Nutritional Assessment of *Vernonia amygdalina* Leaves in Growing Mice

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The elemental analyses of the leaves of *Vernonia amygdalina* and the laboratory experiments with mice, fed diets containing *V. amygdalina* leaves, their alcohol extracts, or purified saponins, were performed. Feeding 2-week-old growing mice of both sexes with the standard diets amended with 25% dry *V. amygdalina* leaves or equivalent amounts of alcohol extracts or crude or purified saponins for 14 days did not alter their feeding performance. However, these treatments caused significant reduction in body weight gain and increased urinary and fecal output, compared with the control group. At necropsy, the liver weights were reduced. The stomachs and small intestines were enlarged, compared to the control groups. It was concluded that care has to be taken when using the leaves for cooking soups and that saponins should be thoroughly washed out during the debittering process. Consumption of tonics containing *V. amygdalina* saponins may create some health hazard.

Keywords: *V. amygdalina; leaves; stigmastane saponins; antinutritional activity; mice*

INTRODUCTION

Vernonia amygdalina (Compositae) is a small tree between 1 and 3 m in height, which grows throughout tropical Africa. Leaves of this plant are used in Nigeria as a green vegetable or as a spice in soup, especially in the popular bitter-leaf soup. To prepare such soups, the freshly harvested leaves are macerated with either cold or hot water to reduce the bitterness of the leaves to a desired level. The bitter water extract is taken as a tonic to prevent certain illnesses.

The tops of this plant are also used in folk medicine as antihelmints, antimalarials, laxatives, and fertility inducers in barren women. Usually decoctions are prepared with local alcohol. In Tanzania, some wild chimpanzees were observed to use this plant for the treatment of parasite-related diseases.

The chemical nature of the active principles of *V. amygdalina* has not been completely documented. Luteolin and its 7-*O*-glucoside and 7-*O*-glucuronide were recently identified in the leaves, and their antioxidant activity was measured (Igile *et al.*, 1994). Some stigmastane-type steroidal saponins were identified (Ohigashi *et al.*, 1991; Kamperdick *et al.*, 1992), and some of them were documented to have bitter taste. No comprehensive biological activity or feeding tests were performed on these compounds. Phillipson *et al.* (1993) reported that some sesquiterpene and steroidal constituents of *V. amygdalina* were active against *Plasmodium falciparum*.

The knowledge of the presence of active constituents in this plant and the wide use of its tops for food purposes or in folk medicine necessitated this study. Hence, the aim of the present work was to study the nutritional performance of the leaves and its saponin constituents in laboratory mice, as a beginning to determining the possible effects of feeding on *V. amygdalina*. The obtained data may serve for adequate nutritional education and awareness for the affected populations.

MATERIALS AND METHODS

Plant Material. The leaves of *V. amygdalina* were harvested fresh from a horticultural garden in Ibadan (Nigeria) and air-dried, according to traditional method. It was botanically identified by Dr. J. Lowe, of the Botany Department, and a voucher sample was deposited in the Department of Biochemistry, College of Medicine, University of Ibadan, Nigeria.

Sample Preparation. Six hundred grams of the dried, powdered leaves was exhaustively extracted with MeOH for 60 h in a Soxhlet apparatus. The methanolic extract was dried *in vacuo* (169 g). Half of this was directly lyophilized and named crude extract (CE). The remaining half was semipurified by column chromatography. The CE (84.5 g) was loaded onto a C_{18} (3 × 5 cm, 25–40 μm, Merck) column and washed with 30% MeOH. The 30% MeOH eluate contained no saponins, as monitored with thin layer chromatography (TLC), and was discarded. TLC was performed on silica gel ready to use plates (Merck) developed in ethyl acetate/acetic acid/water (7:2:2) or chloroform/methanol/water (65:30:5), and saponins were made visible by spraying with methanol/sulfuric acid/acetic anhydride (5:1:1, L-B reagent) followed by heating at 105 °C. Saponins retained on the column were washed out with MeOH. Evaporation of the solvent yielded a brownish powder (8.4 g) recognized as crude saponins (CS).

The CS (4.2 g) was further purified on a C_{18} column (as above). The column was washed subsequently with 200 mL of methanol/water mixtures (50:50; 60:40, and 70:30 v/v). The 50% MeOH eluted a single compound (1.35 g), identified as vernonioside D (Figure 1) by FAB-MS and NMR (Igile *et al.*, 1995) and referred to as compound PS. The 60% and 70% MeOH eluted mixtures of other compounds also identified to be saponins which were not further investigated in the present work.

Elemental Analysis. Levels of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) were determined after mineralization of the dry, powdered leaves with concentrated sulfuric acid and hydrogen peroxide. Nitrogen was determined according to the Kjeldahl method using an autoanalyzer (Technicon), and the protein conversion factor was 6.25.

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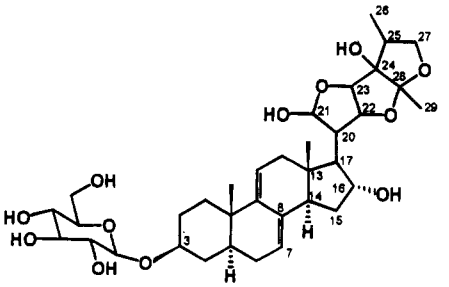

Figure 1. Chemical formula of vernonioside D, dominant stigmastane-type saponin of *V. amygdalina* leaves.

Protein nitrogen content was determined in protein precipitated with copper sulfate ($CuSO_4$).

Ash was measured by calcination at 500 °C to a constant weight.

Fat was determined after CCl_4 Soxhlet extraction of a weighed sample and subsequent gravimetry.

Total dietary fiber content was determined according to the AOAC procedure of Prosky *et al.* (1988) in quadruplicate, so that nitrogen and ash contents of the fiber residues could be determined in duplicate.

Phosphorus was determined with the Technicon autoanalyzer in parallel with nitrogen.

Potassium and sodium were determined by flame photometry (Digital Flame Photometer, Omszov, Hungary). Magnesium and calcium were determined by atomic absorption spectrometry (AAS 1N, Carl Zeiss Jena, Germany). Nitrate (NO_3) content was determined according to the method of Harberts and Thijsen (1960).

Standardization of Animals for the Study. Fifty-five mice of both sexes, weighing between 15 and 18.5 g, aged 2 weeks, were housed in the primate colony of the Department of Biochemistry, IUNG, Puławy, Poland.

Prior to feed modification, each animal was individually housed, in a metabolic cage, in a temperature controlled room (22 °C), completely ambient, with an alternating 12-h light/dark cycle. Three days were allowed for animals to adapt to the diet and experimental conditions. They were fed on mouse standard diet (SD) consisting of protein- and vitamin-enriched pellets (Murigran, Poland; crude protein, 24%; fat, 2.8%; fiber, 3.8%; ash, 3.5%; Ca, 1.2%; P, 0.8%, Mg, 0.18%; no cholesterol), low-fat milk, and distilled water. The pellets were homogenized to powder form and presented as such to the animals (*ad libitum*) so that animals did not select parts of the food while rejecting some. Their body weights and urinary outputs were monitored during this period.

At the end of the conditioning period, animals were randomly distributed into 10 groups of 5 or 6 animals of both sexes. All animals were again housed individually in metabolic cages, and specific diets were presented to them immediately.

Diet Preparation. Nine separate diets were formulated, including the standard mouse feed. Diets were constituted from modifications of those used by Nestares *et al.* (1993), Malinow *et al.* (1980), and Oakenfull *et al.* (1979) to reflect the aim of this study.

Group 1: SD + PL (powdered leaves, 25%).
Group 2: SD + CE (crude extract, 8%).
Group 3: SD + CS (crude saponin, 0.33%).
Group 4: SD + CS (0.7%).
Group 5: SD + CS (1.07%).
Group 6: SD + PS (pure saponin, 0.33%).
Group 7: SD + PS (0.7%).
Group 8: SD + PS (1.07%).
Group 9: SD.

Body Weight and Urinary and Fecal Output Monitoring. Body weights were taken daily on a sensitve, top loading balance (SPA, Poland). Urine was monitored as change in urine weight, while fecal output and texture were visually examined as excess pellets per animal per cage.

Table 1. Composition of *V. amygdalina* Leaves

component	content (% of dry matter)	component	content (% of dry matter)
dry matter	91	ash	10.75
nitrogen (N)	2.94	nitrate (NO_3)	0.065
crude protein (N × 6.25)	18.38	phosphorus (P)	0.26
		potassium (K)	2.88
protein	16.23	magnesium (Mg)	0.35
reducing sugars	5.30	calcium (Ca)	1.08
fiber	8.53	sodium (Na)	0.022
fat	5.83		

Liver Weights and Cholesterol Measurements. At the end of the experiment, animals were ether anesthetized and a lateral section was cut through each.

Plasma blood was taken from the aortic junction of the heart and immediately centrifuged to obtain serum for cholesterol determination.

Livers were excised, blotted dry, weighed, and then freeze-dried. Each liver was separately crushed to fine powder and extracted with 0.3 mL of chloroform. These extracts were centrifuged to obtain supernatant for cholesterol determination. Cholesterol was then determined in blood serum and in liver extracts with the method of Rudel and Morris (1973).

Statistical Analyses. The results from all experiments and analyses were tested statistically by linear regression and by analysis of variance with the one-way procedure of the STATGRAPHICS/PC software package. Means were compared with Tukey's test.

RESULTS

Chemical Composition. The chemical composition of *V. amygdalina* leaves is shown in Table 1. Extraction of the material with MeOH yielded CE with the efficiency of about 30% in relation to the dry matter of the powder. Solid-phase extraction–purification on C_{18} support provided the fraction of crude saponins (CS). On the basis of the efficiency of extraction–purification the amount of saponins in dry matter of *V. amygdalina* leaves was found to be around 2.8%. Further separation of CS using selective solid-phase extraction yielded at the 50% concentration of MeOH the fraction containing a single, dominant compound (PS) showing saponin-like characteristics (foaming, color reaction with L-B reagent), identified as vernonioside D (Figure 1). This saponin was strongly bitter as evaluated by laboratory staff volunteers.

Biological Analyses. *Body Weight Gains.* Daily feed intake during the 3 day period when animals were preconditioned for experiments was determined to be between 2.5 and 3.0 g/day. This daily ration was also consumed throughout the whole experiment regardless of the diet provided *ad libitum*, and thus all of the changes in body weight gains were not due to reduced dietary intake.

After 2 weeks on standard diet, the mice in the control group gained approximately 6 g (Table 2). The linear regression slope for this group was +0.36. The body weights of mice from groups 3 and 6, fed the lowest concentrations of crude saponins or vernonioside D, did not change throughout the duration of the experiment, whereas the body weights of animals in the other groups were strongly reduced. The characteristic sequence observed regardless of the diet was an initial 3–5 day increase in body weight, followed by a sharp drop during the next 5–6 days, and then a plateau reached toward the end of the experiment.

Some deaths of animals were recorded. Groups 1 and 5 each lost a mouse on the 11th day, while two mice died on the 14th day in group 8.

Table 2. Daily Body Weights (Grams) of Mice Fed Diets Amended with *V. amygdalina* Preparations[a]

	group of mice								
days of treatment	1	2	3	4	5	6	7	8	9
0	17.65 a	16.1 a	16.8 a	16.1 bc	18.2 bc	18.5 a	17.6 ab	16.8 bc	16.0 a
1	18.37 a	16.6 a	17.6 a	17.1 b	19.6 a	19.7 a	18.5 ab	18.1 ab	16.9 ab
2	17.00 ab	15.8 ab	17.6 a	17.4 a	19.7 a	19.9 a	18.5 ab	18.4 ab	17.6 ab
3	15.93 b	15.3 ab	18.0 a	17.4 a	20.0 a	21.6 a	19.6 a	19.9 a	19.2 ab
4	15.8 b	15.4 b	18.3 a	17.2 ab	19.4 ab	20.9 a	19.3 ab	18.9 ab	19.5 ab
5	14.4 c	14.8 bc	17.2 a	16.3 bc	18.3 bc	19.0 a	17.8 ab	17.2 bc	19.8 ab
6	15.0 bc	15.8 b	17.7 a	16.9 bc	18.3 a	19.8 a	19.2 ab	18.8 ab	20.3 ab
7	13.9 cd	14.7 bc	17.0 a	15.8 cd	17.3 cd	18.7 a	17.8 ab	17.0 bc	20.66 ab
8	12.8 d	14.2 c	16.7 a	15.7 cd	16.5 cd	18.3 a	18.4 ab	16.8 bc	20.8 ab
9	12.8 d	13.8 cd	16.1 a	15.2 cd	15.2 de	20.9 a	18.2 ab	15.7 c	20.9 ab
10	13.3 cd	13.3 d	16.3 a	14.9 d	14.7 de	18.8 a	17.3 ab	15.6 c	21.1 ab
11	13.3 cd	13.9 c	15.5 a	13.9 d	14.0 e	18.7 a	17.0 b	15.2 c	22.0 b
12	13.2 cd	14.3 c	16.2 a	14.9 d	14.5 de	19.1 a	17.0 b	15.1 c	21.6 b
13	13.9 cd	14.4 bc	16.4 a	15.0 cd	14.4 de	18.9 a	17.0 b	15.4 c	21.7 b
14	13.2 cd	14.2 c	16.0 a	14.8 d	14.7 de	18.9 a	17.2 ab	15.4 c	22.0 b
regression slope value	−0.53	−0.22	−0.14	−0.22	−0.76	−0.07	−0.16	−0.23	+0.36

[a] Values within columns followed by different letters differ (<0.05).

Table 3. Urinary Output of Mice Fed the Diet Amended with *V. amygdalina* Preparations (Milligrams per Mouse per Day)[a]

	group of mice								
days of treatment	1	2	3	4	5	6	7	8	9
0	0.776 ab	0.766 a	0.770 bc	0.726 a	0.806 a	0.840 ab	0.780 a	0.806 a	0.830 a
1	0.750 a	0.773 ab	0.747 a	0.743 ab	0.810 a	0.813 a	0.773 a	0.803 a	0.830 a
2	0.746 a	0.790 ab	0.757 ab	0.753 ab	0.830 ab	0.826 ab	0.780 a	0.837 a	0.833 ab
3	0.746 a	0.820 ab	0.766 bc	0.743 ab	0.840 ab	0.847 bc	0.790 a	0.853 ab	0.830 a
4	0.763 a	0.857 ab	0.766 bc	0.743 ab	0.926 bc	0.846 bc	0.877 b	0.887 ab	0.840 ab
5	0.773 ab	0.910 bc	0.766 bc	0.773 ab	1.020 c	0.843 ab	0.900 bc	0.913 ab	0.843 ab
6	0.806 bc	0.950 cd	0.773 bc	0.793 ab	1.200 d	0.843 ab	0.937 cd	0.983 bc	0.840 ab
7	0.870 c	1.050 d	0.773 bc	0.823 bc	1.223 de	0.843 ab	0.953 cd	1.033 cd	0.843 ab
8	0.943 d	1.233 e	0.760 ab	0.856 cd	1.300 de	0.857 bc	0.967 d	1.037 cd	0.853 bc
9	0.967 d	1.267 e	0.760 ab	0.883 de	1.333 ef	0.850 bc	0.980 d	1.153 d	0.853 bc
10	0.983 d	1.333 e	0.777 c	0.953 e	1.367 f	0.860 c	0.993 d	1.167 d	0.857 c
av STD	0.03	0.06	0.01	0.04	0.05	0.02	0.03	0.09	0.01
Δ output[b]	0.207	0.567	0.007	0.227	0.561	0.020	0.213	0.361	0.017

[a] Values within a column followed by different letters differ (p, 0.05). [b] Urine output difference between last and first day of experiment.

Table 4. Liver Weights and Liver and Plasma Cholesterol Concentration in Mice Fed *V. amygdalina* Preparations

group of mice	liver weight (g + SD[a])	liver cholesterol (% DM[b])	plasma cholesterol (mg/dL)
1	0.89 ± 0.088	0.222 ± 0.04	25.8 ± 8
2	0.80 ± 0.1	0.213 ± 0.06	26.3 ± 8
3	0.96 ± 0.17	0.274 ± 0.06	63.2 ± 14
4	0.95 ± 0.09	0.220 ± 0.09	52.1 ± 21
5	0.93 ± 0.1	0.110 ± 0.04	34.5 ± 16
6	1.37 ± 0.1	0.374 ± 0.1	81.6 ± 20
7	1.35 ± 0.08	0.333 ± 0.1	62.8 ± 17
8	1.2 ± 0.05	0.190 ± 0.07	43.0 ± 15
9	1.51 ± 0.07	0.442 ± 0.17	95.0 ± 17

[a] SD, standard deviation. [b] DM, Dry matter.

Urinary and Fecal Output. The average urinary output, measured before the experiment, was 0.8 g/day. For groups 3, 6, and 9, this stayed almost constant throughout the 10 days of urine observation (Table 3). In all other treatments, urination increased significantly, being highest in groups 2, 5, and 8. Fecal output, as evaluated by the number of pellets, followed a similar pattern.

Liver Weights. At necropsy, the livers from those given the standard diet averaged 1.51 g (Table 4). Liver weights were slightly reduced (1.2−1.37 g) for mice given purified saponin (groups 6−8) but were drastically reduced (0.93−0.96 g) for mice given powdered plant material, CE and CS. There was no significant dependence of liver weights on the concentration of CS and PS.

Liver and Plasma Cholesterol. Plasma cholesterol concentration in the control group averaged 95 mg/100 mL, and liver cholesterol was 0.442% (Table 4). Feeding PS and CS significantly reduced the cholesterol level both in liver and in serum. The reduction was correlated to saponin concentration. The highest reduction in liver and serum cholesterol was observed in mice fed plant material or methanolic extract.

DISCUSSION

V. amygdalina has recently attracted some attention due to its possible pharmaceutical use. It was observed that some apparently sick chimpanzees chewed the shoot of the shrub to extract the bitter juice (Ohigashi *et al.*, 1991). After this feeding habit, the animals seemed to return to their normal activity. In West Africa, e.g. Nigeria, leaves of *V. amygdalina* after debittering are used for soup and the extracts are utilized as a tonic. Indeed, as shown in the present study, leaves of *V. amygdalina* are rich in protein and other nutritionally important components. In countries where the protein supply is limited, leaves of *V. amygdalina* may be a good source of it.

However, *V. amygdalina* contains a wide spectrum of physiologically or biologically active compounds, which could be antifeedant principles. Thus, including

this plant in the diet may cause some unpredictable side effects. In the present feeding experiment, the amendment of basic diet with 25% of *V. amygdalina* caused severe changes in mouse growth performance. Significant body weight reduction accompanied with increase in urinary and fecal output was observed in all animals; in some cases even the death of animals occurred. At necropsy, at the end of the experiment, liver weights and plasma and liver cholesterol concentrations were shown to be reduced drastically. The stomachs and small intestines were larger compared to those of animals fed the basic diet. These symptoms may reflect perturbations in cholesterol and lipid metabolism, as well as in nutrient absorption along the gut. Similar patterns of animal performance were observed when lyophilized methanolic extract was mixed into the diet at a percentage almost equivalent to 25% of dry leaves.

These detrimental effects indicated the presence, both in leaves and in extracts, of some chemical principles showing biological activity. In the present work, attention has been focused on steroidal saponins and their biological effects. Their presence in *V. amygdalina* has been recently reported (Ohigashi *et al.*, 1991; Kamperdick *et al.* 1992; Jisaka *et al.*, 1992, 1993). They were isolated and purified by solid-phase extraction and used in this feeding experiment. Concentration of total crude saponins in plant material estimated on the efficiency of their extraction was about 2.8% in dry matter. Hence, the lowest saponin dose (groups 3 and 6) used in experiments with mice was calculated to be equivalent to half (12.5%) of that of plant material as described above. In the second (groups 4 and 7) and third (groups 5 and 8) doses mice were given the amount of saponins equivalent to 25% and 37.5% of plant material in the diet, respectively.

The single saponin isolated from the mixture was a dominant compound that made up 35% of total saponins. This compound was purified and characterized as vernonioside D by spectroscopic techniques. This single saponin was also used in the feeding experiment at the dose identical to total crude saponins.

The lowest concentrations of both CS and PS had identical influence on mouse growth performance. In these treatments body weights were stable, showing neither increase nor decrease throughout the experiment. Urinary and fecal output showed similar patterns as in the control group. However, liver weights and plasma and liver cholesterol concentrations were significantly reduced, and these effects were more pronounced for CS than for PS.

At higher saponin concentrations all measured parameters were influenced; the body weights were reduced, urinary and fecal output increased, and liver weights and liver and plasma cholesterol concentrations were drastically reduced. At the highest saponin concentration, the measured parameters had values similar to those obtained for plant material and crude extract treatments.

This evident influence of saponins, showing concentration dependent effects, suggests that saponins are one of the predominant principles of *V. amygdalina*, responsible for its physiological effects. Their effect on mouse growth performance was similar to those reported for several species of animals ingesting saponins from different plant sources (Malinow *et al.*, 1981, 1982; Oakenfull *et al.*, 1979; Morgan *et al.*, 1972). However, the activity of *V. amygdalina* steroidal saponins seems to be much higher than that of any other saponins from plant source. In comparison, ingestion of alfalfa top saponins in nonhuman primates at 1.0% and 1.2% levels of substitution in the diet was harmless (Malinow *et al.*, 1982). In the present study *V. amygdalina* saponins were used at 0.33%, 0.7%, and 1.07% levels, and none of the concentrations could be recognized as safe. At the lowest concentration no growth retardation was observed but liver weights and cholesterol concentrations were affected. This lowest dose corresponds to 0.55 g of saponins/kg of body weight. To be exposed to such a dose, considering the reaction is similar to the effect in mice, a 60 kg man would need to consume about 1 kg of *V. amygdalina* leaves per day to be on the safe side. Thus, it seems reasonable to conclude that *V. amygdalina* leaves used for cooking soup, pretreated with water to remove bitterness, create some hazard for the consumer. The debittering process should be completed carefully so that most of the saponins are removed. This special care is necessary if we recognize the fact that *V. amygdalina* saponins are sparingly soluble in water. On the other hand, it seems that the small amount of saponins retained after the debittering process may be enough to regulate the body weights of the consumers, especially those with high susceptibility. Very special care should be recommended when alcohol extracts are used for the treatment of different illnesses. Since the dose and the duration of consumption of extracts is undefined, especially in disease states, it is apparent that excess consumption may be injurious to the consumer. Thus, any medical application of these compounds or plant extracts needs closer study on the long-term effects, so that their application may be precisely established at a safe level.

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LITERATURE CITED

Harberts, C. Z.; Thijsen, U. G. Determination of nitrate in plant material. *Landwirtschaft. Forschung.* **1960**, *13*, 128–134.

Igile, G. O.; Oleszek, W.; Jurzysta, M.; Burda, S.; Fafunso, M.; Fasanmade, A. A. Flavonoids from *Vernonia amygdalina* and their antioxidant activity. *J. Agric. Food Chem.* **1994**, *42*, 2445–2448.

Igile, G. O.; Oleszek, W.; Jurzysta, M.; Aquino, R.; Tommasi, N.; Pizza, C. Vernoniosides D and E, two novel saponins from *Vernonia amygdalina* Del. (Compositae). *J. Nat. Prod.* **1995**, in press.

Jisaka, M.; Ohigashi, H.; Takagaki, T.; Nozaki, H.; Tada, T.; Hirota, M.; Irie, R.; Huffman, M. A.; Nishida, T.; Kaji, M.; Koshimizu, K. Bitter steroid glucosides, vernoniosides A_1, A_2 and A_3 and related B_1 from a possible medicinal plant, *Vernonia amygdalina* used by wild chimpanzee. *Tetrahedron* **1992**, *48*, 625–630.

Jisaka, M.; Ohigashi, H.; Takegawa, K.; Hirota, M.; Irie, R.; Huffman, M. A.; Koshimizu, K. Steroid glucosides from *Vernonia amygdalina*, a possible chimpanzee medicinal plant. *Phytochemistry* **1993**, *34*, 409–413.

Kamperdick, C.; Breitmaier, E.; Radloff, M. A. A new steroid saponin from *Vernonia amygdalina* Del. (*Compositae*). *J. Prakt. Chem.* **1992**, *334*, 425–428.

Malinow, M. R.; McLaughlin, P.; Stafford, C. Alfalfa seeds: effects on cholesterol methabolism. *Experientia* **1980**, *36*, 562–565.

Malinow, M. R.; Connor, W. E.; McLaughlin, P.; Stafford, C.; Lin, D. S.; Livingston, A. L.; Kohler, G. O.; McNulty, W. P.

Cholesterol and bile acids balance in *Macaca fascicularis* effets of alfalfa saponin. *J. Clin. Invest.* **1981**, *67*, 156–162.

Malinow, M. R.; McNulty, W. P.; Houghton, D. C.; Kessler, S.; Stenzel, P.; Goodnight, S. H.; Bardana, E. J.; Palotay, J. L.; McLaughlin, P.; Livingston, A. L. Lack of toxicity of alfalfa saponins in cynomolgus macaques. *J. Med. Primatol.* **1982**, *11*, 106–118.

Morgan, B.; Heald, M.; Brooks, S. G.; Tee J. L.; Green, J. The interactions between dietary saponins, cholesterol and related sterols in the chick. *Poult. Sci.* **1972**, *51*, 677–682.

Nestares, T.; Lopez-Jurado, M.; Sanz, A.; Lopez-Friaz, M. Nutrition assessment of two protein concentrates in growing rats. *J. Agric. Food Chem.* **1993**, *41*, 1282–1286.

Oakenfull, D. G.; Fenwick, D. E.; Hood, R. L.; Topping, D. L.; Illman, R. L.; Storer, G. B. Effects of saponins on bile acids and plasma lipids in the rat. *Br. J. Nutr.* **1979**, *42*, 209–216.

Ohigashi, H.; Jisaka, M.; Takagaki, T.; Nozaki, H.; Tada, T.; Huffman, M. A.; Nishida, T.; Kaji, M.; Koshimizu, K. Bitter principle and related steroid glucoside from *Vernonia amygdalina*, a possible medicinal plant for wild chimpanzee. *Agric. Biol. Chem.* **1991**, *55*, 1201–1203.

Phillipson, J. D.; Wright, C. W.; Kirby, G. C.; Warhurst, D. C. Phytochemistry of some plants used in traditional medicine for the treatment of protozoal disseases. *Abstracts,* International Symposium of the Phytochemical Society of Europe; Lausanne, 1993; p L3.

Prosky, L.; Asp, N. G.; Schweizer T. F.; DeVries, J. W.; Furda, J. Determination of insoluble, soluble and total dietary fiber in foods and food products: interlaboratory study. *J. Assoc. Off. Anal. Chem.* **1988**, *71*, 1017–1023.

Rudel, L. L.; Morris, M. D. Determination of cholesterol using o-phtaladehyde. *J. Lipid Res.* **1973**, *14*, 364–366.

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