In Vitro Binding Receptors Study by *Valeriana adscendens*, *Iresine herbstii* and *Brugmansia arborea* Extracts

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Abstract: In this work we examined the affinity and the selectivity of *V. adscendens*, *Iresine herbstii* Hook. (Amaranthaceae) and *Brugmansia arborea* (L.) Lagerheim (Solanaceae) towards 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} serotononergic, D_1 and D_2 dopaminergic, α_1 and α_2 adrenergic receptors by radioligand assays.

The results show weak affinity to 5-HT_{1A} only for the aqueous extract of *V. adscendens* and no affinity for 5-HT_{2A}, 5-HT_{2C} serotononergic receptors, α_1 and α_2 adrenergic receptors and D_2 receptors. As it regards D_1 receptors, only for the methanolic extract the IC_{50} value was determinable.

The data obtained for *I. herbstii* extracts have shown a low affinity for the 5-HT_{1A} receptor (22.44%) and no affinity for 5-HT_{2A} receptor. Otherwise these extracts showed affinity for 5-HT_{2C} receptor but only for the methanolic extract the IC_{50} value (inhibitory concentration 50%) was: 34.8 μg/ml.

The *B. arborea* aqueous extract displayed weak affinity for all receptors tested, the highest levels of inhibition at the maximum concentration tested (125 μg/ml) were 38% for the 5-HT_{1A} , 16% for the 5-HT_{2A} and 39% for the 5-HT_{2C} receptor.

The results of our experiments indicate that *V. adscendens*, *Iresine herbstii* and *Brugmansia arborea* were able to interact with the central 5-HT receptors thus confirming their ritual use.

Key Words: *Valeriana adscendens, Brugmansia arborea*, *Iresine herbstii*, binding, central nervous system, psychotropic agents, ethnopharmacology, receptors.

INTRODUCTION

Man has always used medicinal plants to cure his diseases, in fact a lot of people has accumulated deep knowledge of natural drugs, how to recognise, gather and prepare them. Today in some countries diagnosing and treating illnesses with plants represents the only medical practice.

In the traditional medicine of the Northern Peruvian Andes, psychoactive plants play a pivotal role, because they are seen as intermediaries between human and supernatural and the Andean shamans associate the action of these plants with a supernatural depersonalization or dissociation of body and spirit [1].

Previous studies have been carried out about the neuropharmacological effects induced by aqueous and methanolic extracts of *V. adscendens* on the central nervous system (CNS) [2,3]. The results obtained in the mouse indicated that *V. adscendens* induced a significant reduction of locomotor activity, motor coordination, stereotyped behaviour and an increase of pentobarbital-induced sleep was also reported [2]. Furthermore the methanolic extracts are able to inhibit GABA uptake and to decrease intracellular neurotransmitters content [3].

Man has always sought and found remedies for his illnesses in nature, usually in the vegetable kingdom. Every people has accumulated deep knowledge of natural drugs, how to recognise, gather and prepare them. One can still find this situation in communities that are culturally and geographically isolated, where it is difficult or impossible to find medical doctors who practice "official" medicine, and in those countries still economically emerging, where there are very few medical and social facilities due to the limitation of economic factors. In these areas, the treatment of diseases is based essentially, and sometimes exclusively, on medicines that have a natural origin; among these, vegetal drugs constitute the majority. The recognition and the use of medicinal plants are an untouchable heritage of most preliterate cultures. Therefore, in the past centuries, and presently in some cultures, the practice of using plants for medicine has assumed a "sacred" characteristic: it is secretly kept and conveyed by priests and other religious figures, who are very knowledgeable about herbs and who combine their botanical, phytotherapeutical and toxicological knowledge with religious elements and rituals based on magic, superstition and ancestral beliefs [4].

In rural communities of the Northern Peruvian Andes, the herbalist or "curandero", the individual who is knowledgeable about all healing and harmful plants, assumes a primary role. He is considered a priest, an intermediate figure between our world and the world of the spiritual forces. At the same time he is also a therapist and an expert on all healing plants, psychotropic plants (used to awaken religious spirits or to gain an altered state of mind) and harmful plants [5]. There is daily contact between the priest and the plant world,

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from which he receives most of his remedies; hence, his power over the rest of the community.

The shamanic culture in the Andean area of Peru is very old. Its origins certainly date back to pre-Columbian eras and, since then, have been enriched by continuous intercultural and interethnic relationships. In relatively recent times, it has also been influenced by Spanish and other European contacts as well as academic medicine. Today, this culture is alive and often represents the only medical practice that a population can refer to daily. This makes the "curandero" the only medical doctor that the Andean people can go to treat an illness.

In the traditional medicine of the Northern Peruvian Andes, psychoactive plants play a pivotal role, because they are seen as intermediaries between human and supernatural [4] and the Andean shamans associate the action of these plants with a supernatural depersonalisation or dissociation of body and spirit. This conception has been reported for other cultures [6].

Independent of the reasons for their consumption and application, and of the psychopharmacological differences between them, psychoactive plants are used in a ritual context intimately related to religion. It is probable that the fundamental property of these plants, the alteration of the habitual state of consciousness, has led them to be considered special, divine or sacred, and appropriate for use in religious and curative ceremonies. The objectives of the rituals centered around psychodysleptics are several. Sacramental practices imply the intention of establishing contact with the sacred to produce ecstasy. Divinatory rituals are carried out quickly to enable the shaman to detect the origin and treatment of a disorder or the whereabouts of a missing object or person. Finally, in the context of magic and sorcery, there are rituals for the purpose of inflicting harms (daño), purification and exorcism which involves the internal or external use of plants [4,7].

The most important psychoactive plants in traditional practices of the Northern Peruvian Andes are the cacti *Tricocereus pachanoi* Britt. *et* Rose and *T. peruvianus* Britt. *et* Rose ("San Pedro"). These cacti contains the alkaloid mescaline [8,9] and other phenethylamine derivatives [10,11] with well known hallucinogenic properties. Very few reports are available in ethnobotanical literature on other hallucinogenic plants, usually used in association with "San Pedro".

The aerial parts of Valeriana adscendens Trel. (Valerianaceae) are used in magical-therapeutic rituals in traditional practices of the Northern Peruvian Andes to enhance hallucinogenic power of "San Pedro" decoction [1].

Iresine herbstii Hook. (Amaranthaceae), "cimora señorita", is used in black magic [4]. Also, it is employed in association with other species, such as *Trichocereus pachanoi* ("San Pedro") for divination [4,12,13], to diagnose diseases, and to take possession of another identity [14]. Its leaves are applied externally as a skin depurative, whereas the aerial part decoction is claimed to be an antipyretic [4].

Also, the South American species of the genus *Brugmansia* are known with the vernacular names "floripondio", "campanchu", "yerba del diablo" and are used in the traditional therapeutic and magical practices of the folkloric Peruvian medicine to reach altered states of consciousness [4]. *Brugmansia* species have been reported to be used also in other ethnic groups during ritual practices for magical and curative purposes in several zones of the amazonic forest [15-24]. In Andean zones, the ritual use of *B. sanguinea* was reported by Schultes [19,25] in the Sibundoy Valley, Colombia.

B. arborea is known in the Northern Peruvian Andes as "misha oso", "misha toro" and "misha galga" and, in addition to ritual use, the decoction of its leaves and flowers, is used externally as an analgesic, antirheumatic, vulnerary, decongestant and antispasmodic and to cure pimples and other skin eruptions [4].

Given the above evidence, the aim of the present study is to evaluate if the central effects of *Valeriana Adscendens*, *Iresine herbstii* and *Brugmansia arborea* could be associated with interaction with SNC serotonin (5-HT) receptors.

The serotoninergic system is known to modulate mood, emotion, sleep and appetite and thus is implicated in the control of numerous behavioural and physiological functions [26]. There is now converging evidence from biochemical, electrophysiological, and behavioral studies that the two major classes of psychedelic hallucinogens, the indoleamines (e.g., LSD) and the phenethylamines (e.g., mescaline) have a common site of action as partial agonists at 5-HT_{2A} and other 5-HT_2 receptors in the central nervous system [27]. In addition we have studied the interaction with noradrenergic and dopaminergic receptors, both of these transmitter systems regulate a variety of physiological, behavioural and endocrine functions.

In this paper two *V. adscendens* extracts (methanolic and aqueous) were tested for *in vitro* affinity on 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} serotoninergic, D_1 and D_2 dopaminergic, α_1 and α_2 adrenergic receptors by radioligand binding assays. Two *I. herbstii* extracts (methanolic and aqueous) and one *B. arborea* aqueous extract were tested for *in vitro* affinity on 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} receptors by radioligand binding assays.

MATERIALS AND METHODS

Plant Materials and Extraction

V. adscendens was collected in September 1990 in the Sierra of Huancabamba, Piura Department, Peru, and identified by Dr. V. De Feo and are deposited at the herbarium of the School of Pharmacy, University of Salerno.

The air-dried aerial part of *V. adscendens* (500 g) were extracted for 10 days sequentially with methanol and water, at room temperature, giving 13.57 and 8.19 g of residues, respectively. The methanol extract was resuspended in methanol (ME), the aqueous dried material was resuspended in distilled water (0.5 mg/ml), obtaining the aqueous extract (AE).

AE and ME were dissolved in water or dimethylsulphoxide (DMSO) 5% respectively at initial concentrations of 1.25 mg/ml and subsequently diluted (0.625, 0.416, 0.313, 0.156,

0.078 mg/ml). These dilutions were tested in duplicate to final tube concentrations between 7.8 and 125 μg/ml.

I. herbstii was collected in September 2001 in the Sierra of Huancabamba, Piura Department, Peru, and identified by Dr. V. De Feo. Voucher specimens of the plants are deposited at the herbarium of the School of Pharmacy, University of Salerno, labelled as DFP 90/88. Leaves and flowers of *B. arborea* were collected in September 1991 near Huancabamba City, Piura Department, Northern Peru. The plant was identified by Dr. V. De Feo. A voucher specimen of the plant (DFP 91/98) is deposited at the herbarium of the School of Pharmacy, University of Salerno (Italy).

The air-dried aerial part of *I. herbstii* (500 g) were extracted sequentially with methanol and water, at room temperature, giving 6.52 g and 7.03 g of residues, respectively.

Air-dried and powdered leaves and flowers of *B. arborea* (500 g) were extracted at room temperature with methanol to give 13.04 g of residue.

The extracts were dissolved in water or dimethylsulphoxide (DMSO) 5% respectively at initial concentrations of 1.25 mg/ml and subsequently diluted. These dilutions were tested in triplicate to final tube concentrations between 7.8 and 125 μg/ml.

Receptor Binding Experiments

5-HT_{1A} serotoninergic assay [28]. Cerebral cortex from male Wistar rats (Harlaan Italy srl) (150-200 g) was homogenized in 20 volumes of ice-cold Tris-HCl buffer (50 mM, pH 7.7 at 22°C) with a Brinkmann Polytron (setting 5 for 15 s) and the homogenate was centrifuged at 50,000 *g* for 10 min at 0°C. The resulting pellet is then resuspended in the same buffer, incubated for 10 min at 37°C, and centrifuged at 50,000 *g* for 10 min at 0°C. The final pellet is resuspended in 80 volumes of the Tris-HCl buffer containing 10 μM pargyline, 4 mM $CaCl_2$ and 0.1% ascorbate. To each assay tube were added the following: 100 μl of the sample extract dilution, 100 μl of [^{3}H]8-hydroxy-2-(di-n.propylamino)tetralin ([^{3}H]-8-OH-DPAT) (Amersham Bioscences Europe GmbH) as radioligand in buffer (containing Tris-HCl, $CaCl_2$, pargyline and ascorbate), to achieve a final concentration of 1 nM and 800 μl of resuspended membranes. The mixture is incubated for 30 min at 37°C. Specific [^{3}H]8-OH-DPAT binding is defined as the difference between binding in the absence or presence of 10^{-5} M serotonin.

5-HT_{2A} and 5-HT_{2C} serotoninergic assay [29]. Frontal cortical regions of male Wistar rats (150-200 g) were dissected on ice and homogenized (1:10 w/v) in ice-cold buffer solution (50 mM Tris-HCl, 0.5 mM EDTA and 10 mM $MgCl_2$, pH 7.4 at 22°C) with a Brinkmann Polytron (setting 5 for 15 s) and the homogenate centrifuged at 3,000 *g* for 15 min at 0°C. The pellet was resuspended in the same buffer (1:30 w/v), incubated at 37°C for 15 min and then centrifuged twice more at 3,000 *g* for 10 min at 0°C. The final pellet is resuspended in the same buffer that also contains 10^{-5} M pargyline and 0.1% ascorbate. Assays were performed in triplicate in 1 mL volume containing 5 mg wet weight of tissue and 1.4 nM [^{3}H] ketanserin (Perkin Elmer Life Sciences s.r.l.) for 5-HT_{2A} receptor and 10 mg wet weight of tissue and 1 nM [^{3}H] mesulergine (Amersham Bioscences) for 5-HT_{2C} receptor. 10^{-4} M cinanserin was used to define nonspecific binding in the 5-HT_{2A} assay and 10^{-4} M mianserin for the 5-HT_{2C} assay, in the presence of 10^{-5} M spiperone, added to block binding to 5-HT_{2A} receptors. In both experiments, incubation was carried on for 15 min at 37°C.

D_1 e D_2 dopaminergic assay [30,31]. Corpora striata were homogenized in 30 vol (w/v) ice-cold 50mM Tris-HCl buffer (pH 7.7 at 25°C) using a Polytron PT10 (setting 5 for 20 s). Homogenates were centrifuged twice for 10 min at 50,000 *g* with resuspension of pellet in fresh buffer. The final pellet was resuspended in 50 mM ice-cold Tris-HCl buffer containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% ascorbic acid and 10 μM pargyline (pH 7.1 at 37°C). Each assay tube contained 50 μl [^{3}H]SCH-23390 to achieve a final concentration of 1.4 nM for D_1 receptor or 50 μl [^{3}H]spiroperidol (Amersham Bioscences) to achieve a final concentration of 1.2 nM for D_2 receptor, 50 μl of the sample extract dilution and 900 μl resuspended membranes (3 mg fresh tissue). The tubes were incubated for 15 min at 37°C. Specific [^{3}H]SCH-23390 (Amersham Bioscences) binding was defined as the difference between binding in the absence or in the presence of 0.1 μM SCH-23390 in the D_1 assay and 10 μM (+)-butaclamol in the D_2 assay.

α_1 adrenergic assay [32]. Brain cortex was homogenized in 30 vol (w/v) ice-cold 50 mM Tris-HCl buffer (pH 7.2 at 25°C) using a Polytron PT10 (setting 5 for 20 s). Homogenates were centrifuged twice for 10 min at 50,000 *g* with resuspension of pellet in fresh buffer. The final pellet was resuspended in 50 mM ice-cold Tris-HCl buffer (pH 7.4 at 37°C). Each assay tube contained 50 μl [^{3}H]prazosin (Amersham Bioscences) to achieve a final concentration of 1.2 nM, 50 μl of the sample extract dilution and 900 μl resuspended membranes (10 mg fresh tissue). The tubes were incubated for 30 min at 25°C. Specific [^{3}H]prazosin binding was defined as the difference between binding in the absence or in the presence of 10 μM phentolamine.

α_2 adrenergic assay [33]. Brain cortex was homogenized in 30 vol (w/v) ice-cold 5 mM Tris-HCl, 5 mM EDTA buffer (pH 7.3 at 25°C) using a Polytron PT10 (setting 5 for 20 s). Homogenates were centrifuged three times for 10 min at 50,000 *g* with resuspension of pellet in fresh buffer. The final pellet was resuspended in 50 mM ice-cold Tris-HCl, 0.5 mM EDTA buffer (pH 7.5 at 25°C). Each assay tube contained 50 μL [^{3}H]yohimbine (Amersham Bioscences) to achieve a final concentration of 1.2 nM, 50 μl of the sample extract dilution and 900 μl resuspended membranes (10 mg fresh tissue). The tube were incubated for 30 min at 25°C. Specific [^{3}H]yohimbine binding was defined as the difference between binding in the absence or in the presence of 10 μM phentolamine.

The incubations were stopped by vacuum filtration using filters Whatman GF/B (Brandel Biomedical Research and Laboratories Inc.). The filters were washed twice with ice-cold Tris-HCl buffer, added to 5 ml of liquid scintillation (Packard BioScience s.r.l.), and the radioactivity bound to the filters is measured by liquid scintillation spectrometry (Packard TRI-CARB 2000CA-Packard BioScience s.r.l.).

We had effected a control test to verify the solvent effect (DMSO 5%) on binding assay. There were not important variations.

Data Analysis

The results are expressed as means ± standard deviation of more than three experiments run in duplicate. For the collection and analysis of data we used SPSS (Statistical Package for the Social Sciences, version 6.0.1).

The IC_{50} value was calculated using commercially available software (Calcusyn; Biosoft, Ferguson, MO) to obtained the concentration of extract that caused 50% inhibition of labelled ligand binding (IC_{50}).

RESULTS AND DISCUSSION

Valeriana Adscendens

The affinity of *Valeriana adscendena* extracts for receptors is definite as inhibition percentage of radioligand/receptor binding and measured as the radioactivity of remaining complex radioligand/receptor.

The affinity of *V. adscendens* extracts about all receptors studied at the maximum concentration tested (125 µg/ml) were reported on Table **1**.

Table 1. The Affinity (Inhibition %) of *Valeriana adscendens* Extracts at the Maximum Concentration Tested (125 µg/ml)

Receptor	Inhibition %	
	***V. adscendens* aqueous extract**	***V. adscendens* methanolic extract**
5-HT_{1A}	34.88	n.a.
5-HT_{2A}	n.a.	n.a.
5-HT_{2C}	n.a.	n.a.
α_1	n.a.	n.a.
α_2	n.a.	n.a.
D_1	39.48	73.73
D_2	n.a.	n.a.

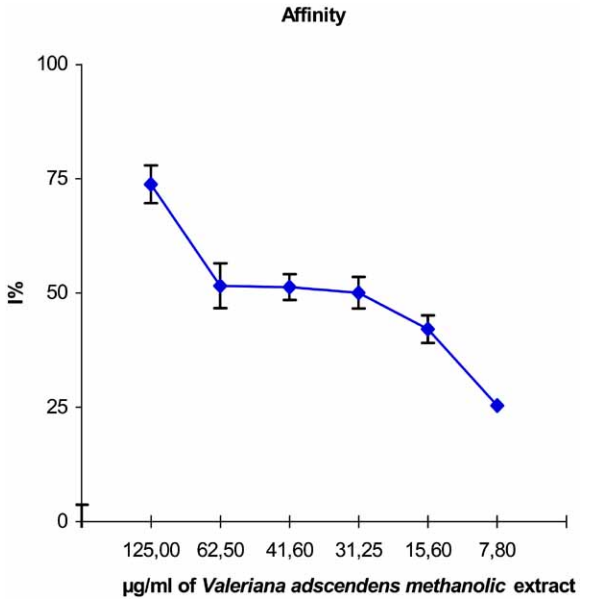
n.a. = the extract shows no affinity for tested receptors.

The results show that only the aqueous extract has affinity for the 5-HT_{1A} receptor, varying according to extract concentration. The highest level of inhibition percentage (Inhibition %) is about 35% at the maximum concentration tested (125 µg/ml).

Both extracts showed no affinity for 5-HT_{2A} and 5-HT_{2C} receptors.

The data obtained for noradrenergic and dopaminergic receptors have shown that these extracts have no affinity for α_1 and α_2 adrenergic receptors and D_2 receptors. Otherwise these extracts showed affinity for D_1 receptors but only for the methanolic extract the IC_{50} value was determinable.

The IC_{50} value (30.14 µg/ml) of *Valeriana adscendens* methanolic extract and the relative curve are reported in (Fig. **1**).



Concentrations (µg / ml)	I % ($\overline{M}$ ± s.d.)	IC_{50} (µg/ml)	C.V.%
125	73.73 ± 5,65	30.14 ± 2.99	7.93
62.5	52.53 ± 4,15		
41.6	51.46 ± 4.89		
31.25	51.03 ± 2,82		
15.6	42.05 ± 3,42		
7.8	25.36 ± 5,01		

Fig. (1). IC_{50} value of *Valeriana adscendens* methanolic extract.

A previous study has demonstrated that methanolic extract (ME) of *Valeriana adscendends* acts through the interaction with the GABA-A receptors [3]; in this paper, we also have shown that this extract binds strongly to D_1 receptors while the aqueous extract (AE) is able to bind weakly to 5-HT_{1A} and D_1 receptors.

The variability of affinity to different CNS receptors of these extracts, seems to be bond to their different extraction techniques.

Actually there are no data about chemical compositions of this plant, it is unknown which are the active components, but surely numerous of them may contribute synergistically.

The affinity for GABA-A receptors agrees with previous data available in literature on other Valerian species [34], the contribute of this study is the affinity for dopaminergic receptors (D_1) actually not find for Valerianaceae family.

Further researches are in progress in order to define the active substance or the components responsible of binding to receptors (valepotriates, lignans).

These results could open the way to other studies on a possible use of *V. adscendens* in common disease the involve D_1 receptors (as Parkinson, psychosis, emesis).

Table 2. The Affinity of *Iresine herbstii* and *Brugmansia arborea* Extracts for Receptors at the Maximum Concentration Tested (125 µg/ml) About All Receptors Tested were is Defined as Inhibition Percentage of Radioligand/Receptor Binding and Measured as the Radioactivity of Remaining Complex Radioligand/Receptor

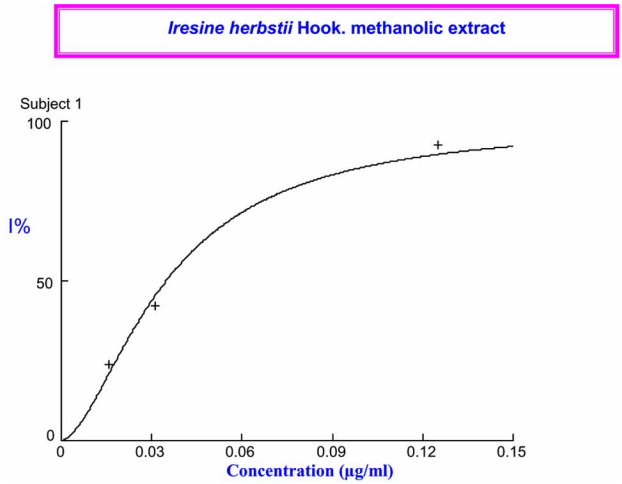
	$5HT_{1A}$	$5HT_{2A}$	$5HT_{2C}$	D_1	D_2	α_1	α_2
Iresine herbstii *Methanolic extract*	M.E. 22,44%	No affinity	IC_{50} 60,27±5,59 µg/ml	IC_{50} 1,39±1,70 µg/ml	No affinity	M.E. 11,76%	Non attivo
Iresine herbstii *Aqueous extract*	M.E. 13,51%	No affinity	M.E. 22,13%	M.E. 48,32%	IC_{50} 2.99±0.02 µg/ml	No affinity	M.E. 25,73%
Brugmansia arborea **Aqueous extract**	M.E. 38,57%	M.E. 16%	M.E. 39,28%	IC_{50} 1.23±0.35 µg/ml	IC_{50} 0.16±0.07 µg/ml	M.E. 21,07%	M.E. 23,83%

The IC_{50} value (34.8 µg/ml) of *Iresine herbstii* methanolic extract and the relative curve are reported in figure 2.

Iresine herbstii and *Brugmansia arborea*

We have evaluated the affinity for the serotoninergic receptors ($5HT_{1A}$, $5HT_{2A}$ and $5HT_{2C}$) of two *Iresine herbstii* extracts and one *Brugmansia arborea* extract.

The extracts affinity for receptors is definite as inhibition percentage of radioligand/receptor binding and measured as the radioactivity of remaining complex radioligand/receptor.



[ ] finale (µg / ml)	I% ($\overline{M}$ ± d.s.)	IC_{50} (µg / ml)	C.V.%
125	92.43 ± 1,65	34.78 ± 3.71	10.61
62.5	42.26 ± 4,95		
31.25	32.41 ± 1,82		
15.6	23.77 ± 3,42		

Fig. (2). IC_{50} curve and value of *Iresine herbstii* extract.

The data obtained for *Iresine herbstii* extracts have shown a very low affinity for the $5\text{-}HT_{1A}$ receptor (Table **2**) and no affinity for $5\text{-}HT_{2A}$ receptor. Otherwise these extracts showed affinity for $5\text{-}HT_{2C}$ receptor but only for the methanolic extract the IC_{50} value (inhibitory concentration 50%) was determinable.

The *Brugmansia arborea* aqueous extract displayed weak affinity for all receptors tested, the highest levels of inhibition at the maximum concentration tested (125 µg/ml) were 38% for the $5\text{-}HT_{1A}$ (Table **2**), 16% for the $5\text{-}HT_{2A}$ and 39% for the $5\text{-}HT_{2C}$ receptor.

The results of our experiments indicate that both *Iresine herbstii* and *Brugmansia arborea* were able to interact with the central 5-HT receptors thus confirming their ritual use their significant CNS effect similar to that observed with some psychotropic agents.

Our data on these psychoactive plants represent a validation of popular beliefs [4]. It appears very important to collect document and save medicinal and ritual plants. The study of plants with medical properties is especially meaningful in tropical lands, due to their great variety of animal and vegetal species, a factor that increases the number of available resources. Tropical areas are also regarded as a primary source of undiscovered pharmaceuticals [35], and ethnobotanic data may constitute the basis for developing new active metabolites, also in the fascinating field of psychoactive plants.

Thus, the results described in this manuscript support the fundamental importance of ethnobotanical knowledge, which Schultes [36] as described as “the prolific and promising treasure-trove of the ethnopharmacological knowledge”.

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