Interaction of Various *Piper methysticum* Cultivars with CNS Receptors *in vitro*

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Abstract: Methanolic leaf and root extracts of the Hawaiian kava (*Piper methysticum* Forst.) cultivars, *Mahakea*, *Nene*, *Purple Moi* and *PNG*, were tested on binding affinities to CNS receptors including $GABA_A$ (GABA and benzodiazepine binding site), dopamine D_2, opioid (μ and δ), serotonin (5-HT_6 and 5-HT_7) and histamine (H_1 and H_2). HPLC analysis was carried out in order to determine the amount of the main kavalactones kavain, 7,8-dihydrokavain, methysticin, 7,8-dihydromethysticin, yangonin and 5,6-demethoxyyangonin. The most potent binding inhibition was observed for leaf extracts to $GABA_A$ receptors (GABA binding site) with IC_{50} values of approximately 3 μg/ml, whereas root extracts were less active with IC_{50} values ranging from 5 μg/ml (*Nene*) to 87 μg/ml (*Mahakea*). Since the leaf extracts generally contained lower amounts of the kavalactones than the root extracts, there might exist additional substances responsible for these activities. Leaf extracts also inhibited binding to dopamine D_2, opioid (μ and δ) and histamine (H_1 and H_2) receptors more potently than the corresponding root extracts with IC_{50} values ranging from 1 to 100 μg/ml vs. $\geq$100 μg/l, respectively. Significant differences in the potential of binding inhibition were also observed between cultivars. Binding to serotonin (5-HT_6 and 5-HT_7) and benzodiazepine receptors was only weakly inhibited by both root and leaf extracts of all four cultivars. In conclusion, our investigation indicates that the $GABA_A$, dopamine D_2, opioid (μ and δ) and histamine (H_1 and H_2) receptors might be involved in the pharmacological action of kava extracts. Since the cultivars contained similar amounts of kavalactones, while their pharmacological activities differed markedly, other constituents may play a role in the observed activities. Additionally, leaves generally exhibited more potent binding inhibition than roots, therefore leaf of *P. methysticum* might be an interesting subject for further pharmacological studies.

Key words: *Piper methysticum*, Piperaceae, leaf extracts, root extracts, styryl pyrones, cultivars, CNS recombinant receptors.

Abbreviations:

BHK:	baby hamster kidney
CHO:	Chinese hamster ovary
CNS:	central nervous system
DMY:	5,6-demethoxyyangonin
DHM:	7,8-dihydromethysticin
DHK:	7,8-dihydrokavain
GABA:	γ-aminobutyric acid
^{3}H-LSD:	^{3}*H*-lysergic acid diethylamide
HPLC:	high performance liquid chromatography
IC_{50}:	50 % inhibitory concentration
SFV:	Semliki Forest Virus

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Introduction

Kava (*Piper methysticum*) has been used for ceremonial and medicinal purposes in the South Pacific islands for centuries (1), (2). Its manifold neurotropic effects, such as anxiolytic, muscle-relaxant, anti-convulsive, local anaesthetic, analgesic, and sleep stimulant have been proven in numerous pharmacological and clinical studies (3). The six major kavalactones – kavain, 7,8-dihydrokavain, methysticin, 7,8-dihydromethysticin, yangonin, 5,6-demethoxyyangonin – have been suggested to be the active principles (1), (2).

The characteristic kavalactones have been observed to be distributed unequally in different tissues, such as stems, roots, and leaves, and between cultivars (1), (4). Meanwhile, the pharmacological effects have been found to vary between cultivars by kava drinkers (1). It is therefore likely that the therapeutic potencies of cultivars might correlate to their distinct chemical properties. Up to now, the rootstock (rhizomes and root-laterals) has been the major part of use (1), (2). Roots generally contain high contents of kavalactones. However, several studies showed that DHM and DMK being the most potent kavalactones for analgesic and anti-convulsive effects are abundantly present in leaves (4), (5).

From the pharmacological point of view, a considerable number of studies have been carried out in order to elucidate the mode of actions of *P. methysticum* extracts. Kavain showed evidences of fast and specific action on the voltage-dependent Na^+ channel site (6). This may explain the local anaesthetic, anticonvulsive and anti-ischemic activities (7), (8). However, for the other prominent neurotropic activities (such as anxiolytic, muscle relaxant, and sleep stimulant) the mechanism of action still remains unclear. Receptors of the central nervous system (CNS) may be potential target sites for these pharmacological effects (9). However, the available receptor binding

assays on *P. methysticum* yielded fragmentary and contrary results. On the $GABA_A$ receptor, Jussofie et al. found a specific interaction between kavalactone-enriched extracts and local $GABA_A$ receptors derived from rat brains (e.g., hippocampus, amygdala), whereas no interaction was observed in the cerebellum (10). Meanwhile, Davis et al. did not see an effect between the kavalactones with either benzodiazepine or $GABA_A$ binding sites (11). In contrast, Boonen and Häberlein indicated the specific enhancement of the binding of ^3H-bicuculin to $GABA_A$ receptors by several kavalactones (12). In regard to other CNS receptors, Kretzschmar stated that kavain did not show interactions with the verapamil-binding site of the Ca^{2+} channel, either with adrenergic ($\alpha1$, $\alpha2$, $\beta1$, $\beta2$), serotonin 5-HT_3, cholinergic (M1, M2), GABAergic ($GABA_A$, GABAuptake), glycinergic or with opioid receptors (13).

The aims of our study were firstly to elucidate which CNS receptors may be involved in the actions of *P. methysticum* and secondly whether extracts derived from different origins and tissues may exert different activities in relation to the kavalactone contents. Therefore, we performed radioligand binding studies with leaf and root extracts of different Hawaiian cultivars to selected CNS receptors including benzodiazepine, $GABA_A$, dopamine D_2, serotonin (5-HT_6 and 5-HT_7), opioid (μ and δ) and histamine (H_1 and H_2) receptors. HPLC analysis was carried out to determine the content of the six major kavalactones. Except for the $GABA_A$ receptor complex, this is the first report on the interaction between extracts of *Piper methysticum* derived from different cultivars to dopamine (D_2), opioid (μ and δ), histamine (H_1 and H_2) and serotonin (5-HT_6, 5-HT_7) receptors.

Materials and Methods

Plant material and constituents of Piper methysticum

Four kava Hawaiian cultivars, namely *Mahakea*, *Nene*, *Purple Moi* and *PNG*, were included in the study. The 3-years-old *Mahakea* plant leaves and roots were received from Wainani Farms (Hawaii). Voucher specimens are registered in the herbarium of the Institute of Pharmacy in Basel (# 98-97/1 – # 98-97/4). Plants belonging to *Nene*, *Purple Moi* and *PNG* cultivars were grown from stem cuttings in the green-house (25 ± 3 °C; photoperiod: 16 h/day) and samples were collected from 18-months-old plants. For the preparation of methanolic extracts, leaves and root laterals were collected and dried in a ventilating drier at 35 °C for 48 hours. The dried samples were pulverised and extracted twice with methanol in an ultrasonic bath for 15 minutes. The solvent was then evaporated to dryness and the residues were diluted in methanol to a final concentration of 50 mg/ml. The extracts were stored at – 20 °C until used for HPLC analysis and receptor binding assays. The final concentration of methanol in the receptor binding studies did not exceed 2%, and had only small influences (< 5%) on the binding experiments.

Analysis of Piper methysticum *extracts by RP-HPLC*

HPLC analysis of the six major kavalactones (kavain, DHK, methysticin, DHM, yangonin and DMY) was carried out according to Ross et al. (14) on an analytical Spherisorb – 5 ODS column (5 µm, 250 × 4.6 mm) using a Jasco HPLC system coupled to a diode array detector (Jasco MD-910). The samples were eluted with 22% acetonitrile, 18% methanol and 60% H_3PO_4 (50 mM) at a flow rate of 0.8 ml/minute at 60 °C within 50 minutes. A standardised kava extract (Addipharma GmbH, Hamburg, Germany. EKP 001 96; Ch.B. 602140) was used as the standard reference. The identification of the six major kavalactones in the extracts was based on comparing the retention times and peak areas between the samples and the standard extract. Yangonin and DMY were detected at the wavelength of 360 nm, whereas the other four kavalactones were measured at 240 nm. Each sample was separately extracted at least twice and analysed by HPLC. Analytical determinations are given as mean ± standard deviation.

Receptor preparation

With the exception of benzodiazepine, $GABA_A$, and dopamine D_2 receptors, recombinant transiently expressed receptors using the Semliki Forest Virus (SFV) system were used. Benzodiazepine receptors were prepared from rat cortex. $GABA_A$ receptors were prepared from rat cerebellum and dopamine D_2 receptors from calf striatum.

Expression of CNS receptor with the SFV system: Receptors were expressed using the SFV system as described previously (15). In brief, the receptor cDNAs were subcloned into pSFV1/pSFV2gen by conventional molecular biology techniques. For generation of receptor virus stocks, RNA was transcribed with SP6 RNA polymerase from plasmids carrying the recombinant receptor and pSFV-Helper2 and electroporated into BHK (baby hamster kidney) cells. After 24 h, the recombinant virus particles were collected (16).

CHO (chinese hamster ovary) infected cells were briefly washed with 5 mM Hepes buffer pH 7.4, 2 mM EDTA and lysed in the same buffer for 20 minutes at 4 °C 16 – 48 h post-infection. The lysed cells were transferred to 10 ml centrifuge tubes, spun at 40,000 g for 15 minutes and re-suspended in 50 mM Tris/HCl buffer pH 7.8, 1 mM EGTA and 5 mM $MgCl_2$ using a polytron homogeniser. After centrifugation at 40,000 g for 15 minutes, the pellet was collected and stored at – 80 °C until used in binding assays (this storage condition is also applied for the other receptor preparations).

Preparation of $GABA_A$ receptor: Rat brains (Wistar rats, from the Biological Research Laboratories Ltd, Füllingdorf, Switzerland) were in-ice transported in the laboratory. The cerebellum was removed and homogenised in 50-fold volume of Tris-HCl buffer (50 mM Tris-HCl, pH 7.4, 0.32 M sucrose, 1 mM EDTA, 0.02% NaN_3, ad 0.1 mM PMSF) with a Polytron homogeniser for 30 seconds, and then spun at 4 °C at 500 g for 10 minutes. Supernatant was then diluted by two-fold volume of the buffer and re-centrifuged at 18,000 g, 4 °C for 45 minutes. The supernatant was discarded and the pellet was washed twice with the buffer. In each washing step, the suspension was centrifuged under the same conditions for 30 minutes and the supernatant was removed to collect the membrane pellet.

Preparation of benzodiazepine receptors: Rat cortex was removed from rat brains and then homogenised in 40-fold volume of a Tris-HCl buffer (50 mM Tris-HCl, pH = 7.4, 118 mM NaCl, 4.8 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$) for 30 seconds. The suspension was then diluted further with 120-fold

volume of the buffer and then centrifuged at 4 °C, 18000 g for 10 minutes. Supernatant was decanted to collect the membrane pellets.

Dopamine D_2 receptor preparation: Calf brain was removed in an abattoir in Basel, Switzerland and kept on ice. The striatum was removed and homogenised in 40-fold volume of a Tris-HCl buffer (50 mM Tris HCl, pH = 7.4, 0.1% ascorbic acid, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$) for 60 seconds. The homogenate was then centrifuged at 4 °C, 18,000 g for 10 minutes to collect the membrane pellets.

Determination of protein concentration: Concentration of total proteins in receptor preparations either prepared from animal brain tissues or expressed by the SFV system was determined by the BCA method (17).

Binding assay

Receptor binding was conducted in triplicates from one to 3 experiments in a total volume of 500 µl under the conditions summarised in Table **1**. Binding was terminated by rapid filtration with GF/C filter under reduced pressure and three washes with 5 ml ice-cold Tris/HCl pH 7.4 buffer. Radioactivity on the filter was determined by liquid scintillation analysis (Tri-Carb 2100 TR, Packard Bioscience Company). Specific binding of different concentrations of extracts and compound was plotted and curve fittings were performed using the program SigmaPlot 5.0. The IC_{50} values were deduced from the most fitted curve and represented herein as the mean ± standard error.

Results

HPLC analysis of the six major kavalactones

Figure **1** represents the kavalactone contents present in both leaf and root extracts. The leaves contained mainly DHK and DHM making up for more than 70% of the total kavalactones, whereas the root extracts contained the six major kavalactones in similar quantities, in which each compound accounted for around 10 to 20% of the total kavalactones. Kavain was found in the roots representing from 1.1% (*Nene*) to 1.9% (*Mahakea*) of dry weight. This compound was only found in trace amounts in leaf extracts (less than 0.2%). Methysticin was also found in roots at concentrations comparable as for kavain, i.e., between 1 and 2%, whereas it was not detectable in leaves. Except for *Mahakea* plant, leaves contained more DHM and DHK than roots. The total kavalactones in the leaf extracts of the *Purple Moi*, *PNG* and *Nene* plants were 2.42, 4.35 and 4.96%, respectively. In roots, the total kavalactones in the extracts ranged from 5.09 ± 0.02% (*Purple Moi*) to 9.12 ± 0.07% (*Mahakea*).

Effects of kava extracts from leaves and roots to selected CNS receptors

Table **2** summarises the IC_{50} values obtained from radioligand binding studies of both leaf and root extracts derived from the four cultivars. The most potent binding inhibition was observed for leaf extracts to $GABA_A$ receptors with IC_{50} values of around 3 µg/ml (Fig. **2**). The root extracts inhibited less potently with IC_{50} values ranging from 5 (*Nene*) to 87 µg/ml (*Mahakea*) (Fig. **3**).

Binding to dopamine D_2, opioid (μ and δ) and histamine (H_1 and H_2) receptors was also inhibited more potently by leaves than by roots. We found moderate to strong affinities for the leaf extracts ($1 < IC_{50}$ values < 100 µg/ml), whereas the root extracts exerted only weak activities (IC_{50} values ranged from 100 µg/ml to more than 1000 µg/ml).

Interestingly, binding inhibition differed markedly among the cultivars. On histamine H_1 and H_2 receptors, the highest activity was determined for the *Mahakea* leaf and the lowest activities were found for the *Purple Moi* and *Nene* roots. On opioid receptors, the extracts revealed different inhibitory actions for μ and δ receptors. On μ-opioid receptors, the highest affinity was found for the *Mahakea* leaf (IC_{50} = 19 ± 5 µg/ml), whereas on δ-opioid receptors the most potent activity was observed for the *PNG* leaf (IC_{50} = 71 ± 23 µg/ml).

Binding to benzodiazepine and serotonin (5-HT_6 and 5-HT_7) receptors was only weakly inhibited by kava extracts. On benzodiazepine receptors, no inhibition was found up to 100 µg/ml of both leaf and root extracts. With the exception of the *Mahakea* leaf (IC_{50} = 127 µg/ml), the other extracts inhibited 50% of ^{3}H-LSD binding to 5-HT_7 receptor only at concentrations higher than 300 µg/ml. No binding inhibition was observed for serotonin 5-HT_6 in the presence of the extracts up to 1000 µg/ml.

Table 1 Receptors, radioligands and conditions used for competition binding studies

Receptor	Species (source)	Protein Conc. (µg)	Origin of ligand	Ligand Conc. (nM)
Opioid				
μ	human (SFV expression)	15–20	^{3}H-Naloxone (NEN)	3.6
δ	human (SFV expression)	15–20	^{3}H-Deltorphin (Amersham)	0.28
Serotonin				
5-HT_6	human (SFV expression)	15–20	^{3}H-LSD (NEN)	1.2
5-HT_7	human (SFV expression)	15–20	^{3}H-LSD	1.2
Histamine				
H-1	guinea pig (SFV expression)	15–20	^{3}H-Pyrilamine (Amersham)	1.6
H-2	rat (SFV expression)	15–20	^{3}H-Tiotidine (NEN)	2.5
$GABA_A$	rat (cerebellum)	15–20	^{3}H-Muscimol (NEN)	2.0
Benzodiazepine	rat (cortex)	200	^{3}H-Flumazenil (Amersham)	1.0
Dopamine D_2	calf (striatum)	120	^{3}H-Spiperone (Amersham)	0.2

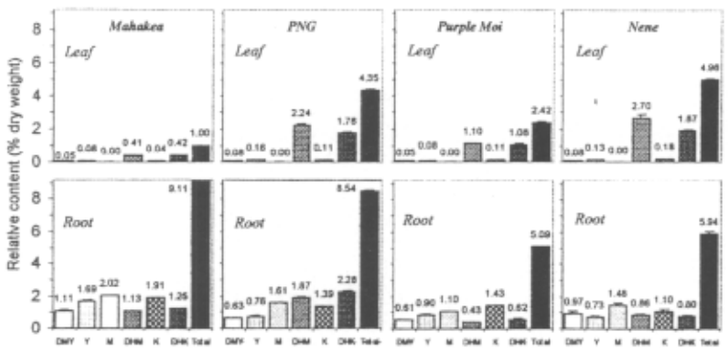

Fig. 1 Quantification of kavalactones of root and leaf extracts from *Mahakea*, *PNG*, *Purple Moi* and *Nene* plants by HPLC analysis. Values represent the means from twice separate extracts and HPLC analysis ± standard deviation. DHK: 7,8-dihydrokavain; DHM: 7,8-dihydromethysticin; DMY: 5,6-demethoxyyangonin; K: kavain; M: methysticin; Y: yangonin.

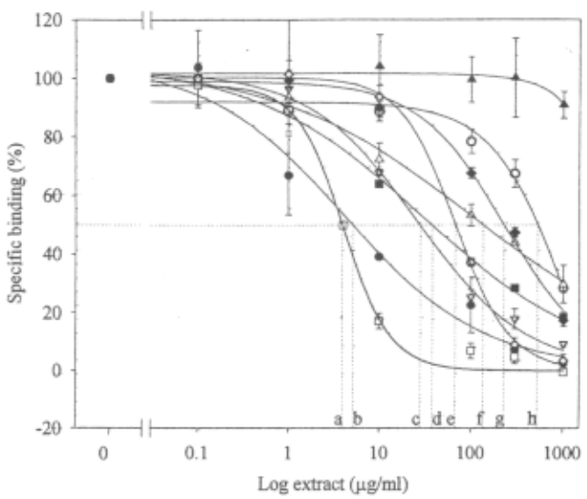

Fig. 2 Competition binding of the *Mahakea* leaf extract on selected CNS receptors. Specific binding and IC_{50} values (in brackets) of the following receptors were determined: ○, benzodiazepine (h); ●, $GABA_A$ (b); ■, histamine H_1 (d); □, histamine H_2 (a); ▲, serotonin 5-HT_6; △, serotonin 5-HT_7 (f); ▽, μ-opioid (c); ◆, δ-opioid (g); and ◇, dopamine D_2 (e).

Discussion

Extracts derived from leaves and roots of four kava cultivars were analysed by HPLC in order to determine the contents of the six major kavalactones. Both quantitative and qualitative differences were observed between leaves and roots. This result was generally in agreement with some previous studies (4). In Fijian kava cultivars, Smith and co-workers found kavain and DMY as the major kavalactones in roots, while DHM and DHK were prominent in leaves (17). In the present study with Hawaiian cultivars, we observed a similar distribution of these four kavalactones. In roots, kavain and DMY accounted for around 20% and 10% of the total kavalactones, respectively, while in leaves only traces of these two compounds were found. In leaves, DHM and DHK made up for more than 70% of the total kavalactones. However, in regard to yangonin, Smith and co-authors failed to find a distinct distribution of this compound between roots and leaves. Our HPLC analysis indicated that yangonin was present mainly in the roots (accounting for 10–20% of the total kavalactones) and appeared only in minor amounts in leaves (less than 6%). Methysticin was only detected in the root extracts.

The significantly lower concentrations of kavalactones, especially DHM and DHK, in *Mahakea* leaf extract compared to the *Nene*, *PNG* and *Purple Moi* plants might be due to the age and growing conditions of the donor plant. The sample of the *Mahakea* cultivar was collected from a 3 years old plant grown in Hawaii, whereas the *Nene*, *PNG* and *Purple Moi* samples were obtained from 18 months old plants grown in a greenhouse in Witterswil, Switzerland.

Kava cultivars differing in the content of kavalactones were used to study the binding potential to selected CNS receptors including benzodiazepine, $GABA_A$, opioid, serotonin, dopa-

Table 2 Effects of *Piper methysticum* Forst. extracts on binding of specific radioligands to selected CNS receptors

IC_{50}-values	Benzodiazepine	Dopamine	$GABA_A$	Opioid		Histamine		Serotonin	
(µg/ml)*		D_2		μ	δ	H_1	H_2	5-HT_6	5-HT_7
Mahakea root extract	860 ± 60	850 ± 22	87 ± 17	592 ± 34	185 ± 61	850 ± 37	806 ± 53	>1000	492 ± 13
Mahakea leaf extract	510 ± 35	68 ± 4	4 ± 1	19 ± 5	240 ± 30	36 ± 7	4 ± 1	>1000	127 ± 32
PNG root extract	556 ± 88	101 ± 32	83 ± 15	256 ± 69	168 ± 16	603 ± 64	630 ± 59	>1000	472 ± 13
PNG leaf extract	710 ± 36	36 ± 18	1 ± 0.5	74 ± 11	161 ± 39	206 ± 33	215 ± 23	>1000	338 ± 17
Purple Moi root extract	900 ± 97	374 ± 61	23 ± 4	980 ± 79	340 ± 32	>1000	>1000	>1000	700 ± 34
Purple Moi leaf extract	860 ± 89	43 ± 16	6 ± 2	263 ± 42	71 ± 23	404 ± 91	240 ± 17	>1000	395 ± 18
Nene root extract	830 ± 89	380 ± 82	5 ± 2	424 ± 16	390 ± 33	>1000	>1000	>1000	905 ± 65
Nene leaf extract	490 ± 68	37 ± 8	3 ± 1	228 ± 22	134 ± 28	337 ± 23	374 ± 80	>1000	326 ± 38

* Values represent means of triplicates from one to three experiments ± Standard Error of the Mean.

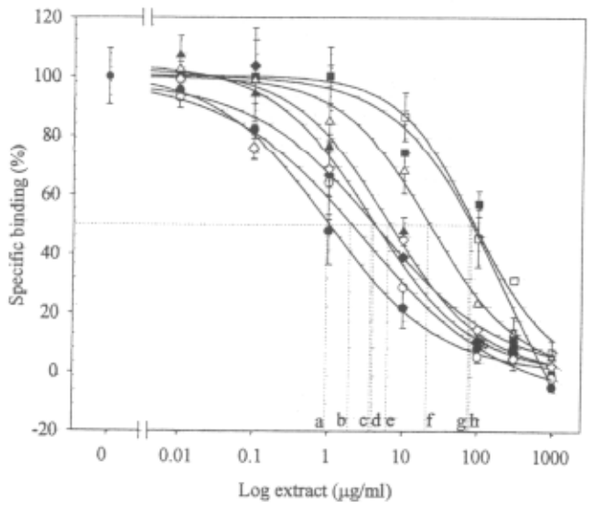

Fig. 3 Inhibition of ^3H-Muscimol binding to $GABA_A$ receptors by *Piper methysticum* extracts. Specific binding and IC_{50} values (in brackets) of the following extracts were determined: ◆, *Mahakea* leaf (c); □, *Mahakea* root (h); ●, *PNG* leaf (a); ■, *PNG* root (g); ○, *Nene* leaf (b); ◇, *Nene* root (d); ▲, *Purple Moi* leaf (e); and △, *Purple Moi* root (f).

mine and histamine receptors. These CNS receptors have been known to play important roles in mental physiology (9), (18), and may represent the target sites for the constituents of various psychoactive medicinal plants (15), (19). As the most prominent pharmacological activities of *P. methysticum* extracts are known as anxiolytic, anticonvulsive, anaesthetic and sleep stimulant, these receptors may be considered as potential target sites.

The benzodiazepine binding site of the $GABA_A$ receptors was only marginally inhibited by both root and leaf extracts of all cultivars. This result was in agreement with previous studies (10), (11). Binding to 5-HT_6 and 5-HT_7 receptors was also only hardly inhibited. The most potent binding inhibition was observed for the GABA-binding site of the $GABA_A$ receptor. This is in contrast to previous studies performed by Jussofie et al. (10) and Boonen et al. (12), who failed to detect a binding inhibition of kava extracts and kavalactones at this binding site. In addition, no enhancement of the specific binding as previously described by Jussofie et al. (10) was observed in the presence of kava extracts up to 1000 µg/ml. One reason for this discrepancy is that they used a kavapyrone enriched extract with 58% kavapyrones, whereas our extracts contained 6 to 50 times less kavapyrones. As a consequence, the kavapyrone concentration at the highest extract concentration tested here (1000 µg/ml) would not be sufficient to induce the effects observed by Jussofie and co-workers using the enriched extract. Nevertheless, kava extracts, especially those from leaves, apparently inhibited the binding of ^3H-muscimol to $GABA_A$ with moderate to strong potencies (IC_{50} values ranged between 1 and 100 µg/ml). However, as we did not see an obvious correlation between the binding inhibition and the kavalactone contents, additional constituents might be also involved in pharmacological ativity. It especially remains to be studied to what extent γ-aminobutyric acid obviously present in leaf extracts is responsible for the observed binding inhibition.

The weak binding inhibition observed for root extracts to dopamine D_2, opioid (µ and δ) and histamine (H_1 and H_2) receptors (IC_{50} > 100 µg/ml) indicated that these receptors may only play minor roles in pharmacological effects. Again, there was no correlation between the kavalactone contents and the binding affinity of the extracts. Since leaf extracts obviously exert higher activities than the corresponding root extracts, there may also exist some additional active compounds in the leaves. A significant variation in the receptor-binding affinities was observed for different cultivars, particularly on opioid and histamine receptors. This observation is in agreement with the distinct psychotropic effects of the cultivars that have been described by kava drinkers (1).

In the present study, the kavalactone content we found in leaves are substantial, especially for *PNG* and *Nene* plants. The total kavalactones of the *PNG* and *Nene* leaf extracts accounted for 4.35 and 4.96% dry weight respectively compared with 8.54 and 5.94% in the root extracts. Although there have been former reports on the use of *P. methysticum* leaves instead of rootstocks as alternative materials for medicinal purposes or for isolating of the kavalactones (especially for DMK and DHM) (20), no significant effort has been made so far concerning a pharmacological study of leaf extracts. The high biological activity *in vitro* of the leaf extracts suggests it as possible new plant material for medicinal purposes.

In summary, our data from both *in vitro* receptor binding assays and HPLC analysis of the kava root and leaf extracts indicate that $GABA_A$, dopamine D_2, opioid (µ and δ) and histamine (H_1 and H_2) might be target sites for certain constituents of kava leaf extracts, though may not associate with the six major kavalactones. Therefore, leaf of *Piper methysticum* may be an interesting object for further pharmacological studies. $GABA_A$ receptors may play a role in psychopharmacological activities of the total root extracts. The pharmacological effects of kava root extracts could not be explained by their interaction with benzodiazepine receptors as well as with dopamine D_2, opioid (µ and δ), histamine (H_1 and H_2) and serotonin (5-HT_6 and 5-HT_7) receptors. The distinct *in vitro* affinities of the extracts derived from different cultivars on histamine and opioid receptors may be another topic for further pharmacological studies of *Piper methysticum*.

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