

Cannabinoid CB1 receptor binding and acetylcholinesterase inhibitory activity of *Sceletium tortuosum* L.

¹Lubbe, A., ^{2,3*}Khatib, A., ^{1,3}Yuliana, N.D., ²Jinap, S. and ¹Verpoorte, R.

¹Division of Pharmacognosy, Section of Metabolomics, Institute of Biology, Leiden University, Einsteinweg 55, P.O. Box 9502, 2300 RA Leiden, The Netherlands.

²Center of Excellence for Food Safety Research, Faculty of Food Science and Technology, University Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia.

³Department of Food Science and Technology, Bogor Agricultural University, Bogor, Indonesia.

Abstract: The whole plant extract of plant *Sceletium tortuosum*, plant native to South Africa, has been known traditionally to have mood enhancing and stimulant properties. These properties have been confirmed before by proving serotonin-uptake inhibition activity. A further confirmation by using CB1 receptor binding assay has been performed in this study. The unfermented alkaloid extract was proved to possess a higher activity to bind CB1 receptor compared to that of the fermented one. GC-MS analysis confirmed that unfermented alkaloid extract contains more alkaloids than the fermented one. The methanol extract was also more active than the fermented one, suggesting that non-alkaloid compounds in this extract could possess this activity. An additional test to check whether this extract can improve cognitive function and memory was performed by acetylcholinesterase inhibitory assay. Both fermented and unfermented alkaloid extracts could inhibit acetylcholinesterase with IC_{50} being 0.303 mg/ml and 0.330 mg/ml, respectively. However, the major alkaloid in the extract, mesembrine, did not show inhibition of the enzyme. A TLC based test proved that other alkaloids in the extract were responsible to the activity.

Keywords: *Sceletium tortuosum*, cannabinoid CB1, acetylcholinesterase, alkaloid, kougoed

Introduction

Sceletium tortuosum (L.) N.E.Br. is a plant native to South Africa, specifically occurring in the southwestern parts of the Cape Province and Namaqualand. The use of *kougoed* (something to chew) or *Kanna*, a concoction prepared from *S.tortuosum* has been recorded in the literature for over 300 years (Scott and Hewett, 2008). The traditional method for preparing *kougoed* involves crushing the whole plant, including the roots, between stones after harvesting. The crushed material is placed in a skin or canvas bag which is closed and left to "ferment" in the sun. After 2-3 days, the bag is opened and the contents mixed, and thereafter sealed and left in the sun for a few more days. Eight days after crushing the material, the bag is opened and the *kougoed* is spread out to dry in the sun. The finished product is light brown and somewhat stringy in appearance (Smith *et al.*, 1996; Smith *et al.*, 1998).

As the name suggests, the fermented and dried material was chewed. There are also some reports of it being used as a tea or a snuff (Watt and Breyer-

Brandwijk, 1962). In the earliest written records of its use, *kougoed* is described as having mood enhancing and stimulant properties (Scott and Hewett, 2008). Most people describe an anxiolytic effect, relaxation with no cognitive impairment, a feeling of tranquil mellowness, enhanced social intercourse and less inhibition. Some say it is good for stomach ailments, can cause anaesthesia of the jaw, can relieve pain and alleviate hunger. Recently a patent has been taken out on pharmaceutical compositions made from *S.tortuosum* for the treatment of depression, anxiety, alcohol and drug dependence, bulimia nervosa and obsessive-compulsive disorder (Gericke and van Wyk, 2001).

In 2001 an US patent was granted for the use of pharmaceutical compositions made from *S.tortuosum* to treat various ailments (Gericke and van Wyk, 2001). The patent was granted on the basis of the potent serotonin-uptake inhibitory activity demonstrated for the crude plant extract, mesembrine, mesembranol and mesembrenone. They have the similar potency as the tricyclic antidepressant imipramine HCl.

The potent serotonin-uptake inhibitory activity

*Corresponding author.

Email: alfikhatib@food.upm.edu.my; alfikhatib@hotmail.com
Tel: +603-8946 8540; Fax: +603-8942 3552

demonstrated for extracts of *S.tortuosum* and its major alkaloids can certainly account for the effects of this plant material and some of its therapeutic potentials. However, secondary metabolites are often multi-functional compounds (van Wyk and Wink, 2004) and they often affect more than one target in the body. Also, crude plant extracts or plant material traditionally consumed by ingestion contains many compounds which may affect many targets in the body simultaneously. Complex mixtures of chemical compounds may have numerous, synergistic effects on various organ systems in the body. The overall effect may be stronger than that of only one compound alone. Minor metabolites can affect targets that have a balancing effect that can for example cause fewer side effects as compared to a synthetic pure pharmaceutical. It is of interest to know which other biological activities a certain plant or compound has, as it could give clues to more potential therapeutic uses besides that already known. Therefore, it is also interesting to investigate other biological activities in an attempt to see through which mechanisms a herbal medicine may have its effect. *Sceletium* and *kougoed* seems to be a promising treatment for mood and anxiety-related disorders, but still requires further studies to understand the activity.

The cannabinoid system is one of the most recently discovered receptor systems in the human body. To date, two receptors CB 1 and CB2 have been identified. Evidence is mounting for the existence of one or more additional cannabinoid receptors (Begg *et al.*, 2005). Cannabinoid receptors CB1 and CB2 are both G-protein coupled receptors. In the brain, endocannabinoids behave as retrograde signaling messengers that stimulate presynaptic CB1 receptors on neurons. This activation results in inhibition of adenylate cyclase activity, regulation of ion channel activities and activation of the mitogen-activated protein kinase cascade (Matsuda *et al.*, 1990). The signal transduction mechanisms have been reviewed by Howlett *et al.* (2004). CB1 receptors are the most abundant receptors in the mammalian brain, and also occur at lower concentrations in other peripheral tissues and cells such as testis, eye, urinary bladder, ileum and adipocytes (Lange and Kruse, 2005; Pertwee, 1997). CB2 is found mostly in cells of the immune and hematopoietic systems (Munro *et al.*, 1993), but has also been found in the brain, liver, pancreas and in bone (Juan-Pico *et al.*, 2005; Julien *et al.*, 2005; Karzak *et al.*, 2005; Van Sickle *et al.*, 2005).

CB1 receptors are found in brain areas related to perception of emotions (Howlett *et al.*, 2004) and control of anxiety (Pistis *et al.*, 2004). Support for

the hypothesis that the endocannabinoid system is important for regulation of mood and anxiety states have been provided by data from neurobiological, pharmacological and genetic studies (Witkin *et al.*, 2005b). Blockage or antagonism of CB1 receptors have produced antidepressant effects, and have also been associated with enhancement of cognition and efficacy against drug addiction disorders, which are often comorbid with depression (Witkin *et al.*, 2005a). CB1 receptors have been found co-distributed with 5-HT transporters in the amygdale of rat brains, and there is evidence to suggest that they mediate 5-HT release (Ashton *et al.*, 2006).

The CB1 receptor therefore seems like a promising target for the treatment of disorders related to mood, anxiety and cognition. The CB1 receptor has also been identified as a possible target for pharmacotherapy of pain and inflammation, other central nervous system disorders, nausea, cardiovascular and respiratory disorders, eye disorders, cancer, gastrointestinal disorders, and musculoskeletal disorders (Pacher *et al.*, 2006).

Another strategy for a treatment of nervous system disorders is inhibition of acetylcholinesterase (E.C. 3.1.1.7; AChE). This enzyme is one of two cholinesterase enzymes that occur in the human central nervous system. AChE hydrolyses acetylcholine at cholinergic synapses to terminate nerve impulse transmission. Inhibition of AChE activity increases the availability of acetylcholine to sustain nerve cell communications, therefore chemical inhibitors as possible leads for nervous system disorders is being searched for (Lenta *et al.*, 2008). Inhibition of AChE has been a strategy for treatment of senile dementia, ataxia, myasthenia gravis and Parkinson's disease (Mukherjee *et al.*, 2007), but probably the most attention has been given to finding AChE inhibitors to treat Alzheimers disease (AD). An overlapping distribution of CB1 receptors with nicotinic acetylcholine (nACh) receptors have been found in brain areas considered important for memory modulation (Cohen *et al.*, 2002).

The goal of this study is to find further scientific evidence for the traditional use of *S.tortuosum* extracts to have enhancing and stimulant properties by testing the extract on CB1 receptor binding assay. The study also included the testing of the extract in the AChE inhibitory assay in order to evaluate the potential of this extract to improve cognitive function and memory.

Materials and Methods

Plant material

Plant material was purchased from a community project where *S. tortuosum* is grown for commercial use. Plants were grown at Helpmekaar, Calvinia, in the Northern Cape province of South Africa. Plants were harvested in October, and *kougoed* was prepared from it according to the traditional method. Plant material was also purchased that had not been processed in the traditional way, but only dried and not ground up.

Preparation of extracts

800 mg *kougoed* was extracted with 4 ml each of *n*-hexane, chloroform and methanol by sonication for 15 min. The extracts were centrifuged for 10 min at 20000x at 24 °C. The supernatant was collected and evaporated to dryness under reduced pressure at 40 °C. The extracts were weighed and redissolved in DMSO to a concentration of 1 mg/ml. Also included in the assay were the alkaloid extracts from *kougoed* and unfermented plant material and mesembrine at concentrations of 1 mg/ml in DMSO. The final concentration of extracts in the assay was 0.037 mg/ml.

For alkaloid extract preparation, 5 g each of *kougoed* and unprocessed plant material was de-fatted by washing with 20 ml *n*-hexane three times. The material was extracted three times with 50 ml 0.05 M H₂SO₄ for one hour. After filtration the acidic water extracts were pooled and basified using NH₄OH to pH 11. The basic aqueous phase was partitioned three times against an equal volume of dichloromethane. The organic fractions were pooled and dried under reduced pressure at 40 °C.

Cannabinoid CB1 receptor binding assay

To each reaction tube 250 µl of incubation buffer (20 mM Hepes; 5 mM MgCl₂; 1 mM EDTA; BSA (0.3%), 20 µl of sample as well as 20 µl of the radioactive ligand [³H]-CP55940 (8x10⁻¹¹ M) was added. After adding 250 µl of CB1 receptor (Cloned Cannabinoid Receptor Subtype 1, Human Sf9 cells, Perkin Elmer) the reaction tubes were incubated for 60 minutes at 30° C in a waterbath. Filters, soaked for one hour in ice cold wash buffer (20 mM Hepes pH 7.4; BSA (0.01%)) with 1% polyethylenimine were placed in a Millipore vacuum manifold and washed once with wash buffer. After incubation, the reaction tubes were filled with wash buffer and the contents of the tubes poured over the filters. The tubes were refilled with wash buffer and the filters washed again. The filters were placed in a counting vial to which 7.5 ml scintillation fluid (Perkin Elmer: Ultima gold) was added. After shaking the counting vial for one minute the vials were placed in a scintillation counter.

For a control to see 100% radioligand binding (total binding), a sample was included with no plant extract and just DMSO. To determine nonspecific binding a sample of excess unlabeled CP55940 (Perkin Elmer) was used. The amount of radiolabel specifically bound in the absence of competing compounds was calculated by subtracting non-specific binding from total binding. The percentage of specific binding was then calculated for the amount of radiolabel bound in the presence of each competing extract or compound. To see how much of the receptors were occupied with each sample the following equation was used:

$$\% \text{ binding of sample} = 100 - \frac{[\text{H}] \text{ CP55940 specific binding in presence of sample}}{[\text{H}] \text{ CP55940 total binding}}$$

Acetylcholinesterase inhibition assay

AChE inhibitory activity was detected by a TLC- and microplate assay based on Ellman's method (Ellman *et al.*, 1961), as described by Rhee *et al.* (2001).

Gas Chromatography-mass spectrometry (GC-MS)

GC/MS was carried out using a Varian Saturn 2000 ion trap Gas chromatography mass spectrometer with 3800 Chromatograph fitted with a VA5 MS column (30m x 0.25mm, 0.25µ film thickness) The mass detector was operated in EI mode at 70eV. The column temperature started at 175°C and increased to 270°C at 3°C /min. Injector temperature was set at 270°C, and detector temperature at 280°C. The column limit was 271°C. For sample analysis, 3µl of a 2 mg/ml methanol solution was injected. Methanol (3µl) was injected as a blank.

Results and Discussion

Cannabinoid CB1 binding

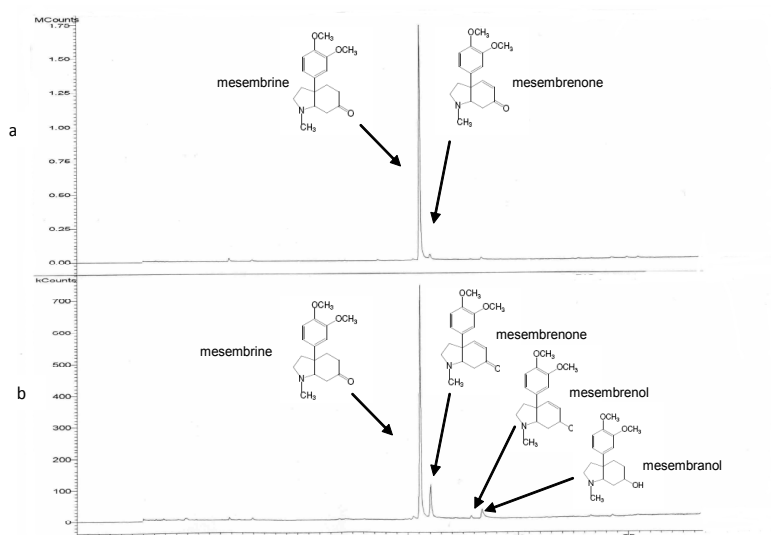
The CB1 receptor binding assay that was performed is a screening test for interaction of extracts or compounds with this receptor. In this assay, experiments were carried out for the different extracts. As shown in Table 1, the methanol extract displaced just over 22% of the radioligand. The *n*-hexane extract displaced more but usually the *n*-hexane extracts of plant material contains non-polar compounds that give false positive results in this assay. The chloroform extract tested gave very weak displacement of the radioligand, confirming that non-polar compounds were most likely not responsible for the activity seen in the crude extracts. The highest activity was shown in the alkaloid extract from unprocessed plant material. Lower activity was

Table 1. Results of cannabinoid CB1 assay. Samples and THC at final concentration of 0.037 and 0.0037 mg/ml, respectively.

Sample	% binding of sample
Control (DMSO)	0
Methanol extract	22.26 ± 0.81
<i>n</i> -hexane extract	33.52 ± 0.90
Chloroform extract	5.55 ± 0.64
Unfermented alkaloid extract	27.58 ± 1.87
Fermented alkaloid extract	17.73 ± 1.54
Mesembrine	11.64 ± 0.80
THC	70.92 ± 0.82
Unlabelled ligand	100

Table 2. Retention time and mass fragmentation of identified constituents in fermented and unfermented alkaloid extracts analyzed by GC-MS.

Compounds	Retention time (min)	Mass Fragmentation
Mesembrine	15.5	m/z: 289 (40%, M+), 288 (72%, M-1), 274 (15%), 218 (100%), 204 (52%), 160 (5%), 96 (45%), 70 (17%).
Mesembrenone	16.0	m/z: 287 (100%, M+), 230 (34%), 219 (33%), 204 (16%), 115 (19%), 70 (92%).
Mesembrenol	17.9	m/z: 289 (18%, M+), 288 (43%), 262 (30%), 261 (37%), 246 (100%), 230 (37%), 218 (30%), 204 (23%), 164 (50%), 77 (31%), 60 (47%).
Mesembranol.	18.3	m/z: 291 (3%, M+), 290 (10%), 234 (100%), 204 (2%), 163 (46%).

**Figure 1.** GC-MS chromatograms of alkaloid extracts prepared from *kougoed* (a) and unprocessed plant material (b). Alkaloids were identified based on fragmentation patterns and molecular mass, and structures are shown.

seen in the alkaloid extract from processed *kougoed*, which contains more of the major alkaloid and less of the minor alkaloids as shown by the results of GC-MS analysis (Figure 1).

The alkaloids were identified by comparing their fragmentation patterns with those published before (Martin *et al.*, 1976). The major alkaloid was identified as mesembrine, and the other minor alkaloids were mesembrenone, mesembrenol and mesembrenol. This identification was considered based on the fragmentation pattern and the retention time during GC-MS analysis as shown in Table 2.

Most *in vitro* activity studies with CB receptor assay have focused on pure synthetic compounds. In one study where a plant extract prepared from fruit juice was tested for CB1 activity, extracts inhibited radioligand binding by 10-14% at concentrations of 1 mg/ml in the assay (Palu *et al.*, 2008). In the present study a crude methanol extract at a of 0.037 mg/ml in the assay inhibited radioligand binding twice as much.

Mesembrine on its own showed less activity, displacing just over 11% of the radioligand at this concentration. Typically, when pure compounds are screened in the CB1 assay in high throughput screening studies, a concentration of around 0.1-10 μ M is tested in a single point experiment (Foloppe *et al.*, 2008; Wang *et al.*, 2008). Usually a range of compounds are tested and only ones displacing a radioligand such as [3 H] CP55940 by more than 50% are investigated further. Since mesembrine did not show a very high displacement of radioligand, further experiments were not carried out to determine IC₅₀ or Ki values.

The *kougoed* alkaloid extract inhibited radioligand binding slightly more than the pure mesembrine in this assay. The unprocessed material alkaloid extract did so even more, inhibiting radioligand binding by more than 27%. The differences in activity could be due to the different compositions of the alkaloid extracts. The alkaloid extract of the unprocessed material has mesembrine as the major alkaloid, but contains relatively more of the other alkaloids (Fig. 1). It is possible that one or more of the minor alkaloids interact with the CB1 receptor, and that the increased activity seen in the unprocessed alkaloid extract is due to their additive effect with mesembrine. The methanol extract also displaced more of the radioligand than the *kougoed* alkaloid extract. The methanol extract contains some alkaloids, but in a much lower concentration than the alkaloid extracts. It is possible that compounds other than the alkaloids present in the methanol extract are responsible for inhibiting the radioligand binding, alone or together

with the alkaloids.

Acetylcholinesterase inhibition

The AchE microplate assay was performed with the alkaloid extracts from *kougoed* and unprocessed plant material, as well as with purified mesembrine. The results are shown in Table 3. The IC₅₀ of the alkaloid extracts were estimated from regression equations using these data. The two alkaloid extracts had similar IC₅₀ values, with that of the *kougoed* and unprocessed material alkaloid extracts being 0.303 mg/ml and 0.330 mg/ml, respectively. Mesembrine was less potent at inhibiting the enzyme, and a 50% inhibition was not reached at the concentrations tested. The inhibition of the enzyme by galanthamine was also tested at three concentrations. It is clear that the potency of galanthamine is much higher.

Mukharjee *et al.* (2007) tested crude extracts of plants known to improve cognitive function and memory for AChE activity. These extracts inhibited the enzyme with IC₅₀ values of between 0.1 mg/ml and 0.15 mg/ml. The activity of these crude extracts was higher than our extract. In the paper of Rhee *et al.* (2001), on which the protocol used in this study was based, crude extracts that caused 85-90% inhibition of the enzyme at a concentration of 1 mg/ml were considered interesting for further investigation. At this concentration the alkaloid extracts tested in this study satisfied this criterion. At the same concentration (1 mg/ml) purified mesembrine had an almost three times lower activity, even though the alkaloid extracts consisted mostly of mesembrine. It is possible that the minor alkaloids present in the alkaloid extracts are responsible for the higher activity, either through a synergistic or additive effect. It was shown in the TLC assay that at least three other alkaloids also have AChE inhibitory activity (data not shown).

Conclusion

The unprocessed alkaloid extract of *S. tortuosum* was the most active in CB1 receptor binding assay, suggesting that a mixture of these alkaloids may have a stronger interaction with the CB1 receptor than the fermented alkaloid extract and the major alkaloid mesembrine. The higher activity of this extract may possibly be due to the synergistic effect of the alkaloids. The Methanol extract showed a higher activity compared to the fermented alkaloid extract. It is possible that compounds other than the alkaloids present in the methanol extract are responsible for the activity.

Both fermented and unprocessed alkaloid extract showed similar activity to inhibit AChE. The

Table 3. Results of AchE microplate assay

Concentration (mg/ml)	% inhibition of acetylcholinesterase compared to blank			
	<i>Kougoed</i> alkaloid extract	Unprocessed material alkaloid extract	mesembrine	Galanthamine
1.000	87.96±6.89	92.93±9.63	29.63±10.08	-
0.500	64.99±8.09	71.54±9.39	-	-
0.250	48.07±6.57	39.53±7.78	-	-
0.100	11.82±7.98	20.90±4.42	-	97.11±2.09
0.010	0	5.03±3.34	-	67.99±4.96
0.001	0	0	-	15.28±4.22

activities of both extracts are higher than the activity of mesembrine alone. It points to the involvement of other alkaloids in the extract to increase the activity.

These findings confirm that the extract may have mood enhancing and stimulant properties which in agreement with the traditional use. Moreover, the extract is a potential candidate for further studies for activity to improve cognitive function and memory.

Acknowledgments

We wish to thank the Netherlands Organisation for International Cooperation in Higher Education (Nuffic) in the Netherlands for the financial support.

References

- Ashton, J. C., Darlington C.L. and Smith, P.F. 2006. Co-distribution of the cannabinoid CB1 receptor and the 5-HT transporter in the rat amygdale. *European Journal of Pharmacology* 537: 70-71.
- Begg, M., Pacher P., Batkai, S., Osei-Hyiaman, D., Offertaler, L., Mo, F-M., Liu, J. and Kunos, G. 2005. Evidence for novel cannabinoid receptors. *Pharmacology and Therapeutics* 106: 133-145.
- Cohen, C., Perrault G., Voltz, C., Steinberg, R. and Soubrie, P. 2002. SR141716, a central cannabinoid (CB1) receptor antagonist, blocks the motivational and dopamine-releasing effects of nicotine in rats. *Behavioural Pharmacology* 13: 451-463.
- Ellman, G. L., Courtney K. D, Andres, V. and Featherstone, R.M. 1961. A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity. *Biochemical Pharmacology* 7: 88-95.
- Foloppe, N., Allen, N. H. , Bentley, C.H., Brooks, T.D., Kennett, G., Knight, A.R., Leonardi, S., Misra, A., Monck, N.J.T. and Sellwood, D.M. 2008. Discovery of a novel class of selective human CB1 inverse agonists. *Bioorganic & Medicinal Chemistry Letters* 18: 1199-1206.
- Gericke, N. P. and van Wyk, B. E. 2001. Pharmaceutical compositions containing mesembrine and related compounds. United States Patent 6288104.
- Howlett, A. C., Breivogel, C. S., Childers, S.R., Deadwyler, S.A., Hampson, R.E. and Porrino, L.J. 2004. Cannabinoid physiology and pharmacology: 30 years of progress. *Neuropharmacology* 47: 345-358.
- Juan-Pico, P., Fuentes, E., Javier Bermudez-Silva, F., Javier Diaz-Molina, F., Ripoll, C., Rodriguez de Fonseca, F. and Nadal, A. 2005. Cannabinoid receptors regulate Ca²⁺ signals and insulin secretion in pancreatic B-cell. *Cell Calcium* 39: 155-162.
- Julien, B., Grenard, P., Teixeira-Clerc, F., Van Nhieu, J.T., Li, L., Karzak, M., Zimmer, A., Mallat, A. and Lotersztajn, S. 2005. Antifibrogenic role of the cannabinoid receptor CB2 in the liver. *Gastroenterology* 128: 742-755.
- Karzak, Cohen-Solal, M., M. Freudenberg, J., Ostertag, A., Morieux, C., Kornak, U., Essig, J., Erxlebe, E., Bab, I. and Kubisch, C. 2005. The cannabinoid receptor type 2 (CNR2) gene is associated with human osteoporosis. *Human Molecular Genetics* 14: 3389-3396.
- Lange, J. H. M. and Kruse C. G. 2005. Medicinal chemistry strategies to CB1 cannabinoid receptor antagonists. *Drug Discovery Today* 10: 693-702.
- Lenta, B. N., Devkota, K. P. Nguela, S., Fekam Boyom, F., Naz, Q., Choudhary, M.I., Tsamo, E., Rosenthal, P.J. and Sewald, N. 2008. Anti-plasmodial and Cholinesterase Inhibiting Activities of some Constituents of *Psorospermum glaberrimum*. *Chemical and Pharmaceutical Bulletin* 56: 222-226.
- Martin, N. H., Rosenthal, D. and Jeffs, P.W. 1976. Mass-Spectra of Sceletium Alkaloids. *Organic Mass Spectrometry* 11: 1-19.
- Matsuda, L. A., Lolait, S. J. and Brownstein, M.J. 1990.

- Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 346: 561-564.
- Mukherjee, P. K., Kumar, V., Mal, M. and Houghton, P.J. 2007. Acetylcholinesterase inhibitors from plants. *Phytomedicine* 14: 289-300.
- Munro, S., Thomas, K. L. and Abu-Shaar, M. 1993. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 365: 61-65.
- Pacher, P., Batkai, S. and Kunos, G. 2006. Endocannabinoid System as an Emerging Target of Pharmacotherapy. *Pharmacological Reviews* 58: 389-462.
- Palu, A. K., Kim, A. H., West, B.J., Deng, S.X., Jensen, J. and White, L. 2008. The effects of *Morinda citrifolia* L. (noni) on the immune system: Its molecular mechanisms of action. *Journal of Ethnopharmacology* 115: 502-506.
- Pertwee, R. G. 1997. Pharmacology of cannabinoid CB1 and CB2 receptors. *Pharmacology & Therapeutics* 74: 129-180.
- Pistis, M., Perra, S., Pillolla, G., Melis, M., Gessa, G.L. and Muntoni, A.L. 2004. Cannabinoids modulate neuronal firing in the rat basolateral amygdala: evidence for CB1- and non-CB1-mediated actions. *Neuropharmacology* 46: 115-125.
- Rhee, I. K., van de Meent, M., Ingkaninan, K. and Verpoorte, R. 2001. Screening for acetylcholinesterase inhibitors from Amaryllidaceae using silica gel thin-layer chromatography in combination with bioactivity staining. *Journal of Chromatography A* 915: 217-223.
- Scott, G. and Hewett, M. L. 2008. Pioneers in ethnopharmacology: The Dutch East India Company (VOC) at the Cape from 1650 to 1800. *Journal of Ethnopharmacology* 115: 339-360.
- Smith, M. T., Field, C. R., Crouch, M.R. and Hirst, M. 1998. The distribution of mesembrine alkaloids in selected taxa of the Mesembryanthemaceae and their modification in the *Sceletium* derived 'Kougoed'. *Pharmaceutical Biology* 36: 173-179.
- Smith, M. T., Crouch, M. R., Gericke, N. and Hirst, M. 1996. Psychoactive constituents of the genus *Sceletium* NEBr and other Mesembryanthemaceae: A review. *Journal of Ethnopharmacology* 50: 119-130.
- Van Sickle, M. D., Duncan, M., Kingsley, P.J., Mouihate, A., Urbai, P., Mackie, K., Stella, N., Makriyannis, A., Piomelli, D. and Davison, J.S. 2005. Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science* 310: 329-332.
- van Wyk, B. E. and Wink, M. 2004. Medicinal plants of the world. Briza Publications.
- Wang, H. W., Duffy, R. A., Boykow, G.C., Chackalamannil, S. and Madison, V.S. 2008. Identification of novel cannabinoid CB1 receptor antagonists by using virtual screening with a pharmacophore model. *Journal of Medicinal Chemistry* 51: 2439-2446.
- Watt, J. M. and Breyer-Brandwijk, M. G. 1962. The Medicinal and Poisonous plants of Southern and Eastern Africa. 2nd edn. London: Livingstone.
- Witkin, J. M., Tzavara, E. T. and Nomikos, G.G. (2005). A role for cannabinoid CB1 receptors in mood and anxiety disorders. *Behavioural Pharmacology* 16: 315-331.
- Witkin, J. M., Tzavara, E. T., Davis, R.J., Li, X. and Nomikos, G.G. 2005. A therapeutic role for cannabinoid CB1 receptor antagonists in major depressive disorders. *Trends in Pharmacological Sciences* 26: 609-617.