BIOGENIC AMINE DETECTION IN SOME THAI TRADITIONAL PLANT

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ABSTRACT

Traditional plant are now very popular for Thai people as other people in many country. Thai people also use traditional plant for protect and heal their healthy. Biogenicamine compound such as histamine and tyramine are important in food. Histamine is an index of freshness of fish ,fish product , fermented plant and induce allergenic symptom.Tyramine is the one biogenic amine found in food and trend to be induce Migraine symptoms. Migraines are believed to be due to a mixture of environmental and genetic factors.The aim of this research was to survey the histamine and tyramine in some Thai traditional plants such as Caesalpinia sappan L., Kaempferia parviflora, Berchemia floribunda Wall., Prunus cerasoides D.Don and Piper ribesioides Wall. All traditional plants as referred have many medicinal properties such as used for Asthma treatment, antiseptic, stimulant herb with a pungent, turpentine-allspice aroma. However, some traditional plant was used to support the strength of body but most of people not awareness about their side effect. From this study, all traditional plant samples were boiled in water and ferment in alcohol and analysed histamine and tyramine to evaluate the risk assessment for health. The results showed that both histamine and tyramine were detected in both boiled water from plant and fermented in alcohol in all plants. However, tyramine showed the highest contant in Caesalpinia sappan L. that bolied in water as17.59±0.59 mg/100g.This presented that, the people who drink the water of Caesalpinia sappan L may have the opportunity to be headace as migraine if they used for a long time.

Keywords: Biogenic Amine, Histamine, Thai traditional plant, Tyramine

INTRODUCTION

Biogenic amines are amine compounds involved in neurotransmitters system(Kalac and Krausova,2005), they are synthesized by plants and animals (Moret. Et al.,2005). The formation of this compound also found in food and relate to the decarboxylation reaction of amino acids by microorganism(Stadnik, J. and Dolatowski,Z.J ,2010). In general ,an interesting biogenic amine in food are histamine, tyramine, putrescine, cadaverine and phenylethylamine. In this work, histamine and tyramine are interesting.

Histamine , shows as in Fig.1

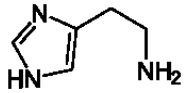

Figure 1. The structural formular of histamine (Songül .et al.,2013)

Histamine is the decarboxylation products of histidine(Songül .et al.,2013) (equation1).

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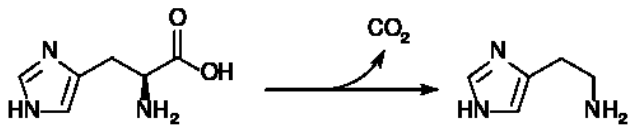
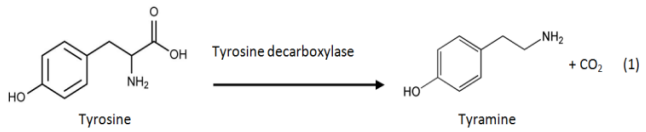


……1)

,it has been implicated as the causative agent in outbreaks of food poisoning where intoxication results from the ingestion of foods containing excessive amounts of histamine(Luliński, et al.,2014). Histamine is grouped with a class of neurotransmitters called "Small Molecule Neurotransmitter Substances (Noszal, et al.,(2004), eating some plant may improve chemical neurotransmitterin human. Histamine intake of 8-40 mg, 40-100 mg and higher than 100 mg may cause slight, intermediate and intensive poisoning, respectively (Maijala,R., and S. Eerola,S,1993)

The other biogenic amines ,tyramine (4-(2-aminoethyl) phenol), that formed by degradation of normal metabolic activity in plants, animals and microorganisms(Bueno-Solano.,et al.,2012). Tyramine is also be the decarboxylase products of tyrosine as in equation2) . In generally, tyramine can be found in many foods and especially in fermented food products such as meat products, cheese, beer, red wine, soy sauce, sauerkraut and chocolate etc. (Yigit , M and Ersoy, L ,2003)



…….2)

Tyramine was synthesized in the brain and the peripheral nervous tissues of human, in very low concentration (0.1–100 ng g^{-1} of tissue) (Shalaby, AR.,1996). It is biologically active amines, which have important physiological effects such as peripheral vasoconstriction, increases the cardiac output, causes lacrimation and salvation, increases respiration, increases blood sugar level and releases noradrenaline from the sympathetic nervous system (Komprda et al.,2008). However, in 1996, Shalaby, A.R. was determined the level of tyramine in foods at 1080 mg kg^{-1} is toxic level and over 100 mg kg^{-1} may cause migraine.

Importance of biogenic amines in foods is mainly due to two reasons:firstly, the intake of foods containing high content of biogenic amines causes health hazard through the direct, toxic effect of these compounds and their interaction with some medicaments (Bardocz, 1995); secondly, they may have a role as indicators of quality and/or acceptability in some foods (Ruiz-Capillas ,C and Moral,A. ,2001)

As above referred, it showed that the biogenic amines found in food may effect on the health of the consumers (Silla-Santos,M.H.1996). Some consumer suffered from allergic reactions and showed many symptoms as difficulty in breathing, itching, rash skin, vomiting, fever, migraine and hypertension (Songület al.,2013). Many works report about foods that contain high levels of biogenic amines such as fish, fish products (Muhammad Zukhrufuz Zaman,et al.,2010) cheese(Guan ,RF et al ,2013) , and fermented foods as beer (Kalac,etal.,2010) (Maijala,R ,1995),wine(Millan, et al.,2007),traditional sausage(Wirachai et al.,2013) However, there has a few work about biogenic amines in plant especially traditional plant. This work aims to analyse histamine and tyramine content in Thai traditional plant to estimate the possibility of people who eat traditional plant and their products.Total phenolic content in all traditional plants was also analysed too.

Currently, traditional plants are widely popular consumption in many countries of Southeast Asian including in Thailand. In this work , the traditional plant such as *Caesalpinia sappan*

L., Kaempferia parviflora, Berchemia floribunda Wall., Prunus cerasoides D.Don and *Piper ribesioides Wall.* were an interesting herb plants. The medicinal properties of these plants concern the some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compound of traditional plants are well known as alkaloids, tannins, flavonoids, and phenolic compounds(Hill AF (1952). All traditional plants have the best properties for treatment of digestive disorders(Yenjai, C ,et al.,2004), asthma (Supinya Tewtrakul et al.,2008) ,gastric ulcer (Rujjanawate .C.,et al.,2005) , modulators of multidrug resistance in cancer cells (Patanasethanont et al., 2007). , anti-inflammatory , antimicrobial, antitumor, antiatherosclerosis, hypoglycemic, and spasmolytic activities and to promote blood flow(Wang,et al.,2011) and also enhance sexual performance (Jaipetch, T, et.al.1983). Thai people use them as many types for eating such as boiling in water , fermented in white spirit liquor.

MATERIALS AND METHOD

Materials

Chemical: All chemical reagents were an AR grade. Tyramine was purchased from Fluka**.** Histamine hydrochloride and sulphanilic acid were purchased from Sigma Aldrich. Sodium nitrite , hydrochloric acid sodium nitrite were purchased from Carlo Erba. Gallic acid standard, Folin-Ciocalteu's phenol reagent, sodium carbonate, sodium acetate, , sodium hydroxide were purchased from Merck Chemical.Co.,Germany). Trichloroacetic acid(AR grade) 2,6-bis (1,1-dimethylethyl) -4-methylphenol(BHT),Thiourea (AR grade), 2-thiobarbituric acid(AR grade),1-butanol, methanol and acetonitrile were purchased from Carlo Erba.

Plant Samples : All plant samples such as *Caesalpinia sappan L., Kaempferia parviflora, Berchemia floribunda Wall., Prunus cerasoides D.Don* and *Piper ribesioides Wall.* (in dried form) were purchased from Thai traditional shop , Thaprachan in Bangkok.

Experimental Methods

Part-1. Preparation on Traditional Plants sample solution.

Traditional plant samples

All plants samples were washed with tap water, left to dried at room temperature for 1 hour. Then all samples were homogenized with blender prior to the extraction step. In this work, also compared the extraction methods such as the following :

Method 1 :Hot extraction (boiling in water) :

The 5.xxxx g of plant samples were placed in 50 ml of deionised water, mixed and boiled on hot plate for 10 mins. The residue plants were filtered through Whatman filter paper no.1 and collected the filtrate.

Method 2: Alcoholic extraction

The 5.xxxx g of plant samples were dipped in 50 ml of white spiritural liqour for 24 hours. The residue plants were treated same as method1.

All extracts filtrate were filtered again passed through a 0.45 micron Nylon filter and kept in eppendorf vessels (1 mL) ,stored at 4 ^{o}C, until analysis in next part.

Part-2. Qualities Evaluation on plants sample extracts

Analysis of Total phenolic compound content

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The analysis method modified from Shoib A. Baba ,& Shahid A. Malik (2015),the clear filtrate from part 1 0.4 ml was mixed with 2 ml of 10 % Folin Ciocalteau reagent and 1.6 ml of 7.5 % Na_2CO_3 and kept at room temperature for 30 mins. The mixing solution was measured an absorbance at 765 nm by Ultraviolet Visible Spectrophotometer(UV -VIS Shimadzu Model UV100). Then Total phenolic compound content was calculated as gallic acid equivalent.

Analysis of histamine and tyramine content

The analysis method modified from Patangec et al.(2005) the clear filtrate from part 1 1.0 ml was mixed with 5.00 ml of 1.1% w/v sodium carbonate , 2.00 ml of *p*-phenyldiazonium sulfonate solution , then shaked for 5 sec. The mixing solution was measured an absorbance at 500 nm for histamine detection and 335 nm for tyramine detection by Ultraviolet Visible Spectrophotometer. Histamine and tyramine content was calculated ,compared with the standard calibration curve of histamine and tyramine standard solutions.

Note: Phenyldiazonium sulfonate solution was prepared by mixing of 1.50 ml of 0.9%(w/v) sulfanilic acid and 1.50 ml of 5.0%(w/v) sodium nitrite in ice bath 15 mins.

Statistical Analysis

In the determinations of all parameters were carried out at least five replicates For all statistics, ANOVA and Microsoft Excel were used for calculate and graph presentation in this work.

RESULTS AND DISCUSSION

After treatment to the traditional plants samples as method 1 and method 2, the filtrate from each method of each plant showed the filtrate color in pale yellow to dark color except the filtrate from *Kaempferia parviflora* Wall. Ex. Baker. The *Kaempferia parviflora* Wall. Ex. Baker gave the pale violet color filtrate that extracts from both methods. However, all filtrate from traditional plants that extract by method 2 (alcoholic extraction) presented the darken color than extraction by method 1 because all pigments in plant more soluble in alcohol or organic solvent better than hot water(Molyneux, et al.,2007). The color of filtrate do not interfere the method of spectroscopic method in analysis of total phenolic compound and both biogenic amines. The quantity of total phenolic compound in all traditional plants samples (as in Table1) showed the different content in each plant and also depend on treatment method

Table 1. Total phenolic content in traditional plant samples by both methods

Plant types	*M1 (mg/100g)*	*M2 (mg/100g)*
S1	9.54±0.44	15.28±0.10
S2	2.07±0.21	4.74±0.18
S3	1.65±0.15	2.65±0.25
S4	1.62±0.19	5.89±0.36
S5	1.75±0.26	8.09±0.12

Note: S1 = *Caesalpinia sappan L.,* S2 = *Kaempferia parviflora,*
S3= *Berchemia floribunda Wall.,* S4 = *Prunus cerasoides D.Don*
S5= *Piper ribesioides Wall.*
M1 = Hot extraction (boiling in water**)** M2 = Alcoholic extraction

As above the total phenolic content in all traditional plants that extraction with white spiritual liquor showed more than using hot water extraction. This concerning that all phytochemical substances in almost plant more soluble in organic solvent such as alcohol , so alcohol could leached through plant cell wall better than water. This results also support the theory of like dissolve like of substance that related to structure of phytochemical compound too(Sarker & Nahar, 2007). The more total phenolic content in plant extracts mean that all important substance from plant appear in drug solution in higher content too and may effective to cure the symptom of human(Hill AF (1952)

After the biogenic amines in all filtrate extracts from traditional plants were measured and calculated as in Table 2 and 3

Table 2. Histamine and Tyramine content in traditional plant samples by M1

Plant types	*Histamine* (mg/100g)	*Tyramine* (mg/100g)
S1	2.44±0.26	17.59±0.59
S2	1.36±0.20	17.43±3.05
S3	0.38±0.00	2.21±0.28
S4	0.36±0.05	1.47±0.41
S5	0.70±0.22	4.45±0.70

Table 3. **Histamine and Tyramine content in traditional plant samples by M2**

Plant types	*Histamine* (mg/100g)	*Tyramine* (mg/100g)
S1	6.33±0.28	15.38±0.57
S2	3.52±0.41	16.05±1.21
S3	1.00±0.21	1.58±0.38
S4	1.73±0.57	1.32±0.49
S5	0.95±0.21	2.21±1.00

From above data : it revealed that both biogenic amine could be detected in all plants at different quantity. Tyramine presented in filtrate of all plants that extraction by hot water more than alcoholic extraction. Since, tyramine is a very reactive amine and could rapidly reacted with other substance in plant. The alcoholic filtrate from plants showed more total phenolic substance than water filtrate, so that tyramine will react with other phytochemical substance and lost its content. It was noticed that S1 which was *Caesalpinia sappan L.,* contained the most tyramine content in filtrate from M1. Histamine was detected in *Caesalpinia sappan L.,* in the highest level approximately as 2.44±0.26 - 6.33±0.98 mg/100g from M1filtrate and M2 filtrate ,respectively.

In the same plant that extracted by M1 and M2 showed the same trend as in Figure2 and 3.

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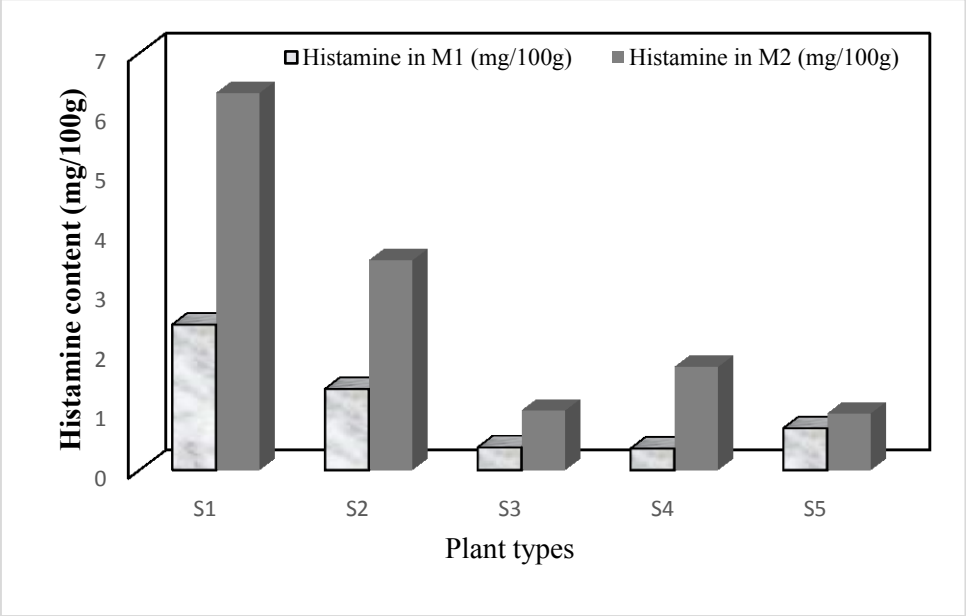

Figure 2. Histamine in traditional plant extract by M1 and M2

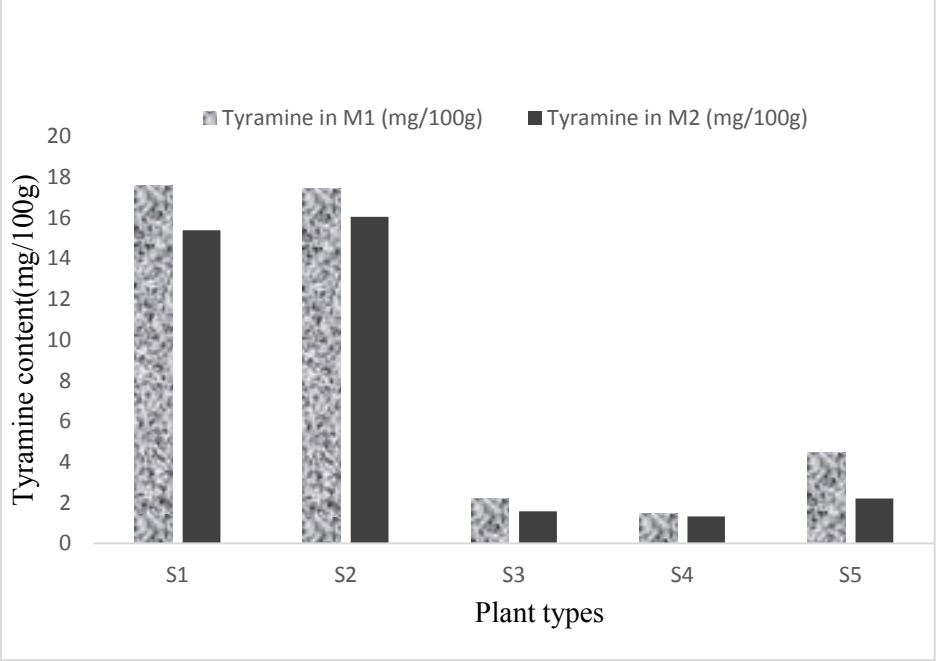

Figure 3. Tyramine in traditional plant extract by M1 and M2

From the figure2 and 3 , tyramine was the biogenic amine that could be found in Thai traditional plant samples in this work higher than histamine. Tyramine and histamine in samples were found in the range of 1.32 ±0.49 – 17.59 ±0.59 mg/100 g of plant , and 0.36 ±0.05 – 6.33 ±0.28 mg/100g plant, respectively. Since histamine is an important amine that index the fresh of plant but these plants may be harvested in freshness condition to prepare as herbal drug.This data gave an important about tyramine which effect on some sickness such as Migraine, so people who eat plant that contain more tyramine content possible to get headache.

CONCLUSION

This work presented that some popular Thai traditional plants contain an important compound such as phytochemical substance which in this work showed in term of total phenolic compound to give a healing property from bad symptom in high content especially *Caesalpinia sappan L.,* How to eat plant such as boiling in water or immerse in white spiritual liquor also get total phenoic compound in difference quantity. However,all people who use these traditional plants should beware the toxic from biogenic amines as tyramine and histamine.Especially tyramine in high level may cause Migraine in their bodies.

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