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DEVELOPMENT AND EVALUATION OF BOTANICAL INSECTICIDAL FORMULATIONS

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ABSTRACT: Herbal insecticidal formulations using two indigenous plants were developed keeping in view disadvantages of the use of synthetic products and to embolden eco-friendly approach. Plants selected were screened for insecticidal and antifeedant activities. Oil in water emulsion-based creams and wettable powder were formulated. Creams were evaluated for different parameters like appearance, pH, spreadability, texture analysis, extrudability, homogeneity, skin irritation, short term stability study and estimation of phytoconstituents while powder granules for appearance, pH, wettability, persistent foaming, suspensibility, spontaneity of dispersion, particle size analysis and flow property. The formulations were found to be stable during stability study according to ICH guidelines. Results indicate that prepared herbal formulations can effectively be used as safe and user convenient products.

INTRODUCTION: Insects are serious pests of stored products such as oilseeds, pulses, and cereals. These pests spread worldwide and cause tremendous economic damages. Their control depends greatly on the use of synthetic insecticides^{1, 2}. But, its extensive use leads to severe issues such as environmental pollution, pest resurgence, eco-toxicity and insect resistance to insecticides³. Therefore, the search for natural products of botanical-origin as natural antifeedant, insect deterrents and repellent is a foremost need of current period^{1, 4}. Because of high volatility, plant extracts or phytochemicals offer a promising biodegradable substitute to synthetic pesticides². Plant extracts also have additional benefit of low cost and convenient to use⁴.

The present study is an effort in this direction incorporating assessment of two medicinal plants *Sphaeranthus indicus* and *Pluerospermum angelicoides* for insecticidal activity using different protocols. Furthermore, two herbal insecticide formulations such as wettable powder and creams were developed. The creams were evaluated for physical parameters like viscosity, pH, texture analysis, acid value, saponification value, short term stability study, content of phyto-constituents like phenolics and flavanoids. Wettable powder was assessed for its physico-chemical parameters, particle size, pH, suspendibility, flowability, wettability, dispersability and stability study.

MATERIALS AND METHODS:

Procurement of Materials: Fresh flowering plants of *Sphaeranthus indicus* were collected from Gujarat university campus area and verified by comparing its morphological and microscopical characteristics available with literature⁵, whereas the fresh roots of *Pleurospermum angelicoides* were collected from Chanda valley, Uttarakhand

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and authenticated by a taxonomist, HRDI, Gopeshwar, Uttarakhand. Dried plant materials were powdered, passed through the 60# and stored properly in airtight containers. Hydro-alcoholic extract (50%) of *S. indicus* and *P. angelicoides* were prepared by reflux method.

The chemicals used during the experiments were of analytical grade. Glyceryl monostearate, cetyl alcohol, stearic acid, liquid paraffin, petroleum jelly, tween 80, triethanolamine, polyethylene glycol etc. were used.

Preparation of Formulations: The hydro-alcoholic extracts of *S. indicus* and *P. angelicoides* were included as active pharmaceutical ingredients (API). The formulas for the cream and wettable powder are given in **Table 1** and **2** respectively. They were prepared according to procedure as follows:

Cream: Three different (O/W) cream formulations (F1-F3) were prepared using 3, 5 and 7% of hydro alcoholic plant extract respectively.

It was prepared after optimizing various cream bases. Ingredients of the oil phase (A) were melted in a beaker using a water bath on constant stirring. Components of aqueous phase (B) were mixed together and warmed to about the same temperature of oil phase (up to 70 ° C). The preservatives propyl paraben and methyl paraben as well as concentrated plant extracts were added into an aqueous phase and heated. Then oil phase was added to water phase little by little on constant stirring. Eucalyptus oil and citronella oil were included as a fragrance to it when the temperature was 35 °C-40 ° C⁶.

Wettable Powder: Wettable powder was prepared after optimization using different water-soluble adherents and wetting agents. Plant extract is adsorbed on clay type material like microcrystalline cellulose:lactose (1:1). This adsorbed clay was allowed to dry in hot air oven until complete drying. Mix it with isopropyl alcohol and pass through sieve no. 80# and dry it completely. Add magnesium trisilicate to avoid cacking⁷.

TABLE 1: FORMULA OF HERBAL CREAM

Ingredients	Quantity (%w/w)		
	F1	F2	F3
Phase A (Oil phase)			
Glyceryl monostearate	8.0	8.0	8.0
Stearic acid	4.0	4.0	4.0
Cetyl alcohol	4.0	4.0	4.0
Petroleum jelly	5.0	5.0	5.0
Liquid paraffin	5.0	5.0	5.0
Tween 80	2.0	2.0	2.0
Phase B (Water phase)			
Water	q.s.	q.s.	q.s.
Tri ethanolamine (TEA)	0.5	0.5	0.5
Polyethylene glycol (PG)	5.0	5.0	5.0
Water	q.s.	q.s.	q.s.
Other ingredients			
Plant extract	3%	5%	7%
Eucalyptus oil	q.s.	q.s.	q.s.
Citronella oil	q.s.	q.s.	q.s.

TABLE 2: INGREDIENTS FOR WETTABLE POWDER

Name	Proportion (%w/w)
Micro crystalline cellulose	25%
Lactose	25%
Magnesium trisilicate	0.1%
Plant extract	More than 50%
Isopropyl alcohol	q.s.

Stability Study and Evaluation of Formulations: The accelerated stability studies of formulated cream and wettable powder were carried out at

40±2 °C /75 %±5% RH for three months according to ICH guideline. The cream formulations were evaluated for different pharmaceutical parameters like after feel, determination of pH, type of smear, dispersion ability, homogeneity, spreadability, texture analysis, extrudability, grittiness, skin irritation, and acid value, saponification value, estimation of phytoconstituents etc⁶. Whereas, the wettable powder formulations were assessed for

appearance, pH, wettability, persistent foaming, suspensibility, spontaneity of dispersion, wet sieve test, particle size analysis and flow property⁷.

Antiradical Activity using DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate): The stock solution of extract was prepared in methanol to achieve concentration of 1mg/ml. From this stock solution, dilutions were made to obtain concentration of 200, 500, 700 and 1000 µg/ml. 0.1 ml solution from each dilution was mixed with 0.9 ml methanolic solution of DPPH (0.4%w/v). The above solution was kept in dark for 30 minutes at room temperature. Absorbance was recorded at 517 nm. Control samples contain only DPPH in methanol. %scavenging was calculated using following equation:

$$\% \text{ scavenging} = (A_c - A_s) / A_c \times 100$$

Where, A_c is absorbance of control (DPPH in methanol); A_s is absorbance of sample.

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of percentage scavenging against extract concentration. Tests were carried out in triplicate⁸.

Insecticidal Activity:

Antifeedant Activity: Antifeedant activity of crude hydro alcoholic extracts was studied by means of the leaf disc with no choice method. The stock concentration of crude extracts (5%) dissolved in acetone and mixed with distilled water. Polysorbate 20 (Tween 20) at 0.05% concentration was used as an emulsifier. Fresh brinjal leaf discs of 3 cm diameter were punched using cork borer and, 2.50%, 3.75% and 5.0% concentrations of crude extracts were applied to different bores. The brinjal leaf discs with acetone treatment and without water were regarded as control. All the leaf discs were kept in petridish containing wet filter paper to prevent early drying of the leaf disc. A single 2 hr prestarved insect was introduced. For each concentration five replicates were taken.

The area of leaf consumed by insects after 24 hr feeding was documented in control and treated discs using graph sheet method, and the area in plant extract treated leaves was rectified from the control parameters⁹. The percentage of antifeedant index was calculated using the formula of,

$$AFI = (C-T)/(C+T) \times 100$$

Where, AFI is antifeedant index; C is area protected in control leaf disc; T is area protected in treated leaf disc.

Insecticidal Activity: Fresh castor leaves were treated with 1.25%, 2.50%, 3.75% and 5.0% concentrations of hydro alcoholic test extracts. Castor leaves with acetone and without solvent treatment were regarded as control. To prevent early drying, petioles of the potato leaves were tied with wet cotton plug and kept in 29 cm × 8 cm round plastic trough. In each concentration 2 hr 10 pre-starved *H. vigintioctopunctata* were placed individually and covered with muslin cloth. Five replicates were recorded for each concentration and the number of dead larvae was counted after 24 hr up to pupation⁹.

Percentage of larval mortality was computed and corrected using Abbott's formula,

$$\text{Corrected Mortality (\%)} = (\%MT - \%MC) / (100 - \%MC) \times 100$$

Where; %MT is % larval mortality in treatment; %MC is % larval mortality in control.

RESULTS AND DISCUSSION:

Extraction: The yield of hydro alcoholic extracts of *S. indicus* and *P. angelicoides* were 9.80%w/w and 13.39%w/w respectively.

Evaluation of Formulations: Physical properties of cream and wettable powder are shown in **Table 3** and **5** respectively. The results obtained showed optimum physicochemical parameters. Percentage of total phenolics and flavanoid content estimated in cream is given in **Table 4**.

TABLE 3: PHYSICOCHEMICAL EVALUATION OF CREAM

Parameter	Result		
	F1	F2	F3
Appearance	Creamish	Creamish	Creamish
Homogeneity	Good	Good	Good
Removal	Easy	Easy	Easy
After feel	Emollient	Emollient	Emollient

Type of smear	Continuous	Continuous	Continuous
pH	6.04	6.21	6.06
Viscosity	30560cp	30018cp	31120cp
Spreadability	Good	Good	Good
Tube extrudability	Satisfactory	Satisfactory	Satisfactory
Acid value	2.61	2.66	2.69
Saponification value	129.75	139.75	134.76
Primary skin irritation test	Nil	Nil	Nil

Stability Studies: Table 6 depicts that all the physicochemical parameters were well maintained during the period of accelerated stability studies in wettable powder. There were no changes found in colour, emolliency, homogeneity etc in all three cream formulations. pH of same was found in the range of 6.02 to 6.04.

TABLE 4: TOTAL PHENOLICS AND FLAVANOID CONTENT OF CREAM

Phyto-constituents	Formulation	Time (day)			
		0	30	60	90
% Flavanoids	F1	0.171± 0.64	0.156± 0.28	0.164± 0.35	0.168± 0.67
	F2	0.195± 0.31	0.211±0.42	0.192± 0.87	0.198± 0.58
	F3	0.213± 0.45	0.243± 0.32	0.231± 0.61	0.240±0.75
% Phenolics	F1	0.321± 0.63	0.306±0.52	0.304± 0.94	0.301±0.23
	F2	0.365± 0.540	0.352± 0.41	0.336± 0.67	0.356± 0.38
	F3	0.421± 0.76	0.378± 0.48	0.408± 0.31	0.416± 0.24

TABLE 5: PHYSICOCHEMICAL PARAMETERS OF POWDER

Parameters	Results
Colour	Brown
Odour	Characteristics
Particle size	≤ 425 µm
Suspensibility	Good
Persistent foam	0.2 ml after 5 minute
Determination of pH	6.07
Flowability	Good
Wettability	Very good
Dispersability	Uniformly dispersed

TABLE 6: STABILITY PARAMETERS OF THE POWDER

Day	Temperature	Parameter			
		pH	Colour	Flowability	Particle size
0	RT	6.56	NC	Good	Good
	40°C±2° C, 75%±5%RH	6.54	NC	Good	Good
30	RT	6.50	NC	Good	Good
	40°C±2° C, 75%±5%RH	6.48	NC	Good	Good
60	RT	6.50	NC	Good	Good
	40°C±2° C, 75%±5%RH	6.48	NC	Good	Good
90	RT	6.50	NC	Good	Good
	40°C±2° C, 75%±5%RH	6.48	NC	Good	Good

NC: Not change.

Antiradical Activity by DPPH Method:

TABLE 7: % SCAVENGING USING DPPH ASSAY

Concentration of test (µg/ml)	<i>P. angelicoides</i>	<i>S. indicus</i>	Concentration of standard (µg/ml)	BHT
200	67.13±0.01	45.27±0.01	20	47.75±0.01
500	70.89± 0.015	59.82±0.01	40	79.42±0.01
700	74.75± 0.010	75.12±0.05	60	93.56±0.01
1000	86.56± 0.022	78.88±0.03	80	96.6±0.01
IC ₅₀	53.62	117.92	-	27.54

Based upon IC₅₀ values, both plants showed good scavenging activity.

Evaluation of Insecticidal Activity:

Antifeedant Activity: Antifeedant activity of the crude extracts of *S. indicus* & *P. angelicoides* were studied at different concentrations and the results are given in **Table 8**. Antifeedant activity of test extracts was determined through antifeedant index. A high value of antifeedant index is suggestive of reduced rate of feeding. In the present investigation, insecticidal activity was found varied

significantly irrespective of concentration and solvents used for extraction. Maximum antifeedant activity was observed at 5%w/w concentration of extract compared to control in both the plants. One-way analysis of variance (ANOVA) followed by least significant difference (LSD) test demonstrated statistical significance ($p < 0.05$) compared to control.

TABLE 8: ANTIFEEEDANT ACTIVITY OF EXTRACTS

Concentration (%w/w)	<i>S. indicus</i>	<i>P. angelicoides</i>
2.50%	46.1±3.61	63.68±12.89
3.75%	65.35±11.24	72.85±4.13
5%	70.21±7.12	88.66±11.12

Data represents mean ± SEM of three different concentration of extracts.

Insecticidal Activity: Insecticidal activity of crude extracts of *S. indicus* and *P. angelicoides* were studied at different concentrations and the results are presented in **Table 9**. Insecticidal activity of sample extracts was calculated using larval mortality after treatment. High larval mortality suggests potential insecticidal activity of plant extracts.

According to the data of the insecticidal activity, hydro alcoholic extract of both the plants were found to have significant activity at 5%w/w concentration compared to control. One-way analysis of variance (ANOVA) followed by least significant difference (LSD) test confirmed statistical significance ($p < 0.0$) compared to control.

TABLE 9: INSECTICIDAL ACTIVITY OF EXTRACTS

Concentration (%w/w)	<i>S. indicus</i>	<i>P. angelicoides</i>
1.25%	35.18±13.98	18.81±5.611
2.50%	64.59±7.89	34.4±4.00
3.75%	72.68±8.81	52.66±14.71
5%	76.84±1.59	64.59±13.97

Data represents mean ± SEM of four different concentrations of extracts.

CONCLUSION: Although a variety of formulations are available in market for insecticidal activity, still appear to be limited in terms of safety and efficacy. Hence, the attempt has been made to formulate polyherbal topical creams and wettable powder along with their physicochemical and phytochemical screening. All the parameters remained unchanged throughout the stability period for the formulations. The 5%w/w hydro-alcoholic extracts of both the plants were found to have potent insecticidal activity. However, further investigation is necessary for isolation of new leads from the above extracts. This study demonstrates that prepared formulations are safe, economic and convenient to use. The study was one of the few endeavours to provide effective herbal insecticidal formulations to the mankind and overcome the drawbacks of existing synthetic products.

CONFLICTS OF INTEREST: The authors declare no conflicts of interest.

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