Evaluation of Antioxidant and Antimicrobial Activities of Ethnic Culinary Herbs and Spices

Ayeza Naeem[1*], Tanveer Abbas[1], Tahira M. Ali[2] and Abid Hasnain[2]

1 Department of Microbiology, University of Karachi, Karachi, Pakistan
2 Department of Food Science and Technology, University of Karachi, Karachi, Pakistan

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Correspondent Author:
Ayeza Naeem
E-mail: ayeza.naeem@gmail.com

KEYWORDS

Nigella sativa; Foeniculum vulgare; Coriandrum sativum; Laurus nobilis; alcoholic extract.

ABSTRACT

The antioxidant, total phenolic content, total flavonoid, total flavonol content and antibacterial activities of selected herbs and spices were examined. The spices extracts were prepared by cold solvent extraction method using two different extractants i.e. methanol and ethanol. The antioxidant activity was determined by using DPPH scavenging ability. The content of phenols was evaluated by using Folin Ciocalteu Micro method and flavonoid and flavonol contents were analysed by UV-Spectrophotometry. These herbal decoctions were also tested against five food-borne isolates by agar well diffusion, drop agar diffusion and macrobroth dilution and simultaneous determination of their minimum inhibitory concentrations (MICs). Significantly higher concentration of bioactive compounds was present in ethanolic extracts of tested herbs and these compounds also displayed higher antibacterial potential against all the tested microorganisms. Results presented here suggested that these extracts can therefore be employed as a natural additive in cosmetic, food and therapeutic industries.

تقييم الأنشطة المضادة للأكسدة وللميكروبات من أعشاب الطهي والتوابل

عايزة نعيم[1*]، تانفير عباس،[1] طاهرة علي[2] وعابد حسنين[2]

1 قسم الميكروبيولوجيا، جامعة كراتشي، كراتشي، باكستان.
2 قسم علوم وتكنولوجيا الأغذية، جامعة كراتشي، كراتشي، باكستان

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بريد الكتروني:
ayeza.naeem@gmail.com

الكلمات الدالة

نيجيلا ساتيفا؛ فوينيكولوم فولغاري؛ كوريانْدروم ساتيفوم؛ لوروس نوبيليس؛ مستخلص كحولي.

المُستلخص

تم فحص مضادات الأكسدة، المحتوى الفينولي الكلي، فلافونويد الكلي، محتوى فلافونول الكلي والأنشطة المضادة للبكتيريا من الأعشاب والتوابل المختارة. تم تحضير مستخلصات التوابل باستخدام طريقة استخراج المذيب البارد باستخدام مستخرجين مختلفين، أي الميثانول والإيثانول. تم تحديد النشاط المضاد للأكسدة باستخدام دبف قدرة الكسح. تم تقييم محتوى الفينول باستخدام طريقة فولين سيوكالتيو مايكرو و تم تحليل محتويات الفلافونويد و الفلافونول بواسطة الأشعة فوق البنفسجية الطيفية. وقد تم اختبار هذه الاستخلاصات العشبية أيضا ضد خمسة عزلات تنتقل عن طريق الأغذية عن طريق نشر آغار بشكل جيد، وانخفاض انتشار أجار وتخفيف الماكروبروث والتقدير المتزامن لتركيزاتها المثبطة الدنيا (ميكس). وكان تركيز أعلى بكثير من المركبات النشطة بيولوجيا موجودة في مقتطفات الايثانول من الأعشاب اختبارها، كما أظهرت هذه المركبات إمكانية مضادة للجراثيم أعلى ضد جميع الكائنات الحية الدقيقة التي تم اختبارها. اقترحت النتائج المقدمة هنا أن هذه المستخلصات يمكن بالتالي أن تستخدم كمواد طبيعية في مستحضرات التجميل والغذاء والصناعات العلاجية.

Introduction

In the recent years, the research is widely conducted on natural products to replace the usage of synthetic compounds in food sector. The consumption of these artificial antioxidants such as BHT, BHA, ascorbic acid has led to pronounced side effects on humans (Saito, Sakagami, & Fujisawa, 2003). These side effects may be cancerous such as enlargement of liver size and alleviating the microsomal enzymatic activities in human beings. During the food production process, a variety of antimicrobials such as antibiotics, decontaminants, fungicides, food preservatives are incorporated in food systems to enhance the quality of the food product and simultaneously food production system. This had led to development of antibacterial resistance which appears to be inveterate but this leading problem should be resolved. Currently the issue of antimicrobial resistance has gained importance on both national and international levels due to the possible implications. The increase demand of organic food is another reason behind the discovery of effective, harmless, and antibacterial compounds from natural reserves. In the last few decades, the exploration of herbal extracts and other natural compounds has gained importance due to their bioactive properties without any possible side effects (Senthilkumar & Venkatesalu, 2013). These herbs are also a part of Traditional Arabic and Islamic Medicine (TAIM) and are used to treat different acute and chronic ailments (Azaizeh, Saad, Cooper, & Said, 2010).

Culinary spices are a major source of phenolics, flavonoids, anthocyanins, carotenoids, widely consumed as flavors and also to improve the keeping quality of dishes and processed food items. They are also used to alleviate nutritional attributes and as food preservatives (Moghaddam, Miran, Pirbalouti, Mehdizadeh, & Ghaderi, 2015). Some researches justify the presence of antioxidant, antimicrobial properties and total phenolic contents in various herbal decoctions (Shan, Cai, Brooks, & Corke, 2007).

The present study deals with the isolation of alcoholic extracts of Nigella sativa linn. Arabic approbation (Alenzi et al., 2013; Bakathir & Abbas, 2011; Mohammed & Al-Hijazi; Saad & Said, 2011a, 2011b), meaning the seed of blessing (Family: Ranunculaceae), Coriandrum sativum L. (Family: Apiaceae/Umbelliferae), Foeniculum vulgare Miller (Family: Apiaceae) and Laurus nobilis L. (Family: Lauraceae) and to evaluate their antioxidant, total phenolic, total flavonoid and total flavonol contents. The antibacterial activities of these decoctions were also examined using different methods against five food borne isolates. The tested herbs were selected due to the ease in their availability, common use in culinary purposes, production throughout the year and because of economic feasibility. The method used in this research to prepare extracts is less time consuming, requires less amount of extractant and target plant material and isolates sufficient amounts of bioactive compounds.

Materials and methods

Chemicals

All chemicals used in this research were of analytical grade and were obtained from Sigma Aldrich (Sigmae Aldrich GmbH, Sternheim, Germany). Mueller Hinton agar and broth were purchased from Thermo Scientific™ Oxoid™.

Seed material

Four commonly used condiments i.e. black seeds, fennel seeds, coriander seeds and bay leaf were procured from a local grocery store in Karachi, Pakistan during the month of February 2015.

Extract preparation

A total of eight extracts namely ethanol and methanol extract of Nigella seeds (EHEN) and (MHEN), ethanol and methanol extract of fennel seeds (EHEF) and (MHEF), ethanol and methanol extract of coriander seeds (EHEC) and (MHEC) and ethanol and methanol extract of bay leaf (EHEB) and (MHEB) were prepared for this study according to the method of Biswas, Chatli, & Sahoo, 2012.

Antioxidant activity

The free radical scavenging abilities were determined using the method of Han, Weng, & Bi, 2008.

Determination of total phenolic content

Total phenolic content was determined by Folin Ciocalteu Micro procedure (Waterhouse, 2002). The content of total phenols was calculated as a gallic acid equivalents from the calibration of gallic acid standard solutions.

Determination of total flavonoid content

Total flavonoid content was determined using the method of Hajlaoui et al., 2009. The flavonoid content was calculated as a quercetin equivalent from the calibration curve of quercetin standard solutions, and expressed as milligrams quercetin/ milliliters.

Determination of total flavonol content

Total flavonols were determined by the method of Hajlaoui et al., 2009. Total flavonol content was calculated as a quercetin equivalent from the calibration curve of quercetin standard solutions, and expressed as milligrams quercetin/milliliters.

For all the analytical tests performed, blank (all the reagents minus the sample) was also prepared and the readings of sample were calculated after subtraction from the blank readings.

Determination of antibacterial activity

The antibacterial activity of extracts was determined using five different concentrations i.e. 1000 µg/mL, 500 µg/mL, 250 µg/mL,125 µg/mL and 62.5 µg/mL. Test microorganisms were food-borne pathogens (Escherichia coli ATCC 8739, Vibrio parahaemolyticus ATCC 17802, Listeria monocytogenes ATCC 13932, Bacillus cereus ATCC 11778 and Vibrio alginolyticus ATCC 17749). Each assay was analyzed in triplicate on three segregated experimental runs.

Agar diffusion method (well)

The antimicrobial activity of extracts was determined by agar well diffusion method (Martins et al., 2013). Respective bacterial cultures were inoculated (0.5 Mcfarland) on MHA. The wells were bored using 6mm sterile borer and filled with 70 µL of diluted extracts. The aqueous solution of DMSO was used as a negative control as 40% DMSO do not inhibit the selected pathogens. The petri plates were subsequently incubated at 37±1 ˚C for 18 h.

Drop agar diffusion method

The evaluation of antimicrobial activity was determined by drop agar diffusion method according to Lopes-Lutz, Alviano, Alviano, & Kolodziejczyk, 2008.

Determination of minimum inhibitory concentration (MIC)

The broth macrodilution method was used to determine the MIC according to the method of Pesavento et al., 2015.

Statistical analysis

Analysis of variance was used to determine significant differences between the means, and Duncan's test at $P \leq 0.05$ was employed to separate means using SPSS software (version 24, SPSS Inc., USA) and IC50 values were calculated using GraphPad Prism version 7.0.

Results

Antioxidant activities of extracts

Antioxidant activities in terms of percent scavenging abilities and their relative IC50 values are presented in Table 1. Three different concentrations of extracts were prepared to determine their antioxidant potential i.e. 10µg/ml, 100µg/ml, 250µg/ml. The highest antioxidant in terms of percent scavenging abilities was observed for EHEB (92.26%) with IC50 value of 269.47µg/mL and the lowest observed for EHEC (32.49%) and IC50 value of 225.36µg/ mL at 250 µg/mL. Ethanol solvent was capable to extract more antioxidant compounds as compared to methanol solvent.

Table1. Determination of % DPPH scavenging effect and IC50 values of extractsa

Solvents	% DPPH scavenging effect (% inhibition)			IC50 values(μg/ml)
	10μg/ml	100μg/ml	250μg/ml	
MHEN	22.80±1.05a	29.57±0.95a	53.77±0.90b	75.21±1.05c
EHEN	11.44±1.07a	26.50±1.05a	32.68±1.05a	64.57±0.90b
MHEF	28.74±1.07b	41.64±1.16c	82.66±0.74c	19.22±1.06a
EHEF	30.76±0.89b	54.41±1.005c	61.58±1.002b	51.72±0.78a
MHEC	31.75±1.11c	34.77±1.12b	39.44±0.96a	870.71±1.18a
EHEC	30.67±1.11b	31.52±0.93b	32.49±1.18a	225.36±0.61c
MHEB	37.55±1.05d	75.64±0.81d	83.43±0.74c	23.68±1.009
EHEB	33.74± 0.90c	64.54±0.92d	92.26±1.18c	269.47±1.05d

aValues are means of triplicates ± SD. Values in the same column with different superscripts are significantly different at $P < 0.05$.

Total phenolic, total flavonoid, total flavonol content and antioxidant activity

The antioxidant assays (DPPH, AEAC) measure the relative antioxidant potencies present in tested herbal extracts to scavenge the free radicals produced in the reagents. Table 2 presented the antioxidant abilities determined by DPPH test methods for MHEC, EHEC, MHEN, EHEN, MHEB, EHEB, MHEB and EHEB extracts. All the dilutions of the tested extracts in their respective extractants and blanks in methanol was prepared. However, total flavonol content was determined of undiluted sample. The highest phenolic content in terms of GAE (mg/L) was observed for MHEB (3.98 GAE mg/L) and the lowest for EHEF (2.85 GAE mg/L) at 250 µg/mL. The antioxidant abilities in terms of ascorbic acid equivalents was also tabulated for all the extracts with the highest activity observed for EHEF (40.07 AAE) and the lowest for MHEN (24 AAE) at 250 µg/mL. Total flavonoid content was quantified in terms of Quercetin equivalents with the highest values observed for EHEN (565 mg/mL) and lowest values was observed for MHEF (447.98 mg/mL). Undiluted extracts were tested for total flavonol contents with the highest values observed for EHEC as 472 mg/mL and lowest as 98 mg/mL for EHEN.

Table 2. Total phenolic, total antioxidant, total flavonoid and total flavonol contents of extracts of condimentsa

Extracts	Total Phenolic content GAE (mg/L)			Total Antioxidant content {AAE (Ascorbic acid equivalent) mg/100g dry weight			Total Flavonoid content (mg/ml)			Total flavonol content (mg/ml)
	10 µg/ml	100 µg/ml	250 µg/ml	10 µg/ml	100 µg/ml	250 µg/ml	10 µg/ml	100 µg/ml	250 µg/ml	Un-diluted
MHEN	2.47	2.76	3.25	17.09	20.65	24.43	434.6	510.40	540.33	324.72
	EHEN	±0.09a	±0.06a	±0.92a	±0.73a	±1.03b	±1.44c	±5.04d	±3.18c	±1.11b
EHEN	1.1398	1.51	3.27	35.58	38.77	40.07	377.03	378.40	565.53	98.51
	EHEF	±0.47a	±0.02a	±1.26b	±0.05a	±0.75a	±0.15a	±1.05a	±5.02b	±1.23a
MHEF	2.73	3.05	3.18	28.14	36.83	39.56	361.56	417.29	447.98	241.71
	EHEC	±0.06b	±0.13a	±0.68b	±1.01b	±1.07b	±0.20a	±8.68b	±3.29a	±0.78a
EHEF	1.73	2.43	2.85	32.52	34.26	40.07	422.26	450.80	484.6	410.51
	EHEB	±0.08c	±0.06a	±1.8b	±1.99c	±0.97b	±3.12c	±3.53c	±1.58a	±1.03b
MHEC	2.28	3.67	3.59	37.41	38.26	39.78	369.76	470.79	483.60	355.54
	±0.12a	±0.11d	±0.09a	±0.86b	±0.76b	±1.40a	±0.32b	±1.38c	±1.21b	±1.16c

EHEC	2.16	2.25	3.29	38.84	38.99	39.17	370.09	408.33	438.26	472.75
	±0.06d	±0.55b,c	±0.8a	±0.16a	±1.13a	±1.03b	±0.74b	±.61b	±0.39a	±0.83c
MHEB	2.55	3.43	3.98	16.56	23.72	33.45	356.2	381.93	451.19	471.61
	±0.10a,b	±0.16c	±1.72a	±0.96c	±1.38c	±0.82c	±0.25d	±2.02a	±0.20a	±1.06d
EHEB	1.43	1.62	3.32	20.10	26.07	38.49	590.20	529.46	590.20	534.65
	±0.008b	±0.06a,b	±0.10a	±1.2c	±1.16c	±0.63c	±5.91d	±8.23d	±5.91b	±0.91d

aValues are means of triplicates ± SD. Values in the same column with different superscripts are significantly different at $P < 0.05$.

Antibacterial activities

Table 3 to 5 concluded the antibacterial activities of MHEC, EHEC, MHEN, EHEN, MHEB, EHEB, MHEB and EHEB extracts against Escherichia coli ATCC 8739, Vibrio parahaemolyticus ATCC 17802, Listeria monocytogenes ATCC 13932, Bacillus cereus ATCC 11778 and Vibrio alginolyticus ATCC 17749. Two of these pathogens were Gram positive others were Gram negative ones. The antimicrobial activities were tested in broth and agar medium to have a clear picture of the bactericidal potencies of these extracts. In well diffusion method, EHEN produced lowest MICs against all the tested pathogens while highest MICs was observed for MHEB. In drop agar diffusion method, MHEB yielded highest zone of inhibition against Vibrio parahaemolyticus ATCC 17802 i.e. 19.69 mm lowest zone of inhibition was observed for EHEB against Escherichia coli ATCC 8739 as 10.33mm. Macrobroth dilution method was employed to determine MICs with the lowest MICs observed for EHEF and highest MICs observed for MHEB against selected food-borne isolates.

Table 3. Antibacterial activity of ethanolic extracts of black seeds, fennel, coriander seeds and bay leaf 70µL (1000 µg/mL, 500

Food pathogens tested	**MIC and MTC of extracts (µg/ml)**											
	MIC		**MTC**		**MIC**		**MTC**		**MIC**		**MTC**	
	MHEN	EHEN	MHEN	EHEN	MHEN	EHEN	MHEN	EHEN	MHEN	EHEN	MHEN	EHEN
Escherichia coli ATCC 8739	1000	250	500	500	1000	N/D	500	1000	1000	500	500	250
Listeria monocytogenes ATCC 13932	N/D	1000	1000	500	1000	N/D	500	1000	1000	1000	500	500
Vibrio parahaemolyticus ATCC 17802	N/D	250	1000	125	N/D	1000	1000	500	N/D	500	1000	250
Vibrio alginolyticus ATCC 17749	1000	250	500	125	N/D	N/D	1000	1000	1000	N/D	500	1000
Bacillus cereus ATCC 11778	N/D	250	1000	125	N/D	N/D	1000	1000	N/D	N/D	1000	1000

µg/mL,250 µg/mL,125 µg/mL and 62.5 µg/mL) by well diffusion methoda

a(N/D) No detection of antibacterial activity. Values are means of triplicates ± SD. In case of Vibrio parahaemolyticus ATCC 17802, ethanolic extract of fennel showed MIC of 500µg/ml and MTC of 250µg/ml. For Escherichia coli ATCC 8739 and Vibrio alginolyticus ATCC 17749, methanolic extract of fennel showed MIC of 1000µg/ml and MTC of 500µg/ml.

Table 4. Antibacterial activity of extracts of black seeds, fennel, coriander seeds and bay leaf (Un-diluted) by drop agar diffusion methoda1

Food pathogens tested	Growth inhibition in mm							
	Extracts							
	MHEN	EHEN	MHEF	MHEF	MHEC	EHEC	MHEB	EHEB
Escherichia coli ATCC 8739	10.66±0.57	11.33±0.58	12.67±0.55	N/D	11.0±0.01	N/D	11.0±0.01	10.33±0.58
Listeria monocytogenes ATCC 13932	N/D	11.34±0.57	N/D	15.0±0.01	9.99±0.0005	15.04±0.01	10.33±0.57	15.0±0.01
Vibrio parahaemolyticus ATCC 17802	N/D	17.03±0.06	13.02±0.05	15.0±0.015	13.02±0.05	N/D	19.69±0.52	15.0±0.01
Vibrio alginolyticus ATCC 17749	11.96±0.06	N/D	14.58±0.71	N/D	12.36±1.10	N/D	13.02±0.05	N/D
Bacillus cereus ATCC 11778	11.00±0.98	19.69±0.52	15.02±0.04	N/D	12.34±0.59	10.33±0.58	16.02±0.05	13.51±0.83

a(N/D) No detection of antibacterial activity. Values are means of triplicates ± SD.

Table 5. Minimum inhibitory concentration and maximal tolerated concentration of methanolic and ethanolic extracts by macrobroth diffusion methoda

Food pathogens tested	MIC and MTC of extracts (µg/ml)															
	MIC		MTC		MIC		MTC		MIC		MTC		MIC		MTC	
	MHEN	EHEN	MHEN	EHEN	MHEF	EHEF	MHEF	EHEF	MHEC	EHEC	MHEC	EHEC	MHEB	EHEB	MHEB	EHEB
Escherichia coli ATCC 8739	62.5	N/D	N/D	1000	500	62.5	250	N/D	500	1000	250	500	1000	1000	500	500
Listeria monocytogenes ATCC 13932	62.5	N/D	N/D	1000	250	62.5	125	N/D	500	62.5	250	N/D	1000	N/D	500	1000
Vibrio parahaemolyticus ATCC 17802	125	N/D	62.5	1000	1000	62.5	500	N/D	500	62.5	250	N/D	1000	1000	500	500
Vibrio alginolyticus ATCC 17749	62.5	N/D	N/D	1000	62.5	62.5	N/D	N/D	125	N/D	62.5	1000	1000	1000	500	500
Bacillus cereus ATCC 11778	62.5	1000	N/D	500	1000	250	500	125	250	1000	125	500	1000	1000	500	500

Discussion

Selection of extraction method

The selection of the extractant is the most crucial step in the isolation of extracts from a specific sample. The qualities of an ideal solvent include non-lethal, eco-friendly, extract large quantities of bioactive compounds without their unwanted loss. Considering these necessary requisites, two conventional solvents i.e. ethanol and methanol were used to prepare the extracts of selected herbs and spices. Results from the quantitative determination of total phenolic content, total antioxidant content, total flavonoid content and total flavonol content are summarized in Table 2.

Antioxidant activity

The antioxidant activity of the methanolic and ethanolic extracts of selected herbs and spices were determined by the potential to remove the free radical i.e. DPPH magnitude. DPPH assay was employed to determine free radical scavenging abilities of the alcoholic extracts and the reaction showed a concentration dependent scheme. The extracts concentrations providing 50% inhibition (IC50) are given in Table 1. The percent inhibition activity was highest in the case of ethanolic extracts. A similar trend was observed in the content of phenolic compounds.

Determination of total phenolic, flavonoid and flavonol contents

The content of phenolic compounds in selected herbs did not vary significantly ($P \leq 0.05$). The phenolic, flavonoid and flavonol contents increased in a parallel manner with the increasing concentration of different extracts. The highest values of TPC were observed for MHEB. Phenolic compounds possess redox potential therefore they function as antioxidants (Soobrattee, Neergheen, Luximon-Ramma, Aruoma, & Bahorun, 2005). Hydroxyl groups present in phenolic compounds greatly enhance their radical scavenging potentials, they may act as a tool to estimate antioxidant capacities. The antioxidant potential of plant secondary metabolites including flavonoids, flavonols, tannins is dependent on the presence of hydroxyl groups especially 3-OH. In vitro, these plant flavonoids display antioxidant activities whereas similar compounds have the potency to demonstrate antioxidant capacities In Vivo conditions (Geetha et al., 2003; Shimoi, Masuda, Shen, Furugori, & Kinae, 1996).

Antibacterial activity of extracts

The antibacterial activity of extracts was evaluated in vitro by agar well diffusion method, broth macrodilution method and drop agar diffusion method against five food-borne pathogens i.e. Escherichia coli ATCC 8739, Vibrio parahaemolyticus ATCC 17802, Listeria monocytogenes ATCC 13932, Bacillus cereus ATCC 11778 and Vibrio alginolyticus ATCC 17749. Table 3 to 5 concluded the antimicrobial efficacies of the extracts. The antibacterial activities of the extracts in different assaying medium depends on their relative polar interaction with the medium components. On general basis, the extracts had similar effectiveness to combat Gram positive bacteria as they possess against Gram negative isolates. Gram positive bacteria are generally more sensitive to the herbal extracts (Ceylan & Fung, 2004; Lopez, Sanchez, Batlle, & Nerin, 2005; Smith-Palmer, Stewart, & Fyfe, 1998; Zaika, 1988). This is due to the differences in the cell wall composition of Gram negative and Gram-positive bacteria. The cell wall of Gram negative bacteria is comprised of outer membrane and a periplasmic space which is absent in Gram positive bacterium (Duffy & Power, 2001; Nikaido, 1994). This outer membrane is rich in lipid polysaccharide moieties making them impermeable to antibiotic compounds. The enzymes present in the periplasmic space break down the molecules which intrude from outside the cell. In Gram positive bacteria, which lacks such type of defense mechanisms, antibiotic molecules easily penetrate from the outer cell wall and destroy them resulting in the leakage of cytoplasm and its coagulation (Kalemba & Kunicka, 2003). These results suggested that these extracts comprise of compounds that can inhibit Gram negative bacteria and did not have selective antibacterial potential on the basis of differences in the cell wall composition of Gram positive and Gram-negative bacteria. By comparing the results of the total antioxidant, TPC, total flavonoid, total flavonol contents and antimicrobial activities, the solvent ethanol had the highest efficacy to extract bioactive compounds from different condiments and these compounds were inhibitory to the tested microorganisms (Alimpić, Oaldje, Matevski, Marin, & Duletić-Laušević, 2014; Badu, Mensah, & Boadi, 2012; Kamkar et al., 2014). Therefore, the use of ethanol can be recommended to be used as an extractant. In addition to its ability to extract different polyphenolic compounds it has highly volatile nature making it safe to be used in food products (Russell & Gould, 2003).

Conclusions

Current study clearly concludes that conventional organic solvent extracts of Nigella, Fennel, Coriander seeds and bay leaf have promising efficacies against the growth of food poisoning, spoilage pathogens. In addition to antimicrobial potential, the alcoholic extracts were also rich in antioxidant activity, TPC, total flavonoid and flavonol contents. Therefore, these extracts can be used as easily available source of natural antioxidants and as a possible therapeutic agent or as a food additive. Therefore, it is suggested that further work could be performed on the application of these decoctions in different ethnic medicinal preparations.

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