

Original Article

SPECTROSCOPIC ANALYSIS AND ANTIBACTERIAL EFFICACY OF BIOACTIVE COMPOUNDS FROM *LIMONIA ACIDISSIMA* L. FRUIT EXTRACT AGAINST CLINICAL PATHOGENS

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ABSTRACT

Objective: To determine the phytochemical composition using spectral analysis and antibacterial activity of *Limonia acidissima* fruit methanol extract.

Methods: Phytochemical analysis was performed to determine the total alkaloids, saponins, total phenols and flavonoids content of fruit extract. Secondary metabolite compounds were determined performed by FT-IR and GC-MS analysis. Antibacterial activity assay was performed using agar well diffusion technique.

Results: The alkaloid and saponins contents of the crude methanol extract were found to be 38.47 g/100 g and 0.13 g/100 g dry matter respectively. The LF methanol extract showed total phenol contents of 33.38 µg/mg and the flavonoids was found to be 33.80 µg/mg extract. FT-IR analysis indicated the presence of phenols, alkanes, amino acids, α, β-unsaturated esters, alkenes, nitro compounds, aromatics, aliphatic amines, carboxylic acid, alkenes, and alkyl halides functional groups. The GC-MS analysis revealed the presence of linoleic acid, octadecanoic acid, hexadecanoic acid, maltol, vinyl guaiacol, furanone, and ascorbic acid. The antibacterial activity assay showed dose dependent inhibition against clinical pathogens.

Conclusion: This study elucidated the prospective value of *L. acidissima* fruit as a nutritional and medicinal source.

Keywords: *Limonia acidissima*, Phytochemical, Bioactive compounds, FT-IR, GC-MS, Antibacterial activity.

INTRODUCTION

Microbial infections and their resistance to conventional therapy have become complicated. This phenomenon has prompted researchers to identify alternative medicines, for the prevention and effective treatment of infections. Plant extracts and biologically active compounds isolated from plants have gained widespread interest, as they have the property to cure a variety of diseases [1]. Medicinal plant compounds are widely accepted as they strengthen the immunity of the host and have minimal side effects. Over the years, the use of medicinal plants has grown consistently, with the population and the emergence of diseases caused by drug resistant pathogens [2, 3].

Limonia acidissima L. belongs to a monotypic genus *Limonia* and is classified under the family Rutaceae. It is an exotic deciduous tree found in India, Pakistan, Sri Lanka and Southeast Asia. The leaves, barks and roots of the plant have medicinal property and are used for diseases related to biological imbalance. The fruits of *L. acidissima* are well documented for its use in Indian traditional medicine and are commonly known as wood apple, elephant-apple, monkey fruit, vilampazham, kath bel and kaitha [4-6]. The fruits are spherical in shape with greyish-white rind and a woody hard outer shell. *L. acidissima* fruit pulp is sweet, sticky, brown, resinous, with peculiar aroma and consists of white seeds [7]. The raw fruit pulp is helpful in the treatment of gastro intestinal problems like flatulence, diarrhoea, dysentery and piles. The plant is reported to have adaptogenic activity against blood impurities, leucorrhoea, dyspepsia, and is used in case of snake bites, throat infections [8-10].

The fruit pulp is known for its medicinal importance in treating tumors, asthma, wounds, cardiac debility, hepatitis and insect stings [11]. Bioactive compounds such as coumarins, tyramine derivatives, tannins, flavonoids have been isolated from the fruit. Further, the plant is extensively studied for its significant anti-fungal [12], anti-larvicidal activity [13], hepatoprotective [14], anti-inflammatory and anti-pyretic [15], antioxidant activity [6, 16]. Although the reports on medicinal properties of this plant are available, studies on the bioactive compounds of *L. acidissima* fruit and its efficacy against

different pathogenic bacteria are still under explored. Our study focuses on the spectroscopic analysis to analyze the bioactive compounds present in *L. acidissima* fruit and determine the *in vitro* antibacterial activity against different clinical pathogens.

MATERIALS AND METHODS

Collection of plant material

Fruits were collected in the month of January, 2014 from the foot hills of Vellingiri, Coimbatore, Tamil Nadu, India. The plant material was taxonomically identified and authenticated (Voucher No. BSI/SC/5/23/09-10/Tech-319.) by Botanical Survey of India, Southern Circle, Tamil Nadu Agricultural University (Govt. of India), Coimbatore, Tamil Nadu, India. The fruits (*L. acidissima*) were thoroughly washed under tap water. The fruit pulp was separated mechanically from the rind and shade dried for a week, coarsely powdered using mixer grinder and stored in an air-tight container for further use.

Reagents

The reagents used for the study were of analytical grade as follows: Folin-Ciocalteu's Phenol reagent (Himedia), Gallic Acid (Himedia), Dimethyl Sulphoxide (Loba Chemie), Aluminium chloride (Fisher), Sodium Hydroxide (Himedia), Ammonia solution (Himedia), Methanol (SD Fine), Ethanol, Catechin (Sigma-Aldrich), Sodium Chloride (Himedia), Di ethyl Ether (Loba Chemie), n-butanol (Himedia).

Solvent extraction

25 g of dried *Limonia* fruit (LF) pulp was extracted in 100 ml methanol (1:4) using cold percolation method. Supernatants were pooled and filtered through Whatmann filter paper No: 1 and evaporated using the rotary vacuum evaporator (Labomed, Model: R150). After evaporation of organic solvents, the extract yield was calculated and the extracts were stored at -20 °C till analysis.

Extract yield

The yield of dried extracts, based on their dry weights was 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.

Estimation of alkaloids

Estimation of crude alkaloid was performed according to Harborne et al. (1973) [17]. About 5 g of LF powder was weighed and 100 ml of 10% acetic acid in ethanol was added and allowed to stand for 4 h. This solution was filtered and the extract was concentrated on a water bath to one part of the original volume. Concentrated ammonium hydroxide solution (15N) was added drop by drop to precipitate alkaloids in the solution.

The solution was allowed to settle, the precipitate was collected and washed with dilute ammonium hydroxide solution (5N) and filtered again. The resulting alkaloid was dried and weighed.

Estimation of saponins

Saponin content was determined by the method of Obadoni and Ochuiko, (2001) [18]. About 5 g of the LF powder was mixed with 30% aqueous ethanol. The sample was heated in a hot water bath at 55 °C for 4 h with continuous shaking. The mixture was filtered and the residue was re-extracted with 50 ml of 20% ethanol. Then the extract was reduced to three fourth of volume by heating in the water bath at 90 °C. The concentrate was transferred to a separating funnel and 20 ml of diethyl ether was added and shaken vigorously.

Then the aqueous layer was subjected to sequential purification process by adding 20 ml of n-butanol and washed with 15 ml of 10 % aqueous sodium chloride. The resulting 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 in percentage.

Determination of total phenol content

Total phenol content was determined by using the Folin Ciocalteu's reagent method [19]. Briefly, 100 µl of extract was mixed with 300 µl of Folin Ciocalteu's reagent and kept at room temperature for 10 min. Then 1.5 ml of 20 % Sodium bicarbonate was added to the solution and incubated at room temperature for 3 h. After incubation absorbance of the sample was measured at 630 nm using a Shimadzu-1610 UV-VIS spectrophotometer. A standard curve was plotted using different concentration of gallic acid and the amount of total phenol was calculated as gallic acid equivalents in µg /mg of dried extract.

Determination of total flavonoids

The total flavonoid content was determined by following the method of Chang et al. (2002), [20] with slight modifications. Briefly, 350 µl of sample was added to 4 ml of distilled water and mixed with 50 µl 5% NaNO₂. The solution was incubated at 25 °C for 5 min and 30 µl of 10% AlCl₃ was added, followed by 2 ml of 1M NaOH. The reaction mixture was then diluted with 10 ml distilled water and Optical density was measured at 510 nm. Catechin was used as standard and the results were expressed as catechin equivalents in µg/mg of dried extract.

Functional group analysis using FT-IR spectroscopy

The FT-IR spectra of *L. acidissima* fruit methanol extract was determined according to Sasidharan et al. (2011) [21]. FT-IR spectrophotometer (Perkin-Elmer 1725x) at the range 4000-400 cm⁻¹ and resolution of 4 cm⁻¹. The number of scan was 20.

Determination of bioactive compounds using GC-MS analysis

Bioactive compound analysis of LF methanol extract was determined by Shimadzu Gas chromatography (Model: QP2010 Plus, Japan), with a 30 m × 0.25 mm RTX-5MS low bleed column with a thickness of 0.25 µm. The carrier gas was helium and maintained at a column flow of 1.0 ml/min (at a pressure of 53.5 kPa). 1 µl of sample from a 100 mg/ml stocks was injected by split injection (1:20) at 260 °C.

The column temperature was maintained at 50 °C/3 min followed by temperature programming at 10 °C/min to 150 °C / 8 min. The

temperature was further increased to 250 °C/ 2 min at the rate of 8 °C/min, and finally to 280 °C/5 min at a rate of 10 °C/min. The Mass Spectrometric Detector (MSD) was used in the ACQ Scan mode with an m/z ranging from 40-600 for the sample.

Screening of volatile and semi volatile compounds was performed using automatic screening software. The spectrum of the unknown components was compared with the spectrum of known components available in Wiley Online Library (Wiley08), NIST Chemistry Web book (NIST08) library. The compound name, molecular weight, and structure of the sample were determined.

Culture of pathogens

Clinical pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Vibrio cholerae*, *Klebsiella pneumoniae*, *Salmonella typhi*, and *Shigella dysenteriae* were obtained from Department of Microbiology, Karpagam University, Tamil Nadu, India. The cultures were maintained in glycerol stock at -20 °C for further use. Routine propagation of microbial cultures were carried out in Nutrient agar medium (Himedia, India).

Antibacterial activity assay

Antibacterial activity was performed by agar well diffusion assay, according to CLSI guidelines [22]. Briefly, 100 µl of the samples were added to the wells punctured on Muller Hinton Agar (Himedia, India) plates (MHA), that were uniformly pre-swabbed with test bacterial suspensions with the cell density equivalent to 0.5 McFarland standard (Himedia, India) units (1 × 10⁸ cells/ml).

DMSO was used as Negative control and Ciprofloxacin (0.01 mg/ml) was used as antibiotic control. The plates were left undisturbed for 15 min for the absorption of excess moisture and then incubated at 37 °C for overnight. After incubation, the diameter (mm) of inhibitory zones were measured.

Statistical analysis

Data were subjected to statistical analysis using SPSS software (Version 21.0, USA). The values were expressed as mean ± S. E.

RESULTS AND DISCUSSION

Phytochemical analysis of *Limonia acidissima* fruit pulp

Phytochemical contents of the pulp *Limonia* fruit (LF) were determined and presented in table 1. The values of saponins and crude alkaloids were determined on dry weight basis (g/100 g). The methanol extract of LF showed an alkaloid content of about 38.47 g/100 g, while the saponins content was estimated to be 0.13 g/100g. Alkaloids have anaesthetic and spasmolytic activity, while saponins are reported to reduce the cholesterol level and enhance immunity [23]. The total phenol content was determined and the results were expressed in terms of standard µg gallic acid (GA) equivalent/mg of LF dry weight.

In our study, methanol extract of *Limonia* fruit showed total phenol content of 33.38 µg GA equivalent /mg of dry weight of fruit. Our results were slightly different from the observation of Shipra et al. (2014) [24] who, determined total phenol content in methanol extract of wood apple pulp. The total phenol contents may vary with different fruits due to some non-phenolic reducing compounds interfering with the phenol contents of the fruit [25].

Phenol compounds are associated with quality and nutritional value of fruit such as colour, taste, aroma and flavour. Further, phenol compounds are known to enhance the defence mechanism to neutralize reactive oxygen species and protect the plants from damage caused by microbes [26].

The present study showed the presence of flavonoids in LF fruit pulp of 33.80 µg catechin equivalent/mg of LF dry weight (table 1). Our results were in affirmation with Shipra et al. (2014) [24] who reported the total flavonoids content of *Limonia* fruit methanol extract as 35.71 µg/mg. Flavonoids were reported to have a wide range of biological activities such as anti-proliferative, enzyme inhibition, induction of apoptosis, antioxidant and antibacterial activity [27].

Table 1: Phytochemical composition and polyphenolic compounds of *Limonia acidissima* fruit methanol extract

Component	Pulp
Yield percentage	7.25
Alkaloids ^a	38.47 ±5.61
Saponins ^a	0.13 ±0.02
Total Phenols ^a	33.38 ±0.20
Flavonoids ^b	33.80 ±1.08

^aValues are measured as g/100g of dry matter. ^aValues are measured as µg Gallic Acid/mg of sample. ^bValues are measured as µg Catechin/mg of sample. Except yield percentage, all values are expressed as mean ± S. E of triplicates.

FT-IR analysis of *Limonia* fruit pulp

The FTIR spectrum was performed to identify the functional group of the active components based on the peak value in the infra red radiation. The presence of intense bands at 2111.39 cm⁻¹, 1725.98 cm⁻¹, 1641.13 cm⁻¹, 1517.70 cm⁻¹, 1403.92 cm⁻¹, 1225.54 cm⁻¹, 1052.94 cm⁻¹ corresponding to -C≡C-/ C=O/C-O/ and N-O/ C-C/C-N with stretch/bend vibration respectively indicate the presence of alkynes, α, β-unsaturated esters, amino acids, nitro compounds, aromatic and aliphatic amines. The spectral range of 500-600 cm⁻¹ indicated the presence of the compound with halides groups containing C-Br/C-Cl bond and stretching vibration. Very strong absorption spectra was observed at 779.101 cm⁻¹, 816.706

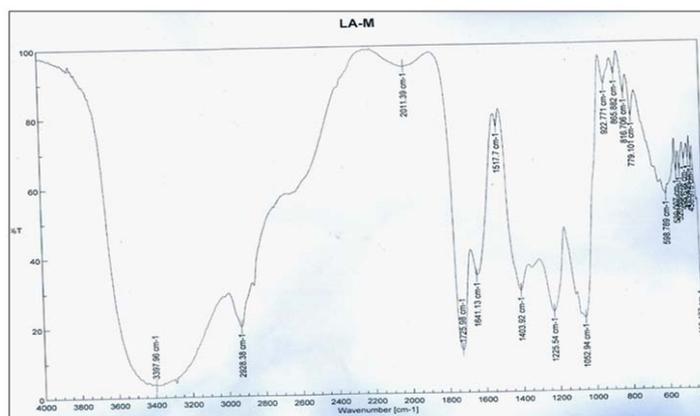
cm⁻¹, 865.882 cm⁻¹, and 922.771 cm⁻¹ corresponding to C-H/=C-H with bending vibrations which indicated the presence of aromatic and alkenes groups. The spectral peaks at 2928.38 cm⁻¹, and 3397.96 cm⁻¹ corresponds to functional groups C-H/ O-H/H with stretching vibration, which indicated the presence of amines, alcohols and phenols. Our results showed that the methanol extract of *Limonia acidissima* fruit contained alcohols, phenols, alkanes, amino acids, α, β-unsaturated esters, alkenes, nitro compounds, aromatics, aliphatic amines, carboxylic acid, alkenes, and alkyl halides compounds which showed major peaks as shown in fig. 1 and table 2.

The results indicated that the methanol extract of LF consist of amino acids which is evident from the strong absorption spectra observed at 1641.13 cm⁻¹. Our results were in affirmation with our previous study which reported the presence of essential amino acids in wood apple fruit [6]. Moreover, the presence of α, β-unsaturated esters, functional group in the LF methanol extract indicated the presence of fatty acids which could be ascertained for its medical significance. There was no absorbance between the regions 2220-2260 cm⁻¹ in the LF methanol extracts which indicated the absence of cyanide in the sample elucidating the absence of toxic substances in the fruit. This study corroborated with Sandhya *et al.* (2006) [28] who studied the functional group analysis of ethno medicinal plants for their medicinal properties. Further, the extract was found to contain compounds with amides, carboxylic acid, phenols, alcohol, and alkanes which serve as a significant pharmaceutical product for jaundice, fever and liver pain, treatment of throat infections, rheumatic joint pain, ulcer, and immune boosters [29].

Table 2: FT-IR absorption frequencies of functional groups in *L. acidissima* fruit methanol extract

S. No.	Frequency (cm ⁻¹)	Bond and type of vibration*	Functional groups
1	418.477	unknown	-
2	458.973	unknown	-
3	473.439	unknown	-
4	495.616	unknown	-
5	520.686	C-Br (m), C-Cl stretch (m)	Alkyl halides
6	539.007	C-Br (m), C-Cl stretch (m)	Alkyl halides
7	598.789	C-Br (m), C-Cl stretch (m)	Alkyl halides
8	779.101	C-H (s), =C-H bend alkenes (s)	Aromatic, Alkenes
9	816.706	C-H (s), =C-H bend alkenes (s)	Aromatic, Alkenes
10	865.882	C-H (s), =C-H bend alkenes (s)	Aromatic, Alkenes
11	922.771	O-H bend	Carboxylic acid
12	1052.94	C-N stretch	Aliphatic amines
13	1225.54	C-N stretch	Aliphatic amines
14	1403.92	C-C stretch (in-ring)	Aromatic
15	1517.70	N-O stretch	Nitro compounds
16	1641.13	C-O stretch (m)	Amino acids
17	1725.98	C=O stretch	α, β-unsaturated esters
18	2011.39	-C≡C- stretch	Alkynes
19	2928.38	C-H stretch	Amines
20	3397.96	O-H stretch, H-bonded (s,b)	Alcohols and Phenols

*Bond strength; m-medium; s-strong; b-broad.

**Fig. 1: Chromatogram showing the FT-IR Spectrum of *L. acidissima* fruit methanol (LA-Me) extract**

Secondary metabolite analysis using GC-MS

The bioactive compounds of LF methanol extract were identified using GC-MS analysis and listed in fig. 2 and table 3. The results showed that the LF pulp contained many saturated fatty acids such as dodecanoic acid, tetradecanoic acid, penta decanoic acid, docosatrienoic acid. Fatty acids had been studied for their antioxidant, anticancer, anti-inflammatory and cardio protective properties. Further, dodecanoic acid was reported for the treatment of acne and increase HDL cholesterol. The present study showed the presence of ocatdecanoic acid ($\omega 6$ fatty acid) and hexadecanoic acid ($\omega 3$ fatty acid). Omega-3 and omega-6 fatty acids are poly unsaturated fatty acids synthesized by plants and reported to influence the metabolic, immunological and cardiovascular functions of host [30].

Dietary omega-3 fatty acids was found to exhibit anti cancer activity and increase the cytokine gene expression patterns in airway epithelial cells [31, 32]. According to Scorletti and Byrne (2013) [33], omega-3 fatty acids enhanced the lipid metabolism in liver and have positive effects on the fatty liver disease. The GC-MS analysis in this study showed the presence of Linoleic acid ($\omega 6$ fatty acid) in wood apple. Linoleic acid (LA) can modulate both innate and adaptive immunity [34]. Further, Omega-6 fatty acids showed

significant development in foetal growth, particularly for the central nervous system, regulating visual acuity. Lack of essential fatty acids promotes skin inflammations and delays wound healing. Moreover, Omega-6 fatty acids was shown to inhibit proliferation of certain cancer cell lines *in vitro* and to reduce the progression of tumours in animals. Dietary supplementation of *L. acidissima* fruit was reported to increase immunoglobulin production in rat spleen lymphocytes and to reduce antigen-induced histamine and prostaglandin-2 release from sensitized guinea pig tracheae [35]. In addition, Linoleic acid was reported for antibacterial activity against different pathogens [36].

Our study showed the presence of Maltol and Vinyl guaiacol in the wood apple extract. Maltol [37], has characteristic sugary flavour and is used as flavour enhancer in candies and processed foods. Vinyl guaiacol is a phenolic substance present in plants and characterized by smoky, roasted flavour. It is used as a flavour enhancer in brewing and pharmaceutical industries [38]. Hunter et al. (2012) [39] studied the biotransformation of ferulic acid as substrates to green synthesize vinyl guaiacol. table 3 shows the presence of Furfural in the pulp which is a natural phenolic compound used in the production of drugs. Further, these furfural derivatives have been reported for their strong bactericidal activity and broad antibacterial spectrum [24, 40].

Table 3: GC-MS analysis of *Limonia acidissima* fruit methanol extract

S. No.	Compound name	RT (min)	% area	CAS number
1.	2,5-Furandione, Dihydro-3-Methylene	7.2	0.60	2170-03-8
2.	[3-(2,3-epoxypropoxy)propyl]ethoxydimethyl- Silane	7.95	0.27	17963-04-1
3.	2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4H-Pyran-4-One	10.8	0.33	28564-83-2
4.	Tetradecanoic acid	25.42	0.11	544-63-8
5.	Pentadecanoic acid	27.46	0.21	1002-84-2
6.	9-Hexadecenoic Acid, Methyl Ester	28.19	0.07	1120-25-8
7.	Hexadecanoic acid, methyl ester	28.59	0.05	112-39-0
8.	2-Hydroxycyclopentadecanone	28.9	0.06	4727-18-8
9.	n-Hexadecanoic acid	29.70	0.05	57-10-3
10.	Heptadecanoic acid, methyl ester	30.11	0.07	1731-92-6
11.	l-(+)-Ascorbic acid 2,6-dihexadecanoate	30.4	0.05	28474-90-0
12.	2-Hydroxycyclopentadecanone	30.47	0.35	4727-18-8
13.	n-Heptadecanoic acid	30.72	0.24	506-12-17
14.	Linoleic acid, methyl ester	31.1	0.11	112-63-0
15.	8,11,14-Docosatrienoic acid, methyl ester	31.22	0.17	56847-02-0
16.	Stearic acid, methyl ester	31.5	2.58	112-61-8
17.	9,12-Octadecadienoic acid (Z,Z)	32.33	0.98	1577-52-2
18.	Octadecanoic acid	32.55	20.92	57-11-4
19.	1,13-Tetradecadien-3-One	32.85	0.15	58879-40-6
20.	9-Octadecenoic Acid, Methyl Ester	33.62	0.39	112-62-9
21.	Eicosanoic acid, methyl ester	33.91	0.32	1120-28-1
22.	18-Nonadecenoic acid	34.14	0.55	76998-87-3
23.	l-(+)-Ascorbic acid 2,6-dihexadecanoate	34.41	2.99	2847490-0
24.	d-Ribose, 2-deoxy-bis(thioheptyl)-dithioacetal	36.133	5.59	123390-22-7
25.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	36.34	1.56	23470-00-0
26.	Docosanoic Acid, Methyl ester	36.55	49.23	929-77-1
27.	9,12-Octadecadienoic acid (Z,Z)-, trimethylsilyl ester	38.18	7.27	56259-07-5
28.	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	38.46	0.40	2716-53-2
29.	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)	38.52	0.15	1191-41-9
30.	Octadecanoic acid, 2,3-dihydroxypropyl ester	38.7	0.30	123-94-4
31.	1,2,3-Propanetriol	9.44	0.51	56-81-5
32.	Maltol	9.75	0.66	118-71-8
33.	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	12.15	0.11	67-47-0
34.	5-(Hydroxymethyl)-2-(dimethoxymethyl)furan	13.02	0.33	90200-14-19
35.	2-Methoxy-4-Vinylphenol (Vinylguaiacol)	13.33	0.12	7786-61-0
36.	3-Furanacetic acid, 4-Hexyl-2,5-Dihydro-2,5-Dioxo	16.62	0.21	39212-21-0
37.	Dodecanoic Acid	18.8	0.92	143-07-7
38.	3',5'-Dimethoxyacetophenone	19.05	0.80	39151-19-4
39.	Quinic acid	21.17	0.21	77-95-2

RT- Retention time; CAS Number- Chemical Abstract Services number

Antibacterial activity test

The antibacterial efficacy of *Limonia acidissima* fruit methanol extract, against different bacterial pathogens was determined using

the agar well diffusion assay (fig. 3; table 4). The assay was performed against gram positive (*S. aureus* and *B. cereus*) and gram negative (*E. coli*, *P. aeruginosa*, *S. typhi*, *S. dysenteriae*, *V. cholerae* and *K. pneumoniae*) at varying concentration (100 to 500 mg/ml). The

CONCLUSION

In conclusion, phytochemical and spectral analysis of Limonia fruit methanol extract confirms the presence of secondary metabolites, helpful in treatment of a variety of illness. The antibacterial activity elucidates that the fruit could be used as an alternative source for treatment against antibiotic resistant pathogens. Our findings substantiate the nutraceutical application of wood apple as it is a rich source of bioactive components. Wood apple fruit could be considered as a potential source for antibacterial drug development. The purification and structural elucidation of the anti microbial compound present in the fruit are under investigation.

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CONFLICT OF INTERESTS

Declared None

REFERENCES

- Guarrera PM. Traditional phytotherapy in central Italy (Marche, Abruzzo, and Latium). *Fitoterapia* 2005;76:1-25.
- Matu EN, Van Staden J. Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. *J Ethnopharmacol* 2003;87:35-41.
- Robert CMJ. Discovering new antimicrobial agents. *Int J Antimicrob Agents* 2011;37:2-9.
- Allen BM. *Malayan Fruits*. An introduction to cultivated species. Donald Moore Press Ltd. Singapore; 1967.
- Khare CP. *Indian medicinal plants: an illustrated dictionary*, Springer Science, Springer Verlag, Berlin/ Heidelberg, Germany; 2007. p. 453.
- Teepica Priya Darsini D, Maheshu V, Vishnupriya M, Nishaa S, Sasikumar JM. Antioxidant potential and amino acid analysis of underutilized tropical fruit *Limonia acidissima* L. *Free Rad Antiox* 2013;3(S):62-9.
- Vidhya R, Narain A. Development of preserved products using under exploited fruit wood apple (*Limonia Acidissima*). *Am J Food Technol* 2011;6(4):279-88.
- Yusuf M, Chowdhury JU, Wahab MA, Begum J. Medicinal plants of Bangladesh BCSIR Lab. Bangladesh; 1994. p. 62.
- Rathayake RMRNK, Sumithra HJ, Fernando MD, Keerthi B, Palipane. Effect of GRAS compounds on *Aspergillus* rot of wood-apple (*Feronia Limonia*). *Pytoparasit* 2009;37:431-6.
- Muniappan Ayyanar, Pandurangan Subash-Babu. *Limonia acidissima* Linn. (L.) Skeels: A review of its phytochemical constituents and traditional uses. *Asian Pac J Trop Biomed* 2012;240-6.
- Senthilkumar KL, Kumawat KB, Rajkumar M, Senthilkumar. Anti-diarrhoeal activity of bark extracts of *Limonia acidissima* Linn. *Res J Pharm Biol Chem* 2010;1(4):550-3.
- Bandara BMR, Gunatilaka AAL, Wijeratne EMK, Adikaram NKB. Antifungal constituents of *Limonia acidissima*. *Planta Med* 1988;54:374-5.
- Rahman AA, Gopalakrishnan G, Ghose BS, Arumugam S, Himalayan B. Effect of *Feronia limonia* on mosquito larvae. *Fitoterapia* 2000;71(5):553-5.
- Kamat CD, Khandelwal KR, Bodhankar SL, Ambawade SD, Mhetre NA. Hepatoprotective activity of leaves of *Feronia elephantum* Correa (Rutaceae) against CCl4-induced liver damage in rats. *J Nat Remed* 2003;3:148-54.
- Ahamed SM, Swamy SK, Jayaverra KN, Rao JV, Kumar VS. Anti-inflammatory antipyretic and analgesic activity of methanolic extract of *Feronia limonia* fruit pulp. *Pharmacologyonline* 2008;3:852-7.
- Ilango K, Chitra V. Wound healing and anti-oxidant activities of the fruit pulp of *Limonia acidissima* Linn. (Rutaceae) in Rats. *Trop J Pharm Res* 2010;37:120-5.
- Herborne JB. *Phytochemical methods*. Chapman and Hall London; 1973. p. 110-3.
- Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extract of some homeostatic plants in Edo and Delta States of Nigeria. *Global J Pure Appl Sci* 2001;8:203-8.
- McDonald S, Prenzler PD, Antolovich M, Robards K. Phenolic content and antioxidant activity of olive extract. *Food Chem* 2001;73:73-84.
- Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoids content in propolis by two complementary colorimetric methods. *J Food Drug Anal* 2002;10, 178-82.
- Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of bioactive compounds from plants' extracts. *Afr J Tradit Complement Altern Med* 2011;8(1):1-10.
- Clinical and laboratory standards institute. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*; approved standard. 7th ed. Clinical and Laboratory Standards Institute document M7-A7. Wayne, PA: Clinical and Laboratory Standards Institute; 2006.
- Vaya J, Belinky PA, Aviram M. Antioxidant constituents from licorice roots: Isolation, structure elucidation and antioxidative capacity towards LDL Oxidation. *Free Rad Biol Med* 1997;23:302-13.
- Shipra Pandey, Gouri Satpathy, Rajinder K Gupta. Evaluation of nutritional, phytochemical, antioxidant and antibacterial activity of exotic fruit *Limonia acidissima*. *J Pharm Phytochem* 2014;3(2):81-8.
- Fu L, Xu BT, Xu X, Qin XU, Gan R, Li HB. Antioxidant capacities and total phenolic contents of 56 wild fruits from South China. *Mol* 2010;15:8602-17.
- Veigas JM, Narayan MS, Laxman PM, Neelwarne B. Chemical nature, stability and bioefficacies of Anthocyanin from fruit peel of *Syzygium cumini* skeels. *Food Chem* 2007;105:619-27.
- Havsteen BH. The biochemistry and medical significance of the flavonoids. *Pharmacol Therap* 2002;96:67-202.
- Sandhya B, Thomas S, Isabel W, Shenbugarathai R. Ethnomedical plants used by the valaiyan community at Piranmalai Hills, Tamil Nadu, India. A pilot study. *Afr J Trad Comp Alt Med* 2006;3:101-14.
- Muruganatham S, Anbalagan G, Ramamoorthy N. FT-IR and SEM-EDS comparative analysis of medicinal plants, *Eclipta alba* and *Eclipta prostrata*. *Rom J Biophys* 2009;19:285-94.
- Das UN, Ramos EJ, Meguid MM. Metabolic alterations during inflammation and its modulation by central actions of omega-3 fatty acids. *Curr Opin Clin Nutr Metab Care* 2003;6:413-9.
- Yong Q Chen. Modulation of prostate cancer genetic risk by omega-3 and omega-6 fatty acids. *J Clin Invest* 2007;117(7):1866-75.
- Zhao YU, Peter VG, Irina AHe, Donghong W, Ting MV, Liliana G, et al. "Regulation of COX-2 Expression and IL-6 release by particulate matter in airway epithelial cells". *Am J Respir Cell Mol Biol* 2009;40(1):19-30.
- Scorletti E, Byrne CD. Omega-3 Fatty Acids, Hepatic Lipid Metabolism, and Nonalcoholic fatty liver disease. *Annu Rev Nutr* 2013;33:231-48.
- Pariza MW, Park Y, Cook ME. Mechanisms of action of conjugated linoleic acid: evidence and speculation. *Proc Soc Exp Biol Med* 2000;223:8-13.
- O'Shea M, Bassaganya-Riera J, Mohede ICM. Immunomodulatory properties of conjugated linoleic acid. *Am J Clin Nutr* 2004;79(Suppl):1199S-206S.
- Choi JS, Ha YM, Joo CU, Cho KK, Kim SJ, Choi IS. Inhibition of oral pathogens and collagenase activity by seaweed extracts. *J Environ Biol* 2012;33:115-21.
- www.fao.org/ag/agn/jecfa-additives/specs/.Additive-182.pdf
- Lasekan OO, Feijao JP, Salva TJG. Volatile flavour compounds of cooked "acha" (*Digitaria exilis*). *Food Chem* 2001;15:131-6.
- Hunter WJ, Manter DK, Van Der Lelie D. Biotransformation of ferulic acid to 4-vinylguaiacol by *Enterobacter soli* and *E. aerogenes*. *Curr Microbiol* 2012;65(6):752-7.
- Koziol JM, Macia JM. Chemical composition, nutritional evaluation and economic prospects of *Spondias purpurea* (Anacardiaceae). *Econ Bot* 1998;52(4):373-80.

41. Patil SP, Kalkar SA, Kulkarni AS. Phytochemical Screening, Antibacterial and Antioxidant activity of *Limonia acidissima* (L). *Bionano Front* 2012;5(2):131-3.
42. Sengul M, Yildiz H, Gungor N, Cetin B, Eser Z, Ercisli S. Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. *Pak J Pharm Sci* 2009;22(1):102-6.
43. Sun CQ, O'Connor CJ, Robertson AM. Antibacterial actions of fatty acids and monoglycerides against *Helicobacter pylori*. *FEMS Immunol Med Microbiol* 2003;36:9-17.
44. Jae-Suk Choi, Nam-Hee Park, Seon-Yeong Hwang, Jae Hak Sohn, Inseok Kwak, Kwang Keun Cho, *et al.* The antibacterial activity of various saturated and unsaturated fatty acids against several oral pathogens. *J Env Biol* 2013;34:673-6.