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A COMPREHENSIVE PHYSICO-CHEMICAL AND SPECTROSCOPIC CHARACTERIZATION OF BENGAL AMLOKI (*PHYLLANTHUS EMBLICA* L.) TOWARDS ITS MEDICINAL POTENTIAL

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ABSTRACT

The present investigation offers a novel approach by scientifically revalidating traditional beliefs surrounding the medicinal value of Bengal amloki (*Phyllanthus emblica* L.) through comprehensive physicochemical, spectroscopic and chromatographic analyses. This study aimed to determine the bioactive potential and establish traditional beliefs within the framework of scientific interpretation, and to characterize the physicochemical and spectroscopic properties of the fruits of Bengal amloki. The fruits' chemical analysis was done to assess their bioactive potential as a source of alternative medicine and their ability to treat ailments. The nutritious fruits of Bengal amloki (also known as amla or Indian gooseberry) revealed C, H, O and N contents of 48.773±0.211%, 4.858±0.222%, 42.411±0.447% and 1.844±0.057%, respectively. Based on Fourier Transform Infrared Spectroscopy (FTIR) results, strong bonds between C-O, O-H, N-H, O=C=O, C-H, and O-H molecules supported the presence of primary alcohol, carboxylic acid, alkene, carbon dioxide, alkane and phenol, respectively. Several significant bioactive phyto-constituents have also been detected by Gas chromatography–mass spectrometry (GC-MS) screening, whose bioactivities are believed to be useful in the management of various disorders. The results showed that biomass with high fixed carbon (FC), high volatile matter (VM) and low Compositional analysis (CA) had the best efficiency, antioxidant properties, and potential for converting energy.

Keywords: *Phyllanthus emblica* L., Physico-chemical analysis, FTIR, TGA, XRD, GC-MS, Bioactivity

INTRODUCTION

Traditionally, plants have been used in the management of a wide range of illnesses and ailments since time immemorial. Due to the fact that medicinal plants are the source of numerous potentially bioactive molecules, there has recently been an increased need for additional medications derived from plant sources (**Bagchi *et al*., 2021a; Darro & Khan, 2023; De & Sharma, 2023; De *et al*., 2023; Chakrabarty *et al*., 2024; Das *et al*., 2025**). Modern tools and the expansion of the pharmaceutical industry can be directly attributed to the development of methods that have made it possible to recognize and isolate the physiologically active phytoconstituents (**Kar *et al*., 2022; Sarkar *et al*., 2022; Shriwas & Sharma, 2023**). The discovery of novel therapeutics (**Pye *et al*., 2017; Bagchi *et al*., 2021a; Dey-Ray et al., 2024; Darro & Khan, 2024; Rai & Sharma, 2024**) is a pressing issue for modern academics to establish ancient beliefs in the context of accurate documentation and scientific interpretation.

In Indian traditional medicine, such as Ayurveda, Bengal Amloki, *P. emblica*, also known as "Indian Goosberry" or "Amla," is one of the most significant plants with several health-improving characteristics. The fruits are more important than any of its other parts in the managemement of a variety of ailments owing to their diuretic, purgative, liver tonic, stomachic, alterative, antipyretic, restorative, anti-inflammatory, hair tonic, peptic ulcer and dyspepsia, to prevent hair loss, and as a digestive aid (**Bhandari & Kamdod, 2012; Acharya, 2016a,b**). The fruits are believed to contain numerous bioactive phytoconstituents, including ellagic acid, tannins, polyphenolic compounds, emblicanin A, emblicanin B, alkaloids, and vitamin C. In preclinical study, Bengal amloki has been reported to exhibit analgesic, antipyretic, antitussive, adaptogenic, cardioprotective, gastroprotective, anti-anaemic, antiatherogenic, gastroprotective, antihypercholesterolemic, wound healing, antidiarrheal, antiatherosclerotic, hepatoprotective, nephroprotective, neuroprotective, anticarcinogenic, chemomodulatory, chemopreventive, radiomodulatory, antioxidant, anti-inflammatory, antimutagenic, free radical scavenging, and immunomodulatory properties (**Bhandari & Kamdod, 2012**). Ethnobotanical study in India has reported the use of the fruit as an anti-emetic, a fever reducer, and for indigestion in Madhya Pradesh, while people in Uttar Pradesh use it for indigestion and bronchitis, in Jharkhand to revive their taste, and in Odisha state, people consume the fruit against thirst (**Acharya, 2015**).

In the deciduous forests of India's West Bengal, the plant is widely grown in the southern parts, mixed plains, as well as lower hill forests in the north of the state. The deciduous tree is believed to produce Bengali amloki fruits of therapeutic value, particularly containing a lot of vitamin C. Therefore, some differences in the phytochemical composition may relate to the differing therapeutic potentialities among fruits from diverse phyto-geographical areas. Using modern techniques such as FTIR (Fourier Transform Infrared Spectroscopy), TGA (XRD (X-ray

Diffraction), and GC-MS (Gas Chromatography-Mass Spectrometry), the fruits of Bengal amloki plant have been analyzed physicochemically and spectroscopically in this controlled study experiment to reveal bioactive chemicals that might be of therapeutic use. Thus, the primary goal of this study was to characterize the fruits of the medicinal plant *P. emblica* L., belonging to the family Phyllanthaceae.

MATERIAL AND METHODS

Study area

Disease free fruit samples were collected from the North Bengal University campus in Siliguri, West Bengal, India (Fig.1). The location's latitude and longitude were recorded as $26^0 42' 18.569''$N to $26^0 43' 2.496''$N and $88^0 20' 39.359''$E to $88^0 21' 43.049''$E, respectively. Photographs of the fruits were also taken (Fig. 2). Impurities such as sand and dust were manually removed.

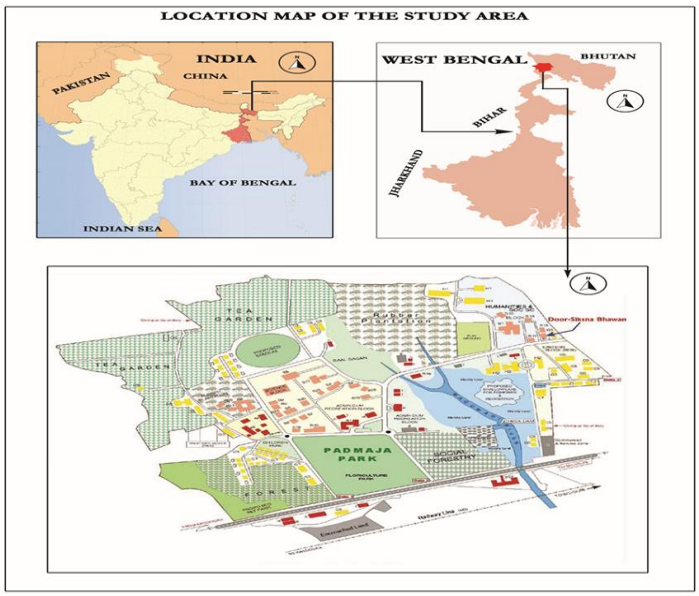

Figure 1 Map of the study area in the North Bengal University Campus in Siliguri, West Bengal, India.

Sample collection

The samples were collected using scissors, knives, high-resolution cameras, a field notebook, a geographic information system (GIS), glue, gloves, and polythene packets for effortless processing. The samples were collected from their natural habitats, which contained no herbicides or pesticides. After collecting plant samples from the area, they were processed as early as possible to prevent the deterioration of secondary metabolites.

Figure 2 Collection of Bengal amloki (*Phyllanthus emblica* L.) fruits.

Herbarium preparation, identification and authentication

Collected twigs were placed in a herbarium sheet, whereas the fruit parts were cleaned under running water from the tap, and extra water, if any, was absorbed with tissue paper. The species was identified and/or authenticated by a qualified botanist from the Department of Life Science, Dr. C. V. Raman University, Kota, Bilaspur, India.

Processing

With a sharp knife, the fruit pulp was divided into equal portions and sun-dried for 7 – 8 days in open air. The dried material was ground into powder to reach a minimum particle size of 1mm. After being sieved, the ground fruit sample was placed in an airtight container.

Size measurement

After the specimen had dried fully, it was ground into a powder using a Wiley Mill grinder (India). A sieve with a mesh size of 40µm was used to sift the powder. The particle sizes were employed to characterize Bengal Amlaki.

Preparation of extracts

Fruits were carefully washed with regular running tap water as soon as they were collected from the field. The fruit pulp was cut into smaller pieces and dried at room temperature in shade. With the aid of a grinding machine, dried fruit pulp was reduced to powder. The powder was kept in an airtight container, with the experimental samples properly labeled. Drying and labelling of the experimental samples collected from various locations, at various times and from diseased as well as healthy fruits were done individually. Then the powdered plant materials weighing 10g by electronic balance were soaked in methanol and distilled water using separate conical flasks. To reduce volatilization/evaporation of the solvent, the mouths of the conical flasks were closed with aluminium foil. Each experimental flask was kept in a rotary shaker for 5 days in laboratory. After five days, each solvent and its solubilized components were gathered and filtered using Whatman No. 1 filter paper. All the chemicals used in this study were of analytical grade NICE Chemical Pvt. Ltd. Kerala, India).

Proximate analysis and higher heating value

For biomass energy utilisation, proximate analysis of fuel is essential because it shows the percentage of material that burns as gas (volatile matter), solid (fixed carbon), and inorganic waste (ash) (**De Marco *et al*., 2014**). The convection oven dry method (**Leonel *et al*., 2018**) was employed to ascertain the biomass sample's moisture content (%). The following formula (**Sluiter *et al*., 2008**) was used to determine the moisture and total solid content:

$$Moisture\ (\%) = \left(1 - \frac{a-b}{c}\right) X\ 100$$

where, a = Weight of the dish plus the dried sample, b = weight of the dish, and c = weight of the sample when received.
Total solid (%) = 100 – moisture (%)
The computation as given below (ASTM D 3175-89, 1989) demonstrates how the variance in weight loss determines the volatile content:

C% = [(a-b)/a)] x 100.

Where, a = weight of the sample before heating, b = weight of the sample after heating, C = weight loss.
Volatile matter in sample (%) = C - D
Where, D = moisture %.
Weight difference determines the percentage of ash content (NREL/TP-510-42622, 2008). Fixed carbon (FC) content was calculated using the empirical formula (Pazo *et al*., 2010) displayed in the following table:
FC = 100 – (moisture % + volatile % + ash %)
The C, H, N, S, and O elemental analyzer was finally used to examine the biomass samples (Eurovector EA3000, India). The analyzer was calibrated with five tin capsules containing a 5L-cystine test. The medication was a tin tablet containing 0.1 mg of biomass powder. It was heated to 98^0C using a constant stream of helium gas that had been augmented with oxygen. The data were analyzed with Callidus® software to ascertain the elemental composition of the biomass.
Using the elemental composition, calorific values of the biomass samples were calculated (**Demirbas, 1997**) as follows:
HHV = [33.5 x % of C + 142.3 x % of H – 15.4 x % of O – 14.5 x % of N] x 10^{-2}
where HHV stands for higher heating value and C, H, O, and N are for carbon, hydrogen, oxygen, and nitrogen percentages.

Thermogravimetric analysis (TGA)

One of the most persuasive technologies to determine the massive mass-reducing kinetics while decomposing the biomass in a pure nitrogen-rich atmosphere is the thermal degradation analysis or thermogravimetric analysis (TGA). On the collected samples, TGA was done using the thermogravimetric analyser (TGA 50H, SHIMADZU, Kyoto, Japan). A 150 ml crucible was made using acetone-dipped tissue paper. The alumina crucible was calibrated with another crucible that served as control. Biomass sample required in the crucible in powder form was 5 - 10 mg and was arranged side by side with care to prevent the related samples from touching. Devolatilization characteristics were recorded between 40 and 90^0C with a heating rate of 10°C per minute and a constant, pure nitrogen gas flow rate of 30 ml/min. As the biomass samples were thermally decomposed, the level of nitrogen gas was checked regularly.

Compositional analysis

Fibra plus automatic fiber estimate tool was used to calculate the biomass sample's polysaccharide content (Pelican, India). In order to determine the neutral detergent fibre (NDF), 0.5 g of sodium sulfite and 100 ml of neutral detergent solution were added to a crucible containing 0.5 – 1 g of powdered biomass sample. The crucible was heated to 400°C for boiling, and after boiling, it was refluxed for 60 minutes. After filtration, the mixer was cleaned with acetone and water. The crucible's weight was determined after 8 hours of drying at 105°C. The following formula (Wang *et al*., 2014) was used to determine the NDF:

$$NDF\% = \frac{(wt\ of\ crucible + NDF) - wt\ of\ crucible}{wt\ of\ sample} X\ 100$$

However, analyzing acid detergent fibre (ADF) required another technique similar to NDF estimate.

$$ADF\% = \frac{(wt\ of\ crucible + ADF) - wt\ of\ crucible}{wt\ of\ sample} X\ 100$$

The ADF was created in a crucible with 72% sulphuric acid (H_2SO_4) added in it, and the mixture was agitated constantly for three hours to produce the acid detergent lignin (ADL). It then proceeded through two water-related processes: washing and filtering.
Weight loss was measured while the crucible was heated to 100ºC for 8 hours. In order to obtain the ash, the crucible was placed in a muffle furnace heated at 500°C for 7 minutes, and the weight loss was noted.
To calculate the proportions of hemicellulose, cellulose, and lignin, the formula as given below was used:
Hemicellulose % = NDF% - ADF%
Cellulose % = (Y-L/W) x 100
Lignin % = (L-A/W) x 100
where, Y = weight of ADF + crucible, L = weight of the crucible + lignin, A = weight of the crucible + ash, W = weight of a sample

Bulk density

Bulk density is a solid's dry weight expressed as a volume (BD) function. The Archimedes principle calculated how much water was moved in a beaker (**Murchana and Purkait, 2021**). Porosity measures a substance's volume percentage of void or air space. The powder's volume quantity depends on how many internal (or closed) or external (or open) pores are present. There is an inverse correlation between porosity and bulk density.

$$Porosity = 1 - (\frac{Bulk\ Density}{Particle\ Density})$$

Swelling index

The swell index test evaluates the bentonite clay's general swelling abilities. A high swell is a good indicator of bentonite quality, although the swelling index test has not been proven to have a proportionate association with hydraulic qualities.

Gc-ms analysis

The GC-MS instrument equipped with a Perkin Elmer Clarus 680 GC/ 600C MS, the capillary column, was used to analyse the biomass and determine its constituent chemical elements (30 mm thick).
INSTRUMENTATION: POAveRnA: MInEitTiaEl RteSm p 60°C for 1 min, ramp 7°C/min to 200°C, hold 3 min, ramp 10°C/min to 300°C, hold 5 min, InjAauto=280°C, Volume = 0 µL, Split=10:1, Carrier Gas = He, Solvent Delay= 8.00 min, Transfer Temp=180°C, Source Temp= 150°C, Scan: 50 to 600Da, Column 60.0m x 250µm.

Ftir analysis

For the purpose of obtaining the infrared spectrum of the absorption, emission, and photoconductivity of solids, liquids, and gases, Fourier transforms infrared spectroscopy (FTIR) technique was employed. Different biomass samples were examined using infrared spectroscopy (Thermo Fisher Scientific, Nicolet TM iS10 FTIR Spectrometer, USA) to identify the existence of different functional groups. FTIR records the interactivity between the infrared radiation and the sample biomass by estimating the frequency in wave number at which the specific sample absorbs specific radiation. Each functional group in the biomass sample absorbs infrared radiation at a specific frequency called the wave number and is recorded as a spectrogram.
Spectroscopy-grade potassium bromide was combined with biomass samples at 200:1 ratio for examination. The resultant pellet was examined and the IR spectrum of different biomass samples was recorded at various wavelengths. Within the 400 – 4000 cm^{-1} wavenumber range, the spectrum was collected at a scan rate of 40 and a step size of 4 cm. The Near-IR part of the electromagnetic spectrum falls between 4,000 - 12,800 cm-1, while the Far-IR portion falls between 10 - 700 cm^{-1}.

XRD analysis

A non-destructive laboratory technique for figuring out crystalline solid lattice spacing is X-ray diffraction (XRD) (**Kasap, 2006**). It is possible to find elastic characteristics, residual stresses, and the identities of unidentified components in a sample by analysing the lattice spacings and distortions of a sample (**Cao & Tan, 2005**). Key material properties may be assessed using XRD to ascertain how a material interacts with a biological tissue(s) after implantation. XRD may determine the lattice spacing by beaming an X-ray into a crystalline material at a specific wavelength and angle and then measuring the intensity of the refracted X-ray (**Jennifer, 2001**). A portion of the X-ray beam will travel through the crystal's atomic plane when it is pointed at it, while the remaining component will hit a lattice and refract back into the diffractometer where it will be measured.
In order to finish the XRD investigation of the biomass samples, a Cu-Ka radiation source that was driven at 18 kW and 250 mA was coupled with a Rigaku TT Rax diffractometer. Angle 2 scans between 10º - 40º and 10º - 60º at a speed of 1º/min. Crystalline indices (CrI) for the biomass sample were calculated as follows (**Pazo *et al*., 2010**):

$CrI = 100 \times [(I_{002} - I_{amorphous})/I_{002}]$

where, I_{002} is the intensity at 2θ = 20 for the crystalline portion (cellulose) and $I_{amorphous}$ is the peak at 2θ = 16.6 for the amorphous portion (cellulose, hemicellulose, and lignin).

Bioinformatics database analysis

To explore the medicinal role of Bengal Amloki on cancer stem cells (CSCs), Cytoscape (Version 3.10.1) bioinformatics database was used (http://www.cytoscape.org/). The cancer stem cell biomarkers were predicted through gene interactions (**Ramos *et al*., 2018; Das *et al*., 2021**) using the database.

RESULTS AND DISCUSSION

Physical analysis

Proximate analysis and hhv

The constituents obtained from the proximate analysis (volatile matter, ash content, fixed carbon content, and higher heat value) of Bengal Amloki fruits are shown in Figure 3. The amount of volatile matter in the biomass used was greater than 70%, suggesting high lignin content, which may be respondible behind high antibacterial characteristic. The assessment of the ash content showed that the biomass is suitable for use as a fuel. The wastes could, therefore, be utilized to showcase a superior energy recovery pathway (**McKendry, 2002**).

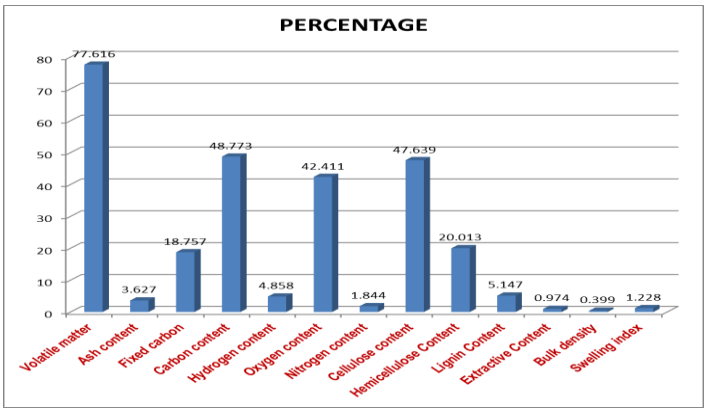

Figure 3 Proximate composition, Ultimate composition and Compositional analysis of Bengal Amloki (*Phyllanthus emblica* L.) showing different percentage values.

Bulk density

The bulk density of biomass revealed its transportability in terms of volume (Fig. 3). It is reasonable to anticipate that the lignin content of the biomass and the volatile matter will be greater if the bulk density is higher. Following the acquisition, biomass waste has little immediate value. This is a source of energy. The bulk density of biomass has a direct impact on its calorific value. Because it has less empty space, a higher packing ratio, and a greater specific energy yield, a substance with a higher bulk density has a higher calorific value (**Nwaiwu Ogueri *et al*., 2006**).

Swelling index

The more hydroscopible the biomass is, the higher the swelling index. Biomass has a large potential to absorb water. As a result, the biomass is less suited for the energy recovery technique and is more vulnerable to microbial activity (**Klemeš, 2020**). In this instance, the biomass has a swelling index of 1.2% (approx.) (Fig. 3). As a result, the biomass is more vulnerable to antibacterial action since it has less moisture. As a result, it is suitable for use as an energy source.

Thermogravimetric analysis (TGA)

There were three separate stages of heat deterioration, as shown by the TGA graph. Clear peaks have been identified with temperatures between 200 and 400^{0}C (Fig. 4).

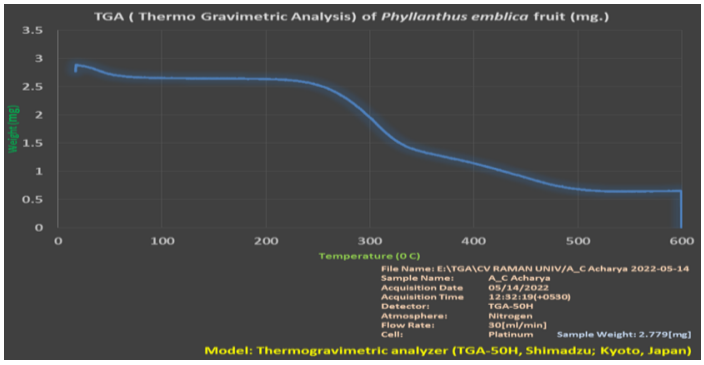

Figure 4 TGA, the Thermal degradation profile of *Phyllanthus emblica* L. fruit sample.

X-ray diffraction (XRD)

The cellulose and hemicellulose components of the XRD graph can be observed at 2Θ between 20 and 25) (Fig. 5). This is explained in the discussion section.

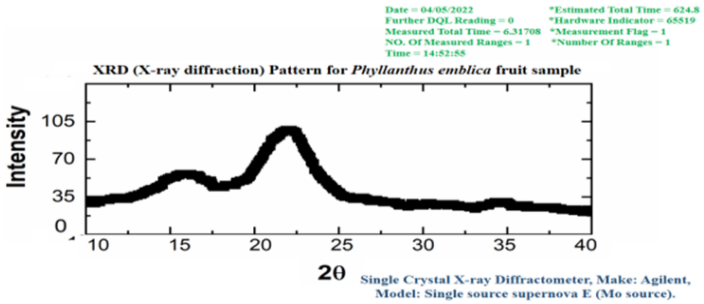

Figure 5 XRD Pattern of *Phyllanthus emblica* L. Fruit sample.

Chemical analysis

Ultimate analysis

As shown in Figure 3, the carbon content is rather large, indicating that biomass has significant energy qualities. The oxygen, hydrogen, and nitrogen ratio reveals a significant result, meaning that the supplied biomass's phenolic content is significant and its volatile substance content is higher. As a result, the supplied biomass has better antibacterial qualities and is a better energy source. The atomic ratios of O/C and H/C are used to calculate energy recovery efficiency (**Milne *et al*., 1992**). Inverse relationships exist between elemental ratios and the energy content of biomass; for example, a low O : C ratio encourages heating value and higher energy recovery.

Ftir analysis

The plant sample underwent FTIR analysis to identify the present functional groups (Table 1). The results of this study also indicate the existence of a specific bioactive component in the plant matter. Fig. 6 shows the FTIR spectra addressed in the discussion section.

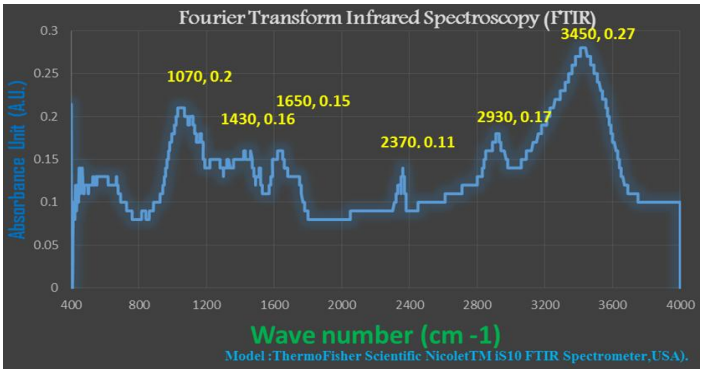

Figure 6 FTIR spectra of *Phyllanthus emblica* L. fruit sample

Table 1 Functional groups and chemical classes were assigned with corresponding *Phyllanthus emblica* fruit sample wave numbers. Source: https://instanano.com/characterization/reference/ftir-functional-group-search/ (accessed April 24th, 2022).

Peak Position	Peak range	Group	Class
1070	1050-1085	C-O stretching	Primary alcohol
1430	1395-1440	O-H bending	Carboxylic acid
1650	1580-1650	N-H bending	Alkene
2370	2349	O=C=O stretching	Carbon dioxide
2930	2840-3000	C-H stretching	Alkane
3450	3200-3550	O-H stretching	Alcohol/phenol

GCMS analysis

Qualitative report of GC-MS analysis (% of Area Vs. Retention Time) of Methanol extract of *Phyllanthus emblica* L. fruit has been depicted in Fig. 7 and the Chromatogram of Methanol extract of *Phyllanthus emblica* L. fruit has been depicted in Fig.8. The individual phytoconstituents found in GCMS peak analysis of fruit has enormous bioactivity potential that has been established through searching Dr. Duke's ethnobotanical database (Table 2 & 3).

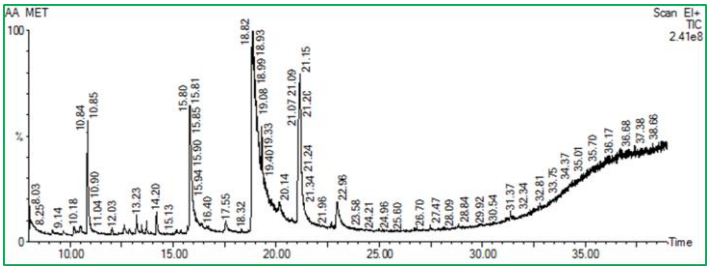

Figure 7. Qualitative report of GC-MS analysis (% of Area Vs. Retention Time) of Methanol extract of *Phyllanthus emblica* L. fruit.

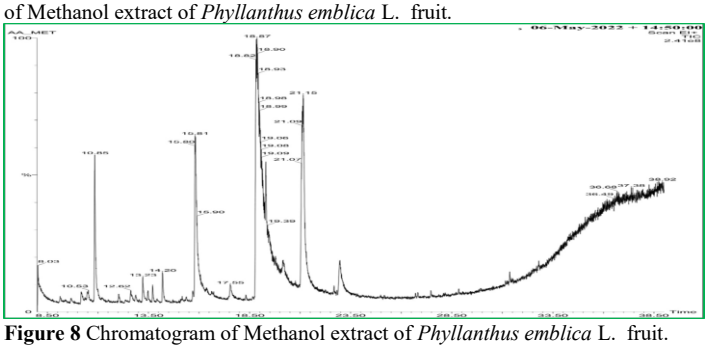

Figure 8 Chromatogram of Methanol extract of *Phyllanthus emblica* L. fruit.

Table 2 Phyto components were identified through GC-MS with their respective peaks, molecular weight, retention time, and area percentage in the methanolic extracts of the *Phyllanthus emblica* L. fruit.

Peak	RT	Name of the Compound	MW (Da)	Scan	Height	Area	Area %	Norm %
1	**10.85**	N-HEXYL ACRYLATE	156	570	130056400	9232467	3.656	13.16
2	**15.812**	5-METHYL-1-NITROPYRAZOLE	127	1562	146371632	20769408	8.224	29.61
3	**18.873**	3-METHYL-2-FUROIC ACID	126	2174	229896640	70136080	27.771	100
4	**21.154**	D-ALLOSE	180	2630	170669440	30198740	11.957	43.06
5	**22.979**	DODECANOIC ACID	200	2995	31033298	5863935.5	2.322	8.36
6	**31.373**	3-METHYL-2-(2-OXOPROPYL)FURAN	138	4673	11922444	578489.1	0.229	0.82
7	**36.495**	CYCLOTRISILOXANE, HEXAMETHYL-	222	5697	11532505	345203.7	0.137	0.49
8	**36.68**	TRICYCLO[4.2.1.0(2,5)]NON-7-ENE, 3,4-DI(TRIS(TRIMETHYLSILYLOXY)SILYL)-	708	5734	15045704	286372.1	0.113	0.41
9	**37.38**	ARSENOUS ACID, TRIS (TRIMETHYLSILYL) ESTER	342	5874	14452315	337400.5	0.134	0.48
10	**38.921**	4-(4-HYDROXYPHENYL)-4-METHYL-2-PENTANONE, TMS DERIVATIVE	264	6182	9025065	181854.1	0.072	0.26

Table 3 Bio-activity of phyto-components identified in the methanolic extracts of the *Phyllanthus emblica* L. fruit through GC-MS.

Peak	RT	Name of the Compound	MW (Da)	Bioactivity
1	**10.85**	N-HEXYL ACRYLATE	156	Antitumor (Nasopharynx), Natriuretic
2	**15.812**	5-METHYL-1-NITROPYRAZOLE	127	Catechol-O-Methyl-Transferase-Inhibitor
3	**18.873**	3-METHYL-2-FUROIC ACID	126	Inhibit Production of Uric Acid
4	**21.154**	D-ALLOSE	180	Anticancer (Duodenum), Detoxicant (Alcohol), Diuretic, **Provide Vit D**
5	**22.979**	DODECANOIC ACID	200	Urine-Acidifier, Inhibit Production of Uric Acid
6	**31.373**	3-METHYL-2-(2-OXOPROPYL)FURAN	138	Catechol-O-Methyl-Transferase-Inhibitor
7	**36.495**	CYCLOTRISILOXANE, HEXAMETHYL-	222	No report found
8	**36.68**	TRICYCLO[4.2.1.0(2,5)]NON-7-ENE, 3,4-DI(TRIS(TRIMETHYLSILYLOXY)SILYL)-	708	Energizer
9	**37.38**	ARSENOUS ACID, TRIS(TRIMETHYLSILYL) ESTER	342	Inhibit Production of Uric Acid
10	**38.921**	4-(4-HYDROXYPHENYL)-4-METHYL-2-PENTANONE, TMS DERIVATIVE	264	Catechol-O-Methyl-Transferase-Inhibitor

Compositional analysis

The given biomass has significant antibacterial capabilities and can be used for energy recovery, as shown by the substantial quantity of lignin, cellulose, hemicellulose and extractable (Fig.3).

Bioinformatics data base analysis: role of *phyllanthus emblica* l. Extract on cancer stem cell biomarkers

The Cytoscape_v3.10.1 and Proteomics Standard Initiative Common QUery InterfaCe (PSICQUIC) data base was used to explore the medicinal role of *P. emblica's* extract to cancer stem cells (CSCs). The data base analysis was accessed on 01/15/2024 to the Reactome Functional Interaction (FI) network and showed the interaction with 69 nodes and 80 edges. Such interactions highlighted *P. emblica*'s extract principally regulates cancer stem cell biomarker UniProtKB P16070 (CD44_HUMAN).

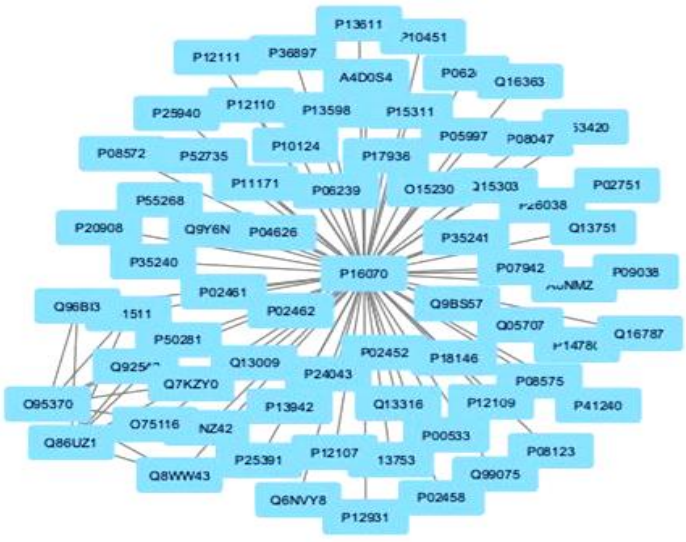

Figure 9 Bioinformatics database analysis showed the role of *Phyllanthus emblica* L. extract on cancer stem cell biomarker CD44.

Proximate analysis, ultimate analysis, compositional analysis, and some assisting tools, including TGA, FTIR, XRD, and GC-MS, have all been accomplished in this study to assess the quality of a plant sample. In this study, *P. emblica's* approximate composition showed that the volatile matter was higher, at 77.616±0.403%, which supported the plant's ethnicity as strong bactericidal, anti-inflammatory, analgesic, and anti-cancer effects of volatile plant components (**Gonzalez-Burgos & Gomez-Serranillos, 2012**). In this case, low ash content encouraged the growth of specific microorganisms, which is why the fruit is highly perishable. Ash content, which was 3.627±0.074%, determines the total amount of mineral matter present in the fruit sample and provides the fruit sample's microbiological stability. The fixed carbon content can determine the amount of char produced during the thermochemical conversion process. After the volatile chemical has been forced out, what is left behind is a residue that is solid and flammable. The char yield produced during the thermochemical conversion process increases as fixed carbon concentration increases. Here, the fixed carbon content was low (18.757±0.343%), resulting in a low level of char formation.

The weight loss kinetics during the breakdown of biomass samples in the presence of nitrogen gas were examined using thermo gravimetric analysis (TGA) on the fruits of *P. emblica*. We found that a 'Z'-shaped curve representing three unique decomposition phases versus a certain weight was derived from TGA curve. The sample that was obtained initially weighed 2.779 mg. At a temperature below 100^0C, during the first drying stage in the TGA graph, the moisture and volatile chemicals (light volatiles) were eliminated.

The second breakdown stage appears as a peak between 200 and 400^0C on the TGA graph, where most of the biomass sample's devolatilization occurred. When compared to the TGA graph, the sharp change in the peak here illustrates the significant loss of biomass content. The significant weight loss occurred during this specific time because volatile hydrocarbons, cellulose, hemicellulose, and lignins were freed (**Singh, 2017**). There was no discernible weight loss in this third stage. When the temperature reaches 600^0C, the remaining lignins and char components stabilise and begin to slowly decompose.

The technique of identifying the various chemical components in a specific substance is known as the analysis of the ultimate composition, which measures the amount of carbon, hydrogen, nitrogen, and oxygen. Compared to the proximate analysis method, this technique produces more thorough data. In the end, it was determined that the proportion of C, H, O, and N contents in the healthy fruits was 48.773±0.211%, 4.858±0.222%, 42.411±0.447%, and 1.844±0.057%, respectively. It is clear that minerals and metals, in the appropriate amounts, are essential to the biological processes of both plants and animals. Since C, H, O, and N make up the majority of the elements in human tissue, their presence in an allowed amount justifies their biological activities for people.

The amorphous cellulose and hemicellulose portions of the provided biomass's XRD pattern could be visible at 2Θ = 22.1°, 22°, or 2Θ = 16.6°. Diffraction happens when a collimated beam of electromagnetic waves interacts with the periodic

arrangement of molecules in a crystal at a wavelength corresponding to the interatomic distance. Incident beams of less than 0.1 nm in wavelength are produced by nuclear reactors, electron weapons, and x-ray sources (neutrons). X-ray diffraction is the most well-known of them (XRD). The electron diffraction of cellulose nanowhiskers proved that cellulose had unit cell symmetry. This experiment is extremely difficult because the tiny cellulose crystals typically sustain significant damage from the electron beam. The positions of the hydroxyl (OH) groups within the cellulose crystal were identified using neutron diffraction data. But a full interchange with deuterated groups was necessary (OD).

FTIR revealed the strong bonds between C-O, O-H, N-H, O=C=O, C-H, O-H of the plant material justified the presence of Primary alcohol, Carboxylic acid, Alkene, Carbon dioxide, Alkane, Alcohol/phenol respectively has been. The values of picks and their functional groups have been depicted in Table 1. The IR peak, 3450 cm -1, in the range of 3200-3550 cm -1 indicates the presence of O-H stretching of Alcohol/phenol compounds, 2930 cm -1, in the range of 2840-3000 cm -1, indicates the presence of C-H stretching of alkane compound, 2370 cm -1, in the range of 2349 cm -1, indicates the presence of O=C=O stretching of carbon dioxide, 1650 cm -1, in the range of 1580-1650, indicates the presence of N-H bending of alkene compound, 1430 cm -1, in the range of 1395-1440 cm -1, indicates the presence of O-H bending of Carboxylic acid and 1070 cm -1, in the range of 1050-1085 cm -1 indicates the presence of C-O stretching of Primary alcohol justifies the bioactive chemical components found in the fruit to cure several disease ailments. Functional groups obtained through FTIR analysis play an important role in forming biological molecules such as protein, DNA, carbohydrates and lipids. Amino acids and amines are important constituents of protein synthesis and play a vital role in living organisms' physiology. Some examples of sulfoxide occurring in nature are allin and allicin, that was found in garlic and are of much Ayurvedic importance. Their Sulphur derivative compounds were used to prepare disinfectants and dermal cream. Functional groups are important components of drug molecule. From the medicinal chemistry perspective, these functional groups provide the specific properties and behaviour that allow the drug molecule to show its pharmacokinetic and pharmacodynamic effects (**Bagchi *et al*., 2021b; Harrold & Zavod, 2018**).

In GC-MS analysis, a total of ten bioactive compounds were obtained from the peaks of methanolic extracts of *Phyllanthus emblica*. The mass spectra of the compounds were compared with the NIST databases and characterised and identified. The bioactive compounds' details, molecular weight, retention time, area percentage, etc, are tabulated (Table 2).

The methanol extract of *Phyllanthus emblica*after GC-MS analysis showed several bioactive compounds including N-hexyl acrylate, 5-methyl-1-nitropyrazole, 3-methyl-2-furoic acid, D-allose, dodecanoic acid, 3-methyl-2-(2-oxopropyl)furan, cyclotrisiloxane, hexamethyl-, tricycle [4.2.1.0(2,5)]non-7-ene, 3,4-di(tris(trimethylsilyloxy)silyl)-, arsenous acid, tris(trimethylsilyl) ester, 4-(4-hydroxyphenyl)-4-methyl-2-pentanone, TMS derivative. Their bioactivities have been searched through Dr. Duke's ethnobotanical and pharmaceutical database and presented in Table 3.

The compositional analysis of the healthy fruits showed that the proportion of cellulose content, hemicellulose content, lignin content and extractive contents were 47.639±0.309%, 20.013 ±0.572%, 5.147 ±0.070% and 0.974±0.006% respectively. Hemicelluloses are the second most abundant polysaccharides in nature after cellulose. They are closely associated with cellulose and lignin and contribute to the rigidity of plant cell walls in lignified tissues. Xylan, hemicellulose, may be extremely useful in the pharmaceutical field, especially for the production of colon-specific drug carriers, such as micro- and nanoparticles and film coatings (**Da Silva *et al*., 2012**). In our study, hemicellulose is present within permissible limits and may impact this bioactivity. Lignins obtained from biological materials are of enormous importance, including their antiviral and antitumor effects. Moreover, recently, the use of lignins in developing NPs for drug delivery has been increasing (**Maria & Montserrat, 2017**). Cellulose is a versatile polymer used in pharmaceutical applications. It can help support gut health in humans, especially when it comes to the large intestine. In our study, cellulose is present within permissible limits and may have an impact on this bioactivity.

The scientific study (**Vadde *et al*., 2016**) mentioned that Indian gooseberry (*Emblica officinalis* L.) controls cell proliferation as well as induces apoptosis in human colon CSCs, defeating Wnt/β-catenin signalling pathway unrelated to p53 stage through inhibition of cyclin D1 and c-Myc. This scientific study showed the extract of Indian gooseberry (*Emblica officinalis* L.) suppresses the production of human colon cancer stem cells (HCCSC) and human colon cancer cells (HCT116) positive for cancer stem cell markers CD133, CD44, CD34, Aldehyde Dehydrogenase, in a p53-independent manner when authors have used the human colon cancer cell lines HCT116 p53 +/+ and HCT116 p53 -/-. Our bioinformatics analysis using Cytoscape highlighted the role of P. emblica extract in modulating the interaction of 69 other genes with the cancer stem cell biomarker UniProtKB P16070 (CD44_HUMAN).

CONCLUSION

This thorough study included advanced instrumental techniques like TGA, FTIR, XRD and GC-MS, as well as proximal, ultimate, and compositional analyses, to completely assess the quality and bioactivity potential of Phyllanthus emblica fruit samples. The proximate analysis showed that the plant has a lot of volatile matter, which fits with what is known about its antibacterial, anti-inflammatory, pain-relieving and cancer-fighting properties. Because the fruit had little ash and fixed carbon, it would spoil rapidly and not make much char when heated. Thermogravimetric analysis indicated three separate stages of breaking down, which showed how the fruit's biomass parts act when heated and how stable they are. The final analysis demonstrated that the fruit includes the proper number of key elements (C, H, O, N) that are needed for biological activity and give the fruit its nutritional and medicinal value.

XRD patterns and FTIR spectra showed that there were significant structural parts, such as cellulose, hemicellulose and lignin, as well as functional groups that are needed to make bioactive molecules. These results are in keeping with what we know about the plant's healing properties, such as its ability to synthesise proteins, DNA, carbohydrates, and lipids that are necessary for healing. The results of GC-MS and bioinformatics profiling, which discovered various bioactive compounds such as anti-cancer of significant medical importance, are supported by ethnobotanical databases. The examination of the composition showed that the levels of cellulose, hemicellulose, and lignin were all appropriate. This means that they might be useful for delivering drugs and in medicine.

Conflict of interest: The authors declare that they have no Conflict of Interest.

Authors contributions: CKA performed the experiments in this study under the guidance of NSK, NRM and JKD. CKA wrote the entire manuscript. BS, MD, AS and SS participate in its design and coordination. All authors read and approve the final manuscript.

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