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دراسة وجود كليكوسيدات سيانوجين والسيانيد في زيت بذور المطاط الماليزي

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الملخص:

في هذا البحث لقد تمت دراسة الكليكوسيدات سيانوجين (لاينامارين) في زيت بذور المطاط والمستخرج باستخدام مذيبات مختلفة مثل الهكسان، مزيج من الكلوروفورم والميثانول، والإيثانول. كما تم تطبيق الطريقة اللونية لتحديد وجود مثل هذه المركبات. أيضا تم إجراء إختبار السمية باستخدام الفئران والذي أكد على عدم وجود مثل هذه المركبات. إن تحديد وجود السيانيد باستخدام الطريقة اللونيه قد بين عدم الإستجابه للسيانيد في زيت بذور المطاط وعدم ظهور أي لون مقارنة مع السيانيد التجاري الذي يبين ظهور اللون الازرق. كما أن زيت بذور المطاط لم تظهر أي احتمالات مسمه للفئران ويمكن أن يعزى ذلك إلى غياب اللاينامارين الخطر في زيت بذور المطاط.

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ORIGINAL ARTICLE

Occurrence of cyanogenic glycoside and cyanide in the Malaysian rubber seed oil

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KEYWORDS

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Abstract The cyanogenic glycoside (linamarin) in rubber seed oil (RSO) extracted using different solvents such as hexane (RSO_h), a mixture of chloroform and methanol ($RSO_{chl+mth}$) and ethanol (RSO_{eth}) was also studied. Colorimetric method was carried out to determine the presence of such compounds. Toxicological test using rats was also conducted to further confirm the absence of such compounds. The determination of cyanide by using colorimetric method was demonstrated by no response of the cyanide in RSO and did not show any color comparing with commercial cyanide which observed a blue color. Rubber seed oil did not show any toxic potential to the rats. This can be attributed to the absence of hazardous linamarin in RSO.

1. Introduction

A rubber seed is an important product of the rubber tree (*Hevea brasiliensis*). The rubber tree is a perennial plantation crop, indigenous to South America and cultivated as an industrial crop since its introduction to Southeast Asia around 1876 (Abdullah and Salimon, 2009). The rubber tree constituent is rubber seed (Abdullah and Salimon, 2009) and latex (Alenius et al., 1995). Fresh seed contains 65% kernel and 35% shell (Joseph et al., 2004). Rubber seeds yield from rubber plantations varies from 100 to 150 kg/ha, depending on soil fertility, crop density (Nwokolo and Smat, 1996), type of planting material, weather conditions, and severity of odium attack during flowering (Chin et al., 1977). Among the ancillary resources obtained from rubber plantations (wood, and seeds), seed has the greatest potential use (Joseph et al., 2003). Rubber seeds are not currently in use and they are found abundantly and wasted (Achinewhu and Akpapunam, 1985), but there are a few uses for the rubber seed, as planting material, oil production, and seed cake which could be used as feed or manure (Chin et al., 1977).

Rubber seed oil (RSO) is a yellow, semi-drying oil (Joseph et al., 2004). The oil does not contain any unusual fatty acids, and its rich source of essential fatty acids $C_{18:2}$ and $C_{18:3}$ make up 52% of its total fatty acid composition (Ghandhi et al., 1990).

However many studies that had been carried out in the rubber seed field indicated that the production of the RSO is facing various vital challenges and one of these challenges is the toxin, which can lead to problems. It is well-known, that

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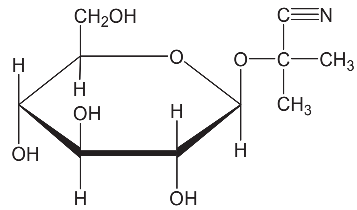

Figure 1 Molecular formula of linamarin.

concentration of poisons may always be found in the seeds of all types of plants such as *Jatropha curcas* whose seed contains the phorbol ester compound (Ahmed and Salimon, 2009). One of these plants that have toxic elements is the seed of the rubber plant (Duke and Ducellier, 1993).

We present the case of the rubber plant whose seeds contain a cyanogenic glycoside called linamarin (Butler, 1965; Lieberei et al., 1986). The molecular formula of linamarin is ($C_{10}H_{17}NO_6$) shown in Fig. 1. The IUPAC name of linamarin is Alpha-hydroxyisobutyonitrile-beta-D-glucopyranoside. The other name of linamarin is Phaseolunatin (Wikipedia 2007). Linamarin has been demonstrated to protect the plant from the herbivore by animals and generalized insect feeders (Siritunga and Sayre, 2004).

2. Materials and methods

2.1. Seed material and oil extraction

Fresh rubber seeds were collected from (RRI) Sungai Buloh. The rubber seeds were milled using a grinder (Okafor and Anyanwa, 2006). The seeds were kept in the refrigerator. RSO was extracted from 500 g rubber seeds by a soxhlet extractor using different solvents such as hexane (RSO_h), chloroform + methanol (RSO_{ch+mth}) and ethanol (RSO_{eth}) in the same extracting conditions at 60°C for 6 h.

2.2. Colorimetric method

2.2.1. Samples extraction and hydrolysis of HCN in sample

The samples of linamarin were extracted from three different RSO (100 g) by using water (20 mL) as a solvent in a separating funnel. After shaking the mixture gently, the mixture was left a few minutes to get two phases the oil phase, and the water phase. The oil phase was removed and the water phase was kept in the fridge. To conserve cyanide, the samples were supplemented with (4 mL) 10 M NaOH. The sample was distilled without further pretreatment. HCN was recovered in the presence of (10 mL) zinc acetate buffer pH 4.5. The remaining cyanide was subsequently recovered by distillation after the addition (5 mL) of $MgCl_2$ and (5 mL) sulfamic acid plus (5 mL) 50% H_2SO_4; the latter added to obtain pH 1–2 for converting to HCN during distillation (ASTM, 1998). After 3 min, 45 mL 50% H_2SO_4 was added and the solution boiled under reflux for 90 min (Bjarnholt et al., 2008; Miller et al., 2006; Dzombak et al., 2006). The released HCN was determined using a colorimetric method.

2.2.2. Determination of CN

The detection of small amounts of cyanide, colorimetric method based on the könig reaction was proposed by Mak et al. (2005). This method involves the oxidation of cyanide to cyanogens chloride with chloramines T. Cyanogen chloride was then reacted with pyridine to form N-cyanopyridinium chloride (könig reaction). N-cyanopyridinium chloride was then reacted with barbituric acid to produce blue color was measured at 578 nm (Mak et al., 2005; Nambisan and Sundaresan, 1984). This method was preferable for cyanide at 3.8×10^{-5} M. The same conditions were used to determine the (C≡N) in sodium cyanide (NaCN) which was used as a standard to compare it with HCN which was hydrolyzed from rubber seed and rubber seed oil samples.

2.3. Rats toxicological test

Male white rats (Rumah Haiwan Laboratories, Universiti Kebangsan Malaysia) weighing between 284 g were used and have been carried by Nwokolo et al., (1988). The male white rats were individually housed in stainless steel cages in a room with controlled temperature (30–35 °C) and lighting (alternative 12 h periods of light and darkness). The male white rats were fed for 3 months. The mortality, color, and the behavior of the male white rats were recoded daily but the food consumption was recorded every two weeks. The food consumption, food efficiency, body waist measurement and body tall measurement were also determined. Two experiments were conducted to determine the toxicological response of rats fed rubber seed oil. In experiment 1, rats were fed rubber seed oil that has been extracted by using hexane. In experiment 2, rats were fed rubber seed oil that has been extracted by using chloroform + methanol. The rubber seed oil was stored at 4°C for the feeding of rats. The toxicological evaluation of the rubber seed oil extracting by using two different solvents was carried out in male white rats by performing an acute oral toxicity limit test to assess its acute toxicity potential. The rats fed RSO were compared with rats fed with normal food.

3. Results and discussion

Three rubber seed oils were studied for cyanide determination. Commercial cyanide was used as a standard and was compared with the rubber seed oil which was extracted using different solvents such as hexane (RSO_h), chloroform + methanol (RSO_{ch+mth}) and ethanol (RSO_{eth}) in the same extracting condition.

The determination of cyanide demonstrated no response of the cyanide in rubber seed oil and did not show any colored comparing with commercial cyanide which observed a blue color. The commercial cyanide showed high response at 578 nm which is reported at Mak et al., (2005). The colorimetric method based on könig reaction showed no response for the detection of cyanide in rubber seed oil.

Toxicological evaluation of the RSO was carried out in white male rats by performing an acute toxicity limit test to assess its acute toxicity potential in 3 months feeding study. Three different types of rubber seed oil were extracted by using different solvents such as hexane (RSO_h), chloroform + methanol (RSO_{ch+mth}) and ethanol (RSO_{eth}) in the same extracting conditions. Table 1 shows the mortality, color and the behavior of male white rats. The results of the current study are in agreement with the above calorimetric method analysis. No

Table 1 Shows the mortality, color, and the behavior of the male white rats.

Activity	Blank/control	RSO_h [a]	RSO_{ch+mth} [b]	RSO_{eth} [c]
Color	White	No changes	No changes	No changes
Behavior	Normal	No changes	No changes	No changes
Mortality	No	No	No	No

[a] RSO was extracted using hexane as the solvent.
[b] RSO was extracted using mixture of chloroform and methanol as the solvent.
[c] RSO extracted using ethanol as the solvent.

Table 2 Shows the food consumption, body weight gain, food efficiency, body waist measurement and body tall measurement.

Activity	RSO_h	RSO_{ch+mth}	RSO_{eth}	Blank/Control
Initial body weight (g)	248	248	248	248
Final body weight (g)	518	517	515	516
Body weight gain (g)	270	269	267	278
Food consumption (g)	1800	1792	1783	1789
Food efficiency ratio	0.13	0.13	0.12	0.15
(weight gain/food intake)				
Initial body waist (cm)	14.5	14.5	14.5	14.5
Final body waist (cm)	20.5	20.5	20.3	20.2
Body weight gain (cm)	6.0	6.0	5.8	5.7
Initial body tall (cm)	23	23	23	23
Final body tall (cm)	24.5	24.2	24.2	24.3
Body tall gain (cm)	1.5	1.2	1.2	1.3
Condition*	Normal	Normal	Normal	Normal

Notes: Condition was assessed by visual appearance.

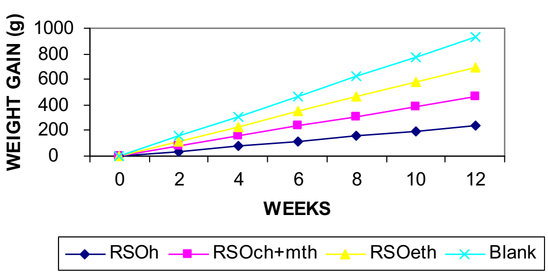

Figure 2 Body weight gain of rats fed RSO and the blank control.

acute toxic potential was observed with RSO. Male white rats did not display any behavioral changes and there was no mortality in any of the groups during the 3 months feeding study. The color of the male white rats did not appear to show any changes during the 3 month feeding study.

The RSO_h, RSO_{ch+mth} and RSO_{eth} had no adverse effect on food consumption. A similar increase in the average daily gain of rats fed RSO_h, RSO_{ch+mth} and RSO_{eth} was also observed; however, the differences between the 3 white male rats was not statistically significant. The 3 male rats showed no significant difference in body weight gain, food efficiency, body waist measurement and body tall measurement. The growth rate of 3 white male rats is shown in Table 2 and Fig. 2. These results would indicate that RSO_h, RSO_{ch+mth} and RSO_{eth} had no toxic or antipalatability effects (Gandhi et al. 1990 & Nwokolo et al., 1988).

4. Conclusion

The current study has shown that RSO could be considered for edible use. These initial results indicate that the use of RSO as an edible oil will not be restricted by toxic factors. The results showed that RSO which has been collected from (RRI) Sungai Buloh, Malaysia do not possess cyanogenic glycosides compared to other Malaysian rubber seed oils.

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