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Inhibitory effects of safflower and bitter melon extracts on biogenic amine formation by fish spoilage bacteria and food borne pathogens

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ABSTRACT

The impact of safflower (*Carthamus tinctorius*) and bitter melon (*Momordica charantia* L.) extracts on growth of fish spoilage bacteria (*Acinetobacter lwoffii, Pseudomonas oryzihabitans, Enterobacter cloacae, Shigella* spp., *Morganella psychrotolerans, Photobacterium phosphoreum)* and food-borne pathogens (*Staphylococcus aureus, Klebsiella pneumoniae, Enterococcus faecalis, Salmonella* Paratyphi A) and their biogenic amine formation were investigated in mackerel infusion decarboxylase broth (MIDB). The broth microdilution method was used to test antimicrobial activity of extracts. *Ent. faecalis* was the most susceptible microorganism with minimum inhibitory concentrations (MIC) of 6.25 mg/mL against safflower extract, whilst bitter melon had the highest inhibitory effect against growth of *Ent. cloacae, Shigella* spp. and *Ent. faecalis* with MIC of 12.5 mg/mL. Differences in ammonia (AMN) and biogenic amine (BA) production among groups were statistically significant ($p < 0.05$). *Ent. faecalis* (2810 mg/L) was the main tyramine producer in MIDB. Although the effect of extracts varied depending on the bacterial strain and specific amine, both extracts generally decreased AMN and BA accumulation by bacteria. Histamine production by *Phot. phosphoreum* was considerably suppressed in the presence of extracts ($p < 0.05$). As a result, safflower and bitter melon extracts could be used as antimicrobial agents to inhibit bacterial growth and their BA formation in food.

1. Introduction

Microbial growth in seafood as a result of the fish spoilage produces many off-odor and off-flavor compounds including hydroxylamine, biogenic amines (BA), ketones, aldehydes, alcohols, and organic acids (Ghaly, Dave, Budge, & Brooks, 2010). BA are considered as toxic when they are above a certain level (Yongjin et al., 2007). Taking into account their presence and toxic effects, histamine (HIS), putrescine (PUT), cadaverine (CAD), tyramine (TYR), tryptamine (TRP), 2-phenylethylamine (PHEN), spermine (SPN), and spermidine (SPD) among BA are of importance in foods (Marcobal, De las Rivas, Landete, Tabera, & Munoz, 2012; Shalaby, 1996; Silla-Santos, 1996). The consumption of high amount of TYR containing food in combination with monoamine oxidase inhibitors can lead to adverse effects such as nausea, headaches, and blood pressure changes (De Palencia et al., 2011; Ladero, Calles-Enríquez, Fernandez, & Alvarez, 2010). CAD, PUT, HIS and TYR are potential spoilage indicators of food (Özogul and Özogul, 2006). Moreover, PUT and CAD also may inhibit muscle function (Flores, Aristoy, & Toldra, 1996; Kongkiattikajorn, 2015).

Scombroid fish including tuna, mackerel, bonito, and saury with high levels of free histidine in their muscle are frequently associated with outbreak of scombroid poisoning (Taylor, 1986). A legal threshold HIS level set by the European Union is 100 mg/kg for raw fish species especially belonging to Scombridae, Engraulidae, Coryfenidae, Pomatomidae and Clupeidae families (EC, 2005).

BA forming microorganisms may be a part of the endogenous microflora of the fish or result from contamination during processing and storage of these fish (Ruiz Capillas and Jimenez-Colmenero, 2010). BA formation in seafood mainly depends on specific bacterial strains, decarboxylase activity, and amino acid substrate (Rivas et al., 2008; Suzzi and Gardini, 2003). Enterobacteriaceae are the most common causative bacterial group for HIS formation in scombroid fish (Biji, Ravishankar, Venkateswarlu, Mohan, & Gopal, 2016). The species of many genera such as *Bacilus, Citrobacter, Clostridium, Klebsiella,*

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Escherichia, Proteus, Pseudomonas, Shigella and *Photobacterium* are capable of decarboxylation of one or more amino acids (Silla-Santos, 1996).

BA accumulation in food has been controlled by inhibiting microbial growth through chilling and freezing as well as hydrostatic pressures, irradiation, controlled atmosphere packaging, or the use of food additives (Naila, Flint, Fletcher, Bremer, & Meerdink, 2010). The demand for naturally preserved food has increased due to consumer concern about synthetic chemical additives (Lucera, Costa, Conte, & Del Nobile, 2012). Edible plants and their essential oils and isolated compounds include a variety of secondary metabolites that have antimicrobial properties (Burt & Reinders, 2003; Chorianopoulos, Giaouris, Skandamis, Haroutounian, & Nychas, 2008; Tiwari et al., 2009).

Safflower are potentially grown in many regions, especially due to its drought resistance with economic return. Bitter melon grow naturally in tropical climates. The cultivation of this plant is increasing in some countries. It may be beneficial to evaluate these plants for antimicrobial and antioxidants properties. Safflower, the corolla of *Carthamus tinctorius* L. (Asteraceae) is a promising food and cosmetic supplement (Dai, Verpoorte, & Choi, 2014). Flavonoid and oil extracts from safflower showed good antibacterial activity at different dilutions against different strains of bacteria (*Escherichia coli* and *Staph. aureus*) (Sabah and Saleh, 2015). Safflower seeds contain various phenolic compounds, such as lignin and flavonoids (Kim et al., 2007, 2008; Yu et al., 2013). The flower of safflower is reported to be effective for the prevention of inflammation, hyperlipemia, arteriosclerosis, gynecological diseases, and osteoporosis (Shi, 1983; Zhao et al., 2009). Yu et al. (2013) suggested that safflower seed extract might be a good source of bioactive compounds that impart functional and natural antioxidant properties. Safflower extracts contain monoamine transporter modulators and would likely improve neuropsychological disorders through regulating mono-amine transporter activity (Zhao et al., 2009).

Momordica charantia, a member of the *Crucurbitaceae* family and also known as bitter melon, karela, balsam pear or bitter gourd, is a plant which contains phenolic compounds with strong antioxidant properties (Horax, Hettiarachchy, & Islam, 2005). Bitter melon is known to have anti-hyperglycemia, anti-cholesterol, immunosuppressive, anti-ulcerogenic, anti-sepermatogenic and androgenic activities anti-HIV, anti-inflammatory, anti-leukemic, anti-microbial, anti-cholesterol, and anti-tumor activities (Bourinbaiar & Lee-Huang, 1995; Patel et al., 2010; Pitipanapong et al., 2007; Scartezzini and Speroni, 2000). This plant has a few constituents responsible for these effects such as proteins, steroids, and phenolic compounds (Budrat & Shotipruk, 2009; Grover & Yadav, 2004). The medicinal value of bitter melon is due to their content of phenols, flavonoids, isoflavones, terpenes, anthroquinones, and glucosinolates with high antioxidant properties (Silva et al., 2016; Snee et al., 2011).

The extracted antimicrobial compounds from safflower and bitter melon may help to reduce health hazards due to food spoilage and food borne bacteria. Thus, the aim of investigation was to evaluate the antimicrobial effectiveness of the safflower and bitter melon extracts on growth of fish spoilage bacteria (*Acinetobacter lwoffii, Pseudomonas oryzihabitans, Enterobacter cloacae, Shigella* spp., *Morganella psychrotolerans, Photobacterium phosphoreum)* and food-borne pathogens (*Staphylococcus aureus, Klebsiella pneumoniae, Enterococcus faecalis, Salmonella* Paratyphi A) as well as bacterial BA production in mackerel infusion decarboxylase broth (MIDB).

2. Material and method

2.1. Preparation of plant extracts

Extraction of safflower and bitter melon was done according to method of Hossain, AL-Raqmi, AL-Mijizy, Weli, and Al-Riyami (2013) with minor modification. One L of ethanol (96%, Merck 1009831011, Darmstadt, Germany) was added to 200 g plant in daily shakeable containers wrapped in aluminum foil. The plant was kept in a dark place for 1 month. Separation of the natural antioxidant extract from the plant was done according to method of Chen, Shi, and Ho (1992). The yields of the ethanolic extract of bitter melon and safflower were about 2 and 4%, respectively. Thirty g of each extracts were produced and their antimicrobial activity was tested. Sensorial properties of the extracts were measured using 4 trained panellist for odor and aroma perception. The color of both extracts was orange. Safflower had a fibery taste and is odorless while bitter melon has a slight sourish taste and pleasing odor.

2.2. Bacterial strains

Acinetobacter lwoffii, Pseudomonas oryzihabitans, Enterobacter cloacae and *Shigella* spp. were isolated from spoiled anchovy and mackerel. Briefly, vacuum packaged spoiled fish muscles stored at 4 °C were aseptically weighed (about 10 g) and mixed with 90 mL of Ringer solution and then mixed well using a Stomacher (IUL, Barcelona, Spain) for 3 min. After that, 0.1 mL of each diluted solutions was pipetted onto the surface of plate count agar plates in triplicate. They were incubated for 2 days at 30 °C. Each of the individually selected bacterial colonies was streaked several times on the agar plate using a sterile loop to obtain pure colonies. Isolates were then identified using API 20E (20100) and API 20NE (2050) test strip system (BioMérieux SA, Marcy-l'Etoile, France). *M. psychrotolerans* (DSM, 17886) and *Phot. phosphoreum* (DSM 15556) were also used, which are known as spoilage and HIS producing bacteria in fish. The bacterial strains were obtained from the German Collection of Microorganisms and Cell Culture (DSMZ, Braunschweig, Germany).

The 4 food-borne pathogens used were *Staph. aureus* (ATCC29213), *K. pneumoniae* (ATCC700603) and *E. faecalis* ATCC29212, which were provided from the American Type Culture Collection (Rockville, MD, USA), and *S.* Paratyphi A (NCTC13) which was obtained from the National Collection of Type Cultures (London, UK). Nutrient broth (Merck 1.05443.0500) was used for growth of all bacterial cultures. Food-borne pathogens were grown at 37 °C, but *M. psychrotolerans* and *Phot. phosphoreum* were incubated at 28 and 20 °C for 24 h, respectively, before analysis of antimicrobial activity.

2.3. Antimicrobial activity of extracts

Clinical and Laboratory Standards Institute's methods (2008) were used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC). One mL of plant extract (with stock solution of 50 mg/mL) was added to the first tube in each series and serially diluted with Mueller Hinton Broth (MHB, Merck 1.10293). Bacterial strains were adjusted to 1 McFarland (10^6 cfu/mL) using a densitometer (DEN-1, BioSan, Warren MI, USA). After that, the inoculum suspension (1 mL) of each bacterial strains was added in each tube containing plant extract and MHB. The final concentrations of the extracts were 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19 mg/mL. Each tube was evaluated for bacterial growth and compared to the control. As a positive control, a tube containing MHB and bacterial suspension without extracts was used. As a negative control, a tube not having MHB was used. The tubes were incubated at 35 °C for 18–24 h after which the MIC was obtained. MBC was determined by subculturing the contents of tubes of MIC showing no growth.

2.4. Culture conditions and BA analysis

Fish infusion broth was made according to method of Okuzumi et al. (1981) with minor modifications. Mackerel (*Scomber scombrus*) were purchased from Akoluk company (Adana, Turkey) which imports frozen mackerel from Norway. Two hundred fifty g of minced white and dark flesh of mackerel was steamed with 2 vol of water (w/v) at 100 °C for 1 h and filtered through a filter paper (Whatman No. 1, Sigma).

Then, the filtrate was enriched with the addition of 1% glucose and 0.5% NaCl. Three mg of pyridoxal HCl (Sigma P9130, Steinheim, Germany) was also added to the fish infusion broth. After propagation, 0.5 mL of bacterial cultures (~10^8 cfu/mL) standardized using a densitometer (DEN-1, Warren MI, USA) was removed and put into 9 mL of the MIDB. Safflower or bitter melon extracts at doses of 0.5% were added into the MIDB. The control was the absence of any of the extracts. Samples were incubated at 37 °C for 72 h, except for *M. psychrotolerans* and *P. phosphoreum* groups which were incubated at 28 and 20 °C for 72 h, respectively. Extraction and derivatisation of BA were made according to the method of Kuley and Özogul (2011). For extraction of bacterial cultures, 5 mL of the MIDB containing the bacterial strains were removed and placed in separate bottles and then 2 mL trichloroacetic acid was added. They were centrifuged at 3000 × *g* for 10 min using a Hettich 32R centrifuge (Tuttlingen, Germany) and then filtered through a Whatman filter paper. After that, 4 mL of bacterial supernatant was taken for derivatisation of the BA obtained from each of the bacterial strain. The confirmation of BA production was carried out using a rapid HPLC method (Özogul, 2004). For AMN and trimethylamine (TMA) analysis, the same analytic method was done. The mobile phase consisted of acetonitrile and high-performance liquid chromatography (HPLC) grade water for the amine analyses.

2.5. Statistical analysis

Mean and standard deviation of three replicates were measured. The significance of differences ($p < 0.05$) was determined using one way ANOVA with the Statistical Package for the Social Sciences (SPSS) version 15.0 for Windows (SPSS Inc., Chicago, IL. USA).

3. Result and discussion

3.1. Antimicrobial activity of safflower and bitter melon extract

MIC of safflower against fish spoilage bacteria was 12.5 mg/mL for *Acinetobacter lwoffii* and 50 mg/mL for *Ent. cloacae*, *Shigella* spp. and *Photobacterium phosphoreum* (Table 1). *Ent. cloacae* and *Shigella* spp. were the most susceptible fish spoilage bacteria against bitter melon. Among food-borne pathogens, *E. faecalis* had the lowest MIC with value 6.25 mg/mL for safflower and 12.5 mg/mL for bitter melon, although *Staph. aureus* was the most resistant bacteria to the extracts. *Salmonella* Paratyphi A and *Kleb. pneumoniae* were more sensitive against safflower extract (25 mg/mL) than bitter melon extract (> 50 mg/mL). Flavonoid and oil extracts from safflower had good antibacterial activity against *E.coli* and *Staph. aureus* (Sabah and Saleh, 2015). Karimkhani, Shaddel, Khodaparast, Vazirian, and Piri-Gheshlaghi (2016) studied antioxidant and antimicrobial activities in safflower methanolic extracts of different cultivars (IL111, Padide, Isfahan-28 and Mahali). The Isfahan-28 cultivar showed the best antimicrobial activity with 30 and 60 mg/mL of the MIC against *Staph. aureus* and *Salmonella enterica* serovar *typhi*, respectively. Abalaka, Olonitola, Onaolapo, and Inabo (2009) also found that MIC and MBC of ethanol extracts from the leaf and stem of bitter melon ranged from 10 to 30 mg/mL to 15 and 45 mg/mL against *E. coli* and *Staph. aureus,* respectively. *Staph. aureus* ATCC 6538 was the most sensitive microorganism against seed oil of bitter melon, while low antimicrobial activities were found against strains of *E. coli* ATCC 25922 and *Candida albicans* ATCC 10231 with corresponding MIC values of 500 µg/mL (Braca, Siciliano, D'Arrigo, & Germanò, 2008). Yaldız et al. (2015) indicated that inhibitory activity of ethanol extract of different parts bitter melon fraction were not the same and fruit ethanol extract of bitter melon had considerable inhibitory effect on *Staph. aureus*, *Salmonella typhi* and *Aspergillus niger*. Both pulp and skin of bitter melon were found to be effective against tested 11 different Gram-negative bacilli strains including *K. pneumoniae, Salmonella* Parathyphi A and *Enterobacter aerogenes,* (Saeed & Tariq, 2005). Yeo et al. (2014) showed the potency of bitter melon fruit extracts towards Gram-negative bacteria (*E. coli* and *K. pneumoniae*) and fungi (*C. albicans*). MBC of safflower was 25 mg/mL for *M. psychrotolerans* and 50 mg/mL for *Phot. phosphoreum,* but MBC of bitter melon against these bacteria was > 50 mg/mL.

Table 1
MIC and MBC of safflower and bitter melon extracts (mg/mL).

Microorganism/Extract	Safflower		Bitter melon	
	MIC[x]	MBC[y]	MIC	MBC
Acinetobacter lwoffii	12.5	50	25	50
Pseudomonas oryzihabitans	> 50	> 50	50	> 50
Enterobacter cloacae	50	> 50	12.5	25
Shigella spp.	50	> 50	12.5	25
Morganella psychrotolerans	25	25	> 50	> 50
Photobacterium phosphoreum	50	50	25	> 50
Staphylococcus aureus	> 50	> 50	> 50	> 50
Klebsiella pneumoniae	25	> 50	> 50	> 50
Enterococcus faecalis	6.25	25	12.5	25
Salmonella Paratyphi A	25	25	> 50	> 50

MIC[x]: minimum inhibitory concentration, MBC[y]: minimum bactericidal concentration.

3.2. Bacterial growth in MIDB

Table 2 shows the bacterial growth in MIDB in the presence of safflower or bitter melon extract. Significant variations ($p < 0.05$) were observed in growth of *A. lwoffii, E. cloacae*, *Phot. phosphoreum* and *Salmonella* Paratyphi A among groups. Bacterial growth was above 7.5 log cfu/mL and the highest inhibitory activity was observed in the existence of bitter melon against *A. lwoffii* with 1.1 log reduction. Bitter melon also led to fewer *Phot. phosphoreum* in the broth. Among food-borne pathogen, a weak growth inhibition was only observed for *S.* Paratyphi A in the safflower and bitter melon extract with 0.4 and 0.5 log reduction, respectively. Bitter melon extract resulted in a decrease in *E. coli* O157:H7 and *Salmonella* spp. population by 4 and 5 log cfu/mL respectively, over the 21 day storage period at 4 °C. On days 0–3, there was no decrease in the cfu in bitter melon extract spiked with *Salmonella*, although *Salmonella* spp. showed a 5 log reduction on days 14 and 21 (Kelly, 2016). Safflower seed meal extract treatment at doses of 0.7% for 3 min led to 1.5 and 1.6 log cfu/g reduction of total viable count and *L. monocytogenes* in lettuce, respectively, compared with the control (Son et al., 2017).

3.3. AMN and BA production by fish spoilage bacteria

Significant differences ($p < 0.05$) in AMN and BA production were found among groups. AMN and BA production changed depending on

Table 2
Bacterial growth in MIDB (log cfu/mL).

Microorganism/Extracts	Control	Safflower	Bitter melon
Acinetobacter lwoffii	8.7 ± 0.3*[a]	8.5 ± 0.1[a]	7.6 ± 0.6[b]
Pseudomonas oryzihabitans	8.6 ± 0.2[a]	8.1 ± 0.3[a]	8.0 ± 0.0[a]
Enterobacter cloacae	7.9 ± 0.04[a]	7.6 ± 0.1[b]	7.7 ± 0.1[ab]
Shigella spp.	8.4 ± 0.03[a]	8.3 ± 0.1[a]	8.3 ± 0.1[a]
Staphylococcus aureus	8.5 ± 0.2[a]	8.3 ± 0.3[a]	8.5 ± 0.0[a]
Klebsiella pneumoniae	8.8 ± 0.01[a]	8.4 ± 0.1[a]	8.5 ± 0.3[a]
Enterococcus faecalis	8.3 ± 0.1[a]	8.2 ± 0.1[a]	8.2 ± 0.1[a]
Salmonella Paratyphi A	8.7 ± 0.02[a]	8.3 ± 0.2[b]	8.2 ± 0.1[b]
Morganella psychrotolerans	8.1 ± 0.2[a]	8.0 ± 0.1[a]	8.1 ± 0.1[a]
Photobacterium phosphoreum	8.3 ± 0.2[a]	8.2 ± 0.3[ab]	7.7 ± 0.0[b]

*Data are expressed as mean value of three samples, Mean value ± Standard deviation.
[a–b] Indicate significant differences ($p < 0.05$) between control and treated group in a column.

Table 3
AMN and BA production by fish spoilage bacteria in MIDB (mg/L).

	AMN	PUT	CAD	SPD	TRP	FEN	SPN	SER	TMA	DOP	AGM	
PP	730 ± 40^{*a}	10 ± 0.5^{b}	25 ± 1^{b}	260 ± 10^{c}	0 ± 0	69 ± 2^{b}	40 ± 0.1^{b}	45 ± 2^{c}	7 ± 1^{c}	40 ± 3^{c}	16 ± 0.3^{c}	C
	470 ± 20^{b}	6 ± 1^{c}	20 ± 1^{c}	910 ± 20^{a}	0 ± 0	86 ± 2^{b}	140 ± 10^{a}	70 ± 10^{b}	20 ± 1^{a}	210 ± 1^{b}	30 ± 2^{b}	S
	570 ± 30^{b}	13 ± 1^{a}	45 ± 0.4^{a}	700 ± 30^{b}	0 ± 0	150 ± 10^{a}	130 ± 10^{a}	120 ± 10^{a}	12 ± 1^{b}	435 ± 10^{a}	40 ± 2^{a}	B
MP	1400 ± 100^{a}	120 ± 6^{a}	34 ± 3^{a}	500 ± 40^{a}	0 ± 0^{c}	122 ± 10^{a}	50 ± 3^{b}	100 ± 2^{c}	15 ± 1^{b}	186 ± 1^{b}	20 ± 1^{b}	C
	420 ± 10^{c}	90 ± 1^{b}	30 ± 3^{ab}	100 ± 2^{b}	13 ± 0.2^{a}	110 ± 10^{a}	190 ± 10^{a}	160 ± 10^{b}	20 ± 1^{b}	784 ± 1^{a}	60 ± 1^{a}	S
	970 ± 80^{b}	30 ± 2^{c}	24 ± 1^{b}	110 ± 10^{b}	2 ± 0.1^{b}	72 ± 3^{b}	50 ± 2^{b}	280 ± 20^{a}	30 ± 3^{a}	140 ± 0.5^{c}	19 ± 1^{b}	B
SH	1300 ± 100^{a}	180 ± 20^{a}	130 ± 3^{b}	40 ± 1^{c}	2 ± 0.2^{c}	2 ± 0.2^{b}	4 ± 0.01^{c}	70 ± 3^{c}	20 ± 1^{b}	60 ± 0.3^{b}	15 ± 1^{b}	C
	870 ± 60^{b}	130 ± 10^{b}	130 ± 10^{b}	130 ± 10^{a}	10 ± 0.3^{a}	0 ± 0^{c}	20 ± 1^{a}	160 ± 5^{a}	60 ± 2^{a}	90 ± 4^{a}	23 ± 1^{a}	S
	800 ± 10^{b}	170 ± 10^{a}	170 ± 10^{a}	60 ± 2^{b}	5 ± 1^{b}	9 ± 0.5^{a}	13 ± 0.5^{b}	90 ± 10^{b}	18 ± 1^{c}	90 ± 5^{a}	24 ± 2^{a}	B
EC	440 ± 20^{b}	10 ± 1^{b}	26 ± 1^{b}	110 ± 10^{b}	6 ± 0.3^{c}	20 ± 0.2^{b}	70 ± 2^{a}	60 ± 4^{b}	15 ± 0.5^{b}	150 ± 10^{a}	26 ± 1^{a}	C
	510 ± 0.4^{b}	7 ± 0.4^{c}	16 ± 0.1^{c}	110 ± 10^{b}	10 ± 0.4^{b}	18 ± 1^{c}	23 ± 2^{c}	65 ± 3^{b}	12 ± 0.5^{b}	100 ± 4^{b}	23 ± 0.2^{b}	S
	890 ± 80^{a}	20 ± 0.3^{a}	54 ± 3^{a}	700 ± 50^{a}	20 ± 1^{a}	83 ± 1^{a}	60 ± 3^{b}	190 ± 20^{a}	70 ± 3^{a}	130 ± 5^{ab}	30 ± 1^{a}	B
PO	1240 ± 10^{b}	150 ± 10^{c}	160 ± 10^{b}	3 ± 0.3^{c}	10 ± 0.1^{a}	10 ± 1^{a}	15 ± 0.5^{a}	50 ± 1^{c}	10 ± 0.3^{b}	135 ± 5^{a}	20 ± 1^{b}	C
	1315 ± 10^{b}	220 ± 10^{b}	145 ± 10^{b}	80 ± 4^{b}	4 ± 1^{b}	7 ± 0.3^{b}	4 ± 0.3^{b}	64 ± 2^{b}	25 ± 2^{a}	130 ± 10^{a}	26 ± 2^{a}	S
	1600 ± 70^{a}	310 ± 3^{a}	280 ± 1^{a}	140 ± 4^{a}	0 ± 0^{c}	5 ± 1^{c}	3 ± 0.4^{b}	70 ± 0.2^{a}	13 ± 1^{b}	130 ± 10^{a}	22 ± 1^{ab}	B
AI	1000 ± 40^{a}	50 ± 1^{b}	20 ± 1^{c}	80 ± 2^{b}	10 ± 0.1^{a}	42 ± 2^{a}	30 ± 0.5^{b}	50 ± 4^{a}	130 ± 1^{a}	50 ± 3^{a}	13 ± 1^{b}	C
	580 ± 30^{b}	120 ± 3^{a}	40 ± 1^{a}	120 ± 10^{b}	6 ± 0.4^{b}	45 ± 2^{a}	40 ± 1^{a}	40 ± 1^{ab}	40 ± 1^{b}	40 ± 2^{b}	20 ± 1^{a}	S
	430 ± 10^{c}	10 ± 1^{c}	25 ± 1^{b}	500 ± 30^{a}	0.3 ± 0.4^{c}	32 ± 2^{b}	30 ± 1^{b}	40 ± 3^{b}	44 ± 0.2^{b}	40 ± 2^{b}	6 ± 0.4^{c}	B

*Data are expressed as mean value of three samples, Mean value ± Standard deviation. AMN, ammonia; PUT, putrescine; CAD, cadaverine; SPD, spermidine; TRPT, tryptamine; PHEN, 2-phenylethyl amine; SPN, spermine; SER, serotonin; TMA, trimethylamine; DOP, dopamine; AGM, agmatine. PP: *Photobacterium phosphoreum; MP: Morganella psychrotolerans, SH: Shigella* spp., *EC: Enterobacter cloacae, PO: Pseudomonas oryzihabitans, Al: Acinetobacter lwoffii,* C: Control, S: Safflower, B: Bitter melon.
[a–c] Indicate significant differences ($p < 0.05$) between control and treated group in a row.

bacteria and extracts used. Fish spoilage bacteria produced AMN more than 445 mg/L in MIDB. On the other hand this bacteria isolated from spoiled anchovy formed less AMN (< 80 mg/L) in anchovy decarboxylase broth (Kuley et al., 2017). AMN formation by bacterial isolates from spoiled sardine was between 110 mg/L for *L. monocytogenes* and 550 mg/L for *Chryseobacterium indologenus* (Houicher, Kuley, Bendeddouche, & Özogul, 2013). In the present study, AMN production by *Phot. phosphoreum, M. psychrotolerans, Shigella* spp. and *A. lwoffii* was significantly inhibited by both extracts. Safflower extract showed 3.2, 1.7 and 1.4 fold lower AMN accumulation by *M. psychrotolerans, A. lwoffii* and *Shigella* spp. than that of control.

PUT, CAD, SPD, DOP, TYR and serotonin (SER) were the main amines produced by fish spoilage bacteria in MIDB (Table 3). Some *Pseudomonas* strains were identified as PUT producers and had the ornithine decarboxylase gene (De Las Rivas et al., 2008; Lakshmann et al., 2002). The main amine-producing bacteria in iced fish and shrimp were *Alcaligenes, Flavobacterium, Acinetobacter, Shewanella* and *Pseudomonas* (Laksmanan et al., 2002). PUT production by bacteria was 10 mg/L for *Phot. phosphoreum* and 175 mg/L for *Shigella* spp. Similarly, safflower extract had a significant effect on reducing PUT formation by *Phot. phosphoreum, M. psychrotolerans, Shigella* spp., and *E. cloacae*. PUT production for *A. lwoffii* and *M. psychrotolerans* decreased 4–5 fold in the presence of bitter melon extract in the broth. *Pseu. oryzihabitans* produced more PUT in the presence of both extract.

CAD production was the lowest (20 mg/L) with *A. lwoffii* and the highest (160 mg/L) with *Pseu. oryzihabitans.* However, *A. lwoffii* (65 mg/L) and *Ser. liquefaciens* (60 mg/L) were found to be the highest PUT and CAD producers in anchovy infusion broth, respectively (Kuley et al., 2017). Safflower extract also led to lower CAD accumulation by *E. cloacae* and *Phot. phosphoreum,* although the presence of safflower extract in the broth statistically did not alter PUT production by *Pseu. oryzihabitans, Shigella* spp., and *M. psychrotolerans.* Rosemary and sage tea extracts had significantly reduced HIS, PUT, CAD and TMA accumulation in vacuum packaged sardine fillets (Özogul et al., 2011). Sodium alginate coating with rosemary extract inhibited the formation of PUT, CAD and TYR in abalone (*Haliotis discus hannai*) stored under chill conditions (Hao et al., 2017). Kongkiattikajorn (2015) found that total BA concentration was 65% less in fermented pork product (Nham) with ginger extract, as compared to control samples. With addition of garlic extract to fish sauces, HIS, PUT, TYR and SPD and the overall BA levels were reduced by 30, 18, 26, 37, and 27%, respectively (Zhou et al., 2016).

M. psychrotolerans and *Phot. phosphoreum* were the main HIS producers with corresponding value of 40 and 30 mg/L, whilst *Shigella* produced the lowest amount of HIS with 2 mg/L (Fig. 1). *Proteus mirabilis* and *E. cloacae* produced the highest HIS in histidine decarboxylase broth (Houicher et al., 2013). Bacterial strains isolated from milk fish formed 370–1260 mg/kg HIS in tryptic soy broth supplemented with 1% L-histidine (Lee, Lin, Liu, Huang, & Tsai, 2015). Extracts significantly inhibited HIS production by *M. psychrotolerans, Phot. phosphoreum* and *Pseu. oryzihabitans*. The presence of safflower or bitter melon extract in broth resulted in 2–4 fold lower HIS accumulation by *M. psychrotolerans* and *Phot. phosphoreum.* However, *E. cloacae* and *A. lwoffii* produced higher HIS in the presence of both extracts. Grape seed extract significantly reduced HIS and PHEN accumulation in smoke-cured bacon during storage (Wang et al., 2018). Kuley et al. (2017) found that olive leaf extract reduced the accumulation of HIS.

TYR has been shown to be a vasopressor amine responsible for some food-borne migraines and hypertensive crisis in sensitive humans (McCabe-Sellers et al., 2006). Among fish spoilage bacteria, *A. lwoffii* was the main TYR producer (2370 mg/L), although TYR production by *E. cloacae* was the lowest (10 mg/L). *Pseu. oryzihabitans* accumulated 160 mg/L of TYR. In tyrosine decarboxylase broth, *Pseu. oryzihabitans* (1650 mg/L) and *Chryseobacterium indologenus* (770 mg/L) formed the highest TYR (Houicher et al., 2013). TYR production by *Shigella* spp. in the presence of safflower extract was about 90 fold less than that observed in control bacteria (Fig. 2). Similarly, the presence of bitter melon extract in broth resulted in 50-fold lower TYR formation by *A. lwoffii.* However, TYR was formed 3–6 fold higher in the presence of bitter melon extract than that of control by *M. psychrotolerans, Phot. phosphoreum* and *E. cloacae.* TYR reduction was 30.7% in smoke-cured bacon treated with grape seed extracts samples compared with control (Wang et al., 2018).

Some bacteria are able to produce TMA from TMAO in anaerobic respiration (Gram & Dalgaard, 2002; Jorgensen et al., 2001; Shewan, 1977). Safflower extract increased TMA formation by *Phot. phosphoreum, Shigella* spp. and *Pseu. oryzihabitans* but did not alter TMA accumulation by *M. psychrotolerans* and *E. cloacae.* SER production by fish spoilage bacteria was above 45 mg/L. Rosemary and sage tea extracts stimulated SER and agmatine formation in sardine muscle

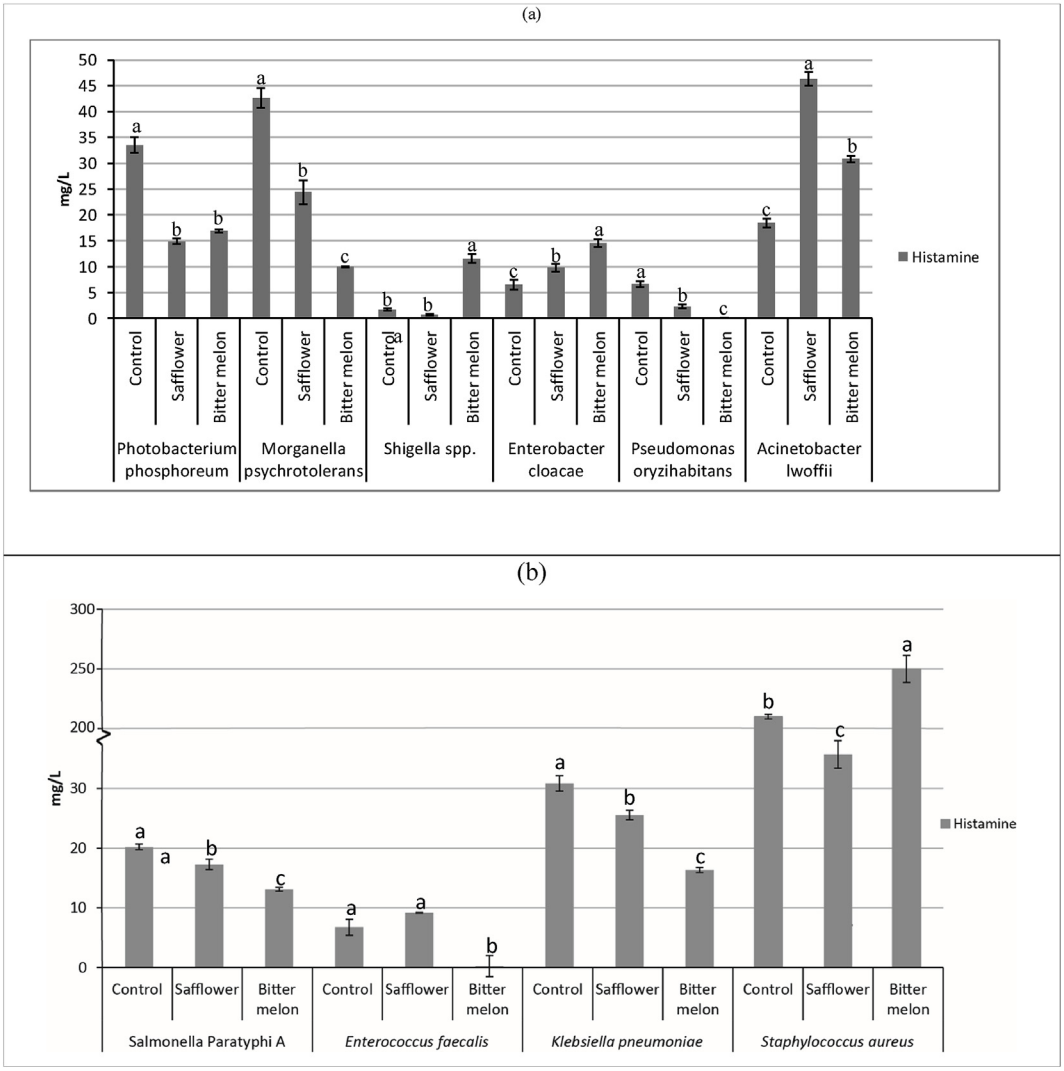

Fig. 1. HIS production by fish spoilage (a) and food-borne pathogen bacteria (b). Data are expressed as mean value of three samples, a–c indicate significant differences ($p < 0.05$) between control and treated group.

(Özogul et al., 2011), which is consistent with current results.

3.4. AMN and BA production by food-borne pathogens

AMN production was the highest with *K. pneumoniae* and the lowest with *Ent. faecalis* with respective level of 720 and 560 mg/L (Table 4). The most significant amounts of AMN were observed for *E. coli* and *Ent. faecalis* with a value of 910 and 685 mg/L, respectively, in histidine decarboxylase broth (Özogul et al., 2015a). Statistically, there was no effect of bitter melon extract on AMN accumulation by *S.* Paratyphi A and *K. pneumoniae.* However, significant increases (about 2 fold) were observed in AMN production by *Ent. faecalis* and *Staph. aureus* in the presence of bitter melon extract.

Food-borne pathogens generally produced all BA but they were mainly TYR, SPD, dopamine (DOP) and SER (Table 4). Significant differences were observed in BA production among groups ($p < 0.05$). The effect of extracts on BA accumulation varied depending on bacteria and specific BA. PUT production by *K. pneumoniae* and *Ent. faecalis* increased from 80 to 130 and 250 mg/L in the presence of bitter melon extract, respectively. *Staph. aureus* produced statistically similar PUT in the presence of both extracts. CAD formed > 25 mg/L with food-borne pathogens and *S.* Parathyphi A and *Staph. aureus* stimulated by both extract. However, there was no effect of safflower extract on *Ent. faecalis* and bitter melon extract on *K. pneumoniae* in terms of CAD accumulation.

HIS production was in range of 6.8 mg/L by *Ent. faecalis* to 210 mg/L by *Staph. aureus.* Similarly, *Staph. aureus* produced the highest amount of HIS (56 mg/L) compared to other bacteria in anchovy infusion decarboxylase broth (Kuley et al., 2017). *Ent. faecalis* produced the lowest HIS (1.6 mg/L) in histidine decarboxylase broth (Özogul et al., 2015b), although *Staph. aureus* did not produce HIS in histidine decarboxylase broth (Gokdogan et al., 2012). Bitter melon extracts significantly inhibited HIS accumulation by bacteria apart from *Staph. aureus* (Fig. 1). Safflower led to 7.5 fold lower HIS formation by *Staph. aureus,* but higher HIS accumulation was observed by bitter melon extract compared to control.

TYR was one of the main amines produced by food-borne pathogens (Fig. 2). Among bacteria tested, *Ent. faecalis* produced considerable amounts of TYR (1170 mg/L) in MIDB, which is consistent with results of a previous report (Burgut, 2019). However, the highest TYR production was found with *P. aeruginosa* (970 mg/L), although the lowest TYR formation was observed for *K. pneumoniae* (6.4 mg/L) in tyrosine decarboxylase broth (Özogul et al., 2015b). TYR production by *S.* Paratyphi A and *K. pneumoniae* was significantly inhibited by both extract, although *Ent. faecalis* and *Staph. aureus* formed very high amounts of TYR in the presence of extract in the broth. TYR production by *S.* Parathyphi A (200 mg/L) dropped 17 and 21 mg/L with safflower and bitter melon extract, respectively. TYR formation by *Staph. aureus*

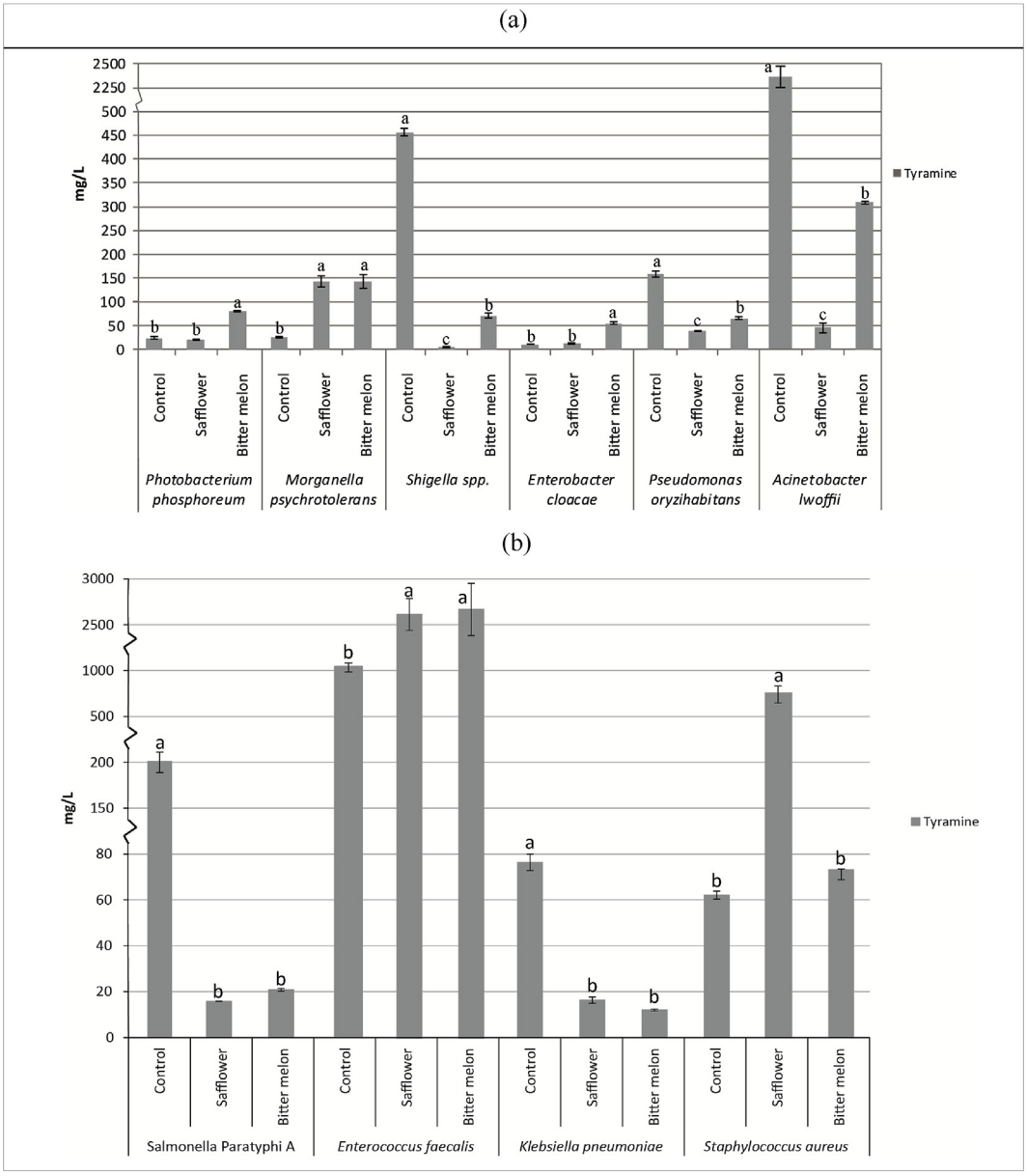

Fig. 2. TYR production by fish spoilage (a) and food-borne pathogen bacteria (b). Data are expressed as mean value of three samples, a–c indicate significant differences ($p < 0.05$) between control and treated group.

Table 4
AMN and BA production by food-borne pathogens in mackerel infusion decarboxylase broth (mg/L).

	AMN	PUT	CAD	SPD	TRP	FEN	SPN	SER	TMA	DOP	AGM	
SP	570 ± 40*[a]	15 ± 1[b]	25 ± 0.2[c]	290 ± 20[a]	8 ± 0.3[b]	32 ± 2[a]	110 ± 4[b]	110 ± 2[b]	16 ± 1[a]	620 ± 20[a]	41 ± 1[a]	C
	380 ± 10[b]	55 ± 3[a]	35 ± 1[a]	160 ± 4[c]	17 ± 1a	18 ± 0.5[c]	140 ± 7[a]	150 ± 10[a]	7 ± 1[c]	220 ± 2[b]	290 ± 2[b]	S
	610 ± 10[a]	10 ± 0.1[c]	30 ± 1[b]	230 ± 10[b]	3 ± 0.3[c]	22 ± 0.04[b]	50 ± 3[c]	100 ± 10[b]	10 ± 1[b]	150 ± 4[c]	32 ± 3[b]	B
EF	560 ± 10[c]	80 ± 6[c]	100 ± 1[b]	17 ± 1[b]	5 ± 0.2[b]	8 ± 0.4[c]	20 ± 2[a]	40 ± 4[c]	56 ± 1[c]	180 ± 5[b]	20 ± 1[b]	C
	860 ± 80[b]	170 ± 3[b]	110 ± 10[b]	15 ± 1[b]	3 ± 0.3[b]	70 ± 10[a]	13 ± 0.4[b]	170 ± 10[b]	170 ± 10[a]	280 ± 3[a]	70 ± 5[a]	S
	1400 ± 100[a]	250 ± 10[a]	160 ± 10[a]	100 ± 4[a]	20 ± 2[a]	40 ± 2[b]	10 ± 0.1[c]	200 ± 10[a]	120 ± 3[b]	170 ± 10[b]	17 ± 0.3[b]	B
KP	720 ± 20[a]	80 ± 7b	30 ± 3[a]	90 ± 8[b]	14 ± 0.1[b]	12 ± 0.52[a]	17 ± 1[b]	66 ± 5[b]	62 ± 4[b]	90 ± 3[a]	24 ± 2[b]	C
	610 ± 50[b]	70 ± 5[b]	20 ± 1[b]	110 ± 0.4[a]	28 ± 0.1[a]	4 ± 0.6[b]	35 ± 2[a]	92 ± 4[a]	110 ± 10[a]	20 ± 1[c]	13 ± 1[c]	S
	650 ± 20[ab]	130 ± 10[a]	30 ± 1[a]	90 ± 3[b]	15 ± 1[b]	0 ± 0[c]	36 ± 1[a]	82 ± 0.5[a]	80 ± 5[b]	30 ± 1[b]	37 ± 0.3[a]	B
SA	700 ± 4[b]	20 ± 0.5[ab]	24 ± 0.03[b]	660 ± 60[a]	23 ± 0.1[a]	22 ± 0.5[b]	50 ± 1[b]	83 ± 2[b]	32 ± 1[b]	90 ± 4[a]	31 ± 2[a]	C
	730 ± 40[b]	20 ± 2[b]	40 ± 1[a]	150 ± 5[b]	19 ± 2[b]	30 ± 2[a]	50 ± 3[b]	110 ± 10[a]	120 ± 10[a]	70 ± 5[b]	26 ± 2[b]	S
	980 ± 70[a]	22 ± 1[a]	34 ± 2[a]	590 ± 40[a]	5 ± 0.4[c]	9 ± 0.5[c]	86 ± 4[a]	120 ± 10[a]	40 ± 1[b]	100 ± 10[a]	32 ± 0.2[a]	B

*Data are expressed as mean value of three samples, Mean value ± Standard deviation. AMN, ammonia; PUT, putrescine; CAD, cadaverine; SPD, spermidine; TRPT, tryptamine; PHEN, 2-phenylethyl amine; SPN, spermine; SER, serotonin; TMA, trimethylamine; DOP, dopamine; AGM, agmatine. SP: *Salmonella* Paratyphi A, EF: *Enterococcus faecalis, KP: Klebsiella pneumoniae, SA: Staphylococcus aureus*, C: Control, S: Safflower, B: Bitter melon.
[a–c] Indicate significant differences ($p < 0.05$) between control and treated group in a row.

(60 mg/L) increased to 790 mg/L in the presence of safflower extract. Özogul et al. (2015a) reported that TYR production by food-borne pathogens was significantly suppressed by addition of carvacrol at levels of 0.5 and 1%, while there was no inhibition with carvacrol at 0.1% on TYR formation by *E. faecalis* and all other *Enterobacteriaceae* species.

4. Conclusion

The extracts led to mostly lower AMN and BA formation by bacteria, although the effect of ethanolic extracts of bitter melon and safflower on AMN and BA were strain-dependent. It has been observed that these extracts have a potential to be used as an antimicrobial food additive for the food industry. However, further research is needed for different food matrices.

Declaration of competing interest

The authors declare no conflicts of interest.

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