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Effect of Dance, Games, and Exercise on the Metabolic Profile of Human Saliva

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Abstract

Volatile organic compounds have an essential role in globally impactful disease activity and an individual's health condition. Saliva also has volatile organic compounds (VOCs) in its composition, and VOCs are being discovered as possible biomarkers for oral diseases. The present study is conducted to evaluate the effect of dance, games, and exercise on the metabolic profile of human saliva. A total of 68 healthy participants were included in the study. Unstimulated saliva (taken as control, n = 29 for exercise, n = 26 for cricket activity, and n = 13 for dance activity) and stimulated saliva (n = 29 for exercise, n = 26 for cricket activity, and n = 13 for dance activity) were taken before and after all three activities. Those who had any metabolic disorder, such as any cardiovascular disease, eating disorder, or any oral disease, such as mouth dryness, any kind of allergy, or smoking habits, were excluded from the study. Metabolic profiling of saliva was done using the GC-MS technique. There were 21 metabolites discovered before the cricket activity, and 24 metabolites were found after the cricket activity. 13 metabolites were discovered before the dance activity, 16 metabolites were discovered after the dance activity. 11 metabolites were discovered before the exercise activity, and 17 metabolites were discovered after the exercise activity. Lately, new metabolic markers of sports performance and exercise tolerance have been identified by salivary metabolomics. Our study found changes in saliva composition before and after different physical activities. Different metabolites were found before and after the different physical activities. Important metabolites distinguishing in our study were triethyl phosphate, geraniol, citronellol, and tetrapentacontane, 1,54-dibromo-. The area percentage of triethyl phosphate was 20.14% before the cricket activity. The area percentage of geraniol was also changed before and after dance and exercise activity. The area percentage of geraniol was 6.62% before the exercise activity, and after the exercise activity, it was 8.30%. Citronellol was formed after the exercise with an area percentage of 5.64%. The Area percentage of geraniol was 8.78% before and after the dance activity; it was 4.05%. Changes have also been found in the area percentage of tetrapentacontane, 1,54-dibromo- in all three activities.

There is a need for research in the field of salivary-derived metabolic biomarkers so that it would be easy to investigate the stage of any disease and further treatment could be done. Saliva is very easy to collect and store, unpainful, compared to blood. Therefore, assessing human health status can be a better biological fluid.

Keywords: Human health status; Metabolic biomarker; Physical activities; Saliva composition

Introduction

Human saliva is used to search for biomarkers for various diseases (Chen *et al.*, 2022; Ning *et al.*, 2021). In whole saliva, the quantity of water is 99%, numerous cellular elements and inorganic and organic matter is 0.5% (Tiwari, 2011; Kaczor-Urbanowicz *et al.*, 2019). Salivary metabolites play an important role in sports physiology by reflecting changes in biochemical composition associated with performance and exercise (Gardner *et al.*, 2020). It has been found that the metabolite levels of saliva are affected by exercise (Zauber *et al.*, 2012). Metabolic and biochemical processes of the human body are affected by certain stimuli like medication or exercise, and in return, the concentration and composition of volatile metabolites are also altered (Coffey and Hawley, 2007; Kofink *et al.*, 2017). In exhaled breath, this metabolite analysis, predominantly of volatile organic compounds (VOCs), provides a picture of a person's health status and disease activity (Buszewski *et al.*, 2007). Globally impacted human diseases are neurological diseases, cancer, metabolic, and cardiovascular diseases, and it has become challenging to diagnose these diseases. Therefore, extra clinical evaluation with laboratory testing is required (Lee and Wong 2009).

Presently, VOCs are being discovered as possible biomarkers for oral diseases, and like the majority of biofluids, saliva also has VOCs in its composition. Moreover, in other biofluids, many biomarkers have also been detected **(**Amann *et al.*, 2014; Milanowski *et al.*, 2017; de Lacy Costello *et al.*, 2014; Al-Kateb *et al.*, 2013; Soini *et al.*, 2010). This evidence adds essential knowledge to our understanding of human metabolism in diseases and health. Also, it has applications for countless medical conditions, such as genetic disorders, cardiovascular problems or infections, and malignancies (Malathi *et al.*, 2014). Higher concentrations of salivary lysozyme are connected with early-stage cardiovascular disorders and hypertension (Qvarnstrom *et al.*, 2008; Janket *et al.*, 2006). Some volatiles, such as indole and acetone, might be altered because of physical exercises like walking and cycling (Yamai *et al.*, 2009; Cooke *et al.*, 2003). Nonanal and hexanal are typical markers of oxidative stress (Al-Kateb *et al.*, 2013).

In medical diagnostics, volatile endogenous compounds to facilitate can be recognized as potential biomarkers consist of oxygen-containing compounds (2-propanol, methanol, acetaldehyde, acetone, and ethanol), hydrocarbons (isoprene, ethane, and pentane) (Lubes and Goodarzi, 2018), nitrogen-containing compounds (ammonia, dimethylamine, and trimethylamine), and sulfur-containing compounds (carbon disulfide, dimethyl sulfide, ethyl mercaptan, and methyl mercaptan) (Miekisch *et al.*, 2004; Buszewski *et al.*, 2007; Rudnicka *et al.*, 2019; Das *et al.*, 2016). Throughout the oxidation of fatty acids, aldehydes, ketones, and alkanes are produced in the individual body. In lipid peroxidation, acetone is produced during the metabolism of carbohydrates (Buszewski *et al.*, 2008). With oxidative stress and inflammation, their concentration increases, which usually goes along with cancer (Saalberg and Wolff 2016). Exhaled air is typically used to find VOCs (Sun *et al.*, 2016), but saliva can also be second-hand for these purposes (Al-Kateb *et al.*, 2013). In terms of volumetric sampling, processing, collection, storage, and delivery, saliva has numerous advantages concerning exhaled air (Campanella *et al.*, 2019). Because of the rapid diffusion equilibrium between dissolved substances in blood capillaries and saliva via thin membranes of the salivary glands, saliva is well thought-out functionally similar to blood in reflecting the physiological state of the human body (Lima *et al.*, 2010). Also, saliva composition shows a relationship well with the substance of polar chemicals present in the urine and blood as 2-propanol (Ernstgård *et al.*, 2003), methanol (Ernstgård 2009), and ethanol (Gubala and Zuba 2003). Endogenously, acetaldehyde and ethanol are produced in the body (Eriksson 2007), and the activity of the enzymes cytochrome p450 and alcohol dehydrogenase (ADH) is connected with the emergence of ethanol (Bouza *et al.*, 2017). All these studies showed that changes in stimuli and physiology affect the metabolic profile of saliva.

The changes in metabolites in saliva composition before and after the three activities, including dance, exercise, and sports, have been studied for the first time. This study highlights the use of saliva as a potential biomarker in an individual's health status and the benefits of physical activities. Research based on salivary metabolomics applications has focused chiefly on diagnostic biomarker discovery and diagnostic value

(Gardner *et al.,* 2020). Some articles have described the role of saliva as a biofluid used in clinical investigations and analytical purposes in the physiological research of sports teams (Papacosta *et al.,* 2011). Also, metabolic profiling of saliva has been partially explored as a potential biomarker concerning other biofluids, such as blood and urine (Issaq *et al.,* 2008; Kochhar *et al.,* 2006). However, a little study was conducted to find the effect of exercise, dance, and games on the metabolic profile of saliva. This study would have high impact changes in the metabolic profile of saliva during different physical activities.

Materials and Methods

Study site and experimental design: This experiment was conducted in Rohtak, Haryana (India). Three different physical activities were chosen for the research. These activities included exercise, dance, and games. Gymnasium exercise, freestyle dance forms, and cricket games were selected for the study. The study sites were Rework Fitness Gym, Haryana (India), Maharshi Dayanand University Cricket Stadium, Haryana (India), and Vibe Dance Studio, Haryana (India). A total of 68 healthy participants were included in the study. Those who had any metabolic disorder, such as any cardiovascular disease, eating disorder, or any oral disease, such as mouth dryness, any kind of allergy, or smoking habits, were excluded from the study. The experiment was explained in detail to all the participants, and all the participants signed the research consent letter. The participants also filled out the questionnaire in which they filled their personal and health details. Twenty-nine healthy participants participated in the gymnasium exercise, 26 participated in cricket game activity, and 13 performed the dance activity. The volunteers performed gymnasium exercises for one hour, cricket for 3 hours, and dance for 45 minutes. All the participants gave saliva twice before and after the activity.

Ethical Clearance

All the individuals who participated in the research work voluntarily participated in the study. No one was forced to participate. They all signed the consent letter before participating in the research and were above 18 years of age; they were also aware of the research work. So, the committee did not need ethical clearance to perform the research.

Collection of saliva samples:

The spitting method was used to collect the saliva from the subjects. The contributors were instructed about the collection procedure a day before the study. Participants were advised not to brush before sample collection and instructed to come on empty stomachs one hour before. Volunteers were asked to rub their cheeks and throat and tilt their heads downwards so that a proper amount of saliva would accumulate on the mouth floor. Participants were requested to spit 3 to 5 ml of saliva in 15 ml falcon tubes. After taking the tubes from the participants, the tubes were immediately placed in a deep-freeze storage box. Saliva samples were stored at -80 degrees Celsius for further GC-MS evaluation (Baliga *et al.,* 2017).

Salivary Volatiles determination using GC-MS Analysis:

For the metabolic study, the samples were sent to the Department of Science and Technology, SAIF, Panjab University, Chandigarh, and the following methodology was used as a reference to fill out the sample analysis form of the SAIF department. GC-MS analysis was made in Thermo Scientific TSQ 8000 Gas Chromatograph - Mass Spectrometer.

Sample analysis:

All samples were thawed at room temperature before analysis. After that, the 1ml of dichloromethane was mixed separately with 1ml of samples in triplicate of the human saliva. The supernatant was filtered through a silica gel column (60-120 meshes) and concentrated under vacuum at 30°C temp for fractionation. Chemical recognition was done by mass spectroscopy correlated with gas chromatography (GC-MS) analysis.

Gas chromatography and mass spectrometry

Two i^l of the extract was injected into GC-MS on a 30 m glass capillary column with a film thickness of 0.25 am. After this, the following temperature program was used. The oven temperature was 40°C, left for 4 minutes, then amplified to 250°C at a rate of 15°C for 10 minutes. GC was equipped with an FID detector and linked to an integrator. The area used quantitative qualifications under each peak. The detection accuracy was about Ing/peak. The percentage amount of the ion current was measured as the relative amount of each component. At 70-ev, the GC-MS was under the computer run. Ammonia was used as a reagent gas at 95-ev

for chemical ionization. Unidentified compounds were identified by making a probability-based matching using a computer library built inside the NICT system **(**Alagendran *et al.*, 2010).

Results

Compounds identified in saliva before and after cricket activity

Twenty-one metabolites were discovered before and 24 metabolites after the cricket activity (Table 1 & 2). Before the activity compounds discovered were: triethyl phosphate, 12-Methyl-E,E-2,13-octadecadien-1-ol, Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate, 5-Heptenoic acid, 6-methyl-4-[(4-methylphenyl), Oxiraneoctanoic acid, 3-octyl-, cis-,(7a-Isopropenyl-4,5-dimethyloctahydroinden-4-yl) Methanol, and Tetrapentacontane, 1,54-dibromo- . After the cricket activity metabolites discovered were: 2-Octen-1-ol, 3,7-dimethyl-, Geraniol, 2,3,4,4a,5,6,6a,7,8,9-Decahydropyrano[3,2-H]chromen-5,5,6,6-tetracarbonitrile8.84, Spiro[2.3]hexan-4-one, 5,5-diethyl-6-methyl-, á-copaene, 6-epi-shyobunol, Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl-, Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methylester, 2-(4a,8-Dimethyl-6-oxo-1,2,3,4,4a,5,6,8a-octahydro-naphthalen-2-yl)-propionaldehyde, (7a-Isopropenyl-4,5-dimethyloctahydroinden-4-yl) methanol, Methacrylic acid, nonadecyl ester, Cyclopenta[g]-2-benzopyran,1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-, Tetrapentacontane, 1,54-dibromo- (Table 1 & 2; Fig. 1 & 2).

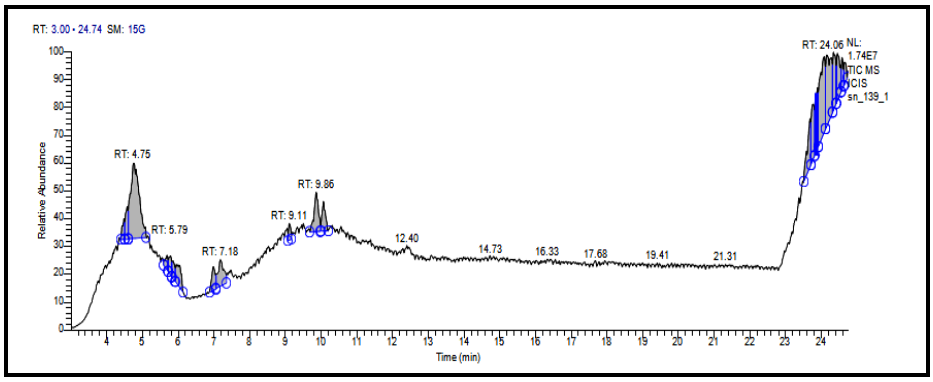

Figure 1. GC-MS chromatogram of saliva samples taken before cricket activity

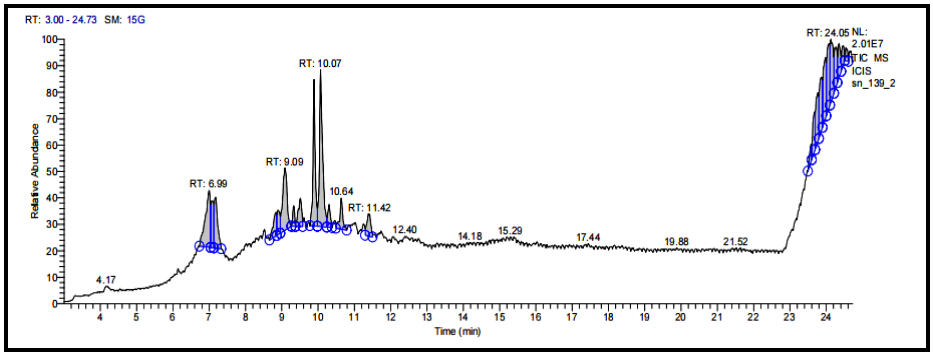

Figure 2. GC-MS chromatogram of saliva samples taken after cricket activity

Table 1. All the metabolites identified before the cricket activity are mentioned below:

Sl. No.	RT value	Compound name	Molecular formula	Area %	Peak Height
1.	4.48	Triethyl phosphate	$C_6H_{15}O_4P$	1.34	1308646.20
2.	4.57	Triethyl phosphate	$C_6H_{15}O_4P$	2.48	2034367.33
3.	4.75	Triethyl phosphate	$C_6H_{15}O_4P$	20.14	4718917.91
4.	5.69	Triethyl phosphate	$C_6H_{15}O_4P$	1.14	933671.77
5.	5.79	Triethyl phosphate	$C_6H_{15}O_4P$	1.58	1184903.06
6.	5.86	Triethyl phosphate	$C_6H_{15}O_4P$	1.36	1100006.60
7.	6.04	Triethyl phosphate	$C_6H_{15}O_4P$	3.32	1387069.28
8.	6.98	12-Methyl-E,E-2,13-octadecadien-1-ol	$C_{19}H_{36}O$	2.34	1451163.85
9.	7.18	Z-(13,14-	$C_{16}H_{28}O_3$	4.75	1671813.01

		Epoxy)tetradec-11-en-1-ol acetate			
10.	9.11	5-Heptenoic acid, 6-methyl-4-[(4-methylphenyl)	$C_{15}H_{20}O_4S$	1.18	1088473.51
11.	9.86	Oxiraneoctanoic acid, 3-octyl-, cis-	$C_{18}H_{34}O_3$	5.16	2458657.46
12.	10.07	(7a-Isopropenyl-4,5-dimethyloctahydroinden-4-yl) methanol	$C_{15}H_{26}O$	3.06	1869572.91
13.	23.68	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	4.94	2995297.60
14.	23.75	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	5.03	3493175.73
15.	23.89	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	5.26	3811687.81
16.	24.06	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	12.87	4771593.89
17.	24.14	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	11.30	4474978.72
18.	24.33	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	4.96	3634762.34
19.	24.44	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	4.69	2966341.59
20.	24.58	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	1.64	2001216.67
21.	24.64	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	1.46	1352793.68

Table 2. All the metabolites identified after the cricket activity are mentioned below:

Sl. No.	**RT value**	**Compound name**	**Molecular formula**	**Area %**	**Peak Height**
1.	6.99	2-Octen-1-ol, 3,7-dimethyl-	$C_{10}H_{20}O$	7.96	4333660.10
2.	7.07	Geraniol	$C_{10}H_{18}O$	3.72	3586680.44
3.	7.17	Geraniol	$C_{10}H_{18}O$	5.00	3886004.07
4.	8.84	2,3,4,4a,5,6,6a,7,8,9-Decahydropyrano[3,2-H]chromen-5,5,6,6-tetracarbonitrile 8.84	$C_{16}H_{14}N_4O_2$	2.42	1818207.55
5.	8.89	Spiro[2.3]hexan-4-one, 5,5-diethyl-6-methyl-	$C_{11}H_{18}O$	1.94	1878194.40
6.	9.09	á-copaene	$C_{15}H_{24}$	8.06	4762738.35
7.	9.33	6-epi-shogunal	$C_{15}H_{26}O$	1.12	1565124.34
8.	9.51	Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl-	$C_9H_9F_3O_2$	2.78	2146964.68
9.	9.89	Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester	$C_{13}H_{22}O_3$	9.41	11168000.81
10.	10.07	2-(4a,8-Dimethyl-6-oxo-1,2,3,4,4a,5,6,8a-octahydro-naphthalen-2-yl)-propionaldehyde	$C_{15}H_{22}O_2$	14.03	11991069.06
11.	10.30	(7a-Isopropenyl-4,5-dimethyloctahydroinden-4-yl) methanol	$C_{15}H_{26}O$	1.69	1746220.32
12.	10.64	Methacrylic acid, nonadecyl ester	$C_{23}H_{44}O_2$	2.73	2383031.35
13.	11.42	Cyclopenta[g]-2-benzopyran,1,3,4,6,7,8-hexahydro-	$C_{18}H_{26}O$	2.51	1701946.87

14.	23.58	4,6,6,7,8,8-hexamethyl-Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	1.15	1389830.07
15.	23.67	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	2.69	3059364.11
16.	23.77	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	4.13	3749443.53
17.	23.85	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	4.62	4193019.93
18.	23.97	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	5.34	4682083.46
19.	24.05	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	5.48	5021387.53
20.	24.12	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	5.21	4837888.69
21.	24.24	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	3.33	3292729.21
22.	24.34	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	2.48	2662334.89
23.	24.44	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	1.42	1640057.13
24.	24.55	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	0.80	1021398.94

Compounds identified in saliva before and after dance

Thirteen metabolites were discovered before the dance, and 16 were discovered afterward (Tables 3 & 4). Before the dance metabolites which were there were 6-Octen-1-ol, 3,7-dimethyl-, (R)-, Geraniol,1-Heptatriacotanol and Tetrapentacontane, 1,54-dibromo- and after the dance were 2-Pentanone, 4-hydroxy-4-methyl-, 2-Propen-1-amine,N,N-bis(1-methylethyl)-, Aziridine,2-(1,1-dimethylethyl)-1-ethyl-3-methyl-,cis, -6-Octen-1-ol,3,7-dimethyl-, (R)-, Geraniol,Tetrapentacontane,1,54-dibromo- (Table 3 & 4; Fig. 3 & 4).

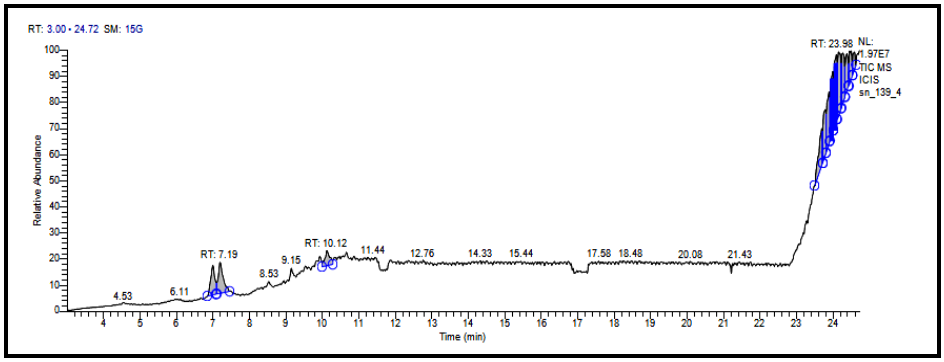

Figure 3: GC-MS chromatogram of saliva samples taken before dance activity

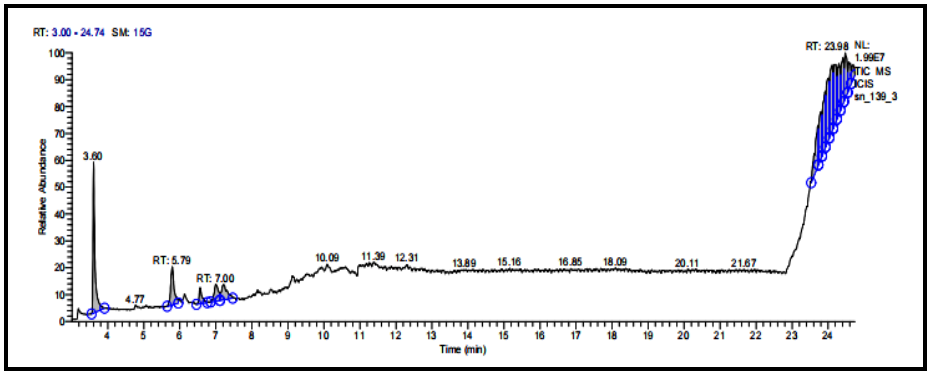

Figure 4: GC-MS chromatogram of saliva samples taken after dance activity

Table 3: All the metabolites identified before the dance activity are mentioned below:

Sl. No.	RT value	Compound name	Molecular formula	Area %	Peak Height
1.	7.00	6-Octen-1-ol, 3,7-dimethyl-, (R)-	$C_{10}H_{20}O$	7.07	2223344.12
2.	7.19	Geraniol	$C_{10}H_{18}O$	8.78	2352508.82
3.	10.12	1-Heptatriacotanol	$C_{37}H_{76}O$	4.57	1173152.19

4.	23.68	Tetrapentacontane, 1,54-dibromo-		$C_{54}H_{108}Br_2$	8.33	2789431.76
5.	23.76	Tetrapentacontane, 1,54-dibromo-		$C_{54}H_{108}Br_2$	7.63	3523570.50
6.	23.89	Tetrapentacontane, 1,54-dibromo-		$C_{54}H_{108}Br_2$	8.98	3952843.93
7.	23.98	Tetrapentacontane, 1,54-dibromo-		$C_{54}H_{108}Br_2$	9.90	4674011.97
8.	24.09	Tetrapentacontane, 1,54-dibromo-		$C_{54}H_{108}Br_2$	12.02	5145571.92
9.	24.14	Tetrapentacontane, 1,54-dibromo-		$C_{54}H_{108}Br_2$	10.69	4851659.74
10.	24.25	Tetrapentacontane, 1,54-dibromo-		$C_{54}H_{108}Br_2$	8.45	3897259.32
11.	24.35	Tetrapentacontane, 1,54-dibromo-		$C_{54}H_{108}Br_2$	6.19	2884338.33
12.	24.45	Tetrapentacontane, 1,54-dibromo-		$C_{54}H_{108}Br_2$	4.82	2461928.85
13.	24.55	Tetrapentacontane, 1,54-dibromo-		$C_{54}H_{108}Br_2$	2.55	1484168.44

Table 4: All the metabolites identified after the dance activity are mentioned below:

Sl. No.	**RT value**	**Compound name**	**Molecular formula**	**Area %**	**Peak Height**
1.	3.60	2-Pentanone, 4-hydroxy-4-methyl-	$C_6H_{12}O_2$	13.67	11192305.98
2.	5.79	2-Propen-1-amine, N,N-bis(1-methyl ethyl)-	$C_9H_{19}N$	6.14	2843831.67
3.	6.56	Aziridine, 2-(1,1-dimethylethyl)-1-ethyl-3-methyl-,cis-	$C_9H_{19}N$	2.30	1210671.77
4.	7.00	6-Octen-1-ol, 3,7-dimethyl-, (R)-	$C_{10}H_{20}O$	3.30	1263038.98
5.	7.21	Geraniol	$C_{10}H_{18}O$	4.05	1124473.73
6.	23.71	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	7.04	3086367.88
7.	23.78	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	6.26	3535507.28
8.	23.91	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	7.52	4258699.36
9.	23.98	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	8.81	4710321.23
10.	24.10	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	9.56	4947592.79
11.	24.16	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	8.48	4556823.25
12.	24.30	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	6.54	3778479.71
13.	24.41	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	6.02	3448813.42
14.	24.47	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	5.41	3399976.85
15.	24.57	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	3.27	2086332.73
16.	24.67	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	1.63	1186042.38

Compounds identified in saliva before and after gymnasium

Eleven metabolites were discovered before the exercise and 17 after (Tables 5 & 6). Before the exercise metabolites which were discovered were: 2-Octen-1-ol, 3,7-dimethyl-2, 6-Octadien-1-ol, 3,7-dimethyl-, Geraniol, á-copaene, Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester, 5-(7a-Isopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-en-1-ol, Methacrylic acid, nonadecyl ester, Tetrapentacontane, 1,54-dibromo and after the exercise metabolites which were discovered were: Citronellol, Geraniol, and Tetrapentacontane, 1,54-dibromo- (Table 5 & 6; Fig. 5 & 6).

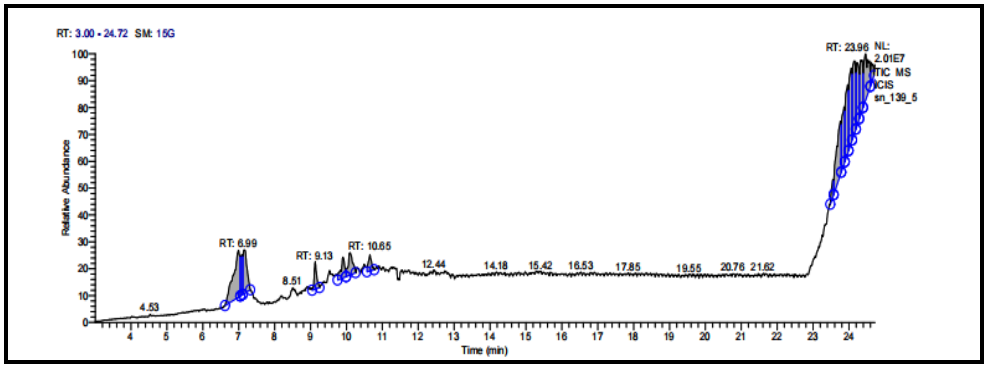

Figure 5: GC-MS chromatogram of saliva samples taken before gymnasium exercise activity

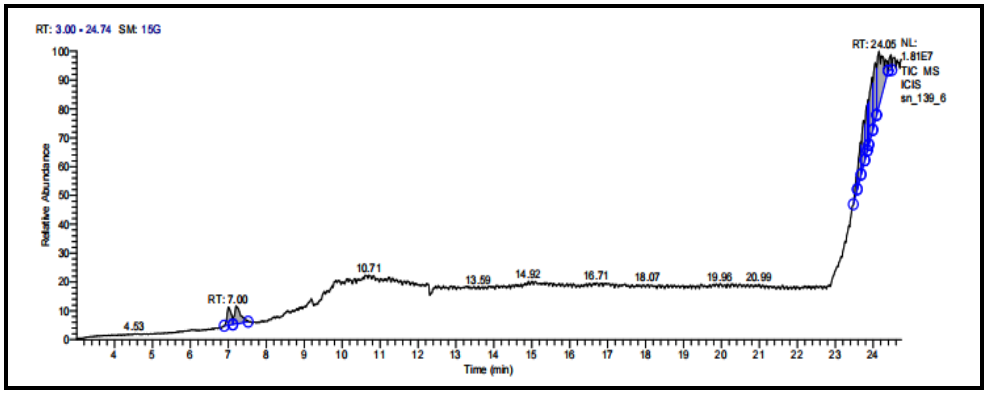

Figure 6: GC-MS chromatogram of saliva samples taken after gymnasium activity

Table 5. All the metabolites identified before the gymnasium exercise activity are mentioned below:

S.No	RT value	Compound name	Molecular formula	Area %	Peak Height
1.	6.99	2-Octen-1-ol, 3,7-dimethyl-	$C_{10}H_{20}O$	12.48	3516056.31
2.	7.09	2,6-Octadien-1-ol, 3,7-dimethyl-	$C_{10}H_{18}O$	3.24	3040837.28
3.	7.15	Geraniol	$C_{10}H_{18}O$	6.62	3279101.38
4.	9.13	á-copaene	$C_{15}H_{24}$	2.55	2040378.00
5.	9.90	Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester	$C_{13}H_{22}O_3$	3.10	1556800.90
6.	10.10	5-(7a-Isopropenyl-4,5-dimethyl-octahydroinden-4-y l)-3-methyl-pent-2-en-1-ol	$C_{20}H_{34}O$	3.91	1683168.64
7.	10.65	Methacrylic acid, nonadecyl ester	$C_{23}H_{44}O_2$	1.95	1198920.25
8.	23.55	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	1.38	1318905.71
9.	23.76	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	9.91	4018804.90
10.	23.86	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	6.38	4268544.23
11.	23.96	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	8.07	5133703.47
12.	24.06	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	8.67	5609253.94
13.	24.13	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	8.51	5505850.39
14.	24.22	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	6.81	4780837.98
15.	24.32	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	6.17	4101812.94
16.	24.45	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	8.46	3529905.63
17.	24.62	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	1.78	1606883.28

Table 6. All the metabolites identified after the gymnasium exercise activity are mentioned below:

Sl. No.	RT value	Compound name	Molecular formula	Area %	Peak Height
1.	7.00	Citronellol	$C_{10}H_{20}O$	5.64	1139445.49
2.	7.20	Geraniol	$C_{10}H_{18}O$	8.30	1113847.57
3.	23.56	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	2.66	1046880.55
4.	23.66	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	7.04	2223872.86
5.	23.74	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	9.88	2870644.08
6.	23.82	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	7.18	2997775.03
7.	23.87	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	4.73	3026425.50
8.	23.97	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	13.01	3506737.80
9.	24.05	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	13.77	3654628.98
10.	24.15	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	25.15	3461476.99
11.	24.47	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	2.65	987092.72

DISCUSSION

Lately, new metabolic markers of sports performance and exercise tolerance have been identified by salivary metabolomics. Taurine and 3-methylhistidine have been identified as metabolites of muscle catabolism and skeletal muscle use (Ra *et al.*, 2014). Our study found changes in saliva composition before and after different physical activities. We discovered salivary metabolic profile changes in all the different physical activities. The area percentage of triethyl phosphate was 20.14% before the cricket activity. The area percentage of geraniol was also changed before and after dance and exercise activity. The area percentage of geraniol was 6.62% before the exercise activity, and after the exercise activity, it was 8.30%. Citronellol was formed after the exercise with an area percentage of 5.64%. The Area percentage of geraniol was 8.78% before and after the dance activity; it was 4.05%. Changes have been found in the percentage of tetrapentacontane, 1,54-dibromo- in all three activities. In our study, we observed citronellol after gymnasium activity. This might be because it has antioxidant, anti-apoptotic, autophagy-modulating properties, and anti-inflammatory properties, which protect dopaminergic neurons (Jayaraj *et al.*, 2022; Table 3). There are changes observed in the area percentage of tetrapentacontane, 1,54-dibromo metabolite in all three activities in our study (Table 1-6). This might be because tetrapentacontane, 1,54-dibromo has potential as an antioxidant compound (Tanod *et al.*, 2019). The area percentage of geraniol changed before and after dance and exercise activity (Table 3-6). A broad spectrum of pharmacological activities, including antioxidant, antimicrobial, anti-cancer, neuroprotective, and anti-inflammatory, are demonstrated by geraniol. It also signifies a capable cancer chemopreventive agent because geraniol has also been revealed to sensitize tumor cells to frequently used chemotherapies together with [DB01248] and [DB00544] (PubChem CID- 637566). Other than that, upon air exposure, the fragrance terpene geraniol is autoxidized (Bäcktorp *et al.*, 2008). Triethyl phosphate was observed in the saliva sample before the cricket activity but was not observed after the activity (Table 1 & 2). This might be due to triethyl decomposition during TEP pyrolysis (Neupane *et al.*, 2019). Triethyl phosphate is a trialkyl phosphate, the triethyester phosphoric acid derivative. It is functionally associated with ethanol (PubChem CID - 6535). Also, saliva composition shows a relationship well with the substance of polar chemicals present in the urine and blood as 2-propanol (Ernstgård *et al.*, 2003), methanol (Ernstgård 2009), and ethanol (Gubala and Zuba 2003). Endogenously, acetaldehyde and ethanol are produced in the body (Eriksson 2007). The activity of the enzymes cytochrome p450 and alcohol dehydrogenase (ADH) is connected with the emergence of ethanol (Bouza *et al.*, 2017).

Conclusion

Our study aimed to observe the effects of different physical activities on the metabolic profile of saliva because the metabolic profile of saliva can give important information about individual health status. In our study, we discovered some potential biomarkers, such as tetrapentacontane, 1,54-dibromo, geraniol, citronellol, and triethyl phosphate, which could be helpful in future research. Other than that, we also observed different metabolites before and after the activities, which indicates that saliva composition is affected by the stimulation of physical activity. There is a need for research in the field of salivary-derived biomarkers so that it would be easy to investigate the stage of any disease and further treatment could be done. Saliva is very easy to collect and store, un-painful, compared to blood. Therefore, assessing human health status can be a better biological fluid.

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Declaration of interests

The authors declare the following financial interests/personal relationships, which may be considered potential competing interests: Dr. Arup Giri reports financial support provided by Baba Mastnath University. Dr. Arup Giri reports a relationship with Baba Mastnath University that includes: employment. Other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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REFERENCES

1. Alagendran, S., Archunan, G., Rameshkumar, K., Kadalmani, B., Rodriguez, J. A., Fernandez, G., ... & Guzman, R. (2010). 2-Nonenal-ovulatory specific volatiles in human saliva through gas chromatography and mass spectrometry analysis throughout the menstrual cycle. Am. J. Biochem. Biotechnol, 6(3), 187-194. https://doi.org/10.3844/ajbbsp.2010.187.194
2. Al-Kateb, H., de Lacy Costello, B., & Ratcliffe, N. (2013). An investigation of volatile organic compounds from the saliva of healthy individuals using headspace-trap/GC-MS. Journal of breath research, 7(3), 036004. https://doi.org/10.1088/1752-7155/7/3/036004
3. Amann, A., Costello, B.deL., Miekisch, W., Schubert, J., Buszewski, B., Pleil, J., Ratcliffe, N., & Risby, T. (2014). The human volatile: volatile organic compounds (VOCs) in exhaled breath, skin emanations, urine, feces, and saliva. Journal of breath research, 8(3), 034001. https://doi.org/10.1088/1752-7155/8/3/034001
4. Bäcktorp, C., Hagvall, L., Börje, A., Karlberg, A. T., Norrby, P. O., & Nyman, G. (2008). Mechanism of Air Oxidation of the Fragrance Terpene Geraniol. Journal of chemical theory and computation, 4(1), 101–106. https://doi.org/10.1021/ct7001495
5. Baliga, S., Chaudhary, M., Bhat, S. S., Bhatiya, P., Thosar, N., & Bhansali, P. (2017). Determination of toral antioxidant capacity of saliva in sickle cell anemic patients - A cross-sectional study. Journal of the Indian Society of Pedodontics and Preventive Dentistry, 35(1), 14–18. https://doi.org/10.4103/0970-4388.199219
6. Bouza, M., Gonzalez-Soto, J., Pereiro, R., de Vicente, J. C., & Sanz-Medel, A. (2017). Exhaled breath and oral cavity VOCs as potential biomarkers in oral cancer patients. Journal of breath research, 11(1), 016015. https://doi.org/10.1088/1752-7163/aa5e76
7. Buszewski, B., Kesy, M., Ligor, T., & Amann, A. (2007). Human exhaled air analytics: biomarkers of diseases. Biomedical chromatography : BMC, 21(6), 553–566. https://doi.org/10.1002/bmc.835
8. Buszewski, B., Ulanowska, A., Ligor, T., Jackowski, M., Kłodzińska, E., & Szeliga, J. (2008). Identification of volatile organic compounds secreted from cancer tissues and bacterial cultures. Journal of chromatography. B, Analytical technologies in the biomedical and life sciences, 868(1-2), 88–94. https://doi.org/10.1016/j.jchromb.2008.04.038
9. Campanella, B., Onor, M., Lomonaco, T., Benedetti, E., & Bramanti, E. (2019). HS-SPME-GC-MS approach for the analysis of volatile salivary metabolites and application in a case study for the indirect assessment of gut microbiota. Analytical and bioanalytical chemistry, 411(28), 7551–7562. https://doi.org/10.1007/s00216-019-02158-6
10. Chen, Y., Liu, F., & Lee, L. P. (2022). Quantitative and ultrasensitive in situ immunoassay technology for SARS-CoV-2 detection in saliva. Science advances, 8(21), eabn3481. https://doi.org/10.1126/sciadv.abn3481

11. Coffey, V. G., & Hawley, J. A. (2007). The molecular bases of training adaptation. Sports medicine (Auckland, N.Z.), 37(9), 737–763. https://doi.org/10.2165/00007256-200737090-00001
12. Cooke, M., Leeves, N., & White, C. (2003). Time profile of putrescine, cadaverine, indole and skatole in human saliva. Archives of oral biology, 48(4), 323–327. https://doi.org/10.1016/s0003-9969(03)00015-3
13. Das, S., Pal, S., & Mitra, M. (2016). Significance of Exhaled Breath Test in Clinical Diagnosis: A Special Focus on the Detection of Diabetes Mellitus. Journal of medical and biological engineering, 36(5), 605–624. https://doi.org/10.1007/s40846-016-0164-6
14. de Lacy Costello, B., Amann, A., Al-Kateb, H., Flynn, C., Filipiak, W., Khalid, T., Osborne, D., & Ratcliffe, N. M. (2014). A review of the volatiles from the healthy human body. Journal of breath research, 8(1), 014001. https://doi.org/10.1088/1752-7155/8/1/014001
15. Eriksson C. J. (2007). Measurement of acetaldehyde: what levels occur naturally and in response to alcohol?. Novartis Foundation symposium, 285, 247–260. https://doi.org/10.1002/9780470511848.ch18
16. Ernstgård L. (2009). Influence of gender on the metabolism of alcohols in human saliva in vitro. Archives of oral biology, 54(8), 737–742. https://doi.org/10.1016/j.archoralbio.2009.04.011
17. Ernstgård, L., Sjögren, B., Warholm, M., & Johanson, G. (2003). Sex differences in the toxicokinetics of inhaled solvent vapors in humans 2. 2-propanol. Toxicology and applied pharmacology, 193(2), 158–167. https://doi.org/10.1016/j.taap.2003.08.005
18. Gardner, A., Carpenter, G., & So, P. W. (2020). Salivary Metabolomics: From Diagnostic Biomarker Discovery to Investigating Biological Function. Metabolites, 10(2), 47. https://doi.org/10.3390/metabo10020047
19. Gubała, W., & Zuba, D. (2003). Gender differences in the pharmacokinetics of ethanol in saliva and blood after oral ingestion. Polish journal of pharmacology, 55(4), 639–644. PMID: 14581724
20. Issaq, H. J., Nativ, O., Waybright, T., Luke, B., Veenstra, T. D., Issaq, E. J., Kravstov, A., & Mullerad, M. (2008). Detection of bladder cancer in human urine by metabolomic profiling using high performance liquid chromatography/mass spectrometry. The Journal of urology, 179(6), 2422–2426. https://doi.org/10.1016/j.juro.2008.01.084
21. Janket, S. J., Meurman, J. H., Nuutinen, P., Qvarnström, M., Nunn, M. E., Baird, A. E., ... & Jones, J. A. (2006). Salivary lysozyme and prevalent coronary heart disease. Possible effects of oral health on endothelial dysfunction. Arteriosclerosis, thrombosis, and vascular biology, 26(2), 433-434.https://doi.org/10.1161/01.ATV.0000198249.67996.e0
22. Jayaraj, R. L., Azimullah, S., Parekh, K. A., Ojha, S. K., & Beiram, R. (2022). Effect of citronellol on oxidative stress, neuroinflammation and autophagy pathways in an in vivo model of Parkinson's disease. Heliyon, 8(11), e11434. https://doi.org/10.1016/j.heliyon.2022.e11434
23. Kaczor-Urbanowicz, K. E., Wei, F., Rao, S. L., Kim, J., Shin, H., Cheng, J., Tu, M., Wong, D. T. W., & Kim, Y. (2019). Clinical validity of saliva and novel technology for cancer detection. Biochimica et biophysica acta. Reviews on cancer, 1872(1), 49–59. https://doi.org/10.1016/j.bbcan.2019.05.007
24. Kochhar, S., Jacobs, D. M., Ramadan, Z., Berruex, F., Fuerholz, A., & Fay, L. B. (2006). Probing gender-specific metabolism differences in humans by nuclear magnetic resonance-based metabonomics. Analytical biochemistry, 352(2), 274–281. https://doi.org/10.1016/j.ab.2006.02.033
25. Kofink, D., Eppinga, R. N., van Gilst, W. H., Bakker, S. J., Dullaart, R. P., van der Harst, P., & Asselbergs, F. W. (2017). Statin effects on metabolic profiles: data from the PREVEND IT (Prevention of Renal and Vascular End-stage Disease Intervention Trial). Circulation: Cardiovascular Genetics, 10(6), e001759. https://doi.org/10.1161/CIRCGENETICS.117.001759
26. Lee, Y. H., & Wong, D. T. (2009). Saliva: an emerging biofluid for early detection of diseases. *American journal of dentistry*, *22*(4), 241–248 PMID: 19824562.
27. Lima, D. P., Diniz, D. G., Moimaz, S. A., Sumida, D. H., & Okamoto, A. C. (2010). Saliva: reflection of the body. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases, 14(3), e184–e188. https://doi.org/10.1016/j.ijid.2009.04.022.
28. Lubes, G., & Goodarzi, M. (2018). GC-MS based metabolomics used for the identification of cancer volatile organic compounds as biomarkers. Journal of pharmaceutical and biomedical analysis, 147, 313–322. https://doi.org/10.1016/j.jpba.2017.07.013
29. Malathi, N., Mythili, S., & Vasanthi, H. R. (2014). Salivary diagnostics: a brief review. ISRN dentistry, 2014, 158786. https://doi.org/10.1155/2014/158786
30. Miekisch, W., Schubert, J. K., & Noeldge-Schomburg, G. F. (2004). Diagnostic potential of breath analysis--focus on volatile organic compounds. Clinica chimica acta; international journal of clinical chemistry, 347(1-2), 25–39. https://doi.org/10.1016/j.cccn.2004.04.023

31. Milanowski, M., Pomastowski, P., Ligor, T., & Buszewski, B. (2017). Saliva - Volatile Biomarkers and Profiles. Critical reviews in analytical chemistry, 47(3), 251–266. https://doi.org/10.1080/10408347.2016.1266925
32. National Center for Biotechnology Information (2023). (PubChem Compound Summary for CID 637566, Geraniol. Retrieved October 3, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/Geraniol.)
33. Neupane, S., Rahman, R. K., Masunov, A. E., & Vasu, S. S. (2019). Theoretical Calculation of Reaction Rates and Combustion Kinetic Modeling Study of Triethyl Phosphate (TEP). The journal of physical chemistry. A, 123(22), 4764–4775. https://doi.org/10.1021/acs.jpca.9b00636
34. Ning, B., Yu, T., Zhang, S., Huang, Z., Tian, D., Lin, Z., Niu, A., Golden, N., Hensley, K., Threeton, B., Lyon, C. J., Yin, X. M., Roy, C. J., Saba, N. S., Rappaport, J., Wei, Q., & Hu, T. Y. (2021). A smartphone-read ultrasensitive and quantitative saliva test for COVID-19. Science advances, 7(2), eabe3703. https://doi.org/10.1126/sciadv.abe3703
35. Papacosta, E., & Nassis, G. P. (2011). Saliva as a tool for monitoring steroid, peptide and immune markers in sport and exercise science. Journal of science and medicine in sport, 14(5), 424–434. https://doi.org/10.1016/j.jsams.2011.03.004
36. Qvarnstrom, M., Janket, S., Jones, J. A., Nuutinen, P., Baird, A. E., Nunn, M. E., ... & Meurman, J. H. (2008). Salivary lysozyme and prevalent hypertension. Journal of dental research, 87(5), 480-484. https://doi.org/10.1177/154405910808700507
37. Ra, S. G., Maeda, S., Higashino, R., Imai, T., & Miyakawa, S. (2014). Metabolomics of salivary fatigue markers in soccer players after consecutive games. Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme, 39(10), 1120–1126. https://doi.org/10.1139/apnm-2013-0546
38. Rudnicka, J., Kowalkowski, T., & Buszewski, B. (2019). Searching for selected VOCs in human breath samples as potential markers of lung cancer. Lung cancer (Amsterdam, Netherlands), 135, 123–129. https://doi.org/10.1016/j.lungcan.2019.02.012
39. Saalberg, Y., & Wolff, M. (2016). VOC breath biomarkers in lung cancer. Clinica chimica acta; international journal of clinical chemistry, 459, 5–9. https://doi.org/10.1016/j.cca.2016.05.013
40. Soini, H. A., Klouckova, I., Wiesler, D., Oberzaucher, E., Grammer, K., Dixon, S. J., ... & Novotny, M. V. (2010). Analysis of volatile organic compounds in human saliva by a static sorptive extraction method and gas chromatography-mass spectrometry. Journal of chemical ecology, 36, 1035-1042. https://doi.org/10.1007/s10886-010-9846-7
41. Sun, X., Shao, K., & Wang, T. (2016). Detection of volatile organic compounds (VOCs) from exhaled breath as noninvasive methods for cancer diagnosis. Analytical and bioanalytical chemistry, 408(11), 2759–2780. https://doi.org/10.1007/s00216-015-9200-6
42. Tanod, W. A., Yanuhar, U., Wahyudi, D., & Risjani, Y. (2019, February). DPPH scavenging property of bioactives from soft corals origin Palu Bay, Central Sulawesi, Indonesia. In IOP Conference Series: Earth and Environmental Science (Vol. 236, No. 1, p. 012121). IOP Publishing. https://doi.org/10.1088/1755-1315/236/1/012121
43. Tiwari M. (2011). Science behind human saliva. Journal of natural science, biology, and medicine, 2(1), 53–58. https://doi.org/10.4103/0976-9668.82322
44. Yamai, K., Ohkuwa, T., Itoh, H., Yamazaki, Y., & Tsuda, T. (2009). Influence of cycle exercise on acetone in expired air and skin gas. Redox report : communications in free radical research, 14(6), 285–289. https://doi.org/10.1179/135100009X12525712409850
45. Zauber, H., Mosler, S., von Heßberg, A., & Schulze, W. X. (2012). Dynamics of salivary proteins and metabolites during extreme endurance sports - a case study. Proteomics, 12(13), 2221–2235. https://doi.org/10.1002/pmic.201100228