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Pharmacognostical and pharmacological study of *Agave attenuata* leaves

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Abstract
The objective of the current study was to investigate several biological functions of an *Agave attenuata* leaf methanol extract. There were 25 chemicals identified by GC-MS in the n-hexane fraction of the extract, with mono-2-ethylhexyl phthalate (10.31%), 1, 2-benzenedicarboxylic acid (5.03%), n-docosane (5.45%), and eicosane (5.04%) serving as the main constituents. The total phenolic content in the leaves was 11.521-39.35 GAE mg/100 g, while the total flavonoid content was 42.35-304.8 CE mg/100g. Moderate antibacterial properties were seen in the extract and several of its components. By measuring DPPH radical scavenging activity and inhibiting lipid peroxidation experiments, leaf extract and fractions also showed good antioxidant ability. Hemolytic effects of the plant were found to range from 2.01% to 3.50%. This plant shows potential as a source of natural antioxidants and functional food nutraceuticals, according to the study's findings.

Keywords: Antibacterial properties, antioxidants, nutraceuticals, hemolytic effects

1. Introduction
Plants possess an almost limitless ability to biosynthesize phytochemicals, which serve as a source for natural antioxidants and as plant defense mechanisms against predation by microorganisms, herbivores and insects [1-2].
Asserted that in comparison to synthetic drugs, antimicrobials and antioxidants of plant origin are not associated with many side effects and has an enormous therapeutic potential to heal many infections and diseases. Many species of the genus *Agave* constitute an important source of steroidal sapogenins, mainly hecogenin [3].
In the pharmaceutical industry, these natural compounds are used for the semisynthesis of medicinal steroids, as corticosteroids, sexual hormones and steroid diuretics [4].
The most investigated species of the genus is *A. americana* L. that has been reported for its antibacterial and anti-inflammatory properties [5].
Some other species possess anticancer properties. Antimicotic, antiviral and antituberculosis properties have also been ascribed to this genus. The antimicrobial activity of some species against pathogens has been previously reported [6-7]. Some species of this genus are used in Chinese Traditional Medicine in treatment of scabies, tumors, dysentery, and as insecticides. *Agave attenuata* Salm-Dyck is native to central Mexico and tropical America. The plant is commonly grown as a garden plant. In fact, the plant has no teeth or terminal spines; this property makes it an ideal plant for areas adjacent to footpaths. It is a hardy survivor of drought, tolerating heat well and moderate salt exposure. Previous literature reports showed the presence in the plant of steroidal saponins. The plant may provide a substitute for niclosamide and be used safely for snail control by rural communities and has been proposed as a contact poison for *Bulinus africanus* [8-10]. Despite its multipurpose uses, very little data exists on its chemical constituents; Therefore the current study reports the chemical composition of n-hexane fraction of *A. attenuata* leaves and the *in vitro* antimicrobial, antioxidant, hemolytic activities of the methanol extract and fractions thereof. This study will provide base-line data for further detailed investigations of various biological activities of this plant and of its use as a functional food.

Fig 1: Plant *Agave attenuata*

2. Materials and Methods

2.1 Materials

2.1.1 Collection of medicinal plants

The selected medicinal plants were collected from different parts of U.P. Some medicinal plants samples were also collected from (CCS University Botany Garden). The list of medicinal plant, their corresponding parts used under this study, month of sample collection and location/place of the sample plants are given in Appendix - B.

2.1.2 Plant Material

The plant *A. attenuata*' s leaves were gathered from the Botanical Garden at CCS University Meerut on April 20, 2023. Vijay from the Department of Botany at CCS University in Meerut, Uttar Pradesh, where a voucher specimen has been deposited, further identified the plant specimens.

2.1.3 Preparation of Extract and Organic Fractions

To get rid of dirt and other impurities, four kilo-grammes of fresh leaves were washed with distilled water. The powdered (80mesh) dried leaves were in the shade. Three litres of methanol were used to extract 500 grammes of powdered leaves over the course of five days at room temperature. The extract was heated to 45 °C in a rotary vacuum evaporator, filtered through Whatman No. 1 filter paper, and then concentrated. After that, the methanol extract was kept at 4 °C. The 85 g of residue was dissolved in distilled water and subsequently extracted with 30 g of n-hexane, 15 g of chloroform, 20 g of ethyl acetate, and 15 g of n-butanol [11].

2.1.4 Gas Chromatography/Mass Spectrometry Analysis

GC 6850 Network gas chromatographic system with 7683 B series auto injector and 5973 inert mass selective detector (Agilent Technologies USA) was used for the GC-MS analysis of the n-hexane fraction. Compounds were separated using an HP-5 MS capillary column with an internal diameter of 0.25 mm and a film thickness of 0.25 m, and a stationary phase of 5% phenyl poly siloxane. 1.0L of sample was injected in split mode with a split ratio of 30:1 at 300 °C with the injector's temperature. A 1.5 ml/min flow rate of helium was used as the carrier gas. The temperature ramped up to 290 °C at a rate of 10 °C/min, then was kept there for 5 minutes as part of the temperature programme. The initial temperature was 150 °C. MSD transfer line was 300 °C in temperature. When determining mass spectra, MSD was run in electron ionisation (EI) mode with ionisation energy of 70e V and a mass range of 3-500 m/z. The MS quadropole had a temperature of 150 °C, while the ion source was 230 °C. By comparing the mass spectra of the components to those in the NIST mass spectral database, the components were identified [12-13].

2.2 Antimicrobial Assay

2.2.1 Test Microorganisms

The bacterial tested organisms included *Pasteurella multocida* locally isolated, *Escherichia coli* ATCC 25922, Bacillus subtilis JS 2004, Staphylococcus aureus API Staph tac 6736153, and Aspergillus niger ATCC 10595, *Aspergillus flavus* ATCC 32612, Alternaria alternata ATCC 20084, and *Rhizoctonia solani*. The Department of TIPER Meerut provided the pure strains of bacteria and fungi. The fungal strains were grown overnight at 28 °C using potato dextrose agar from Oxoid, while the bacterial strains were cultured overnight at 37 °C in nutritional agar (Oxoid, Hampshire, UK).

2.2.2 Disc Diffusion Method

The disc diffusion method was used to assess the antimicrobial activity of the methanol extract and its fractions. In 10% sterile dimethyl sulfoxide, all samples (dry residue) were dissolved. The discs (6mm in diameter) were positioned aseptically over the inoculated agar and impregnated with extract/fractions at a concentration of 20 mg/ml (100 L/disc). As negative controls, discs were injected with 100 L of the appropriate solvents. Rifampin (100 L/disc) and fluconazole (100 L/disc) (Oxoid) were employed as positive references for bacteria and fungus, respectively. For fungi and bacteria, the petri dishes were incubated at 280.3 °C for 40-48 hours and 370.1 °C for 20-24 hours, respectively. The inhibition zones that had formed on the medium at the conclusion of the period were measured. The growth inhibition zone was used to determine the positive antibacterial activity [14].

2.2.3 Resazurin Microtitre-Plate Assay

The minimum inhibitory concentration (MIC) of the plant extract/fractions was evaluated by a modified resazurin microtitre-plate assay reported by Sarker and co-workers with some modifications. Briefly, a volume of 100 µL of each extract and fractions solution in 10% dimethyl sulfoxide (DMSO, v/v) was transferred into the first row of the 96 well plates. To all other wells, 50 µL of nutrient broth and Muller Hinton broth for bacteria and fungi respectively were added. Two-fold serial dilutions were performed using a multichannel pipette such that each well had 50 µL of the test material in serially descending concentrations. To each well, 10 µL of resazurin indicator solution (prepared by dissolving 270mg resazurin tablet in 40ml of sterile distilled water) were added. Finally, 10 µL of bacterial/fungal suspension were added to each well. Each plate was wrapped loosely with aluminum foil. Each plate had a set of controls: A column with broad spectrum antibiotics as positive control, a column with all solutions with the exception of the test samples, a column with all solutions with the exception of the bacterial/fungal solution adding 10 µL of broths instead and a column with respective solvents as a negative control. The plates were prepared in triplicate, and incubated at 37±0.1 °C for 20-24 hrs and 28±0.3 °C for 40-48 h for bacteria and fungi, respectively The absorbance was measured at 620nm by micro quant for fungus and at 500nm for bacteria. The color change was then assessed visually. The growth was indicated by color changes from purple to pink or colorless. The lowest concentration at

which color change appeared was taken as the MIC value [15].

2.2.4 *In vitro* Hemolytic Activity

The plant's hemolytic activity was evaluated using the Powell and colleagues' published approach. A sterile 15ml polystyrene screw-cap tube was filled with three millilitres of freshly drawn, heparinized human blood, which was then gently mixed before being centrifuged at 850g for five minutes. The sticky pellet was rinsed three more times with 5ml of cold (4 °C) sterile isotonic phosphate-buffered saline (PBS) solution, which had been pH-adjusted to 7.4. A haemacytometer was used to count the cells after they had been cleaned and suspended in a final volume of 20ml cold, sterile PBS. For each test, the blood cell suspension was kept on wet ice and diluted to 7.068 108 cells ml1 using sterile PBS. 20 ml of plant extract or fractions were divided into aliquots and aseptically put into 2.0 ml microfuge tubes. The positive, 100% lytic control for each experiment was 0.1% Triton X-100, while the negative, 0% lytic control was PBS. Each 2-ml tube was aseptically filled with aliquots of a 180L diluted blood cell solution, which was then gently mixed three times using a wide mouth pipette tip. The tubes were agitated (80 revolutions per minute) for 35 min at 37 °C. The tubes were immediately centrifuged for 5 min at 1310 g after being incubated for 5 min on ice. A sterile 1.5ml micro-fuge tube was used to dilute aliquots of 100 ml of supernatant with 900 ml of cooled, sterile PBS. After dilution, all tubes were kept chilled on damp ice. Then 200 L were added to 96-well plates, and three replicates one positive and one negative were obtained from each plate. Then, a microquant was used to detect absorbance at 576 nm [16].

The experiment was done in triplicate. Percent hemolysis was calculated by following formula:

% hemolysis = Abs (sample absorbance)/Abs (control absorbance) × 100

2.3 Evaluation of Antioxidant Activity

2.3.1 Determination of Total Phenolic Contents (TPC)

Folin-Ciocalteu reagent procedure was used to determine the amount of TPC. Specifically, 50mg of dry mass of crude extract/fraction was mixed with 0.5ml of Folin-Ciocalteu reagent and 7.5ml of deionized water for 10 min, after which 1.5ml of 20% sodium carbonate (w/v) was added. The mixture was then heated in a water bath at 40 °C for 20 [17].

The results were expressed as gallic acid equivalents (GAE) of dry plant matter.

2.3.2 Determination of Total Flavonoid Contents (TFC)

Following the method outlined by Dewanto and colleagues, the Total Flavonoid Content (TFC) of plant extract and fractions was assessed. Each material's plant extract or fraction (1 ml, 0.1 g/ml) was added to a 10ml volumetric flask along with distilled water. Each volumetric flask was initially filled with 5ml and 0.3ml of 5% NaNO2, and then 0.6 ml of 10% AlCl3 was added after 5 minutes. 2 ml of 1 M NaOH was added and the volume was made up with distilled water after an additional 5 minutes. Solution was then combined. A spectrophotometer was used to measure the reaction mixture's absorbance at 510 nm. The catechin equivalents of TFC were calculated (g/100 g of dried plant materials) [18].

2.3.3 DPPH Radical Scavenging Assay

According to Bozin and colleagues' instructions, the 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) experiment was conducted spectrophotometrically. To create a final volume of 4 ml, the antioxidant activity of the methanol extract and its different fractions (0.1 mg/ml) were combined with 1 ml of a 90 M DPPH solution. The absorbance at 515 nm was measured following a 1 hour incubation period at room temperature [19].

The following formula was used to compute the percentage (%) of free radical inhibition caused by DPPH:

Inhibition (%) = 100 × (Absblank − Abssample/Absblank)

Where,

Abs. blank is the absorbance of the control (Containing all reagents except the test samples), and Abs. sample is the absorbance of the test samples.

2.3.4 Linoleic Acid System Antioxidant Activity

The antioxidant activity of the leaves of *A. attenuata* in methanol extract and its fractions was also tested using the method developed, which involved measuring the% inhibition of peroxidation in the linoleic acid system. Extract/fractions (5 mg) were dissolved in a solution containing 0.13 ml of linoleic acid, 10ml of 99.8% ethanol, and 10ml of 0.2 M sodium phosphate buffer (PH 7.0). The mixture was diluted to a final concentration of 25ml using distilled water. According to the thiocyanate method, the solution was incubated at 40 °C, and the degree of oxidation was assessed by adding 10ml of ethanol (75%), 0.2 ml of an aqueous ammonium thiocyanate solution (30%), 0.2 ml of the sample solution, and 0.2 ml of ferrous chloride (FeCl2) solution (20 mM in 3.5% HCl) solution sequentially. After 3 minutes of churning, the peroxide content was determined by measuring the mixtures' absorbance values at 500 nm. In a control experiment, only linoleic acid was used; no extracts were. Butylated hydroxytoluene (BHT) and ascorbic acid (200 ppm) are artificial antioxidants that were used as a positive control. The sample without any antioxidants was used as a reference point because it had the highest peroxidation level at 360 hours (15 days) [20-21].

The following formula was used to determine the % inhibition of linoleic acid peroxidation.

Percent inhibition of linoleic acid peroxidation = 100 Abs. increase of sample at 360 h/ Abs. increase of control at 360 ×100

2.3.5. Determination of Reducing Power

Little modification was made to the method described by Yen and colleagues in order to determine the extract's reducing power. Equivalent volumes of the crude extracts/fractions from the leaves, each containing 1.0 mg of dry matter, were combined with potassium ferricyanide (5.0ml, 1.0%), sodium phosphate buffer (5.0ml, 0.2 M, pH 6.6), and the mixture was incubated at 50 °C for 20 min. The mixture was then centrifuged at 980 g for 10 min at 5 °C in a chilled centrifuge (Centrifuge H-200NR, Kokusan, Japan) with 5ml of 10% trichloroacetic acid added. In order to measure the absorbance at 700 nm (Hitachi U-2001

Spectrophotometer), the upper layer of the solution (5.0ml) was diluted with 5.0 ml of distilled water and 1.0ml of ferric chloride (1.0 ml, 0.1%) [22].

2.4 Statistical Analysis

Each sample underwent a separate analysis in triplicate, with the results being presented as mean (n = 3 3 1) and standard deviation (n = 3 3). Utilising the statistical programme Minitab 2000 Version 13.2 (Minitab Inc., Pennysalvania, USA), data were analysed using analysis of varia.

3 Results and Discussion

3.1 GC-MS Analysis of n-Hexane

Fraction 31 components, accounting for 90.32% of the total fraction, were identified using GC-MS analysis of the n-hexane fraction from the methanol extract. This volatile fraction was made up of a variety of chemicals from several chemical classes. Mono-2-ethylhexyl phthalate (11.37%), 1, 2-benzene dicarboxylic acid (6.33%), n-docosane (6.30%), and eicosane (6.02%) were revealed to be the main ingredients (Table.3.1). The chemical make-up of a lipophilic extract of *Agave sisalana* fiber was described. The majority of the fatty acids and n-alkenes found in A. sisalana fiber were likewise found in the volatile portion of A. attenuate leaves [23].

Table 1: Chemical constituents of *A. attenuata* leaves n-hexane fraction.

Retention Time	Compounds	Area%
12.430	Eicosane	5.05
13.255	Tetracosane	3.05
14.052	Pentacosane	5.35
14.541	Mono-2-ethylhexyl phthalate	12.17
14.820	n-Hexacosane	4.92
15.657	n-Octacosane	0.40
15.861	Tetracosanoic acid	0.26
17.398	Nonacosane	1.32
18.536	n-Triacontane	1.10
19.366	2,5-Cyclohexadiene-1,4-dione	0.65
19.879	Nonadecane	1.33
3.338	Benzothiazole	0.44
4.186	Tetradecane	0.29
4.686	n-Undecane	2.32
5.005	n-Pentadecane	1.41
5.711	(E)-1-Methoxymethoxy-1-tetradecane-3-ol	0.58
5.915	n-Hexadecane	1.38
6.029	1,2-Benzenedicarboxylic acid	4.13
6.877	n-Heptadecane	1.50
7.856	n-Octadecane	1.31
7.958	Phytane	1.49
8.339	2-Undecanone	0.27
8.823	Nonadecane	1.32
9.090	Hexadecanoic acid	2.48
9.437	Palmitic acid	2.67
Total		74.4

3.2. Antioxidant Potential of *A. attenuata* Leaves

Table.3.2 lists the total phenol content (TPC) and total flavonoid content (TFC) of leaves of *A. attenuata* that were extracted using various solvents. Both of these readings fell between 10.54 and 39.35 mg of gallic acid equivalents (GAE) and between 304.8 and 43.35 mg of catechin equivalents (CE) per 100 g of dry plant matter, respectively. The following was discovered regarding the capacity of several solvents to extract TPC: Chloroform (19.3%), ethylacetate (14.5%), n-butanol (10.65%), and n-hexane (10.54%) are all followed by methanol (39.35%).

Table 2: Antioxidant potential of the methanol extract and its fractions from *A. attenuata* leaves.

Extract or Reference Compound	Total Phenolic Contents (GAE mg/100 g)	Total Flavonoid Contents (CE mg/100 g)	DPPH (% inhibition, 0.1 smg/ml)	Inhibition in Linoleic Acid System (%)	Reducing Power (Absorbance, nm 1 mg/ml)
Methanol	36.30±0.79 a	306.8±5.04a	45.37±1.32b	64.36±1.15b	0.631 ± 0.016 b
Chloroform	16.4±0.52b	76.4±2.52c	62.76±0.52c	52.22±1.04d	0.60±0.011b
Ethylacetate	12.24±1.14c	190.2±4.92b	32.12±0.94b	67.71±1.64b, c	0.35±0.034b
n-Butanol	11.98±1.14d	70.2±2.46c	60.11±0.92c	54.87±1.82c	0.43±0.014b
n-Hexane	09.42±0.30d	41.25±2.39d	58.23±1.04d	48.15±1.12e	0.94±0.043c
Ascorbic Acid	42.12±1.01d	18.43±1.01c	38.56±2.07a	51.13±1.03d	0.68±0.022a
BHT	23.45±1.02c	35.09±2.09a	45.10±2.04a	80.3±2.32a	0.21±1.06d

Data are expressed as the mean ± standard deviation; values having different letters differ significantly ($p< 0.05$).

The following ranking of solvents' effects on TFC values was discovered: Chloroform (79.8%), n-butanol (71.5%), ethylacetate (19.2%), 100% methanol (304.8%), and n-hexane (43.35%). The extract and fractions' DPPH radical scavenging activity (0.1 mg/ml) ranged from 61.41 to 73.97%. The maximum scavenging activity was

demonstrated by the methanol extract (73.97%), which was followed by the ethyl acetate (73.36%), n-butanol (65.21%), chloroform (64.94%), and n-hexane (61.41%) fractions, in that order. BHT produced inhibition at a level of 84.7%, whereas the percent inhibition of linoleic acid oxidation ranged from 50.12% to 70.35%. The highest level of linoleic acid oxidation inhibition was seen in the methanol extract (70.35%), which was followed by the ethylacetate (67.78%), n-butanol (66.23%), chloroform (60.25%), and n-hexane (50.12%) fractions. All of the compounds displayed significantly (p 0.05) modest action when the findings of DPPH scavenging activity and the percent inhibition of linoleic acid oxidation were compared with standard BHT. At doses of 1 mg/ml, the studied extract and fractions' reducing potential was visible. The range of absorbance for the examined materials in this experiment was 0.219-0.664 nm. The n-hexane fraction had the lowest absorbance (0.20 nm), whereas the chloroform fraction had the highest absorbance (0.664 nm). The reducing power of various extracts and fractions reduced starting with chloroform and moving up to 100% methanol, n-butanol, ethylacetate, and finally n-hexane. All of the compounds displayed a minor reducing power when these results were compared to the reference ascorbic acid (0.8 nm) (Table 2). The results showed that the methanolic extract of plant leaves had the highest levels of TPC and TFC and, consequently, the highest antioxidant activity as measured by the inhibition of linoleic acid oxidation and DPPH radical scavenging; in contrast, the n-hexane fraction had the lowest antioxidant activity, probably as a result of its low polarity.

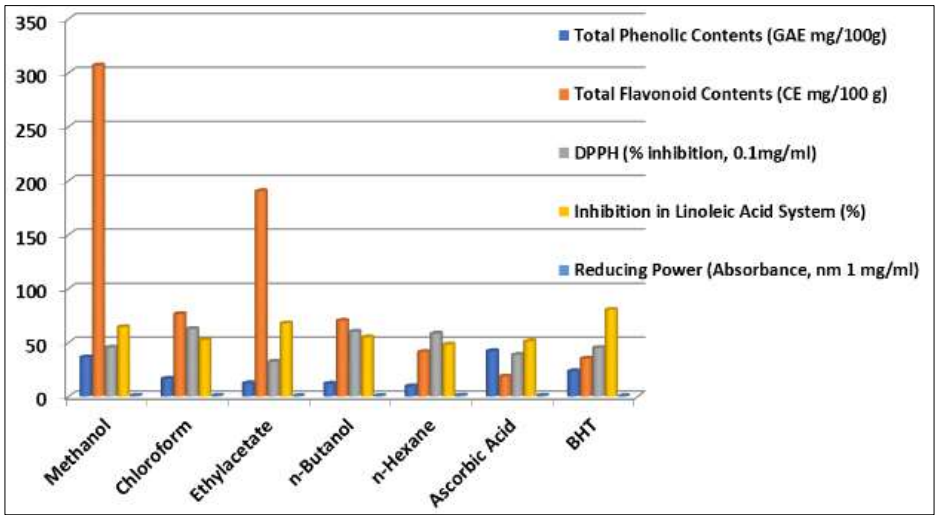

Fig 1: Antioxidant potential of the methanol extract and its fractions from *A. attenuata* leaves.

3.3. *In vitro* Hemolytic Activity:

Even though the plant has strong antioxidant and antibacterial properties, hemolytic activity was researched since without these hemolytic effects associated with saponins, the plant's application in medicine would be impossible. 20 L of Triton X-100 (0.1%) was used to achieve complete hemolysis (100%) in the sample. The highest level of hemolytic activity was demonstrated by the chloroform fraction (2.64%), which was followed by the n-hexane (2.09%), methanol extract (1.46%), ethylacetate (1.40%), and n-butanol (1.01%) fractions. The n-butanol fraction had a less pronounced hemolytic impact than the other fractions (Table. 3.3). The plant extracts may be safe for human use because the data obtained are within safe limits.

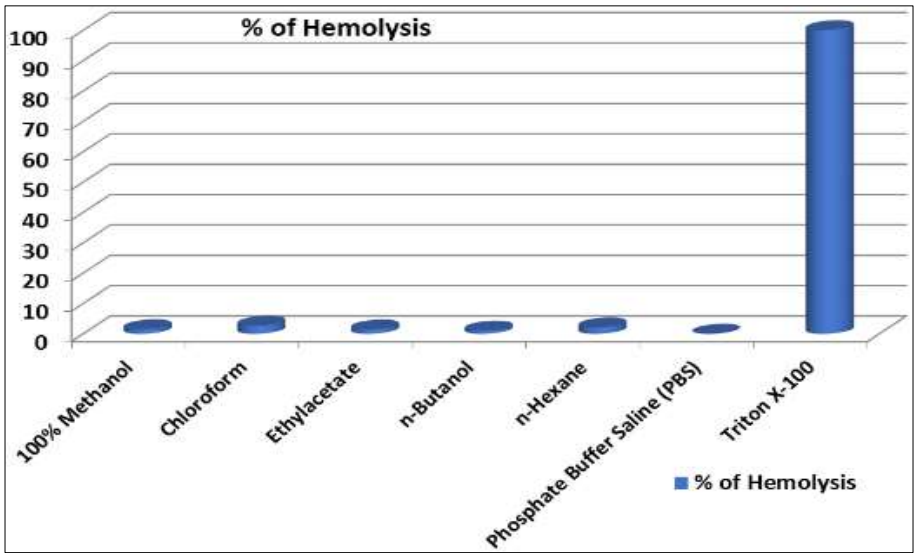

Fig 2: Hemolytic activity, as a percentage of hemolysis caused by A. attenuate leaves methanol extract, its fractions and standard.

Table 3: Hemolytic activity, as a percentage of hemolysis caused by A. attenuate leaves methanol extract, its fractions and standard.

Plant Extract Fraction	% of Hemolysis
100% Methanol	1.46±0.14
Chloroform	2.64±0.11
Ethylacetate	1.40±0.04
n-Butanol	1.01±0.04
n-Hexane	2.09±0.08
Phosphate Buffer Saline (PBS)	0
Triton X-100	100±0.61

3.4. Antimicrobial Activity

According to Table.3.4, the plant demonstrated significant antibacterial activity against all of the tested strains. With the highest inhibition zones (27.5 and 20.75 mm) and the lowest MIC values (18.4 and 69.4 mg/ml), the methanol extract demonstrated strong inhibitory activity against *A. flavus* and *A. alternate*, according to the disc diffusion method results. In tests against *P. multocida* and *A. niger*, methanol extract had little action. *R. solani* and *A. flavus* were strongly inhibited by the chloroform fraction, which had high inhibition zones (26.7 and 19 mm) and lower MIC values (20.4 and 25.1 mg/ml). The activity against *P. multocida* and *S. aureus* was minimal. Only against *E. coli* did the ethylacetate fraction exhibit strong inhibitory activity, with the lowest MIC value (94.2 mg/ml) and an inhibition zone of 18.75 mm. *A. flavus*, *A. alternata*, and *R. solani* did not have their growth inhibited by the ethylacetate fraction. When compared to the conventional medicine rifampicin, whose inhibition zone was of 21.5 mm (MIC 62.1 mg/ml), the n-butanol fraction had a robust activity against *E. coli* with the biggest inhibition zone (26.8 mm) and the lowest MIC value (15.2 mg/ml). With the smallest inhibition zone (13 mm) and highest MIC value (170 mg/ml), *B. subtilis* showed little action. With a high inhibition zone of 25.75 mm and the lowest MIC value of 27.4 mg/ml against *P. multocida*, the n-hexane fraction was ineffective against *B. subtilis*.

Table 4: Antimicrobial activity of a methanol extract and fractions of *A. attenuata* leaves

Tested	Methanol Extract and Its Fractions (Diameter of Inhibition Zone, mm)					Standard Drugs	
Microorganism	**Methanol**	**Chloroform**	**Ethylacetate**	**n-Butanol**	**n-Hexane**	**Rifampcin**	**Fluconazole**
B. subtilis	12.0±1.41b	10.5±1.21c,d	10.0±1.53d	10.0±0.60b,c	0	27.0±1.41a	n.d
P. multocida	0	13.0±0.50d	10.5±1.318d	17.5±1.65 c	28.70±2.58b	26.87±0.52a	n.d
S. aureus	8.65±0.63c	14.0±0.50c	14.35±0.72c	10.45±0.63 b	16.5±0.5c	26.65±1.09a	n.d
E. coli	18.1±1.32b,c	12.2±0.32c	16.58±0.40a	28.8±0.42b	18.20±2.20c	23.9±2.07a	n.d
A. niger	0	11.2±1.37b	13.5±1.15b	16.3±1.65a	17.5±1.66b	n.d	19.5±1.19a
A. flavus	24.5±2.3b	13±0.72c	0	18.2±0.84c	15.0±1.00c	n.d	2.0±1.07a
Tested	**Methanol Extract and Its Fractions (Diameter of Inhibition Zone, mm)**						**Standard Drugs**
Microorganism	**Methanol**	**Chloroform**	**Ethylacetate**	**n-Butanol**	**n-Hexane**	**Rifampcin**	**Fluconazole**
A. alternata	19.55±0.5 b	14.5 ± 1.5 c	0	19.5 ± 0.43 b	12.25 ± 0.5 d	n.d	25.5 ± 1.18 a
R. solani	17.65±1.65d	20.4±2.30 b	0	22.0±0.407 c	16.55±4.91d	n.d	26.15±0.23a
Minimum Inhibitory Concentration (MIC) mg/ml.							
B. subtilis	138±1.11	105±2.71	150±2.10	170±1.15	0	8.48± 0.15	n.d
P. multocida	0	250 ± 2.47	185 ± 1.20	135±1.28	22.4±0.64	9.5±0.40	n.d
S. aureus	262±1.45	255 ± 2.35	210 ± 2.40	59.3 ± 0.54	260 ± 2.15	4.12 ± 0.19	n.d
E. coli	110 ± 0.75	123±0.71	90.2±0.86	15.2 ± 1.15	130±1.40	74.4±0.42	n.d
A. niger	0	212±2.18	221±2.40	125±1.14	114±1.13	n.d	48.8±0.30
A. flavus	12.1±0.55	22.2±0.65	0	99.9±0.59	119±1.28	n.d	6.6±0.23
A. alternata	68.4±0.22	132±0.24c	0	80.4±0.43	180±0.38	n.d	24.4±0.10
R. solani	119±0.76	20.0±0.65	0	88.1±0.27	150±0.36	n.d	7.7±0.13

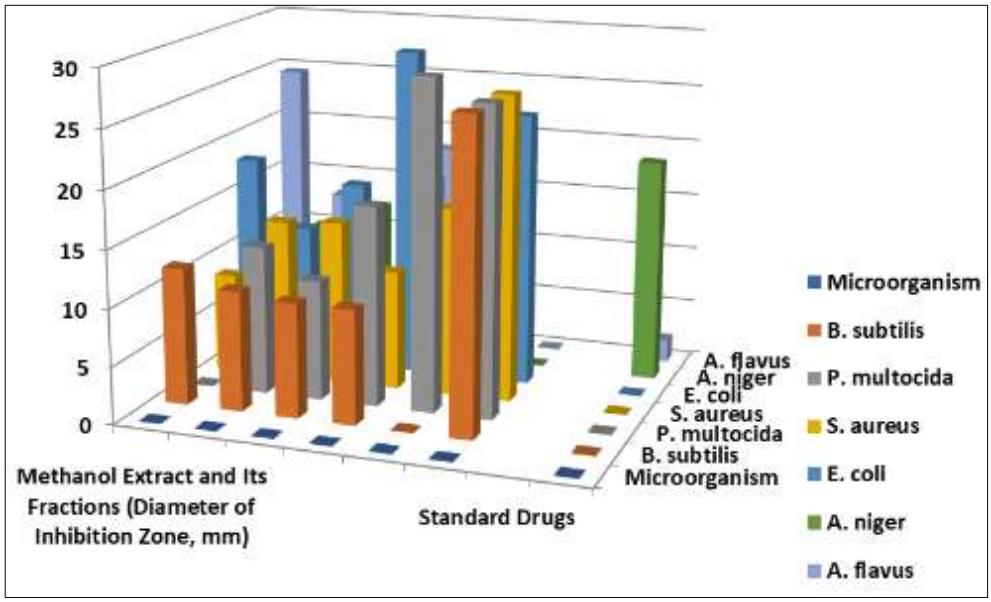

Fig 4: Antimicrobial activity of a methanol extract and fractions of *A. attenuata* leaves.

A. asperrima and *A. striata* flower extracts have been reported to have antifungal action against *A. flavus*, with the *Agave* methanol extracts having a stronger antifungal impact. These outcomes are consistent with our investigation, which showed that methanol extract had good inhibitory effects on all tested fungal strains and a potent inhibitory action against *A. flavus*. Aspergillosis of the lungs and occasionally corneal, otomycotic, and nasoorbital infections are only a few of the illnesses caused by *A. flavus*, which affects both humans and animals Sanchez *et al*.

4. Conclusion: Investigating several biological processes of an *Agave attenuata* leaf methanol extract was the goal of the current investigation. The mono-2-ethylhexyl phthalate (10.31%), 1, 2-benzenedicarboxylic acid (5.03%), n-docosane (5.45%), and eicosane (5.04%) served as the primary ingredients among the 25 compounds discovered by GC-MS in the n-hexane fraction of the extract. The leaves had an overall phenolic content of 11.521-39.35 GAEmg/100 g and an overall flavonoid content of 42.35-304.8 CE mg/100 g. The extract and some of its constituent parts were shown to have mild antibacterial activity. Leaf extract and fractions also shown strong antioxidant potential when lipid peroxidation studies were conducted to measure DPPH radical scavenging activity and inhibit it. The plant was found to have hemolytic effects in the range of 2.01% to 3.50%. The results of the study suggest that this plant has potential as a source of natural antioxidants and functional food nutraceuticals. Data from the current study confirmed that *A. attenuata* has high biological activity. The presence of physiologically relevant phytochemicals in plant extracts may have an impact on both their potential as sources for effective medications and their medical performance. It is possible to process the plant under inquiry for use in pharmaceutical and herbal remedies for human ailments. The antioxidant properties of this plant also suggest a potential use as a functional component for processing into healthy foods in the food industry. This comprehensive study sheds light on the botanical characteristics and chemical composition of *Agave attenuata* leaves. The presence of bioactive compounds with diverse pharmacological properties suggests their potential utility in traditional and complementary medicine. Further research is warranted to elucidate the underlying mechanisms and to explore clinical applications.

5. References

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