Phytochemical Analysis an Antimicrobial Antioxidant Activities of *Azadirachta indica*, *Curcuma longa Arnebia benthamii, Glycyrrhiza glabr*a Polyherbal Planterosomal Gel.

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ABSTRACT
According to ancient system an individual herbs cannot achieve the desired therapeutic result. When it is optimized as multiple herbal compositions in a particular ratio it will give a therapeutic effect in a better way with reduced toxicity. In order to develop such an intervention, the present study was intended to develop a polyherbal planterosomal gel from methanolic extracts of *Azadirachta indica, Curcuma Longa, Glycyrrhiza glabra, Arnebia banthamii*. Furthermore, the study intended to assess the effects of polyherbalism on antioxidant and antibacterial properties; the ratio of distinct plant extracts was subsequently modified to treat the area of damage. Several plant extracts and their phyto-constituents are known as promising alternatives for wound healing agents due to the presence of diverse active components, ease of access, and minimal side effects.
Due to the wide variety of active components, availability, and low risk of side effects, several plant extracts and their phytoconstituents are regarded as potential substitutes for skin care agents.
The majority of the bioactive components found in phyto-medicines are substances that are soluble in water, such as flavonoids, glycosides, and phenolics. However water soluble phytoconstituents are poorly absorbed when applied locally or consumed orally, their effectiveness is limited. A novel emerging technique applied to phytopharmaceutical for the enhancement of bioavailability of herbal extract for medicinal applications. Polyherbal Planterosomal gel is recent advanced forms of herbal formulations that have enhanced absorption rate, producing better bioavailability than conventional herbal extracts. Since they have improved pharmacological and pharmacokinetic parameters, they can be used in the treatment of the acute and chronic liver disease. The methanolic extract was derived from the dried leaves and rhizomes of *Azadirachta indica, Curcuma Longa, Glycyrrhiza glabra, Arnebia benthamii* was subjected to a phytochemical analysis. Four gel formulations of are prepared containing the planterosomes of polyherbal extract in four different concentrations i.e. 2%, 3%, 5%, and 10%. These gels were evaluated for their physical appearance, stability, antimicrobial activity, antioxidant, extrudability, pH, spreadability, and viscosity. The prepared formulas were stable, greenish and homogeneous. The spreadability (g.cm/sec), viscosity (cps), and pH of all three formulations was 28.5, 11231, and 6–7, respectively. Gel-F4 exhibited the highest antimicrobial potential against *E. coli* and *staphylococcus aureus with* a zone of inhibition of 19.2 ± 0.6 mm and 18.7± 0.6 mm, respectively.
An abundant source of phytochemicals, including flavonoids, terpenoids, steroids, tannins, alkaloids, phenolic compounds, carbohydrates, coumarins, proteins, quinines, anthraquinones, and saponins, is the polyherbal extract that was studied in this work. These findings highlight the extract's potential as a useful source of naturally occurring antimicrobial agents, indicating the need for more research into possible medicinal uses.

Key words: Polyherbal gel, phytochemicals, Planterosomes, Phytoconstituents, ethanolic extract, antimicrobial activity

1. INTRODUCTION:

The development and optimization of Polyherbal planterosomal gel for the recovery of abrased skin is an area of growing interest in the field of cosmeceuticals and skin care. Skin abrasion caused by various factors like aging, exposure to environmental pollutants, and injuries can lead to a range of undesirable effects, including wrinkles, scars, and uneven skin tone. Therefore, developing an effective topical product that promotes skin recovery and rejuvenation is of great importance.
Traditional herbal remedies have been used since long for their therapeutic properties in treating various skin ailments. [6]These herbal extracts are known to possess a wide range of bioactive compounds, such as polyphenols, flavonoids, terpenoids, and antioxidants, which contribute to their potential skin-healing properties. Incorporating these herbal extracts into a planterosomal gel formulation can enhance their

bioavailability, stability, and targeted delivery to the skin. Planterosomes, a novel delivery system, have gained significant attention in recent years due to their ability to encapsulate herbal extracts and enhance their penetration into the skin. Planterosomes are lipid-based nanoparticles composed of natural phospholipids, which mimic the skin's lipid structure and promote better compatibility and absorption. By encapsulating herbal extracts within planterosomes, their efficacy can be further improved, ensuring controlled and sustained release of active compounds to the damaged skin [16].
The formulation and evaluation of polyherbal planterosomal gel involve a comprehensive approach, including the selection of appropriate herbal extracts, optimization of planterosome preparation, determination of physicochemical characteristics, and assessment of its skin antimicrobial and antioxidant properties against gram+ve (*staphylococcus aureus*) and gram-ve (*Escherichia coli*) bacteria.[18] The evaluation parameters typically include viscosity, skin permeation, antioxidant activity, and skin regeneration potential. The formulation and evaluation of a polyherbal planterosomal gel involve the development of a topical product that combines multiple herbal extracts within a plantorosomal delivery system. This innovative approach aims to enhance the therapeutic benefits and effectiveness of herbal ingredients for the treatment of various skin conditions. [20]

Various topical formulations such as ointments, gels, creams or wound dressings are available to protect the skin from disease and accelerate wound healing. Gels are simple to apply to the wounds and can be washed easily. Gels are promising drug delivery tools, especially for topical treatments. In this study, gel formulations containing planterosomes and an extract of *Azadirachta indica, Curcuma Longa, Glycyrrhiza glabra, and Arnebia benthamii* were prepared to investigate the antioxidant and antimicrobial potential to cure skin. Gels are popular because of the ease of their application and improved percutaneous absorption compared to other preparations.

2. MATERIAL AND METHODS

Materials
Carbopol 934 Lubrizol Advanced Materials India Private Limited, India, Ethanol Sigma-Aldrich Corporation, triethanolamine, and propylene glycol were obtained from Merck (Darmstadt, Germany). The polyethylene glycol was obtained from Fluka (Steinheim, Germany)

Plant materials and preparation of extract-
Arnebia Benthamii, Azadirachta indicia, Glycyrrhiza glabra, and *Curcuma longa* leaves were authenticated straight from the CSIR (Lucknow). The collected plant materials were cleaned to remove dirt and any foreign materials and then dried under optimal conditions to preserve their bioactive compounds. The drying process varied depending on the plant material's nature; leaves and roots were typically air-dried or shade-dried to avoid the degradation of sensitive compounds by direct sunlight. **[**13]. The leaves and rhizomes part were collected, cleaned and dried in the shade at 25ºC, and a coarse powder of leaves and rhizomes was prepared. The coarse powder obtained from plants was subjected to extraction using the Soxhlet extraction method. 80% of methanol in the proportion of 1:8. Plant materials used in the formulation of polyherbal planterosomal gel are shown in the table below.

S.No	Botanical Plant	Family	Common Name	Part of Plant used
1	*Azadirachta indica*	Meliaceae	Neem	Leaves stems
2	*Arnebia benthamii*	Boraginaceae	Ratanjot	Leaves
3	*Glycyrrhiza glabra*	Leguminaceae	Mulethi/ Licorice	Root/Rhizomes
4	*Curcuma longa*	Zingiberaceae	Turmeric/haldi	Root/Rhizomes

Table -1 Plants used in the formulation of Polyherbal Planterosomal Gel formulation

3. PRELIMINARY PHYTOCHEMICAL ANALYSIS
Phytochemical testing involves screening plant extracts to identify various bioactive compounds. The process typically begins by preparing the plant extract, which is filtered and mixed with distilled water to yield a clear filtrate for the analysis of carbohydrate, alkaloids, flavonoids, glycosides etc. The phytochemical analysis of plant extracts involves a series of qualitative and quantitative assays designed to detect and characterize the primary and secondary metabolites present in the extracts. These metabolites include alkaloids, flavonoids, tannins, saponins, terpenoids, phenolic compounds, and glycosides, among others (Purna et al, 2010). Below is an outline of common phytochemical screening methods applied to the extracts of Neem (*Azadirachta indica*), *Curcuma longa* (Turmeric), *Arnebia benthamii*, and *Glycyrrhiza glabra* (Licorice).[45]

Preparation for phytochemical screening:

Extracts prepared from the selected plants are used for the analysis. It is important to have them in a suitable concentration, typically achieved through evaporation of the solvent and reconstitution in a minimal volume of an appropriate solvent for testing.

Tests for Specific Phytochemicals [25]

Test for Alkaloids:

Reagent Used: Dragendorff's reagent, Mayer's reagent.
Procedure: Treat the extract with the reagent. The formation of a precipitate indicates the presence of alkaloids.
Mayer's test- Took plant extract aqueous added two drops of Mayer's reagent along with side of test tube then white creamy precipitate indicated the presence of alkaloids.
Wagner's test- Took some aqueous plant extract then added few drops of Wagner's reagent and observed for reddish brown precipitate to confirm the test as positive.

Test for Flavonoids:
Reagent Used: AlCl3 solution, NaOH solution.
Procedure: Addition of AlCl3 to the extract causes yellow fluorescence in the presence of flavonoids. The NaOH solution test reveals yellow coloration, which becomes colorless upon addition of dilute acid.

***Test for* Tannins:**
Reagent Used: Ferric chloride (FeCl3).
Procedure: The addition of a few drops of FeCl3 to the extract. A blue-black or green-black colouration indicates the presence of tannins.

Test for Saponins:
Procedure: Shake the extract with water. The formation of stable foam indicates the presence of saponins.

***Test for Terpenoids*:**
Reagent Used: Salkowski test (chloroform and concentrated sulfuric acid).
Procedure: Mix extract with chloroform and carefully add concentrated sulfuric acid. A reddish-brown interface indicates the presence of terpenoids.

Test for Phenolic Compounds:
Reagent Used: Folin-Ciocalteu reagent.
Procedure: The addition of Folin-Ciocalteu reagent and sodium carbonate to the extract. A blue colouration indicates the presence of phenolic compounds.

***Test for Glycosides*:**
Reagent Used: Keller-Kiliani test (hydrochloric acid and ferric chloride).
Procedure: Treat the extract with hydrochloric acid followed by the addition of ferric chloride. The formation of a blue or green colour indicates the presence of glycosides.

Test for Carbohydrates
Procedure: Molish's test- Took 2 ml of plant extract added two drops of alcoholic solution of α-naphthol added then shaken well the mixture and added few drops of concentrated sulphuric acid slowly along the side of test tube, then a violet ring indicated the presence of carbohydrates.
Benedict's test- Took 0.5 ml of filtrate then added 0.5 ml of Benedict's reagent and mixture was heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicated the presence of sugar.

Test for Amino acids
The extract (100 mg) dissolved in 10 ml of distilled water and filtered through Whatmann no. 1 filter paper and then filtrate was subjected to test for amino acids.

Ninhydrin test- Took 2 ml aqueous filtrate then added two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) which given the purple colour indicated the presence of amino acids.

Test for Fixed oils and Fats

Spot test- Took two filter papers and placed a drop of extract on one filter paper then pressed by other filter paper. Oil stains on the paper indicated the presence of fixed oils.

Saponification test- Took some extract and added a few drops of 0.5 N alcoholic potassium hydroxide solutions with a drop of phenolphthalein. Whole mixture was heated over water bath for 2 hours. Formation of soap or partial neutralization of alkali indicated the presence of fixed oils and fats.

Test for Proteins

The 100 mg plant extract dissolved in 10 ml of distilled water was then filtered through Whatmann No. 1 filter paper and the filtrate was subjected to a test for proteins.

Millon's test- To 2 ml of filtrate, added few drops of Million's reagent then a white precipitate indicated the presence of proteins.

Biuret test- 2 ml of filtrate was mixed with 1 drop of 2% copper sulphate solution then 1 ml of ethanol (95%) and an excess of potassium hydroxide pellets. Pink colour ethanolic layer indicated the presence of protein

Test for gum and mucilage

100 mg of plant extract dissolved in 10 ml of distilled water then add 2 ml of absolute alcohol with constant stirring, if a white or cloudy precipitate appeared it indicated the presence of gums and mucilage.

Table: 2 Findings recorded in preliminary phytochemical screening of the methanolic extracts of plants under study:

Test	*Azadirachta indica*	*Curcuma longa*	*Glycyrrhiza glabra*	*Arnebia benthamii*
Carbohydrate	_	+	+	+
Protein/amino acid	+	+	+	+
Fats/ waxes	+	_	_	+
Glycoside	+	+	+	+
Flavonoides	+	+	+	+
Alkaloids	+	+	+	+
Terpenes	+	+	+	+
Steroids	+	_	+	+
Saponins	+	+	+	+
Phenolics/ Tannins	+	+	+	+
Volatile oil	_	+	-	_

+ (presence) - (Absence)

4.Development of Planterosonal Poly herbal Gel Formulation

Carbopol 934 in sufficient quantity was soaked in water for a period of 2 hours and then neutralized by triethanolamine with continuous stirring. The required amount of test extract or planterosome was dissolved in preweighted amounts of propylene glycol and ethanol. Solvent blend was transferred to carbopol container and agitated for additional 20 min. The dispersion was then allowed to hydrate and swell for 60 min, finally adjusted the pH with 98% solution of triethanolamine until desired pH value was obtained (6.8-7) Both of the solutions were mixed in a beaker and tri-ethanolamine was added to the mixture drop wise to obtain the gel consistency. It was stirred by using propeller for 2 hours at 500 rpm to obtain a homogenous gel, devoid of any entrapped air bubbles.

S.NO	**INGRIDIENT**	**FORMULATION POLYHERBAL PLANTEROSOMAL GEL**				
		F1 (2%)	**F2 (3%)**	**F3 (5%)**	**F4 (10%)**	**Role**
1	*Azadirachta indica(%w/w)*	**0.50**	**0.75**	**1.25**	**2.5**	**Planterosome**
2	*Glycyrrhiza glabara(%w/w)*	**0.50**	**0.75**	**1.25**	**2.5**	**Planterosome**

3	*Arnebia benthamii(%w/w)*	**0.50**	**0.75**	**1.25**	**2.5**	**Planterosome**
4	*Curcuma longa*(%w/w)	**0.50**	**0.75**	**1.25**	**2.5**	**Planterosome**
5	Carbopol 934	**48**	**48**	**47**	**47**	**Gelling Agent**
6	Propylene Glycol	**25**	**25**	**20**	**20**	**Preservative**
7	Ethanol	**10**	**10**	**10**	**10**	**Solvent**
8	Triehanolamine		q. s. to neutralize the gel base	q. s. to neutralize the gel base	q. s. to neutralize the gel base	**pH Balance**
9	Water		**q.s to 100 gm**		**q.s to 100 gm**	**Solvent**

Table: 3 (The extract was incorporated into the ointment base at different proportions.)

5. PHYSIOCHEMICAL EVALUATION OF THE POLYHERBAL PLANTEROSOMAL GEL FORMULATION [19]

The gels were assessed for its organoleptic characteristics such as Appearance, Nature, Grittiness, Phase separation. Other physical tests were carried out such as applicability and washability of gel from skin surface.
pH: A calibrated digital pH meter was used to determine the pH of the herbal gels. A total of 1 g of gel was dissolved in 100 mL of distilled water and kept it aside for 1 ht. The pH of each formulation was tested three times and the average results were calculated (Table 2).

Viscosity

For 30 min, herbal gel samples were kept at room temperature. The viscosity of the formulation was measured using a Brookfield viscometer. After attaching Spindle No. 7, the viscosity was measured at 200 rpm. The tests were carried out three times and recorded (Table 2).

Spreadability

A standard-sized glass slide was utilized; on it 0.5 g of gel was spread in a circle 1 cm in diameter on the glass slide, which was then covered with second same glass slide. A 125 g of weight was kept on upper slide to ensure that the gel formed a fine film over both slides. The weight was then withdrawn, as well as any excess gel at corner. The lower slides were fix on their location and upper slide was attached with weight of 20 g. The time was recorded to detach from one another. Formula used to calculate Spreadability is as follow.

$$S=M\times LT$$

Where:
S-Spreadability in grams/seconds.
M-Mass in grams.
L-Length of slide.
T-Time in seconds.

Extrudability

10 g of each formulation were precisely weighed and put into collapsible tubes that were tightly pushed on one side and clamped. After removing the cap to enable the gel to extrude, the gel was collected and weighed, and the gel percentage was determined.

Sensitivity

To the six individuals' specific amount of gel is applied to their forearms and left for 20 min. Any discomfort that developed after 20 min was documented.

Washability

A little amount of gel was applied to the skin surface and left to flow for 10 min under the force of running tap water. It was observed when the gel was fully withdrawn.

Parametr	**F1**	**F2**	**F3**	**F4**
Apperance	Green	Green	Brownish Green	Brownish Green
Nature	Homogenous	Homogenous	Homogenous	Homogenous
Grittiness	Nil	Nil	Nil	Nil

Phase sepration	No	No	No	No
pH	6.5666± 0.05773	6.6±0.1	6.8333± 0.05773	6.6666± 0.5773
Viscosity	11438±1	11460.33±0.5773	11372.33± 56.8712	11231±1
Spreadiity	28.33±0.2081	27.73±0.7767	29.56±0.05773	28.5±0.1
Extrubility	82.40±0.8737	85.24±0.7756	86.15±0.6445	86.25±0.7838

Table: 4 Pharmaceutical Evaluation Parameter of Gel

6. ANTIMICROBIAL ACTIVITIES OF POLYHERBAL PLANTEROSOMAL GEL

Method

The methanolic extract gel anti-bacterial activity will be evaluated by adopting the cup-plate diffusion technique using agar media, and doxycycline as the standard. The agar medium was prepared and sterilized for at least 15 mins at 15 lb/sq inch. An incubator was then used to incubate the prepared agar and placed into the control and test petri plates. Once the agar had solidified, a sterilized borer was used to create cups, filled with the testing solution, and left to incubate for 24 hr. Zone of inhibition demonstrates *Escherichia coli* and *Staphylococcus aureus* sensitivity to prepared samples. [16]

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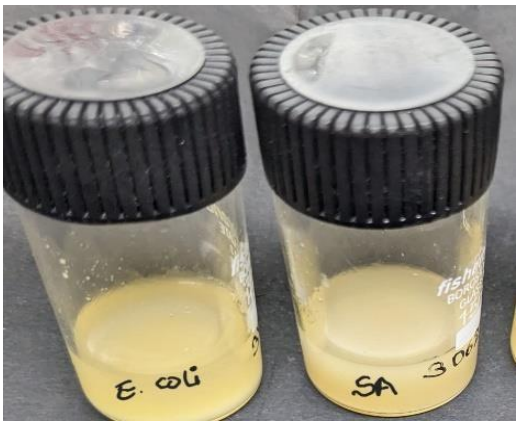


Figure: 1 *Escherichia coli, Staphylococcus aureus*

Result of Antimicrobial activity tests

To determine the anti-bacterial potential of the prepared methanolic extract planterosomal gel formulations, it was examined against gram+ve (*staphylococcus aureus*) and gram-ve (*Escherichia coli*) bacteria. The planterosomal gel formulation exhibited good zone of inhibition against tested bacteria. The zone of inhibition for planterosomal gel formulation was found to be 19.2 (***Stephylococcus aureus)***, 18.7 ***(Escherichia coli)*** and for the marketed formulation 29.5 ± 0.3 mm. The present study data shown in given Table 5 and given Figure, 2 exhibited an acceptable range of zone of inhibition, which is comparable with earlier reports.

Table: 5 Antimicrobial activity of Optimized Planterosomal Gel against *Stephylococcus aureus, Escherichia coli*

Time (in minute)	**Zone of inhibition (cm)**	
	Stephylococcus aureus	***Escherichia coli***
0	0	0
60	3.8	2.9
120	5.9	6.6
180	11.4	10.4
240	13.7	14.2
300	15.3	16.4

1440	19.2	18.7

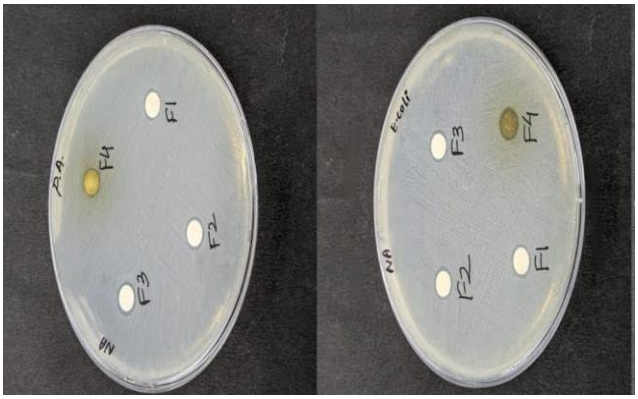


Figure: 2 Zone of Inhibition *Stephylococcus aureus, Escherichia coli*

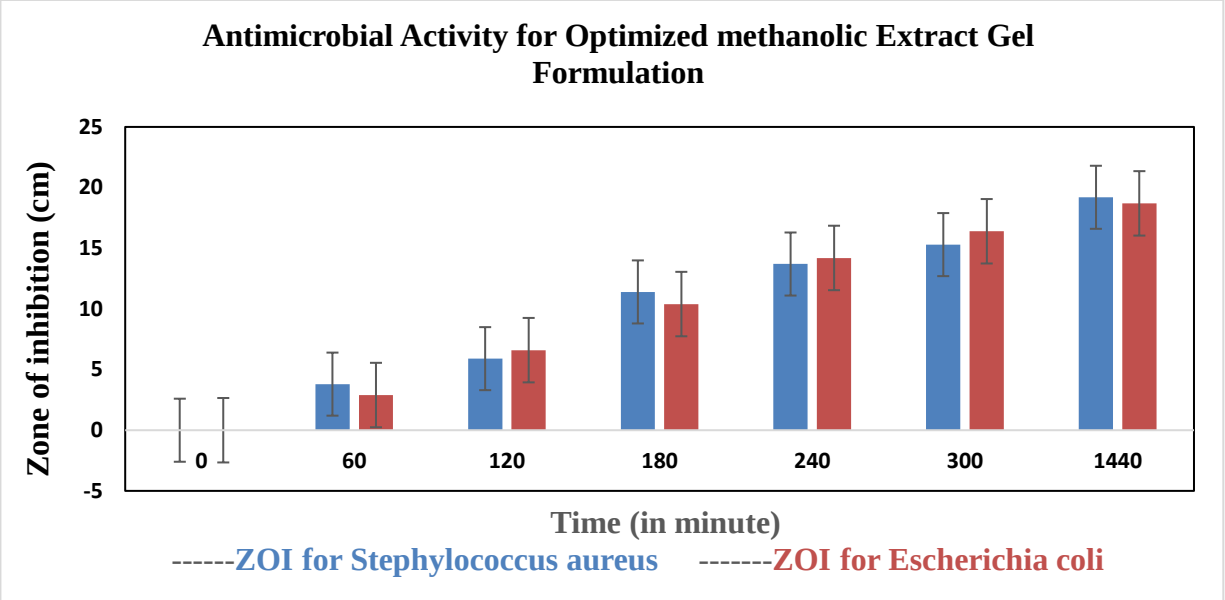


Figure: 3 Antimicrobial activities for optimized methanolic extract gel formulation

7. ANTIOXIDANT ACTIVITIES OF POLYHERBALPLANTEROSOMAL GEL

Determination of Free Radical Activity Methodology: [32]

DPPH is a stable radical and has a violet colour with a maximum absorbance at a wavelength of 517 nm in an ethanolic solution. When DPPH comes in contact with another radical (an antioxidant) it gets reduced. The reduced form of DPPH loses its properties as a free radical and accordingly changes its colour to yellow as represented in **Figure.4** DPPH assay is an easy and rapid way for investigating the antioxidant properties. However, DPPH is not a peroxyl-radicals (physiological radical), therefore Light, O_2, and pH can influence the DPPH absorbance. Furthermore, compounds such as carotenoids with an absorption wavelength of 517 nm can interfere with the absorption maximum of DPPH.

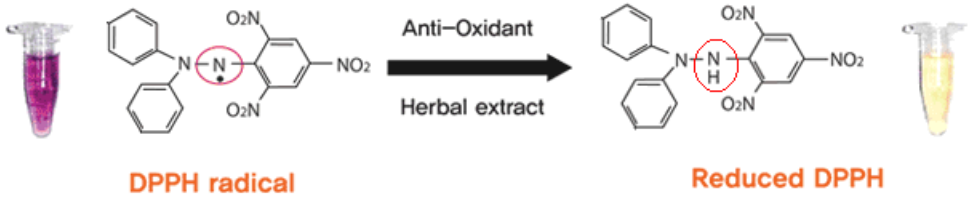


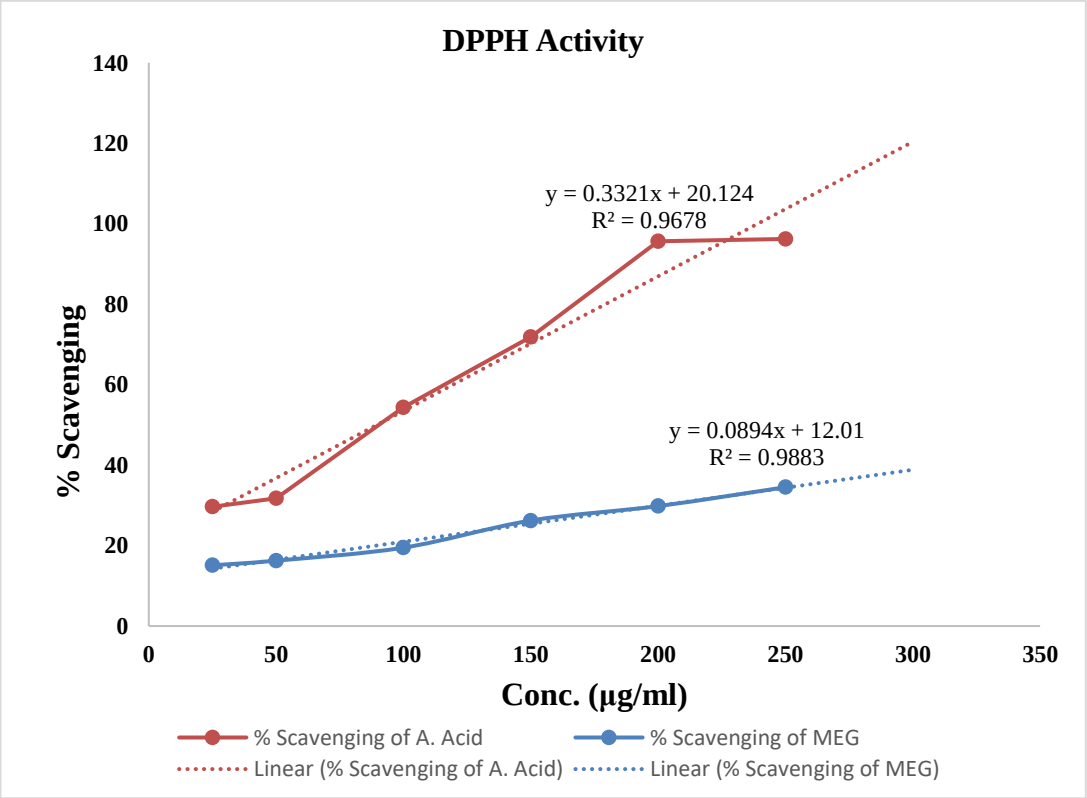
Figure: 4 DPPH assay principle

DPPH% = (A control-A sample) / (A control)

Table: 6 DPPH Activities

Conc. (µg/ml)	% Scavenging of EG	% Scavenging of A. Acid
25	15.1	29.65
50	16.25	31.73
100	19.48	54.36
150	26.22	71.84
200	29.81	95.64
250	34.51	96.22
300	---	---

Figure:5 DPPH Activity Result



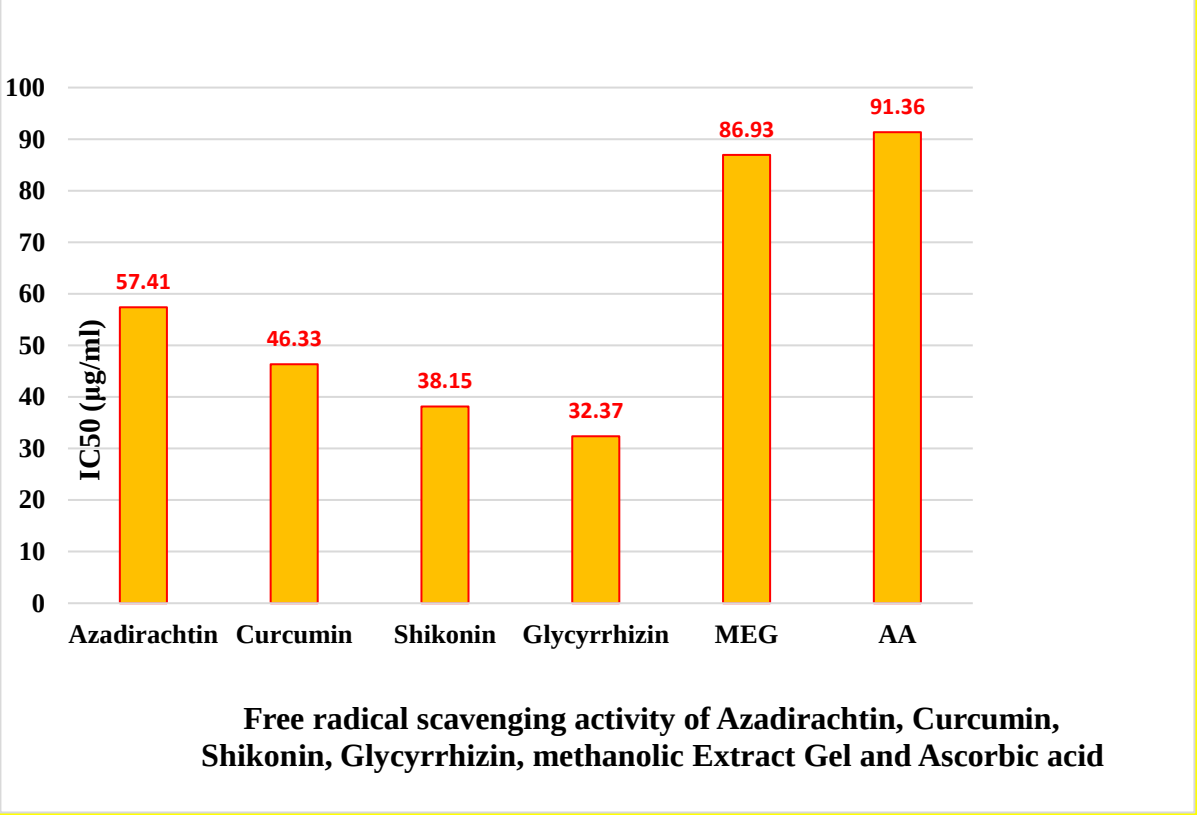
MEG (Methanolic Extract Gel)

*A. Acid (Ascorbic acid)

Table: 7 Free radical scavenging activity of Azadirachtin, Curcumin, Shikonin, Glycyrrhizin, Methanolic Extract Gel and Ascorbic acid

Drug Name	IC50 (µg/mL)
Azadirachtin	57.41
Curcumin	46.33
Shikonin	38.15
Glycyrrhizin	32.37
MEG	86.93
AA	91.36

Figure: 6 Free Radical scavenging activity of Azadirachtin, curcumin, shikonin, glycyrrhizin, methanolic extract gel and Ascorbic acid



8. RESULT AND DISCUSSION

The qualitative phytochemical analysis of extracts from Neem (*Azadirachta indica*), *Curcuma longa* (Turmeric), *Arnebia benthamii*, and *Glycyrrhiza glabra* (Licorice Roots) has provided insightful data on the presence and relative abundance of various bioactive compounds when extracted with different solvents, namely acetone, methanol, ethanol, and chloroform. The efficiency of these solvents in extracting phytochemicals, as indicated by the percentage yield, positions ethanol as the most effective solvent with a yield of 25.75%, followed by acetone, chloroform, and methanol, with yields of 16.10%, 15.50%, and 15.42% respectively. This efficiency is crucial for maximizing the extraction of potentially therapeutic compounds from plant materials. Conducting antimicrobial investigation on polyherbal gel formulation is to evaluate their effectiveness against two distinct bacteria. The gel formulations were also tested for antimicrobial effect

against selective microorganisms. The result shows that all the topical single herb gel and polyherbal Planterosomal gel formulations having the greater antimicrobial activity against *Staphylococcus.aureus, Escheria* coli. Based on zone of inhibitions, polyherbal gel formulation from methanolic extract MEG has better antimicrobial potential against *E. coli* (18.7) *S.aureus* (19.2).

9.CONCLUSION

In conclusion our results indicated that MEG formulations developed from the combination of methanolic extracts of *Azadirachta indica, Curcuma longa, Glycyrrhiza glabra*, *Arnebia benthamii* showed skin healing effect on various stages of bacterial infections.The polyherbal planterosomal gel investigated in this study serves as a potent reservoir of phytochemicals, encompassing a diverse array such as flavonoids, terpenoids, steroids, tannins, alkaloids, phenolic compounds, carbohydrates, coumarins, proteins, quinines, anthraquinones, and saponins. The study's findings underscore the robust antimicrobial properties, antioxidant properties of *Azadirachta indica, Curcuma longa, Glycyrrhiza glabra*, and *Arnebia benthamii*. Polyherbal planterosomal gel. This enhanced activity can be attributed to the presence of significant phenolic compounds, saponins, coumarins, and tannic acid among the active constituents identified. These results emphasize the extract's potential as a valuable source of natural antimicrobial agents, warranting further exploration for therapeutic applications. The poor absorption and poor bioavailability associated with the polar phytoconstituents limit the use of herbal drugs. These hindrances can be overcome by formulating a novel drug delivery system i.e., polyherbal planterosomal gel which has better bioavailability and cost-effective formulation with minimal side effects.

CONFLICT OF INTREST

The Authors declare that there is No conflict of interest

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