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Antibacterial And Antibiotics Resistance Modifying Activity of Oil Extract of Myristica Fragrans's Mace (Aril) Against Multi-Antibiotic Resistant Phenotypes with A Comparative Study Against Cymbopogon Citratus

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ABSTRACT

This study systematically inspects the potent antimicrobial properties inherent in *Myristica fragrance* (Javitri) and *Cymbopogon citratus* (Lemongrass) essential oils. The core point is the targeted action against multiantibiotic-resistant (MAR) bacteria, a critical concern amidst the rising tide of antibiotic resistance. Notably, herbal alternatives are gaining traction as promising solutions. The research involves methodical extraction and assessment of these oils against formidable MAR strains, involving *Escherichia coli* and *Pseudomonas sp*. and through GC-MS analysis, we were able to find out the phytochemicals responsible for outstanding antimicrobial property of essential oil extracted from the mace of *Myristica fragrans*(Javitri). A significant part of this investigation delves into the synergistic interplay of *Myristica fragrans* oil with antibiotics. Through our research, we intend to re-establish the application of traditional herbal components for the purpose of medication and also in synergism with ineffective antibiotics and therapeutic strategies to face the antibiotic resistance crisis.

Keywords: Multi-antibiotic resistance (MAR), Essential oils, Therapeutic properties, Javitri, Lemongrass.

Introduction

The expanding gene pool across various species signifies both evolutionary growth and potential threats, as the increase in genetic variations within species may lead to significant consequences, including the possible extinction of one or more species. While biodiversity is generally considered a positive symbol of evolution, the challenges associated with it could pose a threat to the stability of ecosystems. This intricate relationship between genomic diversity and ecological balance constitutes the broader perspective of micro and macrobiota dynamics (Flandroy et al., 2018). As humanity focuses on resolving issues related to macroorganisms such as animals, birds, and plants, it is crucial not to overlook the threats that microbiota pose to human health. Microbiota, encompassing a wide range of microorganisms, has the potential to pose a substantial risk to the global ecosystem. The continuous evolution and adaptation of microbiota make them increasingly resistant to the antimicrobial drugs currently available in the market (Dhama et al., 2014). The cornerstone of treating microbial infections, both bacterial and fungal, has

traditionally been antibiotics. The discovery of antibiotics was initially celebrated within the medical community, with the anticipation that these drugs would lead to the eventual eradication of infectious diseases. However, the stark reality is the emergence and rapid proliferation of antibiotic resistance, which has become a severe and pervasive issue, particularly in developing nations. This predicament affects not only hospitals but also the general public, resulting in alarming mortality rates annually (Gavazzi et al., 2004).

Multiantibiotic resistance microorganisms (MAR), also known as multidrug-resistant microorganisms (MDR), represent strains of bacteria and fungi that have developed resistance to multiple types of antibiotics. The mechanisms driving this resistance are diverse and can include genetic mutations or the acquisition of resistance genes from other bacteria. Importantly, antibiotic misuse has become a primary contributor to the emergence and dissemination of multi-drug resistance strains across various categories of bacteria (Fernández et al., 2016). A significant therapeutic challenge has arisen due to the widespread prevalence of ß-lactamase producers, such as *E. coli*, *Pseudomonas sps*., *Klebsiella pneumoniae* (*K. pneumoniae*), *Haemophilus*, and other pathogens commonly found in healthcare settings (Sanders et al.,1989). Hospitals are now home to several multidrug-resistant strains of *E. coli* and *Pseudomonas species*, which exhibit resistance to antibiotics such as Ceprofloxacin, Penicillin, and Aminopenicillins. These resistant strains are increasingly isolated not only from hospital-acquired infections but also from diseases contracted in the community (Acar et al., 1997). The challenge of antibiotic resistance necessitates the exploration of alternative medicinal agents derived from plants that exhibit efficiency against bacteria resistant to antibiotics. Such alternatives should not only be effective but also secure and affordable (Kebede et al., 2021).

In response to the urgent need for innovative treatments for multidrug-resistant microbes, researchers are increasingly turning to herbal products. This shift is underscored by the growing evidence of the rapid global expansion of resistant clinical isolates. Certain plants have undergone successful evaluations for their direct antibacterial action and their ability to modulate the effects of antibiotics (Cheeseman et al., 2017). Essential oils (EOs), also known as phytochemicals, emerge as promising candidates in the quest for novel antimicrobial agents. In the historical context, the use of spices in India, prized for enhancing food flavour and preservation, can be attributed to the presence of essential oils in these plants (Sharma et al., 2023). The antimicrobial efficacy of various essential oils has been well-documented over many years. Notable examples include *Syzygium aromaticum* (clove), *Trachyspermum ammi* (ajwain), *Myristica fragrance* (nutmeg), *Cinnamomum verum* (dalchini), *Foeniculum vulgare* (saunf), *Eucalyptus camaldulensis* (Nilgiri), and *Cymbopogon citratus* (lemongrass) (Bajwa et al., 2020). These essential oils, derived from herbs, spices, and plants, present an opportunity to develop antifungal and antibacterial medications that can effectively combat MDR microorganisms. This can be achieved either by enhancing the potency of existing antimicrobial medications or by creating novel medications using various concentrations of essential oils (Chouhan et al., 2017). The focus of this study is to investigate the in vitro antimicrobial and antibiotic resistance-modifying activities of essential oils from *Cymbopogon citratus* and *Myristica fragrance* against multidrug-resistant bacteria and various fungal species isolated from bread. *Myristica fragrance*, an aromatic evergreen plant belonging to the family Myristicaceae, produces seeds known as nutmeg and arils known as mace. These components are utilized as spices in a variety of cuisines and hold significance in Asian Ayurvedic medicine. On the other hand, *Cymbopogon citratus*, commonly known as lemongrass, emits a tropical citrus aroma and possesses various therapeutic properties, including antiamoebic, antibacterial, antifungal, antiinflammatory, antimalarial, antimutagenic,

and antioxidant activities (SE Atawodi et al., 2014). Previous research has demonstrated the efficacy of essential oils in inhibiting multi-resistant bacteria and *Candida* species. For example, essential oils from nutmeg leaves showed inhibitory activity against *K. pneumoniae*, *Acinetobacter* species, *Enterobacter cloacae*, and group A *beta-haemolytic streptococcus*, as well as *Candida* species. Similarly, lemongrass essential oil exhibited higher antibacterial activity than tetracycline against *Streptococcus mutans* and *Staphylococcus epidermis*.

This study aims to expand on existing knowledge by investigating the antifungal and antibacterial activity of essential oils extracted from the dried mace of *Myristica fragrance* and the leaves of *Cymbopogon citratus*. The experimental extraction of essential oils will be coupled with an assessment of their antimicrobial potential against multidrug-resistant bacteria, including *E. coli* and *Pseudomonas,* as well as various fungal variants. In addition to assessing the antimicrobial properties of these essential oils, this study will conduct qualitative and semi-quantitative chemical characterization using GC–MS analysis. This analysis aims to elucidate the phytoconstituents present in the essential oils, providing valuable insights into their chemical composition and potential pharmacological properties.

MATERIALS AND METHOD:

PLANT SAMPLE COLLECTION:

The dried mace of (*Myristica fragrans)* was procured from a local Ayurvedic store situated in Bilaspur city, within the Chhattisgarh state of India. Fresh samples of Lemongrass (*Cymbopogon citratus*) were meticulously gathered from 'The Department of Forestry' at Guru Ghasidas Vishwavidyalaya in Bilaspur, Chhattisgarh. This strategic acquisition and collection process ensured the authenticity and quality of the plant materials, laying a robust foundation for subsequent analyses and investigations in our research study.

EXTRACTION OF ESSENTIAL OIL:

The *Myristica fragrans*, weighing approximately 150 grams, underwent an initial rinsing with distilled water before being placed in a Clevenger apparatus. This apparatus facilitated a hydro-distillation process involving boiling, condensation, and evaporation. Simultaneously, fresh leaves of *Cymbopogon citratus* (approx. 150 grams) were precisely cut into 2-4 cm pieces and introduced into the Clevenger apparatus. The distillation process, operating individually for each sample over 6-8 hours, involved initiating boiling at 60% temperature, followed by regulated heating at 25-30%. Essential oils from both samples were then collected in transparent glass tubes above the water level within the condenser tube. The extracted oils were stored at 4°C for subsequent detailed analysis (Schmidt et al., 2020). After that, the concentrated essential oils were collected and transferred into their respective transparent vials and were stored at about 4°C until used for further experimental process.

Test organisms and isolation

The bacterial strains, specifically *Escherichia coli* (*E. coli*) and *Pseudomonas sp*., were acquired from the Microbiology laboratory located in the Department of Biotechnology, with the sole purpose of conducting in-depth scientific research and analysis.

Bacterial Culture

Bacterial specimens, such as *E. coli* and *Pseudomonas sp*., were intricately isolated from their respective cultures, employing aseptic techniques. These specimens were then introduced into freshly prepared Nutrient Agar Medium (NAM) using a sterile inoculating loop, with rigorous heat sterilization before each

transfer to ensure a sterile environment. The application of the streak plate method facilitated an even distribution of bacterial samples on NAM plates. Subsequently, the inoculated plates underwent incubation in a BOD incubator set at 33ºC for 24-48 hours, fostering optimal conditions for bacterial growth before undergoing meticulous observation.

Selection of Modern antibiotics

Novel antibiotics such as Cefixime (CFM,5 mcg), Levofloxacin (LE,5 mcg), Ampicillin (AMP ,10 mcg), Rifampicin (RIF,5 mcg), Penicillin-G (P,10 mcg), Gentamicin (GEN,10 mcg) were implemented for the identification of multidrug-resistance in the isolated bacterial strains according to (Polsfuss et al., 2012).

Screening For Susceptibility of Bacteria to antibiotics:

Through the implementation of disc diffusion method the susceptibility of bacterial strains against antibiotics (gentamicin, penicillin-G, rifampicin, levofloxacin, cefixime, and ampicillin) were evaluated, prior to it successful introduction of bacterial culture uniformly throughout the agar plate was taken into consideration through a spreader. After all the processes were complete, the petriplates were stored in an incubator for 2-3 days until the evaluation of the result This provided insights into antimicrobial efficacy, guiding potential therapeutic options for infections caused by these strains (McLain et al., 2016).

Screening for efficiency of essential oil against MAR bacteria

The antimicrobial susceptibility test followed the well diffusion assay protocol outlined by Magaldi et al. (2004). NAM plates were utilized, and 0.5 ml of bacterial culture was evenly spread on each plate using a sterilized micropipette and spreader. After 10-15 minutes, wells (6mm in diameter) were created with a sterilized cork borer. Different concentrations (25%, 50%, 75%, and 100%) of ethanol extracts from *M. fragrans* mace and *C. citratus* leaves were prepared and added (100µl) to respective wells. The plates were then incubated undisturbed in a BOD incubator at 36ºC for 24 hours for observing inhibition zones.

Synergistic Antimicrobial Effects of Essential Oil

The potential synergistic effects of oil extracts in combination with the antibiotic three different antibiotics cefixime (5 mcg), penicillin-G (10 units), and ampicillin (10 mcg) against two common pathogenic bacteria, *E. coli* and *Pseudomonas sp*. The disc method was utilized to evaluate the antibiotic resistance modifying activities of different concentrations of the oil extract and antibiotics. The discs of the above mentioned antibiotics were dissolved into some selectively different concentrations of the essential oil of *Myristics fragrans* during a comparative analysis of oil against the antibiotics mentioned above (50% oil and 75% oil), resulting in the demonstration of varying degrees of synergistic effects, suggesting the potential of this oil extracts to enhance the antibiotic efficacy. Two reading were considered for the checking the synergism of essential oil with the above mentioned antibiotics.

GC- MS ANALYSIS

The GC-MS analysis of the essential oil sample (Sample ID: ABJ-01) was conducted utilizing 1.00 mL injection volume with a dilution factor of 1. The analysis was performed on Vial #26, using the n-Alkanes method file. The analysis was executed with an AOC-20i+s instrument, and the method involved multiple rinses and specific settings for plunger and syringe speeds. The GC-2010 parameters included a column oven temperature of 40.0 °C, injection temperature of 250.00 °C, and a split injection mode. The temperature program involved ramping from 40.0 °C to 220.0 °C in 4.00 minutes, and further to 250.0 °C in 15.00 minutes. The GCMS-QP2010 Ultra parameters included an ion source temperature of 200.00 °C and interface temperature of 260.00 °C. The qualitative analysis report from the Central University of Punjab, Bathinda. The analysis involved scanning in the m/z range of 40.00 to 800.00 over 60 minutes,

with a solvent cut time of 4.50 minutes. The detector gain mode was set to Relative, with a detector gain of 1.26 kV. The analysis provided insights into the composition of the essential oil.

RESULT

Extraction yield of Essential oil

Extraction of essential oil of dried mace of *Myristica fragrans* and fresh leaves of *Cymbopogon citratus* were obtained and the total amount of oil extracted from 250g of *M. fragrans* was about 12 ml having colour appearance of transparent soft green hue. The 250g of *Cymbopogon citratus* oil extract was measured to be about 1.7 ml and the appearance was found to be of vibrant mustard yellow colour.

Antibacterial potency of antibiotics

According to this study, the antibiotic susceptibility test emerges as a refined method, designed with precision to discern the potential impact exerted by bacterial strains. As the focal point of scrutiny shifts to *E. coli*, the strain delineates a nuanced hierarchy of sensitivity, with Levofloxacin(LE 5), Gentamicin(GEN 10), and Rifampicin(RIF 5) taking precedence, revealing zone of inhibition of (15±0.1), (13.3±0.2), and (9.9±0.1), respectively. In contrast, *E. coli* robustly resists the influence of Penicillin-G(P 10), Ciprofloxacin(CFM 5), and Ampicillin (AMP 10) (Table 1). Parallel observations in *Pseudomonas* species underscore the unwavering sensitivity pattern across Levofloxacin, Gentamicin, and Rifampicin, evidenced by zone of inhibition of (14.1±0.1), (13±0.1) and (9.8±0.1) as per (Table 2). This steadfast sensitivity profile resounds conspicuously across both bacterial strains (Figure 1).

Antibacterial activity of Essential oil:

The antibacterial efficacy of *M. fragrans* and *C. citratus* oil extracts against *E. coli* and *Pseudomonas* strains was assessed, observing inhibition zones. For *M. fragrans*, varying concentrations (25%, 50%, 75%, 100%) revealed concentration-dependent inhibitory effects which yielded appreciable results among the two. In *E. coli*, the zone of inhibition was to be (8.9±0.1) for 25% and (10.3± 0.2) for 100%, with intermediate concentrations 50% and 75% forming an inhibitory zone of (8.2±0.1) and (11.1±0.1) (Table 3). *Pseudomonas* displayed inhibition zone for 25%, 50%, 75% were (7±0.1), (7.9±0.05) and (8±0.05) while ethanol served as the positive control and 100% oil showed an inhibition zone of (7.9±0.1) (Table 4).

In case of *C. citratus*, the findings comparatively lower antimicrobial potency within its oil extracts against both *E. coli* and *Pseudomonas sp*. The inhibitory impact exhibited variability contingent on the concentration of the extract. At a 25% concentration, the inhibitory zone for *E. coli* measured was (6.1±0.1) (Table 5), whereas for *Pseudomonas sp.*, it registered at (5.2±0.1) (Table 6) . With an escalation to 100%, the inhibitory zones expanded to (6.3±0.1) for *E. coli* and (8±0.05) for *Pseudomonas sp.* For *E. coli*, the inhibitory zones at 50% and 75% concentrations were measured at (6.2±0.1) and (6.2±0.1), respectively. Conversely, for *Pseudomonas sp.*, the inhibitory zones at 50% and 75% concentrations were (6.1±0.1) and (6.3±0.1) (Figure 2).

Synergistic effect of Essential Oil

In this study, the potential synergistic effects of essential oil extracts from *Myristica fragrans* (*M. fragrans*) was explored in combination with three antibiotics (cefexime, penicillin, and ampicillin) against multi-antibiotic resistant strains of *Escherichia coli* and *Pseudomonas sp*. The disc method was employed to assess antibiotic resistance modification at different concentrations. The results revealed varying degrees of synergistic effects, demonstrating the capability of these oil extracts to enhance antibiotic efficacy. For *M. fragrans* oil extract combined with cefixime against *E. coli*, concentrations of 25% and 75% exhibited

enhanced inhibitory effects yielding (7.75±0.3) and (11.2±0.3) zones of inhibition, respectively (Figure 3). However, at 100% oil concentration, the inhibitory activity decreased to (7.3±0.4) (Table 7). Similar synergistic effects were observed against *Pseudomonas sp*., with 25% concentrations resulting in cloudy zone of inhibition and and 75% oil resulting in (9.7±0.3), respectively. Notably, 100% oil concentration led to a decrease in the inhibitory zone to (7.2±0.3) and the antibacterial disc of CFM individually (without synergism) formed hazy/cloudy zone of inhibition, *Pseudomonas* (Table 8). Combining *Myristica fragrans* oil with penicillin against *E. coli* showed enhanced inhibitory effects, with concentrations of 25% and 75% resulting in zones of inhibition of (9.2±0.3) and (9.2±0.3) mm, respectively. However, at 100% oil concentration, the inhibitory zone decreased to (6.7±0.3) and the penicillin disc individually did yield any zone of inhibition of (7.2±0.3) (Table 9). Similar effects were observed against *Pseudomonas sp*., where 25% and 75% oil concentrations with synergism to the ampicillin disc led to zones of inhibition of (10.2±0.3) and (11±0), respectively (Figure 4), while 100% oil concentration resulted in a (7.2±0.3) mm inhibitory zone (Table 10). The synergistic effect of *Myristica fragrans* oil with ampicillin against *E. coli* showed increased antibacterial efficiency, with 25% and 75% oil concentrations resulting in zones of inhibition of (9.2±0.3) mm and (9.2±0.3) mm, respectively. However, at 100% oil concentration, the inhibitory zone decreased to (7±0) and the same synergism of oil extract of *M. fragrans* with ampicillin antibacterial disc (Table 11), in case of *pseudomonas* was observed to form a zone of inhibition (11.2±0.3) at 25% and (10.2±0.3) for 75%, respectively. Adding on to it, 100% oil resulted in the zone formation of (7.2±0.3) and a (8.2±0.3) (Figure 5) for the disc without synergism (Table 12). These findings suggest that the combination of essential oil extracts of *Myristica fragrans* with antibiotics has the potential to address antibiotic resistance in both *E. coli* and *Pseudomonas sp*., offering new avenues for therapeutic strategies.

PHYTO-CHEMICAL COMPOSITION OF *Myristica fragrans*

The GC-MS analysis outcomes provide an intricate portrayal of the composition of the oil sample, depicted in the chromatogram. Thirty-one distinct compounds were identified, each characterized by specific retention times (RT), peak heights, and areas (Figure 6 and 7). Terpinen-4-ol predominated, constituting 21.06% of the peak area. Other notable compounds included Methyleugenol (13.01%), 1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)- (10.70%), and alpha-Pinene (8.97%). This diversity underscores the myriad bioactive compounds within the sample. Compounds like alpha-Pinene, beta-Pinene, and Terpinen-4-ol, recognized for antimicrobial and pest-repelling properties, bear agricultural significance. Additionally, compounds like Linalool and gamma-Terpinene, known for pleasant aromatic qualities, hold potential for applications in fragrance and cosmetics. The intricate GC-MS analysis underscores the complexity and richness of the sample, presenting opportunities for diverse applications, including natural product formulation, agrochemical development, and the exploration of novel bioactive compounds for various industrial purposes. Subsequent research and validation studies are imperative to effectively harness the potential of these compounds.

DISCUSSION

Our comprehensive investigation focused on assessing the antibacterial properties of essential oils derived from dried mace of *M. fragrans* and *C. citratus*. The bacterial strains under scrutiny displayed multidrug resistance, exhibiting resilience against commonly prescribed antibiotics such as penicillin, CFM, and AMP, as confirmed through rigorous antibiotic susceptibility testing.

The well diffusion assay was meticulously conducted to validate the antibacterial efficacy of *M. fragrans* essential oil. Significantly, this oil demonstrated pronounced effectiveness, particularly against *E. coli* and *Pseudomonas*, as further confirmed by a meticulous disc diffusion setup. Conversely, the essential oil from *C. citratus* did not yield satisfactory results in the well diffusion assay, leading to the decision to forego further antimicrobial testing for this specific oil.

To explore the potential synergistic effects of the essential oil from *M. fragrans* with antibiotic drugs, various concentrations of antibiotics to which the bacteria were initially resistant were combined with the oil. Surprisingly, this combination yielded enhanced results, surpassing the efficacy of the oil or antibiotics used individually. Additionally, the essential oil showcased the ability to augment the activity of previously ineffective drugs against opportunistic bacterial strains.

Furthermore, we conducted disc diffusion assays to evaluate the antifungal properties of *M. fragrans* essential oil against self-cultured fungal strains, namely BFG-01AJ and BGF-01AJ. The oil exhibited significant and effective results against these isolated fungal strains, underscoring its potential in combating antimicrobial resistance in both bacterial and fungal domains.

In comparison to the study conducted by Nikolic et al. (2021), our findings regarding the antibacterial effects of *M. fragrans* oil deviated. Our results indicated potent effectiveness against *E. coli* and *Pseudomonas*, highlighting the inherent variability in essential oil effects on different bacterial strains. This variability emphasizes the importance of considering specific bacterial strains and their unique responses when evaluating the potential applications of essential oils in combating multidrug resistance.

In our comprehensive investigation, we expanded the scope beyond the antibacterial and antifungal activities explored in the study to acknowledge the diverse antimicrobial properties found in essential oils from various plants. Tea tree oil (*Melaleuca alternifolia*), for instance, has been extensively studied for its potent antimicrobial effects against bacteria, fungi, and viruses (Carson et al., 2006).

Eucalyptus oil, derived from Eucalyptus species, emerged as another essential oil with notable antimicrobial properties. Research indicates its effectiveness against various bacterial strains, including *Staphylococcus aureus* and *Escherichia coli*, with 1,8-cineole and alpha-pinene identified as key constituents responsible for its antimicrobial effects (Juergens et al., 2011).

Furthermore, lavender oil (*Lavandula angustifolia*) showcased broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as fungi. The antimicrobial prowess of lavender oil is attributed to major components like linalool and linalyl acetate (Cavanagh et al., 2002).

These examples collectively underscore the diverse and potent antimicrobial activities present in essential oils from different plants, suggesting their broad potential applications in combating various microbial challenges. The multifaceted nature of essential oils offers a promising avenue for future research and applications in the field of antimicrobial therapy.

Conclusion

The experimented Javitri sample was found potent not only against MAR strains of bacterias i.e, *E.coli* and *Pseudomonas sp.* but also showed efficacy against cultured strains of *Aspergillus* strains. Phytochemicals present in the aril are confirms the reason behind the aroma and efficiency against microbes which were considered for the experiment. In addition to sabinene, the essential oil also exhibited notable concentrations of methyl eugenol, β-pinene, terpinen-4-ol, and α-pinene. These constituents contribute to the overall aromatic profile and therapeutic properties of the oil, enhancing its efficiency which was confirmed through the GC-MS. Observably the yield of the essential oil obtained through the

hydrodistillation process was highly distinguishable in comparison with *Cymbopogon citritus* and comparatively and, the latter showed less effective result against bacterias and self cultured fungal strains. Variety of healthcare products and hygene utilities can be produced using the crude oil extracts of *M. fragrans* and through this research we can conclude its usage in formulating drugs in different pharmaceutical industries.

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Conflict of Interest:
No potential conflict of interest was reported by the author(s).

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Figures and Tables:

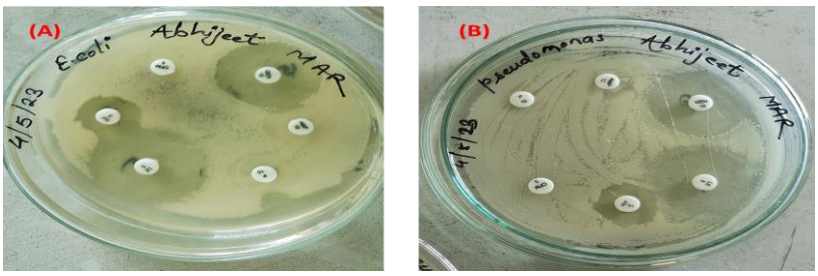

Figure 1- Multiantibiotic resistance along with antibiotic susceptibility test of (A) *E. coli* and (B) *Pseudomonas sp.*

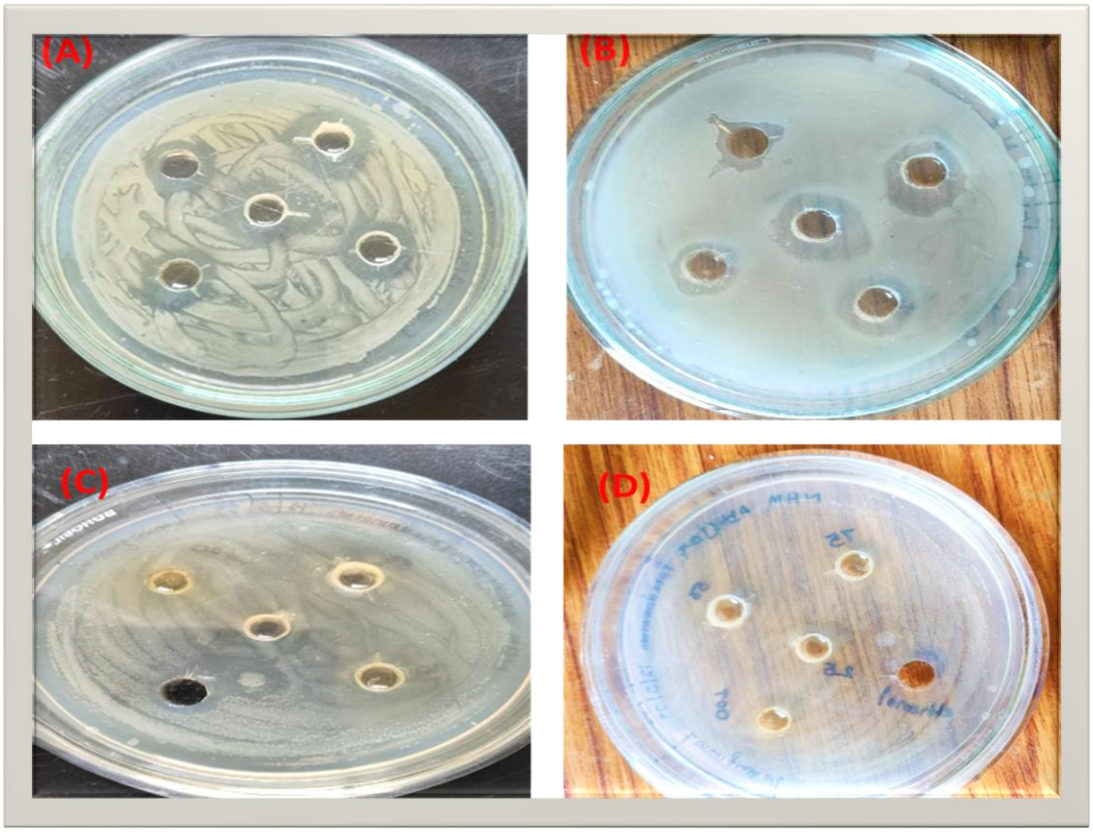

Figure 2. Well diffusion assay of oil extract of M. fragrance against (A) *E. coli* (B) *Pseudomonas* and *C. citratus* against (C) *E. coli* (D) *Pseudomonas sp*

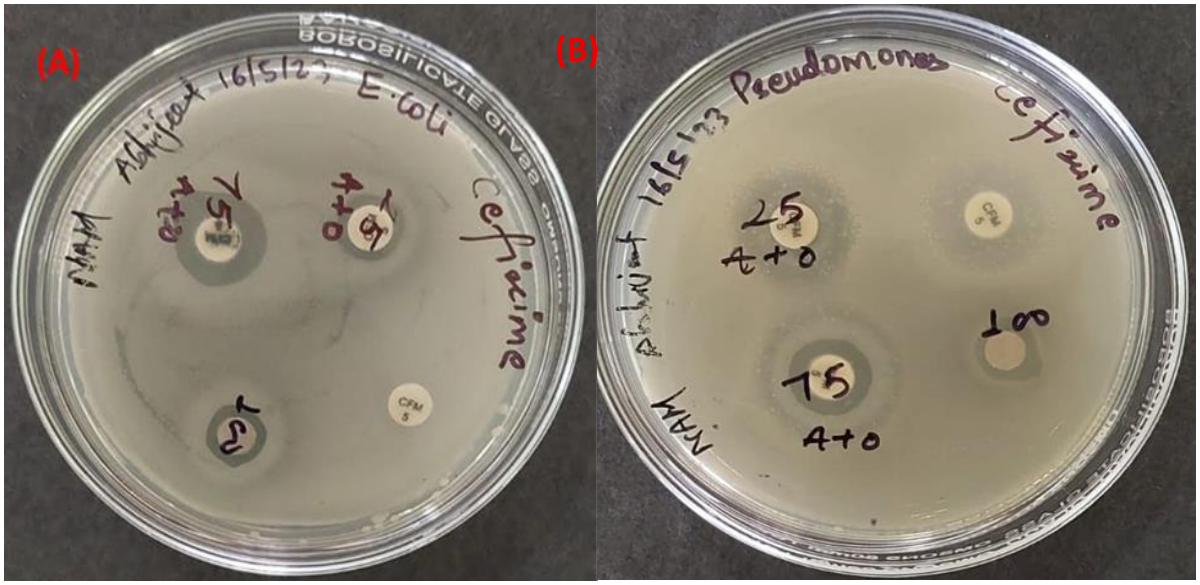

Figure 3. Synergistic effect of Cefixime along with oil against (A) *E. coli* (B) *Pseudomonas sp.*

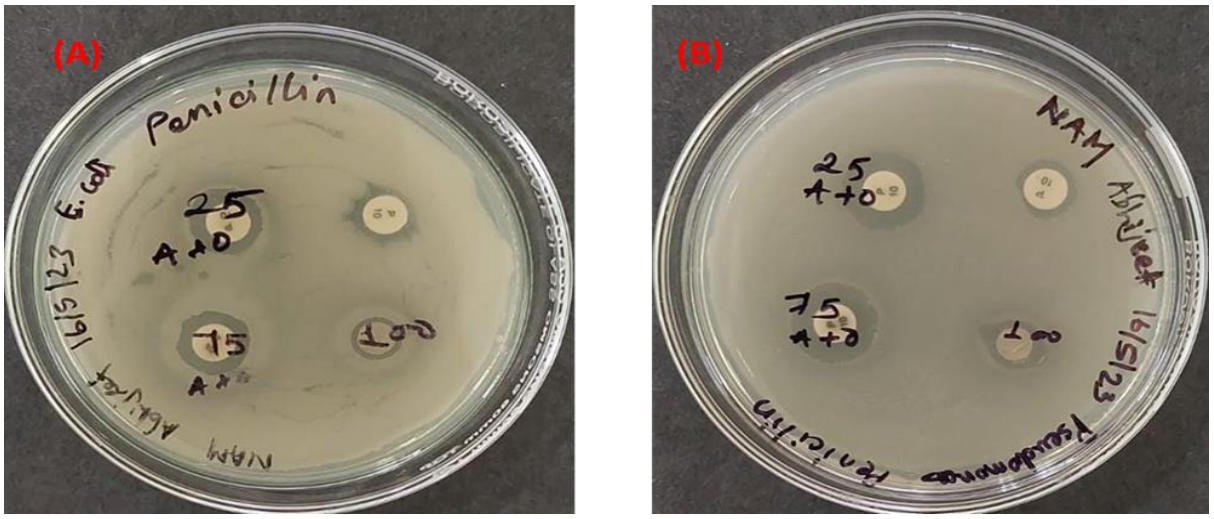

Figure 4. Synergistic effect of Penicillin along with oil against (A) *E. coli* (B) *Pseudomonas sp.*

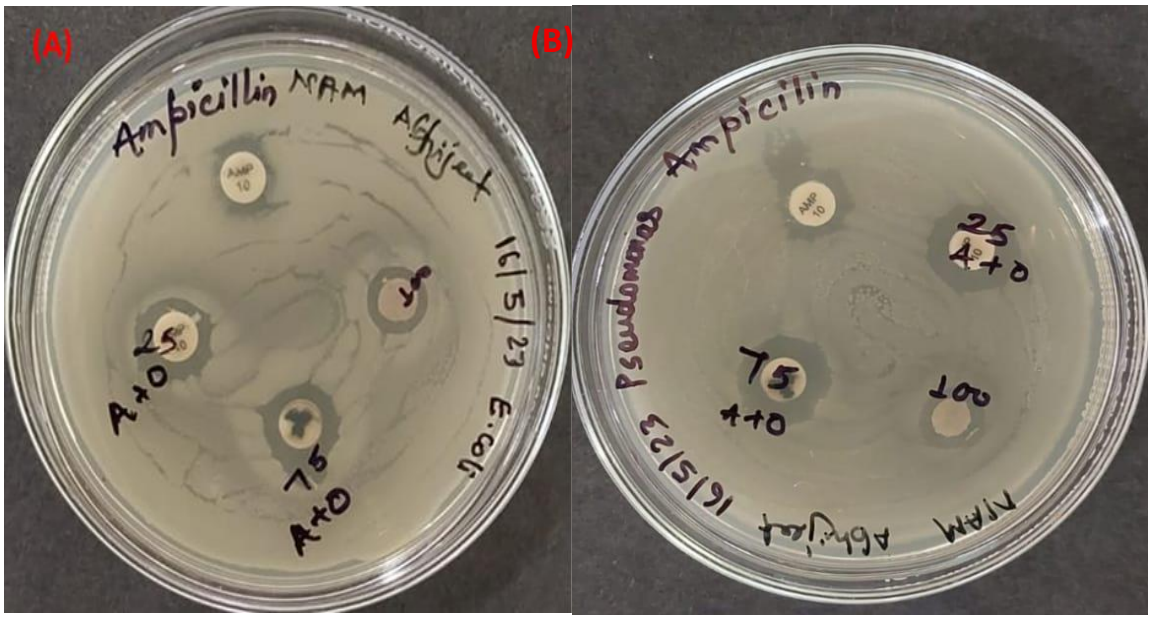

Figure 5. Synergistic effect of Ampicillin along with oil against (A) *E. coli* (B) *Pseudomonas sp.*

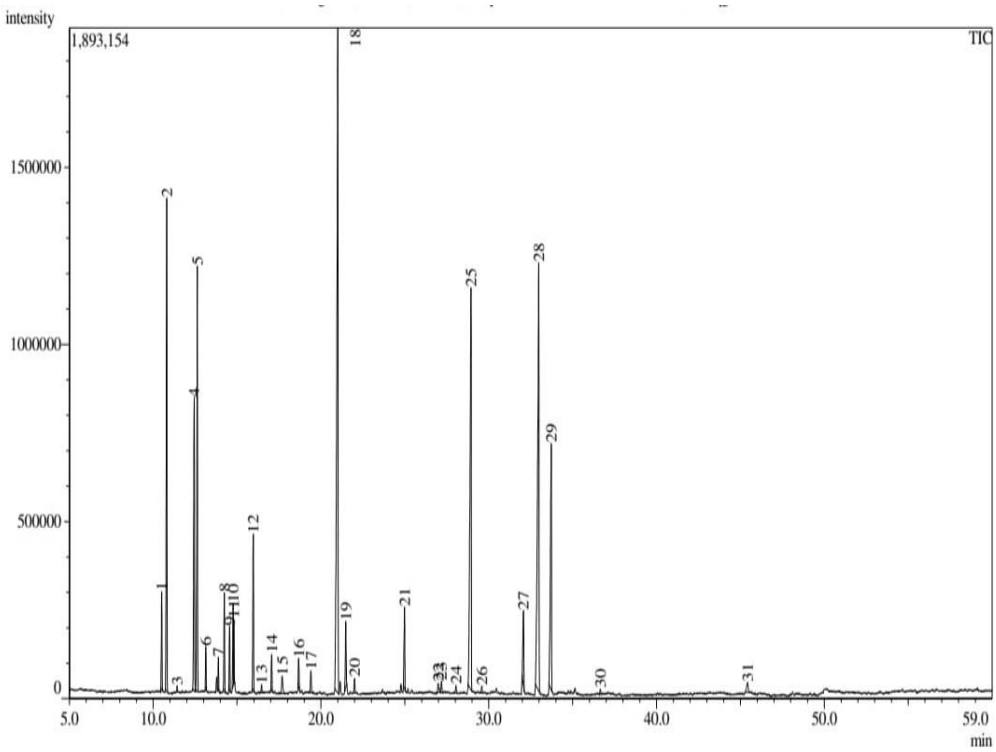

Figure 6. Chromatogram of *M.fragrans* extracts in water.

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	10.503	10.450	10.567	772226	1.71	277147	2.45	2.79		.alpha.-Phellandrene
2	10.804	10.717	10.858	4049442	8.97	1393151	12.32	2.91		.alpha.-Pinene
3	11.423	11.358	11.517	49334	0.11	17943	0.16	2.75	MI	Camphene
4	12.440	12.350	12.508	2871233	6.36	829318	7.33	3.46		Bicyclo[3.1.0]hexane, 4-methylene-1-
5	12.625	12.533	12.683	3533303	7.83	1200381	10.61	2.94		.beta.-Pinene
6	13.133	13.092	13.183	301230	0.67	121352	1.07	2.48		.beta.-Myrcene
7	13.883	13.842	13.933	244184	0.54	93425	0.83	2.61		.beta.-Ocimene
8	14.235	14.175	14.292	771640	1.71	279278	2.47	2.76		Cyclohexene, 1-methyl-4-(1-methylet
9	14.551	14.500	14.608	512624	1.14	181693	1.61	2.82		o-Cymene
10	14.762	14.700	14.792	829534	1.84	232847	2.06	3.56		Cyclohexene, 1-methyl-4-(1-methylet
11	14.800	14.792	14.850	273621	0.61	182632	1.61	1.50	V	.alpha.-Phellandrene
12	15.963	15.892	16.017	1342690	2.97	443091	3.92	3.03		.gamma.-Terpinene
13	16.456	16.375	16.592	78618	0.17	23829	0.21	3.30	MI	Cyclohexanol, 1-methyl-4-(1-methyle
14	17.056	17.008	17.117	305603	0.68	102808	0.91	2.97		Cyclohexene, 1-methyl-4-(1-methylet
15	17.683	17.575	17.858	220025	0.49	49970	0.44	4.40	MI	Linalool
16	18.667	18.625	18.725	244294	0.54	82539	0.73	2.96		4-Isopropyl-1-methylcyclohex-2-enol
17	19.398	19.300	19.500	227178	0.50	61960	0.55	3.67	MI	4-Isopropyl-1-methylcyclohex-2-enol
18	20.999	20.817	21.058	9506624	21.06	1876847	16.60	5.07		Terpinen-4-ol
19	21.476	21.408	21.533	602277	1.33	185870	1.64	3.24		.alpha.-Terpineol
20	21.997	21.892	22.133	146080	0.32	42500	0.38	3.44	MI	2-Cyclohexen-1-ol, 3-methyl-6-(1-me
21	24.976	24.908	25.033	768708	1.70	231910	2.05	3.31		1,3-Benzodioxole, 5-(1-propenyl)-, (Z
22	26.981	26.883	27.125	111638	0.25	27014	0.24	4.13	MI	.alpha.-Terpinyl acetate
23	27.176	27.125	27.258	120184	0.27	32472	0.29	3.70	MI	Phenol, 2-methoxy-4-(2-propenyl)-, a
24	28.040	27.908	28.092	74315	0.16	21344	0.19	3.48	MI	2,6-Octadien-1-ol, 3,7-dimethyl-, acet
25	28.939	28.767	28.992	5874382	13.01	1135655	10.04	5.17		Methyleugenol
26	29.584	29.458	29.642	78463	0.17	20716	0.18	3.79	MI	cis-.alpha.-Bisabolene
27	32.064	31.983	32.125	814959	1.81	210787	1.86	3.87		Benzene, 1,2-dimethoxy-4-(1-propeny
28	32.976	32.775	33.033	6992476	15.49	1210214	10.70	5.78		1,3-Benzodioxole, 4-methoxy-6-(2-pr
29	33.719	33.583	33.775	3174426	7.03	695565	6.15	4.56		Benzene, 1,2,3-trimethoxy-5-(2-prope
30	36.647	36.575	36.742	69594	0.15	16623	0.15	4.19	MI	Benzene, 1,2,3-trimethoxy-5-(1-prope

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6/12/2023

Qualitative Analysis Report

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
31	45.428	45.283	45.508	185664	0.41	28591	0.25	6.49	MI	n-Hexadecanoic acid
				45146569	100.00	11309472	100.00			

Figure 7.- Tabular representation of the data output through GC-MS analysis.

Table 1. Antibacterial Potency of Antibiotics against *E.coli* (in mm as per the measurement through Vernier calipers for three observations).

Observation	GEN 10	LE 5	RIF 5	P 10	AMP 10	CFM 5
1	13.3	15	9	0	0	0
2	13.1	14.9	10.1	0.1	0.2	0
3	13.5	15.2	9.8	0	0	0
Mean	13.3	15.03333	9.966667	0	0.066667	0
SD	0.2	0.152753	0.152753	0	0.11547	0

Table 2. Antibacterial potency of Antibiotics against *Pseudomonas sp.* (in mm as per the measurement through Vernier calipers for three observations.)

Observation	GEN 10	LE 5	RIF 5	P 10	AMP 10	CFM 5
1	13	14	9.7	0	0	0
2	13.2	14.2	10	0	0	0
3	12.9	14.2	9.8	0	0	0
Mean	13.03333	14.13333	9.833333	0	0	0
SD	0.152753	0.11547	0.152753	0	0	0

Table 3- Antibacterial activity of Essential oil(*Myristica fragrans*)against *E.coli*(in mm as per the measurement through Vernier calipers for three observations).

Observations	25%	50%	75%	100%	Ethanol
1	9	8.3	11	10.6	8.2
2	8.8	8.1	11.2	10.4	8.6
3	9.1	8.4	11.1	10.1	8.3
Mean	8.966667	8.266667	11.1	10.36667	8.366667
SD	0.152753	0.152753	0.1	0.251661	0.208167

Table 4- Antibacterial activity of Essential oil(*Myristica fragrans*) against *Pseudomonas*(in mm as per the measurement through Vernier calipers for three observations).

Observation	25%	50%	75%	100%	Ethanol
1	7	7.9	8.1	8	6.8
2	7.2	7.9	8	8	6.5
3	7	8	8.1	7.8	6.7
Mean	7.066667	7.933333	8.066667	7.933333	6.666667
SD	0.11547	0.057735	0.057735	0.11547	0.152753

Table 5. Antibacterial activity of Essential oil*(Cymbopogon citratus)* against *E.coli*(in mm as per the measurement through Vernier caliper for three observations).

Observation	25%	50%	75%	100%	E
1	6.2	6.3	6.2	6.2	5.7
2	6	6.4	6.2	6.3	6
3	6.3	6.1	6.3	6.4	5.8
Mean	6.166667	6.266667	6.233333	6.3	5.833333
SD	0.152753	0.152753	0.057735	0.1	0.152753

Table 6. Antibacterial activity of Essential oil (*Cymbopogon citratus*) against *Pseudomonas sp.* (in mm as per the measurement through Vernier calipers for three observations).

Observation	25%	50%	75%	100%	E
1	5.2	6.1	6.3	8	7
2	5.1	6	6.4	8	7.1
3	5.4	6.3	6.2	8.1	7
Mean	5.233333	6.133333	6.3	8.033333	7.033333
SD	0.152753	0.152753	0.1	0.057735	0.057735

Table 7. Synergistic effect of Essential oil of *Myristica fragrans* with antibiotic disc CFM against *E.coli*.(in mm measured ruler scale due to visible differentiation between the zone of inhibition)

Observation	**25%O+CFM**	**75%O+CFM**	**100%Oil**	**CFM**
1	8	11	7	0
2	7.5	11.5	7.7	0
Mean	7.75	11.25	7.35	0
SD	0.353553	0.353553	0.494975	0

Table 8. Synergistic effect of Essential oil of *Myristica fragrans* with antibiotic disc CFM against *Pseudomonas sp.* .(in mm measured ruler scale due to visible differentiation between the zone of inhibition)

Observation	**25%O+CFM**	**75%+CFM**	**100%Oil**	**CFM**
1	Cloudy zone of inhibition	9.5	7	0
2		10	7.5	0
Mean		9.75	7.25	0
SD		0.353553	0.353553	0

Table 9. Synergistic effect of Essential oil of *Myristica fragrans* with antibiotic disc Penicillin against *E.coli*.(in mm measured ruler scale due to vividly observable zone of inhibition)

Observation	**25%+Penicillin**	**75%+Penicillin**	**100%Oil**	**Penicillin**
1	9	9	6.5	7
2	9.5	9.5	7	7.5
Mean	9.25	9.25	6.75	7.25
SD	0.353553	0.353553	0.353553	0.353553

Table 10. Synergistic effect of Essential oil of *Myristica fragrans* with antibiotic disc Penicillin against *Pseudomonas sp.*(in mm measured ruler scale due to vividly observable zone of inhibition)

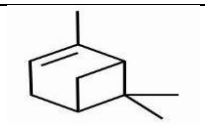
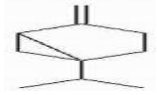
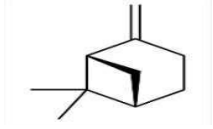
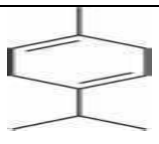
Observation	25%+Penicillin	75%+Penicillin	100%Oil	Penicillin
1	10	11	7.5	7.5
2	10.5	11	7	8
Mean	10.25	11	7.25	7.75
SD	0.353553	0	0.353553	0.353553

Table 11. Synergistic effect of Essential oil of *Myristica fragrans* with antibiotic disc Ampicillin against *E.coli*.(in mm measured ruler scale due to vividly observable zone of inhibition)

Observation	25%+Ampicillin	75%+Ampicillin	100%Oil	Ampicillin
1	9.5	9	7	7.5
2	9	9.5	7	8
Mean	9.25	9.25	7	7.75
SD	0.353553	0.353553	0	0.353553

Table 12. Synergistic effect of Essential oil of *Myristica fragrans* with antibiotic disc Ampicillin against *Pseudomonas sp.* (in mm measured ruler scale due to vividly observable zone of inhibition)

Observation	25%+Ampicillin	75%+Ampicillin	100%Oil	Ampicillin	Ampicillin
1	11	10	7	8	0
2	11.5	10.5	7.5	8.5	0
Mean	11.25	10.25	7.25	8.25	0
SD	0.353553	0.353553	0.353553	0.353553	0

S.No.	Retention time (RT value)	Peak area(%)	Constituent	Structure of Constituent	Molecular formula
1	10.804	8.97	Alpha -Pinene		C10H16
2	12.440	6.36	Sabinen		C10H16
3	12.625	7.83	Beta-Pinene		C10H16
4	15.963	2.97	Gamma-Terpinene		C10H16

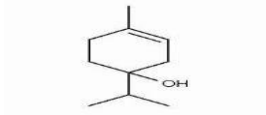
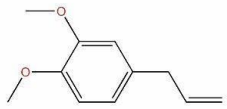
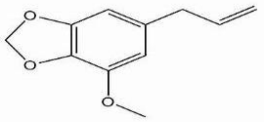
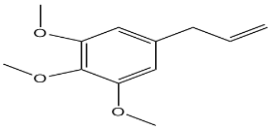
5	20.999	21.06	Terpinen-4-ol		C10H18O
6	28.939	13.01	Methyleugenol		C11H14O2
7	32.976	15.49	Myristicin		C11H12O3
8	33.719	7.03	Elemicin		C12H16O3

Table 13. Tabular representation of Phytochemicals showing highest peak value.