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FORMULATION AND EVALUATION OF ANTIBACTERIAL GEL USING LEAF EXTRACT OF ANDROGRAPHIS PANICULATA

Rashmi D. Zode*, C.M Chakole

Bajiraoji Karanjekar college of pharmacy, Sakoli.

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ABSTRACT

Andrographis Paniculata is an annual herbaceous plant in the family of acanthaceae native to India and Shrilanka. Andrographis Paniculata has shown anti-bacterial property which makes it an essential herb in the treatment of various infections. This study was to check the effectiveness of Andrographis Paniculata against the microbial species Staphylococcus aurens, to observe the zone of inhibition and to develop a topical gel formulation of Andrographis Paniculata for the treatment of acne. The extraction was done by maceration method and continues with phytochemical screening and herbal gel formulation using hydroxypropyl methyl cellulose as a base with different concentration 2%v/v, 4%v/v, 6%v/v and 8%v/v. The gel was evaluated for the physical appearance, ph, homogenicity and antibacterial activity. Different concentration of Andrographis paniculata methanolic extract exhibited relatively good antibacterial activity. It was concluded that the gel formulation having higher concentration (8%) is found to be good consistency compared to 2%, 4%, and 6%v/v and can be used to treat the antibacterial activity.

Corresponding author
Rashmi D. Zode
Bajiraoji Karanjekar College of Pharmacy,
Sakoli
rashmizode@gmail.com

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INTRODUCTION

India has rich tradition of plant based knowledge of healthcare. The use of the plant based medication is gradually becoming popular through out the world. [1]Topical gel preparations are intended for skin application or to certain mucosal surfaces for local action or percutaneous penetration of medicament. Gels are typically semi-solid formulations having a liquid phase that has been thickened with other components. The liquid phase allows free diffusion of molecules through the polymers scaffold and hence release should be equivalent to that from a simple solution .[2]Herbal medicine has become an item of global importance both medicinal and economical. Although usage of these herbal medicines has increased their quality, safety, and efficiency are serious concerns in industrialized and developing countries. Herbal remedies are getting increasing patient compliance as they devoid of typical side effects of allopathic medicines. The present research has been undertaken with the aim to formulate and evaluate the herbal gel containing Andrographis Paniculata leaf extracts. The gel formulation was designed by using carbapol 940, Andrographis paniculata leaf extract, propylene glycol, methyl paraben and required volume distilled water. The skin pH was maintained by drop wise addition of Tri-ethanolamine. The physiochemical parameters of formulation of formulation (pH, Spreadability, viscosity) were determined.[3] Herbal medicines are considered safer than allopathic medicines as allopathic medicines are associated with the side effects. One of the methods for its survival is preparation of extract and their formulation for better absorption and penetration of the active moiety into the systemic circulation.

MATERIALS AND METHODS:

Materials:

Collection of plant material:

The dried leaves of Andrographis Paniculata was collected from Sakoli of August 2017.The plant material was identified and authenticated from Department of Botany, M. B. Patel College , Sakoli

Preparation of Andrographis Paniculata Extract :

The Freshly collected leaves of Andrographis Paniculata Were cleaned and air dried under shade for 48 hours. Then dried leaves were then grounded into powder using an electrical grinder.150 gm of powder was extracted using 250 ml of methanol. The mixture was allowed to stand for 24 hours to ensure that all solvents and samples were completely homogenized. The macerated mixture was then filtered using filtered using filter paper and Buchner funnel. The liquid was concentrated and evaporated using a rotary evaporator under controlled temperature of 55^0c. The resultant extract was then stored in a refrigerator, prior to use.[4]

Fig1 .Extract of Andrographis paniculata extract.

Formulation of Andrographis Paniculata Gel

Briefly, Carbopol 940 was dispersed in 50 ml distilled water. It was kept under magnetic stirrer until a homogenous dispersion was formed. 1 g of methyl paraben was dissolved in 100 ml distilled water in a heated water bath. The solution was later allowed to cool. 2 ml methyl paraben was added to the carbopol dispersion. Then 10 ml propylene glycol 400 and 2 ml glycerin were added to the mixture. Required volume of Andrographis paniculata extract was added to 100 ml of distilled water to produce. Andrographis paniculata solution at various concentrations(2%v/v, 4%v/v, 6%v/v and 8%v/v). The homogenous dispersion was stirred using a magnetic stirrer. The pH of formulation was then adjusted to a neutral pH by titrating triethanolamine which enhances the gelling properties of carbopol 940. For positive control, gentamicin disc was used. For negative control, gel was prepared without M. ptelefolia. Table 1 below shows the various gel formulations. [4]

Table 1. Various gel formulations for the experiment.

a) Gel base A :

Extract concentration	Gel formulation(2%)
Carbapol	1g
Gycerine	2ml
Propylene glycol	10ml
Methyl Paraben	3ml
100% extract of Andrographis Paniculata	2ml
Distilled water	Up to 100ml

b) Gel base B :

Extract concentration	Gel formulation(4%)
Carbapol	1g
Gycerine	2ml
Propylene glycol	10ml
Methyl Paraben	3ml
100% extract of Andrographis Paniculata	4ml
Distilled water	Up to 100ml

c) Gel Base C :

Extract concentration	Gel formulation(6%)
Carbapol	1g
Gycerine	2ml
Propylene glycol	10ml
Methyl Paraben	3ml
100% extract of Andrographis Paniculata	6ml
Distilled water	Up to 100ml

d) Gel Base D.

Extract concentration	Gel formulation(8%)
Carbapol	1g
Gycerine	2ml
Propylene glycol	10ml
Methyl Paraben	3ml
100% extract of Andrographis Paniculata	8ml
Distilled water	Up to 100ml

Fig 2. Andrographis paniculata gel of Conc 8% v/v.

Evaluation of Gel:

Physical Evaluation:

Physical parameters such as color, consistency , appearance , washability and odour were assessed.

pH

pH of 1% aqueous solution of the formulation was measured by using a calibrated digital pH meter at constant temperature.[5]

Spreadability

Spreadability denotes the extent of area to which the gel readily spread on application to skin or the affected part. The bioavailability efficiency of a gel formulation also depends on its spreading value. The spreadability is expressed in terms of time in seconds taken by two slides slip off from the gel, placed in between the slides, under certain load. Lesser the time taken for separation of two slides, better the spreadability. Two sets of glass slides of standard dimensions were taken. The herbal gel formulation was placed over one of the slides. The other slide was placed on the top of the gel, such that the gel was sandwiched between the two slides in an area occupied by a distance of 6 cm along the slide. A 30gm weight was tied to thwe upper slide carefully. The time taken for the upper slide to travel the distance of 6cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated three times both formulated gels and marketed gel and the mean time taken for calculation.[6]

Formula: S=M×L/T

S=Spreadability
M= Mass in gm (30gm)
L=Length of the glass (6cm)
T= Time in sec.

Viscosity:

Viscosities of gels were determined using Brookfield viscometer. Gels were tested for their rheological characteristics at 250C using Brookfield viscometer. The measurement was made over the whole range of speed settings from 10rpm to 100rpm with 30seconds between 2 successive speeds and then in a descending orders.[2]

Drug content uniformity:

About 1 gm of gel was accurately weighed and transferred to 100ml volumetric flask to which about 70ml of methanol was added. After mixing, the volume was made up to 100ml with methanol. The content was filtered using filter paper. A quantity of 1ml was pipette out from the filtrate and suitably diluted with methanol. Then the extract was estimated spectrophotometrically by using Shimadzu UV/VIS spectrophotometer-1700 at respective λ max.[2]

Antimicrobial screening of Andrographis paniculata gel

The anti-bacterial activity of various gel formulations was determined by using modified agar well diffusion method. S. aureus, and Mueller Hinton agar was used in this method. Modified agar well diffusion method is different from disc diffusion method as the bacteria inoculum were mixed with the agar base and solidified before being incubated. Wells were punched into the agar and were filled with the gel sample. The anti-bacterial activity of gel was then determined.[4]

RESULT

Figure shows zone of inhibition exhibited by various gel formulations against Andrographis paniculata. All gel formulations displayed good anti-bacterial activity against Andrographis paniculata. As expected, 2% gel was less sensitive compared to the higher concentrations and 8% gel exhibited the greatest bacteria inhibition. From the physical observation of A B C & D formulation, it was concluded that gel formulation having ”CODE D” is found to be good consistency. Formulation A B & C was found to be thin & very stiff, so formulation D found to be better formula for preparation of hydrogel.

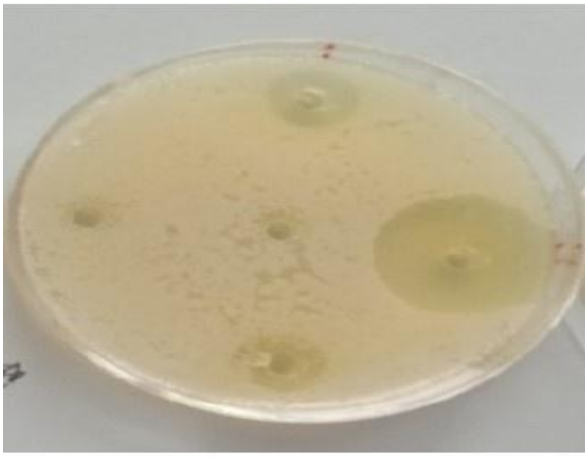

Fig .3 Zone of inhibition of gel of various concentration (2% , 4%,6%, And 8% v/v gel) against s.aureus.

Evaluation of Parameters:

Formulation was greenish in colour. Formulation A,B.C and D was found to have semisolid consistency. All the formulation were homogenous, easily washable and had optimal pH which is well suited with normal skin physiology.

Parameters	Gel A	Gel B	Gel C	Gel D
Color	Greenish	Greenish	Greenish	Greenish
Consistency	Semi-Solid	Semi-Solid	Semi-Solid	Semi-Solid
Odour	No	No	No	No
Appearance	Clear and Transparent	Clear and transparent	Clear and Transparent	Clear and Transparent
Washability	Good	Good	Good	Good
pH	6.52	6.62	6.65	6.73
Phase Separation	No	No	No	No
Spreadability	4.89	8.34	10.27	11.29
Viscosity	1860	1892	1846	1823
Drug Content %	60.5	65.2	80.4	86.9

Amongst all the formulation A, B, C,D had very optimum spreadability. Formulation D showed comparatively more spreadability than A. B and C.

CONCLUSION

Different concentration (25%v/v, 50% v/v, 75% v/v and 100% v/v) of Andrographis paniculata methanolic extract exhibited relatively good anti-bacterial activity against gram-positive bacteria strains S. aureus . There is a direct relationship between the plant extract concentration and the anti-bacterial activity. When incorporated into gel formulations, Andrographis paniculata has shown to be equally effective against both organisms at different concentrations. However, the dose-effectiveness relationship was not apparent for the gel formulations. The gel formulations showed good physicochemical and stability characteristics. From the physical observation of A, B, C and D formulation, it was concluded that gel formulation having “Gel D” is found to be good consistency. Formulation A. B and C was found to be thin and very stiff, so formulation D found to be better formula for preparation of hydrogel.

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Conflict of interest:

No Conflict of interest.

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