Food Chemistry 111 (2008) 433–438

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Antibacterial and anti-PAF activity of lipid extracts from sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*)

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A R T I C L E I N F O

Article history:
Received 2 October 2007
Received in revised form 4 February 2008
Accepted 4 April 2008

Keywords:
Sea bass
Sea bream
Platelet activating factor (PAF)
Antibacterial activity

A B S T R A C T

The anti-PAF and the antibacterial activities of lipid extracts obtained from cultured sea bass (*Dicentrarchus labrax*) and cultured gilthead sea bream (*Sparus aurata*) were evaluated. Total lipids of sea bass and gilthead sea bream exerted PAF-like activity while, in higher amounts they inhibited this PAF activity. Neutral lipids of both sea bass and gilthead sea bream contained only PAF antagonists while the polar lipid fractions contained both PAF antagonists and agonists. Total lipids of sea bass exhibited stronger PAF-like activity than did those of gilthead sea bream; however, neutral lipids of sea bass contained stronger PAF antagonists than did gilthead sea bream.

Total lipids of both sea bass and gilthead sea bream exhibited antibacterial activity only towards *Staphylococcus aureus* (*S. aureus*) with those of sea bass being more potent. Subsequently, neutral lipids of both sea bass and gilthead sea bream also showed antibacterial activity against *S. aureus* and less so towards *Escherichia coli* (*E. coli*), while only neutral lipids of sea bass showed antibacterial activity against *Enterococcus faecalis* (*E. faecalis*). Sea bass neutral lipids were more active against *S. aureus* than were those of gilthead sea bream, while their activity towards *E. coli* was similar. Polar lipids of both sea bass and gilthead sea bream showed antibacterial activity against all bacteria strains. Sea bass polar lipids were more active towards *S. aureus* than were those of gilthead sea bream, while their activities against *E. faecalis* and *E. coli* were the same.

The detected antibacterial activities of the lipid extracts isolated from sea bass and gilthead sea bream were observed in amounts equal to those that exerted either PAF inhibition or PAF-like activity, suggesting that PAF antagonists and agonists of fish lipids may be responsible for the antibacterial activity.

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1. Introduction

Staphylococcus aureus (*S. aureus*), a Gram-positive coccus, appears as grape-like clusters under microscopic observation and has large, round, golden-yellow colonies. *S. aureus* may occur in healthy persons as a commensal on skin or in the nose and throat and, less commonly, may be found in the colon and in urine.

Most *staphylococcus* species are coagulase-negative but *S. aureus* is coagulase-positive. This is medically important because *S. aureus* is much more aggressive and likely to be antibiotic-resistant (Ryan, 2004). This is the reason that *S. aureus* constitutes the most common cause of *staphylococcus* infections. Infections due to *S. aureus* are a main source of morbidity and mortality worldwide and cover a range of illnesses, such as skin infections and other fatal diseases, e.g. pneumonia, endocarditis, toxic shock syndrome (TSS) (Kayser, Bienz, Eckert, & Zinkernagel, 2005), meningitis (Pedersen, Benfield, Skinhoej, & Jensen, 2006) and septicemia (Prasad, Mishra, & Pant, 2007).

Antimicrobial resistance (Chambers, 2001) in strains of *S. aureus*, e.g. methicillin resistance in hospitals, methicillin resistance in community strains, and the recent acquisition of glycopeptide resistance, exacerbate the problem of illness induced by *S. aureus* infection (Bishop & Howden, 2007).

Enterococcus faecalis (*E. faecalis*), is a Gram-positive commensal bacterium which inhabits the gastrointestinal tracts of humans and other mammals. Many life-threatening infections in humans may be caused by *E. faecalis* infection. As in the case of *S. aureus*, intrinsic and acquired resistance of *E. faecalis* to antimicrobial agents (aminoglycosides, aztreonam, cephalosporins, clindamycin, the semi-synthetic penicillins nafcillin, oxacillin, trimethoprim-sulfamethoxazole) comprise a medical problem for its treatment (Ono, Muratani, & Matsumoto, 2005). *E. faecalis* can cause endocarditis (Tsigrelis, Singh, Coutinho, Murray, & Baddour, 2007), as well

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doi:10.1016/j.foodchem.2008.04.011

as bladder (Shiono & Ike, 1999) and prostate infections (Faraj, 1996) and, less commonly nervous system infections (Czaban, Lebkowski, Krajewski, & Borkowski, 2002).

Escherichia coli (*E. coli*), known as gut flora, belong to the main species of bacteria that live in the lower intestines of mammals. This is a Gram-negative organism, and as such is unable to sporulate, and for this reason pasteurization or simple boiling is enough for their eradication. *E. coli* can cause several intestinal and extra-intestinal infections, e.g. urinary tract infections, meningitis, peritonitis (Kayser et al., 2005), mastitis (Schwarz & Rosen, 1974), septicemia (Szymborski & Komar, 1989) and Gram-negative pneumonia (Bermejo et al., 1992). Certain strains of *E. coli*, such as *E. coli* O157:H7, are toxigenic and they can cause food poisoning, usually associated with eating cheese and contaminated meat (Maruzumi et al., 2005).

The root of the antibiotic resistance problem seems to be the result of two practices: first, the overuse of antibiotics in humans and, second, the use of antibiotics as growth promoters in food animals (Johnson et al., 2006).

Platelet-activating factor (PAF) is a potent pro-inflammatory phospholipid mediator that belongs to a family of biologically active, structurally related alkyl phosphoglycerides with diverse pathological and physiological effects. PAF acts by binding to a specific G protein-coupled receptor to activate multiple intracellular signalling pathways. The earliest evidence for the existence of PAF bacteria has been previously reported from our laboratory (Andriotis, Demopoulos, Tournis, Siafaka, & Mavris, 1986). Today it is well known that PAF synthesis is stimulated by *S. aureus* toxins (Suttorp, Buerke, & Tannert-Otto, 1992) or it can be released by *E. coli* (Denizot, Dassa, Benveniste, & Thomas, 1989). Interestingly, PAF has been implicated in most of the aforementioned pathological conditions induced by *S. aureus*, *E. faecalis* and *E. coli*, namely skin inflammation (Archer, 1993), pneumonia (van der Sluijs et al., 2006), meningitis (Denizot, 1991), septicemia, endocarditis (Heuer et al., 1991), peritonitis (Montrucchio et al., 1989), urinary tract infection (Ikeda, Oda, Sakakura, & Yasunaga, 1991), *E. coli* O157-associated hemolytic uremic syndrome (Xu et al., 2000) and neuronal infection (Feuerstein, 1996).

Furthermore, PAF antagonists have been shown to inhibit bacterial-induced inflammatory actions such as cytokine production; therefore, the significance of the PAF signalling system in producing inflammatory molecules induced by bacterial toxins, and live Gram-positive and Gram-negative bacteria is underlined (Ogata et al., 2004). This significant role has also been established in an animal model of sepsis (DeJoy et al., 1994).

Various studies have demonstrated that foodstuffs such as honey, propolis (Miorin, Levy Junior, Custodio, Bretz, & Marcucci, 2003), garlic (Ruddock et al., 2005) and tea (Toda, Okubo, Hara, & Shimamura, 1991), as well as food-originating constituents, e.g. polyphenols (Kusuda et al., 2006), exert antibacterial activities.

So far there are no studies on antibacterial effects of lipid extracts from sea bass (*Dicentrarchus labrax*) or gilthead sea bream (*Sparus aurata*). In a previous work (Nasopoulou, Nomikos, Demopoulos, & Zabetakis, 2007), PAF antagonists have been isolated from wild and cultured specimens of both sea bass and gilthead sea bream. The aim of the present study was to investigate the antibacterial and anti-PAF activities of total, total polar and total neutral lipid extracts from both sea bass (*D. labrax*) and gilthead sea bream (*S. aurata*) towards *S. aureus*, *E. faecalis*, and *E. coli*.

2. Materials and methods

2.1. Chemicals

All reagents, including PAF (β-acetyl-γ-*O*-hexadecyl-L-α-phosphatidylcholine), were supplied by Sigma (Gillingham, UK). All solvents were of analytical grade and supplied by Merck (Merck, Darmstadt, Germany). Mueller-Hinton Broth, Mueller-Hinton Agar, Nutrient Broth and Nutrient Agar were obtained from Oxoid Ltd. (Basingstoke, Hampshire England).

2.2. Materials

Two different cultured species of fish, namely sea bass (*D. labrax*) and gilthead sea bream (*S. aurata*), were cultivated in Nereus marine culture, situated in Chios Island in Greece. The fish were gutted and the head, skin and bones were removed. Three fish from each species were filleted and pieces from each fillet were pooled (this pooling represents each replicate). These pieces were then chopped and weighed (~50 g), prior to homogenization.

2.3. Isolation of fish lipid fractions

Total lipids (TL) were extracted according to the Bligh–Dyer method (Bligh & Dyer, 1959). One tenth of the TL was weighed, then diluted in a specific volume of chloroform, methanol, in the ratio 1:1 and stored at −20 °C while the rest of it was further separated into polar lipids (PL) and neutral lipids (NL) by counter-current distribution (Galanos & Kapoulas, 1962). The PL and NL were weighed, diluted in a specific volume of chloroform, methanol in the ratio 1:1 and stored at −20 °C prior to use.

2.4. Biological assay

Washed rabbit platelets were prepared according to the method of Pinckard, Farr, and Hanahan (1979). Subsequently, total (TL), polar (PL) and neutral (NL) lipids were tested for their biological activity towards washed rabbit platelets according to the method of Demopoulos, Pinckard, and Hanahan (1979), using a Chrono-Log (Havertown, PA, USA) aggregometer coupled to a Crono-Log recorder (Havertown, PA, USA). Briefly, the samples being examined and the PAF were dissolved in 2.5 mg of bovine serum albumin (BSA) per ml of saline. Various amounts of the sample being examined, ranging from 0.001 to 0.434 mg, were placed in the aggregometer cuvette and their abilities to aggregate washed rabbit platelets or to inhibit PAF-induced aggregation were determined.

In order to determine the aggregatory effect of either PAF or the samples being examined, the maximum reversible aggregation was evaluated and 100% aggregation was determined as the increase in light transmission acquired from the concentration that induced the maximum reversible aggregation. The plot of the percentage of the maximum reversible aggregation (ranging from 20% to 80%) vs different concentrations of the aggregatory agent was linear. From this curve, the concentration of the aggregatory agent, which induces 50% of the maximum reversible aggregation, was calculated. This value was defined as EC_{50}, i.e. the equivalent concentration for 50% reversible aggregation.

In order to determine the inhibitory properties of the samples, various amounts of the sample being examined, ranging from 0.001 to 0.434 mg, were placed in the aggregometer cuvette and their ability to inhibit PAF-induced aggregation was determined. The platelet aggregation induced by PAF (2.5×10^{-11} M, final concentration in the cuvette) was measured as PAF-induced aggregation (Fig. 1), in washed rabbit platelets before (considered as 0% inhibition) and after the addition of various amounts of the sample being examined. Consequently, the plot of % inhibition (ranging from 20% to 80%) vs different concentrations of the sample was linear. From this curve, the concentration of the sample, which inhibited 50% PAF-induced aggregation, was calculated. This value was defined as IC_{50}, i.e. inhibitory concentration for 50% inhibition.

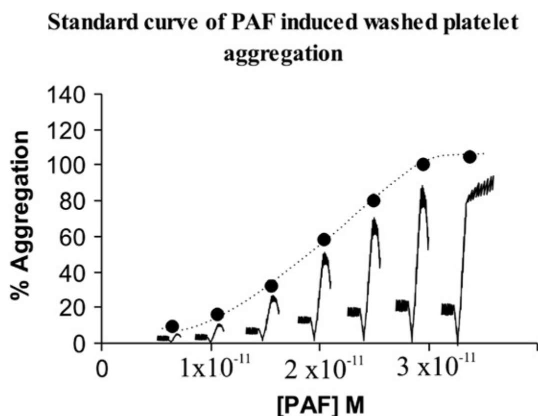

Fig. 1. Standard curve of PAF-induced washed rabbit platelet aggregation, along with aggregation curves at each PAF concentration. Aggregation was expressed as % in terms of increment of light transmission and the concentration of PAF was expressed as final concentration in the cuvette of aggregometer. The concentration 2.5×10^{-11} M of PAF was used for the determination of any inhibition from lipid extracts from sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*). Values correspond to the mean values of two independent experiments.

2.5. Microorganisms and culture conditions

In vitro antibacterial studies were carried out towards three bacteria strains (*S. aureus* ATCC 29213, *E. faecalis* NCTC 775 and *E. coli* NCTC 9001), which were obtained from the Hygiene Laboratory of the Ministry of Health.

The bacterial strains of *S. aureus* and *E. faecalis* were inoculated onto Mueller-Hinton Broth and incubated for 24 h at 36 ± 0.1 °C, while the bacterial strain, *E. coli*, was inoculated onto Nutrient Broth and incubated for 24 h at 36 ± 0.1 °C. Adequate amounts of autoclaved Mueller-Hinton Agar and Nutrient Agar were dispensed into sterile 9 cm diameter Petri dishes and allowed to solidify under aseptic conditions. The counts of bacterial strains were adjusted to yield approximately 1.0×10^7–1.0×10^8 ml^{-1} using the standard McFarland counting method.

One milliliter of the test organisms was inoculated with a sterile swab onto the surface of appropriate solid medium in Petri dishes and then these were incubated at 36 ± 0.1 °C, for 5 h. After the incubation, the developed organisms were tested by Gram painting and microscopic observation.

2.6. Antimicrobial testing

Antimicrobial activities of the lipid extracts (TL, NL and PL) of the two cultured fish species, sea bass (*D. labrax*) and gilthead sea bream (*S. aurata*) were measured by the paper disk diffusion method. Briefly, sterile, 6 mm diameter filter paper discs GF/C Whatman (Whatman International Ltd. Maidstone England) were impregnated with 30 μl of a number of different concentrations of the TL, NL and PL of sea bass and sea bream and dried under a nitrogen stream. A paper disc impregnated with 30 μl of chloroform, methanol in a ratio of 1:1, which was then dried under a nitrogen stream, was used as control. The agar Petri dishes inoculated with the test organisms were incubated for 2 h before placing the lipid impregnated paper discs on the Petri dish. Following this, the sterile paper discs impregnated with the different lipids were placed on the agar Petri dish. The bacterial dishes were incubated at 36 ± 0.1 °C for 24 h. After incubation, all Petri dishes were observed for zones of inhibition and the diameters of the zones were measured in millimetres. All tests were performed under sterile conditions and repeated three times.

2.7. Statistical analysis

Experiments for the determination of IC_{50} and EC_{50} values were performed in duplicate and the values were expressed as the means of two independent measurements. Experiments on antibacterial activity were performed in triplicate and values were expressed as means ± standard deviation (SD) of three independent measurements. The *t*-test for independent samples was used to compare the antibacterial activities of lipids with the three different bacteria. In the cases where antibacterial activity was induced by different amounts of lipids, an analysis of covariance (ANOVA) was used, where the amounts of lipids were entered as a covariate in order to adjust the values of antibacterial activity to the quantity of lipids. Statistical analysis was performed on the data by SPSS 13.0 (SPSS Inc., Chicago, Ill.) with statistical significance determined at $p < 0.05$.

3. Results and discussion

3.1. Biological activity

So far there are no studies regarding the biological activity of fish lipid extracts in relation to PAF, apart from the work of our research team (Nasopoulou et al., 2007; Nomikos, Karantonis, Skarvelis, Demopoulos, & Zabetakis, 2006; Panayiotou et al., 2000; Rementzis, Antonopoulou, Argyropoulos, & Demopoulos, 1996). In the present study, the biological activity of total, total polar and total neutral lipid extracts from both sea bass (*D. labrax*) and gilthead sea bream (*S. aurata*) were examined in order to investigate their antibacterial properties towards *S. aureus*, *E. faecalis* and *E. coli*.

Each lipid fraction (TL, NL or PL) of the sea bass and gilthead sea bream was tested for its biological activity towards washed rabbit platelets, according to the method of Demopoulos et al. (1979). Briefly, the TL, NL, PL of sea bass and gilthead sea bream were tested for whether they induced platelet aggregation (PAF agonists) or inhibited the PAF-induced platelet aggregation (PAF antagonist). The reversible aggregatory and inhibitory activities are reported in Tables 1 and 2.

The TL of sea bass exhibited both aggregatory and inhibitory activities. Such reaction is common since the TL is a mixture of NL and PL, which show different biological activities. The aggregatory effect of TL was observed in lower amounts and was described by a curve of bell shape which indicated two EC_{50} values (EC_{50} = 0.004 mg, $EC'_{50} = 0.021$ mg) (Fig. 2), while the inhibitory properties were observed in higher amounts (IC_{50} = 0.060 mg). The inhibitory activity of the TL is attributed to NL, which contained PAF inhibitors (IC_{50} = 0.237 mg, $IC'_{50} = 0.434$ mg) (curve of bell shape). The aggregatory activity of the TL is attributed to PL, which induced platelet aggregation in lower amounts (EC_{50} = 0.004 mg, $EC'_{50} = 0.072$ mg) (curve of bell shape), while, in higher amounts (IC_{50} = 0.124 mg), it inhibited the PAF-induced platelet aggregation (Table 1). At this point it is important to mention that the aggregation that is induced by the lipid extracts is much weaker than that induced by PAF and that is the reason why these lipid extracts are characterized as PAF antagonists.

In addition, the TL of gilthead sea bream showed similar biological activity to the TL of sea bass. Specifically, in lower amounts (EC_{50} = 0.008 mg, $EC'_{50} = 0.029$ mg), TL induced reversible platelet aggregation while, in higher amounts (IC_{50} = 0.064 mg), it inhibited the PAF-induced platelet aggregation. The NL, which contained PAF inhibitors (IC_{50} = 1.282 mg), are responsible for the inhibitory activity of the TL, while the PL, which induce platelet aggregation in lower amounts ($EC'_{50} = 0.001$ mg, EC_{50} = 0.037 mg) (curve of bell shape) and inhibit the PAF-induced platelet aggregation in higher

Table 1
EC_{50} and IC_{50} values of TL, NL and PL of sea bass

	Biological activity	EC_{50}[a] or IC_{50}[a]
TL	Aggregation	0.004
	Aggregation	0.021
	Inhibition	0.060
NL	Inhibition	0.237
	Inhibition	0.434
PL	Aggregation	0.004
	Aggregation	0.072
	Inhibition	0.124

EC_{50} value expresses the amount (in mg) of lipids which induces 50% of the maximum reversible aggregation.
IC_{50} value expresses the amount (in mg) of lipids which inhibits 50% of the PAF (2.5×10^{-11} M, final concentration in the cuvette)-induced aggregation.
[a] EC_{50} and IC_{50} values are expressed in mg of TL, NL and PL.

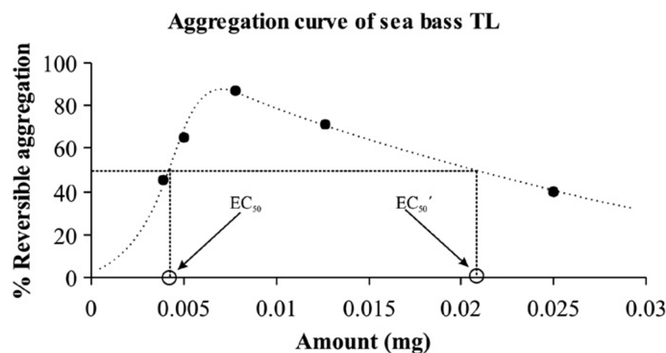

Fig. 2. Representative bell-shaped aggregation curve corresponding to total lipids (TL) of sea bass (*Dicentrarchus labrax*) as % reversible aggregation vs mg of TL. Calculation of the two EC_{50}s is also illustrated. Values correspond to the mean values of two independent experiments.

Table 2
EC_{50} and IC_{50} values of TL, NL and PL of gilthead sea bream

	Biological activity	EC_{50}[a] or IC_{50}[a]
TL	Aggregation	0.008
	Aggregation	0.029
	Inhibition	0.064
NL	Inhibition	1.28
PL	Aggregation	0.001
	Aggregation	0.037
	Inhibition	0.092

EC_{50} value expresses the amount (in mg) of lipids which induces 50% of the maximum reversible aggregation.
IC_{50} value expresses the amount (in mg) of lipids which inhibits 50% of the PAF (2.5×10^{-11} M, final concentration in the cuvette) – induced aggregation.
[a] EC_{50} and IC_{50} values are expressed in mg of TL, NL and PL.

amounts (IC_{50} = 0.092 mg) are responsible for the aggregatory activity of the TL (Table 2). Moreover, the TL of sea bass exhibited stronger biological activity than did the TL of gilthead sea bream and the NL of sea bass contained stronger PAF inhibitors than the ones of gilthead sea bream (Tables 1 and 2).

It is worth mentioning that any washed rabbit platelet aggregation induced by the lipid extracts from sea bass (*D. labrax*) and gilthead sea bream (*S. aurata*) was reversible, meaning that platelets revert to a non-stimulated state, contrary to an irreversible platelet aggregation where platelets maintain a stimulated state. This may have physiological importance in a living organism. Nevertheless, the present results are *in vitro* experiments and *in vivo* studies are now needed.

3.2. Antibacterial activity

Various studies have demonstrated that foodstuffs such as honey, propolis (Miorin et al., 2003), garlic (Ruddock et al., 2005) and tea (Toda et al., 1991), as well as food-originated constituents, e.g. polyphenols (Kusuda et al., 2006) exert antibacterial activities. So far, there are no studies concerning antibacterial effects of lipid extracts from sea bass (*D. labrax*) or gilthead sea bream (*S. aurata*).

Furthermore PAF antagonists have been shown to inhibit bacterial-induced inflammatory actions, e.g. cytokine production underlining the significance of the PAF signalling system in producing inflammatory molecules induced by bacterial toxins, and live Gram-positive and Gram-negative bacteria (Ogata et al., 2004). In this context total, total polar and total neutral lipid extracts, from both sea bass (*D. labrax*) and gilthead sea bream (*S. aurata*), were tested for the antibacterial activity toward *S. aureus*, *E. faecalis* and *E. coli*. The concentrations of the TL, NL and PL that were tested for antibacterial activity were those that corresponded to EC_{50} and IC_{50} values. The tested concentrations and their antibacterial properties are shown in Tables 3 and 4.

The total lipids of both sea bass and gilthead sea bream exhibited antibacterial activity only towards *S. aureus* in amounts of 0.210 and 0.290 mg, respectively, which corresponds to each $10 \times EC'_{50}$. TL of sea bass appear to be more potent than those of gilthead sea bream but failed to reach statistical significance ($p = 0.111$) (Tables 3 and 4).

The neutral lipid extracts of sea bass showed antibacterial activity towards *S. aureus* in the amounts of both $10 \times IC_{50} = 2.37$ mg and $IC'_{50} = 0.434$ mg, with the more potent one being that which

Table 3
Antibacterial activity of the tested amounts of TL, NL and PL of sea bass

	TL					NL		PL					
Amount (mg)	0.210	0.060	0.021	0.004	0.002	2.370	0.434	0.720	0.124	0.072	0.040	0.008	0.004
Amount (times in IC50 or EC50)	$10 \times EC'_{50}$	IC_{50}	EC'_{50}	EC_{50}	$EC_{50} \times 1/2$	$10 \times IC_{50}$	IC'_{50}	$10 \times EC'_{50}$	IC_{50}	EC'_{50}	$10 \times EC_{50}$	$2 \times EC_{50}$	EC_{50}
Inhibition zone (mm)[a]													
S. aureus	7.5 ± 0.3	–	–	–	–	8 ± 0.4	13 ± 0.8[b]	–	–	11 ± 0.6[c]	–	–	–
E. faecalis	–	–	–	–	–	–	7 ± 0.2	–	–	7 ± 0.2	–	–	–
E. coli	–	–	–	–	–	–	7 ± 0.2	–	–	7 ± 0.3	–	–	–

EC_{50} and IC_{50} values are expressed in mg of TL, NL and PL.
EC_{50} value expresses the amount (in mg) of lipids which induces 50% of the maximum reversible aggregation.
IC_{50} value expresses the amount (in mg) of lipids which inhibits 50% of the PAF (2.5×10^{-11} M, final concentration in the cuvette)-induced aggregation.
No antibacterial activity.
[a] Zone of inhibition, including the diameter of the filter paper disc (6 mm). The data are the mean value of three independent experiments ± SD (95% confidence limit).
[b] Denotes higher antibacterial activity of NL (0.434 mg) vs TL and PL towards *S. aureus* ($p < 0.05$), higher antibacterial activity of NL towards *S. aureus* at the amount of 0.434 mg vs 2.37 mg ($p < 0.05$) and higher antibacterial activity of NL (0.434 mg) towards *S. aureus* vs *E. faecalis* and *E. coli*.
[c] Denotes higher antibacterial activity of PL towards *S. aureus* vs *E. faecalis* and *E. coli*.

Table 4
Antibacterial activity of the tested amounts of TL, NL and PL of gilthead sea bream

	TL					NL			PL				
Amount (mg)	0.290	0.064	0.029	0.008	0.001	5.128	1.282	0.321	0.370	0.092	0.037	0.004	0.001
Amount (times in IC_{50} or EC_{50})	$10 \times EC'_{50}$	IC_{50}	EC'_{50}	EC_{50}	$EC_{50} \times 1/10$	$4 \times IC_{50}$	IC_{50}	$IC_{50} \times 1/4$	$10 \times EC_{50}$	IC_{50}	EC_{50}	$EC_{50} \times 1/10$	EC'_{50}
Inhibition zone (mm)[a]													
S. aureus	7 ± 0.3	–	–	–	–	–	8 ± 0.5[b]	–	–	–	7.5 ± 0.4	–	–
E. faecalis	–	–	–	–	–	–	–	–	–	–	7 ± 0.2	–	–
E. coli	–	–	–	–	–	–	7 ± 0.3		–	–	7 ± 0.3	–	–

EC_{50} and IC_{50} values are expressed in mg of TL, NL and PL.
EC_{50} value expresses the amount (in mg) of lipids which induces 50% of the maximum reversible aggregation.
IC_{50} value expresses the amount (in mg) of lipids which inhibits 50% of the PAF (2.5×10^{-11}M, final concentration in the cuvette) - induced aggregation.
No antibacterial activity.
[a] Zone of inhibition, including the diameter of the filter paper disc (6 mm). The data are the mean values of three independent experiments ± SD (95% confidence limit).
[b] Denotes higher antibacterial activity of NL towards *S. aureus* vs *E. coli* ($p < 0.05$).

corresponds to $IC'_{50} = 0.434$ mg ($p = 0.003$). The antibacterial activity against *E. faecalis* and *E. coli* was observed only at the amount of $IC'_{50} = 0.434$ mg and it was less potent than that towards *S. aureus* ($p = 0.004$) (Table 3).

On the other, the neutral lipid extracts of gilthead sea bream showed antibacterial activity towards *S. aureus* and *E. coli* only in the amount of 1.28 mg, that corresponds to a more potent $IC_{50,}$ than that towards *S. aureus* ($p = 0.049$) (Table 4).

The antibacterial activity of NL of sea bass towards *S. aureus* is more potent than that of NL of gilthead sea bream ($p = 0.002$). Considering the fact that the same inhibitory zone (7 mm) was induced by a smaller amount of NL of sea bass (0.434 mg) than that of NL of gilthead sea bream (1.28 mg), the antibacterial properties of NL of sea bass towards *E. coli* appear more potent than those of NL of gilthead sea bream but failed to reach statistical significance ($p = 0.116$). In addition, the NL of sea bass exhibited antibacterial activity towards *E. faecalis* but the NL of gilthead sea bream did not (Tables 3 and 4).

Finally, the PL of both sea bass and gilthead sea bream showed antibacterial activity against all bacterial strains only in the amounts which correspond to $EC'_{50} = 0.070$ mg and $EC_{50} = 0.037$ mg, respectively.

Even though the same inhibitory zone (7 mm) was induced by a smaller amount of PL of gilthead sea bream (0.037 mg) than that of PL of sea bass (0.070 mg), the antibacterial properties of PL of gilthead sea bream, towards both *E. coli* and *E. faecalis*, are equivalent to those of PL of sea bass ($p > 0.05$). in the case of *S. aureus*, the antibacterial activity of sea bass PL was higher than that of gilthead sea bream PL ($p = 0.002$) (Tables 3 and 4).

The antibacterial activity of PL extracts of sea bass, in the amount that corresponds to EC_{50}, was more potent towards *S. aureus* than toward *E. faecalis* and *E. coli* ($p = 0.006$) (Table 3). On the other hand, the antibacterial activities of PL extracts of gilthead sea bream, toward *S. aureus, E. faecalis and E. coli*, were of the same magnitude ($p > 0.05$) (Table 4).

In conclusion, the TL of both fish species showed antibacterial activity only towards *S. aureus* and only in the amount which corresponds to each $10 \times EC'_{50}$ value, with the sea bass being more potent. The NL of sea bass exhibited antibacterial activity towards all bacterial strains, while gilthead sea bream did not exhibit antibacterial activity towards *E. faecalis*. The NL of both sea bass and gilthead sea bream showed antibacterial activity mainly in the amounts of $IC'_{50} = 0.434$ mg and $IC_{50} = 1.28$ mg, respectively, with the NL of sea bass towards all bacterial strains being stronger. The PL of both sea bass and gilthead sea bream exhibited antibacterial activity towards all bacteria strains in the amounts of $EC'_{50} = 0.070$ mg and $EC_{50} = 0.037$ mg, respectively, with the gilthead sea bream being more potent towards *E. faecalis* and *E. coli*.

The fact that the antibacterial activities of the lipid extracts of both fish species were observed in amounts corresponding to the EC_{50} or IC_{50} values, reinforces the idea that PAF antagonists and PAF inhibitors have antibacterial activity. In this study, lipid extracts isolated from sea bass and gilthead sea bream, that contained PAF antagonists or PAF inhibitors, also exhibited antibacterial activity.

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