ANTIMICROBIAL ACTIVITYAND PHYTOCHEMICAL ANALYSIS OF ALOE VERA

A RESEARCH PROJECT

Submitted by

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UNDER THE GUIDANCE OF

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DEPARTMENT OF MICROBIOLOGY

VIVEKANAND COLLEGE, KOLHAPUR

(AN EMPOWERED AUTONOMOUS INSTITUTE)

YEAR 2024-2025

"Dissemination of education for Knowledge, Science and culture"
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OF

RESEARCH PROJECT COMPLETION

This is to certify that MR. NIRANJAN. K. PATIL studying in M. Sc. part II Microbiology at Vivekanand College, Kolhapur (Empowered Autonomous) has sincerely completed research project work entitled "Antimicrobial Activity and Phytochemical Analysis of Aloe Vera" during academic year 2024- 2025

Ms. Mitali M. Nadkarni

Project Guide

Examiner

Dr. T. C. Gaupale

Head of the Department

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Place: Kolhapur

Date:

Mr. NIRANJAN PATIL Mr. OMKAR MADANE

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Ms. ANKITA KESARE.

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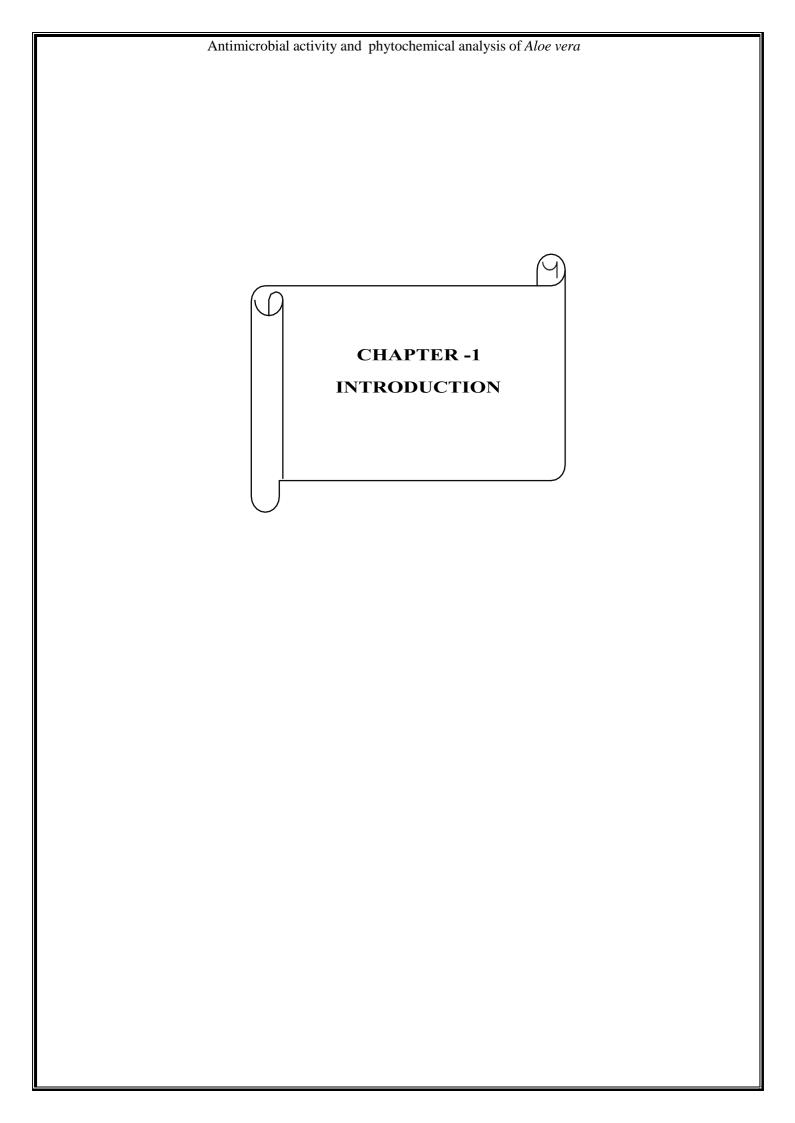
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Antimicrobial activity and	phytochemical	analysis of <i>Aloe vera</i>
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1. Introduction

Plants and herbs have assumed major role in the treatment of numerous disorders caused by pathogens and non-pathogens. Infections brought on by harmful microbes have a high death rate in under developed nations. The utilisation of medicinal plants and herbs for the treatment of pathogenic and non-pathogenic disorders has also been promoted by World Health Organisation (WHO, 2015). According to Santos et al. (2009), these infections are becoming more common in hospitals and have the potential to be invasive. Over 80% of the world's population, particularly in developing nations, still receives medical care through traditional medicine that uses plant extracts, according to Igbinosa et al. (2009). The resistance of pathogenic microorganisms to treatments is a serious concern in recent times, which is developing day by day (Cohen, 2000; Kumar et al., 2013). It is imperative to look for medications that are effective against a variety of pathogens. In order to address this issue, ethnobotanically significant medicinal plants may serve as a source for the identification of novel pharmaceuticals.

Aloe vera has been used as a common folk remedy for as long as civilization has existed. Existing in various parts of Nigeria, Aloe vera is more abundant in the southern than northern part of the country. The plant is thought to have positive effects on the healing of wounds and ulcers, diabetes, constipation due to radiation injury, gastrointestinal issues, skin conditions, and stomach disorders (Bamigboye, 2022). Globally, more than 550 different species of aloe are cultivated. Currently, only two species are cultivated commercially: the most common ones are Aloe barbadensis Miller and Aloe aborescens Miller. One kind of aloe that is especially well-known for its therapeutic qualities is Aloe vera. The Arabic term Alloeh, which means sparkling bitter substance, is the source of the name Aloe vera. Vera, on the other hand, signifies true in Latin. Greek scientists believed Aloe vera to be a universal cure 2,000 years ago. The Egyptians considered Aloe the plant of immortality. The Aloe vera plant is used in dermatology for a number of purposes nowadays (Oxygenetix, 2023).



1.1 History

Aloe vera has been used for medicinal purposes in several cultures for millennia: Greece, Egypt, India, Mexico, Japan and China. Egyptian queens Nefertiti and Cleopatra used it as part of their regular beauty regimes. Alexander the Great, and Christopher Columbus used it to treat soldiers' wounds. The first reference to Aloe vera in English was a translation by John Good yew in A.D. 1655 of Dioscorides' Medical treatise De Materia Medica. By the early 1800s, Aloe vera was in use as a laxative in the United States, but in the mid-1930s, a turning point occurred when it was successfully used to treat chronic and severe radiation dermatitis.

1.2 Taxonomy

Kingdom- Plantae

Division- Spermatophyte

Subdivision- Angiospermae

Class- Monocotyledoneae

Order- Asparagales

Family- Asphodelaceae (Liliaceae)

Genus- Aloe

Species- Barbadensis Mill [24]

The botanical name of *Aloe vera* is *Aloe barbadensis miller*. It is a shrubby or arborescent, perennial, xerophytic, succulent, pea- green colour plant. It grows mainly in the dry regions of Africa, Asia, Europe and America. In India, it is found in Rajasthan, Andhra Pradesh, Gujarat, Maharashtra and Tamil Nadu.

1.3 Anatomy

The plant has triangular, fleshy leaves with serrated edges, yellow tubular flowers and fruits that contain numerous seeds. Each leaf is composed of three layers: An inner clear gel that contains 99% water and rest is made of glucomannans, amino acids, lipids, sterols and vitamins. The middle layer of latex which is the bitter yellow sap and contains anthraquinones and glycosides. The outer thick layer of 15–20 cells called as rind which has protective function and synthesizes carbohydrates and proteins. Inside the rind are vascular bundles responsible for transportation of substances such as water (xylem) and starch (phloem).

1.4 Active components with its properties:

Aloe vera contains 75 potentially active constituents: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids.

1.4.1 Vitamins:

It contains vitamins A (beta-carotene), C and E, which are antioxidants. It also contains vitamin B12, folic acid, and choline. Antioxidant neutralizes free radicals.

1.4.2. Enzymes:

It contains 8 enzymes: aliases, alkaline phosphatase, amylase, Brady kinase, carboxy-peptidase, catalase, cellulase, lipase, and peroxidase. Brady kinase helps to reduce excessive inflammation when applied to the skin topically, while others help in the breakdown of sugars and fats.

1.4.3 Minerals:

It provides calcium, chromium, copper, selenium, magnesium, manganese, potassium, sodium and zinc. They are essential for the proper functioning of various enzyme systems in different metabolic pathways and few are antioxidants.

1.4.4. Sugars:

It provides monosaccharides (glucose and fructose) and polysaccharides: (glucomannans/polymannose). These are derived from the mucilage layer of the plant and are known as mucopolysaccharides. The most prominent monosaccharide is mannose phosphate, and the most common polysaccharides are called glucomannans. Acemannan, a prominent glucomannan has also been found. Recently, a glycoprotein with anti-allergic properties,

called Alprogen and novel anti-inflammatory compound, C-glucosyl chromone, has been isolated from Aloe vera gel.

1.4.5. Anthraquinones:

It provides 12 anthraquinones, which are phenolic compounds traditionally known as laxatives. Aloin and emodin act as analgesics, antibacterials and antivirals.

1.4.6. Fatty acids:

It provides 4 plant steroids; cholesterol, campesterol, β -sisosterol and lupeol. All these have anti-inflammatory action and lupeol also possesses antiseptic and analgesic properties.

1.4.7. Hormones:

Auxins and gibberellins that help in wound healing and have anti-inflammatory action.

1.4.8. Others:

It provides 20 of the 22 human required amino acids and 7 of the 8 essential amino acids. It also contains salicylic acid that possesses anti-inflammatory and antibacterial properties. Lignin, an inert substance, when included in topical preparations, enhances penetrative effect of the other ingredients into the skin. Saponins that are the soapy substances form about 3% of the gel and have cleansing and antiseptic properties.

1.5 Mechanism of actions

1.5.1 Healing properties:

Glucomannan, a mannose-rich polysaccharide, and gibberellin, a growth hormone, interacts with growth factor receptors on the fibroblast, thereby stimulating its activity and proliferation, which in turn significantly increases collagen synthesis after topical and oral Aloe vera. Aloe gel not only increased collagen content of the wound but also changed collagen composition (more type III) and increased the degree of collagen cross linking. Due to this, it accelerated wound contraction and increased the breaking strength of resulting scar tissue. An increased synthesis of hyaluronic acid and dermatan sulphate in the granulation tissue of a healing wound following oral or topical treatment has been reported.

1.5.2. Effects on skin exposure to UV and gamma radiation:

Aloe vera gel has been reported to have a protective effect against radiation damage to the skin. Exact role is not known, but following the administration of aloe vera gel, an antioxidant protein, metallothionein, is generated in the skin, which scavenges hydroxyl radicals and prevents suppression of superoxide dismutase and glutathione peroxidase in the skin. It reduces the production and release of skin keratinocyte-derived immunosuppressive

cytokines such as interleukin-10 (IL-10) and hence prevents UV-induced suppression of delayed type hypersensitivity.

1.5.3. Anti-inflammatory action:

Aloe vera inhibits the cyclooxygenase pathway and reduces prostaglandin E2 production from arachidonic acid. Recently, the novel anti-inflammatory compound called C-glucosyl chromone was isolated from gel extracts.

1.5.4. Effects on the immune system:

Alprogen inhibit calcium influx into mast cells, thereby inhibiting the antigenantibody-mediated release of histamine and leukotriene from mast cells. In a study on mice that had previously been implanted with murine sarcoma cells, acemannan stimulates the synthesis and release of interleukin-1 (IL-1) and tumour necrosis factor from macrophages in mice, which in turn initiated an immune attack that resulted in necrosis and regression of the cancerous cells. Several low-molecular-weight compounds are also capable of inhibiting the release of reactive oxygen free radicals from activated human neutrophils.

1.5.5. Laxative effects:

Anthraquinones present in latex are a potent laxative. It increases intestinal water content, stimulates mucus secretion and increases intestinal peristalsis.

1.5.6. Antiviral and antitumor activity:

These actions may be due to indirect or direct effects. Indirect effect is due to stimulation of the immune system and direct effect is due to anthraquinones. The anthraquinone aloin inactivates various enveloped viruses such as herpes simplex, varicella zoster and influenza. In recent studies, a polysaccharide fraction has shown to inhibit the binding of benzopyrene to primary rat hepatocytes, thereby preventing the formation of potentially cancer-initiating benzopyrene-DNA adducts. An induction of glutathione Stransferase and an inhibition of the tumour-promoting effects of phorbol myristic acetate has also been reported which suggest a possible benefit of using aloe gel in cancer chemoprevention.

1.5.7. Moisturizing and anti-aging effect:

Mucopolysaccharides help in binding moisture into the skin. Aloe stimulates fibroblast which produces the collagen and elastin fibres making the skin more elastic and less wrinkled. It also has cohesive effects on the superficial flaking epidermal cells by sticking them together, which softens the skin. The amino acids also soften hardened skin cells and zinc acts as an astringent to tighten pores. Its moisturizing effects has also been studied in treatment of dry skin associated with occupational exposure where aloe vera gel

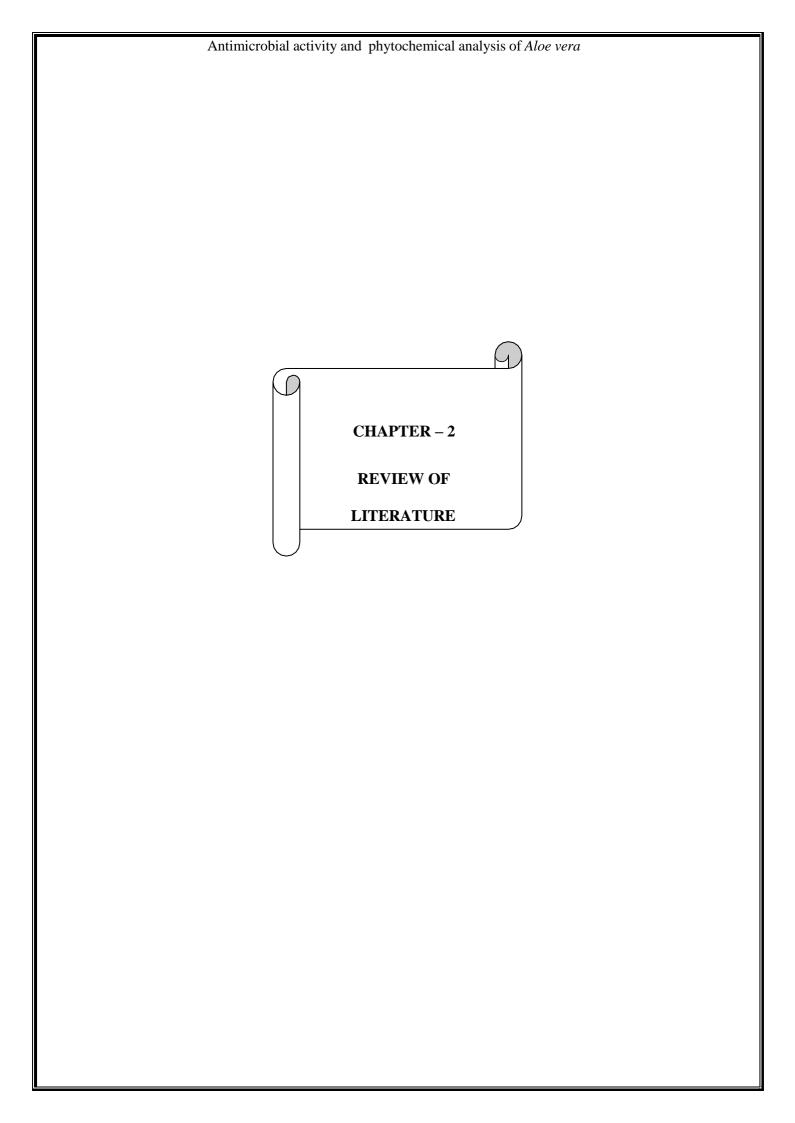
gloves improved the skin integrity, decreases appearance of fine wrinkle and decreases erythema. It also has anti-acne effect.

1.5.8. Antiseptic effect:

Aloe vera contains 6 antiseptic agents: Lupeol, salicylic acid, urea nitrogen, cinnamomic acid, phenols and sulfur. They all have inhibitory action on fungi, bacteria and viruses.



The main limitation of the current clinical knowledge about *Aloe vera* is small clinical studies that often lack rigorous methodology. Several clinical trials are being conducted to further evaluate the use of aloe vera for a variety of disorders, as well as to further confirm traditional uses of the plant extract.



2. Review of literature.

Aloe vera has long been recognized for its medicinal properties, particularly its antimicrobial activity and rich phytochemical composition. Aloe vera acts as an antimicrobial agent that removes or inhibits the growth and development of microorganism such as microorganism (bacteria), fungi, protozoan etc.[12]. The Antimicrobial drugs removes and suppressed the microbes or protect the growth and development of bacteria (micro-biostatic). Various parts of this plant were useful in curing a wide range of health related issue. This plant synthesizes a vast array of secondary metabolites that are important for medicines. Clinical efficacy of many synthetic antibiotics is questioned now days with the emergence of multidrug resistance pathogens.[11] The increasing failures of chemotherapeutics and antibiotics exhibited by pathogenic microbial infection have led to the screening of several medicinal plants for potent microbial activity.

The main limitation of the current clinical knowledge about *Aloe vera* is small clinical studies that often lack rigorous methodology. Several clinical trials are being conducted to further evaluate the use of aloe vera for a variety of disorders, as well as to further confirm traditional uses of the plant extract.[12]

A review of the literature on this topic provides insights into its potential applications in medicine and biotechnology.

2.1 Antimicrobial Activity of Aloe Vera

Aloe vera contains bioactive compounds, such as anthraquinones, flavonoids, alkaloids, and tannins, which contribute to its antimicrobial properties. Several studies have examined the effectiveness of Aloe vera extracts against various bacterial and fungal strains. Research suggests that Aloe vera exhibits significant inhibitory effects on Gram-positive bacteria like *Staphylococcus aureus* and *Bacillus subtilis* as well as Gram-negative bacteria like *Escherichia coli*, *Proteus spp. Salmonella typhi* and *Pseudomonas aeruginosa*. The gel and leaf extracts have also demonstrated antifungal activity against species like *Candida albicans* and *Aspergillus niger*.

The ethanol, methanol and distilled water extracts of Aloe vera leaf were studied for their antimicrobial activity against Gram positive and Gram negative bacteria using paper disc method. (Aida et al., 2001). The extracts showed varied levels of antimicrobial activity against the tested pathogens. The ethanol and methanol extracts showed higher activity while distilled water extract, showed least or no activity against most of the tested pathogens Moreover, Aloe vera has been investigated as a potential alternative to synthetic antimicrobial

agents due to concerns about antibiotic resistance. Studies have explored its mechanism of action, which includes disrupting microbial cell membranes and interfering with metabolic pathways.

2.2 Phytochemical Composition of Aloe Vera

Aloe vera is rich in phytochemicals, which account for its therapeutic effects. The plant contains polysaccharides, flavonoids, saponins, sterols, anthraquinones, and vitamins. The presence of acemannan, a polysaccharide, contributes to its immunomodulatory and wound-healing properties.

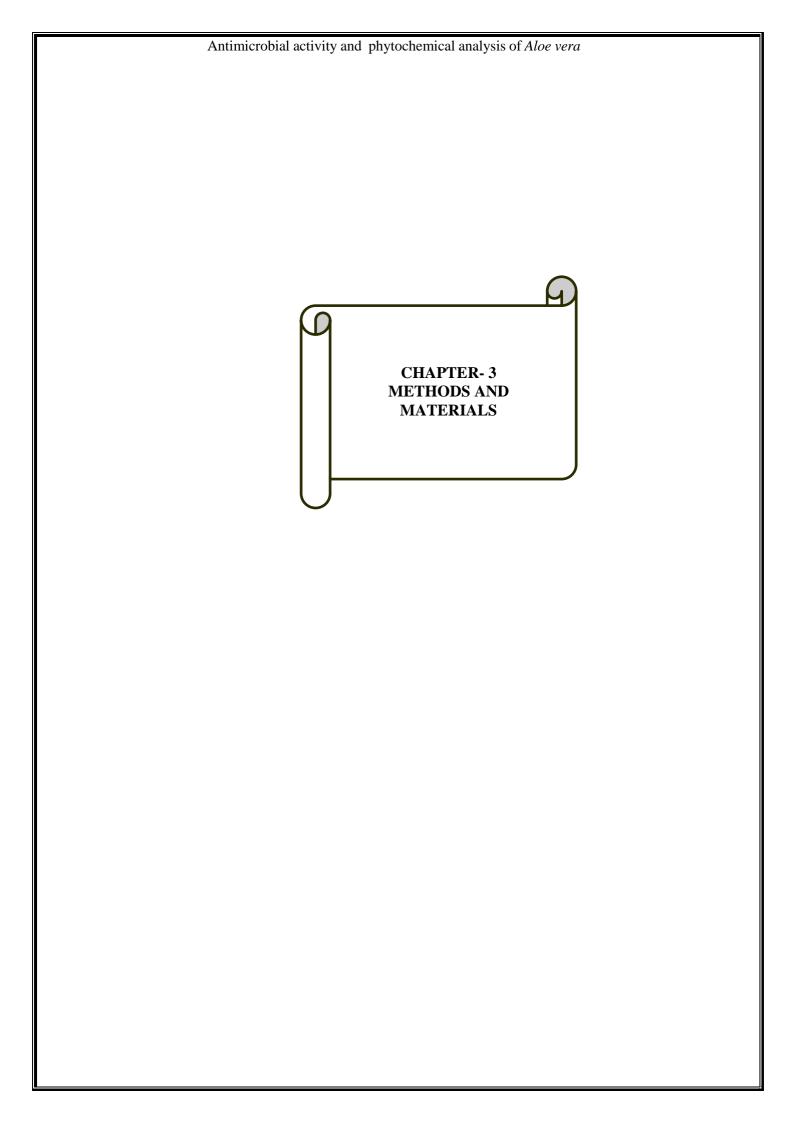
Flavonoids and phenolic compounds in Aloe vera exhibit antioxidant properties, which play a role in preventing oxidative stress-related damage. Additionally, anthraquinones such as aloin and emodin are known for their laxative and antimicrobial effects. The phytochemical diversity of Aloe vera supports its use in skincare, wound healing, and pharmaceutical formulations.

AIM AND OBJECTIVES

❖ AIM: To study the antimicrobial activity and phytochemical analysis of Aloe vera

***** OBJECTIVES:

- **I.** To evaluate the antimicrobial activity of Aloe Vera against various microorganism.
- II. To identify the phytochemical compound present in Aloe Vera.



3. Methods and materials:

3.1 Collection of sample

Aloe Vera – (Aloe barbadensis miller)

The different variety of aloe vera is available. For study, normal aloe vera plant is selected from locality near college.

3.2. Materials Needed

- Microorganisms
- Agar plates
- Nutrient Broth media
- Paper disc
- Soxhlete apparatus

3.2.1. Bacterial cultures

Following bacterial cultures available in our laboratory where used in the study

Gram positive organisms

> Staphylococcus aureus

Gram negative organisms

- > Escherichia coli
- > Salmonella typhi
- > Klebsiella pneumonia
- > Proteus vulgaris

Fungi

> Candida albicans

The microorganisms were reconstituted by sub culturing on to freshly prepared nutrient agar slants. They were incubated at 37° Celsius for 24 hours.

3.3.2. Culture media

Nutrient agar medium was used for cultivation and checking the antibacterial activity of Aloe Vera.

Peptone:	0.5 gm
Meat extract / yeast extract:	0.3gm
Sodium chloride:	0.5gm
Agar:	2gm
Distilled water:	100 ml
pH:	7 to7.2
Distilled water:	100ml

3.3.3. Preparation of the Extracts

Extraction of phytochemical and screening

For the preparation of ethanol and methanol extracts, the areal parts of the Aloe vera plant were used, leaves were cleaned with water and after that dried in shade. An electric blender was used to grind the material into powder form. 0.3 grams of this powder was soaked in 20ml. of each of the solvents namely ethanol and methanol for 24 h. The contents were then filtered through Whattman filter paper no. 1 and the filtrate was evaporated to dryness using rotary evaporator (less than 45°C of temperature). Sterile bottles were used for storage of crude extract of plants under refrigerator for further use of antimicrobial tests [9].



Table -1
Extraction of Aloe Vera.

Sr.No.	Aloe vera extract	Amount (ml)	Aloe vera powder (gm)
1	Methanol	20	0.3
2	Ethanol	20	0.3
3	Distilled water	20	0.3

Defatting of plant material:

Before applying extraction, the powdered plant materials were defatted by soaking it in petroleum ether at room temperature for 24 hours to remove any fatty, oily or lipid content from them. After defatting of plant material the petroleum ether was remove by filtration and the crud drug is again dried that is to be extracted with distilled water and ethanol

Soxhlet extraction:

Like the aqueous extraction about 10-20 grams of defatted dried powder was subjected to Soxhlet extraction with 200 ml of 95% ethanol as extraction solvent till the complete exhaustion of sample material at 65°C. The defatted fine powders of Aloe vera leaves were subjected to Soxhlet extraction with 95% ethanol till its complete exhaustion. The extract so obtained after the process of Soxhlation was subjected to evaporation of solvent to get the extract in form of crystals, slurry or paste. This is done by taking the extracted drug containing solvents in a glass beaker and placing them in a boiling water bath. The contents were kept in boiling water bath till the solvent of extract is evaporated completely. The phyto extracts so obtained after this are now could be used to assess the yield of phytochemical extraction, evaluation of organoleptic properties, phytochemical analysis. After concentration of phytochemical extracts both aqueous and ethanolic the organoleptic properties of extracted drug were evaluated. The extracted drugs were evaluated for colour, texture, smell and yield of extraction.



3.3.4 Staphylococcus aureus:

To study antibacterial activity of aloe vera against Staphylococcus aureus

1. Preparation of suspension of Staphylococcus aureus

A loop full of freshly grown *Staphylococcus aureus* was added in to sterile saline to prepare thick suspension of organism.

2. Preparation of nutrient broth medium

Composition:

Composition	Peptone	Sodium chloride	Agar	Distilled water	Meat/yeast extract	РН
Nutrient broth	0.5	0.5	2gm	100ml	0.3gm	7 to 7.2

3. Preparation of spread plate technique:

A sterilized 20 ml nutrient agar medium was prepared in flasks and poured in sterile petri plate. Plates were allowed to solidify.

4. Inoculation of suspension:

Fresh 0.1 ml *Staphylococcus aureus* suspension was spread on sterile solidified nutrient agar using sterile glass spreader under aseptic condition.

5. Preparation of paper disc.

The paper discs (6mm diameter) are prepared for each aloe vera extract.

6. Addition of paper disc.

Paper disc is dipped into each aloe vera extract under aseptic condition. Then the discs are placed into the plates. In each extraction of aloe vera the paper disc was dipped and added in well-labelled petri plates, and proper labeling was done.

- 7. All plates were kept in refrigerator at 4° c for 15 mints
- 8. After that plates were incubated at 37°c for 24 hrs.
- 9. After incubation plates were examined for inhibition zone around each well.

Similar procedure was followed for all other pathogen

- > Escherichia coli
- Salmonella typhi

- > Candida albicans
- ➤ Klebsiella spp
- > Proteus vulgaris

3.4. Phytochemical analysis:

Chemicals:

1. Mayer's reagent:

Mercuric chloride (1.36 gm)

Potassium Iodide = 5 gm

Distilled water (P.W) = 100 ml

2. Dragendorff's reagent:

Bismuth sub nitrate (1.7 g)

Potassium Iodide = (40 gm)

Citric acid = 20 ml

Distilled water (P.W) = 100 ml

3. Wagner's reagent:

Iodine = 1.27 gm

Potassium Iodide = 2 gm

Distilled water (P.W) = 100 ml

3.4.1. Analysis of Flavonoids compound: (Aloe Vera filtrates in Ethanol)

1. Extract + NaOH (in test tube)

Positive results: Yellow colour turns colourless when addition of acid.

3.4.2. Analysis of Shinods compound: (Aloe Vera filtrates in Ethanol)

1. Extract + 0.5 ml HCL + Mg metal

Positive results: Reddish coloration.

3.4.3. Analysis of saponin compound: (Aloe Vera filtrates in Ethanol)

1. Foam test

0.5 mg (extract) - 20 ml D/W

Shaken 15 min – Foam formation

3.4.4. Analysis of Tannin Compound: (Aloe Vera filtrate in Ethanol)

1. Extract – 5ml D/W

Few drops of 5 % Fec13 – A dark green colour.

3.4.5. Analysis of Glycosides (Aloe Vera filtrate in Ethanol)

Extract + 2ml Glacial acetic acid + 1 drop FeCl3 + 1ml conc. H2SO4

Red violet colour

3.4.6. Analysis of Phenol compound: (Aloe Vera filtrate in Ethanol)

Lead acetate test

Extract + 3ml of 10% lead acetate

White precipitate

3.4.7. Analysis of Protein compound: (Aloe Vera filtrate in Ethanol)

Extract 2ml + metallic mercury in nitric acid

Red precipitate.

3.4.8. Analysis of Triterprnoids compound: (Aloe Vera filtrate in Ethanol)

1. Molish test

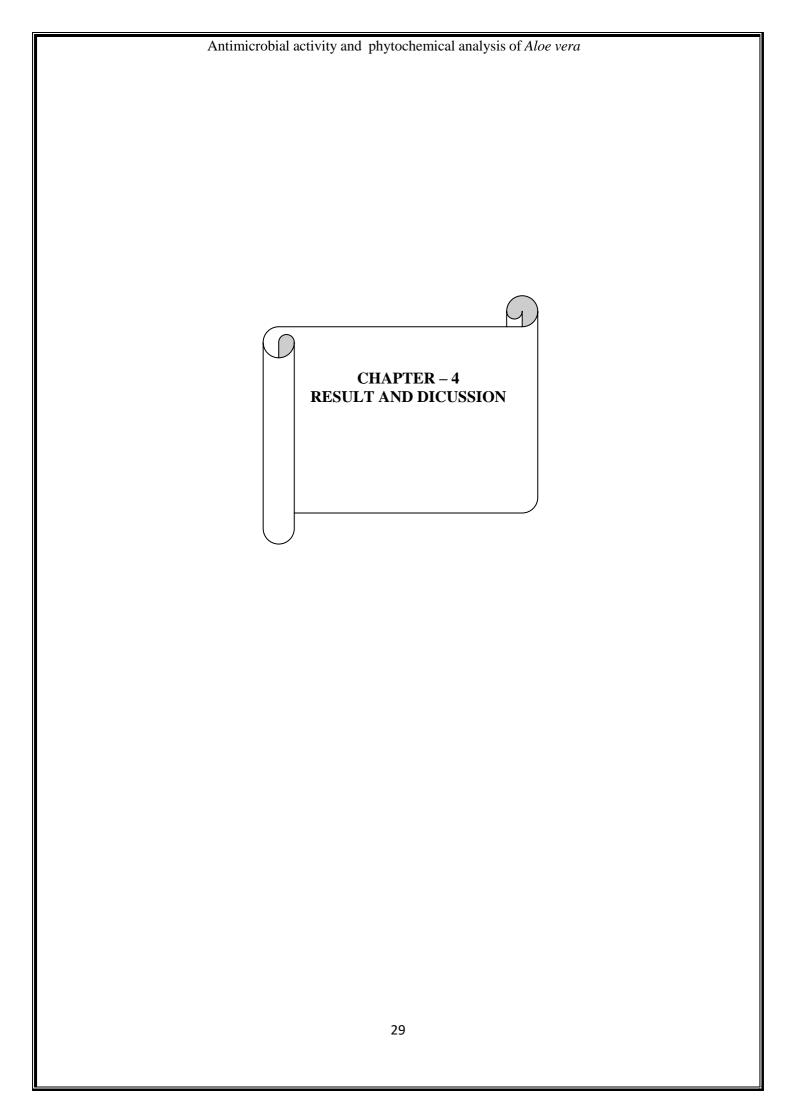
 $Extract\ 2ml + alpha\ naphthol + Shake + Conc.\ H2SO4\ few\ drops$

Violet colour at junction

3.4.9. Analysis of Fixed oil and fats compound: (Aloe Vera filtrate in Ethanol)

1. Spot test

Extract pressed between 2 filter paper Oil stain.



Antimicrobial activity

The antibacterial property of Aloe vera gel extracted using different solvents showed varying degree of response towards the selected pathogens. (Tables 1-4).Generally the extracts showed greater antibacterial activity against Gram positive as compared to Gram negative bacteria. With respect to individual pathogens, ethanol extract showed greater inhibition than methanol extract while, significantly lower inhibition was observed with D/W extract (Martinez et al.)

As stated by Cowan (4), nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds and are most often obtained through initial ethanol or methanol extraction. This explains higher antimicrobial activity of ethanol and methanol extracts observed in the study.

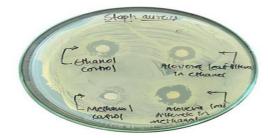
4.1.1 Staphylococcus aureus

Table 1. Effect of Aloe vera filtrate on Staphylococcus aureus.

Aloe Vera leaf filtrate	Methanol		Ethanol	
in	Control	Aloe vera leaf filtrate	Control	Aloe vera leaf filtrate
Zone of inhibition	0.8	1.5	1.3	1.6

From table 1 it is clear that leaf filtrate of Aloe Vera shows zone of inhibition. The higher zone diameter was observed in ethanol 1.6 and lower zone diameter was observed in methanol 1.5.





Antimicrobial activity of Aloe Vera leaf filtrate on Staphylococcus aureus

4.1.2. Escherichia coli

Table 2 Effect of Aloe vera filtrate on, Escherichia coli.

	Methanol		Ethanol	
Aloe Vera leaf filtrate in	Control	Aloe vera leaf filtrate	Control	Aloe vera leaf filtrate
Zone of inhibition (cm)	0.6	0.9	1.2	1.4

From table 2 it is clear that leaf filtrate of Aloe Vera shows a zone of inhibition. The higher zone diameter was observed in ethanol 1.4 and lower zone diameter was observed in methanol 0.9.





Antimicrobial activity of Aloe Vera leaf filtrate on Escherichia coli.

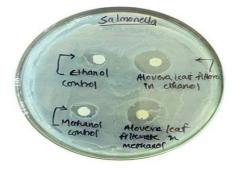
4.1.3. Salmonella spp

Table 3 Effect of Aloe Vera leaf filtrate on Salmonella spp

	Methanol		Ethanol	
Aloe Vera leaf filtrate in	Control	Aloe vera leaf filtrate	Control	Aloe vera leaf filtrate
Zone of inhibition(cm)	1	1.4	0.7	1.5

From table 3 it is clear that leaf filtrate of Aloe Vera shows zone of inhibition. The higher zone diameter was observed in ethanol 1.5 and lower zone diameter was observed in methanol 1.4.





Antimicrobial activity of Aloe Vera leaf filtrate on Salmonella spp.

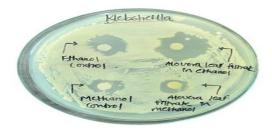
4.1.4. Klebshiella spp.

Table 4 Effect of Aloe Vera leaf filtrate on Klebshiella spp.

Aloe Vera leaf filtrate	Methanol		Ethanol	
in	Control	Aloe vera leaf filtrate	Control	Aloe vera leaf filtrate
Zone of inhibition(cm)	1.1	1.2	1.4	1.8

From table 4 it is clear that leaf filtrate of Aloe Vera shows zone of inhibition. The higher zone diameter was observed in ethanol 1.8 and lower zone diameter was observed in methanol 1.2.





Antimicrobial activity of Aloe Vera leaf filtrate on Klebshiella spp.

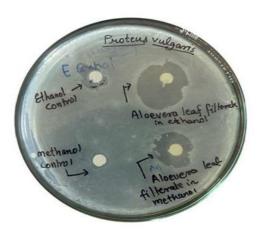
4.1.5. Proteus vulgaricus

Table 5 Effect of Aloe Vera leaf filtrate on Proteus vulgaris

Aloe Vera leaf filtrate	Methanol		Ethanol	
in	Control	Aloe Vera leaf filtrate	Control	Aloe Vera leaf filtrate
Zone of inhibition(cm)	0.8	1.4	1	2.1

From table 5 it is clear that leaf filtrate of Aloe Vera shows zone of inhibition. The higher zone diameter was observed in ethanol 2.1 and lower zone diameter was observed in methanol 1.4.





Antimicrobial activity of Aloe Vera leaf filtrate on *Proteus vulgaris*

4.1.5 Fungi – *Candida albicans*

Table 6 Effect of Aloe Vera leaf filtrate on Candida albicans

Aloe Vera leaf filtrate	Methanol		Ethanol	
in	Control	Aloe Vera leaf filtrate	Control	Aloe Vera leaf filtrate
Zone of inhibition	1	1.2	1.3	1.4

From table 6 it is clear that leaf filtrate of Aloe Vera shows zone of inhibition. The higher zone diameter was observed in ethanol 2.1 and lower zone diameter was observed in methanol 1.4.





Antimicrobial activity of Aloe Vera leaf filtrate on Candida albicans

NOTE:

Distilled water extract, showed least or no activity against the tested pathogens.

4.2 Phytochemical analysis of Aloe vera

The preliminary phytochemical analysis gives valuable information regarding the presence of important classes of phytochemicals present in the extracts. The outcomes of the qualitative phytochemical analysis of various extracts of Aloe vera leaves are given in tables. The result point out the presence of some phytochemical in ethanol as compared to chloroform and distilled water. This might be a reason behind no antimicrobial activity in distilled water against selected microorganism (Arunkumar and Muthuselvam ,2009; Raphael ,2012).

4.2.1. Phytochemical analysis of *Aloe vera* chloroform extract

Components + chloroform	Aloe vera extract	Conformation of the components
Tannin	p	
Glucoside	N	
Molish	N	
triterpenoids	p	

phenol	p	
Saponin	N	Common orientación de la composition del la composition del la composition de la composition de la composition de la composition del la composition de la composition del la composition del la composition della

4.2.2. Phytochemical analysis of $Aloe\ vera$ ethanol extract

Components + Ethanol	Aloe vera extract	Conformation of the components
Tannin	p	
Glucoside	N	
Molish	N	
triterpenoids	p	

phenol	N	
Saponin	N	

4.2.3 Phytochemical analysis of *Aloe vera* ethanol extract by using Soxhlet

Components + Ethanol	Aloe vera extract	Conformation of the components
Flavonoids	P	
Saponins	N	
Tannins	P	
Phenol test	P	

Glycoide	N	
Triterpenoids	P	

Standerd results for phytochemical analysis

Sr	Flavonoids compound	Choroform	Ethanol	Aloevera extract (soxhlate)	d/w
1.	1	-	-	+	_
	2	-	+	+	_
	3	-	_	+	+
2.	Saponins	-	-	-	_
3.	Tannins	+	+	+	+
4.	Phenol	+	+	+	+
5.	Glycoside	-	-	-	-

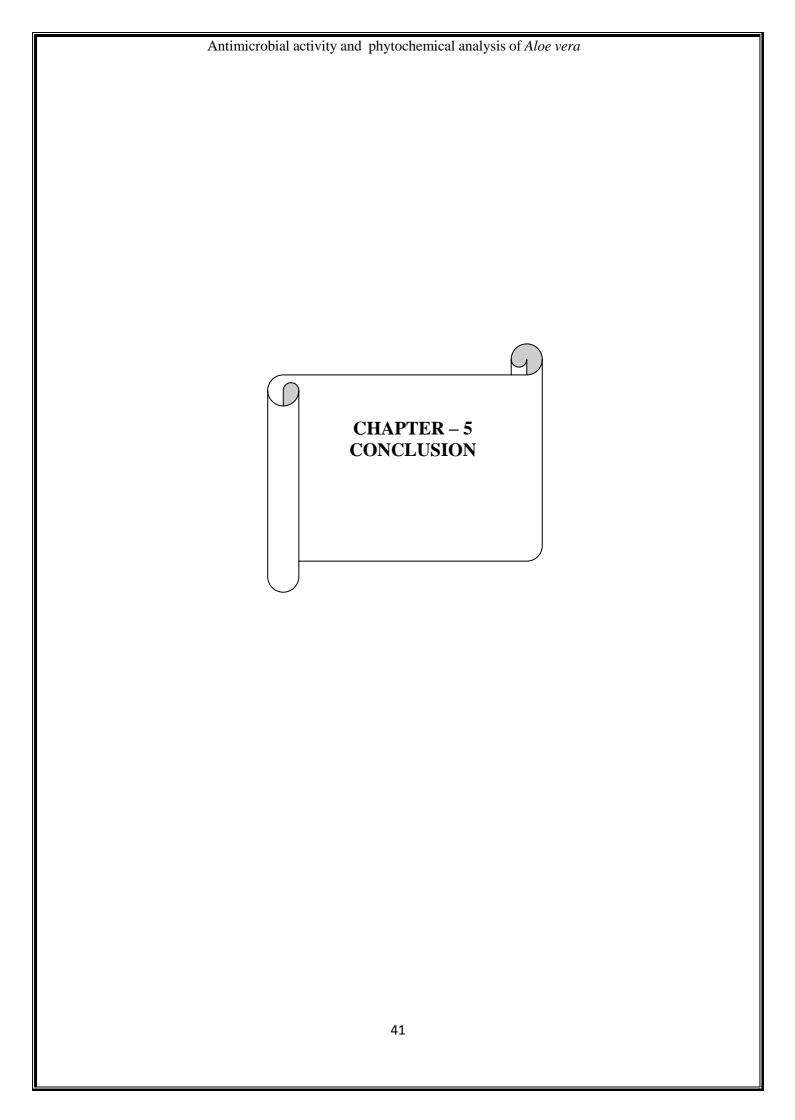
6.	Protein	_	_	_	_
7.	Triterpenoids	+	+	+	+
0					
8.	Carbohydrate	_	_	_	_
9.	Fixed oil and fats	_	_	_	_

Results:

Positive = +

Negative = -

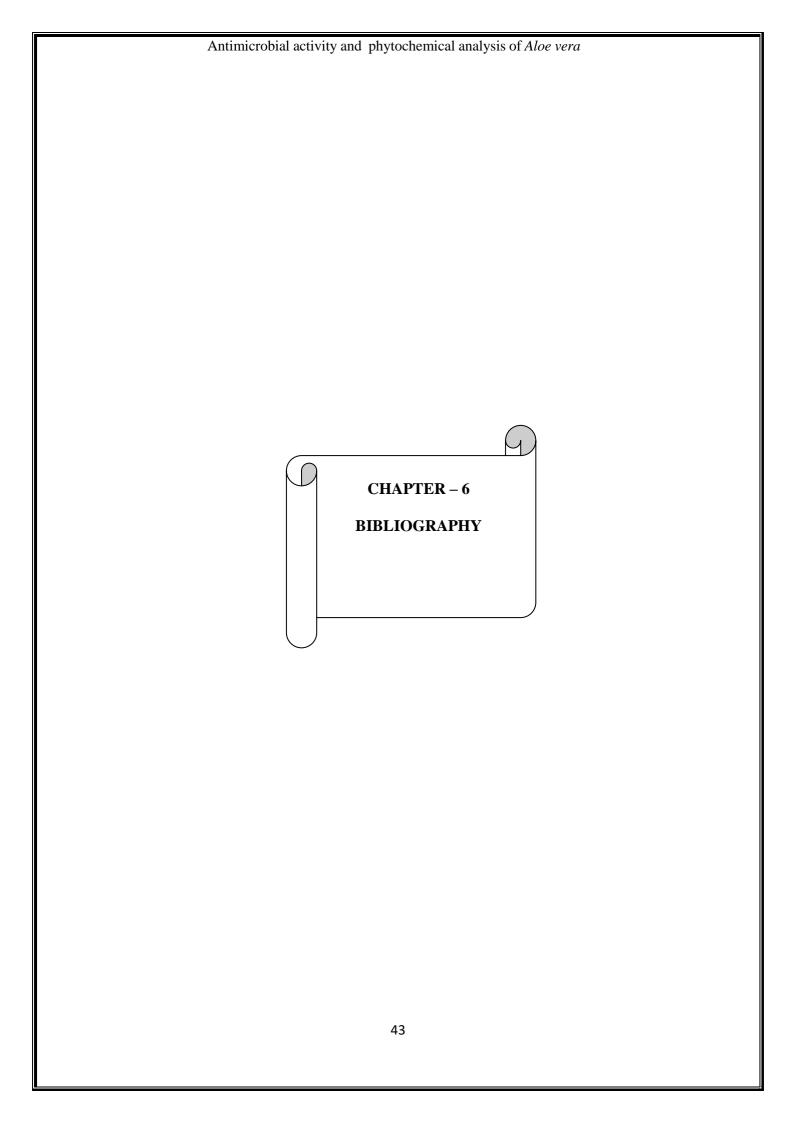
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Conclusion:

The current study revealed that the ethanol extract of Aloe vera leaves possessed more antimicrobial activity followed by methanol however aqueous extract showed no antimicrobial activity.

From the phytochemical analysis of aloe vera it is clear that aloe vera is rich in tannins, phenol, Triterpenoids, Flavonoids. Therefore we can use this Aloe vera as antimicrobial agent against various pathogenic organisms. The aloe vera is active against pathogenic organisms. It is effective in medicinal uses its commonly used to treat skin condition like sunburn, minor, burns, cuts and insect bites.



Bibliography:

- **1.** Eichhorn, B., & Wendel, F. (2009). Aloe vera in ancient Egypt and the Mediterranean: A review of the historical uses of Aloe vera. Journal of Apicultural Research, 48(3), 171-179.
- **2.** Molan, P. C. (1992). The antibacterial properties of Aloe vera. Bee World, 73(1), 5-28.
- **3.** Rasmussen, T. (2008). Hippocrates and the healing powers of honey. Journal of Ancient Medicine, 19(4), 211-218.
- **4.** Lambertsen, C. (2004). Aloe vera as an ancient commodity and trade resource. Historical Review of Economic Practices, 22(1), 45-52.
- **5.** Scully, M. (2016). Aloe vera in medieval Europe: A medical and economic history. Medieval Medicine Journal, 14(2), 88-104
- **6.** Krishnamurthy, K. (2012). Aloe vera in ancient Indian texts and tradition. Journal of Indian History, 34(2), 213-228.Sharma, P. (2007). Ayurvedic applications of Aloe vera: A historical perspectiv Indian Journal of Traditional Medicine, 5(3), 156-162.
- 7. Nadkarni, A. K. (2000). Indian Materia Medica. Popular Prakashan.
- **8.** Schimmel, A. (1975). Aloe vera and its significance in Mughal culture. Historical Studies in Indian Culture, 19(1), 82-95
- **9.** The antimicrobial activity of Aloe vera against common equine wound bacterial isolatesR Carnwath, EM Graham, K Reynolds, PJ Pollock The veterinary journal, 2014 Elsevier
- 10. Antimicrobial properties of honey ZH Israili American journal of therapeutics, 2014- journals.lww.com
- 11. Comparison of methods to determine antibacterial activity of Aloe vera against Staphylococcus aureus. Sandra M. Osés *, Ana Pascual-Maté, Daniel de la Fuente, Ana de Pablo, Miguel A. Fernández-Muino, ~ M Teresa Sancho*Department of Biotechnology and Food Science, University of Burgos, Plaza Misael-Ba~nuelos s/n, Burgos, 09001 Spain
- 12. Antimicrobial Activity of Aloe vera against Oral Microorganisms: Current Reality, Methodological Challenges and Solutions Diego Romário-Silva 1,2, Severino Matias Alencar 3, Bruno Bueno-Silva 4, Janaína de Cássia Orlandi Sardi 2,4, Marcelo Franchin 5,, Rafaela Durrer Parolina de Carvalho 1, Thayná Ellen de Sousa Alves

- Ferreira 1,2, Pedro Luiz Rosalen 1,6,
- 13. Antimicrobial activity of commercial organic Aloe vera against clinical isolates of human pathogenic bacteria Jackie K. Obey. Moses M. Ngeiywa Marjatta Lehesvaara Jussi KauhanenAtte von Wright Carina Tikkanen-Kaukanen
- **14.** Antimicrobial and Antioxidant Activity of Different Aloe vera Samples from Beekeepers and Commercial Producer by Miroslava Kačániová 1,2,*ORCID,Petra Borotová 3ORCID,Lucia Galovičová 1ORCID,Simona Kunová 4,Jana Štefániková 3ORCID,Przemysław Łukasz Kowalczewski 5ORCID andPeter Šedík 6ORCID
- 15. Antibacterial and antioxidant activity of different types of Aloe vera derived from Mount Olympus in Greece Dimitrios Stagos Nikolaos Soulitsiotis Christina Tsadila Stamatina Papaeconomou Charalampos Arvanitis Alexandros Ntontos Fani Karkanta Soultana Adamou-Androulaki Konstantinos Petrotos Demetrios A. Spandidos Demetrios Kouretas Dimitris Mossialos
- **16.** Natural bioactive compounds of Aloe vera and their antimicrobial activity Nesrine Feknous, Mahieddine Boumendjel Czech Journal of Food Sciences 40 (3), 163-178, 2022
- **17.** Investigation of in vitro antimicrobial activity of Aloe vera. Nur Ceyhan, AYSEL Ugur Rivista di biologia 94 (2), 363-371, 2001
- 18. Phytochemical identification of Aloe vera from several regions in Java and Sumbawa Adalina Yelin; Kuntadi Author & Article Information AIP Conf. Proc. 2120, 080024 (20