



## DETERMINATION OF MICROORGANISMS MARKERS BY THE METHOD GC-MS AND EFFICACY EVALUATION of RHINOSINUSITIS

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**ABSTRACT:** The work is devoted to determination of microorganisms markers by the method GC-MS (gas chromatography with ions mass detection) and efficacy evaluation of rhinosinusitis treatment. The microorganisms markers were revealed by the method GC-MS from nasal cavity, and, there were detected such microorganisms as: *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Streptococcus viridans*,  $\beta$ -hemolytic streptococcus group A, *Streptococcus mitis/oralis*, *Streptococcus sanguinis*, *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, *Streptococcus haemolyticus* Gram (-) flora were typical for children of yearly age with acute rhinosinusitis which served as diagnostic criterion. Use of anise ethereal oil as antimicrobial, antiviral, antioxidant medicine led to restore microbial view in patients with acute rhinosinusitis and brought them to normal indices with efficacy 98 %.

**KEYWORDS:** children, early age, acute, sinusitis, ethereal oil, anise, chromatography, marker, quality, quantity, microflora.

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### INTRODUCTION

The problems of acute rhinosinusitis (ARS) having been urgent in connection of widespread of the given disease. Rhinosinusitis became one of the prevalent diagnoses in ambulatory practice. The statistics shows that in the USA about 15% adult populations suffer from various forms of rhinosinusitis [1], as well as in Russia approximately 10 million people suffer from this disease yearly, and, the given pathology composes from 15 to 36% [2] in structure of ENT-hospitals. According to medical statistics of Healthcare Department of Moscow City the prevalence of sinusitis makes up 1 420 cases per 100 thousand of adult population [3].

It is known that ARS etiopathogenesis was mainly caused by rhino-genetic infection of PRN (paranasal sinuses) which were the natural co-ostia, through those the aeration and drainage are carried out [4, 5]. The trigger of ARS was the viral infec

The reduction of muco-ciliary speed transport allows to prolong contact time of pathogenic bacteria with mucous membrane and promote to bacterial infection [2,5].

As show many researches, the main agents of bacterial ARS and ABRS (acute bacterial rhinosinusitis) were: *Streptococcus pneumoniae* (20–43 %), *Haemophilus influenzae* (22–33 %) and *Moraxella catarrhalis*, at adults [7]. The most significant from these microorganisms is pneumococcus *Streptococcus pneumoniae*, as the occurrence of spontaneous eradication of given pathogen at ABRS composes only 30%, whereas, for *Haemophilus influenzae* and *Moraxella catarrhalis* it was 60 and 80% accordingly [6,8]. For other possible agents (anaerobes, streptococcus, including *Streptococcus pyogenes*, and *Staphylococcus aureus*) the possibility of spontaneous eradication composes 50/50 [7].

According to the data of Russian researches, the dominating agents of ABRS in adult patients are also pneumococci and hemophilic bacilli but *Moraxella catarrhalis* is separated in single case. Side by side with this, in some studies there is marked the change of general results picture for microbiologic study at ABRS, and, it is predominance of hemophilic bacillus in some populations, and, also separation from smears for streptococci in different serous groups [9,10,11]. By the information of foreign authors there took place the increase of specific weight of *Staphylococcus aureus* in etiological structure of ABRS agents [12].

Therefore, it can be proved that the most important factors of rhinosinusitis development were inflammatory process and edema of mucous membrane in nasal cavity, in sinuses the disorder of their aeration and drainage, viral and bacterial infections.

One of the alternative approaches to reveal microecological disorders in human organism was analysis of specific micro-biotic markers, making up the wide spectrum of fatty acids with odd number of carbohydrates atoms being branched as chains and hydro-oxyle groups. Now there are known more than 250 such markers (human organism may have only about 25 of them). They are able to spread to all parts of body and can be found even in low concentration with the help of gas chromatography and ions mass-selective detection (GC-MS) [13–15].

The given method was applied in given study. The high sensitivity of GC-MS – method allows quantitatively to detect and find the microbiota markers in very low concentration, which makes possible to make a sample content analysis.

From the other side according to many clinical researches, having been taken for the last decade, the more perspective and purposeful were methods of inhalational therapy with mixture of ethereal oils at treatment acute sinusitis, which made multi-profiled effects: antibacterial, antiviral, anti-inflammatory, sedative, tone up, relieving respiration, immune modifier [16–18]. The interest to aroma therapy has already arisen in ancient civilizations of Egypt, India and Greece. For many ages the natural ethereal oils were applied not only as ones causing the esthetic senses, but as drugs with therapeutic aims [19].

The antimicrobial effect of ethereal oils was almost spread on all groups of pathogenic microbes including antibiotic-resistant strains. The series of authors showed that under influence of ethereal oil steams the qualitative content of respiratory ways microflora was improved, the microbial covering of skin and pathogenicity of auto-flora were reduced. The ethereal oils stimulated humoral cellular immunities, having activated T-cells, synthesis of local immune globulin A, alveolar macrophages and so on.

Moreover, it was marked that inhalation method of aromatherapy with use ethereal oils for prophylaxis and treatment acute infections of respiratory ways had series such positive results:

- safety and comfort of the method, allows to use it in all aged patients' groups;
- maximum physiological introduction of medicine was together with breathing in air;

the straight action on mucous membrane of respiratory organs, arresting of congestive and inflammatory reactions;

alleviating action of oil microparticles on mucous membranes. By the authors' data [20] it was known that the drug of ethereal oils for passive inhalation was oil «Breathe (ZAO «AQION», Russia). It composes of natural pure ethereal oils of vegetable origin: mint (without menthol), eucalyptus, cedar, vintergren, juniper, cloves and levomenthol. The results of randomized clinical studies proved the positive effect of use the given oils «Breathe» at acute infection of upper respiratory ways. The complex effect of natural ethereal oils «Breathe» was caused with its component properties. Use of eucalyptus oil led to reduce headache, stuffy nose, inflammatory expression of mucous membranes in nasal cavities, improvement of general state at patients with rhinosinusitis [21]. Moreover, the eucalyptus oils possess antibacterial and antiviral activity [22].

Stimulation of cold-like receptions in upper respiratory ways, which was carrying out at breathing in menthol steam, decrease discomfort sense, and, by that relieve nasal respiration [23].

Antibacterial effect also was made by cedar oil. Combination with antibiotics was mutually enhanced the antimicrobial effect of both drugs [24].

The juniper oil has antibacterial action in quite high concentrations (from 8%), but the essential fungicide activity it has already shown at concentration from 0,39% [25], which may be used in complex therapy for mycosis in upper respiratory ways.

The anti-inflammatory effect of mint oil was shown in experience with monocytes of healthy volunteers: use of mint oil and menthol led to monocyte suppression of inflammatory mediators [26–28].

The important specific feature of complex of ethereal oils was absence of limitation the course of treatment at daily application.

The researches, having taken in Russian hospitals, demonstrated efficacy and safety of use the complex of ethereal oils «Breathe» at children with different aged groups in therapy and prophylaxis of acute respiratory diseases.

Under of control employees in Pediatric Department at Tumen State Medical Academy the evaluation efficacy of oil «Breathe» in complex therapy of acute respiratory diseases for children was proved [29]. There were 112 children at the age from 5 to 8 years. At the result of study there was shown that use oil «Breathe» for prophylaxis of respiratory infections took to decrease the amount of cases for ARVI on 65% in comparison with control group, though, in often sick children at the first signs of disease it allowed to relieve the course essentially and to prevent the development of complications.

Furthermore, under the control of employees in Nizhniy Novgorod State Medical Academy of Ministry of Healthcare and Social Development in Russia, there was carried the efficacy evaluation of medicine «Breathe» in complex therapy of acute respiratory diseases at children from 3 to 14 years old in ambulatory and polyclinic conditions [30]. There were 45 children with acute respiratory diseases under observation, who had relapsing course in anamnesis. The application of «Breathe» allowed to reach the decrease of ARD length in 1,6 times. It was marked that there was statistically significance decrease of segment and nuclear neutrophils, eosinophils in nasal secretion before and after use «Breathe». There were no registered the unfavorable manifestations at children with allergic diseases in anamnesis, who had the full course of «Breathe».

Besides, for local use the warming gel «Breathe» can be applied for children. The content of given composition has badger fat, levomenthol, ethereal oils of eucalyptus, mint, lavender, fir tree and

turpentine oil. If it is laid on skin the substances make long warming and local irritating effect. The ethereal oils in gel content make analgesic, anti-inflammatory, tone up, antibacterial and antiviral effect.

The warming gel "Breathe" can be used as prophylaxis of acute respiratory diseases after super cooling, the first symptoms of acute respiratory diseases as well as including in coughing.

Thus, coming from the above-mentioned data, it can be spoken about possibility of use phyto-therapeutic medicines, particularly, the complex of ethereal oils "Breathe in combined treatment of rhinosinusitis [31-34].

With the account of anti-inflammatory activity, the medicine may be recommended as the therapy means for post-viral rhinosinusitis at children. The anti-bacterial effect of drug component makes possible to use it together with anti-bacterial medicines which allow to recommend the use complex of ethereal oils "Breathe" and for treatment bacterial rhinosinusitis. Moreover, there is necessity of further study the efficacy and safety of medicine including complex of ethereal oils at treatment acute rhinosinusitis in children, which demands to carry out clinical researches in future. In addition, the aromatherapy with use "Breathe" make favorable influence not only on state of upper respiratory ways, but and on harmonization of mental functions as: life quality and adaptive child's status.

Coming from the above mentioned information it can be proved that at treatment acute sinusitis the most effective and non-hazardous were ethereal oils use, which is very actual in otorhinolaryngology.

**The aim of study** was kind identification of microorganisms at children of early age with acute rhinosinusitis by the gas chromat-mass-spectrometry and efficacy evaluation of use ethereal oil of anise at patients treatment.

#### **MATERIAL AND METHODS OF STUDY**

The research was carried out in clinic of TashPMI (Tashkent Pediatric Medical Institute). The study included 46 sick children.

As the materials were: use washings off from nasal cavity. It was carried out in aseptical conditions. 46 samples of complex analyses were taken from nasal cavities by the method GC-MS.

The presence of microflora at children of early age was detected with special markers for every sort of microorganism, which was determined by GC-MS method. Method of GC-MS was applied to determine microorganisms of fatty acids in the kind of trimethyl-cilil derivatives. The material was gathered with sterile cotton wool was put in Ames' transport coal environment and brought to laboratory not later than 24 hours. At preparation for chromat-mass-spectrum analysis the sample on cotton wool was dried with addition of equal methanol volume and undergone to sour methano-lysis in 1M HCl methanol. The metano-lysis was carried out in 0,4 ml reagent for 10 –15 mg of dry residue during 1 hour at the temperature 80°C. At this stage occur separation of fatty acids and aldehydes from complex lipids of microorganisms and other cells of liquid as methyl ester and de-methyl-acetals. These components get extractable with hexan (400 mcl) for 5 minutes, hexan extract was dried, and, and the dry residue was treated 20 mcl N,O-bis(trimetyl-cilil)-tri-fluorine-acetamide for 15 minutes at 80°C for taking trimetyl-cilil esters,oxy-acids and sterols. To reactional ester mixture were added 80 mcl hexane, and 1-2 mcl solution in injector of GC-MS system.

For detection of microorganisms markers the chromatograph YL6900 GC-MS with capillary column HP 5 30mX0,32mmX0,25mcmwas used.

The conditions of chromatography were: thermostat temperature - primary – 60° 3 min. (isothermal regimen); heating with the speed 15° C/min (regimen of programming temperature) to 250° C

and at 250° C (isothermal regimen) 3 min. Injector temperature was 250° C, Helium gas flow was 1 ml/min, SplitRatio-1/100. Parameters of detector mass were solvent delay 3 minutes, Emission was 50mA, scanning range was 0-350 a.e.m., the speed of scanning was 1600 a.e.m./sec, temperature of ion source is 230°, transfer temperature is 280° C. Time of analyses was 21min.

The components identification was carried out on the comparison base of taken mass spectrum with the library NIST and by the time keeping. The quantitative analysis was performed by the inner normalization and with the methods [35]. Statistical processing of data was carried out by application of package programs Statistica6.0.

### TAKEN RESULTS AND THEIR DISCUSSION

On the base of results analyses on markers detection there were revealed the most characteristic microorganisms in children of wasarly age with acute rhinosinusitis. The results are given in table 1.

As it is seen from the table there were revealed such microorganisms as: Streptococcus pneumoniae, Haemophilus influenzae Staphylococcus aureus, Streptococcus viridans,  $\beta$ -hemolytic streptococcus of group A, Streptococcus mitis/oralis, Streptococcus sanguinis, Staphylococcus saprophyticus, Staphylococcus epidermidis, Streptococcus haemolyticus. Gram (-) flora disappear from microbial landscape.

For normalization of microbial view, the children of early age with acute rhinosinusitis were administered the ethereal oil of anise, having exuded from the ground part of plant (The chemical content of ethereal oil of anise [36] were shown in table 2). The ethereal anise oil was prescribed children as inhalation. For this the inhalator "Micro-life" was suggested. The treatment length was from 5 to 7 days. After treatment they were undergone to secondary control analysis with GC-MS method on detection quantitative microorganisms markers. The results were also given in table 1.

Table 1. Results of GC-MS analysis on reveal microorganisms markers at children of early age with acute rhinosinusitis before and after treatment

	Microorganism Cocci, Bacilli, Caryl-bacteria	Control, Kl/g*10 <sup>4</sup>	Before treatment, Kl/g*10 <sup>4</sup>	After treatment, Kl/g*10 <sup>4</sup>
1	Streptococcus pneumoniae	2298±112	0	2225±96
2	Haemophilus influenzae	120±11	283±14*	128±10
3	Staphylococcus aureus	682±44	1243±78*	643±48
4	Streptococcus viridans	30±4	2152±118*	52±4
5	$\beta$ -hemolytic streptococcus of group A	266±16	480±22*	280±17
6	Streptococcus mitis/oralis,	93±8	0	86±7
7	Streptococcus sanguinis	103±7	0	92±6
8	Staphylococcus saprophyticus	112±9	126±11	116±10
9	Staphylococcus epidermidis	409±24	0*	350±22
10	Streptococcus haemolyticus	5±1	0	4±1
11	Streptococcus anhaemolyticus)	317±18	30±3*	302±16
12	Moraxella catarrhalis	1390±96	2867±185*	1367±77
13	Proteus mirabilis	34±3	534±41*	134±10
14	Stenotrophomonas X	17±2	0	12±1



15	Maltophilia	507±34	16863±875*	863±44
16	Ralstonia pickettii	275±21	21465±198*	465±32

\* $P < 0,001$  authenticity of differences relativity at income

As it is seen from the table 1 the use of ethereal oil of anise led to restore of microbial view in nasal cavity at patients with acute rhinosinusitis to normal indices with the efficacy 98 %.

Table 2. Qualitative and quantitative content of ethereal oil "Pimpinella anise L."

N	Time keeping		Component	Content, %
	Experiment	Calculated		
1	2.12	2.18	3-methyl-butanol	0,15±0,01
2	2.18	2.23	2-methyl-butanol	0,06±0,01
3	6.91	7.09	$\alpha$ - pinene	0,07±0,01
4	9.78	9.62	$\alpha$ - terpinene	0,15±0,01
5	10.14	10.21	para-semen	0,13±0,01
6	10.29	10.09	limonene	0,28±0,02
7	11.48	11.52	$\gamma$ - terpinene	0,21±0,01
8	13.36	13.41	linalool	0,28±0,01
9	16.49	16.41	menthol	0,09±0,01
10	16.72	16.64	terpinene-4-ol	0,61±0,02
11	17.32	17.44	$\alpha$ - terpineol	0,15±0,01
12	17.58	17.44	methylhavicol	1,28±0,08
13	19.41	19.49	cumin aldehyde	0,19±0,01
14	17.68	17.77	carvon	0,27±0,02
15	19.98	20.08	cys-anethol	0,38±0,02
16	20.22	20.36	anise aldehyde	0,65±0,03
17	21.78	21.52	trans-anethole	82,56±1,24
18	23.59	23.66	$\delta$ - elemen	0,13±0,01
19	26.96	26.84	$\beta$ - caryofillen	0,10±0,01
20	27.74	27.82	транс- $\alpha$ - bergamoten	0,08±0,01
21	28.21	28.33	$\alpha$ - chimchalen	0,41±0,02
22	28.65	28.53	$\beta$ - farnesen	0,08±0,01
23	29.46	29.61	chimchalen	3,89±0,11
24	29.71	29.83	ap-curcumen	0,98±0,06
25	30.19	30.28	singiberen	1,05±0,05
26	30.36	30.51	$\beta$ - chimchalen	0,32±0,02
27	30.72	30.84	$\beta$ - bisabolen	0,71±0,03

28	31.34	31.58	$\beta$ –sequiphellandren	$0,16 \pm 0,01$
29	42.98	43.11	Isovalerilisoevhenol	$1,32 \pm 0,02$
30	44.81	44.96	epoxiisovalerilkisoevhenol	$0,51 \pm 0,01$

Note: 6 components are not identified on the chromatogram.

It has been seen from the given data in tables that the content of trans-anethole composes  $82,56 \pm 1,24\%$  and refers to the high qualitative oils.

During the study for the efficacy evaluation of therapy with the ethereal oils from the day of hospitalization up to 10 day of having been in hospital the dynamics of clinical pictures at sick children having been observed. After discharge the sick children were controlled in polyclinic. The safety and tolerance of drug was estimated by the presence or absence of side reactions (table 3).

Table 3. Dynamics of main complaints of patient's parents with traditional (n=22) and suggested methods of treatment (n=24)

Complaints		Amount of patients					
		Before treatment		On the 7-th day		After treatment	
		Abs	%	Abs	%	Abs	%
Stuffy nose	I gr	22	100	13	54,2*	2	8,3
	II gr	24	100	13	54,2*	2	8,3
Discharge from the nose, mucus (rhinorrhea)	I gr	20	90,9	13	54,2*	-	-
	II gr	23	95,6	10	42,6*	-	-
Cough	I gr	7	46,7	1	6,7*	-	-
	II gr	5	33,3	-	-	-	-
Discharge from the ear	I gr	5	33,3	-	-	-	-
	II gr	2	8,3	-	-	-	-
General infectious symptoms (high body temperature, general malaise, muscular weakness).	I gr	19	79,2	-	-	-	-
	II gr	19	79,2	-	-	-	-

\*P<0,001 authenticity of differences before treatment relatively

As it was seen from the table 3, the positive dynamics (P<0,001) of parents' complaints in all indices of both groups, where the ethereal oils have used, was marked. On the 7th day of treatment with ethereal oils the disappearance of coughing, nasal and ear discharge, temperature normalization, as well as general intoxication process. Such complaints as stuffy nose and discharge (rhinorrhea) were visually reduced in dynamics on 5-6 day of treatment.

The next stage estimated the dynamics of clinical manifestations of disease, analyzed the data of frontal rhinoscopy, otoscopy and presence of clinical symptoms (Table 4).

Table 4. Dynamics of clinical symptoms at patients of I and II groups

Clinical symptoms	groups	1 day treatment	7 day treatment	14 day treatment	28 day treatment
Presence of mucous and purulent discharge from the nose	I gr	+++	++	+	-
	II gr	+++	++	+	-
Hyperemia of mucous membrane of nose	I gr	+++	++	+	-
	II gr	+++	++	-	-
Edema of nasal mucous membrane	I gr	+++	++	-	-
	II gr	+++	+	-	-
Disorder of nasal respiration	I gr	+++	++	+	-
	II gr	+++	+	-	-
Paranasal syndrome	I gr	++	+	+	-
	II gr	++	+	-	-
Presence of mucous and purulent discharge from the ear	I gr	++	+	-	-
	II gr	+	-	-	-
Voice hoarseness	I gr	++	+	-	-
	II gr	+	-	-	-

Note: +++ - greatly expressed; ++ middle expressed; + weakly expressed

It is seen from the table 4 that the true improvement of clinical picture was marked at patients, who used ethereal oil.

After therapy the given indices were shortened in some times, getting approach to normalization of clinic. For prophylaxis and improvement of local immunity state for acute rhinosinusitis, at discharge the children were recommended the secondary use of ethereal oils in a month.

Thus, the use of ethereal oil anise takes to restoration of normal view in nasal cavity and clinical picture in children of early age with acute rhinosinusitis.

### CONCLUSIONS

1. There were revealed the microorganisms markers by the method of gas and chromat-mass-spectrometry from the nasal cavity at children of early age with acute rhinosinusitis.
2. The most sharply change is characteristically for the next microorganisms:
3. Streptococcus pneumoniae, Haemophilus influenzae Staphylococcus aureus, Streptococcus viridans.  $\beta$ -hemolytic streptococcus of group A, Streptococcus mitis/oralis, Streptococcus sanguinis, Staphylococcus saprophyticus, Staphylococcus epidermidis, Streptococcus haemolyticus which describe the microbial view at children of early age with acute rhinosinusitis.
4. Use of ethereal anise oil led to restoration of microbial view in nasal cavity and normalization of clinical picture at patient with efficacy 98 %.



## REFERENCES:

1. Mamatova Sh.R., Karabaev H.E., Namahanov A.M. "Ultrasonic study rhinosinusitis at children of yearly age" "Journal of biomedicine and practice" Vol.6, no.2, Tashkent 2021, P. 68-63.
2. Karabaev H.E., Mamatova Sh.R. "Clinical case of orbital complication after rhinosinusitis in children of yearly age." "Eurasian Bulletin" no.3, 2020 Tashkent, P. 78-82
3. Karabaev H.E., Mamatova Sh.R., Abdurahmanov H.N. "Acute rhinosinusitis at children: up to date treatment in 2020" "Tibbiyotda yangi kun" 4 (32) 2020 Tashkent, P. 18-23
4. Anand V.K. Epidemiology and economic impact of rhinosinusitis // Ann Otol Rhinol Laryngol Suppl. 2004. Vol. 193. P. 3-5.
5. Yanov U.K. and et al. Practical recommendations on antibacterial therapy of sinusitis (manual for doctors). Col., 2002 P.
6. Krukov A.I., Stoodoniy M.E., Artemyev M.E. and et al. Treatment of patients with rhinosinusitis: possibilities of conservative and operative influence // Medical Council, 2012, no. 11.
7. Lopatin A.S. Anti-biotic-therapy of acute inflammatory diseases for paranasal sinuses // Consilium medicum. 2003. Vol. 05., no. 4. P. 1-8.
8. Ryazantsev S.V. Acute sinusitis. The approaches to therapy (methodical recommendations). M., 2003. P.16
9. Svistushkin V.M., Shevchik E.A. Acute rhinosinusitis—modern point of view on the problem //PMJ. – 2014. – Vol. 9. – P. 643-648.
10. Hadley J. A., Pfaller M. A. Oral beta-lactams in the treatment of acute bacterial rhinosinusitis // Diagn Microbiol Infect Dis. 2007. Vol. 57 (3 Suppl). P. 47-54.
11. Lopatin A.S., Svistushkin V.M. Acute rhinosinusitis: etiology, pathogenesis, diagnosis and principles of treatment //Clinical recommendations. – 2009. – no. P.12.
12. Kolosov A.V., Guchev I. A., Krechikova O.I. Acute bacterial rhinosinusitis at military service men: etiology, sensitivity to antibiotics and efficacy of antimicrobial therapy // Clinical microbiology and antimicrobial chemotherapy. 2009, no. 11 (1). P. 14-21.
13. Otvagin I.V. Pharma-epidemiological and clinic-bacterial bases of use cephalosporines at patients with acute sinusitis: Synopsis of thesis, cand. of med. scien., Smolensk, 1998. P. 22.
14. Strachinskiy L.S., Tarasov A.A., Krukov A.I. with co-authors. The agents of acute bacterial rhinosinusitis. The results of multicentral microbiological researches of SSSR // Clinical microbiology and antimicrobial chemotherapy. 2005., no.7 (4). P. 337-349.
15. Chow A.W., Benninger M.S., Brook I. et al. IDSA clinical practice guideline for acute bacterial rhinosinusitis in children and adults // Clin Infect Dis. 2012; Vol. 54 (8). P. 72-112.
16. Osipov GA, Verkhovtseva NV. Study of human microecology by mass spectrometry of microbial markers. Benef Microbes. 2011 Mar;2(1):63-78. doi: 10.3920/BM2010.0017
17. Yang Y, Misra BB, Liang L, et al. Integrated microbiome and metabolome analysis reveals a novel interplay between commensal bacteria and metabolites in colorectal cancer. Theranostics. 2019 May 31;9(14):4101-14. doi: 10.7150/thno.35186. eCollection 2019.
18. Ktsoyan ZA, Beloborodova NV, Sedrakyan AM, et al. Profiles of Microbial Fatty Acids in the Human Metabolome are DiseaseSpecific. Front Microbiol. 2011 Jan 20;1:148. doi: 10.3389/fmicb.2010.00148. eCollection 2010.
19. Fokkens W., Lund V., Mullol J. et al. European position paper on rhinosinusitis and nasal polyps

- 2012 (EP3OS) // Rhinology, 2012, vol. 50 (23), p. 1–299.
20. Zaplatnikov A.L. Topical decongestants in pediatric practice: safety and clinical efficacy // Pediatrics, 2006, no. 6, c. 69–75.
  21. Tulupov D.A., Karpova E.P. About the role of nasal vascular constricting medicines in symptomatic treatment of acute sinusitis at children // Russian rhinology, 2011, no. 2. P. 50.
  22. Kolesnikova M.B., Kilina A.V. Efficacy of use ethereal oils in prophylaxis of acute respiratory diseases at preschool children in organized offices // Bulletin on otorhinolaryngology, 2011, no.5. P. 51–54.
  23. Karpova E.P., Vagina E. E. Use inhalation therapy with ethereal oils in prophylaxis and complex treatment of children with acute rhinosinusitis//Pediatrician's practice. – 2015. – no. 1. – P. 21–26.
  24. Karpova E.P., Tulupov D.A. About safety of use nasal vascular-constricting drugs in pediatric practice // Russian rhinology, 2014, no.1, P. 12–14.
  25. Ryazantsev C.V. Comparison of Russian standards at treatment acute sinusitis with international program EPOS // Consilium medicum, 2008, no. 10, P. 87–90.
  26. Kolosova N.G., Geppe N.A. Therapy by nebulizers in pediatric practice // PMJ, 2011, no. 8, P. 514–518.
  27. Karpova E.P., Tulupov D.A. About possibility of nebulizer therapy in treatment of acute rhinosinusitis at children // Russian otorhinolaryngology, 2013, no. 65 (4), P. 160–163.
  28. Kolesnikova M.B., Kilina A.V. Efficacy of use ethereal oils in prophylaxis of acute respiratory diseases at preschool children in organized offices // Bulletin on otorhinolaryngology, 2011, no. 5, P. 51–54.
  29. Karpova E.P., Bojatova M.P. Rational methods of treatment ARVI at children // Pharmateka, 2008; 19: 89–92.
  30. Karpova E.P., Sokolova M.B. Irrigational therapy of allergic rhinitis at children // Bulletin on otorhinolaryngology, 2007, no.5, P. 23–24.
  31. Gramann J., Hippeli S., Dornisch K. et al. Antioxidant properties of essential oils, *Arzneim. Forsch. // Drug. Res.*, 2000, vol. 50 (1), p. 135–139.
  32. Petrushina A.D., Nikogosyan A.C., Kayb I.D. et al. Use inhalation with ethereal oils in complex therapy and for prophylaxis ARVI at children // Polyclinic, 2013; 4: 105–7.
  33. Azova E.A. et al. Experience of inhalation use “Breathe” oil in prophylaxis and complex therapy of ARD at children. Reference book of pediatrician, 2012; 3: 55–61.
  34. Boykova N.E., Garashenko T.I. Rationality of use ethereal oils in therapy of viral rhinosinusitis at children //Attending doctor. – 2018. – no. 9. – P. 36.
  35. Baranov V.M. et al. Evaluation of microecological human status by the method chromat-mass spectrometry //New medical technology MH RF no. HHO-40006. – 2009. – T. 17.- P. 37.
  36. Muhamadiev A.N., Fazlieva N.T., Muhamadiev N.Q. Study of the chemical composition of essential oil extracted from *Pimpinella Anisum* L. by GX-MS method // Scientific bulletin of Samarkand State University. - 2014. - No. 1 (83). – P. 83–87 (Uzb).