UJMR, Volume 4 Number 2, December, 2019, pp 49 - 56 *ISSN: 2616 – 0668*
https://doi.org/10.47430/ujmr.1942.009

Received: 05/07/019 **Accepted:** 07/08/019

Phytochemical Screening and Antimicrobial Activity of Aqueous Stem Extract of *Aloe vera* on Some Common Pathogenic Bacteria

***Baha'uddeen Salisu Dandashire and Sa'adatu Muhammad Shema**
Department of Microbiology, Umaru Musa Yar'adua university, P.M.B. 2218, Katsina, Katsina State, Nigeria
Corresponding Author: bahauddeen.salisu@umyu.edu.ng, Phone: +2348036961624

Abstract

Aloe vera is one of the most popular ethnomedicinal plants that is commonly used in treating many infectious diseases. However, the knowledge of the spectrum of antimicrobial activity of the plant and its bioactive components is still inadequate. In this study, we carried out an automated determination of phytochemical components in aqueous extract (AE) of Aloe vera whole stem by GC-MS (Gas chromatography-mass spectrometry), determined the in vitro antimicrobial activity of the extract on some common pathogenic bacteria which included Escherichia coli, Salmonella typhi, Enterococcus faecalis and Staphylococcus aureus by agar well diffusion method, and determined the minimum inhibitory concentrations (MICs) and minimum Bactericidal concentration (MBCs) by agar dilution method. The result showed that the extract has strong antimicrobial activity against all the test organisms. No resistance was observed. The range for zones of inhibition, MIC and MBC were 12.33 ± 0.33 - 28.67 ± 0.60mm, 25mg/ml - 50mg/ml and 50mg/ml - 100mg/ml respectively. The most susceptible isolate was S. typhi (MBC = 50mg/ml) and the least sensitive isolate was E. faecalis (MBC = 150mg/ml). The GC-MS of the extract revealed 9 different compounds of which 82.82% are Palmitic acid methyl ester, (7Z)-7-Tetradecenal, Palmitin, 1,2-di-, 2-aminoethyl hydrogen phosphate and Decyl hexanoate with peak areas of 34.40%, 20.48%, 15.46% and 12.48% respectively. In conclusion, Aloe vera contained many broad spectrum antimicrobially active compounds that can be further purified to produce new antimicrobial compounds that can be used in the treatment of many diseases especially those caused by S. typhi, S. aureus and E. coli.

Key words: *Aloe vera*; Antimicrobial; Bactericidal, Bioactive; Chromatography, Phytochemical.

INTRODUCTION

Plants play a vital role in the existence of man and are most essential to his well-being. Medicinal plants have been used by humans for many centuries (Pertobuska, 2012; Santic *et al*, 2017) and will continue to serve as alternative medicine especially in the current global campaign for combating drug resistant organisms. Ethnopharmacopoea from olden civilizations have passed from generation to generation (Jamshidi-Kia et al, 2018; Santic *et al.*, 2017) and various modifications were being made as man continue to search for medications against many life-threatening diseases. Today, many ethnomedicinal practices involving the use of variety of medicinal plants to treat various ailments have been proven effective by scientific investigations (Santic, *et al.*, 2017) and variety of them have been incorporated into the modern medicine (Pertobuska, 2012). In fact, research has shown that many natural compounds from plants are even more effective in treatment, control and prevention of diseases than the modern synthetic drugs. For instance, "Patentiflorin A" compound isolated from *Justicia gendarussa* plant have been proven to be more effective in the management of HIV than the well - known antiretroviral drug, azidothymidine (AZT) (Zhang, *et al.*, 2017). Many plants decoctions have been proven effective in the treatment of Tuberculosis (TB) caused by extended spectrum drug resistant *Mycobacterium tuberculosis* (Ram *et al.*, 2018). Thus, the need to search for more natural products from medicinal plants can never be over emphasize

However, research have shown that, despite the numerous medicinal compounds found in plants, some medicinal plants contained highly toxic compounds in addition to the pharmacologically important substances. This has necessitated the need for screening of phytochemical components especially in commonly used medicinal plants with a view to

evaluate their efficacy and safety for human *Aloe vera* is one of such plants with numerous ethnomedicinal applications worldwide. However, the knowledge of its phytochemical components and spectrum of antimicrobial activity is not adequately resolved. Hence, the significance of the present study and its contribution to knowledge.

MATERIALS AND METHODS

Sample Collection and Preparations

The *Aloe vera* stem samples were collected from botanical garden of the Faculty of Natural and Applied Sciences Umaru Musa Yar'adua University, Katsina. The samples were cut into thin pieces, dried in the laboratory in laminar floor with blower, and subsequently grinded in to powdered form using laboratory blender.

Extraction of the Powdered Sample

The stem bark powder was extracted following the method of (Salisu *et al.*, 2017). One hundred gram (100g) of the powdered sample was extracted in 1000ml of sterile distilled water by percolation at room temperature for 168 hours with regular shaking at intervals. The extract was filtered by passing through a sterile cotton wool, re-filtered through a Whatman filter number 1, and then concentrated using a rotary evaporator. The extract was weighed, transferred into sterile air-tight bottle and stored in refrigerator at 4^0C until needed for downstream application. The percentage yield of the extract (% w/w) was calculated as follows:

$$\text{Percentage Yield} = \frac{\text{Mass of the extract}}{\text{Total Mass of the Stem Powder Extracted}} \times 100\%$$

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of the Extract

The GC-MS analysis of *Aloe vera* stem extract was performed using a GCMS-QP2010 plus Shimadzu, Japan at the chemical laboratory of the National Research Institute for Chemical Technology (NARICT) Zaria, Nigeria. The GC-MS was equipped with Shimadzu AOC 20i GC autosampler and alltech AT 502.2 column. The injection volume was 2.0µL at injection temperature of 200.00^0C, column oven temperature of 80^0C, pressure of 108.0 kPa, column flow rate of 1.58mL/min and total flow rate of 6.2mL/min at a linear velocity of 46.3cm/sec. Helium was the carriers gas and the temperature was programmed as 80.0°C (hold time, 2min) to 200.0°C (4.00min) to 280°C(10min). The ACQ of the MS was set to scan between 40.00m/z to 800.00m/z at a scan speed of 1666 with a start time of 3.00min and end time of 30.00min.

Identification of the Phytochemical Components

The phytochemical compounds present in the extract were identified using the database of National Institute Standard and Technology (NIST), WILEY8 and FAME library. The name, structure and molecular weights of all the phytochemical components were obtained from the data base by comparing the mass spectrum of the unknown components with the spectrum of the known components stored in the (NIST), WILEY8, FAME library (Salisu *et al.*, 2017)

Determination of Antimicrobial Activity of the Extract

To determine the antimicrobial activity of the extract by agar well diffusion method, four different dilutions of the extract (200mg/ml, 150mg/ml, 100mg/ml and 50mg/ml) were prepared by serial dilution of the extract in sterile distilled water; sterile Muller Hinton agar plates were prepared according to the manufacturer's instruction; stock solution of the overnight cultures of each of the test isolates were prepared in sterile distilled water and standardized by adjusting the turbidity to that of McFarland turbidity standard. Subsequently, 0.1ml of each stock solution of the test isolates were spread plated in separate plates of the prepared Muller Hinton agar, after which a sterile cork borer (6.00mm in diameter) was used to make 5 wells in each of the inoculated plates. A 0.1ml of sterile distilled water was added to the central well in each plate to serve as negative control, while 0.1ml each of the four extract solutions were added in the remaining four wells separately. Finally, each of the wells was filled with sterile molten agar and allowed to solidify. The plates were replicated in triplicate for each of the test organism and incubated at 37^0C for 24 hours. Millimeters of the zones of inhibition produced by the various concentration of the extract for each of the test isolates were measured and the triplicate values obtained for each concentration were converted to means and standard error of mean using Graphpad Instat statistical softwire.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Extract

Agar-dilution method was used to evaluate the antimicrobial activity of the extract. A 200mg/ml stock solution of the extract was prepared by dissolving 2 grams of the extract 10ml of sterile distilled water. From the stock solution, a serial double dilution was made in to four test tubes containing 5ml of sterile

distilled water by transferring 5ml of the stock the 1st tube was transferred to the next tube and continued until tube four, making 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml solutions of the extract in the 1st, 2nd, 3rd and 4th test tube respectively. For each isolate, 6 plates of Muller Hinton agar, were used. The plates, 1 – 5 were labelled as 200mg/ml – 12.5mg/ml, and the last plate was labelled as "control". Five milliliter (5ml) of the diluted extracts were added aseptically using micropipette in the appropriate plates. Five milliliter (5ml) of sterile distilled water was added to control plate. Then, 15ml of melted Muller Hinton agar, cooled at 50^0c was pipetted into each plate and mixed thoroughly with the extract. The plates were left to set for the medium to solidify at 37^0c for 20-30 minutes in an incubator. They were then inoculated with 0.1ml of the standardized inoculum of the isolates by spread plating. The six set of plates for each of the organism were incubated at 37^0C for 24 hours.

to the 1st tube and subsequently 5ml from The lowest concentration of the extract showing about 99% inhibition was taken as the MIC for the organism. The MBC was taken as the double concentration of the MIC.

RESULTS

Physical Characteristics and Percentage Yield of the Extract

After extraction and solvent evaporation, the extract appeared brownish solid with rough texture. Of the 100g powdered sample extracted, the amount of extract obtained was 19.0g, giving a percentage yield of 19%.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of the Extract

The result of total ion chromatogram (TIC) of the extract by GCMS is presented in Figure 1. As shown in the figure, the extract contained 9 different compounds which are indicated by their peak numbers, peak shapes and retention time.

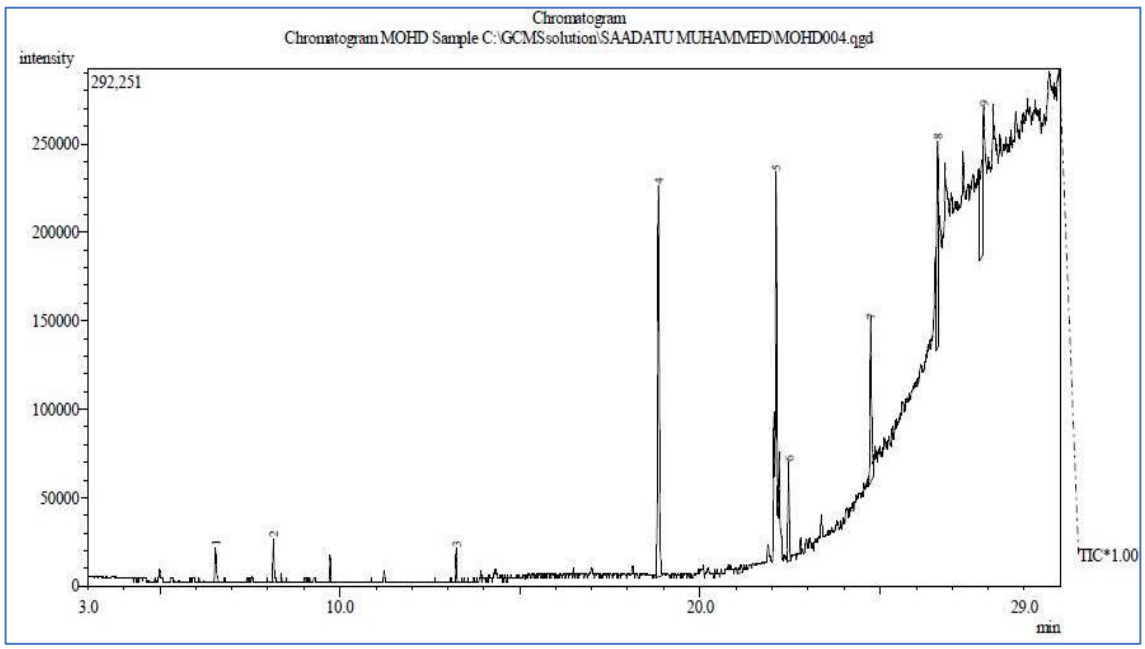
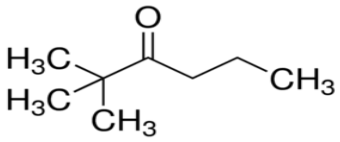
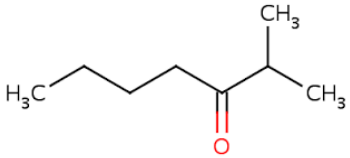
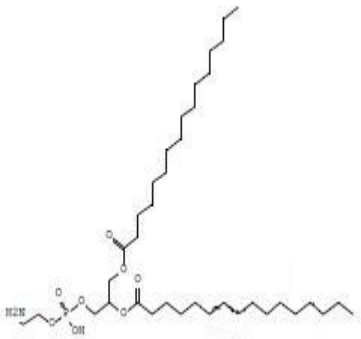


Figure 1: GC-MS Showing the Total Ion Chromatogram (TIC) of Aqueous Extract of *Aloe vera* Stem

The full identity of the detected phytochemical compounds including their IUPAC names, molecular formulae and structures are presented in Table 1. Of the 9 compounds identified, 82.82% are Palmitic acid, (7Z)-7-Tetradecenal, Palmitin, 1,2-di-, 2-aminoethyl hydrogen phosphate and Decyl hexanoate with peak areas of 34.40%, 20.48%, 15.46% and 12.48% respectively. The remaining 5 compounds constitute less than 20% by peak area. They include 15-methyl Hexadecanoic acid methyl ester (7.71%), 2,2-Dimethyl hexan-3-one (3.19%), 2-methyl heptan-3-one (3.00%), 3,3-Bis-tert-butylsulfanyl-2-fluoro-acrylonitrile (1.97%) and Oleic acid methyl ester (1.31%).

Table 1: Phytochemical Compounds Identified form the Aqueous Extract of *Aloe vera* Stem by GC-MS Analysis

Peak No	R. Time	% Area	Molecular Formula	IUPAC Name	Structure
1	6.543	3.19	$C_8H_{16}O$	2,2-Dimethyl hexan-3-one	
2	8.158	3.00	$C_7H_{12}O_2$	2-methyl heptan-3-one	
3	13.233	1.97	$C_8H_{18}C_{18}C_{12}P_{12}$	3,3-Bis-tert-butylsulfanyl-2-fluoro-acrylonitrile	
4	18.850	34.40	$C_{17}H_{34}O_2$	Palmitic acid methyl ester (Hexadecanoic acid methyl ester)	
5	21.150	1.31	$C_{19}H_{36}O_2$	Oleic acid methyl ester	
6	22.462	7.71	$C_{22}H_{44}O_2$	Hexadecanoic acid	
7	24.475	15.46	$C_{10}H_{22}O$	Palmitin, 1,2-di-, 2-aminoethyl hydrogen phosphate	
8	26.600	20.48	$C_{18}H_{31}0_{10}$	(7Z)-7-Tetradecenal	
9	27.828	12.48	$C_{23}H_{42}O_6$	Decyl hexanoate	

Antimicrobial Activity of the Extract

The result of antimicrobial activity of the *Aloe vera* extract against the selected Gram-positive bacteria (*S. aureus and E. faecalis)* and Gram-negative bacteria (*E. coli and S. typhi*) is presented in Table 2. The result showed that the extract has strong antimicrobial activity against all the test organisms. No resistance was observed. The range for zone of inhibition, MIC and MBC were 12.33 ± 0.33 – 28.67 ± 0.60mm, 25mg/ml – 50mg/ml and 50mg/ml – 100mg/ml respectively. The most susceptible isolate was S. typhi (MBC = 50mg/ml) and the least sensitive isolate was E. faecalis (MBC = 100mg/ml).

Table 2: Antimicrobial Activity of the Crude Aqueous Extract of *Aloe vera* Stem

S/N	Test Organisms	*Zones of Inhibition Produced by the Various Concentrations of the Extract against the Test Organisms (mm)				MIC (mg/ml)	MBC (mg/ml)
		200 mg/ml	150 mg/ml	100 mg/ml	50 mg/ml		
1	*S. aureus*	24.00 ± 0.58	22.00 ± 0.58	21.00 ± 0.76	19.67 ± 0.67	50	100
2	*E. faecalis*	16.17 ± 0.44	15.83 ± 0.44	14.17 ± 0.44	12.33 ± 0.33	50	150
3	*E. coli*	24.00 ± 0.29	22.83 ± 0.44	22.83 ± 0.33	21.5 ± 0.00	50	100
4	*S. typhi*	28.67 ± 0.60	27.67 ± 0.73	26.33 ± 1.69	23.33 ± 3.94	25	50

**Values are Means ± Standard Error of Means obtained from the 3 replicates used for each concentration.*

DISCUSSION

The percentage yield of the extract obtained in this study signifies that the stem of *Aloe vera* contained many water-soluble compounds or that water soluble compounds are present in large quantities in the stem of the plant since the weight of the extract recovered represent about a quarter of the weight of the stem powder extracted. It also suggests that water may be a good solvent for the extraction of phytochemicals present in *Aloe vera*, given that the amount of extract recovered during an extraction is directly proportional to the type of phytochemical compounds present and the polarity of the solvent used for the extraction (Salisu *et al.,* 2017). Kordali *et al.,* (2003) reported that the amount of extract obtained from *Pistia spp* was proportional to the polarity of the solvent used for the extraction.

The presence of the important bioactive compounds in *Aloe vera* stem detected by GCMS in this study justifies its worldwide usage in traditional formulations for the treatment of various human ailments. The various compounds identified in the stem of *Aloe vera* in this study are similar to the phytochemical compounds identified by other researchers in Aloe plants (Sathyaprabha *et al.,* 2010; Lakshmi *et al.,* 2011; Alrumman, 2018; Edward & Vaitheeswaran, 2014; Lakshmi & Rajalakshmi, 2011; Saljooghianpour & Javaran, 2013; Sathyaprabha, Kumaravel, Ruffina, & PraveenkumarP, 2016)

Furthermore, all the major components identified have been shown to possess important biological activities. The highest detected phytocomponent, Palmitic acid methyl ester (34.40%) which is a fatty acid ester has been shown to have many pharmaceutical applications due to its various biological properties such as antioxidant, nematicide, hypo-cholesterolemic, anti-androgenic, lubricant, flavor, hemolytic 5-alpha reductase inhibitor and pesticidal properties (Duke, 1992; Komensilan *et al.,* 2012). The second most abundant phytochemical, (7Z)-7-Tetradecenal (20.48%), has been previously identified as part of the major bioactive components of *Pamburus missionis* that contribute to its strong antimicrobial and other pharmacologically important applications (Sree *et al.,* 2015). Palmitin, 1,2-di-, 2-aminoethyl hydrogen phosphate (15.46%) is a phospholipid with strong antimicrobial properties and has been identified as part of the major bioactive compounds in *Fieberiella florii* that has strong curative properties in wide range of medical conditions involving reactive oxygen species as well as muscular dysfunction which may damage membranes or proteins that are required for fueling biochemical processes in the body (Kale, *et al.,* 2018). It has also been identified among the bioactive compounds in the leaf flower of *Piliostigma malabaricum* that has renown medicinal properties (Igwe and Okeke, 2017). In addition, Palmitin, 1,2-di-, 2-aminoethyl hydrogen phosphate was reported for its ability to interact with and enhances the antimicrobial activities of cationic surfactants (Joondan, *et al.,* 2014). Other phytocompounds of the plant include 15-methyl Hexadecanoic acid methyl ester (7.71%) which has both antibacterial and antifungal activity (Chandrasekaran *et al.,* 2011), 2,2-Dimethyl hexan-3-one (3.19%) which is an important volatile organic compound that has been isolated as part of major antimicrobially active compound responsible for the broad spectrum antimicrobial activity of the chloroform crude extract of the medicinal plant *Datura metel* (Hossain *et al.,* 2013) and butanol extract of 3 *Bacillus spp (Bacillus amyloliquefaciens*, *Bacillus thuringiensis* and *Bacillus* sp), isolated from rhizoides of Bamfara nut, with strong antagonism against *B. cereus*, *E. feacalis* and *Fusarium graminearium,* all of which are known to cause infections in humans, animals and plants with high rates of mortalities (Ajilogba *et al.,* 2019).

However, despite its antimicrobial activity, it has been classified by the European Chemicals Agency (ECHA, 2017) as a potential human toxin that can cause eye, skin and gastrointestinal irritations. Its presence in *Aloe vera* stem therefore support the antimicrobial activity of the stem and potential health risks to humans.

The last 3 compounds are 2-methyl heptan-3-one (3.00%) which is a yellow liquid that is commonly used as flavouring agent in food and is relatively considered safe (EFSA, 2004), 3,3-Bis-tert-butylsulfanyl-2-fluoro-acrylonitrile (1.97%) which has anticancer activity (Bachovchin *et al.*, 2011), and Oleic acid methyl ester (1.31%) which has antioxidant and antimicrobial activity (David, 2005).

The observed antimicrobial activity of the *Aloe vera* extract in this study is thus, fully supported by the presence of the various important pharmacologically active components identified in the extract by GCMS. Our result in this study has corroborated the findings of Kedarnath *et al.*, (2013) who reported that *Aloe vera* has strong antimicrobial activity against some pathogenic bacteria (*Staphylococcus aureus, Klebisella pneumonia* and *E.coli*) and fungi (*Aspergillus niger* and *Candida spp*), producing zones of inhibition at 20mg/ml and 40mg/ml higher than that of a standard antibiotic (Gentamicin, 1mg/ml).

In addition, a previous study conducted to determine the ability of *Aloe vera* extract to be used for decontamination of gutta percha cones showed that the extract was able to inhibit the growth of the 3 major contaminants identified in the study (*E. coli*, *E. faecalis* and *S. aureus)* producing a zone of inhibition of 24mm, 21mm and 24mm respectively after 48hours of incubation (Athiban *et al.*, 2012). Some similar other literatures also reported *Aloe vera* as having strong antibacterial and antifungal properties (Subramanian *et al.*, 2006; Thiruppathi *et al.*, 2010; Nejatzadeh-Barandozi, 2013; Jothi *et al.*, 2014; Jain *et al.*, 2016; Saket *et al.*, 2017; Paul *et al.*, 2018).

CONCLUSION

In conclusion, *Aloe vera* contained many broad spectrum antimicrobially active compounds that can be further purified to produce new antimicrobial agents that can be used in the treatment of many diseases especially those caused by *S. typhi, S. aureus* and *E. coli.*

REFERENCES

Alrumman, S. A. (2018). In Vitro Antimicrobial Activity and GC–MS Findings of the Gel of Aloe vacillans Forssk. of Abha Region, Saudi Arabia. *Arabian Journal for Science and Engineering*, *43*(1), 155–162. https://doi.org/10.1007/s13369-017-2785-7

Ajilogba CF, Babalola OO. (2019). GC–MS analysis of volatile organic compounds from Bambara groundnut rhizobacteria and their antibacterial properties. *World J Microbiol Biotechnol*;35(6):83. Available from: http://link.springer.com/10.1007/s11274-019-2660-7

Athiban, P. P., Borthakur, B. J., Ganesan, S., & Swathika, B. (2012). Evaluation of antimicrobial efficacy of *Aloe vera* and its effectiveness in decontaminating gutta percha cones. *Journal of conservative dentistry: JCD*, *15*(3), 246–248. doi:10.4103/0972-0707.97949

Bachovchin, D. A., Zuhl, A. M., Speers, A. E., Wolfe, M. R., Weerapana, E., Brown, S. J., … Cravatt, B. F. (2011). Discovery and optimization of sulfonyl acrylonitriles as selective, covalent inhibitors of protein phosphatase methylesterase-1. *Journal of medicinal chemistry*, *54*(14), 5229–5236. doi:10.1021/jm200502u

Chandrasekaran, M; Senthilkumar, A.; Venkatesalu, V. (2011). Antibacterial and antifungal efficacy of fatty acid methyl esters from leaves of Sesuvium portulacastrum L. *Eur. Rev. Med. Pharmcol. Sci.*, 15, 775–780.

Da-Cheng H. (2019). Genomics and Evolution of Medicinal Plants. In: *Ranunculales Medicinal Plants*; *ScienceDirect*; p. 1–14. Available from: https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/medicinal-plants/pdf

David Tin Win (2005). Oleic Acid – The Anti-Breast Cancer Component in Olive Oil. *Assumption University Journal Thailand* 9(2): 75-78

Duke J. A. (1992). *Handbook of Biologically Active Phytochemicals and their Activities*, CRC Press,.

Edward, D. S., & Vaitheeswaran, M. (2014). Ft-Ir and Gc-Ms Determination of Bioactive Constituents of Aloe Barbadensis Miller (Liliacae). *World Journal of Pharmacy and Pharmaceutical Sciences*, *3*(5), 749–758.

EFSA. (2004). Commission Regulation (EC) No 1565/2000 of 18 July 2000) Adopted on 9 December 2004. *The EFSA Journal* (2004) 164, 1-63, Available from: https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2005.164

EU Food Improvement Agents. (2012). Record Name: 2-Methylheptan-3-one. Available from: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32012R0872

European Chemicals Agency (ECHA). (2017). Record Name: 3-Hexanone, 2,2-dimethyl-. Available from:https://echa.europa.eu/information-on-chemicals/cl-inventory-database/-/discli/details/3310

Hossain MA, ALsabari KM, Weli AM, Al-Riyami Q. (2013) Gas chromatography–mass spectrometry analysis and total phenolic contents of various crude extracts from the fruits of Datura metel L. *J Taibah Univ Sci* 7:209–215CrossRefGoogle Scholar

Igwe O, E Okeke I. (2017). Leaf and Flower Extracts of Piliostigma malabaricum: Phytochemistry and Antibacterial Application. *Journal of Applied Chemical Science International*. Vol., 8.

Inoue M, Hayashi S, E. Craker L. (2019). Role of Medicinal and Aromatic Plants: Past, Present, and Future. In: *Pharmacognosy - Medicinal Plants*. IntechOpen;. Available from: https://www.intechopen.com/books/pharmacognosy-medicinal-plants/role-of-medicinal-and-aromatic-plants-past-present-and-future

Jain, S., Rathod, N., Nagi, R., Sur, J., Laheji, A., Gupta, N., … Prasad, S. (2016). Antibacterial Effect of *Aloe vera* Gel against Oral Pathogens: An In-vitro Study. *Journal of clinical and diagnostic research : JCDR*, *10*(11), ZC41–ZC44. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5198455/

Jamshidi-Kia F, Lorigooini Z, Amini-Khoei H. (2018). Medicinal plants: Past history and future perspective. *J Herbmed Pharmacol*. 7(1):1-7. doi: 10.15171/jhp.2018.01

Joondan N, Jhaumeer-Laulloo S, Caumul P. (2014). A study of the antibacterial activity of l-Phenylalanine and l-Tyrosine esters in relation to their CMCs and their interactions with 1,2-dipalmitoyl-sn-glycero-3-

Paul Njenga Waithaka, Eliud Mugu Gathuru, Benson Muriuki Githaiga and Rachael phosphocholine, DPPC as model membrane. *Microbiol Res*;169(9–10):675–85. Available from: https://www.sciencedirect.com/science/article/pii/S0944501314000263

Jothi Karumari R, Vijayalakshmi K, Tamilarasi L and Ezhilarasi Balasubramanian. (2014). Antibacterial Activity of Leaf Extracts of *Aloe vera*, Ocimum Sanctum and Sesbania Grandiflora against the Gram Positive Bacteria. Asian Journal of Biomedical and Pharmaceutical Sciences; 4(35), 60-63. Available from: https://www.alliedacademies.org/articles/antibacterial-activity-of-leaf-extracts-of-aloe-vera-ocimum-sanctum-and-sesbania-grandiflora-against-the-gram-positive-bacteria.pd.pdf

Kale O, TF O, Uyaiabasi N, Oyekale K, Walker O, Aderounmu A. Privet Leafhopper, Fieberiella florii Aqueous Extract Demonstrates In Vitro and In Vivo Neuromuscular Potentials in Mice and Chickens. J Pharmacol Pharm Res [Internet]. 2018 [cited 2019 Jul 19];1(1):1–9. Available from: www.thebiomedica.org

Kedarnath, Kamble Kaveri M, Vishwanath B Chimkod and C S Patil. (2013). Antimicrobial Activity of *Aloe vera* Leaf Extract. *International Journal of Applied Biology and Pharmaceutical Technology*, 4(4), 286-290. https://www.researchgate.net/publication/257948745_ANTIMICROBIAL_ACTIVITY_OF_ALOE_VERA_LEAF_EXTRACT

Kordali S., Cakir A. and Drum M. (2003). "Antifungal activity of the leave of three Pistacies from Turkey" *Fitoterapia* 74: 164-167

Lakshmi, P. T. V, & Rajalakshmi, P. (2011). Identification of Phyto-Components and Biological Activities of *Aloe vera* through the Gas Chromatography-Mass Spectrometry. *IRJP* (Vol. 2). Retrieved from http://www.irjponline.com

National Center for Biotechnology Information. PubChem Database. CID=545218, https://pubchem.ncbi.nlm.nih.gov/compound/3_3-Bis-tert-butylsulfanyl-2-fluoro-acrylonitrile (accessed on July 17, 2019)

Nejatzadeh-Barandozi F. (2013). Antibacterial activities and antioxidant capacity of *Aloe vera*. *Organic and medicinal chemistry letters*, *3*(1), 5. doi:10.1186/2191-2858-3-5

Zawadi Kazungu. (2018). Antimicrobial Properties of *Aloe vera*, Aloe volkensii and Aloe secundiflora from Egerton University. *Acta Scientific Microbiology* 1(5): 06-10

Petrovska BB. (2012). Historical review of medicinal plants' usage. *Pharmacogn Rev*;6(11):1–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22654398

Ram Sewak Kachhi, Vikas Kumar Saket, Poonam Sharma and Pranveer Singh. (2018). Treatment of Tuberculosis using Ethno-medicinal Plants of Amarkantak Region. *Asian Journal of Animal and Veterinary Advances, 13: 52-6*. Available from: https://scialert.net/abstract/?doi=ajava.2018.52.60

Saket K, Dan S J, Rajesh S. (2017). Evaluating Antimicrobial activity of *Aloe vera* Plant Extract in Human Life. *Biomed J Sci & Tech Res* 1(7). BJSTR. MS.ID.000565. DOI : 10.26717/BJSTR.2017.01.000565, https://biomedres.us/pdfs/BJSTR.MS.ID.000565.pdf

Saljooghianpour, M., & Javaran, T. A. (2013). Identification of phytochemical components of aloe plantlets by gas chromatography-mass spectrometry. *African Journal of Biotechnology*, *12*(49), 6876-6880–6880. https://doi.org/10.5897/AJB11.4307

Salisu, B. D., Magaji, A. M., & Abdulkadir, B. (2017). Phytochemical Determination and In Vitro Antimicrobial Activity of Crude Ethanolic Extract of Stem Bark of Boswellia dalzielii. *International Journal of Science and Research (IJSR)*, *6*(12), 1484–1492. https://doi.org/10.21275/ART20178999

Šantić Ž, Pravdić N, Bevanda M, Galić K. (2017). The historical use of medicinal plants in traditional and scientific medicine. *Psychiatr Danub.*;29 Suppl 4 (Suppl 4):787–92. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29278625

Sathyaprabha, G., Kumaravel, S., Ruffina, D., & PraveenkumarP. (2016). A Comparative study on Antioxidant, Proximate analysis, Antimicrobial activity and phytochemical analysis of *Aloe vera* and Cissus quadrangularis by GC-MS. *Journal of Pharmacy Research*, *3*(12), 2970–2973.

Sermakkani M & Thangapandian V. (2012). GCMS analysis of Cassia italic leaf methanol extract. Asian journal of pharmaceutical and clinical research .vol 5.

Sewak Kach R, Kumar Sake V, Sharma P, Singh P. (2017). Treatment of Tuberculosis using Ethno-medicinal Plants of Amarkantak Region. *Asian J Anim Vet Adv.*;13(1):52–60. Available from: http://www.scialert.net/abstract/?doi=ajava.2018.52.60

Sree JS, Gomathi SK, Helen A MP. (2015). Study on Antimicrobial Activity and Chemical Profiling of Pamburus missionis (Wight) Swingle. *International J Pharm Pharm Res* ;2(2):118–29. Available from: www.ijppr.humanjournals.com

Subramanian S., D. Sathish Kumar , P. Arulselvan and G.P. Senthilkumar , (2006). *In vitro* Antibacterial and Antifungal Activities of Ethanolic Extract of *Aloe vera* Leaf Gel. *Journal of Plant Sciences, 1: 348-355*. Available from: https://scialert.net/abstract/?doi=jps.2006.348.355

Thiruppathi, J. S. Ramasubramanian , V, Sivakumar, T . And Thirumalai Arasu, V. (2010). Antimicrobial activity of *Aloe vera* (L.) Burm. F. against Pathogenic Microorganisms, . *Bio s c i. Re s .*, Vol. 1(4):251-258. https://pdfs.semanticscholar.org/da0b/700213ca0a64d6f569ed8c871858d332a71f.pdf

Zhang H-J, Rumschlag-Booms E, Guan Y-F, Wang D-Y, Liu K-L, Li W-F, et al. (2017) Potent Inhibitor of Drug-Resistant HIV-1 Strains Identified from the Medicinal Plant, Justicia gendarussa. *J Nat Prod* ;80(6):1798–807. Available from: http://pubs.acs.org/doi/10.1021/acs.jnatprod.7b00004