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Original article

Chemical Constituents in Extracts from Leaves of *Lantana trifolia* and Their *In Vitro* Anti-oxidative ActivityPalmer Sivoko Imbenzi^{1, 2}, Yong-zhi He^{1*}, Zhi-xue Yan¹, Eric Kibagendi Osoro^{1, 2}, Peter K Cheplogoi²

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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 IC₅₀ 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), umhengerin (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*

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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-oxidant 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 °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_j) 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% NaNO₂ solution. After 6 min of incubation, 0.15 mL of 10% AlCl₃ 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

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