Pharmacognosy Research [Phcog Res]
Vol 1, Issue 6, Nov-Dec, 2009 Page 336-341
(An Official Publication of Pharmacognosy Network Worldwide)
Received: 25 08, 2009
Modified: 05 09, 2009
Accepted: 18 09, 2009

PHCOG RES.: Research Article

# Bioactivity-Directed Separation of an Anxiolytic Fraction from *Aethusa cynapium* L.

**Shri Richa[1], Singh Manjeet[1], Sharma Anupam[2]**

[1] *Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala*
[2] *Pharmacognosy Division, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh*

*** Corresponding author:** *rshri587@hotmail.com*

## ABSTRACT

The present study evaluated the putative anxiolytic activity of petroleum ether, chloroform, methanol and water extracts of the aerial parts of *Aethusa cynapium* using the widely accepted elevated plus-maze (EPM) model in mice. The bioactive fraction was isolated by bioactivity-directed fractionation, and two chromatographic procedures - column and flash chromatography. Fraction 3.1.3.2 derived from the methanol extract of the plant, showed significant anxiolytic activity at a dose of 50 mg/kg p.o. which was comparable to the standard drug, diazepam (2 mg/kg p.o.). This sub fraction comprised two components as seen in the TLC profile. Phytochemical screening indicated the presence of unsaturated fatty acid in Fraction 3.1.3.2. The study demonstrates that *A.cynapium* has significant anti anxiety activity. This activity may be due to fatty acids present in the bioactive fraction.

**Keywords:** *Aethusa cynapium, L.;* Anxiolytic effect, Elevated plus-maze model, column and flash chromatography.

## INTRODUCTION

*Aethusa cynapium* L. (Apiaceae) is commonly called Fool's Parsley, dog's parsley or lesser hemlock. The plant is an annual (rarely biennial) well-known garden weed native to the United Kingdom (1). *A. cynapium* has been used in folk medicine for gastrointestinal complaints in children, infantile cholera, summer diarrhea, convulsions, anxiety, sleep disorders, delirium, and as stomachic (2–3). In homeopathy *A. cynapium* is used as an intellectual stimulant (4). The plant contains a volatile alkaloid named cynopine which resembles coniine in its physical and chemical characters, as well as physiological actions (3 and 5–7); polyacetylenes (8) including aethusin, aethusanol A & B; essential oil; flavone glycosides such as rutoside, narcissine, and ascorbic acid (2). Fool's Parsley is poisonous when fresh, but is not harmful when dried (9). Toxins like cynopine are destroyed by drying, and hay containing the plant is not poisonous (2, 4).

The anti-anxiety activity of this plant has not been studied. The present study investigated the anti- anxiety activity of different extracts of *A. cynapium* using Elevated Plus Maze (EPM) model. The bioactivity-directed fractionation to isolate the bioactive fraction having anxiolytic effect is described.

## MATERIALS AND METHODS

### Plant material.

Aerial parts of *A.cynapium*, were collected from a cultivated source (Rati Ram Nursery) at village Khurrammpur via Kalsia, district Saharanpur (U.P., India) in March 2006. The identity of the plant was confirmed by Dr H.B.Singh, Head, Raw Materials, Herbarium & Museum at National Institute of Science Communication and Information Resources, (NISCAIR, CSIR), New Delhi 110067. A voucher specimen no: NISCAIR/RHM/

F-3/3/2005/Consult/698//15 is deposited in the same herbarium.

**Animals.**

Swiss albino mice of either sex, weighing 20–24g were procured from the Central Animal House of Panjab University, Chandigarh. The mice were allowed to take standard laboratory feed and water *ad libitum*. The animals were fasted 18 h prior to the biological study (10). Groups of five mice were used in all sets of experiments. All animals used in the study were naive to the elevated plus-maze test. The experiments were conducted in a semi-sound proof laboratory. The biological studies were carried out as per the guidelines of the Institutional Ethical Committee (Reg. no.107/1999/CPCSEA) of Department of Pharmacuetical Sciences and Drug Research, Punjabi University, Patiala, India.

**Chemicals and instruments.**

Solvents namely petroleum ether (60–80 °C), methanol, chloroform and 1-butanol (S.D. Fine-Chem Ltd., Mumbai), all of LR grade were employed for the extraction of the plant material. Silica gel (# 60–120, S.D. Fine- Chem Ltd., Mumbai) was used for column chromatography. For Flash chromatography, Büchi pump module C-601 and Büchi pump controller C-610, silica gel (# 250–400) were used. Pre-coated TLC sheets (Macherey-Nagel D-5160 DUREN, 0.25 mm, Polygram® SiLG) and 2µL capillary tubes (CAMAG) were used for thin layer chromatography (TLC). The chromatograms were visualized under 254/366 nm UV light (DESAGA, Heildberg, Min. UVIS) and also by spraying with 60% v/v aqueous sulfuric acid (BDH). Diazepam I.P. was procured from Triko Pharmaceuticals, Rohtak, Haryana, India.

**Preparation of extracts and evaluation of anxiolytic activity**

*Preparation of extracts* Aerial parts of *A. cynapium* were dried in shade and powdered (#60). One kg of the plant was successively extracted in the Soxhlet apparatus with petroleum ether, chloroform, methanol and water. These extracts were labeled F1, F2, F3 and F4 respectively. Exhaustive extraction with each solvent was ensured. The four extracts were dried using Buchi 461 Rotary Vacuum Evaporator and the dried extracts were preserved in vacuum desiccator containing anhydrous silica gel.

**Elevated plus-maze model of anxiety**

Anxiolytic activity was evaluated using the modified elevated plus-maze (11–13). The plus-maze apparatus consisted of two open arms (16×5 cm) and two closed arms (16×5×12 cm) having an open roof, with the plus-maze elevated (25 cm) from the floor was used to evaluate anxiolytic behavior in animals (14). Vehicle [5% Tween 80 in Simple Syrup I.P. (66.7% w/w sucrose in water)] (0.25ml), extracts of *A. cynapium* (50, 100, 200 and 400 mg/kg) and reference drug (diazepam, 2 mg/kg), both suspended in vehicle, were administered orally using a tuberculin syringe fitted with an oral canula. The dose administration schedule was adjusted so that each mouse was having its turn on the elevated plus-maze apparatus 45 min after the administration of the test extract, diazepam or vehicle.

Each mouse was placed at the center of the elevated plus-maze with its head facing towards the open arms. During the 5 min duration of the experiment, the behavior of the mouse was recorded as (a) the number of entries into the open or closed arms, (b) mean time spent by the mouse in each of the arms. During the entire experiment, the animals were allowed to socialize. Every precaution was taken to ensure that no external stimuli could evoke anxiety in the animals.

**Bioactivity guided fractionation of bioactive extract.**

The extract showing most significant antianxiety activity was subjected to bioactivity guided fractionation by using solvent partitioning, column chromatography and flash chromatography. Each fraction collected was evaluated for anxiolytic activity using the elevated plus maze model.

**Phytochemical screening and TLC profiles**

Extracts and different fractions were subjected to phytochemical screening (15–17) and their TLC profiles were examined (18).

**Statistics**

The anxiolytic activities of test substances, diazepam (standard) and control were analyzed using one-way analysis of variance (ANOVA) and post hoc analysis was done using Tukey's multiple range test.

## RESULTS

**Extracts of *A.cynapium* and their anxiolytic activity.**

The dried aerial parts of *A.cynapium* were subjected to successive extractions with solvents in increasing order of polarity yielding four extracts. The yield of various extracts after exhaustive extraction was: petroleum ether (2.2 %w/w), chloroform (1.8 %w/w), methanol (3.8 %w/w), and aqueous (2.9 % w/w).

The extracts of *A.cynapium* were separately suspended in the vehicle. Four sets of doses viz. 50, 100, 200 and 400 mg/kg of each extract were prepared. Diazepam was used as the standard anxiolytic drug, and the vehicle was used as the control. The relative anxiolytic profile of the four extracts of *A.cynapium* is reported in Table 1. The methanol extract (F-3) at a dose of 400mg/kg significantly increased both the time spent in the open arms and the number of open arm entries compared to the control group. The effect was comparable to that produced by diazepam.

### Anxiolytic activity of different fractions of F-3.

The bioactive methanol extract (F-3) (30 g) was shaken successively with chloroform and butanol (4×10 ml each). The bioactive methanol extract yielded chloroform fraction (F3.1, 36.6%w/w), butanol fraction (F3.2, 34.7%w/w) and remaining methanol soluble fraction (F3.3, 27.4%w/w) The antianxiety profile of each fraction is reported in Table 2. Fraction F-3.1 at a dose of 200mg/kg significantly increased the number of open arm entries and the average time spent in the open arms.

### Column chromatography and anxiolytic evaluation of F-3.1.

The bioactive chloroform fraction (F-3.1, 7 g) was subjected to column chromatography using silica gel. Elution was done with chloroform and chloroform–methanol in increasing order of polarity. The different collected fractions were pooled on the basis of similar TLC profiles. Four fractions were generated namely F-3.1.1 to 3.1.4 (Table 3). Each fraction was subjected to

**Table 1: Anxiolytic effect of different extracts of *A. cynapium* using the EPM**

| Treatment | Dose (mg/kg, p.o) | Number of entries in open arms Mean$^n$ ± S.E.M. | Time spent in open arms (seconds) Mean$^n$ ± S.E.M. |
|---|---|---|---|
| Vehicle (control) | 0.25 ml/kg | 4.8±0.58 | 3.2 ± 0.15 |
| Diazepam (standard) | 2 | 11.2±1.02 | 12.2 ± 0.53 |
| Pet. ether extract (F-1) | 50 | 6.2±0.37 [a, b] | 7.0±0.46[a, b] |
| | 100 | 6.8±0.58[a] | 7.9 ± 0.19[a, b] |
| | 200 | 6.6± 0.51[a] | 7.9 ± 0.36[a, b] |
| | 400 | 5.2±0.74[a] | 5.9 ± 0.44[a, b] |
| Chloroform extract (F-2) | 50 | 7.4±0.51[a, b] | 5.1 ±0.52[a, b] |
| | 100 | 7.0±1.14[a] | 8.1 ± 0.53[a, b] |
| | 200 | 5.8±1.06[a] | 7.6 ±0.43[a, b] |
| | 400 | 7.2±0.58[a] | 7.0 ±0.59[a, b] |
| Methanol extract (F-3) | 50 | 5.8±1.02[a, b] | 7.7 ±0.45[a, b] |
| | 100 | 6.4±0.74[a, b] | 8.2 ± 0.46 [a, b] |
| | 200 | 8.8±1.28[b] | 9.5 ±0.53[a, b] |
| | 400 | 9.4±1.16[b] | 12.7 ± 0.71[b] |
| Water extract (F-4) | 50 | 4.4±0.51[a] | 5.2 ± 0.35 [a, b] |
| | 100 | 5.0±0.71[a] | 8.2 ± 0.77[a, b] |
| | 200 | 9.4±0.25[b] | 8.4 ± 0.66[a, b] |
| | 400 | 7.6±1.20[a] | 7.9 ± 0.64[a, b] |

n = 5. ANOVA followed by Tukey's multiple range test.
$p<0.05$.
[a]= significant with respect to standard,
[b]= significant with respect to control

**Table 2: Anxiolytic effect of different fractions of methanol extract using the EPM**

| Treatment | Dose (mg/kg, p.o) | Number of entries in open arms Mean$^n$ ± S.E.M. | Time spent in open arms (seconds) Mean$^n$ ± S.E.M. |
|---|---|---|---|
| Vehicle (control) | 0.25 ml/kg | 3.0±0.89 | 2.6 ± 0.19 |
| Diazepam (standard) | 2 | 10.0±1.23 | 11.3 ± 0.37 |
| F-3.1 | 100 | 7.0±0.70 [a, b] | 9.3 ± 0.35 [b] |
| | 200 | 10.0±0.95[b] | 10.6 ± 0.36[b] |
| F-3.2 | 100 | 4.8±0.86 [a] | 2.8 ± 0.25 [a, b] |
| | 200 | 8.2±1.32 [b] | 6.6 ± 0.92 [a, b] |
| F-3.3 | 100 | 5.0±0.55 [a] | 4.1 ± 0.19 [a, b] |
| | 200 | 8.0±0.70 [b] | 5.4 ± 0.25 [a, b] |

n = 5. ANOVA followed by Tukey's multiple range test.
$p<0.05$.
[a]= significant with respect to standard,
[b]= significant with respect to control

**Table 3: Results of column chromatography of chloroform fraction (F-3.1) obtained from methanol extract of *A.cynapium***

| Fraction | Obtained by pooling Fractions | Eluants | Yield (g) |
|---|---|---|---|
| F-3.1.1 | 1-10 | chloroform | 1.21 |
| F-3.1.2 | 11-26 | chloroform:methanol (50:50) | 0.96 |
| F-3.1.3 | 27-32 | chloroform:methanol (40:60) | 2.64 |
| F-3.1.4 | 33-50 | chloroform:methanol (20:80) | 0.88 |

**Table 4: Anxiolytic activity profile of the fractions obtained by column chromatography of the bioactive chloroform fraction (F-3.1)**

| Treatment | Dose (mg/kg, p.o) | Number of entries in open arms Mean$^n$ ± S.E.M. | Time spent in open arms (seconds) Mean$^n$ ± S.E.M. |
|---|---|---|---|
| Vehicle (control) | 0.25 ml/kg | 2.8±0.66 | 2.2 ± 0.14 |
| Diazepam (standard) | 2 | 10.0±0.70 | 12.5 ± 0.42 |
| F-3.1.1 | 50 | 4.0±0.63 [a, b] | 3.3 ± 0.28 [a, b] |
| | 100 | 4.2±1.02 [a, b] | 4.6 ± 0.29 [a, b] |
| F-3.1.2 | 50 | 6.0±0.71 [a, b] | 5.7 ± 0.22 [a, b] |
| | 100 | 10.2±0.80 [b] | 6.7 ± 0.34 [a, b] |
| F-3.1.3 | 50 | 5.9±0.71[a, b] | 11.0 ± 0.18 [b] |
| | 100 | 9.8±1.20 [b] | 12.4 ± 0.45[b] |
| F-3.1.4 | 50 | 4.8±0.58 [a, b] | 5.3 ± 0.57 [a, b] |
| | 100 | 7.2±0.86 [a, b] | 6.2 ± 0.45 [a, b] |

n = 5. ANOVA followed by Tukey's multiple range test.
$p<0.05$.
[a]= significant with respect to standard,
[b]= significant with respect to control

anxiolytic screening using the elevated plus maze model (Table 4). Fraction F-3.1.3 showed significant antianxiety activity.

### Flash Chromatographic and antianxiety activity of F-3.1.3.

The bioactive fraction F-3.1.3 (1.8 g) was subjected to flash chromatography using silica gel (# 250–400). Elution was done with chloroform and chloroform–methanol in increasing order of polarity. The eluants were driven through the column by pressurized air (10 psi). The different collected fractions were pooled on the basis of similar TLC profiles. Five sub fractions were collected namely f-3.1.3.1 to 3.1.3.5 (Table 5). Each fraction was subjected to anxiolytic screening using the elevated plus maze model (table 6). F-3.1.3.2 showed significant anxiolytic effect at doses of 50 and 75 mg/kg.

### Phytochemical screening and TLC profiles

Phytochemical screening of the fraction F-3.1.3.2 showed the absence of alkaloids and flavonoids and presence of unsaturated fatty acids. TLC of F-3.1.3.2 using chloroform: methanol (9:1) as the mobile phase showed two distinct spots (Rf 0.50 and 0.67), in the iodine chamber.

**Table 5: Results of Flash Chromatographic separation of F-3.1.3**

| Fraction | Obtained by pooling Fractions | Eluants | Yield (g) |
|---|---|---|---|
| F-3.1.3.1 | 1-3 | chloroform:methanol (97.5:2.5) | 0.435 |
| F-3.1.3.2 | 4 | chloroform:methanol (92.5:7.5) | 0.524 |
| F-3.1.3.3 | 5 | chloroform:methanol (90:10 ) | 0.300 |
| F-3.1.3.4 | 6-7 | chloroform:methanol (50:50) | 0.281 |
| F-3.1.3.5 | 8-10 | chloroform:methanol (20:80) | 0.215 |

**Table 6: Anxiolytic activity profile of subfractions of F-3.1.3, obtained by Flash chromatography, on the time spent by mice in the open arms of the EPM**

| Treatment | Dose (mg/kg, p.o) | Number of entries in open arms Mean$^n$ ± S.E.M. | Time spent in open arms (seconds) Mean$^n$ ± S.E.M. |
|---|---|---|---|
| Vehicle (control) | 0.25 ml/kg | 2.8±0.66 | 2.2 ± 0.14 |
| Diazepam (standard) | 2 | 10.0±0.70 | 12.5 ± 0.42 |
| F-3.1.3.1 | 50 | 3.4±0.51 [a, b] | 7.1 ± 0.87 [a, b] |
| | 75 | 5.2±0.37 [a, b] | 8.6 ± 0.83 [a, b] |
| F-3.1.3.2 | 50 | 5.1±0.37 [a, b] | 11.1 ± 0.26 [b] |
| | 75 | 9.0±1.09 [b] | 12.3 ± 0.32[b] |
| F-3.1.3.3 | 50 | 5.8±0.80 [a, b] | 5.4 ± 0.19 [a, b] |
| | 75 | 7.8± 1.65 [a, b] | 5.5 ± 0.23 [a, b] |
| F-3.1.3.4 | 50 | 3.8±0.66 [a] | 5.1 ± 0.25 [a, b] |
| | 75 | 4.2±0.73 [a] | 5.6 ± 0.21 [a, b] |
| F-3.1.3.5 | 50 | 5.2±1.02[a] | 6.1 ± 0.28 [a, b] |
| | 75 | 7.2±1.98 [a, b] | 6.3 ±0.22 [a, b] |

n = 5. ANOVA followed by Tukey's multiple range test.
$p<0.05$.
[a]= significant with respect to standard,
[b]= significant with respect to control

## DISCUSSION

Traditionally *Aethusa cynapium* has been used as a sedative, intellectual stimulant and for various gastrointestinal complaints (2–4). *A. cynapium* has been of interest because of its neurotoxic toxic effects due to the presence of the volatile alkaloid- cynopine (7, 19). However, no study has investigated the anxiolytic effect of this plant. In this study bioactivity-directed fractionation of *A. cynapium* aerial parts was followed with a view to isolate the anxiolytic fraction. The Elevated Plus Maze model employed in this study is a valid animal model because natural stimuli have been used in this model (20). The average time spent in the open arms of the EPM as well as the number of entries in the open arms is considered an index of anxiety level. Antianxiety drugs increase both the average time spent and the mean number of entries in open arms of EPM. Standard antianxiety drug diazepam increased the percentage of open arm entries and the time spent in the open arms of rodents on the EPM (21–23). In the present investigation of the four extracts of *A. cynapium* viz. petroleum ether, chloroform, methanol and water, methanol extract (F-3) exhibited maximum anxiolytic activity in mice at a dose of 400 mg/kg. This activity was comparable to the effect of the standard drug, diazepam. The methanol extract of *A. cynapium* was further fractionated using chloroform and 1-butanol resulting in three fractions F-3.1 to F-3.3. Relative anxiolytic activity profile of the three fractions showed that significant anxiolytic activity resided in F-3.1. Of the four fractions (F-3.1.1 to F-3.1.4) obtained by column chromatography of F-3.1, F-3.1.3 was observed to be the only fraction with significant anxiolytic activity at doses 50 and 100mg/kg. F-3.1.3 was subjected to flash chromatography and sub fraction F-3.1.3.2 showed significant anxiolytic effect at doses 50 and 75 mg/kg. The present investigation demonstrates that *A.cynapium* has significant anti anxiety activity. No adverse reaction was observed in mice.

*A.cynapium* is reported to contain alkaloid, flavone glycosides and polyacetylenes (2, 3, and 6). Phytochemical screening of F-3.1.3.2 in the present study showed absence of alkaloids and flavonoids and presence of unsaturated fatty acids. Unsaturated fatty acids are widely distributed in human foods including vegetable oils, meat, milk, and soy products (24), and are associated with nutritional and metabolic functions. They are involved in important structural and physiological functions. Polyunsaturated fatty acids (PUFA) have been associated with many health

benefits. The benefits of omega-3 and omega-6 PUFAs have been studied extensively. Consumption of omega-3 and omega-6 fatty acids has been associated with reduced mortality from cardiovascular disease, suppressed arthritis-associated inflammation, and decreased risk of cancer (25–26). A study investigated rodent behaviour on the EPM and demonstrated that omega-3 fatty acids deficient animals were significantly more anxious under stressful conditions (27). Omega 3- fatty acids supplementation during brain development of rats had a beneficial effect on preventing the development of depression-like behavior in adult rats (28). The contribution of fatty acid in depression and mood disorders has been supported by epidemiologists who related negative correlation between fish consumption (high omega-3 polyunsaturated fatty acid consumption) and depression (29–31). Omega-3 PUFAs like Docosahexaenoic acid (DHA) and eicosapentaenoic acid and omega-6 PUFA like arachidonic acid are important constituents in mammalian cell membranes (32) and are crucial to brain and eye development in human infants (33–34). DHA plays an essential role in brain functioning (35–37). In primates and humans DHA plays an important role in anxiety, aggression, depression, attention-deficit/hyperactivity disorder (ADHD) and schizophrenia (38).

It is possible that the anxiolytic activity demonstrated in the present study by the fraction F-3.1.3.2 may be due to the presence of unsaturated fatty acids. The authors are currently involved in bioactivity directed isolation and characterisation of anxiolytic constituent(s) from *A. cynapium.*

## ACKNOWLEDGMENTS

The authors wish to acknowledge the facilities of flash chromatography provided by Dr K.K.Bhutani, Prof and Head, Department of Natural Products, NIPER, Mohali, Punjab, INDIA.

## REFERENCES

1. Bond W. and Turner R.J. The biology and non-chemical control of Fool's Parsley (*Aethusa cynapium* L.). http://www.organicweeds.org.uk (2004).
2. Fleming T. *PDR for herbal medicines*, 2nd edition, (Medical economics company inc, Montvale, NJ07647–1742, 2000)316.
3. Vikramaditya and Joshi P. *A guide to important medicinal plants used in homeopathy*, V2, (Homeopathic Pharmacopoeial laboratory, India, 1997), 4 and 23.
4. Moreau J. Rudiments of Hahnemann's first material medica. *L' Homdopathie EuropFenne* **5**: 19–20 (1996).
5. Clapham A.R., Tutin T.G. and Moore D.M. *Flora of the British Isles*, 3rd edition, (Cambridge University Press, Cambridge, UK., 1987).
6. Tutin T.G. *Umbellifers of the British Isles*. (BSBI Handbook No. 2. Botanical Society of the British Isles, London, UK, 1980).
7. Power F.B. and Tutin F. Chemical examination of Aethusa cynapium. *J. Am. Chem. Soc.* **27**, 1461–1476 (1905).
8. Andreev G. N., Schulz H., Schrader B., Fuchs R., Popov S. and Handjieva N. Non-destructive NIR-Ft-Raman analyses in practice. PartI. Analyses of plants and historic textiles. *Fresenius J.Anal. Chem.* **371**(7): 1009–17 (2001).
9. Salisbury E. J. *Weeds & Aliens*. New Naturalist Series, (Collins, London., Society of the British Isles, London, UK, 1961).
10. Vogel H.G. and Vogel W.H. *Drug discovery and evaluation- Pharmacological assays*. (Springer-Verlag, New York, 1997) 204–245.
11. Montgomery K.C. The relation between fear induced by novel stimulation and exploratory behaviour. *J. Comp. Physiol. Psychol.* **48**:254–260 (1958).
12. Pellow S., Chopin P.H., File S.E. and Briley M. Validation of open:closed arm entries in an elevated plus maze as a measure of anxiety in the rat. *J. Neurosci.Meth.* **14**: 149–167 (1985).
13. Lister R.G. The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology (Berl).* **92**(2):180–185 (1987).
14. Kulkarni S.K. and Reddy D.S. Animal behavioral models for testing anti-anxiety agents *Meth Find Exp ClinPharmacol* **18**:219 (1996).
15. Farnsworth N.R. Biological and phytochemical screening of plants. *J. Pharm.Sci.* **55**: 225–276 (1966).
16. Vishnoi N.K., *Advanced practical organic chemistry*. (Vikas publishing house, India, 2 edition, 2009) 58–59.
17. Mukherji S.M., Singh S.P. and Kapoor R.P., *Organic Chemistry*, Volume **II**. (New age international (P) Ltd Publishers, India, 2008) 638–640.
18. Wagner H., Bladt S. and Zgainski E.M. *Plant Drug Analysis*. Springer-Verlag, New York, p163–172. (1984).
19. Moro P.A., Assisi F., Cassetti F., Bissoli M., Borghini R., Davanzo F., DellaPuppa T., Dimasi V., Ferruzzi M., Giarratana T. and Travaglia A. Toxicological hazards of natural environments:Clinical reports from Poison Control Centre of Milan. *Urban Forestry&UrbanGreening* **8**: 179–186 (2009).
20. Dawson G.R. and Tricklebank M.D. Use of the elevated plus maze in the search for novel anxiolytic agents. *Trends in Pharmacological Science* **16**(2): 33–36 (1995).
21. Moser P.C. An evaluation of the elevated plus-maze using the novel anxiolytic buspirone. *Psychopharmacology* **99**: 48–53 (1989).
22. Helton D. R., Berger J.E., Czachura J.F., Rasmussen K. and Kallman M.J. Central nervous system characterization of new cholecystokinin B antagonist LY288513. *Pharmacology, Biochemistry and Behavior* **53**: 493–502 (1996).
23. Eguchi J., Inomata Y. and Saito K.I. The anxiolytic- like effect of MCI-225, a selective NA reuptake inhibitor with 5-HT3 receptor antagonism. *Pharmacology, Biochemistry and Behavior* **68**: 677–683 (2001).
24. Jacobsen C. Developing polyunsaturated fatty acids as functional ingredients. In: Arnoldi, A. (Ed.), Functional Foods, Cardiovascular Disease and Diabetes. CRCPress, Woodhead Publishers, Cambridge, England, pp. 307–332 (2004).
25. Rizzo M.T., Regazzi E., Garau D., Akard L., Dugan M., Boswell H.S., Rizzoli V., and Carlo-Stella C. Induction of apoptosis by arachidonic acid in chronic myeloid leukemia cells. *Cancer Res.* **59**: 5047–5053 (1999).
26. Freeman M.P., Omega-3 fatty acids and perinatal depression: a review of the literature and recommendations for future research, *Prostaglandins Leukot. Essent. Fatty Acids* **75**: 291–297 (2006).
27. Fedorova I. and Salem N. Omega-3 fatty acids and rodent behavior. *Prostaglandins, Leukotrienes and Essential Fatty Acids* **75**: 271–289, (2006).
28. Ferraz A. C., Kiss Á., Araú jo R.L.F., Maria Hé., Salles R., Naliwaiko K., Pamplona J., Matheussi F. The antidepressant role of dietary long-chain polyunsaturated n-3 fatty acids in two phases in the developing brain. *Prostaglandins, Leukotrienes and Essential Fatty Acids* **78**: 183–188 (2008).
29. Simopoulos A.P. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed. Pharmacotherap.* **56**: 365–379 (2002).
30. Morris M.C., Evans D.A., Tangney C., Bienias J., Wilson R.S., Fish consumption and cognitive decline with age in a large community study, *Arch. Neurol.* **62**: (2005).
31. Timonen M., Horrobin D., Jokelainen J., Laitinen J., Herva A., Rasanen P., Fish consumption and depression: the Northern Finland 1066 birth cohort study, *J. Affect. Disord.* **82**: 447–452 (2004).
32. Sala-Vila A., Campoy C., Castellote A.I., Garrido F.J., Rivero M., Rodriguez-Palmero M. and Lopez-Sabater M.C. Influence of dietary source of docosahexaenoic and arachidonic acids on their incorporation into membrane phospholipids of red blood cells in term infants. *Prostaglandins, Leukotrienes Essential Fatty Acids* **74**: 143–148 (2006).

33. Birch E.B., Birch D. and Uauy R. Visual function and the essentiality of alinolenic acid and docosahexaenoic acid in human infants. In: Yehuda, S., Mostofsky, D.I. (Eds.), *Hand Book of Essential Fatty Acid Biology*. Human press, Totowa, NJ, pp. 183–199 (1997).
34. Zimmer L., Vacanssel S.D., Durand G., Guilloteau D., Bodard S., Besnard J.C., Chalon S., Modification of dopamine neurotransmission in the nucleus accumbens of rats deficient in n-3-polyunsaturated fatty acids, *J. Lipid Res.* **41**: 32–40 (2000).
35. Salem N. Jr., Omega-3 fatty acids: molecular and biochemical aspects, in: Spiller G.A., Scala J. (Eds.), *Current Topics in Nutrition and Disease: New Protective Roles for Selected Nutrients*, Alan R. Liss, New York, pp. 109–228 (1989).
36. Salem N., Kim H- Y. and Yergey J.A Jr.. Docosahexaenoic acid: membrane function and metabolism, in: Simopoulos A.P., Kifer R.R., Martin R. (Eds.), *The Health Effects of Polyunsaturates in Seafoods*, Academic Press, New York, pp. 263–317 (1986).
37. Lauritzen L., Hansen H.S., Jùrgensen M.H. and Michaelsen K.F. The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. *Progress in Lipid Research* **40**: 1–94 (2001).
38. McNamaraa R. K. and Carlson S. E. Role of omega-3 fatty acids in brain development and function: Potential implications for the pathogenesis and prevention of psychopathology. *Prostaglandins, Leukotrienes and Essential Fatty Acids* **75**: 329–349 (2006).