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ANTI-STRESS ACTIVITY OF *OCIMUM GRATISSIMUM* L. EXTRACT

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KEYWORDS: *Ocimum gratissimum*, anti-stress, adaptogenic product, ursolic acid, HPLC

INTRODUCTION

An adaptogenic substance is one that demonstrates a nonspecific enhancement of the body's ability to resist a stressor⁽¹⁾. There are many kind of plants that have adaptogenic properties such as red ginseng, American ginseng, Siberian ginseng, ashwaganda, astragalus, licorice, schisandra and jiaogulan. Plants in *Ocimum* species are also considered to have adaptogenic properties. As a part of our research in developing of a relaxant adaptogenic product, *Ocimum gratissimum* was one of our selected herbs. *O. gratissimum* is a herbaceous plant which belongs to the Lamiaceae family. It's a well-known aromatic plant that has been used extensively as condiment or spice in Thai cuisine. *O. gratissimum* has great medicinal value for treating a variety of ailments and diseases such as headaches, coughs, diarrhea, fever and conjunctivitis⁽²⁾. The flowers and the leaves of this plant are rich in essential oils which contained eugenol and 1,8-cineole. The chemical compositions of ethanolic extract of *Ocimum* species have been reported⁽³⁾. Ursolic acid is one of the commonly compounds found in many plants of this species and has several pharmacological activities such as anti-inflammatory, anti-oxidant, anti-rheumatic and anti-depressant⁽⁴⁾. In this study we extracted *O. gratissimum* with 95% ethanol and determined for active compound and biological activity. Quantitative determination of ursolic acid from the *O. gratissimum* extract was achieved by HPLC method. Anti-stress study was conducted using cold restraint model in rats. The blood cortisol level was measured for stress status consideration.

MATERIALS AND METHODS

Plant Extraction The fresh arial part of *O. gratissimum* was washed, dried in hot air oven at 45°C for 48 hrs., ground into powder and then extracted with 95% ethanol by using percolator at room temperature. The extract was concentrated on rotary evaporator under reduced pressure.

Determination of ursolic acid content The HPLC analysis was performed on a Waters Alliance 2695 LC system connected with a Waters model 2996 photodiode-array detector and an Empower workstation. X-terra C₁₈ Column (5 µm, 150x3.9 mm) was used. The mobile phase consisted of 1% Ortho-Phosphoric acid and acetonitrile, isocratic mode with a flow rate of 0.8 ml/min. The UV detection wavelength was set at 206 nm. The stock solution of standard ursolic acid was prepared by dissolving 10 mg of ursolic acid (>98.5 % purity) in 10 ml methanol. The stock solution was diluted to create a five-point standard curve. *O. gratissimum* extract (150 mg.) was dissolved in 100 ml methanol by sonicator for 30 min. and then filtered through a 0.2 µm syringe filter before injection.

Anti-Stress Activity Twelve male Wistar rats (250-300g) were given orally with *O. gratissimum* extract suspension in 0.5% Sodium Carboxymethylcellulose (CMC, control group) at dose 250, 500 and 1,000 mg/kg body weight for 7 consecutive days. The positive control group was administered by diazepam (DZP) at dose of 2 mg/kg body weight orally. After 1 hr., each rat was individually placed in fix plastic bottle as normal position using adhesive tape and soaked in cold water that was maintained at 10°C for 1 hr. On the 8th day rats were fasted at least for 18 hr. before blood collection by tail vein. Plasma cortisol level of each rat was measured using Cortisol EIA Kit Assay (Cortisol EIA Kit, Assay Designs, Inc., USA).

Analysis of Data The results are expressed as means ± S.E.M. Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA). Statistical significance was assessed as $p < 0.05$.

RESULTS

The dried powder of *O. gratissimum* yielded 6.39 % (w/w) of ethanolic extract. The *O. gratissimum* extract contained 0.93 % (w/w) of ursolic acid.

The analytical method of ursolic was developed and validated. The HPLC chromatogram of the extract was shown in Fig 2. The retention time of ursolic acid was 25.3 minute. Linearity was found in the concentration range between 20-100 ppm with high reproducibility and accuracy. The linear regression equation with correlation coefficient (r^2) of 0.9998 was $y=15888x+15917$.

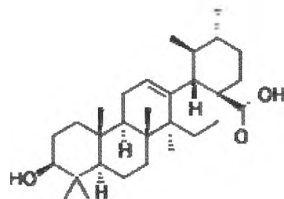


Fig 1. Structure of Ursolic acid

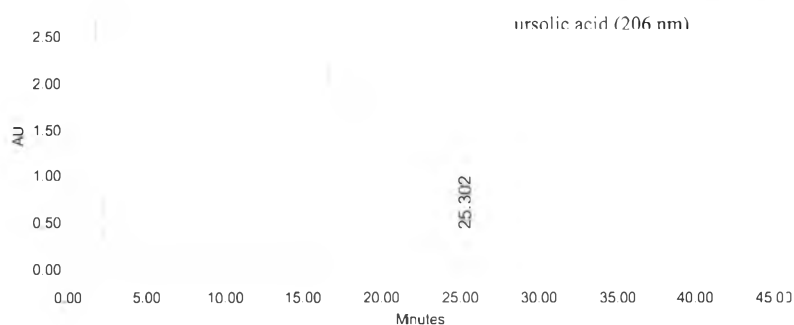


Fig 2. HPLC chromatogram of *O.gratissimum* extracts.

The anti-stress activity of *O. gratissimum* extract in various doses was performed by cold restraint-induced stress model in rats. The results showed that the extract at dose of 1,000 mg/kg body weight exhibited the best activity and could significantly reduce plasma cortisol level in comparison with control group (table 1).

Table 1. Plasma cortisol level in cold restraint-induced stress rat.

Group	Serum Cortisol(ng/dl)
Control	13.1 ± 0.43
Diazepam 5 mg/kg bw	8.6 ± 0.44*
<i>O.gratissimum</i> extract 250 mg/kg bw	12.9±0.93
<i>O. gratissimum</i> extract 500 mg/kg bw	14.7±0.88
<i>O. gratissimum</i> extract 1000 mg/kg bw	8.6±1.30*

* $p<0.05$

DISCUSSION

The development of HPLC method was performed by choosing ursolic acid as a standard marker. The HPLC chromatogram of the extract and standard ursolic acid were well separated without interference by other components under this chromatographic condition. The peak obtained was sharp and had clear baseline separation. The optimum HPLC system was comprised of a C₁₈ reverse phase column (X-terra C₁₈, 5 µm, 150x3.9 mm), gradient elution with 1% Ortho-Phosphoric acid and acetonitrile as mobile phase and PDA detection at 206 nm. The analytical method was validated on precision, accuracy, linearity and range. This simple and rapid HPLC method could be used for the quality control of the *O. gratissimum* extract.

The anti-stress activity, conducted by using cold restraint model showed significantly reduce plasma cortisol level at 1,000 mg/kg bw of *O. gratissimum* extract. Basically, an increment of plasma cortisol level is reversed by anti-stress agents⁽⁵⁾ These results showed that the relaxant adaptogenic product will be developed by using the ethanolic extract of *O. gratissimum* as an active ingredient.

CONCLUSION

O. gratissimum extract contained 0.93 % of ursolic acid and exhibited anti-stress activity by using cold restraint model in rats. *O. gratissimum* extract at dose of 1,000 mg/kg body weight could significantly reduce plasma cortisol level in comparison with control group. These results showed that ethanolic extracts of *O. gratissimum* could be developed as a relaxant food supplement.

REFERENCES

1. Brekhman, I.I. & Dardymov, I.V. "New substances of plant origin which increase nonspecific resistance," *Annu Rev Pharmacol*, 9: 419-30, 1969.
2. K.S. Prabhu¹, R. Lobo¹, A.A. Shirwaikar² and A. Shirwaikar *Ocimum gratissimum*: A Review of its Chemical, Pharmacological and Ethnomedicinal Properties *The Open Complementary Medicine Journal*, 2009, 1, 1-15.
3. Chiang LC,Ng LT,Cheng PW, Chiang W and Lin CC.(2005) Antiviral activities of extracts and selected pure constituents of *Ocimum basilicum*. *Clin Exp Pharmacol Physiol*. 32 (10): 811-6.
4. Taviano, M. F., Miceli, N., Monforte, M. T., Tzakou, O. and Galati, E. M. (2007), Ursolic acid plays a role in *Nepeta sibthorpii* Bentham CNS depressing effects. *Phytotherapy Research*, 21: 382-385.
5. Sen P, Maiti PC, Puri S and Ray A,(1992) Mechanism of anti-stress activity of *Ocimum sanctum* Linn, eugenol and *Tinospora malabarica* in experimental animals, *Ind.J. Exp. Biol.*30:592-96.
6. Atal CK, Zutshi U, Rao PG. Scientific evidence on the role of Ayurvedic herbals on bioavailability of drugs. *J Ethnopharmacol*. 1981 Sep; 4(2):229-32.
7. Ardayfio P, Kim KS. 2006. Anxiogenic-like effect of chronic corticosterone in the light-dark emergence task in mice. *Behavioral Neuroscience*. 120:249-259.
8. Krishna HNG, Sangha RB, Mirsa N, Pai MRS. 2006. Antianxiety activity of NR-ANX-C, a polyherbal preparation in rats. *Indian J Pharmacol*. 38:330-335.