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ASSESSMENT OF *LIPPIA ADOENSIS* HOCHST. VAR. KOSERET, *ROSMARINUS OFFICINALIS* L. AND *RUTA CHALEPENSIS* L. ESSENTIAL OILS AS A POTENTIAL SOURCE OF FUNGITOXIC AND MYCOSPORICIDAL ACTIVITY AGAINST TOXIGENIC *ASPERGILLUS* SPECIES

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Abstract

The objective of the current study was conducted to explore antimycotic activity of 3 odoriferous angiospermic plants (*Lippia adoensis Hochst. var. koseret, Rosmarinus officinalis and Ruta chalepensis*). Essential oils of *Lippia adoensis* Hochst. var. koseret, *Rosmarinus officinalis* and *Ruta chalepensis* were tested against toxigenic strain of *Aspergillus* species isolated from food commodities. The oil of *L. adoensis* showed highest antifungal efficacy. The *L. adoensis* oil absolutely inhibited the mycelial growth and spore germination of *A. flavus, A. fumigatus, A. niger* and *A. parasiticus* at a concentration of 2µl/ml. *L. adoensis* essential oil as antifungal was found superior over chemical synthetic preservative. The present study revealed that *L. adoensis* essential oil can be a promising source of antifungal drugs against post harvest infection of food commodities by *Aspergillus* species. For large scale utilization further studies are recommended to explore its activity on other toxigenic fungal species, mechanism of action and chronic toxicity of the oil.

Keywords: *Aspergillus species*, Essential oils, food spoilage, mycotoxin, Antimycotic activity, Sporicidal activity

Introduction

The genus *Aspergillus*, which includes about 350 species, are kind of fungi important in public health as human and animal pathogens, as toxin-producing food contaminants [1, 2]. They are major threat to human beings worldwide since they are a common contaminant of indoor and outdoor environment [3]. They produce and release millions of spores small enough to be found in air, water, soil, plant debris, rotten vegetation, manure, sawdust waste, bagasse waste, animal feed, on animals and indoor air environment [4]. Humans are frequently exposed to *Aspergillus* and which can lead to life-threatening infections when immunocompromised [4]. Over 95% of the infections are caused by *Aspergillus fumigatus, Aspergillus flavus,* and *Aspergillus niger* [5]. The clinical manifestations and severity of *Aspergillus* species depend upon the host immune status and degree of exposure to Aspergillus spore [1].

From the time when first described 300 years ago the genus *Aspergillus* is an important genus in foods, from the point of view of spoilage or biodeterioration than other fungi [6]. Even currently, in the era of quite technological advancement in food industries, *Aspergillus* food spoilage are major problem of food and feedstuffs during storage [7]. They are extremely common in stored commodities such as grains, nuts and spices, and occur more frequently in tropical and subtropical than in temperate climate [6]. Food contamination of food commodities occurs at almost all stages; starting from field level through storage of raw products, and subsequent transport and trade causing considerable economic losses annually [7]. *Aspergillus* are able to grow on almost all kinds of food: cereals, meat, milk, fruit, vegetables, nuts, fats and products of these [4]. Food spoilage is responsible for 25% of economic losses worldwide and 30% of the global population suffers from food-borne disease each year [1, 8-10]. During their lifecycle, over 40 species of *Aspergillus* have been listed as capable of producing a wide range of mycotoxins harmful to humans and animals that consume them [11], but the *Aspergillus* mycotoxins of greatest public health and agro-economic significance are aflatoxins, ochratoxin A, sterigmatocystin, and cyclopiazonic acid [11]. Aflatoxin has carcinogenic, teratogenic, hepatotoxic, mutagenic, and immunosuppressive properties [11]. Ochratoxin A, which is a nephrotoxin, teratogen, and carcinogen while, Citrinin affect kidney function [11].

Human exposure to mycotoxins may result from consumption of foods commodities that are contaminated with toxins [12]. Consumption of foods containing mycotoxins causes mycotoxicosis [11]. Furthermore, mycotoxins are responsible for generating huge economic losses in the producing countries [13], as 25–40% of cereals consumed in the world are contaminated by these toxic compounds [14]. So far, synthetic preservatives has been used to prevent/control growth of fungi, including *Aspergillus* species, and to control natural spoilage processes (food preservation [15]. However, the application of synthetic preservatives has led to a number of environmental and health problems because they are themselves carcinogenic, teratogenic, and highly toxic with long degradation periods [16]. In addition its indiscriminate use has led to a number of ecological and medical problems due to residual toxicity, hormonal imbalance and spermatotoxicity, etc. [16]. Another problem with the use of synthetic preservatives is that up on the consumption of food containing these substances some individuals produce allergic reactions to these substances may result in adverse reactions [17].

Current consumer opinion suggests a desire for high quality foods that are more natural, minimally processed and preservative free, while remaining safe and with an extended shelf life. Continuous search mechanisms for safe and effective preservatives from natural products that have antifungal and antimycotoxigenic activity are necessitated. One possible source of new drugs is natural product especially essential oils and/or their constituents [18]. Since they seldom accumulate in the ecosystem, they do not lead to development of resistance as a result it could potentially substitute chemical preservative [19, 20]. Essential oils (EOs) are known for their bactericidal, virucidal and fungicidal, and their fragrance, they are used in the preservation of foods [21]. So, EOs with antimicrobial activity are possible candidates for using as natural antimicrobial preservatives to controlling microbial food contaminations, which might replace synthetic fungicide [22]. One of the traditional practices that kept till today in Ethiopia is the use of aromatic plants as aroma-treatment of food commodities and skin diseases [23]. According to our knowledge, fungitoxic and sporicidal activity of essential oils against toxigenic fungi was not investigated and therefore the present study was designed to investigate the fungitoxic activity of essential oils against the toxigenic strain of Aspergillus species. In addition, the essential oil of *Lippia adoensis Hochst. var. koseret* has been investigated regarding its potential to inhibit spore germination production of

Aspergillus species.

Material and method

Test organisms

Aflatoxigenic strain of *Aspergillus flavus* and *Aspergillus parasiticus* were chosen for the present study. The strain was isolated from food commodities in Addis Ababa prior to this study. Some other moulds viz. *Aspergillus niger* and *Aspergillus fumigatus* (isolates from food commodities), and standard organisms (*A. niger* ATCC and *A. flavus* ATCC). Cultures of all test organisms were maintained on potato dextrose agar (PDA) medium.

Plant material collection, identification, and extraction of essential oils

Lippia adoensis Hochst. var. koseret (leaves collected from Sibu-Sire district, East Wellega, Ethiopia), *Rosmarinus officinalis* (aerial part from the botanical garden of TMMRD, EHNRI, Ethiopia) and *Ruta chalepensis* (fruit bought from local market of Addis Ababa from the merchant of Alemgena, Ethiopia) were selected for essential oil extraction. The plants were identified by Taxonomist and Botanist in Traditional and Modern Drug Research Department of the Ethiopian Public Health Institute (EPHI). Plant materials of each plant species was thoroughly washed with 1% Sodium hypochlorite (NaOCl) followed by distilled water. Areal parts of *R. officinalis*; leaves of *L. adoensis Hochst. var. koseret* and fruits of *R. chalepensis* were used for the extraction of essential oils. Fresh plant materials (250 g) were placed in a 5 L round-bottom distillation flask and the plant material was wetted with 3 L distilled water. The essential oils were obtained by hydrodistillation using Clevenger-type apparatus for continuous 3 h. The volatile oil was taken from the upper layer. The excess aqueous layers were further portioned using dichloromethane extract and enrich the essential oil from the water layer. The organic layer (dichloromethane extract) was filtered and dried with anhydrous sodium sulfate and concentrated using rotary evaporator to give the crude essential oil.

Antifungal screening of essential oils

The fungitoxicity of essential oils were tested against *Aspergillus* species following poisoned food technique [24], using potato dextrose agar as nutrient medium. Five-day old fungal cultures were punched aseptically with a sterile cork borer of generally 5mm diameter.

The fungal discs were then put on the gelled agar plate. The agar plates were prepared by impregnating desired concentration of essential oils (2µl/ml) at a temperature of 45 - 50˚C. The plates are then incubated at temperature 26 ± 2˚C for fungi.

Colony diameter was recorded by measuring the two opposite circumference of the colony growth. Percentage inhibition of mycelial growth is evaluated by comparing the colony diameter of poisoned plate (with plant essential oils) and non-poisoned plate (with distilled water) and calculated using the formula given below [24]:

$$\% \text{ MI} = \frac{\text{Mgc} - \text{Mgt X 100}}{\text{Mgc}}$$

Where: Mgc is mycelial growth in control
Mgt is mycelia growth in test

Impact of essential oils on Aspergillus spore production and spore germination

Sporicidal activity was conducted using spore germination assay on *L. adoensis Hochst. var. koseret*, that have showed better fungicidal activity by using the above screening program [24]. Desired concentration and volume of the essential oil were tested for their potential of spore germination inhibition on tested *Aspergillus* species. The test organisms were grown on PDA medium for sporulation and spores were harvested when the cultures were fully sporulated; which was achieved after 10days of incubation. Spores were collected by adding 5 ml of sterile water containing 0.1% (V/V) Tween 80 (for better spore separation) to each Petri dish and rubbing the surface with a sterile L-shaped spreader (3 times). The suspension was collected and then centrifuged at room temperature at 2000 rpm for 5 min. The supernatant was discarded and re-centrifuged until 1ml of highly concentrated spore solution remained. A haemocytometer slide was used to count spore production to haveapproximately 10^8spore/ml [25].

Various concentrations: 0.25µl/ml, 0.5µl/ml, 1µl/ml, 2µl/ml, 4µl/ml and 8µl/ml of *L. adoensis Hochst. var. koseret* essential oils was prepared in 5ml of sabouraud dextrose broth in 100ml flask and then 1ml of the spore suspension were added to each flask. The flasks were then incubated for 24 h at 25˚C on a rotary shaker (60 rpm) as to evenly disperse the oil throughout the broth. At the end of the incubation period, germinated spores were observed using a light microscope at 400X magnification. Experiment was performed in triplicate and the extent of spore germination was assessed by looking

for the presence of germ tubes. Results were expressed in terms of the percentage of spores germinated as compared to the control from the average of the triplicates. Percentage spore germination inhibition is calculated according to the following formula:

$$\% \text{ Spore Germination ihnibition} = \frac{(sc - st)}{(sc)} X\ 100$$

Where: sc, average number of spore germinated in control set; st, average number of spore germinated in test set.

Minimum inhibitory concentration of essential oils

The minimum inhibitory concentrations (MIC) were evaluated by the agar dilution methods [24, 26]. Twofold serial dilution of each EOs in Sabouraud dextrose agar was made by adding two milliliter of each dilution of the desired concentrations of EOs into each 18 milliliter of agar in a test tube which was well mixed and poured in to 90mm Petri dish. As the concentration of essential oils was diluted 1:10 in media the pre stock solutions of EOs should be made in ten times the required final concentration to be tested. The experiments were performed in triplicates. The agar was permitted to solidify in the plates on a level surface at room temperature. Control plates, containing no essential oils were run simultaneously. The agar surface of the plates containing the dilution of EOs and the control plate are inoculated five millimeter discs of the test fungi taken from advancing edge of 7-day-old cultures. The plate containing the lowest concentration of EOs was seeded first. Control plates were seeded last to insure that viable organisms were present throughout the procedure. Incubate the inoculated plates at 26±2^{O}C for seven days before being read. End-points for each EOs are best determined by placing plates on a dark background and observing for the lowest concentration that inhibits visible growth, which is recorded as the MIC. The MIC of each antimicrobial agent is usually recorded in micro liter per milliliter.

Statistical analysis

All the measurements were replicated three times for each treatment and data were entered into excel spreadsheet and are presented as mean ± SE/SD. One way analysis of variance (ANOVA) followed by Dunnett's test was used for statistical evaluation. P-values less than 0.05 ($p<0.05$) were considered significant.

Results

Yields of crude essential oils extracts

Different parts of six species of plants belonging to five different families were extracted by hydrodistillation for 3h using a Clevenger type apparatus. The hydrodistillation of *R. chalepensis L.* fruits yielded light green colored oils with the highest percentage yield of essential oils (3%), followed by the light green colored essential oils extracted from fruits of *R. officinalis* L. (2.5%), while *L. adoensis Hochst. var. koseret* leaves yielded the minimum amount of yellow colored essential oil when compared to the other five plants used in the study (0.7) (Table 1).

TLC profile of essential oils

TLC fingerprint was used for screening of essential oils as bioactive compounds were separated in a sequence of different zones and characterized by the value of Retention Factors (R_f) in Toluene: Ethyl acetate (9.3:0.7) solvent system and the color of zone they produce after being treated with detection reagent (Vanillin-sulfuric acid). Each plant essential oils have shown quite different thin layer chromatography finger print (Table 2). TLC screening indicated the presence of many terpenoids in the essential oil tested which was confirmed by the presence of different colored spots. Highest number of spots was obtained in the chromatogram of *Lippia adoensis Hochst. var. koseret* essential oils that showed distinctive 8 spot/bands, *Rosmarinus officinalis* were observed visually having 7 spot/bands, while *Ruta chalepensis* essential oil have separated in four different spot/bands when visually observed after treatment with Vanillin-sulphuric acid reagent.

Antifungal screening of plant essential oils

Table 3 shows the fungitoxic activity screening program of essential oils from *L. adoensis Hochst. var. koseret, R. officinalis,* and *R. chalepensis* at a concentration of 2µl/ml against toxigenic *A. flavus*, *A. fumigatus A. niger* and *A. parasiticus* strains isolated from food commodities. In order to access the antifungal activity of the six essential oils, food poisoning techniques were used; and their average scores were compared. It can be clearly seen that all tested essential oils affected the growth of *Aspergillus* species (Table 3). Absolute fungitoxicity against all tested organisms were shown by the essential oils of *L. adoensis Hochst. var. koseret*. The highest number of mycelial expansion meaning the lowest to moderate antifungal activity were seen in the essential oils of both *R. officinalis* and *R.*

chalepensis at the tested concentration.

Effect of essential oils on spore germination of toxigenic *Aspergillus* species

Table 4 shows the inhibition effect of *L. adoensis Hochst. var. koseret* oils on spore germination of *Aspergillus* species. Tween20 (5%v/v) used as a control did not inhibited the spore germination. There was a significant inhibition of fungal spore germination by each concentration of *L. adoensis Hochst. var. koseret* essential oil in comparison to control plates after 24 hour incubation (P<0.05). Sixty nine percent spore germination inhibitions at the lowest concentration of the oil tested (0.25µl/ml) and 100% inhibition at 2µl/ml for all tested strains of *Aspergillus* species was observed. It has also been microscopically observed that those spores which germinated in presence of low concentrations of essential oil produced small and/or short germ tubes as compared to the control.

Effect of essential oils on the growth of toxigenic Aspergillus species (MIC)

Strong evidence of antifungal efficacy was found by *L. adoensis Hochst. var. koseret* essential oil during our investigation to determine MIC (Table 5). It can be clearly seen that the fungitoxic activity of this plant essential oil affected the growth of all tested toxigenic *Aspergillus* species on sabouraud dextrose agar. Statistical results showed that concentration of essential oils have significant effect (P<0.05). Compared to the control, a significant reduction of growth of *Aspergillus* species were observed after exposure to *L. adoensis Hochst. var. koseret* essential oil at concentration of ≥0.5µl/ml (P<0.05). These reductions were concentration dependent since the higher concentration resulted in higher suppression of growth of the fungal mycelia extensions. As this table indicated the MIC was recorded at 2µl/ml which showed complete inhibition for up to seven days against all toxigenic *Aspergillus* species.

The oil have documented to have better fungitoxic activity against aflatoxigenic *Aspergillus* species when compared to synthetic chemical preservative sodium benzoate (Table6). Statistical results showed that both the kind and concentration of essential oil have significant effect (P<0.05).

Discussion

In this millennium infection from *Aspergillus* species become the major public health problem of modern mycology [4]. They have a capability to cause directly infection and indirectly mycotoxicosis especially upon the consumption of food contaminated with *Aspergillus* species. Many chemical preservative have been used for the control of *Aspergillus* food contamination [27]. The widespread use of chemical preservative has significant drawbacks including increased cost, handling hazards, concern about pesticide residues on food, and threat to human health and environment [28]. Public awareness of these risks has increased interest in finding safer alternatives natural products to replace currently used synthetic chemical preservatives to control *Aspergillus* food contamination. One such alternative is the use of essential oils with antifungal and antiaflatoxigenic activity, since they tend to have low mammalian toxicity, less environmental effects and wide public acceptance [29]. *L. adoensis Hochst. var. koseret*, *R. officinalis* and *R. chalepensis* are common economic food spices in Ethiopia and thus, it is an advantage to develop safe botanical food preservative against toxigenic *Aspergillus* species that have strong affinity to colonize various food commodities due to its secretion of hydrolytic enzymes [23].

The essential oil yield and constituents were different in the tested aromatic medicinal plants [30]. Our TLC analysis confirmed the presence of various components of essential oils which were characterized by the distance they travel in a particular TLC system and their appearance (color) after visualization of the spots. Essential oils are very complex natural mixtures which can contain about 20–60 components at quite different concentrations. They are characterized by two or three major components at fairly high concentrations (20–70%) compared to other components present in trace amounts [30]. This chromatogram developed from essential oils with the distinctive spot R_f and color were due to the presence of major component of essential oils i) Alcohols: borneol in *R. officinalis* and linalool in *L. adoensis Hochst. var. koseret*; ii) Ketones: fenchone in *F. vulgare;* and undecanone and nonanone in *R. chalepensis*; iii)Ethers: 1,8-cineole in *R. officinalis*; essential oils [31]. The presence of various constituent in the essential oils are relevant for clinical application as it is impossible for the organism to develop resistance to all components of the oils. TLC finger print is the most common chromatographic technique widely available for phytochemical analysis of plant essential oils. It is used in standardizing the constituents of essential oils that are classified as generally recognized as safe by FDA for their use as food additives in controlling food spoilage [29].

Our antifungal screening results confirm that essential oils have fungitoxic potentials against the tested organisms. The essential oil of *L. adoensis Hochst. var. koseret* showed higher inhibitory effects on the growth of aflatoxigenic *Aspergillus* species at a concentration of 2µl/ml. The essential oil composition of plants varies significantly in different genera and species [32]. Antifungal activity of tested plant essential oil against toxigenic strain of *Aspergillus* species may be due to major constituents' linalool *L. adoensis Hochst. var. koseret* [33]. Piperitone, 1,8-Cineole and a-Pinene have been reported as major components of *R. officinalis* [34, 35]. Undecanone and decanone have been reported as major components of *R. chalepensis* [36]. Our finding of antifungal activity of these essential oils support the finding of Bakkiali and his coworker [37], on the biological effect of essential oils. Screening program using food poisoning technique helps us to reduce the wastage of resources and focus our effort on valuable experiment by providing valuable information. The minimum amount of essential oils to inhibit the fungal population was studied by our MIC to save and/or minimize the wastage of essential oils.

Our results from spore germination assay of *L. adoensis Hochst. var. koseret* essential oil confirms that it have spore germination inhibition potential against *Aspergillus* species. Complete inhibitions of spore germination were recorded at the concentration of 2µl/ml which could be a suggestive of the fungicidal action of the oil the specified concentration. Our finding that has shown *L. adoensis Hochst. var. koseret* oil have mycosporicidal activity against *Aspergillus* species was in agreement with the reputation of this plants in the traditional practices by Oromo and Gurage people in Ethiopia as reported by previous researcher [23]. The vapor action exerted by volatile constituents of this essential oil on surface mycelial development (and thus the 'platform' to support spore production) and/or the transduction of signals involved in the switch from vegetative to reproductive development could be responsible for the spore germination inhibition activity. Moreover, essential oils sporicidal activity could be due to the interference with the amino acid involved in germination or denaturation of the enzymes responsible for spore germination [38].

From other plants our result confirmed the fungitoxic potentials on the growth *Aspergillus* species was shown by essential oils of *L. adoensis Hochst. var. koseret*. A complete inhibition of growth were recorded at a concentration of 2µl/ml against all aflatoxigenic organism tested. This finding of efficacy of this plant against storage organism were supported the report of traditional use of the plants by Oromo and Gurage people in Ethiopia by Tadeg [23].

Even though, there was no previous studies conducted reporting the antifungal activity of this essential oil, there exists the antifungal activity of the major constituents' linalool from other plants. It may be responsible for the fantastic fungitoxic activity of the plant. Moreover, the vapor action exerted by volatile constituents of this essential oil may support the fungitoxic activity. As the traditional use of this plant is most common in Ethiopia, having growth inhibitory effect at small amount was promising finding that will avoid the risk of residual effect of the oil on health and environment upon use as botanical preservative.

In our study we tried to compare the preservative potentials of plant essential oils with the prevalent synthetic preservative by using their MIC value. For easy of comparison we took the weight of each essential oils For easy of comparison we took the weight of 1µl of each essential oil and found to be 1.10mg, 1.04mg, 1.01mg and 1.09mg for *L. adoensis Hochst. var. koseret*. The four essential oils were more active than sodium benzoate.

These results that indicated essential oils have better fungitoxic activity than synthetic preservative are in accordance with the finding of previous study [30]. The authors of have showed highest activity of sodium benzoate at pH 3.5. This may be due to mode of action of the chemicals, i.e. salt of sodium and benzoic acid. Weak acid compounds are more lipophilic in their non dissociated form which enables them to cross the cell membrane that led to pH lowering of cytoplamic cell with rupture of certain metabolic reactions of the microorganism, permeabilization of the cytoplasmic membrane and cell death. Other authors' demonstrated benzoic acid has membrane-perturbing potentials. In addition, these acids induce loss of mitochondrial function, and one possibility that we entertained was that this could be the result of mitochondrial autophagy [39].

Moreover, essential oils are complex mixtures of numerous molecules, and one might wonder if their biological effects are the result of a synergism of all molecules or reflect only those of the main molecules present at the highest levels. In the literature in most cases, only the main constituents of certain essential oils were analyzed [30]. Thus, synergistic functions of the various molecules contained in an essential oil, in comparison to the action of one or two main components of the oil,

seems questionable. However, it is possible that the activity of the main components is modulated by other minor molecules [30]. Moreover, it is likely that several components of the essential oils play a role in cell penetration, lipophilic or hydrophilic attraction and fixation on cell walls and membranes, and cellular distribution [30].

Conclusion

On the bases of antifungal and sporicidal activity, we found essential oils of *L. adoensis Hochst. var. koseret* has promising broad spectrum fungitoxic potency. *L. adoensis Hochst. var. koseret* essential oils has superior antimycotic activity over the most prevalent synthetic preservative, we recommend that this plant essential oil may serve as potential source of alternatives for the control of toxigenic *Aspergillus* species. Because of volatile nature, easy applicability, pronounced antifungal and sporicidal activity we recommend *L. adoensis Hochst. var. koseret* oil as food preservative. Exploring the efficacy of *L. adoensis Hochst. var. koseret* essential oil using other toxigenic organism that contaminate food commodities. Mechanism of action and chronic toxicity studies to determine its safety on term usage.

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Table 1. Essential oil yield of aromatic plants used in the study

Plant Species	Family	Part Extracted	Percentage Yield (W/W)	Color of EOs
Ruta chalepensis L.	Rutaceae	Fruit	3.0 ± 0.058	Pale yellow
Lippia adoensis Hochst. var. koseret	Verbenaceae	Leaf	0.7 ± 0.116	Yellow
Rosmarinus officinalis L.	Lamiaceae	Leaf	2.5 ± 0.115	Light green

Values are mean ± SD

Table 2. TLC fingerprint of essential oils of aromatic plants

plant	Spot (bands) R_f value and corresponding colors							
	1st	2nd	3rd	4th	5th	6th	7th	8th
La	0.96 (blue violet)	0.85 (brown)	0.71 (gray)	0.63 (brown)	0.54 (violet)	0.45 (green)	0.44 (blue)	0.36 (blue)
Rc	0.94 (blue violet)	0.73 (violet)	0.59 (gray)	0.25 (brown)	-	-	-	-
Ro	0.96 (violet)	0.65 (blue)	0.56 (violet)	0.48 (blue)	0.39 (blue)	0.3 (blue)	0.23 (blue)	-

La, *Lippia adoensis Hochst. var. koseret*; Ro, *Rosmarinus officinalis*; Rc, *Ruta chalepensis*

Table 3. Antifungal screening of some plant essential oils against the toxigenic strain of *Aspergillus* species using food poisoning technique at a concentration of 2µl/ml.

***Aspergillus* species**	**Antifungal activity**		
	Lippia adoensis	***Rosmarinus officinalis***	***Ruta chalepensis***
AFST	100.0 ± 0.0	8.7 ± 0.0	29.0 ± 0.8
AF001	100.0 ± 0.0	14.1 ± 0.3	12.5 ± 0.8
AF006'	100.0 ± 0.0	22.3 ± 0.3	32.9 ± 1.2
AF009	100.0 ± 0.0	22.7 ± 1.2	28.0 ± 0.0
AP037	100.0 ± 0.0	22.3 ± 0.7	23.7 ± 0.8
ANST	100.0 ± 0.0	32.9 ± 0.3	37.0 ± 0.8
AN002	100.0 ± 0.0	26.5 ± 0.3	29.7 ± 0.0
AFU037	100.0 ± 0.0	45.5 ± 0.6	69.7 ±0.3

AFST, *Aspergillus flavus* (ATCC 13697); AF, *Aspergillus flavus*; AP, *Aspergillus parasiticus*; ANST, *Aspergillus niger* (ATCC 10535); AN, *Aspergillus niger*; AFU, *Aspergillus fumigatus*. Values are mean (n=3)±standard error.

Table 4. Percent of Spore Germination Inhibition of toxigenic *Aspergillus* species by *Lippia adoensis Hochst. var. koseret* essential oil, using fungal spore germination assay

Aspergillus	**Antifungal activity**					
species	**0.25µl/ml**	**0.5µl/ml**	**1µl/ml**	**2µl/ml**	**4µl/ml**	**8µl/ml**
AFST	71.0 ±1.1	83.3 ±1.6	93.1 ±0.6	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0
AF001	76.2 ±0.6	87.8 ±0.3	94.5 ±0.0	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0
AF006'	69.0 ±0.5	80.0 ±1.3	94.0 ±0.9	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0
AF009	70.8 ±1.1	83.5 ±3.1	95.2 ±0.8	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0
AP037	75.5 ±0.0	88.3 ±1.1	95.5 ±0.0	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0
ANST	82.2 ±1.4	91.2 ±1.2	97.5 ±1.0	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0
AN002	84.3 ±0.7	92.8 ±1.2	98.2 ±0.3	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0
AFU037	91.2 ±0.7	99.3 ±0.2	99.8 ±0.3	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0

AFST, *Aspergillus flavus* (ATCC 13697); AF, *Aspergillus flavus*; AP, *Aspergillus parasiticus*; ANST, *Aspergillus niger* (ATCC 10535); AN, *Aspergillus niger*; AFU, *Aspergillus fumigatus;* Values are mean (n=3) ± standard deviation.

Table 5. Fungitoxic spectrum of *Lippia adoensis Hochst. var. koseret* essential oil against the toxigenic strain of *Aspergillus* species using agar dilution technique

Aspergillus	**Antifungal activity**					
species	**Control***	**0.25µl/ml**	**0.5µl/ml**	**1µl/ml**	**2µl/ml**	**4µl/ml**
AFST	26.0 ± 0.6	23.0 ± 0.0	21.3 ± 0.8	17.7 ± 0.7	0.0 ± 0.0	0.0 ± 0.0
AF001	22.0 ± 0.0	21.3 ± 0.3	21.0 ± 0.0	18.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
AF006'	24.7 ± 0.7	24.3 ± 0.3	22.7 ± 0.3	19.0 ± 0.6	0.0 ± 0.0	0.0 ± 0.0
AF009	24.7 ± 0.3	23.7 ± 0.3	20.3 ± 0.3	19.7 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
AF037	26.3 ± 0.3	22.7 ± 1.5	21.3 ± 0.3	18.7 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
ANST	25.0 ± 0.6	22.7 ± 0.7	20.3 ± 0.3	18.7 ± 0.7	0.0 ± 0.0	0.0 ± 0.0
AN002	21.7 ± 0.3	20.7 ± 0.3	18.3 ± 0.3	17.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
AFU037	11.0 ± 0.0	11.3 ± 0.3	11.0 ± 0.6	10.0 ± 0.3	0.0 ± 0.0	0.0 ± 0.0

AFST, *Aspergillus flavus* (ATCC 13697); AF, *Aspergillus flavus*; AP, *Aspergillus parasiticus*; ANST, *Aspergillus niger* (ATCC 10535); AN, *Aspergillus niger*; AFU, *Aspergillus fumigatus;* *, 5% Tween 20; Values are mean (n=3) ± standard error.

Table 6. Comparative mycotoxic activity of *Lippia adoensis Hochst. var. koseret* essential oil with Sodium Benzoate

Aspergillus	**MIC activity (mg/ml)**	
species	***Lippia adoensis***	**Sodium Benzoate**
AFST	2.00	>16.00
AF001	2.00	>16.00
AF006'	2.00	>16.00
AF009	2.00	>16.00
AP037	2.00	>16.00
ANST	2.00	16.00
AN002	2.00	16.00
AFU037	1.00	8.00

AFST, *Aspergillus flavus* (ATCC 13697); AF, *Aspergillus flavus*; AP, *Aspergillus parasiticus*; ANST, *Aspergillus niger* (ATCC 10535); AN, *Aspergillus niger*; AFU, *Aspergillus fumigatus*