# Quantitative High Performance Liquid Chromatographic Analysis of Simple Terpenes, Carotenoids, Phytosterols and Flavonoids in the Leaves of *Acalypha wilkesiana* Muell Arg.

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## ABSTRACT

The simple terpenes, carotenoids, phytosterols, and flavonoids compositions of the leaves of *Acalypha wilkesiana* were determined by high performance liquid chromatography (HPLC). The leaves were rich in flavonoids (about 251.3mg/kg dry weight), carotenoids (76.3mg/kg dry weight), and phytosterols (16.6mg/kg dry weight), but very low in simple terpenes (0.0084mg/kg dry weight). Three flavonoids (artemetin, luteolin, and vitexicarpin) were detected, with luteolin (about 35.85% of the flavonoid extract), having the highest concentration. Five carotenoids were detected, with carotene (56.76%), and lutein (21.80%) having the highest concentrations. Of the six phytosterols detected, the levels of stigmasterol (37.55%) and β-sitosterol (24.80%) were the highest. Myrcene (2.99%), β-amyrin (2.96%), lupeol (2.50%) and thujene (2.44%) were the highest amongst the twelve simple terpenes detected. These results indicate that the leaves are potential sources of allelochemicals and nutraceuticals, and in addition, justify their use in traditional medicine.

(Keywords: *Acalypha wilkesiana*, carotenoids, flavonoids, phytochemical, phytosterols, terpenes, HPLC, high performance liquid chromatography)

## INTRODUCTION

The three broad categories of plant secondary metabolites include: terpenes (or terpenoids), alkaloids, and phenolic compounds [Zwenger and Basu, 2008]. Terpenes are compounds built up from isoprene subunits. They are volatile substances which give plants and flowers their fragrance, and are manufactured by plants in response to herbivory or stress factors, to attract beneficial mites, which feed on the herbivorous insects [Kessler and Baldwin, 2001]. They are also emitted by flowers to attract pollinating insects [Kappers *et al*., 2005; Zwenger and Basu, 2008]. Less volatile but strongly bitter-tasting or toxic terpenes also act as anti-feedants (i.e., they protect some plants from being eaten by animals).

Terpenes play an important role as signal compounds and growth regulators (phytohormones) of plants [Breitmaier, 2006], accessory pigments during photosynthesis [Dillard and German, 2000], and have shown antimicrobial activities [Islam e*t al*., 2003]. They include monoterpenoids, iridoids, sesquiterpenoids, sesquiterpene lactones, diterpenoids, triterpenoid saponins, steroid saponins, cardenolides and bufadienolides, phytosterols, cucurbitacins, nortriterpenoids, other triterpenoids, and carotenoids [Dillard and German, 2000; Breitmaier, 2006].

The carotenoids are a very popular family of terpenoids found in all parts of the plant, but are usually most noticeable in the flowers. Because of their ability to attract insects and birds, these plant pigments mediates pollination and seed dispersal, thus playing an important ecological role [Tinoi *et al.,* 2006]. Several different classes of pigments are responsible for coloration but in many yellow/orange/red flowers, the pigments synthesized by the plant are carotenoids. In photosynthetic organisms, specifically flora, they play a vital role in the photosynthetic reaction centre, either by participating in the energy-transfer process, or protecting the reaction center from auto-oxidation. They are linked to

photosynthesis, photoprotection and plastid structure [Tinoi *et al.,* 2006].

Phytosterols, another important terpene subclass [Dillard and German, 2000], are a group of naturally occurring steroid alcohols found in plants and their oils. They are a key structural component of plant cell membranes, assuming the role that cholesterol plays in mammalian cells [Piironen *et al*., 2000]. They are primarily encountered in the plasma membrane, the outer membrane of mitochondria and the endoplasmic reticulum, and to a large extent determine the properties of these membranes. They participate in the control of membrane-associated metabolic processes, which involves the action of a few specific sterols. They also play an important role in cellular and developmental processes in plants as precursors to the brassinosteroids [Rao *et al*., 2002; Sasse, 2003; Ayad *et al*., 2009]. In addition, they act as precursors for a wide variety of secondary metabolites such as the glycoalkaloids, cardenolides, and saponins.

The flavonoids subclass of phenolics are primarily recognized as the pigments responsible for the autumnal burst of hues and the many shades of yellow, orange, and red in flowers and food [Dillard and German, 2000, Middleton *et al*., 2000]. They participate in the light-dependent phase of photosynthesis during which they catalyze electron transport [Das, 1994]. They are found in fruits, vegetables, nuts, seeds, herbs, spices, stems, flowers [Middleton *et al*., 2000]. They are of particular importance in the human diet as there is evidence that they act as antioxidants, antiviral, anti-inflammatory agents [Soetan, 2008], and are associated with reduced risk of cancer and cardiovascular disease [Middleton *et al*., 2000; Grubešić *et al*., 2007].

Earlier workers [Akinde, 1986; Gill, 1992; Adesina *et al*., 2000; Yusha'u *et al*., 2008] have reported the presence of terpenes, steroids and flavonoids in the leaves of *Acalypha wilkesiana*, a common Nigerian ornamental and medicinal plant. However, these reports were either on the qualitative or evaluation of the total content of each group. Little or nothing has been reported about the individual components of each group. Therefore, in the present study, we investigated by HPLC, the detailed simple terpenes, carotenoids, phytosterols and flavonoids contents of the leaves of *Acalypha wilkesiana*.

## MATERIALS AND METHOD

### Collection of Plant Samples

Samples of fresh leaves of *Acalypha wilkesiana* were collected from within the Abuja and Choba Campuses of University of Port Harcourt, Port Harcourt, Nigeria. They were duly identified at the University of Port Harcourt Herbarium, Port Harcourt, Nigeria, after which they were rid of dirt and stored for subsequent use. All reagents used were HPLC-grade purity.

### Calibration, Identification and Quantification

Standard solutions were prepared in methanol for flavonoids, acetone for carotenoids, and methylene chloride for phytosterols and terpenes. The linearity of the dependence of response on concentration was verified by regression analysis. Identification was based on comparison of retention times and spectral data with standards. Quantification was performed by establishing calibration curves for each compound determined, using the standards. The result of the calibration of the HPLC system is shown in Table 1.

### Determination of Simple Terpenes Composition

3.0g of the pulverized sample was extracted three times with 30mL of ethylacetate solvent for 15minutes at $40^0$C. The resultant mixture was filtered into a pre-cleaned borosilicate 100mL beaker. After filtration, the acidic compounds were extracted three times with 10mL of 5% aqueous potassium hydroxide. This was followed by the extraction of the basic compounds with another 10mL of 5% aqueous hydrochloric acid. The organic fraction that contained the neutral compounds was washed with 30mL of deionized water.

The extract was concentrated using a rotary evaporator, to 30mL. The concentrated mixture was centrifuged at 6000rpm for 10minutes. After the removal of the suspended particles, the solvent was removed by evaporation. The residue was later dissolved in methylene chloride for chromatographic analysis.

**Table 1:** Calibration Data of the HPLC System.

| Compounds | Correlation Constant | Relative Resolution (%) | Equation |
|---|---|---|---|
| **a.** | | | |
| Flavonoids | | | |
| ➢ Artemetin | 0.99935 | -4.255 | Area=0.0064*Amt-0.0066667 |
| ➢ Luteolin | 0.99834 | 6.452 | Area=0.04*Amt+0.0666667 |
| ➢ Vitexicarpin | 0.99878 | -5.882 | Area=0.07*Amt-0.1 |
| **b.** | | | |
| arotenoids | | | |
| ➢ Neo-xanthine | 0.99915 | -8.256 | Area=4.409*Amt+0 |
| ➢ Viola-xanthine | 0.99915 | -8.256 | Area=4.409*Amt+0 |
| ➢ Anthera-xanthine | 0.99872 | -10.109 | Area=3.9014*Amt+0 |
| ➢ Carotene | 0.99959 | -5.738 | Area=0.6901*Amt+0 |
| ➢ Lutein | 0.99921 | -7.976 | Area=3.4806*Amt+0 |
| **c.** | | | |
| Simple Terpenes | | | |
| ➢ Camphene | 0.99935 | -4.223 | Area=227952291*Amt-23568.259 |
| ➢ Sabinene | 0.99886 | -5.494 | Area=227811657*Amt-30448.865 |
| ➢ Limonene | 0.99886 | -5.494 | Area=227811657*Amt-30448.865 |
| ➢ α-Pinene | 0.99886 | -5.494 | Area=227811657*Amt-30448.865 |
| ➢ β-Pinene | 0.99933 | -4.286 | Area=227946806*Amt-23913.056 |
| ➢ Ocimene | 0.99813 | -6.941 | Area=37552689.7*Amt-6354.1585 |
| ➢ Myrcene | 0.99981 | -2.281 | Area=45494.8168*Amt-2.5656645 |
| ➢ Thujene | 0.99728 | -8.572 | Area=8.78612E11*Amt-180576397 |
| ➢ α-Amyrin | 0.99931 | -4.216 | Area=2.27814E12*Amt-234999687 |
| ➢ β-Amyrin | 0.99589 | -10.480 | Area=2.27379E12*Amt-566014632 |
| ➢ Taraxerol | 0.99911 | -4.686 | Area=2.1766E12*Amt-248511898 |
| **d.** | | | |
| Phytosterols | | | |
| ➢ Cholesterol | 0.99950 | -3.704 | Area=12.7*Amt-11.546667 |
| ➢ Cholestanol | 0.99927 | -4.512 | Area=15*Amt-16.546667 |
| ➢ Campesterol | 0.99991 | -1.580 | Area=8.2*Amt-3.2133333 |
| ➢ Stigmasterol | 0.99992 | -1.446 | Area=0.13*Amt-0.0466667 |
| ➢ β-Sitosterol | 0.99973 | -2.703 | Area=0.07*Amt-0.0466667 |
| ➢ Tocopherol | 0.99783 | -7.913 | Area=0.55*Amt-1.0466667 |

Amt = amount.

2µL of each sample was injected into the HPLC instrument consisting of a Hewllet-Packard 6890 HPLC with flame ionization detector (FID), powered by HP Chemstation Rev A 09.01 (1206) software. The column was ZB-5 Column (30cm × 0.32mm × 0.25µm film thickness) eluted at a flow rate 1mL/min with methanol/water/tetrahydrofuran (94:5:1, v/v/v), pH 5 (adjusted with acetic acid) as mobile phase; the detection wavelength set at 220nm.

## Determination of Carotenoid Composition

All procedures were carried out in a laboratory equipped with yellow lights that did not include the ultraviolet end of the spectrum. 10.0g of the pulverized sample was weighed into a pre-cleaned borosilicate beaker, and homogenized with 150mL of acetone for 1hour. The resultant mixture was then filtered by suction. The filtrate was concentrated and recovered residue was extracted with 50mL of a mixture of diethyl ether and petroleum ether. The extract was concentrated after drying with anhydrous sodium sulphate and made up to 10mL with acetone. The carotenoid extracts were analyzed using a Hewllet-Packard HP6890 HPLC with flame ionization detector (FID; range scanned, 300 to 600nm) and powered with HP Chemstation Rev A 09.01 (1206) software. The capillary column was a ZP-5 Column (30cm × 0.32mm × 0.25µm

film thickness) operating at 22°C, detected at 450nm. The eluent was a mixture of acetonitrile and methanol (65:35, v/v) with a flow rate of 1.0mL/min. The injection volume was 25µL.

**Determination of Phytosterol Composition**

3.0g of the pulverized sample was extracted three times with 30mL of ethylacetate solvent for 15minutes at $40^0$C. The resultant mixture was filtered into a pre-cleaned borosilicate 100mL beaker. After filtration, the acidic compounds were extracted three times with 10mL of 5% aqueous potassium hydroxide. This was followed by the extraction of the basic compounds with another 10mL of 5% aqueous hydrochloric acid. The organic fraction that contained the neutral compounds was washed with 30mL of deionised water. The extract was concentrated to 30mL, before centrifuging at 6000rpm for 10minutes. After the removal of the suspended particles, the solvent was removed by evaporation, and the residue was dissolved in methylene chloride for chromatographic analysis.

2µL of each sample was injected into the HPLC instrument consisting of a Hewllet-Packard 6890 HPLC with flame ionization detector (FID), powered by HP Chemstation Rev A 09.01 (1206) software. The column was ZB-5 Column (30cm × 0.32mm × 0.25µm film thickness) eluted at a flow rate 1mL/min with methanol/water/tetrahydrofuran (94:5:1, v/v/v), pH 5 (adjusted with acetic acid) as mobile phase; the detection wavelength set at 220nm.

**Determination of Flavonoid Composition**

The method reported by Markowski and Płocharski [2006] was adopted. 1.0g of the pulverized sample was extracted with 10.0mL of methanol, at $60^0$C for 15minutes. The extract was filtered with Whatman No. 1 filter paper and concentrated to 1.0mL for chromatographic analysis. 20µL of this solution was injected for HPLC analysis.

HPLC analysis was carried with an HP 6890 (Hewlett Packard, Wilmington, DE, USA), HPLC apparatus, fitted with a flame ionization detector (FID), and powered with HP Chemstation Rev A 09.01 (1206) software. The capillary column was a HP-5 Column (30m × 0.32mm × 0.25µm film thickness). The mobile phase consisted of 10.2% acetic acid in 2mM sodium acetate (solvent A) and acetonitrile (solvent B). The flow rate was kept constant at 0.5 ml/min for a total run time of 72min at $25^o$C. The system was run with a gradient program: 3% B (0-20min); 3-35% B (45min); 35-90% B (3min); 90-90% B (4min); 90-0% B (1min). The column was equilibrated for ten minutes at initial conditions.

**Data Analysis**

Comparisons were based on simple percentages.

**RESULTS AND DISCUSSION**

Table 2 shows the flavonoids composition of the leaves of *A. wilkesiana*. The leaves have high flavonoids contents, 106.5551mg total flavonoid/kg wet weight and 251.3092mg total flavonoid/kg dry weight. Three flavonoids, artemetin (28.16%)[1], luteolin (35.85%) and vitexicarpin (27.73%) and an unidentified component (8.26%) were detected, with luteolin having the highest concentration. Flavonoids are known to act as allelochemicals [Yoshioka *et al*., 2004]. Luteolin is reported to have anti-mutagenic, anti-inflammatory, antioxidant, immune-modulating and antibacterial activities [Dillard and German, 2000]. Vitexicarpin is an anti-inflammatory and immune-modulating agent [You *et al*., 1998]. Artemetin has anti-inflammatory and anti-edematous activity [Sertie *et al*., 1990].

The carotenoids composition of the leaves of *Acalypha wilkesiana* is given in Table 3. The leaves have moderate levels of carotenoid content, 32.34217mg total carotenoid/kg wet weight and 76.27870mg total carotenoid/kg dry weight. The main carotenoids were carotene (56.76%) and lutein (21.80%), with lower levels of neo-xanthine (11.69%), viola-xanthine (4.59%) and anthera-xanthine (5.17%). Lutein has anti-oxidant [Tinoi *et al.,* 2006] and photo-protective activities [Pintea *et al*., 2003]. Carotenes have pro-vitamin A and antioxidant activities [Tinoi *et al.,* 2006].

The simple terpenes composition of *Acalypha wilkesiana* is given in Table 4. The leaves had very low terpenes content, 0.00035648mg total terpenes/kg wet weight and 0.00084074mg total terpenes/kg dry weight.

**Table 2:** Flavonoid Composition of the Leaves of *Acalypha wilkesiana.*

| Compounds | R. time (min) | Composition (mg/kg) /Wet weight | /Dry weight |
|---|---|---|---|
| Total flavonoids | - | 106.5551 | 251.3092 |
| ➢ Artemetin | 19.111 | 30.0032 | 70.7623 |
| ➢ Luteolin | 16.267 | 38.2042 | 90.1042 |
| ➢ Vitexicarpin | 17.714 | 29.5443 | 69.6799 |
| ➢ Unidentified component | 8.599 | 8.8034 | 20.7629 |

R. time = retention time

**Table 3:** The Carotenoid Content of *Acalypha wilkesiana*.

| Compounds | R. time (min) | Composition (mg/kg) /Wet weight | /Dry weight |
|---|---|---|---|
| Total carotenoids | - | 32.34217 | 76.27870 |
| ➢ Neo-xanthine | 22.404 | 3.78225 | 8.92039 |
| ➢ Viola-xanthine | 23.979 | 1.48339 | 3.49857 |
| ➢ Anthera-xanthine | 25.484 | 1.67046 | 3.93976 |
| ➢ Carotene | 26.935 | 18.35685 | 43.29445 |
| ➢ Lutein | 28.252 | 7.04922 | 16.62552 |

R. time = retention time

**Table 4:** The Simple Terpenes Content of *Acalypha wilkesiana.*

| Compounds | R. time (min) | Composition (mg/kg) /Wet weight | /Dry weight |
|---|---|---|---|
| Total simple terpenes | - | 0.0035648 | 0.0084074 |
| ➢ Camphene | 7.715 | 0.0000438 | 0.0001034 |
| ➢ Sabinene | 9.592 | 0.0000571 | 0.0001346 |
| ➢ Limonene | 11.141 | 0.0000568 | 0.0001339 |
| ➢ α-Pinene | 11.356 | 0.0000567 | 0.0001338 |
| ➢ β-Pinene | 12.813 | 0.0000446 | 0.0001052 |
| ➢ Ocimene | 14.176 | 0.0000720 | 0.0001699 |
| ➢ Myrcene | 14.902 | 0.0001065 | 0.0002513 |
| ➢ Thujene | 15.337 | 0.0000871 | 0.0002055 |
| ➢ α-Amyrin | 15.405 | 0.0000437 | 0.0001031 |
| ➢ β-Amyrin | 16.364 | 0.0001055 | 0.0002489 |
| ➢ Lupeol | 16.569 | 0.0000891 | 0.0002101 |
| ➢ Taraxerol | 17.665 | 0.0000484 | 0.0001142 |
| ➢ Unidentified component | 2.922 | 0.0027532 | 0.0064935 |

R. time = retention time.

The main terpenes were myrcene (2.99%), β-amyrin (2.96%), lupeol (2.50%) and thujene (2.44%); others detected include camphene (1.23%), sabinene (1.60%), limonene (1.59%), α-pinene (1.59%), β-pinene (1.25%), ocimene (2.02%), α-amyrin (1.23%) and taraxerol (1.36%), and an unidentified component (77.24%). In a bioremediative study [Suttinun *et al*., 2004], limonene and pinene were found to increase the uptake and subsequent degradation of trichloroethylene by bacteria. Limonoids have anti-cancer properties [Dillard and German, 2000]. Limonene promotes

glutathione-S-transferase and cancer cell apoptosis.

The leaves have moderate levels of phytosterols (Table 5), and this is mainly made up of stigmasterol (37.55%) and β-sitosterol (24.80%), with relatively lower levels of cholesterol (8.03%), cholestanol (7.25%), campesterol (5.76%), and tocopherol (16.60%). Stigmasterol belongs to brassinosteroids which are one of growth regulator and signaling molecules essential for normal plant growth [Rao *et al*., 2002; Ayad *et al*., 2009]. Brassinosteroids have the ability to confer resistance to plants against various abiotic stresses [Priti, 2003]. As a group of plant hormones, steroids have a significant regulatory function in cell elongation and division, vascular differentiation and other diverse developmental processes [Sasse, 2003].

Plant sterols modulate the activity of ATPase [Piironen *et al*., 2000]. Cholesterol and stigmasterol stimulate the export of $H^+$ at low concentrations, whereas all other sterols act as inhibitors. α-Tocopherol (vitamin E) is an antioxidant which mainly protect membrane from oxidative damage. Tocopherols function prominently in the protection of polysaturated fatty acids from lipid peroxidation. A positive correlation has been reported between α–tocopherol content and shoot or root growth under drought [Ayad *et al*., 2009]. In animals, β-sitosterol and its glycoside exhibit anti-inflammatory, anti-neoplastic, anti-pyretic, immune-modulating, cholesterol lowering and atheroprotective activity [Dillard and German, 2000].

## CONCLUSIONS

In conclusion, the above results indicate that the leaves are rich in flavonoids, carotenoids and phytosterols, and so can serve as good sources of them. It also justifies their use in traditional medicine.

## ENDNOTES

1. Percentages are based on the weight of the compounds per its total extract, whether flavonoids, carotenoids, phytosterols, or terpenes.

## REFERENCES


1. Adesina, S.K., O. Idowu, A.O. Ogundaini, H. Oladimeji, T.A. Olugbade, G.O. Onawunmi, and M. Pais. 2000. "Antimicrobial Constituents of the Leaves of *Acalypha wilkesiana* and *Acalypha hispida*". *Phytotherapy Research*. 14:371-374.

2. Akinde, B.E. 1986. "Phytochemical and Microbiological Evaluation of the oils from the Leaves of *Acalypha wilkesiana*". In: A. Sofowora, (ed.), *The State of Medicinal Plant Research in Nigeria*. University of Ibadan Press: Nigeria, pp 362-363.

3. Ayad, H.S., K.M. Gamal El-Din, and F. Reda. 2009. "Efficiency of Stigmasterol and α-Tocopherol Application on Vegetative Growth, Essential Oil Pattern, Protein, and Lipid Peroxidation of Geranium (*Pelargonium Graveolens* L.)". *Journal of Applied Sciences Research*. 5:887-892.


**Table 5:** The Phytosterol Composition of *Acalypha wilkesiana.*

| Compounds | R. time (min) | Composition (mg/kg) /Wet weight | Composition (mg/kg) /Dry weight |
|---|---|---|---|
| Total phytosterols | - | 7.05204 | 16.63218 |
| ➢ Cholesterol | 19.568 | 0.56622 | 1.33542 |
| ➢ Cholestanol | 20.620 | 0.51150 | 1.20636 |
| ➢ Campesterol | 21.504 | 0.40647 | 0.95866 |
| ➢ Stigmasterol | 23.412 | 2.64793 | 6.24511 |
| ➢ β-Sitosterol | 24.820 | 1.74922 | 4.12552 |
| ➢ Tocopherol | 26.046 | 1.17071 | 2.76110 |

R. time = retention time

4. Das, D.K. 1994. “Naturally Occurring Flavonoids: Structure, Chemistry, and High Performance Liquid Chromatography Methods for Separation and Characterization”. *Methods in Enzymology*. 234: 410–420.

5. Dillard, C.J. and J.B. German, 2000. “Phytochemicals: Nutraceuticals and Human Health”. *Journal of the Science of Food Agriculture*. 80:1744–1756.

6. Gill, L.S. 1992. *Ethnommedical uses of Plants in Nigeria*. Uniben Press, Benin City, Nigeria. 276.

7. Grubešić, R.J., J. Vuković, D. Kremer, and S. Vladimir-Knežević. 2007. “Flavonoid Content Assay: Prevalidation and Application on Plantago L. Species”. *Acta Chim. Slov.* 54:397–406.

8. Islam, A.K., M.A. Ali, A. Sayeed, S.M. Salam, A. Islam, M. Rahman, G.R. Khan, and S. Khatun. 2003. “An Antimicrobial Terpenoid from *Caesalpinia pulcherrima* Swartz.: Its Characterization, Antimicrobial, and Cytotoxic Activities”. *Asian Journal of Plant Science*. 2:17-24.

9. Kappers, I.F., A. Aharoni, T. Van Herpen, L. Luckerhoff, M. Dicke, and H.J. Bouwmeester. 2005. “Genetic Engineering of Terpenoid Metabolism Attracts Bodyguards to Arabidopsis”. Science. 309: 2070-2072.

10. Kessler, A. and T. Baldwin 2001. “Defensive Function of Herbivore-Induced Plant Volatile Emission in Nature”. *Science*. 291: 2141-2144.

11. Markowski, J. and W. Płocharski, 2006. “Determination of Phenolic Compounds in Apples and Processed Apple Products”. *Journal of Fruit and Ornamental Plant Research*. 14:133-142.

12. Middleton, R. Jr., C. Kandaswami, and T.C. Theoharides. 2000. “The Effects of Plant Flavonoids on Mammalian Cells: Implications for Inflammation, Heart Disease and Cancer”. *Pharmacological Reviews*. 52:673-751.

13. Piironen, V., D.G. Lindsay, T.A. Miettinen, J. Toivo, and A.-M. Lampi. 2000. “Plant Sterols: Biosynthesis, Biological Function and Their Importance to Human Nutrition”. *Journal of the Science of Food Agriculture*. 80:939-966.

14. Pintea, A., C. Bele, S. Andrei, and C. Socaciu. 2003. “HPLC Analysis of Carotenoids in Four Varieties of *Calendula officinalis* L. flowers”. *Acta Biologica Szegediensis*. 47:37-40.

15. Priti, K. 2003. “Brassinosteroid Mediated Stress Responses”. *Journal of Plant Growth Regulators*. 22: 289 – 297.

16. Rao, S.S.R., B.V.V. Vardhini, E. Sujatha, and S. Anuradha. 2002. “Brassinosteroids – A new class of Phytohormones”. *Current Science*. 82:1239–1245.

17. Sasse, J.M. 2003. “Physiological Actions of Brassinosteroids: An Update”. *Journal of Plant Growth Regulators*. 22:276–288.

18. Sertie, J.A., A.C. Basile, S. Panizza, A.K. Matida, and R. Zelnik. 1990. “Anti-Inflammatory Activity and Sub-Acute Toxicity of Artemetin”. *Planta Medica*. 56:36-40.

19. Soetan, K.O. 2008. “Pharmacological and Other Beneficial Effects of Antinutritional Factors in Plants: A Review”. *African Journal of Biotechnology*. 7:4713-4721.

20. Suttinun, O., P.B. Lederman, and E. Luepromachai. 2004. “Application of Terpene-Induced Cell for Enhancing Biodegradation of TCE Contaminated Soil”. *Songklanakarin Journal of Science and Technology*. 26:131-142.

21. Tinoi, J., N. Rakariyatham, and R.L. Deming. 2006. “Determination of Major Carotenoid Constituents in Petal Extracts of Eight Selected Flowering Plants in the North of Thailand”. *Chiang Mai Journal of Science*. 33:327–334.

22. Yoshioka, T., T. Inokuchi, S. Fujioka, and Y. Kimura. 2004. “Phenolic Compounds and Flavonoids as Plant Growth Regulators from Fruit and Leaf of *Vitex rotundifolia*”. *Z. Naturforsch*. 59c: 509-514.

23. You, K.M., K.H. Son, H.W. Chang, S.S. Kang, and H.P. Kim. 1998. “Vitexicarpin, a Flavonoid from the Fruits of *Vitex rotundifolia*, Inhibits Mouse Lymphocyte Proliferation and Growth of Cell Lines *in vitro*”. *Planta Medica*. 64:546-50.

24. Yusha’u, M., B.S. Aliyu, and S.O. Olonitola. 2008. “Preliminary Screening of Acalypha Extracts for In Vitro Inhibitory Activity against Extended-Spectrum B-Lactamase Producing Enterobacteriaceae”. *International Journal of Pure and Applied Science*. 2:1-5.

25. Zwenger, S. and C. Basu. 2008. “Plant Terpenoids: Applications and Future Potentials”. *Biotechnology and Molecular Biology Reviews*. 3: 001-007.

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## SUGGESTED CITATION

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