International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Profiling of Bioactive Compounds in Aegle Marmelos (Bael) Fruit Pulp Powder Using Modern Analytical Techniques

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Abstract:
Aegle marmelos (Bael) is a medicinally important plant widely used in traditional medicine. This study focused on the characterization and solvent extraction of Bael fruit pulp powder to evaluate its nutritional composition, phytochemical constituents, and biological activity. The pulp was dried, powdered, and extracted using different solvents such as ethanol, ethanol–acetone, and acetone. Phytochemical screening revealed the presence of important bioactive compounds including alkaloids, flavonoids, tannins, phenols, steroids, and terpenoids. Spectral analyses using FT-IR, ^{1}H NMR, and ^{13}C NMR confirmed the presence of functional groups and structural features associated with these compounds. The extracts also showed antibacterial activity against both gram-positive and gram-negative bacteria. These findings suggest that Bael fruit pulp powder is a rich source of bioactive compounds with potential therapeutic and nutritional applications.

Keywords: Aegle marmelos, Bael fruit pulp powder, phytochemical screening, solvent extraction, antibacterial activity, bioactive compounds.

1. INTRODUCTION

Aegle marmelos (L.) Correa, commonly known as Bael, is a member of the Rutaceae family. [1] This plant has been traditionally used in folk medicine for centuries to treat various ailments. Native to India, Bael is widely distributed across the Himalayan regions, Bengal, and central to southern parts of the country. It is a medium-sized tree that thrives in forests at elevations of up to 1200 meters. Nearly all parts of the tree, including the root, stem, bark, leaves, flowers, and fruit, exhibit medicinal properties at different stages of maturity. [1,2]

The fruit of Bael has a hard outer shell that changes color from green to brown as it ripens. Its pulp is soft, fragrant, and slightly sweet, resembling cooked pumpkin. An oily, translucent mucilage surrounds the seeds. Various bioactive compounds, including coumarins, alkaloids, sterols, and essential oils, are found in different parts of the plant. Studies have highlighted its potential in managing blood sugar levels, reducing cholesterol, and lowering blood pressure.

Among its medicinal benefits, Bael fruit is traditionally used in Ayurveda for treating chronic diarrhea and is considered a tonic for the heart and brain. It exhibits antiviral, antibacterial, hypoglycemic, and anti-inflammatory properties. [5] The ripe fruit is known for its cooling and laxative effects, making it beneficial for conditions such as constipation, dysentery, and indigestion. Additionally, consuming Bael marmalade has been linked to the prevention of cholera outbreaks. When dried and processed into powder,

the fruit pulp serves as a febrifuge, antiscorbutic, stimulant, and antipyretic. The powdered form of unripe Bael fruit is particularly effective against intestinal parasites like *Entamoeba histolytica* and *Ascaris lumbricoides*.

The medicinal value of Bael is attributed to its bioactive compounds, which exert physiological effects on the human body. Key phytochemicals found in Bael include alkaloids, terpenoids, steroids, glycosides, tannins, and phenolic compounds. Limonene, a major component of Bael, constitutes approximately 82.4% of its composition. Research suggests that Bael extract plays a role in reducing lipid peroxidation, lowering blood urea and cholesterol levels, and enhancing antioxidant enzyme activity in diabetic conditions. [4] Furthermore, Bael leaves (patra) have demonstrated potential in controlling hyperglycemia, as studies indicate a significant decrease in blood glucose and urine sugar levels after eight weeks of treatment.

Besides its medicinal properties, Bael is valued for its nutritional benefits. It is a rich source of carbohydrates, dietary fiber, essential minerals, and vitamins. The fruit, in its various stages of ripening, is utilized for different therapeutic purposes. Unripe and semi-ripe Bael fruits are known to help in preventing scurvy, a condition caused by vitamin C deficiency. Globally, Bael is incorporated into various food products. In Thailand, for example, dried Bael fruits are used to prepare tea, while preserved Bael is included in desserts and cakes. Additionally, Bael seeds contain high-quality protein, making them a potential supplement for enhancing protein intake in meals.

This study aims to analyze the nutritional composition and spectral properties of Bael fruit pulp. The findings could contribute to the broader utilization of this valuable medicinal plant, not only for its therapeutic benefits but also as a nutritious food source.

2. MATERIALS AND METHODS

2.1. Collection of Plant Material

Fresh Aegle marmelos (Bael) fruits were sourced from the ("Ahmednagar, Maharashtra, India"). The fruits were thoroughly washed with clean water to remove any debris and impurities. After washing, the mature fruits were broken open, and the pulp, along with the seeds and fibers, was scooped out. The pulp was then separated from the fiber and homogenized. To prepare Bael fruit powder, 2g of sodium carbonate was added to the pulp, which was then spread into a thin layer and cut into smaller pieces. These pieces were dried in a cabinet dryer at 60 ± 5°C until the moisture content was reduced to below 4%. The dried pulp was then ground into a fine powder and stored in airtight containers for subsequent analysis.

Proximate Analysis

A sterilized crucible was used for sample preparation. Initially, the crucible was dried and weighed before adding the sample. After placing the sample, it was oven-dried at **110°C** and repeatedly weighed until a constant weight was obtained. Each weighing was conducted after cooling the crucible in a desiccator to ensure accuracy. The proximate analysis included moisture, fat, ash, and vitamin C estimation in the fruit pulp powder. Crude fat content was determined using the Soxhlet extraction method with petroleum ether as the solvent. Total ash content was measured by incinerating the sample in a muffle furnace at 550°C for 12 hours. The crude protein content was estimated using the micro-Kjeldahl distillation method. The percentage of carbohydrates was determined by the difference method.

Spectral Analysis

The extracted compounds were characterized using Fourier-transform infrared (FT-IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and mass spectrometry to confirm its structural identity.

The FT-IR spectra were recorded on a Bruker instrument to identify characteristic functional groups. The ^{1}H and ^{13}C NMR spectra were obtained using a Brucker NMR spectrometer at frequencies of 400 MHz for ^{1}H and 100 MHz for ^{13}C. Chemical shifts were reported in ppm relative to tetramethylsilane (TMS) as an internal standard. Distinct shifts indicative of the aromatic protons and the amino group confirmed the compound's identity. Mass spectra were recorded on a Brucker mass spectrometer to verify the molecular weight and fragmentation pattern of the synthesized compound, further confirming its purity and structure.

Biological Activity Evaluation

Antibacterial Activity

The antibacterial activity of extracted compounds were evaluated using the agar well diffusion method against selected gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacterial strains. Briefly, each bacterial strain was cultured in nutrient broth and adjusted to a turbidity of 0.5 McFarland standard before being spread onto Muller-Hinton agar plates. Wells were made in the agar, and each well was filled with 50 µL of the test compound at a concentration of 1 mg/mL[29]. After incubation at 37 °C for 24 hours, the inhibition zones around each well were measured to determine antibacterial efficacy.

Ampicillin was used as a standard antibiotic control[30].

Preparation of Extract

Decoction Method

To prepare the decoction, 1g of dried fruit pulp powder was boiled in 16ml of double-distilled water until the volume reduced to 4ml. The resulting decoction was filtered and used for further analysis.

Soxhlet Extraction

For crude extract preparation, the Soxhlet extraction method was employed. 50g of Bael fruit powder was uniformly packed into a thimble and extracted using 250ml of different solvents (ethanol, 1:1 ethanol: acetone, and acetone). The extraction continued until the solvent in the siphon tube turned clear, indicating complete extraction. The obtained extract was transferred to a beaker and heated on a hot plate at 30–40°C until all the solvent evaporated. The dried extract was then stored in a refrigerator at 4°C for later spectral analysis.

Phytochemical Screening

The obtained extracts and decoctions were tested for the presence of bioactive compounds such as tannins, phenols, flavonoids, saponins, alkaloids, steroids, and terpenoids using standard phytochemical screening methods as described by Trease and Evans (1989) and Harborne (1973).

3. RESULTS AND DISCUSSION

Phytochemical analysis of the ethanol extract of Bael fruit pulp powder confirmed the presence of tannins, phenols, flavonoids, alkaloids, steroids, and terpenoids. However, saponins were not detected in the ethanol extract. The presence of these bioactive compounds in ethanolic extracts has been linked to significant antibacterial activity, as supported by previous studies. The ethanol: acetone (1:1) extract of the fruit pulp powder indicated the presence of tannins, flavonoids, and alkaloids, whereas the acetone extract contained only phenols and alkaloids. Phytochemical screening revealed that the crude extracts contained key secondary metabolites, including alkaloids, flavonoids, phenols, and tannins, which contribute to various medicinal and physiological benefits.

Tannins are known to interact with proline-rich proteins, forming irreversible complexes that inhibit cell protein synthesis. Fruits rich in tannins exhibit astringent properties and are traditionally used to treat

digestive disorders such as diarrhea and dysentery. The presence of saponins in Bael fruit suggests its potential application in managing inflammatory conditions.

Flavonoids have been widely recognized for their diverse biological properties, including antimicrobial, antioxidant, anti-inflammatory, anti-allergic, and cytostatic effects. Studies indicate that tannins and flavonoids contribute to anti-diarrheal effects by reducing intestinal motility and secretion. Additionally, steroids and triterpenoids exhibit antibacterial activity, with triterpenoids specifically disrupting microbial cell walls, leading to cell lysis. Alkaloids, on the other hand, are known for their analgesic, antispasmodic, and antibacterial properties.

Table 1: Phytochemical screening of Bael fruit pulp powder in different solvents

Sr. No.	Phytochemical Compounds	Ethanol	Acetone
1	Tannin	Present	Absent
2	Phenol	Present	Absent
3	Flavonoids	Present	Present
4	Alkaloids	Present	Present
5	Steroids	Present	Absent
6	Terpenoids	Present	Absent

3.1. Spectral studies:

3.1.1. ^{1}H NMR spectral data

The ^{1}H NMR spectral data of the ethanolic extract of *Aegle marmelos* fruit pulp powder provided crucial insights into the presence of bioactive compounds, particularly alkaloids, terpenoids, and steroids. The observed chemical shifts and corresponding functional groups indicate the structural diversity of phytochemicals within the extract.

A singlet at δ 1.487 ppm (6H) corresponds to methyl protons (-CH_3) attributed to alkaloids, suggesting the presence of methyl-substituted nitrogen-containing compounds. Additionally, a singlet at δ 5.342 ppm (1H) is indicative of an amide (-NH-) proton, which further supports the presence of alkaloidal structures. These findings align with previous reports where alkaloids in medicinal plants exhibited similar chemical shifts due to the presence of nitrogen-containing heterocycles.

The presence of terpenoids in the extract was confirmed by the characteristic doublet at δ 0.862 ppm (2H), corresponding to a terminal methylene group (=CH_2). Terpenoids are known for their diverse biological activities, including antimicrobial and anti-inflammatory properties, reinforcing the medicinal significance of the extract. Furthermore, the signal observed at δ 2.744 ppm (2H) corresponds **to** methylene (-CH_2-) protons associated with steroidal structures. This shift is consistent with previously reported data for naturally occurring steroids, which contribute to various pharmacological effects, such as anti-inflammatory and hormone-regulating properties. The presence of steroids in the extract suggests potential therapeutic applications, particularly in managing inflammatory conditions. The ^{1}H NMR spectral analysis confirms the presence of key bioactive compounds in the ethanolic extract, supporting its traditional medicinal use. The identification of alkaloids, terpenoids, and steroids highlights the phytochemical richness of *Aegle marmelos*, making it a valuable source of natural therapeutic agents.

The ^{1}H NMR spectrum of the ethanol: acetone (1:1) extract of *Aegle marmelos* fruit pulp powder revealed the presence of key bioactive compounds, including alkaloids, terpenoids, and steroids, based on their characteristic chemical shifts. A singlet signal at δ 1.487 ppm was attributed to the presence of methyl (-CH_3) protons, which are characteristic of alkaloid structures. Additionally, a downfield shift at δ 5.315 ppm was observed, indicating the presence of an -NH- proton, further confirming the alkaloid nature of the extract. Alkaloids are known for their significant pharmacological properties, including antimicrobial and analgesic effects. The presence of terpenoids was confirmed by the signal at δ 0.850 ppm, corresponding to methylene (=CH_2) protons. Terpenoids are biologically active compounds widely recognized for their antimicrobial, anti-inflammatory, and antioxidant properties. A peak observed at δ 2.520 ppm was assigned to methylene (-CH_2-) protons, characteristic of steroid frameworks. Steroids play an essential role in various physiological functions and have been reported to exhibit antibacterial and anti-inflammatory activities.

The ^{1}H NMR spectrum of the acetone extract of *Aegle marmelos* fruit pulp powder provided insights into the presence of various bioactive compounds, including alkaloids, terpenoids, and steroids. The spectral analysis confirmed distinct chemical shifts corresponding to characteristic functional groups, which support the presence of secondary metabolites with potential biological significance. A singlet at δ 1.977 ppm corresponds to the methyl (-CH_3) protons associated with alkaloids, indicating the presence of nitrogen containing heterocyclic compounds. The presence of an amide (-NH-) proton in alkaloids was observed as a singlet at δ 5.326 ppm, which is characteristic of hydrogen bonding within the molecular framework. These findings align with previous reports on alkaloid-rich plant extracts, suggesting their possible role in antimicrobial and pharmacological activities.

A chemical shift at δ 0.850 ppm, attributed to the methylene (=CH_2) protons of terpenoids, confirms the existence of unsaturated hydrocarbon structures, which are often linked to antioxidant and anti-inflammatory properties. Terpenoids have been widely recognized for their role in modulating biological pathways and enhancing therapeutic efficacy in traditional medicine. Furthermore, a distinct peak at δ 2.508 ppm, corresponding to methylene (-CH_2-) protons in steroids, signifies the presence of steroidal compounds in the extract. The presence of steroids has been correlated with anti-inflammatory, immunomodulatory, and antimicrobial effects, contributing to the pharmacological potential of *Aegle marmelos*. The ^{1}H NMR data collectively indicate that the acetone extract of Bael fruit pulp powder contains significant quantities of alkaloids, terpenoids, and steroids, supporting its medicinal and nutraceutical applications. These findings reinforce the phytochemical diversity of *Aegle marmelos* and highlight its potential as a source of bioactive molecules for pharmaceutical and therapeutic purposes.

3.1.2. ^{13}C NMR spectral data

The ^{13}C Nuclear Magnetic Resonance (NMR) spectrum of Bael fruit pulp powder extracts provided valuable insights into the chemical composition of its bioactive compounds. The spectral data revealed distinct peaks corresponding to different functional groups, indicating the presence of various organic constituents. A characteristic signal was observed in the range of 14.28–14.33 ppm, which corresponds to methyl (-CH_3) carbon resonances. This suggests the presence of aliphatic or terminal methyl groups in the extract. Additionally, the peaks appearing at 22.23–22.60 ppm were indicative of N-methyl (-N-CH_3) functionalities, which are commonly associated with alkaloid structures.

The spectral data further displayed signals at 29.07–29.21 ppm, corresponding to methylene (-CH_2-) groups, which are typically found in long-chain alkanes and fatty acids. A distinct peak at 31.78–31.79 ppm was assigned to aromatic methyl (Ar-CH_3) groups, suggesting the presence of methyl-substituted

aromatic systems, which could be linked to phenolic or flavonoid compounds. A significant downfield shift was noted in the 174.80–
174.89 ppm range, corresponding to carbonyl (C=O) groups. This signal is characteristic of esters, carboxylic acids, or lactones commonly found in plant-derived secondary metabolites with potential bioactive properties.

3.1.3. FTIR Spectral Analysis of Bael Fruit Pulp Powder Extract

FTIR spectroscopy was employed to identify the functional groups present in the Bael fruit pulp powder extract. The spectral data revealed key absorption bands corresponding to various functional groups, indicating the presence of significant bioactive compounds. A broad absorption band observed in the range of 3328–3360 cm^{-1} is attributed to the O–H stretching vibrations, which suggest the presence of hydroxyl (-OH) functional groups. These groups are commonly found in phenolic and alcoholic compounds, which contribute to the antioxidant properties of the extract. The absorption peak at 3009 cm^{-1} corresponds to NCH_3 stretching, indicating the presence of methylated nitrogen-containing compounds. This suggests the possible existence of alkaloids or other nitrogenous biomolecules within the extract.

The bands appearing at 2917–2918 cm^{-1} are assigned to aromatic C-H stretching, which signifies the presence of methyl ($-CH_3$) groups attached to aromatic rings. Similarly, the peaks observed at 2843–2851 cm^{-1} correspond to $=CH_2$ stretching, indicating the presence of aliphatic methylene groups, which are typically associated with fatty acids and terpenoids. A significant absorption band at 1707 cm^{-1} is characteristic of C=O stretching, suggesting the presence of carbonyl functional groups. This peak is typically associated with aldehydes, ketones, esters, and carboxylic acids, which are commonly found in bioactive phytochemicals such as flavonoids and organic acids. The spectral range between 1272–1377 cm^{-1} corresponds to C-O-C stretching vibrations, indicating the presence of ether linkages. This suggests the existence of glycosidic bonds, which are prevalent in carbohydrates, flavonoids, and other polyphenolic compounds.

The identification of hydroxyl (-OH), carbonyl (C=O), and ether (C-O-C) groups strongly suggests the presence of phenolic compounds, flavonoids, and carbohydrates in the Bael fruit pulp powder extract. These bioactive components are well-known for their antioxidant, antimicrobial, anti-inflammatory, and therapeutic properties. The presence of aromatic methyl ($-CH_3$) and aliphatic methylene ($=CH_2$) groups further supports the existence of essential phytoconstituents such as terpenoids, which have been widely recognized for their medicinal benefits. The FTIR spectral analysis confirms the presence of diverse functional groups associated with bioactive secondary metabolites. These findings highlight the potential pharmacological applications of Bael fruit pulp powder extract, reinforcing its traditional medicinal use.

3.1.4. Powder XRD:

X-ray diffraction patterns of the Bael fruit pulp powder extract were obtained in the 2θ = 20-80◦ range. The lack of peaks observed in the Bael fruit pulp powder extract indicates their amorphous nature.

4. Conclusion

This results from above study indicates that Bael (Aegle marmelos) fruit pulp powder as a rich source of bioactive compounds, including alkaloids, flavonoids, tannins, phenols, steroids, and terpenoids, which contribute to its medicinal and nutritional value. Ethanol was found to be the most effective solvent for extracting these compounds. Spectral analyses (FT-IR, ^1H NMR, ^{13}C NMR, and XRD) confirmed their functional groups and structural features, validating the phytochemical results. The extracts exhibited

significant antibacterial activity against both gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria, supporting the traditional use of Bael in treating infections and digestive disorders. Overall, Bael fruit pulp powder demonstrates considerable therapeutic and nutraceutical potential, making it a promising candidate for pharmaceutical and dietary applications.

Fig: - 1: - FTIR Spectral Analysis Graph

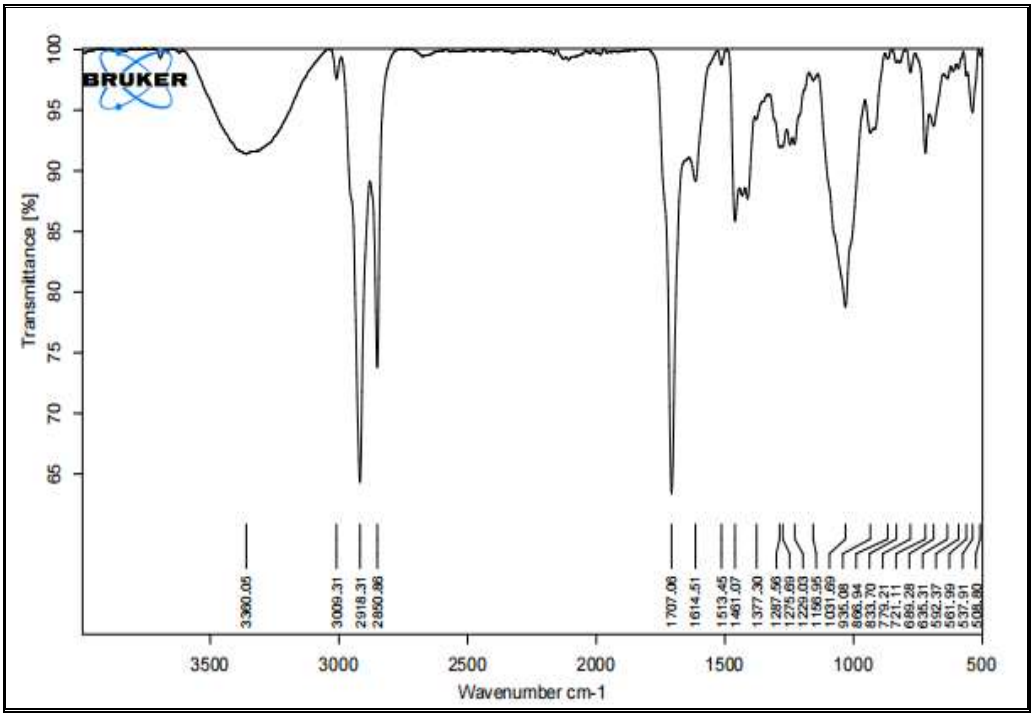


Fig: - 2: A) **^{13}C NMR spectral Graph**

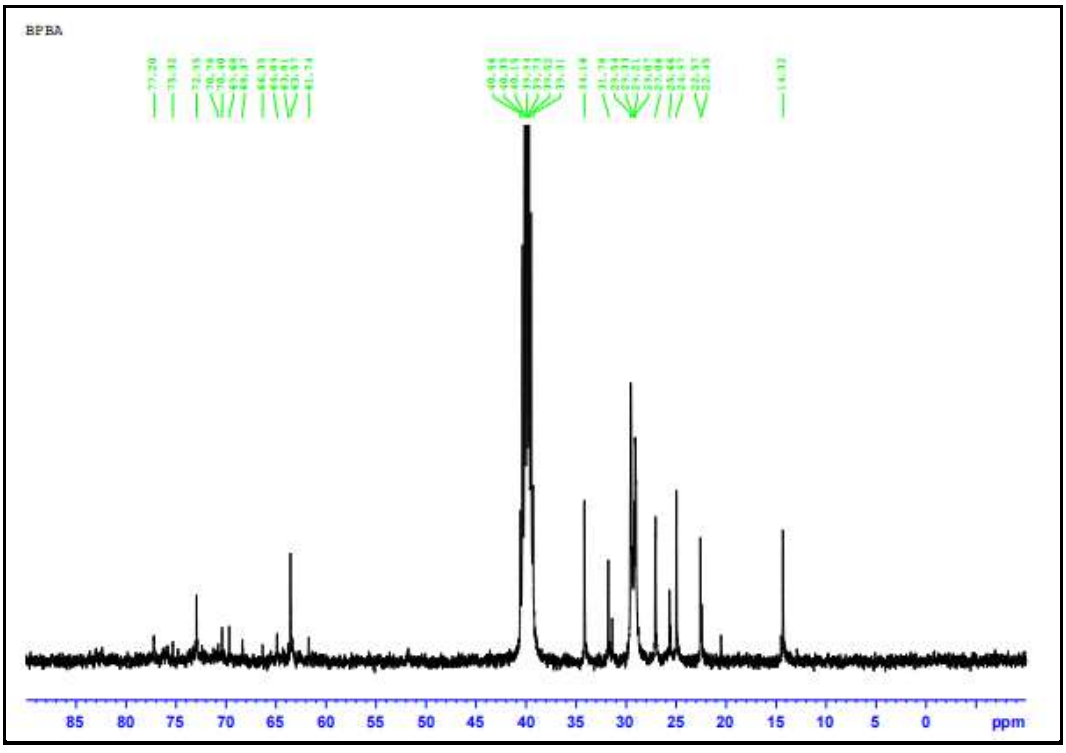


Fig: - 2: B) ^{13}C NMR spectral Graph

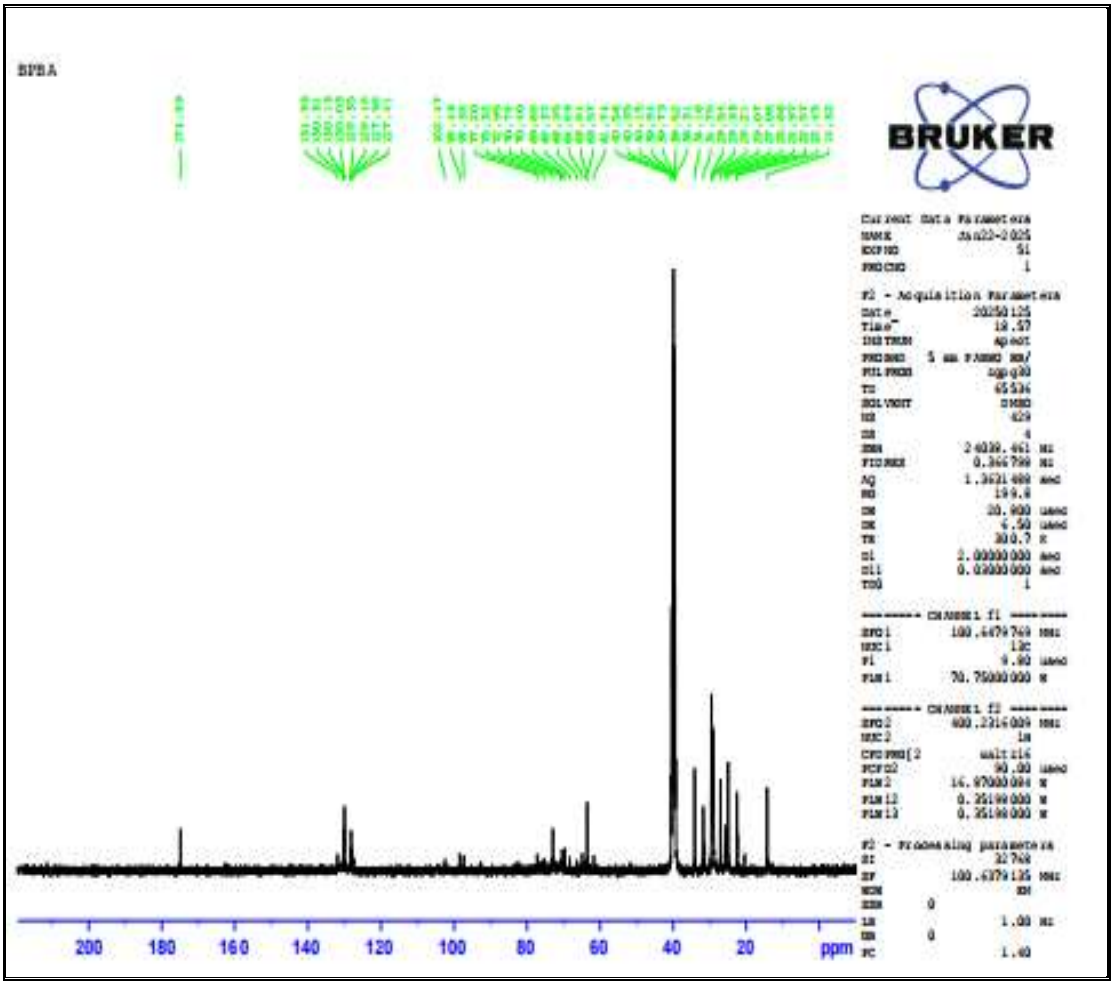
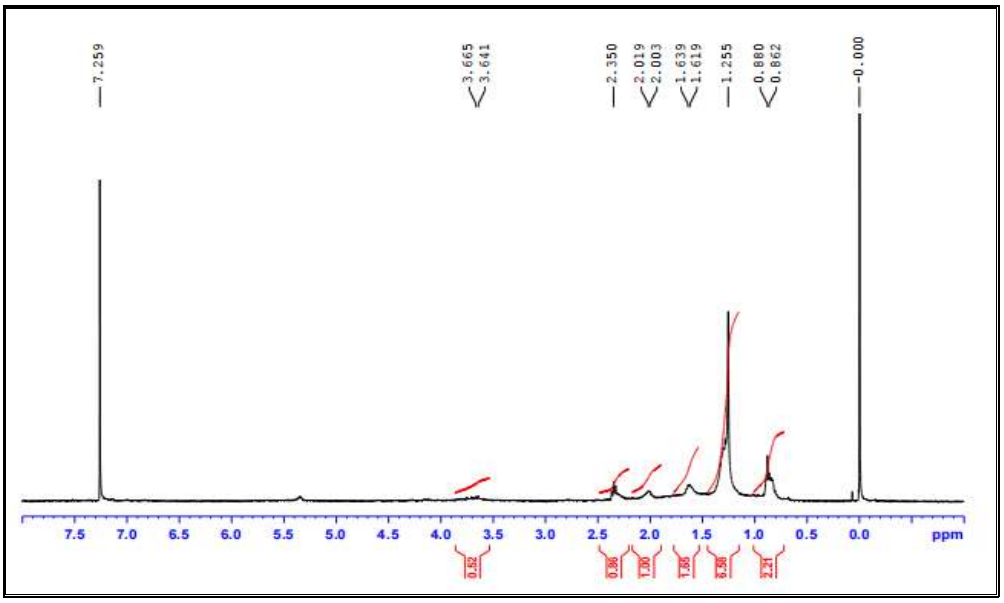


Fig 3: - ^{1}H NMR spectral Graph



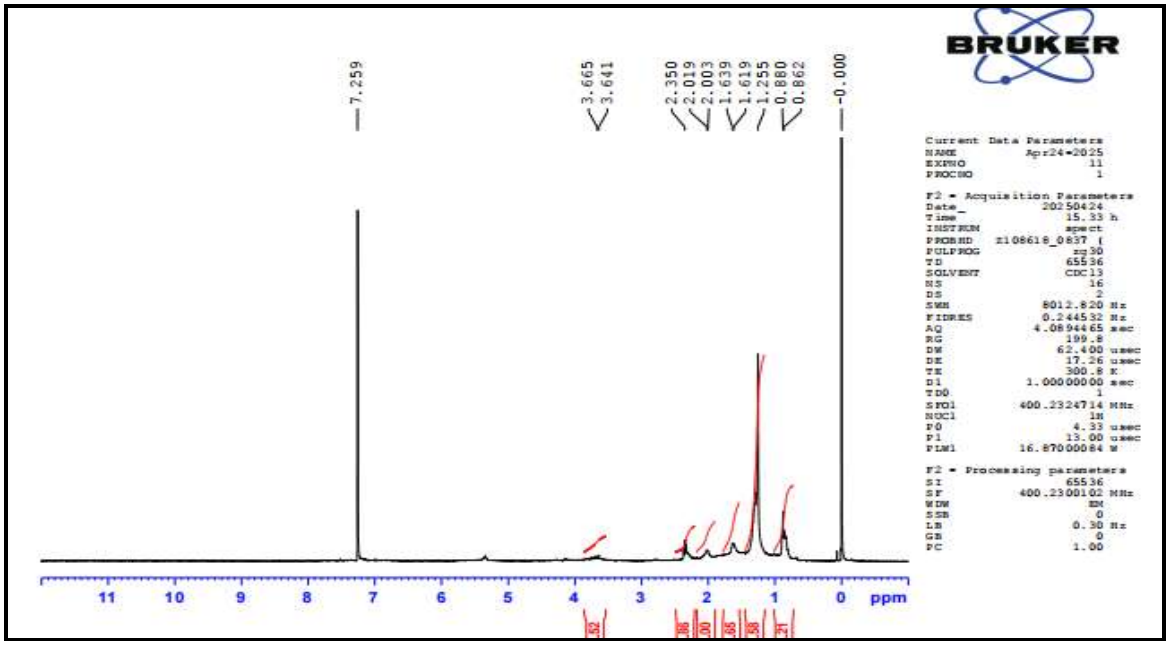
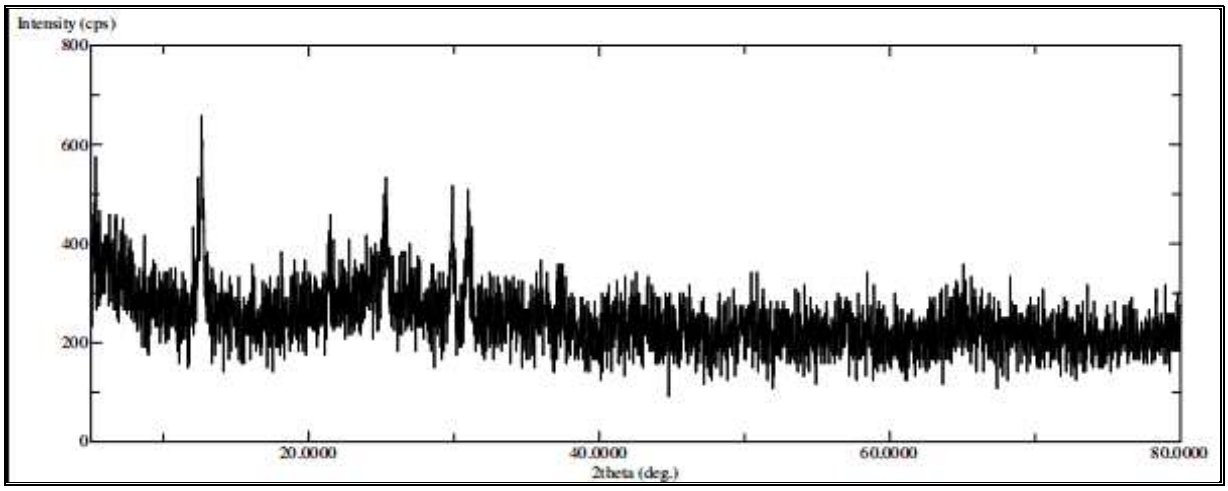
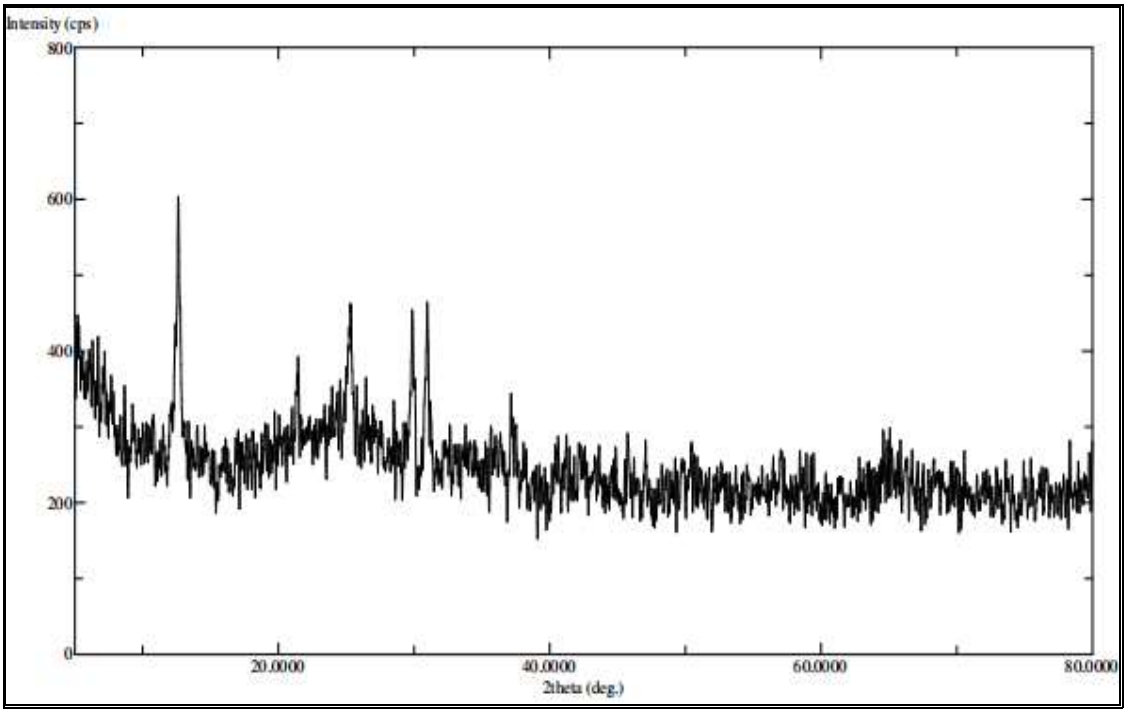


Fig 4: - Powder XRD

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400
200
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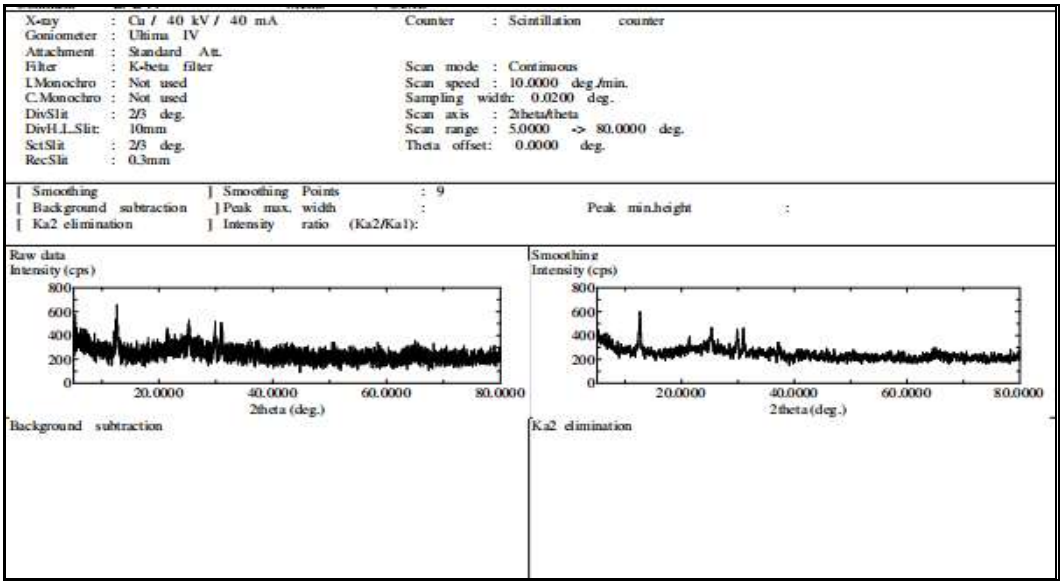
20.0000
40.0000
60.0000
80.0000
2theta (deg.)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Goniometer : Ultima IV
Attachment : Standard Att.
Filter : K-beta filter
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C.Monochro : Not used
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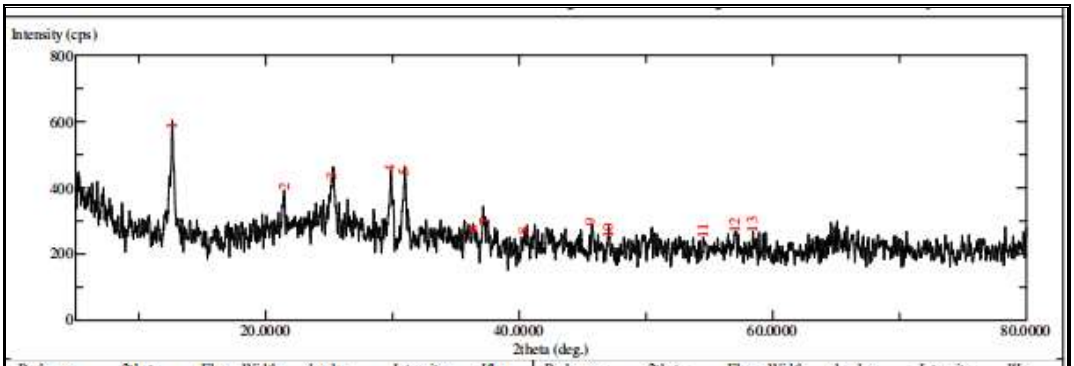
DivH.L.Slit: 10mm
SctSlit : 2/3 deg.
RecSlit : 0.3mm
Counter : Scintillation counter
Scan mode : Continuous
Scan speed : 10.0000 deg./min.
Sampling width: 0.0200 deg.
Scan axis : 2theta/theta
Scan range : 5.0000 -> 80.0000 deg.
Theta offset: 0.0000 deg.
[Smoothing] Smoothing Points : 9
[Background subtraction] Peak max. width :
[Ka2 elimination] Intensity ratio (Ka2/Ka1):
Peak min.height :
Raw data
Intensity (cps)
2theta (deg.)
Background subtraction
Smoothing
Intensity (cps)
2theta (deg.)
Ka2 elimination



Peak no.	2theta	Flex Width	d-value	Intensity	I/Io	Peak no.	2theta	Flex Width	d-value	Intensity	I/Io
1	12.600	0.565	7.0195	579	100						
2	21.460	0.565	4.1373	393	68						
3	25.260	0.635	3.5228	430	75						
4	29.880	0.541	2.9878	449	78						
5	31.000	0.612	2.8824	440	77						
6	36.440	0.612	2.4636	268	47						
7	37.320	0.565	2.4075	287	50						
8	40.440	0.494	2.2287	260	45						
9	45.740	0.565	1.9820	292	51						
10	47.120	0.494	1.9271	250	44						
11	54.520	0.494	1.6817	250	44						
12	57.120	0.565	1.6112	267	47						
13	58.440	0.518	1.5779	269	47						

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