Antimicrobial activity and Screening of Herbal gel using Extract of Blueberry and Banana

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Abstract

The aim of present work is to study of antimicrobial activity of Herbal gel. The Antimicrobial herbal gel is developed with the use of Blueberry and banana Extract and tried for various microorganisms like Staphylococcus aureus, Corynebacterium, staphylococcus, Brevibacterium we found the inhibitory concentration of these microbes. Herbal medicine now become an important item for both medicinally and economically. The quality, safety, and effectiveness of these herbal products have improved along with their use. Due to the some side effects of allopathic medications, patients are becoming more and more compliant with herbal therapies. Herbal medications are considered safer than allopathic medicines as allopathic medicines are associated with the side effects. The formulas for Polyherbal Gel are created. To create an optimum formulation, the concentration of extracts in Polyherbal formulations is maintained at the chosen level while the quantity of other ingredients is adjusted. Each formulation is then evaluated to see which the best.

Keywords: Gel, *Blueberry* (fruit) and *Banana* (fruit), Staphylococcus aureus, Corynebacterium, staphylococcus, Brevibacterium, Disk Diffusion Method.

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Introduction:

In the past, people have used both artificial and natural remedies to cure these infections[1]. Herbal therapies are growing more and more popular today because they are less harmful or have no adverse effects than manufactured pharmaceuticals[2]. Infectious illnesses have traditionally been treated with medicinal herbs like tulsi, aloe vera, neem, turmeric, and turmeric. [3-8] In order to cure skin diseases, herbal topical medicines became more and more popular. Numerous plant compounds called phytochemicals have been found by scientists to inhibit a wide range of

microorganisms through a variety of mechanisms and function as secure, broad-spectrum antibiotics in the treatment of microbial strains that are resistant to antibiotics and other synthetic antimicrobials. [9]. Topical antimicrobial agent application at the infection site is more advantageous than systemic therapy. [10–11.] First off, topical dosing makes it straightforward to get the medicine into the right concentration at the target spot for antibacterial activity. Second, after topical treatment, systemic levels of the active components are greatly lowered or almost undetectable[19]. Thirdly, it can avoid exposing the gut flora to antimicrobial medications unnecessarily, which could lead to drug resistance or a decline in the GIT's natural bacterial population. Therefore, topical application of antimicrobial drugs is considered to be a substantial alternative to systemic antibiotic administration for the treatment of skin diseases[17]. The Antimicrobial herbal gel is developed with the use of Blueberry and banana Extract and tried for various microorganisms like Staphylococcus aureus, Corynebacterium, staphylococcus, Brevibacterium we found the inhibitory concentration of these microbes.

There are two types of skin infections: primary and secondary. Primary infections frequently begin in healthy skin, develop in a certain way, and are caused by solitary organisms. Corynebacterium, streptococcus pyogenic, and staphylococcus aureus are the most often found offenders. Typical symptoms include impetigo, folliculitis, boils, and erythrismal. Skin symptoms of a possible systemic infection. The most frequent reason for future infections is skin issues. Herbal remedies are thought to be less likely to have negative effects than those created with synthetic ingredients. Market data show that a key factor in the upward trend in the herbal trade, which is raising demand for herbals internationally, is the herbal cosmetic industry. Products made from herbs have been touted for their efficacy, inherent acceptance, and lack of the adverse effects frequently associated with synthetic products. [12-18]

Material and Methods

Fresh fruits of blueberry and banana were purchased from market suppliers from Chhatarpur in May 2019, and authentication of plant material was done by taxonomist Dr. Manjusa Saxena at the Department of Botany, Govt. Maharaja College, Chhatarpur (M.P.).

A suitable amount of polysorbate was dissolved in 5 ml of hot water to make each formulation. The quantity of various ingredients needed to prepare gel bases was determined through experimental design. The supplied quantity of carbopol 940 was then thoroughly mixed with 50

ml of deionized water for 20 minutes. This mixture was retained for soaking the next day. In another beaker, ethanol, and deionized water were combined with the necessary amounts of propylene glycol, isopropyl myristate, and cremophor. The second beaker received the necessary concentration of a Blueberry fractionated extract that was diluted in ethanol 90% in consideration of its MIC.

Formula of Polyherbal gel

In polyherbal formulation concentration of extracts was kept fixed as selected and the quantity of other ingredients varied to get an optimized formulation and evaluation of each formulation was performed to select the best formulation. The final formulations were selected based on the results of the evaluation.

Ingredient /Formulations	F1	F2	F3	F4	F5	F6	F7	F8
Carbapol (g)	1	0.25	0.5	0.75	0.4	0.6	1	0.5
Propylene glycol (ml)	5	5	5	5	5	5	5	5
Potassium sorbate (ml)	0.5	0.5	0.5	0.25	0.25	0.25	0.25	0.25
Isopropyl myristate (ml)	5	5	5	5	5	5	5	5
Blueberry extract fraction (g)	2	2	2	2	2	2	2	2
Banana extract active fraction (g)	3	3	3	3	3	3	3	3
Alcohol (ml)	25	25	25	25	25	25	5	25
Cremophor (g)	2	2	2	2	2	2	2	2
Water Q.S (ml)	100	100	100	100	100	100	100	100

Antimicrobial Activity of Herbal Gel

Screening of antimicrobial activity

- **Preparation of culture media and culture plates:**

Agar	-	20 gms
Yeast extract	-	10 gms
Peptone	-	10 gms
Sodium chloride	-	5 gms
Distilled water	-	to make 1000 ml.

Method of preparation: Agar medium was dissolved in distilled water and boiled in conical flask of sufficient capacity. Dry ingredients are transferred to flask containing required quantity of distilled water and heat to dissolve the medium completely. pH 7 was maintained by adding buffer solution. The flask containing medium was cotton plugged and was placed in autoclave for sterilization at 15 lbs /inch^2 (121^{o}C) for 15 minutes. Disk diffusion method was used for the determination of antimicrobial activity.

List of microorganisms

S.No.	Name of microorganism	Strain	Characteristics
1	Staphylococcus aureus	NCTC-9002	Gram positive
2	Corynebacterium	NCTC-6017	Gram positive
3	staphylococcus epidermidis	ATCC-6538	Gram positive
4	Brevibacterium	ATCC-9027	Gram positive
5	Candida albicans	ATCC-6832	Fungi
6	***A***ctinomycetoma	ATCC-4042	Actinomycetes
7	**s**treptococcus	ATCC- 10231	Gram Positive

Disk diffusion method

Screening of antimicrobial activity of extracts and standard drugs (chloramphenicol gentamicin and fluconazole) was done by disk diffusion method. It was performed using 24 hours incubation

(for bacterial culture) and 48 hours (for fungal culture) at 37^{o}C in 20 ml of agar medium. Bacterial and fungal inoculums were spread over the plates containing agar medium using a sterile cotton swab in order to get a uniform microbial growth on both control and test plates. The extracts were dissolved in ethylene glycol and sterilized by filtration under aseptic conditions; empty sterilized discs (what man no. 5, 6 mm diameter) were impregnated with 100µl of each of the extracts of different concentration and left to dry under laminar flow cabinet and placed on the agar surface. Paper disk moistened with ethylene glycol was placed on the seeded Petri dish as a vehicle control. Standard discs containing chloramphenicol (10µg/ml), gentamicin (10µg/ml) and fluconazole (10µg/ml) were used as reference control. All Petri dishes were sealed with sterile laboratory paraffin to avoid contamination and eventual evaporation of the test samples. The dishes were left for 30 minutes at room temperature to allow the diffusion of test drugs and kept for incubation on 37^{o}C.(Mehta *et al* 2011, Badria *et al* 2004, Chairandy *et al* 1999)

Incubation of plates

The dishes containing the bacterial culture and fungal culture were incubated at 37^{o}C for respectively 24 hours and 48 hours. After the incubation time all dishes were examined for the presence of zones of inhibition. The antibacterial activity was interpreted from the size of the diameter of zone of inhibition measured to the nearest millimeter (mm).As observed from the clear zones surrounding the discs.

Minimum inhibitory concentration Assay

The agar dilution method recommended by the National Committee for Clinical Laboratory Standards was used. A series of two fold micro dilution of isolated fraction with ethylene glycol was prepared. Plates were dried at room temperature for 30 min prior to spot inoculation. Firstly plates were Inoculated and then theses plates were incubated at 37°C for 18-24 hours after that minimum inhibitory concentration was determined. Inhibition of bacterial growth in the plates containing test extract was judged by comparison with growth in blank control plates. The lowest concentration of the extracts in the wells of the microliter plate that showed no turbidity after 24 hours of incubation at 37° C was considered as Minimum inhibitory concentration. (National Committee for Clinical Laboratory Standards)

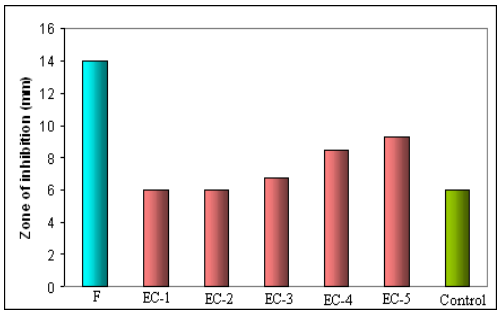
Screening of Antimicrobial activity of blue berry extract

The screening of anti-microbial activity was performed with the help of disc diffusion method. Following tables shows anti-microbial activity of mentholic extract of *Blueberry* at different concentration against gram positive bacteria and fungi.

Micro-Organism ↓ **Name of drug**	Staphylococcus aureus		Candida albicans		*A*ctinomycetoma	
	In mm Mean	**As %**	**In mm Mean**	**As %**	**In mm Mean**	**As %**
Gentamycin (10 mg/ml)	17.67±1.47	100	16.88±0.87	100	19.34±0.68	100
Chloromphenicol (20 mg/ml)	16.33±0.33	92	10.45±0.87	61	11.56±0.63	59
MeOH Extract of *Blue berry* (mg/ml)						
15	6.00± 00	00	6.00± 00	00	6.00± 00	00
30	6.00± 00	00	6.00± 00	00	6.00± 00	00
45	6.00± 00	00	8..44± 00	50	7.42±0.54	38
60	8.00± 00	45	9. 34±0.85*	55	8.20±0.52	42
90	9.36± 0.47*	52	9. 49±0.46*	56	9.89±0.38*	51
Control	6.00±00	00	6.00±00	00	6.00±00	00

Zone of inhibition for various concentrations of *Blue berry extract* compared to reference drugs: activity against bacteria.

Methanolic extract of *Blue berry* have shown the significant activity against Staphylococcus aureuson the concentration 90 mg/ml, against Candida albicans on the concentration 60 and 90 mg/ml and against ***A***ctinomycetoma it has shown significant activity on 90 mg/ml concentration. On the concentration of 15, 30 mg/ml no zone of inhibition was observed.



Zone of inhibition for various concentrations of *Blue berry* compared to reference drugs

Antimicrobial activity of methanolic extract of *Banana*

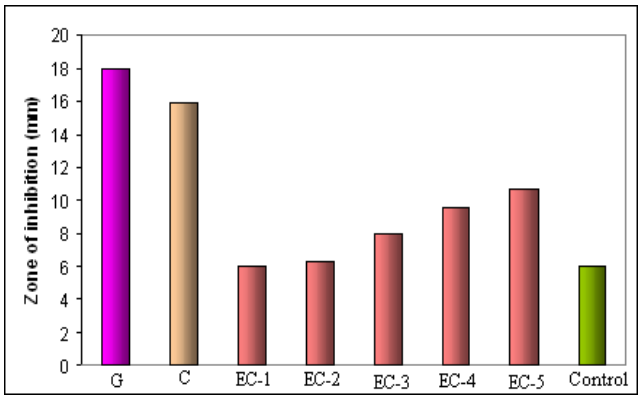
The screening of anti-microbial activity was performed with the help of disc diffusion method. Following tables shows anti-microbial activity of methenolic extract of *Banana* at different concentration against gram positive bacteria and fungi.

Micro-Organism → / **↓ Name of drug**	Staphylococcus aureus		Candida albicans		***A***ctinomycetoma	
	In mm Mean	**As %**	**In mm Mean**	**As %**	**In mm Mean**	**As %**

Gentamycin (10 mg/ml)	17.67±1.47	100	16.88±0.87	100	19.34±0.68	100
Chloromphenicol (20 mg/ml)	16.33±0.33	92	10.45±0.87	61	11.56±0.63	59
MeOH Extract of *Banana* (mg/ml)						
20	6.00± 00	00	6.00± 00	00	6.00± 00	00
30	6.00± 00	00	6.00± 00	00	6.00± 00	00
40	7.00± 00	00	8..44± 00	51	7.52±0.54	39
50	9.00± 00	49	9. 44±0.85*	57	8.30±0.52	43
60	9.88± 0.47*	54	9. 49±0.46*	59	9.69±0.38*	52
Control	6.00±00	00	6.00±00	00	6.00±00	00

Zone of inhibition for various concentrations of *Banana* compared to reference drugs

Methanolic extract of *Banana* have shown the significant activity against Staphylococcus aureus on the concentration 40 mg/ml, against Candida albicans on the concentration 40 mg/ml and 50 mg/ml and against ***A***ctinomycetoma it has shown significant activity on 50 mg/ml concentration. On the concentration of 20, 30 mg/ml no zone of inhibition was observed.



Zone of inhibition for various concentrations of *Banana* compared to reference drugs

Conclusion

In my present work two plants Blueberry and Banana fruits are extracted and used the disc diffusion method to test them for antibacterial activity against a variety of oral infections. It was discovered that both plants' methanolic extracts had good and moderate antimicrobial activity. These extracts were then fractionated according to their bioactivity. The acetone fraction (fraction-III) of the methanolic extract of blueberries and the ethyl acetate fraction of the methanolic extract of bananas were shown to be active against skin infections after each fraction's antibacterial activity was tested .These active fractions were once more exposed to the isolation of the substance that may be in charge of these plants' antibacterial activity against some oral infections.

We created our own Polyherbal Gel compositions, and many measures were employed to standardise and assess them. The formulations' testing results against a number of parameters were satisfactory, thus their antibacterial activity was assessed once again. Additionally, a study on accelerated stability and temperature- and humidity-dependent degradation for compound stability was carried out. The findings suggest that these formulations will be able to prevent or treat a variety of skin issues without causing any negative side effects. The plant extract used in the experiment demonstrated antibacterial activity against fungal, Gram-positive, and Gram-negative strains, which raises the possibility that it could serve as a source for the creation of drugs with a variety of effects. The study's findings also lend support to the plants' traditional uses and imply that chemicals found in plant extracts may have antibacterial characteristics and serve as potential antimicrobial agents. Future challenges will include more research and testing of these herbal

dental formulations, including the separated chemicals, on a commercial scale, as well as clinical and toxicological studies. **References**

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