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# Chemical Constituents in Extracts from Leaves of *Lantana trifolia* and Their *In Vitro* Anti-oxidative Activity

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ABSTRACT

**Objective** To isolate, purify, and analyze the anti-oxidants from the leaves of *Lantana trifolia*. **Methods** The anti-oxidative activities of the crude extracts from liquid-liquid extraction of *L. trifolia* leaves were assayed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method to assess their radical scavenging and reducing abilities. The total flavonoids and phenol contents in the ethyl acetate fraction were determined by colorimetric and Folin-Ciocalteu methods, respectively. Chemical constituents were isolated from the ethyl acetate fraction and repeatedly purified using silica gel, Sephadex LH-20 column chromatography, and HPLC, respectively. The chemical structures isolated were identified by spectral analysis and chemical evidence. **Results** Ethyl acetate partition from liquid-liquid extraction exhibited the highest anti-oxidative activity with an $IC_{50}$ value of 4.94 μg/mL, close to that of the standard (vitamin C, VC, 4.23 μg/mL). The extract was proved to contain total flavonoids and phenol contents with values of (39.0 ± 1.6) and (29.27 ± 1.46) mg/g, respectively. Six compounds were isolated and identified as kaempferol-3,7-dimethyl ether (**1**), verbascoside (**2**), apigenin (**3**), umuhengerin (**4**), ladanetin (**5**), and scutellarein-7-*O*-β-*D*-apiofuranoside (**6**). **Conclusion** The ethyl acetate extract from the leaves of *L. trifolia* possesses the potent anti-oxidative and free radical scavenging activities which are directly proportional to the concentration of phenolic contents. The anti-oxidative activity of the extract from the leaves of *L. trifolia* is due to its proton donating ability that converts free radicals to more stable products and terminates chain reactions. Compound **1** is isolated from the plants of *Lantana* Linn. for the first time. The mechanisms may be related to the therapeutic benefits of the certain traditional claims of wild *L. trifolia*.

*Key words*

anti-oxidative activity; diphenyl picryl hydrazine; *Lantana trifolia*



## 1. Introduction

*Lantana* Linn. (from the Latin *lento*, to bend) probably derives from the ancient Latin name of the genus *Viburnum* Linn., which resembles a little foliage and inflorescence (Lu and Atkins, 2004). The plants in *Lantana* linn. produce a number of metabolites in high quantities and some possess useful biological activities. *Lantana* Linn. is free from diterpenoids and rich in essential oils. Monoterpenes, triterpenes, flavones, coumarin, steroids, iridoid, glycosides, and caffeic acid derivatives are also reported with triterpenes and flavones being the most abundant secondary metabolites. The

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plants in *Lantana* Linn. are used in folk medicine in many parts of the world (Nagao et al, 2002; Hayashi et al, 2004; Akhtar et al, 2006).

From the methanol extract of the dried leaves of *L. trifolia* L., a new antimicrobially active and polymethoxylated flavone was isolated and named as umuhengerin (Rwangabo et al, 1988). The methanol extract of the aerial parts of *L. trifolia* had previously been assessed for the anti-inflammatory, antinociceptive, and antipyretic effects in experimental animals. The extract produced an inhibitory effect on carrageenan-induced edema in the rat paw over a dose range of 10−300 mg/kg. The extract also produced small but significant increase in the response latency of rats subjected to the hot plate, and a thermal pain test only detected analgesia by high efficacy agents. Therefore, the *L. trifolia* extract could have therapeutically relevant anti-inflammatory and analgesic properties in human (Uzacátegui et al, 2004). In a present study, phytochemical investigation of the ethyl acetate extract of *L. trifolia* led to the isolation and identification of flavonoids and phenylpropanoids, which were compounds reported to have sedative properties. The sedative effect of *L. trifolia* extracts on mice can not be attributed to the direct activation of the central benzodiazepine site but by the action of flavones, phenylpropanoids, and verbascoside (Julião et al, 2009). The healing properties of medicinal plants have been typically attributed to their phenolic content, mostly flavonoids and phenolic acids, and their probable role in the prevention of disease associated with oxidative stress (Scalbert et al, 2005). The screening of plant extracts using the DPPH free radical method proved to be effective for the selection of those components with anti-oxidative activity. These extracts may be rich in radical scavengers, such as flavonoids, known as anti-oxidants. Further more detailed studies on the chemical composition of those extracts, as well as studies with other models such as lipid peroxidation and *in vivo* assays are essential to characterize them as biological anti-oxidants (Mensor, 2001).

There is an increasing interest in anti-oxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, and to prevent the deterioration of fats and other constituents of food stuffs. In both cases there is preference for anti-oxidatant from natural rather than from synthetic sources (Abdalla and Roozen, 1999). *Lantana* Linn. is mostly native to subtropical America, but a few taxa are indigenous to tropical Asia and Africa. In Kenya, it is mainly distributed at Kambi ya moto region of Nakuru county where the leaves are medicinally used in the form of infusions to treat respiratory inflammatory diseases.

## 2. Materials and methods

### *2.1 Preparation of extracts*

The leaves of *Lantana trifolia* L. (5 kg), were collected at Kambi ya moto, Nakuru county, Kenya in January 2011. The plant was identified by Thomas Rotich, Botany Department of Egerton University, Kenya. A voucher specimen (PSI-TUTCM) has been retained at the herbarium of Tianjin University of Chinese Traditional Medicine. The dried leaves from *L. trifolia* were ground into fine powder using an electric hammer mill. The pulverized sample (3.5 kg) was exhaustively extracted with 95% and 75% ethanol at room temperature. The extracts were concentrated separately under reduced pressure using rotary evaporator to afford brown syrup. TLC experiment of both 95% and 70% crude ethanolic extracts gave the same result, so they were combined and placed on water bath to evaporate the remaining traces of ethanol. The extract was partitioned between water and organic solvents of increasing polarities to afford the new separate extracts in petroleum ether, chloroform, ethyl acetate, and *n*-butanol.

### *2.2 DPPH radical-scavenging activity*

DPPH radical-scavenging activity of the extract was determined according to the methods by Blois (1958). An ethanol solution of DPPH radical was prepared by weighing 10 mg of DPPH into 250 mL volumetric flask and diluting with absolute ethanol to 250 mL, then stored at 10 $^{o}$C in the dark. An ethanol solution of the test compound (vitamin C, VC) was prepared by weighing 0.1 g of VC powder into 25 mL volumetric flask and diluting with absolute ethanol to 25 mL. Five consecutive serial dilutions were prepared by transferring 12.5 mL from each of the newly prepared 25 mL solution. An ethanol solution of each organic partition from liquid-liquid extraction was prepared by weighing 250 mg to 25 mL volumetric flask and diluting with absolute ethanol to 25 mL. Five consecutive serial dilutions of each sample were prepared by transferring 12.5 mL from each newly prepared 25 mL solution. DPPH solution (1 mL) was diluted with 1 mL absolute ethanol and absorbance ($A_c$) measurements were immediately recorded with a UV-visible spectrophotometer under 517 nm. The decrease in absorbance at 517 nm was continuously determined, with data being recorded at 1 min intervals until the absorbance stabilized. VC sample solution (2 mL) starting from low concentration was added to 2 mL DPPH solution into three different test tubes and set aside for 30 min, then their absorbance ($A_i$) was detected under 517 nm. DPPH solution (2 mL) was added to 2 mL of each sample solution of the serial dilutions. Their absorbance ($A_j$) was detected under 517 nm. All the determinations were performed in three replicates and averaged.

### *2.3 Determination of total flavonoids*

The total flavonoid content of the ethyl acetate partition was determined according to colorimetric method. The sample solution (0.5 mL) was mixed with 2 mL of distilled water and subsequently with 0.15 mL of 5% $NaNO_2$ solution. After 6 min of incubation, 0.15 mL of 10% $AlCl_3$ solution was added and then allowed to stand for 6 min, followed by adding 2 mL of 4% NaOH solution to the mixture. Immediately, water was added to the sample to bring the final volume to 5 mL. The mixture was thoroughly mixed and

allowed to stand for another 15 min. The absorbance of the mixture was determined at a wavelength of 510 nm. The total flavonoids content was calculated from a calibration curve.

### *2.4 Determination of total phenols*

The total phenol content of ethyl acetate partition was analyzed according to the Folin-Ciocalteu method as described by Wu et al (1994). After 2 g of sample was well mixed with 2.5 mL of distilled water, 0.5 mL of the Folin-Ciocalteu stock reagent and 1.0 mL of $Na_2CO_3$ reagent (75 g/L) were added to the mixture. They were then incubated at room temperature for 30 min. The absorbance of the mixture was spectrophotometrically measured at a wavelength of 765 nm. The total phenol content was determined and expressed in gallic acid equivalent (GAE).

### *2.5 Isolation and purification*

Column chromatography technique was used to fractionate the crude extract into enriched fractions and eventually purify the actual active compounds. The ethyl acetate fraction (73 g) was separated using gradient elution approach on silica gel column with a biphasic solvent mixture composed of dichloromethane-ethyl acetate (100:1 to 1:5) at a flow rate of 3 mL/min. Fractions (30 mL) were collected and concentrated using a rotary evaporator. The experiment was monitored by TLC experiment to monitor the change of the polarity of the eluting solvent in relation to elutes which were later pooled according to their retention factor on the TLC plate. Gradient elution on silica gel was not very sufficient for isolation, therefore the collected fractions were further purified using Sephadex LH-20 column with methanol as the eluent at a flow rate of 2 mL/min. HPLC analysis ($C_{18}$ column, 150 mm × 4.6 mm, 5 μm) using LC solution machine and preparative HPLC (PHPLC) was employed to distinctively isolate more compounds. The structures of the isolated compounds were elucidated by Bruker 600 MHz NMR technique ($^1$H-NMR, $^{13}$C-NMR, and DEPT).

## 3. Results and discussion

### *3.1 DPPH radical-scavenging activity*

As shown in Table 1, the $IC_{50}$ values for DPPH radical-scavenging activity of the extracts were 4.23, 57.30, 17.37,

**Table 1 DPPH essay**

| Samples | $A_i$ | $A_j$ | Concentration / (μg·mL$^{-1}$) | Scavenging rates / % | $IC_{50}$ / (μg·mL$^{-1}$) |
|---|---|---|---|---|---|
| VC | 0.065 | 0.037 | 33.00 | 95.25 | 4.23 |
| (positive control) | 0.068 | 0.037 | 17.00 | 94.75 | |
| | 0.131 | 0.036 | 8.00 | 83.89 | |
| | 0.384 | 0.036 | 4.00 | 41.02 | |
| | 0.520 | 0.036 | 2.00 | 17.97 | |
| | 0.581 | 0.036 | 1.00 | 7.63 | |
| sample I | 0.311 | 0.289 | 1253 | 96.27 | 57.30 |
| (chloroform extract) | 0.170 | 0.145 | 626 | 95.76 | |
| | 0.103 | 0.075 | 313 | 95.25 | |
| | 0.123 | 0.040 | 157 | 85.93 | |
| | 0.308 | 0.020 | 78 | 51.19 | |
| | 0.426 | 0.010 | 39 | 29.49 | |
| sample II | 0.082 | 0.013 | 157 | 88.06 | 17.37 |
| (*n*-butanol extract) | 0.080 | 0.007 | 78 | 87.37 | |
| | 0.119 | 0.004 | 39 | 80.10 | |
| | 0.274 | 0.003 | 19 | 53.11 | |
| | 0.409 | 0.002 | 9 | 29.58 | |
| | 0.479 | 0.002 | 5 | 17.47 | |
| sample III | 0.107 | 0.059 | 401 | 91.70 | 79.74 |
| (petroleum ether extract) | 0.148 | 0.033 | 201 | 80.10 | |
| | 0.294 | 0.006 | 101 | 50.17 | |
| | 0.421 | 0.000 | 50 | 27.16 | |
| | 0.483 | −0.004 | 25 | 15.74 | |
| | 0.507 | −0.004 | 13 | 11.59 | |
| sample IV | 0.051 | −0.005 | 50 | 90.31 | 4.94 |
| (ethyl acetate extract) | 0.056 | −0.007 | 25 | 89.10 | |
| | 0.075 | −0.008 | 13 | 85.64 | |
| | 0.219 | −0.006 | 6 | 61.07 | |
| | 0.382 | −0.009 | 3 | 32.35 | |
| | 0.460 | −0.004 | 2 | 19.72 | |

DPPH absorbance ($A_c$) = 0.590

79.74, and 4.94 μg/mL for VC (positive control), samples I−IV (chloroform, *n*-butanol, petroleum ether, and ethyl acetate extracts), respectively. The results showed that sample IV (ethyl acetate extract) was the most effective in reducing the stable radical DPPH to the yellow colored diphenyl picryl hydrazine, indicating that it was very active in DPPH radical scavenging. This was attributed to its $IC_{50}$ value (4.94 μg/mL) that was close to that of the positive control (4.23 μg/mL). We discovered that the radical scavenging effects of partitioned *L. trifolia* extract and synthetic anti-oxidants used as positive control on DPPH radical were concentration dependent. The scavenging of DPPH radicals increased with increasing anti-oxidant concentration (Figure 1). Proton radical-scavenging action is one mechanism for oxidation. DPPH has a proton free radical and shows characteristic absorption at 517 nm (purple). When it encounters proton radical scavenger, its purple colour fades rapidly, suggesting that the anti-oxidative activity of *L. trifolia* leaf extract is due to its proton donating ability.

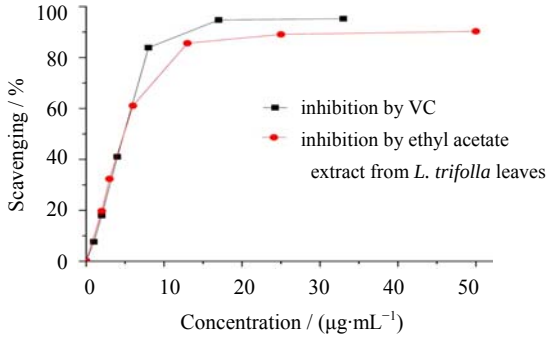



**Figure 1 Radical scavenging effect of ethyl acetate partition from *L. trifolia* leaves and VC by DPPH colouring method**

### 3.2 Total flavonoid and phenol contents

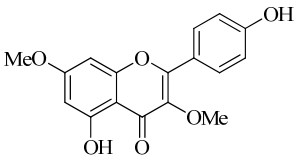
The total phenolic content of ethyl acetate extract from *L. trifolia* leaves measured by Folin Ciocalteu reagents in terms of GAE was (29.27 ± 1.46) mg/g, while the total flavonoids content was (39.0 ± 1.6) mg/g.

### 3.3 Isolation and identification of compounds

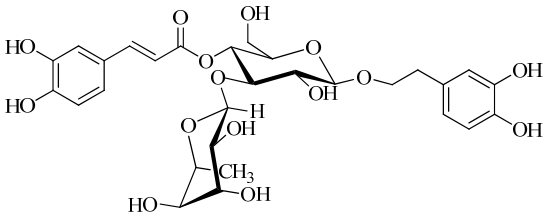
The chemical structures of the compounds separated by column chromatography, HPLC, and PHPLC respectively were identified. Comparing with reference data, compounds **1**−**6** (Figure 2) were identified as kaempferol-3,7-dimethyl ether (**1**, Julião et al, 2009), verbascoside (**2**, Wu et al, 2004), apigenin (**3**, Julião et al, 2009), umuhengerin (**4**, Rwangabo et al, 1988), ladanetin (**5**, Alam et al, 1986), and scutellaein-7-*O*-β-*D*-apiofuranoside (**6**, Julião et al, 2010).

## 4. Conclusion

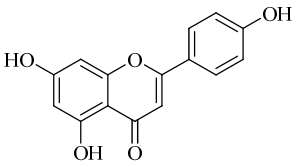
In conclusion, ethyl acetate has the highest extract yield and total phenol recovery, as well as the highest anti-oxidative activity when determined by DPPH assay. Therefore our



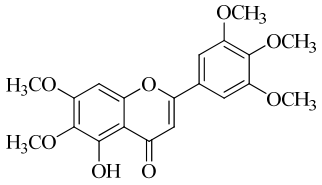
kaempferol-3,7-dimethyl ether (**1**)



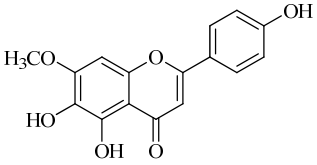
verbascoside (**2**)



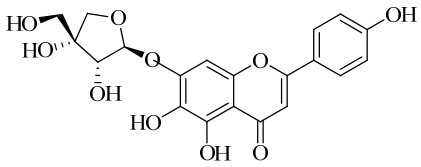
apigenin (**3**)



umuhengerin (**4**)



ladanetin (**5**)



scutellaein-7-*O*-β-*D*-apiofuranoside (**6**)

**Figure 2 Chemical structures of isolated compounds 1−6**

results indicate that the selective extraction from natural materials by an appropriate solvent is important for obtaining the fractions with high anti-oxidative activity. The ethyl acetate extract from the leaves of *L. trifolia* possesses the potent anti-oxidative and free radical scavenging activities which are directly proportional to its phenol contents. The anti-oxidative activities of the extract from the leaves of *L. trifolia* may be due to its proton donating capability as shown in DPPH radical scavenging results. Acting as an electron donor that can react with free radicals, it converts them to

more stable products and terminates radical chain reactions. These mechanisms could have contributed, at least partly, to the therapeutic benefits of *L. trifolia* for the treatment of inflammatory and respiratory disorders as a sedative.

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