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Research Article

Evaluation of Antimicrobial Activity of Polyherbal Gel Prepared from *Artemisia nilagirica* (Clarke. Pamp) and *Murraya koenigii* (Linn.) Leaves

*Shivani Bhardwaj, Chandra Shekhar Tailor

Department of Pharmacognosy, School of Pharmaceutical Sciences, Shri Guru Ram Rai University, Patel Nagar, Dehradun (Uttarakhand) 248001

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ABSTRACT

The Practice of herbal medicine has been noted as a major form of medicine since Prehistoric times. In this space age Where the Technology is so advanced, herbal medicines is still thriving and is highly accepted in both the developing and the developed countries due to it`s natural origin and low side effects.

Many of the Pharmaceuticals currently available have a long history of use as herbal remedies including opium, aspirin, digitalis and quinine. While purification and quantification of these plant extracts makes them more predictable and chemical based processing can sometimes modify their effects in affecting ways, herbal remedies tend to have a more complex and subtle mix of chemicals, and can sometimes offer access to drugs or combinations of drugs, that the pharmaceutical industry has not exploited. Increased focus on plant based remedies as a source of medication for a wide range of human afflictions in the result of factors including population growth, insufficient drug supply, prohibitive cost of treatments, side effects of several synthetic drugs, and the emergence of drug resistance to currently prescribed medications for infectious diseases.

Herbal drugs are getting popularity and there Pharmacological properties are reported from different parts of the world. In the current study, the Poly herbal gel of both *Artemisia nilagirica* and *Murraya koenigii* alcoholic extract of leaves showed good anti microbial potential.

Herbal medicine have received much attention as a source of new anti microbial drugs since they are considered as a time tested and comparatively safe both for human use and for environment.Overall Result of Current Study demonstrate that the Polyherbal gel prepared from these plant species has good Antimicrobial potential against *Escherichia coli*, *Pseudomonas* and *Staphylococcus aureus* microbial species.The Present study was designed to search for newer, safer and more potent Antimicrobial component which may be accomplished over present need.

Keywords : *Artemisia nilagirica*, *Murraya koenigii*, Polyherbal gel, Antimicrobial potential etc.

INTRODUCTION :

The Practice of herbal medicine has been noted as a major form of medicine since Prehistoric times. In this space age Where the Technology is so advanced, herbal medicines is still thriving and is highly accepted in both the developing and the developed countries due to it`s natural origin and low side effects. In addition to the Widespread use of Botanical as Medicinal products in developing countries, Such products are fast becoming a part of the integrated medical systems of the developed countries, known as Complementary and Alternative systems of medicine.[1]

Many herbal traditions dominate the practice of Alternative medicine. These include the Western herbal traditions based on Greek, Roman and Medieval sources, the Ayurvedic tradition of India and the Chinese herbal medicine. The Chinese medicine continues as a distinct area of modern medical practice. The traditional herbal remedies as alternative medicine plays a specific role in South Africa also, where it forms a part of the culture and beliefs of the indigenous generation and also features significantly in primary health care. Botanicals or Phytomedicines have always been a major component of traditional systems of healing in developing countries, which have also been an integral part of their history and culture. In the ancient Indian system of medicine of medicine, Ayurveda and Siddha and such examples.[2]

Since time immemorial, plants have been known to be rich sources of a wide variety of chemicals with nutritional and therapeutics properties. Herbs belong to general botanicals of various types which are also often the aromatic plants used especially in medicine or as seasoning. Herbs may be used directly as teas or extracts and they may be used in the production of drugs.[3]

A drug or preparation made from a plants and used for any of such purpose is better known as herbal drug. Many of the Pharmaceuticals currently available have a long history of use as herbal remedies including opium, aspirin, digitalis and quinine. While purification and

*Author for Correspondence: shivaanibhardwaj97@gmail.com

quantification of these plant extracts makes them more predictable and chemical based processing can sometimes modify their effects in affecting ways, herbal remedies tend to have a more complex and subtle mix of chemicals, and can sometimes offer access to drugs or combinations of drugs, that the pharmaceutical industry has not exploited.

Herbs hold promise not only for prevention but also for the treatment of various types of diseases. The drugs of natural origin constitute very important and valuable segment of modern medicine. Traditional medical practitioners and scientists are turning towards medicinal plants for curing ailments such as inflammation, rheumatoid arthritis, cancer, diabetes and many more because of the fact that they possess lesser side effects owing to their natural origin. These extracts are formulated into different formulation for ease of administration.[4-5]

Pharmacological Effects of Herbal Drugs : The medicinal value of plants lies in some chemical active substances that produce define physiological action on the human body. Plants are considered as a rich source of bioactive chemicals. The bioactive constituents or plant extracts may be used for treatment of various diseases and these would be used as a new formulation for the novel drugs discovery in pharmaceutical industries.[6] Many diseases related to infections have been known to be treated with Remedies of herbal origin throughout the history of mankind. This action is due to the presence of Phytochemical components like glycosides, tannins, alcohols, aldehydes etc. Herbal medicines such as Brahmi and Ashwagandha helps to increase nutrients, restore body cells, and enhance a person's immunity. Herbs such as black pepper, cinnamon, myrrh, aloe, sandalwood, ginseng, red clover, burdockand bayberry are used to heal wounds, sores and boils. Herbs such as marshmallow root and leaf serve as antacids. Herbs like aloe, sandalwood, turmeric and khare khasak are commonly used as antiseptic[7].

Antimicrobial activity -

It can be defined as a collective term for all active principles (agents) which inhibit the growth of bacteria, prevent the formation of microbial colonies and may destroy microorganism. In the field of antimicrobial finishes, many common terms are used including antibacterial, bacteriostatic, bactericidal, fungicidal, fungistatic or biocidal and biostatic.[8]

Aromatic and medicinal plants are known to produce certain bioactive molecules which can inhibit the growth of different micro-organism.Many extracts from medicine plant have been known to possess antimicrobial effects and used for the purpose of food preservation and medicinal purposes. Considered as time and comparatively safe both for human used and for environment the herbal extracts have received much attention as a source of new antibacterial drugs. Traditional medicines is still first choice for treatment of various disease by many peoples.[9]

The extracts of *Murraya koenigii* L. were tested against bacterial strains *Bacillus subtilis*, *Staphyloccous aureus*, *Escherichia coli*, *Salmonella typhi* and fungal strains are *Aspergillus niger*, *Candida albicans* and *Trichophyton rubrum*. There are various extracts are used such as hexane, methanol and chloroform extracts of the roots of *Murraya koenigii* L. was effective on all tested strains. In these strains methanol strain is more reactive and significant antimicrobial activity as compared to other types of extracts with maximum inhibitory effects on *Staphyloccous aureus* and *Trichophyton rubrum*.[10]

M. koenigii roots showed antibacterial effect against *B. subtilis*, *S. aureus*, *C. pyrogenes*, *P. vulgaris*, *P. asteurella multicida*. The acetone roots of *M. koenigii* shown mosquitocidal, antimicrobial and topisomerase I and inhibition activities.The ethanolic extract of leaves showed fungitoxicity against *Colletoyrichum falctum* and *Rhizoctonia solani*, *Cryptococcus neoformans*, *Tricophyton mentagrophytes* and *Microsporum canis*.[11]

The diethyl ether extract of the leaves of *Artemisia nilagirica* showed antimicrobial activities against the soil-borne plant pathogen Phytophthora capsici, which causes footrot in pepper used disc diffusion method to assess the antioomicetical activity.[(12)] The result showed mycelial growth of *P.capsici* was completely inhabited by a concentration of 100 ppm of the oil in the carrot agar medium. The inhibitory property against *P.capsici* is due to thujones, which is present in high amount (41.9%). They also studied the same activity using cedar leaf oil and found that *Artemisia nilagirica* oil was as equally effective as the cedar leaf oil.[13]

This Study was aimed to evaluate antimicrobial activity of Polyherbal gel prepared from *Artemisia nilagirica* (Clarke) Pamp. & *Murraya koenigii* Linn. Leaves alcoholic extracts.

MATERIALS AND METHODS:

PLANT MATERIAL- The Medicinal Plant material were collected from the locality of Shimla Bypass, Dehradun in the month of March –April 2022 and freshly collected leaves of *Artemisia nilagirica* (Clarke) Pamp & *Murraya koenigii* Linn. were dried under shade. Dried leaves were ground to coarse powder with the help of grinder[14-15].

AUTHENTIFICATION OF PLANT SPECIES -

The Medicinal Plant materials were authenticated from the Botanical Survey of India (BSI), Dehradun under Accession Number: 922 on dated 9/06/2022 and Accession Number: 868 on dated 6/05/2022.

Figure 1: *Murraya koenigii* Linn.

Figure 2: *Artemisia nilagirica* (Clarke) Pamp.

PREPARATION OF PLANT EXTRACT

The fresh leaves of *Artemisia nilagirica* and *Murraya koenigii* were collected and washed several times with water to remove the dust particles and then air dried to remove the residual moisture. Then, the air dried leaves crushed and extracted by hot continuous successive percolation method (Soxhalation) with different solvents of increasing polarity order, starting with non-polar solvents i.e. Petroleum ether followed by Chloroform and Ethanol (95%). Water extract was prepared by maceration method[16].

PROCEDURE FOR EXTRACTION- About 100 gm dried powder of leaves of *Murraya koenigii* and *Artemisia nilagirica* were packed into Soxhlet apparatus separately and extracted firstly with Petroleum ether to remove fatty material. After that Marc was collected, dried for complete removal of Petroleum ether & again Marc was extracted with Chloroform and Ethanol using same Soxhlet assembly until the solvent in the thimble became clear. After complete extraction, the extract was concentrated in water bath. While in maceration process whole coarsely powdered plant drug is kept in contact with the Solvent water in a stoppered container for 24 hours until soluble matter is dissolved. After that this aqueous extract was filtered and Concentrated[17].

Figure 3 : Extraction of leaves using Hot Continuous Percolation method by Soxhlet apparatus and by Maceration method

POLYHERBAL GEL PREPARATION[18]

In this study, 1% w/w Poly herbal gel formulations were prepared (F1-F9), which comprises Alcoholic extracts of *Artemisia nilagirica* &*Murraya koenigii* in the ratio of 50:50, respectively in a base. The base was prepared using Carbopol 940, Propylene glycol-400, Ethanol,

Methyl paraben, Propyl paraben, Triethanolamine & Distilled water in a sufficient quantity to prepare 10gm.
Out of all Nine types of formulations the F2 and F3 Polyherbal gel formulations showed good performance upon basic evaluation parameters by a wide margin and demonstrate their superior efficacy over the other formulations.

Figure 4 : Polyherbal gel formulation

BASIC EVALUATION PARAMETERS[19]

1) Appearance – The developed individual and polyherbal gels were evaluated for Physical appearance and homogeneity by visual observation.

2) Extrudability – The gel formulations were filled in standard capped collapsible aluminium tubes and sealed by crimping to the end. The weights of the tubes were recorded. The tubes were placed between two glass slides and were clamped. 0.5 gm was placed over the slides and then the cap was removed. The amount of the extruded gel was collected and weighed. The percent of the extruded gel was calculated.

3) Spreadability – The Spreadability of the gel formulations was determined by measuring the spreading diameter of 1gm of gel between two horizontal plates (20 cm x 20 cm) after one min. The standard weight applied on the upper plate was 100 gm.

DEVELOPMENT OF STANDARDIZATION PARAMETERS FOR LEAVES OF BOTH PLANT SPECIES[20-21]:

Artemisia nilagiricia (Clarke) Pamp &*Murraya koenigii* Linn.

Determination of Physico-chemical parameters

- Moisture Content / Loss on drying (LOD)
- Total Ash & Acid insoluble ash value
- Water soluble extractive value
- Alcohol soluble extractive value

Physico-Chemical Analysis- Determination of Loss on drying (LOD) :

10gm.of the sample (without preliminary drying) was weighed and placed in a tarred evaporating dish. It was dried at 105°C for 5 hours and at 1 hour interval until difference two successive weighing corresponded to not more than 0.25%. % Loss on drying= Loss in weight of sample/ Weight of sample X 100

Determination of Total Ash and Acid Insoluble ash value :

About 2 to 3 gm. of sample was accurately weighed in a tarred silica dish at a temperature not exceeding 450°C until it was free from carbon. Then it was cooled and weighed. The percentage of total ash was calculated with reference to the air dried drug.

% Total ash value= Wt. of total ash / Wt. of crude drug taken X 100

After this, total ash obtained was dissolved in 25 ml. of Dil. HCl to dissolve any acid soluble impurity and filtered then using ash less filter paper / What man filter paper. At last, this ashless filter paper was again kept in silica dish and burnt at same temperature as previous. This time acid insoluble ash was obtained which was cooled and weighed with reference to air dried drug.

% Total Acid Insoluble ash value= Wt. of acid insoluble ash / Wt. of crude drug taken X 100

Determination of Water Soluble Extractive value :

5gm. of test sample was weighed and macerated with 100ml of Chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing standing for eighteen hours. It was filtered rapidly, taking precautions against the loss of solvent. 25ml of the filtrate was taken and evaporated to dryness in a tarred flat bottomed shallow dish at 105°C, to constant weight and weighed the percentage of water soluble extractive was calculated with reference to the air dried sample.

% Water Soluble Extractive value= Wt. of residue X 80

Determination of Alcohol Soluble Extractive value :

Procedure of Water Soluble Extractive was followed for the determination of Alcohol Soluble Extractive but 90% ethanol was used instead of chloroform water.

% Alcohol Soluble Extractive value= Wt. of residue X 80

PHYTOCHEMICAL ANALYSIS / SCREENING/ TESTS OF PLANT EXTRACT[22-25]:

Presence of Phytochemicals are determined on the basis of Standard qualitative test procedures and these procedures are as follows:

TEST FOR ALKALOIDS:

Dragendorff's test: 2-3ml extract was added with few drops of Dragendroff's reagent.
Orange brown ppt. formed.

Mayer's test: 2-3ml of extract was added with few drops of Mayer's reagent gives cream ppt.

Wagner's test: 2-3ml of extract was added with few drops of Wagner's reagent. Reddish brown ppt. formed.

Hager's test: 2-3ml of extract was added with few drops of Hager's reagent gives yellow colour precipitate.

TEST FOR CARBOHYDRATES:

Fehling's Test

Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

Molisch's Test: Crude extract was mixed with 2ml of Molisch's reagent and the mixture was shaken properly. After that, 2ml of concentrated H_2SO_4 was poured carefully along the side of the test tube. Appearance of a violet ring at the interphase indicated the presence of carbohydrate.

Iodine Test: Crude extract was mixed with 2 ml of iodine solution. A dark blue or purple coloration indicated the presence of the carbohydrate.

Benedict's test (for Reducing sugar): Equal volume of Benedict's reagent and test solution was mixed and heated in boiling water bath for 5min. Solution appeared green, yellow or red.

Barfoed's test (for Monosaccharide): Equal volume of Barfoed reagent & extract was taken & heated for 1-2min in boiling water bath and cool. Red ppt observed.

Tollen's Phloroglucinol test (for hexose sugars): 2.5ml Conc. HCl & 4ml 0.5% phloroglucinol were mixed and added 1-2 ml test solution, heated the mixture. Yellow to Red color appeared.

Tannic acid: With 20% of tannic acid test solution gives ppt.

TEST FOR PROTEINS :

Ninhydrin Test

Crude extract when boiled with 2 ml of 0.2% solution of Ninhydrin reagent violet colour appeared suggesting the presence of amino acids and proteins.

TEST FOR FLAVONOIDS:

Alkaline reagent test: Crude extract was mixed with 2 ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

Sulphuric acid test: On addition of H_2SO_4 flavons and flavonols dissolve and give deep yellow solution.

Lead acetate test: To the residue, lead acetate solution was added. Yellow coloured precipitate formed.

Zinc HCl test: Test solution was heated with Zinc dust & HCl. Pink to red color appeared.

Shinoda test: Test solution was treated with few fragments of magnesium ribbon and Conc. HCl showed pink to magenta red colour.

TEST FOR SAPONINS :

Crude extract was mixed with 5 ml of distilled water in a test tube and shaken vigorously. The formation of stable foam was taken as indication for the presence of Saponins.

TEST FOR GLYCOSIDES:

A. Test for Cardiac Glycosides

1. **Killer- Killani test:** the extract was added with glacial acetic acid, 1 drop 5% $FeCl_3$ & Conc. H_2SO_4. Reddish brown color appeared at junction of two liquid layers & upper layer appeared bluish green.
2. **Legal's test:** the extract was added with 1ml pyridine and 1ml Sodium Nitro prusside. Pink to red color appeared.
3. **Baljet's test:** the extract was added with Sodium picrate gives yellow to orange colour.
4. **Raymond's test:** the extract was added with Dinitrobenzene in hot Methanolic alkali gives violet colour.
5. **Bromine water test:** the extract was added with Bromine water gives yellow precipitate.

B. Test for Anthraquinone Glycosides:

1. **Borntrager's test:** the extract was added with Dil. H_2SO_4, boiled & filtered. To cold filtrate, equal volume of chloroform or benzene was added, shake well. the organic solvent was separate and added with ammonia. Ammonical layer turned pink or red.
2. **Modified Borntrager's test:** 5ml extract was added with 5ml 5% $FeCl_3$ then 5ml Dil. HCl and heated for 5 min in water bath then, cooled. After this, chloroform or benzene was added and shake well. organic layer was separated and added equal volume of ammonia. Ammonical layer showed pinkish red color.

C. Test for Saponin Glycosides:

1. **Foam test:** Extract was shaked vigorously with water. Foam observed.
2. **Haemolytic test:** Extract was added to one drop of blood placed on glass slide. Haemolytic zone appeared.

D. Test for Cyanogenetic Glycosides:

Grignard's test: Strips of Sodium picrate filter paper were inserted between split cork stoppers which were fitted into the neck of the test tube containing a small amount of powdered drug in water. Care was exercised that the paper didn't touch the inner side of the test tube. The content was warmed for half an hour. The red colour of the strips indicated the presence of Cyanogenetic glycosides.

E. Test for Coumarin Glycosides:

Alcoholic extract made alkaline, showed blue or green fluorescence.

Test for Gums & Mucilages : Hydrolyzed the test solution using Dil. HCl & performed Fehling's or Benedict's test. Gums & mucilages were present, as Red colour was developed.

Test for Phenols & Tannins : 2-3 ml of extract was added with few drops of following reagents:

1. **Lead acetate solution:** White ppt. observed.
2. **5% $FeCl_3$ solution:** Deep Blue- black colour observed.
3. **Acetic acid solution:** Red color solution observed.
4. **Dil. HNO_3 Test:** Reddish to yellow colour observed.
5. **Dil. Iodine test:** Transient Red color solution observed.
6. One drop NH_4OH, excess 10% $AgNO_3$ solution, heated for 20 min in boiling water bath. White

precipitate was observed, then dark silver mirror deposited on wall of test tube.

Test for Vitamins :

Test for Vitamin A: A quantity equivalent to 10- 15 units was dissolved in 1ml of chloroform & 5 ml of antimony (II) chloride solution was added then, a transient blue colour was produced immediately.

Test for B complex :

1. **Test for B_1 (Thiamine HCl):** 20mg in 10ml of water was dissolved and added with 1ml of 2M acetic acid & 1.6 ml of 1M NaOH then, heated on water bath for 30 min. It was allowed to cool. 5ml of 2M NaOH , 10ml of Potassium ferri cyanide solution and 10 ml of n- butanol were added then and shaken for 2 min. The upper layer exhibited an intense light blue fluorescence in UV 365nm.

a) **Test for B_2 (Riboflavin):** 1mg in 100ml of water was dissolved. The solution had a pale greenish yellow colour by transmitted light and an intense yellowish green fluorescence by reflected light, which disappeared on addition of mineral acids or alkalis.

b) **Test for Nicotinic acid**

- Heated a small quantity with twice its weight of soda lime, pyridine was evolved.
- Dissolved 50mg in 20ml water, neutralized with 0.1M NaoH. Added 3ml of copper sulphate solution, a blue ppt was formed.

Test for Vitamin C:

a) Diluted 1ml of 2% w/v solution with 5ml of water and added 1 drop of freshly prepared 5% w/v solution of sodium nitroprusside and 2ml of diluted NaOH solution. Added 0.6ml of Conc. HCl drop wise & stirred. The yellow colour turned blue.

b) To 2ml of 2% w/v solution, added 2ml of water, 0.1gm of sodium bicarbonate & about 20mg of ferrous sulphate. Shaken & allowed to stand. A deep violet colour was produced. Added 5ml of 1M H_2SO_4, the violet colour was disappeared.

Test for Vitamin D: Dissolved a quantity equivalent to 1000 units of vitamin D activity in chloroform and added 10ml of antimony (II) chloride solution, pinkish- red color appeared.

FORMULATION - THE POLYHERBAL GEL PREPARATION :

In this study, 1% w/w Poly herbal gel formulations were prepared (F1-F9), which comprises Alcoholic extracts of *Artemisia nilagirica* &*Murraya koenigii* in the ratio of 50:50, respectively in a base. The base was prepared using Carbopol 940, Propylene glycol-400, Ethanol, Methyl paraben, Propyl paraben, Triethanolamine & Distilled water in a sufficient quantity to prepare 10gm.as per given formula.

Table 1 : Formulations of Poly herbal gel (Composition table)[26-28]

S. N.	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
1.	**Carbopol 934**	1	1.5	2	1	1.5	2	1	1.5	2
2.	**Propylene glycol**	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
3.	**Methyl paraben**	1	1	1	1	1	1	1	1	1
4.	**Propyl paraben**	1	1	1	1	1	1	1	1	1
5.	**triethanolamine**	1	1	1	1	1	1	1	1	1
6.	**Distilled water (Q.S)**	100	100	100	100	100	100	100	100	100
7.	***Murraya koenigii* extracts (Alc.)**	5	5	5	2.5	2.5	2.5	1	1	1
8.	***Artemisia nilagirica* extracts (Alc.)**	5	5	5	2.5	2.5	2.5	1	1	1

ANTIMICROBIAL ACTIVITY: The bacterial cultures were obtained from the Shri Guru Ram Rai Institute of Medical and Health Sciences, Patel Nagar, Dehradun. The Agar well diffusion method was used for testing Antimicrobial activity. For the cultivation of the bacterial strains, Nutrient Agar medium (NAM) was prepared using 8% nutrient broth in double distilled water and agar-agar. It was subjected to autoclaving at 15 Lbs psi for 25–30 minutes. Agar test plates were prepared by pouring 15 ml of NAM into Petri dishes under aseptic conditions and allowed to stand at room temperature for sterilization. Bacterial cell cultures were maintained in peptone saline solution by regular subculturing and were incubated at 37^0C for 24 hrs extracts at a concentration of 50 µg as well as a 10 and 20-ppm solution of Streptomycin at the concentration of 50 µg. The filter Petri plates were placed equidistantly on inoculated media and diffusion of the solution was allowed to occur for 30 minutes at room temperature. Plates were incubated at 37˚C for 24 hours. The average Zone of inhibition was recorded. Extracts were also mixed with an equal proportion of antibiotics and tested against the test organisms. The bacteria *Staphylococcus aureus* gram-positive and *Escherichia coli* gram-negative and *Pseudomonas aeruginosa* have been used for the study[29].

AGAR WELL DIFFUSION METHOD: Agar diffusion refers to the movement of the molecules through the agar matrix that is formed by the gelling of agar. The degree of the molecule`s movement is related to the concentration of the molecules. This phenomenon forms the basis of the agar diffusion assay that is used to determine the susceptibility or resistance of a bacterial strain to an anti-bacterial agent. The antibiotic compound moves from higher concentration to the surrounding lower concentration regions and from the zone of inhibition by inhibiting microbial colonies. This diffusion was the basis of the agar diffusion assay.

To prepare Nutrient broth and Agar medium (containing in g 1-1: peptic digest of animal tissue, 5; NaCl, 5; Beef extract, 1.5; Yeast extract, 1.5; Agar, 15 and PH 7.4) was

prepared by dissolving 28 g of NAM powder (Hi Media, M001-500G) in 1000 ml distilled water. The solution was heated to completely dissolve the solid components of the medium and sterilized by autoclaving.

A bacteria suspension was spread onto the surface of the agar. Then a different concentration of anti-microbial compound was applied to several wells in the plate. Then incubate the plate for 24-48 hours. After incubation, the plates were checked. If there was a clearing around the well, then the bacteria have been adversely affected by the compound. The size of the inhibition zone could be measured and related to standards, to determine whether the bacterial strain is sensitive to the antibiotic [30].

Table 2 : Composition of Nutrient broth

S. No.	Ingredients	Quantity
1.	Beef extract	1gm
2.	Yeast extract	2gm
3.	Sodium chloride	5gm
4.	Peptone	5gm
5.	Distilled water	1000ml

RESULTS & DISCUSSION :

Table 3: Physiochemical standard values of both *Artemisia* and *Murraya* Plant species

S. No.	Parameters	Physiochemical Standards Value / Inference	
		Artemisia nilagirica	***Murraya koenigii***
a)	**Ash value (A.V.)**	**Value % w/w**	**Value % w/w**
*	Total ash value	7.85%	12.5%
*	Acid Insoluble Ash value	0.25%	0.1%
b)	**Extractive value (E.V.)**		
*	Water soluble ext. value	9.28%	4.5%
*	Alcohol soluble ext. value	2.08%	6.8%
c)	**Loss on drying (L.O.D.)**	0.96%	1.04%

Table 4 : Physiochemical attributes or Basic evaluation parameter values

PHYSIOCHEMICAL ATTRIBUTES OF COMBINED *Artemisia nilagirica* & *Murraya koenigii* Polyherbal gels			
Formulations Code	**Extrudability**	**Spreadability (g.cm / sec)**	**Appearance**
F1	Good	5.6	Light Green
F2	Excellent	7.63	Light Green
F3	Excellent	9.21	Light Green
F4	Excellent	6.38	Light Green
F5	Good	4.3	Light Green
F6	Good	2.0	Light Green
F7	Good	3.2	Light Green
F8	Good	3.46	Light Green
F9	Good	3.81	Light Green

Table 5 : Phytochemical Screening test observations of *Artemisia nilagirica* various extracts

ARTEMISIA NILAGIRICA CLARKE PAMP LEAVES **PHYTOCHEMICAL SCREENING TEST RESULTS**						
S.N.	**Phytochemical Constituents**	**Phytochemical Tests**	**Extracts**			
			P.E.E.	**Chl.E.**	**Alc.E.**	**Aq.E.**
1.	Alkaloids Test	Mayer's Test	+	+	+	+
		Wagner's Test	+	+	+	+
2.	Flavonoids	Alkaline Acetate Test	-	-	+	+
3.	Carbohydrates	Fehling's Test	+	+	-	+
		Molish Test	-	+	+	+
		Iodine Test	+	+	+	+
4.	Glycosides	Salkowaski Test	-	-	-	+
		Saponins Foam test	-	-	+	+
5.	Protein	Ninhydrin test	-	-	-	-
6.	Terpenoids	Salkowaski Test	-	-	-	+

Table 6 : Phytochemical Screening test observations of *Murraya koenigii* various extracts

***MURRAYA KOENIGII LINN. LEAVES* HYTOCHEMICAL SCREENING TEST RESULTS**						
S.N.	**Phytochemical Constituents**	**Phytochemical Tests**	**Extracts**			
			P.E.E.	**Chl. E.**	**Alc. E.**	**Aq. E.**
1.	Alkaloids Test	Mayer's Test	+	+	+	+
		Wagner's Test	+	+	+	+
2.	Flavonoids	Alkaline Acetate Test	-	-	+	+
		Fehling's Test	+	+	-	+
3.	Carbohydrates	Molish Test	-	+	+	+
		Iodine Test	+	+	+	+
4.	Glycosides	Salkowaski Test	-	-	-	+
		Saponins Foam test	-	-	+	+
5.	Protein	Ninhydrin test	-	-	-	-
6.	Terpenoids	Salkowaski Test	-	-	-	+

ANTI MICROBIAL ACTIVITY OF POLYHERBAL GEL BY AGAR WELL DIFFUSION METHOD :

Table 7 : Evaluation of Antimicrobial potential Showing Diameter of Zone of Inhibition by two best Polyherbal gel formulation in low concentration

S. No.	**Bacterial Strain**	**Diameter of Zone of Inhibition (In mm)**			
		N.C. (50ul)	**T1(10ul)**	**T2(10ul)**	**P.C (50ul)**
1	E. coli	0	0	0	16
2	S. aureus	0	0	0	19
3	Pseudomonas	0	0	0	19

N.C.= Negative control (Dist. water), P.C= Positive control (Streptomycin), T1=formulation1, T2=formulation 2

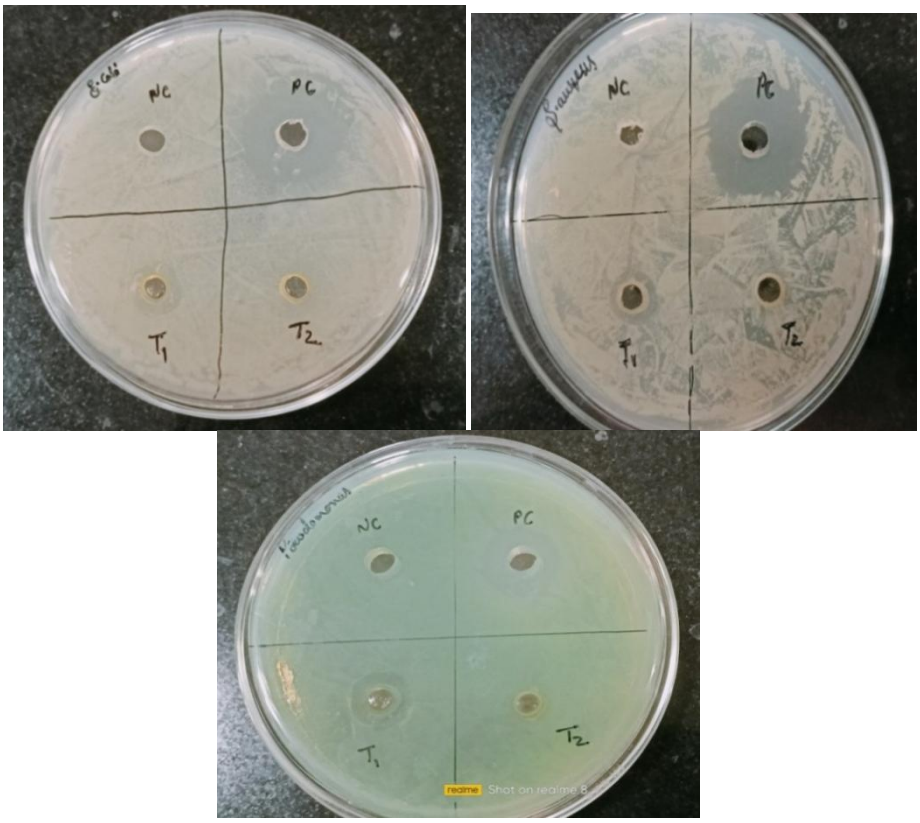

Figure 5 : Antimicrobial activity of two best Polyherbal gel against three types of microbial organisms viz. E.Coli, S. aureus & Pseudomonas

Table 8 : Evaluation of Antimicrobial potential showing diameter of Zone of Inhibition by two best Polyherbal gel formulation in high concentration

S. No.	Bacteria Strain	Diameter of zone of inhibition (mm)			
		N.C. (50ul)	T1 (20ul)	T2 (20ul)	P.C. (50ul)
1	E. coli	0	6	0	19
2	S.aureus	0	6	0	19
3	Pseudomonas	0	7	0	11

N.C.= Negative control (Dist. water), P.C.= Positive control (Streptomycin), T1=formulation1, T2= formulation 2

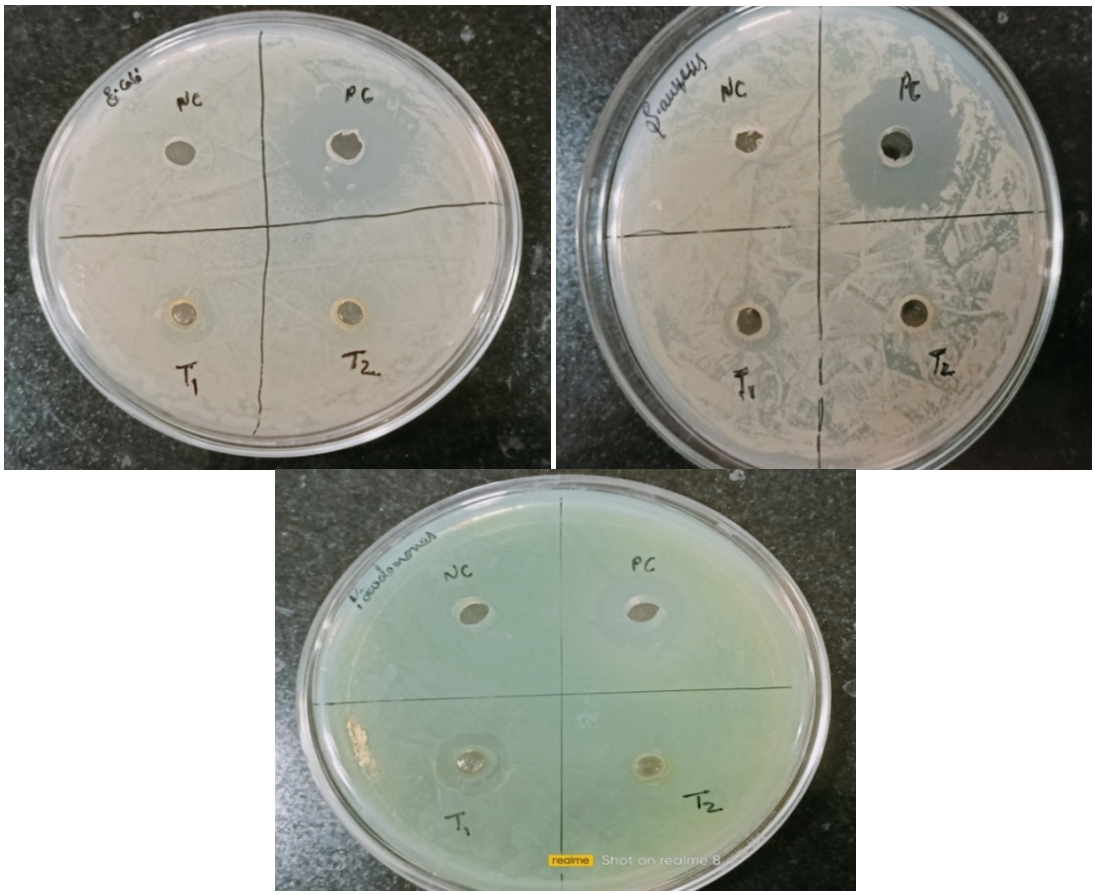

Figure 6 : Antimicrobial activity of two best Polyherbal gel against three types of microbial organisms viz. E. Coli, S. aureus & Pseudomonas

DISCUSSION :

Herbal drugs are getting popularity and there Pharmacological properties are reported from different parts of the world. In the current study, the Poly herbal gel of both *Artemisia nilagirica* and *Murraya koenigii* alcoholic extract of leaves showed good anti microbial potential. In vitro study of Anti microbial potential showed that, out of nine Polyherbal gel formulations two were found best which were formulated using ethanolic leaf extracts of both *Artemisia nilagirica* and *Murraya koenigii* plant species and tested against the growth of E. coli, Pseudomonas and Staphylococcus aureus at the different concentration.

The Significant Anti microbial activity was determined by measuring the diameter of Zone of inhibition and compared with the standard drug Streptomycin.

As per observations noted it was proved that the formulation F3 of *Artemisia nilagirica* and *Murraya koenigii* ethanolic leaf extracts showed best result in 20 micro litre concentration against E. coli, Pseudomonas and Staphylococcus aureus when compared with standard drug Streptomycin.

CONCLUSION :

In the Present investigation of various Standardization parameters, such as Physiochemical standards like Total Ash and Acid Insoluble Ash value, Extractive value both Water soluble and Alcohol Soluble were used to check the presence of various inorganic and organic matter in the drug. Further, Practical Loss on drying and Phytochemical analysis were done to investigate that moisture content present or absent in the *Artemisia nilagirica* and *Murraya koenigii* medicinal plant species.

The Percentage yield of Water soluble extractive value of leaves *Artemisia nilagirica* and *Murraya koenigii* were found to be 9.28% and 4.5%.

The Percentage yield of Alcohol soluble extractive value of leaves *Artemisia nilagirica* and *Murraya koenigii* were found to be 2.08% and 6.8%.

The Percentage yield of Total Ash value of leaves *Artemisia nilagirica* and *Murraya koenigii* were found to be 7.85% and 12.5%.

The Percentage yield of Acid Insoluble Ash value of leaves *Artemisia nilagirica* and *Murraya koenigii* were found to be 0.25% and 0.1%.

The Percentage yield of Loss on drying (LOD) leaves powder of *Artemisia nilagirica* and *Murraya koenigii* were found to be 0.96% and 1.4%.

The Goal of the current study is to find newer, safer and more effective Antimicrobial component that can meet current needs.

According to Phytochemical screening results of various extracts of leaves *Artemisia nilagirica* a variety of Phytoconstituents were determined as Alkaloids, Flavanoids, Carbohydrates and Glycosides and various extracts of leaves *Murraya koenigii* determined Phytoconstituents were Alkaloids, Glycosides, Carbohydrates, Flavonoids and terpenoids etc.

Herbal medicine have received much attention as a source of new anti microbial drugs since they are considered as a time tested and comparatively safe both for human use and for environment.

As per the Result obtained by Antimicrobial potential assessment, It was also concluded that the Polyherbal gel prepared by these plant species possess antimicrobial potential too in higher concentration. Thus, It can be used in the treatment of topical infectious disease. The Current study also appreciate Antimicrobial activity of *Artemisia nilagirica* and *Murraya koenigii* leaves which were widely used in Uttarakhand. Overall Result of Current Study demonstrate that the Polyherbal gel prepared from these plant species has good Antimicrobial potential against Escherichia coli, Pseudomonas and Staphylococcus aureus microbial species.

The Present study was designed to search for newer, safer and more potent Antimicrobial component which may be accomplished over present need. Herbal medicine have received much attention as a source of New Anti microbial drugs since they are considered as a time tested and comparatively safe both for human use and for environment. So, Still there is much more scope for Research in these type of Polyherbal formulations. Many more activities can be explored in future.

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