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RESEARCH ARTICLE

Phytochemical Profiling and Therapeutic Potential of *Acacia sinuata* (Lour.) Merr.: Insights into *In Vitro* Haemolytic, Antioxidant and Cytotoxic Activities

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Abstract

Background: *Acacia sinuata* (*Shikakai*) has several traditional and conventional benefits, including its use as a leprosy treatment, as well as for malaria, diabetes, constipation, and jaundice. Etc. Due to the presence of saponin and other phytochemicals contents, it exhibits therapeutic properties. **Aim:** The current investigation seeks to analyse the phytochemicals and *in vitro* haemolytic activity of the extracts of *A. sinuata* successively and screening of its antioxidant properties and cytotoxic potential. **Methods:** Pods of *A. sinuata* were gathered, desiccated and ground. The Soxhlet extraction was carried out with the powdered material. Thus, the crude extracts were utilised to further screen the phytochemicals and fluorescence characteristics and *in vitro* haemolytic properties. *In vitro,* anti-oxidant investigations were conducted for the plant's ethanolic extract *A. sinuata*. The MCF-7 cell line was subjected to *in vitro* cytotoxic assay with ethanolic extract. **Results:** *Acacia sinuata*'s phytochemical analysis demonstrated that polar solvents are more effective in extracting the majority of secondary metabolites than non-polar ones. Every *Acacia sinuata* extract has a notable quantity of phytochemicals and showed significant haemolytic activity. Ethanolic extracts showed minimum haemolytic activity and chloroform-water extract showed maximum haemolytic activity. The ethanolic extract has potent antioxidant properties and showed better cytotoxic properties compared to acetone and aqueous extracts. **Conclusion:** This study investigates and documents the haemolytic activity of *A. sinuata* pod extracts. The current study's data indicate that *A. sinuata*'s ethanolic extract possesses strong anticancer and antioxidant capacities. In future, the ethanol-based extract can be taken for formulating different kinds of dosage forms for therapeutic uses.

Major Findings: This research of *Acacia sinuata* pod extracts shows the haemolytic activity and anti-cancer activity which is reported for the first time in this article along with antioxidant capacity.

Keywords: *Acacia sinuata*, Antioxidant, Cytotoxicity, Haemolytic Activity

Abbreviations: ACE: Acetone; AQ: Aqueous; BEN: Benzene; CH: Choroform; °C: Degree Celsius; ETH: Ethanol; IC50: Inhibitory concentration; MTT: Microculture tetrazolium test; µg: Micro-gram; PBS: Phosphate buffer saline; PE: Petroleum ether; Rpm: Revolutions per minute; TCA: Trichloroacetic acid.

1. Introduction

Common names for *Acacia sinuata* include *Reetha*, *Kochi*, *Banritha*, *Ritha*, *Shikai*, *Chikaka*, *Shikaya Sige* and *Shikakai*. This is a climbing tree/shrub that is most known for its fruit, which is used to make natural shampoo. When it ages, the flower heads have a diameter of around 1cm. The flowers are pink with or without diminished subtending bracts. Pods are 8-10cm long by 1.6–1.8 cm diameter, thick, stalked and slightly flattened[1]. *S. rugata, A. sinuata* or *A. concinna*, a ubiquitous shrub in the humid plains of central and south India, belonging to the family *Leguminosae*; commonly known as *shikakai* is a spiny climbing shrub native to tropical Asia and China. *Shikakai* is referred to as "fruit for hair" as it has a naturally moderate pH that

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gently cleanses the hair without removing its organic oils. *Shikakai* is beneficial for controlling dandruff, promoting hair growth and strengthening hair roots as a hair-washing agent, the powder or extract from the leaves, bark and pods is used. In indigenous medicine, *shikakai* has been utilised to treat skin issues, diarrhoea and jaundice. Leaves and pods can be used to cure cuts, wounds and dental issues because of their astringent properties[2]. India's tropical jungles are home to an abundance of soap nut, or *shikakai*, plants, particularly in the Deccan region. It is highly regarded as a scalp cleaner and is utilised as a cleansing agent in different regions of India. Skin diseases can be effectively treated by the tonic and astringent qualities of soap nut fruit. The tannins, rutin, alkaloids and citric acid found in soap nut leaves are what give the tree its medicinal properties[3]. Surface-active glycosides found naturally with a characteristic foaming behaviour are called saponins. Their name comes from the soapwort shrub (*Saponaria*), the root was historically used as soap (*sapo* means soap in Latin). Although some bacteria and lesser marine species can also make them, plants are the primary source of these components[4].

Saponin's capacity to foam is increased when hydrophilic, or water-soluble, sugar is combined with hydrophobic, or fat-soluble, sapogenin. Triterpenoid saponins are typically more abundant in farmed crops, whereas steroidal saponins are more frequently found in plants that are utilised as herbs or for their perceived health benefits. Saponin can rupture Red Blood Corpuscles (RBC) (erythrocytes). As a result, haemolytic assays have been developed to identify the occurrence of saponins in drugs or plant extracts. The chemical reaction between the erythrocyte membrane's sterols and saponins is typically responsible for the haemolytic qualities. The membrane bursts as a result, increasing permeability and resulting in haemoglobin loss[5]. Saponin = Sugar + Sapogenin.

The impact of saponins on human erythrocyte hemolysis on the membrane structure demonstrated that erythrocytes are lysed by no saponin reseal, suggesting that the lipid bilayer is irreparably damaged by saponins[6]. One of the saponins' pharmacological effects is the augmentation of immunological responses[7-11]. Research has demonstrated that saponins can mitigate oxidative stress's repercussions[12,13], prevent apoptosis and so shield cells from dying[14-17]. However, saponins might be useful in the fight against malignancy[18-29]. Prior to haemolysis, erythrocytes may undergo eryptosis or cell suicide., thus resulting in the removal of damaged erythrocytes before the haemoglobin is released[30].

2. Materials and Methods

2.1 Collection, Identification and Authentication of Plant Samples

The pod parts of the plant *A. sinuata* were collected from the Alipurduar forest, West Bengal, India in January, 2022. The shrub has been identified and authenticated by the Botanical Survey of India, Howrah, West Bengal, India; bearing number- BSI/PLANT CHEM/0008-2022/ dated 18th of April, 2022.

2.2 Preparation of Plant Materials

The plant samples (pods) were air-dried in the Himalayan Pharmacy Institute's laboratory. Using a mechanical grinder, the plant components were ground into a coarse powder, which was subsequently utilised for investigation.

2.3. Pharmacognostic Evaluation[31]

2.3.1 Macroscopic Assessment

The shape, size, taste, colour, odour, fracture, and texture of this crude drug were all assessed macroscopically using standard techniques (Figure 1).

2.3.2 Microscopical Evaluation

2.3.2.1. Transverse Section (TS) of Fruit (Pods)

A blade was utilised to section the plant's fruit, first observed in distilled water to locate the crystal system. Then with glycerine followed by staining with Phloroglucinol and concentrated Hydrochloric acid to observe lignified components. Other staining reagents



Figure 1. Pods of *Acacia sinuata*.

such as safranin, iodine etc. are also used but only phloroglucinol HCl has shown better results.

2.3.2.2 Powder Microscopy

The powder examination of the crude drug was performed by collecting and then washing thoroughly with water to remove any unwanted matter, this was further air dried in the shade. Using glycerine and distilled water as mounting media, a finely powdered drug was prepared and various tissue and cell components were checked.

2.3.3 Extractive Value

The extracts that are produced when crude drugs are used up are a good way to determine the amounts of their chemical components. 150gm of air-dried coarse powder of pods of *A. sinuata* was extracted by Soxhlet extraction (hot continuous extraction) with 300ml of each solvent successively according to their polarity i.e., Petroleum Ether (PE), followed by Benzene (BEN), Chloroform (CH), Acetone (ACE) and Ethanol (ETH) in Soxhlet apparatus for 6 hours. Thereafter, it was filtered by muslin cloth and the solvent was gently separated from the extract by rotary evaporator apparatus. After drying, the dried extract was weighed and the air-dried drug powder that was taken for extraction was applied as an allusion for determining the extractive value. The following formula is used for calculating extractive values[32].

$$\text{Extractive value} = \frac{\text{Weight of dried extract}}{\text{Weight of plant materials}} \times 100$$

2.3.3.1 Determining the Extractive Value That is Soluble in Water

120gm of previously used *A. sinuata* powder was macerated with 200ml of water (1% chloroform water) in a beaker for 48 hours with occasional stirring during the first 6 hours and then allowed to stand for 12 hours Thereafter, it was filtered by muslin cloth and solvent was gently separated from the extract using rotary evaporator. After drying, the dried extract was weighed and the extractive value was determined with the reference to powder taken for extraction.

2.3.4 Determination of Moisture Content

In a tarred watch glass, precisely 5g of the air-dried crude powdered material was weighed. The materials were dried for a while at 105°C in a hot air oven until their weight remained consistent. The drug's moisture content is determined by the weight differential using the following formula[33].

$$\text{Moisture Content}(\%) = \frac{(\text{Ww} - \text{Wd})}{\text{Ww}} \times 100$$

Where, Ww- Wet weight of the sample, Wd- Weight of the sample after drying.

2.3.5 Determination of Ash Value

2.3.5.1 Total Ash

A tar-coated platinum or silica dish containing precisely two grams of air-dried medicament was ignited at a temperature of not more than 700°C until the substance was carbon-free[34]. After cooling, the weight was recorded. If a carbon-free ash could not be obtained, the burned mass was cleansed with hot water and the remnant was gathered. On ashless paper, the remnant was burned together with the filter paper until the ash was white or almost whitish. Once the filtrate had evaporated, it was put into the dish. It was calculated as a proportion of the overall ash content of the medication.

2.3.5.2 Acid-insoluble Ash

Using 25 millilitres of 2M HCL acid to bring the ash to a boil (from total ash) for five minutes, the insoluble debris was taken in an ashless filter paper or Gooch crucible. Following a hot water cleaning, it was ignited, let to cool in a desiccator and then weight was noted. The percentage of acid-insoluble ash was determined using the drug base that was dried.

2.3.5.3 Ash Soluble in Water

The ash (total ash) was heated for five minutes with 20cc of water, and the insoluble materials were taken out in a Gooch crucible/on an ashless filter paper. Following a fifteen-minute burning at a temperature not exceeding 800°C, the mixture was washed with hot water. Water-soluble ash displays the weight differential following the deduction of the insoluble material's weight from the ash's weight. Next, using dry medications as a base, the percentage of water-soluble ash was computed[35].

2.3.6 pH Value

The pH was checked using pH papers and a pH meter while dipping it in the extract solution. Then the values were calculated separately for all the extracts.

2.4 Qualitative Analysis

Following standard protocols, the extracts of *A. sinuata* were put through qualitative phytochemical analysis to determine the various Phyto-constituents present in the extracts.

2.4.1 Preliminary Screening for Phytochemicals

The concentrated extracts were subjected the chemical tests for the analysis of various phytochemicals as per the standard methods[36].

2.4.2 Fluorescence Analysis

To determine the type of fluorescence, the powder was treated with several chemicals, and the combination was examined in both daylight and ultraviolet light. Many of the chemical components found in plant material exhibit the phenomena of fluorescence. Many natural substances, including alkaloids like berberine, illuminate when exposed to ultraviolet light but do not glow in the presence of daylight. It is usually possible to convert non-fluorescent compounds into fluorescent derivatives or breakdown products using other reagents. Because of this, several crude medications are regularly assessed qualitatively, which is a crucial pharmacognostic indicator. The high sensitivity of fluorescence analysis techniques, which offer detection limits at picomolar levels and lower as well as the vast range of sample presentation options, have led to their widespread use today.

2.5 Haemolytic Activity

The spectrophotometric method was used to measure the *in vitro* haemolytic activity. Blood was drawn from five millilitres of *Tilapia* fish (*Oreochromis niloticus*). For three minutes, the blood was centrifuged at 1500rpm. Centrifugation at 1500rpm for 5 minutes was used to wash the pellet three times with sterile Phosphate Buffer Saline (PBS) solution (pH 7.2±0.2). The cells were again suspended in 0.5% normal saline. A volume of 0.5 millilitres of the extracts (concentrations of 125, 250, 500 and 1000 *µg*/ml in saline) was combined with 0.5 millilitres of cell suspension. The mixtures were centrifuged for 10 minutes at 1500rpm after 30 minutes of 37°C incubation. In the Ultra Violet (UV)-Vis spectrophotometer, the free haemoglobin in the supernatants was determined at 540nm. PBS and deionised water were employed as the lowest and highest levels of haemolysis. Every experiment was run three times for every concentration. It was determined what percentage of haemolysis the extracts caused according to the formula[37]:

$$\%\text{Haemolysis} = \frac{(\text{At} - \text{As})}{\text{Ac} - \text{As}} \times 100$$

Here:

At is the test sample's absorbance. As is the control's absorbance (saline control). Ac is the control's absorbance (water control).

2.6 Antioxidant Study of *A. sinuata* Ethanolic Extract

2.6.1 1, 1, Diphenyl-2- Picrayl hydroxyl Assay (DPPH Assay)

DPPH radical scavenging ability of the extract was assessed using the Blois method, 1958[38]. Test tubes containing *Acacia sinuata* crude extracts at several concentrations (125, 250, 500, and 1000 µg/ml) were used. Each test tube holding five millilitres of a 0.1mM ethanolic solution of 1, 1, Diphenyl-2-Picryl hydrazyl (DPPH) was thoroughly shaken before being left to stand at 37°C for twenty minutes in the absence of light. At 517nm, the absorbance was calculated by UV spectrophotometer. A positive control was ascorbic acid. There were three test runs conducted. DPPH free radical scavenging ability (%) was calculated by using the following formula-

$$\%\text{ of inhibition} = \frac{\text{absorbance of control-absorbance of sample}}{\text{absorbance of control}} \times 100$$

2.6.2 Ferric Reducing Antioxidant Potential Assay (FRAP Assay)

Acacia sinuata crude extracts at varying dilutions (125, 250, 500, and 1000 µg/ml) were placed in test tubes. 2.5ml of sodium phosphate buffer and 2.5ml 1% potassium ferric cyanide solution were incorporated into the test tubes. After thoroughly mixing, these ingredients were incubated for 20 mins at 50°C. 2.5ml of 10% Trichloroacetic Acid (TCA) was incorporated after incubation and the test samples were centrifuged for 10 minutes at 3000rpm. Following centrifugation, 5ml of supernatant liquid was extracted, and 5ml of distilled water was added. After adding around 1mL of 1% ferric chloride, this was incubated for 20 minutes at

35°C. At 700nm, the absorbance (O.D.) was measured. The blank was produced by mixing all other solutions with ferric chloride (0.1%) and no extract, whereas the control solution was made by mixing all other solutions without extract. Tests were performed on triplicates[39].

2.7 *In Vitro* Cytotoxic Activity of *Acacia sinuata* Extracts by MTT Assay

2.7.1 Cell Culture Procedure

In this work, a continuous line of Human breast carcinoma (MCF-7) was the target cells. The cells have been grown in an environment with increased humidity with 5% CO2 and maintained at 37°C under 100% humidity in Dulbeccos Modified Eagle Medium (DMEM). The cells have been supplemented with RPMI-fetal bovine serum to a final volume of 10% and antibiotics (100 U/milliliters penicillin and 100 µg/millilitres streptomycin). Every other day, the medium changed using 0.2% (w/v) trypsin, the cells were separated when they reached confluence and then moved to fresh culture flasks. Once the cells had grown sufficiently for testing, they were trypsinised and placed at a density of 1×10^5 cells/well on 96-cluster culture well plates. To generate a monolayer culture, the plates were incubated for 24 hours at 37°C with 5% CO2 and contained 100µl of cell suspension per well. Following 24 hours of incubation, the old media was extracted from every well. Afterwards, the cancer cells were treated with a range of concentrations of each solvent extract of *A. sinuata* – ACE, ETH and AQ such as 10, 25, 50, 100 and 200µg/mL. The tests were conducted in triplicate. The plates' cell survival was evaluated after a 24-hour incubation at 37°C with 5% CO_2[40,41].

2.7.2 MTT Assay

From each well of the first plate, the test samples were extracted. After that, 50µL of Microculture Tetrazolium Test (MTT) reagent (5 mg/mL) was added, and the CO2 incubator was allowed to incubate for two hours at 37°C. After that, 100µL of isopropanol was incorporated and the MTT solution was disposed of. The purple crystal formazan forms were dissolved by shaking the plates. A microplate reader operating at 570nm was used to measure the absorbance. A graph of the % inhibition versus extract concentrations was created using the data[42]. To evaluate the safety of ethanolic extract (chosen after MTT assay), primary macrophage cells of mice were taken to evaluate the % viability against different concentrations of ethanolic extract. The formula below was applied to evaluate the percentage of cytotoxicity or suppression of cell growth[43]:

$$\%\text{Growth inhibition} = 100 - \frac{\text{Mean OD of ondividual Test Group}}{\text{Mean OD of control group}} \times 100$$

3. Results and Discussion

The pharmacognostical constants for the fruit of this plant and the qualitative microscopy are clearly explained for its identification and authentication. The numerical criteria and diagnostic microscopic features described in this study may prove helpful in compiling an appropriate monograph for accurate identification. One crucial factor in identifying drug adulteration or inappropriate handling is the physical ongoing evaluation of the drugs.

3.1 Macroscopical Evaluation

- Length: 6-10cm
- Thickness: 0.5cm
- Taste: Sour
- Odour: Characteristic
- Colour: Reddish brown/ blackish brown

3.2 Microscopical Evaluation

Fruit (pod) was transversely sectioned using free-hand sections treated with chloral hydrate. The crystal system was first identified by observation in distilled water. To see lignified components, stain with fluoroglucinol and concentrated HCl and seen under a Corl Zeiss binocular microscope. Distilled water and glycerin were used as mounting media to create several slides of the finely powdered drugs, which were then examined for various tissue and cell contents. Additionally, the powder was cleaned with a chloral hydrate solution and examined under a microscope to examine various tissues. To distinguish between lignified and non-lignified tissues, the powders were treated with phloroglucinol and then HCl. Iodine solution was also used to test crude powder for the presence or absence of starch granules (Figures 2 and 3).

3.3 Extractive Value

Pharmacognostical investigations, which included macroscopic and proximal analysis were applied to

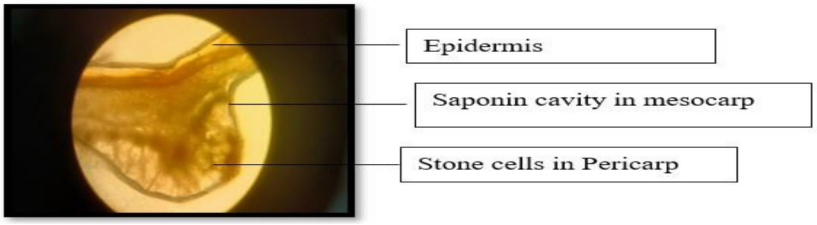


Figure 2. T. S of *Acacia sinuata* pod.

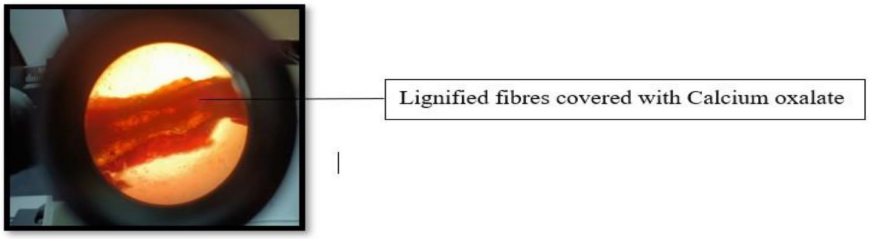


Figure 3. Powder microscopy of *Acacia sinuata* pod.

identify the crude drug. When determining whether a medicine is contaminated or exhausted, extractive values can be helpful. Therefore, the identification and verification of the plant material will be aided by extractive value, ash value, and moisture content analysis. Typically, these criteria are examined to verify the plant material's purity. The results suggest that the crude drug has a higher water-soluble extractive value in comparison to organic and alcohol-soluble extractive values. In accordance with their increasing polarity, it describes the physical characteristics and colour of the extracts. The extractive values in percentage were calculated using the following formula and recorded (Table 1).

3.4 Moisture Content

Because the medication's moisture content wasn't excessively high, it might inhibit the growth of germs, fungi or yeast. It was reported that the crude powder had a moisture content of 6.37%w/w (Table 2).

3.5 Ash Value

The examination of drug purity, which involves determining whether or not inorganic foreign components like silica or metallic salts are present, depends heavily on the overall ash content. The ash value is shown in the Table 3.

3.6 pH Value

pH values were measured by pH meter and pH paper (Table 4).

Table 1. Extractive values of various solvent extracts of *Acacia sinuata*

Sl. No.	Extract	Colour	Extractive Value (%w/w)
1	Ethanol soluble extract	Brown	14.6%w/w
2	Aqueous soluble extract	Dark brown	27.15%w/w

Table 2. Moisture content of crude powder of *A. sinuata* pods

Pods (extract)	Wet Weight (gram)	Dry Weight (gram)	Moisture Content(%w/w)
Acacia sinuata	1.10	1.03	6.37

Table 3. Different types of ash values

Sl. No.	Type of Ash	Ash value (%w/w)
1	Total ash	7.5 %
2	Acid insoluble ash	2.5 %
3	Ash soluble in water	4 %

Table 4. pH values of different solvent extracts

Sl. No.	Solvent for extraction	pH Values
1	Benzene	9.06
2	Chloroform	8.10
3	Acetone	6.69
4	Ethanol	5.65
5	Aqueous	6.21

3.7 Preliminary Screening for Phytochemical

The concentrated extracts were subjected the chemical tests for the analysis of various phytochemicals and the results are shown in Table 5.

3.8 Fluorescence Analysis

The qualitative phytochemical analysis provided insightful information about the numerous phytoconstituents found in the various extracts of the same plant material, information that may be useful in choosing a specific extract for more research into isolating the active constituent. The examination of fluorescence of powdered crude drug showed various colours depending upon the treatment of chemicals with the powder under visible and Ultraviolet light (Table 6).

3.9 Haemolytic Activity

The presence of components with therapeutic activity was discovered during the current study's

Analysis of plant samples. Fish blood was taken to test the *A. sinuata* pod extracts' haemolytic activity in various solvents. Extracts displayed low to moderate haemolytic effect toward erythrocytes. The haemolytic action of the plant is indicated in % haemolysis. The result indicated that the ethanolic extract (at dose 125 µg/ml) possessed minimum haemolytic activity (7.98%) with an IC_{50} value of 388µg/ml, whereas water extract (at dose 1000 µg/ml) possessed the highest haemolytic activity (89.18%) with an IC_{50} value 401.71µg/ml. It showed that the haemolytic percentage increased as the dosage increased (Table 7). This is the first-ever report on the haemolytic properties of *A. sinuata* pods. The haemolytic activity was observed

Table 5. Phytochemical screening of variant polarity solvent extracts of *Acacia sinuata*

Phyto chemicals	Performed Chemical Test	Solvents					
		PE	BEN	CH	ACE	ETH	AQ
Carbohydrate	Molisch's test	-	-	-	+	+	+
	Fehling's test	-	-	-	+	+	+
	Barfoed test	-	-	-	-	-	+
Alkaloid	Dragendorff's test	-	-	-	-	+	+
	Mayer's test	-	-	-	-	+	+
	Hager's test	-	-	-	-	-	-
Tannin and Phenol	Ferric Chloride test	-	-	+	+	+	+
	Lead acetate test	-	-	+	+	+	+
	Iodine test	-	-	-	+	+	+
Flavonoid	Vanillin HCL test	-	-	+	+	+	+
	Alkaline reagent test	-	-	+	+	+	+
	Lead acetate test	-	-	+	+	+	+
	Shinoda test	-	-	-	-	+	+
Saponin	Foam test	-	-	-	-	+	+
	Froth test	-	-	-	-	+	+
	Haemolysis test	-	-	-	-	+	+
Glycoside	Keller-Killiani test	-	-	-	+	+	+
	Borntrager's test	-	-	-	+	+	+
Phytosterol	Liebermann Burchard's test	-	-	-	-	-	+
	Salkowski's test	-	-	-	-	+	+
Protein	Millon's test	-	-	-	-	-	-
	Ninhydrin test	-	-	-	-	-	-

(+) indicates Presence and (-) indicates Absence

Table 6. Fluorescence analysis of pod powder of *Acacia sinuata*

Sl. No.	Treatments	Observations	
		Visible light	UV light (254nm)
1	Powder + 1N NaOH	Brown	Dark brown
2	Powder + 1N H_2SO_4	Yellowish brown	Dark brown
3	Powder +50% HNO_3	Brown	Reddish brown
4	Powder + Conc. HNO_3	Pale brown	Brown
5	Powder + 10% HNO_3	Brown	Dark brown
6	Powder + Ammonia	Greenish brown	Brown
7	Powder + Acetic acid	Reddish brown	Blackish brown
8	Powder + 50%Iodine	Dark green	Fluorescent green
9	Powder + 5% $FeCl_3$	Greenish brown	Dark brown
10	Powder + Methanol	Brown	Black
11	Powder + water	Brown	Dark brown

Table 7. Haemolytic activity of *A. sinuata* pods

Test Extracts	% Haemolysis				IC_{50} Value (µg/ml)
	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml	
PE	40.54	46.32	56.85	59.10	433.44
ACE	9.71	43.37	46.44	51.18	818.97
ETH	7.98	45.22	85.54	87.50	388
AQ	10.75	46.03	75.47	89.18	401.71

under UV Spectrophotometer for different extracts when treated with fresh fish blood in PBS solution and the haemolytic potency of the plant extracts is expressed in % haemolysis. The maximum haemolysis is shown by the chloroform-water extract (89.18%) and the minimum activity by ethanolic extract (7.98%). The value of IC_{50} was determined from the equation of the standard graph plotted in an MS Excel sheet.

3.10 Antioxidant Activity

To determine *A. sinuata*'s antioxidant activity, ethanolic extracts were assessed at several doses (125–1000 µg/ml). Calibration curves that were dependent on dosage were created. The calibration curves for the sample and standard were used to calculate the IC_{50} values. The ethanolic extract of *A. sinuata* (sample) was a powerful DPPH scavenging agent (IC50 257.6 µg/ml), which was followed by ascorbic acid (standard) (IC_{50} 170.8 µg/ml). In the case of the FRAP assay the IC_{50} value for extract was 535.2 µg/ml and the IC_{50} value for Ascorbic acid was 191.8 µg/ml. Only ethanolic extract has been chosen as the extractive value came maximum in the case of ethanol-soluble extract of pods of *A. sinuata*.

Table 8. Antioxidant activity of *Acacia sinuata* (DPPH assay)

Test Sample	% Radical Scavenging Activity				IC_{50} Value (µg/ml)
	125µg/ml	250µg/ml	500µg/ml	1000µg/ml	
Ethanolic Extract	38.26	46.4	58.62	81.48	257.6
Ascorbic Acid	48.36	56.2	68.6	90.24	170.8

3.10.1 DPPH Assay

DPPH scavenging action data from *A. sinuata* ethanolic crude extract were tabulated along with standard (Ascorbic acid) and the IC50 for these results was calculated (Table 8, Figure 3). The findings demonstrated that *A. sinuata* extract could donate hydrogen and have antioxidant properties. The substrate concentration that leads to a 50% reduction in DPPH activity (colour) is referred to as the IC_{50} parameter, which is used to interpret the outcomes of the DPPH technique. The absorbance was determined with a UV spectrophotometer (517nm).

3.10.2 FRAP Assay

The FRAP assay determines how strongly antioxidants block reactive oxygen species' oxidative effects. Higher levels of antioxidant activity are indicated by a high

FRAP score and these levels rise with absorption. In the reducing effect experiment, the ethanolic extract of *A. sinuata* gave concentration-dependent antioxidant activity. The antioxidants' presence in the extract induces inhibition of the ferric cyanide complex in the ferrous form in this assay, changing the colour of the test sample solution from yellow to various shades of green and blue, depending on the reduction capability of the investigated extract. Ferrous concentration can thus be monitored by looking for the production of Pearl's Prussian blue (700nm). A rise in reducing power is indicated by increased absorbance at 700nm. Table 9 and Figure 4 show the outcomes of the FRAP experiment.

3.11 *In Vitro* Cytotoxic Activity of *A. sinuata* Ethanolic Extract by MTT Assay

Natural compounds with anticancer properties from different Indian medicinal plants include flavones, alkaloids, saponins, cardiac steroids and crude extracts. This emphasises the significance of conventionally used therapeutic herbs as extraordinarily valuable resources for researching anticancer compounds. Except for the ACE extract, the other two extracts of the *A. sinuata* showed good cytotoxic potential on the MCF-7 cell line as indicated by the results shown in Table 10.

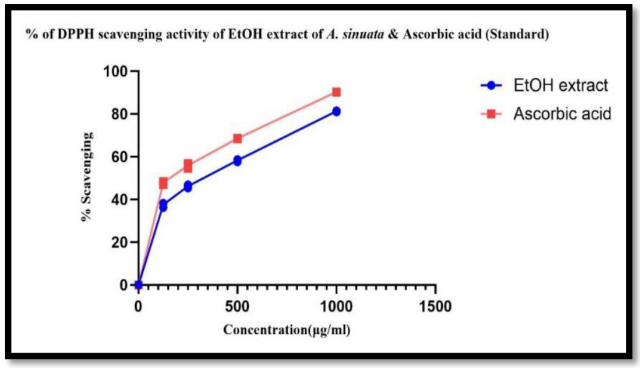


Figure 3. DPPH scavenging antioxidant activity of *Acacia sinuate*.

Only ethanolic extract is used for the mouse macrophage study as the IC_{50} value (35.92 µg/ml) came minimum among the three solvent extracts. After assessing the safety study with mouse primary macrophages, it has been found that up to the 200µg/ml concentration, the ethanolic extract of *A. sinuata* is safe to use and produces above 87% cell viability, as shown in Figure 5. Therefore, it can be said that the anti-cancer activity of *A. sinuata* pods against the cell line for breast cancer is a solid indicator that the plant contains pharmacological compounds that may be used for the treatment of breast cancer. For treating breast cancer, more research work needs to be incorporated to study the lead compounds that can be isolated from plant components. Because the used cell line possesses traits common to cancer, its cell death directly adds to the anti-cancer property.

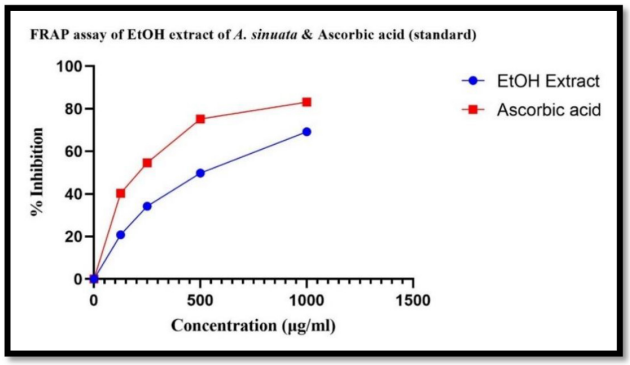


Figure 4. FRAP antioxidant activity of *Acacia sinuate*.

Table 9. Antioxidant activity of *A. sinuata* (FRAP method)

Test Sample	% Radical Scavenging Activity				IC_{50} Value (µg/ml)
	125µg/ml	250µg/ml	500µg/ml	1000µg/ml	
Ethanolic Extract	20.8	34.26	49.8	69.27	535.2
Ascorbic Acid	40.3	54.62	75.26	83.14	191.8

Table 10. Cytotoxicity (% inhibition) of various solvent extracts against MCF-7

Test Extracts	% Inhibition					IC_{50} Value (µg/ml)
	10µg/ml	25µg/ml	50µg/ml	100µg/ml	200µg/ml	
ACE	6.57654	9.54623	18.78614	21.32604	30.19208	468.08
ETH	6.426955	18.62538	62.56964	72.72174	78.89781	35.92
AQ	9.250398	19.32589	40.72456	58.24553	69.27953	74.72

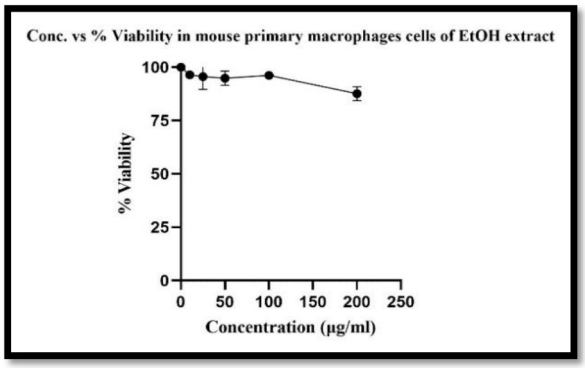


Figure 5. Concentration vs % viability of mouse primary macrophage with ethanolic extract.

3.12 Statistical Analysis

Data were calculated in all sets of experiments using Graph Pad Prism version 4.03 and Graph Pad Instat software (Graph Pad Software Inc., San Diego, CA, USA). Interpolation was also performed using Graph Pad Prism version 4.03 considering force zero, (X=0; Y=0) using the graph Concentration *vs.* % Inhibition/ viability. Data are represented as MEAN±SEM where n=3.

4. Conclusion

Utilising powdered plant material and its extracts, the current physicochemical and phytochemical screening process yielded valuable information on the plants' identity. World Health Organization (WHO) promotes the identification and provision of safe and efficacious remedies for use in public and private health services, as there is growing awareness of the benefits of many traditional medicine components. The study revealed that the selected plant contains saponin, tannin, flavonoids, glycosides, carbohydrates, alkaloids, phytosterols and phenols. *A. sinuata* is a medicinal plant, that is widely used by people as a traditional medicine all over India Although significant research has not been done in this area, it has been noted that this plant has some strong therapeutic properties. As a result, pharmacognostical research was done to identify and confirm the crude medication, which can then yield helpful information for creating other formulations from the basic medication. These mixtures will aid in treating numerous illnesses and conditions. *A. sinuata* is a significant medicinal plant with a variety of applications in ethnic communities and traditional medicinal systems. In this investigation, the haemolytic activity of *A. sinuata* pods extract with different solvents (PE, ACE, ETH and AQ) has been documented. The results unveiled that the pods possess very little haemolytic activity in the case of ethanolic extract and very high haemolytic activity for aqueous extract. The plant's ethanolic extract exhibited promising antioxidant properties. The MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test was utilised to measure the cytotoxic potential of the plant sample using the human cell line for breast carcinoma MCF-7 (Michigan Cancer Foundation-7). The ethanolic extract of *A. sinuata* is safe to use up to a concentration of 200µg/ml and produces more than 87% cell viability, according to an assessment of the safety study conducted with mouse primary macrophages.

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6. Author Contribution

Shreetama Roy: responsible for the selection of the research work and major data collection by trial and error methods. Rajat Das: contributed to drafting, designing, and referencing the manuscript. Jyochhana Priya Mohanty: responsible for the guidance of the work till the end. Nihar Ranjan Bhuyan: responsible for data formatting and validation. S.R, plays centre part in the overall formatting and drafting of this scientific research article.

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