Available online at www.sciencedirect.com
SCIENCE DIRECT®
Phytomedicine 12 (2005) 39–45
Phytomedicine
www.elsevier.de/phymed

Pharmacological studies on the sedative and hypnotic effect of *Kava kava* and *Passiflora* extracts combination

A. Capasso[a], L. Sorrentino[b,*]

[a]*1st Department of Pharmaceutical Sciences, University of Salerno, Italy*
[b]*Department of Experimental Pharmacology, University of Napoli-Federico II, Via Domenico Montesano 45, 80131 Napoli, Italy*

Received 15 September 2003; accepted 5 March 2004

Abstract

Kava kava extract, *Passiflora* extract and a combination of both extracts, administered to mice, caused a significant decrease of the amphetamine-induced hypermotility and significant prolongation of sleeping phase induced by subcutaneous injection of barbiturates. Due to a synergism of both extracts, simultaneously administered the pharmacologically registered effect in both *in vivo* experiments was found to be superior over the sum of the single separately administered extracts.

Keywords: *Passiflora incarnata*; *Kava kava* extracts; Sedative and hypnotic effects; Pharmacological synergism

Introduction

Kava and *Passiflora* extracts have been used for a long time as pharmaceutical preparations with a sedative, hypnotic effect, and the synergism which is achieved with concomitant administration of the two plant drugs is known from the literature.

An extensive literature exists on the sedative effect of kava; the chemical (Smith, 1983; Smith et al., 1984; Klohs et al., 1959) and pharmacological (Haensel, 1959, 1964; Meyer, 1962, 1979; Kretzschmar and Meyer, 1969; Jamieson et al., 1989; Jamieson and Duffield, 1990a, b) properties of its components have been described.

Scientific studies on the effects of the active substances both on the central nervous system and on the peripheral nervous system have been carried out. In this connection, evidence of the sedative, anxiolytic, and muscle relaxant effect with mild antipsychotic effects has been provided (Jamieson et al., 1989; Meyer and Kretzschmar, 1969).

A direct effect on the muscular contractility and a local anesthetic effect have also been observed (Jamieson et al., 1989). A number of clinical studies have shown that the active substances of the kava plant promote physiological sleep, exert an anxiolytic effect, and lead to an improvement of psychovegetative and psychosomatic symptoms (Singh, 1983; Dona et al., 1986; Warnecke et al., 1986).

Recently, controlled clinical studies were carried out in which the therapeutic effect of kava on anxiety states was demonstrated (Kinzler et al., 1991). Clinical studies confirming the psychotherapeutic effect of kava (Volz and Kieser, 1997) and the available literature were reviewed in 1999 and in 2000; the clinical studies were carried out also in comparison with synthetically produced anxiolytic drugs. Additionally, the clinical efficacy of *Kava* extracts was also confirmed by a clinical

*Corresponding author. Fax: +39 081 678 403.
E-mail address: sorrent@cds.unina.it (L. Sorrentino).

0944-7113/$ - see front matter © 2004 Published by Elsevier GmbH.
doi:10.1016/j.phymed.2004.03.006

study (Mittmann et al., 2000). There are also numerous scientific studies concerning extracts from *Passiflora incarnata* (Pittler and Ernst, 2000; Della Loggia et al., 1981; Sopranzi et al., 1990).

As early as 1981, Della Loggia et al., investigated the sedative effect of *P. incarnata* in comparison with other drugs and concluded from their studies that these *P. incarnata* extracts show potentiated anxiolytic properties in combination with other plant extracts (chamomile, valerian, hawthorn, Jamaican dogwood, belladonna, and henbane) with a less potent hypnotic component. The synergism of the pharmacological effect of passiflora was shown for combinations with other plant extracts.

Passiflora also showed a sedative, anxiolytic effect in rats without exerting negative effects on weight, rectally measured body temperature, pain sensitivity, motor coordination ability, and normal electocerebral activity (Della Loggia et al., 1981).

Recently, the sedative and anxiolytic effect of *P. incarnata* extracts and their most important efficacious principles was confirmed (Sopranzi et al., 1990).

For carrying out our experimental studies, we were provided with the following substances from Harras Pharma, Munich, Germany:

(1) *Kava* soft extract from root and rhizome (extract medium ethanol 96% v/v) standardized to 50 mg kavalactones HPLC in 100 mg extract/inert substance macrogol 3000 ~single dose 100 mg extract = 50 mg kavalactones.
    Method: HPLC determination (normal phase) against desmethoxy-yangonin, dihydrokawain, yangonin, kawain, dihydromethysticin, methysticin as reference substances (test specification no. 151) Company Gehrlicher GmbH.
(2) *P. incarnata* soft extract from herb 8:1 as native extract (extract medium ethanol 70% v/v) standardized to 4% m/m flavonoids HPLC ~single dose 250 mg native extract (corresponding to 2.0 g Herba Passiflorae).
    Method: HPLC determination (reversed phase) against apegenin as reference substance and calculated as apegenin.
    Test specification no. 381 Company Gehrlicher GmbH.
    Separation column: LiChrospher 100, RP-18.5 µm; 250 × 4.6 mm.
    Flow: 1.2 ml/min; application value: 20 µl; detection: 340 nm.
    Eluent A: Acetonitril/water = 55/345 (m/m); pH = 2.8.
    Eluent B: Acetonitril/water = 120/180 (m/m); pH = 2.8.
    Gradient: 13.5 min long 100% A, from 13.5 to 16.5 min to 100% B, afterwards 7 min equilibration to 100% A.
(3) Combination of the kava and passiflora soft extracts (100 mg kava soft extract ~50 mg Kavalacton + 250 mg passiflora soft extract = 350 mg combined soft extract) ~single dose 350 mg.

In the following sections, the extracts from kava, passiflora, and the combination will be referred to with the abbreviations K, P, and KP, respectively.

The following studies were carried out with these substances:

(A) Effect on amphetamine-induced hypermotility.
(B) Prolongation of the barbiturate-induced sleeping time.

All experimental studies described in the present report were carried out with the extracts provided by the company; analytic reports for batch numbers V1462000 (Kava), 7039 (Passiflora), and V1562000 (Kava and Passiflora) were available for these extracts. The analytic reports as well as portions of the extracts used were, as stipulated, stored in our Department.

Material and methods

General experimental conditions/good laboratory practice

The studies were carried out in accordance with the guidelines of good laboratory practice (GLP) applied in our laboratory.

All tests were supervised by a scientific employee with a doctorate; the staff members responsible for the tests were qualified for this job by their training and they were informed about the aim of the studies.

A person who did not belong to the study group monitored the correct application of the procedure and following of the experimental plan.

Until the time of the experiments, the experimental animals were kept in cages under a room temperature that was kept constant by an air-conditioning system. The animals were kept in Makrolon cages with automatic drinkers.

Each cage was provided with a label on which the experimental group was specified in a clearly readable way. Before the experiments were carried out, the cages and other equipment were thoroughly cleaned and the litter was changed at the required intervals.

Origin, room conditions, and feeding of the experimental animals

Origin

Male Swiss mice from the breeding colony of Charles River, Calco (Como, Italy) were used.

Feeding

The experimental animals were fed Altromin feed in the form of pellets and allowed to drink ad lib.

Housing

The animals were housed in cages containing four or five animals each in an air-conditioned environment ($22 \pm 2\,^{\circ}C$) with a humidity of $60 \pm 10\%$ and artificial lighting with a light-dark cycle of 12:12 h.

Extracts

Extract: *Kava kava* e rhiz. et radice spissum standardized to 50 mg kavalactones HPLC in 100 mg extract/inert substance macrogol 3000 (Ph. Eur.) (desmethoxyyangonin, dihydrokawain, yangonin, kawain, dihydromethysticin, and methysticin):

Botanical name: *Piper methysticum* G. Forster.
Extracted part of the plant: roots and rhizomes.
Extracting medium: ethanol 96% v/v Ph. Eur./
100 mg kava extract = 1 dose ~50 mg kavalactones.

Extract: Passiflorae e herb. spissum 8:1 (native extract) standardized to 4% m/m flavonoids HPLC:

Botanical name: *P. incarnata* L.
Extracted part of the plant: Herba.
Extracting medium: Ethanol 70% v/v DAB 250 mg native extract = 1 dose corresponding 2 g Herba.

Extract: *Kava/Passiflora* spissum conc. (combination) consisting of 100 mg kava soft extract ~50 mg kavalactones HPLC and 250 mg passiflora soft extract 8:1 350 mg extract combination = 1 dose.

Pharmacological experiments

Results

Effect on amphetamine-induced hypermotility

A total of 40 mice with a body weight of 20–25 g were subdivided into four groups (10 animals/group). The extracts were administered at the following doses: kava extract (K) at a dose of 100 mg/kg (~50 mg kavalactones), passiflora extract (P) at a dose of 250 mg/kg, and the combination of the two extracts (KP) at a dose of 350 mg/kg (100 + 250 mg/kg). The above-mentioned doses were dissolved in macrogol 400 so that a quantity of liquid of 10 ml/kg was administered via a stomach tube. The same quantity of vehicle liquid was also administered via stomach tube to the control group. The amphetamine dose (amphetamine sulfate from Sigma Tau, Rome, Italy) of 5 mg/kg was administered subcutaneously in physiological saline to all animals 1 h after the stomach tube administration of the extracts and immediately before the measurement of spontaneous motility.

The activity of the animals was measured with an "activity cage" from U. Basile, Milan, Italy. The floor of these cages consists of metal bars that are insulated from one another; the movement of the animals triggers off impulses which are recorded by an electronic counter. The hypermotility produced by amphetamines was monitored over a period of 2 h; the activity was recorded at 30-min intervals.

The data for the individual animals are presented in Tables 1–4, and Fig. 1 shows the means. Based on the results, it can be seen that there was a clear increase in the mean motility in the control group. The groups treated exclusively with K or P showed a significant reduction of the motility compared with the control group. The decrease was far greater in the group simultaneously treated with both pharmaceutical preparations. When the percent decrease was calculated with reference to the control values (i.e. the values of the group that was treated only with amphetamine), the results showed a reduction of 47% in the group treated with K and 39% in the group treated with P 2 h after the injection of the amphetamine. The highest percentage for the reduction of hypermotility was achieved by the concomitant administration of both extracts.

In this case, the reduction was 83% compared to the control values.

The motility values of the groups treated with K, P, or KP were significantly different from those of the control group.

Table 1. Amphetamine-induced hypermotility in mice

Experimental animals number	Motility values/step impulses (counts) at			
	30 min	60 min	90 min	120 min
1	2653	4373	8676	12,115
2	3729	6736	4939	8436
3	4328	5111	7773	13,339
4	3713	4127	9026	13,447
5	1750	6185	10,112	10,731
6	3480	3476	8344	8449
7	5437	4598	6381	9478
8	2348	6113	6134	8634
9	3363	5551	7350	9919
10	3302	7934	9994	7990
Mean	3410.3	5420.4	7872.9	10,253.8
Standard error	±327	±428	±536	±653

Note: The control group ($n = 10$) was only treated with the vehicle liquid macrogol 400 (10 ml/kg) administered via stomach tube 2 h before subcutaneous administration of amphetamine sulfate (5 mg/kg).

Table 2. Amphetamine-induced hypermotility in mice

Experimental animals number	Motility values/step impulses (counts) at			
	30 min	60 min	90 min	120 min
1	879	2449	4912	5437
2	1130	4730	5673	6778
3	1867	5429	3844	8436
4	3410	2870	3778	5112
5	2316	3112	4821	5031
6	2843	2836	5050	4986
7	1115	4331	5133	4800
8	1826	2896	6541	7099
9	1534	4887	2600	5829
10	2813	4437	3002	8134
Mean	1973.3	3797.7	4535.4	6164.2
Standard error	±269	±338	±385	±429
Compared with control group	**	*	***	***

Note: The group ($n = 10$) was treated with 100 mg/kg kava extract corresponding to 50 mg kavalactones in 10 ml/kg of the vehicle liquid macrogol 400 administered via stomach tube 2 h before subcutaneous administration of amphetamine sulfate (5 mg/kg).
$^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

Table 3. Amphetamine-induced hypermotility in mice

Experimental animals number	Motility values/step impulses (counts) at			
	30 min	60 min	90 min	120 min
1	2834	4733	8166	9120
2	3012	4422	7434	7839
3	1632	2748	6229	6224
4	1837	2637	5437	5736
5	1005	5035	3962	8330
6	2878	3713	4873	9211
7	3046	4117	8310	6347
8	2138	2054	6772	7004
9	2115	2733	3845	9122
10	2691	3939	5046	6147
Mean	2318.8	3613.1	6007.4	7508
Standard error	±217	±320	±517	±436
Compared with control group	*	**	*	**

Note: The group ($n = 10$) was treated with 250 mg/kg passiflora solid extract in 10 ml/kg of the vehicle liquid macrogol 400 administered via stomach tube 2 h before subcutaneous administration of amphetamine sulfate (5 mg/kg).
$^{*}p < 0.05$, $^{**}p < 0.01$.

In our study, the results clearly showed the sedative characteristics of the stomach tube-administered kava extract and passiflora extract as well as the synergistic effect of both components for the tested dosage ratio.

Table 4. Amphetamine-induced hypermotility in mice

Experimental animals number	Motility values/step impulses (counts) at			
	30 min	60 min	90 min	120 min
1	732	2122	3386	2284
2	847	1243	1829	3126
3	1233	1003	2831	3237
4	1627	940	1894	1870
5	639	1237	1637	1925
6	786	1115	2448	2627
7	999	2083	2826	1044
8	1050	1640	4093	2200
9	1335	1658	3031	1521
10	1329	903	2314	1500
Mean	1057.7	1394.4	2628.9	2133.4
Standard error	±100	±144	±242	±225
Compared with control group	***	***	***	***

Note: The group ($n = 10$) was treated with the combination kava extract (100 mg/kg) corresponding to 50 mg kavalactones and passiflora native extract (250 mg/kg) in 10 ml/kg of the vehicle liquid macrogol 400 administered via stomach tube 2 h before subcutaneous administration of amphetamine sulfate (5 mg/kg).
$^{***}p < 0.001$.

Statistical methods

Effect on amphetamine-induced hypermotility

Comparison of motility values/step impulses (counts) at 30 min, 60 min, 90 min, 120 min in four groups (control, kava extract, passiflora extract, both kava + passiflora extract).

Method used: ANOVA-repeated measures.
Four levels of within-subject factor (four times repeating).
Four levels of between-subject factor (groups).
Null hypothesis—equality of four groups was rejected with high significance $p < 0,0005$.
Scheffe's method was used to compare control vs. kava, control vs. passiflora and control vs. kava + passiflora in particular times (30, 60, 90, and 120 min).
Results in Tables 2–4.

Prolongation of the barbiturate-induced sleeping time

For this experiment, a total of 40 mice with an average weight of 20–25 g were used; they were subdivided into four groups ($n = 10$ animals/group).

Pentobarbital sodium dissolved in physiological saline was subcutaneously administered at a dose of 35 mg/kg body weight. Ninety minutes before the barbiturate injection, one group received only 10 ml/kg of the vehicle liquid (group C) via a stomach tube and the

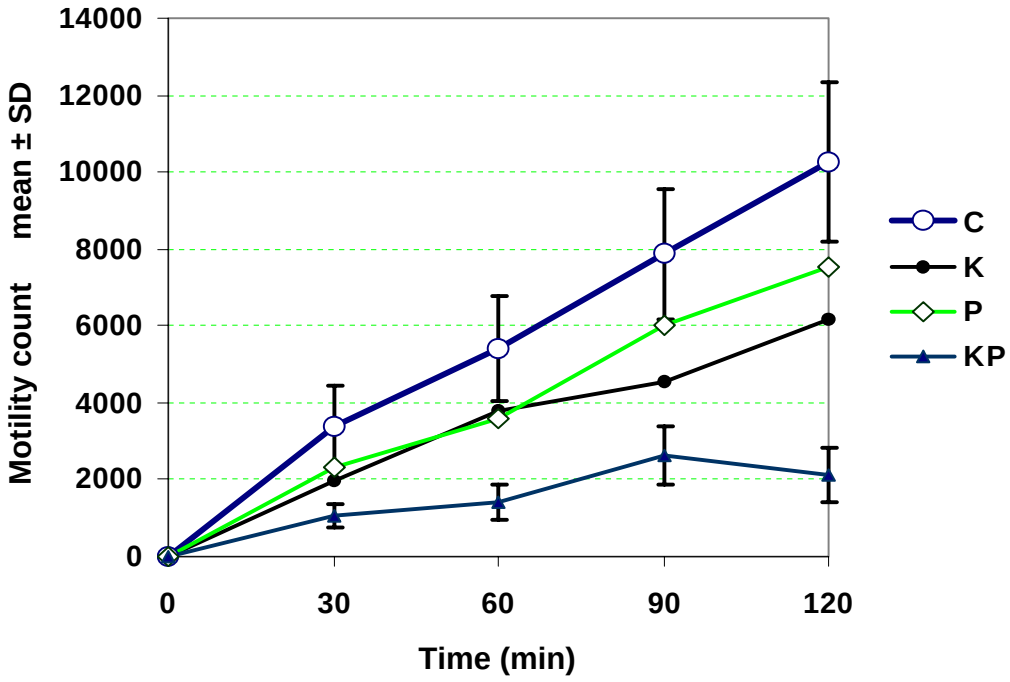


Fig. 1. Reduction of the amphetamine-induced hypermotility produced by extracts from K, P, and KP. C = experimental animals from the control group (5 mg/kg amphetamine sulfate administered subcutaneously), K = pretreatment with kava extract (100 mg/kg) 2 h before amphetamine administration, P = pretreatment with passiflora extract (250 mg/kg) 2 h before amphetamine administration, and KP = pretreatment with the combination kava extract and passiflora extract (100 mg/kg + 250 mg/kg) 2 h before amphetamine administration.

other three groups were administered the same quantity of vehicle liquid containing 100 mg/kg (corresponding to 50 mg kavalactones) of kava extract (group K), 250 mg/kg of passiflora extract (group P), or both extracts at the above-mentioned doses (combination product) (group K + P) in the same way. The sleep duration was measured based on the loss and regaining of the righting reflex. In Table 5, the individual data for all animals are presented, and Fig. 2 shows the means. It can be concluded from the results that the mean sleep duration of the control group (i.e. the animals that received no pharmacological treatment) was 17.8 min, while the sleep duration was prolonged by 45.5% and 53.4% in the groups which were administered a dose of 100 mg/kg kava extract or 250 mg/kg passiflora extract, respectively, via a stomach tube 90 min before the barbiturate injection. When both extracts were administered simultaneously, a prolongation of the sleep duration by 91.6% was achieved. This provides evidence of the hyperadditive synergism of the effects of the two extracts combined.

Sleep duration

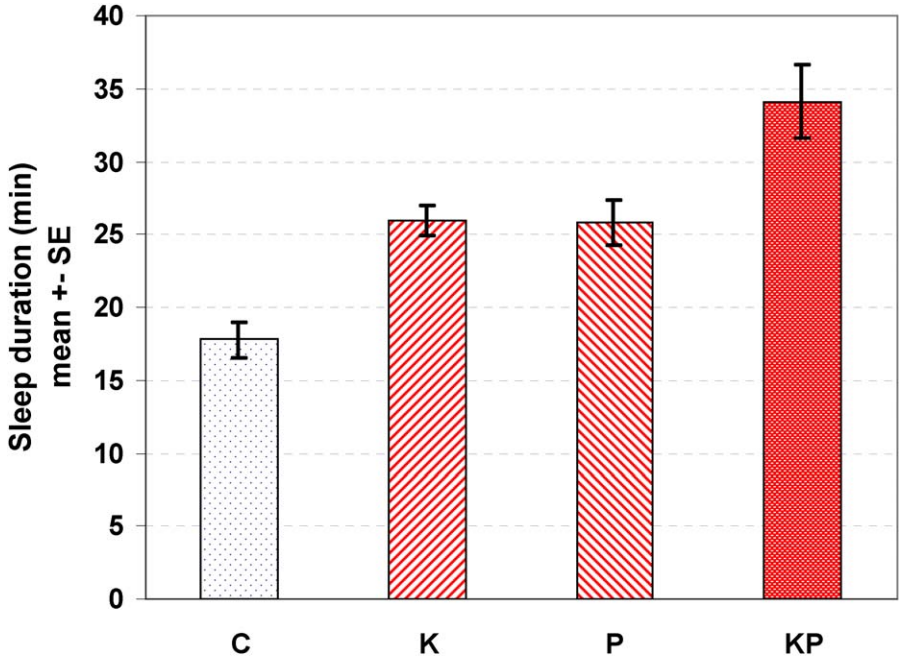
Comparison of four groups:

Method used: one-way ANOVA.
Multiple comparisons—Sheffe.

Table 5. Prolongation of the barbiturate-induced sleep in mice

Experimental animals number	C	K	P	K + P
1	16.7	28.4	23.9	41.6
2	14.2	29.7	31.4	34.8
3	15.4	26.4	28.4	24.1
4	18.3	27.3	27.2	31.9
5	27.2	27.9	25.8	42.5
6	18.1	28.4	13.6	41.2
7	17.9	25.3	28.4	44.7
8	14.3	24.2	27.4	28.2
9	20.4	18.7	24.3	24.3
10	15.3	22.4	27.1	28.1
Mean	17.8	25.9	25.8	34.1
SEM	1.2	1.1	1.5	2.5
Compared with control group		*	*	***

Note: All groups of 10 experimental animals each received 35 mg/kg of subcutaneously injected pentobarbital sodium (Sigma-Tau, Rome). One hour before the injection, the groups were administered via a stomach tube 10 ml/kg of pure vehicle liquid (group C), vehicle liquid containing 100 mg/kg of kava extract (group K), vehicle liquid containing 250 mg/kg of passiflora extract (group P), or vehicle liquid containing 100 mg/kg of kava extract and 250 mg/kg of passiflora extract (group K + P). In the table above, the individual values, means, and standard errors are presented.
$^{*}p < 0.05$ and $^{***}p < 0.001$.



C = Control group

K = Group treated with kava extract

P = Group treated with passiflora extract

KP = Group treated with kava and passiflora extracts

Fig. 2. Prolongation of the barbiturate-induced sleep phase in mice. C = control group, K = group treated with kava extract, P = group treated with passiflora extract, and KP = group treated with kava and passiflora extracts.

The null hypothesis—all groups are equal—is rejected with high significance $p < 0.0005$.

Multiple comparisons (Sheffe):

C vs. K	*	$p = 0.016$
C vs. P	*	$p = 0.018$
C vs. KP	***	$p < 0.0005$
K vs. P	NS	
K vs. KP	*	$p = 0.013$
P vs. KP	*	$p = 0.012$

Discussion

This conclusion is also confirmed in the relevant literature. For example, it should be recalled that it was already demonstrated in 1981 (Della Loggia et al.) that the passiflora extracts suitable for long-term therapy mentioned showed "specific anxiolytic effects" and the combination with other plant extracts led to great potentiation of the anxiolytic (at low doses) and sedative (at high doses) effects. According to Della Loggia et al., the therapeutic response for the combination with various plant extracts was "greater than for the individually administered monoextracts".

Also the publication of Williamson (2001) describes in a review synergistic effects—positive as well as negative—of phyto-extracts in relation to the single substances contained. Examples of hypericum, kava and gingko are interpreted. As a rule a stronger pharmacological effect of the active substance is observed in relation to the single substances.

Our study results with kava and passiflora confirm the results of Della Loggia et al., which provided evidence of a synergism of the extracts from passiflora and other plant extracts like chamomile, valerian, hawthorn, belladonna, etc., whereas our studies showed that there is a synergistic interaction between the extracts from passiflora and kava. These pharmacologically received results in our tests with passiflora and kava extracts have relevance for the clinical application as the synergistic effect of the combination stands also for a lower dosage of the single substances in the therapeutic application.

The quantitatively assessed effect in the pharmacological test shows an approximately 50% higher efficacy of the combination passiflora/kava in comparison with the single substances. In case of the barbiturate sleeping time the effect of the combination is as well approximately 50% higher compared with the single substances.

References

Della Loggia, R., Tuburo, A., Redaelli, C., 1981. Valutazione dell'attività sul S.N.C. del topo di alcuni estratti vegetali e di una loro associazione. Riv. Neurol. 51, 297–310.

Dona, G., Cuzzoni, G., Pecorini, M., 1986. Die Wirkung von Kavain bei älteren Patienten mit neurovegetativen und psychischen Symptomen. Therapiewoche 26, 2836–2844.

Haensel, R., 1959. Einige neue Ergebnisse der Arzneipflanzen-Forschung. Dtsch. Apoth. Ztg. 99, 1037–1042.

Haensel, R., 1964. *Piper methysticum*, der Rauschpfeffer – Geschichte und gegenwärtiger Stand der Wirkstoff-Forschung. Dtsch. Apoth. Ztg. 104 (15), 459–464 and 104(16), 496–501.

Jamieson, D.D., Duffield, P.H., 1990a. Positive interaction of ethanol and kava resin in mice. Clin. Exp. Pharmacol. Physiol. 17, 509–514.

Jamieson, D.D., Duffield, P.H., 1990b. The antinociceptive actions of kava components in mice. Clin. Exp. Pharmacol. Physiol. 17, 495–508.

Jamieson, D.D., Duffield, P.H., Cheng, D., Duffield, A.M., 1989. Comparison of the central nervous system activity of the aqueous and lipid extract of kava. Arch. Int. Pharmacodyn. 301, 66–80.

Kinzler, E., Kromer, I., Lehmann, E., 1991. Wirksamkeit eines Kava-Spezial-Extraktes bei Patienten mit Angst-Spannungs-und Unruhezuständen nicht psychotischer Genese. Arzneim. Forsch. 41 (6), 584–588.

Klohs, M.W., Keller, F., Williams, R.E., Toekes, M.I., Cronheim, G.E., 1959. A chemical and pharmacological investigation of *Piper methysticum* Forster. J. Med. Pharm. Chem. 95, 103.

Kretzschmar, R., Meyer, H.J., 1969. Vergleichende Untersuchungen über die antikonvulsive Wirksamkeit der Pyronverbindungen aus *Piper methysticum* Forster Arch. Int. Pharmacodyn. 177 (2), 261–276.

Meyer, H.J., 1962. Pharmakologie der wirksamen Prinzipien des Kava-Rhizoms (*Piper methysticum* Forster). Arch. Int. Pharmacodyn. 138, 505–536.

Meyer, H.J., 1979. Pharmacology of Kava. In: Efron, D.D., et al. (Eds.), Ethnopharmacological Search for Psychoactive Drugs. Raven Press, New York, pp. 133–140.

Meyer, H.J., Kretzschmar, R., 1969. Untersuchungen über Beziehungen zwischen Molekular-Struktur und pharmakologischer Wirkung C6-arylsubstituierter 4-Methoxy-Alfa-Pyrone vom Typ der Kava-Pyrone. Arzneim. Forsch. 19, 617–623.

Mittmann, V., Schmidt, M., Vrastyakova, J., 2000. Akut anxiolytische Wirksamkeit von *Kava spissum* Extrakt und Benzodiazepinen als Pramedikation bei chirurgischen Eingriffen, Ergebnisse einer randomisierten, referenzkontrollierten Studie. J. Pharmacol. Ther. 9, 99–108.

Pittler, M.H., Ernst, E., 2000. Efficacy of kava extract for treating anxiety: systematic review and meta-analysis. J. Clin. Psychopharmacol. 20, 84–89.

Singh, Y.N., 1983. Effects of kava on neuromuscular transmission and muscle contractility. J. Ethnopharmacol. 7, 267–276.

Smith, R.M., 1983. Kavalactones in *Piper methysticum* from Fiji. Phytochemistry 22 (4), 1055–1056.

Smith, R.M., Thakrar, H., Arourolo, T.A., Shafi, A.A., 1984. High-performance liquid chromatography of kavalactones from *Piper methysticum*. J. Chromatogr. .283, 303–308.

Sopranzi, N., De Feo, G., Mazzanti, G., Tolu, L., 1990. Biological and electroencephalographic parameters in rats in relation to *Passiflora incarnata* L. Clin. Ther. 132, 329–333.

Volz, H.P., Kieser, M., 1997. *Kava kava* extract WS 1490 versus placebo in anxiety disorders. A randomized, placebo-controlled, 25-week outpatient trial. Pharmacopsychiatry 30, 1–5.

Warnecke, G., Gerster, G., Jager, H., 1986. Anxiolyse mit einem Phyto-Tranquilizer in der Frauenheilkunde. Med. Welt 37, 1379–1383.

Williamson, E.M., 2001. Synergy and other interactions in phytomedicines. Phytomedicine 8, 401–409.