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Central Nervous System Receptor Activities of Some Malaysian Plant Species

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Abstract

In this investigation, 185 plant samples representing more than 30 plant families collected from the Malaysian forests were assessed for their ability to inhibit specific radioligand binding to 5HT1a, $GABA_B$, and dopamine (D2S) receptors. For this study, 96-well microplate filtration assays were adopted, and the screening parameters including screening window factor (z factor) and z′ factor indicated that the assays adopted were robust and suitable for medium-throughput screening (MTS). z factor also indicated that data on plant extracts at 10 μg/well were more reliable compared to those obtained from 100 μg/well. Therefore, only data at 10 μg/well in duplicate were used in the determination of actives. In the preliminary screen, 23 plant extracts were found to show activity (50% or higher level of inhibition over the mean of all samples for a given plate) in either one or both of the duplicates. Of these, seven were reconfirmed to be active on 5HT1a receptor in the hit confirmation. The active plant extracts were isolated from *Popowia odoardoi* Diels (Annonaceae) (leaf and stem), *Artabotrys roseus* Boerl. (Annonaceae) (bark), *Litsea elliptibacea* Merr. (Lauraceae) (bark), *Decaspermum fruticosum* Forst. (Myrtaceae) (bark), *Dyera costulata* (Miq.) Hook. f. (Apocynaceae) (leaf), and *Irvingia malayana* Oliv. (Simaroubaceae) (leaf). However, none of the plant extracts tested were active on either $GABA_B$ or D2S receptors.

Keywords: Central nervous system, dopamine receptor, GABA receptor, Malaysian plants, receptor binding assay, serotonin receptor.

Introduction

Disorders of the central nervous system (CNS) are the cause of a number of common diseases throughout the world, which include migraine, sleeping disorders, obsessive disorders, schizophrenia, Alzheimer disease, epilepsy, and Parkinson disease (Laurence et al., 1997; Lefkowitz et al., 1990). Some of these disorders are related to neurotransmitters such as acetylcholine, glutamic acid, dopamine, 5-hydroxytryptamine, γ-aminobutyric acid, glycine, benzodiazepine, noradrenaline, and histamine, and their receptors (Lefkowitz et al., 1990).

Historically, plants have provided important CNS active compounds including morphine, codeine, reserpine, and caffeine (Cragg et al., 1997; Grabley & Thiericke, 1999; Evans & Evans, 2002). However, the majority of the plants have not been investigated to any great extent for their pharmacological activities, and it is believed that plants can provide new drug leads for the treatment of CNS diseases (Zhu et al., 1996). The recent developments in radioactive ligand-receptor binding assays offer a rapid turnover of the screening process and hence may expedite the process in the search of novel drug molecules or templates (Zhu et al., 1996; Marks et al., 2002). These developments include the miniturization and automation of the screening process (Oldenburg et al., 2001; Menke, 2002). In the current study, we evaluated CNS activities of some Malaysian plants using competitive radioligand receptor binding assays, and the receptor activities assessed were 5-hydroxy tryptamine (5HT), GABA, and dopamine. The aim was to qualitatively identify plants that exhibit significant CNS activities for further bioassay-guided isolation of active constituents.

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Materials and Methods

Materials

[^{3}H]-Spiperone, [^{3}H]-CGP 54626, and [^{3}H]-8-OH-DPAT were obtained from Amersham Pharmacia Biotech (Little Chalfont, Buckinghamshire, UK), Tocris (Ellisville, MO, USA), and Perkin Elmer (Boston, MA, USA), respectively. Haloperidol, GABA, and metergoline were purchased from RBI (Natick, MA, USA). Unless stated otherwise, all other reagents of analytical grade were obtained through standard commercial sources.

Deep-well titer plate polypropylene (Beckman Coulter, Fullerton, CA, USA), Multiscreen Harvest plates-GF/C (Millipore, Billerica, MA, USA), TopSeal-A (Packard, Meriden, CT, USA), Bottom Seal (Millipore), and MicroScint-O (Packard) were purchased. SignalScreen membranes from cells expressing dopamine (D2S: co-expressed in sf9 cells with $G\alpha 3\beta 1\gamma 2$; no. 6110138), $GABA_B$ 1a (co-expressed in HEK 293 cells with $GABA_B$ 2; no. 6110557), and 5-HT1a (expressed in CHO cells; no. 6440501) human receptors were supplied by Biosignal (Montreal, Quebec, Canada).

Plant samples and crude extracts dilution

A total of 185 plant samples were collected from Forest Research Institute Malaysia, Kuala Lumpur, Malaysia (voucher no.: 5-digit series) and Tabun Wildlife Reserve, Sabah, Malaysia (voucher no.: 6-digit series). The voucher specimens were kept at the herbaria of Forest Research Institute Malaysia, Kuala Lumpur, Malaysia (5-digit series), and Forest Research Center, Sepilok, Sandakan, Sabah, Malaysia (6-digit series). The plant samples were dried, ground, and macerated (100 g) with sufficient methanol in conical flasks for 7 days with sonication (2×30 min). The methanolic solution was filtered, solvent removed *in vacuo* and freeze-dried, giving a dried residue that was weighed and kept at -20°C in sample vials until use.

DMSO (1.25 ml) was added to 5 mg of crude plant extracts and vortexed vigorously, giving an initial concentration of 4 mg/ml. The extracts were tested at 100 µg/assay point and 10 µg/assay point.

Receptor binding assays

The receptor binding assays were carried out according to the recommended protocols (Biosignal). Briefly, the membranes were thawed on ice and diluted to 1 SignalScreen Unit per 500 µl with the appropriate binding buffer (D2S: 50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 1 mM EDTA, 120 mM NaCl; $GABA_B$ 1a + 2: 50 mM Tris-HCl, pH 7.4, 2.5 mM $CaCl_2$; 5HT1a: 50 mM Tris-HCl, pH 7.4, 10 mM $MgSO_4$, 0.5 mM EDTA, 0.1% ascorbic acid). The reference ligands and radioligands were diluted at $22 \times$ the final concentration in 100% DMSO and in binding buffer, respectively (Table 1).

The diluted membranes (500 µl) were added to each well of the deep-well plate, followed by addition of 25 µl of DMSO (total value, 5 wells), reference compound (nonspecific value, 3 wells) or crude extracts to the corresponding well in the deep-well plate. The reaction was initiated by adding 25 µl of radioligand to each well. TopSeal-A was applied to the plate, vortexed gently, and incubated at 27°C while shaking for 60 min. During incubation, the Multiscreen Harvest plates were preincubated in 0.3% aqueous polyethyleneimine (D2S), 50 mM Tris pH 7.4 ($GABA_B$ 1a + 2), or 0.3% polyethyleneimine in binding buffer (5HT1a). The reaction mixture was then filtered over the presoaked Multiscreen Harvest plate using a Tomtec Harvester and washed 9-times with 500 µl of cold 50 mM Tris-HCl, pH 7.4, at 4°C and air-dried for 30 min at room temperature under a fume hood. A bottom seal was applied to the Multiscreen Harvest plate, 25 µl of MicroScint-O added to each well, and followed by sealing of the top using TopSeal-A. The plate was then counted for 60 s per well using TopCount NXT (Packard) with a count delay of 60 s.

Data analysis

Data analysis on saturation experiment using the nonlinear least-squares regression method was performed using PRISM Software (GraphPad Software, Inc., San Diego, CA, USA) and the results are presented as mean ± SEM. z' factor analysis, originally described by Zhang et al. (1999) to evaluate the quality of the screening assays, was performed on replicate (>150) test wells and NSB wells and the conditions were as described above.

To calculate the percentage of inhibition of specific binding to 5HT, GABA, or dopamine receptors in the presence of the test compounds, a standard data reduction algorithm was used as shown below:

$$\%\ \text{Inhibition of specific binding} = \frac{[T - NSP] - [B - NSP]}{[T - NSP]} \times 100$$

Table 1. Final concentrations of radioligands and reference ligands.

Receptor	Radioligand	Final concentration	Reference ligand	Final concentration
D2S	[^{3}H]-Spiperone	0.2 nM	Haloperidol	10 µM
$GABA_B$ 1a + 2	[^{3}H]-CGP 54626	5.1 nM	GABA	10 mM
5HT1a	[^{3}H]-8-OH-DPAT	0.25 nM	Metergoline	10 µM

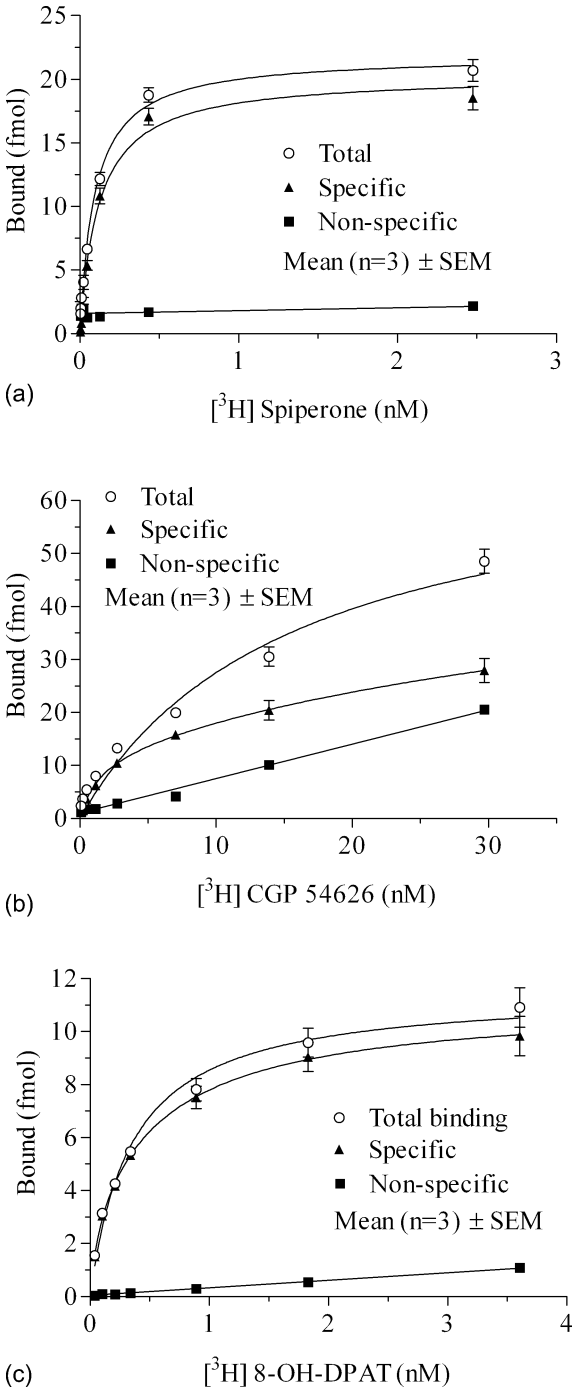

Figure 1. (a) Saturation curve for D2S receptors. (b) Saturation curve for $GABA_B$ 1a + 2 receptors. (c) Saturation curve for 5HTla receptors.

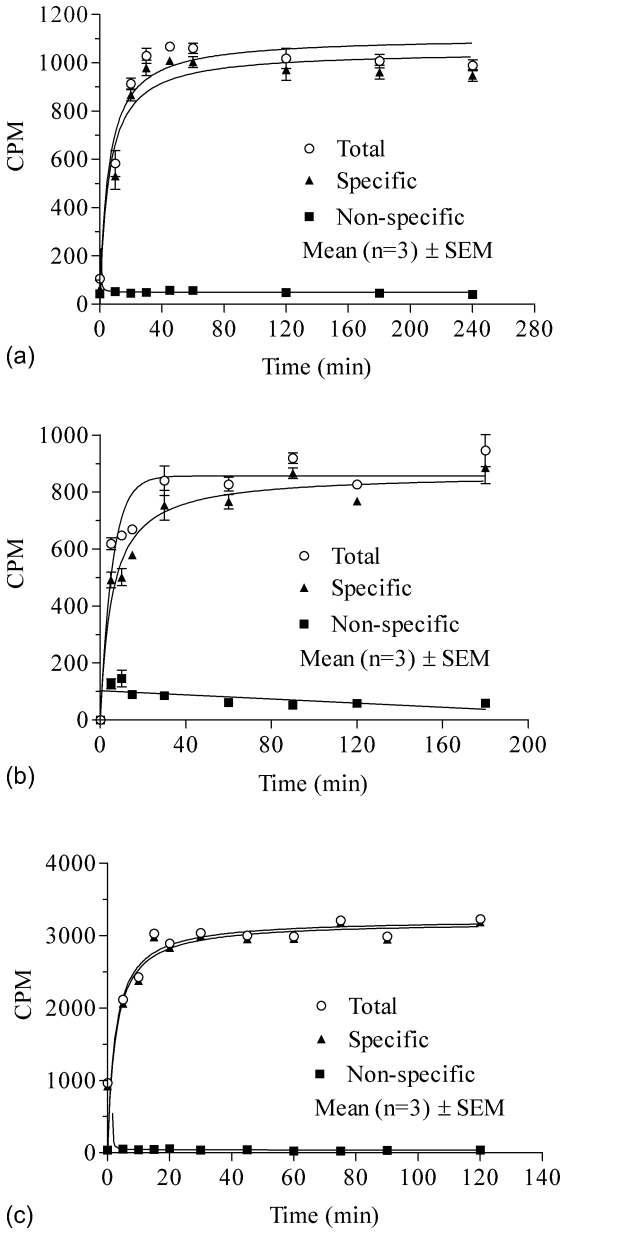

Figure 2. Time course of association of [^{3}H]-spiperone to D2S receptors. (b) Time course of association of [^{3}H]-CGP 54626 to $GABA_B$ 1a + 2 receptor. (c) Time course of association of [^{3}H]-6-OH-DPAT to 5HTla receptors.

Table 2. K_d of radioligands and K_i of competitive ligands.

Receptor	Radioligand	K_d	Reference ligand	K_i
D2S	[^{3}H]-Spiperone	0.12 nM	Haloperidol	13.6 nM
$GABA_B$ 1a + 2	[^{3}H]-CGP 54626	6.22 nM	GABA	22.8 nM
5HT1a	[^{3}H]-6-OH-DPAT	0.3 nM	Metergoline	6.3 nM

Table 3. Interplate receptor binding variations.

Receptor	Total binding	Specific binding	Nonspecific binding
D2S	1155.8 ± 116.1 (10)	1109.3 ± 108.6 (9.8)	46.5 ± 19.8 (42.6)
$GABA_B$ 1a + 2	705.0 ± 55.1 (7.8)	597 ± 57.5 (9.6)	108.0 ± 16.6 (15.4)
5HT1a	980.2 ± 28.6 (2.9)	925 ± 50.8 (5.5)	55.2 ± 28.3 (51.3)

The values of total binding, specific binding, and nonspecific binding are in cpm and are expressed as mean (n = 6) ± SD. Values in parentheses represent %CV.

where B = binding in the presence of test extract, NSP = nonspecific binding in the presence of excess inhibitor, and T = total binding.

Results and Discussion

The respective binding of [^{3}H]-8-OH-DPAT, [^{3}H]-CGP 54626, and [^{3}H]-spiperone to 5HT1a, $GABA_B$ 1a + 2, or D2S receptors were with high affinity and saturable (Fig. 1). The dissociation constants (K_d) were estimated to be 0.12 nM ([^{3}H]-spiperone; D2S), 6.22 nM ([^{3}H]-CGP 54626; $GABA_B$), and 0.3 nM ([^{3}H]-6-OH-DPAT; 5HT1a) (Table 2). K_i for the respective competitive/reference ligands were 13.6 nM (haloperidol), 22.8 nM (GABA), and 6.3 nM (metergoline). In the time-course experiments, association of radioligands were completed at about 30 min; this clearly suggests 60 min incubation time was adequate (Fig. 2).

Interplate variations of total binding, specific binding, and nonspecific binding were analyzed from 5 points per microplate of a total of 12 microplates. For total binding and specific binding for each receptor binding assay, the %CV is less than 10, whereas the values were higher for nonspecific binding (Table 3). The results show interplate variation is minimal and acceptable for the purpose of high throughput screening (HTS) and is further supported by the z′ factor (see below).

Table 4. S/N and z′ factor.

Receptor	Concentration of plant extracts (μg/well)	S/N	z′
D2S	100	27.2	0.76
	10	50.1	0.80
5HT1a	100	21.1	0.71
	10	21.3	0.68
$GABA_B$ 1a + 2	100	6.7	0.56
	10	4.1	0.60

The quality of the assays was tested by performing z′-factor analysis as described by Zhang et al. (1999). Assays with a z′ factor between 0.5 and 1.0 are considered to be reliable, robust, and suitable for HTS. In each case, the z′ factor determined was more than 0.5 (Table 4), indicating the assays adopted are suitable for HTS purposes (Zhang et al., 1999; Oldenburg et al., 2001).

In the preliminary screening, the plant extracts were tested at 10 and 100 μg/assay point as, under these conditions, the samples remained soluble and proper filtration was achieved. The samples were screened in duplicate at both concentrations for the three receptors and the percent inhibition averaged. The calculated values of z factor at 10 μg/well were generally larger compared to those of 100 μg/well for the assay procedures; this indicates the screening results of 10 μg/well are more reliable. Furthermore, at 100 μg/well, most of the samples showed a high level of inhibition, thus making the determination of actives difficult. For these reasons, only the data at 10 μg/well were used in the determination of actives. Actives were determined by choosing any extract that showed 50% or greater inhibition over the mean of all samples for a given plate.

From the preliminary screens, 23 plant extracts were found to show activity (none on D2S, 9 on 5HT1a, and 14 on $GABA_B$ 1a + 2) in either one or both of the duplicates (Table 5). All the plant extracts that exhibited activity in only one of the duplicates were found to be false positives. Of those extracts that showed activity in the preliminary screen, seven were reconfirmed to be active (showed 50% or greater inhibition) on 5HT1a receptors in the hit confirmations. The active plants are *Popowia odoardoi* Diels (Annonaceae), *Artabotrys roseus* Boerl. (Annonaceae), *Litsea elliptibacea* Merr. (Lauraceae), *Decaspermum fruticosum* Forst. (Myrtaceae), *Dyera costulata* (Miq.) Hook. f. (Apocynaceae), and *Irvingia malayana* Oliv. (Simaroubaceae) (Table 6). However, none of the plant extracts tested show high receptor binding activity against $GABA_B$ and D2S receptors. The active plants have now been selected for further testing and bioassay-guided fractionation to identify active constituents.

Table 5. Preliminary screening on CNS receptor activities of plant extracts of some Malaysian plant species.

No.	Voucher no.	Family	Plant	Part	Receptors		
					5HT1a	$GABA_B$	D2S
1	43264	Alangiaceae	*Alangium ebenaceum*	Leaf	−	−	−
2	143521		*Alangium griffithii*	Bark	−	−	+
3	143521			Leaf	−	−	−
4	43303	Anacardiaceae	*Bouea macrophylla*	Leaf	−	−	−
5	43304		*Bouea oppositifolia*	Leaf	−	−	−
6	43304			Stem	−	−	−
7	143516		*Buchanania insignis*	Bark	−	−	−
8	143516			Leaf	−	−	−
9	143523	Annonaceae	*Artabotrys roseus*	Bark	++	−	+
10	143523			Leaf	−	−	−
11	143506		*Neouvaria acuminatiisima*	Bark	−	−	−
12	143506			Leaf	−	−	−
13	143509		*Orophea corymbosa*	Bark	−	−	−
14	143509			Leaf	−	−	+
15	143511		*Polyalthia insignis*	Bark	−	−	−
16	143511			Leaf	−	−	+
17	143502		*Polyalthia longipes*	Bark	−	−	−
18	143502			Leaf	−	−	−
19	145376		*Polyalthia microtus*	Bark	−	−	−
20	145376			Leaf	−	−	−
21	143524		*Polyalthia rumphii*	Leaf	−	−	−
22	143524			Root	−	−	−
23	145365		*Popowia odoardoi*	Leaf	++	−	−
24	145365			Stem	++	−	−
25	143507		*Xylopia malayana*	Bark	−	−	−
26	143507			Leaf	−	−	−
27	43125	Apocynaceae	*Dyera costulata*	Leaf	++	−	−
28	145362		*Kopsia dasyrachis*	Leaf	−	−	+
29	145362			Stem	−	−	−
30	43323	Bixaceae	*Flacourtia rukam*	Fruit	−	−	−
31	43323			Leaf	−	−	−
32	145361	Burseraceae	*Canarium denticulatum*	Bark	−	−	+
33	145361			Leaf	−	−	−
34	143522		*Canarium hirsutum*	Bark	−	−	−
35	143522			Fruit	−	−	−
36	143522			Leaf	−	−	−
37	43302		*Dacryodes rugosa*	Leaf	−	−	−
38	43306		*Santiria griffithii*	Leaf	−	−	−
39	43127		*Santiria laevigata*	Leaf	−	−	−
40	43127			Stem	−	−	−
41	43350	Caesalpiniaceae	*Peltophorum pterocarpum*	Leaf	−	−	−
42	43123	Clusiaceae	*Mesua ferrea*	Leaf	−	−	−
43	43320	Combretaceae	*Terminalia superba*	Leaf	−	−	−
44	43344	Coniferae	*Dacrydium becarii*	Leaf	−	−	−
45	43344			Stem	−	−	−
46	43345		*Dacrydium elatum*	Leaf	−	−	−
47	43345			Stem	−	−	−
48	143503	Dichapetalaceae	*Dichapetalum gelonioides*	Bark	−	−	−
49	143503			Leaf	−	−	−
50	43308	Dipterocarpaceae	*Anisoptera costata*	Leaf	−	−	−
51	43124		*Hopea dryobalanoides*	Leaf	−	−	−
52	43124			Twigs	−	−	−
53	43112		*Neobalanocarpus heimii*	Leaf	−	−	−
54	43134		*Shorea parvifolia*	Leaf	−	−	−

(*Continued*)

Table 5. Continued.

No.	Voucher no.	Family	Plant	Part	Receptors		
					5HT1a	$GABA_B$	D2S
55	43134			Stem	–	–	–
56	43324		*Upuna borneensis*	Leaf	–	–	–
57	43324			Stem	–	–	–
58	143504	Ebenaceae	*Diospyros cauliflora*	Bark	–	–	–
59	143504			Leaf	–	–	–
60	143519		*Diospyros tuberculata*	Bark	–	–	–
61	143519			Leaf	–	–	–
62	43273	Elaeocarpaceae	*Elaeocarpus petiolaris*	Leaf	–	–	–
63	145393	Euphorbiaceae	*Borneodendron aenigmaticum*	Bark	–	–	–
64	145393			Leaf	–	–	–
65	145400		*Mallotus griffithianus*	Bark	–	–	–
66	145400			Leaf	–	–	–
67	145371		*Mallotus wrayi*	Bark	–	–	–
68	145371			Leaf	–	–	–
69	43108		*Phyllanthus emblica*	Leaf	–	–	–
70	43108			Twigs	–	–	–
71	43338		*Phyllanthus pectinata*	Leaf	–	–	–
72	43137	Fagaceae	*Castanopsis inermis*	Leaf	–	–	–
73	145388	Flacourtiaceae	*Homalium panayanum*	Bark	–	–	–
74	145388			Leaf	–	–	–
75	145398	Guttiferae	*Calophyllum blancoi*	Bark	–	–	–
76	145377		*Calophyllum gracilipes*	Bark	–	–	–
77	145377			Leaf	–	–	–
78	145385		*Calophyllum nodosum*	Bark	–	–	–
79	145385			Leaf	–	–	–
80	133842		*Garcinia brianii*	Bark	–	–	–
81	133842			Leaf	–	–	–
82	133846		*Garcinia cuspidata*	Bark	–	–	–
83	142698		*Garcinia parvifolia*	Leaf	–	–	–
84	145380	Lauraceae	*Litsea elliptibacea*	Bark	++	–	–
85	145380			Leaf	–	–	–
86	145368		*Litsea garciae*	Bark	–	–	–
87	43339		*Persea americana*	Leaf	–	–	–
88	43129	Leguminosae	*Milletia atropurpurea*	Leaf	–	–	–
89	43138		*Sindora echinocalyx*	Leaf	–	–	–
90	43138			Twigs	–	–	–
91	43279	Linaceae	*Ixonanthes reticulata*	Leaf	–	–	–
92	43144	Magnoliaceae	*Aromadendron elegans*	Leaf	–	–	–
93	43105	Meliaceae	*Aglaia korthalsii*	Leaf	–	–	–
94	143512		*Chisocheton erythrocarpus*	Bark	–	–	–
95	143512			Fruit	–	–	–
96	143512			Leaf	+	–	–
97	145367		*Chisocheton macranthus*	Bark	–	–	–
98	145367			Fruit	–	–	–
99	145367			Leaf	–	–	–
100	143513		*Chisocheton pentandrus*	Bark	–	–	–
101	143513			Leaf	–	–	–
102	142663		*Chisocheton polyandrus*	Leaf	–	–	–
103	43104		*Sandoricum koetjape*	Leaf	–	–	–
104	43104			Twigs	–	–	–
105	43143		*Walsura chrysogene*	Leaf	–	–	–
106	145378	Menispermaceae	*Fibraurea chloroleuca*	Bark	–	–	–
107	145378			Fruit	–	–	–

(*Continued*)

Table 5. Continued.

No.	Voucher no.	Family	Plant	Part	Receptors		
					5HT1a	$GABA_B$	D2S
108	145378			Leaf	–	–	–
109	143518	Moraceae	*Ficus septica*	Leaf	–	–	–
110	145397	Myrtaceae	*Decaspermum fruticosum*	Bark	++	–	–
111	145397			Leaf	–	–	–
112	145392	Ochnaceae	*Gomphia serrata*	Bark	–	–	–
113	145392			Leaf	–	–	–
114	43111	Olacaceae	*Ochanostachys amentacea*	Leaf	–	–	–
115	145396	Oleaceae	*Chionanthus crispus*	Bark	–	–	–
116	145396			Leaf	–	–	–
117	43307	Polygalaceae	*Xanthophyllum stipitatum*	Leaf	–	–	–
118	43329	Rhamnaceae	*Maesopsis eminii*	Leaf	–	–	–
119	145373		*Vantilago dichotoma*	Leaf	–	–	–
120	145373			Stem	–	–	–
121	143525	Rhizophoraceae	*Carallia borneensis*	Bark	–	–	–
122	143525			Leaf	–	–	–
123	43348		*Carallia suffruticosa*	Leaf	–	–	+
124	43309	Rosaceae	*Maranthes corymbosa*	Stem	–	–	–
125	145363	Rubiaceae	*Gardenia tubifera*	Bark	–	–	–
126	145363			Leaf	–	–	–
127	145395		*Morinda rigida*	Bark	–	–	–
128	145395			Leaf	–	–	–
129	145395			Stem	–	–	–
130	145395			Stem	–	–	–
131	145364		*Praravinia suberosa*	Bark	–	–	–
132	145364			Leaf	–	–	+
133	145382		*Psychotria sarmentosa*	Bark	–	–	–
134	145382			Leaf	–	–	–
135	145384		*Timonius flavescens*	Bark	–	–	–
136	145384			Leaf	–	–	–
137	145366	Rutaceae	*Clausena excavata*	Bark	–	–	–
138	145366			Leaf	–	–	–
139	145390		*Melicope subunifoliolata*	Bark	–	–	–
140	43325	Sapindaceae	*Amesiodendron chinense*	Leaf	–	–	–
141	43325			Stem	–	–	–
142	43337		*Dimocarpus longan*	Leaf	–	–	–
143	43337			Stem	–	–	+
144	43327		*Lepisanthes alata*	Fruit	–	–	–
145	43327			Stem	–	–	–
146	43106		*Nephelium lappaceum*	Twigs	–	–	–
147	43310		*Nephelium maingayi*	Leaf	–	–	–
148	43341		*Nephelium rambutanake*	Leaf	–	–	–
149	43341			Stem	–	–	–
150	43110		*Pometia pinnata*	Leaf	–	–	+
151	43110			Twigs	–	–	–
152	143514		*Walsura pinnata*	Bark	–	–	–
153	143514			Leaf	–	–	–
154	43334	Sapotaceae	*Mimusops elengi*	Leaf	–	–	–
155	43334			Stem	–	–	–
156	43347		*Palaquium maingayi*	Leaf	–	–	–
157	43347			Stem	–	–	–
158	143520	Scyphostegiaceae	*Scyphostegia borneensis*	Bark	–	–	–
159	143520			Leaf	–	–	–
160	43145	Simaroubaceae	*Irvingia malayana*	Leaf	++	–	–

(*Continued*)

Table 5. Continued.

No.	Voucher no.	Family	Plant	Part	Receptors		
					5HT1a	$GABA_B$	D2S
161	133822		*Quassia indica*	Bark	–	–	–
162	43311	Sterculiaceae	*Heritiera simplicifolia*	Stem	–	–	–
163	145381		*Sterculia stipulata*	Bark	–	–	–
164	145381			Leaf	–	–	–
165	145383	Theaceae	*Schima wallichii*	Bark	–	–	–
166	145383			Leaf	–	–	–
167	43275	Tiliaceae	*Pentace triptera*	Leaf	–	–	++
168	145391	Ulmaceae	*Gironniera subaequaelis*	Leaf	–	–	–
169	145375	Urticaceae	*Dendrocnide elliptica*	Bark	–	–	–
170	145375			Leaf	–	–	–
171	145387		*Leucosyke winklerii*	Bark	–	–	+
172	145387			Leaf	–	–	–
173	106702	Verbenaceae	*Callicarpa erioclona*	Bark	–	–	–
174	133849		*Callicarpa fulvohirsuta*	Bark	–	–	–
175	145389		*Callicarpa havilandii*	Bark	–	–	–
176	145389			Leaf	–	–	–
177	145374		*Callicarpa longifolia*	Bark	–	–	–
178	145374			Leaf	–	–	–
179	106701		*Callicarpa stapfii*	Bark	–	–	–
180	106701			Leaf	–	–	–
181	145386		*Stachytarpeta jamaicencis*	Stem	–	–	–
182	43322		*Vitex pubescens*	Leaf	–	–	–
183	145370	Vitaceae	*Leea indica*	Bark	–	–	–
184	145370			Leaf	–	–	–
185	145379	Zingerberaceae	*Alpinia fraseriana*	Stem	–	–	–

Each plant extract was tested at 10 µg/well in duplicate for each receptor type. The result for each data point was expressed in % inhibitory specific binding in the presence of test extract. – : Both data points were below 50% inhibition over the mean of all samples for a given plate, + : One data point above 50% inhibition, whereas the other was below 50% inhibition over the mean of all samples for a given plate, ++ : Both data points were above 50% inhibition over the mean of all samples for a given plate.

Table 6. Hit confirmation on 5HT1a receptor activity of plant extracts of some Malaysian plant species.

No.	Voucher no.	Family	Plant	Part	% Inhibition Mean (n = 3) ± SD
1	143523	Annonaceae	*Artabotrys roseus*	Bark	72 ± 1
2	145365	Annonaceae	*Popowia odoardoi*	Leaf	89 ± 1
3	145365	Annonaceae	*Popowia odoardoi*	Stem	84 ± 2
4	43125	Apocynaceae	*Dyera costulata*	Leaf	90 ± 1
5	145380	Lauraceae	*Litsea elliptibacea*	Bark	84 ± 1
6	145397	Myrtaceae	*Decaspermum fruticosum*	Bark	86 ± 2
7	43145	Simaroubaceae	*Irvingia malayana*	Leaf	65 ± 1

The active plant extracts were tested at 10 µg/well in triplicate. The results were expressed as % inhibitory specific binding in the presence of test extract.

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