

## Formulation and Evaluation of Poly herbal Ointment of Aloe Vera, Azadirachta Indica, Lycopersicon Esculentum.

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### Abstract

Herbal extract play an important role in the design and development of the herbal ointment from the ancient era using the Mortar and pestle. In the research work we have to formulate the herbal ointment using the poly herb like aloevera, indica and lycopersicon. The plants under investigation have been found to be excellent facial creams with diverse applications. Water soluble ash, acid insoluble ash and total ash value of Aloe Vera was discovered to be 4.2 %, 0.89 % and 85.90 % respectively, whereas the water soluble ash, acid insoluble ash, and total ash value of *Azadirachta Indica* was discovered to be 11.9 %, 1.2 % and 11.2 % respectively. Extractive value (water and alcohol soluble) of Aloe Vera were discovered to be 5.20 % and 6.26 % respectively. The loss on drying of *Aloe Vera*, *Azadirachta Indica* and *Lycopersicon Esculentum* were discovered to be 4.54 % w/w, 15.5 % w/w and 14.5 % w/w correspondingly. The saponins and polyphenols derivatives prevail among the herbal formulations as compared to the chemical entities of the active pharmaceutical ingredients. The aqueous (water) and alcoholic (methanol) extract of Aloe Vera was discovered to be 9.12 % and 11.0 % respectively. The aqueous (water) and alcoholic (methanol) extract of *Azadirachta Indica* was discovered to be 12.15 % and 7.55 % respectively. The alcoholic (methanol) extract of Aloe Vera indicates the existence of tannins, flavonoids, saponins, carbohydrates, terpenoids, phenolic compounds, steroids, proteins and amino acids. The methanolic concentrate of *Lycopersicon Esculentum* indicates the existence of flavonoids, alkaloids, tannins, glycosides, saponins, steroids, carbohydrates, proteins, amino acid, vitamins and phenolic compounds.

**Keywords:** *Lycopersicon Esculentum*, Herbal ointment, *Indica*, Antimicrobial Activity, Zone Reader.

## INTRODUCTION

Plants are the most necessary elements for daily life on earth.<sup>1</sup> It was a vital aspect in the evolution of modern civilization.<sup>2</sup> Primitive man saw and valued the enormous range of flora accessible to him.<sup>3</sup> The plants supply food, clothes, shelter and essential medicines. Finding medicinal abilities in plants is an old belief. It is firmly believed that there is a treatment from someplace in nature for every sickness.<sup>4</sup> It is estimated that there are between 2,500 and 50,000 different species of plants on our planet.<sup>5</sup> Only a very tiny portion between 5 and 10 percent of them are consumed as meals by either human beings or other animal species.<sup>6</sup> Life is not feasible without the innate metabolic capabilities of plants that autonomously transform carbon dioxide and water into sugars, and nitrogen to amino acids and nucleic acids.<sup>7</sup> The vital role of the plants is not only restricted to the synthesis of carbohydrates and proteins but also includes an almost endless potential to manufacture countless chemical substances.<sup>8</sup> To meet their most basic medical needs, an estimated eighty percent of the global population relies on traditional forms of treatment, says the WHO.<sup>9</sup> Biochemical studies have shown several health benefits, including anti-tumor and wound-healing properties as well as anti-diarrhea and analgesic effects. In many circumstances, individuals say that some natural or herbal products have a positive effect on their health.<sup>10</sup> Ancient traditional therapeutic understanding about plants and the chemical components responsible for their activities has been supported by research on medicinal plants, which has identified and identified the chemical components responsible for their activities. The use of medicinal herbs to combat germs has been documented by several ethnic groups across the globe. The pharmacological effects of these antibacterial medicinal plants have been studied, and various active components have been identified. There are several folkloric uses for aloe, including curing boils, wounds, and burns, as well as treating a variety of other maladies.

## Definition

Natural medicine, also known as herbalism, botanical medicine, herbal remedies, Phytomedicines, phytotherapeutic agents (or HMPs), or herbal medicine products (HMPs), is the use of herbs for their medicinal or therapeutic value.<sup>12</sup> Herbal medicine is a broad term that encompasses a variety of practices and disciplines.<sup>13</sup> An herb is a plant or plant component that is prized for its medicinal, fragrant, or savory properties.<sup>14</sup> Herbs include a variety of plants and plant parts. Herbs generate and contain a wide range of chemical compounds that have physiological effects on the body.<sup>15</sup> Herbal medications are made up entirely of plants that function as active components.<sup>16</sup> Herbal medicines are complicated concoctions that typically include at least 50 chemical elements, with the majority of them including hundreds or thousands of them. The active ingredients of the vast majority of these combinations are currently unknown for the most part.<sup>17</sup>

**Advantages of Herbal Drugs**

1. High low/minimum cost
2. Complete accessibility
3. Enhanced tolerance
4. More protection
5. Fewer side effects
6. Potency and efficacy is very high

**ALOE VERA GEL.**

Aloe's medicinal properties are affected by a variety of circumstances, including its age, harvesting, care, distribution, and gel processing methods. After harvesting, it is critical to preserve the Aloe vera's organic characteristics and keep the gel from hydrolysis and oxidation. Separation of the gel from the outer cortex must also be done correctly. Incorporating a cellulose enzyme helps to speed up the gel extraction process. Aloe juice is run through activated carbon in order to remove anthraquinones, with aloin being the major component that has laxative effects. Excessive heat degrades the medicinal efficacy of Aloe gel during the sterilising process. Thus, the aloe's most potent energy molecules, the MPS and other active elements, are mostly deactivated, and the plant's once-promising properties are no longer present. Microorganisms cannot grow aerobically if enzymes such as glucose oxidase or catalase are used.<sup>18</sup>



**Figure no. 1: Alovera Plants**

**Table no .1. Botanical name Aloe vera**

<b>Languages</b>	<b>Names</b>
Nepali	Gwarpatha
Sanskrit	Ghritkumari
Hindi	Musabar
Marathi	Korphad
kannada	kathaligida

### **Azadirachta Indica**

*Azadirachta indica* (*A. indica*) belongs to the botanic family Meliaceae, commonly known as Neem. It is used in traditional medicine as a source of many therapeutic agents. *A. indica* (leaf, bark and seeds) are known to contain antibacterial and antifungal. *Azadirachta indica*, or *A. indica*, is a member of the Meliaceae plant family. The majority of people refer to this species as Neem. In conventional medical practice, it is used as a source of a number of different therapeutic compounds. Antiviral capabilities against vaccinia, chikungunya, measles, and the Coxsackie B virus are well-known, as are the antibacterial and antifungal characteristics of *A. indica* (leaf, bark, and seeds). Antibacterial and antifungal properties of *A. indica* (leaf, bark, and seeds) are well-known. It has been shown that the leaf, the bark, and the seeds of the neem tree all exhibit a variety of medicinal advantages.<sup>19</sup> These benefits include the ability to combat free radical damage and malaria, as well as antimutagenic and anticarcinogenic properties. Its biological activity may be attributed to the presence of a large number of bioactive molecules within its diverse component parts. The aqueous extract of neem leaf has antihyperglycemic effects that show promise in both insulin-dependent and non-insulin-dependent diabetic mellitus. Since the dawn of time, people in several parts of Europe and Asia have been exploring the therapeutic potential of various plant species. A significant amount of work has been completed, much of which has avoided the notice and understanding of the general population. Because of the growing prevalence of the use of technology in today's world, human suffering has been given a new name in this century. It is an excellent natural insect repellent because of its antibacterial, antifungal, antiviral, and anti-inflammatory effects. Neem oil includes fatty acids that aid to the healing of wounds, the preservation of the pliability of the skin, and the creation of collagen.<sup>20</sup>



**Figure no 2. Neem Plant**

**Table no 2. Azadirachta Indica**

LANGUAGES	NAME
English	Paradisetree
Sanskrit	Aristha
Urdu	Neem
Hindi	Nim
Marathi	Kadu-limba

**Lycopersicon Esculentum:**

Solanum Lycopersicon, popularly known as tomato, originated in South America and now is used and cultivated in various parts of the world. This product is cultivated in warm climate regions, but can also be planted inside a greenhouse during winter. Tomatoes are full of vitamins and antioxidants essential to a healthy body. Tomatoes can be consumed in several ways from salads up to sauces and easily harvested; making it the second most consumed vegetable of the American diet and has China being the main country that producer tomatoes in 31% of the total produce in the world. It belongs to the nightshade family or Solanaceae or nightshade family, which includes tomatoes. Many people like the versatility of the tomato. The leaves of the tomato plant contain harmful alkaloids, thus only the fruits may be eaten. Vitamin C, calcium, phosphorus, and iron are all found in abundance in this fruit, making it a wonderful source of nutrition. When it comes to keeping your skin healthy, tomato juices a common ingredient in many homemade skin care products. A wide range of human ailments, including cancers of the lung, prostate, esophagus, and pancreas; high blood pressure; kidney and liver disorders.

**Figure no.4 :Lycopersicone sculentum****Table no.3. L. Sculentum**

Language	Name
English	Tomato
Tamil	Takkali
Hindi	Tamatar
Kashmiri	Ruvangum

## OINTMENTS

Semisolid dosage forms such as ointments may be used for topical application to the skin, placement on the surface of the eye, or for nasal, vaginal, or rectal use for medicinal or protective activities or cosmetic purposes. These preparations are used for the limited effects they have on the skin or mucous membrane at the moment of application due to drug penetration, and they are why they are used. In the treatment of cutaneous illnesses, these devices are intended to deliver the medication directly into the skin, with the skin serving as the target organ. Emulsions of oil and water are used to create the semi-solid substance known as an ointment. Both oil-in-water (O/W) and water-in-oil (W/O) ointments are composed of very small droplets of oil and water that are distributed uniformly across a continuous oily or watery phase, respectively. Ointments may either be oil-in-water or water-in-oil, and this distinction is based on how the oil is distributed throughout the product. Because they are less oily and can be removed with water more readily, ointments that include oil and water are more pleasant to use and are appropriate for cosmetic use. It is more difficult to work with water-in-oil ointments, but because many of the medications that are included in ointments are hydrophobic, they will be released more easily from a water-in-oil cream than from an oil-in-water cream. Water-in-oil ointments have the disadvantage of being less stable. Because they create an oily barrier, water-in-oil ointments are also more moisturizing. Traditional medical practices are being championed by both the World Health Organization (WHO) and the United States of America due to the fact that they are less costly, readily accessible, and comprehensive, particularly in poor nations.

## METHODOLOGY

### Equipment/Apparatus

“Pipettes, capillary tubes, graduated dropper, pipette filler, Microtech Gloves, Watsman filter paper, glass wool, spreader, bleach, measuring cylinder, TLC plates, test tubes, calibrated ruler, plates, sterilized wire loop, column tube, beakers, cotton wool, aluminum foil, 5mm borer, glass bottles, Bunsen burner, measuring cylinder.

### List of Reagents

Hexane laboratory reagent of 95% purity from Thomas Baker (chemicals) chemical laboratory NRI Bhopal.

### Preparation of *Aloe barbadensis*, *Lycopersicon Esculentum*, *Azadirachta indica*

After being brought to the laboratory in plastic bags, the leaves were washed under running water to remove any traces of dirt that may have remained on them. Before being dried in the oven, the leaves were first dried in the sun for a period of four weeks. This was done in order to eliminate any remaining moisture in the leaves. Before utilizing the powdered plant material, the

dried leaves were pulverized using a mortar and pestle and then kept in an airtight container. The powdered plant material was used.

### **Extraction of the Leaf of *Aloe barbadensis*, *Lycopersicon Esculentum*, *Azadirachta indica***

In an ethanol solution that was 100 percent, 1.5 kg of powdered leaves were macerated for 72 hours at room temperature (270 C). In order to get the most efficient extraction of the bioactive phytochemicals, the mixture was vigorously shaken on many occasions. After the solvent in the combination was evaporated using a rotary evaporator set to 40 degrees Celsius, the resulting residue weighed 155.61 gram and was sticky and black. In order to ensure that all of the solvent was extracted, a tube made of aluminum foil and covered in aluminum foil was put inside of a desiccator. The tube was labeled with the words "ethanol extract." Following the drying process, the unprocessed extract was stored in the refrigerator, where it would stay until it was required once again.

### **Collection and authentication of Plant Materials**

In the present study, the leaves of *Aloe vera* collected from the surrounding area of Bhopal district and authenticated by Bhopal Botanical Garden. Soon after collection the leaves were cleaned, gel was collected using sharp knife and dried in hot air oven at 400C, powdered to coarse using mortar and pestle. A container that prevented air from getting in was used to store the powder until it was needed again. Bhopal Botanical Garden verified the authenticity of *Azadirachta indica* leaves and *Esculentum*, collected in the Bhopal Botanical Garden leaves were washed, dried in the shade, and ground into a fine powder before being kept in an airtight container for future use. Then the leaves and fruit were taken for the size reduction using cutter mill in order to get coarser to a fine powder. After size reduction, the powder passed through 40# sieve to get uniformly sized powder. The final powdered material is kept in air tight wide mouth bottle for future use.

### **A] Formulation of ointment**

At 70-75 degrees Celsius, the emulsifying wax, white soft paraffin, and liquid paraffin were heated to a homogeneous mass, and the mixture was continually stirred. The extracts were included into the ointment base, the ointment formulation, and the ointment formulation itself.

**B]** Ointment may be made by dissolving the polymer in water and glycerol, then adding methyl paraben as a preservative. The extract was used to make it. In order to make the mixture more viscous and neutral, triethanolamine was added to the mixture after that step had been completed. Ointment formulation necessitates the combination of a plethora of different substances.



**Table 4: - 1 Formulation of Ointment****1) Formulation of ointment base: -**

S. No.	Name of Ingredient	Quantity to be taken
1	Wool fat	2 gm
2	Cetostearyl alcohol	2.5gm
3	Hard paraffin	1.5gm
4	Yellow soft paraffin	4 gm

**Table 5:- 2 Formulation of ointment 20 gm**

S. No.	Name of Ingredients	Quantity to be taken
1	Azadirachta Indica	2 gm
2	Aloe Vera	5 gm
3	Lycopersicon Esculentum	3 gm
4	Ointment base	10

**RESULTS AND DISCUSSIONS****Pharmacognostic Study****Anatomical Study:**

The various morphological characters like colour, odour, taste, size, shape, etc. has been studied for all three plant materials. All the plant parts are greenish to dark green in colour with characteristic and aromatic odour. The taste of *Aloe Vera* and *Azadirachta Indica* are bitter in taste while *Lycopersicon Esculentum* are acid in taste. The compiled results of macroscopical study are shown in table 6.

**Table 6. Macroscopical features of plant materials**

Sr.no.	Parameters	<i>Aloe Vera</i>	<i>Azadirachta Indica leaf</i>	<i>Lycopersicon esculentum. leaf</i>
1	Colour	Depends on variety dark brown, Brownish Black or black	Greenish brown color Powder	Redun even with light inclusion
2	Odour	Characteristic	Typical	Typical
3	Taste	Intensely bitter and Nauseating	Bitter	A bitsour with as mack of Overripe tomatoes
4	Size	Masses of various sizes	20-40 cm long	1 – 10 cm long
5	Shape	N/A	Oblong	Oval



**Histological Study:*****Aloe Vera*****Phytochemical Parameters****Ash Values:**

Water soluble ash, acid insoluble ash and total ash value of *Aloe Vera* was discovered to be 4.2 %, 0.89 % and 85.90 % respectively, whereas the water soluble ash, acid insoluble ash, and total ash value of *Azadiracta Indica* was discovered to be 11.9 %, 1.2 % and 11.2 % respectively. In the case of *Moringa oleifera*, water soluble ash, acid insoluble ash value and total ash value was found to be 4.80 %, 2.90 % and 19.56 % respectively.

**Extractive Values**

Extractive value (water and alcohol soluble) of *Aloe Vera* were discovered to be 5.20 % and 6.26 % respectively. Extractive value (water and alcohol soluble) of *Azadiracta Indica* were discovered to be 29.5 % and 16.6 % respectively. Extractive value (water and alcohol soluble) of *Lycopersicon Esculentum* were discovered to be 29.60 % and 13.8 % correspondingly.

**Loss on Drying**

The loss on drying of, *Aloe Vera*, *Azadiracta Indica* and *Lycopersicon Esculentum* were discovered to be 4.54% w/w, 15.5% w/w and 14.5% w/w correspondingly.

All the compiled results are shown in table 7.

**Table 7. Physical constants for *Lagenaria siceraria*, *Azadiracta Indica* and *Moringa oleifera***

Parameters	Results		
	<i>Aloe Vera</i>	<i>Azadiracta Indica</i>	<i>Lycopersicon Esculentum</i>
<b>Ash Values</b>			
1) Water Soluble Ash	4.1 % w/w	11.8 % w/w	4.7 w/w
2 ) Acid Insoluble Ash	0.88 % w/w	1.1 % w/w	2.80 % w/w
3) Total Ash	85.80 % w/w	11.1 % w/w	19.46 % w/w
<b>Extractive Values</b>			
1) Alcohol Soluble Extractives	6.25 %w/w	16.5 %w/w	13.6 % w/w
2 ) Water soluble extractive	5.10 %w/w	28.5 %w/w	28.60 % w/w
<b>Loss on Drying</b>	4.44 % w/w	14.5 % w/w	13.5 % w/w

Ash value indicates the presence of inorganic and calciferous matter present in the collected sample. Extractive values show type of compounds and quantity of compounds presents in

samples. Loss on drying indicates amount of moisture present in samples. Higher % value of water soluble extractive as compared to alcohol soluble extractive indicates that the plants contain a high amount of water soluble constituents. This extractive value also supports our hypothesis for the presence of the higher amount of water soluble compounds in plants. The results obtained were comparable with the standard literature values collected. These values have the potential to be regarded as standard values, and as a result, they may be used in the future for the purpose of standardising the aforementioned plant-based medications.

**Table 8. Percentage yield of extracts of *Aloe Vera*, *Azadiracta Indica*, and *Lycopersicon Esculentum* extracts**

Sr.no.	Plant name	Extracts	% yield w/w	Physical state of extract
1	<i>Aloe Vera</i>	Aqueous (200g)	9.22 %	Semisolid viscous
2		Methanol (200 g)	11.2 %	Semisolid viscous
3	<i>Azadiracta Indica</i>	Aqueous (200g)	12.25 %	Semisolid viscous
4		Methanol (200 g)	7.15 %	Semisolid viscous
5	<i>Lycopersicon Esculentum</i>	Aqueous (200g)	25.60 %	Semisolid viscous
6		Methanol (200 g)	9.8 %	Semisolid viscous

#### QUALITATIVE CHEMICAL INVESTIGATION OF EXTRACTS

The fruits of *Aloe Vera*, leaves of *Azadiracta Indica* and *L. Esculentum* were evaluated for the existence of numerous phytoconstituents. (E.g. Glycoside, Terpenoids, Steroids, Tannins, Flavonoids, Carbohydrates, Saponins, Protein, Alkaloids and Aminoacid)

**Table 9. *Aloe Vera* extracts qualitative phytochemical study**

Sr.No.	Phytoconstituents	<i>Aloe Vera</i>	
		Methanolic extract	Aqueous extract
1	Flavonoids	+	+
2	Alkaloids	+	+
3	Glycoside	+	+
4	Saponins	+	+
5	Tannins and phenolic compounds	+	+
6	Steroids and terpenoids	+	+
7	Carbohydrates	+	+
8	Proteins and Aminoacids	+	+
9	Vitamins	+	+

(+) : Present, (-) : Absent

The alcoholic (methanol) extract of *Aloe Vera* indicates the existence of tannins, flavonoids, saponins, carbohydrates, terpenoids, phenolic compounds, steroids, proteins and aminoacids. The aqueous extract of *Aloe Vera* indicates the existence of phenolic compounds, flavonoids, tannins, carbohydrates proteins, amino acids and vitamins.

**Table 10. *Azadiracta Indica* extracts qualitative phytochemical study**

Sr.No.	Phytoconstituents	<i>Azadiracta Indica</i>	
		Methanolic extract	Aqueous extract
1	Flavonoids	+	+
2	Alkaloids	+	-
3	Glycoside	+	+
4	Saponins	-	+
5	Tannins and phenolic compounds	+	+
6	Steroids and terpenoids	+	+
7	Carbohydrates	-	-
8	Proteins and aminoacids	+	+
9	Vitamins	+	+

(+) : Present, (-) : Absent

**Table 11. *Lycopersicon Esculentum* extracts qualitative phytochemical study**

Sr.No.	Phytoconstituents	<i>L. Esculentum</i>	
		Methanolic extract	Aqueous extract
1	Flavonoids	+	+
2	Alkaloids	+	+
3	Glycoside	+	+
4	Saponins	+	+
5	phenolic compounds and Tannins	+	+
6	Steroids and terpenoids	+	+
7	Carbohydrates	+	+
8	Proteins and Aminoacids	+	+
9	Vitamins	+	+

(+) : Present, (-) : Absent

### 6.1. Physical Appearance

Ointment formulations were pale green viscous preparation with a smooth homogeneous texture. The Physical Appearance of the ointment were given in

**Table no. 12. Physical Appearance**

S.No	Formulations	Colour	Homogeneity	Consistency
1	2%w/w	pale green	Excellent	+++
2	4%w/w	pale green	Excellent	+++

### Determination of pH:

The pH of the ointment solution was measured with the help of pH meter. 0.5g of ointment was dissolved in 50ml of distilled water and stored for two hours. The measurement of pH each formulation was done in triplicate.

**Table no. 13. pH of ointment**

S.no.	Formulation	pH
1	2%w/w	6.36±0.3
2	4%w/w	6.27±0.1

### Extrudability:

Ointments were filled into collapsible tubes after formulating them. The extrudability of the formulation has been checked.

**Table no. 14. Extrudability of ointment**

S.no.	Formulation	Extrudability
1	2%w/w	Easily Extrudable
2	4%w/w	Easily Extrudable

**Spreadability**

Spreadability of the ointment on skin surface of human volunteers were

**Table no . 15. Spreadability**

S.no.	Formulation	Spreadability
1	2%w/w	Good
2	4%w/w	Good

**Skin sensitivity test**

The skin sensitivity showed no irritation ,redness or erythema indicating that both ointments were non-irritant.

**Table no. 16. Skin sensitivity test of ointments**

S.no.	Formulation	Skin test
1	2%w/w	Non irritant
2	4%w/w	Non irritant

**Viscosity**

Ointment viscosity resistance to flow of a liquid can be measured by Bork field viscometer.

**Table no. 17. Viscosity**

S.no.	Formulation	Viscosity poise
1	2%w/w	2691 poise
2	4%w/w	2700 poise

**Antimicrobial studies**

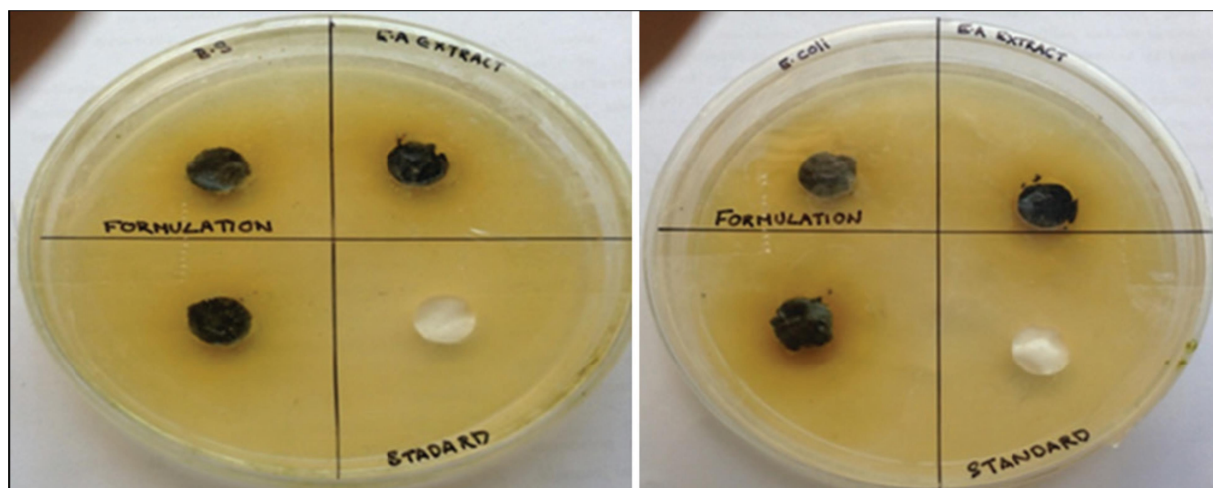
Antimicrobial activity can be determined by *B. subtilis* and *E.coli*. Ointment formulations and standard cup plate methods used for the study. The nutrient agar solution poured into the previously sterilized Petri dishes up to 5 mm thickness. Each microorganism added to Petri dishes with a sterilized loop and plates were allowed to solidify for 5 min. On each Petri dish,

five perforations made with a metal tube with a 4 mm diameter to receive test materials. The standard (neomycin sulfate ointment) and test materials (prepared formulations) added immediately into the wells and kept for incubation at 37°C for 24 h to allow the microorganism to grow and reagents to diffuse through the culture medium. At the end of the incubation, the zone of diameter can be measured by using the zone reader.

### Antimicrobial study

**Table no.18. Antimicrobial study**

Sr.No	Formulation	Zone of inhibition for <i>E.Coli</i> (MM)SD	Zone of inhibition for <i>B. Subtilis</i> (MM)SD
1	F1	12.60±0.18	10.20±0.41
2	F2	14.70±0.15	18.50±0.52



**Fig.NO.6. Zone of inhibition shown by the ethyl acetate extract formulations for E.coli (a) and B. subtilis (b)**

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