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Evaluation of nutritional, phytochemical, antioxidant and antibacterial activity of exotic fruit "*Limonia acidissima*"

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ABSTRACT
Limonia acidissima (L.), Wood-Apple, is an underexploited and edible fruit of family Rutacae which can be consumed either in raw or ripe form. In northern parts of India, which is locally called Kaitha. In the present work, we have attempted to study different characteristics of pulp and rind (outer shell) of Kaitha fruit, to understand its health benefits. Various studies were done to estimate phytochemicals like alkaloids, saponins, flavonoids, total phenolics. Nutritional analysis of pulp proved it to be a potential source of energy, proteins, dietary fiber, vitamins; ascorbic acid, thiamine, riboflavin, and betacarotene and minerals; phosphorous, magnesium, calcium, iron and zinc. The extracts displayed a moderate antioxidant activity. The pulp exhibited good antibacterial activity against gram positive bacteria. This study demonstrates that *L. acidissima* fruits may be used as nutraceuticals for disease prevention and health promoting benefits.

Keywords: *L. acidissima*, Kaitha, Nutritional, Phytochemical, Antioxidant, Antibacterial, GC-MS, ICP-OES.

1. Introduction
Limonia acidissima (L.) of family Rutaceae (Citrus family) belongs to the monotypic genus *Limonia,* 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. This plant is given as a medicine for the treatment of various disorders [2]. *L. acidissima* is a deciduous, slow-growing, erect tree with a few upward-reaching branches bending outwards near the summit where they are subdivided into slender branchlets drooping at the tips. Its fruit is spherical in shape with 5-12.5 cm diameter. The rind is greyish-white in color and 6 mm thick. It has woody and extremely hard outer shell (called as rind) which is very difficult to crack open. Hammer is used to crack the hard rind of wood-apple fruit. The Pulp is brown, mealy, aromatic, resinous, sour or sweetish with many small white seeds embedded in it. Syrups, drinks, jellies and jams can be prepared from its sticky pulp [3, 4]. The valuable parts of the plant include its roots, fruits, bark and the leaves which are used for various therapeutic purposes [5].
Wood-apple is useful in preventing and curing scurvy and in relieving flatulence. Mashed seedless pulp of the raw fruit is beneficial in the treatment of dysentery, diarrhoea and piles.
L. acidissima, considered to be a hepatoprotectant, possess different biological activities namely adaptogenic activity against blood impurities, leucorrhoea, dyspepsia and jaundice. Traditionally, all parts of the plants are given as natural medicine as a cure for various ailments [3]. It is very often used against snakebites [6]. People use it as a tonic for liver and heart, in diarrhoea and dysentery. This fruit is considered to be an effective treatment for hiccups and for problems of throat and gum [7]. The pulp is applied onto bites and stings of deadly insects. It is also protective against skin cancer as it can block UV rays. A paste "Thanaka", prepared from the pulp of *L. acidissima*, is used as face cream to remove small spots and lesions on the skin [8]. The fruits and stem bark of *L. acidissima* possess larvicidal and antimicrobial activity [9]. Ahmed *et al.* [10] screened the fruit pulp of *L. acidissima* for anti-inflammatory, antipyretic and analgesic activities as well as for the anthelmintic activity of its leaves. The hepatoprotective activity of the leaves of *F. limonia* was studied by Kamat *et al* [11]. A study by Darsini *et al.* 2013, revealed potent antioxidant activity of the fruit and its ability for being used in food and pharmaceutical applications [12]. The aim of the present study is to evaluate the nutritional, phytochemical constituents, antioxidant and antibacterial activity of the non-commercial or underutilized raw fruits of *L. acidissima*.

2. Material and methods

2.1 Materials

Fruits were collected in the month of July, 2013 from a local market in Uttar Pradesh, India. The fruits (*L. acidissima*) were taken and thoroughly washed under tap water. All the clean samples were separated mechanically into fruit pulp and rind. They were dried in an oven at 40 ˚C and coarsely powdered using a mixer grinder and stored in an air-tight container for further use.

2.2 Reagents

Folin-Ciocalteu's Phenol reagent (SRL), Gallic Acid (HiMedia), Dimethyl Sulphoxide (SRL), Aluminium chloride (Fisher), Sodium Hydroxide (SRL), Ascorbic Acid (SRL), 2,2'-Azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) (Sigma), Trolox (Aldrich),TPTZ (Fluka), Ammonia solution (SRL), Ferrous Chloride (Thomas Baker), Petroleum Ether (Loba Chemie), Methanol (Thomas Baker), Dichloromethane (Fisher), Ethanol, Ethyl Acetate, Acetone, ICP Multielement Standard (Qualigens), Distilled water.

2.3 Extraction

Both the dried pulp and rind of the fruits were extracted in 100 ml methanol (double stage extraction) using incubator shaker at 60˚C and 150 rpm. Supernatants were pooled and combined filtrates were filtered through Whatman filter paper # 1 and evaporated in hot air oven at ≤ 65 ˚C. After evaporation of organic solvents, the extract yield (EY) was calculated and the extracts were stored at -20 ˚C till analysis.

2.4 Extract Yield (EY)

The yield of dried extracts (pulp and rind) based on their dry weights were calculated using the following equation:

Yield (g /100 g of dry plant material) = (W1 × 100) / W2

Where, W1 was the weight of the extract after the evaporation of solvent, and W2 was the weight of the dry plant material.

2.5 Nutritional Analysis

Macro kjeldahl method was used for the estimation of crude protein content [13]. Powdered fruit samples were put in an oven at 105 °C for 24 h. Difference in weight determines the moisture content [14]. Ash content was analyzed by AOAC method Ref. 942.05. Fat content of the samples was determined by using petroleum ether as a solvent. Total carbohydrate [13] and energy calorific value [13] were also calculated. Crude fiber content was evaluated by using AOAC method [15]. Mineral content was analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES). Vitamins content were estimated by using HPLC with Fluorescence and UV detection using reverse phase column C18 (5 µm, 250 mm x 4, 6 mm) as per AOAC 19th Edition method [15]. For estimation of water soluble vitamins (B_1 & B_2), 2g of sample was dissolved in 50 ml of 0.1 N hydrochloric acid and autoclave the test portion at 121 ^{0}C for 30 min. Adjust the pH of the solution to less than 4.00 using 2.5 M sodium acetate. Make up to 100 ml with water, shake the solution and filter through a filter paper and 20 µl of filtrate injected to HPLC by using isocratic pump using Methanol: phosphate buffer, pH 3,5 (1 g/l tetraethyl ammonium chloride and 5 mmol/l sodium heptanesulfonate (35:65)) as mobile phase. Flow rate: 1, 0 ml/min The fluorescence detection was done with wavelength; Excitation: 468 nm; Emission: 520 nm for B_2. And B_1 was detected with Excitation: 366 nm; Emission: 435 nm by using Methanol: acetate buffer as mobile phase. For beta carotene estimation 2g of sample was extracted by using petroleum ether at 80^{0}C. Evaporate and dissolve the residue with mobile phase and inject to HPLC- UV at 450 nm by using mobile Phase; Acetonitrile + methanolic ammonium acetate solution + Dichloromethane (75+20+5)(volume parts) containing 0.1% of butylatedhydroxytoluene and 0.05% by mass of triethylamine. Vitamin C was estimated by iodometric titration using starch indicator.

2.6 Phytochemical Analysis

2.6.1 Determination of Crude Alkaloids

2.5g of the sample was weighed in a 250 ml beaker and 100 ml of 10% acetic acid in ethanol was added. It was covered and allowed to stand for 4h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle, then the precipitate was collected and washed with dilute ammonium hydroxide and filtered again. The resulting alkaloid was dried and weighed [16].

2.6.2 Determination of Saponins

The method of Obadoni and Ochuko [17] was used to determine saponins. To 5g of the powdered sample, 50 ml of 20% aqueous ethanol was added in a conical flask. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55 °C .The mixture was filtered and the residue 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 the oven to a constant weight and the saponin content was calculated as percentage.

2.6.3 Determination of Total Phenolics

Folin Ciocalteu's reagent method was used to determine total phenols [18]. An aliquot(100µl) of extract was mixed with 250 µl of Folin Ciocalteu's reagent and allowed to stand at room temperature for 5 min. Sodium bicarbonate (20%, 1.5 ml) was added to the mixture and incubated at room temperature for 120 min. Absorbance was measured at 765nm using a spectrophotometer. A standard curve was plotted using different concentrations of gallic acid and the amount of total phenolics was calculated as gallic acid equivalents in µg/ mg of dried extract.

2.6.4 Determination of Total Flavonoids

The total flavonoid content was also determined using the aluminium chloride colorimetric method, with Catechin as a standard by Chang *et al,* (2002). To the sample extract (250 µl) was added to 4.5 ml distilled water followed by 5% $NaNO_2$ (0.03 ml). After 5 min at 25 °C, $AlCl_3$ (0.03 ml, 10%) was added. After another 5 min, the reaction mixture was treated with 2 ml of 1M NaOH. Finally the reaction mixture was

diluted to 10 ml with distilled water and absorbance was measured at 510 nm. The results were expressed as catechin equivalents (CE) in µg/ mg of dried extract.

2.7 Determination of Secondary Metabolites

Secondary metabolites were determined by GCMS analysis. An Agilent 5975B mass spectrometric detector (MSD) was used in the scan mode (m/z 35-1050) for all the samples. Screening of volatiles and semi volatiles were performed using the automatic RTL screener software in combination with the Agilent NIST'05 library. 1 µl of the sample from a 100 mg/ ml stock was injected by split injection (1:20) at 280 °C.

2.8 Antioxidant activity

2.8.1 ABTS radical scavenging assay

ABTS radical scavenging activity of the hydrophilic fractions was determined by a procedure reported by Re *et al* [19]. The ABTS solution was prepared by mixing 8 mM of ABTS salt with 3 mmol of potassium persulfate in 25 ml of distilled water. The solution was held at room temperaturein the dark for 16 h before use. The ABTS solution was diluted with ethanol in order to obtain an absorbance between 0.8 and 0.9 at 734 nm. Fresh solution was prepared for each analysis. Antioxidant or standard solutions, 10 µl were mixed with 990 µl of diluted ABTS solution and incubated for 10 min. The absorbance at 734 nm was read. Ethanol was used as a blank. The Ascorbic acid was used as a standard. The concentration of extracts required to scavenge 50% of ABTS radicals, called inhibitory concentration (IC_{50}) was also calculated.

2.8.2 Ferric reducing antioxidant power (FRAP) assay

FRAP solution (900 µl) was mixed with a certain concentration of the plant extract (100 µl) and incubated at 37 °C for 4 min. The absorbance of the reaction mixture was measured at 593 nm BHT (butylatedhydroxytoluene) was used as a standard.

2.9 Determination of antibacterial activity.

Antibacterial activity was assessed using agar well diffusion method against three gram positive bacteria (*Staphylococcus aureus, Staphylococcus epidermidis*, *Bacillus subtilis*) and a gram negative bacterium (*Proteus mirabilis*). Wells were punched on to the seeded nutrient agar plates with the help of 1ml micro pipette tips (6 mm diameter). 100 µl of the samples were added into the wells under strict aseptic conditions and all the plates were incubated at 37 °C overnight. Antimicrobial activity was determined by measuring the diameter of zone of inhibition and the mean values were calculated.

3. Results and Discussion

3.1 Nutritional analysis of the fruit pulp

In the present study, potential benefits were shown by nutritional attributes of kaitha fruit (Table1). Moisture content and dry matter analysis reporting during nutritional analysis is very important because it directly affects the nutritional content of fruit. The moisture content was quite low (6.4%) which may be advantageous in view of increasing the sample's shelf life. Kaitha pulp was found to be very rich in carbohydrates (70.14%). There was an appreciable amount of protein (13.8% by wt) making it a good source of protein, while its fiber content is also good. There is an evidence that dietary fiber has a number of beneficial effects related to its indigestibility in the small intestine [20]. Pulp has low amount of fat (4.38%) which makes it an ideal diet for overweight people. The energy value of dried pulp was calculated and the value obtained was 375.18 kcal. Pulp was found to contain calcium, magnesium, iron and zinc in high amounts followed by many other beneficial nutrients (Table 2). A high content of phosphorous can play an important role in bone formation and other essential metabolic activities of the body. Calcium can play a crucial role in providing rigidity to the skeleton besides its involvement in the neuromuscular functions, blood clotting, and many other metabolic processes [21]. It also contains iron which is used against anaemia, tuberculosis and disorders of growth [22]. Zinc supplementation in diabetes mellitus proved to have antioxidant effect [23]. The ascorbic acid content (180 µg/ g of dry matter) would fulfill the recommended dietary allowance (RDA) of National Research Council (1989). It has been reported for its antioxidant and electron donor properties for eight important enzymes in humans (Moncada & Higgs, 1993) [24]. It also protects against the oxidative damage of eyes caused by light and plays an important role in sperm maturation. Its antihistamine effect can minimize the formation of carcinogenic substances from dietary material and can be used as herbal medicine for the treatment of common cold and other diseases like prostate cancer (Okwu & Emenike, 2006) [25]. The presence of vitamin B_1 and B_2 can help to convert carbohydrates into energy and these are also essential for growth, production of red blood cells, and healthy skin and eyes (Table 2). For such an uncommon vitamin, it's pretty important, especially if you want healthy skin and eyes. The beta-carotene (a precursor of Vitamin A) also helps to build and maintain teeth, bones and mucous membranes.

Table 1: Nutritional analysis of dried pulp.

Constituents	Dried pulp (%)
Moisture	6.4
Ash	5.28
Protein	13.8
Fat	4.3
Carbohydrate	70
Dietary fibre	1.7

Table 2: Mineral and Vitamins content of kaitha pulp.

Minerals (µg/ g)	
Analyte	**Concentration(µg/ g)**
P	1137.35
Mg	852.5
Ca	711.8
Fe	23
Zn	23.84
Cu	6.67
Mn	3.64
Sb	0.626
As	ND
Be	ND
Cd	ND
Cr	1.543
Co	ND
Pb	0.163
Li	0.241
Mo	0.263
Ni	0.819
Se	0.768
Sr	ND
Tl	1.930
Ti	0.257
Sn	0.474
Vitamins(µg/ g)	
Vitamin C	180
Riboflavin(B_2)	0.23
Thiamine(B_1)	0.31
Beta-carotene	0.04

3.2 Phytochemical analysis of dried pulp and rind

Phytochemical content of the pulp and the rind of kaitha was analyzed and was found to be very promising. The values of saponins and crude alkaloids were determined on dry weight basis (g/100 g) (Table 3). A relatively high amount of alkaloids were found to be present in both the pulp and the rind. Saponins were found in moderate quantity. Alkaloids are good spasmolytic and anesthetic agents while saponins help in boosting the immunity system, in lowering cholesterol levels in the blood and reducing the risk of getting intestinal cancer.

Various reports have shown that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma and flavor and also in providing beneficial health effects. Therefore, total phenolic and flavonoid content of the pulp and rind extracts of the fruit were estimated (Table 4). The total phenolic content was found in high amounts in both the pulp and rind extracts. Phenolics provide the plants with defense mechanisms to neutralize reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by microorganisms, insects, and herbivores [26].

Flavonoids show a wide range of biological activities such as inhibition of cell-proliferation, induction of apoptosis, inhibition of enzymes and other antibacterial and antioxidant effects [27-29]. The flavonoid content of the extracts was also found to be quite high.

Table 3: Phytochemical analysis of pulp and rind of kaitha.

Components	Pulp	Rind
Alkaloids (g/100 g DM)	36	13.27
Saponins (g/100 g DM)	0.16	0.5

Table 4: Polyphenolic compounds of pulp and rind of kaitha.

Polyphenolic compounds	Pulp	Rind
Total phenols(µg GAE/mg)	35.72	33.8
Flavonoids (µg CE/mg)	35.51	39.51

3.3 Antioxidant activity

Antioxidant capacity of the extracts was evaluated against ascorbic acid as percent inhibition of ABTS free radicals. ABTS radical is a blue chromophore produced by the reaction

between ABTS and potassium persulfate. The antioxidant activity (IC_{50} value) as determined by ABTS assay was found to be 0.7 mg/ ml and 0.8mg/ ml for methanolic extracts of the pulp and rind of kaitha, respectively.

In FRAP assay, reduction of the ferric-tripyridyltriazine to the ferrous complex forms an intense blue colour which can be measured at a wavelength of 593 nm. The intensity of the colour is related to the amount of antioxidant reductants in the samples. FRAP activity was found to be much higher in the rind (46.03 µg BHTE/ mg) as compared to the pulp (42.95 µg BHTE/ mg) of the fruit.

3.4 Characterization of GC/MS analysis

The details of all the identified compounds (Figure 1(A) and (B)) present in the pulp and the rind are grouped according to their chemical nature (Table 5 and 6). Furfural is found in the pulp which is used as a raw material in the production of drug 'atropine' and has many other pharmaceutical uses as well as these furfural derivatives have been reported for their strong bactericidal capacity and rather broad antibacterial spectrum [30]. In pulp many saturated fatty acids: Hexadecanoic acid (used in cosmetics, soaps, antioxidant), Dodecanoic acid (acne treatment, increases HDL cholesterol), Pentadecanoic acid, Eicosanoic acid, Tridecanoic acid, Docosanoic acid, gamma-Tocopherol (anticancer, antioxidant, anti-inflammatory, cardioprotective) were also found. Maltol (flavour enhancer) and isosorbide (treats chest pain) were also observed in the pulp. In rind, some saturated fatty acids like Dodecanoic acid, Tetradecanoic acid (cosmetic usage), Pentadecanoic acid, Hexadecanoic acid and oleic acid (monounsaturated fatty acid, omega-9) were found. Germacrenes known for antimicrobial and insecticidal properties was also found. The presence of phytosterol and campesterol in both the pulp and rind which are well known for their medical, cosmetic, and functional food applications may contribute towards the antimicrobial and antioxidant activities of the fruit. They are also known for their saturated fat reducing and cholesterol lowering activity and thus, may reduce the risk of heart diseases [31]. In rind, Phytol was observed which can be used as a precursor for manufacturing synthetic forms of vitamin E and vitamin K. Phytol is also used in various industries like fragrance, cosmetics, shampoos, toilet soaps, household cleaners, and detergents. Phytol and 6, 10, 14-trimethyl-2-Pentadecanone have anticancerous properties. Rind extract also revealed the presence of caryophyllene which is an FDA approved food additive.

Table 5: GCMS profiling of methanolic extract of pulp of kaitha

Compound name	RT(min)	% Area	Cas #
Furfural	4.444	0.39	000098-01-1
2,5-Furandione, 3-methyl	6.530	24.67	000616-02-4
2-furancarboxaldehyde, 5-methyl-	6.676	1.66	000620-02-0
Butanedioic acid, methylene-, dimethyl ester	8.997	0.99	000617-52-7
Maltol	9.356	1.24	000118-71-8
4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl-	9.951	1.68	028564-83-2
Benzofuran, 2,3-dihydro-	11.117	0.48	000496-16-2
Isosorbide	12.306	1.10	000652-67-5
2-Methoxy-4-vinylphenol	12.687	2.56	007786-61-0
DL-Proline, 5-oxo-, methyl ester	13.820	2.62	054571-66-3
Dodecanoic acid	15.951	0.15	000143-07-7
L-Phenylalanine, N-acetyl-, methyl Ester	17.790	0.75	003618-96-0
Tridecanoic acid, 12-methyl-, methyl ester	17.835	0.12	005129-58-8
Tetradecanoic acid	18.216	0.18	000544-63-8
Pentadecanoic acid, methyl ester	18.912	0.06	007132-64-1
Hexadecanoic acid, methyl ester	19.955	2.35	000112-39-0
2-Propenenitrile, 3-(3,4-dimethoxy phenyl)-	20.246	0.22	006443-72-7
n-Hexadecanoic acid	20.347	3.13	000057-10-3
11,14-otadecadienoic acid, methyl Ester	21.603	1.67	056554-61-1
Cyclopropaneoctanal, 2-octyl	22.074	8.64	056196-06-6
13-Tetradecenal	23.409	0.15	085896-31-7
Eicosanoic acid, methyl ester	23.611	0.16	001120-28-1
cis-9-Hexadecenal	24.845	0.72	056219-04-6
Docosanoic acid, methyl ester	25.226	0.13	000929-77-1
.gamma.-Tocopherol	30.497	0.25	007616-22-0
Campesterol	33.660	0.82	000474-62-4
Stigmasterol	34.344	1.13	000083-48-7
Stigmasterol, 22,23-dihydro	35.667	1.72	1000214-20-7

Table 6: GCMS profiling of methanolic extract of rind of kaitha.

Compound name	RT(min)	% Area	Cas #
2,5-Furandione, dihydro-3-methylene-	6.194	0.71	002170-03-8
2-Decenal, (E)-	11.812	0.08	003913-81-3
2-Methoxy-4-vinylphenol	12.665	1.45	007786-61-0
4-Methylproline methyl ester	13.584	0.25	145730-69-4
Benzaldehyde, 3-hydroxy-4-methoxy	13.921	0.43	000621-59-0
Caryophyllene	14.269	0.95	000087-44-5
4-Hydroxy-3-methoxybenzyl alcohol	14.605	0.20	000498-00-0
1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]-	15.087	0.28	023986-74-5
Dodecanoic acid	15.940	0.44	000143-07-7
Caryophyllene oxide	16.411	0.61	001139-30-6
10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5.beta.-ol	17.028	0.47	019431-80-2
Benzaldehyde, 4-hydroxy-3,5-dimeth Oxy-	17.207	0.35	000134-96-3
1-Methyl-6-methylenebicyclo[3.2.0] heptanes	17.398	0.21	1000210-90-0
4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	18.082	0.92	1000297-95-5
Tetradecanoic acid	18.228	0.84	000544-63-8
Guaifenesin	18.676	0.24	000093-14-1
2-Pentadecanone, 6,10,14-trimethyl	19.136	0.24	000502-69-2
Pentadecanoic acid	19.271	0.39	001002-84-2
Cyclopentadecane	19.484	0.15	000295-48-7
9-Octadecenal, (Z)-	19.663	0.20	002423-10-1
Hexadecanoic acid, methyl ester	19.944	2.11	000112-39-0
2-Propenenitrile, 3-(3,4-dimethoxy phenyl)-	20.224	0.37	006443-72-7
n-Hexadecanoic acid	20.392	10.14	000057-10-3
Heptadecanoic acid, methyl ester	20.919	0.28	001731-92-6
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	21.592	0.44	000112-63-0
Phytol	21.749	0.39	000150-86-7
Cyclopropaneoctanal, 2-octyl-	22.086	15.42	056196-06-6
2-(1-Hydroxy-1-methylethyl)-2,3-dihydrofuro[3,2-g]chromen-7-one	23.701	0.64	1000210-83-0
Cyclopentadecanone, 2-hydroxy	24.845	0.98	004727-18-8
Cyclotetracosane	24.934	0.80	000297-03-0
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	25.103	0.96	023470-00-0
1,2-Benzenedicarboxylic acid, diis ooctyl ester	25.439	0.20	027554-26-3
Oleic acid, 3-hydroxypropyl ester	26.527	2.56	000821-17-0
2(1H)-Naphthalenone, octahydro-4a- methyl-7-(1-methylethyl)-, (4a. alpha, 7.beta, 8a. beta.)-	26.583	1.12	054594-42-2
Campesterol	33.716	5.76	000474-62-4
Stigmasterol	34.445	9.46	000083-48-7
9,19-Cycloergost-24(28)-en-3-ol, 4 ,14-dimethyl-, (3.beta.,4.alpha.,5 .alpha.)-	36.576	1.62	000469-39-6

Table 7: Antibacterial activity of dried pulp and dried rind of *L. acidissima* (500 mg/ml)

Bacterial strains	Diameter of zone of inhibition(mm)	
Gram positive	**Pulp**	**Rind**
Staphylococcus aureus	18.3	12
Staphylococcus epidermidis	25.3	16.6
Bacillus subtilis	15	--
Gram negative		
Proteus mirabilis	--	--

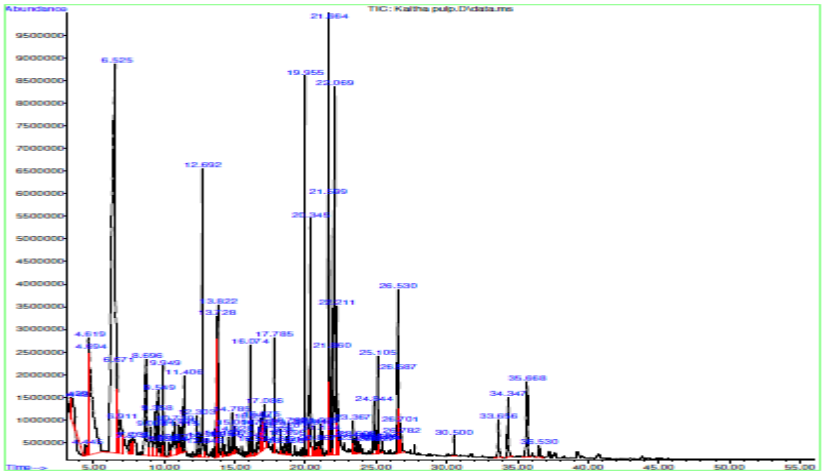

Fig 1(A): GCMS chromatogram of methanolic extract of pulp of kaitha.

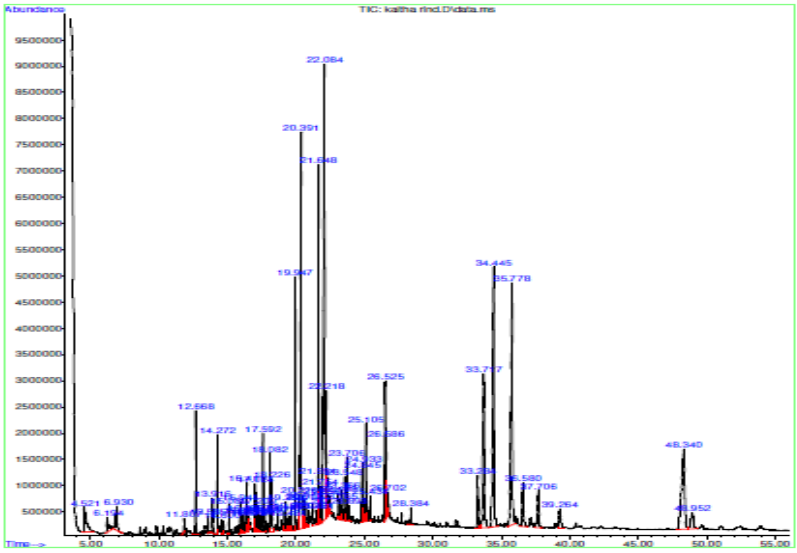

Fig 1(B): GCMS chromatogram of methanolic extract of rind of kaitha

3.5 Antibacterial activity

Three gram positive (*Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis*) and a gram negative bacterium (*Proteus mirabilis*) were used to evaluate the antibacterial activity of dried pulp and rind. Inhibiting concentrations used for both samples was 500 mg/ml. The methanolic extract of pulp was found to possess highest antibacterial activity against *Staphylococcus epidermidis* followed by *Staphylococcus aureus* and *Bacillus subtilis.*

The rind of kaitha also revealed antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*. The antibacterial activity can be attributed to the phenolic content of the sample extracts. The samples having higher phenolic content were found to be better in inhibiting the growth of bacteria hence were giving zone of clearance of greater diameter. The GC/MS screening confirms the presence of hydrocarbons, sterols, aldehydes, carboxylic acids and their esters, phenolic acids, and flavonoids which might be contributing towards these antimicrobial properties.

4. Conclusion

"Kaitha" was analyzed for nutritional, phytochemical, antioxidant and antibacterial activity for use as functional foods and nutraceutical and flavoring agents to provide health benefits. There are substantial anecdotal reports indicating that the consumption of kaitha could ameliorate a wide range of illnesses. In addition, it can be used as a food ingredient to make processed products like jams, jellies, sweets and savory chutneys. Rind of *L. acidissima* can also be used as animal feed as it does not contain any toxic compound. These results also support beneficial health claims. Thus, there is enormous scope for future research and further pharmacological investigation on *L. acidissima.*

5. Acknowledgement

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