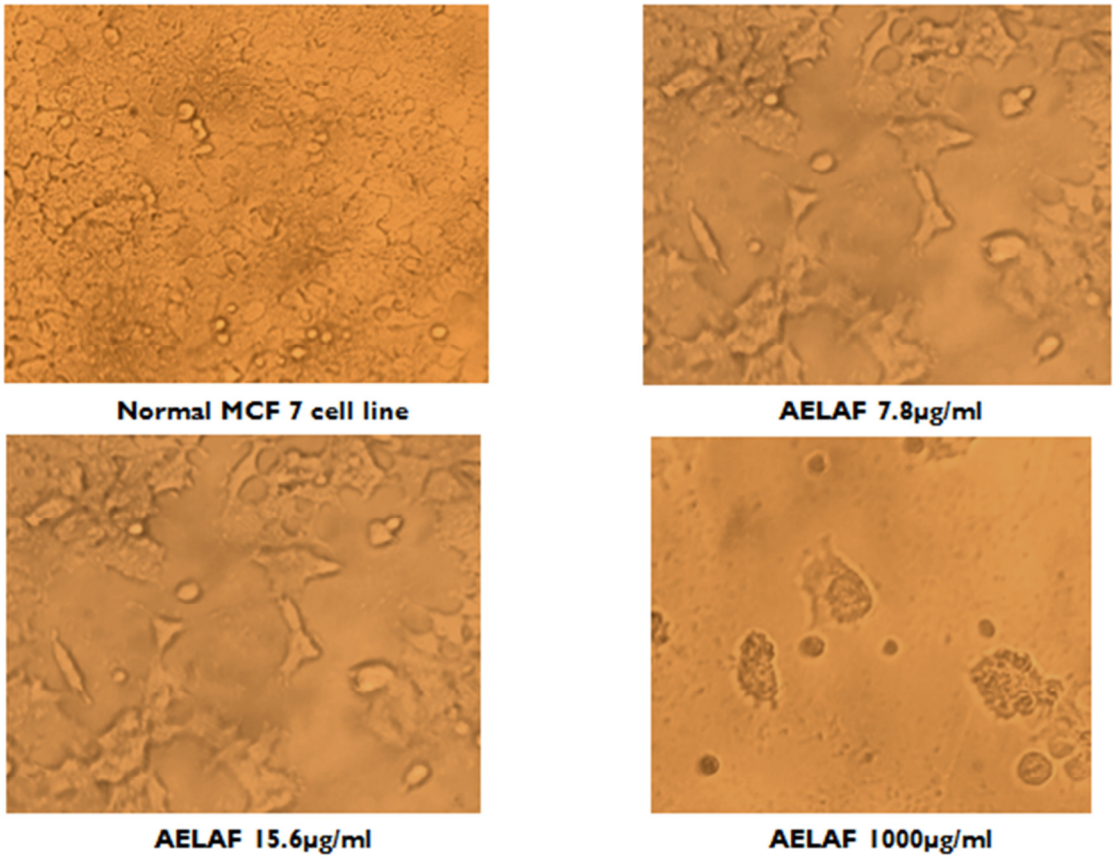


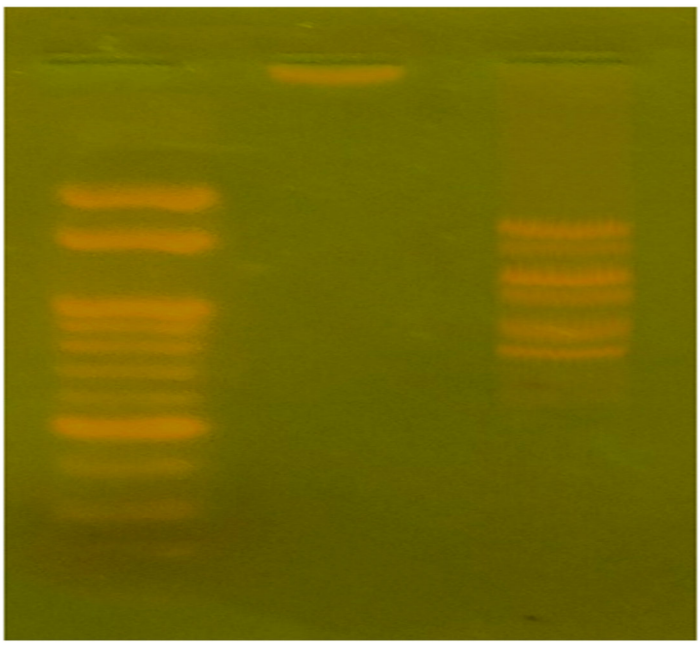
Fig.4: Effect of AELAF on cell viability of MCF7 cell line



(AELAF-Aqueous extract of L. acidissima Fruit)

Fig 5. *In vitro* cytotoxic effect of AELAF on MCF 7 cell line

A distinctive feature of apoptosis at the biochemical level is DNA fragmentation.[45] This method is used as a semi-quantitative method for measuring apoptosis. DNA fragmentation study was performed by Agarose Gel Electrophoresis. DNA migrated as discrete bands which were compared to DNA markers, giving a ladder of approximately 100 base pairs. Such DNA ladders formed due to cleavage of chromosomal DNA into oligonucleosome fragments is a hallmark of apoptosis. This cleavage of DNA or its fragmentation can be visualized by DNA Laddering assay.[46-47] In the present study, DNA ladders appeared in AELAF treated cells but not in control cells which ascertains the in vitro cytotoxic activity of the wood apple extract through the induction of apoptosis and subsequent DNA fragmentation (Fig.6). The data of the results obtained indicate that AELAF has induced significant inter nucleosomal DNA fragmentation in the MCF-7 cell line. This activity might be contributed by the phytochemical constituents.



Lane 1: DNA Marker Lane 2: Control Lane 3: Sample

Fig.6: Effect of AELAF on DNA fragmentation of MCF-7 cell

GC-MS analysis of *L. acidissima* fruit extract revealed the presence of 35 compounds. The data of the results obtained were presented in Table 3 and Fig. 7.

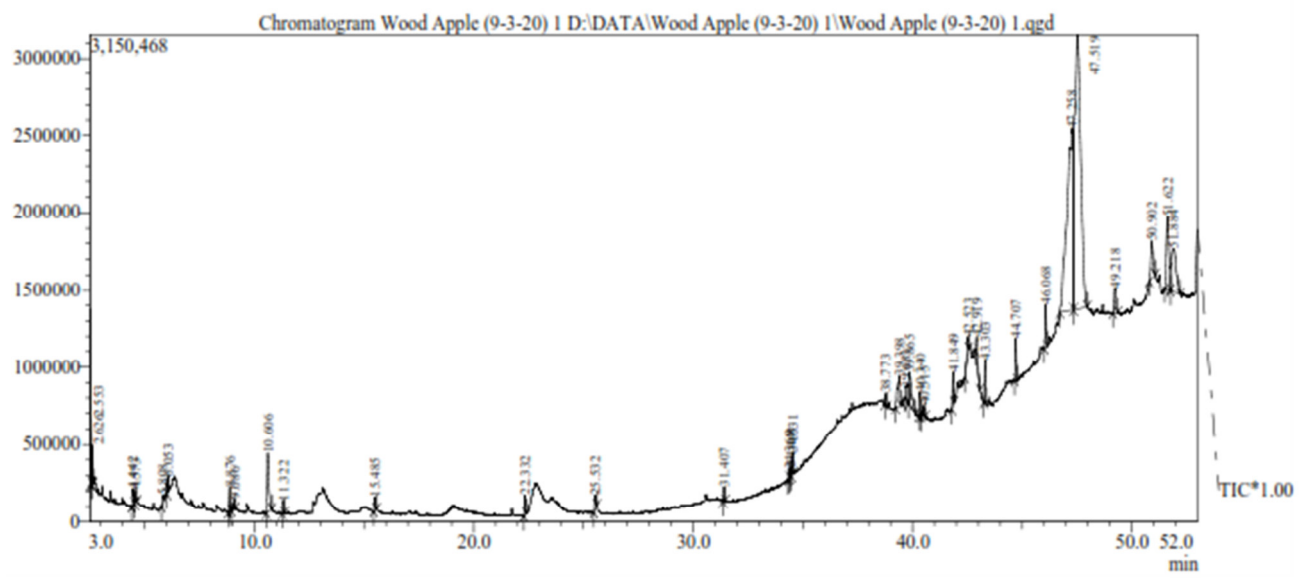


Fig.7: GCMS chromatogram of *L. acidissima* fruit extract

GCMS analysis revealed the presence of several phytochemicals present in the wood apple fruit pulp and rind which might have been responsible for the therapeutic potential of the extract. It is evident from the research reports that compound 1,3,5-Triazine-2,4,6-Triamine is cytotoxic on lung cancer cell line [48],Eicosane is effective in human prostate cancer [49]; Hexatriacontane is cytotoxic on MCF7 and MDA-MB-231[50]; Hexatriacontane and Tetratriacontane have shown anticancer activity [51]; Compounds such as Triarachine[52]; Nonacosane[53]; Dotriacontane (anti melanoma) [54], 22-Tetratetracontane [55]; Tetra Penta Contane, 1,54-Dibromo [56] have reported anticancer activity which was evident from the previous studies. It is already reported that compounds such as flavonoids and tannins possess significant antimutagenic [57] and anti-malignant activity. [58] They play an important role as a chemopreventive agent in cancer because of their effects on signal transduction in cell proliferation [59] and angiogenesis. [60] Such pharmacologically active phytoconstituents induce cell death of cancer cells by concentration-dependent decrease of ATP and a deterioration of cellular gross morphology.[61-62] These substances not only inhibit growth of cancer cells [63] but also protect the normal cells and thereby considered as efficient candidates to enhance opportunities for DNA repair, immune stimulation, anti-inflammation and cancer prevention.[64] Plant derived phytochemicals inhibit the tumor growth by altering signal transduction pathways.[65-66] In the present study, presence of such pharmacologically active phytoconstituents in the aqueous extract of wood apple might have been responsible for significant anticancer activity.

Table 3: GC-MS profile of *L. acidissima* fruit extract

S. No.	Peak Name	Mol. Weight	Mol. Formula	Retention Time	%Peak Area
1.	METHYL 2-OXOPROPANOATE	102	C4H6O3	2.553	1.19
2.	PROPANAL, 3-ETHOXY-	102	C5H10O2	2.626	0.56
3.	3-METHYL ENEDIHYDRO-2,5-FURAND	112	C5H4O3	4.44	0.62
4.	2-CYCLOPENTEN-1-ONE-2-HYDROXY	98	C5H6O2	4.573	0.31
5.	3-CYCLOPENTEN-1-OL	84	C5H8O	5.808	0.43
6.	2,4-DIHYDROXY-2,5-DIMETHYL -3(2H)-FURAN-3	144	C6H8O4	6.053	0.29
7.	1,3,5-TRIAZINE-2,4,6-TRIAMINE	126	C3H6N6	8.876	0.77
8.	PROPANE,1,1,3-TRIETHOXY	176	C9H20O3	9.086	0.34
9.	4H-PYRAN-4-ONE, 2,3-DIHYDRO-3,5-DIHYDROXY	144	C6H8O4	10.606	2.50
10.	BICYCLO [2.2.1] HEPTAN-2-OL, 1,7,7-TR	154	C10H18O	11.322	0.27
11.	2-METHOXY-4-VINYLPHENOL	150	C9H10O2	15.485	0.37
12.	1,2-BENZENEDICARBOXYLIC ACID, D	222	C12H14O4	22.332	0.44
13.	4-((1E)-3-HYDROXY-1-PROPENYL)-2-METHOXY	180	C10H12O3	25.532	0.48
14.	HEXADECANOIC ACID, ETHYL ESTER	284	C18H36O2	31.407	0.27
15.	9,12-OCTADECADIENOIC ACID (Z,Z)	294	C19H34O2	34.369	0.18
16.	ETHYL (9Z,12Z)-9,12- OCTADECADIEN	308	C20H36O2	34.440	0.24
17.	9-OCTADECENOIC ACID (Z)-, ETHYL E	310	C20H38O2	34.531	0.47
18.	2.49 EICOSANE	282	C20H42	38.773	0.23
19.	9-OCTADECENOIC ACID,1,2,3-PROPANETRIYL EST	884	C57H104O6	39.398	1.79
20.	STIGMAST-4-EN-3-ONE	412	C29H48O	39.683	0.98
21.	HEXADECANOIC ACID, 2-HYDROXY-	330	C19H38O4	39.865	2.08

22.	HEXATRIACONTANE	506	C36H74	40.340	0.52
23.	PENTADECANAL	226	C15H30O	40.515	0.32
24.	TETRATRIACONTANE	478	C34H70	41.849	0.75
25.	9-OCTADECENOIC ACID(Z)-2,3-DIHYDROXYPRO	356	C21H40O4	42.523	0.69
26.	TRIARACHINE	974	C63H122O6	42.919	0.72
27.	HEXATRIACONTANE	506	C36H74	43.303	0.94
28.	NONACOSANE	408.6	C29H60	44.707	0.96
29.	DOTRIACONTANE	450	C32H66	46.068	1.07
30.	22-TRITETRACONTANONE	618	C43H86O	47.258	28.02
31.	TETRAPENTACONTANE,1,54-DIBROMO	758	C54H110	47.519	39.17
32.	HEXATRIACONTANE	507	C36H74	49.218	0.97
33.	ERGOST-5-EN-3-OL, (3. BETA.,24R)-	400.7	C28H48O	50.902	1.88
34.	STIGMASTA-5,23-DIEN-3-OL, (3. BETA)	412.7	C28H48O	51.622	4.26
35.	OLEYLALCOHOL, TRIFLUOROACETATE	268.5	C18H36O	51.884	4.91

5. CONCLUSION

Taken together it could be concluded that wood apple is bestowed with numerous health benefits such as antioxidant, anti-inflammatory and anticancer properties which are evident from the results obtained through *invitro* experiments. Hence inclusion of this fruit in our diet should be practiced regularly to reap the health benefits of this forgotten fruit. Many of the younger generation might not have heard of this fruit. They should be educated in such a way to make them consume indigenous fruits that are rich in medicinal values. These immunity boosting fruits will surely help to build a disease-free nation in future.

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7. AUTHORS CONTRIBUTION STATEMENT

S. Sujitha, who is the principal investigator of this work, has contributed to data collection, processing and interpretation of results. Dr. P. Venkatalakshmi, research guide has given valuable inputs, suggestions and guidance for the successful completion of this work and in designing the manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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