WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

FORMULATION AND EVALUATION OF ANTIMCROBIAL ACTIVITY OF POLYHERBAL FORMULATION

K. Saritha *, A. Ahalya Bindhu , R. Madhusudhan , G. Mounika, K. Parshuram, S. Haritha

K.V.K College of Pharmacy, Surmaiguda, Abdullapurmet, Rangareddy(Dist), Telangana-501512,India.

ABSTRACT

In the recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries because herbal medicines have been reported safe with minimal adverse side effect especially when compared with synthetic drugs. In the present study we prepared gel formulations which comprised of ethanolic extract of Bauhinia tomentosa, Tephrosia villosa and Citrullus colcoynthis. The results clearly indicated that the three extracts when mixed at different proportions showed superior antimicrobial activity against all the organisms when compared to single extracts of the same plants. The results obtained from this study supported the preference and effectiveness of mixed extracts by traditional healers in management of opportunistic infections. From the results it was clear that both the single extracts and multi extracts of all three plants showed differentiating antimicrobial activity against all the selected bacterial and fungal strains. The formulations were also evaluated for appearance and homogeneity, pH, viscosity and rheological studies, spreadability, drug content uniformity, skin irritation test (Patch test) and washability

Keywords*: Antibacterial Activity, Patch test, Polyherbal gel.*

1. INTRODUCTION

A Wide variety of herbal remedies can benefit the skin when applied topically. They usually heal the skin by fighting inflammation, promoting healthy circulation, or soothing irritations. Many have antibacterial, antiviral, antifungal, or antiseptic properties. Some act as astringents and others as analgesics. Topically applied herbal remedies can promote the healing of wounds and burns, treat common skin conditions, and support healthy skin. In skin care formulations blends of herbs complement each other and produce even more powerful results36. The use of multi-plant extracts in traditional healing systems is a common ethnomedicinal practice in many parts of the world. In Venda (Cyprus), mixed root extracts of *Tabenaemontana elegans*, *Terminalia sericea, Euclea natalensis, X*

single plant extracts37. Basedon the above information, in the present study three medicinal plants viz. *Bauhinia tomentosa, Tephrosia villosa and Citrullus colcoynthis* which have proved to have many pharmacological activities including antimicrobial, antioxidant, anti-mycotic, antihyperglycemic, anti-spermatogenic are selected. Among the above mentioned activities, it was found that anti-microbial activity is a common pharmacological activity shown by the three plants. Hence in this work, the antimicrobial properties of mixed and single extracts of three plants are studied and novel herbal cream formulations with marked antimicrobial activity is prepared and compared with a standard drug with a view to confirm the use of the prepared formulation as a remedy for skin disease.

2. MATERIALS AND METHODS

Collection of Plant Materials:

The three medicinal plants viz. *Bauhinia tomentosa, Tephrosia villosa and Citrullus colocynthis* were selected based on ethnomedical importance. Healthy, disease free plants were selected from local areas of Kerala, Karnataka and Rajasthan. It was authenticated by the Botanist T. Nagendra, Head of the Department of Botany, Bharathi College of Pharmacy.

Preparation of Extracts:

Collected plants were washed thoroughly and chopped into small pieces, shade dried and grinded into powdered form. After this, the coarse powder of three plants (600g *Bauhinia tomentosa*, 750g *Tephrosia villosa*, and 400g *Citrullus colocynthis*) were packed well in thimble and was exhaustively extracted with methanol individually for 72 hours in a Soxhlet apparatus. Evaporation of the solvent under reduced pressure gave crude extracts of BT, TV and CC separately. The extractive values of three plants were calculated as follows.

$$\% \text{ Extractive value (yield \%)} = \frac{\text{Weight of dry extract}}{\text{Weight taken for extraction}} \times 100$$

The extracts obtained were placed in sterile screw capped bottles and stored at 4°C until further use[68]. Subsequently a known weight of each air-dried extract was dissolved in known volume of 5% DMSO to give the desired concentration of each extract and was used for the present study. Later the three plant extracts were used to conduct preliminary phytochemical analysis, antimicrobial studies and preparation of herbal formulations.

Qualitative Phytochemical Analysis:

The methanolic extracts of *Bauhinia tomentosa*, *Tephrosia villosa* and *Citrullus colocynthis* were subjected to chemical tests for identification of the various active constituents.

Antimicrobial Screening of Extracts:

Microorganisms: Bacterial and fungal strains were obtained from NCIM. The following Gram-positive, Gram-negative and fungal organisms were used in the present study to determine the antimicrobial activity of the three plant extracts.

- Gram Positive OrganismsBacillus coagulans Bacillus subtilis Staphylococcus aureus
- Gram Negative Organisms Escherichia coli Kleibseilla pneumonia Pseudomonas aeruginosa
- Fungi
  (NCIM 2313)
  (NCIM 2063)
  (NCIM 2079)
  (NCIM 2345)
  (NCIM 2239)
  (NCIM 2200)
  Aspergillus flavus (NCIM 0535)
  Candida albicans (NCIM 3471)
  Cryptococcus neoformans (From CMC)

The bacterial and fungal stock cultures were maintained on Nutrient agar and Sabouraud-dextrose agar respectively which were stored at 4oC.

Antibacterial Activity:

B. Preparation of test inoculum

(a) Sub culture (Preparation of seeded broth)

The strains of bacteria were inoculated into conical flasks containing 100 ml of sterile nutrient broth. These conical flasks were incubated at 37°C ± 1°C for 24 hours. This was stored as seeded broth.

(b) Viable count

(i) Dilutions

1 ml of 24 h seeded broth of each strain was diluted with 9 ml of sterile water. 1 ml of this was further diluted to 10 ml with sterile water. This was continued till 10^{-2} to 10^{-7} dilutions of the seeded broth were obtained

(ii) Incubation of nutrient agar Petri dishes

The dilution was studied by inoculating 0.1 ml of each dilution on to the solidified nutrient agar medium by spread plate method. After incubation at 37°C ± 1°C for 24h, the number of well formed colonies on the plates were counted. The seeded broth was then suitably diluted to contain between 10^{-6}-10^{-7} microorganisms per ml or Cfu/ml. This was designated as the working stock, which was used for antibacterial studies.

Antifungal activity:

The medium was sterilized by autoclaving at 15 lb/sq. inch pressure at 121°C for 15min.

B) Preparation of test inoculums:

(a) Sub-culture (Preparation of seeded broth): The strains of fungi were inoculated into conical flasks containing 100 ml of sterile SDB. These conical flasks were incubated at 28° ± 1°C for 48 hours. This was stored as seeded broth.

(b) Viable count

(i) Dilutions

1 ml of 24 h seeded broth of the strain was diluted with 9 ml of sterile water and 1ml of this was further diluted to 10 ml with sterile water. This was continued till 10^{-2} to 10^{-7} dilutions of the seeded broth were obtained.

(ii) Incubation of SDA Petri dishes

The dilutions were studied by inoculating 0.1 ml of each dilution on to the solidified SDA by spread plate method. After incubation at 28° ± 1°C for 48 h, the number of well formed colonies on the plates were counted. The seeded broth was then suitably diluted to contain between 10^{-6}-10^{-7} microorganisms per ml or Cfu/ml. This was designated as the working stock, which was used for antifungal studies.

Preparation of extracts at different concentration:

The solution of three plant extracts (*BT, TV* and *CC)* were prepared by dissolving each of the extracts in DMSO and stored in a refrigerator. The solutions were removed from the refrigerator 1 hour prior to their use and allowed to warm up to room temperature. The solution of the extracts at different concentrations such as 1000µg/ml, 500µg/ml, 250µg/ml, 125µg/ml and 62.5µg/ml were prepared separately. Standard drug penicillin for gram positive bacteria and gentamycin for gram negative bacteria at a

Concentration of 100 µg/ml and griseofulvin for fungi at a concentration of 200µg/ml were also prepared.

Procedure:

Petri dishes were cleaned, dried and sterilized. The sterilized nutrient agar media for gram positive and gram negative bacteria and SDA media for fungi was poured in the plates with uniform thickness and allowed to solidify. After solidifying, the plates were inoculated by adding 0.1 ml of the above mentioned gram positive, gram negative bacteria and fungi. Three holes were made in the inoculated plates by means of a stainless steel sterile borer with a height of 10 cm and an internal diameter of 6 mm. In the first plate 1000µg/ml of crude extract solution of *Bauhinia tomentosa*, *Tephrosia villosa and Citrullus colocynthis* were added separately to each hole. Similarly in other plate's 500µg/ml, 250µg/ml, 125µg/ml and 62.5µg/ml of three plant extracts were added to the three holes. Each of the three plant extracts at different concentrations were screened against all 3 gram positive, 3 gram negative bacteria and 3 fungi separately three times. All the plates were incubated at 37°C for 24h except the fungal inoculated plates. The fungal inoculated plates were kept in room temperature. All the standard drugs were also tested against the same organisms. The inhibition zone for each hole or well were noted and recorded. From the obtained results the least concentrations in which the three plant extracts showed anti-microbial activity was selected i.e. 125 µg/ml and 250 µg/ml. The three extracts in these concentrations were prepared and mixed in different proportions 1:1:2 (MPE-1), 1:2:1 (MPE-2) and 2:1:1 (MPE-3) to study the effect of multi-plant extract over single plant extract.

Preparation of herbal Cream Formulations:

After selecting the least concentrations in which the three plant extracts showed antimicrobial activity, formulations in the form of cream was prepared by mixing the three extracts in different proportions using a suitable cream base.

Composition of cream base

S.No	Ingredients	% w/w
1.	Stearic acid	16.00
2.	Potassium hydroxide	02.00
3.	Cetosteryl alcohol	05.00
4.	Liquid paraffin	03.50
5.	Petroleum jelly	03.50
6.	Methyl paraben sodium	00.16
7.	Propyl paraben sodium	00.04
8.	Glycerine	07.00
9.	Purified water	62.80

Procedure:

Aqueous cream seems to be as effective as the test agents because of its easy penetration through the skin. Stearic acid, cetosteryl alcohol, liquid paraffin and petroleum jelly were melted on a steam bath at 75^{O}C. The remaining ingredients were dissolved in water and boiled at 75^{O}C. This aqueous solution was then added to the above oily phase with agitation. Potassium hydroxide reacts with a portion of stearic acid to form potassium stearate, which emulsifies the unreacted stearic acid as dispersed phase. Excessive agitation was avoided so as to avoid air getting entrapped within the cream. Glycerin was added finally and mixed.[72]

After selecting the least concentrations in which the three plants showed antimicrobial activity, i.e 125 µg/ml and 250µg/ml the extracts were prepared and mixed with the above prepared cream base in different proportions i.e 1:1:2 (Formulation-1), 1:2:1 (Formulation-1) and 2:1:1 (Formulation3) with uniform stirring using a mechanical stirrer. The formulated cream was filled in suitable plastic containers.

Antimicrobial study of the cream formulations and multi-plant extracts: Procedure:

Petri dishes were cleaned, dried and sterilized. Then they were filled with Nutrient Agar medium for gram positive and gram negative bacteria and SDA medium for fungi with uniform thickness. After solidifying, the plates were inoculated with the above mentioned organisms of Gram positive, Gram negative bacteria and fungi. Three holes were made in the inoculated plates by means of aluminum or stainless steel sterile borer with a height of 10 cm and an internal diameter of 6 mm. In the first plate cream base, MPE-1 and Formulation-1 was added. In the second plate cream base, MPE-2 and Formulation-2 was added and in the third plate cream base, MPE-3 and Formulation-3 was added. Similar procedure was done against all the 3 gram positive bacteria, gram negative bacteria and 3 fungal strains. Then the plates were kept in the refrigerator for 2 h for diffusion. All the plates were incubated at 37^{o}C for 24 h except the fungal inoculated plates. The fungal inoculated plates were kept in room temperature. The zone of inhibition for each hole or well were noted and recorded.

Accelerated Stability Studies:

The purpose of stability testing is to provide evidence on how the quality of an active substance or medicinal product varies with time under the influence of a variety of environmental factors such as temperature, humidity, light and to establish a re-test

period for the active substance or a shelf-life for the finished product and recommended storage conditions. As per the ICH guidelines, the recommended temperature for long term stability is 25^{o} ± 2^{o}C and 60% ± 5% RH, for accelerated stability studies 40^{o} ± 2^{o}C and 75% ± 5% RH (at least 15^{o}C warmer than proposed long-term storage condition) and for refrigerated storage conditions 5^{o} ± 3^{o}C with no corresponding humidity condition is

imposed. Finally for the intermediate storage condition the temperature recommended is 30° ± 2°C and 60% ± 5% RH (intermediate storage condition is only required when a significant change occurs prior to the completion of 6 months accelerated stability studies)[73].

For long term stability studies, the time required for analysis of samples are once in three months for first one year and once in six months for the second year and further. The time required for accelerated stability studies are month 0, 3 and 6. The samples to be analyzed for its quality and for the data to be maintained, the shelf-life of the product is to be fixed as per the stability of the product at a specified temperature.

In present study, the formulated creams (individual collapsible tubes containing 10g each) were subjected to accelerated stability studies along with refrigerated storage conditions.

Physical appearance:

The formulated cream was observed for its visual appearance, transparency, colour, consistency and feel upon application such as stickiness, smoothness and cooling effects.

Determination of pH:

The pH of the prepared cream formulations and the base were determined using a pH meter (Eutech – cyber scan pH 310) by preparing 1 gm of cream formulation dissolved in 100 ml of water and maintaining a temperature of 25°C. The average of the triplicate observations was recorded.

Determination of Extrudability:

Extrudability is a useful empirical test to measure the force required to extrude the material from a tube. Since packaging in collapsible tubes has gained considerable importance in delivery of the desired quantity of cream, measurement of extrudability becomes an important critical data for creams. In the present study, the method adopted for evaluating cream formulation for extrudability was based upon the quantity (in percentage) of the cream extruded from a collapsible tube on application of certain load. More the quantity extruded, better is the extrudability.

The cream formulation was filled in a standard capped collapsible sealed tube. The tube was weighed and recorded. The tube was placed between two-glass slides and was clamped. A 500 g weight was placed over the glass slide and then the cap was opened. The amount of cream extruded was collected and weighed. The percentage of cream extruded was calculated and recorded as per grades allotted.

Determination of syneresis:

Many creams undergo a contraction upon standing and the interstitial liquid is expressed and collected at the surface of the cream. This process is referred as syneresis. Freeze thaw encycling method can be used to check whether separation or syneresis will occur. In the present study, the presence of interstritial liquid was observed by subjecting the product to a temperature of (4-10°C) for 12 h, then at room temperature (25°C) for 12 h and then 40°C for 12 h (freeze thaw cycling).

3. RESULTS

Table No.1: Data showing the Extractive values of Bauhinia *tomentosa, Tephrosia villosa and Citrullus colcoynthis*

S.no	Plant Name	Methanolic extract
1.	*Bauhinia tomentosa*	12.66% w/w
2.	*Tephrosia villosa*	14.93% w/w
3.	*Citrullus colcoynthis*	11.75% w/w

Table No.2: Data showing the phytochemical analysis of Bauhinia tomentosa, Tephrosia villosa and Citrullus colcoynthis

Constituents	***Bauhinia tomentosa***	***Tephrosia villosa***	***Citrullus colcoynthis***
Alkaloids	+	_	+
Carbohydrates	_	_	+
Glycosides	+	+	+
Protein & Amino acid	+	_	+
Saponins	+	+	+
Phenol	+	+	+
Flavanoids	+	+	_
Steroids	_	+	_
Sterols	_	_	_
Tannins	_	+	_

Table No.3: Data Showing the Anti-bacterial activity of methanol extract of *Bauhinia tomentosa, Tephrosia villosa, Citrullus colocynthis* and standard antibiotic Penicillin against Gram positive Bacteria.

Samples	Conc. (µg/ml)	Diameter of zone of Inhibition (mm)		
		B. coagulans	*B. subtilis*	*S. aureus*

I. Plant Extract	62.5	—	—	—
a) Bauhinia tomentosa	125	9.6±0.5[c]	8.3±0.6[c]	9.4±0.4[c]
	250	12.6±0.5[b]	10.3±1.2[c]	13.6±0.6[c]
	500	14.2±1.0[b]	12.3±0.5[a]	19.2±1.1[a]
	1000	17.3±0.6[a]	16.6±0.6[a]	21.8±1.4[a]
b) Tephrosia villosa	62.5	—	—	—
	125	8.3±0.5[c]	8.3±0.5[c]	9.3±1.1[c]
	250	12.3±0.3[c]	10.3±0.5[b]	13.2±0.4[b]
	500	14.4±0.8[b]	12.3±0.1[b]	19.3±0.5[a]
	1000	16.6±1.4[a]	14.6±0.4[a]	21.6±0.8[a]
c) Citrullus colocynthis	62.5	—	—	—
	125	8.2±1.4[c]	8.2±1.1[c]	9.6±0.5[c]
	250	9.3±0.5[c]	9.33±1.4[b]	14.3±0.1[b]
	500	12.6±1.0[b]	11.6±0.4[b]	19.6±0.4[a]
	1000	16.3±1.2[a]	17.3±0.5[a]	21.3±1.0[a]
	100	18.3 ± 0.57	15.8 ± 0.10	20.6 ± 0.57
II. Standard Drug				
a) Penicillin				

- Values are mean ± SD of three separate experiments.
- No Inhibition Zone.
- Statistical value [a]P <0.001; [b]P<0.01; [c]P<0.05 when compared to the standard.

Table No.4: Data Showing the Anti-bacterial activity of methanol extract of *Bauhinia tomentosa, Tephrosia villosa, Citrullus colocynthis* and standard drug Gentamycin against Gram negative Bacteria.

Samples	Conc. (µg/ml)	Diameter of zone of Inhibition (mm)		
		E-coli	*K. pneumonia*	*P. aeruginosa*
I. Plant Extract				
a) Bauhinia tomentosa	62.5	—	—	—
	125	8.6±1.1[c]	9.6±0.8[c]	9.3±0.4[c]
	250	12.3±0.5[b]	13.3±0.5[c]	11.6±0.6[c]
	500	18.3±0.8[a]	16.3±1.2[c]	14.2±1.1[b]
	1000	21.3±1.2[a]	22.2±0.6[a]	17.6±1.4[b]
b) Tephrosia villosa	62.5	—	—	—
	125	9.3±0.5[c]	9.3±0.4[c]	8.3±0.1[c]
	250	12.3±0.5[b]	12.3±0.5[c]	11.6±0.4[b]
	500	15.4±1.0[a]	17.2±1.2[a]	14.6±0.5[b]
	1000	19.6±1.2[a]	22.3±1.4[a]	17.3±0.8[a]
c) Citrullus colocynthis	62.5	—	—	—
	125	8.6±1.0[b]	9.2±0.6[c]	9.6±0.5[c]
	250	13.6±0.5[b]	13.3±0.4[c]	10.3±0.1[c]
	500	19.3±1.0[a]	18.6±0.8[a]	14.6±0.4[b]
	1000	22.3±1.2[a]	22.3±1.5[a]	18.8±1.4[a]
II. Standard Drug				
Gentamycin	100	21.6 ± 0.57	22.6 ± 1.0	18.6±0.5

- Values are mean ± SD of three separate experiments.

- No Inhibition Zone.
- Statistical value [a]P <0.001; [b]P<0.01; [c]P<0.05 when compared to the standard.

Table No.5: Data Showing the Anti-fungal activity of methanol extract of *Bauhinia tomentosa, Tephrosia villosa, Citrullus colocynthis* and Standard drug Griseofulvin.

Samples	Conc. (µg/ml)	Diameter of zone of Inhibition (mm)		
		A. flavus	*C. albicans*	*C. neoformans*
I. Plant Extract				
a) Bauhinia tomentosa	62.5	—	—	—
	125	8.3±0.5[c]	7.9±0.2[c]	—
	250	11.4±0.5[b]	10.4±1.2[c]	8.75±0.5[c]
	500	14.6±0.8[b]	13.6±0.5[a]	13.6±1.1[b]
	1000	16.8±1.0[a]	17.6±1.2[a]	17.6±1.2 [a]
b) Tephrosia villosa	62.5	—	—	—
	125	8.6±0.5[c]	9.3±0.5[c]	—
	250	11.8±0.4[c]	11.6±0.5[c]	8.9±0.4[c]
	500	14.4±0.8[b]	14.3±0.1[b]	10.8±1.2[c]
	1000	17.6±1.0[a]	17.2±0.4[a]	14.6±1.2[b]
c) Citrullus colocynthis	62.5	—	—	—
	125	9.3±0.5[c]	9.6±1.1[c]	8.8±0.5[c]
	250	10.6±0.5[b]	11.8±1.4[c]	10.7±0.1[b]
	500	14.8±1.0[b]	14.6±0.4[b]	14.8±0.4[b]
	1000	18.8±1.2[a]	20.3±0.5[a]	19.6±1.0[a]
	200	19.1 ± 0.57	19.2 ± 0.42	18.3 ± 1.5

II. Standard Drug Griseofulvin				

- Values are mean ± SD of three separate experiments.
- No Inhibition Zone.
- Statistical value aP <0.001; bP<0.01; cP<0.05 when compared to the standard.

Figure no. 4: Anti-microbial activity of standard drug Penicillin against Gram positive bacteria

Figure No. 5: Anti-microbial activity of standard drug Gentamycin against Gram negative bacteria

Figure No. 6: Anti-microbial activity of standard drug Griseofulvin against Fungal strains

Table No.6: Data showing the Anti-microbial activiy of the standard drugs, Multi-Plant Extract-1 and formulation-1 obtained from *Bauhinia tomentosa, Tephrosia villosa and Citrullus colocynthis*

S.no	Microorganism	Zone of Inhibition (in mm)		
	Gram positive bacterial organisms	Standard Penicillin	Multi-Plant Extract-1	Formulation -1
1.	*B. coagulans (NCIM -2313)*	18.3	14	10
2.	*B. subtilis (NCIM -2063)*	15.8	13	10
3.	*S. aureus (NCIM -2079)*	20.6	14	11
	Gram negative bacterial organisms	Standard Gentamycin	Multi-Plant Extract -1	Formulation -1
1.	*E. coli (NCIM -2345)*	21.6	13	09
2.	*K. pneumonia (NCIM -2239)*	22.6	11	08
3.	*P. aeruginosa (NCIM -2200)*	18.6	12	09
	Fungal organisms	Standard Griseofulvin	Multi-Plant Extract-1	Formulation -1
1.	*A. flavus (NCIM -0535)*	19.1	15	13
2.	*C. albicans (NCIM -3471)*	19.2	15	12
3.	*C. neoformans (from CMC*)	18.3	16	14

- **Formulation-1 = 1:1:2 Propotion of plant extracts of *Bauhinia tomentosa*(125µg/ml), *Tephrosia villosa*(125µg/ml) *and Citrullus colocynthis*(250µg/ml)**

Figure no. 7: Antimicrobial activity of *multi plant extract-1* and *formulation* against Gram positive bacteria

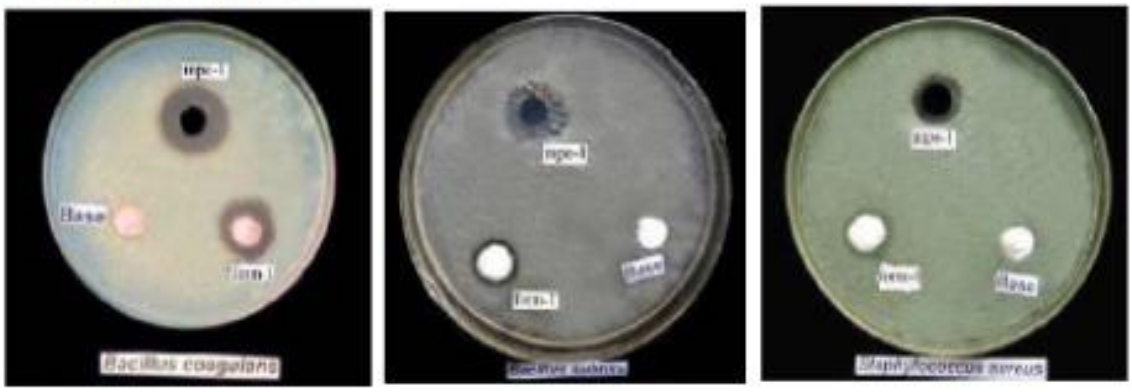

Figure no. 8: Anti-microbial activity of *multi plant extract-1* and *formulation-1* against Gram negative bacteria

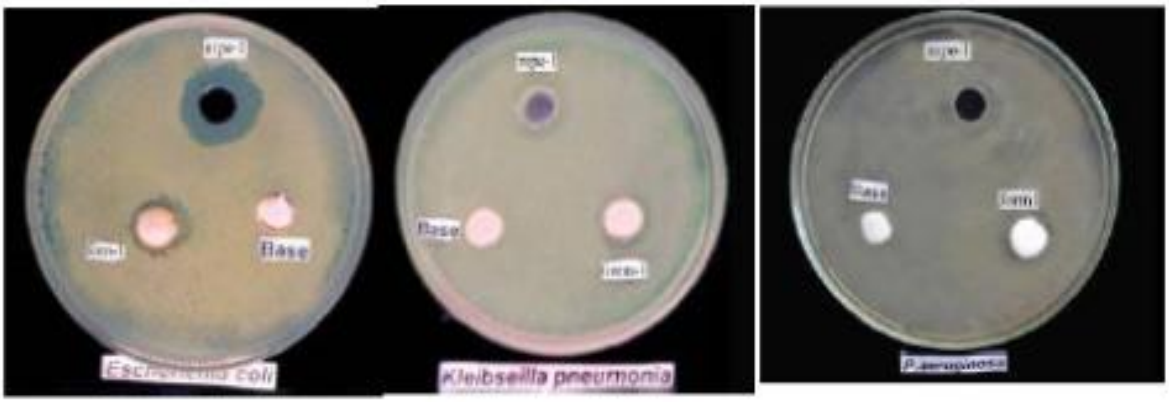

Figure no. 9: Anti-microbial activity of *multi plant extract-1* and *formulation-1* against Fungal strains

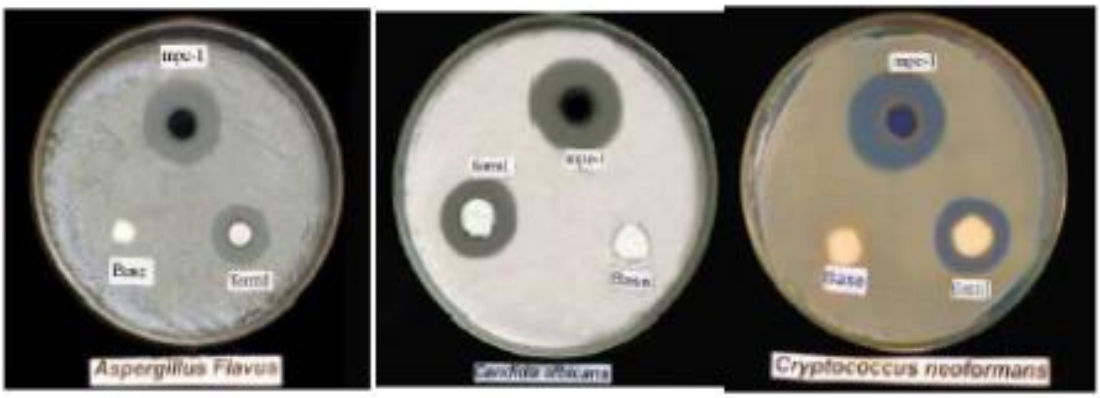

Table No.7: Data showing the Anti-microbial activity of the standard drugs Multi-Plant Extract-2 and formulation-2 obtained from *Bauhinia tomentosa, Tephrosia villosa and Citrullus colocynthis*.

S.no	Microorganism	Zone of Inhibition (in mm)		
	Gram positive bacterial organisms	Standard Penicillin	Multi-Plant Extract -2	Formulation -2
1.	*B. coagulans (NCIM -2313)*	18.3	11	09
2.	*B. subtilis (NCIM -2063)*	15.8	10	08
3.	*S. aureus (NCIM -2079)*	20.6	12	08
	Gram negative bacterial organisms	Standard Gentamycin	Multi-Plant Extract -2	Formulation -2
1.	*E. coli (NCIM -2345)*	21.6	11	08
2.	*K. pneumonia (NCIM -2239)*	22.6	10	09
3.	*P. aeruginosa (NCIM -2200)*	18.6	11	08
	Fungal organisms	Standard Griseofulvin	Multi-Plant Extract -2	Formulation -2
1.	*A. flavus (NCIM -0535)*	19.1	12	10
2.	*C. albicans (NCIM -3471)*	19.2	13	10
3.	*C. neoformans (from CMC*)	18.3	13	09

- Formulation-2 = 1:2:1 Propotion of plant extracts of *Bauhinia tomentosa*(125µg/ml), *Tephrosia villosa*(250µg/ml) *and Citrullus colocynthis*(125µg/ml)

Table No.8: Data showing the Anti-microbial activity of the standard drugs, Multi-Plant Extract-3 and formulation-3 obtained from *Bauhinia tomentosa, Tephrosia villosa and Citrullus colocynthis.*

S.no	Microorganism	Zone of Inhibition (in mm)

	Gram positive bacterial organisms	Standard Penicillin	Multi-Plant Extract -3	Formulation -3
1.	*B. coagulans (NCIM -2313)*	18.3	10	08
2.	*B. subtilis (NCIM -2063)*	15.8	10	07
3.	*S. aureus (NCIM -2079)*	20.6	11	08
	Gram negative bacterial organisms	Standard Gentamycin	Multi-Plant Extract -3	Formulation -3
1.	*E. coli (NCIM -2345)*	21.6	12	07
2.	*K. pneumonia (NCIM -2239)*	22.6	10	08
3.	*P. aeruginosa (NCIM -2200)*	18.6	11	08
	Fungal organisms	Standard Griseofulvin	Multi-Plant Extract -3	Formulation -3
1.	*A. flavus (NCIM -0535)*	19.1	12	10
2.	*C. albicans (NCIM -3471)*	19.2	14	11
3.	*C. neoformans (from CMC*)	18.3	112	09

Formulation-3 = 2:1:1 Proportion of plant extracts of *Bauhinia tomentosa*(250µg/ml), *Tephrosia villosa*(125µg/ml) *and Citrullus colocynthis*(125µg/ml)

4. DISCUSSIONS

In the present study three medicinal plants *viz*. *Bauhinia tomentosa*, *Tephrosia villosa* and *Citrullus colocynthis* with known antimicrobial activity was selected initially and their extraction was done exhaustively with methanol. Traditional healers use primarily water as the solvent for extraction. But earlier studies conducted by (Jigna Parekh) and team have proved that plant extracts in organic solvents provided more consistent antimicrobial activity compared to those extracted in water. Hence in this study methanol was selected as the solvent. The crude methanol extracts of three plants were obtained by using the soxhlet apparatus. The result of extractive values obtained from the three plants is given in **Table no 1**.

Secondly, qualitative phytochemical screening of three plants was done. The phytochemical screening of crude methanolic extracts of *BT, TV* and *CC* revealed that alkaloids, glycosides, proteins, saponins, phenols, flavanoids are present in *BT*. Glycosides, saponins, phenols, flavanoids, steroids and tannins are present in *TV* and alkaloids, carbohydrates, glycosides, proteins, phenols and saponins are present in *CC*. The results of phytochemical screening of three plants are given in **Table no.2**.

In the present study a variety of Gram positive and Gram negative bacterial and Fungal stains were selected for the screening of antimicrobial activity exhibited by the three selected plant extracts to perceive their antimicrobial spectrum and to find out the various concentration's in which the three plant extracts showed activity. **Table's 3-5** show the inhibitory effects of three plants *viz BT, TV* and *CC* individually on Gram positive and gram negative bacterial and fungal strains at different concentrations varying between 62.5µg/ml and 1000µg/ml. The results were compared with the antimicrobial activities shown by the standard drugs, Penicillin

(100 µg/ml) for Gram positive bacteria, Gentamycin (100 µg/ml) for gram negative bacteria and Griseofulvin (200 µg/ml) for fungal strains. The anti-microbial activity shown by standard drugs Penicillin, Gentamycin and Griseofulvin against gram positive, gram negative and fungal strains are demonstrated in **Figure 4, 5 and 6.** The results showed that three plant extracts have varying Anti-microbial activities against the tested organisms. These results supported the findings of (Mythreyi, Vijayan S and Usman Memon). From the results it was found that the three plant extracts showed activity even at the least concentrations of 125µg/ml and 250µg/ml. Hence, these particular concentrations were selected for further study.

The studies conducted by Joseph Nicolao Otieno and team clearly show that the multi-plant extracts were more superior over single plant extracts. The present study supports the above. After selecting the least concentrations in which the three plant extracts showed activity, i.e. 125µg/ml and 250µg/ml, the three extracts were prepared and mixed in different proportions. The results of inhibitory effects of the three plant extracts when mixed in different proportions are given in **Table no. 6, 7** and **8.** From the present investigation it was clearly evident that both single and multi- plant extracts in this study were effective against all the test organisms. However the result of the analysis of variance ($P<0.05$) implied that mixing of plant extracts in different proportions i.e., 1:1:2 (MPE-1), 1:2:1 (MPE-2) and 2:1:1 (MPE-3) lead to higher bioactivity or broad activity against all the tested organisms than individual extracts of the same plants.

Finally in the present work, novel herbal cream formulations were prepared by mixing the three plant extracts in different proportions as above i.e. 1:1:2 (formulation 1), 1:2:1 (formulation 2) and 2:1:1 (formulation 3) and its antimicrobial activity was studied with a view to confirm the use of the multi- plant extract as a remedy for skin disease. The results of antimicrobial activity shown by the cream formulations are also given in **Table no 6, 7 and 8**. The antimicrobial activity shown by MPE-1 and formulation-1 against Gram positive, Gram negative and fungal strains are demonstrated in **Figure 7, 8 and 9.** The results showed that among the three prepared formulations, formulation 1 showed better activity when compared to the other two. At last, the accelerated stability studies of the formulated creams were done. The results are tabulated in **Table no. 9, 10, 11, 12, 13 and 14**. Thus in search of a novel broad spectrum antimicrobial agent, the mixed plant extract and the formulation comprising different proportions of three plant extracts proved to be very good.

5. CONCLUSION

In conclusion, the results of this investigation reveals that the crude methanolic extracts of all three plants *viz. BT, TV* and *CC* showed differentiating antimicrobial activity against all the selected bacterial and fungal strains. By comparing the antimicrobial activity shown by single and multi-plant extracts it can be concluded that the three plants extracts when mixed in different proportions showed higher bioactivity or broad activity against all the tested organisms than individual extracts of the same plants. The differentiating activities of the three extracts against variety of microorganisms encouraged to develop novel broad spectrum antimicrobial herbal cream formulations with these extracts. The antimicrobial activity of the prepared cream formulation showed that formulation 1 has better antimicrobial activity when compared to the other two. The mixture of plant extracts in this study may be developed further into standard antibiotics for a broader application against diverse types of resistant pathogenic microbes. Despite the relevance of multi-extract therapy, from this study, it is recommended that proper standardization of the regimens should be carried out by conducting pharmacological and toxicological tests for safe dispensing.

It is necessary to carry out a screening of these plants in order to reveal the active principles by isolation and characterization of their antimicrobials constituents. Pharmacological and toxicological studies of the prepared herbal cream formulations also must be performed to ensure the safety of their use as a remedy for skin diseases. The discovery of a potent remedy from plant origin will be a great advancement in fungal and bacterial infection therapies. The results in this study support the preference and effectiveness of mixed extracts by traditional healers in management of opportunistic infections. The study suggests that the prepared multi- plant extract could be exploited in the management of diseases.

REFERENCES

[1] Kokate CK, Purohit AP, Gokhale SB. Text book of pharmacognosy. 20th ed.Pune: Nirali Prakashan; 1996.

[2] Bhushan Patwardhan, Ashok Vaidya DB, Mukund Chorghade. Ayurveda andnatural products drug discovery. Current science. 2004;86(6):789-799

[3] Malireddy S, Damavarapu Radha Krishna, Prasad V, Naraparaju A. Herbal andpharmaceutical drugs enhanced with probiotics; 2000

[4] Hajra PK. A manual of Ethanobotany – plants in magico Religious Beliefs and inSanskrit literature; 1987. p . 117.

[5] Importance of Medicinal Plants. Ayuvedaherbs; 2009.

[6] National Policy for Assessments of Herbal Products. Ministry of Health andPopulation; 2007. p. 1-59.

[7] Mukherjee PK, Shau M, Suresh B. Indian Herbal Medicines. Eastern Pharmacist.1998; 42 (8):21-4.

[8] Tandon V, Kappor B, Gupta BM. Herbal drug research in India: A trend analysisusing IJP as a marker. Indian Journal of pharmacology. 2004;36(2):99-100.

[9] Iyengar MA, Bibliography of investigated Indian Medicinal Plants. First edition,Manipal Power Press, Manipal;1976

[10] Iwu MW, Duncan AR, Okunji CO. New antimicrobials of plant origin. In: J.Janick (ed.), Perspectives on new crops and new uses. ASHS Press, Alexandria,VA; 1999:457–462.