Ashraf *et al. J Pure Appl Microbiol,* **13**(**4**), 2545-2552 | December 2019
Article 5864 | https://doi.org/10.22207/JPAM.13.4.69

Print ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

GC-MS Analysis of Commercially Available *Allium sativum* and *Trigonella foenum-graecum* Essential Oils and their Antimicrobial Activities

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Abstract

Food preservation and safety is drawing more attention globally due to the increasing prevalence of food-borne diseases. The natural methods of food preservation are considered safer compared to methods using synthetic preservatives. The essential oils with natural preservative properties could be useful for food safety and preservation. The objective of this study was to analyze the chemical composition of commercially available *Allium sativum* and *Trigonella foenum-graecum* essential oils by gas chromatography-mass spectroscopy (GC-MS). The antimicrobial activities of *Allium sativum* and *Trigonella foenum-graecum* essential oils were determined by agar well diffusion technique. The GC-MS analysis of Garlic essential oil (GEO) revealed that, Allyl methyl trisulfide (13.10%), Di-allyl sulfide (9.47%) and Di-allyl tetrasulfide (4.38%) were the major components, while methanolic extract of Fenugreek essential oil (FEO) showed limonene (12.92%), Silane trimethylphenyl (10.71%), carvone (4.57%) and Trigolline (0.38%) as major components. The results of our study showed a significant antimicrobial activity of GEO and FEO against the tested microbial strains, which indicates the presence of broad-spectrum antimicrobial constituents in GEO and FEO. However, further studies are needed for individual bioactive components and safety aspects for their application in food preservation.

Keywords: Bio-active components, Essential oils, GC-MS; Agar well assay; Antimicrobial activity.

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(Received: 19 September 2019; accepted: 19 December 2019)

Citation: Syed Amir Ashraf, Mushtaq Ahmad Khan, Amir Mahgoub Awadelkareem, Shaikh Tajuddin, Md Faruque Ahmad and Talib Hussain, GC-MS Analysis of Commercially Available *Allium sativum* and *Trigonella foenum-graecum* Essential Oils and their Antimicrobial Activities, *J Pure Appl Microbiol*., 2019; **13**(**4**):2545-2552. https://doi.org/10.22207/JPAM.13.4.69

INTRODUCTION

The role of essential oils (EOs) in food preservation has been extensively studied due to the undesirable effects of synthetic preservatives used in food industries[1]. Furthermore, rise in food-borne diseases worldwide put people at the risk of health hazards, calling for more effective, safe and natural source of food preservation. To improve the food safety, one of the options in modern time is to study and investigate the antimicrobial activities of the bioactive compounds and use them in food industry. Antimicrobial properties of naturally occurring bioactive components restrict the use of chemical antimicrobial agents, which may possess a potential human health hazard[2]. The main bioactive components of EOs are mono and sesquiterpene, which are believed to be responsible for their biological activity; therefore, the identification of these bioactive components from various plant sources has become meaningful task. Gas chromatography (GC) or gas chromatography-mass spectrometry (GC-MS) is used exclusively for the qualitative analysis of the volatiles compounds[3]. Additionally, the antimicrobial properties of volatile components are known for decades and because of their potential microbial growth inhibition activity, these volatile components are being investigated as a substitute to synthetic chemical preservatives in food industries[4]. Since ancient times, herbs and spices are added in food and food products, not only as seasoning agent but also as a method of preservation[5]. Plant materials such as Garlic essential oil (GEO) and Fenugreek essential oil (FEO) with antibacterial properties could have a possible application in food preservation, for example, several studies have proved that garlic (*Allium sativum*) possesses a significant antimicrobial activity. Antimicrobial activity of garlic extracts has been reported against bacteria and fungi[6]. GEO have been reported to contain antibiotic, immunomodulatory, antioxidant, anti-inflammatory, cardiovascular-protecting and hypoglycemic effects[7, 8]. Garlic can be used as an effective source for food preservation and also as a natural herbal antibiotic[9]. Moreover, fenugreek seed (*Trigonella foenum*) oil has been known for their antimicrobial properties against food borne pathogens, thus it could be potentially useful in increasing shelf life of food products. FEO has been reported to have antimicrobial, anti-diabetic, anti-cancer, anti-fertility, and anti-parasitic activity[10].

Thus the growing demand of natural ingredients like essential oils in food preservation appears as a viable and healthy alternative to synthetic preservatives. Based upon our literature survey, we did not find any reports on GC-MS analysis as well as antimicrobial activity of commercially available essential oils. Therefore, this work was designed to evaluate the chemical composition and antimicrobial activity of commercial sample (GEO and FEO) against various microbial pathogens.

MATERIALS AND METHODS

Sample Collection

Garlic essential oil (GEO) and fenugreek essential oil (FEO) were procured from the local market of Ha'il, Kingdom of Saudi Arabia in December 2018. Selected samples were chosen based upon literature survey and their possible application in food industry. Quality of the oils was ascertained to be more than 98% pure.

Gas chromatography mass spectrometry (GC-MS)

GC-MS (Thermo Scientific, Triple quadropole MS, TSQ 8000) analysis were performed for the GEO and FEO using two fused silica capillary column TG-5MS, (30 m x 0.25 mm x 0.25µm). Moreover, temperature for detector and injector were fixed at 250°C as well as 220°C and helium (1 mL/min) as a carrier gas was employed for this study. Samples (1µL) dissolved in methanol were introduced into column which was initially fixed at 50°C for 1 min and concurrently raised to 280°C by slowly raising temperature of 5°C/min. Both the samples were run for 30 minutes and analysis of obtained chromatograms was performed. Identification and characterization of various compounds were made by comparing relative retention time (RT) and mass spectra of samples with reference standards by using National Institute of Standards and Technology (NIST) library database[5].

Test Organisms

Micro-organism used in this study was procured from ATCC *(American Type Culture Collection from* LGC Promochem, Banglore INDIA as well as MTCC strains were procured from Institute of Microbial Technology (IMTECH), Chandigarh, INDIA. Fungal strains used in this study

was *Aspergillus niger* (MTCC 2196), *Penicillium pinophilium* (MTCC 2192), *Candida albicans* (ATCC 10231), *Aspergillus flavus* (MTCC 2798), and *Saccharomyces cerevisiae* (MTCC 786). However, bacterial strains selected for this study was *Rhodococus equi* (ATCC 6939), *Listeria innocua* (ATCC 33090), *Listeria monocytogenes* (ATCC 19111), *Vibrio parahaemolyticus* (ATCC 17802), *Enteococcus hirae* (ATCC10541), *Escherichia coli* (ATCC 15597), *Cronobacter sakazakii* (ATCC 29544), *Listeria ivanovii* (ATCC 19119), *Bacillus cereus* (MTCC 430), *Shigella* (MTCC 1457), *Enteococcus faecalis* (MTCC 439), *Salmonella enterica* (MTCC 733), *Staphylococcus aureus* (MTCC 96), *Clostridium perfringens* (MTCC 450), *Vibrio cholera* MTCC (3906), *Enterobacter aerogenes* (MTCC 111), *Salmonella typhi* (MTCC 733), *Klebsiella pneumonia* (MTCC 109), *Micrococcus luteus* (MTCC 2470), *Pseudomonas aeruginosa* (MTCC 741) and *Citrobacter freundii* (MTCC 1658).

Culture medium and inoculum preparation

Pure test organisms of all the selected micro-organism (Bacteria and fungus) were sub-cultured onto fresh plates of Mueller-Hinton agar (Hi Media laboratories) for 24 h and Saboraud dextrose agar (Hi Media laboratories) for 5-7 days at 37°C for bacteria and fungi, respectively. All the test organisms were incubated as specified for each organism for a period of 18- 24 h[11].

Agar well diffusion assay

Antimicrobial activity of GEO and FEO were analyzed by using agar well diffusion assay techniques[5]. Muller-Hinton Agar and Sabouraud Dextrose Agar plates were used for antibacterial and antifungal activity, respectively. 100µL standard bacterial and fungal inoculums were spread over the sterile plates and subsequently 8mm diameter wells were borer over the respective agar plates. Afterwards, 100µL GEO and FEO were filled into the Muller-Hinton agar and Sabouraud Dextrose agar plate wells and kept at room temperature for 1 hour for proper diffusion and incubated for 37C for 24 hours and 30 C for 3–5 days respectively[12]. All the samples were prepared in triplicates, essential oils having antimicrobial activity inhibited the microbial growth and the clear zones were formed. The zones of inhibition were measured in millimeters[13].

Statistical analysis

All the experimental results were carried out in triplicates and expressed as mean ± SEM (Standard Error of Means) of three independent experiments (n = 3).

RESULTS AND DISCUSSION

Characterization of chemical constituents using Gas Chromatography-Mass Spectrometry

Identification of various chemical components present in GEO and FEO were determined by GC-MS. The full scan GC-MS chromatograms are presented in Fig. 1A and 1B. Based upon the GC-MS chromatogram analysis, major components identified are presented in table 1. The identification of bioactive components are determined by comparing the chromatogram peak obtained in our samples with reference peaks as mentioned in National Institute of Standards and Technology (NIST) Library[5]. Furthermore, reference peaks were directly compared with the retention time and mass spectral data obtained in GEO and FEO sample chromatogram. According to GEO chromatogram (figure 1A), nineteen distinctive peaks were analyzed by GC-MS. Moreover, the major bioactive components analyzed by GC-MS were found to be Allyl methyl trisulfide (13.10%), Diallyl sulfide (9.47%), c-Sitosterol (6.15%), Diallyl tetrasulfide (4.38%) and Allyl methyl disulfide (3.40).. The results of the current study were comparable to previous studies[14-16]. Previously, allicin degradation has been proved responsible for the presence of sulphide compounds in oils. Moreover, formation of allicin in garlic occurs due to release of allinase enzyme after crushing garlic bulb. Subsequently, allicin is converted into alliin and because allicin is very unstable compound, it suddenly undergo reactions to form sulphur derivative components[17]. The GC-MS analysis of Chinese commercial GEO sample, which reports diallyl disulphide (45.1–63.2%) as highest components compared to other diallyl sulfide (4.5–11.4%), and diallyl tetrasulfide (6.3–10.5%)[18]. However, our results showed that GEO sample had highest amount of Allyl methyl trisulphide followed by diallyl sulphide and diallyl tetra sulphide. The differences obtained in sulphide content could be due the geographical as well as the process of distillation to obtain the essential oils.

On the other hand, FEO chromatogram showed seventeen distinctive peaks analyzed by

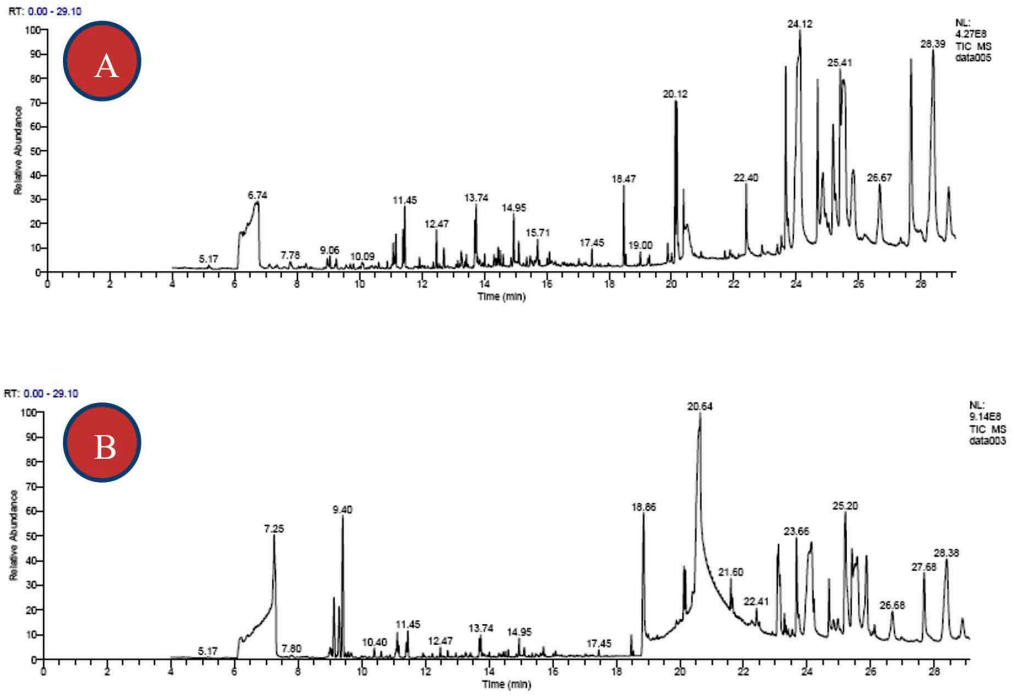

Fig. 1. GC-MS chromatogram of the bioactive compounds present in (A) Garlic essential oil and (B) Fenugreek essential oil

GC-MS and all the major components identified are presented in table 1. The major components present in the fenugreek EO were identified as limonene (12.92%), c-Sitosterol (9.58%), Carvone (4.57%), Campesterol (3.60%), Stigmasterol (2.83%), Cedrane-8-propoxy (1.50%) and Trigolline (0.38%). The previous scientific reports revealed that, stolones- furanones are the principle volatile compounds present in fenugreek oils[19]. The bioactive component sigmasterol present in FO have been reported to decrease blood cholesterol level[20].

Antimicrobial activity of essential oils

Both Garlic and Fenugreek essentials oils were investigated for *in-vitro* antimicrobial activity and both GEO and FEO showed positive antibacterial activity against Gram-negative, Gram-positive bacteria and few fungal strains tested in this study[21]. Antimicrobial activities of tested microorganisms are presented in Table 2 & Table 3. GEO showed antimicrobial activity against all the tested strains. Among the fungal strains, *Aspergillus flavus* showed the maximum inhibition zone (18.3 ± 0.29) followed by *Candida albicans* (18.1 ± 0.41). *Saccharomyces cerevisiae* showed least zone of inhibition (12.5 ± 0.29). Clotrimazole (50µg/ml) were tested against the stated fungal strains and their inhibition zone found to be in the range of 26.4 ± 0.42 - 35.2 ± 0.47. *Micrococcus luteus* showed the highest sensitivity (20.8 ± 0.28) followed by *Escherichia coli, Bacillus cereus, Enteococcus hirae* and *Listeria monocytogenes* were the least sensitive (5.2 ± 0.21) to GEO. Tetracycline (50µg/ml) antibacterial activity varied from (9.6 ± 0.48 - 31.1 ± 0.38). Clotrimazole and tetracycline antimicrobial standards were compared with GEO, which showed t GEO had a broad antimicrobial potential. The antibacterial activity of GEO has been attributed to the presence of allicin[21]. Additionally, allicin contains thiosulfnate group (-S(O)-S- group) found in the GEO extract is proved to possess antimicrobial properties[22]. One of the previous studies have revealed that, SH group of cellular proteins react with -S(O)-S- group, for the production of mixed disulfides[23]. Furthermore, the antimicrobial activity of GEO has been reported mainly because of the presence of organosulfur compounds such as allicin, ajoene and diallyl sulfides[24]. In addition, Mousumi & Prabir also reported that, garlic

Table 1. Garlic and fenugreek essential oil composition obtained by gas chromatography mass spectrophotometry

RT	Compound Name	Molecular Formula	Area %	Identification
		Garlic Essential Oil (GEO)		
7.25	Benzyl alcohol	C_7H_8O	5.79	MS, RI
9.13	l-Menthone	$C_{10}H_{18}O$	1.82	MS, RI
9.29	p-Menthan-3-one	$C_{10}H_{18}O$	1.63	MS, RI
9.67	a-Terpineol	$C_{10}H_{18}O$	0.19	MS, RI
10.40	Pulegone	$C_{10}H_{16}O$	0.26	MS, RI
28.38	c-Sitosterol	$C_{29}H_{50}O$	6.15	MS, RI
9.40	Diallyl tetrasulfide	$C_{10}H_{20}O$	4.38	MS, RI
11.12	Menthyl acetate	$C_{12}H_{22}O_2$	1.05	MS, RI
11.45	1-Triethylsilyloxy-heptadecane	$C_{23}H_{50}OSi$	0.99	MS, RI
12.70	2,4,7,9-Tetramethyl-5decyn4,7diol	$C_{14}H_{26}O_2$	0.19	MS, RI
15.71	1-Monolinoleoylglycerol Tri-methylsilyl ether	$C_{27}H_{54}O_4Si_2$	0.33	MS, RI
18.47	Hexadecanoic acid, methylester	$C_{17}H_{34}O_2$	0.43	MS, RI
18.86	l-(+)-Ascorbic acid 2, 6-dihexadecanoate	$C_{38}H_{68}O_8$	5.66	MS, RI
20.60	Allyl methyl tri-sulfide	$C_{18}H_{32}O_2$	13.10	MS, RI
23.09	Oleic acid, 3-hydroxypropylester	$C_{21}H_{40}O_3$	5.61	MS, RI
23.66	Allyl methyl disulfide	$C_{19}H_{38}O_4$	3.40	MS, RI
24.05	2,6-Bis(3,4methylenedioxyphenyl)-3,7-dioxabicyclo (3.3.0)octane	$C_{20}H_{18}O_6$	5.16	MS, RI
24.13	2,6-Bis(3,4-methylenedioxyphenyl)-3,7-dioxabicyclo(3.3.0)octane	$C_{20}H_{18}O_6$	4.12	MS, RI
25.20	Diallyl sulfide	$C_{21}H_{40}O_4$	9.47	MS, RI
		Fenugreek Essential Oil (FEO)		
12.70	2,4,7,9-Tetramethyl-5-decyn-4,7-diol	$C_{14}H_{26}O_2$	0.35	MS, RI
13.27	Dimethyl phthalate	$C_{10}H_{10}O_4$	0.44	MS, RI
13.43	n-Cetyl alcohol	$C_{16}H_{34}O$	0.33	MS, RI
18.47	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	1.19	MS, RI
19.29	5,8,11-Heptadecatriynoicacid, methyl ester	$C_{18}H_{24}O_2$	0.28	MS, RI
19.88	Trigolline	$C_{28}H_{58}O_9$	0.38	MS, RI
20.39	Methyl stearate	$C_{19}H_{38}O_2$	1.64	MS, RI
20.49	Cedryl propyl ether	$C_{18}H_{32}O$	1.50	MS, RI
22.90	1-Monolinoleoylglycerol-trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	0.29	MS, RI
23.66	Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	4.04	MS, RI
24.12	Limonene	$C_{20}H_{18}O_6$	12.92	MS, RI
24.68	Nonaethylene glycol	$C_{18}H_{38}O_{10}$	3.57	MS, RI
25.83	Campesterol	$C_{28}H_{48}O$	3.60	MS, RI
26.67	Stigmasterol	$C_{29}H_{48}O$	2.83	MS, RI
27.68	Carvone	$C_{18}H_{38}O_{10}$	4.57	MS, RI
28.39	c-Sitosterol	$C_{29}H_{50}O$	9.58	MS, RI

Table 2. Antifungal activity of essential oils using Agar diffusion method

Microorganisms	Zone of Inhibition in diameter (mm)		
	GEO	FEO	Clotrimazole (50µg/ml)
Aspergillus niger	16.1 ± 0.43	4.9 ± 0.26	28.2 ± 0.37
Aspergillus flavus	18.3 ± 0.29	6 .1± 0.62	26.4 ± 0.42
Candida albicans	18.1 ± 0.41	8.1 ± 0.56	27.1 ± 0.33
Penicillium pinophilium	14.6 ± 0.13	3.1 ± 0.17	37.5 ± 0.19
Saccharomyces cerevisiae	12.5 ± 0.29	5.2 ± 0.08	35.2 ± 0.47

Values are expressed as Mean ± SEM (Standard Error of Means)

extracts possesses a very strong antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* [25]. In addition to that, garlic juice is also reported to shown antibacterial activity against, *Escherichia coli* and *Staphylococcus aureus*[26,27]. Yin et al., reported that, *Salmonella typhimurium* growth in ground beef were effectively inhibited by GEO derived organosulfur compounds[28]. Antimicrobial activity of FEO showed poor results against the tested microorganism, when compared with standard clotrimazole and tetracyclines (50µ/ml). Zone of inhibition for both the standards varied from (26.4 ± 0.42- 35.2±0.47) and (9.6 ± 0.48 - 31.1 ± 0.38) respectively. Among the fungal strains, *Aspergillus flavus* had maximum zone of inhibition (6.1± 0.62). Moreover, least zone of inhibition was found for *Penicillium pinophilium* (3.1 ± 0.17). Bacterial strains had similar results

Table 3. Antibacterial activity of essential oils using Agar diffusion method

Microorganisms	Zone of Inhibition in diameter (mm)		
	GEO	FEO	Tetracycline (50 µg/ml)
Rhodococus equi	13.2 ± 0.18	3.0 ± 0.41	22.3 ± 0.31
Bacillus cereus	18.1 ± 0.31	6.8 ± 0.26	31.1 ± 0.38
Enteococcus faecalis	9.7 ± 0.53	5.2 ± 0.13	24.7 ± 0.27
Staphylococcus aureus	15.4 ± 0.19	4.2 ± 0.08	27.1 ± 0.32
Listeria monocytogenes	5.2 ± 0.21	1.5 ± 0.06	19.8 ± 0.22
Escherichia coli	18.5 ± 0.28	5.1 ± 0.24	21.6 ± 0.39
Enterobacter aerogenes	17.3 ± 0.41	4.0 ± 0.08	25.2 ± 0.41
Cronobacter sakazakii	10.3 ± 0.61	3.6 ± 0.23	22.6 ± 0.37
Klebsiella pneumonia	8.7 ± 0.46	3.7± 0.0	18.8 ± 0.26
Pseudomonas aeruginosa	13.5 ± 0.39	3.7 ± 0.14	19.4 ± 0.32
Citrobacter freundii	11.7 ± 0.32	4.0 ± 0.00	23.4 ± 0.41
Clostridium perfringens	4.5 ± 0.35	4.0 ± 0.45	20.7 ± 0.26
Micrococcus luteus	20.8 ± 0.28	5.0 ± 0.37	29.5 ± 0.53
Salmonella typhi	14.6 ± 0.29	3.5 ± 0.36	18.3 ± 0.28
Vibrio parahaemolyticus	5.1 ± 0.25	3.9 ± 0.09	NT
Vibrio cholerae	5.7 ± 0.24	6.1 ± 0.34	NT
Salmonella enterica	12.7 ± 0.23	2.5 ± 0.08	18.2 ± 0.31
Shigella	10.6 ± 0.67	2.0 ± 0.00	9.6 ± 0.48
Enteococcus hirae	18.5 ± 0.41	4.3 ± 0.19	19.8 ± 0.36
Listeria ivanovii	5.6 ± 0.35	1.6 ± 0.29	NT
Listeria innocua	6.3 ± 0.24	1.8 ± 0.08	NT

Values are expressed as Mean ± SEM (Standard Error of Means), NT; Not Tested

varying from (1.5 ± 0.06 - 6.8 ± 0.26). The antimicrobial activity of GEO and FEO could be due to the collective effect of tannins, phenolic compounds, flavonoids, alkaloids, and terpenoids present in oil[29].

CONCLUSIONS

The antimicrobial activity against the broad range of tested microorganism indicates the presence of wide spectrum of antimicrobial compounds in Garlic and Fenugreek essential oils. Additionally, antimicrobial components showed by GC-MS/MS analysis could be a good source of food preservation rendering the growth of microbes. In conclusion, it is suggested that these plant-derived products could be valuable to find out natural bioactive compounds. More importantly, these can be incorporated in the list of food preservation system due to their antimicrobial activity and lesser side effects. Hence, essential oils and their chemical components can be recommended for food preservation.

ACKNOWLEDGEMENTS

We are grateful to the Department of Clinical Nutrition, College of Applied Medical Sciences, Hail University and Department of Food technology, Hamdard University, New Delhi, and for encouragement and providing facilities to carrying out the present study.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

None.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript and/or the Supplementary Files.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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