Detecting Biogenic Amines in Food and Drug Plants with HPLC: Medical and Nutritional Implications

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Abstract: *Background*: This work reports the results of the initial stage of the project aimed at detecting neuroactive substances in tropical plants that are widely used as food and/or drugs.

Methods: The content of neuroactive biogenic amines, e.g, dopamine (DA), norepinephrine (NE), epinephrine (E), serotonin (5-HT), and others was determined using high-performance liquid chromatography (HPLC) with amperometric detection in leaf samples from *Plumeria rubra* L. cv. *acutifolia, Syzigium jambos* (L.) Alston, *Buxus megistophylla (*or *Euonymus japonicas* cv. *aureoma*), and *Cinnamomum bodinieri* Levl.

Results: The total fraction of disintegrated leaves contained (sub)micromolar concentrations of DA, NE, and 5-HT. They lacked E and the catecholamine precursor 2,3-dihydrophenylalanine (DOPA).

Conclusions: From the data obtained, it is evident that heretofore unexplored tropical plants used in drug preparations (*P. rubra* and *S. jambos*) and as desserts (*S. jambos)* and spices (*C. bodinieri*) contain physiologically active concentrations of neurochemicals. The neurochemicals are expected to produce a significant effect on the people who consume preparations and food additives made from the aforementioned plants. Moreover, such plant preparations can potentially be used as psychoactive drugs for the purpose of intentionally manipulating human behavior.

Keywords: Neurochemicals, biogenic amines, serotonin, dopamine, norepinephrine, epinephrine, drug plants, food plants, tropical medicine, HPLC.

INTRODUCTION

This work sums up the data obtained during the first stage of a large-scale research project that is focused on evaluating the neurochemical effects produced by popular medicinal plants (often also used as food) in the tropical region, as exemplified by the area around the Shenzhen-Hong-Kong conurbation (South China).

The brain cannot operate without neurochemicals (neuromediators, neurotransmitters, neuromodulators, etc.), which are involved in transmitting information among neurons or between neurons and the cells of glands or muscles. Neurochemicals are classified into several groups: (i) biogenic amines including catecholamines (dopamine, norepinephrine, and epinephrine), serotonin, histamine, octopamine, and others; (ii) amino acids (aspartic acid, glutamic acid, γ-aminobutyric acid (GABA), glycine, etc.); (iii) neuropeptides, such as endorphins, encephalins, dynorphins, substances P and Y, and others; (iv) gaseous neurochemicals, such as nitric oxide, carbon monoxide, hydrogen sulfide, etc.; and (v) purines including adenosine, AMP, ADP, ATP, and others.

A large number of neurochemicals are multifunctional agents: they combine the role of neurochemicals with that of hormones or local tissue factors (histohormones). For instance, many neurochemicals perform communicative and regulatory functions in various animals [1], plants [2-5], fungi, and protozoans [4]. It was revealed that many neurochemicals significantly influence microbial growth, cell aggregation, respiratory electron transfer, membrane potential generation, toxin synthesis, and other processes in microorganisms (reviewed: [6, 7]). Therefore, these substances, apart from being called *neurochemicals*, should also be referred to as *biomediators* [3].

In particular, the group of neurochemicals known as *biogenic amines* function as signals and regulators in diverse plant systems [2-5]. Suffice it to say that catecholamines are released not only by animals but also by plants in response to stress. Stressed tomatoes, e.g., those incubated at low temperatures, produce significant catecholamine amounts [8]. Serotonin accelerates the development of plants as

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exemplified by radish seed germination [2], and this is attributable to its similarity to the plant hormone auxin, or indole-3-acetic acid.

The development of the vegetative microspores of the common horsetail *Equisetum arvense* is stimulated by serotonin, dopamine, and norepinephrine [3]. Dopamine and serotonin also promote the development of the pollen of *Hippeastrum hybridum* [3]. Epinephrine and norepinephrine slow down cyclosis in the cells of this charophyte [3]. It is known that plant species of various taxonomic groups synthesize neurochemicals, including catecholamines (dopamine, norepinephrine, and epinephrine), serotonin, histamine, and acetylcholine; they contain enzymes that are involved in their biosynthesis, transport, and degradation [7]. In response to touching, mimosa leaves collapse, and this response involves a norepinephrine-dependent system [9].

In spite of the aforementioned recent findings, insufficient data are available in the literature on the neurochemicals contained in many widely used species of food and/or drug plants. Needles to say, this information would be of paramount importance in terms of their effects on human physical and mental health.

Filling this gap in our knowledge is one of the main goals of a large-scale research project currently carried out under the aegis of the recently founded Shenzhen MSU-BIT University. The stage of this project summed up in this work deals with biogenic amines that are important plant signals and regulators.They were quantitatively determined in the leaf tissue of several tree species growing in the Dayun Park of the town of Shenzhen.

MATERIALS AND METHODS

The goal of the project stage described in this work is to screen a number of popular food and drug plants for neurochemicals, such as biogenic amines. The plant species used for measuring biogenic amine concentrations were as follows:

1. *Plumeria rubra* L. cv. *acutifolia.*
2. *Syzigium jambos* (L.) Alston
3. *Buxus megistophylla* (or *Euonymus japonicas* cv. *aureoma*)
4. *Cinnamomum bodinieri* Levl.

P. rubra L. cv. *acutifolia,* originally imported from Central America and Mexico, is used in China as a medicinal plant, apart from being cultivated as a decorative species. Several parts of the *S. jambos* tree are used as a tonic or a diuretic [10]. The leaf decoction is applied to sore eyes, it also serves as an expectorant and is used in the treatment of rheumatism [11]. The ever-green shrub *B. megistophylla* (box, or boxwood) is only used in topiary and wood carving. *C. bodinieri* belongs to the group of plants used to produce the spice cinnamon (from the inner bark [12]).

Based on the data presented in the literature [1-6], we assumed that maximum concentrations of biomediators are typically present in plant tissues during the hot tropical summer season. The summer weather promotes plant metabolic activities and, therefore, maximizes the involvement of regulatory substances that include biogenic amines. Therefore, plant leaf samples were collected in the Dayun Park (Shenzhen) at the beginning of June (in 2019). It is also known that the biomediators tend to accumulate in plant tissues towards the end of the day, as a result of the functioning of the photosynthetic machinery in the daytime. This is the reason why we harvested the leaves between 6 and 7 pm local time. To minimize the effect of storage conditions on biogenic amine concentrations, we stored the leaf samples, after collecting them, at -10^{o}C for maximally 2 days in polyethylene bags.

In this work, frozen leaf samples were disintegrated using a mortar with Quartz sand at 0^{o}C in 0.1 N HCl; the debris were removed by centrifugation (20,000 g, 20 min).The biogenic amines, i.e., dopamine (DA), norepinephrine (NE), and serotonin (5-HT) were separated by high-performance liquid chromatography (HPLC) with an amperometric detection system [13]. A LC-304T chomatographer (BAS, West Lafayette, USA) with a Rheodyne 7125 injector was used; the volume of the loop used for applying samples was 20 μl. The tested biogenic amines were separated on a reverse-phase ReproSil-Pur column (ODS-3, 4x100 mm, 3 μ; Dr. Majsch GMBH, Elsico, Moscow). A PM-80 pump (BAS, USA) was used; the elution rate of the mobile phase was 1.0 ml/min at a pressure of 200 atm. The mobile phase contained 0.1 M citrate-phosphate buffer with 1.1 mM octanesulfonic acid, 0.1 mM EDTA, and 9% acetonitrile (pH 3.0). The measurements were carried out using an LC-4B electrochemical detector (BAS, USA) with a glass-carbon electrode (+0.85 V) against an Ag/AgCl reference electrode. The samples were scanned with the Multichrome 1.5 (Ampersand) hardware-software system. All reagents used for the assay were of analytical grade. The chromatographer was calibrated using a mixture of the tested biogenic amines; the concentrations of all these substances were 0.5 μM. The amine concentrations contained in the samples were calculated by the internal standard method that is based on determining the ratio between the peak area in the standard mixture and that in the samples [13]. The data presented in the Table **1** below are the mean values obtained using 4-5 independent repeats of each measurement.

RESULTS AND DISCUSSION

Using HPLC with an amperometric detector (Table **1**), it was demonstrated that the total fraction of disintegrated leaves of the tree species *Plumeria rubra* L. cv. *acutifolia, Syzigium jambos* (L.) Alston, *Buxus megistophylla (*or *Euonymus japonicas* cv. *aureoma*), and *Cinnamomum bodinieri* Levl. contained relatively high concentrations of biogenic amines, i.e., serotonin (5-hydroxytryptamine, 5-HT), norepinephrine (NE), and dopamine (DA). The leaves of the tested plants, except *B. megistophylla,* also contained 3-methyltryptamine (3-MT). These concentrations were in the order of micromoles per 1 kg of leaf weight (Table **1**).

The tested leaves lacked detectable amounts of epinephrine (E) and the catecholamine precursor 2,3-dihydrophenylalanine (DOPA).

The data obtained indicate that biogenic amine concentrations in the plant leaf samples exceed those in many other biological systems. For instance, human blood is known to contain 0.5-1.5 μM serotonin [14], 0.001-0.01 μM dopamine (in the free form; human blood also contains ~0.2-0.3 μM sulfoconjugated dopamine) and about 0.1 μM norepinephrine [1, 15]. Therefore, the biogenic amine concentrations detected by us in the plant leaves are physiologically significant, and the neurochemicals are expected to perform important functions in the interaction among cells within a plant organism as well as between the plant cells and the microbiota that overgrows the plant leaf surface and/or grows inside leaves.

These findings are also of practical interest, since some of the tested tropical plants are used as medicines and/or as food. Their consumers should experience the neurochemical effects of the neuroactive substances they contain.

In more general terms, the present work deals with some of the communication signals that are widely used to coordinate the activities of living cells, organisms, and their groups, including plants and their associations. The specific signals addressed in this project are biogenic amines. In conformity with earlier work on plant neurochemicals [2, 3, 7], we established that the leaves (the total fraction) of a number of heretofore unexplored tropical plants, including those used in drug preparations (*P. rubra* and *S. jambos*) and as desserts (*S. jambos)* and spices (*C. bodinieri*) contain physiologically active concentrations of the neurochemicals dopamine, norepinephrine, and serotonin.

These substances are known to stimulate growth and regulate a wide variety of physiological processes in diverse microbial species, including the bacterium *Pseudomonas aeroginosa* and the yeast *Saccharomyces cerevisiae* (reviewed, [7, 8, 16, 17]). For example, the important neurotransmitter serotonin behaves as the signal of the *lasIR* quorum sensing system in *Pseudomonas aeruginosa*. It increases virulence and stimulates biofilm formation [18].

In light of our findings in conjunction with relevant recent literature data, the neurochemicals should play an important role in the ecological interaction between the plants and the microorganisms that overgrow their leaves and other parts. Studies on this plant-microbial interactivity constitute the subject of the next stage of the current research project; its initial stage is summed up in the present work.

Table 1: Concentrations (means ± *standard deviations*) of serotonin (5-hydroxytryptamine, 5-HT), epinephrine (E), norepinephrine (NE), dopamine (DA), 2,3-dihydrophenylalanine (DOPA), and 3-methyltryptamine (3-MT) in the total fraction obtained by disintegrating plant leaves. All concentrations are expressed in micromoles per 1 kg of wet biomass

Species	E	NE	DA	5-HT	DOPA	3-MT
Plumeria rubra L. cv. *acutifolia*	0	0.43±0.03	0.11±0.05	1.41±0.07	0	2.24±0.09
Syzigiumjambos(L.) Alston	0	0.34±0.01	0.09±0.04	1.18±0.06	0	1.87±0.07
Buxus megistophylla	0	0.27±0.02	0.06±0.01	0.25±0.02	0	0
Cinnamomum bodinieri Levl.	0	1.21±0.14	3.09±0.12	1.21±0.09	0	0.33±0.03

Besides, the neurochemicals are expected to produce a significant effect on the people who consume preparations and food additives made from the aforementioned plants. These important neurochemicals regulate brain processes involved in locomotion, affection, sociable and dominant behavior, as well as aggression [19]. Such plant preparations can potentially serve as behavior-modifying drugs for the purpose of intentionally manipulating human social and political behavior.

CONSENT FOR PUBLICATION

The authors confirm that all experiments with plants were performed in accordance with the national and international standards and guidelines. This article does not contain any studies involving animals performed by any of the authors.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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