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Full Length Research Paper

# Identification of 1-decyne as a new volatile allelechemical in baobab (*Adansonia digitata*) from Sudan

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Leaf, fruit, wood, and gum of fifty-five plants collected in Sudan were evaluated by Dish pack method for their allelopathic activity through volatile chemicals using lettuce (*Lactuca sativa*) as a receptor plant. Several potential plants with high allelopathic activity, such as *Terminalia brownie, Euphorbia hirta, Diospyros mespiliformis, Corchorus olitorius, Adansonia digitata, Hibiscus sabdariffa,* were determined. Baobab (*A. digitata*) leaves demonstrated relatively higher inhibition (23.9 and 21.5% of hypocotyl and radicle, respectively) than most of the screened plant species. Identification of the volatile compounds using headspace gas chromatography-mass spectrometry revealed 1-decyne as the main volatile compound naturally released from dried baobab leaves. EC<sub>50</sub> (50% growth inhibition) of radicle and hypocotyl growth of lettuce seedlings by authentic 1-decyne was determined in the headspace air using by GC-MS with Cotton Swab method at the concentration of 0.5 ng/ml. The obtained results could fully explain the plant growth inhibitory activity of baobab volatiles by the presence of 1-decyne. Thus, in this study, we first identified 1-decyne as a new volatile allelochemical from baobab leaves. 1-decyne might be an important allelochemical for the survival of baobab in Africa and our findings may also offer a potential for future development of new volatile plant growth modulator.

Key words: Adansonia digitata, baobab, 1-decyne, allelopathy, volatile, GC-MS, Sudan.

#### INTRODUCTION

Recent intensification of cropping and rapid conversion of vast areas into intensive agricultural production has led to serious weed problems in all areas in Sudan (Hamada, 2000; Mahgoub, 2014). Another problem of the last decades is rapidly evolving resistance of weeds, for example, *Cyperus rotundus* (Nagulendran et al., 2007) and *Panicum turgidum* (Williams and Farias, 1972), to a

number of herbicides (Green and Owen, 2011). As an alternative to synthetic herbicides, allelopathy offers the potential for bio-rational weed control through the production and release of allelochemicals from plant materials (Weston, 1996) along with using allelochemicals as growth regulators, insecticides, and antimicrobial crop protection products (Cheng and Cheng, 2015).

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Screening of plants is the first essential step in the identification of the allelopathic potential of plant material followed by allelochemicals identification, laboratory bioassays, and field tasting (Cheng and Cheng, 2015). Previous research on the allelopathic activity of African plants has reported about trimethyl allo-hydroxycitrate and β-sitosterol from *H. sabdariffa* (Piyatida et al., 2013; Suwitchayanon et al., 2015) and phenolic antioxidants from C. olitorius leaves (Azuma et al., 1999). Aqueous and methanol extracts of Capparis spinosa and Cleome arabica reduced mitotic index triggered oxidative damage and caused the disruption in membrane permeability (Ladhari et al., 2014). The study of the phytotoxic activity of Vachellia sieberiana, Albizia adianthifolia, Buddleja saligna, Combretum kraussii, Halleria lucida, and Rapanea melanophloeos from South Africa showed significant inhibition germination, chlorophyll in accumulation, and growth of lettuce (Sunmonu and Van Staden, 2014). Despite some studies, African plants remain poorly studied as a source of allelopathic compounds that can be used directly in weed control or be a source for new non-synthetic herbicides. Therefore, the objectives of our study were to evaluate the allelopathic potential of Sudanese plants and to identify volatile allelochemicals from baobab leaves.

### **MATERIALS AND METHODS**

## Collection of plant samples

Plant material (fruit, stem, leaves) of 55 species was collected from different places of Sudan (Sinnar, Kassala, Elgezira, Algedaref, and Kordofan) in August 2015 and from the international botanical garden in Khartoum, Sudan in September 2016. All plant species were identified in the international botanical garden (Khartoum, Sudan). The leaves were dried at 60°C for 24 hrs and stored at room temperature.

# Dish pack method

Dish pack method was used to determine allelopathic activity through volatile compounds emitted from plant material (Fujii et al., 2005). Briefly, 200 mg of dried leaves were placed into one of the holes in multi-dish with 6 holes, except control. Filter papers (33 mm) were placed and 0.7 ml of distilled water was added to another five dishes. Seven seeds of lettuce (*Lactuca sativa*), variety great Lakes 366 (Takii seed Co., Japan) were placed on the filter paper. The multi-dish plates were sealed and incubated at 22°C under the dark condition. After 3 days the length of radicle and hypocotyl of lettuce seedlings was measured.

# Gas chromatography-mass spectrometry (GC-MS)

1 g of baobab leaves was placed in 20 ml glass vial and sealed. Headspace air was collected after 24 and 48 hrs of incubation at room temperature. The composition of volatile compounds was detected by GC-MS-QP 2010 plus system (Shimadzu, Japan) equipped with EQUITY-5 column  $(0.25 \times 30 \times 0.25 \text{ mm})$  with helium gas as a carrier. The oven temperature was increased from 50 to

200°C at a rate of 10°C/min. The injection was set to split modes. Mass spectra were recorded at 70 eV with a mass range of m/z 50 to 400, compared to a mass spectral library (NIST and Wiley), and confirmed against spectra of authentic 1-decyne, 1-nonyne, and 1-undecyne (Tokyo Chemical Industry, Japan).

### Cotton swab method

To determine the inhibitory activity of authentic 1-decyne, cotton swab method (Mishyna et al., 2015) was used. Agar solution was prepared at the concentration of 0.75% w/v and sterilized in an autoclave at 115°C for 15min. 10 ml of agar were added to each glass vial and five lettuce seeds were placed on solidified agar. The prepared cotton swab was inserted in the agar and 1-decyne solution at different volumes (0.5, 1, 10, 20, 50 and 100  $\mu$ l) was added. To identify EC $_{50}$  of volatiles from baobab leaves, 500, 700, and 900 mg of plant material were added instead of the cotton swab. Measurement of the length of radicle and hypocotyl of lettuce seedlings were done after three days of incubation at 22°C. All treatments were replicated three times and repeated twice.

### Statistical analysis

ANOVA test (level of significance is P<0.05) was used to evaluate the effect of volatiles on the growth of radicles and hypocotyl of tested plants.

## **RESULTS AND DISCUSSIONS**

# Screening of allelopathic activity of plant volatiles

Table 1 shows the results of testing of allelopathic activity through volatile compounds of 55 plants from Sudan. Hypocotyl growth of the lettuce seedlings was inhibited from 0.4% (leaves of *Xanthium brasillicum*) to 30.5% (wood of *Terminalia brownii*) compared to control. Top 5 plants with the strongest allelopathic activity (21 - 30.5% and 19.4 - 38.7% for hypocotyl and radicle, respectively) was identified including *T. brownie*, *E. hirta* (leaves), *D. mespiliformis* (leaves), *A. digitata* (leaves), and *C. olitorius* (leaves).

The volatiles from shaf (T. brownii) wood showed the strongest inhibition of the hypocotyl growth (30.5%) of lettuce seedlings in this study. It has been previously reported that shaf contains antimycobacterial compounds of diverse polarities and can be used for tuberculosis treatment (Salih et al., 2018). Mbiri et al. (2016) demonstrated an antinociceptive activity of the barks of shaf and rationalize the traditional use of the barks in the management of pain. The volatiles from *E. hirta* leaves showed secondary strongest inhibition (27.8 and 38.7% of hypocotyl and radicle growth of lettuce, respectively). Very little is known about the allelopathic potential of volatiles of E. hirta, but the analysis of essential oil from dried leaves E. hirta demonstrated major compounds such as 3,7,11,15-tetramethyl-2-hexadecen-1-ol, 6,10,14trimethyl-2-pentadecanone, hexadecanal, phytol, and nhexadecanoic acid and its potential for medication of asthma (Ogunlesi et al., 2009). Volatiles from gogan (D.

 Table 1. Assessment of allelopathic activity of plant volatiles from Sudanese plants.

N	Family name	Local name in Sudan	Scientific name	Parts used	Inhibition (%)	
					Hypocotyl	Radicle
	Mimosaceae	Shaf	Terminalia brownii	wood	30.5	24.1
<u> </u>	Euphorbiaceae	(Euphorbia)	Euphorbia hirta	leaf	27.8	38.7
}	Ebenaceae	Gogan	Diospyros mespiliformis	leaf	27.4	19.4
	Malvaceae	Baobab	Adansonia digitata	leaf	23.9	21.5
,	Malvaceae	Nalta Jute	Corchorus olitorius	leaf	21.0	24.8
6	Malvaceae	Karkade	Hibiscus sabdariffa	fruit	20.1	29.6
,	Lythraceae	Henna	Lawsonia inermis	leaf	18.2	13.8
3	Aristolochiaceae	Um Galagel	Aristolochia bracteolate	leaf	17.8	11.0
)	Poaceae	Ankoj	Ischaemum afrum	leaf	17.4	18.2
0	Cyperaceae	Cyperus	Cyperus rotundus	leaf	17.4	15.9
1	Poaceae	Allambri	Abutilon figarianium	leaf	16.7	15.1
2	Euphorbiaceae	Tabar	Ipomoea cardiosepala	leaf	16.0	12.6
3	Malvaceae	Guddaim	Grewia tenax	fruit	16.0	4.26
4	Lythraceae	Henna	Lawsonia inermis	leaf powder	15.7	20.6
5	Amaranthaceae	Doneb	Chenopodium ambrosioides	leaf	14.6	13.8
6	Convolvulaceae	Dareyah	Merremia emarginate	leaf	14.6	9.43
7	Aizoaceae	Rabah	Trianthema portulacastrum	leaf	14.2	17.5
8	Malvaceae	Guddiam	Grewia tenax	leaf	14.1	-0.5
		Henna	Lawsonia inermis		13.8	2.8
9	Lythraceae			leaf powder		
20	Fabaceae	Pohoniya	Ceratonia siliqua	leaf	13.7	19.8
!1	Fabaceae	Arad	Albizzia sericocepala	leaf	13.7	17.9
2	Poaceae	Shleyma	Phragmites australes	leaf	13.5	20.8
3	Euphorbiaceae	(Euphorbia)	Euphorbia buraspastoris	leaf	13.5	13.1
4	Malvaceae	Baobab	Adansonia digitata	fruit powder	13.2	17.0
25	Fabaceae	Senna Mekka	Cassia senna	fruit	12.9	16.4
26	Lamiaceae	Rehan	Ocimum basilicum	leaf	12.7	23.6
27	Zygophyllaceae	Hegleg	Balanites aegyptiaca	leaf	12.3	16.0
8.	Poaceae	Taman	Panicum turgidum	leaf	11.9	16.6
9	Acanthaceae	Tgtag	Ruellia patula	leaf	11.4	20.1
0	Fabaceae	Senna Mekka	Cassia senna	leaf	11.2	3.8
1	Poaceae	(Johnson grass)	Sorghum halepense	leaf	11.0	24.9
2	Asteroideae	Sheeh	Artemisia seiberi	leaf	10.7	13.7
3	Mimosaceae	Hashab	Acacia senegal	gum	10.4	18.1
4	Mimosaceae	Shaf	Terminala bornii	leaf	10.3	18.6
35	Poaceae	(Millet)	Pennisetum glaucum	leaf	9.3	1.8
6	Mimosaceae	Kiter	Acacia melofera	leaf	7.7	3.5
7	Mimosaceae	Telih	Acacia seyal	leaf	7.3	2.9
8	Poaceae	Durra	Sorghum bicolor	leaf	7.2	19.3
9	Orobanchaceae	Buda	Striga hermonthica	leaf	6.8	22.3
0	Mimosaceae	Hashab	Acaica senegal	gum	6.0	7.4
1	Mimosaceae	Hashab	Acacia senegal	leaf	5.6	0.6
2	Asteraceae	Moleta	Sonchus comutas	leaf	5.3	13.4
.3	Arecaceae	Doum Palm	Hyphaena thebaica	leaf	5.2	3.6
4		Karkade	Hibiscus sabdariffa	leaf	4.5	6.7
	Malvaceae					
5	Malvaceae	Guddiam	Grewia tenax	leaf	4.3	-0.8
6	Malvaceae	Baobab	Adansonia digitata	leaf	4.1	16.8
7	Rhamnaceae	Sidr	Ziziphus spinachristi	leaf	4.1	9.22
8	Leguminosae	Alsurib	Phyllanthus maderaspatensis	leaf	3.9	14.3
.9	Fabaceae	(Peanut)	Arachis hypogaea	leaf	3.0	11.3
0	Fabaceae	Aradeeb	Tamarindus indica	leaf	3.0	5.9
1	Asteraceae	Rantook	Xanthium brasillicum	leaf	0.4	13.8
2	Zygophyllales	Dresa	Tirbulus terrestris	leaf	0	-3.8
3	Poaceae	Um Koaat	Brachiaria eruciformis	leaf	-1.1	7.9
54	Salvadoraceae	Arak	Salvadora peresica	leaf	-4.3	4.4
5	Pedaliaceae	(Sesame)	Sesamum indicum	leaf	-5.2	4.3

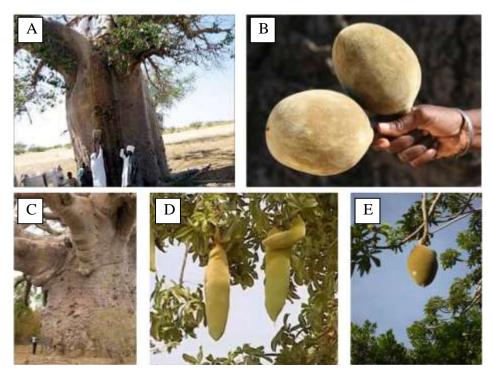


Figure 1. Baobab tree (a, c) and fruits (b, d, e) in Sudan.

mespiliformis) inhibited the growth of hypocotyl and radicle of lettuce seedlings by 27.4 and 19.4% respectively. Leaf volatiles from C. olitorius showed the relatively close inhibition of hypocotyl growth to the results observed for baobab leaf volatiles (21 and 23.9%, respectively). Essential oil from flowers and leaf of C. olitorius was previously analyzed by gas chromatography/mass spectrometry and main components benzaldehvde (56%),methyl methoxysalicylate (6.55%) and carvacrol (4.75%) were identified (Driss et al., 2015), however there is no information about allelopathic potential of *C. olitorius*.

Fruit volatiles of kardade (H. sabdariffa) suppressed the radicle and hypocotyl growth of lettuce by 29.6 and 20.1%, respectively. (Farag et al., 2015) previously identified 104 volatiles from hibiscus flower aroma using solid-phase microextraction coupled to GC-MS. Moreover, it has been previously reported the presence of two allelochemicals, trimethyl allo-hydroxycitrate and β-sitosterol, in hibiscus fruits (Piyatida et al., 2013). Despite not much is known about allelopathy of hibiscus though volatile compounds (Piyatida and Kato-Noguchi, 2011) and (Piyatida et al., 2013) isolated and identified active substance β-sitosterol with I<sub>50</sub> values of 16.2 and 406.7 µM for cress and lettuce, respectively.

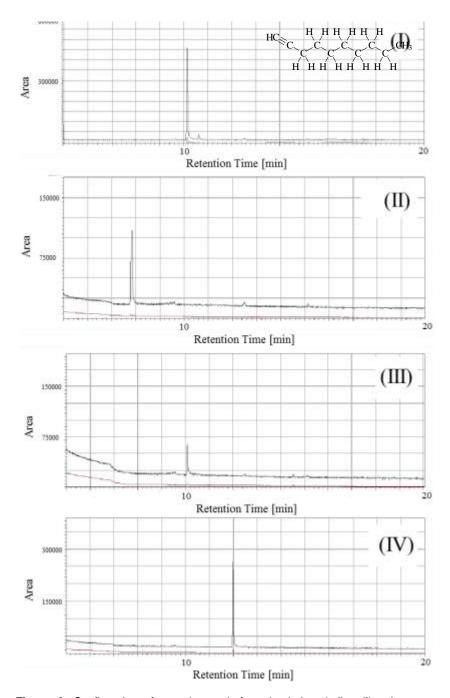
## Allelopathic activity of volatiles from baobab leaves

Volatiles from baobab (A. digitata) leaves inhibited 23.9%

of hypocotyl growth and 21.5% of radicle growth. Plant growth inhibitory activity by baobab through volatile compounds was not previously studied, but it was reported that baobab root extracts may inhibit the germination and seedling growth of *L. sativa*, *H. sabdariffa*, and *S. bicolor* (Suleiman and Banagab, 2016).

Baobab is an indigenous tree of Malvaceae family growing in the arid and semi-arid areas of Africa (Cissé et al., 2013; Rahul et al., 2015) as a solitary plant or occasionally in groups (Gebauer et al., 2016) (Figure 1). In Sudan, baobab is mainly occurring in the southern area, which can be considered as the extreme northern part of the East African distributional range of the species (El Amin, 1990; Gebauer et al., 2016). Baobab was also introduced in India and Sri Lanka centuries ago by Moslem traders, and into Asian and North American countries as an ornamental plant (Sidibe et al., 2002; Rahul et al., 2015). Since ancient time baobab has been widely utilized by indigenous people for medicinal and nutritional purposes (Kamatou et al., 2011; Cissé Ibrahima et al., 2013; Coe et al., 2013 ); leaves and seeds are used for soups, while locals prepare a drink from baobab pulps dissolving them in water or milk (Becker, 1983; Sidibe et al., 2002).

While there is limited information on volatile allelochemicals for all screened plants and some of them can be considered as the promising source of allelopathic compounds, in this research we focused on the identification of volatile compounds that caused allelopathic activity from baobab leaves due to their

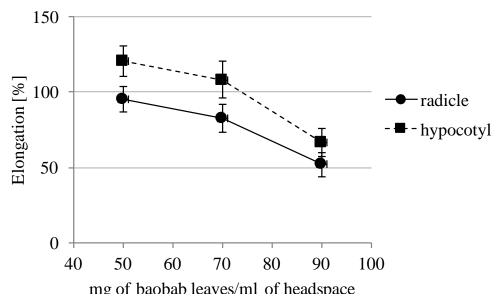


**Figure 2.** Confirmation of a major peak from baobab volatiles (I) using pure standard compounds: 1-nonyne (II), 1-decyne (III) and 1-undecyne (IV).

availability for locals and high biomass.

# Identification of volatile allelochemicals from baobab leaves

Headspace GC-MS analysis of volatiles naturally emitted from baobab leaves showed the presence of one major peak with retention time of 10.00 min, corresponding to 1decyne from in-house library. The compound was confirmed by using an authentic 1-decyne in comparison with 1-undecyne and 1-nonyne, which revealed a retention time of 11.99 and 7.84 min, respectively. The chromatograms of 1-decyne, 1-undecyne, and 1-nonyne are displayed in Figure 2. The concentration of 1-decyne in the headspace (0.43 ng/ml) was calculated based on the calibration curve of authentic 1-decyne and the peak area of 1-decyne emitted from baobab leaves.



**Figure 3.** Effect of volatiles from baobab leaves on the radicle and hypocotyl growth of lettuce seedlings.

1-Decyne is an alkyne hydrocarbon with the chemical formula C<sub>10</sub>H<sub>18</sub>. This compound has been previously identified in the essential oil of A. racemosa leaf which showed the larvicidal activity against three mosquito species, Aedes aegypti, Anopheles stephensi, and Culex quinquefasciatus (Arun et al., 2015). (Ahmad et al., 2016) reported 1-decyne as one of 123 components of essential oil Rumex possess from hastatus which anticholinesterase and antioxidant potentials. GC-MS analysis of methanol extract of flowers of Viola odorata showed the presence of 1-decyne (Jasim et al., 2018). Moreover, 1-decyne was reported as one of the major volatile from photo-oxidized cottonseed oil (Fan et al., 1983) volatile 1-decyne was previously also determined in the defensive secretions of male and female of Pyrrhocoris tibialis and Scantlus aegyptlus (Krajicek et al., 2016). Despite the fact that 1-decyne has been previously identified as natural chemicals from plants, there was no report on the plant growth inhibitory activity by 1-decyne.

# A contribution of 1-decyne into the total inhibitory activity of volatiles from baobab

Naturally released volatiles from dried leaves of baobab significantly inhibited growth of radicle and hypocotyl of lettuce seedlings at  $EC_{50} > 90$  mg of baobab leaves/ml of headspace (Figure 3). The plant growth inhibitory activity of authentic 1-decyne in headspace was expressed as  $EC_{50}$  value (50% of radicle or hypocotyl growth inhibition) and was detected at  $\approx 0.5$  ng of 1-decyne/ml of headspace (Figure 4). Based on it the contribution of 1-

decyne released from baobab leaves into the total inhibitory activity of leave volatiles in shown in Figure 5 and demonstrates that 1-decyne is responsible for the total allelopathic activity. 1-Decyne is a compound with the triple bond (Figure 2), and it is well known that *cis*-dehydromatricaria ester, an allelochemical reported from *Solidago* and *Erigeron* spp, also contains triple bond in their structure.

Our results demonstrated stronger inhibitory activity of 1-decyne compared to the previously published EC $_{50}$  value for volatile allelochemicals octanal and safranal obtained by the same method. So, it was previously reported that *Heracleum sosnowskyi* fruits emit octanal at concentration 20  $\text{ng/cm}^3$  (Mishyna et al., 2015), while EC $_{50}$  of safranal, an allelochemical from *C. sativus* is 1 and 2  $\mu$ g/l for lettuce radicle and hypocotyl, respectively (Mardani et al., 2015). Moreover, allyl isothiocyanate and methyl isothiocyanate, the major volatiles released form chopped plants as allelopathic green manure crops, completely inhibited the germination of all tested species at a headspace gas concentration of 1 ppm (Vaughn and Boydston, 1997).

## Conclusion

Evaluation of allelopathic activity through volatile compounds of 55 plants from Sudan revealed several potential plants with high plant growth inhibitory activity (*T. brownie, E. hirta, D. mespiliformis, A. digitata, C. olitorius*). Allelopathic activity of volatiles emitted from baobab leaves can be explained by the presence of 1-decyne, a newly determined volatile allelochemical. 1-

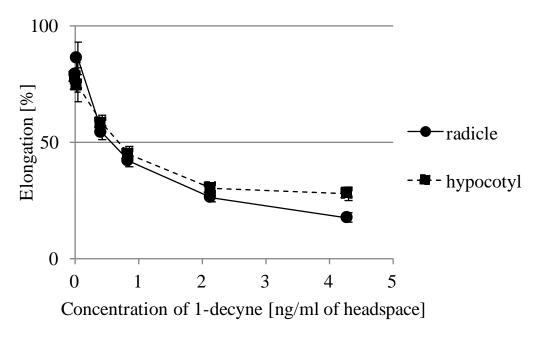


Figure 4. Effect of authentic 1-decyne on the radicle and hypocotyl growth of lettuce seedlings.

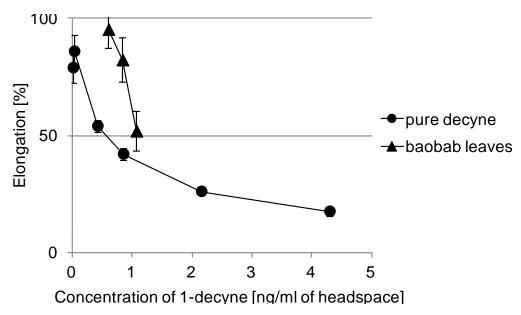


Figure 5. Contribution of 1-decyne to the radicle inhibition of lettuce seedlings by volatiles from baobab leaves.

decyne could be an important allelochemical for the survival of baobab in Africa and may offer a potential for future development of new volatile plant growth modulator.

# **CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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