The Oil of *Adenanthera pavonina* L. Seeds and its Emulsions

Robert Zarnowski[a,§,*], Anna Jaromin[b], Milan Certik[c], Tibor Czabany[c],
Joël Fontaine[d], Tibor Jakubik[e], Mohamed C. M. Iqbal[f],
Anne Grandmougin-Ferjani[d], Arkadiusz Kozubek[b], and Stanislaw J. Pietr[a]

[a] Department of Agricultural Microbiology, Agricultural University, Wroclaw, Poland
[§] Present address: Department of Medical Microbiology and Immunology, 424 Medical Science Centre, University of Wisconsin – Madison, 1300 University Ave, Madison, WI 53706, USA. Fax: +1-(608)-2656717. E-mail: robert@plantpath.wisc.edu
[b] Department of Lipids and Liposomal Technology, Institute of Biochemistry and Molecular Biology, University of Wroclaw, Wroclaw, Poland
[c] Department of Biochemical Technology, Slovak University of Technology, Bratislava, Slovak Republik
[d] Laboratoire de Mycologie/Phytopathologie/Environment, Université du Littoral-Côte d'Opale, Calais Cedex, France
[e] Central Laboratories of Faculty of Chemical and Food Technology, Slovak University of Technology, Bratislava, Slovak Republik
[f] Plant Reproductive Biology, Institute for Fundamental Studies, Kandy, Sri Lanka

* Author for correspondence and reprint requests

The oil of *Adenanthera pavonina* L. seeds was analysed by chromatographic and instrumental means. The oil was found to be rich in neutral lipids (86.2%), and low in polar lipids (13.8%). The neutral lipids consisted mainly of triacylglycerols (64.2%). Unsaturated fatty acids were found as high as 71%, while the percentage of saturated fatty acids was only 29%. GC and GC/MS analyses revealed linoleic, oleic and lignocerotic acid to be predominant among all fatty acids in the *A. pavonina* oil, whereas stigmasterol was the major steroid identified within this study. Subsequently, the oil was used for preparation of submicron oil-in-water (o/w) lipid emulsions. Lipid emulsions were formulated by using soybean lecithin (SL) to investigate their particle size, Zeta potential and stability at the different oil and SL ratios. The results obtained indicate possible applications of the tested oil in pharmaceutical and medical fields as drug and cosmetic active ingredient carriers.

Key words: Indian Red Wood, Leguminosae, Nanoemulsions

Introduction

Continuing our research on dicotyledonous plant seed lipids (Zarnowski and Kozubek, 1999; Zarnowski *et al.*, 2001), we have focused our attention on a tropical deciduous tree *Adenanthera pavonina* (L.) (family Leguminosae, subfamily Mimosoideae). This species is endemic to Southern China and India. It has been widely naturalized in Malaysia, Western and Eastern Africa as well as in most islands of both the Pacific and Caribbean regions. This plant is extensively cultivated as a valuable agroforestry species esteemed for fuelwood production, however there are also some historical accounts of using this tree for traditional medicines (Burkill, 1966; Watt and Breyer-Brandwijk, 1962). Its wood is hard and has a red-coloured heartwood and a light-grey sapwood making it useful for constructing decorative wood products (Benthall, 1946; Clark and Thaman,

1993). *A. pavonina* is also known as a "food tree", because its seeds are often eaten by people. Nutritional studies have proven one quarter of the seed to be oil with a high percentage of proteins (Burkill, 1966). To our best knowledge, there have been very little studies published previously that examined chemicals accumulated in *A. pavonina* seeds. Those investigations led only to the isolation of a few protein trypsin inhibitors (Prabhu and Pattabiraman, 1980; Richardson *et al.*, 1986; Lam *et al.*, 1999), flavonoids (Gennaro *et al.*, 1972), fatty acids (FAs) (Kabele-Ngiefu *et al.*, 1975; Balogun and Fetuga, 1985; Sotheeswaran *et al.*, 1994), triterpenoids (Yadav *et al.*, 1976) and carbohydrates (Moreira *et al.*, 1998). In the present work, we show detailed compositions of lipids present in the oil of *A. pavonina* seeds. Moreover, a possible use of this oil for a submicron oil-in-water (o/w) lipid emulsion formulation is also described.

Materials and Methods

Plant material and extraction

Mature seeds of *A. pavonina* were collected in the Kandy district, Sri Lanka. A 750-g sample of seeds was extracted with acetone as described elsewhere (Zarnowski and Kozubek, 1999). Briefly, the volume of the solvent sufficient to soak the seeds completely was used. The extraction was carried out three times for 24 h at room temperature each time using a fresh solvent. The obtained extracts were combined, filtered through a filter paper to remove any solid contaminating particles and concentrated under reduced pressure. This procedure yielded about 30 ml of the oil that was stored at – 20 °C until further laboratory analyses.

Lipid fractionation

Total lipids (TL) were fractionated by TLC. To separate polar lipids (PL), 5% solution of the extract dissolved in $CHCl_3/MeOH$ (1:1, v/v) was applied to TLC plates (Silica gel 60, 20 cm × 20 cm, 0.5 mm layer; Merck, Darmstadt, Germany) and developed with solvent system $CHCl_3/(CH_3)_2CO/MeOH/CH_3COOH/H_2O$ (50:20:10:10:5, v/v) (Certik and Shimizu, 2000). The spots were visualized by I_2 vapor and identified by comparison of their R_f values with the known standards. Neutral lipids (NL) were removed from the plates, eluted from silica gel with $CHCl_3/MeOH$ (1:1, v/v) and applied to TLC plates. NL were separated by solvent system $C_6H_6/(C_2H_5)_2O/CH_3COOH$ (70:30:1, v/v/v) (Certik *et al.*, 1996). After visualization with I_2 vapor the spots were identified by comparison of their R_f values with known standards. All visible polar and neutral components were scraped off (I_2 vapor and water traces were removed under reduced pressure) and extracted from silica gel twice with $CHCl_3/MeOH$ (1:1, v/v). Organic phases were pooled (silica gel was removed by centrifugation), the solvent was evaporated under reduced pressure and lipids were further analyzed.

Fatty acid analysis

Triplicates of individual lipid classes were prepared during a lipid fractionation procedure to assess reproducibility of TL composition and fatty acid (FA) determination as well. FAs from TL and lipid structures were analyzed as their methyl esters (FAME) (Christopherson and Glass, 1969) by GC using a CHROM 5 column (1.8 m × 2 mm)

packed with diethyleneglycol succinate (15% w/w + 3% w/w H_3PO_4 on Chromaton NAW DMCS; Supelco, Bellefonte, PA, USA) (Sajbidor *et al.*, 1994). Identification of the FAME peaks was performed using the authentic standards (Supelco). FAs were also verified by GC/EI-MS with a KRATOS Analytical (Manchester, UK) MS 25 RFA mass spectrometer equipped with a Carlo Erba (Milan, Italy) model 5160 gas chromatograph. A J & W Scientific (Folsom, CA, USA) fused-silica capillary column (ϕ 0.32 mm × 30 m) with DB-5MS coating (0.25 μm) was used. He (1 ml min^{-1}) was used as a gas carrier. The injector temperature was 250 °C. The GC oven was programmed as follows: 100 °C, then at rate of 8 °C min^{-1} up to 250 °C. For EI-MS, the ion source temperature was 250 °C, the electron energy was 70 eV, ionization current 100 μA and the scan speed was 0.6 s per decade. The reproducibility was in the range 92–96%.

Sterol isolation and analysis

The oil obtained from studied seeds was re-extracted and subsequently analysed according to a procedure described elsewhere (Grandmougin-Ferjani *et al.*, 1999). Briefly, sterols (STs) from the oil were extracted by direct saponification with 2 ml of 6% (w/v) KOH in MeOH for 1 h at 90 °C. After addition of 1 vol of H_2O, the unsaponifiable fraction was extracted three times with 3 vol of C_6H_6. STs were acetylated and steryl acetates in the final extract were analyzed by FID-GC equipped with a glass capillary column (DB5, ϕ 0.25 mm × 30 m, 2 ml of H_2 min^{-1}). The temperature program used includes a fast rise from 60 °C to 270 °C (30 °C min^{-1}), then a slow rise from 270 °C to 310 °C (2 °C min^{-1}). Steryl acetates were quantified with cholesterol as an internal standard. Each compound was identified by its retention time with regard to free cholesterol.

Preparation of emulsions

The preparation of lipid o/w emulsions was performed as follows. Appropriate amounts of soybean lecithin (SL) (Lipoid S 75; Lipoid, Ludwigshafen, Germany) were dissolved in the oil extracted from *A. pavonina* seeds. Then, 2 ml of aqueous phase comprised of 2.25% (w/v) glycerol (Sigma, Poznan, Poland) was mixed with the oil phase, gently heated and vortexed for 5 min. The sample was further sonicated for 10 min with an

ultrasonic cell disruptor (Microson™) at 4 W. The sonicated preparations were filtered through a sterile Acrodisc® 0.2 μm filter (Gelman Sciences, Ann Arbor, MI, USA). Each formulation was produced 3-fold.

The formulations were submitted to a 4-week stability test at 4 °C. Determinations of the size of the emulsions, stored in the dark, were evaluated at time intervals of 7, 14, 21 and 28 d or until instability was detected.

Measurement of particle size and Zeta potential

The mean particle size and the size distribution of the emulsion dispersion were determined by photon correlation spectroscopy (PCS) using a ZetaSizer 5000 (Malvern, Warsaw, Poland) and the volume distribution algorithm. For the analysis 15 μl of emulsions were added to 2 ml of 2.25% (w/v) aqueous glycerol solution.

The surface charge (Zeta potential) of the emulsions was measured using a Malvern ZetaSizer 5000. 3 μl of each emulsion formulation was diluted with 2 ml of 2.25% (w/v) aqueous glycerol solution.

Results and Discussion

The oil extracted from *A. pavonina* seeds had a pale yellowish colour and fragrant odour. It became solid when stored at or below 4 °C. The compounds present in this oil could be identified unambiguously by direct comparison of the data obtained with standards as well as with on-line

available chemical databases. This acetone-extracted oil consisted mainly of NL (86.2%), and amongst them triacylglycerols (TAG) were the most abundant group (64.2%) of the components. Free FAs represented 9.7% of TL, whereas 1,2-diacylglycerols (1,2-DAG) and 1,3-diacylglicarols (1,3-DAG) were present in minor amounts (5.9 and 3.7%, respectively). The content of PL amounted only 13.8%. In this case, the most abundant fraction consisted of phosphatidylcholine (PC) (9.5%). Phosphatidylethanolamine (PE) and lysophosphatidylcholine (LPC) represented only 3.0 and 1.0%, respectively, of total *A. pavonina* lipids isolated.

The subsequent examination of FAs revealed their different distribution among various lipid classes (Table I). The presence of lignoceric acid ($C_{24:0}$) was the most characteristic feature of the oil analyzed. This data are in a good agreement with previously published reports (Kabele-Ngiefu *et al.*, 1975; Balogun and Fetuga, 1985), however cerotic acid ($C_{26:0}$) was not detected in the present study. This fact may be a direct consequence of the use of different methodological approaches in our and previously reported studies. On the other hand, it may be also considered as an indication of various chemotypes existing within the *A. pavonina* species. The extract we have obtained contained mainly mono- and diunsaturated FAs, what explained its oily nature. Linoleic acid ($C_{18:2}$) was found to be the most predominant compound within the majority of lipid classes examined, ex-

Table I. Fatty acid composition in *A. pavonina* seeds. Abbreviations used: TL, total lipids; NL, neutral lipids; TAG, triacylglycerols; FFA, free fatty acids; 1,2-DAG, 1,2-diacylglycerols; 1,3-DAG, 1,3-diacylglycerols; PL, polar lipids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; LPC, lysophosphatidylcholine.

Lipid	Fatty acids[a] (%)											
	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:0}$	$C_{20:1}$	$C_{20:2}$	$C_{22:0}$	$C_{24:0}$
TL	*t*	*t*	10.0	0.2	2.2	17.8	51.1	*n.d.*	1.9	*t*	3.1	13.5
NL	0.1	0.1	11.4	0.2	2.7	18.8	44.0	*n.d.*	2.8	0.3	3.6	16.0
TAG	0.2	0.1	15.4	0.1	3.7	22.5	30.4	*n.d.*	2.8	*n.d.*	6.1	18.7
FFA	1.2	0.3	30.1	0.1	6.8	11.2	22.1	2.8	0.8	*n.d.*	6.2	18.4
1,2-DAG	0.5	0.3	25.4	*t*	4.4	9.1	23.6	*n.d.*	1.6	*n.d.*	6.6	28.5
1,3-DAG	1.5	0.8	40.4	1.5	11.0	19.2	14.8	*n.d.*	*t*	*n.d.*	10.8	*n.d.*
PL	2.6	0.9	34.0	*t*	5.1	21.6	24.2	*n.d.*	0.4	0.2	1.1	9.8
PC	2.4	1.1	32.6	3.9	4.4	25.1	17.3	*n.d.*	*n.d.*	*t*	2.4	11.0
PE	3.1	1.0	37.5	5.1	3.8	20.2	28.4	*n.d.*	1.1	*n.d.*	*n.d.*	*n.d.*
LPC	3.5	1.5	35.3	6.5	4.1	16.3	22.1	*n.d.*	1.0	0.5	*t*	9.1

[a] The reproducibility was in the range 92–96%.
t = Trace (less than 0.05%).
n.d. = not detected.

cept pools of 1,2-DAG and free FAs. In this case, lignocerotic acid ($C_{24:0}$) was the most prominent chemical. Interestingly, we could not detect its presence in 1,3-DAG and PE fractions. Oleic acid ($C_{18:1}$) was also widely distributed, particularly in all PL (contents varied from 16.3 to 25.1%) and within free FAs. Such a diverse distribution indicated diverse saturation of particular lipid classes. Indeed, we found free FAs, 1,2-DAG and 1,3-DAG fractions to be very rich in saturated FAs among all analyzed lipids, whereas the content of saturated FAs in the TAG pool was the lowest. The remaining FAs (myristic $C_{14:0}$, pentadecanoic $C_{15:0}$, palmitic $C_{16:0}$, palmitoleic $C_{16:1}$, stearic $C_{18:0}$, eicosanoic $C_{20:0}$, eicosenoic $C_{20:1}$, eicosadienoic $C_{20:2}$, docosanoic $C_{22:0}$ acid) were present in considerably smaller amounts.

The *A. pavonina* seed oil was also analysed as regards the presence of steroidal lipids (STs), polyamines (PAs) and 5-*n*-alkylresorcinols (ARs) (procedures for the two last groups not shown). In this case, stigmasterol was identified as the main ST compound (62%), whereas the remaining STs identified were sitosterol (10%), 24-methyl cholesterol (9%), 24-epiclerosterol (4%) and isofucosterol (3%). The pool of non-identified STs comprised 13%. The ST pattern found in *A. pavonina* seeds was typical for all plants. We have not detected the occurrence of PAs as well as ARs, but their absence was expected. A lack of PAs could be easily explained by the use of $(CH_3)_2CO$ for the extraction procedure. This solvent is too polar and is not recommended for such a purpose (J. M. C. Geuns, personal communication). However, $(CH_3)_2CO$ has been demonstrated to be also one of the most efficient solvents for the isolation of ARs (Zarnowski and Suzuki, 2004). In this case, the absence of these phenolic non-isoprenoid polyketides could be judged on a basis of our previous studies (Zarnowski *et al.*, 2001). Namely, we have proved previously that dicotyledonous plants belonging to the Mimosoideae subfamily did not produce ARs. It should be also noted that these two types of chemicals possess many biological activities including haemolytic properties against red blood cells (Kozubek and Tyman, 1999). Taking these facts together, the described composition of the oil obtained from the seeds of *A. pavonina* as well as the absence of haemolytic compounds within suggest its possible usefulness in the oil-in-water (o/w) emulsions *e.g.*, as drug carriers for the *i.v.* applications.

Nowadays there is a big interest in this type of drug carriers, especially with the use of lecithin and vegetable oils. Submicron lipid emulsions are biocompatible, biodegradable, physically stable, minimize the side effects of the incorporated drugs and can easily be produced in a large scale. This type of colloidal drug carriers can incorporate compounds of poor water solubility, what is very important, because many newly developed drugs are poorly water-soluble. The haemolysis of erythrocytes caused by them can be also avoided by their incorporation into the lipid emulsion (Jumaa and Müller, 2000). Till now only several poorly water-soluble drugs have been formulated in o/w emulsions including such drugs as paclitaxel (Kan *et al.*, 1999b; Lundberg *et al.*, 2003), cyclosporin A (Kim *et al.*, 2002), piroxicam (Klang *et al.*, 1996), clarithromycin (Lovell *et al.*, 1995), antifungal drugs (Piemi *et al.*, 1999) and oligonucleotides (Teixeira *et al.*, 2001).

The o/w emulsions with different amounts of oil from *A. pavonina* seeds (40–60 mg) were obtained by mixing them with 10–20 mg of SL as an emulsifier in 2.25% aqueous glycerol solution. The emulsion diameter changed significantly corresponding to the amount of the oil and SL added as shown in Table II. When the SL content was fixed (10, 15, and 20 mg, respectively) the increased amounts of the oil resulted in larger emulsion particle sizes. The exceptions were emulsions containing 40 and 60 mg oil and 10 mg SL, because two populations of emulsion particles were obtained.

Table II. Variations in particle size and Zeta potential of the emulsions with different *A. pavonina* L. seed oil and emulsifier ratios (wt/wt). Abbreviation used: SL, soybean lecithin.

Oil [mg]	SL [mg]	Size [nm]	Zeta potential [mV]
40	10	178.2 ± 15.2 371.8 ± 19.3	− 24.9 ± 0.8
50	10	288.7 ± 20.1	− 26.2 ± 0.4
60	10	201.1 ± 16.9 376.8 ± 19.2	− 27.6 ± 0.4
40	15	201.1 ± 26.8	− 26.6 ± 0.5
50	15	209.8 ± 22.1	− 26.9 ± 0.5
60	15	262.1 ± 20.5	− 27.0 ± 0.3
40	20	196.3 ± 13.9	− 25.7 ± 0.3
50	20	207.2 ± 34.7	− 26.2 ± 0.4
60	20	210.7 ± 15.2	− 26.9 ± 0.4

In the case of the emulsion containing 10 mg of SL, the homogeneous populations of particles for 40 and 60 mg oil was not received. However, the composition of 50 mg of the oil and 10 mg of SL resulted in the largest emulsion particle size (288 nm). Contrary to this, if the amount of lecithin was kept at the level of 15 mg we have obtained a well-defined emulsion, but with a wide range of size (201–262 nm). On the other hand, the very narrow size range and the smallest particle size (196 nm) was obtained for the emulsion containing 40 mg of the oil and 20 mg of SL. We conclude that the size of emulsion particles is generally a function of the oil to emulsifier ratio in the tested compositions. Table II also shows the influence of the oil and emulsifier ratio on the Zeta potential. It can be seen that all emulsion compositions exhibited a negative Zeta potential indicating, thereby, the possible stabilizing effect due to electrostatic repulsion. Increasing the volume of the oil phase in the emulsion caused only slight increase in the Zeta potential at the constant amount of emulsifier (SL).

As a stability indicator, the changes of size of the emulsions were monitored for 4 weeks at 4 °C. The data presented in Fig. 1 show that the stability of emulsions strongly depends on tested formulations. The mean particle size for both emulsions containing 50 mg of oil and 15 or 20 mg of SL showed almost no changes within a period of 4 weeks. In contrast, the data obtained for formulations containing 20 mg of SL (40 and 60 mg, respectively) demonstrate a strong increase in the particle size in comparison with initial values. However, the emulsion produced from 40 mg of
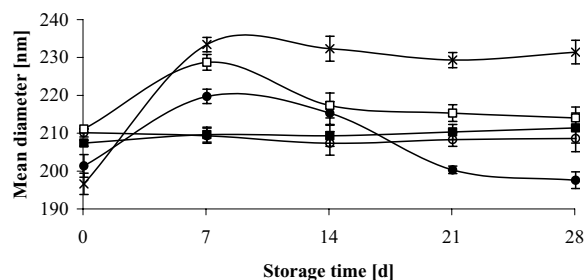

Fig. 1. Stability of emulsions in 28-day storage. The amount of oil and soybean lecithin used were (mg/mg): (●) 40/15, (○) 50/15, (×) 40/20, (■) 50/20, (□) 60/20, respectively.

the oil and 15 mg of SL was found to decrease the size during the storage. Formulations containing 50 mg of the oil and 10 mg of SL and 60 mg of the oil and 15 mg of SL, which were of the largest size, were completely unstable during storage (data not shown). These findings are substantially in agreement with the other results (Chung *et al.*, 2001) showing the relationship between emulsion stability and initial emulsion particle size.

The compositions of the emulsion containing 50 mg of oil and 15 or 20 mg of lecithin are suggested for further investigation. The postulated use of additions of pharmaceutically accepted non-ionic surfactant (Kan *et al.*, 1999a) would also be considered.

Balogun A. M. and Fetuga B. L. (1985), Fatty acid composition of seed oils of some members of the Leguminosae family. Food Chem. **17**, 175–182.

Benthall A. P. (1946), Trees of Calcutta and its Neighborhood. Thacker Spink and Co., Calcutta, India.

Burkill I. H. (1966), A Dictionary of the Economic Products of the Malay Peninsula, 2nd ed., vol. 1, A–H. Government of Malaysia and Singapore, Kuala Lumpur, Malaysia.

Certik M. and Shimizu S. (2000), Kinetic analysis of oil biosynthesis by arachidonic acid-producing fungus, *Mortierella alpina* 1S-4. Appl. Microbiol. Biotechnol. **54**, 224–230.

Certik M., Andrasi P., and Sajbidor J. (1996), Effect of extraction methods on lipid yield and fatty acid composition of lipid classes containing γ-linolenic acid extracted from fungi. J. Am. Oil Chem. Soc. **73**, 357–365.

Christopherson S. W. and Glass R. L. (1969), Preparation of milk fat methyl esters by alcoholysis in an essentially nonalcoholic solution. J. Dairy Sci. **52**, 1289–1290.

Chung H., Kim T. W., Kwon M., Kwon I. C., and Jeong S. Y. (2001), Oil components modulate physical characteristics and function of the natural oil emulsions as drug or gene delivery system. J. Control. Release **71**, 339–350.

Clark W. C. and Thaman R. R. (1993), Agroforestry in the Pacific Islands: Systems for Sustainability. United Nations University Press, Tokyo, Japan.

Gennaro A., Merlini L., and Nasini G. (1972), Flavonoids from *Adenanthera pavonina*. Phytochemistry **11**, 1515.

Grandmougin-Ferjani A., Dalpé Y., Hartmann M.-A., Laruelle F., and Sancholle M. (1999), Sterol distribution in arbuscular mycorrhizal fungi. Phytochemistry **50**, 1027–1031.

Jumaa M. and Müller B. W. (2000), Lipid emulsions as a novel system to reduce the hemolytic activity of lytic agents: mechanism of the protective effect. Eur. J. Pharm. Sci. **9**, 285–290.

Kabele-Ngiefu C., Vieux A., and Paquot C. (1975), An oil rich in lignocerotic and cerotic acids. The oil of *Adenanthera pavonina* L. seeds. Oleagineux **30**, 119–120.

Kan P., Chen Z. B., Kung R. Y., Lee C. J., and Chu I. M. (1999a), Study on the formulation of o/w emulsion as carriers for lipophilic drugs. Colloid. Surf. **15**, 117–125.

Kan P., Chen Z. B., Lee C. J., and Chu I. M. (1999b), Development of nonionic surfactant/phospholipid o/w emulsion as a paclitaxel delivery system. J Control. Release **58**, 271–278.

Kim S. J., Choi H. K., and Lee Y. B. (2002), Pharmacokinetic and pharmacodynamic evaluation of cyclosporin A O/W-emulsion in rats. Int. J. Pharm. **249**, 149–156.

Klang S. H., Baszkin A., and Benita S. (1996), The stability of piroxicam incorporated in a positively-charged submicron emulsion for ocular administration. Int. J. Pharm. **132**, 33–44.

Kozubek A. and Tyman J. H. P. (1999), Resorcinolic lipids, the natural non-isoprenoid phenolic amphiphiles and their biological activity. Chem. Rev. **99**, 1–26.

Lam J. M., Pwee K. H., Sun W. Q., Chua Y. L., and Wang X. J. (1999), Enzyme-stabilizing activity of seed trypsin inhibitors during desiccation. Plant Sci. **142**, 209–218.

Lovell M. W., Johnson H. W., and Gupta P. K. (1995), Stability of a less-painful intravenous emulsion of clarithromycin. Int. J. Pharm. **118**, 47–54.

Lundberg B. B., Risovic V., Ramaswamy M., and Wasan K. M. (2003), A lipophilic paclitaxel derivative incorporated in a lipid emulsion for parenteral administration. J. Control. Release **86**, 93–100.

Moreira R. A., Castelo-Branco C. C., Monteiro A. C. O., Tavares R. O., and Beltramini I. M. (1998), Isolation and partial characterization of a lectin from *Artocarpus incisa* L. seeds. Phytochemistry **47**, 1183–1188.

Piemi M. P. Y., Korner D., Benita S., and Marty J. P. (1999), Positively and negatively charged submicron emulsions for enhanced topical delivery of antifungal drugs. J. Control. Release **58**, 177–187.

Prabhu K. S. and Pattabiraman T. N. (1980), Natural plant enzyme inhibitors. Isolation and characterization of a trypsin/chymotrypsin inhibiotor from Indian red wood (*Adenanthera pavonina*) seeds. J. Sci. Food Agric. **31**, 967–980.

Richardson M., Campos F. A. P., Xavier-Filho J., Macedo M. L. R., Maia G. M. C., and Yarwood A. (1986), The amino sequence and reactive (inhibitory) site of the major trypsin inhibitor (DE5) isolated from seeds of the Brazilian Carolina tree (*Adenanthera pavonina* L.). Biochim. Biophys. Acta **872**, 134–140.

Sajbidor J., Certik M., and Grego J. (1994), Lipid analysis of baker's yeasts. J. Chromatogr. **665**, 191–195.

Sotheeswaran S., Sharif M. R., Moreau R. A., and Piazza G. J. (1994), Lipids from the seven Fijian plant species. Food Chem. **49**, 11–13.

Teixeira H., Dubernet C., Rosilio V., Laigle A., Deverre J. R., Scherman D., Benita S., and Couvreur P. (2001), Factors influencing the oligonucleotides release from O-W submicron cationic emulsions. J. Control. Release **70**, 243–255.

Watt J. M. and Breyer-Brandwijk M. G. (1962), The Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd ed. E & S Livingstone Ltd., London, England.

Yadav N., Misra G., and Nigam S. K. (1976), Triterpenoids from *Adenanthera pavonina* bark. Plant Med. **29**, 176–178.

Zarnowski R. and Kozubek A. (1999), Alkylresorcinol homologs in *Pisum sativum* L. varieties. Z. Naturforsch. **54c**, 44–48.

Zarnowski R. and Suzuki Y. (2004), Expedient Soxhlet extraction of resorcinolic lipids from wheat grains. J. Food Compos. Anal. (doi: 10.1016/j.jfca.2003.09.007) (in press).

Zarnowski R., Zarnowska E. D., and Kozubek A. (2001), Alkylresorcinols in the family Fabaceae. Acta Soc. Bot. Polon. **70**, 25–29.