Indian Journal of Experimental Biology
Vol.49, May 2011, pp. 352-356

Anxiolytic effects of *Equisetum arvense* Linn. extracts in mice

Navdeep Singh, Sarabjit Kaur*, P M S Bedi & Divneet Kaur
Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar 143 005, India

Received 18 March 2010; revised 5 January 2011

The petroleum ether (PE), chloroform (CH), ethanol (ETH) and water extracts of *E. arvense* stems were evaluated for anti-anxiety activity in mice using elevated plus maze model. Ketamine induced hypnosis and actophotometer was used to evaluate sedative effect with various extracts in mice. The results were compared with standard drug diazepam. The ethanolic extract of *E. arvense* (50 and 100 mg/kg) significantly increased the time-spent and the percentage of the open arm entries in the elevated plus-maze model which was comparable to diazepam. Ethanolic extract (100 mg/kg) prolonged the ketamine-induced total sleeping time and decreased the locomotor activity in mice. The results suggest that the ethanolic extract of *E. arvense* seems to possess anxiolytic effect with lower sedative activity than that of diazepam. The results could be attributed to the flavonoid content of the ethanolic extract.

Keywords: Anti-anxiety, *Equisetum arvense*, Ethanol extract, Sedative

Anxiety disorders are the most common mental illness in the world and became a very important area of research interest in psychopharmacology. Interest in alternative medicine and plant-derived medications that affect the 'mind' is growing[1]. Anxiety affects one-eighth of the total population worldwide and has become an important area of research in psychopharmacology during this decade[2]. Benzodiazepines (BZDs) are the major class of compounds that are used in anxiety and they remain the most commonly prescribed treatment for anxiety. However, the realization that BZD have a narrow safety margin has prompted many researchers to evaluate new compounds in the hope of identifying other anxiolytic drugs with fewer unwanted side effects[3]. The use of herbal medications by physicians in Europe and Asia is becoming very common and researchers are exploring the traditional remedies to find a suitable cure for these 'mind affecting diseases[4].

Equisetum arvense Linn. (Equisetaceae) commonly known as field horsetail is a plant showing aerial stems, branched with regular verticilies 2–23 mm in diameter, terminal strobile in the branches and the main stem is 10 mm long and 4 mm in diameter. It grows in several regions of Europe and North, Central and South America[5]. Hypoglycemic[6] and diuretic activity[7] of horsetail have been reported. The plant presents a popular use as an anti-inflammatory agent in bathing for skin disease in Europe, Asia and America, as well as antiseptic in Turkey and America[8,9]. Water and ethanolic extract of *E. arvense* extract possess free radical scavenging activity so it is used as antioxidant[10]. Despite the widespread traditional use of *E. arvense* for treating various disorders there are no reports of scientific evaluation of its anxiolytic activity, therefore the present study has been undertaken to explore anxiolytic potential of the plant in three different models in mice.

Materials and Methods

Plant material—E. arvense dried sterile stems were brought from Himalaya Herb Store, Saharanpur, U.P, India. The taxonomic identity of the plant was confirmed by Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar. A voucher specimen no S.R.BotSci/0349 herb has been deposited in department herbarium.

Animals—Male swiss albino mice weighing 20-25 g were procured from the Central Research Institute (CRI), Kasauli, Himachal Pradesh. The animals were given standard laboratory feed and water. Groups of six mice (20-25 g) were used in all sets of experiments. The experiments were conducted in a semi-sound proof laboratory. The animal studies were carried out as per the guidelines of institutional animal ethical committee (IAEC).

*Correspondent author
Telephone: +91 09878916333
E-mail: gndu_sarabjit@yahoo.co.in

Chemicals—Solvents viz., petroleum ether (60º-80º Merck), chloroform (Merck) and Ethanol (S.D. Fine-Chem. Ltd, Mumbai), all of LR grade were employed for the extraction of the plant material. Diazepam hydrochloride I.P (2 mg/kg) was procured from Ranbaxy Laboratories Ltd, Mumbai.

Preparation of extracts and doses—Stems of *E. arvense* were dried in oven at 37°C and reduced to moderately coarse powder by passing through sieve no. 22. The powdered stems (500 g each), were extracted separately with petroleum ether, chloroform and ethanol successively using soxhlet apparatus[11]. The ultimate dried marc of stems was macerated with distilled water for 24 h and filtered. The extractives were obtained on evaporation of solvents using Rotary vacuum evaporator and dried extracts were preserved in vacuum dessicator. Simple syrup I.P. + carboxy methyl cellulose (0.5% w/v) was used as a vehicle for preparation of suspensions of various test doses of different extracts. The animals are divided into 5 groups of 6 mice each and given following treatment—Group I: vehicle treated animals served as control; Group II: animals received diazepam (2 mg/kg, ip), Group III, IV V were treated with different extracts of *E. arvense* at a dose of 25, 50 and 100 mg/ kg respectively.

Elevated plus maze (EPM)—Anxiolytic activity was measured using the elevated plus- maze test[12]. The maze consisted of two open (30 × 5 × 0.2 cm) and two closed (30 × 5 × 15 cm) arms, extending from a central platform (5 × 5 cm) and elevated to a height of 45 cm above the floor. Mice were individually placed on the center of the maze facing an open arm, and the number of entries and the time spent in closed and open arms were recorded during a 5 min observation period. The percentage of open arm entries (100 × open/total entries) was calculated for each animal. The experimental animals were intraperitoneally treated with diazepam (2mg/kg,) and the extracts (25, 50 or 100 mg/kg), 30 min respectively, before evaluation in the maze. The *E.arvense* extract at doses higher than 100mg/kg caused a marked decrease in motor activity that interfered with an accurate evaluation of anxiolytic effect. Therefore, higher doses of the extract were not included in the plus-maze test. Similar observations were recorded for the standard group (diazepam 2 mg/kg) as well as the control group (vehicle).

Locomotor activity—The most active extract (ethanolic) in anxiolytic models was further evaluated for sedative action by measuring its locomotor activity in mice using actophotometer. The breaking of infrared beams by mice[13] after 30 min of injecting them with control, standard drug and ethanolic extract was measured for 5 min.

Ketamine-induced sleeping time—The effect of the ethanolic extract on ketamine-induced sleeping time was measured[14]. After 30 min pre-treatment with the extract (25, 50 and 100 mg/kg, ip) or vehicle, animal (6 in each group) were injected with ketamine (100 mg/kg, ip). The interval between the administration of ketamine until the loss of the righting reflex was recorded as onset of sleep. The time from the loss to regaining of the righting reflex was considered as duration of sleep[15]. Diazepam (2 mg/kg) was used as standard drug.

Phytochemical screening—The various extracts of *E. arvense* were subjected to screening for the presence of various phytoconstituents like alkaloids, glycosides, flavonoids and saponins in accordance with the standard procedures[16,17].

Statistical analysis—The anxiolytic activities of the extracts, diazepam and control were analyzed by one way ANOVA. The test groups were compared with standard/control by Tukey's Multiple Range Test. The groups treated with extracts were compared with the vehicle group. Difference were considered significant at $P<0.05$. A SIGMA STAT® version 2.3 software program was used.

Results and Discussion

Anxiety and depressive disorders are common in all regions of the world[18]. They constitute a substantial proportion of the global burden of disease, and are projected to form the second most common cause of disability by 2020[19]. This increased importance of non-communicable diseases such as anxiety and depressive disorders. Such diseases present a particular challenge for low income countries, where infectious diseases and malnutrition are still high and only a low percentage of gross domestic products is allocated to health services[20]. The role of herbal medicine in the treatment of various psychological disorders has become well established over the past two decades. Many herbal products used to treat anxiety, cognitive disorders, and insomnia contain chemicals that influence the function of ionotropic receptors for the brain's major inhibitory neurotransmitters GABA, serotonin and BZD[21]. Despite the widespread traditional use of *E. arvense* for treating various disorders there are no reports of scientific evaluation of its anxiolytic activity, therefore the present study was undertaken to explore

anxiolytic potential of the plant. The elevated plus-maze test has been in use as a rodent model of anxiety for a decade, and is representative of those tests that are based upon the study of spontaneous behaviour patterns and which have high ecological validity. The fear due to height induces anxiety in mice when placed on the elevated plus-maze. The ultimate manifestation of anxiety and fear then is exhibited by decrease in motor activity, which is measured by the time spent by mice in the open arms. Anxiolytic compounds, by decreasing anxiety, increase the open arm exploration[22]. The ethanol extract at dose levels of 50 and 100 mg/kg showed significant increase in time spent in open arm and percentage of open arm entries ($P < 0.05$; Fig. 1 A and B). Since the petroleum ether, chloroform and water extracts (25, 50 and 100 mg/kg) of the plant did not produce meaningful effects on EPM, it was eliminated from further pharmacological investigation in this study. The anti-anxiety response was partially reverted when doses were increased from 100 mg/kg due to the presence of sedative effects (data not shown) and were not considered suitable for further evaluation. It is generally believed that locomotor activation results from brain activation, which manifests as an excitation of central neurons and as an increase in cerebral metabolism. While different neurochemical metabolisms are involved in brain activation, dopamine (DA) appears to play an essential role[23]. Locomotor activity is considered as an index of alertness and a decrease leads to sedation as a result of reduced excitability of the central nervous system[24]. Locomotor activity in actophotometer was significantly decreased in animals injected with *E. arvense* ethanol extract at the dose of 100 mg/kg, compared with vehicle treated controls ($P < 0.05$, Fig. 2). The reduction in locomotor activity was evident during activity measurement. Administration of diazepam at 2 mg/kg suppressed the locomotor activity to greater extent ($P< 0.05$, Fig. 2)

The anxiolytic effect of the plant extract was accompanied by a decrease in locomotor activity producing sedation at a higher dose (100 mg/kg). It is well known that drugs such as benzodiazepines and phenobarbital possess anxiolytic and sedative

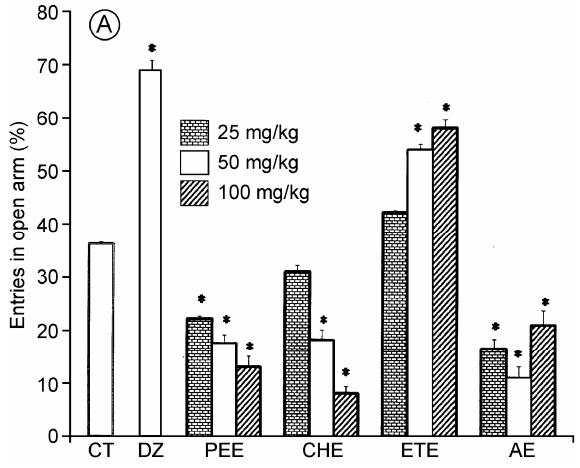

Fig. 1(A)—Effects of *E. arvense* extracts on the percentage of open arm entries of the elevated plus-maze (EPM). Values are mean ± SE from 6 mice each. *P<0.05 compared with vehicle-treated control. CT- Control; DZ- Diazepam; PEE- Petroleum ether extract; CHE- Chloroform extract; ETE- Ethanol extract; AE- Aqueous extract.

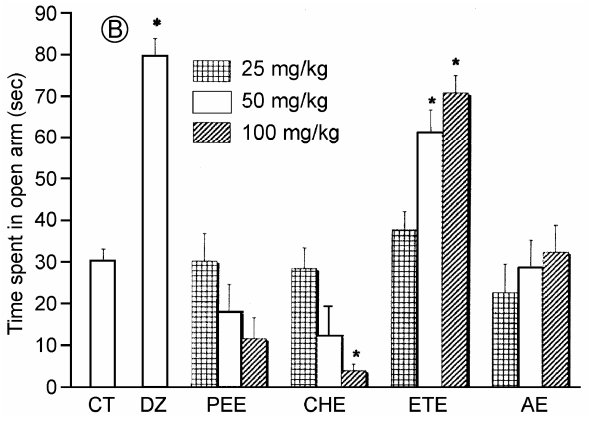

Fig. 1(B)—Effects of *E. arvense* extracts on the time spent in the open arm (sec). Values are mean ± SE from 6 mice each. *P<0.05 compared with vehicle-treated control. CT- Control; DZ- Diazepam; PEE- Petroleum ether extract; CHE- Chloroform extract; ETE- Ethanol extract; AE- Aqueous extract.

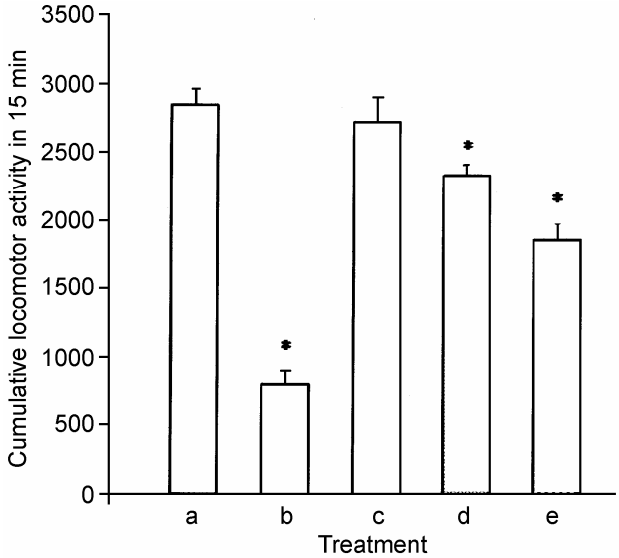

Fig. 2—Effects of *E. arvense* ethanolic extract on locomotor activity in mice. Values are mean ± SE from 6 mice each. *P<0.05 compared with vehicle-treated control. [a = control, b = diazepam (2 mg/kg), c = ETE (25 mg/kg), d = ETE (50 mg/kg), e = ETE (100 mg/kg)].

effects[25]. In the present study, the sedative effect of the plant was much lower than those produced by diazepam, thus showing a better profile as anxiolytic mrdicine. It is generally well accepted that the sedative effect of drugs can be evaluated by measurement of ketamine induced sleeping time in laboratory animals[26]. Injection of plant extract (30 min prior to ketamine) at doses of 25, 50 and 100 mg/kg, significantly suppressed the latency to sleep by 8, 26 and 37%, respectively ($P< 0.05$, Fig. 3). Injection of the diazepam (2 mg/kg) 30 min prior to ketamine reduced the latency to sleep by 56% ($P< 0.05$, Fig. 3). In animals pretreated with *E. arvense* ethanolic extract, only 100 mg/kg dose significantly prolonged the duration of sleep by 32% ($P< 0.05$). In a similar fashion, but much more pronounced, diazepam pretreatment increased (by 112 %) the total sleep time induced by ketamine ($P<0.05$, Fig. 4). Administration

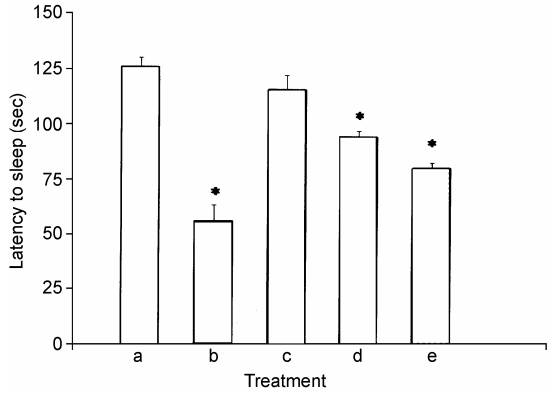

Fig.3—Effect of *E. arvense* ethanol extract on the latency to loss of righting reflex. The interval between the administration of ketamine until the loss of the righting reflex was recorded as onset of sleep. Values are mean±SEM from 6 mice. *$P<0.05$, compared with vehicle-treated control. [a = control, b = diazepam (2 mg/kg), c = ETE (25 mg/kg), d = ETE (50 mg/kg), e = ETE (100 mg/kg)].

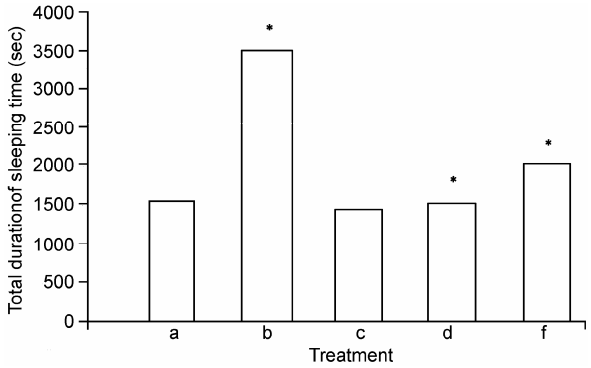

Fig.4—Effect of *E. arvense* ethanol extract on duration of sleeping time. Values are mean ± S.E.M from 6 mice. *$P<0.05$, compared with vehicle-treated control. [a = control, b = diazepam (2 mg/kg), c = ETE (25 mg/kg), d = ETE (50 mg/kg), e = ETE (100 mg/kg)].

Table 1—Results of phytochemical screening

Test	PEE	CHE	ETE	AE
Alkaloids	—	—	+	+
Carbohydrates	+	+	+	+
Proteins and amino acids	—	—	+	+
Phytosterols	—	+	+	—
Saponins	—	—	+	—
Flavonoids	—	—	+	—
Triterpenoids	—		+	—

+ Positive test; — Negative test

PEE- Petroleum ether extract; CHE- Chloroform extract; ETE- Ethanol extract; AE- Aqueous extract.

of ethanol extract of *E. arvense* (100 mg/kg) prolonged the duration of sleep and shortened the latency to loss of righting reflex. Similar results were obtained with diazepam, however in case of diazepam the time required for loosing the righting reflex was much shorter than that of plant extract, thus exhibiting lesser sedative properties by *E. arvense*.

The results of this study showed that the ethanolic extract of *E. arvense*, possess anxiolytic effects at therapeutically acceptable doses. This effect may be due to the interaction of the extract with the neural substrates or chemical mediators like noradrenaline, serotonin, GABA and BZD which are implicated to be responsible for anxiety-like condition. The mechanism of its anxiolytic action may be by interacting with some of the natural endogenous mediators in the body[27,28]. Also, there could be a linkage in the interaction of the extract with serotonergic pathway since serotonin had been widely implicated in aggressive behavior[29]. Phytochemical screening showed the presence of flavonoids, saponins and triterpenoids in ethanolic extract (Table 1). Earlier reports available stated the presence of several flavonoids, the major one being apigenin[30-33] in the plant. It has been observed that flavones like apigenin specifically recognise the central BDZ receptors and it has been found that flavones bind with high affinity BZD site of the $GABA_A$ receptor[34,35]. All these reports support the fact that apigenin act as ligand for the central benzodiazepine receptors exerting anxiolytic and slight sedative effects. Therefore the anxiolytic effects of ethanolic extract of *E. arvense* could be related at least in part to flavonoids or specifically to apigenin.

In summary, the present results demonstrated an anxiolytic effect from *E. arvense* ethanolic extract with a mild sedative action. Further pharmacological investigations are underway to identify the active constituents of the plant extract responsible for the reported activities.

Acknowledgement

This study was supported by the Department of Pharmaceutical Sciences, Guru Nanak Dev University Amritsar, Punjab, India.

References

1 Spinella, M., Herbal Medicines and Epilepsy: The potential for benefit and adverse effects, *Epilepsy Behav,* 2 (2001) 524.

2 Cha, H Y, Seo J J, Park J H & Oh K W, Anxiolytic effects of total saponin fraction from Ginseng radix rubra on the elevated plus maze model in mice. *J Ginseng Res*, 28 (2004) 132.

3 Grundman O, Nakajima J, Seo S & Butterweck V, Anti-anxiety effects of *Apocynum venetum* L. in the elevated plus maze test, *J Ethnopharmacol,* 110 (2007) 406.

4 Rabbani M, Sajjadi S E & Zarei H R, Anxiolytic effects of *Stachys lavandulifolia* on the elevated plus-maze model of anxiety in mice, *J Ethnopharmacol*, 89 (2003) 271.

5 Joly A B, *Botanica Introducão ã taxonomia vegetal*, (Nacional, São Paulo) 1979,152.

6 Soleimani S, Azarbaizani F F & Nejati V, The effect of *Equisetum arvense* L. (Equisetaceae) in histological changes of pancreatic β-cells in streptozotocin induced diabetes in rats, *Pak J Biol Sci,* 10 (2007) 4236.

7 Mamedova K T & Gysejnova ID, Effect of *Equisetum arvense* L. on diuresis, *Doklady – Akademiya Nauk Azerbaidzhana,* 51 (1996) 175.

8 Ody P & Kindersley D, *The complete medicinal herbal* (DK Publishing House, New York) 1993, 3.

9 Mineo S, Takayasu M, Kaori H, Yoshiro T, Taisuke S & Masaaki M, Studies on bathing agent: anti- inflammatory effect of bathing agent which used for skin disease, *Shoyakugaku Zasshi*, 47 (1993) 1.

10 Nagai T, Myoda T & Nagashima T, Antioxidative activities of water extract and ethanol extract from field horsetail (tsukushi) *Equisetum arvense*, *Food Chem,* 91(2005) 389.

11 Evans W, Quality control in *Trease and evans pharmacognosy* (WB Saunders Company Ltd, London) 1996, 98.

12 Lister RG, The use of a plus-maze to measure anxiety in the mouse, *Psychopharmacology*, 92 (1987) 180.

13 Rabbani M, Wright E J & Little H J, Tolerence to competitive NMDA antagonists, but no cross tolerence with barbirurates, *Pharmacol Biochem Behav,* 50 (1995) 9.

14 Mimura M, Namiki A, Kishi R, Ikeda T & Miyake H, Antagonistic effect of physostigmine on ketamine-induced anesthesia, *Psychopharmacology,* 102 (1990) 399.

15 Bastidas Ramirez B E, Navarro R N, Quezada Arellano J D, Ruiz M B, Villanueva M T & Garzon P, Anticonvulsant effects of *Magnolia grandiflora* in the rat, *J Ethnopharmacol,* 61 (1998) 143.

16 Farnsworth N R, Biological and phytochemical screening of plants, *J Pharm Sci,* 55 (1966) 225.

17 Khandelwal K R, Techniques and experiments in *Practical pharmacognosy* (Nirali Prakashan, Pune) 2003, 149.

18 Mirza, I & Jenkins, R, Risk factors, prevalence, and treatment of anxiety and depressive disorders in Pakistan: Systematic review, *Brit Med J,* 328 (2004) 794.

19 Murray C & Lopez A, The global burden of diseases: a comprehensive assessment of mortality and disability from diseases, injuries and risk factors in 1990 and projected to 2020. Boston: Harvard School of Public Health, WHO and World Bank, 1996.

20 World Health Organization, Macroeconomics and health: investing in health for economic development, Geneva, 2001.

21 Sarris J, Herbal medicines in the treatment of psychiatric disorders: a systematic Review, 21 (2007) 703.

22 Kumar S & Sharma A, Anti-anxiety activity studies of various extracts of *Turnera aphrodisiaca* Ward, *J Herb Pharmacother*, 5 (2005) 13.

23 Salamone J D, Correa M, Mingote S M & Weber S M, Beyond the reward hypothesis: Alternative functions of nucleus accumbens dopamine, *Curr Opin Pharmacol*, 5 (2005) 34.

24 Thakur V D & Mengi S A, Neuropharmacological profile of *Eclipta alba* (Linn.) Hassk, *Ethnopharmacol*, 102 (2005) 23.

25 Treit D, Animal models for the study of anti-anxiety agents: A review, *Neurosci Biobehv Rev*, 9 (1985) 202.

26 Ayoka, A E, Akomolafe R O, Iwalewa E O Akanmu M A & Ukponmwan O E, Sedative, anti-epileptic and antipsychotic effects of *Spondias mombin* L (Anacardiaceae) in mice and rats, *J Ethnopharmacol,* 103 (2006) 166.

27 Siqueira I R, Lara D R, Silva D, Gaieski F S, Nunes D S & Elisabersky, E, Psychopharmacological properties of *Ptychopetalum Olacoides Bentham* (*olacaceae*), *Pharmaceut. Bio*, 36 (1998) 327.

28 Shih J C, Ridd M J, Chen K, Meehan, W P, Kung M & Self I, Ketanserin and tetrabenazine abolish aggression in mice lacking mono amine oxidase A, *Brain Res*, 835 (1999) 104.

29 Sanchez C, Arnt J, Hyttel J & Moltzen, Z K,The role of serotonergic mechanisms in inhibition of isolation-induced aggression in rats and mice, *Psychopharmacol*, 110 (1993) 53.

30 Carnet A, Petitjean-Freytet C, Muller D & Lamaison J L, Content of major constituents of horsetails, *Equisetum arvense, Plantes medicinales et Phytotherapy,* 25 (1991) 32.

31 Pietta P, Mauri P, Bruno A, Rava A, Manera E & Ceva P, Identification of flavonoids from *Ginkgo biloba* L., *Anthemis nobilis* L. and *Equisetum arvense* L. by high-performance liquid chromatography with diode-array UV detection, *J Chromatography,* 553 (1991) 223.

32 Veit M, Bauer K, Geiger H & Czygan F C, Flavonoids of Equisetum hybrids in the subgenus Equisetum, *Planta Med*, 58 (1992) A 697.

33 Oh H, Kim D H, Cho J H & Kim Y C, Hepatoprotective and free radical scavenging activities of phenolic petrosins and flavonoids isolated from *Equisetum arvense, J Ethnopharmacol,* 95 (2004) 421.

34 Adeyemi O O, Yemitan O K & Taiwo A E, Neurosedative and muscle-relaxant activities of ethyl acetate extract of *Baphia nitida, J Ethnopharmacol*, 106 (2006)312.

35 Huang X, Liu T, Gu J, Luo X & Cao Y, 3D-QSAR model of flavonoids binding at benzodiazepine site in $GABA_A$ receptors, *J Med Chem*, 44 (2001)1883