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Justicia anselliana (Nees) T. Anders Essential Oils Compounds and Allelopathic Effects on Cowpea Vigna unguiculata (L.) Walp Plant

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Abstract

Analysis of *Justicia ancelliana* essential oils by GC and GC/MS revealed the presence of β-phellandrene (51.2%), δ-2-carene (43.4%), dihydrotagetone (0.4%), propyl butyrate (0.3%) in the aerial parts and phenylacetaldehyde (39.2%), δ-3-carene (31.7%), 6-methyl-5-hepten-2-one (16.4%) and α-phellandrene (12.6%) in the roots. These oils and their compounds induced a reduction in the growth of the roots, the apparition of yellow leaves after two weeks of the treatment and the death of the *Vigna unguiculata* young plants and showed a specific action on the germination and the growth of *Zea mays*, *Vigna unguiculata* and *Arachis hypogeae* plants. β-Phellandrene and phenylacetaldehyde showed maximum inhibition to germination and growth of *Vigna unguiculata*.

Key Word Index

Justicia anselliana, essential oil composition, *Vigna unguiculata*, allelopathy.

Introduction

Vigna unguiculata (L.) Walp (Cowpea) is cultivated over more than nine million hectares in all the tropical area, in the Mediterranean basin and in the United States (1). Worldwide, it is estimated that 37 million tons are annually produced (2–10). Cowpea is a source of relatively low cost, high quality protein (11) which contained adequate levels of most essential amino acids for pre-school children and all essential amino acids for adults. Their digestibility is higher than that of other common legumes (12).

West and Central Africa annually produce about 26 million tons of cowpea on 7.8 million hectares, accounting for about 69% of the word production. Nigeria, Burkina Faso, Benin, Mali, Cameroon, Chad and Senegal are net exporters; Ghana, Togo, Côte d'Ivoire, Gabon and Mauritania are net importers (13). It is the most consumed leguminous: its seeds reduced into flour are used to make fritter, particularly in Benin and in some of the west African countries.

In south Benin, the Cowpea cultivation takes the lead on the economical level because of the economical guarantee it offers to farmers. Its production occupies 90% of the working population and was done during the drop in water level, on the grounds set free by the water (14).

In the Ouémé valley (south Benin) *Justicia anselliana* (Nees) T. Anders was identified as being very dangerous for cowpea. It belongs to the family of Acanthaceae and helps to grass over the grounds for a percentage of 12 to 18% (14). The almost glabrous, upright or drooping, herbaceous plant is 30 cm in height and can sometimes reach up to 60 cm (15).

According to the empirical observations described by farmers, this grass forms a very intimate association with cowpea and at an advanced stage of its development, it leads to the discoloration of its leaves. It thus puts an end to the cowpea development. Allelopathy was defined as the effect of one plant (including microorganisms) on the growth of another plant through the release of chemical compounds into the environment (16,17). A contribution to the characterization of allelopathic

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potential of *J. ansellianа* using some extracts from the dried material of the weed proved that alcoholic extracts produced more significant effects on growth parameters such as seedlings elongation and weight of the cowpea small plant (18).

To protect cowpea, it is important to know the substances inducing damages and to establish their relationship with the inhibition of the cowpea growth. As volatile compounds may diffuse and have allelopathic effects, we decided to identify the major compounds of the *Justicia anselliana* aerial parts and root oils and study their allelopathic effects on the cowpea growth by comparison with corn and groundnut.

Table I. Percentage composition of *Justicia anselliana* aerial parts and root oils

Compound	RI[a]	Aerial Parts Oil	Root Oil
propyl butyrate	909	0.3	-
6-methyl-5-hepten-2-one	990	-	16.4
δ-2-carene	998	43.4	-
α-phellandrene	1005	-	12.6
δ-3-carene	1010	-	31.7
β-phellandrene	1033	51.2	-
phenylacetaldehyde	1045	-	38.2
dihydrotagetone	1061	0.4	-
Total		95.3	98.9
Undentified		4.7	1.1

[a]RI = retention indices on DB-5 Column.

Experimental

Vegetable material: Aerial parts and roots of *Justicia anselliana* were collected in Ouémé valley (south R. of Bénin) and identified by the National Herbarium of the University of Abomey - Calavi (Republic of Benin). Seeds of cowpea (*Vigna unguiculata)* IT86D-719 variety, corn (*Zea mays*) KPM variety and groundnut (*Arachis hypogeae)* Glazoué variety were obtained from the International Institute of Tropical Agriculture (IITA) station of Benin.

Essential oil isolation and floral water preparation: Fresh aerial parts and roots were separately isolated by hydrodistillation for 3 h with a Clevenger-type apparatus (19). Oils were dissolved in xylene for their chemical analysis. The mixture of the oils and floral water (water that remains after producing an essential oil via steam distillation) was obtained by the steam distillation method.

Chemical analysis: Analysis of the oils was performed by GC-FID and GC/MS (20) and relative retention times on three GC columns (21).

GC-FID analysis: The gas phase chromatography analysis was firstly carried out on a DELS/IGC 121C equipped with a CP WAX 52 CB (25 m x 0.3 mm, df: 0.25μm) column, an injector (split/splitless) and a flame ionization detector (FID). The temperature of the injector and the detector were 240°C and 250°C, respectively. The temperature programming was as follows: isothermal at 50°C for 5 min followed by 2°C/min gradient until 220°C. Carrier gas was N with a flow rate of 1 mL/min under 0.8 bar pressure. The volume of oil injected was 0.5 μL. The GC was a Hewlett Packard 5890 equipped with an ionization detector (FID) and a split/splitless injector was secondly used for compound separation with a glass capillary column (30 m x 0.25 mm) coated with DB-5 phase thickness 0.25 μm or glass capillary column (30 m x 0.25 mm) coated with Supelcowax (0.20 μm film thickness). The conditions were the same.

GC/MS: The oils were analyzed on a Hewlett Packard gas chromatograph model 6890 coupled to a Hewlett-Packard MS Model 5873 equipped with an HP5 column (30 m x 0.25 mm, df: 0.25 μm) programmed from 50°C (5 min) to 300°C at 5°C/min, and 5 min hold. The carrier gas was He (1 mL/min), injection in split mode (1/10); injector and detector temperatures 250°C and 320°C, respectively. The MS ran in electron impact at 70 eV, electron multiplier 2200 V, ion source temperature 230°C. Mass spectral data were acquired in the scan mode in the *m/z* range 33–450.

The oil constituents were identified by matching their mass spectra and retention indices with reference libraries (21–27).

Allelopathic tests: Cowpea, groundnut and corn seeds were used in the tests. The seeds were surface sterilized with 2% sodium hypochlorite solution for 5 min and washed with distilled water three times and distributed onto sterile Petri-dishes (four seeds by each dish). The stratum was Whatman filter papers (N°1). To limit the activities of microorganisms, the filter papers were put into steam at 120°C for 45 min, after which they were retrieved and set out in sterile Petri-dishes (9 cm x 2 cm). A mixture of oils and floral water (5 mL) was used for each Petri-dish. For the selected components (β-phellandrene and phenylacetaldehyde) and pure essential oil (600 ppm concentration) bioassay, 3 mg of each compound and the root and aerial parts oils of *Justicia anselliana* were poured on to Whatman filter paper in Petri-dishes after which, 5 mL of water, which was used to prepare the mixture of oils and floral water, was added. The control Petri-dishes contained 5 mL of water. Each test was replicated eight times. All the Petri-dishes were closed, packed up in aluminium foil and kept in an incubator at 25° ± 1°C (28,29) for five and seven days according to the case.

To study the influence of the *J. anselliana* volatile components on the growth and height of the cowpea, seeds were divided into 2 groups of 20 pots (30) containing each 200 g of lay soil. To each pot in the first group, 50 mL of the oil/floral water mixture was added the first day, after which a further was added 25 mL every four days until the end of the observations. At the same time the pots of the second group, 20 pots used as controls were treated under the same conditions with the same water additions as the test group. Four seeds were put into each pot and four pots of each group were analyzed every five days.

The percentage of inhibition or stimulation of germination, emission of rootlets, length and fresh weight of the small plants have been calculated by considering the control as zero with the formula i-100% (with i the percentage of the parameter concerning the treatment, reported to the one of control).

Statistical analysis: The statistical analysis was realized with the software package Statistical Analysis Systems (SAS). The data were analysed by ANOVA (Analysis of variance). When the statistical revealed a significant difference at the doorway of 5%, the test of Newman Keuls was used to separate the averages (31).

Table II. Results of the germination of cowpea, groundnut and corn in water

Repetitions	Cowpea		Groundnut		Corn	
	Amount of germinated seed out of 25	Germination rate (%)	Amount of germed grains out of 25	Germination rate (%)	Amount of germed grains out of 25	Germination rate (%)
1st repetition	25	100	25	100	25	100
2nd repetition	25	100	25	100	25	100
Average	25	100	25	100	25	100

Table III. Effects of the oil and floral water mixture of the *Justicia. anselliana* on the development parameters of groundnut, corn and cowpea

	$Log(X_1+1)$	$Log(X_2+1)$	Average length of the small plant (cm)	Average wet weight of the small plant (g)
		Groundnut (Ar)		
EAr	0.70 ± 0.00 a	1.09 ± 0.13 a	5.03 ± 1.08 a	1.17 ± 0.08 a
EFAr	0.66 ± 0.08 a	1.11 ± 0.29 a	4.74 ± 1.29 a	1.16 ± 0.13 a
CV	12.77%	30.14%	31.51%	9.58%
		Corn (M)		
EM	0.69 ± 0.03 a	0.86 ± 0.03 a	25.34 ± 4.37 a	0.81 ± 0.11 a
EFM	0.62 ± 0.13 a	0.85 ± 0.04 a	23.95 ± 3.96 a	0.82 ± 0.09 a
CV	24.28%	4.74%	19.25%	13.22%
		Cowpea (N)		
EN	0.70 ± 0.00a	1.15 ± 0.05 a	12.32 ± 1.66 a	0.99 ± 0.05 a
EFN	0.70 ± 0.00a	1.21 ± 0.09 a	12.4 ± 1.19 a	0.99 ± 0.09 a
CV	25.68%	25.76%	22.23%	12.21%

X_1 : Average amount of germinated grains out of 4; X_2 : Average amount of rootlets emitted by small plants; The average followed by the same letter are not significantly different at the level of 5% according Newman-Keuls test; CV : coefficient of variation; E : water; EF : floral water.

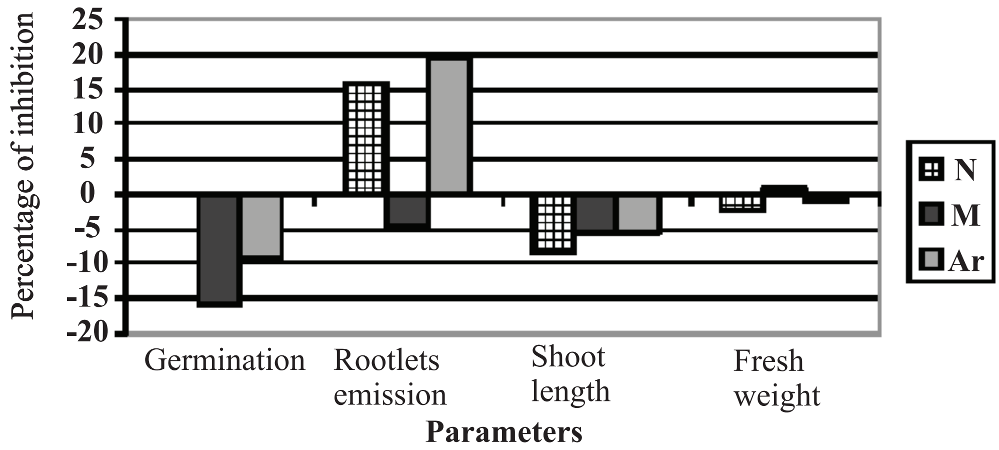
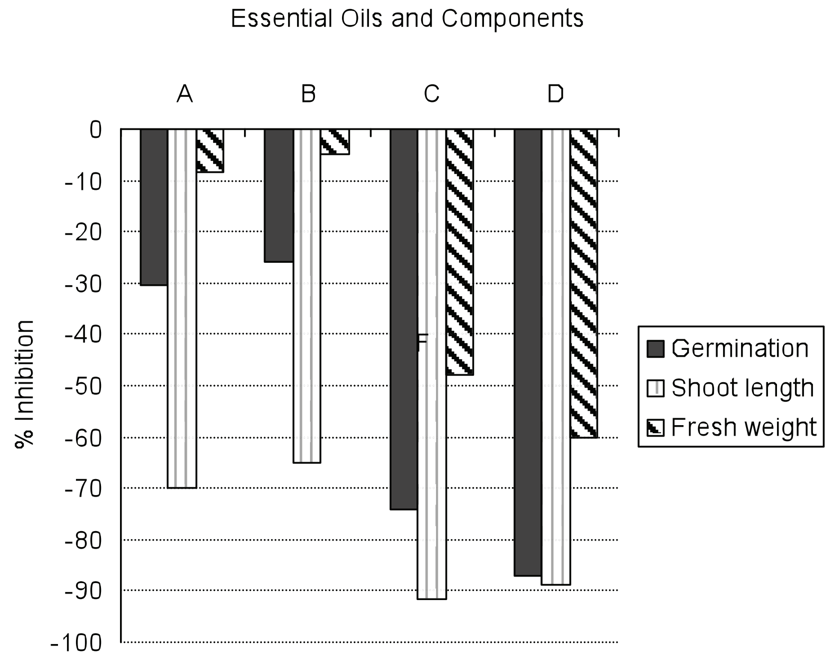


Figure 1. Effects of the mixture of oil and floral water on the cowpea (N), the corn (M) and the groundnut (Ar)



A: Aerial parts oil; B: Root oil; C: Phenylacetaldehyde; D: β-Phellandrene

Figure 2. Effect of oils and selected components (600 ppm) from *Justicia anselliana* on Cowpea *Vigna unguiculata* seedling growth

Plants of cowpea treated with the mixture of the essential oil and the floral water at left and with water at right.

Figure 3. Effects of the mixture of oil and floral water on growth of the cowpea aged 20 days

Results and Discussion

The oil from the aerial parts of *J. anselliana* (Table I) contained monoterpenes (94.6%) as major compounds: β-phellandrene (51.2%), δ-2-carene (43.4%), where as δ-3-carene (31.7%), α-phellandrene (12.6%) were the major monoterpenes in the root oil. It also contained phenylacetaldehyde (38.2%) and 6-methyl-5-hepten-2-one (16.4%). These compounds belong to chemical families known as that having allelopathic effects (16,18,32,33).

Justicia anselliana contained a low percentage of essential oils: 25 µL/100 g in the aerial parts and 20 µL/100 g in the roots.

The vitality test of all seeds used in different assays showed 100% germination (Table II) in comparison to 70% of recommended percentage germination (34).

The oil/floral water mixture, showed no effect on the cowpea seed germination (0.0%), 15.6% and 9.4% germination inhibition on corn and groundnut seeds, respectively (Figure 1). We observed a stimulating effect (14. 8%) on the emission of rootlets whereas it slightly inhibited the length (8.4%) and the fresh weight (1%) of the cowpea 5 day old plant (Figure 1). The same effects were observed at 15.7% on groundnut and at 4.8% on corn (Figure 1) by a specificity test, respectively. Globally , the effect of the volatiles *J. anselliana* compounds on small 5 day old cowpea, corn and groundnut plants was not net significant at the dose used (500 ppm) (Table III).

At the concentration of 600 ppm, the aerial parts and root oils of *J. anselliana* both inhibited the germination (30.6% and 26.0%), shoot length (70.1% and 65.1%) and the fresh weight of the 5 day old cowpea plants, respectively (Figure 2).

The bioassay of selected components revealed that β-phellandrene and phenylacetaldehyde showed maximum

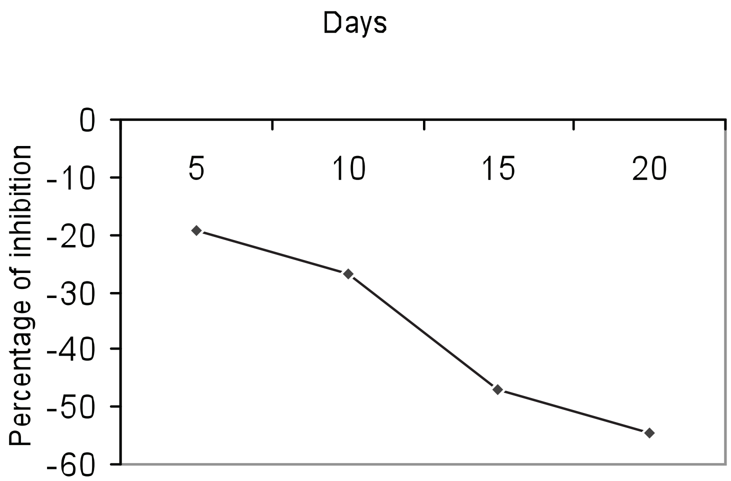


Figure 4. Inhibition of the root length of the cowpea between 0 and 20 days

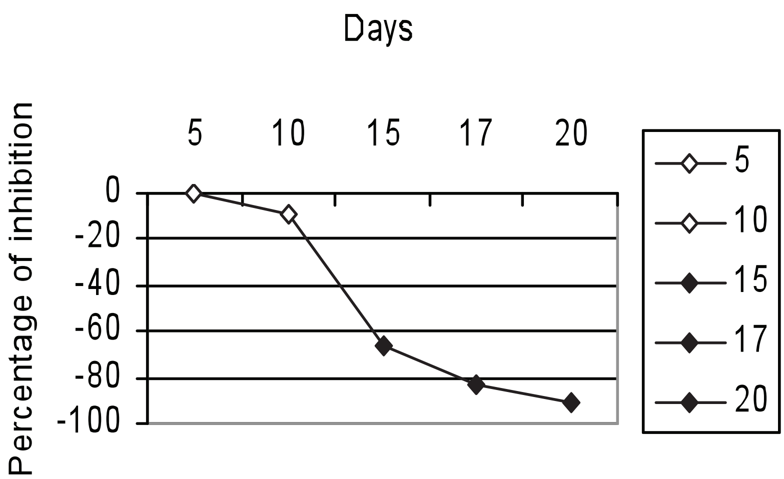


Figure 5. Inhibition of the emission of the cowpea leaves between 5 and 20 days
◆ = significantly different to the control.

inhibition to germination (87.0% and 74.0%), shoot length (89.0% and 91.6%) and fresh weight (60.2% and 48.0%) of 5 day old cowpea plants at the concentration of 600 ppm, respectively (Figure 2).

It was observed that the mixture of oil and floral water inhibited root development in cowpea plants (Figure 3) 19.2% (5th day), 26.8% (10th day) and 57.7% (20th day) (Figure 4). Inhibition of root development in cowpea plants by the mixture of oils and floral water became very significant in the second week of germination.

The mixture of oil and floral water caused a yellowing of the leaves of the cowpea plant after which they dried up and fell off (Figure 3). The effect on cowpea leaves was not evident on the fifth day, weak on the tenth day (8.9%) and became very intense (66.6%) on the fifteenth day, 82.5% on the seventeenth day and almost 100% by the twentieth day (Figure 5).

The inhibition of the root development in the cowpea plant during the second week was therefore accompanied in the same period with the inhibition of the leaf emission by the mixture of oil and floral water of *J. anselliana*.

These allelopathic effects are considered to be due to the terpenes (30,35,36) and the phenylacetaldehyde which constituted the major compounds of the oils of *J. anselliana* and revealed the maximum inhibition in Petri-dish allelopathic tests.

In conclusion, the aerial parts oil of *Justicia anselliana* contained β-phellandrene (51.2%), δ-2-carene (43.4%) and δ-3-carene (31.7%), α-phellandrene (12.6%) and phenylacetaldehyde (38.2%) in the roots. These oils and components were found to have a toxic effect on the growth of the Cowpea plant which translated in to a reduction in development of the roots and the yellowing of the leaves of the Cowpea from the second week onwards.

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