BJMHR
British Journal of Medical and Health Research
Journal home page: www.bjmhr.com

Formulation & Evaluation of Anti- Headlice Activity of Herbal Gel

Vikas P. Patil*, Prakash H. Patil
KVP's Sanstha's Institute of Pharmaceutical Education, Boradi 425 428, Tal-Shirpur, Maharashtra, India.

ABSTRACT

Gels are transparent to opaque semisolid containing gelling agent that merges or entangles to form a three dimensional colloidal network structure. Its responsible for a gel resistance to deformation and its visco-elastic properties. Gels have better potential as a vehicle to administer rug topically in comparison to ointments because they are non-sticky, require low energy during formulation, have aesthetics value and are stable. The physical appearance was simply checked by colour, odour, taste. In this a homogeneity and texture of prepared gel was also determined. Viscosity was determine using Brookfeild digital viscometer. 5gm prepared gel sample was placed in sample holder of B. Viscometer using spindle no.6 at 25 ^{0}c and allowed it to settle for 5 min and viscosity measured by rotating it at different rpm. Antiheadlice activity was determined by using antibacterial method by diffusion assay or cylinder plat or cup- plate method.

Keywords: Diffusion assay, anti-headlice activity, antibacterial activity.

*Corresponding Author Email: vp_57@rediffmail.com
Received 28 May 2019, Accepted 07 June 2019

Please cite this article as: Patil VP *et al.*, Formulation & Evaluation of Anti- Headlice Activity of Herbal Gel. British Journal of Medical and Health Research 2019.

INTRODUCTION

Herbal drugs have great growth potential in the global market. The R & D thrust in the pharmaceutical sector is focused on development of new innovative indigenous plant-based drugs through investigation of leads from the traditional system of medicine[1]. According to World Health Organization (WHO) more than 80% of the world's population, mostly in poor and less developed countries depend on traditional plant based medicines for their primary healthcare needs [2].

Among the estimated 400,000 plant species, only 6% have been studied for biological activity, and about 15% have been investigated phytochemically. This shows a need for investigation of various chemical constituents, its activity and phyto pharmacological evaluation of herbal drugs. The efficacy and safety of herbal medicine have turned the major pharmaceutical population towards medicinal plant's research [3].

MATERIALS AND METHOD

Formulation of Gel

Method of formulation

Different combination of Annona squamosa and Azadirachta Indica plant part extract were tried with different Types of polymers (carbomer934, HPMC) using various formulae. The following combination with sodium alginate resulted in a best gel formulation which was suitable and stable.

Procedure:

1. 2.5 g of sodium alginate was dispersed in 50 ml distilled water kept the beaker aside to swell sodium alginate for some time.
2. Then stirring should be done to mix the polymer in water.
3. Take a 0.5 g of methyl paraben and propyl paraben as preservative in warm water then add in the dispersion
4. Place the beaker in water bath and stir continuously until gel like consistency will obtain.
5. Further 2.5ml of Annona squamosa extract and Azadirachta indica add with 1 g of camphor. Again stir well.
6. Finally add the rose water as perfume and adjust the pH of gel with help of sodium hydroxide.

Formula for preparation of gel:

Table 1: Composition of Herbal gel

Sr. No.	Ingredients	Weight %
1.	Annona squamosa seed extract	2.5
2.	Azadirachta indica leaf extract	2.5
3.	Camphor	1
4.	Sodium alginate	2.5
5.	HPMC	1
6.	Methyl paraben	0.5
7.	Propyl paraben	0.5
8.	Rose water	q.s
9.	Sodium hydroxide	0.2
10.	Distilled water	100

A. Evaluation Parameters

1. Physical appearance:

The physical appearance was simply checked by colour, odour, taste. In this a homogeneity and texture of prepared gel was also determined [4.]

2. Measurement of Viscosity:

It was determine using Brookfield digital viscometer. 5gm prepared gel sample was placed in sample holder of B. Viscometer using spindle no.6 at 25 ^{0}c and allowed it to settle for 5 min and viscosity measured by rotating it at different rpm.

3. Determination of pH:

The pH of herbal gel was determined by using digital pH meter. 1gm of prepared herbal gel was dissolved in 25ml of distilled water. Then electrode was dipped in solution for 30 min until the constant reading was obtained and noted the constant reading [4, 9].

4. Wash ability:

Formulation applied on the skin. Then the ease and extent of gel washing with water were observed manually [5].

5. Spread ability:

Two glass slides of standers dimension (6×2) were selected. The gel was placed on slide and second slide placed on first slide in such a way that gel was sandwich between them across a length of 6cm of slide. 100 gm of weight was placed up on upper slide so that the gels between two slides were traced and uniform layer was formed. The weight was removed and excess of gel from the slides were scrapped off. The upper slide was then subjected to a pull of 50 gm with the help of string attached to the hook and the time (sec) required by upper slide to cover a distance of 10 cm was noted [4, 9.]

The spreadability was calculated by using formula-

$$S = ml/t$$

Where, S= Spreadability

m= weight tied to the upper slide

l= length of slide

t= time in second.

6. **Anti head lice activity**

Human head lice *Pediculus humanus capitis* (Trichodectidae) were collected from tribal children between the age of 3-12, with the approval of their guardians, residing in tribal pada near Boradi town in Dhule district. The insects were collected by combing the children scalps. The children had not been treated with any licicidal solution for at least the preceding month, using only the louse comb.

For testing the licicidal activity, a filter paper diffusion bioassay was made. After careful selection of lice under a dissecting microscope, a filter paper discs (Whatman No 1; 9-cm diameter) coinciding with internal diameter of petri dish were cut and placed in petri dishes, 0.25 gms of each test solution was spread over the lice and filter paper by using brush in each group. The sample solutions of gel formulation were tested for the licicidal activity.

Negative control lice were placed directly on the filter paper spread with 1 % w/w lindane solution in coconut oil was simultaneously run as a positive control, 1 % lindane topical lotion is commonly used synthetic insecticide to treat lice infestation hence licicidal activity of these test sample were compared with lindane. The numbers of lice were same in gel sample in negative control group and positive control group. The criteria used for survival of lice were extremely strict. If any minor signs of life, such as movements of antennae or minimal leg movements were observed (with or without stimulation by a forceps), the lice were categorized as alive. The lice were judged as dead if there were no vital signs at all. The test was done in duplicate and average considered [6, 11, 13, 14].

Anti bacterial activity:

1. Diffusion assay or Cylinder plat or Cup- plate method:

This method depends on the diffusion of an antibiotic from a vertical cylinder or cavity through the solidified agar layer of Petri dish or plat to an extent such that growth of added microorganism is presented entirely in a circular area or “zone” around the cavity.

Sample Compound:1

Media used:

Microbiology media used for bacteria (*Staphylococcus aureus and Escherichia coli)* is

Nutrient agar(Hi-media)

Inoculum Size: Bacteria: 1 X 10^8 bacteria per ml

Concentration of compound:

Stock solution [1000 microgram per ml] of each compound was prepared in DMSO. Assay carried taking concentration 100 microgram per disk.

Hi-media antibiotics disk: Ciprofloxacin (10 microgram/disk, Amphotericin-B (100 Unit/Disk) moistened with water are used as standard.

Method used:

Agar diffusion assay (Disc method, Disc size 6 mm)

Microbially cultures :

Micro-organism	Strain Name	Strain reference
Gram positive bacteria	*Staphylococus aureus*	NCIM 2079
Gram negative bacteria	*Escherichia coli*	NCIM 2109

Method of Screening:

The organisms were found to grow on nutrient agar. The sub culturing of the organisms was done on nutrient agar and the growth after 24 hours was taken for the sensitivity testing of the different compounds.

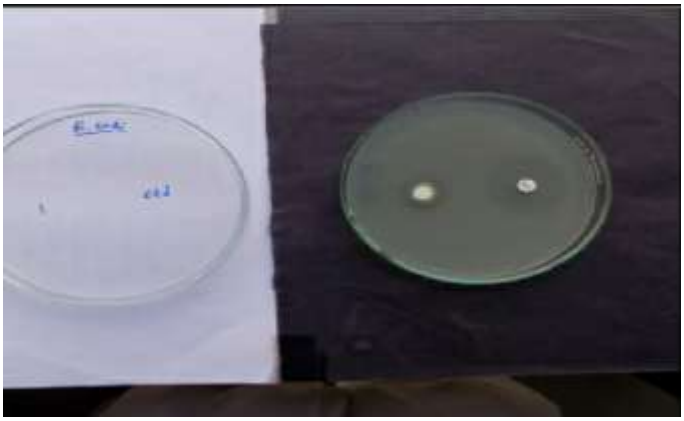
Table 2: Composition of Nutrient Agar media:

Sr.No.	Ingredients	Quantity
1.	Peptone	10.0 gm.
2.	Beef extract	10.0 gm.
3.	Sodium chloride	5.0 gm.
4.	Agar	3%.
5.	Distilled water	100 ml.
6.	pH	7.2.

Procedure:

Peptone, Yeast extract and Sodium chloride were dissolved in Purified water and the pH of the media was adjusted to 7.2 with 5M sodium hydroxide solution. To this solution Agar was added, boiled and stirred thoroughly until the agar was dissolved. Then 5 – 20 ml of this nutrient agar medium was transferred into each boiling tube and plugged with non-absorbent cotton. The tubes containing the nutrient agar medium were sterilized by pressure controlled heat sterilization technique using an autoclave at 15 Ibs and 115o C for 20 mints. After the sterilization the nutrient agar medium was melted, cooled and inoculated with one G (+ ve) organism viz. *Staphylococcus Aureus* and one G (- ve) organism viz. *E.Coli* are poured into sterile petridish to get a uniform thickness of 5 – 6 mm. Cups were made out in the other plate using sterile cork borer (6 dm).

The standard anti-bacterial agent Chloramphenicol (10µg/ml) and solvent control (10% v/v DMSO) suspension and the newly compound (10µg/ml) were added with the sterile micro pipette into each cups. The plates were kept in the refrigerator for 6 hrs and then incubated at 37oC for 24 hrs and the diameters of zone of inhibition were measured [7, 8, 10, 15].



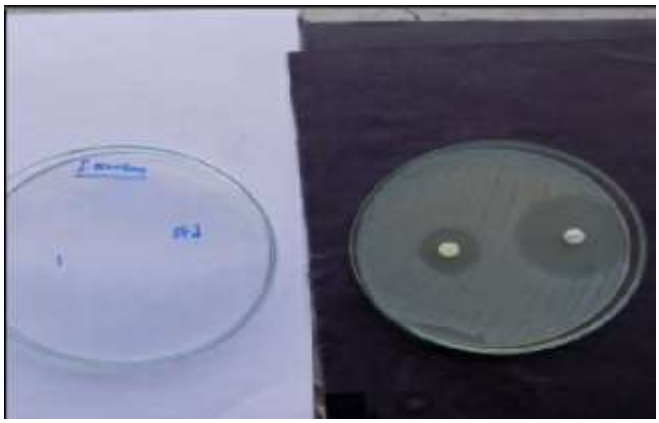


Figure 1 & 2: Zone of Inhibition of against *E Coli* and *S.aureus organism.*

RESULTS AND DISCUSSION

Evaluation of herbal gel formulation.

1. Physical Evaluation:

The prepared herbal gel was evaluated for its physical appearance. That is the physical characteristics like colour, odour, taste and texture were checked visually and tabulated in given table.

Table 3: Physical Evaluation

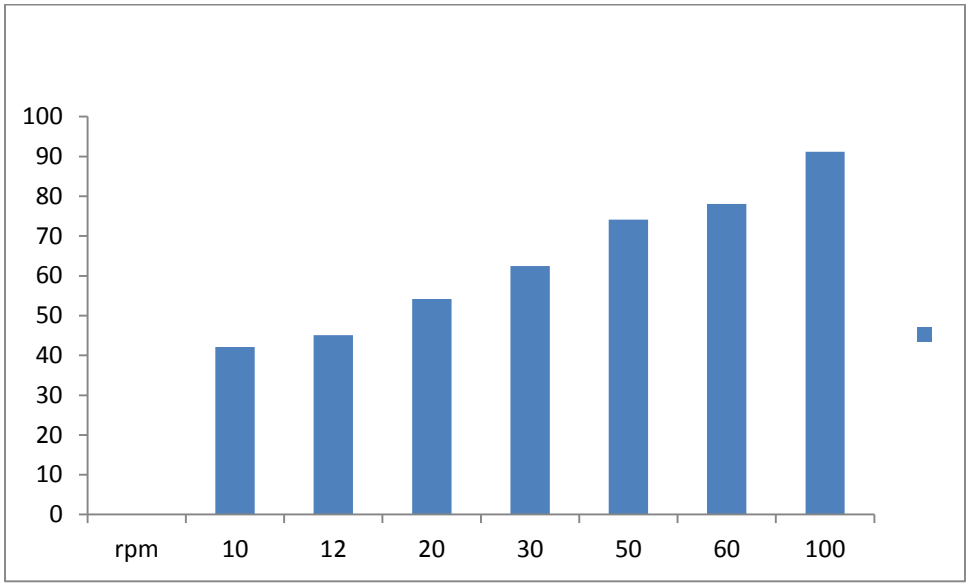
Physical Identification	Characteristics
Colour	Green
Odour	Aromatic
Taste	Bitter
Texture	Smooth

2. Viscosity:

Viscosity is the rheological parameters of the gel and controlled by the different polymers used in the formulation. The viscosity of prepared herbal gel differs in accordance with the use of polymers in the formulation. Thus the viscosity of given prepared gel by using Brookfield viscometer was found is tabulated as below-

Table 4: Viscosity of gel formulation

Sr no.	Rpm	Viscosity in %	Viscosity in cp
1	10	42.1	42100
2	12	45.1	27100
3	20	54.2	21300
4	30	62.5	20870
5	50	74.1	14800
6	60	78.1	13050
7	100	91.2	9130



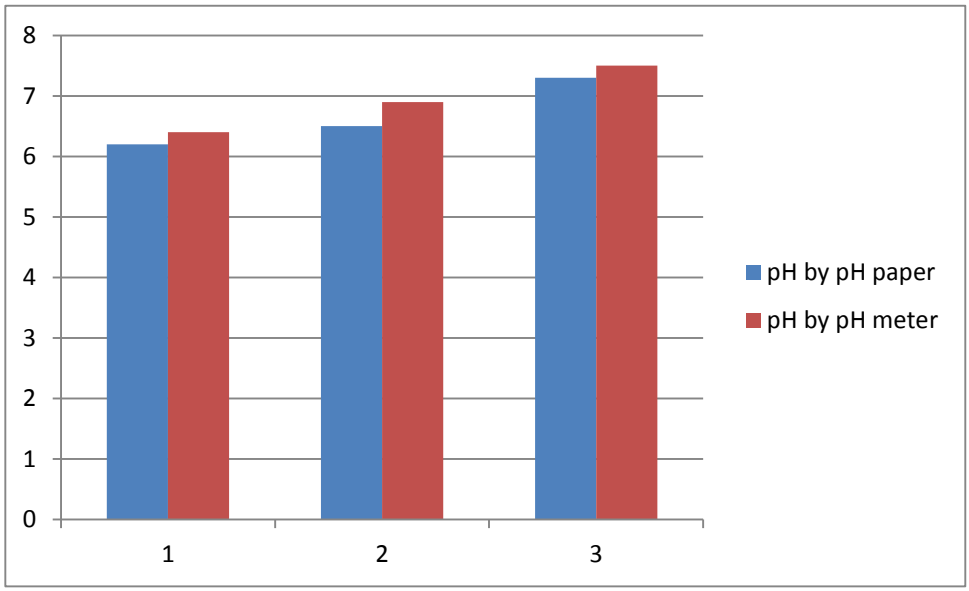
Graph 1: Viscosity of gel

3. pH:

The pH is determine by using pH paper or by digital pH meter. The pH of formulation differs mainly due to the difference of pH of polymers used due to the neutralization of the formulation and its same in all. Thus the pH of given prepared herbal gel by using pH paper was found to be 7.00 and by using pH meter it was found to be 7.2 which are between the range the of skin pH.

Table 5: pH determined by different method

Sr. No.	pH by pH paper	pH by pH meter
1	6.2	6.4
2	6.5	6.9
3	7.0	7.2



Graph 2: pH of gel formulation

4. Wash ability:

The wash ability is simply check by washing with water. Thus the given prepared formulation of gel was easily wash with water and found to be good wash ability.

5. Spread ability:

Spreadability plays important role and helps in uniform application of gel on hair. A good gel takes less time to spread and have higher spreadability. Thus the spreadability of given prepared formulation of gel took less time to spread and was found to be 82.1%.

6. Anti head lice activity:

The sample of gel showed significant decrease in the mean time required to kill lice with sample gel formulation when compared to 1 % lindane. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of *Azadirachta indica* A. and *Annona squamosa* L. gel for controlling head lice by tribals in this area, which are difficult to control because of their resistance to the currently used anti-louse agents.

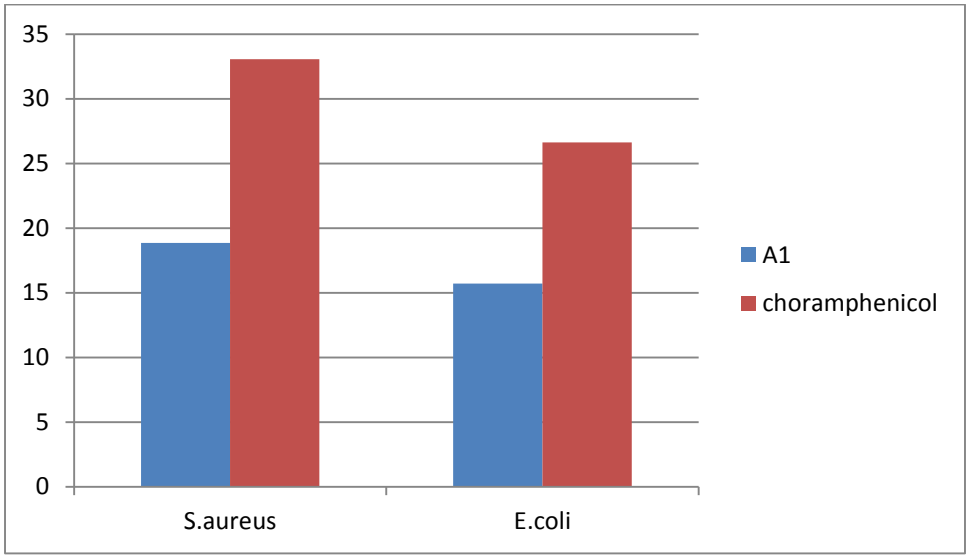
Table 6: Licicidal activity of gel formulation

Drugs	Concentration	Mean time
Sample gel formulation	2.5%	4 min 36 sec
Lindane solution	1%	53 min 40 sec

7.Anti-Bacterial Activity:

All compounds were screened for antibacterial activity against Staphylococcus Aureus and Escherichia coli at 10µg/ml. The synthesized compounds showed significant anti-bacterial activity against *Escherichia coli* and *Staphylococcus Aureus* at 10µg/ml.

Sr. No.	Sample code	*S.aureus*	E.coli
1.	1	18.86	15.71
2.	Chloramphenicol	33.06	26.64



Graph 3: Antibacterial activity of sample and standard drug

REFERENCES

1. Rajsekhar Saha, International journal of pharmacy and life science, Pharmacognosy and Pharmacology of A. squamosa, Vol-II, issue 2, Oct 2011.
2. Ashok Kumar J, Rekha T, Jaswanth, Gopal V, Insecticidal Activity of Ethanical Extract of Leaves of A. squamosa, Journal of Chemical and Pharmaceutical Research, Res., 43:141-150, special issue, Dec-2011.
3. Trease and Evans, Pharmacognosy, fifteenth edition, published by Elsevier, pg no. 470.
4. Kosalge S. B. and Fursule R. B., Investigation of Licicidal activity of some plants from Satapuda hill, International Journal of Pharma Tech Research, Vol-1, No.- 3, pg no. 564- 567, July-Sep – 2009.
5. Sharma M., Bhowmick M., Pandey G.K., Joshi A., Dubey B., Formulation and Evaluation of Hair Gel, International Journal for Pharmaceutical Research Scholars, Vol-2, I-4, 2013.
6. William Hewitt., 2004 Microbiological Assays for Pharmaceutical Analysis, a Rational Approach, Interpharm / CRC, Washington Page no. 684.
7. Michigan Head Lice Manual, A Comphrensive Guide to Identify, Treat, Manage and
8. Prevent Head Lice, published by Michigan Department of Community Health, pg no. 2, 6.
9. Drawn H. Gouge, Pediculosis capitis(lice), The University Arizona, Collage of Agriculture and Life science, pg no. 4.
10. Kokate C. K., Purohit A. P., Gokhale S. B., Pharmacognosy, 44th edition-2008, Nirali Prakashan Pune, pg no.20.5, 11.17.
11. Pelzar, M.V., Chan, E.C.S, Krieg, M.R**.,** Microbiology, 5thEd. Mac Grow Hill Publishing Co. Ltd., New Delhi, 1983, Page no. 535.

12. Sharma M., Bhowmick M., Pandey G.K., Joshi A., Dubey B., Formulation and Evaluation of Hair Gel, International Journal for Pharmaceutical Research Scholars, Vol-2, I-4, 2013.
13. Michigan Head Lice Manual, A Comphrensive Guide to Identify, Treat, Manage and Prevent Head Lice, published by Michigan Department of Community Health, pg no. 2,6.
14. Junya Intaranongpai, Warinthorn Chavasiri, Wandee Gristasanapan, Anti head lice effect of A. squamosa, Vol-37, No.3, May 2006.
15. Gajlakshami S, Divya R, Divya V, Mythili S, Pharmacological activities of Annona squamosa -A Review, Vol-II, issue 2, 2011.