Formulate and Evaluate the Antimicrobial Activities of Hydrogel by Using Alangium *Salvifolium* Leaf

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Abstract: **The antimicrobial activity of ethanolic extract of dried leaves of *Alangium Salvifolium* is formulated and evaluated. The preliminary invitro antimicrobial activity of the extract at various concentration and those of their Hydrogel were determined against some microorganisms using the Mueller-Hilton agar plates for anti bacterial activity and Sabouraud's dextrose agar (SDA) for anti fungal activity. The growth inhibition zones of the extracts on the micro-organisms were noted. The physical properties of the Hydrogel formulated with the extract were evaluated using standard procedures. The main object of present investigation to antimicrobial activity of Alangium *Salvifolium.* It has the potential for anti-diabetic, anticancer, diuretic, antiinflammatory, antimicrobial, laxative, anthelmintic, and antiepileptic activities.**

Key Words: *Alangium salvifolium,* Anti bacterial activity - *Staphylococcus aureus* (MTCC 87) and *Escherichia coli* (MTCC 443), Anti fungal activity - *Candida albicans* (MTCC 183) and *Aspergillus flavus* (MTCC 277) and Herbal Hydrogel.

I. INTRODUCTION

Topical drug delivery system is the most preferred route for local delivery of therapeutic agents especially in pain and inflammation management, hormonal therapy, treatment of diseases of the cardiovascular and central nervous systems. Because of its convenience and affordability, it also prevents drug loss due to first-pass metabolism and maximizes the therapeutic effect without interference of pH, enzyme, and intestinal bacteria. Hydrogel is one of the semi-solid topical dosage forms, which includes cross-linked three-dimensional networks of hydrophilic polymer chains; therefore, it can entrap a larger amount of water. Hydro-gels have the advantages of increased biocompatibility, porous structure, tunable biodegradability, and proper mechanical strength as compare to other type of topical drugs. A hydrogel is a three-dimensional (3D) network of hydrophilic polymers that can swell in water and hold a large amount of water while maintaining the structure due to chemical or physical cross-linking of individual polymer chains. Hydrogels were first reported by Wichterle and Lím (1960). Ankol (*Alangium lamarkii*) synonym *Alangium salvifolium* (Linn. f.) wang.

It belongs to the family Alangiaceae and is commonly known as sage-leaved alangium. The genus Alangium consists of 22 species that are growing throughout India. All parts of plants such as stems, leaves, roots, and bark, are being used to make medicines that are used to cure illnesses. Ankol oil is used in Ayurvedic medicine. Ankol seed is used to improve stamina and physical performance. While Ankol oil is also valuable in itching, herpes, and other skin disease. The ankol leaf paste is used in treating pain linked to rheumatic and Osteoarthritis and also treated asthma. This tree grows up to 20 m in height, clusters of 4 to 8 flowers, at times only a single flower, which is dense and fragrant, cream color. Ankol oil is also valued for healing snakebites, scorpion bites as well as a dog bites. *Alangium salvifolium* is the most useful medicinal plant having a wide spectrum of biological activity. It has the potential for anti-diabetic, anticancer, diuretic, antiinflammatory, antimicrobial, laxative, anthelmintic, and antiepileptic activities. The plant was also reported for its antifungal activity, antimicrobial activity, cardiac activity, and antifertility activity

II. MATERIALS AND METHODS

Plant Material:

Collection, identification and authentication of raw *Alangium Salvifolium* was done. Fresh leaves of

Alangium Salvifolium were collected from in and around Ariyalur. Collected leaves are authenticated by Botanist, Department of Botany, National college,Trichy. Then the leaves cleaned properly and shade dried at room temperature.

Alcoholic Extraction process of Leaves of *Alangium Salvifolium*:

The Leaves are collected, cleaned and dried under shade, then dried leaves are pulverized by a mechanical grinder and passed through a 40 mesh seive. Then the *Alangium salvifolium* leaves were stored in an air-tight container .Then the 20 gm powdered leaves were successively extracted with 200 ml ethanol by hot continuous percolation method in Soxhlet apparatus.Then the temperature was mainatined between 70-75^{0}C for 8 hour.Then the extract was concentrated by using a evaporating the ethanol.Now the crude extract of *Alangium Salvifolium* was obtained.The flask was kept in the dark to avoid effect of the light on the active constituents of the *Alangium salvifolium*. Then the extract are filtered through a muslin cloth after a week of extraction. The extract are concentrate till dryness. The use of water bath maintain the room temparature the extract are heated for evaporation till the gryness.

Figure 1 SOXHLET EXTRACTION PROCES

III. METHOD OF PREPARATION

Weigh accurately about 10g of Sodium Hydroxide and dissolved in 100ml of deionized water. Accurately weighed Carbopol 934 on analytical balance and then dispersed in distilled water using mechanical stirrer for two hour at 100 rpm. The resultant dispersion was converted into gel base by neutralization with 10 % NaOH w/w. Viscous mixture of poly ethylene glycol 400, glycerol and methyl paraben is prepared. Then the crude drug i.e *Alangium Salvifolium* extract is dissolved in the viscous mixture. This viscous solution was thoroughly mixed into the viscous carbomer gel base. Sufficient addition of distilled water to make 100g of Hydrogel.

S. NO	INGREDIENT	FORMULATION CODE		
		F1	F2	F3
1.	Alangium Salvifolium Extract (gm)	0.1	0.1	0.1
2.	Carbopol 934 (gm)	0.25	0.5	1
3.	Sodium Hydroxide (gm)	Qs*	Qs*	Qs*
4.	Poly ethylene glycol 400 (ml)	5	5	5
5.	Methyl paraben (gm)	0.1	0.1	0.1
6.	Glycerol (ml)	10	10	10
7.	Distilled water (ml)	Qs*	Qs*	Qs*

Table No.1: Formulation of Hydrogel

IV. EVALUATION OF HYDROGEL

Determination of Antimicrobial activity:

Test microorganisms

The following bacterial and fungal strain were used for screening of the antibacterial and antifungal activity of sample were exhibited against one gram positive bacterial strains *Staphylococcus aureus* (MTCC 87) and one gram negative bacterial strains *Escherichia coli* (MTCC 443) and for fungal culture used in the study are *Candida albicans* (MTCC 183) and *Aspergillus flavus* (MTCC 277) were prepared as test microorganisms. All the bacterial strains were purchased from the Microbial Type Culture and Collection (MTCC) at Chandigarh, India and the fungal strains from National Chemical Laboratory (NCL), Pune, Maharashtra, India.

Preperation of test and standard solution

The test sample are prepare by dissolving the prepared hydrogel in ethanol (95%v/v) the prepared Hydrogel and herbal extract are taken as test sample. 10 mg of sample are taken and dissolved with 1 ml of ethanol and taken 100 micro liter as final volume. The amoxicillin are used as standard for antibacterial activity 10mg are dissolved in 1 ml ethanol and final volume taken as 100 micro liter. The fluconazole are used as standard for antifungal activity 10mg are dissolved in 1 ml ethanol and final volume taken as 100 milli litre.

Determination of antibacterial activity by disc diffusion method

The disc diffusion method is used to evaluate the antibacterial activity of the sample. Ten ml of Mueller-Hilton agar medium was poured into sterile petri dishes (diameter 60 mm) and inoculated with test organism. Sterile filter paper dics loaded with various concentrations of

sample of 60, 80 and 100 µg/ml were placed on the top of Mueller-Hilton agar plates. Filter paper disc loaded with 5 µg of amoxicillin was used as positive control. The plates were incubated at 37 ºC for 24 hours and the zone of inhibition was recorded in millimeter and the experiment was repeated twice.

Determination of antifungal activity by disc diffusion method

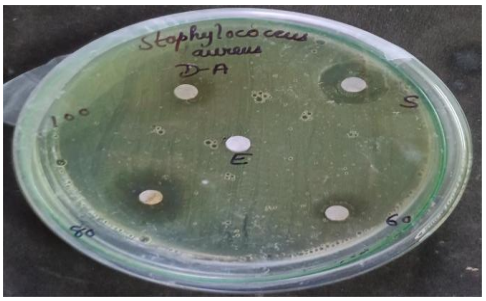
Disc diffusion method in order to test the antifungal activity of sample against test pathogens was carried out. In petri dishes (60 mm) filled with Sabouraud's dextrose agar (SDA) and seeded with a 0.3 ml of test organism, a sterile filter paper disc (diameter 6 mm, whatmann paper no.3) was placed. The sterile disc was impregnated with 10 µl of samples at varying concentration of 60, 80 and 100 µg/ml respectively. The zones of growth inhibition around the disc were measured after 24 h of incubation at 37ºC. while, Fluconazole was used as a positive control.

V.RESULT AND DISCUSSION

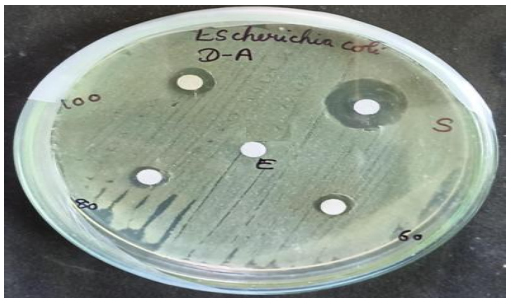
Screening of Antimicrobial activity of *Alangium Salvifolium*

The prepared herbal hydrogel of various concentration and alcoholic extract of *Alangium Salvifolium* leaves are exhibited for antimicrobial activity against various microorganism such as Gram-negative bacteria, viz Escherichia coli (MTCC 443), Gram-positive bacteria Staphylococcus aureus (MTCC 87) and fungi viz., Aspergillus flavus (MTCC 277) and Candida albican (MTCC 183).

ANTIBACTERIAL ACTIVITY OF F1 [FORMULATION]

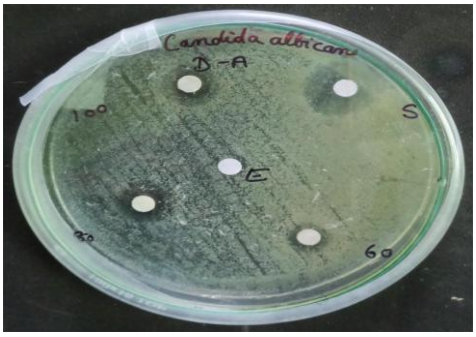


Staphylococcus aureus

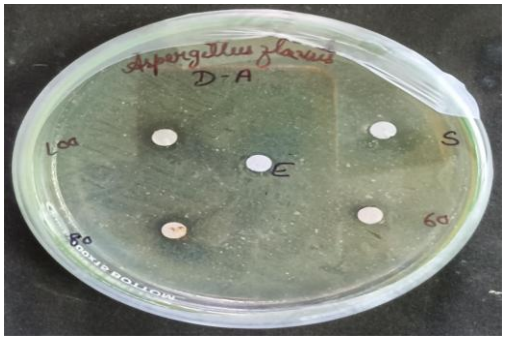


Escherichia coli

ANTIFUNGAL ACTIVITY OF F1 [FORMULATION]

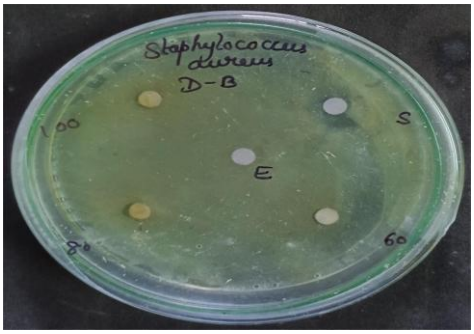


Candida albicans

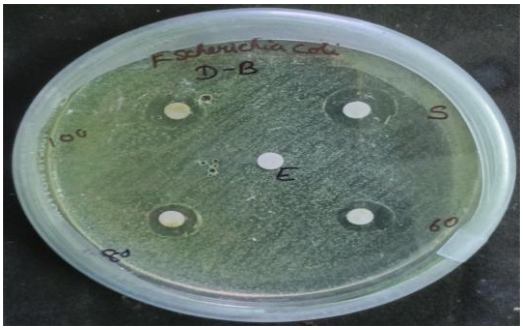


Aspergillus flavus

ANTIBACTERIAL ACTIVITY OF F2 [FORMULATION]

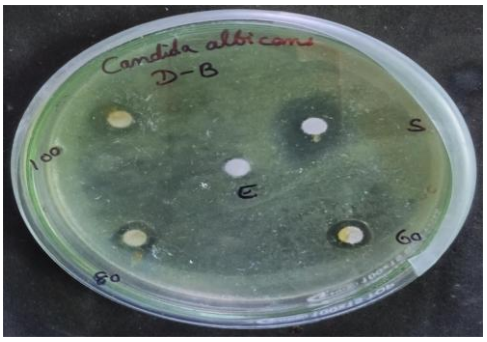


Staphylococcus aureus

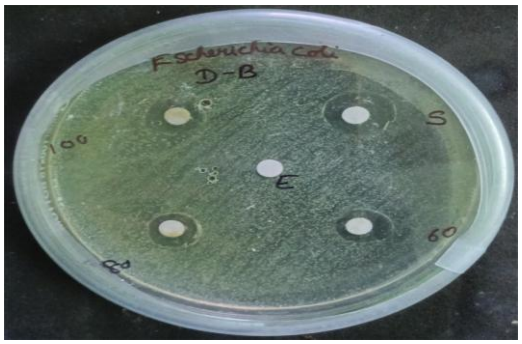


Escherichia coli

ANTIFUNGAL ACTIVITY OF F2 [FORMULATION]

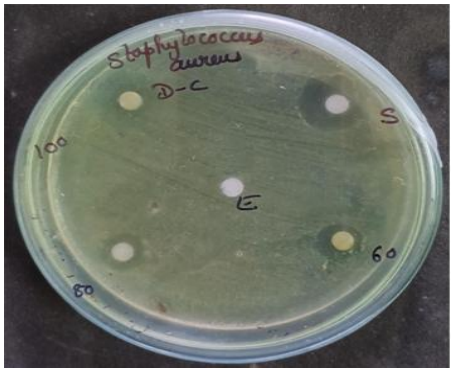


Candida albicans

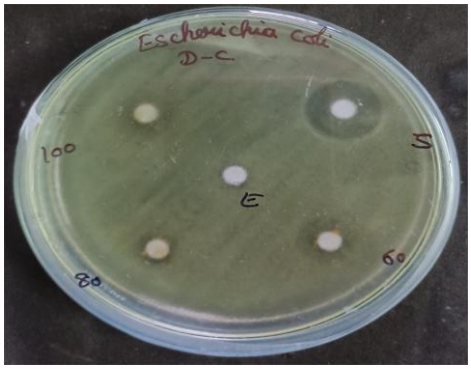


Aspergillus flavus

ANTIBACTERIALACTIVITY OF F3 [FORMULATION]

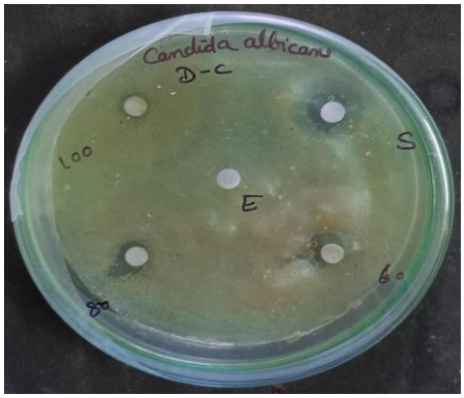


Staphylococcus aureus

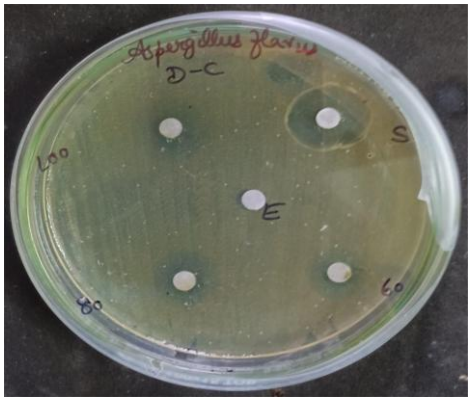


Escherichia coli

ANTIFUNGAL ACTIVITY OF F3 [FORMULATION]



Candida albicans



Aspergillus flavus

Screening of Antibacterial activity of F1, F2 and F3[Formulation]

Samples	Concentrations (µg/ml)	Organism/Zone of inhibition(mm)					
		Staphylococcus aureus			*Escherichia coli*		
		F1	F2	F3	F1	F2	F3
Samples	60	3	0	4	1	3	5
	80	5	0	5	2	4	6
	100	6	0	6	3	6	7
Standard(std)(Amoxicillin)	10 µl/disc	8	7	8	9	8	9
ETHANOL	10 µl/disc	0	0	0	0	0	0

Table No.2: Screening of Antibacterial activity of F1, F2 and F3

The antibacterial activity of F1 , F2 , F3 and Herbal extract are evaluated. The herbal extract are produce a better inhibition activity than prepared formulation, but prepared formulation produce better activity in minimum concentration of extract.

In comparision with F1 ,F2 and F3, F3 showed greater inhibition against *Staphylococcus aureus* ssand *Escherichia coli.* The prepared formulation are compared with standard

Screening of Antifungal activity of F1, F2 and F3[Formulation]

Samples	Concentrations (µg/ml)	Organism/Zone of inhibition(mm)					
		Candida albicans			*Aspergillus flavus*		
		F1	F2	F3	F1	F2	F3
Samples	60	3	2	1	1	4	3
	80	5	3	2	2	5	6
	100	7	5	3	3	7	9
Standard(std) (Amoxicillin)	10 µl/disc	9	9	8	8	8	10
ETHANOL	10 µl/disc	0	0	0	0	0	0

Table No.3: Screening of Antifungal activity of F1, F2 and F3

The antifungal activity of F1 , F2 , F3 and Herbal extract are evaluated. The herbal extract are produce a better inhibition activity than prepared formulation, but prepared formulation produce better activity in minimum concentration of extract. In comparision with F1 ,F2 and F3, F3 showed greater inhibition against *Candida albicans* and *Aspergillus flavus.* The prepared formulation are compared with standard

UV Spectrum of Alangium Salvifolium

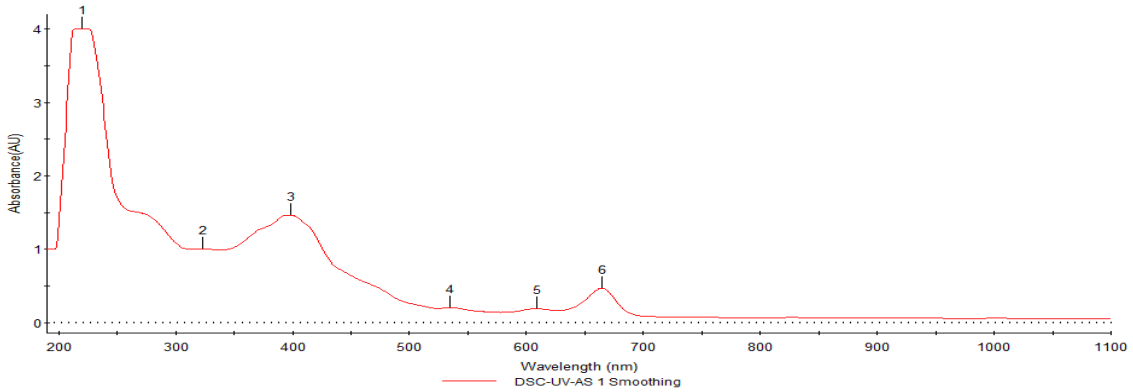


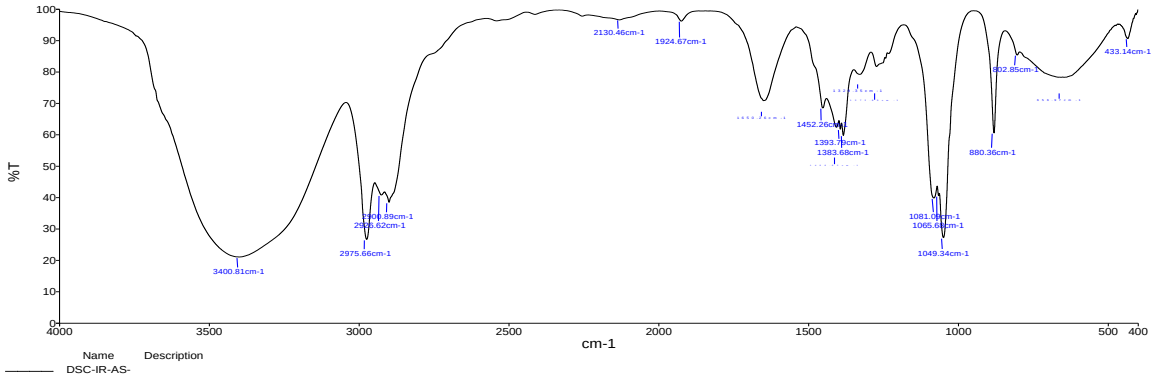
Figure 2 :UV Spectrum of Alangium Salvifolium

The UV-Visible absorption spectra for the sample were taken in the range of 200 - 800nm. The Maximum absorption of *Alangium salvifolium* leaves extract was found at 219nm.

S.NO	WAVELENGTH (nm)	ABSORBANCE
1.	219.9	4.000
2.	323.3	1.008
3.	397.6	1.465
4.	535.5	0.205
5.	608.7	0.191
6.	664.5	0.473

Table 4 :UV Spectrum of Alangium Salvifolium

FTIR Studies: FTIR Spectrum and interpretation of Ethanolic Extract of Alangium Salvifolium



FTIR Spectrum of Ethanolic Extract of Alangium Salvifolium

S.NO	WAVE NUMBER(cm^{-1})	FUNCTIONAL GROUP
1.	3400.81	O-H Stretching
2.	2975.66	C-H Stretching
3.	2130.46	C≡C Stretching
4.	1924.87	C≡N Stretching
5.	1452.26	-COO- Stretching
6.	1065.63	C-O Stretching
7.	883.36	C-H Bending

Table 5: FTIR Spectrum of Ethanolic Extract of Alangium Salvifolium

VI. CONCLUSION

The study determine the good antimicrobial activities and the desired physical properties of the Hydrogel formulations containing the extract. These could make them potential topical antimicrobial agents effective in the treatment of skin infection. The use of alcoholic herbal extract produce a effective antimicrobial property. The prepared formulation (F3) show effective in both bacteria and fungi. The zone of inhibition is more in Formulation 3 in both anti microbial and anti fungal activity. So the prepared Hydrogel have better antimicrobial and antifungal property.

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