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# EVALUATION OF ANTIFUNGAL PROPERTIES OF *Ageratum conyzoides, Spilanthes filicaulis* and *Tithonia diversifolia* LEAF EXTRACTS AND SEARCH FOR THEIR COMPOUNDS USING GAS CHROMATOGRAPHY - MASS SPECTRUM

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**ABSTRACT**

Leaf extracts from *Ageratum conyzoides, Spilanthes filicaulis* and *Tithonia diversifolia* (Asteraceae) were evaluated for their antifungal activities against three leafspot fungi such as *Cochliobolus lunatus*, *Fusarium lateritium* and *Fusarium solani.* In this study, ethanol was used as an extraction solvent and bioactivity screening was done by poisoned food technique. All the extract concentrations ranging from 8 - 120 mg/ml in Potato Dextrose Agar (PDA) medium were significantly ($P<0.05$) toxic to the test fungi *in vitro* with their inhibition potentials being concentration and species dependent. The phytochemical screening of the extracts reveals the presence of alkaloids, anthraquinones, flavonoids, phenols, saponins, tannins and terpenes in varying degrees. Furthermore, the extracts were analyzed using Gas Chromatography - Mass Spectrometry (GC-MS). Results indicated a mixture of components ranging from 10 in *T. diversifolia,* 14 in *S. filicaulis* and 18 in *A. conyzoides* with varied percentage of abundance in each extract. The main components of *T. diversifolia* extracts were Diethyl Phthalate (18.05%), Octane, 1-chloro- (11.04%), Palmitic acid ethyl ester (10.67%) and Undecanoic acid 2-methyl-, methyl ester (10.67%), and those in *S. filicaulis* were Palmitin, 2-mono- (9.47%), Caryophyllene (8.99%), Palmitic acid ethyl ester (8.50%) and 1-Pentadecene (8.02%). 2, 4, 6-Tri-tert-butyl phenol (12.14%), 7-t-Butyl-3,3-dimethyl -1-indanone (9.88%), 1(2H)-Naphthalenone - (1, 1-dimethylethyl) -3,4-dihydro- (9.19%) and Demethoxyageratochromene (8.60%) were found abundant in *A. conyzoides* extracts. These compounds could be responsible for the toxic activity of the extracts. The finding of this study therefore unlocks the potentials of these Asteraceae for bio-pesticide production in Nigeria.

**Keywords:** antifungal properties, leaf extract, *A. conyzoides, S. filicaulis, T. diversifolia,* leafspot fungi, GC-MS analysis.

## INTRODUCTION

The overzealous and indiscriminate use of most synthetic fungicides has created different types of environmental and toxicological problems. In different parts of the world, attention has been paid towards exploitation of higher plant products as novel chemotherapuetants in plant protection (Gurjaret *et al*., 2012). Biological control has attained importance in modern agriculture to curb the hazards of intensing use of chemicals for pest and disease control (Seema *et al*., 2011). The ultimate aim of recent research in this area has been the development of alternative control strategies to reduce our dependency on synthetic fungicides. Plants have limitless ability to synthesize aromatic secondary metabolites most of which show antimicrobial effects and serve as plant defense mechanism against pathogenic microorganisms (Das *et al*., 2010; Gurjar *et al*., 2012).

Extracts obtained from many plants have recently gained popularity and scientific interest for their antifungal activities (Chiejina and Ukeh, 2013).

*Ageratum conyzoides* (goat weed), *Spilanthes filicaulis* (Brazil cress) and *Tithonia diversifolia* (Mexican sunflower) are common weeds in the family of Asteraceae found growing abundantly in tropical and subtropical African including Nigeria (Akobundu and Agyakwa, 1998). *Ageratum conyzoides* is widely utilized in traditional medicine hence several pharmacological investigation has been conducted to determine efficacy (Kamboj and Ajay, 2008). Variability in secondary metabolites of *A. conyzoides* has been reported (Ming, 1999). Its antifungal activities have been reported (Adekunle, 2001; Ogbebor *et al*., 2007). *Spilanthes filicaulis* has anti-ageing properties and cures various diseases of tooth and gums including *Pyorrhoea* (Rai *et al*., 2004). It contains tannins, alkaloids, saponins, sequiterpenoids and pungent amide called spilanthol (Sabitha and Suryanarayana, 2006). *Tithonia diversifolia* leaves are used for treatment of various diseases including skin disease and possesses many chemical components (Perera and Perera, 2003; Rapasa *et al*., 2008).

Therefore the present study was designed to investigate the following:

a) Evaluate the antifungal potentials of *A. conyzoides, S. filicaulis* and *T. diversifolia* (Asteraceae) in three leafspot fungi.
b) Search for the phytochemical constituents of these extracts via GC-MS analysis with the view of exploiting an interesting lead components for biofungicide production in Nigeria.

## MATERIALS AND METHODS

### Source of fungi

The fungi used in this study were previously isolated and identified from leafspot disease of sweet

potato (Ilondu, 2013). The fungi included *Cochliobolus lunatus* R.R Nelson and F.A. Haasis. Anamorph: *Curvularia lunata* (Wakker) Boedgin (1M1394871); *Fusarium lateritium* Nees. Teleomorph: *Gibberella baccata* (Wallr.) Sacc. (1M1394869) and *Fusarium solani* (Mart.) Sacc. (1M1 394872). The cultures of these fungi were maintained on potato Dextrose Agar (PDA) slant at 4°C in the laboratory until needed. The isolates were revived twice on PDA before use.

**Collection and extraction of plant sample**

*Ageratum conyzoides* Linn., *Spilanthes filicaulis* (Shum and Thonn.) C.D. Adams and *Tithonia diversifolia* (Hemsl.) A. Gray used in this study (Figure-1) were obtained from the premises of site II, Delta State University, Abraka. The plants were properly identified using Akobundu and Agyakwa (1998). The leaves were plucked and washed in flowing tap water, shade dried following the method of Rai and Acharya (1999). Dried leaves were ground into powder using electric blender (Philip Confort HR 1727) before extraction. The method of extraction was a modification of Oyewale and Audu (2007). 100g of pulverised sample was put into the Soxhlet extractor and 300ml of methanol was added at the ratio of 1:3 and extracted for 8 hours. Each batch of the extract was evaporated on a rotary evaporator at 40$^{o}$C to remove excess solvent leaving behind the solidified extracts which were put into sterile bottles and stored in the refrigerator until needed.

**Preparation of plant extract-agar medium**

For every set of experiment the plant extracts were each dissolved in a solvent, dimethyl sulphoxide (DMSO) in the ratio of 1 g of extract to 10 ml of DMSO (1:10) to give the 100mg/ml concentration (Onyeke and Maduewesi, 2006). Different concentrations (8mg/ml, 16mg/ml, 24mg/ml, 32mg/ml ….. 120mg/ml) were prepared from each of the extracts. One milliliter of each level of concentration was aseptically incorporated into 20ml of cool molten PDA in sterile test tube. Three test tubes were used for each extract concentration and the fourth test tube without the extract served as control. Each medium was thoroughly homogenized by gentle agitation before dispensing into 9 cm diameter sterile Petri dishes. The plates were allowed to set on the laboratory bench for 3 hrs.

**Effects of extract on radial growth**

This was done by inoculating at the centre of 9cm Petri plates with a mycelia disc (4mm) obtained from the colony edge of 7-day old culture of the test fungi. Three replicates of both the control and PDA-extract plates per isolate were incubated at room temperature (28±2$^{0}$C) and radial growth was measured with a metric ruler daily for seven days. The inhibition activity to the radial growth (IR) was determined according to the following formula (Ni Putu and Suprata, 2012);

$$IR\ (\%) = \frac{dc - dt}{dc} \times \frac{100}{1}$$

Where: IR = inhibitory activity to the radial growth
dc = average increase in mycelia growth in control plates
dt = average increase in mycelia growth in treated plates

**Phytochemical screening**

For preliminary phytochemical analysis, the ethanol extracts were tested for the presence or absence of secondary metabolites using standard phytochemical procedures according to Trease and Evans (2002).

**Extract analysis**

GC-MS analysis was done at National Research Institute for Chemical Technology (NARICT) Zaria, Kaduna state, Nigeria. A SHIMADZU GCMS-QP 2010 Plus system was used. The GC-MS was operated under the following conditions: Column oven temperature: 70$^{o}$C; Injection temperature: 250$^{o}$C; Injection mode: split; Pressure: 104.1kPa; Total flow: 6.2ml/min; Column flow: 1.59ml/min; Linear velocity: 46.3cm/sec; Purge flow: 3.0.

**Data analysis**

Data obtained were subjected to analysis of variance (ANOVA) using statistical package for social science SPSS version 17.0 and means were separated according to Duncan's Multiple Range Test (DMRT) at 5% probability level.

*a. Spilanthes filicaulis* (Shum and Thonn.) C.D. Adams

*b. Tithonia diversifolia (Hemsl.) A. Gray*

*c. Ageratum conyzoides* Linn.
**Figure-1.** Plants used for the study.

## RESULTS

The inhibitory effects of *A. conyzoides, S. filicaulis, T. diversifolia* and their percentage inhibition on the test fungi are presented in Tables 1 and 2. The result showed that the extracts significantly ($P<0.05$) differed in their potential to inhibit the mycelia growth of the fungal pathogens. In effect the inhibitory activity of the extract is concentration and species dependent. Minimum inhibitory concentration (MIC) where there was no observed fungal growth on each of the leaf extract is shown in Table-3. Complete inhibition of growth of *C. lunatus, F. lateritum* and *F. solani* was achieved with *A. conyzoides* extract at 88, 120 and 80 mg/ml concentrations respectively, while *S. filicaulis* extracts completely inhibited the three fungi at 72, 80 and 72 mg/ml concentration respectively. For *T. diversifolia* extract 100% inhibition was achieved for *C. lunatus* at 56, *F. lateritum* at 72 and *F. solani* at 72 mg/ml concentrations.

The phytochemical screening of the extracts revealed the presence of various secondary metabolites (Table-4). Tannins were found in moderate concentrations in both *S. filicaulis* and *T. diversifolia* extracts while alkaloid and terpenes occur in moderate concentrations in the extracts from *A. conyzoides* and *T. diversifolia* respectively.

The GC-MS analysis clearly showed a mixture of compounds in the extracts. The chromatograms of the peak compounds detected in the extracts are shown in Figures 2-4. Various constituents with their retention time (RT), molecular weight, molecular formula and concentration (% abundance) are presented in Tables 5-7. Eighteen compounds were identified in the *A. conyzoides* leaf extract with 2, 4, 6-Tri-tert-butyl phenol (12.14%) being the most abundant followed by 7-t-Butyl-3, 3-dimethyl -1-indanone (9.88%), 1(2H)-Naphthalenone - (1, 1-dimethylethyl) -3, 4-dihydro- (9.19%) and Demethoxyageratochromene (8.60%) (Table-7); fourteen compounds were observed in *S. filicaulis* extracts with palmitin, 2-mono (9.47%) being the most abundant followed by Caryophyllene (8.99%), Palmitic acid ethyl ester (8.50%) and 1-Pentadecene (8.02%), while ten compounds occurred in *T. diversifolia* with diethyl phthalate (18.05%) being the most abundant, this was followed by Octane, 1-chloro- (11.04%), Palmitic acid ethyl ester (10.67%) and Undecanoic acid 2-methyl-, methyl ester (10.67%).

**Table-1.** Effects of leaf extract of *A. conyzoides, S. filicaulis* and *T. diversifolia* on Day 7 mycelial diameter (cm) of three leafspot fungi of sweet potato (*Ipomoea batatas* (*L.*) Lam)

| Extract concentration (mg/ml) | Leafspot fungi | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Ageratum conyzoides* | | | *Spilanthes filicaulis* | | | *Tithonia diversifolia* | | |
| | ***C. lunatus*** | ***F. lateritium*** | ***F. solani*** | ***C. lunatus*** | ***F. lateritium*** | ***F. solani*** | ***C. lunatus*** | ***F. lateritium*** | ***F. solani*** |
| 0 | $4.30^{a}$ | $4.30^{a}$ | $4.30^{a}$ | $4.30^{a}$ | $4.30^{a}$ | $4.30^{a}$ | $4.30^{a}$ | $4.30^{a}$ | $4.30^{a}$ |
| 8 | $2.77^{b}$ | $3.17^{b}$ | $3.60^{b}$ | $2.93^{b}$ | $4.10^{b}$ | $4.30^{a}$ | $3.37^{b}$ | $4.07^{b}$ | $3.53^{b}$ |
| 16 | $1.77^{c}$ | $3.10^{bc}$ | $3.00^{c}$ | $1.90^{c}$ | $3.80^{c}$ | $4.27^{a}$ | $2.23^{c}$ | $3.90^{c}$ | $3.03^{c}$ |
| 24 | $1.40^{d}$ | $2.83^{c}$ | $2.40^{d}$ | $1.50^{d}$ | $3.00^{d}$ | $3.43^{b}$ | $1.27^{d}$ | $2.10^{d}$ | $2.53^{d}$ |
| 32 | $1.07^{e}$ | $2.43^{d}$ | $1.97^{e}$ | $0.90^{e}$ | $1.90^{e}$ | $2.73^{c}$ | $1.00^{e}$ | $1.83^{e}$ | $1.83^{e}$ |
| 40 | $0.93^{f}$ | $2.20^{e}$ | $1.53^{f}$ | $0.67^{f}$ | $1.50^{f}$ | $1.27^{d}$ | $0.47^{f}$ | $1.17^{f}$ | $1.47^{f}$ |
| 48 | $0.80^{g}$ | $2.10^{e}$ | $0.97^{g}$ | $0.50^{g}$ | $1.33^{g}$ | $0.73^{e}$ | $0.20^{g}$ | $0.97^{g}$ | $1.33^{g}$ |
| 56 | $0.73^{g}$ | $1.83^{f}$ | $0.73^{h}$ | $0.50^{g}$ | $0.93^{h}$ | $0.23^{f}$ | $0.00^{h}$ | $0.50^{h}$ | $0.80^{h}$ |
| 64 | $0.33^{h}$ | $1.70^{f}$ | $0.63^{hi}$ | $0.16^{h}$ | $0.50^{i}$ | $0.07^{g}$ | $0.00^{h}$ | $0.20^{i}$ | $0.36^{i}$ |
| 72 | $0.23^{hi}$ | $1.37^{g}$ | $0.53^{i}$ | $0.00^{i}$ | $0.30^{j}$ | $0.00^{g}$ | $0.00^{h}$ | $0.00^{j}$ | $0.00^{j}$ |
| 80 | $0.13^{i}$ | $1.27^{g}$ | $0.00^{j}$ | $0.00^{i}$ | $0.00^{k}$ | $0.00^{g}$ | $0.00^{h}$ | $0.00^{j}$ | $0.00^{j}$ |
| 88 | $0.00^{j}$ | $0.73^{h}$ | $0.00^{j}$ | $0.00^{i}$ | $0.00^{k}$ | $0.00^{g}$ | $0.00^{h}$ | $0.00^{j}$ | $0.00^{j}$ |
| 96 | $0.00^{j}$ | $0.53^{i}$ | $0.00^{j}$ | $0.00^{i}$ | $0.00^{k}$ | $0.00^{g}$ | $0.00^{h}$ | $0.00^{j}$ | $0.00^{j}$ |
| 104 | $0.00^{j}$ | $0.37^{j}$ | $0.00^{j}$ | $0.00^{i}$ | $0.00^{k}$ | $0.00^{g}$ | $0.00^{h}$ | $0.00^{j}$ | $0.00^{j}$ |
| 112 | $0.00^{j}$ | $0.27^{j}$ | $0.00^{j}$ | $0.00^{i}$ | $0.00^{k}$ | $0.00^{g}$ | $0.00^{h}$ | $0.00^{j}$ | $0.00^{j}$ |
| 120 | $0.00^{j}$ | $0.00^{k}$ | $0.00^{j}$ | $0.00^{i}$ | $0.00^{k}$ | $0.00^{g}$ | $0.00^{h}$ | $0.00^{j}$ | $0.00^{j}$ |

Values with the same superscript(s) in the same column are not significantly different at $P>0.05$ by DMRT.

**Table-2.** Percentage inhibition of the leafspot fungi after 7 days of inoculation on agar plate.

| Extract concentration (mg/ml) | Leafspot fungi | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Ageratum conyzoides* | | | *Spilanthes filicaulis* | | | *Tithonia diversifolia* | | |
| | *C. lunatus* | *F. lateritium* | *F. solani* | *C. lunatus* | *F. lateritium* | *F. solani* | *C. Lunatus* | *F. lateritium* | *F. solani* |
| 0 | $0.00^{j}$ | $0.00^{l}$ | $0.00^{j}$ | $0.00^{i}$ | $0.00^{k}$ | $0.00^{g}$ | $0.00^{h}$ | $0.00^{j}$ | $0.00^{j}$ |
| 8 | $35.66^{i}$ | $26.36^{k}$ | $16.28^{i}$ | $31.78^{h}$ | $4.65^{j}$ | $0.00^{g}$ | $21.71^{g}$ | $5.43^{i}$ | $17.83^{i}$ |
| 16 | $58.92^{h}$ | $27.91^{j}$ | $30.23^{h}$ | $55.81^{g}$ | $11.63^{i}$ | $0.78^{g}$ | $48.06^{f}$ | $9.30^{h}$ | $29.46^{h}$ |
| 24 | $67.44^{g}$ | $34.11^{i}$ | $44.19^{g}$ | $65.12^{f}$ | $30.23^{h}$ | $20.16^{f}$ | $70.54^{e}$ | $51.16^{g}$ | $41.09^{g}$ |
| 32 | $75.20^{f}$ | $43.41^{h}$ | $54.26^{f}$ | $79.07^{e}$ | $55.81^{g}$ | $36.43^{e}$ | $76.74^{d}$ | $57.36^{f}$ | $57.36^{f}$ |
| 40 | $78.30^{e}$ | $48.84^{g}$ | $64.34^{e}$ | $84.50^{d}$ | $65.12^{f}$ | $70.54^{d}$ | $89.15^{c}$ | $72.87^{e}$ | $65.89^{e}$ |
| 48 | $81.40^{d}$ | $51.16^{g}$ | $77.52^{d}$ | $88.37^{c}$ | $73.64^{e}$ | $82.95^{c}$ | $95.35^{b}$ | $77.52^{d}$ | $73.64^{d}$ |
| 56 | $82.95^{d}$ | $57.36^{f}$ | $82.95^{c}$ | $88.37^{c}$ | $78.30^{d}$ | $94.57^{b}$ | $100.00^{a}$ | $88.37^{c}$ | $81.40^{c}$ |
| 64 | $92.25^{c}$ | $60.47^{f}$ | $85.27^{bc}$ | $96.12^{b}$ | $88.37^{c}$ | $98.45^{a}$ | $100.00^{a}$ | $95.35^{b}$ | $91.47^{b}$ |
| 72 | $94.57^{bc}$ | $68.22^{e}$ | $87.60^{b}$ | $100.00^{a}$ | $93.02^{b}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ |
| 80 | $96.90^{b}$ | $70.54^{e}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ |
| 88 | $100.00^{a}$ | $82.95^{d}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ |
| 96 | $100.00^{a}$ | $87.60^{c}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ |
| 104 | $100.00^{a}$ | $91.43^{b}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ |
| 112 | $100.00^{a}$ | $93.80^{b}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ |
| 120 | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ |

Values with the same superscript(s) in the same column are not significantly different at P>0.05 by DMRT.

**Table-3.** Minimum inhibition concentration (mg/ml) of plant extracts on leafspot fungi.

| Leafspot fungi | Minimum inhibitory concentration | | |
|---|---|---|---|
| | *A. conyzoides* | *S. filicaulis* | *T. diversifolia* |
| *C. lunatus* | 88 | 72 | 56 |
| *F. lateritum* | 120 | 80 | 72 |
| *F. solani* | 80 | 72 | 72 |

**Table-4.** Phytochemical screening of the plant extracts used in the study

| Phytochemicals | *Ageratum conyzoides* | *Spilanthes filicaulis* | *Tithonia diversifolia* |
|---|---|---|---|
| Alkaloids | ++ | + | - |
| Saponins | + | + | + |
| Flavonoids | + | + | + |
| Anthraquinones | + | + | + |
| Terpenes | + | + | ++ |
| Steroids | + | + | - |
| Tannins | + | ++ | ++ |
| Cardiac glycosids | - | - | + |
| Phenols | + | - | - |

Key: + low concentration, ++ moderate concentration,
+++ high concentration, - absent

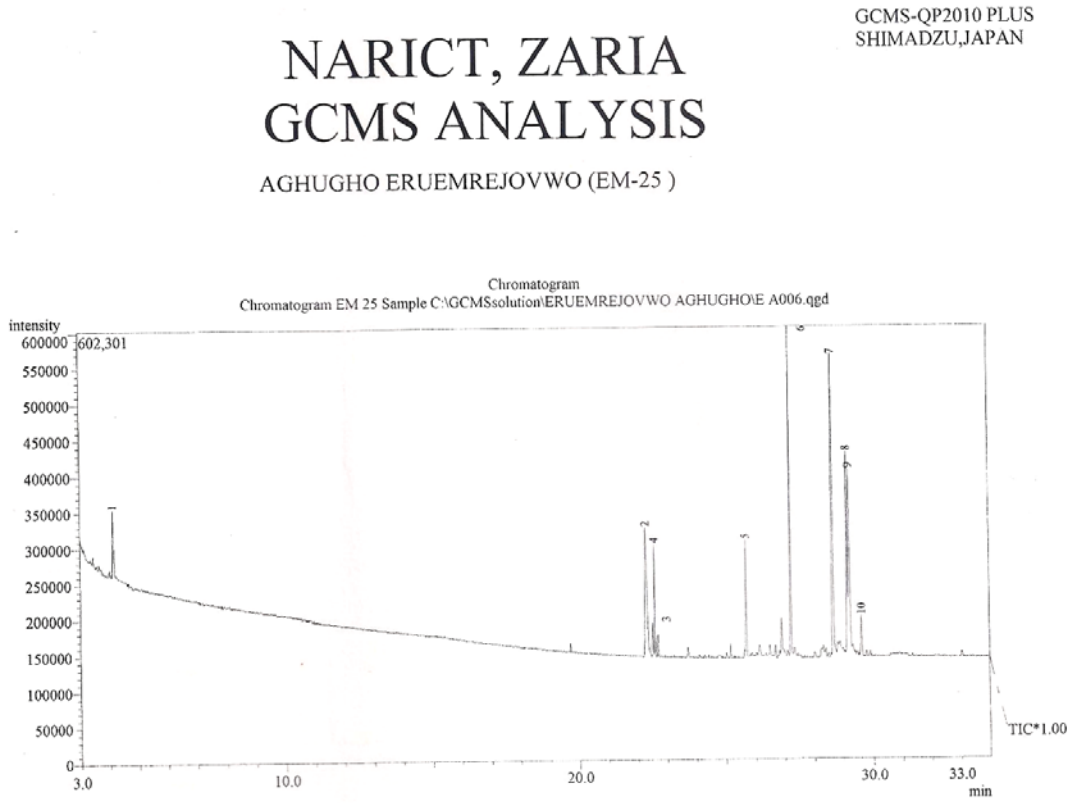


**Figure-2.** GC-MS chromatogram of extract from *Tithonia diversifolia.*

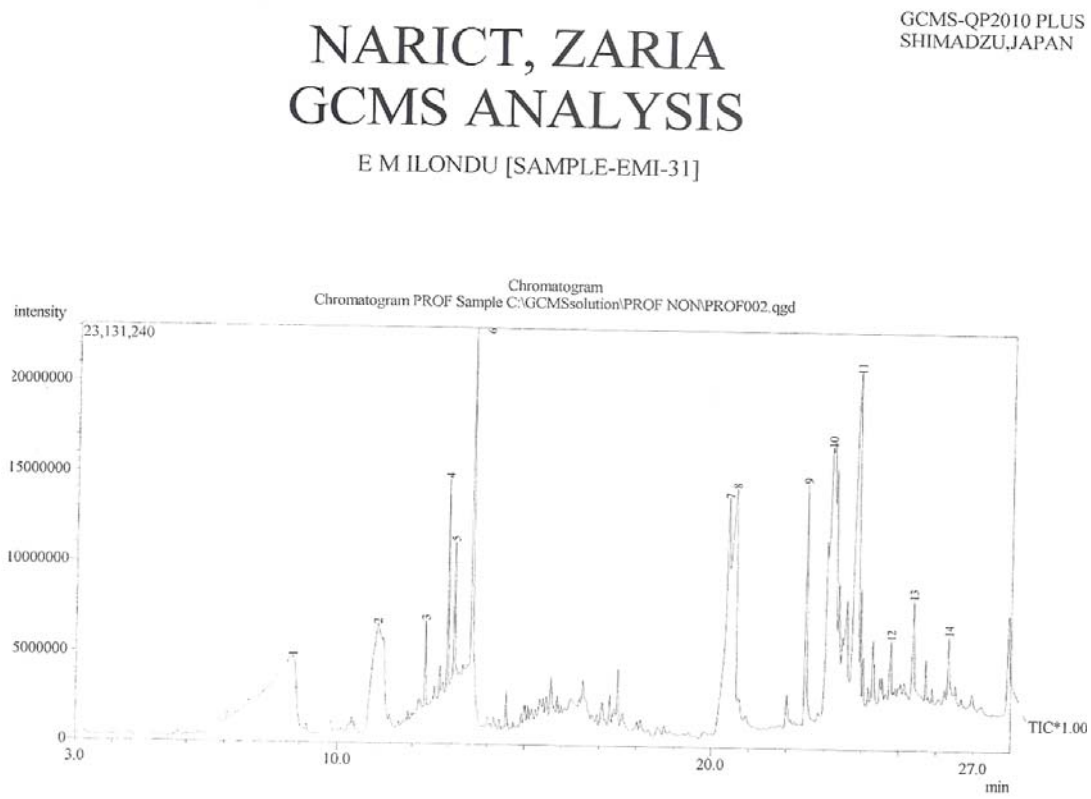


**Figure-3.** GC-MS chromatogram of extract from *Spilanthes filicaulis.*

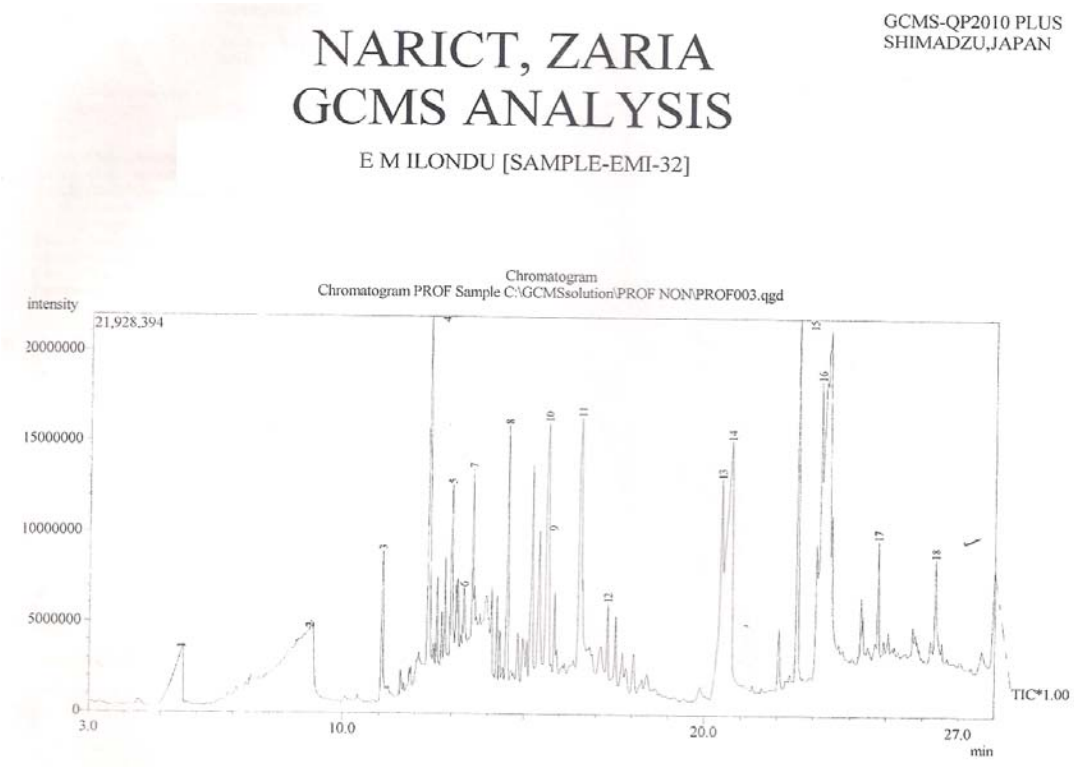


**Figure-4.** GC-MS chromatogram of extract from *Ageratum conyzoides.*

**Table-5.** Major identified constituents of leaf extract from *Tithonia diversifolia.*

| Peak No. | Retention time (min) | Molecular weight | % Abundance | Name of compound | Compound formula |
|---|---|---|---|---|---|
| 1 | 4.133 | 118 | 5.43 | Ethane, 1,1-diethoxy- | $C_6H_{14}O_2$ |
| 2 | 22.300 | 222 | 18.05 | Diethyl Phthalate | $C_{12}H_{14}O_4$ |
| 3 | 22.525 | 148 | 11.04 | Octane, 1-chloro- | $C_8H_{17}OL$ |
| 4 | 22.600 | 220 | 9.58 | Caryophyllene | $C_{15}H_{24}O$ |
| 5 | 25.708 | 250 | 8.24 | 1-Octadecyne | $C_{18}H_{34}$ |
| 6 | 27.217 | 284 | 10.67 | Palmitic acid ethyl ester | $C_{18}H_{36}O_2$ |
| 7 | 28.658 | 296 | 8.61 | Phytol | $C_{20}H_{40}O$ |
| 8 | 29.150 | 222 | 8.12 | 7-Hexadecyne | $C_{16}H_{30}$ |
| 9 | 29.217 | 278 | 9.58 | Linolenic acid | $C_{18}H_{30}O_2$ |
| 10 | 29.617 | 214 | 10.67 | Undecanoic acid 2-methyl-, methyl ester | $C_{13}H_{26}O_2$ |

**Table-6.** Major identified constituents of leaf extracts from *Spilanthes filicaulis.*

| Peak No | Retention time (min) | Molecular weight | % Abundance | Name of compound | Compound formula |
|---|---|---|---|---|---|
| 1 | 8.808 | 92 | 5.89 | Glycerin | $C_3H_8O_3$ |
| 2 | 11.050 | 130 | 4.15 | 1-Butanol, 3-methyl acetate | $C_7H_{14}O_2$ |
| 3 | 12.333 | 204 | 8.99 | Caryophyllene | $C_{15}H_{24}$ |
| 4 | 12.933 | 210 | 8.02 | 1-Pentadecene | $C_{15}H_{30}$ |
| 5 | 13.108 | 198 | 5.60 | 2-Tridecanone | $C_{13}H_{26}O$ |
| 6 | 13.583 | 204 | 6.67 | Beta-Sesquiphellan-drene | $C_{15}H_{24}$ |
| 7 | 20.433 | 284 | 8.50 | Palmitic acid ethyl ester | $C_{18}H_{36}O_2$ |
| 8 | 20.625 | 256 | 7.05 | Palmitic acid | $C_{16}H_{32}O_2$ |
| 9 | 22.517 | 296 | 6.86 | Phytol | $C_{20}H_{40}O$ |
| 10 | 23.158 | 306 | 7.63 | Linolenic acid ethyl ester | $C_{20}H_{34}O_2$ |
| 11 | 23.892 | 247 | 7.83 | N-Isobutyl-(2E, 4Z, 8Z, 10E)-dodecatetraenamide | $C_{16}H_{25}NO$ |
| 12 | 24.775 | 261 | 7.83 | N-(2-Methylbutyl) (2E, 4E, 8Z, 10E)-dodeca tetraenamide | $C_{17}H_{27}NO$ |
| 13 | 25.375 | 243 | 5.51 | N-(2-Methylbutyl) Undeca- (2E, 4Z)-diene-8-diynamide | $C_{16}H_{21}NO$ |
| 14 | 26.317 | 330 | 9.47 | Palmitin, 2-mono- | $C_{19}H_{38}O_4$ |

**Table-7.** Major identified constituents of leaf extract from *Ageratum conyzoides.*

| Peak No | Retention time (min) | Molecular weight | % Abundance | Name of compound | Compound formula |
|---|---|---|---|---|---|
| 1 | 5.567 | 100 | 4.92 | Methyl-2-butenoic acid | $C_5H_8O_2$ |
| 2 | 9.092 | 92 | 3.00 | Glycerin | $C_5H_8O_2$ |
| 3 | 11.092 | 150 | 6.63 | 2-Methoxy-4-vinyl phenol | $C_9H_{10}O_2$ |
| 4 | 12.383 | 204 | 4.58 | Caryophyllene | $C_{15}H_{24}$ |
| 5 | 12.992 | 190 | 8.60 | Demethoxyagerato chromene | $C_{12}H_{14}O_2$ |
| 6 | 13.325 | 204 | 5.16 | Alpha-Muurolene | $C_{15}H_{24}$ |
| 7 | 13.567 | 204 | 3.39 | Beta-Sesquiphellandrene | $C_{15}H_{24}$ |
| 8 | 14.542 | 220 | 3.86 | Caryophyllene oxide | $C_{15}H_{24}O$ |
| 9 | 15.417 | 202 | 9.19 | 1(2H)-Naphthalenone -(1,1-dimethylethyl) -3,4-dihydro- | $C_{14}H_{18}O$ |
| 10 | 15.650 | 216 | 9.88 | 7-t-Butyl-3,3-dimethyl -1-indanone | $C_{15}H_{20}O$ |
| 11 | 16.567 | 262 | 12.14 | 2,4,6-Tri-tert-butyl phenol | $C_{18}H_{30}O$ |
| 12 | 17.317 | 296 | 3.34 | 3,7,11,15-Tetramethyl -2-hexadecen-1-ol | $C_2H_{40}O$ |
| 13 | 20.458 | 284 | 4.33 | Palmitic acid ethyl ester | $C_{18}H_{36}O_2$ |
| 14 | 20.725 | 256 | 3.59 | Palmitic acid | $C_{16}H_{32}O_2$ |
| 15 | 22.558 | 296 | 3.49 | Phytol | $C_{20}H_{40}O$ |
| 16 | 23.183 | 306 | 3.89 | Linolenic acid ethyl ester | $C_{20}H_{34}O_2$ |
| 17 | 24.767 | 325 | 5.16 | 5-Benzamido-4-oxo-6-phenylhexanoic acid | $C_{19}H_{19}O_4$ |
| 18 | 26.350 | 330 | 4.82 | Palmitin, 2-mono | $C_{19}H_{38}O_4$ |

## DISCUSSIONS

The sensitivity of the three leafspot fungi to leaf extract of *A. conyzoides, S. filicaulis* and *T. diversifolia* were investigated in this study. The results of the antifungal activity assay showed that the plant extracts had inhibitory effects on the growth of the test fungi. These results revealed that antifungal activity of the extracts was enhanced by increasing concentration; hence the inhibition activity of the extracts was concentration dependent. This is in agreement with the report of Ilondu (2012), Chiejina and Ukeh (2013) that increase in the antifungal activity was observed with corresponding increase in concentration of plant extracts.

Several reports on the antifungal activities of plant extracts from *A. conyzoides* have been documented in literature. Adekunle (2001) reported that ethanolic extracts of *A. conyzoides* effectively inhibited all the eight fungi studied including *Curvularia lunata* and *Fusarium solani* compared to other plant extracts assessed. Singh *et al*. (1986) found that mycelia discs of *Epidermophyton floccosum*, *Microsporum canis* and *Trichophyton mentogrophytes* were completely killed when dipped in the oil of *A. conyzoides*.

Among the 31 plant species of the family Asteraceae screened for antimycotic activities, Rai and Acharya (1999) listed *A. conyzoides* among those found to be effective inhibitor of mycelia growth of *F. oxysporium*. Extracts from various Asteraceae including those used in this study *(T. diversifolia, A. conyzoides* and *S. acmella*) were effective inhibitors of the mycelia growth of *F. oxysporium* and *Trichophyton mentagrophytes* (Rai and Acharya 1999). The antifungal activities of *Tithonia diversifolia* (Obafemi *et al.*, 2006; Ragasa *et al.*, 2008) and *Spilanthes* sp. (Rai *et al.,* 2004; Sabitha and Suryanaraya, 2006) have been reported. The inhibitory activities of the extracts are of the order: *T. diversifolia* > *S. filicaulis* > *A. conyzoides.*

The differences in the fungitoxic potential among these plant extracts may be attributed to the susceptibility of the fungal pathogens to different concentrations of the extracts or due to the diverse structural and compositional aspect of the natural products hence variations in the degree of activity. This is in accordance with the report of Dellavalle *et al.,* (2011), Ilondu (2013a). The GC-MS analysis clearly showed a mixture of compounds in the extracts. The presence of these phytochemicals have been reported by other researchers including Odemena *et al.,* (2008), Rai *et al.,* (2004), Ragasa *et al.,* (2008). Ming (1999) reported that there is a high variability in the secondary metabolites of *A. conyzoides*.

Ethanolic extracts of many plants in the family Asteraceae have been subjected to GC-MS analysis (Abirami and Rajendra, 2012; Ilondu, 2013a, 2013b). Although the GC-MS analysis clearly showed a mixture of compounds in the extracts, the antifungal activity of plant extracts might not be due to the action of a single compound but the synergistic effect of several compounds that are in minor proportions in the extract. In our study, the extract from *T. diversifolia* reveals presence of 10 compounds and showed more antifungal activity against all the fungi tested compared to *A. conyzoides* with 18 compounds. According to Dellavalle *et al.,* (2011), this may be due to numerous compounds within the extract interfering with the actions of one another. Therefore the appropriate extract concentration to show a specific effect depends on the plant used and nature of the extract. Some of the identified compounds in the extract have been reported to have antifungal activities. Senthilkumar *et al.* (2011) isolated six compounds including diethyl phthalate from culture filtrate of *Trichoderma harzianum* which inhibited the growth of *Fusarium oxysporum.* Yang and Kang (2013) identified eight compounds in the essential oil from peels of *Citrus unshiu* which included diethyl phthalate. They demonstrated that the oil of *Citrus unshiu* peel had a damaging effect on the morphology of *Bacillus subtilis*. Deba *et al*., (2008) reported that β-caryophyllene and caryophyllene oxide were very fungitoxic against *Fusarium solani* and *Fusarium oxysporum.* According to Deba *et al.,* (2008) these chemical components exerts their toxic effects against the fungi through the destruction of membrane integrity. Choi *et al* (2010) have reported the antifungal activity of methyl end ethyl esters of fatty acids against plant pathogenic fungi. Similarly, Ilondu (2013a) reported that phytol caused inhibitory effects on the pathogen by damaging the cell membrane to allow the leakage of $K^+$ ions from the cell.

As reported by several researchers (Dellavalle *et al*., 2011; Abirami and Rajendra, 2012; Ilondu 2013a and Ilondu, 2013b), crude plant extracts are generally a mixture of active and non-active compounds. MIC values of less than 100mg/ml suggest good antimicrobial activity (Webster *et al.,* 2008). Excepting for the MIC (120mg/ml) for *A. conyzoides* against *F. lateritum,* all the MIC values against the other fungi and from other extracts were less than 100mg/ml demonstrating strong antifungal activity of the extracts in our study.

Although plant extracts with antifungal potential in *in-vitro* tests are not always effective under field conditions (Astiti and Suprata, 2012), several works showed the effectiveness of plant extracts in controlling plant diseases in the field. Enikuomehin (2005) reported that *Cercospora* leafspot disease of sesame (*Sesanum indicum* L.) was controlled with plant extracts including *Tithonia diversifolia.* Field evaluation of leaf extracts of some Asteraceae especially *T. diversifolia* were effective in the control of leafspot disease of sweetpotatoes in Abraka (Ilondu *et al.,* 2014).

## CONCLUSIONS

The extracts from *A. conyzoides, S. filicaulis* and *T. diversifolia* contain various constituents which are valuable source of new and biologically active molecules possessing antifungal properties.

The results of this study are important steps towards developing plant based fungicides which are cheaper and ecofriendly for the management of foliar diseases of crops and the development of commercial formulations of botanicals.

The extracts should be subjected to continuous *in-vivo* testing to evaluate the efficacy in controlling incidence of leafspot disease in agricultural crops.

Effective collaborations with plant pathologists, microbiologists, pharmacologists and chemists are crucial to see the complete development of interesting lead components into exploitable products.

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**REFERENCES**

Abirami P. and Rajendran A. 2012. GC-MS analysis of methanol extracts of *Vernonia cinera*. European Journal of Experimental Biology. 2(1): 9-12.

Adekunle A. A. 2001. Ethnobotanical studies of some medicinal plants from Lagos State of Nigeria. Nigerian Journal of Botany. 14: 71-79.

Akobundu I. O. and Agyakwa C. W. 1998. A Handbook of West African weeds 2nd edition. International Institute of Tropical Agriculture, Ibadan, Nigeria. p. 564.

Chiejina N.V. and Ukeh J.A. 2013. Efficacy of *Aframomum melegueta* and *Zingiber officinale* extracts on fungal pathogens of tomato fruits IOSR Journal of Pharmacy and Biological Sciences. 4(6): 13-16.

Choi J.G., Jang K.S., Choi Y.H., Yu J.H. and Kim J.C. 2010. Antifungal activity of lower alkyl fatty acid esters against powdery mildews. Plant Pathol. J. 26(4): 360-366.

Das K., Tiwari R.K.S. and Shrivastava D.K. 2010. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. Journal of Medicinal Plant Research. 4: 104-111.

Deba F., Xuan T.D., Yasuda M. and Tawata S. 2008. Chemical composition and antioxidant, antimicrobial and antifungal activities of the essential oils from *Bidens pilosa* Linn. var. Radiata. Food Control. 19: 346-352.

Dellavalle P.D., Cabrera A., Alem D., Larranaga P., Ferreira F. and Rizza M.D. 92011. Antifungal activity of medicinal plant extracts against phytopathogenic fungi *Alternaria* spp. Chilean Journal of Agricultural Research. 7(2): 231-239.

Enikuomehin O.A. 2005. Cercospora leafspot disease management in sesame (*Sesamum indicum* L.) with plant extracts. Journal of tropical Agriculture. 4(1-2): 19-23.

Evans W.C. and Trease G.E. 2002. Pharmacognosy. 5th Edition. Cambridge University Press, London. pp. 336-393.

Gurjar M.S., Ali S., Akhtar M. and Singh K.S. 2012. Efficacy of plant extracts in plant disease management. Agricultural Sciences. 3(3): 425-433.

Ilondu E.M. 2012. Fungitoxic activity of leaf extracts from four Asteraceae against *Sclerotium rolfsii* Sacc, an isolate of sweet potato (*Ipomoea batatas* (L.) Lam) vine rot disease. Journal of Agricultural and Biological Sciences. 3(2): 287-295.

Ilondu E.M. 2013a. Chemical constituents and comparative toxicity of *Aspilia africana* (pers) C.D Adams leaf extractives against two leafspot fungal isolates of Paw-paw (*Carica papaya* L.). Indian Journal of Science and Technology. 6(9): 5242-5248.

Ilondu E.M. 2013b. Phytochemical composition and efficacy of ethanolic leaf extracts of some *Vernonia* species against two phytopathogenic fungi. Journal of Biopesticides. 6(2): 165-172.

Ismet A., Shinwari M.M.A., Rashed S.A. and Bakir M.A. 2013. Evaluation of antimicrobial properties of two different extracts of *Juplan regia* tree bark and search for their compounds using Gas Chromatography - Mass Spectrum. International Journal of Biology. 5(2): 92-102.

Kamboj A. and Ajay K.S. 2008. *Ageratum conyzoides* L.: A review on its phytochemical and pharmacological profile. International Journal of Green Pharmacy. 2: 59-68.

Ming L.C. 1999. *Ageratum conyzoides*: A tropical source of medicinal agricultural products. In: Janick, J. (Ed). Perspective on new crops and new uses. ASHS Press Alexandra. pp. 469-473.

Ni Puta A.A. and Saprata D.N. 2012. Antifungal activity of teak (Tectona grandis L.F.) leaf extract against *Arthranium phaeospermum* (Corda), M.B. Ellis, the cause of wood decay on *Albiria falcataria* (L.) FOSBERG Journal of ISSAAS. 18(1): 62-69.

Ogbebor N., Adekunle A. T. and Enobakhare D. A. 2007. Inhibition of *Colletotrichum gloeosporiodies* (Penz) Sac. causal organism of rubber (*Hevea brasiliensis* Muell. Arg.) leafspot using plant extracts. African Journal of Biotechnology. 6(3): 213-218.

Perera A.N.F and Perera E.R.K. 2003. *Tithonia diversifolia*, A valuable Multipurpose Shrub: A review. Sri Lankan Journal of Agricultural Science. 40: 88-109.

Ragasa C. Y., Tepora M. M. and Rideout J. A. 2008. Terpenoids from *Tithonia diversifolia*. Journal of Research in Science, Computing and Engineering. 4(1): 1- 7.

Rai M. K., Varma A. and Pandey A. K. 2004. Antifungal potential of *Spilanthes calva* after inoculation of *Piriformospora indica*. Mycoses. 47: 479-481.

Sabitha A. R. and Suryanarayana U. M. 2006. Antifungal potential of flower head extract of *Spilanthes acemella* Linn. African Journal of Biomedical Research. 9: 67-68.

Seema M., Sreenivas S.S., Rekha N.D. and Devaki. 2011. In-vitro studies of some plant extracts against *Rhizoctonia solani* Kuhn infecting FCV tobacco in Karnataka Light soil, Karnataka, India. Journal of Agricultural Technology. 7(5): 1321-1329.

Senthilkumar G., Madhanraj P. and Panneccreselvam A. 2011. Studies on the compounds and its antifungal potentials of fungi isolated from Paddy Field soil of Jenbagapuram Village, Thanjavur District and South India. Asian Journal of Pharmaceutical Research. 1(1): 19-21.

Webster D., Taschereau P., Belland R.J. Sand C. and Rennie R.P. 2008. Antifungal activity of medicinal plant extracts. Preliminary screening studies. Journal of Ethnopharmacology, 115:140-146.

Yang X.N. and Kang S.C. 2013. Chemical composition, antioxidant and antibacterial activities of essential oil from Korean *Citrus unshiu* peel. Journal of Agricultural Chemistry and Environment. 2(3): 42-49.