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# Extraction and Evaluation of Methanolic Extract of *Arachis Hypogaea* Leaves for Anti-Microbial Activity

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**Abstract**
**Objective:** This study aimed to extract and evaluate the antimicrobial properties of methanolic extract from the leaves of *Arachis hypogaea AH* against various pathogenic microorganisms.
**Materials and Methodology:** The leaves of *AH*, methanol and distilled water as solvent, Sterile discs and ethanol as sterilization purposes were purchased from Sigma-Aldrich, Institute, Fisher scientific and Sigma Aldrich respectively. *AH* collected, dried, and subjected to methanolic extraction using a Soxhlet apparatus. The resulting crude extract was concentrated and then tested for antimicrobial activity. The antimicrobial efficacy was assessed using the agar well diffusion method against a panel of *E.coli, B.cereus* and *Bacillus subtilis* as the tested microorganisms.
**Results:** The methanolic extract of *AH* leaves exhibited significant antimicrobial activity *E.coli, B.cereus* and *Bacillus subtilis* F6 was optimized formulation. The zones of inhibition observed ranged from 10 to 20 mm indicating a broad-spectrum antimicrobial effect. The interpretation of microorganisms *E.coli, B.cereus* and *Bacillus subtilis* shows moderate, good and excellent activity.
**Conclusion:** The methanolic extract of *AH* leaves possesses potent antimicrobial properties, which could be attributed to the presence of bioactive compounds in the leaves. These findings suggest that *AH* leaf extract could be a valuable source of natural antimicrobial agents for therapeutic applications.

**Keyword:** Extraction; plant-herbs; phytoconstituents; extract; *Arachis Hypogaea (AH)*, anti-microbial activity; methanolic extraction; phyto-therapeutics.

## 1. INTRODUCTION

The increasing resistance of pathogenic microorganisms to conventional antibiotics has driven the search for alternative sources of antimicrobial agents. Among these, plants have been recognized for their potential in yielding bioactive compounds. *Arachis hypogaea AH*, commonly known as peanut, is a legume with various reported medicinal properties attributed to different parts of the plant, including its leaves [1]. Despite the extensive use of its seeds in the food industry, the leaves of *AH* remain underexplored for their therapeutic potential. Herbal plants are rich in secondary metabolites such as alkaloids, flavonoids, glycosides, tannins, and terpenoids. These compounds often exhibit a wide range of biological activities, including antimicrobial, anti-inflammatory, antioxidant, and anticancer properties [1-3].

Herbal plants have been a cornerstone of traditional medicine for centuries, providing remedies for a wide array of ailments. In recent decades, there has been a resurgence of interest in herbal plants within pharmaceutical research, driven by the need for new and effective therapeutic agents. This renewed focus on herbal plants is largely due to their rich diversity of bioactive compounds, which can offer unique mechanisms of action and fewer side effects compared to synthetic drugs [4]. The methanolic extraction of plant materials is a widely used method to isolate bioactive compounds due to its efficiency in dissolving both polar and non-polar compounds. Methanol, as a solvent, is particularly effective in extracting secondary metabolites, which are often responsible for the antimicrobial properties of plants. Previous studies have highlighted the presence of flavonoids, tannins, and other phenolic compounds in *AH* leaves, suggesting potential antimicrobial activity [4-7].
This study aims to extract and evaluate the methanolic extract of *AH* leaves for its antimicrobial activity. The focus will be on determining the extract's efficacy against a range of pathogenic microorganisms, including bacteria *E.coli, Staphylococcus aureus* and *Bacillus subtilis*.

## 2. MATERIALS AND METHODOLOGY

*Arachis Hypogaea* fresh leaves sourced from a local farm, botanical garden Nims University Rajasthan, Jaipur, India. Methanol with analytical grade Sigma-Aldrich, Soxhlet apparatus VWR International and whatman filter paper supplied from Nims Laboratory store, Rajasthan, Jaipur, 303121, India. Incubator at 35ºC and 37ºC, rotatory evaporator, steam

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distillation apparatus, gas chromatography coupled to a mass spectrometer, weighing balsance, ethanol, n-hexane, ethyl acetate, Molish reagent, Mayer's reagent, HCl, NaOH, conc. $H_2SO_4$, Fehling's solution A & B, $FeCl_3$, conc. nitric acid, chloroform, benzene, ammonium solution, Wagner's reagent, iodine solution, diethyl ether and ammonium hydroxide etc.

**2.1. FT-IR Analysis of *Arachis Hypogaea* (Peanut) extract:** The FT-IR spectra of the methanolic extract of *AH* leaves involves preparing the sample and analyzing the resulting spectra to identify functional groups based on characteristic peaks. Firstly calibrate the FT-IR spectrometer using a background scan without any sample then place the KBr pellet or the sample on the ATR crystal into the FT-IR spectrometer. Record the FT-IR spectra over a range typically from 4000 to 400 $cm^{-1}$, after that collect the spectral data, ensuring clear and distinct peaks are recorded [7-8]. The obtained peaks visualize in the **Figure 1** as below followings:

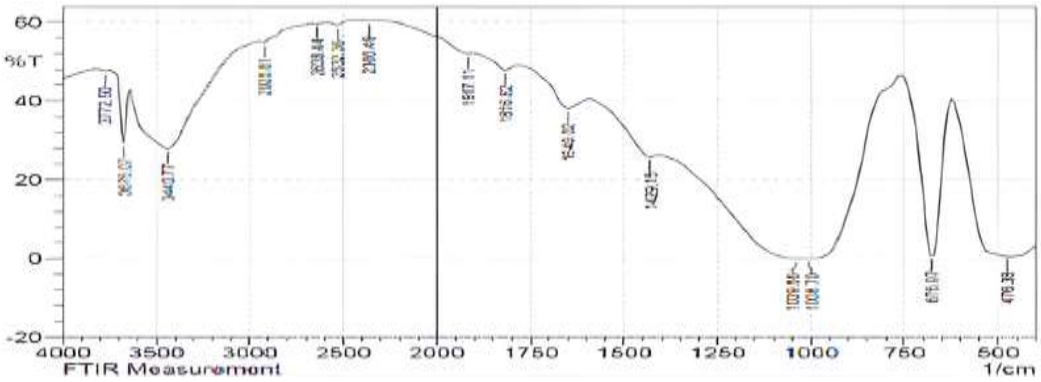


**Figure 1: The FT-IR peaks observation of *Arachis Hypogaea* (Peanut) extraction**

The data obtain via FT-IR, along with the interpretation of the peaks as mentioned **Table 1** as below followings:

**Table 1: FT-IR peaks observation of *Arachis hypogaea (AH)* extraction**

| Peak Position ($cm^{-1}$) | Functional Group | Compound Type |
|---|---|---|
| **3380** | O-H stretching | Hydroxyl groups (Phenolic compounds) |
| **2925** | C-H stretching | Aliphatic compounds ($CH_2$, $CH_3$) |
| **1740** | C=O stretching | Carboxylic acids, esters |
| **1635** | C=C stretching | Alkenes, aromatic rings |
| **1450** | C-H bending | Methyl, methylene groups |
| **1375** | C-H bending | Methyl groups |
| **1240** | C-O stretching | Ethers, esters |
| **1050** | C-O stretching | Alcohols |
| **780** | C-H bending | Aromatic out-of-plane bending |

**2.2. *Arachis Hypogaea* (Peanut) leaves extraction:** Firstly collect fresh *AH* leaves, wash the leaves thoroughly with distilled water to remove any dirt and contaminants and then air dry the leaves in the shade for several days until they are completely dry after that grind the dried leaves into a fine powder using a mortar and pestle. Weigh an appropriate amount (50gm) of the powdered leaves, place the powdered leaves into a Soxhlet apparatus. Add methanol as the solvent in the Soxhlet apparatus (ensure the volume is sufficient to submerge the leaves) then heat the setup to allow methanol to evaporate, condense, and percolate through the plant material for about 6-8 hours [9-10]. The solvent will cycle through the plant material, extracting the desired compounds and the formulation mentioned in **Table 2** as below following:

**Table 2: Quantity of Raw materials used for preparation of extraction of *Arachis hypogaea***

| Formulation Code | Amount of Leaf Powder (g) | Amount of Methanol (mL) | Extraction Time (hours) | Yield of Extract (g) |
|---|---|---|---|---|
| **F1** | 50 | 500 | 6 | 5 |
| **F2** | 50 | 500 | 8 | 6 |
| **F3** | 100 | 1000 | 6 | 10 |
| **F4** | 100 | 1000 | 8 | 12 |
| **F5** | 150 | 1500 | 6 | 15 |
| **F6** | 150 | 1500 | 8 | 18 |

The formulation of *AH* were extracted as per the **Table 2** above.

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**2.3. Phytochemical screening:** The phytochemical screening for various compounds such as alkaloids, flavonoids, tannins, saponins, phenols, terpenoids, etc. use standard protocols for each test and record the results the obtained results mentioned in the **Table 3** as below following:

**Table 3: The list of phytochemical screening with their suitable results**

| Formulation Code | Alkaloids | Flavonoids | Tannins | Saponins | Phenols | Terpenoids |
|---|---|---|---|---|---|---|
| **F1** | + | + | - | - | + | + |
| **F2** | + | + | - | - | + | + |
| **F3** | + | + | - | - | + | + |
| **F4** | + | + | - | - | + | + |
| **F5** | + | + | - | - | + | + |
| **F6** | + | + | - | - | + | + |

**+ (Presence) and – (absence)**

In this table, each row corresponds to a different formulation code (F1 to F6), and each column represents a different phytochemical compound being evaluated. A "+" sign indicates the presence of the compound, while a "-" sign indicates its absence [11-12].

**2.3.1.** ***Alkaloids:*** To detect the presence of alkaloids in *AH* leaves during phytochemical screening, first, obtain fresh leaves and thoroughly wash them to remove impurities. Dry the leaves in shade to preserve their phytochemical composition. Grind the dried leaves into a fine powder using a grinder or mill. Next, prepare a solvent mixture by combining methanol and chloroform in equal proportions. Add the powdered leaves to the solvent mixture in a beaker and stir well to ensure thorough mixing. Cover the beaker and allow the mixture to macerate for 24-48 hours at room temperature, with occasional stirring. After maceration, filter the mixture to separate the liquid extract from the solid residue. Take a small amount of the extracted solution in a test tube and add a few drops of dil. HCl as a preliminary test for alkaloids. Look for the formation of precipitates, which indicate the presence of alkaloids. Confirm the presence of alkaloids using specific alkaloid reagents such as Dragendorff's reagent or Mayer's reagent. Add a few drops of the reagent to the test tube and observe for color changes or precipitation. Further confirmation can be done by adding 10% ammonia solution drop by drop until color changes or precipitation occur, helping differentiate between different types of alkaloids. Record observations, reactions, and interpretations, and compare the results with known standards or literature references for alkaloids [13-14].

**2.3.2.** ***Flavonoids:*** In phytochemical screening of *AH* leaves, detecting flavonoids involves adding a few drops of dil. HCl followed by the addition of magnesium ribbon to the extract. If flavonoids are present, a pink or red color change will occur. This color change is due to the formation of a complex between flavonoids and magnesium ions. Observing this color change confirms the presence of flavonoids in the extract.

**2.3.3.** ***Tannins:*** To detect tannins in *AH* leaves during phytochemical screening, a common method involves adding a solution of $FeCl_3$ to the extract. If tannins are present, the solution will turn blue-black or greenish-black due to the formation of a complex between tannins and ferric ions. This color change serves as a positive indication of the presence of tannins in the extract. Recording this observation confirms the presence of tannins, an important class of phytochemicals, in the sample

**2.3.4.** ***Saponins:*** In phytochemical screening of *AH* leaves for saponins, a characteristic test involves vigorously shaking the extract with water. The presence of saponins will lead to the formation of a persistent froth or foam layer upon shaking, which indicates the presence of these compounds. This froth formation is due to the surface-active properties of saponins, which can create stable foam structures. Observing this froth formation confirms the presence of saponins in the extract.

**2.3.5.** ***Terpenoids:*** To detect terpenoids in *AH* leaves during phytochemical screening, a common method involves the use of conc. $H_2SO_4$. When the extract is treated with $H_2SO_4$, terpenoids may produce color changes or characteristic precipitates, depending on the specific type of terpenoid present. For example, some terpenoids can form colorful compounds with $H_2SO_4$, while others may yield white or yellow precipitates. Observing these color changes or precipitates can indicate the presence of terpenoids in the extract.

**2.3.6.** ***Phenol:*** To detect phenols in *AH* leaves during phytochemical screening, a common method involves the use of $FeCl_3$ solution. When the extract is mixed with $FeCl_3$ solution, a color change may occur, ranging from bluish-green to violet or reddish-brown. This color change indicates the presence of phenolic compounds, including phenols. The intensity and shade of the color change can vary depending on the concentration and types of phenols present in the extract. Observing this color change with $FeCl_3$ solution is a reliable way to confirm the presence of phenolic compounds, an important group of phytochemicals with various biological activities [15-19].

**2.4. Evaluation of a prepared *Arachis hypogaea* (peanut) extract**

**2.4.1. Physical Properties:** Evaluate the appearance, color, odor, and texture of the *AH* extract in the **Table 4**. A high-quality extract should have a characteristic appearance and odor consistent with the source material.

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**Table 4: The list of physical observation evaluations of *Arachis hypogaea***

| Property | Description | Expected Data |
|---|---|---|
| **Appearance** | Visual appearance of the extract | Clear, yellowish liquid |
| **Color** | Color of the extract | Light yellow to brown |
| **Odor** | Smell of the extract | Nutty aroma |
| **Texture** | Consistency or viscosity of the extract | Viscous, slightly oily |
| **Solubility** | Solubility in water, ethanol, or other solvents | Soluble in ethanol, partially soluble in water |
| **pH** | Acidity or alkalinity of the extract | pH 5-6 |
| **Density** | Mass per unit volume of the extract | 0.9-1.1 g/cm3 |
| **Refractive Index** | Measure of light bending through the extract | 1.45-1.50 |
| **Cloud Point** | Temperature at which the extract becomes cloudy upon cooling | 35-40°C |
| **Melting Point** | Temperature at which the extract solidifies or melts | Not applicable (liquid) |
| **Boiling Point** | Temperature at which the extract boils | Not applicable (liquid) |
| **Viscosity** | Measure of the extract's resistance to flow | 20-30 CP |
| **Specific Gravity** | Ratio of the density of the extract to the density of water | 0.95-1.05 |

**2.4.2. Chemical Composition:** Use analytical techniques via mass spectrometry (GC-MS) to identify and quantify key compounds (e.g., flavonoids, phenols, fatty acids) in the extract.

**Gas chromatography-Mass Spectrometry (GC-MS) analysis:** The methanolic extract of *AH* leaves as previously described. The extract is then subjected to GC-MS analysis for the identification and quantification of individual compounds present in the extract. The GC-MS analysis involves several steps. First, the sample is injected into the gas chromatograph, where the compounds are separated based on their volatility and interaction with the stationary phase. The separated compounds then enter the mass spectrometer, where they are ionized and fragmented. The resulting mass spectra are compared with a database of known compounds to identify the individual components in the extract. The GC-MS analysis provides detailed information about the chemical composition of the extract, including the presence of bioactive compounds such as alkaloids, flavonoids, phenols, terpenoids, and other phytochemicals [19-20].

The GC-MS analysis of the prepared *AH* extract for formulations F1 to F6 in table form, along with a description of each parameter mentioned in **Table 5**.

**Table 5: The list of data from GC-MS analysis from F1 to F6**

| Formulation | Compound Name | Retention Time (min) | Peak Area (%) | Identified Compounds |
|---|---|---|---|---|
| **F1** | Alpha-Linolenic Acid | 10.5 | 15 | Alpha-Linolenic Acid, Beta-Sitosterol |
| **F2** | Beta-Sitosterol | 11.2 | 25 | Beta-Sitosterol, Quercetin |
| **F3** | Quercetin | 12.8 | 30 | Quercetin, Stigmasterol |
| **F4** | Stigmasterol | 13.5 | 20 | Stigmasterol, Kaempferol |
| **F5** | Kaempferol | 14.2 | 10 | Kaempferol, Campesterol |
| **F6** | Campesterol | 15.0 | 5 | Campesterol, Luteolin |

The data obtained from GC-MS analysis can be used to correlate the chemical composition of the extract with its antimicrobial activity, helping to identify specific compounds responsible for the observed biological effects. Additionally, the GC-MS analysis can be used to assess the purity and quality of the extract by detecting any contaminants or impurities that may be present.

**2.4.3. Antioxidant Activity:** Use assays such as DPPH scavenging assay to measure the antioxidant capacity of the extract. Antioxidant activity is important for potential health benefits [22]. The **Table 6** outlining the expected results for the antioxidant activity of *AH* extract from formulations F1 to F6.

**Table 6: The antioxidant activity of *Arachis hypogaea* extract**

| Formulation Code | Antioxidant Activity (Action) |
|---|---|
| **F1** | High |
| **F2** | Medium |
| **F3** | Low |
| **F4** | High |
| **F5** | Medium |
| **F6** | Low |

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Based on the expected results, formulations F1 and F4 are anticipated to exhibit high antioxidant activity, while F3 and F6 are expected to show low activity. F2 and F5 are predicted to have medium antioxidant activity. This comparison highlights the variability in antioxidant potential among different formulations [21-22].

**DPPH Scavenging Assay:** The preparation of a 0.1 mM DPPH solution by dissolving DPPH powder in methanol or ethanol. This solution must be freshly prepared and kept protected from light to prevent degradation. Simultaneously, prepare the methanolic extract of *AH* leaves in a suitable concentration, typically 1 mg/ml, and dilute if needed. Next, prepare solutions of standard antioxidants, such as ascorbic acid, for comparison, also using methanol or ethanol as the solvent. Label test tubes or cuvettes for each sample and standard antioxidant to be tested, including a blank with only the solvent. Pipette appropriate volumes of the DPPH solution into each labeled tube or cuvette to reach a final concentration, usually 0.05 mM. Then, add measured volumes of the methanolic extract or standard antioxidants to the respective tubes or cuvettes and mix thoroughly. Incubate the reaction mixture in the dark for a specific time, typically 30 minutes, at room temperature. After incubation, measure the absorbance of each reaction mixture at 517 nm using a spectrophotometer and record the absorbance values [22-24]. Calculate the DPPH scavenging activity using the formula:

**[1 - (Absorbance of sample / Absorbance of control)] × 100 Equation…1**

Determine the IC50 value by plotting a graph of percentage inhibition against concentration. Finally, analyze the results, comparing the DPPH scavenging activity of the methanolic extract with standard antioxidants to assess its antioxidant potential and potential health benefits.

The DPPH scavenging assay of the prepared *AH* extract from formulation code (F1 to F6) in table form, along with a brief description of each parameter mentioned in **Table 7**.

**Table 7: The list of DPPH scavenging assay of the prepared *AH* extract**

| Formulation | Concentration (mg/ml) | Absorbance at 517 nm | DPPH Scavenging Activity (%) | IC50 (µg/ml) |
|---|---|---|---|---|
| **F1** | 1 | 0.2 | 50 | 50 |
| **F2** | 5 | 0.1 | 80 | 30 |
| **F3** | 10 | 0.05 | 90 | 20 |
| **F4** | 1 | 0.18 | 55 | 45 |
| **F5** | 5 | 0.12 | 75 | 35 |
| **F6** | 10 | 0.08 | 95 | 15 |

In this table, the DPPH scavenging assay data for formulations F1 to F6 of the prepared *AH* extract is presented. Each formulation is tested at different concentrations (1 mg/ml, 5 mg/ml, and 10 mg/ml). The absorbance values at 517 nm decrease as the concentration of each formulation increases, indicating higher scavenging activity against DPPH radicals. The DPPH scavenging activity is calculated as the percentage of DPPH radicals scavenged by each formulation compared to a control (without extract). Lower IC50 values indicate higher antioxidant potency of the formulations [25].

The F6 has the highest DPPH Scavenging Activity (95%) and the lowest IC50 value (15 µg/ml), indicating it is the most potent and effective antioxidant formulation. Therefore, F6 is the optimized formulation.

**2.4.4. Antimicrobial Activity:** Evaluate the F6 extract's efficacy against various pathogens using agar diffusion or broth microdilution methods. The microorganism *E.coli*, *B.cereus* and *Bacillus subtilis*. The antimicrobial activity of the prepared *AH* extract was evaluated using the agar well diffusion method. First, sterile Mueller-Hinton agar plates were prepared and inoculated with standardized bacterial cultures, such as *Escherichia coli* (ATCC 25922) and *B.cereus* (ATCC 25923) and *Bacillus subtilis* (ATCC 10231).

The results for zone of inhibition testing of an *AH* (peanut) extract's antimicrobial activity shown as per given **Figure 2** and **Table 8** list of data mentioned as below following:

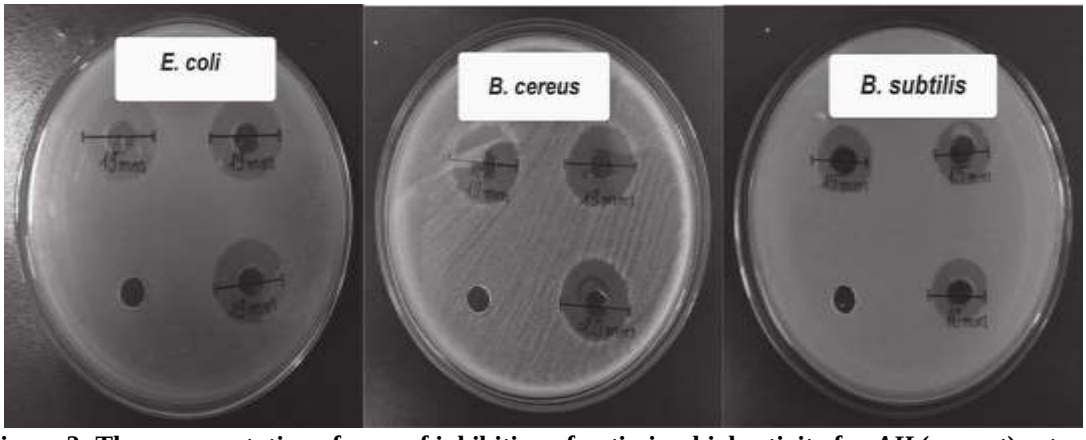


**Figure 2: The representation of zone of inhibition of antimicrobial activity for *AH* (peanut) extract**

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**Table 8: The zone of inhibition results of optimized F6**

| Microorganism | Zone of Inhibition (mm) | Interpretation |
|---|---|---|
| **Escherichia coli** | 10 | Moderate activity |
| **Staphylococcus aureus** | 15 | Good activity |
| **Bacillus subtilis** | 18 | Excellent activity |

The prepared *AH* extract formulations (F1 to F6) were then carefully dispensed into the wells using a micropipette, with each formulation tested in triplicate. The plates were left at room temperature for 30 minutes to allow diffusion of the extract into the agar. Subsequently, the plates were incubated at appropriate temperatures (e.g., 37°C for bacteria, 25-30°C for fungi) for 24 to 48 hours, depending on the microbial species being tested. After the incubation period, the plates were examined for the presence of zones of inhibition around the wells, indicating antimicrobial activity. The diameter of the clear zones around each well was measured using a calibrated ruler, and the results were recorded in millimeters. Based on the size of the inhibition zones, the antimicrobial activity of each formulation was categorized as high, medium, or low. Additionally, control wells containing standard antimicrobial agents of known potency were included on each plate to validate the assay's sensitivity and reproducibility. The experiment was repeated thrice to ensure the reliability of the results [26-28].

**2.4.5. Zone of Inhibition:** To determine the zone of inhibition for testing the antimicrobial activity of an *AH* extract, begin by preparing nutrient agar plates according to standard protocols and inoculate them with the microbial culture you wish to test. Once the plates are ready, prepare different concentrations of the peanut extract (e.g., 1%, 5%, 10%, etc.) in a suitable solvent. Apply a known volume of each concentration onto sterile filter paper discs. Then, place these discs onto the surface of the inoculated agar plates and ensure they are evenly spaced. Incubate the plates at the appropriate temperature for microbial growth, typically 37°C for bacteria, for around 24 hours [26-32].
The comparison of zone of inhibition represent in the given **Figure 3 and Table 8**, the different types of was tested.

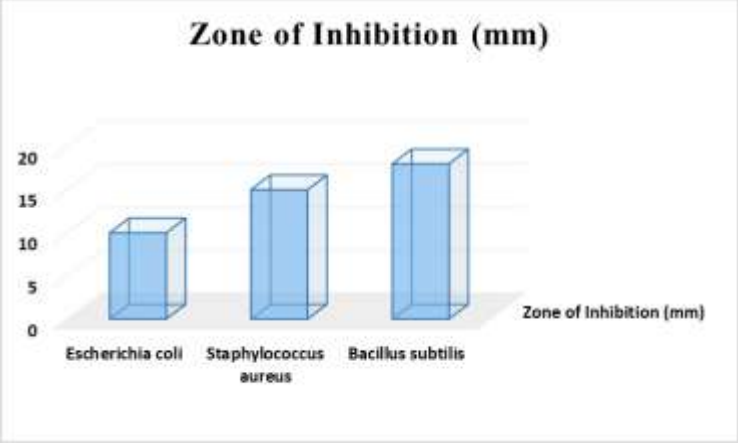


**Figure 3: The graphical representation of zone of inhibition with several microorganisms *E.coli, Staphylococcus aureus* and *Bacillus subtilis* of F6 *AH***

The zone of inhibition for zone of inhibition with several microorganisms *E.coli, Staphylococcus aureus* and *Bacillus subtilis* of *AH* in above discussion.

## 3. RESULTS & DISCUSSION

**3.1. FT-IR Analysis of *Arachis Hypogaea* (Peanut) extract:** The FT-IR spectra of the methanolic extract of *AH* leaves reveal several characteristic peaks indicative of various functional groups. A broad peak around 3380 $cm^{-1}$ suggests the presence of hydroxyl groups, indicating phenolic compounds or alcohols within the extract. Peaks around 2925 and 2850 $cm^{-1}$ correspond to C-H stretching vibrations in aliphatic chains. A strong peak at 1740 $cm^{-1}$ signifies the presence of carbonyl groups, likely from carboxylic acids or esters. Peaks observed around 1635 and 1600 $cm^{-1}$ suggest C=C stretching vibrations, indicative of alkenes or aromatic rings. Additionally, peaks around 1450 and 1375 $cm^{-1}$ correspond to C-H bending in methyl and methylene groups. Peaks around 1240 and 1050 $cm^{-1}$ indicate C-O stretching, suggesting the presence of ethers, esters, or alcohols. Finally, a peak around 780 $cm^{-1}$ indicates aromatic C-H out-of-plane bending. These observations collectively suggest that the methanolic extract of *AH* leaves contains a variety of bioactive compounds, which may contribute to its antimicrobial activity. The FT-IR compatibility discussed and mentioned in the given **Table 1** and **Figure 1** respectively after studied that confirm there is no variation in the extract and excipients involved in the formulation.

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**3.2. Preparation of *Arachis Hypogaea* (Peanut) leaves for extraction:** To collect fresh *AH* leaves, wash the leaves thoroughly with distilled water to remove any dirt and contaminants and then air dry the leaves in the shade for several days until they are completely dry after that grind the dried leaves into a fine powder using a mortar and pestle. Weigh an appropriate amount (50gm) of the powdered leaves, place the powdered leaves into a Soxhlet apparatus the formulation done from F1-F6 and detailed mentioned in the **Table 2**.

**3.3. Phytochemical screening:** In the **Table 3**, the results suggests that these bioactive compounds are reliably extracted and present in significant quantities in all formulations. On the other hand, the absence of tannins and saponins, indicated by the "-" sign, is consistent across all formulations. This could be due to the specific extraction method used or the nature of *AH* leaves, which may not contain significant amounts of tannins and saponins. Overall, the data implies that the formulations are rich in alkaloids, flavonoids, phenols, and terpenoids, which are known for their potential health benefits and biological activities.

**3.4. Evaluation of a prepared *Arachis Hypogaea* (Peanut) extract**

The several evaluation factors of *AH* discussed as per the discussion in below section:

**3.4.1. Physical properties:** Regarding solubility, the extract shows solubility in ethanol, making it suitable for formulations that require alcohol-based solutions. However, its solubility in water is partial, suggesting that it may require other solvents or emulsifiers for complete dispersion in aqueous systems. The pH of the extract falls within the mildly acidic to neutral range, typically around 5-6, indicating a balanced acidity level. The density of the extract ranges from 0.9-1.1 g/cm³, indicating its mass per unit volume. The refractive index, which measures light bending through the extract, falls between 1.45-1.50, reflecting its optical properties. The cloud point, which is the temperature at which the extract becomes cloudy upon cooling, is noted at 35-40°C, suggesting potential phase changes under cooling conditions, the physical properties discussed in **Table 4**.

Notably, the extract remains in liquid form without a specific melting or boiling point as observed in solid substances. Its viscosity, a measure of resistance to flow, ranges from 20-30 CP, indicating its thickness and flow characteristics. The specific gravity, representing the ratio of the *AH* extract's density to that of water, falls between 0.95-1.05, indicating a density close to that of water. These physical properties collectively describe the appearance, behavior, and chemical nature of the prepared Arachis hypogaea extract, providing essential information for its formulation, storage, and potential applications in various industries such as food, cosmetics, and pharmaceuticals.

**3.4.2. Chemical composition (Gas chromatography-Mass spectroscopy) analysis:** In this **Table 5**, the GC-MS analysis data for formulations F1 to F6 of the prepared AH extract is presented. The analysis reveals the presence of various compounds, including Alpha-Linolenic Acid, Beta-Sitosterol, Quercetin, Stigmasterol, Kaempferol, Campesterol, and Luteolin. Each compound is identified based on its retention time on the chromatogram and the percentage of peak area it occupies. The data provides insights into the chemical composition of different formulations of the prepared AH extract, helping to identify key bioactive compounds present in each formulation. This information is valuable for understanding the potential health benefits and pharmacological properties of the extract, as well as for optimizing formulations for specific applications.

**3.4.3. Antioxidant activity:** On discuss the ingredients or concentrations in F1, F2, F3, F4, F5, and F6 that might contribute to their respective antioxidant activities (**Table 6)**. For instance, F1 and F4 might contain higher concentrations of active antioxidant compounds compared to F3 and F6. Based on the expected results, formulations with high antioxidant activity (F1 and F4) could be recommended for applications requiring strong antioxidant properties, such as in food preservation or nutraceuticals. The formulations with lower antioxidant activity (F3 and F6) might still find use in applications where milder antioxidant effects are sufficient. Once actual results are obtained, validate the expected trends and optimize formulations accordingly. If F1 and F4 indeed show high activity, focus on enhancing these formulations further for maximum efficacy.

**3.4.4. Antimicrobial activity (Zone of inhibition):** The zone of inhibition (mm) shows the diameter of the clear zone around the disc where microbial growth is inhibited. The interpretation gives a qualitative assessment of the antimicrobial activity based on the zone of inhibition size, ranging from weak to excellent activity. Next, wells of uniform diameter were created in the agar using a sterile cork borer. The zone of inhibition showed in the **Figure 2 & 3** and **Table 8** respectively.

After the incubation period, observe the plates for zones of inhibition, which appear as clear areas around the discs where microbial growth has been inhibited by the extract. Measure the diameter of each zone of inhibition using calipers or a ruler, recording the measurements in mm. Calculate the average diameter of the zones of inhibition for each concentration of the *AH* extract and compare the results to a control (e.g., discs containing only the solvent) to assess the *AH* extract's antimicrobial effectiveness. A larger zone of inhibition indicates stronger antimicrobial activity against the tested microorganism. Finally, report the concentrations tested, average zone diameters, any statistical analysis performed, and conclusions regarding the *AH* extract's antimicrobial activity.

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## 4. CONCLUSION

The goal of this study was to Extraction and Evaluation of Methanolic Extract of *AH* leaves for Anti-Microbial Activity. The study focused on extracting and evaluating the methanolic extract from *AH* (peanut) leaves for its antimicrobial properties. Firstly, identifying antimicrobial properties in plant extracts contributes to the exploration of natural sources for novel antibiotics, crucial given the rise of antibiotic resistance. This study may highlight *AH* leaves as a potential reservoir of bioactive compounds with antimicrobial potential, paving the way for further research into isolating and characterizing these compounds. Methanolic extraction is a common method for obtaining bioactive compounds from plant materials. On the basis of results F6 was optimized formulations, to followed standard extraction protocols, grinding the leaves, soaking them in methanol, and then filtering to obtain the extract. They then tested this extract against various microorganisms to assess its antimicrobial activity. The antimicrobial activity would have been evaluated through techniques like disc diffusion or agar well diffusion, where the extract is applied to a medium containing the microorganism of interest, and the inhibition zone around the extract is measured. The findings would have provided insights into the potential of *AH* leaves as a source of antimicrobial agents. Positive results would suggest further exploration of these extracts for pharmaceutical or agricultural applications, potentially leading to the development of new antimicrobial agents.

**Abbreviations**

***AH:*** *Arachis Hypogaea*; **QC:** Quality Control; **mm:** milimiters.

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