ANTIOXIDANT ACTIVITY OF THE MEDICINAL PLANT *LANTANA CAMARA L.*

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خلاصہ

اس مطالعہ کا مقصد Methanolie مادے اورLantana camara کے حوائی حصوں سے حاصل کئے ہوئے 3مرکبات یعنی olenolic , A lanatadeneکا تیزاب اور Lantanilic تیزاب کیoxidentکی مخالف صلاحیوں کا اندازہ لگانا تھا۔ اسمیں DPPHطریقہ کار استعمال کیا گیا۔ ہمارے نتائج یہ ظاہر کرتے ہیں کہ Methanolicسے نکالا گیا مادہ اسکے ماحول اسکا غیر حل شدہ پیڑولیم اور حل ہونے والے ایتھائل ایسی ٹیٹ کافی اثر انگیز ہوتے ہیں۔ خالص مرتب oleanolicتیزاب بھی oxidantمحل کو ظاہر کرتا ہے۔جبکہ Aserbicتیزاب کو مثبت کنڑول کے لیئے استعمال کیا جاتا ہے

Abstract

The propose of this study was to estimate the antioxidant activity of methanolic extract, its fractions and three pure compounds lantadene A (**1**), oleanolic acid (**2**) and lantanilic acid (**3**) of the aerial parts of the *Lantana camara* Linn. by using the DPPH assay. Our results demonstrated that methanolic extract, its aqueous, petroleum ether insoluble and ethyl acetate soluble fractions were found to be active. The pure compound oleanolic acid also showed antioxidant activity while ascorbic acid was used as positive control.

Introduction

Biological reactions often generated reactive oxygen species (ROS) including hydrogen peroxide, hydroxyl radicals, superoxide radicals and singlet oxygen as by products. ROS produced by ionizing radiation, chemical reactions, ultraviolet light, metabolic processes and DNA damage, sunlight causes carcinogenesis and several deteriorating syndromes such as neuro-degenerative diseases, cardiovascular diseases and aging (Patel *et al*., 2010; Zahin*et al.*, 2009). In cell metabolism including phagocytosis, intercellular signaling and energy production some of these ROS play an significant role. During last three decades antioxidant based formulations and drugs for the treatment and prevention of multifarious diseases have appeared. Recent advances have revealed that a various plant products containing terpenes, polyphenols and several plant extracts applied an antioxidant action (Taj Ur Rahman*et al*., 2016;Khalaf*et al*., 2008)

Lantana camara L. (*L. camara*) (Verbenaceae) is a significant weed. Conventional healers have used for the cure of a range of human diseases such as bilious fever, cuts, catarrh, eczema, headaches, itches, malaria, rheumatism, swellings, tetanus, tumor, and ulcers(Ross, 2003; Sousa and Costa, 2012). Several terpenes, steroids and alkaloids have been report earlier from this plant(Begum *et al.*, 2015). Hence the current study was selected to assess the antioxidant activity of extracts of airborne parts of *L. camara* by using DPPH radical scavenging activity.

Materials and Methods

Plant Material

Different airborne parts of *L. camara* were unruffled from the University of Karachi region. The identity of plant was confirmed by senior taxonomist Mr. Abdul Ghafoor in the (Department of Botany) University of Karachi. In the herbarium of the university a voucher No. 63482 KUH was deposited.

Extraction and Isolation

The aerial parts of *Lantana camara* were dried in the laboratory. This material was repetitively extracted with methanol. The solvent was evaporated by using a rotary evaporation the concentrated extract (**LC**), obtained. The extract was suspended in water and take out with ethyl acetate. The EtOAc phase after usual workup was treated with charcoal, filtered and freed of the solvent by rotary evaporator. The filtrate (**LC-EAR**, 204 g) was distributed into petroleum ether-soluble (**LC-PES**) and petroleum ether-insoluble (**LC-PEI**) portions. The petroleum ether-insoluble portion was again separated into ether soluble (**LC-ES**) and ether

insoluble (**LC-EI**) fractions. The ether insoluble fraction was further alienated into ethyl acetate soluble (**LC-EA**) and ethyl acetate insoluble (**LC-EAI**) fractions.

The main ether soluble fraction (**LC-ES**, 114 g) was again estranged into pet.etherinsoluble and pet.ether soluble fractions. This pet.ether insoluble part was gradient subjected to vacuum liquid chromatography by using petroleum ether-EtOAc which yielded 11 fractions, Fr-I to Fr-XI.

Fr-V-7 (petroleum ether-EtOAc, 8.75:1.25 eluate) was further applied to silica gel column and eluted with inclined petroleum ether-EtOAc which yielded 11 fractions Fr-V-7-1 to Fr-V-7-11. Fr-V-7-5 (petroleum ether-EtOAc 8.5:1.5 eluates) provided lantadene A(**1**, 15.5 mg)**.** Fr-V-9 was separated into ten fractions by column chromatography usingsilica gel with gradient petroleum ether-EtOAc. Fraction eight was further divided into 16 fractions by CC. Fraction no. 5 yielded oleanolic acid (**2,** 70.4 mg) as colorless crystallizate on keeping overnight in $CHCl_3$-MeOH (1:1)at room temperature. Fr-VII (9.6 g) (petroleum ether-EtOAc 8.25:1.75 eluate) afforded 9 fractions (Fr-VII-1 to Fr-VII-9) when chromatographed on VLC by using petroleum ether- EtOAc, in order of increasing polarity. Fr-VII-3 was chromatographed on VLC gradient petroleum ether-EtOAc which provided 13 fractions. Fraction 9 gave lantanilic acid (**3**, 911.7 mg), through crystallization from $CHCl_3$-MeOH (1:1) at room temperature.

Infrared bands were acquired on a JASCO A-302 spectrophotometer. Ultraviolet spectra were taken on a Hitachi-U-3200 spectrophotometer. The HREI-MS and EI-MS were chronicled on JMS HX-110 and Finnigan MAT-112 spectrometers, respectively. The ^{1}H-NMR spectra were taken on Bruker Avance spectrometers operative at 400 MHz The Chemical shifts are articulated in δ (ppm) referenced to the remaining solvent signal and *J* (the coupling constants) are in Hz.

Silica gel PF_{254} (Merck) was used for vacuum liquid chromatography (VLC) (Coll and Bowden, 1986) and silica gel 9385 (Merck) was used for flash column chromatography (FCC, Model Eyela EF-10)(Still *et al*., 1978). Kieselgel 60 F_{254} precoated aluminium cards (Merck, 0.2 mm thickness) were used for taking TLC and spots were envisioned underneath UV light at 254 and 365 nm and by squirting with 5% H_2SO_4.

Antioxidant Activity

Antioxidant activity of (**LC**)methanolic extract, its fractions and purified compounds **1-3** was determined following the procedure as described earlier by Lee *et al*. (1998). Solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was prepared in ethanol (333 μM) and stock solutions of samples were prepared in dimethylsulfoxide. Reaction mixtures containing 10 μL of test samples and 90 μL of DPPH were added in 96-well microtiter plates (final concentration of test sample was 500 μg/mL for fractions and 200 μg/mL for pure compounds and final concentration of DPPH in the well was 300 μM) and incubated at 37 ^{o}C for 30 minutes. Absorbance was measured at 515 nm using spectrophotometer (Spectra Max 340). Percent inhibition by sample treatment was determined by comparison with a DMSO treated control group.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of the control} - \text{Absorbance of test sample}}{\text{Absorbance of the control}} \times 100$$

Ascorbic acid was used as positive control. The EC_{50} value were calculated as the concentration (in μg/mL) of sample required to scavange 50% of DPPH radical.

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Results and Discussion

The methanolic extract (**LC**) of aerial parts of the plant and its fractions (**LC-AQ**, **LC-EAR**, **LC-PES**, **LC-PEI**, **LC-ES**, **LC-EI**, **LC-EAS** and **LC-EAI**) were practiced for antioxidantfree radical scavenging activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) at 500 μg/mL concentration. The methanolic extract (**LC**) showed 67% inhibition of DPPH free radical with EC_{50} value 375 μg/mLwhile the fractions (**LC-AQ**, **LC-PEI** and **LC-EAS**) were also active and exhibited 70%, 72% and 65% inhibition respectively with EC_{50} value 375 μg/mL (Table 1).

Pure compounds lantadene A (**1**), oleanolic acid (**2**) and lantanilic acid (**3**) (Fig. 1) were identified by comparing spectral data (UV, IR, ^{1}H-NMR) with that of reported values (Begum, *et al*., 2014) Compounds **1-3**also screened for DPPH free radical scavenging activity at 200μg/mLconcentration. Compound **2** showed 65% inhibition with EC_{50} 187μg/mL while **1** and **3** were found inactive (Table 2).

Conclusion

The methanolic extract, its some fractions and oleanolic acid inhibited DPPH radical. It is concluded that further work should be performed to isolate and id.entify the antioxidative components of the plant.

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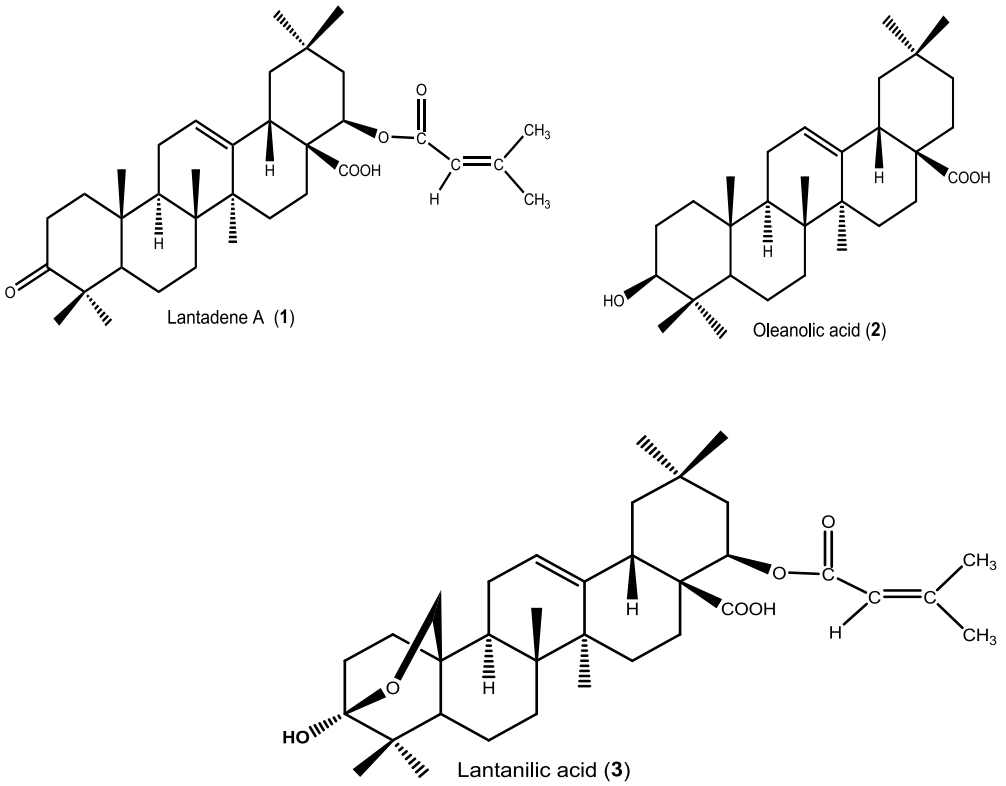

Fig. 1. Three pure compounds isolated from *Lantana camara* and tested for antioxidant activity

Table1.*In vitro* DPPH radical scavenging activity of extract (LC) and its fractions.

Samples	% Inhibition[a]	EC_{50}(*µ*g/mL)
LC	67	375
LC-AQ	70	375
LC-EAR	55	>500
LC-PES	22	>500
LC-PEI	72	375
LC-ES	33	>500
LC-EI	53	>500
LC-EAS	65	375
LC-EAI	29	>500
Ascorbic acid (+ve control)	87[b]	9.4

[a]conc= 500 *µ*g/mL, [b] At 200 *µ*g/mL

Table 2.*In vitro* DPPH radical scavenging activity of pure compounds.

Samples	% Inhibition[a]	EC_{50}(*µ*g/mL)
Lantadene A (**1**)	17	>200
Oleanolic acid (**2**)	65	187
Lantanilic acid (**3**)	17	>200
Ascorbic acid (+ve control)	87	9.4

[a]conc.= 200 *µ*g/mL

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