



## INVESTIGATION OF PHYTOCHEMICAL AND ANTI-BACTERIAL ACTIVITY ON *AGAVE AMERICANA* METHANOLIC EXTRACT FOR MEDICAL APPLICATIONS

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### ABSTRACT

The *Agave Americana* plant is one of the very important natural resource which is available in the tropical areas with plenty of hygienic properties. The plant contains saponin useful for medical and pharmacological importance. Based on the view, the Americana plant leaves were collected and extracted by soxhlet extraction method. The extract was tested under qualitative phytochemical screening and the test results revealed the presence of phytochemical constituents such as proteins, steroids, alkaloids, flavanoids, saponin and tannins. The anti-bacterial property of *agave* extract was tested using standard AATCC 147 qualitative and AATCC 100 quantitative tests against both gram positive bacterial pathogens *Staphylococcus aureus*, *Klebsiella pneumoniae* and gram negative bacterial pathogens *Pseudomonas auruginosa*, *Escherichia coli* and the antifungal activity were assessed against *Aspergillus nigar* and *Aspergillus fumigates*. The test result shows that the *Agave Americana* leaf extract has good antibacterial activity against the gram negative bacteria than gram positive bacteria.

**KEYWORDS:** *Agave Americana*, Methanolic extract, Phytochemical constituents, Antibacterial activity, Microorganisms, Agar well diffusion. Broth dilution.



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## INTRODUCTION

Medicinal plants play a key role in world health care systems. These plants constitute an important natural wealth of a country. The serviceable requirements of medical textiles have led to the innovative use of a variety of natural fibers with enhanced comfort and hygienic properties in the development of new products for medical applications. The various ranges of natural fibers and biodegradable polymers are being utilized for developing new products into medical textiles. The medical textile applications are directly related to the skin and also life of human being, those are required to undergo stringent testing and hygienic criteria, which led to innovative use of variety of fibers and a lot of developments taking place in this area. Different parts in the plant kingdom has own different uses. They play a significant role in providing primary health care services to rural people and serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine<sup>1</sup>. From the plant kingdom, one of the abundant sources of strong natural fiber is *Agave Americana*<sup>2-3</sup>. It belongs to the *Agavaceae* family. Common names include Century plant or American Aloe. It is not, however, closely related to the genus *Aloe*<sup>4</sup>. According to a report by Moris (2006) the plant grows well in acidic, neutral or basic alkaline soils that are well drained, either light (sandy) or medium (loamy). *A. americana* plant grows well in Lanet and Mbaruk areas in Nakuru district, Kenya where it's being planted for its aesthetic purposes<sup>5</sup>. *Agave americana* is an exotic plant, which is widely cultivated in the world for aesthetic purposes. It belongs to the family *Agavaceae* and in most varieties the leaf has a white or yellow marginal or central stripe from the base to apex. It provides also stable economic return to local communities especially through the sale of wild harvested material<sup>6</sup>. *Agave americana* plant is probably originated from Eastern Mexico. It was introduced into Europe about the middle of the 16th century and is now widely cultivated for its aesthetic appearance<sup>7</sup>. The natural fibres produced under different environmental conditions such as soil type and weather

can significantly affect the fibre properties<sup>8</sup>. *Agave Americana* plant is found to possess better antibiotic properties under laboratory conditions, the leading researchers to think this fiber may be effective in the treatment of both staphylococcus and streptococcus bacteria strains. Recent studies on antibacterial activity, it is more effective in inhibiting growth of various bacteria. Apart from medical applications, the *agave americana* fibre also used to develop apparel products. Hence the present research work aims at developing antibacterial medicated apparels for medical and healthcare applications.

## MATERIALS AND METHODS

### Materials

*Agave Americana* fiber is a natural fiber, which are vastly available in and around the area of central and North America. It has various medicinal and disease curing properties and it has been used for screening the phytochemical constituents and analyzing the antimicrobial properties.

### Collection of plant material

The leaves of *Agave Americana* were selected for the study on the basis of their medicinal and skin disease curing properties and it was collected from Ayurvedic centre, Coimbatore.

### Preparation of plant extract

The *Agave Americana* leaves were shadow dried for about seven days then chopped and converted into powder form by using automatic machines. 150 gms of the fine powder was mixed with 500 ml of methanol for seven hours using soxhlet apparatus by extraction method in order to obtain the yield of extraction. After the extraction process, the extracted solution was kept at room temperature for solvent evaporation. The obtained precipitate of the developed extract was stored in a refrigerator with the help of tight containers at 4°C. Based on the investigation, the extract was diluted and utilized for further end use<sup>9-11</sup>



Figure 1  
*Agave Americana* Leaves for extraction

**Methods**

The various methods are used for identifying the phytochemical constituents and antimicrobial activity in the *Agave Americana* leaf extracted solution<sup>12-14</sup>.

**Preliminary phyto-chemical screening**

The phytochemical analysis method is used to identify the presence of active phytoconstituents in *Agave Americana* Leaf extract such as carbohydrates, steroids, alkaloids, flavanoids, saponins and tannins. The preliminary test was conducted as per standard test procedures.

**Test for Carbohydrates****Fehling's Test**

The plant leaf extract of 2.5 ml was mixed with 1.0 ml of Fehling's solution. The mixed solutions were boiled for a few minutes. The formation of red or brick red precipitate indicates the presence of reducing sugar.

**Benedict's Test**

The 1.5 ml of the americana extract was added with 5.0 ml of Benedict's reagent and boiled in hot water bath for about 5 minutes. The appearance of red, yellow or green color precipitate showed the presence of reducing sugar.

**Test for Steroids****Salkowki's Test**

The concentrated sulphuric acid 1.0 ml was added with 2.0 ml of the extract carefully along the sides of the test tube then the red color was formed in the chloroform layer which indicates the presence of steroids.

**Test for Alkaloids****Wagner's Test**

The 1.7% v/v of hydrochloric acid and a few drops of Wagner's reagent were added to acidify the plant extract of 1.0 ml then the formation of brown or reddish precipitate indicates the presence of alkaloids.

**Meyer's Test**

Few drops of Meyer's reagent were mixed with 1.0 ml of the extract. A yellow **creamy** precipitate indicates the presence of alkaloids.

**Test for Flavanoids****Alkaline Reagent Test**

A few drops of the americana leaf extract were added with 4 to 5 drops of sodium hydroxide solution. Intense yellow color was formed and turned into colorless on addition of few drops of dilute hydrochloric acid indicates the presence of flavanoids.

**Test for Saponins****Foam Test:**

A few drops of sodium bicarbonate solution were added with 1.0 ml of the vetiver extract, shaken vigorously and kept for 3 minutes. A honey comb like froth will be formed and this indicates the presence of saponins.

**Test for Tannins****Ferric Chloride Test**

A few drops of aqueous 5% Ferric chloride were mixed with 1.0 ml of the extract. A bluish black color was formed which then gets disappeared in addition of few drops of dilute sulphuric acid and an yellowish brown precipitate was formed which indicates the presence of tannins.

**Lead Acetate Test**

A few drops of 1% solution of lead acetate was added with 5.0 ml of the extract then the formation of yellow or red precipitate indicates the presence of tannins<sup>15-18</sup>

**Assessment method of antibacterial activity on Agave Americana methanolic extracts**

The antibacterial activity of the *Agave Americana* leaf extract was analyzed using both qualitative antibacterial assessment method of Agar well diffusion test (AATCC-147) and quantitative assessment method of Broth dilution test (AATCC-100)

**Preparation of gram positive and negative bacterial cultures**

The four different bacterial cultures and two different fungal cultures were developed from Microbial Type Culture Collection (MTCC), Department of biotechnology Laboratory, Kumaraguru College of Technology, Coimbatore, India. The developed bacterial cultures for the study were namely gram positive bacterial pathogens *Staphylococcus aureus*(MTCC-724) and *Klebsiella pneumoniae*(MTCC-107), gram negative bacterial pathogens namely *Pseudomonas auruginosa* (MTCC-421) and *Escherichia coli*(MTCC-442) respectively. The developed bacterial cultures were maintained on nutrient agar slant and stored separately in a refrigerator at 4°C.

**Qualitative antibacterial activity assessment by agar well diffusion method( AATCC- 147)**

The antibacterial activity of *Agave Americana* leaf extract was evaluated using agar well diffusion method.<sup>19</sup> 30 ml of nutrient agar was prepared and allowed for sterilization at 121°C for about 15 minutes. The petri plates were autoclaved in hot air oven at 121°C for 45 minutes. The developed ethanolic extract has been converted into 150 µg/ml concentration. The nutrient agar of 25 ml was dropped into the petri plates and was allowed to solidify. Then the plant extract was poured in the developed well and the plates were incubated for 24 hours at 37°C. After 24 hours, the antibacterial activity was assessed against the test organisms *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas auruginosa* and *Escherichia coli* by measuring the zone of inhibition.

**Quantitative antibacterial activity assessment by broth dilution test method( AATCC -100)**

The *agave americana* leaf extracts were taken for broth dilution test. In each conical flask, the 30ml of Nutrient broth powder was weighed and mixed with distilled water. Then the solution was stirred well. 150µl *Staphylococcus aureus* bacteria were added into 2 conical flasks which contain the *Agave Americana* leaf extract and standard. Similarly 150µl *Escherichia coli* bacteria were added into 2 conical flasks. Then the

flasks were kept in a shaker for 24 hours under medium speed at room temperature. Then the 3gms of Nutrient broth was diluted with distilled water to measure the blank which was used to set as zero calibration. Before calculating the readings, the machine was calibrated at 600 nm and the readings were noted and then reduction percentage of the absorption values was calculated against standard antibiotic.

## RESULTS AND DISCUSSION

The preliminary phytochemical screening and antibacterial activity test results were discussed.

### **Preliminary phytochemical screening of *Agave Americana***

The qualitative phytochemical screening of *Agave Americana* leaf methanolic extract test results are shown in the table 1.

**Table 1**  
**Qualitative Phytochemical analysis of the methanolic extract of *Agave Americana***

S.No	Plant constituents	Methanolic extract
1	Carbohydrates	
	A) Fehling's test	+
	B) Benedict's test	+
2	Steroids	
	A) Salkowki's test	+
3	Alkaloids	
	A) Wagner's test	+
	B) Meyer's test	+
4	Flavanoids	
	A) Alkaline reagent test	+
5	Saponins	
	A) Foam test	+
6	Tannins	
	A) Ferric chloride test	+
	B) Lead acetate test	+

+ symbol represents presence of phytochemical constituents

The test results revealed the presence of phytochemical constituents in the extract such as carbohydrates, flavanoids, steroids, tannins, alkaloids and saponins were present in the *agave* extract. The obtained precipitate color represents each compounds present in the *Agave Americana* plant leaf extract. The presence of these components induces either individually or in combination to posse's antimicrobial activity. The presence of tannin and saponins in the leaves of *Agave Americana* inferred that these two compounds may be the active compound which was responsible for antimicrobial activity in this study. The component tannin expresses better antibacterial activity<sup>20-21</sup>.

### **Qualitative Assessment method of antimicrobial activity on *Americana* plant leaf methanolic extract (AATCC-147 and AATCC 100 Test Method)**

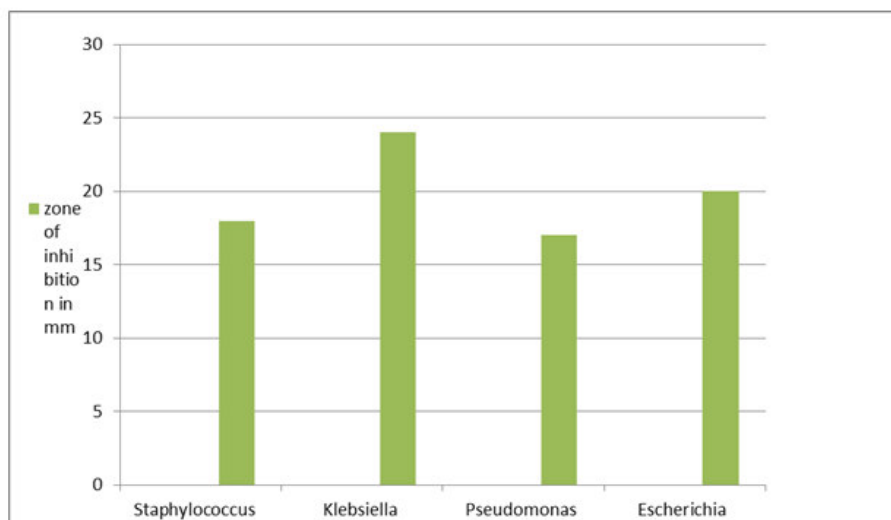
The qualitative and antibacterial activity of the *Agave Americana* leaf extract has been shown in the table 2 and 3.

### **Qualitative antibacterial activity assessment by agar well diffusion method**

The antibacterial activity zone of inhibition test results of *Agave Americana* leaf extract against gram positive bacterial pathogens namely *Klebsiella pneumoniae*, *Staphylococcus aureus* and gram negative bacterial pathogens namely *Pseudomonas auruginosa* and *Escherichia coli* by agar well diffusion method were shown in table 2

**Table 2**  
**Antibacterial zone of inhibition in (mm) against gram positive and gram negative bacterial pathogens on methanolic leaf extract of *Agave Americana* plant**

Test organisms	Bacterial growth control by Zone of inhibition ( in mm)
<i>Staphylococcus aureus</i> - MTCC 724	19
<i>Klebsiella pneumoniae</i> - MTCC 107	24
<i>Pseudomonas auruginosa</i> - MTCC 421	17
<i>Escherichia coli</i> -MTCC 442	20



**Figure 2**  
**Assessment of antibacterial activity**  
**zone of inhibition in mm**

The zone of inhibition test results of *Agave Americana* methanolic leaf extract showed good antibacterial activity against gram positive pathogens namely *Staphylococcus aureus* (18 mm) and *Klebsiella pneumoniae* (24 mm) than gram negative pathogens namely *Pseudomonas auruginosa* (17 mm) and *Escherichia coli* (20 mm). The extract proved that it has better control over the positive pathogens than compared to the negative pathogens.

#### **Quantitative antibacterial activity assessment by broth dilution method**

The quantitative antibacterial activity of the *Agave Americana* leaf extract by broth dilution method was shown in table 3. The absorbance value was measured at 600 nm on the conical flasks for both gram positive and negative pathogens namely *Staphylococcus aureus* and *Escheirchia Coli*.

**Table 3**  
**Quantitative analysis of test results of Agave Americana extract (Broth dilution test)**

Samples	Antibacterial activity (Absorbance value OD at 600 nm)					
	Bacterial reduction value of <i>Staphylococcus aureus</i> gram positive bacteria (nm)			Bacterial reduction value of <i>Escheirchia Coli</i> gram negative bacteria (nm)		
	24 hrs	48 hrs	value of gram positive	24 hrs	48 hrs	value of gram negative bacteria
<i>Agave Americana</i> leaf extract	1.71	1.61	1.61	1.76	1.80	1.80
Standard Antibiotic	1.50	1.81	1.81	1.46	0.73	0.73

From the Table-3, the quantitative result shows that the *Agave Americana* leaf extract has good absorbance value in 24 and 48 hrs time treatments against gram negative and positive bacteria when compared to standard antibiotic. The OD value of *agave americana* extract showed that better bacterial reduction percentage and good activity against gram negative E.Coli (1.76 & 1.80 nm) than gram positive *Staphylococcus aureus* (1.71nm & 1.61nm) bacteria strains.

## **CONCLUSION**

From the test results, we have concluded that the phytochemical constituents present in the extract include carbohydrates, flavanoids, steroids, alkaloids, saponin and tannins. The presence of phytocomponents such as saponin and flavanoids in the extract provides better antimicrobial activity. The both qualitative and quantitative antimicrobial test results also showed greater levels of antibacterial activity against gram

positive bacterial pathogens namely *Staphylococcus aureus* and *Klebsiella pneumoniae* than gram negative bacterial pathogens *Pseudomonas auruginosa* and *Escherichia coli* and the OD value of *agave americana* extract showed that better bacterial reduction percentage and good activity against gram negative E. Coli than gram positive *Staphylococcus aureus* bacterial strains. Based on the phytochemical screening and antimicrobial activity assessments, the extract proved that it will be more suitable for the medical applications. Hence, this research work will give a nutshell about the sustainable, antimicrobial medicated product developments for medical practitioner and also hospital workers in healthcare field as well as raw material is from 100 % natural resources, it is renewable, nature friendly, economic, social and environmental benefits.

## **CONFLICT OF INTEREST**

Conflict of interest declared none.

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