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ALLELOPATHIC POTENTIL OF *Cymbopogon citratus*L. AGAINST DIFFERENT WEED SPECIES

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ABSTRACT:

The chemical compositionof essential oil isolated from *Cymbopogon citratus* L. by hydrodistillation was determined by gas chromatography -mass spectrometry (GC/MS) analysis, 34 compounds were identified. The majorcomponentswere Neral 25.4% and Geranial 22.7 %. Allelopathic potential of isolated essential oils was tested on germination and seedling growth of different species of weeds, namely, *Panicum virgatum*, *chloris barbata, Euphorbia hirta and Stachytarpheta indica.. C. citratus* essesntialoil was shown high effectiveness in inhibiting seed germination and suppressing seedling growth in all targeted weeds. A high efficacy in inhibiting germination and seedling growth completely was achieved at higher concentration 5 µL/ml. Results showed that*C. citratus*essential oil could be an interesting alternative to conventional synthetic herbicides.

KEYWORDS: *Cymbopogon citratus* L; Essential oil;Chemical composition; Allelopathic potential

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INTRODUTION:

Weed competition is considered the most important problem facing the agricultural field. It caused losses more than the losses that comes from pathogens and insects together[1]. Chemical weed control comes to reduce weed competition by using chemical compounds which have ability to kill weed plants in a high efficiency compared with conventional methods. Nowadays,weed chemical control (syntheticherbicides) became one of the most technical in modern agricultural production and have been accepted as a standard tool of the trade by farmers throughout the world[2].
Recently, Negative impacts of using chemical compounds represented by synthetic herbicides had begun worrying lately. Influence of synthetic herbicides directly or indirectly from their residues by affecting of soil organisms and soil structure. In addition, it became one of contaminated factors of the environment and drinking water sources[3].
Moreover, using of synthetic herbicides constantly lead to increase of weed resistance, which in turn reduce the efficiency and effectiveness of these materials against weeds[4].Hence, we need to find alternatives to synthetic herbicides, effective against weeds and less dangerous residue become a priority Indispensable in order to avoid the implications for the use of those materials[5]. Taking advantage of the biological defenses of some plants that are characterized by releasing compounds able to inhibit growth organisms in surrounding environment which are known as allelopathy phenomenon considered good sources of natural alternatives to synthetic herbicides[6].
These compounds are known as allelochemical or allelchemics and produces from donor plants as a secondary metabolisms. Allelochemical compounds released to the environment by different ways like leaching, root exudation, volatilization and residue decomposition[7]. Allelochemical compounds are synthesized by shikimite pathway or in isoprene pathway is case of essential oil.
Tropical plants characterized by a high diversity depending on their content and differences of bioactive compounds including allelochemical compounds. So, its widely used in traditional medicine for the maintenance of health. Natural plant products by tropical plants proved good effectiveness against bacteria, fungi.
Previously, Natural extracts of tropical plants have been widely used as alternatives attractive to synthetic pesticides. Forthermore, extracts of these plant have been tested its phytotoxicity against weed, especially essential oils which is a consist of mixture of compounds mainly terpenes and phenols.
Cymbopogon citratus L. An aromatic tropical perennial tall grass with rhizomes and densely tufted fibrous root belongs to Poaceae family. This plant is used widely in traditional medicine in many countries around the world, duo to its properties that related of its effectiveness as an anti-bacterial and Antifungal [8]. The aims of present study to identify chemical composition of essential oil isolated from*C. citratus*and determine their allopathic potential against different species of weeds in order to evaluate the possibility to be alternative to synthetic herbicides.

METHODS AND MATERIALS

Collecting of plant material :

Freash plants of *C. citratus* were purchased from the wet market in kanger, Perlis, Malaysia and dried for two weeks at room temperature. Then, chopped into small pieces approxometely 1cm in order for isolaton.

Isolation of Essential oil :

The essential oil was isolated from dry material of *C. citratus* byhydrodistillation using modiefied Clevenger-type apparatus[9]. Briefly, two hundred grams of each sample were soaked in 1L of distilled water for three hours. The isolated oil was dried over using anhydrous sodium sulfate (a pinch/10 ml^{-1}) and stored in sealed glass vials at 4°C until use. Essential oil yield was calculated as mean of five replications based on the weight of the sample.

Table 1. Chemical composition of *C. citratus* essential oil

	Compound	RI	Area %
1	Thujene	9.30	tr
2	Camphene	9.53	tr
3	Myrcene	9.93	3.35
4	1,8-cinole	10.34	2.7
5	γ-Terpinene	10.57	tr
6	Linalol	10.97	1.63
7	Citronellal	11.43	2.3
8	Camphor	11.45	1.3
9	Citronellol	12.13	1.79
10	Nerol	12.28	0.57
11	Neral (Citral A)	12.35	25.4
12	Cis-Geraniol	12.55	2.6
13	Geranial (Citral B)	12.70	22.7
14	Generyl Formate	13.01	tr
15	Generyl actate	13.79	0.9
16	Eugenol	13.53	tr
17	Methyl eugenol	13.69	tr
18	Caryophyllene	14.18	3.1
19	β-Cedrene	14.24	0.74
20	γ-Elemene	14.26	tr
21	Farnesol	14.45	0.65
22	α- Humelene	14.55	1.5
23	Muurolene	14.77	0.58
24	Germacrene D	14.80	0.29
25	Selinene	14.86	1.46
26	Cadinene	15.18	tr
27	Elemicin	15.45	0.31
28	γ-Eudesmol	16.13	0.39
29	α- Cadinol	16.53	5.5
30	Geranyl tiglate	17.00	0.43
31	Abietal	22.61	0.55
32	Palmitic acid	19.84	9.9
33	Muurolol	21.45	3.65
34	Hexadecane	21.52	2.19
Total monoterpenes			65.24%
Total sesquterpenes			17.73%
Other components			13.38%
Total identified components			96.35%

RI: Retention Index on non polar Elite-5MS column. tr: trace amounts <0.05. Values are means ± standard error of Four samples.

Identify the chemical composition of the essential oil:

The chemical composition of the isolated essential oils was investigated by Gas chromatograph -mass spectrophotometer using Elite-5MS non-polar fused silica capillary column (30 m × 0.25 mm ID, film thickness of 0.25 mm). The column temperature was increased from 40 ºC to 280 ºC at a rate of 5 ºC/min; injector temperature, 250 ºC; injection volume, 1 µL; transfer temperature, 280 ºC. Mass spectra were taken over the m/z 40–600 and interface line temperature of 280 °C. The constituents of essential oils were identified based on their Kovats Index, calculated in relation to the retention time of a series of alkanes (C4- C28) as reference products compared with the chemical compounds gathered by Adams table(Adams,2007)[10], and the mass spectra of chemical compounds gathered in the NIST-MS and Wily Library.

Seed germination and seedling development bioassay:

Weed seeds of *Panicum virgatum*, *chloris barbata, Euphorbia hirta and Stachytarpheta indica* were extracted from healthy mature plants growing inthe Agrotechnology research station in Sg Chuchuh, which belongs to the University Malaysia Perlis UniMAP, Padang Besar, Perlis, Malaysia. CollectedWeed seeds weresterilized by sodium hypochlorite solution 15% for 20 min. Then seeds rinsed with water several times. Healthy seeds were selected by floating test[11].

The effect of isolation of essential oils on seed germination and seedling development of weed seeds were tested under laboratory condition. Briefly, Petri dishes 9mm diameter were lined with a layer of filter paper Whatman No. 1 and filled with 20g of thin, sterilized sand. Twenty seeds of each weed species were distributed regularly in petri dishes. Ten ml of each concentration 1.25, 2.5, and 5 µl/ml were added to the sand. Petri dishes were covered and sealed with Parafilm® to Prevent volatile compounds to come out. Distilled water was served as controls.Each treatment including concentration and weed species were replicated four times. Then, Petri dishes were placed in a dark place at 25±2 temperature. At 7 days after sowing, seed germination was measured and seedling were thinned to five seedlings in each treatment in order to measure shoot length and dry weight in 14days after sowing.

Statistical analyses:

Data of seed germination and seedling development assays were subjected to one-way analysis of variance (ANOVA) using the SASS system,version(9). The experiment was conducted in completely randomized design (CRD) with four replications. The differences between mean values were determined using Fisher's least significant difference (LSD) range test (P≤ 0.05).

RESULTS AND DISCUSSION:

Chemical composition of the essential oils:

The chemical composition of *C. citratus* oils, area percentage of the individual components, retention indices and yield percentage are shown in Table 1. The essential oil by steam distillation gave 0.95% as a final yield depending on the part of the plant analyzed. 34 compounds were identified accounting 96.35% of the total oil . The monoterpene fraction amounted 65.24%, while squiterpenes accounted for 17.73%, Neral and Geranial were the dominant monoterpene compounds 25.4 and 22.7 respectively.

The result in current study comes in agreement with most of the previous studies, like conducted to identifythe chemical composition of *C. citratus*[12,13,14] and found Neral and Geranial were the major components. These compounds conjugated to consist a double bond isomer compound known as Citral.

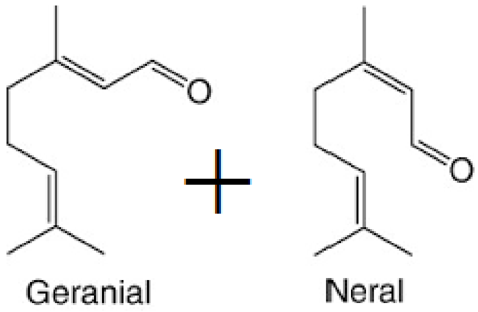

Figure 1. Structure of Citral

Seed germination and seedling growth:

Allelopathic potential of *C. citratus* essential oils were tested on germination and seedling growth of *P. virgatum*, *C. barbata, E. hirta and S. Indicate* which are very invasive weeds in experimental areas. Providing statistical analysis shown in (figure 2), seed germination of weeds under study was significantly inhibited by different concentration of oil testing. Complete inhibition in germination of all targeted weeds was observed in higher concentration 5µl/ml, while lower concentration 1.25 and 2.5µl/ml decreased germination partially. The result obtained from a current study come in the lane with results obtained in previous studies[15,16,17,18]which confirmed the effectiveness of essential oils against seed germination of weeds. As well as, essential oils which characterized by high content of monoterpene compounds have a good efficacy against weed seed germination. At the same time high content of monoterpens in essential oils could be a good indicator to evaluate their effectiveness against weed seed germination[9]. On the other hand, individual components could play an important role in biologicalactivity, including the allelopathic effects towrds weed plants . Previous studies have reported that essential oils and individual monoterpenes, such as α-pinene, limonene, terpinen-4-ol, camphor, 1,8- cineole, thymol, eugenol and carvacrol strongly inhibit seed germination and seedling growth of some weed species[19,20,21,22].

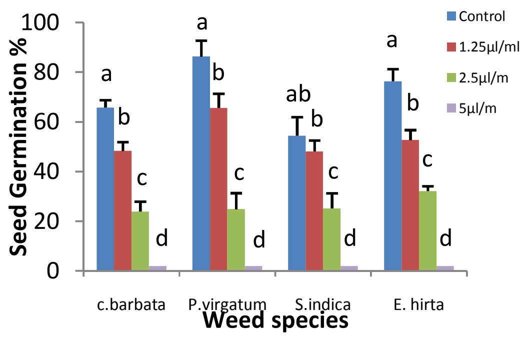

Figure 2. Allelopathic potential of different concentration of *C. citratus*essential oilon Seed germination% of some species of weeds

Regarding of weed seedling growth, the result obtained in (Figure 2and3), showed that Seedlinggrowth (Shoot length and dry weight) were significantly reduced by treating with different concentration of *C. citratus* essential oil.

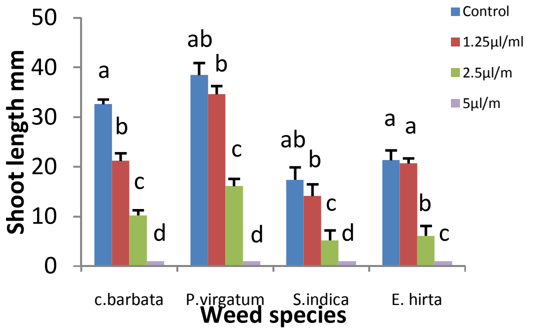

Figure 3. Allelopathic potential of different concentration of *C. citratus*essential oilon Shoot length (mm) of some species of weeds

In general, a relationship was observed between increasing of essential oil concentration and reducing of shoot length and dry weight in weed seedling. At the lower concentration 1.25 and 2.5 µl/ml, seedling growth was partially suppressed by lower concentration. While higher concentration 5µl/ml was suppressed seedling growth completly.Our results support the findings[9,11,23,24] in which the essential oils isolated from different plants suppressed seed germination and seedling development of weed plants.

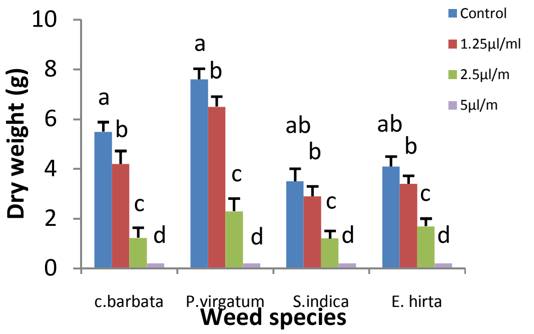

Figure 4. Allelopathic potential of different concentration of *C. citratus*essential oilon Shoot length (mm) of some species of weeds

Based on result obtained from current study, althougth the lower concentration, especially 2.5 µl/ml didn't reach to complete inhibiting of weed seed germination, it was able to reduce seedling growth. Hence, weed plants will be not able to grow healthy.

CONCLUSION

From the present study, *C. citratus* essential oil show strong allelopathic potential against diferent species of weedsand could be useful for developing as a bioherbicide. Furthermore, studies are required to evaluate the allelopathic potential of *C. citratus*essential oil (as a post emergence bioherbicide)under field conditions and determine the effect onnon-target weed species.

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