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Apigenin: The Anxiolytic Constituent of *Turnera aphrodisiaca*

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Abstract

Turnera aphrodisiaca Ward (Turneraceae) has been traditionally used for the treatment of anxiety neurosis and as aphrodisiac, but no attempts have been made to investigate the plant systematically for its traditional claims. The current investigation was carried out ot isolate the bioactive constituent(s) from *T. aphrodisiaca* using bioactivity-guided fractionation. Antianxiety activity-guided fractionation of methanol extract of the plant led to isolation of 5,7,4′ -trihydroxy flavone apigenin. Its structure was elucidated by UV and NMR data. Apigenin exhibited significant anxiolytic activity at a dose of 2 mg/kg, p.o., in mice using elevated plus maze model of anxiety. It is concluded that apigenin is responsible for anxiolytic effects of this traditionally used plant.

Keywords: Anxiolytic, aphrodisiac, apigenin, *Turnera aphrodisiaca*, Turneraceae.

Introduction

Anxiety and insomnia are biological disorders that affect one-eighth of the total population of the world (NIMH, 2002). Pharmacotherapeutic approaches for the management of these ''modernization-borne diseases'' include psychotropic drugs such as barbiturates, benzodiazepines, azaspirones, norepinephrine and serotonin-reuptake inhibitors, monoamine oxidase inhibitors, and phenothiazines (Baldessarini, 2001). Among these, benzodiazepines are the most widely prescribed synthetic chemical drugs for the treatment of anxiety, insomnia, epilepsy, and stress. Regular use of benzodiazepines causes deterioration of cognitive functioning, addiction, physical dependence, and tolerance (Council Report, 1997; Longo & Johnson, 2000; Baldessarini, 2001). In the light of adverse effects associated with the synthetic drugs, researchers of today are exploring natural resources to discover safer and effective drugs. Investigating plants, based on their use in traditional systems of medicine, is a sound, viable, and cost-effective strategy to develop new drugs (Dhawan, 1995). A benzoflavone from *Passiflora incarnata* Linn. (Passifloraceae) aerial parts has been reported, from our laboratory, to be a potential anxiolytic moiety (Dhawan et al., 2001a,b,c). Continuing the endeavour, *Turnera aphrodisiaca* Ward was selected for evaluating its anxiolytic potential.

T. aphrodisiaca (synonym *T. diffusa* willd). (Turneraceae) is commonly known as ''Damiana.'' The leaves of *T. aphrodisiaca* have been used traditionally as a stimulant, aphrodisiac, tonic, diuretic, nerve tonic, laxative, and in kidney, menstrual, and pregnancy disorders (Hocking, 1955; Parfitt, 1999). The leaf infusion of Damiana has been used as a traditional remedy in the diseases related to the gastrointestinal and respiratory systems (Caceres, 1996), reproductive organs (Saggese, 1959), and for the treatment of gonorrhea in Latin American societies (Koch, 1936). Damiana has achieved some repute in the treatment of sexual impotence where it is used in conjunction with strychnine, phosphorus, or some other stimulants in homeopathic formulations (Osol et al., 1947). Mother tincture (85% ethanol extract) of Damiana is an important homeopathic medicine for the treatment of sexual debility and nervous prostration (Boericke, 1988). The *British Herbal Pharmacopoeia* (1983) lists specific indications for Damiana as anxiety neurosis associated with impotency and includes other indications such as depression, nervous dyspepsia, atonic constipation, and coital inadequacy.

Phytochemical reports on *T. aphrodisiaca* indicate that the plant contains tetraphyllin B (cyanoglycoside) (Spencer & Seigler, 1981); gonzalitosin I (flavonoid) (Dominguez & Hinojosa, 1976); arbutin (phenolic glycoside) (Auterhoff & Hackle, 1968); damianin (Steinmetz, 1960); tricosan-2-one, hexacosanol (hydrocarbons)

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(Fryer, 1965); a volatile oil containing α-pinene, β-pinene, *p*-cymene, and 1,8-cineole (Auterhoff & Hackle, 1968); and β-sitosterol (phytosterol) (Dominguez & Hinojosa, 1976).

A survey of the literature on *T. aphrodisiaca* revealed only three pharmacological reports on the plant. The aqueous extract of *T. aphrodisiaca* whole plant has been reported to exhibit significant hypoglycemic activity in alloxan-diabetic male mice (Perez et al., 1984) and in rabbits (Aguilara et al., 1998). The aqueous extract of the plant was reported to exhibit aphrodisiac activity in sexually sluggish male rats at a dose of 1 ml/kg (Arletti et al., 1999).

Despite a long history of use of *T. aphrodisiaca* as a traditional medicine for the treatment of various ailments, especially CNS disorders, the plant has never been subjected to CNS activity studies. Recently, authors have reported that among various extracts, viz., petroleum ether, chloroform, methanol, and water, of *T. aphrodisiaca* aerial parts, only the methanol extract (25 mg/kg, p.o.) exhibited significant antianxiety activity on elevated plus maze model (Kumar & Sharma, 2004). Thus, it was considered worthwhile to subject the methanol extract of *T. aphrodisiaca* to antianxiety activity-guided fractionation studies.

Materials and Methods

Plant material

T. aphrodisiaca Ward was procured from a cultivated source [Rati Ram Nursery, Village Khurrampur, district Saharanpur (U.P.), India] in August 2002 and was dried in the shade. Identity of the plant was confirmed through Botanical Survey of India (Howrah, India). A voucher specimen of the plant has been deposited in the Herbarium-cum-Museum of the University Institute of Pharmaceutical Sciences, Panjab University (Chandigarh, India).

Animals

Laca mice (either sex), bred at the Central Animal House, Panjab University, Chandigarh, were allowed a standard pellet diet (Ashirwad, Chandigarh, India) and water *ad libitum*. Groups of five mice (20–24 g) were used in all sets of experiments. The animals were fasted for 18 hours before use. Approval from the Institutional Animal Ethical Committee of Panjab University (Chandigarh, India) was received before carrying out the antianxiety studies.

Solvents

Chloroform (Ranbaxy Laboratory Chemicals, New Delhi, India), methanol, acetonitrile (s.d. Fine Chemicals, Mumbai, India), toluene and acetone (E. Merck, Mumbai, India), all of LR grade, distilled under normal atmospheric pressure, were employed for column and preparative/thin-layer chromatography.

Chemicals and instruments

Keiselguhr white (Loba Chemicals, Mumbai, India) was used for column chromatography. Precoated aluminum TLC sheets (silica gel G, 0.2 mm; E. Merck) and 2 µl capillary tubes (CAMAG) were used for TLC studies. The thin-layer chromatograms were visualized under 254/366 nm UV light (DESAGA, Heidelberg, Germany, Min. UVIS [Ultraviolet Imaging Spectrograph]) and also by spraying with 60% v/v aqueous sulfuric acid (E. Merck). Melting point was determined with Rescholar melting point apparatus (Ambala, India). UV spectra was obtained on a Perkin Elmer Hitachi 330, Switzerland (Lambda 15 UV/VIS) spectrophotometer. NMR spectra were run on a Bruker spectrometer (Switzerland) at 300 MHz.

Preparative thin-layer chromatography

Preparative thin-layer chromatography was performed using 20×20 glass plates coated with 0.5-mm silica gel G (E. Merck).

Vehicle and standard

Simple syrup I.P. + Tween 80 (5%) was used as vehicle for preparing the suspension of various test doses. Diazepam (2 mg/kg, p.o.) (Triko Pharmaceuticals, Rohtak, Haryana, India), suspended in vehicle, was used as standard anxiolytic drug.

Preparation of doses

Test doses of various fractions and subfractions of methanol extract of *T. aphrodisiaca* were prepared by suspending in the vehicle in such concentrations as to treat mice with a volume ranging from 0.20 to 0.24 ml per oral route.

Statistics

The results were expressed as mean ± standard error of mean (SEM). The test doses were compared with diazepam and control by analysis of variance (ANOVA) followed by Studentized Tukey's test (Scheffer, 1980).

Elevated plus maze model of anxiety

The plus maze apparatus, consisting of two open arms (16×5 cm) and two closed arms ($16 \times 5 \times 12$ cm) having an open roof, with the plus maze elevated (25 cm) from the floor, was used to observe anxiolytic behavior in animals (Kulkarni, 2003). Each mouse was placed at the center of the elevated plus maze with its head facing the open arms. During a 5-min experiment, the behavior

of the mouse was recorded as (a) the number of entries into the open arms, and (b) average time spent by the mouse in the open arms (average time = total time spent in open arms/number of entries in open arms). Various fractions and bioactive constituent of *T. aphrodisiaca* methanol extract were administered orally using a tuberculin syringe fitted with oral canula. Dose administration schedule was so adjusted that each mouse was having its turn on the elevated plus maze apparatus 45 min after the administration of the dose. During the entire experiment, the animals were allowed to socialize. Every precaution was taken to ensure that no external stimuli, other than the height of plus maze, could invoke anxiety in the animals.

Preparation of methanol extract of *T. aphrodisiaca*

Powdered aerial parts (2.5 kg) of *T. aphrodisiaca* were successively extracted in a Soxhlet apparatus using the solvents in order of increasing polarity, viz., petroleum

Table 1. Fractionation of methanol extract of *T. aphrodisiaca*, and antianxiety activity of various fractions using elevated plus maze apparatus.

Treatment		Dose (mg/kg)	Mean[a] number of entries ± SEM	Mean[a] time[b] (s) ± SEM
Control		Vehicle	2.4 ± 0.51	3.18 ± 0.22
Fraction	Eluant			
F_1 (7.80 g)	$CHCl_3$	5	2.0 ± 0.45	3.34 ± 0.23
		10	2.2 ± 0.49	2.89 ± 0.16
		15	2.4 ± 0.40	3.31 ± 0.24
		20	2.8 ± 0.58	3.57 ± 0.18
		25	2.2 ± 0.58	3.72 ± 0.33
F_2 (2.60 g)	$CHCl_3$	5	2.2 ± 0.37	3.04 ± 0.17
	$CHCl_3$ + MeOH (49:1)	10	2.4 ± 0.51	3.26 ± 0.48
	$CHCl_3$ + MeOH (19:1)	15	2.4 ± 0.60	3.16 ± 0.24
		20	2.2 ± 0.58	3.30 ± 0.34
		25	2.6 ± 0.51	3.50 ± 0.60
F_3 (7.58 g)	$CHCl_3$ + MeOH (9:1)	5	2.0 ± 0.45	2.98 ± 0.27
		10	2.4 ± 0.40	3.21 ± 0.16
		15	2.8 ± 0.49	3.55 ± 0.25
		20	2.8 ± 0.58	3.08 ± 0.24
		25	3.0 ± 0.55	3.82 ± 0.41
F_4 (11.68 g)	$CHCl_3$ + MeOH (4:1)	5	2.2 ± 0.37	3.21 ± 0.14
		10	2.6 ± 0.68	3.09 ± 0.44
		15	2.6 ± 0.51	3.35 ± 0.15
		20	3.0 ± 0.78	3.53 ± 0.28
		25	3.2 ± 0.58	3.84 ± 0.23
F_5 (76.85 g)	$CHCl_3$ + MeOH (4:1)	5	4.4 ± 0.68*	6.18 ± 0.33*
	$CHCl_3$ + MeOH (1:1)	10	6.6 ± 0.81*	11.87 ± 0.56*
		15	4.6 ± 0.68*	5.74 ± 0.44*
		20	1.8 ± 0.37	2.59 ± 0.14
		25	0.0 ± 0.00	0.00 ± 0.00*
F_6 (48.05 g)	$CHCl_3$ + MeOH (1:1)	5	2.2 ± 0.37	3.03 ± 0.25
		10	2.4 ± 0.51	3.63 ± 0.35
		15	3.6 ± 0.51	3.33 ± 0.26
		20	2.8 ± 0.58	3.65 ± 0.32
		25	3.2 ± 0.37	4.05 ± 0.20
F_7 (34.60 g)	MeOH	5	2.2 ± 0.37	3.02 ± 0.16
	MeOH + acetonitrile (1:1)	10	2.6 ± 0.51	3.38 ± 0.49
		15	2.4 ± 0.51	3.32 ± 0.22
		20	2.8 ± 0.37	3.55 ± 0.53
		25	2.8 ± 0.74	4.14 ± 0.60

[a] $n = 5$.
[b] Average time each animal spends in open arms = total duration in open arms/number of entries in open arms.
* $p < 0.05$ vs. control; ANOVA followed by Studentized Tukey's test.

Table 2. Fractionation of F_5, and antianxiety activity of various subfractions.

Treatment		Dose (mg/kg)	Mean[a] number of entries ± SEM	Mean[a] time [b] (s) ± SEM
Control		Vehicle	2.8 ± 0.66	3.06 ± 0.26
Fraction	Eluant			
$F_{5.1}$ (3.42 g)	$CHCl_3$	1	2.2 ± 0.37	2.94 ± 0.30
		2	2.6 ± 0.51	3.14 ± 0.28
		5	2.6 ± 0.25	3.48 ± 0.29
		10	3.0 ± 0.32	3.14 ± 0.38
		20	3.2 ± 0.20	3.50 ± 0.13
$F_{5.2}$ (0.89 g)	$CHCl_3$	1	2.2 ± 0.37	2.99 ± 0.33
	$CHCl_3$ + MeOH (49:1)	2	2.6 ± 0.25	3.45 ± 0.24
		5	2.8 ± 0.37	3.63 ± 0.25
		10	3.0 ± 0.55	3.38 ± 0.33
		20	3.2 ± 0.58	3.64 ± 0.61
$F_{5.3}$ (2.78 g)	$CHCl_3$ + MeOH (49:1)	1	3.0 ± 0.32	3.33 ± 0.22
	$CHCl_3$ + MeOH (19:1)	2	4.2 ± 0.58*	5.32 ± 0.31*
		5	6.4 ± 0.93*	12.34 ± 0.74*
		10	3.4 ± 0.51	3.52 ± 0.26
		20	0.0 ± 0.00*	0.00 ± 0.00*
$F_{5.4}$ (5.84 g)	$CHCl_3$ + MeOH (19:1)	1	3.0 ± 0.45	3.15 ± 0.29
		2	3.0 ± 0.55	3.52 ± 0.23
		5	3.8 ± 0.58	4.20 ± 0.28
		10	4.6 ± 0.51*	3.32 ± 0.31
		20	6.4 ± 1.03*	4.14 ± 0.35
$F_{5.5}$ (9.23 g)	$CHCl_3$ + MeOH (19:1)	1	2.6 ± 0.40	3.09 ± 0.22
	$CHCl_3$ + MeOH (9:1)	2	2.8 ± 0.37	3.26 ± 0.32
		5	3.2 ± 0.58	3.24 ± 0.44
		10	3.0 ± 0.55	3.46 ± 0.42
		20	3.2 ± 0.58	3.16 ± 0.19
$F_{5.6}$ (6.72 g)	$CHCI_3$ + MeOH (9:1)	1	3.0 ± 0.45	2.92 ± 0.22
		2	3.0 ± 0.55	3.22 ± 0.23
		5	3.6 ± 0.51	3.22 ± 0.17
		10	3.0 ± 0.63	3.62 ± 0.38
		20	3.4 ± 0.75	3.52 ± 0.27
$F_{5.7}$ (11.1 g)	$CHCI_3$ + MeOH (17:3)	1	2.6 ± 0.40	3.39 ± 0.21
	$CHCI_3$ + MeOH (4:1)	2	3.0 ± 0.55	3.19 ± 0.29
	$CHCI_3$ + MeOH (1:1)	5	3.0 ± 0.32	3.63 ± 0.30
		10	3.6 ± 0.51	2.89 ± 0.61
		20	3.6 ± 0.40	3.28 ± 0.22

[a] $n = 5$.
[b] Average time each animal spends in open arms = total duration in open arms/number of entries in open arms.
*$p < 0.05$ vs. control; ANOVA followed by Studentized Tukey's test.

ether (60–80°C), chloroform, and methanol. The yield of methanol extract of *T. aphrodisiaca* aerial parts was found to be 12.10% w/w.

Fractionation of methanol extract

The methanol extract (250 g) of *T. aphrodisiaca* was loaded onto a column packed with Keiselguhr white and eluted with chloroform, chloroform-methanol, and methanol-acetonitrile solvent systems. A total of 130 fractions, 250 ml each, were collected. These were pooled, based on similar thin-layer chromatograms, to get 7 fractions ($F_{5.1}$–$F_{5.7}$), which were evaluated for anti-anxiety activity at various doses (5, 10, 15, 20, or 25 mg/kg, p.o.) using elevated plus maze apparatus.

The bioactive fraction F_5 (75 g) was subjected to phytochemical screening (Farnsworth, 1966) and column chromatographed over Keiselguhr white. Elution was done with chloroform and chloroform-methanol to get 7 pooled subfractions ($F_{5.1}$–$F_{5.7}$). These were evaluated

Table 3. Antianxiety activity of K_1 and K_2 isolated from $F_{5.3}$.

Treatment	Dose (mg/kg)	Mean[a] number of entries ± SEM	Mean[a] time[b](s) ± SEM
Control	Vehicle	$2.6 \pm 0.40^{\dagger}$	$3.33 \pm 0.29^{\dagger}$
Diazepam	2.00	$6.6 \pm 0.93^{*}$	$12.13 \pm 0.66^{*}$
K_1	0.5	$3.4 \pm 0.51^{\dagger}$	$3.30 \pm 0.30^{\dagger}$
	1.00	$4.2 \pm 0.66^{*\dagger}$	$5.40 \pm 0.66^{*\dagger}$
	2.00	$6.4 \pm 1.03^{*}$	$12.49 \pm 0.63^{*}$
	5.00	$4.0 \pm 0.32^{\dagger}$	$6.06 \pm 0.46^{*\dagger}$
K_2	0.5	$2.6 \pm 0.40^{\dagger}$	$3.66 \pm 0.33^{\dagger}$
	1.00	$2.8 \pm 0.37^{\dagger}$	$3.03 \pm 0.23^{\dagger}$
	2.00	$2.8 \pm 0.49^{\dagger}$	$3.33 \pm 0.32^{\dagger}$
	5.00	$3.2 \pm 0.58^{\dagger}$	$3.50 \pm 0.39^{\dagger}$

[a] $n = 5$.
[b] Average time each animal spends in open arms = total duration in open arms/number of entries in open arms.
$^{*}p < 0.05$ vs. control; $^{\dagger}p < 0.05$ vs. standard; ANOVA followed by Studentized Tukey's test.

for anxiolytic activity at various doses (i.e., 1, 2, 5, 10, or 20 mg/kg, p.o.).

Bioactive subfraction 7.3 ($F_{5.3}$) was also subjected to phytochemical screening (Farnsworth, 1966). Thin-layer chromatography of $F_{5.3}$ using mobile phase toluene: chloroform: acetone: 8:5:7 showed two spots. Repeated preparative thin-layer chromatography of $F_{5.3}$ using the solvent system toluene:chloroform:acetone, 8:5:7, yielded two pure isolates K_1 (72 mg) and K_2 (13 mg), which were evaluated for antianxiety activity at the dose levels of 0.5, 1.0, 2.0, or 5.0 mg/kg, p.o.

Characterization of K_1

K_1 was characterized on the basis of its melting point, UV, ^{1}H NMR, and ^{13}C NMR spectra, and by comparison with that of the reference standard of apigenin (Sigma-Aldrich, St. Louis, USA).

Results

Results of column chromatography of methanol extract of *T. aphrodisiaca* and the antianxiety activity profile of F_1–F_7 are shown in Table 1. Phytochemical screening of F_5 showed that the fraction contained alkaloids and flavonoids. Results of column chromatography of F_5, and antianxiety activity of $F_{5.1}$–$F_{5.7}$ are shown in Table 2. Subfraction $F_{5.3}$, when subjected to qualitative phytochemical screening, tested positive only for flavonoids. Antianxiety activity profile of K_1 and K_2 is reported in Table 3.

Discussion

Antianxiety activity of various fractions and bioactive constituent of methanol extract of *T. aphrodisiaca* aerial parts was evaluated employing a widely used model (i.e., elevated plus maze). The model was chosen because it is effective, cheap, simple, less time consuming, requires no preliminary training for the mice, and does not cause much discomfort to the animals while handling. The model is principally based on the observations that exposure of animals to an elevated and open maze results in approach-avoidance conflict, which is manifested as an exploratory-cum-fear drive. The fear due to height (acrophobia) induces anxiety in the animals when placed on the elevated plus maze. The ultimate manifestation of anxiety and fear in the animals is exhibited by decrease in motor activity, which is measured by the time spent by the animal in the open arms.

Out of the four extracts, viz., petroleum ether, chloroform, methanol, and water, of *T. aphrodisiaca*, the methanol extract exhibited significant anxiolytic activity (Kumar & Sharma, 2004). Therefore, the methanol extract of the plant was selected for bioactivity-guided fractionation. Column chromatography of the methanol extract afforded bioactive fraction F_5, which tested positive for alkaloids and flavonoids. F_5 exhibited significant antianxiety activity at a dose of 10 mg/kg, p.o., using elevated plus maze model of anxiety (Table 1). Column chromatography of F_5 yielded 7 subfractions out of which only $F_{5.3}$ exhibited significant anxiolytic activity at a dose of 5 mg/kg, p.o. (Table 2). $F_{5.3}$ gave positive test only for flavonoids. Repeated preparative thin-layer chromatography of $F_{5.3}$ led to the isolation of two pure compounds, K_1 (72 mg) and K_2 (13 mg). K_1 exerted significant anxiolytic effects, which were comparable with those of the control as well as the standard (diazepam), at a dose of 2 mg/kg, p.o. (Table 3). K_2 was found to be devoid of antianxiety activity (Table 3). A dose dependent decrease in antianxiety activity was observed at higher doses of F_5 (15, 20, or 25 mg/kg, p.o.), $F_{5.3}$ (10 or 20 mg/kg, p.o.) and K_1 (5 mg/kg, p.o.). We suggest that these effects might be due to mild sedation in mice

at higher doses. The structure of K_1 was elucidated by UV, ^{1}H NMR, and ^{13}C NMR spectral data and characterized as 5,7,4′-trihydroxy flavone, that is, (apigenin) (Mabry et al., 1970; Viola et al., 1995; Owen et al., 2003). Further, the identity of K_1 was confirmed by comparison of its spectral data with that of reference standard of apigenin. Because the quantity of K_2 generated, following column and preparative thin-layer chromatography, was not sufficient to perform its spectral analysis, its structure was not established. Our findings are in agreement with those of Viola et al. (1995) and Salguiero et al. (1997) who have reported that apigenin exhibits anxiolytic activity at a dose of 3 mg/kg, i.p., and mild sedative activity at higher doses. In the light of the above findings, it is concluded that apigenin is responsible for the antianxiety activity of *T. aphrodisiaca*.

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References

Aguilara FJA, Ramos RR, Gutirrez SP, Contretras AA, Weber CCC, Saenz JLF (1998): Study of the antihyperglycaemic effect of plants used as antidiabetics. *J Ethnopharmacol 61*: 101–110.

Arletti R, Benelli A, Cavazzuti E, Scarpetta G, Bertolini A (1999): Stimulating property of *Turnera diffusa* and *Pfaffia paniculata* extracts on the sexual behavior of male rats. *Psychopharmacology 143*: 15–19.

Auterhoff H, Hackle HP (1968): Components of Damiana drug. *Arch Pharm 301*: 537–544.

Baldessarini R (2001): Drugs and the treatment of psychiatric disorders. In: Hardman JG, Limbird LE, eds., *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 10th ed. New York, McGraw-Hill, pp. 399–427.

Boericke W (1988): *Pocket Manual of Homoeopathic Materia Medica*. New Delhi, India, B. Jain Publisher Private Limited, p. 659.

British Herbal Pharmacopoeia (1983): Monograph: *Turnera diffusa*. West Yorks, British Herbal Medicine Association, p. 29. Available at www.phytotherapies.org/monograph_detail.cfm?id=222

Caceres A (1996): Damiana. In: Giron L, Caceres A, eds., *Plantas de Uso Medicinal en Guatemala*, Ist Ed. Editorial Universataria San Carlos de Guatemala, pp. 160–162.

Council Report (1997): Benzodiazepines: Risks, benefits or dependence. London, Royal College of Psychiatrists, London, pp. 1–10. Available at www.rcpsych.ac.uk/publications/cr/council/cr59.pdf

Dhawan BN (1995): Centrally acting agents from Indian plants. In: Koslovo SH, Srinivasa MR, Coelho GV, eds., *Decade of the Brain: India/USA Research in Mental Health and Neurosciences*. Rockville, MD, National Institute of Mental Health, pp. 197–202.

Dhawan K, Kumar S, Sharma A (2001a): Comparative biological activity study on *Passiflora incarnata* and *P. edulis*. *Fitoterapia 72*: 698–702.

Dhawan K, Kumar S, Sharma A (2001b): Anxiolytic activity of aerial and underground parts of *Passiflora incarnata*. *Fitoterapia 72*: 922–926.

Dhawan K, Kumar S, Sharma A (2001c): Anti-anxiety studies on extracts of *Passiflora incarnata* Linneaus. *J Ethnopharmacol 78*: 165–170.

Dominguez XA, Hinojosa M (1976): Mexican medicinal plants. XXVIII. Isolation of 5-hydroxy-7, 3′ 4′-trimethoxy flavone from *Turnera diffusa*. *Planta Med 30*: 68–71.

Farnsworth NR (1966): Biological and phytochemical screening of plants. *J Pharm Sci 55*: 225–286.

Fryer FA (1965): Chemical investigation of damiana (*Turnera diffusa*). *Specialities 1*: 21–23.

Hocking GM (1955): *A Dictionary of Terms in Pharmacognosy and Other Divisions of Economic Botany*. Springfield, IL, Charles C. Thomas, p. 234.

Koch L (1936): Drug collection from Bolivia systematically, anatomically and chemically examined. *Arch Pharmacol 274*: 343–369.

Kulkarni SK (2003): *Handbook of Experimental Pharmacology*, 3rd ed. Pitampura, New Delhi, Vallabh Prakashan, pp. 135–140.

Kumar S, Sharma A (2004): Anti-anxiety activity studies of various extracts of *Turnera aphrodisiaca* Ward. *J Herbal Pharmacother 5*: 13–21.

Longo L, Johnson B (2000): Addiction: Part I. Benzodiazepines-side effects, abuse risk and alternatives. *Am Fam Physician 61*: 2121–2128. Available at http://ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=Pubmed&list_uids=10779253&dopt=Abstract

Mabry T, Markham K, Thomas M (1970). *The Systematic Identification of Flavonoids*. New York, Springer-Verlag, pp. 81, 280.

NIMH (2002): *Anxiety Disorders*. Bethesda, MD, National Institute of Mental Health, Office of Communications and Public Liaison.

Osol A, Farrar GF, Leuallen EE, Youngken HW, Detweiler DK (1947): *Dispensatory of United States of America*, 24th ed. Philadelphia, J. B. Lippincott Company, pp. 1422–1423.

Owen RW, Haubner R, Mier W, Giacosa A, Hull WE, Spiegelhalder B, Bartsch H (2003): Isolation, structure elucidation and antioxidant potential of the major phenolic and flavonoid compounds in brined olive drupes. *Food Chem Toxicol 41*: 703–717.

Parfitt K (1999): *Martindale, the Complete Drug Reference*, 32nd ed. London, Pharmaceutical Press, p. 1570.

Perez RM, Ocegueda A, Munoz JL, Avita JG, Morrow WW (1984): A study of the hypoglycaemic effect of some Mexican plants. *J Ethnopharmacol 12*: 253–262.

Saggese D (1959): *Medicinal Herbs of Argentina*, 10th ed. Argentina, Antoghazzi & Co., Rosario Argentina, pp. 1–189.

Salgueiro JB, Ardenghi P, Dias M, Perreira MBC, Izquierdo I, Medina JH (1997): Anxiolytic natural and synthetic flavonoid ligands of the central benzodiazepine receptor have no effect on memory tasks in rats. *Pharmacol Biochem Behav 58*: 887–891.

Scheffer WC (1980): *Statistics for the Biological Sciences*. Reading, MA, Addison-Wesley, pp. 121–141.

Spencer KC, Seigler DS (1981): Tetraphyllin B from *Turnera diffusa*. *Planta Med 43*: 175–178.

Steinmetz EF (1960): Damianae folia. *Acta Phytotherapeut 7*: 1–2.

Viola H, Wasowski C, Stein MLd, Wolfman C, Silveira R, Dajas F, Medina JH, Paladini AC (1995): Apigenin, a component of *Matricaria recutita* flowers, is a central benzodiazepines receptors-ligand with anxiolytic effects. *Planta Med 61*: 213–216.