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Chemical Comparison by GC/MS Chromatography of Two Chemotypes of Essential Oils of *Canaga odorata* Against *Colletotrichum gloeosporioides*

Alexander Perez-Cordero*, Jaime de la Ossa-V and Donicer Montes-Vergara

Departamento de Zootecnia, Facultad de Ciencias Agropecuarias, Universidad de Sucre, Colombia; alexander.perez@unisucre.edu.co, jaime.delaossa@unisucre.edu.co, donicer.montes@unisucre.edu.co

Abstract

Objectives: To determine the antagonistic activity of the essential oils of *Cananga odorata* flowers as a phytosanitary alternative against the phytopathogen *Colletotrichum gloeosporioides*. **Methods/Statistical Analysis:** The essential oils were obtained from *C. odorata* yellow flowers in the municipalities of Sincelejo and La Union, Sucre, by microwave-assisted hydrodistillation. The tests of antagonistic activity were carried out on the mycelial growth of *C. gloeosporioides*. The chemical profiles of the oils were made by GC/MS. With the results, the variance analysis and the Tukey test (p-value≤0.05) were performed for the significant differences. **Findings:** The results show that the flowers of *C. odorata* are a potential source of the raw material for the production of essential oils, due to their high yield, for which commercial exploitation is proposed. Presented the essential oil collected in Sincelejo the highest yields with 0.86% (v/w). In addition, the two essential oils presented antagonistic activity against *C. gloeosporioides*, being the one collected in Sincelejo the one that showed the highest averages of inhibition with 62.17%, followed by La Union with 57.38%. But at concentrations of 20,000 mg/L, 100% inhibition was found, similar to the positive control with benomyl. The MIC was 17,000 mg/L and 18,000 mg/L with 88.47% and 84.65% for Sincelejo and La Union, respectively. While the MFC was 100% at concentrations of 18,000 mg/L and 19,000 mg/L for Sincelejo and La Union, respectively. The analysis by GC/MS showed that the flowers of *C. odorata* collected in Sincelejo correspond to the Linalool chemotype and La Union al Bencil Benzoato. **Improvements/Applications:** Natural products represent a biotechnological alternative for phytosanitary problems, in this highly polluted planet, thanks to its high antagonistic capacity, zero environmental pollution and high biodegradability.

Keywords: Bioactive, Chromatography, Phytopathogen, Yam

1. Introduction

Latin America and the Caribbean are the regions with the greatest biological diversity on the planet presenting almost half of tropical forests and home to several of the mega-diverse countries in the world, and this exceptional biodiversity allows countries like Colombia reap its benefits in order to promote social and economic growth[1].

Colombia is called by the United Nations Environment Program (2011) as one of the 17 mega-diverse countries, hosting 70% of the world's biodiversity in only 10% of the territory, where there have been more than 54,000 species according to dataGlobal Biodiversity Information Facility, sharing with Brazil the first place in terms of global biodiversity, further characterized by the importance of natural forests, which cover 53% of the continental territory and accounts for roughly half of species of animals and plants on the planet, so it takes first as a country rich in diversity of birds and orchids, the second in diversity of plants, amphibians, freshwater fish and butterflies, the third in diversity of reptiles and palms, and the fourth in a variety of mammals[2].

While all this great diversity of plants present in the country, yet have little Ethnobotanical and phytochemical research, therefore, this raw material constitutes a phytosanitary alternative to counter the diseases that afflict the

**Author for correspondence*

health of people and animals and mainly economically important crops, because since ancient times agriculture has been the basis for social and economic development of territories, but this is hampered by the high incidence of infectious diseases caused by pathogenic microorganisms.

In the Colombian Caribbean region there are many crops that are affected by these pathogens, such as the yam (*Dioscorea*spp.). *Dioscorea* is one of the 6 genera nested in Dioscoraceae family where there are about 600 species of which only 12 are edible. In Colombia the main production centers in the Caribbean which is of great socio-economic importance, so that in 2010, Colombia was among the 12 countries with the highest yam production worldwide with 395,374 tons and ranked first in performance with 28.3 ton per planted hectare[3].

In 2012 the GDP of the department of Sucre grew 5.50%, while agriculture, hunting, forestry and fishing slowed to 6.40%, but in 2013 the agricultural sector and hunting grew by 131.60% over the previous year, presenting 68.90% of exports by the Department[4]. Many grown in Sucre agricultural products, including varieties of criollo and hawthorn yams are planted, being the municipalities of Ovejas, Los Palmitos and Sincelejo the highest production[6], generating sources of direct and indirect jobs in its supply chain, but this tuber has been the target of numerous pathogens such as the fungus *C. gloeosporioides* causes anthracnose and the Colombian Caribbean region has caused losses up to 85%[6]. This disease manifests itself in tubers, leaves, stalks and/or stems[7,8]. During the onset of the disease, the leaves present in the beam reddish spots cloven appearance with yellow halo and on the undersides blackening of the ribs is observed, and over time, the lesions grow irregularly and are joined together eventually causing leaf necrosis, also causes necrosis on the stem and eventually dieback of the plant[8].

C. Gloeosporioides is widely distributed in tropical and subtropical regions of the world[9], and is causing great economic losses in various crops such as fruit, cereals, grasses, vegetables, legumes and perennial crops[10]. In addition to causing damage to yam crops also attacks soursop (*Annona muricata* L.) causing up to 90% of losses in non-technified orchards[11], tree tomato [*Cyphomandra betacea* (Cav.) Sendt.] apple (*Malus domestica* Borkh), blackberry (*Rubus glaucus* Benth) causes losses exceeding 50%[12] and anthracnose avocado (*Persea americana* Mill).

Today, farmers use continuous dose of fungicide to counteract the effects of this fungus on the cultivation, mainly yam. Studies and published in bibliographies show that these compounds cause environmental problems, human health, animal and microbial diversity. Also the improved varieties tolerant to the diseases exhibit unsatisfactory results in the field.

Based on this scenario, it is necessary to develop new alternatives such as integrated disease management where agroecology plays an important role by promoting the balance between man and nature by adopting a production system that will improve the quality of soil and obtain clean products for human consumption. The use of bioproducts is a practical solution, namely use of products derived from biodiversity for controlling phytopathogenic, such as essential oils of plant origin.

Essential oils in recent years have positioned themselves as a great agronomic alternative to replace synthetic pesticides on the market today for possessing antifungal, antibacterial, antiviral, etc. Besides this, they can replace synthetic food additives, favoring their stability and protection against lipid disorders by its antioxidant activity[13]. Therefore, it is intended to evaluate in vitro activity of plant essential oils against *C. gloeosporioides* (Penz.) Penz. & Sacc Causes. Anthracnose in crops of yam in the department of Sucre.

2. Material and Methods

2.1 Study Area

Sampling was conducted in two municipalities in the department of Sucre, Colombia, one located in La Union belonging to the subregion of San Jorge located at a northern latitude of 8° 50' 46.8" west longitude and 75° 17' 01.12"to 73 m.a.m.s.l and the other in the town of Sincelejo located in the subregion Montes de Maria north latitude of 9° 17' 10.1" and west longitude 75° 22' 45.1" to 183 m.a.m.s.l.

2.2 Raw Material

The plant material used was mature flowers of *Cananga odorata*. These were collected during sunset; to prevent high temperatures could volatilize some components present in the samples. The plant material was packed in a container of expanded polystyrene (styrofoam) previously containing ice cubes to maintain a temperature of 25°C[14]. The collection was made in October 2017, in rainy period.

2.3 Taxonomic Identification

The collected plant materials were sent to the Herbarium of the University of Sucre, national register of biological collections formaking taxonomic identity.

2.4 Processing Plant Material

The collected flower was washed with distilled water and selected to ensure good condition; then the petals are separated, chopped, dried, weighed and the extraction process.

2.5 Extraction of Essential Oils

It was performed by the microwave-assisted hydrodistillation method (MWHD), equipment hydrodistillation type Clevenger balloon distillation capacity to 2L, where they were introduced approximately 300g of plant material with 250mL of distilled water and the system was brought to a boil for 45 min divided into three cycles of 15 minutes each using was used as a heat source of the microwave conventional oven, essential oils collected radiation into a Dean Stark type container, then dehydrated with anhydrous sodium sulfate and stored in amber vials type at 4°C until further use[15].

2.6 Yield Calculation

To determine the yield of essential oils formula was used:

$$Yield(\%) = \left(\frac{V}{V}\right) * 100$$

Y: yield (%), V: volume of the essential oil (mL) and M: mass of plant material (g)[16].

2.7 Fungus

The phytopathogen used for antifungal test was identified by the Group Bioprospecting Agricultural Research at the University of Sucre as *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc., a causative agent of the disease known as anthracnose in crops of yam in the department of Sucre.

2.8 Antagonistic *in vitro* Assays of Essential Oils against Plant Pathogen

The determination of the antagonistic activity of essential oils of flowers *C. odorata* was performed by the method of direct seeding with pure growth of the isolates of about 7 mm diameter area growth[17]. Which were sown on the surface of potato dextrose agar medium (PDA) supplemented with antibiotics chloramphenicol, ampicillin and rifampicin. Each isolate was added with 50mL of each essential oil at different concentrations in mg/L dissolved in a mixture of Tween 0.6% and DMSO0.3%. A positive control with the fungicide benomyl (2.5g/L), and a negative control solution was used Tween 0.6% and DMSO0.3% and an absolute control without any treatment. Assays were incubated at 30±2°C for 8 days in light and dark intervals. Antifungal activity was evaluated by measuring the radial growth of each isolate with different concentrations after the eighth day. The result was interpreted as a percentage of antifungal index:

$$Percentage\ of\ antifungal\ Index(\%A.I) = \left[1 - \left(\frac{D_a}{D_b}\right)\right] x100$$

Where D_a corresponds to the growth of each treatment and D_b to the absolute control growth[18].

Of the oils and 6 concentrations 1,000 mg/L, 2,000 mg/L, 4,000 mg/L, 10,000 mg/L, 15,000 mg/L and 20,000 mg/L they were prepared. This assay was performed in triplicate for a total of 45 experimental units.

2.9 Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

To determine the MIC and MFC concentrations 15,000 mg/L, 16,000 mg/L, 17,000 mg/L, 18,000 mg/L, 19,000 mg/L and 20,000 mg/L dissolved in Tween 20 0.6% and DMSO0.3% and an absolute control are prepared. Each treatment was done in triplicate for a total of 39 experimental units.

MIC values were calculated MFC and the radial growth of the fungus inoculated into potato dextrose agar medium (PDA) and incubated at 30±2°C for 8 days at intervals of 12 hours light and 12 hours dark. The MIC is defined as the lowest concentration of the treatments it showed ≥90% growth inhibition at 8 days of incubation[19]. The MFC was defined as the lowest concentration of the treatments showed no visible growth that fungus or caused growth inhibition around 100% after 14 days of incubation.

2.10 Analysis by Gas Chromatography Coupled to Mass Spectrometry (GC/MS)

The determination of the chemical components of the essential oils was performed by instrumental technique of Gas Chromatography coupled to Mass Spectrometry (GC/MS), using a kit from Agilent 6890N gas chromatograph coupled to a mass selective detector Agilent 5973N. Kovats indices were determined on a column DB_5MS 30mx320µmx0.5µm, using helium as carrier gas at a pressure of 0.27psi and an average flow rate of 40cm/sec. The initial oven temperature was 150°C and temperature final of 350°C. The injector temperature was 250°C and detector 300°C was. The identity of the compounds was assigned by comparison of the experimentally obtained mass spectrum for each component, with those reported in the databases NIST98.L, NIST02.L and NIST5a.L[15].

2.11 Statistic Analysis

Assays were performed in triplicate. An analysis of variance (ANOVA) factorial arrangement was performed to establish the correlation of antifungal activity against oils according to the concentration used and the area of collection, using software InfoStat free version to determine the criteria of ANOVA test Shapiro-Wilks normality was applied and the Tukey test (p-value≤0.05) for significant differences.

3. Results and Discussion

3.1 Taxonomic Identification

Taxonomic identity of the plant species used in this study was confirmed, which was identified as *Cananga odorata* (Lam.) HOOK.F. & Thomson stored in the herbarium collection of the University of Sucre under registration No. 000832 voucher.

3.2 Yield Calculation

The yield of essential oil extracted from flowers *C. odorata* collected in the city of Sincelejo was 0.86% and La Union was 0.76% (v/w) respectively (Graph 1. A). This performance obtained from *C. odorata* collected in Sucre department indicates that may be proposed commercial exploitation of its essential oils because yieldare superior to 0.1% which is the minimum value of yield limit[20]. These essential oils are of great importance in industry and as a source of employment such as the archipelago of Indonesia, where production of ylangylang is of great cultural and economic value and is the second largest export after oil the Comoros islands.

3.3 Antagonistic *in vitro* Assays of Essential Oils against Plant Pathogen

The concentrations of essential oils evaluated showed antagonistic activity against *C. gloeosporioides* (Figure 1).

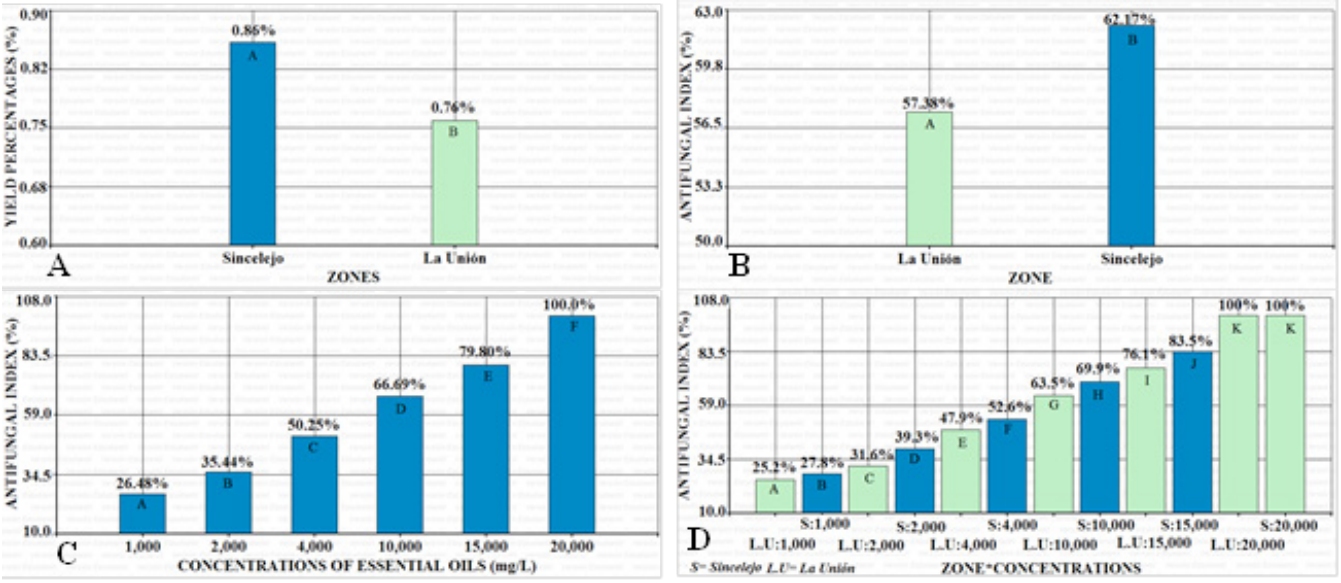

Figure 1. Essential oils of Canangaodorata. (A): Yield Percentages (%). Percent of antifungal index. (B): By collection zone. (C): By concentration. (D) Interaction between zones and concentrations.

All the ANOVA criteria were met. The variance analysis indicates statistically significant difference (p-value<0.05) between the zones of collection of plant material and the concentrations of essential oils of *Cananga odotata* evaluated and between their interaction. The results of the multiple range test Tukey statistical differences shown significant (p-value<0.05) between the zones of collection of plant material, presenting higher average antifungal activity essential oil of *C. odorata* collected in the city of Sincelejo with 62.17% de %AI against mycelial growth of *C. gloeosporioides*, followed essential oil collected in La Union with %IA of 57.38% (Figure 1.B). While with respect to concentrations which showed the highest inhibitory activity was average concentration of 20,000 mg/L with %A.I of 100% (Figure 1. C), both the essential oil collected in Sincelejo and La Union, similar to the positive control with benomyl (Graphic 1. D). While the concentration of 1,000 mg/L showed the lowest average antifungal activity (Figure 1. D).

According to reports, *C. odorata*, used against pitta, stomach ailment, fever, swelling, burning sensation, malaria fever, aromatherapy asthma, hypertension, anxiety, depression and as a sexual stimulant[21].

3.4 Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

The results obtained indicated that the minimum inhibitory concentration for the essential oil collected in Sincelejo was 17,000 mg/L with %A.I of 88.47% and La Union was 18,000 mg/L with %A.I of 84.65% and minimum fungicide was 100% for both essential objectives for 18,000 mg/L and 19,000 mg/L for the essential oil of La Union and Sincelejo, respectively. These results are similar to that presented by the positive control with Benomil to 2.5g/L. The negative control showed similar growth to the control, indicating that the solvent used does not inhibit mycelial growth (Figure 2).

3.5 Analysis by Gas Chromatography/Mass Spectrometer (GC/MS)

C. odorata have identified about 146 secondary metabolites including the β-are mircyno, α-phellandrene, β-limonene, linalool, neral, geraniol, geranial, eugenol, α- and β-ylangeno, among others[22].

The above results are consistent with the results obtained in the essential oil of Sincelejo 30 compounds flower were identified, while for La Union found 29. The essential oils of the two municipalities presented small Eugenol amounts, however, in the Sincelejo presented as the major secondary metabolite concentrations Linalool to 39.52%, followed byto the Benzyl benzoate 17,12% (Table 1 A), while that in the LaUnion presented the major secondary metabolite concentrations were Benzyl benzoate 26.76 % and Nerilo Acetate 13.84% (Table 1. B). Results found suggest that the presence and quantity of secondary metabolites present in *C. odorata*, depends on geographical area and environmental conditions where the plant grows.

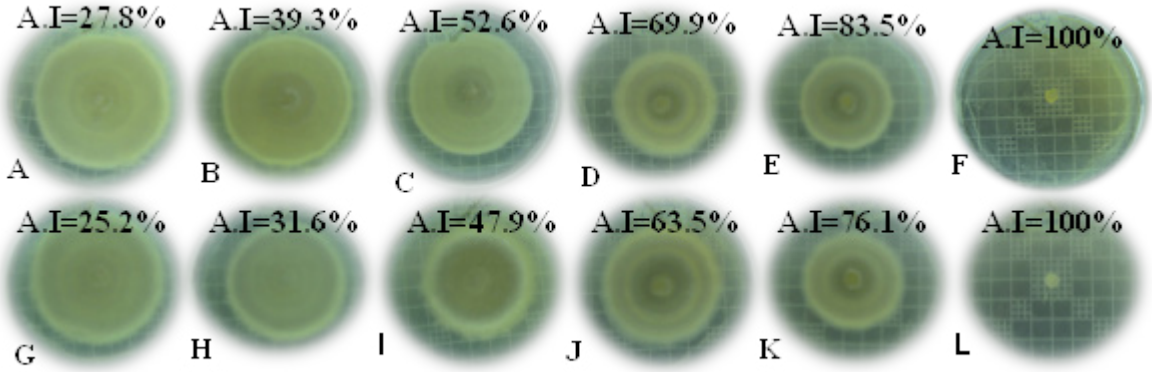

Figure 2. Test of antifungal activity of essential oils from *Cananga odorata* against *Colletotrichum gloeosporioides*. **Sincelejo essential oil**. A. 1,000 mg/L. B. 2,000 mg/L. C. 4,000 mg/L. D. 10,000 mg/L. E. 15,000 mg/L. F. 20,000 mg/L. **La Union essential Oil**. G. 1,000 mg/L. H. 2,000 mg/L. I. 4,000 mg/L. J. 10,000 mg/L. K. 15,000 mg/L. L. 20,000 mg/L.

Table 1. Chromatograms of the essential oils of *Cananga odorata* collected in municipality Sincelejo (**A**) and The Union (**B**)

Sincelejo				(B) La Unión			
Pk#	**RT**	**% Área**	**Identificación**	**Pk#**	**RT**	**% Área**	**Identificación**
1	4.018	0.08	Acetato de Isoamilo	1	4.858	0.05	Not
2	4.134	0.34	Not	2	5.013	0.44	α-Pineno
3	4.858	0.47	Not	3	5.685	0.12	Benzaldehido
4	5.019	0.45	Pineno	4	5.911	0.19	L-β-Pineno
5	5.685	0.18	Benzaldehido	5	6.299	0.11	Not
6	5.911	0.23	β-Pineno	6	6.732	0.24	Not
7	6.299	0.08	β-Mirceno	7	6.932	0.22	Not
8	6.738	0.18	Not	8	7.378	6.79	p-Metoxitolueno
9	7.41	9.24	Anisol	9	7.682	0.21	α-Toluenol
10	8.011	0.11	Alcohol Bencílico	10	7.96	0.29	α-Pineno
11	9.853	39.52	Linalool	11	9.446	15.52	Not
12	10.661	0.23	Veratrol	12	9.601	4.43	Linalool
13	11327	8.5	Plastolin	13	11.314	10.71	Acetato de Bencilo
14	11.469	0.89	Metil Benzoato	14	11.423	0.35	Benzoato de etilo
15	11.714	0.19	Not	15	11.682	0.17	Not
16	12.012	0.07	Metil Salicilato	16	13.039	0.11	Not
17	13.11	0.2	3-Metil Veratrol	17	13.259	0.89	Not
18	13.175	0.09	Not	18	13.407	0.29	Feniletilmetacrilato+Fenetil Acetato
19	13.394	0.22	Not	19	13.847	0.23	Geraniol
20	14.409	0.48	Not	20	14.409	0.77	Anetol
21	14.461	0.3	Not	21	14.493	0.39	Not
22	15.624	0.16	Dimetil Salicilato	22	14.823	0.38	Carvacrol
23	16.664	1.8	Acetato de Geranilo	23	15.643	0.1	Not
24	17.511	0.69	Cariofileno	24	16.968	13.84	Acetato de Nerilo
25	18.364	1.41	γ-Fenilalil Acetato	25	17.582	0.98	Cariofileno
26	18.655	0.71	Eugenol	26	17.866	0.07	(Z,E)-α-Farneseno
27	19.101	2.83	Germacreno	27	18.422	1.32	Acetato de Cinamilo
28	19.366	0.58	Not	28	18.674	4.51	Acetato de (E)-Cinamilo
29	19.618	0.37	α-Farneseno	29	18.816	0.78	Isoeugenol
30	19.863	3.58	Isoeugenol	30	19.172	3.16	D-Germacreno
31	20.639	0.08	Hedicariol	31	19.43	0.2	Not

32	21.291	0.24	β-Bourboneno+Linderol	32	19.637	0.28	α-Farneseno
33	22.997	0.18	τ-Cadinol+2-Isopropil-5-metil-9-metileno-biciclo-1-deceno(4.4.0)	33	19.837	1.93	Isoeugenol
34	23.107	0.07	α-Copaeno+ (-)-Cedreanol	34	19.954	0.19	β-Cadineno
35	23.437	0.24	α-Cadinol	35	20.658	0.14	Not
36	25.311	1.76	Farnesol	36	21.324	0.45	β-Bourboneno
37	27.437	17.12	Benzoato de Bencilo	37	23.01	0.15	τ-Cadinol+epi-Biciclosesquifelandreno
38	28.852	0.41	(E)-Farnesil Acetato	38	23.12	0.07	α-Copaeno
39	30.287	5.6	Salicilato de Bencilo	39	23.443	0.19	α-Cadinol+ Elixeno
40	34.83	0.11	Not	40	24.393	0.07	Not
				41	25.298	1.48	Not
				42	27.695	26.76	Benzoato de Bencilo
				43	28.904	0.36	Acetato de (E)-Farnesilo
				44	32.497	0.1	Not

The essential oil extracted from *C. odorata* Sincelejo showed higher average efficiency antifungal against *C. gloeosporioides* 42.6%, in contrast to *C. odorata* La Union 39.9%. This is possibly due to the high concentration of linalool present in the flower essential oil Sincelejo, since this metabolite has been attributed for pharmacological activities as antileishmaniasis[23], antimicrobial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Candida albicans*[24]. Although, the essential oil of La Union contains Linalool in considerable quantities, this oil showed antifungal activity possibly combining cinnamyl acetate, Isoeugenol, Geraniol, Benzyl Benzoate and other minor components responsible for this activity[25].

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