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# RESEARCH ARTICLE

## DETERMINATION OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF SYRUPS USED FOR UPPER RESPIRATORY TRACT INFECTIONS IN CHILDREN

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**ABSTRACT**

**Background:** It is known that no treatment has yet been developed for upper respiratory tract infections (URTI) caused by many different pathogenic mechanisms and the immune system must be strong in order to overcome the symptoms. **Aim:** To research the antimicrobial effects against clinically significant bacterial and fungal organisms and antioxidant activities of some syrups sold with and without prescriptions and commonly used for URTI in children. **Design and setting:** This study analyzed the syrups recommended by pediatricians for upper respiratory infections. **Method:** The antimicrobial efficacy of 10 syrups, 5 of which are chemical-based and 5 of which are herbal-based, against microbial species was tested with the disc diffusion method and obtained results were statistically analyzed. Additionally, the antioxidant activities of the syrup samples were evaluated based on different methodand total phenolic contents. **Results:** Found herbal-based syrups had mean activity (11 mm) that was higher compared to chemical-based syrups (9.42 mm) according to inhibition diameters. While the total phenolic content of a syrup with chemical origin was higher than that of all other syrups, the antioxidant activity of another syrup containing many herbal extracts at the same time was considerably higher as a result of 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay. **Conclusion:** The strongest antimicrobial activity was identified in syrups with herbal-based active material of *Pelargonium sidoides* root extract and thyme fluid extract. The antioxidant activities of herbal syrups containing extracts of different parts of herbal species such as thyme, licorice root, echinacea, ginger, African geranium, barberry, and acerola were found to be more effective other syrups.

## INTRODUCTION

The most commonly encountered infections in the pediatric period are upper respiratory tract infections (URTI).[1] The most commonly observed URTI are nasopharyngitis, tonsillopharyngitis, acute otitis media and acute rhinosinusitis.[2] Annually, children under the age of 5 experience 6-7 URTI and disease symptoms may last one to two weeks.[3] For symptomatic treatment, most of the time analgesic-antipyretics, decongestants, antihistamines and expectorants or mucolytics are sufficient alone or with combined use.[4] A mixture of a pure chemical substance with biological activity or equivalent vegetable or animal origin, containing a standard amount of active substance, is known as medicine and is used for the prevention or treatment of diseases. These reports suggest that the drugs used may have different positive effects. In order to make a useful contribution to this confusion regarding drugs and their use, this study aimed to research the antimicrobial and antioxidant efficacy of syrups with different types of active ingredients, both chemical and herbal, used for treatment of URTI and the most common symptom of URTI of cough.

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## MATERIALS AND METHODS

**Tested syrups:** Medications were approved by the Ministry of Health and supplementary foods were permitted by the Ministry of Agriculture and Forestry and obtained from pharmacies (Table 1).

**Bacterial and Fungal Strains and Growth Conditions:** In this study the antimicrobial activity of the samples of cough syrup (chemical and herbal drugs) were studied against (ATCC®) *P. aeruginosa* 27853 Gram (-), *E. coli* 25922 Gram (-), *K. pneumoniae* 13883 Gram (-), *C. freundii* 43864 Gram (-), *S. aureus* 6538 Gram (+), *B. cereus* 10876 Gram (+), *E. faecalis* 29121 Gram (+), *L. monocytogenes* 7677 Gram (+), *S.enterica* 14028 Gram (-) and *A. niger* 9642.[5]

**Antibacterial and Antifungal Assay:** Bacterial suspensions with a turbidity of 0.5 McFarland and fungal suspension with a turbidity of 1.0 McFarland standards were prepared.

Inhibition zones were determined after incubation at 27 °C for 48 h. Inhibition zones were measured with the help of digital caliper[5]. All measurements were performed on triplicate samples.

**Minimum Inhibition Concentration (MIC):** The minimum inhibitory concentration (MIC) values were determined by micro-well dilution method. Each of the cough syrups was tested at concentrations of (100- 0.781 µl). The inoculums were incubated at 37 °C for 24 hours. Then, 30 µL of 3-(4, 5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) at 0.5 mg/mL was added to each well and incubated for 30 min. The change to red color indicated that the bacteria were biologically active. [5]

**Total Phenolic Contents (TPCs):** To determine total phenolic contents of the studied syrups, the Folin-Ciocalteu method based on the principle of spectrophotometric measurement of phenolic substances in syrup to form a blue complex by reducing the phosphomolybdic-phosphotungistic solution contained in the Folin-Ciocalteu reagent was followed and TPC values were calculated as gallic acid equivalent (mg GAE/5mL).[6]

**Antioxidative Activity Tests:** The scavenging activity of the DPPH radical of each syrup sample was tested by monitoring the decolorization at 517 nm when sufficient amount of syrup solution was added to the methanolic solution of DPPH. For this purpose, a series of syrup samples in different volumes were added to the DPPH solution. The amount of the syrup sufficient to clean half of the DPPH solution in the medium was calculated and the related value was expressed as $SC_{50}$. To determine the FRAP values of syrup samples as an indicator of antioxidant activity,[7] FRAP values for samples were expressed as Trolox equivalents (mMTrolox/5 mL). Inhibition potentials of the syrup samples on 2,2′-azobis-(2-amidinopropane)-dihydrochloride. (ABAP)-induced lipid peroxidation were also investigated. For this purpose, linoleic acid solution was combined with 0.1µL volume of each syrup samplein the presence of ABAP.[8]

## RESULTS

The values related to antimicrobial activity of cough syrups tested in the scope of this study were summarized in Table 2 Syrup numbered 7 with chemical-derived oxolamine phosphate active agent had high degree of antimicrobial efficacy with 19 mm zone diameter against *E.coli* and *C. freundii* and 26 mm zone diameter against *A. niger* and moderate degree of antimicrobial efficacy with 23 and 22 mm zone diameter against *L. monocytogenes* and *E. faecalis*. Syrup numbered as 9 with chemical-derived levodropropizine active agent showed high degree of sensitivity with 27 mm zone diameter against *E. faecalis*. Syrup numbered as 4 containing acetaminophen, phenylephrine hydrochloride, and chlorpheniramine maleate showed high degree of antimicrobial effect with 40 mm zone diameter against *E.coli*. The other chemical-derived syrups of numbered as 3 containing ambroxol active agent and numbered as 5 containing butamirate citrate were observed not to have antimicrobial effect in general. Herbal-based syrup number 1 had high degree of activity against *L. monocytogenes, E. faecalis*and *P.aeruginosa* with zone diameters of 56, 35 and 30 mm. Syrup number 8 containing *P. sidoides* root extract showed very high degree of activity against the same organisms with zone diameters of 46, 37 and 39 mm, respectively. Syrup number 8 also had high degree of antifungal effect with 16 mm zone diameter for *A.niger*. In second place was activity against fungus with 10.56 mm zone diameter, and lowest effect forming against gram negative bacteria with mean 9.12 mm zone diameter. According to active agents, syrups number 3, 5 and 10 had closest activity to each other and also lowest activity. The minimum inhibitory concentration (MIC) results for syrups tested against microorganisms are shown in Table 2 as mean and minimum value. Syrup number one with largest zone diameter (56 mm) against *Listeria monocytogenes* was shown to have lowest minimum inhibition concentration (MIC) value of <3.25 mg/mL. For other microorganisms, MIC values were in the range of 12.5-100 mg/mL.

**Finding about the phenolic contentes and antioxidative activities of the tested syrups:** In the present study, the total phenolic content followed by Folin-Ciocalteu method was calculated as the gallic acid equivalent for 5 mL of each syrup sample (mgGAE / 5 mL syrup) and the results are listed in Table 3. When the values in Table were examined, the syrup with the number 4 was calculated as the richest in terms of total phenolic. This syrup was followed by the other two syrup samples with the numbers 9 and 10. The DPPH free radical scavenging potentials of the samples were investigated to determine the amount of syrup sweeping 50% of the DPPH radical in the medium.When the values in Table 3 are examined, it can be said that the syrup numbered as 8 with the smallest $SC_{50}$ value (0.28 µL)has the highest sweeping efficacy of DPPH radical, while the syrup numbered as 7 with the highest $SC_{50}$value (1540.12 µL) has the lowest potential among the tested syrups in terms of cleaning the DPPH radical.

## DISCUSSION

Cough, a commonly observed symptom of viral URTI generally passes within two weeks.[9] As a result, the effect of agents like plant roots, honey, vitamins and trace elements on cough has come to be researched more in recent years. One of the tested active ingredients of ambroxol [trans-4- (2) amino-3,5-dibromobenzylamino) -cyclohexane hydrochloride] stimulates surfactant synthesis and secretion from type II pneumocytes in the lungs and prevents sodium absorption by respiratory tract epithelial cells. Thus, the viscosity of secretions from bronchial glands reduces and mucolytic effect occurs. A study found that ambroxol has antibacterial effect against *Aggregatibacter actinomycetemcomitans* and *Streptococcus mutans*.[10] In this study, syrup number 3 containing ambroxol as active agent generally did not show antimicrobial effectAnother chemical syrup contained the active ingredient of one of the synthetic derivatives of oxolamine 3,5-disubstituted 1,2,4 –oxadiazole known to have anti-inflammatory effect in experimental animals. A placebo-controlled study on humans showed that oxolamine reduced cough sensitivity. [11] There was no study about the antimicrobial effect of this active agent encountered in the literature. Syrup number 7 containing oxolamine phosphate as active agent had high degree sensitivity against *E.coli* and *C. freundii and* moderate degree of sensitivity against the gram positive *bacteria L. monocytogenes* and *E. faecalis*. Additionally, it had high degree of sensitivity against *A. niger*. Levodropropizine is a peripherally effective antitussive agent showing inhibitory effect at respiratory tract sensory nerve levels. Syrup number 9 containing levodropropizine as active ingredient was observed to have antibacterial effect against the

**Table 1. Contents of syrups used in the study (given for 5 mL)**

| Product No | Active agent | Assisting material |
|---|---|---|
| 1[x] | Thyme fluid extract 6.372 gr Vitamin C 60 mg Zinc 7.5 mg | |
| 2[x] | *Pelargonium sidoides* root fluid extract in 100 grams (= 93.985 mL) 0.2506 gr Extraction agent ethanol | Maltodextrin, xylitol, glycerol, citric acid anhydrite, potassium sorbate, xanthan gum, pure water |
| 3 | Ambroxol HCl 15 mg | Benzoic acid, cherry essence, sorbitol |
| 4 | Acetaminophen120 mg Phenylephrine hydrochloride 5 mg Chlorpheniramine maleate 2 mg | |
| 5 | Butamirate citrate 15 mg | Sodium saccharin 10 mg, carboxymethylcellulose sodium 12.5 mg, sorbitol (70%) |
| 6[x] | *Hedera helix* folium extract | Potassium sorbate, citric acid, xanthan gum, flavoring, sorbitol, distilled water |
| 7 | 35.5 mg oxolamine-based equivalent 50 mg oxolamine phosphate | Saccharose, methyl paraben, citric acid monohydrate, ponceau 4 R, tutti frutti flavoring, deionized water |
| 8[x] | South African geranium (*Pelargonium sidoides*) root extract 40 mg Licorice (*Glycyrrhiza glabra*) root extract 25 mg Echinacea (Echinacea sp.) root extract 10 mg Propolis extract 10 mg Ginger (*Zingiber officinale*) rhizome extract 5 mg Barberry (*Berberis vulgaris*) root and bark extract 0.5 mg | Pure water, sugar, honey, glycerol, sorbitol |
| 9 | Levodropropizine 30 mg | Methyl paraben, propyl paraben, saccharose, cherry flavoring, monohydrate citric acid, sodium hydroxide, pure water |
| 10[x] | Beta-Glucan 1.3/1.6 10 mg Acerola fruit powder 450 mg | |

[x] Supplementary foods of vegetable origin permitted by the Ministry of Agriculture and Forestry, Herbal-based syrups

**Table 2. Antimicrobial effects of syrups on some pathogenic microorganisms (zone= in mm) (MIC) on microorganisms (in mg / mL)**

| | Syrups | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Microorganism | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Ampicillin | Cephazolin | Nystatin | Mean |
| *P. aeruginosa* | 30.55±0.23 | 6.00±0.00 | 9.60±0.00 | 10.86±1.65 | 6.00±0.00 | 6.00±0.00 | 8.90±0.87 | 39.70±0.87 | 6.00±0.00 | 6.00±0.00 | 33.67±0.15 | 27.33±0.23 | NT | 12.86±0.95 |
| *E. fecalis* | 35.45±0.00 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 6.97±0.00 | 15.00±0.12 | 22.75±0.45 | 37.45±0.19 | 27.75±0.00 | 13.75±0.00 | 33.50±0.023 | 24.27±0.21 | NT | 17.67±0.45 |
| *C. freundii* | 6.00±0.00 | 9.63±0.84 | 6.00±0.00 | 8.16±0.95 | 6.00±0,00 | 6.00±0.00 | 19.67±0.85 | 10.87±0.63 | 6.00±0.00 | 6.00±0.00 | 15.23±0.12 | 17.86±0.59 | NT | 8.56±0.76 |
| *A. niger* | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 26.00±0.87 | 16.75±0.34 | 6.00±0.00 | 6.00±0.00 | 29.76 ±0.67 | 33.67±0.78 | 17.56±0.78 | 10.56±0.56 |
| *E. coli* | 6.00±0.00 | 9.45±0.87 | 11.39±0.00 | 40.06±0.40 | 6.00±0.00 | 6.00±0.00 | 19.00±0.00 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 20.00±0.23 | 19.00±0.00 | NT | 11.76±0.23 |
| *B. subtilis* | 16.00±0.00 | 8.65±0.43 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 33.56±0.45 | 35.67±0.42 | NT | 6,72±0,55 |
| *L.monocytogenes* | 56.67±0.22 | 7.52±0.65 | 7.06±0.73 | 7.87±0.66 | 9.34±0.56 | 7.77±0.88 | 23.87±0.00 | 46.00±0.00 | 6.00±0.00 | 6.00±0.00 | 29.76 ±0.67 | 33.67±0.78 | NT | 17.86±0.23 |
| *K. pneumoniae* | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 9.90±0.00 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 15.34±0.23 | 17.27±0.10 | NT | 6.43±0.87 |
| *S. aureus* | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 10.76±0.54 | 6.00±0.00 | NT | 6.00±0.00 |
| *S. enteric* | 6.00±0.00 | 6.53±0.00 | 9.32±0.12 | 7.23±0.12 | 6.00±0.00 | 7.30±0.13 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 6.00±0.00 | 34.68 ±0.34 | 36.23±0.35 | NT | 6.80±0.56 |
| MIC values (µg /mL Mean and smallest value | 43,75/ 3.25≤ | 44,075/ 3.25≤ | 44,075/ 3.25≤ | 41,575/ 3.25≤ | 45,325/ 3.25≤ | 37,825/ 3.25≤ | 24,125/ 3.25≤ | 30,65/ 3.25≤ | 23,15/ 3.25≤ | 31,9/ 3.25≤ | 3.25≤ | 3.25≤ | 3.25≤ | |
| Mean | 16.76±0.12 | 6.87±0.24 | 6.84±0.23 | 10.55±1.12 | 6.59±0.42 | 7.43±0.56 | 14.87±0.17 | 17.21±0.42 | 8.26±0.14 | 6.80±0.33 | | | | |

(ATCC®)P. aeruginosa 27853 Gram (-), E. feacalis 29121 Gram (+), C. freundii 43864 Gram (-), A. niger 9642, E. coli 25922 Gram (-), B. subtilis B209 Gram (+), L. monocytogenes 7677 Gram (+), K. pneumoniae 13883 Gram (-), S. aureus 6538 Gram (+), S.enterica 14028 Gram (-). NT: No test

**Table 3. Total phenolic contents (TPCs) and antioxidative activity values of the syrup samples**

| | TPC (mg GAE/5 mL syrup) | DPPH ($SC_{50}$; µL) | Lipid Peroxidation Inhibition (for 0.1µL syrup) | FRAP (mM TX/5 mL syrup) |
|---|---|---|---|---|
| 1 | 5.01 | 2.6 | 18.93805 | 119.25 |
| 2 | 2.10 | 11.88 | 0.884956 | 20.64 |
| 3 | 0.75 | 539.52 | 7.256637 | 1.32 |
| 4 | 57.13 | 1.825 | 6.548673 | 78.27 |
| 5 | 1.21 | ND | 0.530973 | 0.37 |
| 6 | 1.89 | 12.76 | 15.04425 | 22.52 |
| 7 | 2.17 | 1540.12 | 13.9823 | 1.03 |
| 8 | 5.53 | 0.28 | 9.734513 | 36.32 |
| 9 | 22.88 | 234.64 | 3.716814 | 3.02 |
| 10 | 24.06 | 1.41 | 13.9823 | 65.46 |

gram positive bacteria of *E. faecalis*. Acetaminophen with analgesic and antipyretic activity was evaluated for antibacterial activity against seven bacterial isolates in a 2010 study and showed sensitivity against the gram-negative bacteria *E. coli* and *S. typhi*, and the gram-positive bacteria *B. subtilis.*[12] Syrup number 4 with acetaminophen as active agent showed similar sensitivity against *E. coli*. El Astal et al. researched the effect of thyme extract on 10 pathogenic microorganisms and found it showed antibacterial activity against *S.aureus and Enterococcus sp*.[13] In this study, the sensitivity of syrup number 1 containing thyme against *L. monocytogenes, E. faecalis* and *P. aeruginosa* supports the literature. Many studies about *Pelargonium sidoides* have shown it is effective against microorganisms like *K. pneumoniae, E. coli, P. aeruginosa, Proteus mirabilis* and *S aureus*.[14,15] In this study, syrup number 8 containing *Pelargonium sidoides* root extract showed high degree of sensitivity against *L. monocytogenes, E. faecalis* and *P.aeruginosa* the mean activity of the herbal-derived syrups (11 mm) was found to be higher than chemical-derived syrups (9.42 mm). Among all syrups, the syrup containing *P. sidoides* root extract had highest activity against all tested microorganisms.

High phenolic content of the drug numbered as 4 can be attributed to the paracetamol content because paracetamol (N-4-hydroxy acetamide; acetaminophen) contains a phenolic group and is known to react with the folin reagent.[16] The high phenolic content of syrup numbered as 9, which contains 30 mg of Levodropropizine in 5 mL as active ingredient (Table 1), can be attributed to the presence of methyl parahydroxybenzoate (E-218) and propyl parahydroxybenzoate (E-216) parabens, which are used as preservatives. On the other hand, it is expected that the total phenolic content of the drug number 10 containing Acerola (Barbados cherry; Malphighia glabra) fruit powder, which is known to have intense vitamin C content (Table 1), is high. Because it is reported that *Malpighia glabra* plant has contain phenolics such as rutin, quercetin, gallic acid, epicatechin, caffeic acid and so on.[17] Syrup number 1 containing thyme liquid extract, Syrup number 2 containing African geranium extract and syrup number 6 containing wall ivy leaf extract as well as syrup number 8 containing extracts from different parts of many plant sources (African geranium, licorice root, echinacea, propolis, ginger, barberry) were expected to show a higher total phenolic content than calculated. The antioxidant potential of medicinal plants is known to be related to the type and concentration of phenolics they contain. [18] DPPH analysis is considered as a measure of the ability of such compounds to present hydrogen atoms.[19] It may not be right to expect a high degree of correlation with the phenolic content at all times.

Namely, for syrup 9, total phenolic content of which was found to be higher than the other drugs, the volume required to sweep 50% of the DPPH radical was calculated to be much higher than the others. In contrast, the $SC_{50}$ value of the syrup numbered as 8, total phenolic content of which was found to be equivalent to 5.53 mg gallic acid in 5 mL of syrup, the indicator of DPPH free radical scavenging potential, was determined to be very low (0.28 µL) and we can say that the antioxidant activity is quite high according to this method. Apart from these two situations, when we look at Table 3, we can see that for syrup sample numbered as 3, $SC_{50}$ value (539.32 µL) as a result of DPPH test and total phenolic content (0.75 mg GAE/5mL şurup) are highly proportional. The high antioxidant activity of the syrup 8 determined by the DPPH test can be attributed to the plant extracts it contains. Because the antioxidant activity of African geranium (*Pelargonium sidoides*) is presented in the literature.[18,20] Likewise, licorice (Glycyrrhiza glabra L.), another herbal ingredient in the content of the syrup, contains valuable and commercial glycyrrhizic acid, glabridin and others flavonoid compounds of interest to the food and pharmaceutical industries. Antioxidant activity data of the echinacea plant, which is another component of the syrup 8, based on DPPH test, were also listed in a review.[21] Russo et al. had reported that in addition to these knowledge, the antioxidant activity of propolis is particularly dependent on caffeic acid phenethyl ester and galangine.[22] Ginger, another component of this herbal medicine, has been the subject of many studies. Root and bark extracts of Berberis vulgaris L. from the Berberidaceae family known as barberry or female saline are among the components of the medicine. It is known that water and alcohol extracts prepared under suitable conditions of Berberis vulgaris, collected from Iran's Southern Khorasan province, has the efficacy of sweeping the DPPH radical. Therefore, when all these literature information is collected and evaluated, it is considered that the syrup numbered as 8, which is completely herbal, has such high DPPH radical scavenging activity in accordance with the literature. On the other hand, it has been demonstrated in a thesis that the antitussive oxalamine component of the syrup 7, which has a high sweeping activity of DPPH when compared with other drugs, has analgesic, antiinflammatory, local anesthetic and antispasmodic properties and has protective effect against renal damage caused by doxorubicin.[23] Antioxidant activity of the syrups examined in this study has also been demonstrated by the FRAP method. According to Albayrak *et al*. report, there is a linear relationship between the results obtained by different electron transfer based methods to measure the reducing capacity of antioxidants.[24] However, a linear relationship between the values obtained as a result of the calculations made with this expectation could not be observed ($R^2$ = 0.0258).

The FRAP values for the 5 mL portions of syrups as numbered 4 and 10,which have a low $SC_{50}$ value obtained by DPPH test and which are more rich in phenolic content than other syrups, are quite high. On the other hand, although the total phenolic content was not high, the FRAP value of syrup 1 was the highest in the Table 3 (119.25 mM TX / 5 mL).This high FRAP value of syrup 1, known to contain about 6.372 g liquid thyme extracts in a 5 mL portion, can be attributed to phytotherapeutic molecules (such as Thymol and Carvacrol) contained in the thyme essential oil composition. Because of their role in many diseases and deaths, intensive efforts are being made to identify and develop compounds that improve the toxic effects of lipid peroxides.[25] It is clinically important to test whether the drugs tested have the potential to prevent the formation of lipid peroxides that can damage the integrity of the cell membrane or whether they can destroy them.It has been shown that very small portions (0.1 µL) of the syrups inhibit lipid peroxidation very effectively.In particular, it can be seen from Table 3 that syrups 1, 6, 7 and 10 provide 15% inhibition in compliance with FRAP values.

**Conclusion**

Each syrup had different degrees of antimicrobial effect linked to differences in their content. According to the results obtained, syrups containing Pelargonium sidoides root extract and thyme fluid extract had highest degree of antimicrobial effect. In light of this knowledge, the tested syrups may be used to benefit from antimicrobial effects when necessary in upper respiratory tract diseases.

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