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N. S. Vasudewa, D. T. U. Abeytunga & W. D. Ratnasooriya

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Activity-Directed Fractionation of *Pleurotus ostreatus* in the Search for Analgesics

N. S. Vasudewa,[1] D. T. U. Abeytunga,[1] and W. D. Ratnasooriya[2]

[1]Department of Chemistry, and [2]Department of Zoology, University of Colombo, Colombo, Sri Lanka

Abstract

Pleurotus ostreatus (Jacquin: Fries) P. Kummer (Tricholomataceae) was subjected to an antinociceptive activity-guided fractionation procedure in order to identify the fraction having highest activity. Three extracts, namely, acetone, dichloromethane, and hexane, were prepared at room temperature using fresh *P. ostreatus,* and 500 mg/kg was orally administered to male rats. The acetone extract showed significant antinociceptive activity with the hot-plate assay (male, 68%; $p < 0.05$), whereas dichloromethane and hexane extracts did not show significant activity. The same dose of acetone extract with female rats in the diestrous stage also showed prolongation of reaction time using the hot-plate test (female, 54%; $p < 0.05$). None of the extracts showed a significant increase in reaction time with the tail-flick test. The acetone extract was further fractionated by solvent partition to obtain four fractions (hexane, dichloromethane, ethyl acetate, and aqueous). Of these extracts, only the aqueous fraction showed marked prolongation in reaction time with the hot-plate test (500 mg/kg dose) on both male rats and on female rats in the diestrous stage (male, 37%; female, 26%). The aqueous fraction, following purification on a reverse phase column, yielded AqFrA-1, AqFrA-2, and AqFrA-3. Oral administration of a 500 mg/kg dose of these three fractions also showed marked prolongation in reaction time with the hot-plate test after 1 h of treatment (AqFrA-1, 26%; AqFrA-2, 69%; AqFrA-3, 101%). The effect was highest in AqFrA-3 at 1 h of treatment, and the effect lasted for 3 h. Hence, we can conclude that the compounds responsible for the activity have very high polarity. The acetone extract of *P. ostreatus* did not induce a membrane-stabilizing effect and did not cause prostaglandin inhibition. Naloxone blocked the antinociceptive activity in the hot-plate test upon feeding of 500 mg/kg of acetone extract of *P. ostreatus*, indicating the mechanism of action is via opioid receptor mediation.

Keywords: Antinociceptive activity, bioassay-guided fractionation, edible mushroom, opioid receptor mediation, *Pleurotus ostreatus.*

Introduction

Several mushroom extracts and compounds isolated from them have special effects on the central nervous system, and some have been shown to contain antinociceptive properties. Erinacin E from *Hericium coralloides* (Scop.: Fr.) S.F. Gray (Hericiaceae) is a highly selective agonist at the kappa opioid receptor (Saito et al., 1998). Screening investigations of selected basidiomycetes—*Piptoporusbetulinus* (Bull.) P. Karst (Fomitopsidaceae), *Ganoderma applanatum* (Pers.) Pat. (Ganodemataceae), *Heterobasidion annosum* (Fr.) Bref. (Bondarzewiaceae), *Fomitopsis pinicola* (Sw.:Fr.) P. Karst (Poriceae), and *Daedaleopsis confragosa* (Bolton) J. Schröt (Polyporaceae)—indicate inhibitory effects on neutral endopeptidase (enkephalinase). The inhibition of this endopeptidase could be useful in the treatment of pain with a spectrum of activity similar to that of opioids (Melzig et al., 1996). Scutigeral, a triprenyl phenol isolated from the fruiting bodies of *Scutiger ovinus* (Schaeff.: Fr.) Murrill (Albatrellaceae), has affinity to the brain dopamine D1 receptors and may act as an orally active pain killer targeting vanilloid receptors (Szallasi et al., 1999). Albaconol, a drimane-type sesquiterpenoid from the fruiting bodies of *Scutiger confluens* (Alb. & Schwein.: Fr) Bondartsev & Singer (Albatrellaceae), is an antagonist at the VR1 receptor (Liu, 2002). Muscimol, structurally related to the brain

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Address correspondence to: D.T.U. Abeytunga, Department of Chemistry, University of Colombo, Colombo 3, Sri Lanka. E-mail: thusitha@chem.cmb.ac.lk

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neurotransmitter GABA (γ-aminobutyric acid) and which was isolated from the toxic mushroom *Amanita muscaria* (Linnaeus) Hook (Amanitaceae) (Brehm et al., 1972), has been developed (Krogsgaard-Larsen et al., 2004) as an analgesic called gaboxadol.

The antinociceptive activity of *Pleurotus ostreatus* (Jacquin: Fries) P. Kummer (Tricholomataceae), commonly known as American oyster mushroom, was reported by us in an earlier study (Vasudewa et al., 2007) where the freeze-dried mushroom was proved to possess analgesic activity. The previous study further illustrated that the crude extract of the mushroom acted via opioid receptors. The main objective of the current study is to subject *P. ostreatus* to a bioassay-directed fractionation in order to obtain the fraction containing the active ingredient with antinociceptive potential.

Materials and Methods

Animals

Healthy adult Wistar male (150–225 g) and female (150–225 g) rats purchased from Medical Research Institute (Borella, Colombo, Sri Lanka) were used. All rats were kept under standardized animal house conditions with free access to pelleted food (Vet House Ltd., Colombo, Sri Lanka) and tap water at all times. All experiments were conducted in accordance with internationally accepted laboratory animal use and care guidelines and the rules of the Faculty of Science, University of Colombo, for animal experiments.

Mushrooms

Fresh *P. ostreatus* mushroom was collected in August 2006 from a farmer using the spawn provided by the Mushroom Cultivation Center, Export Research Board (Ratmalana, Sri Lanka). The identification and authentication was performed by Prof. R.L.C. Wijesundera, Department of Plant Science, University of Colombo, Sri Lanka. A voucher specimen (A-T-Po, 2006) was deposited at the research laboratory, Department of Chemistry, University of Colombo, Sri Lanka.

Extracts of *Pleurotus ostreatus*

Acetone extract of P. ostreatus *(A)*

Fresh *P. ostreatus* mushroom chopped into pieces (3 kg) was extracted two-times with 2 L of distilled acetone (Hemsons Int. Pvt. Ltd., Colombo, Sri Lanka) using a homogenizer (TR-50; Janke & Kunkel, IKA-WERK, Japan). The solution was filtered (No. 1, Whatman Int. Ltd., Maidstone, England), and it was evaporated using a rotary evaporator (R-114; Büchi, Switzerland). The crude extract was freeze-dried (LFD-600EC; Laytant Life Science Co. Ltd, Tokyo, Japan) to obtain 65 g of dark-brown solid. A solution of freeze-dried acetone extract was prepared with water prior to the oral administration to rats by dissolving 0.5 g in 10 mL of tap water.

Dichloromethane extract of P. ostreatus *(D)*

The residue, after extracting with acetone, was again extracted twice with 1.5 L of distilled dichloromethane (Countryman Pvt. Ltd., Colombo, Sri Lanka) to enhance the percentage extraction, and it was filtered using Whatman filter papers. The solvent was evaporated using a rotary evaporator and freeze-dried to obtain 1.9 g of a brown gum. Freeze-dried dichloromethane extract (0.5 g) was suspended in 10 mL of 5% DMSO (Fluka Chemica, Buchs, Switzerland) and sonicated to obtain a homogeneous solution.

Hexane extract of P. ostreatus *(H)*

The residue, after extracting with dichloromethane, was again extracted twice with 1.5 L of distilled hexane (Sigma Chemical Pvt. Ltd., Colombo, Sri Lanka) to enhance the percentage extraction, and the extract was filtered. The solvent was evaporated using a rotary evaporator and freeze-dried to obtain a yellow gum (1 g). The hexane extract (0.5 g) was suspended in 10 mL of 5% DMSO and sonicated to obtain a homogeneous solution.

Evaluation of analgesic activity

All three extracts were subjected to the hot-plate test and tail-flick test ($n = 6$) on male rats ((Langerman et al., 1995) upon oral administration of a 500 mg/kg dose of each extract. A group of female rats in the diestrous stage ($n = 9$) was orally administered 500 mg/kg of acetone extract and subjected to the hot-plate and tail-flick tests. A group of male rats ($n = 6$) and of female rats ($n = 6$) in the diestrous stage was orally given 2.5 mL of tap water and served as the control group for the acetone extract. A group of male rats ($n = 6$) was orally treated with 2.5 mL of 5% DMSO, and it served as the control group for the dichloromethane and hexane extracts.

Evaluation of prostaglandin synthesis inhibition activity

This was done as described by Dharmasiri et al. (2003). Female rats in the diestrous stage were selected by microscopic examination of vaginal smears. They were sacrificed with an overdose of anesthetic ether (State Pharmaceutical Corporation, Colombo, Sri Lanka). Their uterine horns were excised carefully and cut into approximately 1-cm pieces. These uteri were individually placed in a 50-mL organ bath containing Kreb's Henseleit solution having the following composition (mmol/L): Na^+ 143, K^+ 5.8, Ca^{2+} 2.6, Mg^{2+} 1.2, Cl^- 128, $H_2PO_4^-$ 1.2, HCO_3^- 25, SO_4^{2-} 1.2, and glucose 11.1 at pH of 7.4. The organ bath was maintained at 37°C and aerated with a gas mixture of 95% O_2 and 5%

CO_2. The spontaneous activity of the uteri was recorded isometrically under a resting tension of 1 g until contractions became regular using an isometric sensor (Star Medicals, Tokyo, Japan). After the contractions became regular, the normal activity of the uteri was recorded for a further 10 min. After this, acetone extract of *P. ostreatus* was added into the organ bath so that the final concentrations of organ bath became 0.25, 0.50, and 1.00 mg/mL (n = 3/dose). The amplitude and the frequency of contractions were recorded.

Evaluation of plasma membrane stabilization activity

This property of the acetone extract of *P. ostreatus* was investigated (5.00, 2.50, 1.67, and 1.25 mg/mL concentrations) using the heat-induced hemolysis test of rat erythrocytes (Dharmasiri et al., 2003). Uncoagulated fresh rat blood (20 μL) was added into vials containing 1.00 mL of 0.15 M phosphate-buffered saline (PBS). The aforementioned acetone extract (15 μL) was added to these vials (n = 3/dose). PBS (15 μL) was used as the control (n = 3). Thereafter, the vials were mixed well and incubated at 37°C for 15 min followed by 25 min at 54°C. The vials were centrifuged at 3200 × *g* for 2 min. Absorbance of the supernatant was measured at 540 nm.

Investigation of involvement of opioid receptors

Twelve male rats were fasted overnight and randomly divided into two equal groups (n = 6/group). Those in group 1 were intraperitoneally injected with 1 mg/kg of naloxone (Bodene Ltd, Port Elizabeth, South Africa), an opioid antagonist, and those in group 2 with isotonic saline. After 45 min, rats in both groups were orally treated with 500 mg/kg of the freeze-dried acetone extract (A) of *P. ostreatus*. These rats were subjected to the hot-plate test, and the reaction time was determined (Ratnasooriya & Dharmasiri, 1999) before treatment and 1 h after the administration of extract A of *P. ostreatus*.

Fractionation of acetone extract

Solvent extraction method

Freeze-dried acetone extract (60 g) of *P. ostreatus* was dissolved in 350 mL of water:methanol (80:20) mixture. Hexane fraction of acetone extract (HeFrA). The above solution was placed in a separatory funnel, and 200 mL of hexane was added. This extraction to hexane was repeated, and the two hexane layers were combined, evaporated, and weighed (1.0 g).

Dichloromethane fraction of acetone extract (DMFrA). The aqueous layer from the extraction above was placed in a separatory funnel and extracted to dichloromethane (2 × 200 mL) twice. Both fractions were combined, evaporated, and weighed (0.72 g).

Ethyl acetate fraction of acetone extract (EAFrA). The remaining aqueous layer from the step above was rotary evaporated to remove methanol. The remaining solution was again extracted with ethyl acetate (2 × 200 mL) twice. Both fractions were combined, evaporated, and weighed (0.38 g).

Aqueous fraction of acetone extract (AqFrA). Aqueous layer was rotary evaporated to remove traces of organic solvents. It was freeze-dried to remove all the water (55 g).

DMFrA and EAFrA (0.5 g each) were dissolved while sonicating in 10 mL of 5% DMSO. The AqFrA (0.5 g) was dissolved in 10 mL of tap water. In animal experiments, a 500 mg/kg dose of each fraction was orally administered to male rats (n = 6). A group of female rats in the diestrous stage (n = 9) was orally treated with 500 mg/kg of AqFrA, and the reaction times were recorded on the hot-plate test.

Morphine was used as the reference drug, and male rats (n = 5) were orally treated with 15 mg/kg of morphine.

Chemical components of AqFrA

Freeze-dried AqFrA (4 g) was mixed with 4 mL of anhydrous acetic anhydride (Park Scientific Ltd., Northampton, UK) and 4 mL of pyridine (Park Scientific Ltd.) and stirred for 24 h at room temperature. The products were dissolved in 10 mL of dichloromethane and washed with 5% $CuSO_4$. The dichloromethane layer was again washed with 5% aqueous NaOH followed by a wash with distilled water. The organic layer was separated, dried with anhydrous Na_2SO_4, and rotary evaporated. The resultant yellow oil was purified on a silica gel column with a gradient of hexane and ethyl acetate. The fraction eluted with hexane:ethyl acetate (6:4) was rotary evaporated, and ^{13}C NMR was recorded.

Fractionations of AqFrA

The active fraction (AqFrA) was fractionated on a reverse phase column (C-18, 60-120 mesh, BDH Chemical Ltd., Poole, UK) into three large fractions using methanol:water (3:7, 50 mL), methanol:water (1:1, 50 mL) and methanol:water (7:3, 50 mL) and labeled as AqFr-1, AqFr-2, and AqFr-3. Methanol in these fractions was rotary evaporated and freeze-dried.

Male rats in three separate groups (n = 6/group) were orally treated with 500 mg/kg of AqFr-1, AqFr-2, and AqFr-3.

Statistical analysis

Data are given as mean ± standard error of mean. Data were analyzed with Mann-Whitney *U*-test. Significance was set at $p \leq 0.05$.

Table 1. Effect of oral administration of *P. ostreatus* extracts on the hot-plate reaction time of rats.[a]

	Hot-plate reaction time (s) (mean ± SEM)					
		Treatment				
Treatment (mg/kg)	Pretreatment	1 h	2 h	3 h	4 h	5 h
Male						
Control: H_2O (n = 6)	7.8 ± 0.4	7.7 ± 1.0	4.9 ± 0.4	5.5 ± 0.8	5.7 ± 1.0	5.1 ± 0.4
Control: 5% DMSO (n = 6)	9.7 ± 0.9	9.4 ± 0.5	8.8 ± 0.9	8.5 ± 0.9	7.5 ± 0.7	6.5 ± 0.4
500 (n = 6) M-A	9.0 ± 1.4	15.1 ± 2.7*	10.6 ± 2.5	8.8 ± 1.6	7.7 ± 2.2	7.0 ± 1.4
500 (n = 6) M-D	7.8 ± 0.5	7.7 ± 0.8	7.3 ± 0.9	7.3 ± 0.4	6.6 ± 0.8	6.3 ± 1.0
500 (n = 6) M-H	8.1 ± 0.8	7.4 ± 0.4	5.9 ± 0.9	7.5 ± 1.0	6.0 ± 0.8	5.7 ± 0.8
Female						
Control: H_2O (n = 9)	6.8 ± 0.6	7.6 ± 0.7	8.0 ± 0.9	7.1 ± 0.6	6.8 ± 0.5	7.1 ± 0.4
500 (n = 9) F_D-A	6.3 ± 0.6	9.7 ± 1.3*	7.0 ± 0.5	6.9 ± 0.8	6.9 ± 0.7	7.0 ± 0.9

[a]Males and females in the diestrous stage are abbreviated as M and F_D, respectively. The extract used in each experiment is given after the hyphen as A, D, and H, for acetone, dichloromethane, and hexane, respectively. The acetone extract was dissolved in water, and the dichloromethane and hexane extracts were dissolved in 5% DMSO.
*$p < 0.05$ compared with the respective pretreatment.

Results

Evaluation of analgesic activity of extracts of *Pleurotus ostreatus*

The increase in the reaction time (Table 1) after 1 h of treatment with acetone extract of *P. ostreatus* (500 mg/kg) in male rats (68%, $p < 0.05$) and in female rats in the diestrous stage (54%, $p < 0.05$) was marked and significant on the hot-plate test. However, oral administration of 500 mg/kg of hexane extract and dichloromethane extract to male rats showed no marked or significant increase in the reaction time after 1 h of treatment. None of these extracts showed a marked increase in reaction time in the tail-flick test ($p > 0.05$) (data not shown).

Prostaglandin synthesis inhibition activity

As compared with the control, solutions (0.25, 0.50, and 1.00 mg/mL) of acetone extract did not significantly ($p > 0.05$) alter the mean amplitudes (cm) or the mean frequency of uterine contractions (for 15-min intervals) in the organ bath. Control versus 0.25, 0.50, and 1.0 mg/mL of acetone extract: mean amplitude (cm) 4.1 ± 0.5 versus 4.7 ± 0.6, 5.1 ± 0.8, and 5.4 ± 0.6, respectively; mean frequency of uterine contraction (per 15 min) 12.0 ± 0 versus 11.7 ± 0.9, 11.3 ± 1.5, and 11.0 ± 1.2, respectively.

Plasma membrane stabilization activity

All four concentrations of the acetone extract of *P. ostreatus* did not significantly ($p > 0.05$) change the absorbance in the heat-induced hemolysis test of rat erythrocytes. Mean percentage inhibition of hemolysis: positive control (aspirin 100 μg/mL) versus 1.25, 1.67, 2.50, and 5.00 mg/mL of acetone extract was 80.3% versus 1.2%, 1.85%, 29.6%, and 9.4%, respectively.

Investigation of involvement of opioid receptors

The opioid receptor antagonist naloxone significantly ($p = 0.05$) impaired the reaction time induced by 500 mg/kg of acetone extract of *P. ostreatus*, as shown in Table 2.

Fractionation of acetone extract (A)

The increase in the reaction time after 1 h of treatment with DMFrA, EAFrA, or AqFrA is given in Table 3. The oral administration of AqFrA to male rats showed a marked (37%) increase in the reaction time on the hot-plate test. There was no significant increase in the reaction time after 1 h of treatment with DMFrA or EAFrA (500 mg/kg) to male rats. The oral administration of AqFrA (500 mg/kg) to female rats in the diestrous stage also showed significant prolongation in reaction time (26%, $p < 0.05$) after 1 h of treatment.

Chemical components of AqFrA

^{13}C NMR (500 MHz) in $CDCl_3$ gave signals at δ 20.4, 20.5, 20.6, 61.6, 68.1, 68.4, 69.7, 69.8, 92.1, 169.4, 169.5, 169.7, 170.4. ^{13}C NMR data exactly matches with the literature

Table 2. Effect of naloxone on the hot-plate reaction time of male rats, induced by 500 mg/kg of acetone extract of *P. ostreatus.*

	Reaction time on hot-plate test (s) (mean ± SEM)	
Treatment (mg/kg)	Pretreatment	First hour
500 (n = 6) no naloxone	6.7 ± 0.6	10.8 ± 0.9*
500 (n = 6) with naloxone	8.3 ± 0.6	8.8 ± 0.7

*Values are significant at $p < 0.05$.

Table 3. Effect of oral administration of fractions of the acetone extract on the hot-plate reaction time of rats.[a]

	Hot-plate reaction time (s) (mean ± SEM)					
		Treatment				
Treatment (500 mg/kg)	Pretreatment	1 h	2 h	3 h	4 h	5 h
Male						
AqFrA-M (n = 6)	6.8 ± 0.5	9.3 ± 1.2	8.2 ± 1.3	7.6 ± 1.3	5.2 ± 0.8	5.1 ± 0.8
EAFrA-M (n = 6)	7.3 ± 0.4	8.5 ± 1.0	7.6 ± 0.8	6.3 ± 0.9	6.4 ± 1.1	6.2 ± 0.9
DMFrA-M (n = 6)	6.5 ± 0.6	6.6 ± 0.8	6.9 ± 0.7	6.6 ± 0.6	5.2 ± 0.7	5.3 ± 0.7
Female						
AqFrA-F_D(n = 9)	9.3 ± 0.7	11.7 ± 0.8*	10.0 ± 0.8	7.1 ± 0.9	5.7 ± 0.6	6.3 ± 0.6

[a]Males and females in the diestrous stage are abbreviated as M and F_D, respectively. The fractions obtained from solvent partition of acetone extract to dichloromethane, ethyl acetate, and aqueous are indicated as DMFrA, EAFrA, and AqFrA, respectively.
*Values are significant at $p < 0.05$ compared with pretreatment.

reported (Perera et al., 2001) octaacetyl trehalose, which is derived from trehalose present in the extract.

Fractionations of AqFrA

Reaction times in the hot-plate are given in Table 4. The first two fractions showed marked increase in the reaction time on the hot-plate assay at 1 h after the oral administration (AqFr-1 [26%, $P < 0.05$], AqFr-2 [69%, $P < 0.05$]). The third AqFr-3 had almost 100% prolongation in the increase in reaction time after 1 h of administration (101%, $p < 0.01$), and this antinociceptive activity lasted for 3 h (at 2 h: 50%, $p < 0.05$; at 3 h: 73%, $p < 0.05$).

Discussion

The results obtained here undoubtedly strengthen our previous results on the antinociceptive activity of *Pleurotus ostreatus* (Vasudewa et al., 2007). The initial fractionation and the bioassays showed that the antinociceptive activity was retained in the acetone fraction. The activity was observed both in males and in the females in the diestrous stage. The experiments on females in the other stages of the estrous cycle were not performed as antinociceptive activity was not observed in those females in our previous study (Vasudewa et al., 2007). This activity has a quick onset and a relatively short duration of action.

The antinociceptive activity of the acetone extract of *P. ostreatus* was observed in the hot-plate test but not in the tail-flick test, indicating supraspinally mediated activity. Prostaglandins induce pain, and prostaglandin synthesis blockers are potent analgesics (Rang et al., 2003). However, antinociception in the acetone extract of *P. ostreatus* is unlikely to be mediated via prostaglandin synthesis inhibition as the acetone extract failed to suppress contractions of uterine horns isolated from female rats in the diestrous stage. Antinociceptive activity can be brought about by membrane stabilization (Thabrew et al., 2003). However, this mode of action is unlikely in the acetone extract of *P. ostreatus* as it failed to inhibit heat-induced hemolysis of rat erythrocytes *in vitro*. In our previous study, antinociception of freeze-dried *P. ostreatus* was blocked by the opioid receptor antagonist naloxone (Vasudewa et al., 2007). The same experiment, using acetone extract of *P. ostreatus*, resulted in abolishing the antinociception, proving opioid

Table 4. Effect of oral administration of AqFr-1, AqFr-2 and AqFr-3 on the hot-plate reaction time of male rats.

	Hot-plate reaction time (s) (mean ± S.E.M.)					
		Treatment				
Treatment (500 mg/kg)	Pretreatment	1 h	2 h	3 h	4 h	5 h
Male						
AqFrA-1 (n = 6)	8.1 ± 0.9	10.2 ± 0.7	8.4 ± 0.8	6.8 ± 0.7	6.0 ± 0.5	6.3 ± 0.5
AqFrA-2 (n = 6)	8.1 ± 1.1	13.7 ± 3.3*	11.2 ± 2.8	7.5 ± 0.7	6.4 ± 1.0	6.0 ± 0.4
AqFrA-3 (n = 6)	8.0 ± 1.1	16.1 ± 1.2**	12.0 ± 1.6*	13.8 ± 0.8*	10.1 ± 1.5	9.0 ± 0.9
Morphine (n = 6): 15 mg/kg	7.5 ± 0.5	13.8 ± 0.8**	16.7 ± 1.5**	11.5 ± 2.6	12.6 ± 1.4**	9.4 ± 1.4

Values are significant at **$P < 0.01$ and at *$P < 0.05$ compared with pre-treatment.

receptor mediation is the key factor in bringing about the pain-relieving property.

Morphine, a potent opioid analgesic, is soluble in water, but its solubility in lipids is poor (Christrup, 1997). In order to investigate the polarity of the compound responsible for the antinociceptive activity, the acetone extract of *P. ostreatus* was subjected to a liquid-liquid partition using hexane, dichloromethane, ethyl acetate, and water with varying polarities. The amount of the hexane fraction was not sufficient to treat six rats with the 500 mg/kg dosage. The bioassay of dichloromethane, ethyl acetate, and water fractions indicated that the activity was retained in the water fraction (AqFrA) suggesting the compound responsible for the activity is polar in chemical nature. The reduction of antinociceptive activity in this extraction protocol may be due to the coexistence of components that may block pain inhibition pathways of the brain. Such a mode of action is proposed for opioid analgesics such as morphine (Roumy & Jean-Marie, 1998). During the investigation of chemical compounds present in AqFrA, trehalose was isolated as one of the components. Trehalose, which contains two D-glucose units connected by an α, α-1,1 linkage, functions in many organisms as an energy source or a protectant against the effects of freezing or dehydration (Richards et al., 2002). Trehalose is also known to increase the density of solutions resulting in hyperbaric solutions (Iadarola et al., 2004), which can be used to administer analgesics.

Studies of plain versus hyperbaric solutions in intrathecal application of analgesics have shown a similar effect (Srivastava et al., 2004), less effect (Rofaeel et al., 2007), and less side effects (Caute et al., 1988) in the hyperbaric application. However, the effect of trehalose in oral administration of analgesics is not known. The decrease in analgesic action in AqFrA as oppose to the original acetone fraction in the current study may be due to the increase of constituents such as trehalose in the AqFrA fraction, as the solvent partition protocol used in our study would increase trehalose in AqFrA.

The use of reverse phase silica gel in the purification of polar compounds is well-known (Morden & Wilson, 1999). Purification of the active AqFrA fraction on a C-18 reverse phase silica gel resulted in obtaining AqFrA-3 fraction, which had extremely high antinociceptive activity with the 500 mg/kg dose. This activity had a rapid onset and lasted for 3 h. The increase in analgesic activity in AqFrA-3 could possibly be due to the elimination of trehalose from the active ingredient by the reverse phase column. However, the activity of AqFrA-3 is lower than that of the reference drug, morphine. It is interesting to note that antinociceptive activity at the first hour of administration with the 15 mg/kg dose of morphine is lower than that of AqFrA-3 with a 500 mg/kg dose.

Several chemical constituents have been isolated from *Pleurotus* species and reported in the literature. Polysaccharides, such as pleuran, a β-1,3 glucan (Bobek & Galbavy, 2001) and glycopeptides (Li et al., 1994) are known to be present in *P. ostreatus*. An antifungal peptide, eryngin (Wang et al., 2004), has been isolated from *P. eryngii* (De Cand.) Gillet (Pleurotaceae). Pleurotin, with a quinonoid skeleton, was isolated from *P. griseus* (Robbins et al., 1947) and shown to have antimicrobial properties and anticancer properties (Welsh et al., 2003). We were unable to prove the presence of any such chemical constituents in AqFrA-3.

These results indicated that the antinociceptive activity-guided fraction of *P. ostreatus* was very successful in identification of the active fraction. In conclusion, the active components of *P. ostreatus* possessing analgesic activity lie in a relatively high polar fraction resulting from the liquid-liquid partition.

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