BVG Trust

J. Sci. Trans. Environ. Technov. 2007, 1(2): 88 - 91

Scientific Transactions in Environment and Technovation

Pharmacognostic Standardisation of an Eco-Friendly Herbal Insecticide

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Abstract

Pharmacognostic standardization of an eco-friendly bio-insecticide having *Acorus calamus* (Sweet flag-rhizome), *Nigella sativa* (Black cumin-seeds), *Cinnamomum zeylanicum* (Cinnamon-bark), *Piper nigrum* (Pepper - fruit) and *Eugenia caryophyllaea* W. (Clove - flower buds) as its ingredients was carried out. The phytochemical ingredients of the bioinsecticide possessed antifeedant, antilarval, insecticidal activity. Besides, it also possessed chemicals that induced sedation. The parameters studied include macroscopic and microscopic features, physicochemical constants, fluorescence characters and assay of alkaloids. Level of total alkaloids was estimated because the major chemical constituent of this herbal formulation is alkaloid, which is also a proven antifeedant principle.

Keywords: alkaloids, eco-friendly, herbal bio-insecticide, standardisation, phytochemicals

INTRODUCTION

The indiscriminate use of insecticides like synthetic pyrethroids and organo phosphates had led to complete resistance among key pests. In addition to insecticide resistance, the effect of these compounds on beneficial insect populations frequently results in secondary pest outbreaks and pest resurgence, which are increasingly recognized as adversely affecting our ability to manage pest populations in general. Besides the use of chemical insecticides adversely affects human health and contaminates the ecosystem as well. In this scenario herbal preparation in controlling insects would be more desirable. Consequently the percentage of harmful chemicals in the environment could also become less. In this regard, in recent years there has been concerted international efforts at developing plant derived insect control agents which are relatively safe, biodegradable and eco-friendly.

The paper based library materials are organic in nature and are subjected to deterioration by various factors including physical factors such as light, heat, moisture, water and fire, biological factors that include fungi, insects and rodents and chemical factors like dust and ink stains. To prevent such damages and deteriorations, chemical preservatives such as ammonia, calcium hydroxide, barium hydroxide, thymol solution, mercuric chloride, arsenic oxide and methyl bromide are being used. But these chemical preservatives for books and files may result in damages, papers may become brittle and decolored, pictures and photographs may fade and black and brown stains may appear on the papers. They may even cause human health hazards such as headache, fatigue, dizziness and asthma.

The present paper aims at developing and standardizing a plant based agent to control the biological factors, particularly insects that cause damages and deteriorations to books and files.

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MATERIALS AND METHODS

To develop a bioinsecticide formulation, the following raw drugs were procured from the local markets of Trichy, India, identified and authenticated (Chopra *et al.*, 1999) (Table 1).

Table 1. Constituents and proportions of herbals used in preparing the bioinsecticide

Botanical Name	English Name	Vernacular Name	Parts Used	Proportion
Acorus calamus L.	Sweet flag	Vasambu	Rhizome	(1 part)
Nigella sativa L.	Black cumin	Karuncheeragam	Seeds	(1 part)
Cinnamomum zeylanicum Blume.	Cinnamon	Elavangapattai	Bark	(1 part)
Piper nigrum L.	Pepper	Milagu	Fruits	(1/4parts)
Eugenia caryophyllata ea W.	Clove	Kirambu	Flower buds	(1/4parts)

The above raw materials were coarsely powdered using mixer grinder and mixed thoroughly. This herbal mixture was subjected to standardization studies as per the standardization protocols listed in Table 2.

RESULTS

The finished product is a coarse brown powder with bitter taste and camphoraceous odour (Table 3). Microscopic analysis of the powder confirmed the presence of various ingredients in the end product (Table 4). Reticulate and annular vessels confirmed the presence of *Acorus calamus* L., elongated stone cells confirmed the addition of *Piper nigrum* L. and endospermcells characterized *Nigella sativa* L., and seeds. Acicular crystals is characteristic of *Cinnamonum zeylanicum* Blume. and rosette crystals must have come from *Eugenia caryophyllaea* W. Thus presence

Table 2. Standardization protocols used for the novel bio-insecticide formulated

S.No.	Characteristics Analyzed	Standardization protocols employed
1.	Macro and Microscopic features	as per Esau 1898 and Johnson, 1940
2.	Preliminary Phytochemical Tests	as per Harborne and Mabry (1984).
3.	Behaviour of drug powder with chemical reagents	as per Dey and Sitaraman (1957).
4.	Physico chemical constants	as per Anonymous, 2001.
5.	Fluorescence analysis	as per Chase and Pratt (1949).
6.	Alkaloids	as per the standard procedure Handa and Kapoor (2003).

Table 3. Macroscopic characters and organoleptic features of the herbal mixture formulated

S.No.	Features	Observations
1.	Nature	Coarse powder
2.	Mesh	80-100 mesh
3.	Colour	Brown
4.	Odour	Camphoraceous
5.	Taste	Bitter

of all ingredients were confirmed in the end product.

Hexane extractions confirmed the presence of aromatic oils (Table 5). Presence of alkaloids and terpenes biomolecules responsible for insecticidal activity were confirmed through preliminary phytochemical screening and behavior of the product with various chemical reagents (Tables 6 & 7). Fluorescence analysis also revealed characteristic brown and green fluorescence in UV light (Table 8 &9).

Table 4. Microscopic characters of the herbal mixture formulated

S.No	Features	Observations
1.	Fibers	Present in single as well as in groups; Spindle shaped
2.	Vessels	Annular and reticulate
3.	Trichomes	Non-glandular
4.	Stone cells	Elongated
5.	Parenchyma	Present in groups
6.	Crystals	Minute acicular and rosette
7.	Endosperm cells	Present
8.	Oil cavities/ oil globules	Present
9.	Spiral tracheids	Present
10.	Starch grains	Present, compound type

Table 5. Physico-chemical constants of the herbal mixture

S.No	Parameter	Value % (w/w)
1.	Total Ash	3.7
2.	Water Soluble Ash	0.36
3.	Acid insoluble Ash	2.14
	Successive Extractive values	
1.	Hexane	14.3
2.	Chloroform	1.97
	Solubility	
1.	Alcohol	1.55
2.	Water	7.44

Assay for alkaloid = 0.3%

DISCUSSION

A novel eco-friendly herbal insecticide is prepared and taken up for present study. Main ingredients of the product are *Acorus calamus* L. (Sweet flag - rhizome), *Nigella sativa* L. (Black Cumin-seeds), *Cinnamomum zeylanicum* Blume. (Cinnamon -bark), *Piper nigrum* L. (Pepper - fruit), and *Eugenia caryophyllaea* W. (Clove - flower buds).

From the literature survey it is observed that *Acorus calamus* possess asarone, β- asarone and have sedative properties (Rasgoti and Mehrotra, 1999a). *Nigella sativa* yield α, β-hydroxy ketones possessing antifeedant activity (Rastogi and Mehrotra, 2001). The chief essential component of *Cinnamomum zeylanicum* are eugenol, caryophyllene and p-cymene. Alkaloids such as piperine, piperetine, chavicine are present in *Piper nigrum* seeds which inhibit the development of larva of *Drosophilla* and also possess insecticidal activity (Rastogi and Mehrotra, 2001). Flower buds of *Eugenia caryophyllaea* W. also possessed eugenol, pinene and vanillin (Rastogi and Mehrotra, 1999b). To sum up, the ingredients of the novel herbal insecticide formulated in the present study had phyto-chemicals possessing antifeedant or antilarval or insecticidal activity.

Table 6. Behaviour of the drug powder with various chemical reagents

S.No	Test for	Reagents	Reaction	Result
1.	Gums	Powder + drop of water	No reaction	-ve
2.	Saponins	Water + Shake	No reaction	-ve
3.	Proteins	Powder + Picric acid	Yellow colour	-ve
4.	Tannins	Powder + Lead acetate solution	No reaction	-ve
5.	Sterols	Powder + Acetic anhydride + sulphuric acid	Green colour	-ve
6.	Terpenes	Powder + Tin + Thinoyl chloride	Pink colour	+ve
7.	Sugars	Powder + Anthrone + Sulphuric acid	Green colour	-ve
8.	Phenols	Powder + Ferricchloride	No reaction	-ve
9.	Flavonoids	Powder + Mg bits+ HCl	No reaction	-ve
10.	Coumarins	Powder + dil. NaoH	Yellow colour + Conc. H_2SO_4—Substance regenerated	+ve
11.	Quinone	Powder + Conc. H_2SO_4	Red colour	+ve
12.	Lignin	Powder + Alcoholic solution of phloroglucinol+dil. HCl	No reaction	+ve
13.	Starch	Powder + Iodine in KI	No reaction	-ve
14.	Alkaloids	Powder + Dragendroff's reagent	Orange red colour	+ve

Table 7. Preliminary phytochemical screening of the drug powder formulated

S.No	Test for	Hexane	Benzene	Chloroform	Ethyl acetate
1.	Steroids	-ve	-ve	-ve	-ve
2.	Terpenes	+ve	+ve	+ve	+ve
3.	Sugars	-ve	-ve	-ve	-ve
4.	Alkaloids	+ve	+ve	+ve	+ve
5.	Phenols	-ve	-ve	-ve	-ve
6.	Acids	-ve	-ve	-ve	-ve
7.	Tannins	-ve	-ve	-ve	+ve
8.	Saponins	-ve	-ve	-ve	-ve
9.	Quinone	+ve	+ve	+ve	+ve
10.	Coumarins	+ve	-ve	-ve	+ve

Table 8. Fluorescence analysis of the drug powder formulated

S.No.	Treatment	Day light	UV light
1.	Drug powder	Brown colour	Brown colour
2.	Drug powder + aq.1N NaOH	Brown colour	Brownish green colour
3.	Drug powder +alc.1N NaOH	Pale yellow colour	Light green colour
4.	Drug powder + 1N HCl	Colourless	Colourless
5.	Drug powder + 50% H_2SO_4	Brown colour	Brownish green colour

Table 9. Fluorescence analysis of extracts of the drug powder formulated

S.No.	Treatment	Day light	UV light
1.	Hexane	Pale yellow	Greenish tinge
2.	Benzene	Brown	Green
3.	Chloroform	Pale yellow	Dark green
4.	Acetone	Pale yellow	Light green
5.	Ethyl acetate	Light green	Green
6.	Alcohol	Brown	Light green
7.	Water	Brown	Dark green

Thus in the present work an eco-friendly herbal insecticide is prepared and standardized. This could be used to protect the books, manuscripts, inscriptions and paintings which have to be maintained with constant care and keen observation as they are our national heritage and culture. But this should be confirmed by field trials to test the efficacy of this new formulation.

ACKNOWLEDGEMENTS

We place on record our deep sense of gratitude to The Principal and The Management of Srimad Andavan Arts & Science College, for providing necessary funds, equipments and constant encouragements through out the work.

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