International Journal of Advanced Research in Engineering Technology & Science
ISSN: 2394-2819
Impact Factor: 7.10
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Email: editor@ijarets.org Volume-13, Issue-1 January – 2026 www.ijarets.org

Characterization and Estimation of Phyto-constituents in Anti-fungal Formulations

1. **Deepika Arya, Research Scholar School of Pharmacy , YBN University, Ranchi**
2. **Ms. Umang Shahu, Assistant Professor School of Pharmacy, YBN University, Ranchi**
3. **Prof. (Dr.) Ashish Sarkar, Dean School of Pharmacy , YBN University, Ranchi**

ABSTRACT

The selected gum arabic based nanoemulsion were further formulated into a nanogel by using two different gelling agents i.e. xanthan gum and guar gum. Nine different ratios of xanthan gum and guar gum were formulated with five different ratios of oils i.e (1%, 3%, 5% and 10%). On the basis of physical evaluation, stability parameters, Droplet size and polydispersity index characterization. Out of all prepared herbal formulations, the guar gum based *B. aristata* nanogel showed good results with least particle size of 206.3 nm and 0.24 PDI value as compared to other herbal nanogel. The pH was near to pH of the skin which revealed nonirritating nature of the formulation. *In vitro* cumulative drug released was found to be highest in *B. aristata* guar gum nanogel (81.50 ± 1.29) at the end of 24 h respectively. *In vitro* antidermatophytic activity of optimized xanthan gum and guar gum nanogels prepared from herbal extracts was carried out against *T. rubrum* and *T. mentagrophytes.* The results of antidermatophytic effect revealed that the *B. aristata* guar gum nanogel showed significant zone of inhibition against *T. rubrum* as compared to other herbal formulations. After optimization and *in vitro* analysis, the formulated *B. aristata* based xanthan gum and guar gum nanogel was further evaluated for zeta potential, TEM, FESEM, spreadability, extrudability, rheological behavior, texture analysis,

.

Keywords: *Berberis aristata*, *Citrullus lanatus*, Skin infections, Nanoemulsion, Herbal nanogel, and antidermatophytic

1 Introduction

Herbal Drugs

Herbs have been used by all cultures throughout the history. It was an integral part of the development of modern civilization. Primitive man observed and appreciated the great diversity of herbs available to him. The herbs provide food, clothing, shelter and medicine. Much of the uses of plants seem to have been developed through observations of primitive men by trial and error. As time went on, each tribe added the magic power of herbs in their area to its knowledge base. They methodically collected information on herbs and developed well-defined herbal knowledge.

Herbs are staging a comeback and herbal renaissance is happening all over the globe. The herbal products have been symbolizing safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs have been priced for their medicinal, flavoring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, due to serious side effects. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security.

Tribe man is a vanishing relic of early man in our society. He confined to widely scattered and completely restricted pockets, chiefly in the hills to which he has restricted under the increasing pressure of civilizations. The primitives men during the course of their struggle for existence in the forest must have encountered the miseries of pains and sickness, sustained injuries and to liberate themselves from these suffering should have looked towards their fellow friends – the plants. And thus, the discovery of healing properties of herbs comes into existence.

The knowledge of herbal products flourished in our country since ancient past and has been handed down through generation and has survived among the ethnic communities. The circumstances under which the people lived – abject poverty, disease and hunger, combined with their natural curiosity towards their closest neighbor, the forest in which they lived and sought help in mitigating their woes and sorrows, must have been the essential factor in preserving their knowledge of herbs and usefulness to mankind.

2 Methodology

Formulation of Guar gum and Xanthan gum based nanogels

Nanogels were finally formulated from above prepared different concentration of oil based nanoemulsion formulations. In general, two different hydrophilic polymers like guar gum and xanthan gum were used to formulate the gel for topical application. Different concentrations of natural and semisynthetic polymers were postulated by adding pectin, carboxymethylcellulose (CMC) which was used as a gelling agent. Finally, the solution was plasticized with addition of glycerol. The composition of formulation was tabulated in Table 3.3. The gel stage is mixed into the nanoemulsion stage to procure nanogel. Incorporation of above prepared nanoemulsion into gel base with steady blending. The mixture was subjected to magnetic stirrer at 1200 rpm up to 30 minutes to form nanogel. Same procedure was used to formulate the xanthan gum based nanogels. The pH of prepared nanogel formulas was adjusted using triethanolamine (TEA) with stirring until desired pH value was approximately reached (6.1-6.5) . Prepared samples were stored in glass vials at room temperature (25℃) for further analysis.

Physical evaluation and characterization of formulated Nanogel

Homogeneity

After placing the gels in the container, all formulations were tested for color, homogeneity (aggregates presence and appearance) by inspecting visually.

Droplet size and polydispersity index

The droplet size and PDI was determined using Zetasizer Nano ZS Particle Sizer (Malvern Instruments Ltd, Malvern, WR14 1XZ, UK) at a temperature of 25°C. The 1% nanogel was suitably diluted in triple distilled water before taking the droplet size and three measurements were acquired for each sample.

In vitro drug release study of formulated nanogels

In vitro drug release for the nanogel formulation was performed in phosphate buffer (pH 6.8) using dialysis membrane. The membrane (pore size: 12 KD, Sigma Chemical Co., USA) was

activated by keeping it in phosphate buffer solution overnight. It was exposed to running water for few hours to remove glycerin based contents. Quantity of 5 g of all prepared hydrogel formulations were individually packed into dialysis tube with the ends being tightly fastened and placed in a 150 ml of 10 mM phosphate buffer solution (pH 7.4) in a shaking incubator (maintained at 100 rpm and 37
± 0.5°C). 1ml sample was withdrawn at predetermined intervals and replaced with same volume of the fresh buffer solution. The samples were then filtered and assayed for the drug content at 210 nm on UV/Visible spectrophotometer against the control. Absorbance was converted to drug concentration using a linear equation of calibration curve and then the cumulative percentage released was calculated taking into consideration the dilution factor. All measurements were performed in triplicate (n = 3).

***In vitro* antidermatophytic activity of optimized nanogel against dermatophytes**

The antifungal potential was evaluated by agar well diffusion method. In brief, 25 ml of sterilized SDA media poured into the Petri plates. 10 µl of fungal suspension was uniformly spread over solidified SDA plates by using a sterilized spreader. The wells having a diameter of 6 mm were made in the center of these SDA plates by sterilized cork borer. The 100 mg of formulated nanogel was loaded into the wells and permitted to diffuse at room temperature for an hour. Pure DMSO was used as a negative control, and for positive control, Ketoconazole marketed gel was used. Then, for 3 days, the plates were incubated at 28- 30°C. Antifungal activity was evaluated around the well that was filled with the oil by measuring the diameter of the inhibition zone.

Characterization of selected nanogel formulations

Zeta potential measurement

Zeta potential of the samples was measured as an indicator of physical stability. It was carried out in a folded capillary cell using a Zetasizer Nano. 1% of sample was suitably diluted in triple distilled water before taking the zeta potential measurement. Each sample was measured in triplicates.

The prepared nanogel was analyzed by field emission scanning electron microscope

(FESEM). A small amount of nanogel sample was first fixed on carbon coated copper grid. The fixed sample on the grid was allowed to dry for 10 to 15 minutes prior to FESEM imaging. at high tension range 200 to 30,000 volts.

In-vitro evaluation of nanogels

Spreadability test of prepared nanogels

A sample of 0.5 g of each developed nanogel formulations were pressed between two slides and left for about 5 min where no more spreading was expected. Diameters of spreader circles were measured in cm and were taken as comparative values for spreadability. The results obtained were average of three determinations. The following formula was used for the determination of spreadability:

$S = ML/T$

Where, S indicated the spreadability (g.cm/sec), M indicates the mass (g), L indicates the length (cm), and T indicates time (sec).

Stability studies

The optimized nanogel was stored at 4°C, 25℃ and 40°C for 3 months and samples were evaluated for physical parameters like turbidity, color change, pH and physicochemical parameters, drug content at one month interval. Any changes in evaluation parameters, if observed were noted.

Statistical analysis

All experiment was performed in triplicates with at least three to four concentration and results were expressed as Mean ± standard error mean (SEM). IC_{50} was calculated by excel and graph was plotted using prism 5.02. The data was analyzed by one-way ANOVA followed by Dunnett's test and $P < 0.05$ considered as significant.

Plant Extract Preparation

The yield of *C. lanatus* seed oil, *Pipper longum* (whole plant), *Phyllanthus niruri* (whole plant) and *Berberis aristata* (Bark) were found out to be 4.1%, 15.2%, 9.1% and 10.5 respectively. The

percentage yield of all the plants extract may depend on several factors which includes polarities of solvents, type of plant extract, solubility of plant and physical assets of the plants.

Table : Percentage yields of *C. lanatus* seed oil, *Pipper longum, Phyllanthus niruri* and *Berberis aristata*

S. No	Plant name	Part used	Extraction process	Final % yield
1.	*C. lanatus* seed oil	Seeds	Water Distillation	4.1
2.	*Pipper longum*	Fruit	Solvent distillation using methanol	15.2
3.	*Phyllanthus niruri*	Whole plant	Solvent distillation using methanol	9.1
4.	*Berberis aristata*	Bark	Solvent distillation using methanol	10.5

Qualitative screening of Phytoconstituent

The phytochemical screening of the obtained plant extracts was qualitatively screened out for the presence of alkaloids, flavonoid, phenolics, amino acids, proteins, carbohydrates, saponins, glycosides, and phytosteroids. It was observed from qualitative analysis that the plant extracts consist of the most of the phytoconstituents listed.

Physiochemical characterization of *Citrullus lanatus* seed oil

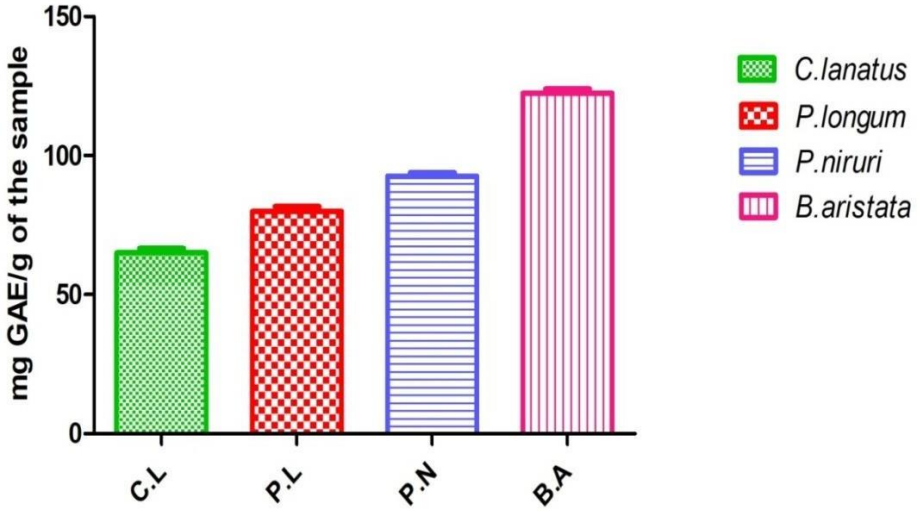
C.lanatus seed oil was evaluated for its physicochemical characteristics and the results are represented in (Table 4.3). The appearance of oil was light yellow in colour and was stable at room temperature and the oil acid value was found to be 7.60 mg/Na OHg. However, *C.lanatus* oil has 6.6% content of low free fatty acid; oil with low free fatty acids considered to be of better quality. However, the peroxide value of oil was found to be 18.5 (meq/kg) which is slightly higher and this can be due to the presence of higher amounts of unsaturated fatty acids whereas, the iodine value was observed 58.4 (g/100g) which measures the unsaturation in fats and oils.

3 Result and Discussion

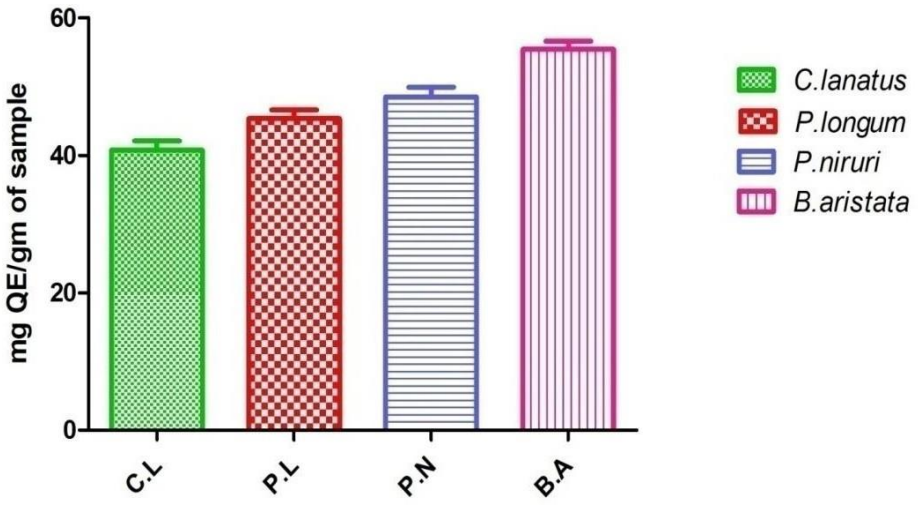
Quantitative analysis of Phenolic and Flavonoid content in plants

The total phenolic content (TPC) and total flavonoid content (TFC) was carried out and results are represented in Table 4.5. Total flavonoid content and phenolic content was calibrated by using the standard curve of gallic acid and quercetin using equation respectively (TFC- Y = 0.2052x - 0.0521 and TPC- Y = 1.3939x + 0.4682). The total phenolic content was found to be highest *in Berberis aristata* (122.5 ± 1.47 mg GAE/ gm), followed by *Phyllanthus niruri* (92.6 ± 1.24 mg GAE/ gm) and *Pipper longum* (79.9
±1.71 mg GAE/ gm). Whereas, the *C. lanatus* seed oil showed (65 ± 1.63 mg GAE/ gm) of total phenolic content (Fig 4.2 A & B).

Furthermore, the total flavonoid content was also found to be highest in *Berberis aristata* (55.4 ± 1.17 mgQE/ gm) followed by *Phyllanthus niruri* (48.4 ± 1.40 mgQE/ gm) and least in *Pipper longum* (45.3 ± 1.24 mgQE/ gm). Whereas, the *C. lanatus* seed oil showed (40.7 ± 1.32 mgQE/ gm) of flavonoid content. The results indicated that the methanolic extract of *Berberis aristata* consists of high amount of total phenolic and flavonoid content.



(A)



(B)

Figure (A) Quantitative analysis of Phenol (GAE/gm) and (B) Quantitative analysis of Flavonoids (mgQE/ gm) in *Citrullus lanatus* seed oil (C.L), *Pipper longum* (P.L), *Phyllanthus niruri* (P.N) and *Berberis aristata* (B.A)

***In vitro* Antioxidant activity (DPPH assay)**

The free radical scavenging activities of the plant extracts and *C. lanatus* seed oil were estimated. Among the plant extracts undertaken, the *Berberis aristata* showed percentage inhibition of 82.1% (IC_{50} value 54.8 µg/mL) followed by *Phyllanthus niruri* having inhibition of 72.80% having (IC_{50} value (61.9 µg/mL) and *Pipper longum* showed inhibition of 61.1% (IC_{50} value 79.2 µg/mL). The *C. lanatus* seed oil showed percentage inhibition of 69.2% with IC_{50} value 64.3 µg/mL. While the ascorbic acid taken as positive control showed the inhibition of 89.8% and IC_{50} value of 49.7 µg/mL (Fig 4.3). It is well known that lesser the value of IC_{50}, more is the antioxidant potential. Based on the free radical scavenging activity the IC_{50} value of *Berberis aristata* (54.8 µg/mL) were observed closed to IC_{50} of positive control (49.7 µg/mL). Thus *Berberis aristata* could be better scavenger of free radicals followed by *Phyllanthus niruri*.

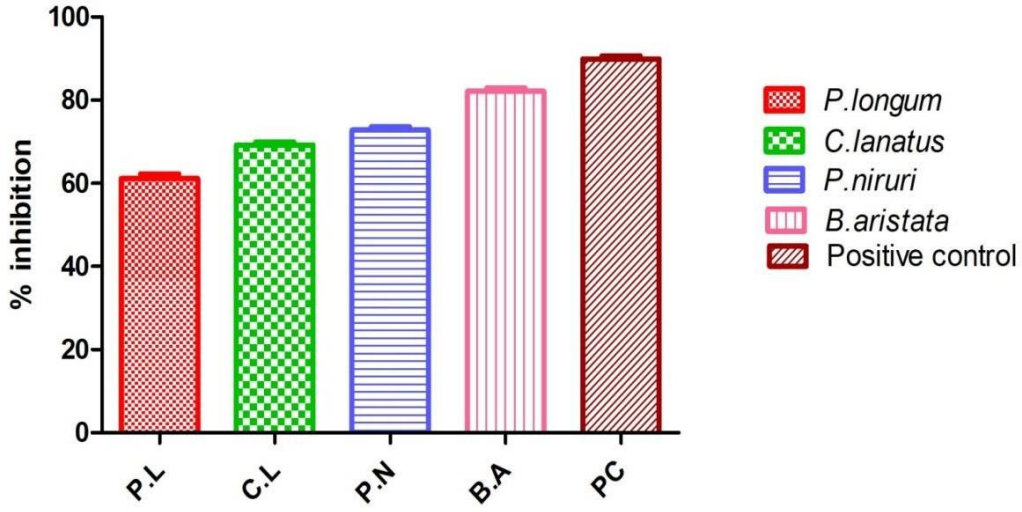


Figure : DPPH free radical scavenging activity of *Citrullus lanatus* seed oil (C.L), *Pipper longum* (P.L), *Phyllanthus niruri* (P.N) and *Berberis aristata* (BA). Value are expressed as Mean ± SEM (n=3)

Bovine serum albumin (BSA) anti-denaturation assay

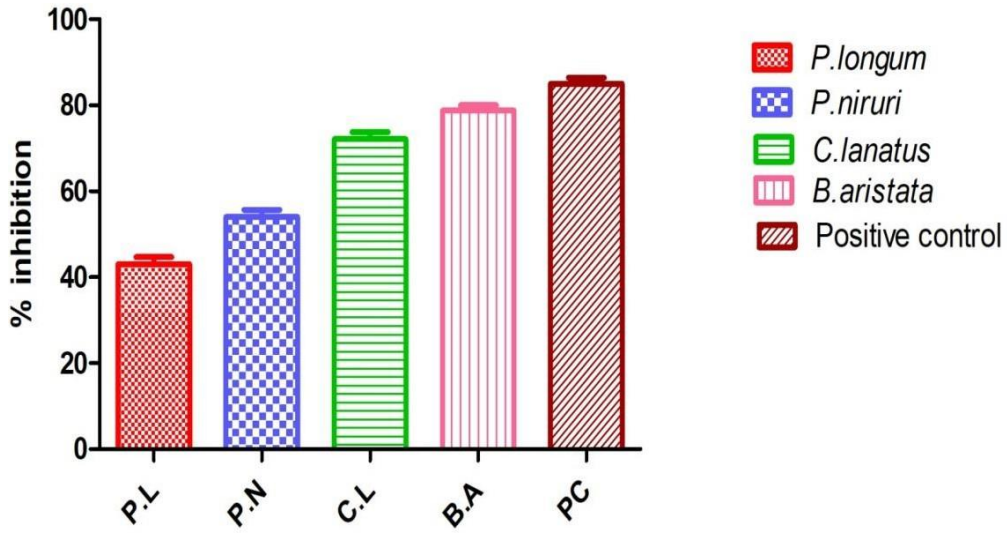
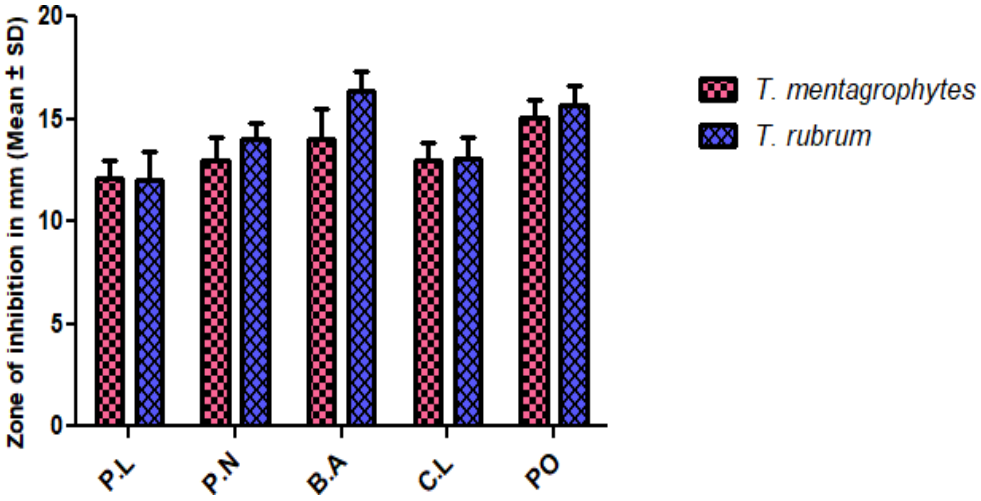


Figure: Anti-denaturation activity of *Citrullus lanatus* oil (C.L), *Pipper longum* (P.L),

***Phyllanthus niruri* (P.N) and *Berberis aristata* (BA) and Positive control (PC). Value are expressed as Mean ± SEM (n=3)**

Antidermatophytic activity of *C.lanatus* seed oil and plant extracts



Preparation of plant extract with sodium alginate, gum arabic and chitosan based nanoemulsion formulation

The plant extracts *Pipper longum*, *Phyllanthus niruri* and *Berberis aristata* based nanoemulsions were successfully prepared by the low energy method. The nanoemulsion was prepared with different biopolymers i.e sodium alginate, gum arabic and chitosan in a ratio of 1% and non ionic surfactant tween 80 (0.5%). Different combinations ratios of all three biopolymers, *C. lanatus* oil and plant extract were enlisted in table no. 4.1. The blank nanoemulsion as a control was also prepared without plant extract. Nanoemulsion loaded with *P. niruri* extract showed light brown color, *P. longum* showed light greenish appearance and *B. aristata* loaded nanoemulsion showed light yellowish color while nanoemulsion prepared without plant extract i.e. control showed milky white color.

Droplet size and polydispersity index (PDI)

Globular size and PDI are considered essential parameters for the nanoemulsion formulation in terms of its stability and efficacy. The results of globule size and PDI for different ratios of both xanthan gum and guar gum based control formulations (i.e without plant extracts) are shown in table 4.11 and 4.12. It was observed that out of all prepared ratios of formulations the 1:1:1:1 of xanthan gum nanogel exhibited low particle size 184 nm and PDI (0.26) value. Whereas, in guar gum nanogel least particle size was showed in ratio 2:1:1:1 having mean particle size 172 nm i.e. < 200 nm and PDI values remained between 0.35 respectively.

The formulated nanogel with different plant extracts showed different mean particle size which was ranged between 206- 262 nm. Out of all, the guar gum based formulations of the *B. aristata* nanogel showed least particle size of 206.3 nm followed by *P. niruri* nanogel 223 nm and *P. longum* based nanogel showed 249 nm particle size . On the other hand, in case of xanthan gum based formulations the *B. aristata* gel showed less particle size 226 nm in comparison to *P. niruri* gel 243 nm and *P. longum* showed 262 nm particle size. The PDI value for all the formulations were ranged between 0.24 - 0.38 respectively. The pH for all the formulations were ranged between 5.5- 6.1 which is entirely compatible with the pH of the skin.

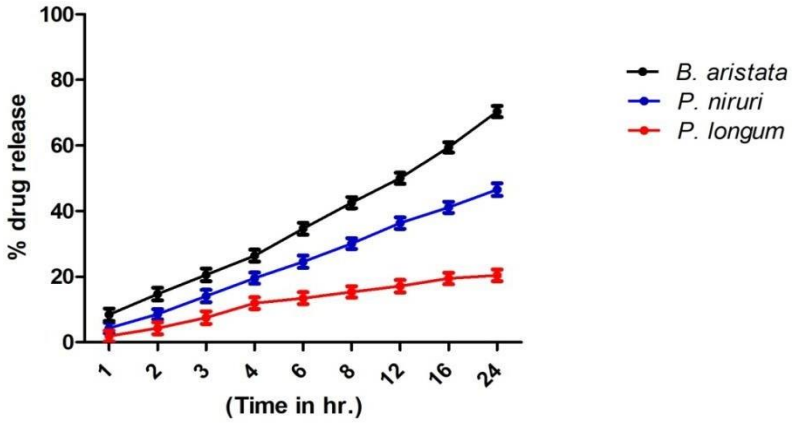
Table : Particle size distribution of herbal nanogel with (GG) Guar gum and (XG) Xanthan gum

Formulations	***Pipper longum***		***Berberis aristata***		***Phyllanthus niruri***	
Ratios	**Mean particle size (nm)**	**PDI value**	**Mean particle size (nm)**	**PDI value**	**Mean particle size (nm)**	**PDI value**
2:1:1:1 (GG)	249	0.38	206.3	0.24	223	0.31
1:1:1:1 (XG)	262	0.29	226	0.44	243	0.25

In vitro drug release study of formulated nanogel

The percent cumulative drug release of xanthan gum and guar gum loaded nanogel and marketed gel was investigated by *in vitro* over a period of 24 h using a dialysis

membrane. The *in vitro* release of1% Xanthan gum and 2% guar gum from the nanogel was studied. The release of drug was in following ascending order *P. longum*, *P. niruri*, and *B. aristata* in both the prepared formulations. Where amount of % release in xanthan gum was 20.40% < 46.57 %< 70.34% and 21% <51.5 %< 81% release in guar gum formulations. From the study, it was concluded that guar gum nanogel formulated with *B. aristata* plant extract showed better drug release in comparison within 24 h. The results of drug release are reported.



(A)

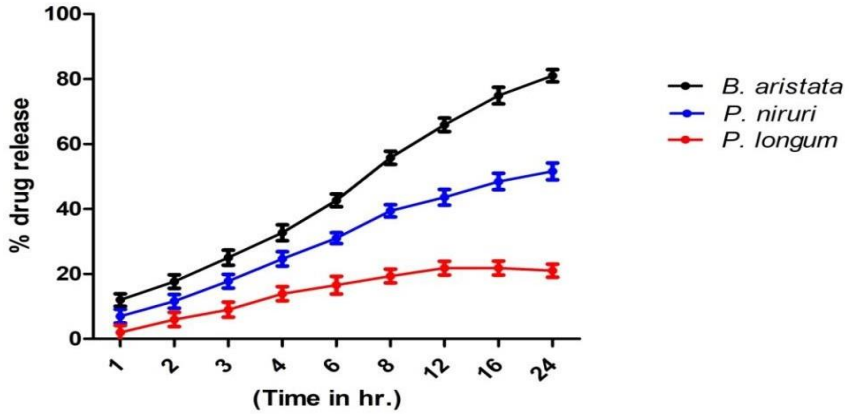


Fig : *In vitro* drug release study of *P. niruri, P. longum* and *B. aristata* based nanogel formulations (A) Xanthun gum (B) Guar gum

Drug Release Kinetics

Release kinetics from different plant extracts loaded with xanthan and guar gum nanogel formulation was compared to marketed gel from different kinetic models reported . Sustained drug release was observed during the entire period of the study showed a delayed burst release. The release kinetics was computed by fitting the release rate data to zero-order, first-order, second-order, Higuchi, and Hixson & Korsmeyer Peppas models. The best fit with the highest r^2 value was found to be shown by first order permeation for *B. aristata* for both xanthan and guar gum nanogel formulations which were near to 0.99, thus; release of drug from nanogel formulations followed first order release kinetic with $R^2 = 0.99$.

It was observed that *B. aristata* guar gum based nanogel showed better drug release with respect to marketed gel. To analyse the release mechanism of drug from nanogel formulations, the data was fit to Korsmeyer Peppas model, where the n varies from 0.2 to
0.5 representing change in drug transport mechanism. It is due to the changes in polymeric ratio. Among the above formulations drug release from is best described by Quasi Fickian and Fickian diffusion release model (n<0.5 and 0.5 respectively) i.e drug release mechanism is non swellable matrix- diffusion.

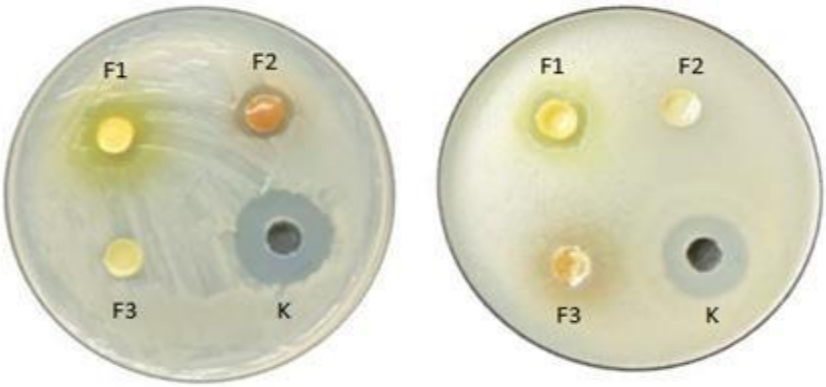
***In vitro* antidermatophytic activity of optimized nanogel against dermatophytes**

The antifungal susceptibility assay of both xanthan gum and guar gum nanogels was performed by using agar well diffusion method. The results of zone of inhibition obtained for different treatments including commercial Ketoconazole as a control and selected ratio of nanogel with different plant extracts are shown. All prepared formulations showed inhibitory activity against both *T. rubrum* and *T. mentagrophytes* except formulations prepared from *P. longum* guar gum and xanthan gum compared with the positive control. For xanthan gum based nanogels, having *B. aristata* extract showed the zone of inhibition of 10.3±0.47 mm against the both dermatophytic

strains. Whereas, in case of guar gum based nanogels, the 1% *B. aristata* nanogel exhibited higher antifungal activity against *T. rubrum* strain having zone of inhibition of 16±0.45 while 10.1±0.62 inhibition was showed in case of *T. mentagrophytes* strain. The Ketoconazole gel showed 16.4±0.49 mm zone of inhibition against *T. rubrum* and 15.3±0.49 mm in *T. mentagrophytes* (Fig 4.34 ,4.35). Out of all herbal formulations the guar gum nanogel formulated with *B. aristata* showed superior inhibition zone diameter in *T. rubrum* strain than the rest of formulations.

A **B**

Fig : Representation of antidermatophytic activity of (A) xanthan gum and (B) guar gum based nanogel on *trichophyton rubrum* strain: F1- *Phyllanthus niruri;* F2-*Berberis aristata*; F3-*Pipper longum*; K- Ketoconazole



A **B**

Fig : Representation of antidermatophytic activity of (A) xanthan gum and (B) guar

gum based nanogel on *trichophyton mentagrophytes* strain: F1- *Berberis aristata*; F2- *Pipper longum*; F3- *Phyllanthus niruri;* K- Ketoconazole

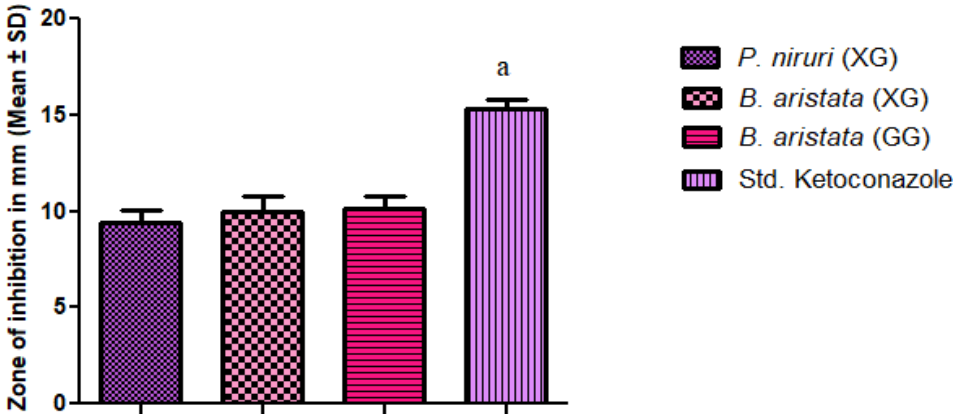


Fig : Antidermatophytic effect of different nanogel formulations against fungal strains *Trichophyton mentagrophytes*. Value are expressed as Mean ± SEM (n=3); $^{a}p<0.05$ (standard ketoconazole show significant difference in comparison to *P. niruri* (XG) (GG) and *B. aristata* (GG) (XG); one-way ANOVA followed by Dunnett's test.

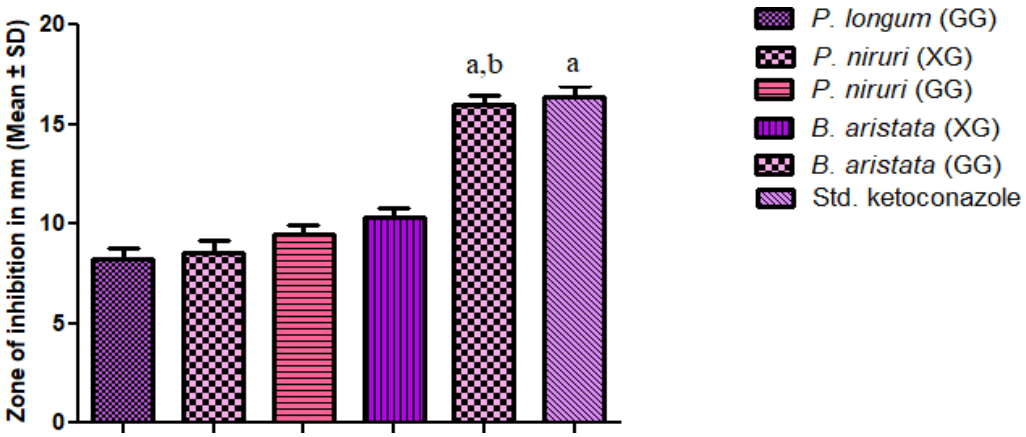


Fig : Antidermatophytic effect of different nanogel formulations against fungal strains *Trichophyton rubrum*. Value are expressed as Mean ± SEM (n=3); [a] $p<0.05$ (*B. aristata* (GG) and standard ketoconazole show significant difference

Stability studies

Formulation has to be remained stable for sufficient period of time. Accelerated stability studies have to be carried out to ensure stability of the optimized formulation at even adverse, variable conditions. Results of stability study of optimized nanoemulsion formulation for parameters like clog formation, phase separation, drug content and Ph after keeping at 4°C, 25°C room temperature and 40°C for 3 months are tabulated. There was no significant change in the viscosity, drug content and pH found during the stability study. It shows that the formulation passed all the tests and efficiently stable up to 3 months at variable temperature conditions.

Results of TEM were found to be in agreement with the results obtained from particle size analysis. From FESEM it was revealed that some of the prepared nanogel was in the form of discrete spherical clusters and mostly uniform dispersion was found. Results are well in line with the findings of who observed spherical morphological structures with prepared tea tree oil nanogel. prepared xanthan gum nanogels which showed droplet size in the range of 117– 615 (nm) depending on pH and SEM images exhibited ellipse shapes with narrow distribution. The spreadability and extrudability tests of nanogel observed that both nanogel formulations showed good spreadability which is required for easier topical application of the formulation. The spreadability of xanthan gum based nanogel was found to be slightly higher than guar gum nanogel formulation due to the lower concentration of biopolymer present in the xanthan gum formulation. Similarly, the extrudability of both the formulation was found to be satisfactory.

Good spreadability is one of the criteria for gel to meet ideal qualities. It is the term expressed to denote the extent of area to which gel readily spreads on application. Therapeutic efficacy of a gel formulation also depends on its spreading value. Additionally, sufficient extrudability is an important requirement for easy removal of the gel from the tube, while it is being used by the patient. However, more spreadability and extrudability values of *B. aristata* xanthan gum nanogel indicate towards its lower viscosity and higher flow which may not be acceptable for topical gel with good consistency. It can be concluded that the prepared guar gum nanogel fulfilled the requirement of gel based formulations for dermatological use which should have several favorable properties such as greaseless and ease of spreadability. Further, these observations were confirmed by rheological studies and texture analysis. Rheological study confirms that the *B. aristata* guar gum based nanogel formulation obtained from rheogram (viscosity vs. rate of shear) exhibited pseudoplastic flow behavior with shear thinning. However, no evidence of thixotropy was observed. Shear thinning property which is important for easy extrudability from the tube and better spreadability during topical application of the formulation . Formulations intended for topical application, must show acceptable mechanical characteristics,

4 Discussion

. The result agreed with the previous study as they prepared ketoconazole loaded nanoemulgel for the topical treatment of onychomycosis which was found to be effective against *T. rubrum* and *C. albicans* and was non-sensitizing and safe for topical use, untreated infected tissues showed a thick film of fungal colonization and partial skin structure damage. After treated with herbal guar gum based *B. aristata* nanogel there is a complete reduction in fungal bioburden with increased intercellular gap in comparison to conventional ketoconazole. This could be attributed to the extended drug release of formulation which assisted drug penetration to skin tissue and increased the duration of action of the drug and its bioavailability.

The present study provides a novel herbal nanoformulation for the treatment of fungal skin infections. In the present study, extraction of the medicinal plants (*Pipper longum*, *Phyllanthus niruri* and *Berberis aristata*) and *C. lanatus* seed oil was carried out. After the extraction process, the extracts were used for quantitative and qualitative assessment of phytoconstituent, their antioxidant and anti-inflammatory potential. Among the selected plants *Berberis aristata* showed

highest phenolic, flavonoid, antioxidant and anti-inflammatory content. Moreover, the GCMS profiling of the oil extracted from *C. lanatus* seeds identified eight main components from which ç-Tocopherol is recorded as a fat-soluble vitamin E bearing potential antioxidant, antimicrobial, antifungal and anti- inflammatory activity. After that, plant extracts of *Pipper longum*, *Phyllanthus niruri* and *Berberis aristata* with *C. lanatus* seed oil based nanoemulsion formulation was prepared with different biopolymers namely sodium alginate, chitosan and gum arabic. On the basis of physical evaluation, stability parameters and low droplet size, gum arabic based nanoemulsion with varying concentration of oils was selected to formulate into nanogel with two gelling agents i.e xanthan gum and guar gum. Nine different ratios of xanthan gum and guar gum were formulated with five different ratios of oils i.e (1%, 3%, 5% and 10%). After that on analyzing physical evaluation, droplet size and polydispersity index characterization. Out of total 72 ratios, 36 ratios of xanthan gum and 36 ratios of guar gum it was found that 2:1:1:1 ratio of guar gum nanogel and 1:1:1:1 ratio of xanthan gum nanogel showed good stability and least particle size ranged between 172 – 184 nm and PDI values remained between 0.26- 0.35 respectively. After optimizing, the 5% ratio of *C. lanatus* seed oil was selected for formulation with ratio 1:1:1:1 of xanthan gum and ratio 2:1:1:1 of guar gum for formulating herbal nanogel. Out of all herbal formulations, the guar gum based *B. aristata* nanogel showed good results with least particle size of 206.3 nm and 0.24 PDI value as compared to other herbal nanogel. The pH was near to pH of the skin which revealed nonirritating nature of the formulation. In addition to this, *in vitro* drug release studies showed that the *B. aristata* guar gum based herbal nanogel showed higher drug release rate which permitted a faster rate of drug dissolution into the aqueous phase. The release kinetics fitting data indicated that drug release followed the Quasi Fickian and Fickian diffusion release model. Further, *in vitro* antidermatophytic activity of optimized herbal nanogel against *T. rubrum* and *T. mentagrophytes* was carried out**.** The results revealed that guar gum nanogel formulated with *B. aristata* showed significant zone of inhibition as compared to *B. aristata* xanthan gum nanogel and ketoconazole against *T. rubrum*.

After optimization and *in vitro* studies, *B. aristata* based xanthan gum and guar gum based nanogel was selected further for zeta potential, TEM, FESEM, spreadability, extrudability, rheological behavior and texture analysis. Furthermore, the negative value of zeta potential i.e (-23.7 mV and -29.9 mV) indicate that the prepared nanogels showed

5 References

1. Abeer K, Soha I. Formulation and evaluation of oxiconazole nitrate mucoadhesive nanoemulsion based gel for treatment of fungal vaginal infection. International Journal of Pharmacy and Pharmaceutical Sciences. 2016; 8:33-40.
2. Acosta E. Bioavailability of nanoparticles in nutrient and nutraceutical delivery. Current opinion in colloid & interface science. 2009; 14(1):3-15.
3. Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry. Carol Stream, IL: Allured Publishing Corporation; 2007.
4. Afshari MA, Shams-Ghahfarokhi M, Razzaghi-Abyaneh M. Antifungal susceptibility and virulence factors of clinically isolated dermatophytes in Tehran, Iran. Iranian Journal of Microbiology. 2016; 8(1):36.
5. Ahmad M, Faruk R, Shagari KA, Umar S. Analysis of essential oil from watermelon seeds. SosPoly Journal of Science & Agriculture. 2017; 2.
6. Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nature protocols. 2007; 2(4): 875-7.
7. Akbar E, Yaakob Z, Kamarudin SK, Ismail M, Salimon J. Characteristic and composition of *Jatropha curcas* oil seed from Malaysia and its potential as biodiesel feedstock feedstock. European journal of scientific research. 2009; 29(3):396-403.
8. Akinyemi KO, Oladapo O, Okwara CE, Ibe CC, Fasure KA. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. BMC complementary and alternative medicine. 2005; 5(1):6.
9. Albataineh MT, Sutton DA, Fothergill AW, Wiederhold NP. Update from the laboratory: clinical identification and susceptibility testing of fungi and trends in antifungal resistance. Infectious Disease Clinics. 2016; 30(1):13-35.
10. Albishri HM, Almaghrabi OA, Moussa TA. Characterization and chemical composition of fatty acids content of watermelon and muskmelon cultivars in Saudi Arabia using gas

chromatography/mass spectroscopy. Pharmacognosy magazine. 2013; 9(33):58.

11. Almeida KB, Araujo JL, Cavalcanti JF, Romanos MT, Mourão SC, Amaral AC, Falcão DQ. *In vitro* release and anti-herpetic activity of *Cymbopogon citratus* volatile oil-loaded nanogel. Revista Brasileira de Farmacognosia. 2018; 28(4):495-502.
12. Alves MP, Scarrone AL, Santos M, Pohlmann AR, Guterres SS. Human skin penetration and distribution of nimesulide from hydrophilic gels containing nanocarriers. International journal of pharmaceutics. 2007; 341(1-2):215-20.
13. Ameen M. Epidemiology of superficial fungal infections. Clinics in Dermatology. 2010; 28(2):197–201.
14. Anandharamakrishnan C. Nanoencapsulation of food bioactive compounds. InTechniques for Nanoencapsulation of Food Ingredients. Springer, New York, NY. 2014; 1-6.
15. Andriole VT. Current and future antifungal therapy: new targets for antifungal therapy. International journal of antimicrobial agents. 2000; 16(3):317-21.
16. Anton N, Benoit JP, Saulnier P. Design and production of nanoparticles formulated from nano-emulsion templates—a review. Journal of controlled release. 2008; 128(3):185-99.
17. Anton N, Vandamme TF. The universality of low-energy nano-emulsification. International journal of pharmaceutics. 2009; 377(1-2):142-7.
18. AOAC, Official methods of analysis, Association of official analytical chemists, 18th edition Washington, DC, U.S.A, 2005.
19. Asz-Sigall D, Martinez-Velasco MA, Arenas R. Infections and Infestations. InHair and Scalp Treatments.Springer, Cham. 2020; 197-216.
20. Ayoola GA, Lawore FM, Adelowotan T, Aibinu IE, Adenipekun E, Coker HA, Odugbemi TO. Chemical analysis and antimicrobial activity of the essential oil of Syzigium aromaticum (clove). African Journal of Microbiology Research. 2008; 2(7):162-6.
21. Azeem A, Rizwan M, Ahmad FJ, Iqbal Z, Khar RK, Aqil M, Talegaonkar S. Nanoemulsion components screening and selection: a technical note. Aaps Pharmscitech. 2009; 10(1):69-76.