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TOPICAL GEL FORMULATION FROM *CASSIA AURICULATA* FLOWER AND *ALOE VERA* LEAF EXTRACT AGAINST SKIN INFECTION CAUSING BACTERIA AND *STAPHYLOCOCCUS AUREUS* CAUSED WOUND SCAR

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ABSTRACT

This study was aimed to formulate the gel using four different extracts obtained from *Cassia auriculata* flower and *Aloe vera* leaf against skin infection causing bacteria. The extract was collected using soxhlet, and the extracts were characterized by FTIR analysis. Gel was formulated in two different formulations and tested against skin infection causing bacteria (*Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Pseudomonas aeruginosa, Klebshilla pneumonia)*. From the study it shows that the formulated gels possess the antibacterial property against the tested organisms.

KEYWORDS: *Cassia auriculata, Aloe vera,* antibacterial activity.

INTRODUCTION

Skin is the major organ of the human body. It acts as a protective barrier to the human body. It helps on maintaining the body temperature and pH. Skin flora is usually non pathogenic and either not harmful to their host or offer a benefit.

The skin consists of three layers: the outer epidermis, the dermis where many hair follicles and sweat glands and the fatty subcutaneous layer are found. These layers protect the connective tissues, muscles and bones. Wound can penetrate any of these layers and can cause skin infections. Skin infections are generally caused by Bacteria, fungi, parasites and virus. These can be either mild or severe depending on the infecting agents. Bacterial skin infections develop when bacteria enter through the hair follicles or through the small breaks in the skin that result from scrapes, punctures, surgery, burns, sunburn, animal or insect bites, wounds and pre existing skin disorders. These infections can occur while swimming in contaminated water, gardening in contaminated soil (Valarmathi, S *et al*., 2013).

There are many type of bacteria that can infect the skin but the most common type of bacteria are *Staphylococcus* and *Streptococcus.* MRSA (Methicillin-resistant *Staphylococcus aureus*), which is resistant to most of the antibiotics, is the most common bacteria that causing skin infections (Harsent, R *et al*., 2022). Other bacteria that causing skin infections are *Klebshilla, Pseudomonas, Corynebacterium, E.coli* etc. Initial infectins caused due to bacteria. Fungal infections caused due to *Candida, Aspergillus, Fusarium,* and other infections may arise as they are not inhibited by antibacterial treatment. Viral infections such as those caused by the herpes simplex virus, may also occur.

Some people are at particular risk of developing skin infections. People with diabetes, are likely to have poor blood flow (especially at hands and feet), due to the high level of sugar in their blood, decreases their immune response against skin infections; people who are hospitalized; people who are older; people who have AIDS or other immune disorder; people who are undergoing chemotherapy or treatment with other drugs that suppress the immune system can develop skin infections. Skin that is inflamed or damages can become infected. (Nowicka, Danuta, *et al*., 2022)

Cassia auriculata commonly known as Tanner's cassia (Avaram in tamil) is a shrub belongs to Caesaepiniaceae family. It is distributed throughout the hot deciduous forests of India. Wild in dry regions of Madhya Pradesh, Tamil Nadu, Rajasthan and also in other parts of India. It is a traditional medicinal plant, widely used for treatment of various ailments in Ayurveda and siddha system of medicine in India.

Flower of this plant is used in the treatment of skin disorders and body odour. Tea prepared from this flower treats diabetes (Meena, Vandana, *et al.*, 2019). The plant is known well for its phytochemical compositions,

pharmaceutical applications and Therapeutic potential. Flower of *cassia auriculata* showed the significant amount of phytochemical compositions such as flavonoids, phenols, tannin, terpenoids, alkaloids, carbohydrates and steroids.(Kanthimathi, M., and R. Soranam., 2014).

Extract of dried flower shows antimicrobial activity against bacteria and fungi. Ethanol and aqueous extractions of flower shows antibacterial activity against *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Klesiella pneumoniae, E.coli, Proteus mirabila* and *vibrio cholorae* and antifungal activity against *Candida albicans, Aspergillus niger*. The ethanol and methanol extracts of *C. auriculata* flower shows antioxidant activity. (Maneemegalai, S., and T. Naveen., 2010).

The botanical name of Aloe vera is *Aloe barbadensis miller*. It belongs to Asphodelaceae (Liliaceae) family and is a shrubby or arborescent, perennial, xerophytic, succulent, pea-green color plant. It grows mainly in the dry regions of Africa, Asia, Europe and America. In India, it is found in Rajasthan, Andhra Pradesh, Gujarat and Tamil nadu.

Aloe vera has been traditionally used to treat skin infections such as burns, cuts, insect bites and eczemas and digestive problems because of its anti-inflammatory, antimicrobial, and wound healing properties (Liang, Jiaheng, *et al.*, 2021).

Because of their glucomannan, a mannose-rich polysaccharide and gibberellins it increases collagen synthesis. Aloe vera gel increase the collagen content of the wound and prevent from formation of scar tissue. Aloe vera gel has been reported to have a protection against radiation damage to the skin (Joseph, *et al*., 2010). Anthraquinones present in latex are a potent laxative. It increases intestinal water content, stimulates mucus secretion and increases intestinal peristalsis. Mucopolysaccharides that present in aloe vera help in binding moisture into the skin. It stimulates fibroblast which produces the collagen and elastin fibers making the skin more elastic and less wrinkled. It decreases erythema and also has anti-acne effect (Sharma, P., *et al*., 2014).

Aloe vera contains 6 antiseptic properties: Lupeol, salicylic acis' urea, nitrogen, cinnamonic acid, phenols and sulfur. They all have inhibitory action on fungi, bacteria and viruses. Aloe vera shows significant effect against both gram positive and gram negative bacteria. One of the most studied bacteria are *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Hence, the aquoues extract of aloe vera reduced growth and biofilm formation against methicillin resistant *Staphylococcus aureus.* Moreover this bacteria has been inhibited by aloe vera gel (50% and 100% concentration), along with this other oral pathogens obtained from patients with periapical and periodontal abscess including *Actinobacillus actinomycetemcomitans, Clostridium bacilli* and *Streptococcus* mutants. Aloe vera extracts inhibit the growth of multidrug-resistant *Pseudomonas aeruginosa* isolated from burned patients wound (Kamble, K. M., Chimkod, V. B., & Patil, C. S. 2013).

Beeswax plays an important role also in Ayurvedic medicine. It used as a main ingredient for ointments and creams preparations, to treat burns and wounds. In ancient time, many used to apply a cream containing olive oil, beeswax and rose water for the treatment of burns, wounds, cuts, bruises and fractures.

Crude beeswax showed antibacterial activity against several bacterial strains and against the *Candida albicans*. Beeswax was effective against both Gram-positive bacteria, in particular *S. aureus, Staphylococcus epidermidis* and *Streptococcus pyogenes* and against Gram-negative bacteria, in particular *Bacillus subtilis, Pseudomonas aeruginosa*, *Escherichia coli.*

Cradle cap is a condition resulting in dry scaly skin, most commonly found on the head, it can be cured by liquid paraffin. Sodium benzoate is commonly used as a preservative in cosmetics and personal care items, such as hair products, baby wipes, toothpaste, and mouthwash (Khan, Ishfaq Shafi, *et al.*, 2022). Tween 80 used as a surfactant in soaps and cosmetics.

MATERIALS AND METHODS

Collection Of Samples - The sample *Cassia auriculata* fresh flower and Aloe vera leaf was collected from Perumanallur, in Tirupur.

Collection Of Clinical Pathogens - The Clinical pathogens such as *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Pseudomonas aeruginosa, Klebshilla pneumonia* were collected from the Bioline Laboratory, RS Puram, Coimbatore.

Sample Preparation - The collected fresh flower of *Cassia auriculata* was allowed to show dried for 3 days and grained into coarsed powder. The collected fresh Aloevera leaf was cut into thin slices and allowed to shadow dried for 3 days and grained into powder.

Extraction of Plant Material

Extract From Dried *Cassia Auriculata* Flower - Extract of dried flower was carried out by continuous hot percolation method using Soxhlet apparatus (Raja *et al*., 2013). 25g of powder was extracted with 200ml of solvent (Ethanol & water). Then both the ethanol extract and aqueous extract was concentrated to dryness using rotary evaporator and the crude extract was collected and stored at 4c

Extract From Dried *Aloe Vera* Leaf - Extract of dried Aloevera leaf was carried out by continuous hot

percolation method using Soxhlet apparatus (Raja *et al*., 2013). 25g of powder was extracted with 200ml of solvent (Ethanol & Water). Then both the ethanol extract and aqueous extract was concentrated to dryness using rotary evaporator and the crude extract was collected and stored at 4c**.**

FTIR (Fourier Transform Infrared Spectroscopy) - Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Dried powder of different solvent extracts of each plant materials were used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each plant specimen was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a Scan range from 400 to 4000 cm 1 with a resolution of 4 cm 1. (Parag A. Pednekar, Bhanu Raman. 2013).

Antibacterial Analysis

Preparation Of Broth Cultures - All the five skin infection causing bacteria (*Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Pseudomonas aeruginosa, Klebshilla pneumonia) were cultured in nutrient broth at 37*˚c for 24 hours; it was used for antibacterial analysis.

Media Preparation - 3.8gram of MHA (Mueller Hinton Agar) was dissolved in 100ml of distilled water and then autoclaved for 20 minutes at 121˚C. Once the medium was about 40˚ - 50˚ C, it was poured into sterile petri plate and allowed to settle completely.

Antibacterial Activity - Antibacterial activity was performed using MHA by disc diffusion method. The prepared overnight broth cultures were swabbed on MHA media plates with a sterile cotton swabs and allow the plates for 2-3 minutes. 20µL of crude extract (ethanol and water extract of flower and aloe vera leaf) was loaded in a sterile disc. Then the loaded discs was placed on the surface of the medium and left for 30 minutes at room temperature for compound diffusion. Negative control was prepared using respective solvent. PenicillinG was used as a positive control for gram positive organisms and Ciprofloxin was used as a positive control for Gram negative organism (Sharmeen *et al.*, 2012). Then the plates were incubated at 37˚c for 24 hours. After incubation, the diameter of zone of inhibition (mm) was measured and recorded (kainsa *et al*., 2012, Saranraj *et al*., 2010).

Formulation Of Cream - The nature of cream is water in oil emulsion and two different creams were prepared, one with crude extract of *Cassia auriculata* flower and Aloe vera leaf obtained using ethanol as a solvent(F1) and other one with crude extract of *Cassia auriculata* flower and Aloe vera leaf obtained using water as a solvent(F2).

Preparation Of Oil Phase - 2 grams of bee wax and 6 ml of liquid paraffin were taken mixed together in a porcelain dish and melted at 75˚c (Nikita D Gidde., 2021).

Preparation Of Aqueous Phase - 2ml of water, preservative (Sodium benzoate) along with 1g of extracts were added and heated at 70˚c in another porcelain dish (Nikita D Gidde., 2021).

Addition Of Aqueous Phase To Oil Phase - At 75˚C, the aqueous phase was added to the oil phase by stirring continuously. After transfer was complete, it was allowed to cool to room temperature while being stirred constantly. Then move the cream to the air tight container and stored.

Antibacterial Activity Of Formulated Cream - Antibacterial activity of formulated cream were performed against the clinical pathogens (*Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Pseudomonas aeruginosa, Klebshilla pneumonia)* by swabbing the culture broth in MHA media and loaded sterile disc (dipped in cream for 6 hours before use) was placed over the surface of the media(Sahana et al., 2014). Then the plates were incubated at 37˚C for 24 hours. After incubation plates were observed foe zone if inhibition and the zone was measured and recorded.

RESULTS AND DISCUSSION

Plant Extract – The plant extracts were taken and the colour of the extracts was identified (Fig 1)

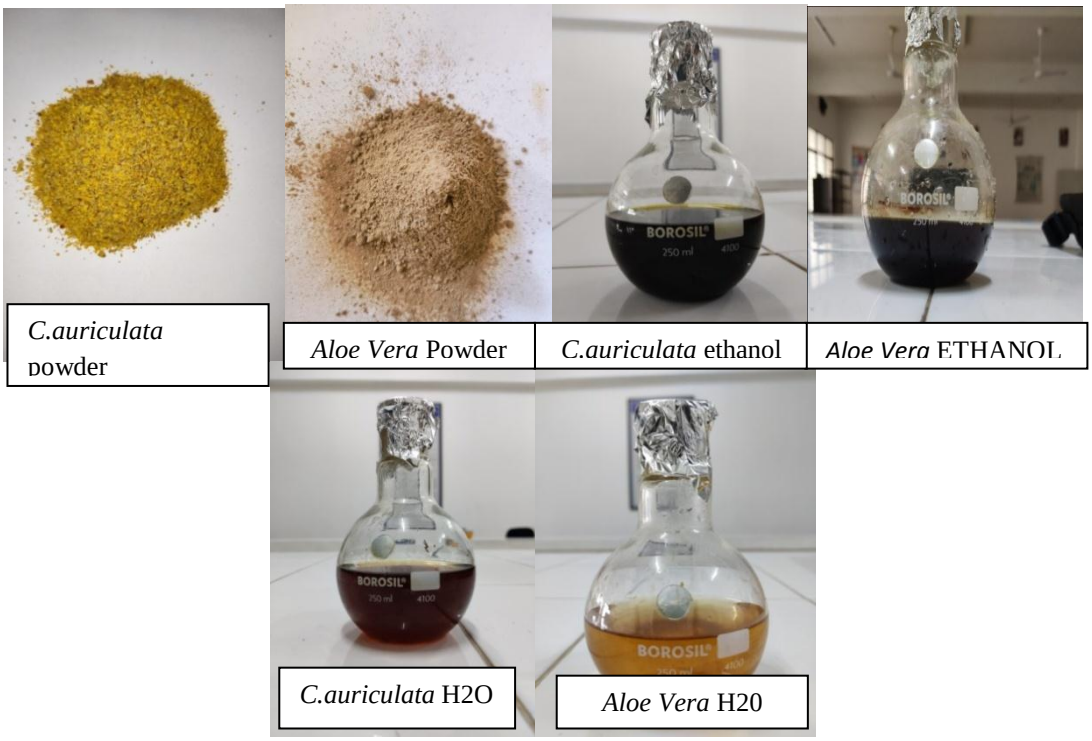


Fig. 1: Plant extraction process.

Antibacterial effect of two extracts and formulated creams against skin infection causing bacteria

The Ethanol and aqueous extract of *Cassia auriculata* and Aloe vera shows maximum inhibitory activity against *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes* and minimum inhibitory activity against *Pseudomonas aeruginosa, Klebshilla pneumonia* (Table-1&2 & Fig -2). The Penicillin and Ciprofloxin, which serves as a control shows resistance against five of the clinical pathogens. (Table-1&2).

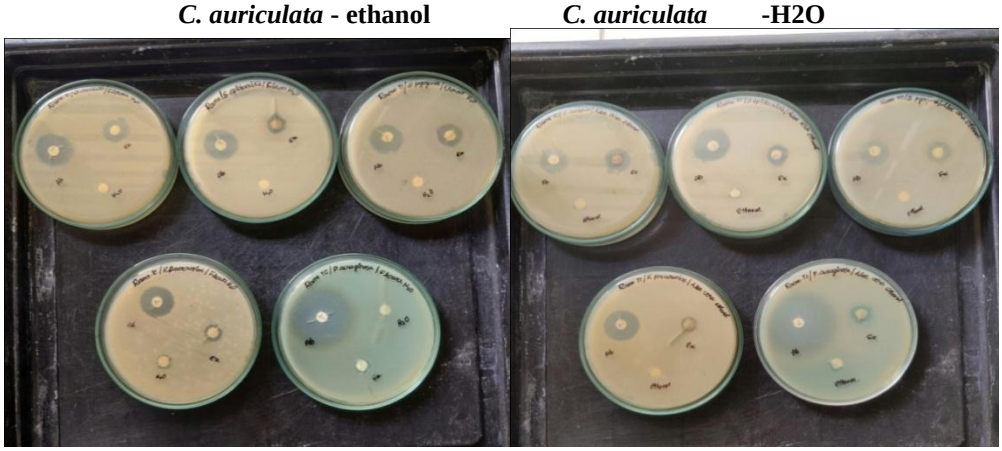
Table 1: Antibacterial Activity Of *Cassia Auriculata* Extracts.

S. No.	Organisms	ZONE OF INHIBITION (mm)		
		Ethanol Extract	Aqueous extract	Control (penicillin & ciproflaxin)
1.	*Staphylococcus aureus*	15	12	25
2.	*Staphylococcus epidermidis*	13	8	23
3.	*Streptococcus pyogenes*	12	11	20
4.	*Klebshilla pneuminiae*	9	8	28
5.	*Pseudomonas aeruginosa*	7	5	30

Table 2: Antibacterial Activity Of Aloe Vera Extracts.

S. No.	Organisms	Zone of inhibition (mm)		
		Ethanol Extract	Aqueous extract	Control (penicillin & ciproflaxin)
1.	*Staphylococcus aureus*	12	9	24
2.	*Staphylococcus epidermidis*	10	11	21
3.	*Streptococcus pyogenes*	9	5	22
4.	*Klebshilla pneuminiae*	4	9	25
5.	*Pseudomonas aeruginosa*	5	7	28

***C. auriculata* - ethanol** ***C. auriculata* -H2O**



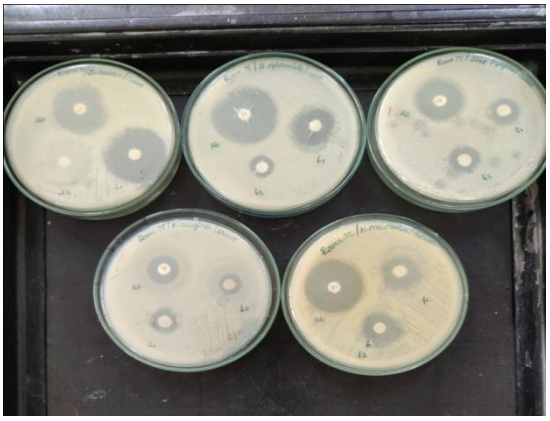
***Aloe vera*-ethanol** ***Aloe vera* – H20**

Fig. 2: Antibacterial assay plates.

Formulated gel shows maximum inhibition against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Klebshilla pneumonia* and minimum inhibition against *Pseudomonas aeruginosa.* (Table 3 & Fig - 3)

Table 3: Antibacterial Activity Of Cream.

S. No.	ORGANISMS	ZONE OF INHIBITION (mm)		
		F1	F2	CONTROL(PENICILLIN & CIPROFLAXIN)
1.	*Staphylococcus aureus*	16	-	26
2.	*Staphylococcus epidermidis*	12	3	21
3.	*Streptococcus pyogenes*	9	6	20
4.	*Klebshilla pneuminiae*	11	10	23
5.	*Pseudomonas aeruginosa*	9	5	27



Formulated cream

Fig. 3: Antibacterial Activity Of Formulated Cream.

FTIR Analysis

Cassia auriculata

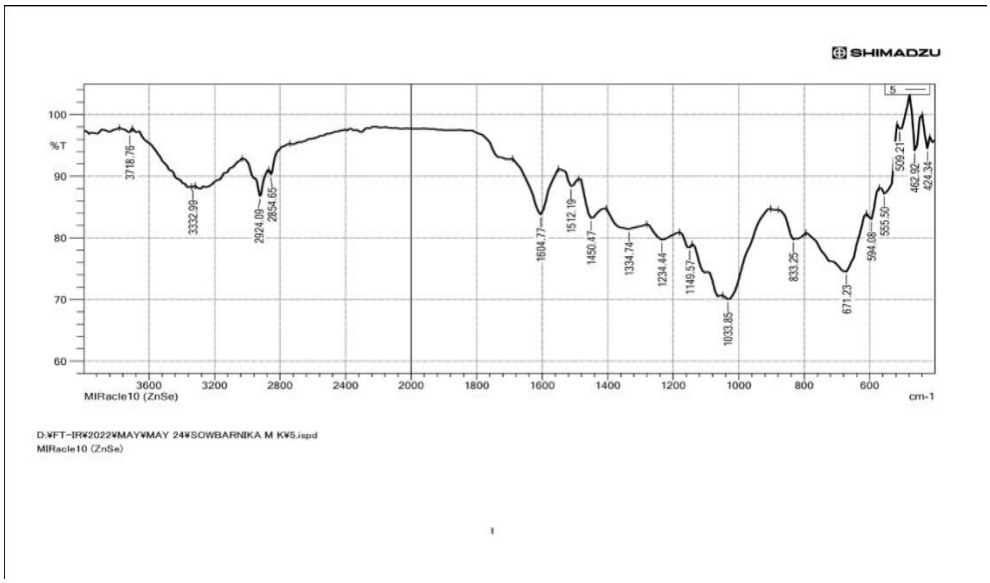
The ethanol and aqueous extract of *C. auriculata* contains the main functional groups such as alcohol group, alkane group, aromatic compound, amine group, nitro group, amine salt. (Table 4 & Fig 4)

ALOE VERA

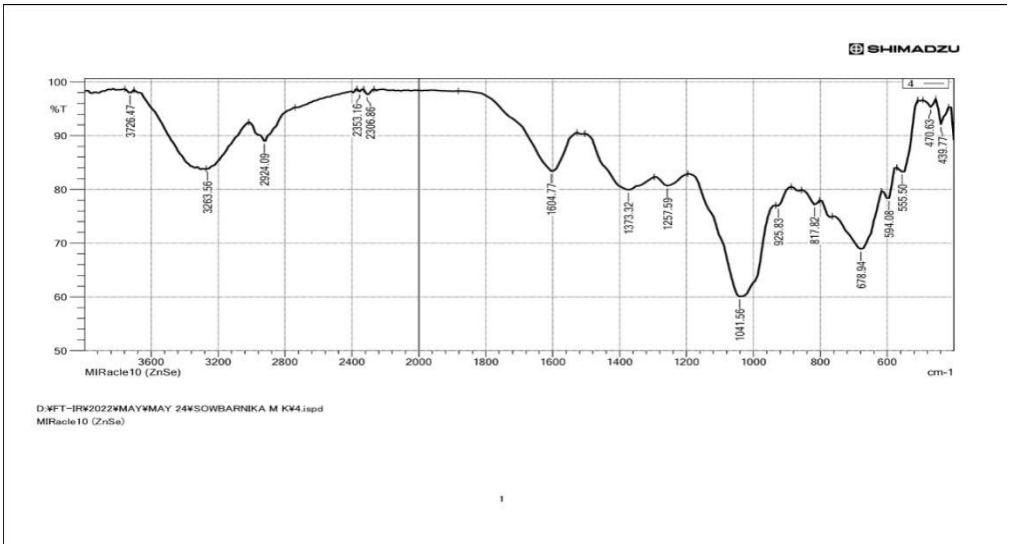
The ethanol and aqueous extract of Aloe vera contains the main functional groups such as alcohol group, carboxylic acid, amine group, alkene group, phenol, fluoro compound, halo compound. (Table 4 & Fig 4)

Table 4: FTIR.

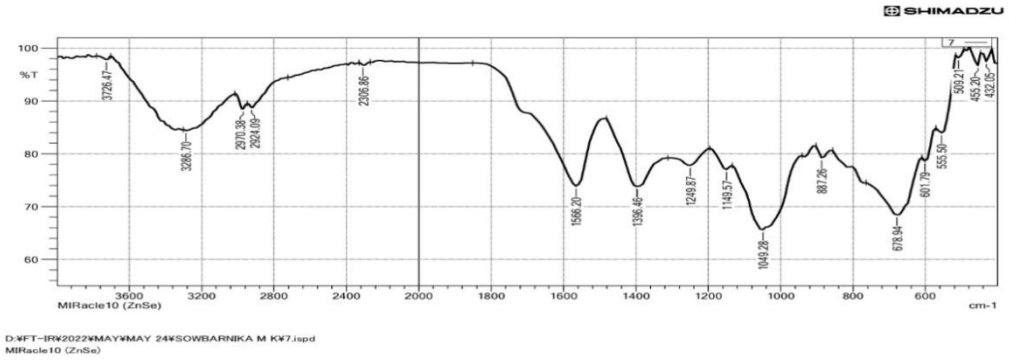
Samples	Functional Groups	Wave Number Ranges
Cassia auriculata (Ethanol)	alcohol group, alkane group, aromatic compound, amine group, nitro group, amine salt.	3718, 3286, 2924, 1720, 1249, 887.
Cassia auriculata (aqueous)	alcohol group, alkane group, aromatic compound, amine group, amine salt.	3718, 1450, 2854, 1512, 1033.
Aloe vera (Ethanol)	alcohol group, carboxylic acid, amine salt, amine group, alkene group, halo compound.	3726, 3286, 2970, 1049, 887, 678.
Aloe vera (Aqueous)	alcohol group, carboxylic acid, amine group, alkene group, phenol, halo compound, fluoro compound.	3726, 3263, 2353, 1604, 1373, 1041, 817.



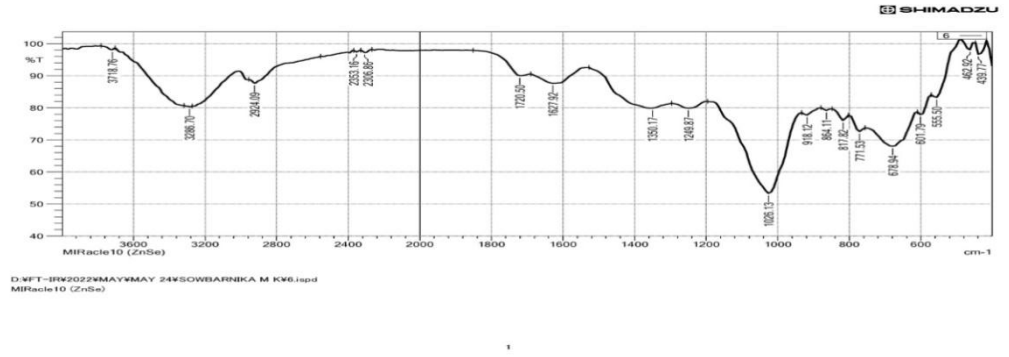
***C.auriculata* (ETHANOL)**



***C.auriculata* (H20)**



ALOE VERA (ETHANOL)



ALOE VERA (H20)
Fig. – 4. FTIR.

DISCUSSION

The present study was carried out to formulate the cream using *C.auriculata* flower extract and *Aloe vera* leaf extract, and to analyse its antibacterial activity against *Staphylococcus aureus, Streptococcus pyogenes, Staphylococcus epidermidis Klebshilla pnuemoniae, Pseudomonas aeruginosa.* The extracts were characterized by specteral analysis by FTIR analysis, the high peaks indicates the presence of antimicrobial properties. Formulated f1 cream shows high activity against gram positive bacteria and minimum activity against gram negative bacteria. F2 shows less activity against both gram positive and gram negative bacteria. This shows the efficiency of cream against the skin infection causing bacteria.

CONCLUSION

From the study I conclude that the formulated cream have promising antibacterial activity against gram positive skin infection causing bacteria then gram negative skin infection causing bacteria.

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