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Antimicrobial Activity of Aloe-Vera Extract and Developing Anti-Acne Gel Using Aloe-Vera and Other Herbal Drugs

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ABSTRACT
The preliminary phytochemical analysis of aqueous extract of Aloe-Vera showed presence of proteins, carbohydrate, phenol, steroids, flavonoids, glycosides. The preliminary phytochemical study of ethanolic extract of Aloe-Vera showed presence of anthraquinone. The study showed Aloe-Vera is a good source of phytochemical and can be used as medicinal herb.The antimicrobial activity of Aloe-Vera extract was tested against pathogenic bacteria like E. coli, Staphylococcus aureus and fungi Aspergillus Niger using agar well diffusion method. Ethanolic extract shows higherantimicrobial activity against E. coli and Aspergillus Niger. The aim of the study was to overcome the antibiotic resistance and side effects of synthetic drugs. Hence extract of aloe vera, neem and ocimum sanctum were used in the work because natural medicine are more acceptable with the belief that they are safe with less side effects. The topical anti- acne gel was formulated and tested for physical parameters.

Keywords: Antimicrobial, Aloe-Vera, Pathogens, Phytochemicals, Anti-acne.

I. INTRODUCTION

An antimicrobial is an agent an agent that kills microorganisms or stops their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibiotics are used against bacteria, and antifungals are used against fungi. They can also be classified according to their function. Agents that kill microbes are bactericide, while those that merely inhibit their growth are called bacteriostatic agents[(1)]Antimicrobials are necessary to treat various diseases. However, they cause adverse effects, such as allergic reactions, in addition to increased bacterial resistance. All common antimicrobials create harms that must be considered when choosing whether to prescribe.[(2)]

Herbs can be used in the form of plant extract or as their as their active components. Furthermore, most of the world's population used herbal materials due to their strong antimicrobial properties and primary healthcare benefits.[(3)]

There are at least 420 different plant species of aloe. Aloe- Vera sometimes described as wonder plant. It is a short stemmed succulent shrub growing to 60-100cm. The plant can survive at 40ºC temperature. [(4)] The aloe is derived from the Arabic word 'Alooh'. Alooh means shinning bitter substance while vera means true[(5)] Aloe gel is gellylike substance found in inner part of aoloevera plant leaf (parenchyma), that has been used since ancient times to treat burns and other wounds.[(6)] Aloe latex comes from plants skin vascular layer and is yellow in colour, containing a high concentration of anthraquinone compound which has been throught the centuries as cathartic abd for medicinal purposes.[(5)]

- Kingdom : Plantae
- Botanical name : Aloe-Vera, aloe barbedensis
- Synonym : Aloe barbedensis, aloe indicaroyle, aloe homilies, Ghrit kumara
- Family :Asphodalaceae
- Genus : Aloe
- Species : A. Vera

There are number of formulations are available in market with various pharmaceutical ingredients for the treatment of acne. Topical formulations, available in the market are as follows: gel, cream, lotion, face wash, cleansers, face pack, face mask. Aloe vera, Neem leaves, Tulsi are reported to have very beneficial effect on acne due to anti-microbial, anti-inflammatory and anti-oxidant activities of different chemical constituents.

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II. LITERATURE REVIEW

1. **Ibrahim Kahramanoglu,et. al., (2019).** Active constituent of the Aloe vera plant extract are chromone and antraquinone and its glycoside derivatives, alongside others such as phenyl pyrone derivatives, flavonoids, phenyl propanoids, coumarins, phytosterols, naphthalene analogs, lipids, and vitamins.(6)
2. **M. Malmir,et. al., (2017).** The antimicrobial activities of anthraquinone have been extensively studied in vitro on pure compounds or in crude plant extracts containing these class of constituents as marker compounds. Most of them exibit positive activity against a large panel of reference of the most common pathogens, including the main causative agents of currently no tratable infections.(7)
3. **Kametani,et. al., (2007).** Reprted the presence of pyrocatechol in Aloe vera, is comparable with the presenct study. Further, the antimicrobial action of pyrocatechol was illustrated by Cowan. Pyrocatechol is a hydroxylated phenol, known to be toxic to microorganisms.(8)
4. **RadhaM.H.,et. al., (2014).** Aloe-Vera is a succulent plant popularly recognized for its health promoting effects and used traditionally for its purgative effect and fresh gel used in different formulation and cosmetic preparations. It is used worldwide mainly for the treatment of dermatological problems and maintaince of healthy skin due to its healing, emollient, antioxidant, anti-inflammatory, anti-microbial, anti-cancer effects etc., it also used in food.(11)
5. **Kamal A.,et. al.,(2016).** The main featureof aloe-vera plant is its high water content ranging from 99% to 99.5% while remaining 0.5% solid material it contains 200 different potential active compounds, including vitamins, minerals, enzymes, simple and complex polysaccharides, phenolic compound and organic acids. Polysaccharides in aloe-vera gel consist of linear chain of glucose and mannose molecules. Phenolic compound consists phenolic acids, flavonoids, catechins, quinons, coumarins, tannins, etc. Nitrogen compound consists alkaloids, amines.(10)
6. **Samiha Mohsen,et. al.,(2020).** Antimicrobial are necessary to treat various diseases. However, they cause adverse effects such as allergic reaction, in addition to increased bacterial resistance. All antimicrobial assessed can cause gastrointestinal effects.(2)
7. **Dr. MahtabAlamKhan,et. al., (2014).** Medicinal plants such as aloe vera, tulsi, neem cure several common ailments. These considered as home remedies in many parts of India. Apart from the medicinal uses, herbs are also used in natural dye, food, perfume, tea and so on.()
8. **L.O. Orafidiya, et. al., (2004).** Studies the effect of aloe vera gel on the anti-acne properties of essential oil of Ocimumgratissimum Linn leaf- a preliminary clinical investigation (13)
9. **LoveleenPreet Kaur, et. al., (2016).** Studied the recent approach for Novel Drug delivery i.e Topical gel. He concluded the gel formulation provides better application property and stability in comparison to cream and ointment.(14)

III. MATERIALS AND METHOD

Drugs and materials used in dissertation work is listed in table

Sr.No.	Materials	Use
1	Aloe vera	Antimicrobial Agent
2	E. Coli	Bacteria
3	S. Aureus	Bacteria
4	Aspergillus nigar	Fungi
5	Ethanol	Solvent for extraction
6	Chloroform	Solvent for extraction
7	Distilled water	Vehicle
8	Yeast extract	For preparation of agar media
9	Peptone	For preparation of agar media
10	Sodium Chloride	For preparation of agar media
11	Agar	For preparation of agar media
12	Ninhydrin solution	For phytochemical test
13	Benedict reagent	For phytochemical test

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14	Ferrous chloride	For phytochemical test
15	Sodium hydroxide	For phytochemical test
16	Conc. Hydrochloric acid	For phytochemical test
17	Dil. HCl	For phytochemical test
18	Benzene	For phytochemical test
19	Ammonia	For phytochemical test
20	Neem powder	Antimicrobial and Antioxidant
21	Ocimum sanctum powder	Antimicrobial,Anti-allergic,Anti-aging
22	Tea tree oil	Anti-inflammatory, Antimicrobial agent
23	Carbapol	Gelling agent
24	Polyethylene Glycol 400	Thickener
25	Propylene Glycol	Emollient
26	Methyl paraben	Preservative
27	Propyl paraben	Preservative
28	Triethanolamine	pH adjuster

METHODS:

1. Collection of plant :

The plant Aloe vera leaves were collected from in and around the city of Kolhapur, Maharashtra.

2. Drying of plant :

The fresh leaves of aloe vera were well washed with distilled water and then air dried till all plant parts become well dried.

3. Preparation of powdered plant material :

After drying, the plant material are then powered by using grinder and placed into a well closed container.

4. Preliminary Qualitative Phytochemical Screening of Plant Specimen :

A. Preparation of Aqueous Extract

The aqueous extraction is done by taking 5 grams of the plant powder and mixed with 200 ml of distilled water in a beaker. The mixture is heated on a plate at30°C-40° C and mixed with continuous stirring for 20 min. The mixture is filtered using Whatmann filter paper and the filtrate is used for further preliminary phytochemical analysis.

B. Qualitative Analysis for Phytochemicals

1. Test for Proteins : Ninhydrin test
2. Test for carbohydrates: Benedict's reagent test
3. Test for Phenol and Tannins
4. Test for Flavonoids: Alkaline reagent test
5. Test for Saponins
6. Test for Glycosides: Salkowski's test
7. Test for Steroid

Preparation of extract by using soxhlet:

The powered Aloe vera was subjected to soxhlet extraction with solvent with solvent like ethanol and chloroform respectively.This extraction was done by taking 20gm of dried plant powder and was placed into a thimble then extracted with 250ml of different solvent separately (ethanol and chloroform).The extraction process carry on till the solvent in siphon tube of Soxhlet apparatus become colorless.

Drying of extract:

The extracts obtained from soxhlet were concentrated to dryness in a thermostatic water bath under controlled temperature.

Preliminary Qualitative Phytochemical Screening of aloe vera extract in solvent:

Modified anthraquinones test

Antimicrobial assay:

Well diffusion method was used to check the antimicrobial activity of sample against Gram-positive and Gram-negative bacteria. For this Sterile Nutrient agar plate was prepared. Then 24 hrs old culture of test organism were spread aseptically on Sterile Nutrient agar plate after that wells were prepared aseptically having 0.7 cm diameter and then 100 sample was added in to the well. Kept for diffusion in Refrigerator for 5 min. The plates were incubated at 37° C for 24 hrs.

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Preparation of extract by using soxhlet for neem and ocimumsanctum:

The powdered extract of herbal drugs were weighed and were individually subjected to soxhlet extraction separately. This extraction was done by taking 40 gm of dried plant powder and was placed into a thimble then extracted with 250 ml of solvent ethanol. The extraction processes carry on till the solvent in siphon tube of soxhlet apparatus become colorless.

Development of Formulation:

The desired conc. of gelling agent was weighed accurately and dispersed in hot purified water (not more than 60°C; 50% weight of the batch size) with moderate stirring, avoiding air entrapment and allowed to soak overnight. Desired quantity of methyl paraben and propyl paraben was dissolved in remaining amount of water by gentle heating. Desired quantity of polyethylene glycol, propylene glycol and herbal extract were added to the above mixture. This was finally mixed with previously soaked gel formulation. Triethanolamine was added at last to adjust the pH. Prepared formulations were filled in a suitable container and labeled accordingly.

Composition of developed formulations

Quantity taken per 100gm gel

INGREDIENS	FORMULATION
Ethanolic Aloe vera extract	1.40gm
Ethanolic Neem extract	0.70gm
EthanolicOcimumSantum extract	0.70gm
Tea tree oil	0.5ml
Carbapol	1.0gm
PEG 400	15ml
Propylene Glycol	3ml
Methyl Paraben	0.2gm
Propyl Paraben	0.2gm
Triethaolamine	Q.S
Purified water	Q.S

Evaluations of Formulations

1. Color:

Color of gel was checked visually

2. Odor:

The odor of the gel was checked by mixing the gel in water and taking the smell.

3. Consistency:

The consistency was checked by applying gel on skin.

4. Greasiness:

The greasiness was assessed by application on the skin.

5. Homogeneity:

Homogeneity was tested by visual inspection after allowing them to set in a container.Theywere evaluated for their appearance and presence of aggregates.

6. Washability:

Formulations were applied on the skin and then ease and extent of washing with water were checked manually.

7. pH:

pH of 1% aqueous solution of the formulation was measured by using a calibrated digital pH meter at constant temperature.

8. Speadiability:

In this method slip and drag characteristic of gel involve. Formulated (20mg) placed on the ground slide under study. The formulated gel placed like sandwich between this slide and another glass slides for 5 min to expel air to provide a uniform film of the paste between slides. Excess of the paste was scrapped off from the edges. The top plate was then subjected to pull of 80g with the help of string attached to the hook and time (sec) required by the top slide to cover a distance of 7.5cm was noted.

IV. RESULTS AND DISCUSSION

Percentage yield:

A. For Ethanol Extract Of Aloe vera:

20gm of powdered extract gives 1.15gm of extract in ethanol

Percentage yield = 5.75%

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B. For chloroform extract of Aloe vera:
20gm of powdered extract gives 0.55gm of extract in chloroform
Percentage yield =2.75%

The yield of extract of Aloe vera obtained by soxhlet with ethanol and chloroform solvent has been compared. The yield of ethanolic extract (5.75%) obtained was much higher than the chloroform extract (2.75%).

C. For Ethanol Extract of Neem:
40gm of powdered extract gives 1.52gm of extract in ethanol
Percentage yield =3.8%

D. For Ethanol Extract of OcimumSanctum:
40gm of powdered extract gives 1.46gm of extract in ethanol
Percentage yield =3.65%

Preliminary Qualitative Phytochemical Screening of Aloe vera aqueous extract:
The aqueous extract of aloe vera was prepared to conduct preliminary phytochemical analysis. The table shows result of phytochemical screening of aloe vera.

Preliminary Qualitative Phytochemical Screening of aqueous extract

Sr.No.	Test	Aqueous extract
1	Protein	Present
2	Carbohydrate	Present
3	Phenol	Present
4	Steroid	Present
5	Saponin	Absent
6	Flavonoids	Present
7	Glycosides	Present

Preliminary Qualitative Phytochemical Screening of Aloe vera Extract in solvents:

Preliminary Qualitative Phytochemical Screening of Aloe vera Extract in solvents

Sr.No.	Test for	Inference
1	Ethanolic extract	Anthroquinone present
2	Chloroform extract	Anthroquinone present

The phytochemical screening of aqueous extract of Aloe vera showed that bioactive compounds such as steroid, flavonoid,protein,phenol,carbohydrate,glycoside were detected to be present in leaves of Aloe vera where as saponin negative as shown in table. These findings are in total agreement with those existing in the literature.Other studies have shown that the presence of saponin depends on extraction solvent.since this plant had been used in the treatment of different ailment such as malaria,dysentery,diarrhea,skin burn etc.,the medicinal roles of these plant could be related to such a identified bioactive compounds. The presence of these biologically active compounds in extract has made the plant to be known for its medicinal use specially for antimicrobial activity against pathogenic organism

Antimicrobial Assay:-
The antimicrobial study of ethanol extract sample showed antibacterial and antifungal activities against E.coli, Staphaylococcus aureus, Aspergillus Niger.

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Data AntimicrobialAssay:-

Organisms	Samples {Zone of inhibition in mm}	
	Ethanol extract	Chloroform extract
Escherichia Coli	3.0	00
Staphalycoccus Aureus	2.0	00
Aspergillus Niger	3.0	00

Antibacterial and Antifungal activities of Aloe vera was evaluated on the basis of zone of inhibition by well diffusion method in the present study.Theseethanolic extract showed different degree of inhibitory effect against test pathogens . Where chloroform extract does not show any activity.

Evaluation of Formulation:-

Data of Evaluation of Formulation:-

Sr.No.	Test	Observation
1	Colour	Golden Yellow
2	Odour	Characteristics
3	Consistency	Semi Solid
4	Greasiness	Non grasy
5	Homogeneity	Homogenous
6	Washability	Good

pH Determination:

The pH formulation of anti-acne gel was studied by using digital ph meter
The observations of ph are mention in given below given table

Data of ph determination:

Sr. No.	Observation Time	pH Observed
1	After 15 min	5.7
2	After 24 hrs	5.7

There is no change of pH of formulation which was stored at room temperature.

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Volume 7, Issue 4 July-Aug 2022, pp:1912-1918 www.ijprajournal.com ISSN: 2456-4494

Spreadability
Data of spreadability

Sr .No.	Test	Observation
1	Spreadability	Easily spreadable

V. CONCLUSION

The result showed Aloe vera is potential plant containing phytochemicals and antimicrobial properties of it can utilized in various medicinal preparations and the control of various life-threatening diseases. The study has showed the importance of natural products to control antibiotic resistance bacteria, which have been threat to human health. Study has revealed that the presence of many secondary metabolites in the leaves of Aloe vera. It has the confirmed that confirmed that the aloe vera extract could be used for treatment of various infections. The result shows that Aloe vera could be exploited for new potent antimicrobial agent. The present study was done with formulation and evaluation of anti-acne gel. The sample materials used for the manufacturing of the formulation were from extracts of aloe vera, neem and tulsi. The main reason behind this study was to formulate a stable and safe anti-acne gel without using any type of synthetic additive. Different evaluation test were performed to check the performance of gel. From the results of performed tests we conclude that gel formulation is safe to use.

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