PHYTOTHERAPY RESEARCH
Phytother. Res. **21**, 1061–1065 (2007)
Published online 18 July 2007 in Wiley InterScience
(www.interscience.wiley.com) **DOI**: 10.1002/ptr.2212

Antidepressant and Antistress Activity of GC-MS characterized Lipophilic Extracts of *Ginkgo biloba* Leaves

Satyan S. Kalkunte[1], Ajay P. Singh[2,3], Fabio C. Chaves[2], Thomas J. Gianfagna[2], Vishal S. Pundir[4], Arun K. Jaiswal[5], Nicholi Vorsa[2,3]* and Surendra Sharma[1]*

[1]Kilguss Research Institute, Women and Infants Hospital, Brown University, Providence, RI, USA
[2]Department of Plant Biology and Plant Pathology, Rutgers University, New Brunswick, NJ, USA
[3]Philip E. Marucci Center for Blueberry and Cranberry Research and Extension, Rutgers University, New Brunswick, NJ, USA
[4]Department of Botany, TDPG College, Purvanchal University, Jaunpur, India
[5]Institute of Medical Science, Banaras Hindu University, Varanasi, India

Lipophilic extracts of *Ginkgo biloba* L. leaves were tested for their possible role on rodent models of depression and stress. Lipophilic extracts of Ginkgo leaves (LEG) at (50 and 100 mg/kg, p.o.) exhibited dose dependent, significant antidepressant activity in the behavioral despair test and learned helplessness rodent model of depression. The activities were comparable to that of imipramine (15 mg/kg) and EGb 761 (50 mg/kg). In the cold immobilization stress induced gastric ulcer model of stress, only the LEG showed a significant reduction in the ulcer index. GC-MS characterization of this bioactive extract was found to be rich in a group of 6-alkyl salicylates (6-AS), along with a fatty alcohol, fatty acids and cardanols. The *n*-heptadecenyl salicylate represented 60% of the 6-AS. Notable was the absence of dihydroxy alkylphenols which are linked to allergic reactions similar to the urushiols present in poison ivy. In commercial products of Ginkgo, these dihydroxy phenols as well as the favorable 6-AS are removed during enrichment of flavonol glycosides and terpenic lactones. The current findings suggest that intact carboxylic acid groups containing 6-AS are the bioactive components of the lipophilic extract of Ginkgo leaves with antidepressant and antistress activities. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: Ginkgo biloba; depression; stress; 6-alkyl salicylates; GC-MS.

INTRODUCTION

Ginkgo biloba L., often referred to as the 'living fossil tree' is the oldest phytopharmaceutical known to mankind. *G. biloba* is the only living species within the Ginkgoaceae, surviving from the Jurassic and Cretaceous eras (Harrison, 1962). Ginkgo preparation is common in the Chinese System of Medicine and is cited in the Chinese Pharmacopoeia of 2800 B.C. for its use in the treatment of asthma and aging (Tang and Einsenbrand, 1989). Commercially available formulations based on extract EGb 761 are therapeutically used in cerebrovascular insufficiencies and as geriatric care medicine. The chemistry of Ginkgo is unique containing tertiary butyl terpenic lactones (Ginkgolide – A, B, C, D, J and M), diterpenes such as forskolin, complex flavonol glycosides, biflavonoids, proanthocynidins (DeFeudis, 1971) and ginkgolic acid(s) such as 6-alkyl salicylates and its phospholipid conjugates known as ginkgsomes (Ghosal, 2000; Ghosal *et al.*, 1997). Ginkgolic acids and other alkylphenol derivatives analysed using different approaches including LC-MS and GC-MS exhibited variation in contents depending on the method used (He and Xie, 2001; Ndjoko *et al.*, 2000; Casal and Moyna, 1979; Chengzhang *et al.*, 2000; Weihong *et al.*, 2001). The alkylphenol derivatives differ in the number and position of hydroxyl groups and in the presence or absence of the carboxyl group and the alkyl chain can have 13, 15 or 17 carbons with up to 3 unsaturations (van Beek and Wintermans, 2001). 6-AS are removed from majority of Ginkgo preparations during enrichment of flavonol glycosides (to 24% w/w) and terpenic lactones (to 6% w/w) (DeFeudis, 1971). However, simple alcohol tinctures of leaves available commercially such as Ginkgo Meckel® contain 6-AS (>2% w/w, Satyan, unpublished data). 6-AS rich lipophilic extracts of Ginkgo leaves were earlier reported to have anxiolytic, free radical captodative, antiallergic and antiinflammatory activities (Ghosal, 2000; Ghosal *et al.*, 1997). 6-AS along with catechins and procyanidins are also found sequestered in the cage of ginkgolide-B (Ghosal *et al.*, 1997). It is proposed that extracts of Gingko rich in 6-AS containing an intact carboxylic acid group do not cause harmful manifestations and contribute to the neuroprotective activity of Ginkgo. Herein we report for the first time the antidepressant, antistress activity of the lipophilic extract of Ginkgo leaves and its characterization by GC-MS.

* Correspondence to: Dr S. Sharma, Department of Pediatrics, Kilguss Research Institute, Women and Infants Hospital, Brown University, Providence, RI 02905, USA.
E-mail: ssharma@wihri.org
* Correspondence to: Dr N. Vorsa, Department of Plant Biology and Plant Pathology, Rutgers University, New Brunswick, NJ, USA.
E-mail: vorsa@aesop.rutgers.edu

MATERIALS AND METHODS

Animals. Adult Charles Foster rats (100–150 g) of either sex supplied by the Central Animal House of the

Received 5 September 2006
Revised 24 April 2007
Accepted 26 April 2007

Institute were used in the present study. The animals were housed in colony cages (4–5 per cage) at an ambient temperature of 25 ± 1 °C and 45–55% humidity with a 12 h light/12 h dark cycle. The rats, unless otherwise mentioned, had free access to standard pellet chow (Lipton) and drinking water. Experiments were conducted between 09.00–14.00 h and animals were acclimatized for 1 week before experimentation. Guidelines of the principles of the laboratory animal care manual (NIH, 1985) were followed.

Drugs. A lipophilic extract from *Ginkgo biloba* leaves enriched in 6-AS as reported earlier (Ghosal, 2000) was administered at doses of 50 and 100 mg/kg. Ginkocer (Ranbaxy Ltd, India) and EGb761 (containing 24% flavonol glycosides and 6% terpenic lactones, a gift from Dr Willmar Schwabe, Germany) were used at a dose of 50 mg/kg. All the drugs were administered by oral intubation as a 0.3% carboxymethyl cellulose suspension. Imipramine (Antidep, Torrent, India) was administered at 15 mg/kg i.p. as a standard antidepressant. All administrations were performed 1 h prior to experimental observations.

Chemicals. HPLC grade hexane and diethyl ether were purchased from Fischer Scientific (Fairlawn, New Jersey, USA). Diazald, potassium hydroxide, heptadecanoic acid (Sigma-Aldrich, St Louis, MO, USA) and Tri-sil 'Z' from Pierce (Rockford, IL, USA) were used in the experiment.

Extraction. Green leaves collected during the months of May–June were air dried at room temperature, pulverized and extracted three times with hexane. Solvent was evaporated under reduced pressure prior to chemical analysis.

Derivatization. Leaf extracts (20 mg) in methanol (2 μg/μL) were methylated with diazomethane (1 mL) synthesized as reported (Hudlicky, 1980). After 30 min, the mixture was evaporated, diluted in hexane (0.5 mL) and injected in the GC (2 μL). Heptadecanoic acid (50 μL) was added as an internal standard before derivatization.

For trimethylsilylation, sample (20 mg), internal standard (100 μL) and Tri-sil 'Z' (1 mL) were added, heated at 60 °C for 5 min and directly injected in the GC.

Analytical conditions. GC-MS analyses were performed using a HP GC 6890 series, equipped with an Agilent column 122-0162 DB-1 ms (60 m × 250 μm i.d. × 0.25 m film thickness) with helium as carrier gas at a flow rate of 0.5 mL/min. The oven temperature was set at 80 °C and increased to 250 °C at 30 °C/min then held for 25 min, when the temperature was raised to 280 °C at 70 °C/min, and held for 35 min. Total run time was 66.2 min. Spectral data were obtained from an HP MS 5973 series with ionization energy of 70 eV at source temperature of 230 °C and Quadrupole at 150 °C. Compounds were identified by comparisons to the library (NIST 98) and published data.

Behavioral despair test. The rodent behavioral despair model of depression was carried out as published earlier (Bhattacharya *et al.*, 1999). Briefly, each rat was placed in a plexiglass chamber (45 × 20 × 20 cm) containing 25 cm water (27 ± 2 °C) so that the rat could not touch the bottom of the chamber with its hind limbs or tail or climb over the edge of the chamber. Two swim sessions were conducted, an initial 15 min pretest, followed by 5 min test after 24 h. Various treatments were administered after pretest and 1 h before the test session. The period of immobility during the 5 min test period was defined when the rat floated on water without any movement or making any attempt to escape.

Learned helplessness. This model is based on the principle that exposure to uncontrollable stress associated with repeated experiences of failure, produces a 'helpless' situation resulting in performance deficits in subsequent learning tasks (Bhattacharya *et al.*, 1999). Briefly, animals received inescapable shock treatment in a Plexiglas chamber (20 × 10 × 10 cm). A constant current shocker was used to deliver 60 scrambled randomized inescapable shocks (15 s duration, 0.8 mA) every minute with 15 s interval between each shock session through stainless steel grid (1.5 cm mesh) floor. Inescapable shock pretreatment was performed on the morning of day 1. The animals were then subjected to conditioned avoidance training (CAT) in a Plexiglas shuttle box consisting of two chambers (60 × 21 × 30 cm) separated with 7 × 7 cm gate with stainless steel grid floor (1.0 cm mesh). Single animals were placed and allowed to adapt for 5 min for the first session only and subjected to 30 avoidance trials with inter-trial spacing of 30 s. During the first three of each trial, a light signal was presented, allowing the animals to avoid shock. If a response did not occur within this period, a 0.8 mA shock (3 s) was applied via the grid floor. In the case of no escape during this period, shock and conditioned stimulus were terminated. The escape response required by the rat was to cross into the 'safe' chamber. The number of escape failures, referred to as no crossing response during shock delivery was recorded 48 h later (day 1) after inescapable shock pretreatment in the prenoon period. The escape failures in rats pre-exposed to inescapable shocks were significantly higher than sham trials. Various drug treatments were administered 1 h before the CAT on day 1.

Cold immobilization stress induced gastric ulcers. The antistress activity was investigated by cold immobilization stress-induced gastric ulceration, after acute treatment in albino rats (Bhattacharya *et al.*, 1987). Thus, rats were immobilized on wooden planks following the Freglys method of immobilization. Fore and hind limbs of fully stretched rats were strapped with adhesive plaster on a wooden plank. The whole body was then strapped to the plank leaving the face and tail free. Animals were immobilized for 2 h at 4 ± 1 °C. All the animals were starved for 24 h with free access to water before immobilization. At the end of this period, the rats were killed, the stomach was slit open along the greater curvature and the numbers of discrete ulcers were noted with the help of a magnifying glass. The severity of the ulcers was scored after histological confirmation as 0, no ulcers; +, changes limited to superficial layers of mucosa with no congestion; ++, half of mucosal thickness showing necrotic changes; +++, more than two-thirds of mucosal thickness showing necrotic changes and ++++, complete destruction of mucosa with

DOI: 10.1002/ptr

Table 1. Effects of Ginkgo extracts and imipramine in behavioral despair test and the learned helplessness paradigm in rats

Group (route of administration)	Dose (mg/kg/day)	Despair test Immobility (s)	Learned helplessness Escape failures
Vehicle (p.o.)	—	119.75 ± 6.53	18.75 ± 1.67
LEG (p.o.)	50	73.64 ± 3.33[a]	16.38 ± 1.82
LEG (p.o.)	100	70.55 ± 2.35[b]	13.14 ± 0.83[b]
Ginkocer (p.o.)	50	93.34 ± 8.45	16.13 ± 1.55
EGb 761 (p.o.)	50	78.27 ± 6.22[b]	9.19 ± 0.42[c]
Imipramine (i.p.)	15	90.82 ± 2.59[b]	11.28 ± 0.42[c]

In all the groups 8 animals per group was used. The control group (not receiving inescapable shock) in the learned helplessness model consisted of six animals. The mean (± SEM) number of escape failures (EF) in this group were 5.8 ± 1.2. Route of administration indicated in parenthesis. Statistically significant differences from the corresponding vehicle treated groups [a] $p < 0.05$; [b] $p < 0.01$ or [c] $p < 0.001$ respectively. All values expressed are mean ± SEM. LEG, lipophilic extract of Ginkgo leaf.

hemorrhage. The severity of ulcer was then calculated and the ulcer index was determined by adding the number of ulcers as reported earlier (Bhattacharya *et al.*, 1987).

Statistical analysis. All the data were subjected to non-parametric multiple group comparison using Mann-Whitney U test.

RESULTS AND DISCUSSION

The lipophilic extract of Ginkgo leaves (LEG) showed significant dose dependent antidepressant activity as reflected by a reduction in the immobility time in Porsolts behavioral despair test and a reduced number of failures to escape from shock chamber to safe chamber (escape failure) compared with vehicle-treated animals in learned helplessness model of depression (Table 1). The activities were comparable to the activity of the known antidepressant imipramine and EGb 761. Ginkocer treatment did not show any significant activity in either of the models tested. On the cold immobilization stress model, only LEG showed significant antistress activity (Fig. 1). GC-MS analysis of lipophilic extract showed a complex mixture of compounds comprising fatty acids, alkylsalicylates, cardanols (alkyl phenols) and derivatives of phytol (Table 2). The long chain alkanol 10-nonacosanol as reported by others and free fatty acids ranging in carbon chain length from 14 to 28 were also detected (Casal and Moyna, 1979; Tu *et al.*, 2001). Most of the fatty acids were saturated with the exception of the C18 linoleic (18:2) and linolenic (18:3) acids.

The alkylsalicylates in this lipophilic extract consisted of a series of compounds with saturated alkyl chain lengths of 13:0, 15:0, two monounsaturated C15 compounds with the double bond at the 8 or 10 positions, and a C17:1 analog. The latter compound was present in the highest concentration in the extract at 48 mg/kg and represented about 60% of the total 6-alkylsalicylate content. Five cardanols were present in a similar series to the alkylsalicylates. A characteristic fragment for the silylated 6-AS was *m/z* 219 (Tu *et al.*, 2001) and a base peak representing the loss of a methyl group (from the trimethylsilyl) (Fig. 2). In the case of silylated cardanols the diagnostic peak was *m/z* 180 (Tu *et al.*, 2001), which is also the base peak. Most notable was the absence of dihydroxy alkyl phenols. Verotta and Peterlong (1993), reported that resorcin derivatives also called bilobols were likely those responsible for the strong allergic and

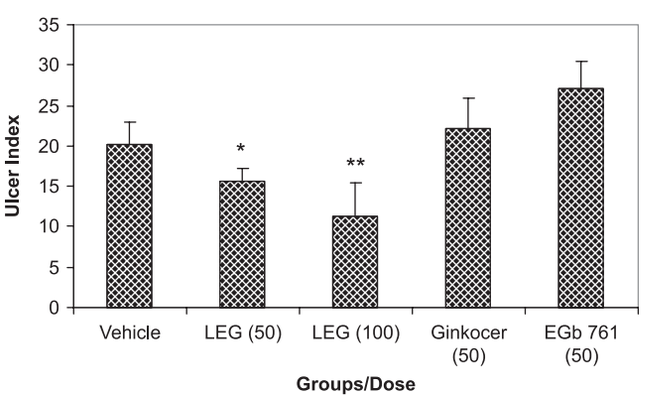

Figure 1. Effect of Ginkgo extracts on cold immobilization induced stress in rats. Statistically significant differences from the corresponding vehicle ($n = 8$) treated groups ($n = 6$) are marked with * and ** representing $p < 0.05$ and $p < 0.01$, respectively. The doses in parenthesis are mg/kg and were orally administered. All values expressed are mean ± SEM. LEG, lipophilic extract of Ginkgo leaf.

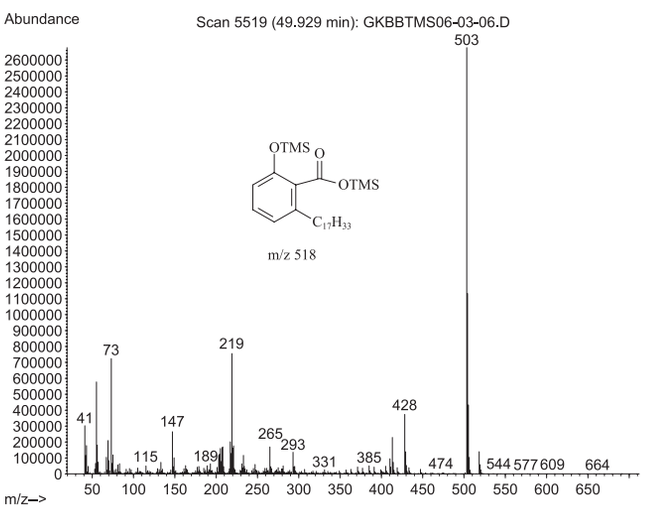

Figure 2. Characteristic MS fragmentation pattern of the silylated alkylsalicylate (17:1) present in the lipophilic extract of Ginkgo leaves.

DOI: 10.1002/ptr

Table 2. Identified chemical entities from the lipophilic extract of Ginkgo leaves

Peak #	Compound[b]	Major fragments (relative abundance)
1	Tetradecanoic acid	74(100), 87(70), 143(20.1), M+242(10.5)
2	Phytadiene I	43(100), 55(70.3), 68(86.1), 82(56.3), 95(63.8), 109(21.6), 123(41.1), 137(8.5), M+278(3)
3	Phytadiene II	43(100), 55(57.4), 68(48.3), 81(77.6), 95(48.5), 109(15.9), 123(30.7), 137(6.4), M+278(14.2)
4	Hexadecanoic acid	74(100), 87(70.1), 143(19.6), 227(15.6), M+270(15.3)
5	Heptadecanoic acid (internal std)	74(100), 87(71.4), 143(22.4), 241(18.5), M+284(20.7)
6	9,12-Octadecadienoic acid	67(100), 81(70.9), 95(47.4), 109(20.9), 263(8.1), M+294(10.4)
7	9,12,15-Octadecatrienoic acid	79(100), 95(47.8),108(28.8), 261(2.7), M+292(4)
8	Dihydro-phytol	71(100), 81(24), 95(13.9), 111(7.2), 123(24.1), M+298(1.8)
9	Octadecane	43(74.5), 57(100), 71(57.1), 85(40.6), 99(11), M+254(1.4)
10	Eicosanoic acid	74(100), 87(74), 143(20.6), 283(16.7), M+326(31.25)
11	Docosanoic acid	74(100), 87(66.6), 143(19.8), 311(17.2), M+354(28.7)
12	Tetracosanoic acid	74(100), 87(70.5), 143(20.2), 339(19), M+382(50.3)
13	Hexacosanoic acid	74(100), 87(58.5), 143(19.7), 367(17.3), M+410(58)
14	Octacosanoic acid	74(100), 87(66.7), 143(23.1), 395(20.8), M+438(80.6)
20	10-Nonacosanol[a]	73(29.2), 229(100), 369(84.5), 481(5.1), M+496(1)
21	Beta-Sitosterol[a]	129(99.9), 275(16), 357(93.2), 381(22.5), 396(100), 471(23), M+486(41.1)
	6-AS/Ginkgolic acids[a]	
15	C13:0 [b]	161(100), 180(35.2), 317(32.2), M+348(23.6)
16	C15:1(8)	207(31.5), 219(23.4), 235(17.1), 382(17.3), 400(40.9), 417(100), M+432(3.1)
17	C15:1(10)	207(31.5), 219(23.4), 235(17.1), 382(17.3), 400(40.9), 417(100), M+432(3.1)
18	C15:0	207(44.4), 219(62.4), 235(17.8), 382(11.5), 400(49.9), 417(100), M+432(3.9)
19	C17:1	207(39.9), 219(63.8), 235(18.9), 382(8.8), 400(42.2), 419(100), M+434(1.5)
	Cardanols[a]	207(36.5), 219(68.1), 235(15.8), 413(23.3), 428(66.8), 445(100), M+460(4.7)
22	C13:0	165(28.6), 180(100), 193(12.6), 333(2.4), M+348(42.1)
23	C15:1(8)	165(22.9), 180(100), 193(9.9), M+374(26.8)
24	C15:1(10)	165(23), 180(100), 193(12.2), 359(0.4), M+374(28.2)
25	C15:0	151(9.6), 165(17.2), 180(100), 361(3.5), M+376(30.5)
26	C17:1	55(53), 73(26.7) 165(23.3), 180(100), 193(11.3), M+402(37.9)

[a] Silylated; [b] Methylated.

contact dermatitis similar to poison ivy. This method was able to detect 6-AS and cardanols, unreported in the extracts analysed by Casal and Moyna (1979). While most LC-MS methods focus on the trace analysis of residual ginkgolic acid congeners in formulations (Ndjoko *et al*., 2000), the use of GC-MS analysis after derivatization allows a more inclusive determination of the alkylphenols derivatives as well as the major fatty acids present in the extracts without the need for prior purification. Therefore a careful determination of all the different alkylphenol derivatives including 6-alkyl salicylates or ginkgolic acids or anacardic acids, cardanols, cardols or bilobols and urushiols is essential in order to avoid confusion in attributing the causal allergen.

Although used world wide in disorders related to circulatory insufficiency, very limited pharmacological evidence is available for Ginkgo leaf extracts. Most investigations focused on the standardized extract EGb 761 which is rich in flavonol glycosides (24% w/w), terpenic lactones (6% w/w) and procyanidins but is depleted of lipophilic constituents such as 6-alkyl salicylates and fatty acids (DeFeudis, 1971). In the present study, the major constituents of lipophilic extracts of Ginkgo leaves are the 6-alkyl salicylates (6-AS) commonly grouped as ginkgolic acid(s) or anacardic acids. These 6-AS (free and phospholipid conjugates known as Ginkgosomes) occur consistently and abundantly throughout the vegetative cycle in leaves (Ghosal, 2000) and in ginkgo fossils (Ghosal *et al.*, 2001). These compounds are also found tenaciously entrapped in caged molecules such as ginkgolide-B and bilobalide and in alcohol tinctures such as Ginkgo Meckel (Satyan *et al*., 1996). Ghosal and co-workers (Ghosal *et al.*, 1997) established that as long as the carboxyl function in the 6-AS was intact, these unique salicylates have significant neuropharmacological properties such as antianxiety activity (Satyan *et al.*, 1998) and brain monoamine modulating properties (Satyan *et al.*, 1997). We report for the first time the novel antidepressant activity of these lipophilic constituents of Ginkgo leaves. An aqueous-methanol extract devoid of 6-AS, failed to show antidepressant activity in the behavior despair test (data not shown). White *et al.* (1996) attributed the monoamine oxidase (MAO A and MAO B) inhibitory activity of crude Ginkgo leaf extract to the unidentified low molecular weight entities in the extract. While Continella and Drago (1985) reported no significant antidepressant activity after acute administration of EGb 761, Porsolt *et al.*, 1990 reported antidepressant activity with EGb 761 only in the learned helplessness model of depression. Our findings in the present study confirm the activity of EGb 761 on behavioral despair and learned helplessness model of depression. Contrary to the reported increase in serotonin uptake *in vitro* (Ramassamy *et al.*, 1992), and increase in 5 HIAA levels and 5 HT content in the cerebral cortex by EGb 761 (Petkov *et al.*, 1993), the ginkgolic acid conjugates from the lipophilic extract produced significant decrease in 5-HT, 5 HIAA levels with a trend toward increase in norepinephrine and dopamine (DA) levels in different regions of the brain (Satyan *et al.*, 1998). Attenuated

DOI: 10.1002/ptr

dopamine is reported in depression and animal studies indicating that the activation of the mesolimbic dopamine system may represent a 'final common pathway' in antidepressant action of clinical antidepressants (Willner *et al*., 2005). The lipophilic extract of Ginkgo leaves, have earlier shown to increase DA levels in the mesolimbic system (Satyan *et al.*, 1998) and this at least in part explains the antidepressant activity seen with 6-AS in the current study.

Serotonin is one of the neurotransmitter involved in immobilization stress-induced activation of the hypothalamic–pituitary–adrenal (HPA) axis. Activation of the HPA axis during stress and with serotonin agonists lead to the release of serotonin (Cassano and D'mello, 2001). Peripherally released serotonin in response to stress such as immobilization is known to reach high gastric mucosal tissue levels (Heuser *et al.*, 1979). 6-AS rich LEG shows antistress activity against stress-induced ulceration (Fig. 1) probably by decreasing the central 5-HT levels (Satyan *et al.*, 1998) leading to attenuated peripheral serotonin. Our findings strengthen the fact that the 6-alkylsalicylates are important components of the lipophilic Ginkgo extract and to our knowledge this is the first report of antidepressant, antistress activity of these bioactives.

Acknowledgements

The authors (KSS, APS and AKJ) greatly acknowledge the insightful discussions and dedicate this work to the Late Professor S. K. Bhattacharya (SKB), Neuropharmacology Laboratory. We are also thankful to Professor Shibnath Ghosal for his insight on the work and Professor A. B. Ray, Department of Medicinal Chemistry, Banaras Hindu University, Varanasi 221005, India for providing anacardic acid standard for carrying out the work. The authors acknowledge the gift sample of EGb761 to SKB by Dr Willmar Schwabe GmBH. Germany.

REFERENCES

Bhattacharya SK, Das N, Sarkar MK. 1987. Inhibition of carrageenin-induced pedal odema in rats by immobilization stress. *Res Exp Med (Berl)* **187**: 303–313.

Bhattacharya SK, Satyan KS, Ramanathan M. 1999. Experimental methods for evaluating psychotropic agents in rodents: II-Antidepressants. *Indian J Exp Biol* **39**: 117–123.

Casal HL, Moyna P. 1979. Components of *Ginkgo biloba* leaf wax. *Phytochemistry* **18**: 1738–1739.

Cassano WJ Jr, D'mello AP. 2001. Acute stress-induced facilitation of the hypothalamic-pituitary-adrenal axis: evidence for the roles of stressor duration and serotonin. *Neuroendocrinology* **74**: 167–177.

Chengzhang W, Zhaobang S, Weihong T, Qing Y. 2000. Chemical constituents of essential oil from the leaves of *Ginkgo biloba. J Trop Subtrop Bot* **8**: 329–332.

Continella G, Drago F. 1985. *Effect of Ginkgo biloba Extract on Organic Cerebral Impairment*, Agnoli A, Rapint R, Scapagniai V, Weitbrecht WV (eds). John Libbey: London, 35.

DeFeudis FV. 1971. *Ginkgo biloba* extract (EGb 761). Elsevier: Paris, 12–18.

Ghosal S. 2000. Research in *Ginkgo biloba*: Recent developments. *J Indian Chem Soc* **77**: 517–522.

Ghosal S, Muruganandam AV, Satyan KS, Chauhan S. 2001. The chemistry of fossils of Ginkgo and its ancestors. *Indian J Chem* **41B**: 845–853.

Ghosal S, Sundaram R, Muruganandam AV *et al.* 1997. The chemistry and actions of 6-alkylsalicylates. *Indian J Chem* **36**: 257–261.

Harrison SG. 1962. *A Handbook of Coniferae and Ginkgoaceae.* Edward Arnold: London, 228.

He JR, Xie BJ. 2001. Separation of ginkgolic acids by secondary chemical equilibrium in reversed phase high performance liquid chromatography. *Nat Prod Chem Res* **59**: 1153–1157.

Heuser L, Gregg R, Max MH. 1979. The association of pyloric antral tissue levels of 5-hydroxytryptamine and restraint stress ulceration in the rat. *Am Surg* **45**: 771–773.

Hudlicky M. 1980. An improved apparatus for the laboratory preparation of diazomethane. *J Org Chem* **45**: 5377–5378.

Ndjoko K, Wolfender J-L, Hostettmann K. 2000. Determination of trace amounts of ginkgolic acids in *Ginkgo biloba* L. leaf extracts and phytopharmaceuticals by liquid chromatography-electrospray mass spectrometry. *J Chromatogr B: Biomed Sci Appl* **744**: 249–255.

NIH 1985. Guide for the care and use of laboratory animals NIH 85-23, Bethesda, MD, USA.

Petkov VD, Hadziivano U, Petkov VV, Milanov S, Visheva N, Boxadijieva N. 1993. Effects of standardized extracts GF 501, from *Ginkgo biloba* L., G115 from Panax ginseng C.A. Meyer and their combination Ginkosan (PHL-00701) on the brain levels of biogenic monoamines and on the serum content of prolactin, growth homones and ACTH. *Phytother Res* **7**: 139–145.

Porsolt RD, Martin P, Lenegre A, Fromage S, Drieu K. 1990. Effects of an extract of *Ginkgo biloba* (EGb 761) on 'learned helplessness' and other models of stress in rodents. *Pharmacol Biochem Behav* **36**: 963–971.

Ramassamy C, Nausin B, Christen Y, Clostre F, Costentin J. 1992. Prevention by *Ginkgo biloba* extract (EGb 761) and trolox C of the decrease in synaptosomal dopamine or serotonin uptake following incubation. *Biochem Pharmacol* **44**: 2395–2401.

Satyan KS, Bhattacharya SK, Jaiswal AK, Sunderam R, Murganandam AV, Ghosal S. 1996. *Ginkgo biloba*: newer perspectives. In *Proceedings of the First Annual Conference on Chemistry, Biology and Health Care, Lucknow, India,* Abr.PB-1:53. CDRI, India.

Satyan KS, Jaiswal AK, Ghosal S, Bhattacharya SK. 1997. Effect of ginkgolic acid conjugates on the brain monoamines and metabolites in rodents. *Biogenic Amines* **13**: 143–151.

Satyan KS, Jaiswal AK, Ghosal S, Bhattacharya SK. 1998. Anxiolytic activity of ginkgolic acid conjugates from Indian *Ginkgo biloba*. *Psychopharmacology* **136**: 148–152.

Tang W, Einsenbrand G. 1989. *Chinese Drugs of Plant Origin*, Tang W, Einsenbrand G (eds). Springer-Verlag: Berlin, 556–565.

Tu TTN, Derenne S, Largeau C, Mariotti A, Bocherens H. 2001. Evolution of the chemical composition of *Ginkgo biloba* external and internal leaf lipids through senescence and litter formation. *Org Geochem* **32**: 45–55.

van Beek TA, Wintermans MS. 2001. Preparative isolation and dual column high-performance liquid chromatography of ginkgolic acids from *Ginkgo biloba. J Chromtogr A* **930**: 109–117.

Verotta L, Peterlongo F. 1993. Selective extraction of phenolic components from *Ginkgo biloba* extracts using supercritical carbon dioxide and off-line capillary gas chromatography/mass spectrometry. *Phytochem Anal* **4**: 178–182.

Weihong T, Zhaobang S, Chengzhang W, Qing Y. 2001. Isolation and identification of alkylphenols from *Ginkgo biloba* leaves. *Chem Indust Forest Prod* **21**: 1–6.

White HL, Scates PW, Cooper BR. 1996. Extracts of *Ginkgo biloba* leaves inhibit monoamine oxidase. *Life Sci* **58**: 1315–1321.

Willner P, Hale AS, Argyropoulos S. 2005. Dopaminergic mechanism of antidepressant action in depressed patients. *J Affect Disord* **86**: 37–45.