IJPSR (2021), Volume 12, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 17 March 2020; received in revised form, 15 June 2020; accepted, 28 June 2020; published 01 March 2021

PHYSICOCHEMICAL AND PHYTOCHEMICAL ANALYSIS OF METHANOLIC EXTRACT OF LEAVES AND FRUITS OF *FLACOURTIA JANGOMAS* (LOUR.) RAEUSCH

Akshaya Pai and K. Chandrakala Shenoy *

Department of Biosciences, Mangalore University, Mangalagangothri, Dakshina Kannada - 574199, Karnataka, India.

Keywords:

Physicochemical parameters, Phytochemical screening, GC-MS, EDS-SEM, *Flacourtia jangomas*

Correspondence to Author:
Dr. K. Chandrakala Shenoy

Professor,
Department of Biosciences,
Mangalore University,
Mangalagangothri, Dakshina Kannada - 574199, Karnataka, India.

E-mail:chandrakalashenoy2020@gmail.com

ABSTRACT: Plants have been used extensively as medicine for the treatment of various ailments for centuries. The present study was conducted to analyze physicochemical and phytochemical parameters of leaves and fruit of *Flacourtia jangomas* (Lour.) Raeusch Physicochemical profile showed the high total ash, water-soluble ash, and acid-soluble ash content in *F. jangomas* leaf when compared to the fruit. The water extractive value of *F. jangomas* leaves and fruit was more a better extractive compared to alcohol. Moisture content, swelling index, and foaming index of leaves were found to be 6.22%, 5.466 ml, and >100 units, whereas fruit was 8.973%, 3.433 ml, and <100 units, respectively. Phytochemical constituents of dried leaf and fruit were analyzed using qualitative and quantitative methods. Preliminary phytochemical screening showed the presence of alkaloids, glycosides, tannins, flavonoids, steroids, phenols, quinones, lignin, resins, coumarins, carbohydrates and proteins. Quantitative analysis showed high flavonoid content (868.04 ± 3.185, 80.91 ± 0.796 mg QE/g) followed by tannin (253.10 ± 0.738, 23.96 ± 0.071 mg TAE/g) then phenol (144.35 ± 0.99, 13.226 ± 0.035 mg GAE/g) and alkaloids (4.98 ± 1.161, 7.130 ± 0.317 %) in both leaf and fruit of *F. jangomas* respectively. GC-MS analysis showed the presence of 19 phytoconstituents in leaf and 7 phytoconstituents in fruit extract. EDS-SEM analysis showed the presence of N, O, K and C, Ca in leaf and N, O, K, C in fruit. The results provide referential information of the plant, and various phytoconstituents suggest that this plant exhibit pharmaceutical significance.

INTRODUCTION: India has one of the oldest, richest, and most diverse traditions associated with the use of medicinal plants [1]. Plants have been used extensively for the treatment of various ailments. According to the World Health Organization (WHO), around 85-90% of the world's population uses herbal medicines [2]. Plants used in traditional herbal medicines may not be as useful as asserted or may have therapeutic properties in addition. Therefore, proper scientific understanding is requiredto explore the medicinal plants for curative purpose [3]. Due to the lack of inadequate synthetic drugs WHO has recommended the evaluation of physicochemical and phytochemical parameters of medicinal plants for its efficacy. These parameters help in identification and authentication of the plant material [4].

Flacourtia jangomas (Lour.) Raeusch (family Salicaceae) is commonly known as Paniala or Indian plum. Leaves are alternate, spirally arranged, rarely ovate-lanceolate, and pale pink when young. The fruits are dark-red when ripe. Traditionally, various parts of the plant are used for the treatment of numerous ailments. Sasi *et al*.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(3).1671-78

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(3).1671-78

reported that the leaves are astringent and stomachic. The fruits are used to overcome various disorders such as quench thirst, biliousness, fevers, stomach upset, and diarrhea [5]. Therefore, the aim of the present work was to ascertain chemical standards like physicochemical and phytochemical testing of the leaves and fruits of *Flacourtia jangomas* (Lour.) Raeusch.

MATERIALS AND METHODS:

Plant Material: *Flacourtia jangomas* (Lour.) Raeusch was collected from Bellikoth, Kasargod District of Kerala, India. It was taxonomically identified and authenticated by Dr. T. S. Swapna, Professor, and Head, Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram, and the voucher specimen was deposited with Accession number KUBH10169. The voucher specimen of the plant *F. jangomas* (Lour.) Raeusch was preserved in the Herbarium of Department of Botany, University of Kerala, Kariavattom.

Physicochemical Analysis: The leaves and fruits of *F. jangomas* were collected, cleaned and shade dried for about 3 weeks and 7 weeks, respectively. Dried leaves and fruits were then homogenized to obtain coarse powder and stored in an airtight container for further use.

The various physicochemical parameters such as moisture content, ash values including total ash, acid insoluble and soluble ash, water-soluble and insoluble ash and sulphated ash, water-soluble extractive value, alcohol soluble extractive value, foaming index, swelling index, and pH of powdered plant material were determined by the methods described in Indian Pharmacopoeia [6] and WHO guidelines [7].

Fluorescence analysis: The leaf and fruit powder of *F. jangomas* was subjected to fluorescence analysis, after treating with various chemical reagents like methanol, chloroform, ethanol, diethyl ether, petroleum ether, acetone, benzene, glacial acetic acid, sulphuric acid, hydrochloric acid, nitric acid, 1N sodium hydroxide and distilled water against visible and UV light (312 nm) [8].

Phytochemical Screening:

Qualitative Analysis: For preliminary phyto-chemical screening, the powdered leaf and fruit material were extracted with methanol in Soxhlet apparatus, and the solvent extract was concentrated using a rotary vacuum evaporator and stored at 4ºC until further use. The extract thus obtained was subjected to different qualitative analyses to discern the bioactive components. The presence of alkaloids, glycosides, tannins, flavonoids, fat and fixed oils, steroids, phenol, quinones, lignin, resins, saponins, coumarins, chalcones, proteins, and carbohydrates were determined according to the Harborne method described by Joshi *et al*., [9] and Irum *et al*.,[10].

Quantitative Analysis:

Determination of Total Phenolic Content: The total phenolic content was determined by the method of Jose and Anilkumar [11] with slight modifications. Gallic acid was used as a standard, and the total phenolics were expressed as mg/g gallic acid equivalents (GAE). Briefly, 1 ml of a standard solution of different concentrations (10-100 µg/ml) of gallic acid and 1 mg/ml of plant extract were prepared in methanol. 0.5 ml of sample mixed with 2.5 ml of a 10% Folin-Ciocalteu's reagent and 2ml of 7.5% sodium carbonate. The tubes were covered and allowed to stand for 30 min at room temperature, and the absorbance was read at 760 nm spectrometrically.

Determination of the Total Flavonoid Content: Total flavonoid content was determined using the Aluminum chloride method of Jose and Anilkumar [11] with slight modifications. In this method quercetin was used as standard, and flavonoid contents were measured as quercetin equivalent (QE). Briefly, 1 ml of standard solution (100-500µg/ml) and 1mg/ml extract were prepared in methanol. 0.5 ml of sample was taken into a test tube containing 4ml of distilled water. 0.3 ml of 5% $NaNO_2$ added to the test tube. After 5 min, 0.3 ml 10% $AlCl_3$ and 2 ml of 1M NaOH were added to the mixture and made up the volume to 10 ml with distilled water. The absorbance was noted at 510 nm using a UV-Visible spectrophotometer. Standard was prepared in the same manner.

Determination of the Total Tannin Content: The total tannin content was determined by Folin-Ciocalteu method of Chandran and Indira [12] with slight modifications. Tannic acid was used as standard, and tannin content was expressed as mg/g

of tannic acid equivalents (TAE). Briefly, 1 ml of standard solution (10-150 µg/ml) was prepared in distilled water, and 1mg/ml extract was prepared in methanol. 0.5 ml of sample was taken into a test tube; containing 7.5 ml of distilled water.0.5 ml of Folin-Ciocalteu's phenol reagent and 1 ml of 35% sodium carbonate solution were added to the test tube and diluted to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. Standard was prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 700 nm with an UV/ Visible spectrophotometer.

Determination of Alkaloid Content: Alkaloid content was determined gravimetrically by the method of Roghini and Vijayalakshmi [13]. One gram of the sample was taken in a beaker. 200 ml of 10% acetic acid in ethanol was added to it and left as such for 4 h. It was then filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated NH_4OH was added drop wise to the extract until the precipitation was complete. The whole solution was left to settle and the precipitate was collected and washed with dilute NH_4OH and then filtered. The residue is the alkaloid, which was dried and weighed.

GC-MS analysis: The phytochemical investigation of methanolic extract of both leaf and fruit of *F. jangomas* was performed on Shimadzu GC-MS (Model Number: QP2010S) instrument and GC-MS Software - GCMS Solutions was used for analysis. Experimental conditions of the GC-MS system were as follows: Rxi-5Sil MS column, dimension: 30 Mts, ID: 0.25 mm, film thickness: 0.25 µm. A flow rate of mobile phase (carrier gas: He) was set at 1.0 ml/min. In the gas chromatography part, the temperature program (oven temperature) was 80 °C raised to 280 °C at 5 °C / min, and injection volume was 1 µl. Samples dissolved in methanol were run fully at a range of 50-500 m/z and the results were compared by using Wiley Spectral library search program (Wiley 8) and NIST mass spectral database (NIST 11).

EDS Analysis: The semi-quantification elemental analysis of leaf and fruit of *F. jangomas* was performed using Scanning Electron Microscope-Energy Dispersive Spectrometer (SEM-EDS) equipment. OXFORD INCA software was used for Digital Imaging and Spectral Collection.

Statistical Analysis: Values have been expressed as mean ± standard deviation (n=3). Statistical comparisons of physicochemical and phyto-chemical parameters *F. jangomas* leaf and fruit were made with Student t-test

RESULTS AND DISCUSSION:

Physicochemical Analysis: Evaluation of physicochemical parameters is important in identifying adulteration or inappropriate handling of drugs [4]. The physicochemical profile of leaf and fruit of *F. jangomas* is shown in **Table 1**.

TABLE 1: PHYSICO-CHEMICAL STANDARDIZATION OF LEAVES AND FRUITS OF *F. JANGOMAS*

S. no.	Parameters	*F. jangomas* Leaf (%)	*F. jangomas* Fruit (%)
1	Moisture content	6.228±0.241	8.973±1.040*
2	Total ash	9.848±0.070	2.993±0.490***
3	Acid insoluble ash	0.163±0.282	0.83±0.285*
4	Acid soluble ash	9.286±0.576	2.163±0.282***
5	Water insoluble ash	7.166±0.763	1.153±0.574***
6	Water soluble ash	2.166±0.288	0.803±0.265**
7	Sulphated ash	14.353±0.495	3.64±0.277***
8.	Water soluble extractives	22.695±1.945	62.333±0.577***
9.	Alcohol soluble extractives	19.76±0.915	36.666±1.154***
10.	Foaming index	>100units±32.995	<100units
11.	Swelling index	5.466ml±0.057	3.433ml±0.057***
12.	Foreign matter	Nil	Nil

The values represent Mean ± SD of three replicates. *F. jangomas* fruit *vs F. jangomas* leaf: * p<0.01 ** p<0.003 *** p<0.0001 (Student t- test).

The total ash content, water-soluble ash and acid-soluble ash of *F. jangomas* leaf was found to be more when compared to the fruit.

Ash values are used to ascertain the quality and purity of crude drug. The water-soluble ash is used to evaluate the amount of inorganic matter and acid

insoluble ash measures the amount of silica present and it shows contamination with earthy material. Water soluble ash is the water-soluble part of the total ash [14]. Water extractive value of *F. jangomas* leaves and fruit was more, a better extractive compared to alcohol. Moisture content, swelling index and foaming index were found to be 6.22%, 5.466 ml and >100 whereas fruit was 8.973%, 3.433 ml and <100 units respectively. Moisture content of drug was found to beless, thus prevents the growth of microorganisms during storage [14].

Fluorescence Analysis: In fluorescence analysis characteristic color changes were observed with various chemical reagents in both leaf and fruit of *F. jangomas* under visible and UV light (312 nm), it showed different colors of the powder in the presence or absence of chemical constituents **Table 2.**

When the substances are not fluorescent by themselves, the chemical reagents promote their transformation into fluorescent derivatives [4].

TABLE 2: FLUORESCENCE ANALYSIS OF F. JANGOMASLEAF AND FRUIT POWDER

Powdered Drug	*F. jangomas* Leaf		*F. jangomas* Fruit	
	Visible/ Day Light	UV (312 nm)	Visible/ Day Light	UV (312 nm)
Powder + Methanol	Dark green	Pink	Light brown	White
Powder + Chloroform	Brown	Neon pink	Colourless	Bluish green
Powder + Ethanol	Dark green	Neon pink	Colourless	Dull white
Powder + Diethyl ether	Venom green	Neon pink	Colourless	Dull white
Powder + Petroleum ether	Light green	Neon pink	Colourless	Dull white
Powder + Acetone	Dark green	Neon pink	Colourless	Dull white
Powder + Benzene	Brown	Neon pink	Colourless	Bluish green
Powder + Glacial acetic acid	Brown	Pink	Light red	Violet
Powder + Sulphuric acid	Niger brown	Cyan blue	Black	Violet
Powder + Hydrochloric acid	Niger brown	Light purple	Brown	Violet
Powder + Nitric acid	Orange brown	Light purple	Light brown	Violet
Powder +1N Sodium hydroxide	Dark brown	Light purple	Black	Violet
Powder + Distilled water	Reddish brown	Light purple	Light red	Bluish green

Phytochemical Screening: Methanolic extract of leaf and fruit of *F. jangomas* were subjected to qualitative chemical tests. The preliminary phytochemical analysis has revealed the presence of various phytochemicals such as alkaloids, glycosides, tannins, flavonoids, steroids, phenols, quinone, lignin, resin, coumarin, carbohydrates and proteins but trace amounts of fats and fixed oils were present and saponins were absent in both leaf and fruit extract **Table 3A** and **3B**. Quantitative analysis of methanolic extract of *Flacourtia jangomas* leaf and fruit was found to possess phenols (144.35 ± 0.99, 13.226 ± 0.035 mg GAE/g), flavonoids (868.04 ± 3.185, 80.91 ± 0.796 mg QE/g), tannins (253.10 ± 0.738, 23.96 ± 0.071 mg TAE/g); alkaloids (4.98 ± 1.161, 7.130 ± 0.317 %) respectively **Table 4**. When compared to fruit extract, leaf extract possesses more phenol, flavonoid, tannin, alkaloid content ($p<0.0001$, Student t-test). Thus, results indicate that the plant holds high profile values and can be used to cure various ailments.

TABLE 3A: QUALITATIVE PHYTOCHEMICAL SCREENING OF METHANOLIC EXTRACT OF *F. JANGOMAS* LEAVES AND FRUITS

Phytochemical tests	*F. jangomas* leaf	*F. jangomas* fruit
Alkaloids		
Dragendorff's test	+++	+++
Mayer's test	+	++
Wagner's test	++	++
Hager's test	++	++
Glycosides		
Legal's test	+++	+++
Keller kiliani test	++	+++
Borntrager's test	+++	-
Tannins		
Ferric chloride test	+++	+++
Lead acetate test	+	+

Alkaline reagent test	+++	+++
Flavonoids		
Shinoda's test	-	-
Lead acetate test	++	++
Alkaline reagent test	+++	+
Ferric chloride test	+++	+
Fats and Fixed Oils		
Stain test	+	+
NaOH	-	+
Saponification	-	-
Steriods		
Salkowski test	+++	+++
Phenols		
Phenol test	+++	+
Ellagic test	-	-
Quinone	+++	+
Lignin	+++	+++
Resin	+++	+
Saponin	-	-
Coumarin	+++	+
Chalcones	+++	-

++ present in moderate; +++ present in more quantity; - Absent

TABLE 3B: QUALITATIVE PHYTOCHEMICAL SCREENING OF METHANOLIC EXTRACT OF *F. JANGOMAS* LEAVES AND FRUITS

Phytochemical tests	*F. jangomas* leaf	*F. jangomas* fruit
Carbohydrates		
Molisch's test	+++	+++
Benedict's test	++	+++
Fehling's test	+	++
Barfoed test	+	+++
Proteins		
Biuret test	-	-
Ninhydrin test	+++	++
Protein containing S	+++	+

++ present in moderate; +++ present in more quantity; - Absent

TABLE 4: QUANTITATIVE PHYTOCHEMICAL ESTIMATION OF METHANOLIC EXTRACT OF *F. JANGOMAS* LEAVES AND FRUITS

Phytochemical Constituents	*F. jangomas* Leaf	*F. jangomas* Fruit
Total phenolic (mg/g) content (in GAE*)	144.35±0.99	13.226±0.035**
Total flavonoid (mg/g) content (in QE*)	868.04±3.185	80.91±0.796**
Total tannin (mg/g) content (in TAE*)	253.10±0.738	23.96±0.071**
Total alkaloids (%)	4.98±1.161	7.130±0.317*

Results are mean of triplicate determinations on the basis of reference standard ± standard deviation. *F. jangomas* fruit *vs F. jangomas* leaf: * p<0.03 **p<0.0001 (Student t-test). GAE*- Gallic acid equivalent; QE*- Quercetin equivalent; TAE*- Tannic acid equivalent.

GC-MS Analysis: The spectrum profile of GC-MS analysis confirmed the presence of nineteen major constituents in the methanolic extract of *F. jangomas* leaves and seven major constituents in the methanolic extract of *F. jangomas* fruits as presented in **Table 5** and **Table 6,** respectively.

Fig. 1 and **Fig. 2** revealed the retention time and the spectral peaks of the bioactive compounds present in *F. jangomas* leaf and fruit extract respectively.

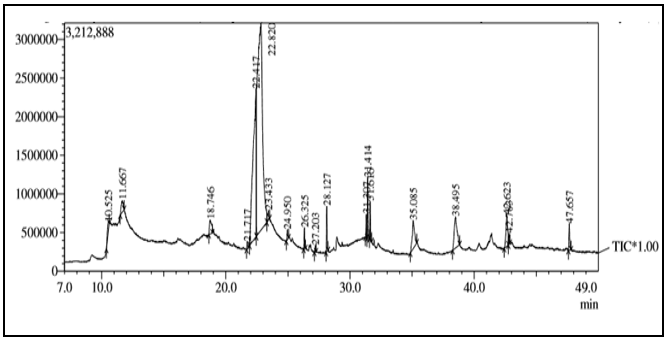

FIG. 1: GC-MS CHROMATOGRAM OF METHANOLIC EXTRACT OF *F. JANGOMAS* LEAF

TABLE 5: PHYTOCONSTITUENTS IDENTIFIED IN THE METHANOL EXTRACT OF *F. JANGOMAS* LEAF

S. no.	Retention time	Name of the Compound	Molecular Formula	Area Percentage	Nature of the Compound
1	10.525	Benzoic Acid	$C_7H_6O_2$	0.84	Aromatic carboxylic acid
2	11.667	2,3-Dihydro-Benzofuran	C_8H_8O	1.16	Coumaran
3	18.746	Decanal	$C_{10}H_{20}O$	1.52	Saturated fatty aldehyde
4	21.717	Megastigmatrienone	$C_{13}H_{18}O$	0.32	Ketone
5	22.417	4-Nitro-5-Hydroxy-1,2-Dimethylindole	$C_{10}H_{10}N_2O_3$	23.42	-
6	22.820	Chinasaure	$C_7H_{12}O_6$	54.19	Cyclic polyol
7	23.433	Blumenol	-	0.68	
8	24.950	Calendin	$C_{11}H_{16}O_3$	0.39	Monoterpenoid lactone
9	26.325	Phytol, Acetate	$C_{22}H_{42}O_2$	0.52	-
10	27.203	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol	$C_{20}H_{40}O$	0.17	Acyclic diterpene alcohol
11	28.127	Hexadecanoic Acid, Methyl Ester	$C_{17}H_{34}O_2$	1.22	Fatty acid methyl esters.
12	31.307	Methyl Octadeca-9,12-Dienoate	$C_{19}H_{34}O_2$	0.67	Fatty acid methyl esters
13	31.414	Linolenic Acid Methyl Ester	$C_{19}H_{34}O_2$	2.02	Fatty acid methyl esters
14	31.616	Phytol, Acetate	$C_{22}H_{42}O_2$	1.05	-
15	35.085	2-Octyl Benzoate	$C_{15}H_{22}O_2$	3.29	Benzoic acid esters
16	38.495	Stigmast-5-En-3-Ol, (3.Beta.)-	$C_{29}H_{50}O$	4.43	Phytosterol
17	42.623	Lupeol	$C_{30}H_{50}O$	2.64	Pentacyclic triterpenoid
18	42.783	Squalene	$C_{30}H_{62}$	0.40	Steriods
19	47.657	Vitamin E	$C_{29}H_{50}O_2$	1.09	Vitamins

TABLE 6: PHYTOCONSTITUENTS IDENTIFIED IN THE METHANOL EXTRACT OF *F. JANGOMAS* FRUIT

S. no.	Retention Time	Name of the Compound	Molecular formula	Area Percentage	Nature of the Compound
1	9.141	4H-Pyran-4-One, 2,3-Dihydro-3,5-Dihydroxy-6 Methyl-	$C_6H_80_4$	3.07	Flavonoid fraction
2	11.809	5-Hydroxymethylfurfural	$C_6H_6O_3$	69.63	Sugar
3	22.600	Quinic acid	$C_7H_{12}O_6$	24.04	Cyclic polyol
4	24.049	Chinasaure	$C_7H_{12}O_6$	1.67	Cyclic polyol
5	28.127	Methyl Palmitate	$C_{17}H_{34}O_2$	0.50	Fatty acid methyl ester
6	31.308	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}0_2$	0.55	Fatty acid methyl ester
7	31.436	9-Octadecenoic Acid (Z)-, Methyl Ester	$C_{19}H_{36}O_2$	0.55	Fatty acid methyl ester

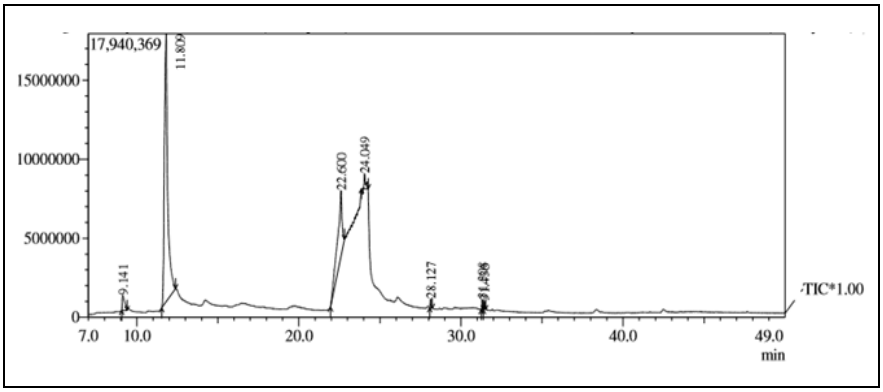

FIG. 2: GC-MS CHROMATOGRAM OF METHANOLIC EXTRACT OF F. *JANGOMAS* FRUIT

EDS Analysis: Results of the elemental composition of *F. jangomas* leaf and fruit powder using SEM-EDS technique is showed in **Table 7** and SEM-EDX spectra are showed in **Fig. 3**.

F. jangomas leaf sample showed a high concentration of Oxygen and Carbon, a moderate amount of calcium, and trace quantities of nitrogen and potassium. While *F. jangomas* fruit sample showed a high concentration of Oxygen and Carbon and a moderate amount of nitrogen and potassium.

TABLE 7: THE PERCENTAGE OF TRACE ELEMENTS PRESENT IN THE LEAF AND FRUIT OF *F. JANGOMAS*

F. jangomas Plant Parts	Elements	Weight Percentage	Atomic Percentage
Leaf	Nitrogen	0.81	3.07
	Oxygen	18.91	62.77
	Potassium	0.77	1.05
	Calcium	1.30	1.72
	Carbon	7.10	31.39
Fruit	Nitrogen	0.52	2.01
	Oxygen	19.23	64.52
	Potassium	0.88	1.21
	Carbon	7.22	32.26

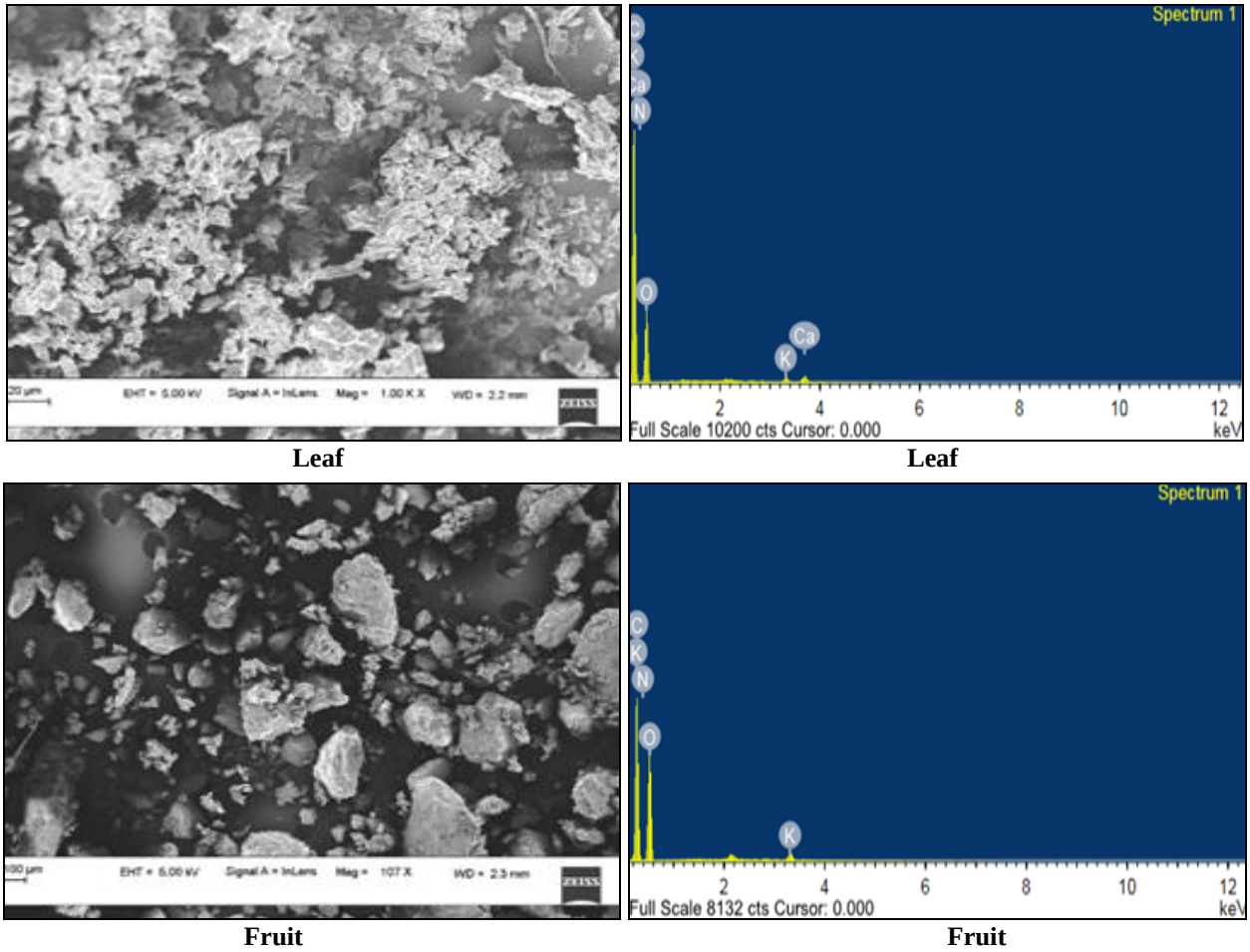

FIG. 3: SEM-EDS SPECTRUM OF *F. JANGOMAS* LEAF AND FRUIT

CONCLUSION: The present study provides information on physicochemical parameters and phytochemicals present in methanolic extract of leaves and fruit of *Flacourtia jangomas* (Lour.)

Raeusch can be used for the identification and authentication of plant material. Quantitative and GC-MS analysis reveals that the leaves and the fruit are a rich source of phytoconstituents indicating that the plant material can be further subjected to elucidate the possible mechanism of action in order to explore its medicinal use.

ACKNOWLEDGEMENT: The author's acknowledge the Department of Biosciences, Mangalore University, for providing DST-FIST and UGC-SAP supported laboratory facilities and also acknowledge DST-PURSE laboratory Mangalore University.

The authors wish to acknowledge the Council of Scientific and Industrial Research University Grants Commission (CSIR-UGC), New Delhi, for financial assistance.

CONFLICTS OF INTEREST: Authors have no conflicts of interest.

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How to cite this article:
Pai A and Shenoy KC: Physicochemical and phytochemical analysis of methanolic extract of leaves and fruits of *Flacourtia jangomas* (lour.) raeusch. Int J Pharm Sci & Res 2021; 12(3): 1671-78. doi: 10.13040/IJPSR.0975-8232.12(3).1671-78.