

Determination of the contents of the main biochemical compounds of Adlay (*Coxi lachrymal-jobi*)

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Received 16 June 2006; received in revised form 27 December 2006; accepted 22 February 2007

Abstract

Adlay (*Coxi lachrymal-jobi*), an annual crop, has long been used in traditional Chinese medicine for its biological activity and as a nourishing food. Its phytochemical composition has been extensively studied; however, information on its policosanols (PC) and phytosterol content is scarce. The objective of this study was to examine and compare the PC, phytosterol and oleamide contents of different fractions of adlay collected from Laos, Thailand, Vietnam and Taiwan. Biochemical compositions of the samples were identified using a gas chromatograph coupled with a mass spectrometer (GC–MS). The adlay bran had higher contents of policosanols (246 mg/kg), phytosterols (4733 mg/kg) and oleamide (45.8 mg/kg) than had hulled and polished fractions. Although plant sterols reduce cholesterol absorption, policosanols may inhibit endogenous cholesterol synthesis. Although adlay contains beneficial phytochemicals that justify its use as a food ingredient or dietary supplement, research is still needed to confirm its traditional use in Asian medicine.

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Keywords: Adlay; Phytochemicals; Policosanols; Phytosterols

1. Introduction

Policosanols (PC), a mixture of high molecular weight (20–36 carbon) aliphatic primary long-chained alcohols containing hexacosanol (C₂₆), octacosanol (C₂₈), triacontanol (C₃₀) and other alcohols, are constituents of plant epicuticular waxes with roles in decreasing serum cholesterol levels. Numerous studies have reported that 5–20 mg/day PC consumption is effective in lowering total cholesterol (TC) by 17–21% and low-density lipoprotein (LDL) by 21–29% and increasing high-density lipoprotein (HDL) by 8–15%. This is accomplished both by inhibiting cholesterol biosynthesis and by increasing LDL processing (Aneiros, Mas, & Calderon, 1995; Menendez et al., 2001). Recent research has shown the benefits to serum lipids in healthy individuals, patients with type II diabetics (Crespo

et al., 1997) and type II hypercholesterolemia (Aneiros et al., 1995). Besides improving serum lipid profiles, PC reduces blood pressure, enhances anti-inflammation and the oxidation of LDL, protects the liver and prevents liver peroxidation and decreases platelet aggregation (Valdes, Aruzazabala, & Fernandez, 1996), smooth muscle cell proliferation and reduces neuronal damage (Borg, 1991).

Phytosterols are cholesterol-like compounds that are structural components of plant cell membranes. In cereal grains they are mostly found in bran and are extractable as part of bran oil waxes. There is a considerable interest in these compounds due to their promotion of cardiovascular health, especially through their cholesterol-lowering properties. Cereal brans reported to have high levels of these compounds include rice (Fang, Yu, & Badger, 2003), corn (Singh, Moreau, & Hicks, 2003) and rye (Piironen, Toivo, & Lampi, 2002).

Primary fatty acid amides (PFAMs) are a group of biologically highly active compounds involved in regulating

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numerous biological functions by free fatty acids and their derivatives. Some members of this group, especially oleamide, have been studied (Hanu, Fales, Spande, & Basile, 1999) with respect to their roles in biological systems. Among the PFAMS are a number of important endogenous biologically active agents, including palmitoylethanolamide (an anti-inflammatory agent), anandamide (arachidonylethanolamide, an antinociceptive and possibly neuroprotective agent, among other functions) and oleamide (a sleep-regulating agent). *cis*-9,10-Octadecenamide or 'oleamide' is a primary fatty acid amide which, *in vitro*, affects a variety of targets, including gap junction communication, serotonin 5-HT_{1A}, 5-HT_{2A/2C}, 5-HT₇ and GABA_A and cannabinoid receptors (Bradshaw & Walker, 2005). It has an effect on food intake and sexual behaviour of rats (Bradshaw & Walker, 2005) and is an anticonvulsant (Dougalis, Lees, & Ganellin, 2004). Oleamide was first isolated from the cerebrospinal fluid of sleep-deprived cats (Bradshaw & Walker, 2005), and has been characterized and identified as the signalling molecule responsible for regulating sleep, supposedly representing a brain lipid. Leggett, Aspley, Beckett, D'Antona, and Kendall (2004) provided evidence that oleamide inhibits agonist and antagonist ligand-binding to cannabinoid receptors, indicating the roles of oleamide and other endogenous lipids that are associated with cannabinoid system physiology. However, the mechanisms among these lipids through which they produce cannabimimetic effects remain to be elucidated. This also applies to the constituent phytochemicals or their functions and the biological activity of adlay.

Adlay (*Coxi lachrymal-jobi*) is an annual crop that has long been used in traditional Chinese medicine and as a nourishing food. The seed of adlay has been reported to exhibit stomachic, diuretic, anti-inflammatory, antispastic effects *in vivo*. A number of benzoxazinones, isolated from the seed of adlay, are reported to exhibit anti-inflammatory activity (Nagao, Otsuka, Kohda, Sato, & Yamasaki, 1985). Coixan A, B, and C, isolated from the seed of adlay, had hypoglycemic activity in rats (Takahashi, Konno, & Hikino, 1986). Wistar rats fed with an adlay mixed diet exhibited decreased fibrinolytic activities of blood plasma (Check & K'Ombut, 1995). Numerous other reports have indicated that the consumption of adlay seed is beneficial to humans and have shown that some extracts of adlay seed possess anti-allergic (Shyu, Lin, & Chiang, 1998), anti-mutagenic (Huang & Chiang, 1999), hypolipidemic effects (Yang et al., 1998), and they could reduce liver fat accumulation (Tsai, Yang, & Hsu, 1999).

The phytochemicals of adlay reported in the literature include benzoxazinones, lignan, phenolic acids, phenolic alcohols, phenolic aldehydes, phenolic glycerides, phenolic ketones, flavonoids (naringenin, tricin), phytin, polysaccharides (coixan A, B, C and glucan), diol lipid (coixenolide), fatty acids, phospholipids (phosphatidyl choline, phosphatidyl inositol, phosphatidyl serine), sphingolipid (cerebrosides) and steroids (campestanol, campesterol, β -sitosterol, stigmasterol) (Kondo, Nakajima, Naeoe, &

Suzuki, 1988; Nagao et al., 1985; Takahashi et al., 1986). Nevertheless, policosanols and phytosterols have not been extensively studied in other cereals, and most of the available literature is based on wheat, sugar cane wax and sorghum wax. There are no published data on the main biochemical compound contents of adlay. The objective of this study was to examine and compare the main biochemical compound contents of adlay found in some Asian countries.

2. Materials and methods

2.1. Material preparation

Adlay whole grains were dehulled and polished to obtain hull, bran and polished adlay grain samples. Adlay samples, collected from four Asian countries (Thailand, Laos, Vietnam and Taiwan) were obtained from the Shang-Sheng Agricultural Improvement Station, Kaohsiung (Taiwan). All adlay grain samples were powdered and stored at 4 °C. Chemical standards (docosanol, tetracosanol, hexacosanol, octacosanol, oleamide, squalene, tocopherol, stigmasterol, β -sitosterol, and campesterol) were purchased from Sigma (99% GC purity). Triacontanol (93% purity) and friedelin (99% purity) were purchased from ACROS, USA and Extrasynthese, France, respectively.

Adlay samples of Laos were converted to the various fractions and used to compare with the other country samples.

2.2. Proximate analysis

Moisture content was determined according to the procedure in AAAC (44-15A, 2000). Ash content was determined by dry-ashing in a furnace oven at 550 °C (AAAC 08-01 2000). The crude protein was calculated by converting the nitrogen content determined by the Kjeldahl method ($N \times 5.75$) (AAAC 46-10, 2000). Crude lipid, using a Soxhlet apparatus (AACC 30-25, 2000), was also determined. All analytical methods were carried out in triplicate.

2.3. Extraction of lipid

Lipid was extracted from samples using a solvent containing a minimum of 95% *n*-hexane (HPLC grade, TEDIA, Inc., USA). Hexanes and adlay samples were refluxed together for 3 h at approximately 70 °C. After refluxing, the mixture was vacuum-filtered. The filters were vacuum-concentrated to remove *n*-hexane.

2.4. Preparation of the unsaponifiable samples

Five gram of the dry-filtered samples (Oil (g)) were weighed and then transferred into a 250 ml round-bottom flask containing 50 ml of 2 M potassium hydroxide ethanolic solution. The flask was fitted with a condenser and

heated to slight boiling, with continuous stirring until the solution became clear (indicating saponification completion). After heating for a further 30 min, the contents of the flask were transferred to a 500 ml separatory funnel, washed several times with 50 ml of distilled water; afterwards, 80 ml of ethyl ether were added and shaken for approximately 1 min and the mixture was allowed to settle. The lower aqueous phase was separated off (3 times) and collected in a second separatory funnel. The ethyl ether extracts were combined in a separatory funnel and washed with distilled water until the wash water gave a neutral reaction. The wash water was discarded from the extracts, dried with anhydrous sodium sulphate and filtered into a pre-weighed bottle (W_0). The ethyl ether was then distilled, then brought to complete drying in an oven at 100 °C for approximately 1 h, and then weighed after cooling in a desiccator (W_1). The contents of unsaponifiables were calculated as follows:

$$\text{Unsaponifiables (\%)} = \frac{W_1 - W_0}{\text{Oil (g)}} \times 100$$

2.5. Preparation of standard solution

One milligram of standards (mixed standards) was dissolved in 1 ml chloroform (1000 ppm). Standard solutions were analyzed by gas chromatography–mass spectrometry (GC–MS).

2.6. Determination of PC, phytosterols and oleamide content in lipids extracted from adlay

Unsaponifiable fractions (30 mg) were dissolved in 1 ml of chloroform, and the contents of long-chain fatty alcohols and phytosterols were determined using GC–MS. GC–MS analyses were performed using a capillary column (DB-5 ms, 0.25 mm × 30 m, 0.25 μm film thickness), on an Agilent 6890N Network GC system and HP-5973 mass-selective detector (Agilent Technologies).

The oven temperature was programmed from 200 to 300 °C at 10 °C/min, and then held for 30 min. Data were processed with the aid of a computing integrator. The mass spectrum was continuously acquired from m/z 20 to 500 in full scan mode.

3. Results and discussion

3.1. Proximate composition of adlay

Adlay hull samples collected from Laos, an important adlay-exporting country to Taiwan, contained 7.98% moisture, 2.55% crude fat, 8.21% protein, 27.0% crude fibre and 14.96% crude ash. Bran samples contained 8.78% moisture, 23.54% crude fat, 9.15% protein, 1.10% crude fibre and 7.89% crude ash. Polished adlay samples contained 11.19% moisture, 6.89% crude fat, 12.2% protein, 2.43% crude fibre and 2.16% crude ash (Table 1). Polished adlay

Table 1
Comparison of proximate compositions of adlay

	Moisture (%)	Crude fat (%)	Protein (%)	Crude fibre (%)	Crude ash (%)	Unsaponifiable matter (%)
<i>Sample^a</i>						
Hull	7.98	2.55	8.21	27.0	15.0	0.24
Bran	8.78	23.5	9.15	1.10	7.89	0.70
Polished adlay	11.2	6.89	12.2	2.43	2.16	0.19
<i>Country^b</i>						
Thailand	10.59	7.22	12.1	2.11	2.30	0.19
Vietnam	12.38	6.20	13.4	2.02	1.62	0.13
Taiwan	12.34	6.29	14.2	1.78	1.78	0.20
Laos	11.19	6.89	12.2	2.43	2.16	0.19

^a Laos adlay samples.

^b Polished adlay samples.

samples from four countries (Thailand, Vietnam, Taiwan and Laos) contained 10.59–12.38% moisture, 6.20–7.22% crude fat, 12.1–14.2% protein, 1.78–2.43% crude fibre and 1.62–2.30% crude ash (Table 1). The proximate compositions of adlay, among the four countries, showed no apparent differences.

3.2. Unsaponifiables matter content of adlay

Unsaponifiable contents of different fractions extracted from adlay (Laos) are shown in Table 1. The content of unsaponifiables of hull, bran and polished adlay were 0.24%, 0.70% and 0.19%, respectively. Adlay bran had the highest percentage of unsaponifiables (0.70%); the polished adlay had the lowest unsaponifiable content (0.19%). The unsaponifiable content of polished adlay from Vietnam, Thailand, Laos, and Taiwan were 0.13%, 0.19%, 0.19%, and 0.20%, respectively. Taiwan had the highest percentage of unsaponifiables contents (0.20%), whereas Vietnam had the lowest unsaponifiables contents (0.13%). The total ion chromatogram (TIC) of the unsaponifiables extracted from adlay is illustrated in Fig. 1.

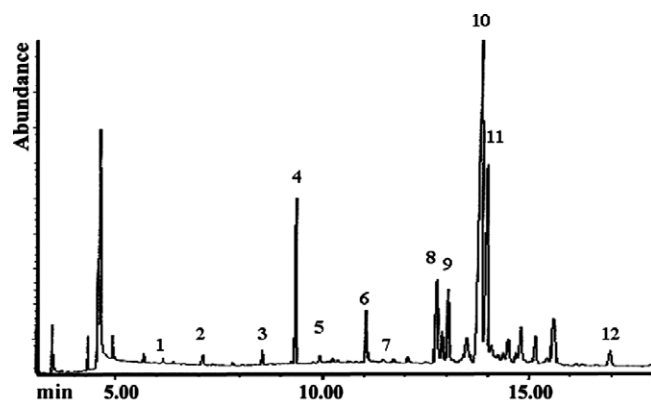


Fig. 1. GC–MS total ion chromatogram of unsaponifiable matter of adlay. 1, Oleamide; 2, docosanol; 3, tetracosanol; 4, squalene; 5, hexacosanol; 6, tocopherol; 7, octacosanol; 8, campesterol; 9, stigmata-5,22-dien-3-ol; 10, β -sitosterol; 11, ergostanol; 12, friedelin.

3.3. Policosanol (PC) content of adlay

Content and composition of PC extracted from different fractions of adlay (Laos) are illustrated in Fig. 1 and summarized in Table 2. The main constituents of PC are docosanol (C₂₂), tetracosanol (C₂₄), hexacosanol (C₂₆) and octacosanol (C₂₈). This translates to approximately 89–247 mg of PC in each 1 kg adlay fraction. The PC content of adlay bran (246 mg/kg) is higher than that of adlay hull (186 mg/kg) and polished adlay (90.1 mg/kg). Table 2 compares the contents and compositions of PC extracted from adlay of the four Asian countries. This translates to approximately 75–90 mg of PC in each 1 kg of polished adlay. The PC content of Laos adlay (90.1 mg/kg) is higher than those of Thailand (74.9 mg/kg), Taiwan (82.6 mg/kg) and Vietnam (76.5 mg/kg).

PC, extracted from sorghum wax represents 19–46% of the wax material, with octacosanol (C₂₈) and triacontanol (C₃₀) as the most abundant constituents. This translates to approximately 380–920 mg of PC in each 1 kg of sorghum grain (Avato, Bianchi, & Murelli, 1990). Sugar cane is the major source for the production of commercial PC-enriched products. The PC contents of sugar cane peel, leaves and whole sugar cane were 270 mg/kg, 181 mg/kg and 17.4 mg/kg, respectively. Octacosanol (C₂₈) was the main component in all the sugar cane samples (about 81%) (Irmak, Dunford, & Milligan, 2006). The amounts of PC in wheat germ and brans were 10 and 30 mg/kg, respectively. About 36% of the total PC in wheat bran was tetracosanol (C₂₄). The main components of wheat germ PC were docosanol (28%) and octacosanol (29%) (Irmak et al., 2006).

This study shows that the PC content of adlay bran was the highest of the adlay fractions. It was found that the adlay from Laos had the highest PC content among the four countries (Table 2). The main constituents of adlay PC were docosanol (C₂₂), tetracosanol (C₂₄), hexacosanol (C₂₆) and octacosanol (C₂₈). The high PC concentration of adlay also makes this variety a good candidate as a source of PC for functional foods and nutraceutical applications. Small variations were observed in the PC composition of adlay varieties examined in this study. In

general, C₂₄ and C₂₆ were the most abundant PCs present in bran fractions of all the adlay varieties (Table 2). Total C₂₄ + C₂₆ contents of bran adlay fractions were higher (~66% and 77% of total PC, respectively) than those of the other fractions (Table 2). Hull and bran adlay grain fractions contained large amounts of C₂₈, C₂₆ and C₂₄. Adlay varieties of Taiwan and Laos had the highest PC content; C₂₄ and C₂₆ were the major components of PC (Table 2). These two varieties also contained very high amounts of C₂₈, indicating that adlay is a potential PC source for functional foods and nutraceutical applications and justifying its traditional use in Chinese medicinal culture.

This study showed that PC was concentrated in the bran fraction of adlay. It was found that significant variations existed in both PC content and composition among the adlay fractions. Intrada and Trego were two varieties that had the highest PC contents. C₂₆ and C₂₈ were the major components of PC in all adlay varieties. The hull and bran fractions also contained a very high amount of C₂₄, indicating that these varieties may be potential PC sources for functional foods and nutraceutical applications. The study of adlay surface waxes is also important for agronomic reasons because of the significance of these compounds in water loss, agricultural spray efficiency, and mechanical damage. It is well-documented that mutations affect the chemical composition of epicuticular waxes of several plant families (Wettstein-Knowles, 1979).

Adlay can be a major source of policosanols, long-chained alcohols, which have beneficial physiological activities. The effect of chemical composition (including PC content and composition of adlay extracts) on lipid oxidation requires further research. The high PC concentration of adlay also makes this variety a candidate as a source of PC for functional foods and nutraceutical applications. The adlay has been used as a food or a Chinese medicine for a long time. The extract thereof has the actions of removing swelling, pain relief, anti-inflammatory and detoxifying agent.

3.4. Phytosterol content of adlay

The contents and composition of phytosterols extracted from different fractions of adlay (Laos) are summarized in Table 3. The main constituents are squalene, tocopherol, campesterol, stigmasta-5,22-dien-3-ol, β -sitosterol, ergostanol and friedelin, which were identified by GC-MS (Fig. 1). The most significant sterols were ergostanol (Fig. 2a) and campesterol (Fig. 2b). Ergostanol accounted for 16.7–30.3% and campesterol, for 7.38–12.4 % of total sterols. This translates to approximately 1114–4733 mg of phytosterols in each 1 kg adlay fraction. In all adlay samples investigated, β -sitosterol (Fig. 2c) was the main plant sterol, ranging from 43.2% to 55.3% of the total sterol contents. Adlay bran had a higher content of phytosterols (4733 mg/kg) than had hull (1114 mg/kg) or polished adlay (1154 mg/kg). The contents and composition of phytoster-

Table 2
Policosanols content (mg/kg) and composition of adlay fractions

	C ₂₂	C ₂₄	C ₂₆	C ₂₈	Total PC
<i>Sample</i> ^a					
Hull	21.5	33.9	59.3	71.5	186
Bran	50.7	65.5	76.7	53.5	246
Polished adlay	18.5	22.6	23.9	25.1	89.1
<i>Country</i> ^b					
Thailand	14.7	19.9	18.0	22.3	74.9
Vietnam	14.2	20.7	20.2	21.5	76.5
Taiwan	14.7	21.7	23.3	22.9	82.6
Laos	18.5	22.6	23.9	25.1	90.1

^a Laos adlay samples.

^b Polished adlay samples.

Table 3
Phytosterol contents (mg/kg) and composition of adlay fractions

	Squalene	Tocopherol	Campesterol	Stigmata-5,22-dien 3-ol	β -Sitosterol	Ergostanol	Friedelin	Total phytosterols
<i>Sample</i> ^a								
Hull	18.1	14.0	138	139	505	186	114	1114
Bran	82.4	227	349	263	2215	1434	163	4733
Polished adlay	61.2	51.9	106	79.7	638	213	4.63	1154
<i>Country</i> ^b								
Thailand	53.6	47.7	69.4	57.3	343	150	5.52	726
Vietnam	41.0	36.4	58.0	53.4	232	112	3.92	536
Taiwan	53.5	38.0	87.2	88.8	398	196	6.44	867
Laos	61.2	51.9	106	79.7	638	213	4.63	1154

^a Laos adlay samples.

^b Polished adlay samples.

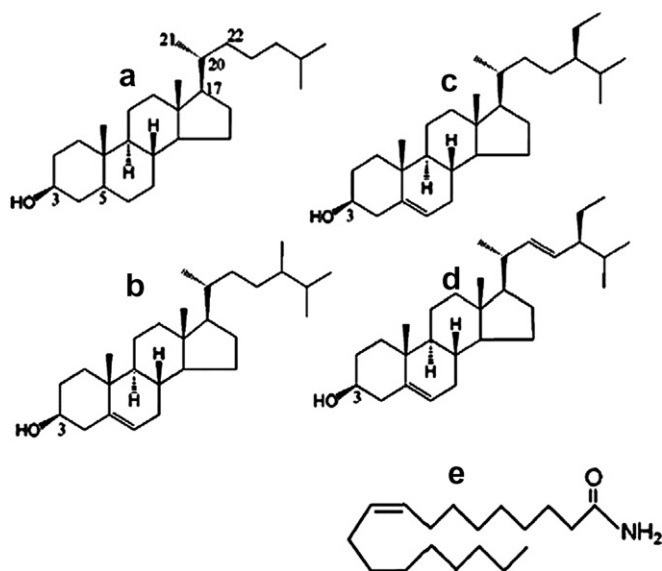


Fig. 2. Chemical structures of (a) ergostanol, (b) campesterol, (c) sitosterol, (d) stigmasterol and (e) oleamide, found in Adlay.

ols extracted from adlay samples, among the four countries, are summarized in Table 3. The phytosterol content of Laos (1154 mg/kg) is higher than those of Taiwan (867 mg/kg), Thailand (726 mg/kg) and Vietnam (536 mg/kg) (Table 3). Singh et al. (2003) reported total phytosterols levels of 500 mg/kg from sorghum grain, compared to 900 mg/kg for corn. Piironen et al. (2002) reported that the average contents in rye, barley, wheat and oats were 955, 761, 690 and 447 mg/kg, respectively. The fibre fraction isolated from sorghum by a corn wet-milling procedure had 700–800 mg/kg of phytosterols. Weihrach and Gardner (1978) reported that rice bran was extremely rich in phytosterols (13,250 mg/kg) compared with bran and whole wheat (890–154 mg/kg).

The possibility of using phytosterols as food additives to reduce absorption, by the human body, of cholesterol has led to numerous research works on therapy properties of phytosterols (Normen, Shaw, Fink, & Awad, 2004). Depending on the chemical structures of molecules and the conditions processed, some species of phytoster-

ols may act as antioxidants or possess antioxidant activities (Guillen & Manzanos, 1998). Yoshida and Niki (2003) reported that β -sitosterol, campesterol and stigmasterol (Fig. 2a, b and d, respectively) exerted antioxidant effects on the oxidation of methyl linoleate oil solution. Because phytosterols are naturally present in daily diets, it is reasonably believed that an increased intake of phytosterols from daily diets may be a practical way to reduce the risk of coronary heart disease (CHD) (Ostlund, 2004). The findings in this study indicate that adlay has a higher phytosterol content than has sorghum and shows promising potential for dietary and therapeutic treatments.

Intake of phytosterols (and -stanols) has been shown to decrease the level of low-density lipoprotein cholesterol and thus protect against development of cardiovascular diseases. Therefore, studies on the fractional phytosterol distribution of adlay, although not conclusive, indicate the need to further investigate the functions of these phytochemicals.

3.5. Oleamide content of adlay

The contents of oleamide extracted from different fractions of adlay (Laos) and the relationship between oleamide content and country of sample origin are summarized in Table 4. Oleamide's structure is illustrated in Fig. 2e and its GC profile is illustrated as abundance (%) vs. time (min) in Fig. 1. This translates to approximately 18.9–45.8 mg of oleamide in each 1 kg adlay fraction. The oleamide content of bran (45.8 mg/kg) is higher than those of hull (18.9 mg/kg) and polished adlay (20.7 mg/kg). The oleamide content of polished adlay from Taiwan (28.1 mg/kg) is higher than those of Thailand

Table 4
Oleamide content (mg/kg) of adlay fractions

Fractions	Country						
	Thailand	Vietnam	Taiwan	Laos			
Hull	18.9	45.8	20.7	22.6	19.3	28.1	20.7

(22.6 mg/kg), Laos (20.7 mg/kg), and Vietnam (19.3 mg/kg), indicating adlay to be a potential source of oleamide.

The elucidation of the biochemistry and pharmacology of these compounds may provide therapeutic targets for the use of adlay for a variety of conditions, including sleep dysfunction, eating disorders, cardiovascular disease, inflammation and pain.

Among the adlay fractions, adlay bran had a high content of oleamide and Taiwan's adlay variety had a high content of oleamide. Based on the above data, adlay bran fractions appear to be the form in which the bioavailability of the phytochemical studied are the highest and recommendations for use of adlay in the bran form are being put forth, based on the data presented in this study. Bran is one of the richest sources of dietary fibre and its inclusion in the diet is reportedly related to preventing heart attacks, intestinal disorders and cancers of the breast, colon, prostate and uterus. The determination of oleamide in plant material is scant in the literature and the technique using GC–MS in adlay fractions is, for the first time, reported in this study.

4. Conclusions

The data presented reveal adlay to be a rich source of important phytochemicals and justify its traditional use in dietary and therapeutic treatments in Asian culture. Adlay bran and adlay varieties of Laos and Taiwan contained more PC, phytosterols and oleamide. The adlay fractions exhibited higher PC contents than did sugar cane and wheat bran, for instance, signifying adlay's high potential in the dietary supplement market. However, since the possible anti-oxidative properties of the plant remain largely unknown, as well as the effects of chemical composition, including PC content and composition of adlay extracts on lipid oxidation, further research is required to confirm the place of adlay in human diets and therapeutic treatments. One major benefit of utilizing policosanols present in adlay as a commercial source of policosanols can be envisioned. This relates to the abundance of policosanols in adlay bran. Another benefit comes from not needing a saponification process, which is a method for producing policosanols from wax esters. Moreover, obtaining policosanols from adlay adds value to an underutilized by-product. The high yield of long-chained lipids and high level of policosanols in adlay compared with adlay bran and the differences of policosanols composition between adlay hull and polished grain can be additional benefits of the use of adlay for policosanols production. From the data, we recommend the Laos adlay varieties, due to their higher bran contents of the phytochemicals studied.

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