FORMULATION AND EVALUATION OF HERBAL GEL SHOWING ANTIOXIANT ACTIVITY

❖ Abstract:

The present project has been undertaken with the aim to formulate and evaluate of polyherbal gel containing Neem (Azadirachta indica),Hemidesmus indicus and Wtightia tinctoria . Natural remedies are more acceptable in the belief that they are suffer with fewer side effects than the synthetic ones. Herbal formulation has growing demand in the world market. The plant has been reported in literature having good antimicrobial, anti-inflammatory, refreshing activity, cleansing agent and anti-oxidant. Formulations are prepared by using varied concentration of extract prepared formulation where evaluated for various parameters like color, appearance, consistency, wash ability, pH and Spreadability, Extrudabilty, skin irritation. It has wide spectrum of antioxidant activity against acne prone skin. The prepared gel is formulated by using carbopol- 934 as gelling agent, herbal extracts are the medicinal agents in formulation. Polyethylene glycol used as a co-solvent, methyl- paraben as a preservative and required quantity of distilled water as a vehicle. On the basis of the results obtained in the present study we conclude that the gel formulation of polyherbal contents showed good activities towards the declared evaluations.

❖ Introduction to Herbal medicine-

Herbal medicine containing plants which are rich in variety of compounds such uses are very effective and safe to administration to human body. Herbal plants are rich in secondary metabolite which does not directly involve in the growth and development but play important role in survival and defense .Plants also contain aromatic substances and their oxygen-substituted derivatives.

Herbal medicine are complementary or alternative medicine used widely as a safe and effective method of treatment which the mainstream healthcare systems increasingly gaining widespread popularity all over the world and gradually streaming toward integration into the mainstream healthcare system. Formulations containing herbal medicine are mainly depend upon the adequate knowledge about their activity and harmful side effects of herbal plants used to prepare herbal medicine.

In case of preparation of herbal medicine isolation of single active ingredient is essential but in case of isolation practitioner sometimes believe that there is loss of activity of single active ingredient when isolated from plants.

Herbal medicine containing many active ingredients which give action in making herbal preparations as- Antioxidant, Anti-inflammatory, Antileprotic, Anti-acne, Analgesic, Anti-pyretic, Anti-hyperlipidemic, Anti-carcinogenic, antifungal, antibacterial, insecticidal.[1,2]

❖ Skin-

Skin made up of water, protein, fats and minerals and it is largest organ of the body. Skin protect the body from germs and help to maintain the body temperature. Nerves present in skin helps to feel the sensation like hot and cold. Antioxidant activity, both naturally occurring and from topical applications, primarily acts on the epidermis, particularly the stratum corneum, and can also penetrate into the deeper layers of the skin.

❖ Gel-

Gels are polymer-based materials with three-dimensional (3D) network structures that can absorb and retain solvents without dissolving in them. They exhibit properties that are intermediate between solids and liquids. Gels can be categorized based on the type of cross-linking that forms their 3D networks, their origin (natural or synthetic), their shape and size, and the nature of the solvent they contain.[3]

❖ Antioxidant activity-Why it is necessary

-It is important to neutralize activity of harmful free radicals.

-It is important to reduce risk of chronic disease.

-It is important for protecting the cell form harm and damage.

-It produces anti-inflammatory effects.

-It reduces the oxidative stress and produce anti aging effect generally beneficial in cosmetics.

-It reduces the oxidative stress and improving blood vessel function and help to treat the cardiovascular diseases.

-It is beneficial in treating conditions such as-diabetes, neurodegenerative disease, eye disease.

❖ Antioxidant role in skin diseases-

Skin cells are constantly exposed to environmental stress like-pollutants, UV radiations. Antioxidant reduces the skin damage by-

1) Neutralizing reactive oxygen species (ROS)-

-The enzymatic antioxidant such as superoxide dismutase (SOD), Catalase and glutathione Peroxidase which convert the ROS into less harmful molecules and reduce harm to skin.

2) Protecting DNA and cell function –

-Protect DNA by reducing the oxidative damage to DNA.

-Reactive oxygen species can damage the DNA and leading to the mutation that lead to the skin cancer.

-So it reduces damage to DNA by reducing ROS.

3) Reduce the inflammation by modulating inflammatory pathways-

-In case of many skin inflammations and inflammatory diseases the inflammatory response can produced by oxidative stress and imbalance between the ROS and antioxidant. So antioxidant reduces the inflammatory response by modulating many inflammatory pathways.

-Certain antioxidant reduces cytokine level and modulates signaling pathways involved in inflammation.

-Hence antioxidant produces the anti-inflammatory response in many skin diseases[4].

❖ DRUG PROFILE-

➢ Azadirachta indica:

Family- Meliaceae

Genus- Azadirachta

Activity- Antioxidant, antifungal, antibacterial, insecticidal, anti-inflammatory, antimalarial, neuroprotective.

Azadirachta indica containing antioxidant molecules which will supplement the work of body's natural antioxidant defense mechanism containing molecule such as Superoxide dismutase (SOD), Catalase(CAT), Glutathione Peroxidase(GPX), Glutathione(GSH),Nitric oxide dioxygenase(NOD).

Inflammation is caused by imbalance between the free radicals or reactive oxygen species (ROS) and oxidative stress that exerting damage. Therefore there is need to provide antioxidant to stabilize or neutralize free radicals. Neem plant is one of the simplest and cost effective way to introduce antioxidant in many skin disease condition.

Neem produces antioxidant activity and naturally boosts our defense mechanism of body.[6]

Fig.no.1

- **Hemidesmus indicus L-**

Family-Apocynaceae.

Genus-Hemidesmus.

Activity-Antioxidant, Anti-inflammatory, Antileprotic, Anti-acne, Analgesic, Anti-pyretic, Anti-hyperlipidemic, Anti-carcinogenic.

The activity of hemidesmus indicus primarily seen in the condition of the coronary heart disease which is primarily lipid disorder .Coronary heart disease mainly caused due to abnormal level of lipids in the blood which cause plaque formation in the arteries. It also initiated with the intracellular increased level of reactive oxygen species which leads to many types of tissue injury and also may cause heart problems such as - ischemia, thrombosis, inflammation, atherosclerosis.

So in such condition formation of free radicals or reactive oxygen species are controlled by antioxidant present in the hemidesmus indicus .It has wide therapeutic value play major role as anti-hyperlipidemic and anticoagulant in treating many cardiovascular disease.[7]

Fig.no. 2

- **Wrightia tinctoria**-

Family-Apocynaceae.

Genus- Wrightia

Activity- anti-microbial, anti-psoriatic, anti-diarrhoeal, anti-Helmintic, anti-oxidant, anti-cancer, anti-inflammatory analgesic, anti-diabetic, diuretic, hepatoprotective and anti-ulcer

A small deciduous tree having medicinal properties and have a long history of use by indigenous communities in India. This plant's medicinal properties for treating a wide range of human ailments are mentioned in Ayurveda, Siddha, Unani, and traditional folk medicine.[8]

Fig.no.3

- **Mentha Piperita l-**

Family-Lamiacae

Genus-Mentha

Activity- antibacterial, anti-inflammatory, antioxidant, and antispasmodic properties

Peppermint (Mentha piperita) is a hybrid plant created by crossing spearmint (Mentha spicata) and water mint (Mentha aquatica). Its leaves are popular worldwide, especially in Western and Middle Eastern countries, where they are used in teas, tinctures, infusions, extracts, and essential oils.

Peppermint is known for its many health benefits, including its ability to relieve pain, fight bacteria and fungi, eliminate parasites, and act as an antioxidant. These benefits come mainly from menthol, its key active ingredient, which is widely used in medicine and industry.[9]

Fig.no.4

❖ Experimental methods-

➢ Preformulation Study:

Preformulation studies play a crucial role in drug development by providing a strong scientific foundation, simplifying regulatory processes, and making better use of resources. They help ensure public safety and improve the quality of medications. Every drug has unique chemical and physical properties that must be carefully considered before creating a pharmaceutical product. These properties determine how well the drug will work with other ingredients in the final formulation.[10]

Goals of Preformulation studies are-

- Identification of Physical & Chemical Properties.
- To determine Compatibility with Excipient

FORMULATION TABLE-

NAME OF INGREDIENT	BATCH 1	BATCH 2	BATCH 3
Wrightia tinctoria extract	0.4gm	0.2gm	0.2gm
Hemidesmus indicus extract	0.4gm	0.2gm	0.1gm
Azadirachta indica extract	0.2gm	0.2gm	0.2gm
Menthol	0.2gm	0.7gm	--
Eucalyptus oil	--	--	1ml
Carbapol 934	1 gm	1.5gm	2gm
Triethanolamine	1.2ml	1.5ml	1.8ml
Polyethylene glycol	5ml	7.5ml	10ml
Methyl paraben	0.5gm	0.5gm	0.5gm
Glycerol	7ml	7ml	7ml
Distilled water	q.s.100ml	q.s.100ml	q.s.100ml

❖ Pharmacognostic Investigation-

Extractive Values:

➢ **Alcohol soluble extractive value:**

20gm coarse powder of Azadirachta indica, Wrightia tinctoria and Hemidesmus indicus is weighed and placed in 250ml conical flask and macerate it with 100ml Ethanol. It kept aside for 7 days. All extracts are filtered and shade dried.

➢ **Determination of Foreign Matter:**

The sample shall be free from visible signs of mold growth, sliminess, stones, rodent excreta, insects or any other noxious foreign matter when examined as given below.
Take a representative portion from a large container, or removed the entire contents of the packing if 100 g or less, and spread in a thin layer in a suitable dish or tray. Examined in daylight with unaided eye. Transfer suspected particles, if any, to a petri dish, and examined with 10x lens in daylight.

❖ Preparation of gel :

To prepare the gel, the required amount of gelling agents was carefully measured and dissolved in distilled water. Once fully mixed, triethanolamine was slowly added drop by drop while continuously stirring, helping to balance the pH and begin forming the gel. Stirring was maintained throughout to ensure a smooth and even consistency as the gel started to take shape**.**

Once the gel had formed, measured amounts of extracts were added and thoroughly mixed in for about 30 minutes to make sure everything blended evenly. Glycerin was included to help keep the gel moist, and preservatives—methyl paraben was dissolved in water and added slowly while stirring, to help keep the gel stable and safe from microbial growth.

Stirring continued until the gel reached a smooth, semisolid texture. The thickness (viscosity) was checked along the way to make sure the final product had the right feel and consistency. Finally, the volume was topped up with distilled water, and the mixture was stirred thoroughly until a smooth, uniform gel was achieved.[11]

Fig.no.5

❖ Evaluation tests of gel :

➢ Physical Appearance:

The formulated gels containing Azadirachta indica, Hemidesmus Indica and wrightia tinctoria were visually examined to assess their color, uniformity, consistency, and any signs of phase separation.

➢ Homogeneity:

Once the gels were formed in the container, they were visually examined to assess uniformity. The inspection focused on their overall appearance and the detection of any visible aggregates.

➢ pH:

pH measurement of the gel was carried out using a digital pH meter by dipping the electrode completely into the gel system to cover the electrode. The measurement was carried out in triplicate and the average of the three readings was recorded.

➢ Extrudability:

A closed collapsible tube containing approximately 10 grams of gel was compressed firmly from the crimped end and a clamp was applied to prevent the gel from rolling back. After removing the cap, the gel was extruded. The extruded portion was then collected and weighed. The percentage of gel expelled from the tube was subsequently calculated.

➢ Rheological study:

Viscosity of gel was determined using Brookfield viscometer at 25c with spindle speed of the viscometer rotated at 100 rpm.

➢ Spreadability:

1. Take about 1 g of gel and place it at the center of one glass slide.
2. Place the second glass slide on top of the gel to sandwich it.
3. Put a standard weight (500 g) on top of the upper slide for 1 minute to spread the gel.
4. Measure the diameter or area of the spread gel in cm or cm^2.
5. You can repeat with different weights for comparison.

$$S = \frac{M \times L}{T}$$

Where:

- S = Spreadability
- M = Weight tied to the upper slide (g)
- L = Length of glass slide (cm)
- T = Time taken to separate slides (sec)

➢ **Skin irritancy test:**

A small amount of test substance is applied on surface of adult volunteers hand and observe the effect and either irritancy for 1 hour.[12,13]

❖ Anti-oxidant activity (Hydrogen peroxide scavenging assay):

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (50 mM, pH 7.4). The concentration of hydrogen peroxide was determined by measuring the absorbance at 230 nm using UV spectroscopy. Different concentrations of the extract were then added to the phosphate buffer, and the absorbance at 230 nm was measured using UV spectroscopy, with the blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging activity was calculated using the following formula:

% Scavenging Activity = [(Ai – At) / Ai] × 100

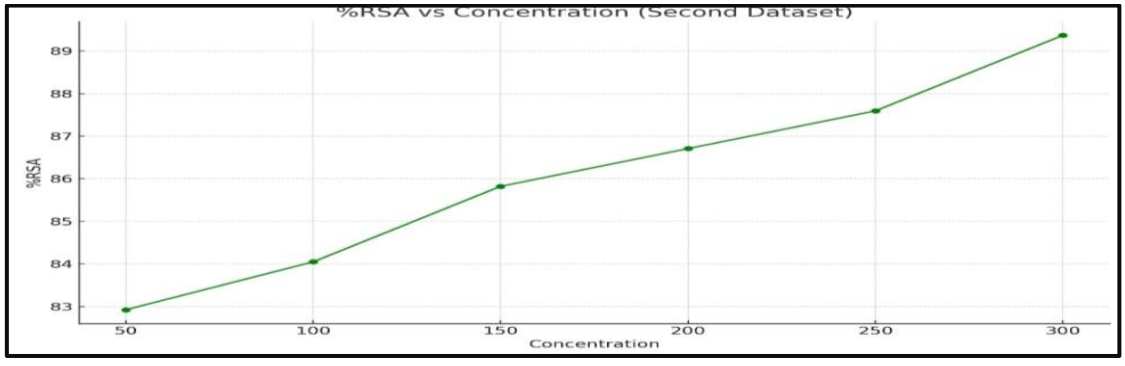
Where;

Ai is the absorbance of the control (without extract)

At is the absorbance of the test sample (with extract)[14]

Fig.no.6

➢ **Graph of antioxidant activity:**



- **Anti microbial test:**
  The Antimicrobial Susceptibility Testing (AST) plate method on MacConkey agar involves inoculating a plate with a bacterial culture, followed by placing antimicrobial disks on the agar surface, incubating the plate, and measuring the zone of inhibition around each disk. This technique helps determine which antibiotics are effective against the tested bacteria. [15,3]

Fig.no.7

❖ Result and discussion:

➢ Organoleptic characteristics of extracts:

Powder	color	odour
Azadirachta indica	Green	pungent
Hemidesmus indicus	Brown	aromatic
Wrightia Tinctoria	Brown	Distinct aroma

Result of evaluation tests:

➢ pH:

pH of the formulation was found to be

Batch	pH
1	4.76
2	5.11
3	4.06

Fig.no.8

- **Extrudability:**

Percentage Extrudability = $\frac{\text{weight of extruded gel}}{\text{total weight of gel in the tube}} \times 100$

$= \frac{7.2}{10} \times 100$

$= 72\%$

The Extrudability of the formulation was found to be 72%

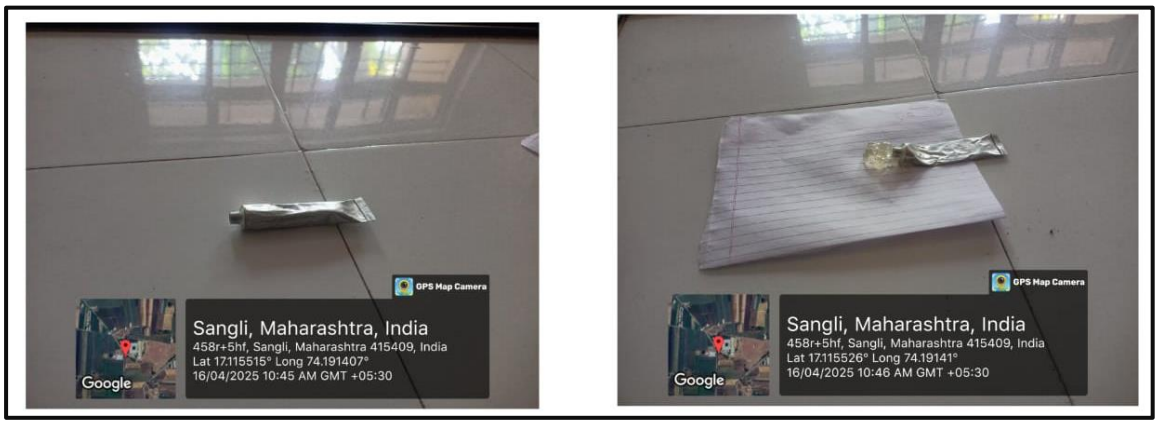


Fig.no.9

➢ Viscosity :

Viscosity of the formulation was found to be

Batch	Viscosity
1	377cp
2	189cp
3	123cp

Fig.no.10

➢ Spreadability:

Spredability(S) = Mass ×Area

Time

$$= \frac{500 \times 4.5}{20}$$

=112.5g·cm/sec

The spredability of he gel was found to be 112.5g.cm/sec.

Fig.no.11

➢ Skin irritancy test:

The gel applied and observed for 1 hour and no observation of any unwanted effect or irritancy.

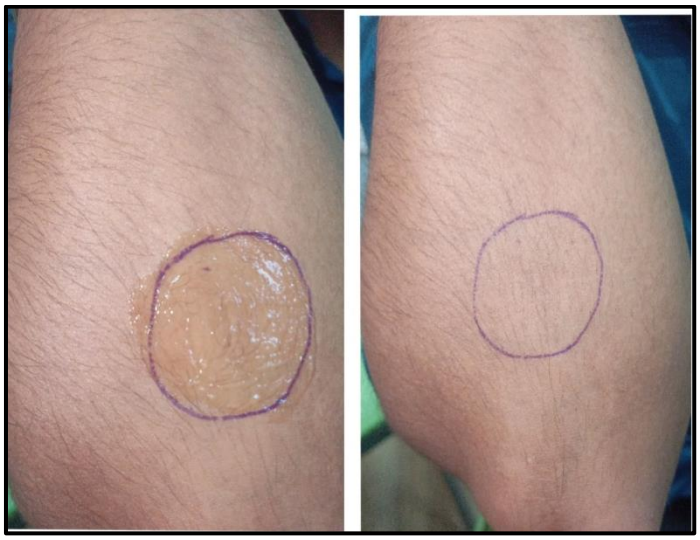
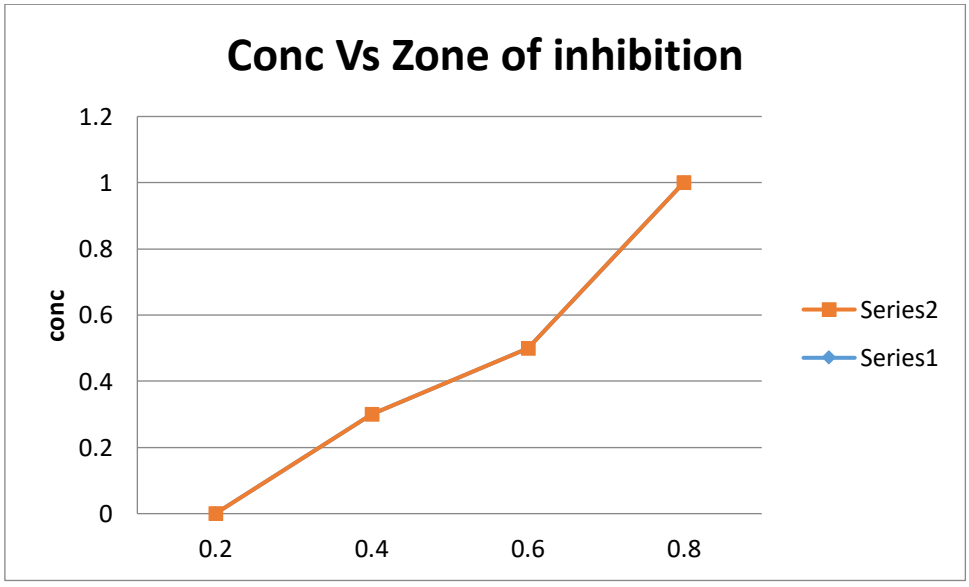


Fig.no.12

➢ Anti microbial test:

Observations:

Conc. of gel	Zone of inhibition
0.2	0.3
0.4	0.5
1	1



As the conc. of gel increases the zone of inhibition also increases. It shows antimicrobial activity.

❖ Conclusion:

The present study demonstrated that the potential of Azadarichta indica, wrightia tinctoria, Hemidesmus indicus in the formulation of an effective gel preparation with antioxidant activity ,antimicrobial activity.

The formulated gel was characterized by various tests including appropriate pH(4.76),Viscosity(377cp), Extrudability(70%), Spreadability(112.5g.cm/sec).It was smooth, homogenous, nonirritant and cosmetically acceptable. The Antioxidant activity evaluation via hydrogen peroxide assay, antimicrobial tests by cup plate method.

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