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Research Paper

Preparation and Evaluation of Ant Repellent Herbal Gel Containing the Extract of *Tagetes Erecta*

Sudipta Santra, Ritam Chatterjee, Sanchari Bhattacharya*

Department of Pharmacy, Guru Nanak Institute of Pharmaceutical Science and Technology, 157F/Nilgunj Road, Panihati, Sodepur, Kolkata 700114

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ABSTRACT

Objective - Ant repellent properties refer to the ability to deter ants from landing on orbiting the treated surface area. Alternative methods of integrated pest management are urgently needed. Using hazardous chemicals as pesticides to control insects and other pestiferous organisms has raised public concerns about environmental and human health effects. **Methods-** Herbal gel formulation prepared from aqueous extract (containing leaf part of Azadirachta indica, Ageratina adenophora, and flower petals of Tagetes erecta) with Carbopol 940 and Xanthan gum as a gel base, which exhibited high homogeneity, no skin irritation, good stability profile, and anti-inflammatory action.**Results-** Herbal plant extract shows minimal adverse effects. Phytochemical screening of Tagetes erecta, Azadirachta indica, and Ageratina adenophora showed the presence of various chemical constituents like tannins, glycosides, phenols, flavonoids, alkaloids, saponins, and carbohydrates. Prepared herbal gel pH, Viscosity, spreadability, and stability conditions were determined. A compatibility study was done to prove it is incompatible with formulation excipients and ensure the safety profile. Data on percentage ant repellence values of herbal plant extracts and their various combination were checked and calculated to study repellent activity. **Conclusion -** However, the Xanthan gum-based gel showed the highest percentage of extrudability, good spreadability, and rheological properties. Here, the effectiveness of the Herbal gel was evaluated to determine whether this clothing is protective against ants by measuring changes.

INTRODUCTION

On a global scale, herbal medicines are a significant part of health care systems. For medical care, almost 75% of the worldwide population

***Corresponding Author:** Sanchari Bhattacharya
Address: *Department of Pharmacy, Guru Nanak Institute of Pharmaceutical Science and Technology, 157F/Nilgunj Road, Panihati, Sodepur, Kolkata 700114*
Email ✉: sanchari.bhattacharya@gnipst.ac.in
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depends on or trusts herbs and herbal remedies due to their diverse classes of phytochemicals and range of pharmacological effects. Non-steroidal ant repellents, or synthetic opioids, are commonly used to treat a variety of inflammations, but they have serious adverse effects [1]. According to the survey, there are a variety of plants which deals with repellent properties dispersed throughout the plant kingdom. They can serve as attractants, deterrents, phagostimulants, antifeedants, or alter oviposition. They can also alter key metabolic processes and induce fast mortality. They may also disrupt the insect's life cycle in various ways or prevent its development. Furthermore, it has been shown that compounds from a variety of floral species can serve as antifeedants, toxicants, and repellents to a variety of Coleoptera (Insects) that prey on stored goods [2]. A few well-known examples are *Ageratina adenophora, Azadirachta indica,* and *Tagetes erecta* [3-5]. A gel is a system comprising at least two parts that is solid or semisolid and is made up of an expanded mass that is surrounded as well as permeated by a liquid. Gels and jellies are made up of few numbers of solids scattered among a vast volume of liquid. [6-8]. Long-lasting insecticide-impregnated nets, or LLINs, provide defense against insects that might irritate, itch, or cause inflammation. Some protection is offered by repellents like diethyltoluamide. Better personal protection against the dominant group of social insects belonging to the order Hymenoptera of class Insecta is thus required. Permethrin-impregnated clothing may be sprayed or dipped to defend against insects in laboratory settings. Military and recreational wearers have also utilized this kind of clothing to protect themselves against arthropod bites [1].

Fig.1 - Ants: A Model for Studying Insect Repellent Effects

These studies showed that ant-repellent clothing protected activity for outdoor workers against self-reported tick bites. This study conducted an open-labeled study performed by extracting natural herbal extracts, which are easy to access such as *Ageratina adenophora, Azadirachta indica,* and *Tagetes erecta*. Subject volunteers are found a protective efficacy over a short period of time. This study suggests that treated clothing had not lost its efficacy over a period. Ants often inhabit areas with dust or food and can even be found on decaying organic matter. Ant infestations are a common problem for homeowners. Despite efforts to clean a room thoroughly, ants can appear in unexpected places and initiate new invasions within the home. While poisons and chemicals are effective in controlling ants, they can pose significant health risks to children and humans [7]. A gel is easily applicable as well as easily removable from the cloth or any surface area by

simple washing with water. The bases and compatible excipients maintain dermatological and nontoxic factors [8].

MATERIALS AND METHODS:

Preformulation study:

To ensure the development product of a reliable, safe, stable, and efficient dosage form, preformulation studies are required. During this phase of development, we studied the physicochemical characteristics and incompatibility of the pharmaceutical ingredients and how they interact with different formulation elements [9,10].

Pharmacognostic investigation:

a. Collection and Authentication: For the collection of *Tagetes erecta* (Marigold), *Ageratina Adenophora* (Crofton weed), and *Azadirachta indica* (Neem), the fresh flower and leaves were collected at the flowering stage in September 2023 from the local community market, West Bengal state, India [11].
b. Organoleptic Characterization: Colour, odour, taste, and size of the leaf and flower were observed [8].
c. Physicochemical Characterization: After botanical evaluation, the shade-dried plant material was subjected to kept for 3 weeks before going for the extraction process. size reduction was done to get coarse particles, and then passed through a sieve no. 40 to get uniform particles. Then, the uniform size was subjected to standardization with different parameters as per the literature [12,13].
d. Solvent selection: Choose a solvent system that is composed of 90% distilled water and 10% methanol or chloroform because this prevents oxidation and hydrolysis and contains more amounts of antioxidant moieties than other solvent systems. The solvent used for the extraction is menstruum [14].

Table 1. **Organoleptic Characterization for Leaves and Flowers**:

Characteristics	***Tagetes erecta* (Marigold)**	***Azadirachta indica* (Neem)**	***Ageratina adenophora* (Crofton weed)**
Colour	Yellow	Green	Green
Oduor	Characteristic	Characteristic	Characteristic
Size	Avg . 5 cm	Avg. 4 cm	Avg. 4 cm
Taste	Astringent	Bitter	Astringent

Extraction and Storage:

a. Pouring: After performing the initial steps and allowing the system to settle for a while, these two were placed on a Rotary Shaker which maintained 100 rpm and the required strength should be left in a closed flask for twenty-four hours, shaking regularly for the first six hours and then remaining upright for eighteen [15].
b. Filtration: The extract solution was filtered through Whatman filter paper and a Buchner Funnel, and the filtrate was collected again in a 250 mL conical flask. For rapid filtration, vacuum filtration is used [16].
c. Solvent Evaporation: The filtered solvent collects in a petri dish. These were then placed on a Hot plate for solvent evaporation. Temperature is maintained at approximately 80°C. These were gently heated until complete removal of the solvent. To remove the solvent completely, utilize 2 days, about 4 hours of each day [17].
d. Extract Collection: The dried and solidified extract was scraped out from the Petri Plates

(used during the solvent evaporation process) using a sharp Scrapper [14].

e. Extractive Yields: The weight of the dried and scraped materials was measured to calculate the extractive yields, and these were then stored in a freezer for future usage. The extractive yields measured were 0.242g and 0.284g, 0.461 g of *Azadirachta indica*, *Tagetes erecta*, *and Ageratina adenophora*, respectively per gram of dry mass [18].

Moisture content:

The moisture content of taken crude drug for the herbal gel preparation is measured by the loss of drying method to prevent oxidation. To measure the moisture content also requires a weighing balance and a Hot air oven [11]

Method / Process: Take an empty porcelain dish or Petri dish to take the sample. Weigh the empty porcelain dish or Petri dish by using the weighing balance and note the weight on gram scale. Tare the weighing balance to get 0 value, then add a specific amount of crude drug sample. Note the initial desired weight on the gram scale. Placed the loaded drug sample in the drying chamber for a certain period of time. Check the weight after 15- or 30-minute intervals. After complete drying, the dry content gives a constant weight. Note the final weight on gram scale.

$$Moisture\ content\ = \frac{Intial\ weight - final\ weight}{Initial\ weight} \times 100\ \%$$

Phytochemical Screening:

Phytochemical screening is essential in the study of medicinal plants because it helps to identify and understand the bioactive compounds present in plants.

a. Test for Tannins:

3 ml of water extract of the sample in separate test tubes. After that, in the sample solution, 2 mL of 5% ferric chloride ($FeCl_3$) solution was added. Any change in colour or precipitate formation was observed [19].

b. Test for Saponin:

1-2 ml aqueous extract sample in separate test tubes. 5 mL of distilled water was added to each sample. Test tubes are shaken vigorously for 5 minutes and allowed to stand for 20 minutes. After that observed whether the honeycomb froth (foam) layer is present or not, which indicates the presence of saponin [11,20].

c. Test for Carbohydrates:

2 ml aqueous extract sample in separate test tubes. 2 mL of Benedict reagent was added to each sample. Then that solution was heated in a boiling water bath for 3-5 minutes. Any change in colour or precipitate formation was observed [11,21].

d. Test for Alkaloids:

Mayer's reagent test: Take 2 mL of the aqueous extract sample in test tubes. A few drops of Mayer's reagent (potassium mercuric iodide) were added to each sample. Any change in colour or precipitate (White / pale yellow) formation was observed [11,12].

Wagner's reagent test: Take 2 mL of aqueous extract sample in test tubes. A few drops of Wagner's reagent (2g of iodine and 6g of potassium iodide in 100 ml of distilled water) were added to each sample. Any change in colour (reddish) formation was observed [11,12].

e. Test for Phenolics:

To the 3 mL aqueous extracts, a freshly prepared aqueous solution of Ferric Chloride ($FeCl_3$) was added and observed for any colour change. [11,22]

f. Test for Flavonoid:

2 ml of the aqueous extracted sample solutions were taken in separate test tubes. Add prepared sodium hydroxide (NaOH) solution. If a yellow colour is observed, then add concentrated hydrochloric acid (HCl). Colourless sample solution indicates the presence of flavonoid groups [11,23].

g. Test for Glycoside:

Take 1 ml of aqueous sample in a test tube. Added 5-10 ml of dilute Hydrochloric acid (HCl). Then that solution was heated in a boiling water bath for 10 minutes. After that, carbon tetrachloride and an equal amount of ammonia were added. Shake well to mix properly. A sample solution colour change to pink or red indicates a positive result [11,18].

h. Test for Coumarins:

2 ml of water extract of the sample in separate test tubes. After that, in the sample solution added 3 ml of 10% Sodium hydroxide (NaOH) solution was added. The sample solution changes colour to yellow indicating the presence of coumarins [11].

i. Test for Quinones:

Take 2 ml of water extract of the sample solution with 1 ml of concentrated sulphuric acid(H_2SO_4) solution. The sample solution's colour changes to red indicates the presence of quinones [23].

j. Test for Anthocyanins &Betacyanin:

Take 2 ml of water extract of the sample in each separate test tube. After that, in the sample solution 1 ml of 1N Sodium hydroxide (NaOH) solution. A sample solution colour change into blue or bluish-brown colour indicates the presence of anthocyanins and betacyanin's [24].

Herbal Gel Preparation:

Two gelling agents were utilized in the formulation process at two different concentrations *Azadirachta indica 20%, Tagetes erecta 50%, and Ageratina adenophora 30%*, resulting in the manufacture of two distinct batches of gels. Both Xanthan gum and Carbopol 934 were used as gelling agents in this instance. Preparation of gel with Carbopol 934: accurately measured four grams of Carbopol 934 and mixed it with 50 ml of purified water. After setting the beaker aside to allow the Carbopol to expand for 30 minutes, stir the mixture with a mechanical or lab stirrer set at 450 rpm for 20 minutes. Add extract aqueous solution, approx. 20 ml, then slowly pour 2 ml of glycerol mix and fill it with weighed liquid paraffin, stirring thoroughly. After all of the Carbopol had dispersed added the remaining distilled water was added, drop by drop triethanolamine was added to the formulations to balance pH (4.8–6) and get the gel at the desired consistency. Preparation of gel with Xanthan gum: A beaker containing 3 g of Xanthan gum was filled with 50 ml of distilled water. Set aside the beaker to let the Xanthan gum swell for ten minutes and then stirring should be done continuously to prevent floc formation. Take 5 ml of propylene glycol and the small amount of extract into a separate beaker and thoroughly mix in the propyl and methylparaben. Then add glycerine as a preservative. Mix it properly and allow it to stand for 6 hours to get a semi-solid concentration.

Evaluation and standardization of gel:

a. PH: Using a digital pH meter, the formulation's conductivity and pH were measured in millivolts (mV). The glass electrode was calibrated using the equipment-specific

solutions (pH of 4.00 and 7.00). The preparation was allowed to reach equilibrium while being measured for approximately five minutes. Average values were computed when the formula's conductivity and pH were analysed in triplicate [9].

b. Appearance and Homogeneity: A visual examination was used to assess the generated individual and polyherbal gels for homogeneity and physical appearance (colour, odour, etc) [25].

c. Viscosity: Using a Brookfield viscometer, the prepared herbal gels' viscosity was determined. Spindle No. 6 is used to rotate the gel at 100 revolutions per minute. [17]

d. Spreadability: Spreading 0.5 g of the gel over a circle between two horizontal planes with a 2 cm diameter pre-marked on a glass plate allowed for the measurement of the gel's spreadability. A second glass plate was then used. Five minutes were allowed for a half-kilogram of weight to lie on the upper glass plate. After the gel was spread, the circle's diameter was measured. [26]

e. Extrudability: Standard capped collapsible aluminium tubes were filled with gel compositions, and the ends were sealed with a crimp. The tube weights were noted. The tubes were clamped after being positioned between two glass slides. After covering the slides with 500 grams, the cap was taken off. Weighing was done on the amount of extruded gel that was collected. The extruded gel's percentage was determined as follows: >80% extrudability is good, >90% extrudability is excellent, and >70% extrudability is fair [25].

f. Stability study: The gel stability investigations are conducted at 37°C for 3 months. [27]

***In vivo* insect repellent study:**

By 2013, the EU had made it a priority to stop using animals in cosmetics testing, providing human in vivo research a boost, especially non-invasive research. [28] Because of this, the study researchers paid close attention to a variety of imaging methods, including confocal microscopy, tomography, and fluorescence investigations [1, 3-5]. Ant is selected as a candidate to perform this study. Due to their jointed legs and exoskeleton, ants belong to the phylum Arthropoda. They have colonized almost every continent on Earth, making it simple to gather data using the manual technique. Ants transport food particles and frequently return them to their nest or mound. Sugar powder is used to gather and sample. Ant bites and stings may result in swelling, irritation, allergic reactions, and in extreme cases, anaphylaxis. This collection is a little dangerous. A clean cotton cloth sample (10 cm × 10 cm) was taken and cut into 3 pieces. Each and every piece was equal in size. Those clothes were kept in a chamber without contact with observers at room temperature. In the centre, sugar powder was kept to attract ants. The experimental chamber maintained environmental conditions equivalent to atmospheric pressure, temperature, humidity, etc. Herbal gel was applied to different sides and corners of the cloth, and after 5 minutes to dry, leave it. The different batch formulation is compared with the standard marketed repellent and blank (in which no repellent is applied). Ticks can either enter or remain on the surface treated with plant extract gel. Tick counts were recorded after 10 min.

RESULTS:

Table 2. Extractive values for Leaves and flowers:

Sample	Extraction method	Solvent use (ml)	Wt. of sample (g)	Extraction values(%w/w)
Tagetes erecta	Solvent extraction	200	50	0.284
Azadirachta indica	Maceration	200	50	0.242
Ageratina adenophora	Maceration	200	50	0.461

Table 3. Moisture content:

Sample	Initial weight(g)	Final weight(g)	Moisture content (%)
Tagetes erecta	10.00	6.87	3.13
Azadirachta indica	107.10	101.00	5.70
Ageratina adenophora	178.60	177.20	1.40

Table 4. Phytochemical screening [11]**:**

PPhytochemical test	Colour	*Tagetes erecta*	*Azadirachta indica*	*Ageratina adenophora*
Tannin	Greenish black indicates the presence of catechol.	+	+	+
Saponins	Honeycomb front or foam layer	+	+	++
Carbohydrates	Blue / Greenish blue	+	+	+
Alkaloids	pale yellow precipitate	+	-	-
Alkaloids	Reddish colour	+	+	+
Phenolics	Reddish brown	+	+	+
Flavonoid	Colourless	+	+	+
Glycoside	Pink or red	+	+	+
Coumarins	Yellow colour	+	+	+
Quinones	Reddish brown	+	+	+
Anthocyanins & Betacyanins	Blue / Bluish brown	+	+	+

Table 5. Physical Observations of Compatibility Study:

Batch	Cake / Flocs	Discoloration	Liquification
extract	No	No change	No change
Carbopol 934	No	No change	No change
Extract + Carbopol 934	Yes	No change	No change
Xanthan gum	No	No change	No change
Extract+ xanthan gum	No	No change	No change

Table 6. Physiochemical Analysis of Herbal Gel:

Physicochemical parameters	Observation
Colour	Brownish green
pH	5.4 ± 0.2
Viscosity	0.38±0.03 poise
Spreadability	22±1.0
Stability	Stable with pH 6.3, 37°C

Table.7: Data of % ant repellence values of herbal plant extracts at 15 min intervals

Treatment (Concentration)	No. of Ants	% Ant Repellence values at 15-minute intervals		
		15 min.	30 min	2h

Neem 10% w/v	25	15	42	53
Neem 20% w/v	25	22	51	72
Marigold 10% w/v	25	10	26	48
Marigold 25% w/v	25	25	43	78
Marigold 50% w/v	25	35	62	100
Crofton weed 10% w/v	25	25	48	75
Crofton weed 30% w/v	25	30	72	100

Table.8: Data of % ant repellence values of herbal plant extracts combination at 15 min intervals

Treatment (Concentration)	No. of Ants	% Ant Repellence values at 15-minute intervals		
		15 min.	30 min	2h
Neem 20% w/v + Marigold 50% w/v	25	25	53	75
Neem 20% w/v + Crofton weed 30% w/v	25	22	62	81
Marigold 50% w/v + Crofton weed 30% w/v	25	35	68	85
Neem 20% w/v + Marigold 50% w/v + Crofton weed 30% w/v	25	67	100	100

Fig. 2 - Ant repellent study conducted by an in vivo study on a cloth using different formulations: a) marketed product, b) blank formulation, c) F_1 formulation, d) F_2 formulation

DISCUSSION:

Ant-repellent activity was checked in various concentrations. Whenever concentration was increased, the repellent activity also increased with time intervals. [Table. 7 shows the percentage and repellence values of herbal plant extracts of neem, marigold, and Crofton weed at 15-minute intervals in concentrations ranging from 10% to 50%. Data on the percentage ant repellence values of herbal plant extract mixtures of neem + Marigold, Crofton weed + Marigold, and neem + Marigold were examined at 15-minute intervals for all plant extracts in concentrations ranging from 20% to 50% (Table 8). The highest percentage of repellence was found in 20% of *Azadirachta indica*, 50% of *Tagetes erecta*, and 30% of *Ageratina adenophora*. The combination of *Ageratina adenophora* and *Azadirachta indica* extracts demonstrated the lowest percentage of repellence. The herbal gel where prepared and evaluated for repellent activity on the cloth by using carbopol 934 and xanthan gum gelling agent. In case of formulation 1 (F_1), the gelling agent carbopol 935 was used, and in formulation 2 (F_2), the gelling agent xanthan gum was used. Then it was evident that an *in vivo* model study was

needed to evaluate the repellent potential of the plant extract. Here ant is selected as a candidate to perform the study because and belongs to the order Hymenoptera of class Insecta. Noticed that after applying the tick on clothes due to the strong odour, candidates do not surround the clothes. Both formulations show good potency and activity for a prolong duration of time. Experimental results were recorded up to 2 hours in 15-minute intervals. It is evaluated that candidates collect sources from blank but not from formulation cloth or marketed sample applied cloth. This is reported that the herbal gel is a nonsensitive, nontoxic, and clinically effective repellent agent. The analytical study showed that repellent activity by a formulation containing an extent significantly differed from the control group at all the concentrations tested. The result shows that formulation 2 (F_2) showed better activity. Both formulations were compared with the marketed formulation and a blank. Both are effective against the arthropod family belonging to the order Hymenoptera of class Insecta but in the case of formulation 1 (F_1), flocs formation occurred; in the case of xanthan gum, it is easy to prepare it also shows good spreadability on the cloth.

CONCLUSION:

The current research concludes that the aqueous extract of *Tagetes erecta, Azadirachta indica, and Ageratina adenophora* has strong repellent properties, which might be described as the presence of flavonoids, triterpenoids, saponins, glycosides, and tannins. This plant's components can be employed as a convenient source of natural ant repellent. The finished product can be used as a topical gel which spreads easily on the surface due to its hygroscopicity, can also be used on any rough surface in one or two tick forms, has no irritating impact, is diffused effectively, and is stable at varied temperatures and pressures. It is also necessary to do a study on the phytochemical and pharmacological aspects. This herbal gel is free from parabens, sulfates, SLS, Synthetic fragrance, or any synthetic colour, so drug excipient interaction produces favourable interactions with the active pharmaceutical ingredient (API). More research is needed to identify the unique bioactive molecule that can lead to greater effectiveness in a variety of biological activities.

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AUTHOR'S CONTRIBUTION

Conceptualization of work and manuscript editing: Dr. Sanchari Bhattacharya

Methodology and data curation: Sudipta Santra

Data analysis and writing: Sudipta Santra and Ritam Chatterjee.

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