Anti-methicillin-resistant *Staphylococcus aureus* (MRSA) activity of Rubiaceae, Fabaceae and Poaceae plants: A search for new sources of useful alternative antibacterials against MRSA infections

M. Sharifi-Rad¹, M. Iriti², M. Sharifi-Rad³, S. Gibbons⁴, J. Sharifi-Rad⁵, ⁶*

¹ Zabol University of Medical Sciences, Zabol 61663335, Iran  
² Department of Agricultural and Environmental Sciences, Milan State University, via G. Celoria 2, Milan 20133, Italy  
³ Department of Range and Watershed Management, Faculty of Natural Resources, University of Zabol, Zabol 98615-538, Iran  
⁴ Research Department of Pharmaceutical and Biological Chemistry, UCL School of Pharmacy, 29–39 Brunswick Square, London WC1N 1AX, UK  
⁵ Zabol Medicinal Plants Research Center, Zabol University of Medical Sciences, Zabol, Iran  
⁶ Department of Pharmacognosy, Faculty of Pharmacy, Zabol University of Medical Sciences, Zabol, Iran

**Abstract:** In this study, we evaluated the effects of the extracts of the leaves of species from the Rubiaceae (*Galiun aparine* L. and *Asperula arvensis* L.), Fabaceae (*Lathyrus aphaca* L. and *Vicia narbonensis* L.) and Poaceae (*Digitaria sanguinalis* (L.) Scop. and *Hordeum murinum* L.) plant families on a wide and extensive panel of isolated methicillin-resistant *Staphylococcus aureus* strains (MRSA). The effects of the methanolic leaf extracts of Rubiaceae, Fabaceae and Poaceae plants on MRSA were evaluated by the disc diffusion assay and the broth dilution method. Among a total of 177 *S. aureus* isolates, 92 (51.97%) were found to be methicillin-resistant in an antibiogram and this was confirmed by the presence of the meca gene in polymerase chain reaction method. All MRSA isolates were sensitive to all extracts. There were dose-dependent inhibitions on tested microorganisms for all plant extracts which showed maximum inhibition zones at a concentration of 300 mg/L. *L. aphaca*, *G. aparine* and *H. murinum* exhibited the highest antibacterial activity on the MRSA strains compared to the positive control (*P* < 0.05), as well as higher total polyphenol and flavonoid contents than other plant extracts. Minimum inhibitory concentrations on MRSA isolates ranged from 388.4 ± 0.2 mg/L in *D. sanguinalis*, to 5.5 ± 0.1 mg/L in *L. aphaca*. The methanolic extracts of *L. aphaca* (Fabaceae), *G. aparine* (Rubiaceae), and *H. murinum* (Poaceae) proved to have high antibacterial activity on MRSA isolates, thus representing promising antimicrobial agents in clinical settings.

**Key words:** Antibiotic resistance, antibiotic therapy, nosocomial infections, medicinal plants, total polyphenol content, total flavonoid content.

**Introduction:** Antibiotic resistance, antibiotic therapy, nosocomial infections, medicinal plants, total polyphenol content, total flavonoid content. Meticillin resistant *Staphylococcus aureus* (MRSA) was first identified almost 50 years ago shortly after the introduction of the antibiotic meticillin (1). MRSA infections are caused by *S. aureus* strains resistant to a number of antibiotics; they are highly contagious and can be spread through direct contact with an infected person. Infection can also be contracted by coming into contact with an object or surface that an infected person has touched. MRSA infections are classified as either hospital-acquired (HA-MRSA) or community-acquired (CA-MRSA). HA-MRSA is associated with infections that are contracted in healthcare settings such as hospitals or nursing homes. It can cause severe problems, such as septicemia and pneumonia. CA-MRSA is associated with infections that are transmitted through close personal contact with an infected person or through direct contact with an infected wound. This type of MRSA infection may also develop as a result of poor hygiene such as infrequent or improper hand-washing (2). In spite of the fact that superficial skin and soft tissue infections remain the most common manifestation of CA-MRSA, severe diseases such as necrotizing fasciitis, necrotizing pneumonia, pyomyositis, septic embolism, venous thrombosis, and osteomyelitis have been described and previously caused death in healthy children (3,4). Despite the availability of new antibiotics, MRSA continues to threaten the world population. Therefore, there is a continuous necessity for novel antimicrobial compounds due to the rapid appearance of MRSA (5, 6).

In developing countries, due to economic constraints, nearly 80% of the population still depends on plant extracts as a source of natural remedies (7). Nevertheless, though plants are greatly exploited in traditional healing systems, only in some cases their therapeutic potential in human has been substantiated (8-10). The need of herb-based medicines, cosmetics, food supplements, pharmaceuticals and health products is progressively increasing all over the world, because, in some cases, natural products *i*) are non or low toxic, *ii*) show low side effects and *iii*) are available at affordable costs (11). Not the least, plant extracts can reduce the occurrence of resistant microbial strains because they consist of many bioactive phytochemicals with different molecular and biochemical targets.

Plants are historically used to treat infectious diseases. In addition, antimicrobial compounds from...
plants may be effective in counteracting infections caused by antibiotic-resistant bacterial strains (12-14). Due to the increasing phenomenon of bacterial resistance to antibiotics, in this study, we investigated the in vitro antibacterial activity of Rubiaceae (*Gailum aparine* L. and *Asperula arvensis* L.), Fabaceae (*Lathyrus aphaca* L. and *Vicia narbonensis* L.) and Poaceae (*Digitaria sanguinalis* (L.) Scop. and *Hordeum murinum* L.) plants against MRSA bacterial strains, in order to find new strategies to control antibiotic-resistant bacteria.

**Materials and Methods**

**Plants and extraction**

The leaves of Rubiaceae (*Galium aparine* L. and *Asperula arvensis* L.), Fabaceae (*Lathyrus aphaca* L. and *Vicia narbonensis* L.) and Poaceae (*Digitaria sanguinalis* (L.) Scop. and *Hordeum murinum* L.) plants (Table 1) were collected between April-May 2013 from the area of Hamun Lake of Zabol, Sistan and Baluchestan Province, Iran. The specimens were identified by a plant taxonomist at the Department of Pharmacognosy, Faculty of Pharmacy, Zabol University of Medical Sciences, Zabol, Iran. The plant leaves were dried in an oven at 60 °C for 72 h. Twenty grams of leaves for each plant were powdered separately and then suspended in 200 mL of methanol 80%, water 20% using a shaker water bath for 24 h at 25 °C. After filtration with Whatman No. 1 filter paper, the resulting solutions were concentrated by a rotary evaporator at 40 °C for 35 min to remove solvent from the extracts. Solid extracts (residues of plant extracts) were stored at -20 °C until further analyses.

**Total phenol content**

Total phenols were assayed based on the method of Dewanto et al. (15). An aliquot of each diluted extract was added to 0.5 mL of distilled water and 0.125 mL of Folin-Ciocalteu reagent. The mixture was shaken and allowed to stand for 10 min, before addition of 1.25 mL of 5% NaCO₃. The solutions were then adjusted with distilled water to a final volume of 4 mL and mixed thoroughly. Absorbance was read at 760 nm versus blank. The total phenolic concentration of each plant was expressed as milligrams of catechin per gram of dry weight (mg CE/g DW) from a calibration curve with gallic acid.

**Total flavonoid content**

The colorimetric assay was used for the assay of total flavonoids according to Dewanto et al. (15). An aliquot of diluted sample or standard solution of (+)-catechin was added to 50 mL of NaNO₂ solution (5%) and mixed for 5 min before the addition of 0.15 mL AlCl₃ (10%). After 5 min, 0.5 mL of NaOH was added. The final volume was adjusted to 2.5 mL with distilled water and mixed thoroughly. Absorbance was determined at 510 nm against blank. The total flavonoid concentration was expressed as milligrams of catechin per gram of dry weight (mg CE/g DW) against the calibration curve of (+)-catechin, from 0 to 400 mg/L.

**Bacterial isolation and culturing**

One hundred and seventy-seven clinical specimens including burns, wounds, urines, pus, and throat swabs were collected from patients who attended the emergency Hospital and Internal Laboratory of Hospital and Central Laboratory in Zabol, Iran, for different infections. Standard isolation protocols were used for all the samples. Identification of *Staphylococcus aureus* was approved by standard techniques accord to diagnostic tests, such as catalase test, culturing on mannitol salt agar, coagulase tube test and DNase.

**Determination of MRSA isolates and antibiotic susceptibility**

For MRSA differentiation from other *S. aureus* isolates, Muller Hinton Agar medium (Oxoid Oxoid Ltd, UK) was used. The strains in a liquid medium of 0.5 McFarland standard concentrations were grown in Muller Hinton Agar medium and antibiotic discs with ampicillin (10 μg), erythromycin (15 μg), penicillin (10 U), ciprofloxacin (5 μg), clindamycin (2 μg), vancomycin (30 μg), methicillin (5 μg) and gentamycin (10 μg) (Mast Group Ltd, UK) were placed on the medium and incubated for 24 h at 37 °C. Then, the diameter of the clear zone around the discs was measured by standards of Clinical Laboratory Standard Institute (17).

**DNA extraction, polymerase chain reaction (PCR) assay and electrophoresis**

For DNA extraction the phenol-chloroform method was used (18). DNA extracted samples were dissolved in Tris acetate-ethylenediaminetetraacetic acid (Tris-EDTA) buffer (HCl 10 mM Tris, 1 mM EDTA, pH = 7.4), and DNA concentration (µg/mL) was determined by a spectrophotometer at A₂₆₀. The quantity of DNA samples used ranged from 10 to 1000 ng. DNA obtained was conserved at -20 °C for further assays. All MRSA isolates were tested for the presence of the mecA gene by polymerase chain reaction (PCR) using previously described primers (19, 20). The standard strain used for MRSA was S. aureus ATCC14458; distilled water was used as the negative control. For PCR, forward and reverse primers (mecA-1 GTGAA-GATATACCAAGTGATT; mecA-2 ATGCCGTATA-GATTGAAAAGGAT) were diluted to reach a concentration of 100 pM. After preparing the PCR mix, amplifications were performed. PCR products were mixed with 1 μL of loading buffer solution and carefully loaded in the wells of the agarose gel (1.5%) and electrophoresed at 75 V for 90 min. The gel was then stained with ethidium bromide (Merck, Germany) solution for 15 min and observed under a UV transilluminator (UV doc, England).

**Disc diffusion assay**

Antimicrobial tests were carried out by the disc diffusion method using 100 μL of bacteria suspension (containing 2.0 × 10⁸ CFU/mL of bacteria) dispersed on Mueller-Hinton agar in sterilized Petri dishes (60 mm in diameter). To the discs (6 mm in diameter, HI Media Laboratories Pvt. Ltd., Mumbai, India) placed on the inoculated agar 10, 25, 50, 100, 150, and 300 mg/L of each leaf plants extracts were added. The inoculated plates were maintained at 4 °C for 2 h and later incubated at 37 °C for 24 h. Antimicrobial activity was determined by measuring the zone of inhibition (mm) against the test bacterial (MRSA isolates and MRSA ATCC14458).
<table>
<thead>
<tr>
<th>Plant name</th>
<th>Family</th>
<th>Common names</th>
<th>Synonyms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galium aparine</td>
<td>Rubiaceae</td>
<td>Cleavers, Clivers, Goosegrass, Catchweed,</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stickyweed, Robin-run-the-hedge, Sticky</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>willow, Velcro weed, Grip grass</td>
<td></td>
</tr>
<tr>
<td>Asperula arvensis</td>
<td>Rubiaceae</td>
<td>Blue woodruff</td>
<td></td>
</tr>
<tr>
<td>Lathyrus aphaca</td>
<td>Fabaceae</td>
<td>Yellow pea, Yellow vetchling</td>
<td></td>
</tr>
<tr>
<td>Orobus aphaca</td>
<td>Fabaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vicia narbonensis</td>
<td>Fabaceae</td>
<td>Narbon pea</td>
<td></td>
</tr>
<tr>
<td>Vicia sativa</td>
<td>Fabaceae</td>
<td>Narbon pea</td>
<td></td>
</tr>
<tr>
<td>Hordeum murinum</td>
<td>Poaceae</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Species names, common names, synonyms and botanic families of the plants used in the study.
Distilled water and vancomycin (30 µg) were used as negative control and positive control, respectively.

**Determination of minimum inhibitory concentration**

For determination of minimum inhibitory concentration (MIC) the broth dilution method was used: 500 µL of 24 h culture of the test organisms (10⁶ CFU/mL) adjusted to McFarland’s turbidity standard, were incubated in distilled water for 24 h at 37 °C in serial dilutions ranging from 3.9 to 500 mg/L of each plant extract. The concentration of the lowest dilution with no detectable bacterial growth was considered as the MIC. Growth absence was confirmed by the absence of turbidity and inoculation into agar (21).

**Statistical analysis**

Diameters of inhibition zones caused by each plant extracts and MICs on different bacteria were analyzed by SPSS v11. Data were compared by one-way ANOVA and Dunnett’s post hoc test. P values less than 0.05 were considered as statistically significant. Each test was performed with three replicates.

**Results**

The total polyphenol and flavonoid contents in methanol leaf extracts are shown in Table 2. Total polyphenols in *G. aparine*, *A. arvensis*, *L. aphaca*, *V. narbonensis*, *D. sanguinalis* and *H. murinum* leaf extract were 76.4 ± 0.2, 8.4 ± 1.1, 160.2 ± 0.5, 6.75 ± 0.2, 4.8 ± 0.3, and 65.9 ± 0.5 GAE/g DW, respectively. Flavonoid contents were 83.7 ± 1.2, 5.5 ± 0.2, 94.6 ± 0.4, 3.9 ± 0.4, 2.3 ± 0.1, and 31.3 ± 0.2 GAE/g DW for *G. aparine*, *A. arvensis*, *L. aphaca*, *V. narbonensis*, *D. sanguinalis* and *H. murinum* leaf extracts, respectively.

Antibiotic resistance/sensitivity of MRSA isolates is reported in Table 3. Ninety-two (51.97%) out of 177 isolates were found to be methicillin-resistant in the antibiogram. In the antibiotic profile of MRSA strains, very high resistance was observed against ampicillin, penicillin and methicillin (100%). A lower degree of resistance was observed against the other antibiotics (ciprofloxacin, clindamycin, erythromycin and gentamicin). In addition, 6 isolates were resistant to vancomycin (Table 3). These results were confirmed by the presence of *mecA* gene in the 92 isolates, as indicated by the 147 bp fragment observed in electrophoresis (Figure 1). The results of the disk diffusion assay, i.e. the inhibition zones of each plant extract, negative control (water) and positive control (vancomycin 30 µg/disc) are shown in Tables 4, 5, and 6. There were

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Total phenolic content (GAE/g DW)</th>
<th>Total flavonoid content (mg CE/g DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Galium aparine</em> L.</td>
<td>76.4 ± 0.2 b</td>
<td>83.7 ± 1.2 b</td>
</tr>
<tr>
<td><em>Asperula arvensis</em> L.</td>
<td>8.4 ± 1.1 d</td>
<td>5.5 ± 0.2 d</td>
</tr>
<tr>
<td><em>Lathyrus aphaca</em> L.</td>
<td>160.2 ± 0.5 a</td>
<td>94.6 ± 0.4 a</td>
</tr>
<tr>
<td><em>Vicia narbonensis</em> L.</td>
<td>6.75 ± 0.2 e</td>
<td>3.9 ± 0.4 e</td>
</tr>
<tr>
<td><em>Digitaria sanguinalis</em> (L.) Scop.</td>
<td>4.8 ± 0.3 f</td>
<td>2.3 ± 0.1 f</td>
</tr>
<tr>
<td><em>Hordeum murinum</em> L.</td>
<td>65.9 ± 0.5 c</td>
<td>31.3 ± 0.2 c</td>
</tr>
</tbody>
</table>

*Gallic acid equivalents per gram of dry weight.

†Milligrams of catechin per gram of dry weight.

§Values are expressed as mean ± SD; different letters show significant differences for each plant in each column at a P < 0.05.

**Figure 1.** Patterns of agarose gel electrophoresis showing PCR amplification products for methicillin-resistant *Staphylococcus aureus* (MRSA) isolates. Lane M: DNA 100 basepair molecular size marker; lane 1: MRSA isolate; lane C+: positive control (reference strain *S. aureus* ATCC14458); lane C-: negative control.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Number of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitive</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>60</td>
</tr>
<tr>
<td>Penicillin</td>
<td>0</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>44</td>
</tr>
<tr>
<td>Methicillin</td>
<td>0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>65</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>86</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>52</td>
</tr>
</tbody>
</table>
dose-dependent inhibitions on tested microorganisms for all plant extracts. All extracts exhibited maximum inhibition at 300 mg/L on MRSA isolates and standard strain (S. aureus ATCC14458). Inhibition haloes on MRSA isolates at 300 mg/L of *G. aparine*, *A. arvensis*, *L. aphaca*, *V. narbonensis*, *D. sanguinalis* and *H. murinum* leaf extracts were 29.3 ± 0.2, 9.9 ± 0.6, 65.5 ± 0.4, 8.8 ± 0.2, 5.4 ± 0.1 and 25.5 ± 0.3 mm in diameter, respectively. The standard strain showed smaller zones of inhibition than the MRSA isolates, and diameters of

dose-dependent inhibitions on tested microorganisms for all plant extracts. All extracts exhibited maximum inhibition at 300 mg/L on MRSA isolates and standard strain (S. aureus ATCC14458). Inhibition haloes on MRSA isolates at 300 mg/L of *G. aparine*, *A. arvensis*, *L. aphaca*, *V. narbonensis*, *D. sanguinalis* and *H. murinum* leaf extracts were 29.3 ± 0.2, 9.9 ± 0.6, 65.5 ± 0.4, 8.8± 0.2, 5.4 ± 0.1 and 25.5 ± 0.3 mm in diameter, respectively. The standard strain showed smaller zones of inhibition than the MRSA isolates, and diameters of
inhibition haloes were 27.8 ± 0.5, 8.5 ± 0.4, 63.3 ± 0.1, 7.8 ± 0.5, 4.8 ± 0.5 and 24.5 ± 0.1 mm for G. aparine, A. arvensis, L. aphaca, V. narbonensis, D. sanguinalis and H. murinum leaf extracts, respectively. The positive control (vancomycin) showed inhibition zones of 18.5 ± 0.1 and 19.8 ± 0.5 mm on MRSA isolates and the standard strain, respectively. Among all plants, the inhibition zones of G. aparine, L. aphaca and H. murinum were more significantly different than the positive control (P < 0.05).

The results of the broth dilution test of plant extracts are shown in Tables 4, 5, and 6. MICs on MRSA isolates were 65.5 ± 0.3, 245.4 ± 0.2, 5.5 ± 0.1, 288.3 ± 0.1, 388.4 ± 0.2 and 83.8 ± 0.2 mg/L for G. aparine, A. arvensis, L. aphaca, V. narbonensis, D. sanguinalis and H. murinum leaf extracts, respectively. The MICs of the extracts against the standard S. aureus strain were 77.9 ± 0.5, 264.3 ± 0.2, 7.8 ± 0.3, 299.5 ± 0.4, 394.9 ± 0.5 and 96.7 ± 0.8 mg/L for G. aparine, A. arvensis, L. aphaca, V. narbonensis, D. sanguinalis and H. murinum leaf extract, respectively and these values were higher than the MICs of the MRSA isolates.

For all the plant extracts, a correlation analysis based on simple linear regression was performed on the assayed variables (total polyphenol content and total flavonoid content vs. MIC) at the 95% confidence level (Figure 2A,B). Both polyphenols and flavonoids were highly correlated with MIC values, with high linear correlation coefficients (R² = 0.787 and R² = 0.776, respectively).

**Figure 2.** Correlation analysis based on simple linear regression at the 95% confidence level between (A) total polyphenol content expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW) or (B) total flavonoid content expressed as milligrams of catechin per gram of dry weight (mg CE/g DW) and minimum inhibitory concentration (MIC, mg/L) of methanol leaf extracts of Galium aparine L., Asperula arvensis L., Lathyrus aphaca L., Vicia narbonensis L., Digitaria sanguinalis L. Scop. and Hordeum murinum L. on methicillin-resistant Staphylococcus aureus (MRSA) strains.

Discussion

The results of our study illustrated that plants with higher levels of total polyphenols and flavonoids in the methanol leaf extracts, i.e. L. aphaca (Fabaceae), G. aparine (Rubiaceae) and H. murinum (Poaceae), showed higher antibacterial activity against the MRSA strains (Figure 2). Antimicrobial activity of plant phenolics, including flavonoids, has been demonstrated since decades (22, 23). Eight flavonoids were isolated in the whole plant of G. aparine: chrysoeriol, apigenin, luteolin, quercetin, chrysoeriol-7-O-β-D-glucoside, apigenin-7-O-β-D-glucoside, luteolin-4’-O-β-D-glucoside and luteolin-7-O-β-D-glucoside (24). More recently, other phenolic compounds were identified in this species, such as coumarin, esculetin, chlorogenic acid, cinnamic acid, p-coumaric acid and quercitrin (25). Two iridoid glycosides were also isolated from the aerial parts of G. aparine, asperulosidic acid and 10-deacetylasperulosidic acid (26), as well as lipophilic compounds including fatty acids, phytosterols, sesquiterpenoids and other isoprenoids (27, 25, 28). In a previous study, a tincture of G. aparine showed a mild inhibitory activity on S. aureus, producing an average zone of inhibition of 2 mm (29). Conversely, S. aureus and Pseudomonas aeruginosa were highly sensitive to the lipophilic extract of this plant (27). Anticancer activity of G. aparine was also reported in leukemia and breast cancer cells (25, 28). A number of flavonoid, namely larcyctin, syringetin-3-O-rutinoside-7-O-β-D-glucoside, quercetin and quercetin-3-O-β-D-glucoside were found in different tissues (leaves, flowers, seeds and pod) of L. aphaca (30, 31). Noteworthy, the butanol extract of L. aphaca seeds exhibited inhibitory activity on S. aureus, Escherichia coli and Klebsiella pneumoniae, with MICs of 76.25, 90.50 and 98.50 µg/mL, respectively (32). To the best of our knowledge, no information is available on phytochemical profile (in terms of polyphenols or flavonoids) and biological activities of H. murinum.

As regards other species, flavonoids isoquercitrin, hyperin, quercetin-7-O-β-D-galactoside, quercetin-4’-O-β-D-galactoside, isorhamnetin-3-O-β-D-galactoside, isorhamnetin-5-O-β-D-galactoside, dihydroykaempfero-7,4’-dimethylether-3-O-β-D-glucoside and isorhamnetin-3-O-a-D-ramnosil-(1’’’→6’’’)-β-D-glucoside were identified in the aerial parts of A. arvensis (33). Similarly, a number of quercetin and kaempferol glycosides were isolated from V. narbonensis fruits and leaves (34), whereas veratic acid (a phenolic compounds), maltol and loliolide (a monoterpenic lactone) were found in D. sanguinalis (35).

In conclusion, this study provides new information on the antimicrobial potential of L. aphaca (Fabaceae), G. aparine (Rubiaceae), and H. murinum (Poaceae) to MRSA strains. As known, mecA gene encodes the protein PBPs2A (penicillin binding protein 2A) in the bacterial cell wall with a low affinity for β-lactam antibiotics (36). In this view, it will be pivotal to investigate the mechanism(s) involved in the observed phenomena, in order to develop new, promising antibacterial agents.

**References**

1. Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H,


23. Raesig S, Sharifi-Rad M, Quek SY, Shabanpour B, Shariri-Rad J. Evaluation of antioxidant and antimicrobial effects of shallot (Allium ascalonicum L.) fruit and ajwain (Trachyspermum ammi (L.) Sprague) seed extracts in semi-fried coated rainbow trout (Oncorhynchus mykiss) fillets for shelf-life extension. LWT-Food Sci Technol 2016; 65:112-121.


