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Research Article

Extraction, Characterization and Antibacterial Potential Assessment of Polar Phytoconstituents of *Caesalpinia bonducella* Seeds Extract

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Abstract: Plant-based medicine has been utilized to cure ailments at a low cost all over the world, medicinal plants are the primary source of medicines and the healthcare system. Traditional medicine has long utilized the seeds of *Caesalpinia bonducella* to cure a variety of symptoms and afflictions, including malaria, colic, fever, edema, leprosy, and abdominal pain. The current investigation aimed to identify the polar phytoconstituents and their antibacterial activity in *Caesalpinia bonducella* seed extracts using polar solvents (methanol, ethanol). The extraction of the phytoconstituents of seed powder of *Caesalpinia bonducella* was carried out by using Soxhlation method. Then the extract was examined by FT-IR, RP-HPLC, and the traces were confirmed by using the GC-MS technique. Antibacterial studies of the extract showed that the active constituents present in the extract have considerable activities against microbes like *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger*, and *Salmonella typhi*. Perhaps it could serve as a substitute for the commercially available synthetic antibiotics. A microbial assay has been performed to assess the antibacterial potency of the identified phytochemicals.

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1. Introduction

Traditional medical practices are used to treat more than 64% of the population and 82% of those are in emerging countries, demonstrating the expanding acceptance of traditional medicine as it is accessible and affordable. Traditional medicine uses a wide range of plant parts, such as bark, roots, seeds, stems, fruits, and leaves for the treatment and prevention of various diseases. In Pune, India, flowering and fruiting take place between July–April and November–December. In Asia's tropical and subtropical regions, such as India, Sri Lanka, Bangladesh, Burma, Myanmar, China, and Vietnam, *C. bonducella* is a prickly shrub or woody vine. It can be found in wastelands, hills, plains, forests, seaside areas, and the Himalayas up to 1000 meters. After sowing, *C. bonducella* grows swiftly, reaching a height of 26 cm in 40 days can grow in a wide range of soil pH and loves full sun to mild shade. It scrambles onto low trees and bushes and grows well in grassy and herbaceous areas. Beach vegetation,

coastal dunes, and mangrove forests are examples of distributed areas where *C. bonducella* flourishes. To create a hedge, seeds are sown at 50 cm intervals after being soaked overnight at the start of the rainy season. After 3–4 weeks, the plants sprout, and in 2–2½ years, reach their optimum height. In order to induce complete germination when exposed to blue light, the naturally occurring plant might be treated with strong sulfuric acid for a duration of 30 to 90 minutes (Sasidharan et al., 2021). On the other hand, modern medicine uses active constituents that are isolated from various plant parts, with 80% of these active components being effective in treating particular diseases with their anticancer, hepatoprotective, antioxidant, antimalarial, antibacterial, antipyretic, antifertility, and anti-inflammatory qualities (Kannur et al., 2006; Moon et al., 2010). In India, the traditional Ayurvedic medical system is used by 76% of the rural population. With over 20 000 medicinal plants and 250 000 registered Ayurvedic practitioners, India is the world's greatest producer of medicinal plants. Traditional medical systems are used by more than 1.5 million practitioners, and more than 7800 industrial facilities produce natural health products and conventional plant-based medications (Ali et al., 2009; Subbiah et al., 2019). Herbal medicine can reduce the adverse effects of pharmaceutical medications by addressing the root cause of pain or discomfort. By switching from prescription medicines to natural remedies, patients can speed up their recovery and benefit from the help of naturopathic physicians (Ghosh and Khamkat, 2021). Herbal medications contain vitamins, antibodies, and other health-improving ingredients, addressing problems from the inside out (Mehra et al., 2015). Additionally, using herbal medications offers financial savings and better, more economical healthcare. *Caesalpinia bonducella*, also known as the bonduc bean or bonduc nut, is an Ayurvedic medicine plant with numerous beneficial effects on the human body (Kannur et al., 2012; Gadakh et al., 2020). Its seeds are used in Ayurvedic medicine, and the plant grows to 10 meters tall and has sharp spines (Billah et al., 2013). The leaves are around 30- to 60-cm-long bipinnate having thorny petioles, with small yellow flowers and clusters of leaflets. The calyx has lobes that are obovate-oblong and obtuse, and it is 6–8 mm long, fulvous, and hairy. The petals have declinate, flattened filaments at the base, and are yellow and oblanceolate. The pods of fruits with 10 seeds are hard and brown with a bitter taste. The treated seeds are extracted from 1–1.25 mm testa in a dry state and have a firm, glossy coat. Their colour is pale yellowish white, their texture is ridged, and their flavour is bitter (Sundare et al., 2007; Kakade et al., 2017). The common name of *Caesalpinia bonducella* plant seed is Bonduc nut, fever nut. It belongs to the family of Fabaceae/Caesalpiniaceae. Katkaliji, Gataran, Karanju, Gajaga, Gajjuga, and Heggejjuga are some of its common names in Hindi. It is also referred to as Gatchakai in Sanskrit and Lata Karanja in Telugu. It is referred to as Kazhanchikkuru Kalechikai, Kazharchikkaai in Tamil (Arindam et al., 2007). This Bonduc nut is useful to cure a variety of symptoms and afflictions, including diabetes mellitus, malaria, colic, fever, edema, leprosy, and abdominal pain. Although this plant has several medical properties, the major chemical constituents and the effects of its 'seeds (Bonduc nut)' have not yet been studied properly. The major phytoconstituents in the alcoholic extract (methanol and ethanol) and their potential for microbiological evaluation have been analyzed using a unique analytical approach in this work.

2. Materials and Methods

2.1. Materials

2.1.1. Plant material

Caesalpinia bonducella seeds were collected from an authentic shop in the local market of Barasat, Kolkata-700125. The seeds were identified and authenticated by Acharya Jagadish Chandra Bose Indian Botanical Garden, Shibpur, Howrah- 711103.

2.1.2. Chemicals

Here, the ingredients were used for the extraction and characterization of phytoconstituents of Caesalpinia seeds. Ethanol (Oxford Lab Fine Chem LLP), Methanol (Oxford Lab Fine Chem LLP), Petroleum ether (Oxford Lab Fine Chem LLP), Chloroform (Oxford Lab Fine Chem LLP), Ethyl acetate (Nice Chemical Pvt Ltd), Sulphuric acid (Nice Chemical Pvt Ltd), Formic acid (Nice Chemical Pvt Ltd), Toluene (Nice Chemical Pvt Ltd), Mayer's reagent (Universal Chemicals), Dragendroff's reagents (Universal Chemicals), Benedict's reagent (Stanbio Reagents Pvt Ltd), Ninhydrin reagent (Universal Chemicals), Fehling's A (Universal Chemicals), Fehling's B (Universal Chemicals), Iodine (Nice

Chemical Pvt Ltd), Potassium iodide (Nice Chemical Pvt Ltd), Ferric chloride (Oxford Lab Fine Chem LLP), Ammonia (Nice Chemical Pvt Ltd), Acetic acid (Loba Chemie Pvt Ltd), Anisaldehyde (Loba Chemie Pvt Ltd), Millon's reagent (Universal Chemicals), Olive oil (Nice Chemical Pvt Ltd) were obtained from Brainware University, Barasat.

2.1.3. Instruments

GC-MS manufactured by Perkin Elmer GC Clarus 680 MS Clarus 600 (EI), Electronic balance manufactured by Mettler Toledo ME204, Water bath manufactured by Vinayak Enterprise, pH meter manufactured by Mettler Toledo, Hot air oven manufactured by Vinayak Enterprise, UV spectroscopy manufactured by Shimadzu UV-1900I, RP-HPLC manufactured by Waters 1525 & 2998 PDA, IR spectroscopy manufactured by Bruker Alpha II, UV Cabinet manufactured by Vinayak Enterprise, Autoclave manufactured by Vinayak Enterprise, Laminar flow manufactured by Vinayak Enterprise, BOD Incubator manufactured by Vinayak Enterprise, were utilized in the research**.**

2.1.4. Microorganisms

Microorganisms like *Salmonella Typhi* (Gram Negative bacteria), *Escherichia coli* (Gram Negative bacteria), *Staphylococcus aureus* (Gram Positive bacteria), and *Aspergillus niger* (Fungi) were used for determining the inhibitory activity of antibacterial agents for the study.

2.2. Method

2.2.1. Preparation of the extract

In this study, the process of extraction and preservation of *C. bonducella* seed extracts was meticulously carried out to ensure the purity and potential therapeutic value of the obtained extracts. Initially, the seed kernels were separated from the outer seed shell using a mortar and pestle, allowing for precise isolation of the desired material. 50g of *C. bonducella* seeds were ground into a powder, and 500 ml of 95% methanol and ethanol (polar in nature) were used as the extracting solvent (Joshi et al., 2016; Sembiring et al., 2018). This method included maceration, percolation, and Soxhlet extraction. The extraction method, which lasted 16 hours, effectively dissolved the beneficial chemicals in the powdered seeds. Particulate debris and contaminants were then filtered out using Whatman filter papers. Evaporation was used to further concentrate the filtrate, producing a highly concentrated C. bonducella seed extract. Finally, to ensure the preservation of the extract's potency and quality, it was carefully stored in a sterile glass container at a temperature of 4°C, providing an optimal environment for future utilization and potential therapeutic applications (Kannur et al., 2006; Ali et al., 2009).

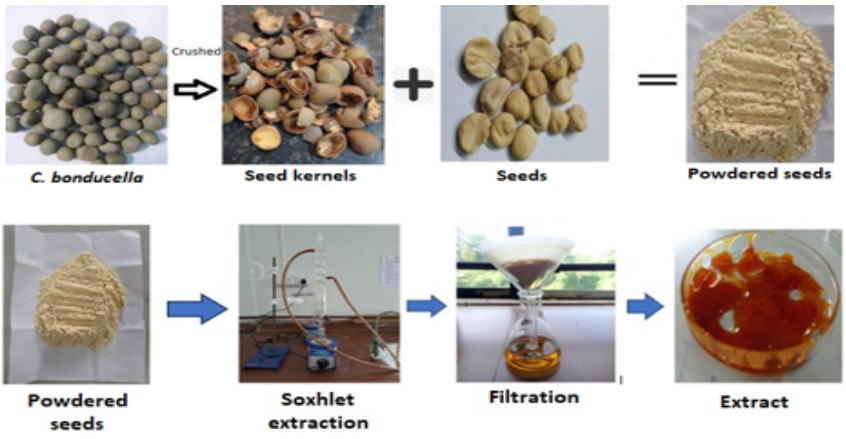

Figure 1. Extraction of *Caesalpinia bonducella* through Soxhlet apparatus.

2.2.2. Preparation and sterilization of Agar Plate

The ingredients used for the preparation of nutrient agar media are beef extract, peptone, sodium chloride, distilled water, and agar (Simin et al., 2001; Sabu et al., 2003).

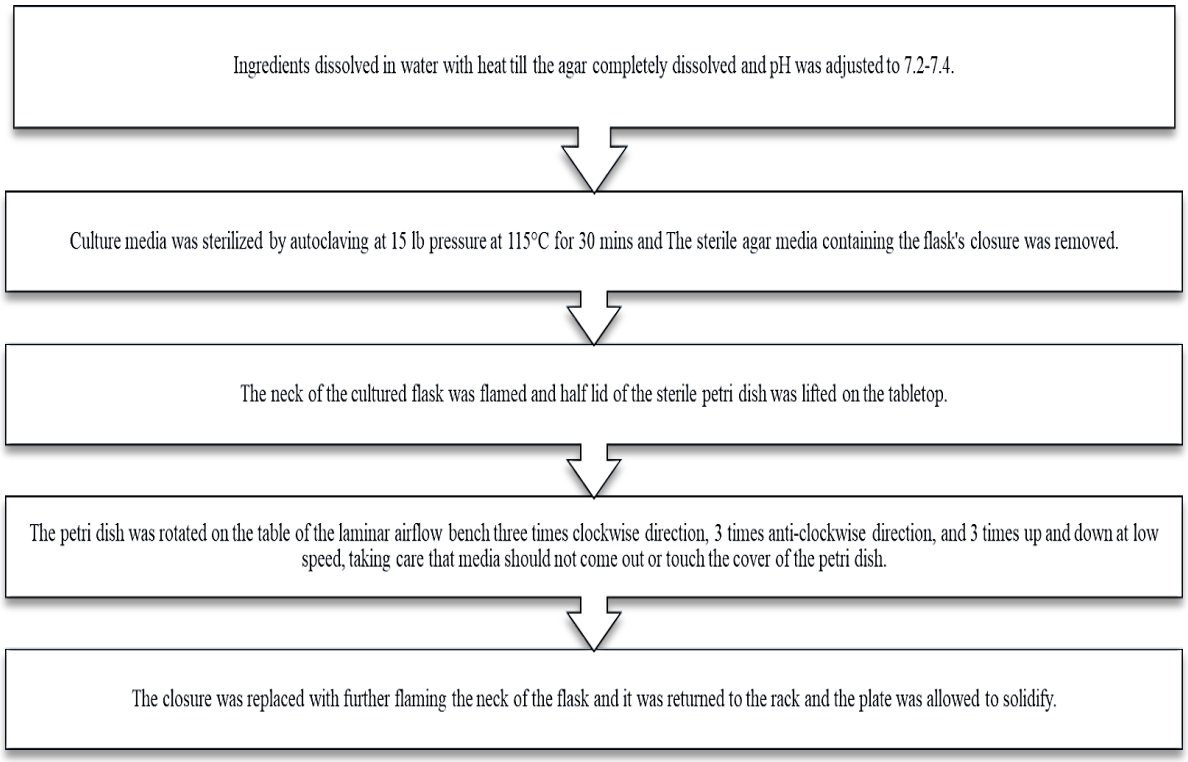

Figure 2. Sterilized agar plate preparation for microbial assay.

2.2.3. Characterization studies of seeds

Organoleptic properties like colour, odour, shape, size, and taste of *C. bonducella* seeds and extract have been assessed.

2.2.4. Chemical studies for identifying phytoconstituents

Standard techniques were applied when subjecting the methanolic extracts to a range of chemical analyses for the detection of phytoconstituents (Shalini and Ilango, 2021).

2.2.4.1. Test for alkaloids

Dragendroff's Test: 1 ml of Dragendroff's reagent was added to 2 ml of the extract along the test tube's edge. Alkaloids were present when an orange or orange reddish-brown precipitate formed.

Wagner's Test: Wagner's reagent (2 mL) was combined with 1 ml of crude extract. Alkaloids are indicated by reddish-brown precipitate, which is a marker of their presence. A solution of 2.5 g of iodine and 12.5 g of potassium iodide (KI_2) in 250 ml of water was created to create Wagner's reagent.

2.2.4.2. Test for cardiac glycosides

Keller-Kiliani Test: 2 ml of glacial acetic acid with a single drop of ferric chloride solution were added to 5 ml of extract. 1 ml of sulfuric acid that had been concentrated came next. The presence of carotenoids' deoxy sugar is suggested by the interface's rich brown colour. Under the brown ring, a violet ring may show up, and as the acetic acid layer steadily grows, a greenish ring may form.

2.2.4.3. Test for carbohydrates

Fehling's Test: Equal amounts of Fehling A and B reagents were added, and the mixture was then heated slightly before adding 2 ml of crude extract. Because a brick-red precipitate developed at the test tube's bottom, reducing sugars were discovered.

Benedict's Test: 1 ml of crude extract and 2 ml of Benedict's reagent were combined and heated. Carbohydrates were present because a reddish-brown precipitate formed (Manikandaselvi et al., 2016).

2.2.4.4. Test for flavonoids

Shinoda Test: 2 ml of the extract was mixed with 1 ml of a 1% ammonia solution. The easiest way to identify whether flavonoids are present visually is by the colour yellow.

2.2.4.5. Test for saponins

Foam Test: 2 ml of natural extract and 5 ml of distilled water were mixed and agitated vigorously in a test tube. Add some olive oil drops. The formation of steady foam was thought to indicate the presence of saponins.

2.2.4.6. Test for free amino acid

Millon's Test: 1 ml of crude extract and 2 ml of Millon's reagent are combined to generate a white precipitate. This precipitate turns red when it is slowly heated, signifying the presence of protein.

Ninhydrin Test: 1 ml of the natural extract and 2 ml of Ninhydrin 0.2% solution were combined and heated. The presence of proteins and amino acids was indicated by the violet precipitate that appeared.

2.2.4.7. Test for tannins

5% Ferric chloride Test: 0.5 ml of 5% ferric chloride was added to 5 mg of extract. Tannins are present when a dark bluish-black colour develops (Nakajima et al., 2005).

2.2.5. Quantitative estimation of phytoconstituents

2.2.5.1. Thin layer chromatographic study

For isolating, identifying, and quantifying plant components, chromatography techniques are essential. To establish the existence of early phytochemicals, thin-layer chromatography (TLC) methanol and chloroform in the ratios of 9:1, 8.8:1.2, and 9.2:0.8 served as the mobile phase. Compounds are separated and classified using TLC depending on how well they adhere to the stationary and mobile phases. A TLC plate is used to apply the prepared sample, and as the mobile phase passes through the stationary phase, different places on the plate represent various phytochemicals (Juvatkar and Jadhav, 2021). The relative polarity and other features of these spots can be learned from them. TLC is a useful approach for analyzing plant extracts and helps characterize and identify bioactive substances (Singh and Raghav, 2012).

2.2.5.2. Absorbance maxima determination

Regarding the domain of characterizing natural products, the utilization of ultraviolet-visible (UV-Vis) spectrophotometry to determine absorbance maxima has become a crucial technique. UV-Vis spectrophotometry precisely helped to identify what was performed. A TLC plate serves as the stationary phase, while a solvent combination includes and quantifies the absorbance maxima at specific wavelengths that correspond to the phytoconstituents like the presence of flavonoids (Pandey et al., 2018).

2.2.5.3. RP-HPLC analysis

Utilization of Reverse Phase HPLC for the comprehensive characterization of *Caesalpinia bonducella* seed extract was done. The RP-HPLC was carried out with the help of a Waters 1525 series chromatograph equipped with a gradient pump, and photodiode array detector (2998) and the sample injection volume of 20 µl. The Rhseodyne sample injector was utilized for the study. The analytical column diameter was 4.6 x 250 mm C18 (Waters, USA), and the particle size of the packed silica is 5 microns, it was 1 ml min^{-1} for the mobile phase flow rate. A binary pump gradient program was carried out in the process. Reservoir A contained acetonitrile, and Reservoir B contained water. Prior to injecting into the column, the extract is diluted twenty times and filtered through a 0.22 µm syringe filter. The gradient software utilized was as follows: 0–5 min 20% A; 5–8min: 30% A; 8–12 min: 40% A; 12–18 min: 50% A; 18–24 min: 60% A, 24-30 min: 70% A, 30-40 min: 80%. The total analysis time was 40 minutes. The peaks were recorded at 280nm. The scanning of the UV spectrum was performed from 200 to 800nm (Kumar et al., 2015; Mondal et al., 2023).

2.2.5.4. IR study

The methanolic extract has selective absorption in the infrared region, which makes it suitable for the structural investigation of its functional groups using infrared spectroscopy. (Khamkat et al., 2022).

2.2.5.5. GC-MS Study

Helium was used as a carrier gas at a constant flow rate of 1 ml min^{-1} to separate the components of a fused silica column packed with Elite-5MS (5% biphenyl, 95% dimethylpolysiloxane; 30 m × 0.25 mm ID × 250 µm df) that was utilized in the Clarus 680 GC analysis. During the chromatographic run, the injector temperature was set to 260 °C. The apparatus was filled with 1 µL of methanolic extract sample, and the oven temperature was set to 60 °C for two minutes, then 300 °C at a rate of 10 °C per minute; finally, 300 °C was maintained for six minutes. Conditions for the mass detector were a 240 °C transfer line, a 240 °C ion source, an ionization mode electron impact at 70 eV, a 0.2 s scan time, and a 0.1 s scan interval. The objects range was 40–600 Da. A database of component spectrums kept up to date in the GC-MS NIST (2008) library was compared to the component spectrums.

2.2.6. Microbial assay

Through uniformly circular zones of inhibition, the antimicrobial agents were permitted to spread into a plate (İnci et al., 2021). In order to assess the fungicidal and inhibitory concentrations' potency, the cup plate method was employed (Gupta et al., 2003; Ata et al., 2009).

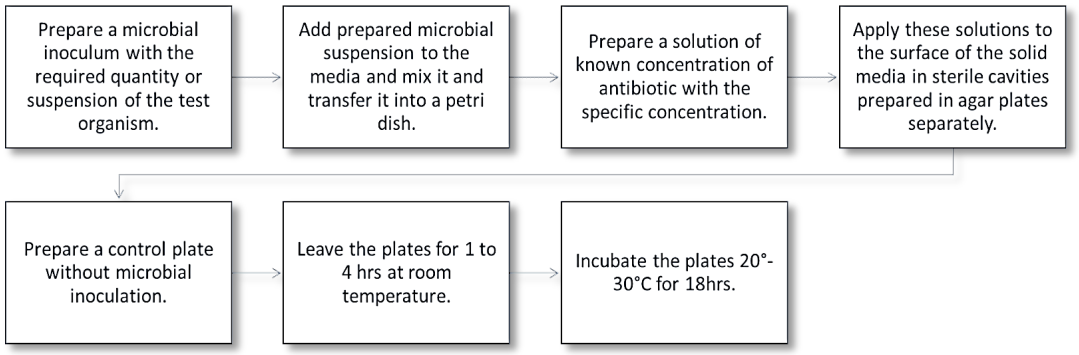

Figure 3. Steps involved in cup plate method for microbial assay.

3. Results

3.1. Characterization studies of seeds extract

Different extraction methods of *C. bonducella* seeds with different yield values are mentioned in Table 1.

Table 1. Different extraction methods by using different solvents

Sl no	Physical nature of seeds	Solvent	Method of extraction	Observation
1	Solid Powder	Methanol, ethanol	Soxhlet Extraction	More Yield
2	Solid Powder	Methanol, ethanol	Percolation	Least Yield
3	Solid Powder	Methanol, ethanol	Maceration	Moderate Yield

3.2. Organoleptic characters

The organoleptic properties of *C. bonducella* seeds and extract are described in Table 2.

Table 2. Observation of organoleptic properties of *C. bonducella* seeds and their powder form

Sl no	Properties of seeds		Sl no	Properties of powdered seeds	
1	Colour of seeds:	Off-white	1	Nature of powder:	Coarse powder
2	Taste:	Bitter	2	Colour of powder:	Off-white
3	Shape of seeds:	Globular	3	Colour of extract:	brown and dark
4	Size:	1-2 cm in diameter and 2.2-4 cm in length	4	Odour:	Characteristics
5	Odour:	Characteristics	5	Taste:	Bitter

3.3. Chemical studies for identifying phytoconstituents

Polar and nonpolar extract for the phytochemical tests. The polar extracts are ethanolic and methanolic extract and the nonpolar extract is the petroleum ether extract. The polar extracts have shown positive results in Table 3 for the chemical constituents like alkaloids, glycosides, tannins, flavonoids, etc.

Table 3. Qualitative analysis of phytochemicals presents in *Caesalpinia bonducella* seeds in different solvents

Sl no	Phytochemical tests	Methanolic extract	Ethanolic Extract
1	Alkaloids	+	+
2	Cardiac Glycosides	+	+
3	Flavonoids	+	+
4	Tannins	+	+
5	Saponins	-	-
6	Phenols	+	-
7	Steroids	-	-
8	Terpenoids	-	-
9	Quinones	-	-
10	Proteins	-	-

"+" indicates positive result and "-" indicates negative result.

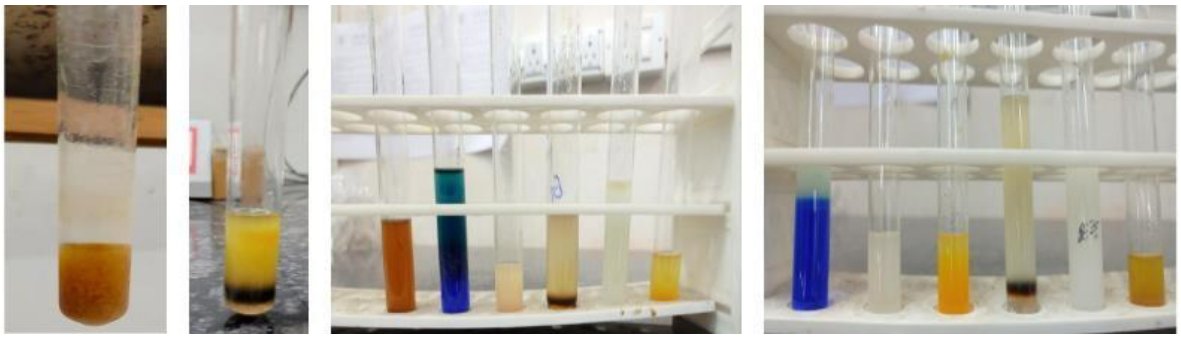

Figure 4. Identification tests of phytochemicals present in *Caesalpinia bonducella* seeds extract.

3.4. Thin layer chromatography

Methanolic and ethanolic extracts have undergone TLC. In Table 4, the methanolic extract has demonstrated the separation of eight distinct compounds, whereas the ethanolic extract has demonstrated the separation of two to three distinct compounds by displaying distinct colors on the TLC plate when viewed at 366 and 254 nm.

Table 4. Thin Layer Chromatography of methanolic and ethanolic extract from *Caesalpinia bonducella* seeds

Sl no	Chemical Constituent	Solvent System	Spraying Reagent	Observation	Retention Factor
1.	Methanolic extract	Formic acid: toluene: methanol: ethyl acetate: (5:4.5:4.5:1)	Aniline-Sulphuric acid	Showed the presence of 2-3 compounds	0.292,0.414,0.853
2.	Methanolic extract	Chloroform: methanol (9:1/8.8:1.2/ 9.2:0.8)	Anisaldehyde - Sulphuric acid	Showed the presence of 8 compounds	0.243,0.452,0.707,0.829,0.951
3.	Ethanolic extract	Methanol: acetic acid: ethyl acetate (6:4:1)	Anisaldehyde - Sulphuric acid	Showed the presence of 2 compounds	0.2, 0.709

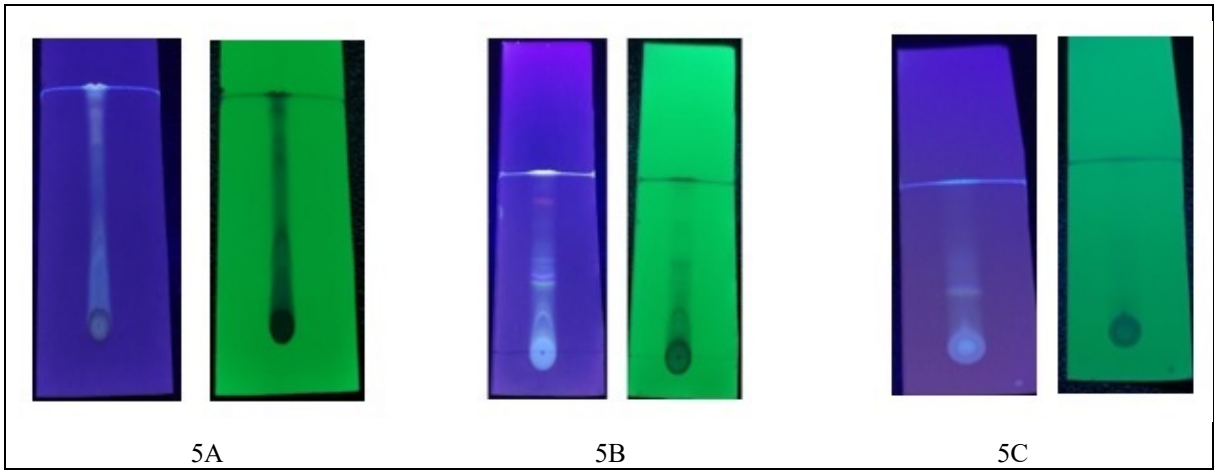

Figure 5. Separation of compounds visible at different wavelengths (366nm & 254nm) of methanolic extract using toluene, ethyl acetate methanol, and formic acid as solvent (5A), methanolic extract using chloroform and methanol as solvent (5B), ethanolic extract using ethyl acetate, methanol, and acetic acid as solvent (5C).

3.5. Infrared Spectroscopy

FT-IR Spectroscopy is done for functional group investigation of the present compounds. Here, the methanolic extract has been used, different functional groups of different compounds are shown at their peak in Figure 6A and interpretation of IR spectra is given in Table 5.

Table 5. Interpretation of IR spectra of methanolic extract of *Caesalpinia bonducella* seeds

Wavenumber(cm^{-1})	Functional Groups
3331.10	O-H Stretching
2914.97	C-H Stretching (Nearby Unsaturation point)
2833.41	C-H Stretching (Nearby saturation point)
1655.89	R-COOR´(Ester)

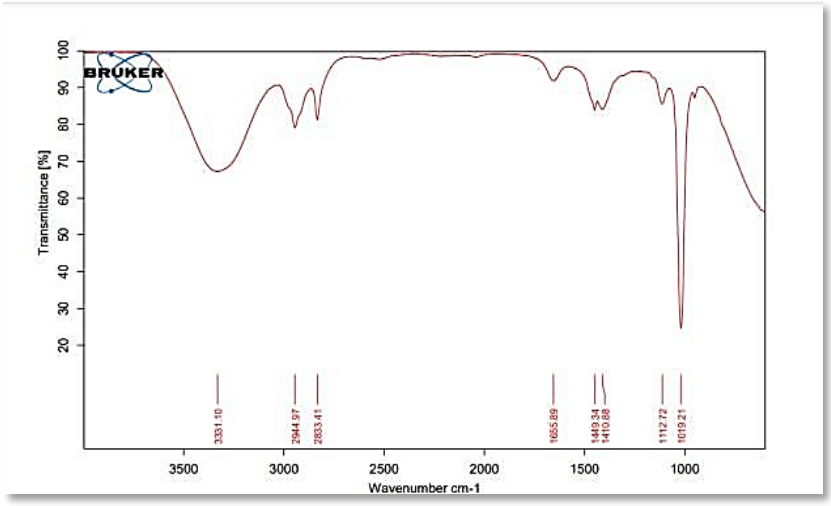

Figure 6. Graphical representation of specific absorbance of methanolic extract of *Caesalpinia bonducella* seeds in IR Spectroscopy.

3.6. RP-HPLC

The concentrated methanolic extract has been diluted 20 times with methanol and the dilute sample has been used in RP-HPLC for the analysis of different compounds and their concentrations are shown in Figure 8A and the data is given in Table 6.

Table 6. RP-HPLC chromatogram data of methanolic extract of *Caesalpinia bonducella*

Retention Time (min)	Area (µV*sec)	%Area	Height (µV)
2.011	4668353	27.65	212940
2.287	2981797	17.66	335308
2.444	4487020	26.58	224078
3.272	694613	4.11	188697
3.383	1537591	9.11	475799
3.812	754089	4.47	79587
4.083	250316	1.48	15139
4.426	327079	1.94	12293
12.915	399487	2.37	49358
21.087	170005	1.01	11584
26.824	165148	0.98	19226
27.187	243846	1.44	22640
27.895	204674	1.21	21567

3.7. GC-MS study

It helps to identify the number of components and the types of compounds present in the methanolic extract showed in Figure 8B. Table 7 and Figure 7 indicate the structural representation of compounds identified in the *Caesalpinia Bonducella* Seeds extract by GC-MS.

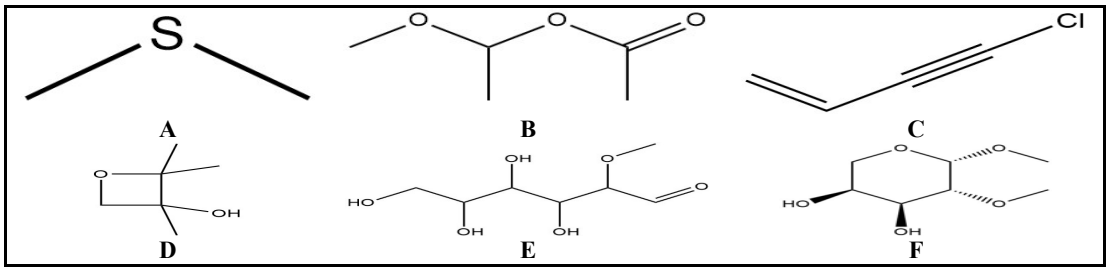

Figure 7. Compounds (A–F) found in the *Caesalpinia bonducella* seed extract and their structural representation using GC–MS.

Table 7. Data of GC-MS study of methanolic extract from *Caesalpinia bonducella* seeds

Compounds	Retention Time(min)	Peak Area %	Compound Name	Molecular formula	Molecular weight(m/z) (Dalton)	Compound nature
A	1.123	5.467	Dimethyl sulfide	C_2H_6S	62	Thioether
B	1.168	2.998	Ethanol, 1-methoxy-, acetate	$C_5H_{10}O_3$	118	Ester
C	1.188	8.373	4-chlorobuten-3-yne	C_4H_3Cl	86	Choloro alkyne
D	1.253	5.395	3-oxetanol, 2,2,3-trimethyl	$C_5H_{10}O$	116	Alcohol
E	16.875	56.294	2-o-methyl-d-mannopyranosa	$C_7H_{14}O_6$	194	Polyhydroxy alcohol
F	16.945	21.473	Methyl-2-o-methyl beta l-arabinopyranoside	$C_7H_{14}O_5$	178	Carbohydrate

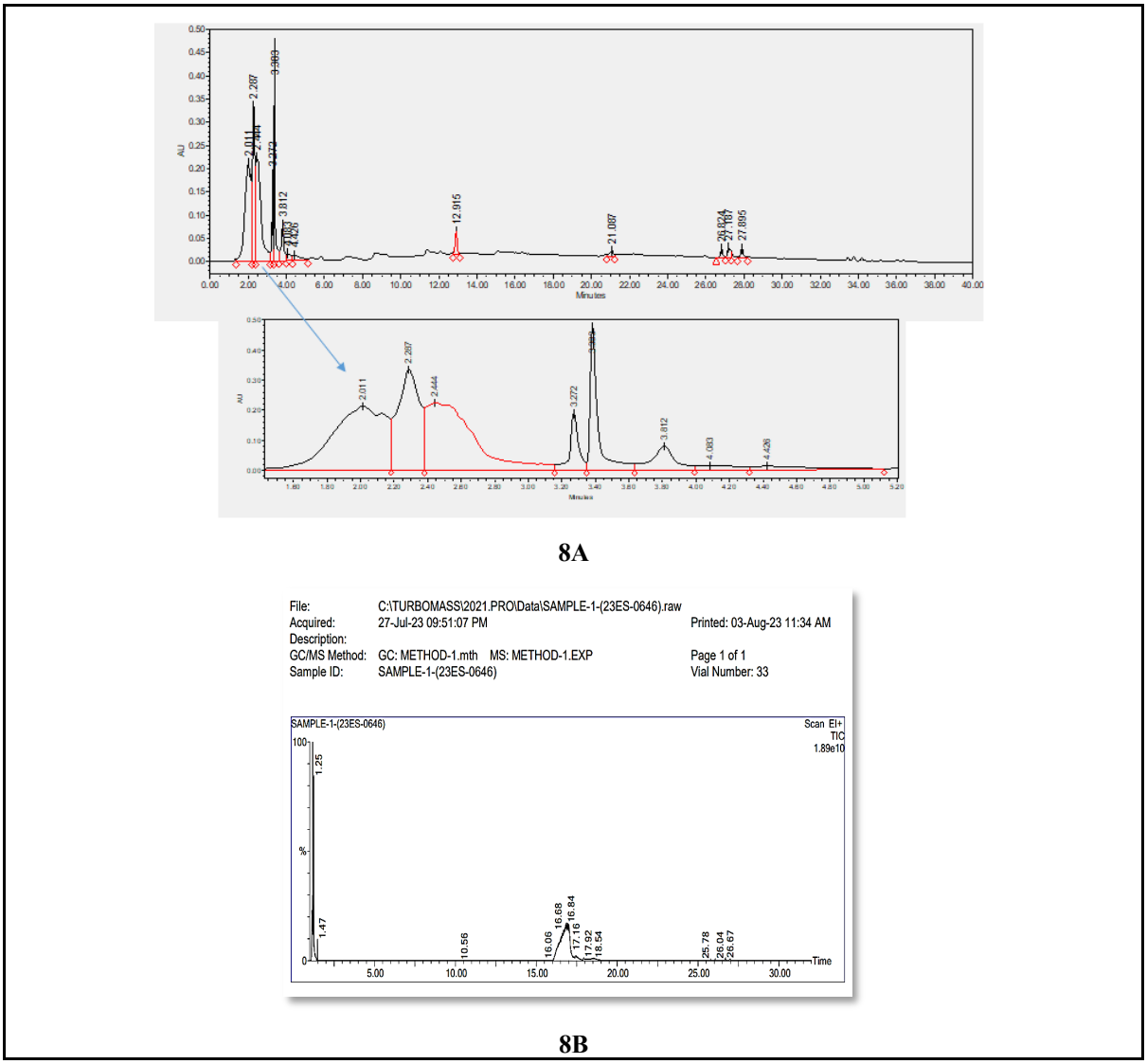

Figure 8. Methanolic extract of *Caesalpinia bonducella* seeds obtained by RP-HPLC (8A) and GC-MS chromatography (8B).

3.8. Microbial assay

Microbiological experiment has been carried out to identify the area where microorganisms undergo inhibition by the methanolic and Ethanolic extract using the Soxhlet extraction method. The

methanolic extract has the highest antibacterial activity shown in Table 8 and Figure 9 shows a zone of inhibition for determining the potency.

Table 8. Ethanolic and methanolic extract's Antibacterial Properties

Microbes	Number of tested isolates	Concentrations of ethanolic extract	Diameter of inhibition zone of Ethanolic extract	Concentrations of Methanolic extract	Diameter of inhibition zone of Methanolic extract
Salmonella typhi	4	100,150,200,250	12±0.53	100,150,200,250	28±8.0
E. coli	5	100,150,200,250	16±0.54	100,150,200,250	28±8.2
Staphylococcus aureus	5	100,150,200,250	12±0.54	100,150,200,250	30±7.9
Aspergillus niger	4	100,150,200,250	17±0.58	100,150,200,250	20±5.0

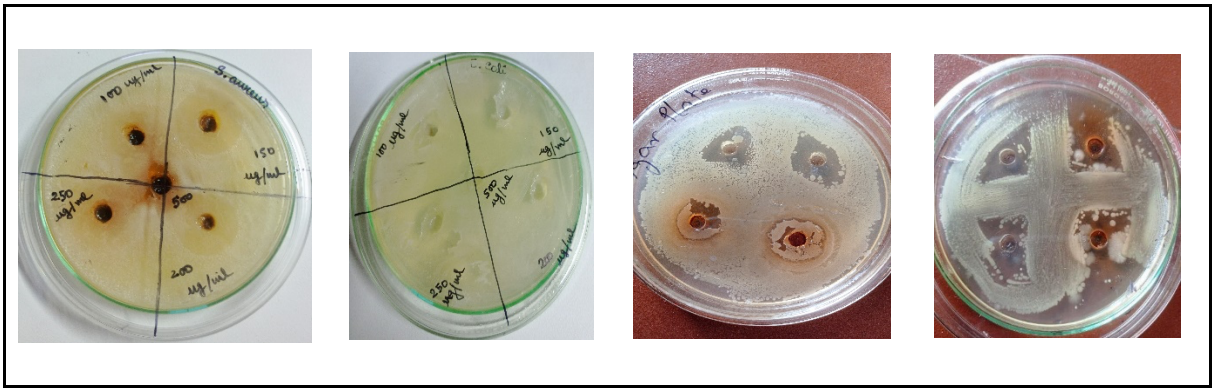

Figure 9. Zone of inhibition of antibacterial microorganisms against methanolic extract.

4. Discussion

The extraction process of the *Caesalpinia bonducella* seeds was carried out using several extraction techniques, including soxhlation, percolation, and maceration. Among all these techniques from the soxhlation process, the yield was the highest. When the methanolic and ethanolic extracts were examined chemically, the methanolic extract gave a superior result. Then further study is carried out with reference to methanolic extract. TLC for the methanolic extract showed eight significant spots, which provisionally indicates the presence of several polar phytoconstituents. Then further FTIR, HPLC, and GC-MS were carried out to determine those polar phytochemicals. The existence of "alcohol", "ether", and unsaturated "alkanes" has been detected in the IR spectroscopy report. Then RP-HPLC was also done to confirm more evidentially the presence of polar constituents in the extracts, and it showed a positive result when the peaks were recorded at 280nm in the investigation. To confirm it, again GC-MS study was carried out. In 2021 Sasidharan et al. reported about the antibacterial activity of *Caesalpinia bonducella* plant (Sasidharan et al., 2021). Finally, a microbiological assay was carried out for both the extracts (methanolic and ethanolic), and it showed a more significant area of inhibition for the methanolic extract.

Conclusion

Caesalpinia bonducella is a valuable therapeutic plant, as demonstrated by the phytochemical investigations done on its seeds. Percolation, maceration, and the Soxhlet apparatus were used to get the total methanolic extract. The extract from the Soxhlet apparatus was more suitable for TLC. Thin Layer Chromatography was performed to isolate compounds. By using UV spectroscopy to quantify phytoconstituents for phytochemical substances, the highest absorbance of two distinct molecules was discovered. at 271.0 and 210.0 nm. IR spectroscopy method has been carried out and it shows the presence of "Alcohol", "Ester" & "Unsaturated alkanes" and later we confirmed their presence with the GC-MS Study. As part of the examination, RP-HPLC was also used to prove the presence of major

chemical constituents. When peaks were recorded at 280 nm, the RP-HPLC test yielded a positive result, and a GC-MS analysis was used to confirm it. Numerous gram-positive and gram-negative bacteria as well as fungi have been used in microbial assays for ethanolic and methanolic extract. It can be said that *Caesalpinia bonducella* seeds containing different phytochemicals have antibacterial and antifungal activity which can be determined by the zone of inhibition. *Caesalpinia bonducella* can be used as an antibacterial agent alternative to synthetic compounds and methanolic extract showed better antibacterial activity. Cultivation of *Caesalpinia bonducella* should be increased in India to treat microbial infections.

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