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Efficacy of *Glycyrrhiza glabra* as an antibacterial agent against isolates that produce biofilm and beta-lactamases

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Abstract
When it comes to human relationships with the natural world, the use of herbal remedies in Asia symbolizes a long history. Antibiotic resistance has emerged as a primary therapeutic dilemma around the globe in recent years, in addition to the rise in the number of diseases. It is common knowledge that plants are a source of active metabolites; hence, they serve as the foundation for a wide variety of pharmacological products. In addition to having anti-inflammatory and expectorant characteristics, *Glycyrrhiza glabra* is a highly pleasant, moist, and soothing plant. It also has the ability to reduce coughing and has hormonal effects. Individuals of *Staphylococcus aureus* and *Klebsiella pneumonia* that have developed resistance to multiple drugs. Ethanol extract was used for the GC-MS analysis of these three different extracts. In the ethanol extract, tannins, saponins, phenols, and terpenoids were found. Proteins were not present in either of the extracts. The ethanol extract had the maximum amount of phytochemicals compared to the chloroform extract. An advantageous component was found in *Glycyrrhiza glabra*, and it demonstrated antibacterial action against clinical isolates that produced beta-lactamases. Therefore, it has the potential to be utilized as an antibacterial agent against a wide variety of microbes, which is superior to the usage of traditional antibiotics.

Keyword: *Glycyrrhiza glabra,* phytochemicals, *S.aureus, K.pneumoniae.*

Introduction

Since the majority of antibiotics now in use were discovered before 1970 (Ayaz *et al.,* 2016) [1] multi-drug-resistant bacteria and their accompanying biofilms have become a global concern (Bayramov *et al.,* 2018) [4] (Thangaraj N, Sivanantham S 2015) [23]. Therefore, to prevent the rise of bacteria that are resistant to multiple treatments, it is imperative to find and develop new antimicrobial medications with unique mechanisms of action. (Ayaz *et al*.,2017) [2] (Indhu *et al*., 2014) [21]. According to estimates from the World Health Organization from 2003, 80% of people living in developing nations depend on traditional remedies because they cannot afford pharmaceutical pharmaceuticals. *Glycyrrhiza glabra* roots have anti-ulcer, anti-inflammatory (Yokota *et al.,* 1998) [19], antioxidant (Ju *et al*.,1989) [11], expectorant, diuretic, laxative, and sedative (Lata 2011) [13] (Indhu *et al*., 2017) [22] properties.

They also possess antipyretic, antiherpes, antiviral, antimicrobial and anxiolytic activities (Geetha and Anita 2013) [9], (Chakravarthi 2012) [6] (Sasikala P 2018) [20]. *Glycyrrhiza glabra* has also been shown to have a direct hepatoprotective and free-radical quenching effect (Fukai *et al.,* 2003) [8]. *Glycyrrhiza* exerts antiviral activity in vitro toward a number of viruses, including hepatitis A (Crance *et al*., 1990,) [7] varicellazoster (Baba and Shiget 1987) [3], HIV (Ito *et al.,* 1987) [10], herpes simplex type 1, Newcastle disease, and vesicular stomatitis viruses (Chakravarthi 2012) [6].

An infection caused by bacteria is an example of the type of biotic stress that a plant must suffer. In order to protect themselves from the effects of biotic stress, plants develop a resistance mechanism, which often involves the production of a number of different metabolites. As a result, the objective of this research was to ascertain whether or not the dry bark extract of *Glycyrrhiza glabra* had any antimicrobial components and whether or not these components possessed any antibacterial activity against bacterial isolates that formed biofilm and betalactamases.

Materials and methods

Components of Plants

In Namakkal, the species of licorice known as *Glycyrrhiza glabra* was collected from its natural habitat. In a mixer grinder, the leaves were ground into a powder after they had been dried. The powdered drug was stored for later usage in an airtight container after being sealed in a polybag and then transported to the storage location.

Using the following formula, we determined the percentage yield by weighing the dried extracts and calculating the percentage.

$$\text{Percent yield of extract} = \frac{\text{Weight of the extract after evaporating solvent and drying x 100}}{\text{Dry weight of the plant material}}$$

Quality assessment of phytochemicals

In each and every one of the *Glycyrrhiza glabra* bark extracts, the levels of alkaloids, carbohydrates, flavonoids, phenolics, saponins, tannins, quinones, steroids, terpinoids, and proteins were analyzed.

Collecting of single-use medical devices

Isolates of *Klebsiella pneumonia* and *Staphylococcus aureus* that were resistant to multiple drugs were used in the research project. These isolates came from the Microbiology Laboratory at Microtech in Coimbatore. In order to verify the isolates that had been collected, chromogenic and selective medium were taken into consideration.

The process of isolating the organisms that will generate biofilms

Before the brain heart infusion agar was manufactured and autoclaved for fifteen minutes at 121 degrees Celsius, Congo red (0.08 g/l) and 5% sucrose were added to it. At a temperature of 35 degrees Celsius, the clinical isolates were injected and stored in an aerobic environment for a period of twenty-four to forty-eight hours. Biofilm producers have colonies that are black and have a dry, crystalline quality, in contrast to biofilm non-producers, which remain pink and occasionally display darkening at the center of the compound.

Identifying the beta lactamases that are produced by the isolates

At a volume of 0.5 milliliters, penicillin solution was poured into small test tubes. Using a loop, test bacteria were isolated from an overnight culture on solid medium and then suspended in the Penicillin solution. This was done in order to produce a density of at least 104 colony-forming units (CFU) per milliliter. One drop of iodine reagent and two drops of starch indicator were added to the suspension after it had been allowed to stabilize at room temperature for a period of one hour. A favorable reaction was shown by the blue tint disappearing almost immediately. On the other hand, the test was deemed unsuccessful if the color remained blue for more than ten minutes.

***Glycyrrhiza glabra* has substances that have antibacterial effects**

Turning the plate through a full sixty degrees will ensure that the microorganisms being examined are dispersed uniformly across the surface of the plate. The inoculums that were employed had 108 colony-forming units per milliliter. Through the utilization of a borer, a well with a diameter of 6 millimeters was created utilizing agar plates. In one of the wells, 10 micrograms per milliliter of chloramphenicol was administered, while in another well, 100 microliters of the equivalent solvent was administered. After the zone of inhibition has been located, the plate should be immersed in 37 degrees Celsius for a period of twenty-four hours.

A combination of gas chromatography and mass spectrometry analysis (GC/MS)

GC/MS detection was carried out with the assistance of an electron ionization apparatus that had an ionizing energy of 70 eV. The injector temperature was 250 degrees Celsius, and the ion source temperature was 280 degrees Celsius. The injection volume was 2 milliliters, and the split ratio was 10 to 1. Helium gas, which was 99.9 percent, was used as the carrier gas at a constant flow rate of 1 milliliter per minute. With pieces ranging from 45 to 450 Da and a scan interval of 0.5 seconds, mass spectra were acquired at a potential energy of 70 electronic volts. For a total of thirty-six minutes, the GC was in operation.

Results

For the purpose of determining the extraction yield, the present experiment made use of organic solvents such as ethanol and chloroform. On the other hand, the output of ethanol was significantly higher (71.1 mg/5g) than that of chloroform (51.6 mg/5g). From this point forward, the examination that is now being conducted revealed that the maximal extraction value percentile was attained with methanol, which was 1.42%, and chloroform, which was 0.03 percent.

Analysis of phytochemicals from a qualitative perspective

In the presence of favorable results, the presence of carbohydrates, flavonoids, and quinones was found by employing two different solvents, namely ethanol and chloroform. The existence of sterols and alkaloids was brought to light by the chloroform extract, as well. Tannins, saponins, phenols, and terpenoids were among the phytochemicals that were present in the ethanol extract, which contained a greater quantity of these compounds than the chloroform extract. It was determined that neither of the extracts contained any proteins.

Collection of clinical isolates and verification of their composition

With the help of chromogenic and selective medium, *S. aureus* and *K. pneumoniae* isolates were collected and analysed. *S. aureus* and *K. pneumoniae* were found to be present in ten of the twelve isolates that were collected, according to morphological assessment performed on both mediums.

The isolating of individual isolates that produce beta-lactamases

Following an incubation period of twenty-four hours, the medium exhibited a color change from blue to colorless, which was indicative of a good reaction. Within the scope of this investigation, a total of ten isolates were put through the process of determining whether or not they produced betalactamase. Out of those ten, six were found to be positive for betalactamase production. It was more common for *K. pneumoniae* to be present than *S. aureus*.

Isolation of biofilm-producing isolates

S. aureus was used more frequently than *K. pneumoniae* among the ten isolates, and eight of them gave favorable results. A black coloration was observed on the congored agar plate medium, which served as an indication of the outcome.

Betalactamase and biofilm generating isolates were resistant to the antibacterial action of *Glycyrrhiza glabra*

Ethanol extract of *Glycyrrhiza glabra* was shown to have antibacterial action, as demonstrated in Table 1. Between 10±1.6 mm and 24±0.51 mm was the range of the zone of inhibition. *S. aureus* 5 was the most suppressed of the five isolates, with a zone of inhibition of 24±0.51 mm. It was followed by *K. pneumonia* 5 in terms of high suppression. Using a concentration of 2.5 milligrams of plant extract reduced the growth of all four isolates. *Glycyrrhiza glabra* ethanol extract was found to have a more potent effect against *S. aureus* than it did against *K. pneumoniae* in this particular investigation. While the use of ethanol as a control agent did not result in any of the isolates being suppressed, the use of the conventional antibiotic Ciproflaxcin resulted in the suppression of *S. aureus* 3 and *S. aureus* 7.

The chloroform extract of *Glycyrrhiza glabra* exhibited effective antibacterial activity.

It was shown in Table 2 that the chloroform extract of *Glycyrrhiza glabra* was used. 10±1.6mm to 20±1.6mm was

the range of the zone of inhibition that was observed. It was shown that *S. aureus* 3 had the strongest inhibitory action, followed by *K. pneumonia* 4 in terms of performance. It was observed that *K. pneumonia* 5 had the least amount of activity. Isolates were suppressed when using a concentration of 2.5 milligrams, and when using a concentration of 5 milligrams, all of the isolates were suppressed. The chloroform control agent was not successful in inhibiting the growth of the bacteria tested. MIC for *S. aureus* 2, *S. aureus* 3, *K. pneumoniae* 4, and *K. pneumoniae* 5 was determined to be 5 milligrams of concentration.

The GCMS Procedure

Only the ethanol extract of *Glycyrrhiza glabra* was chosen for the GCMS analysis because of its antibacterial activity and the phytochemicals that it contained. An examination using GC-MS showed that the extract of *Glycyrrhiza glabra* included a number of chemicals, which are listed in Table 3. The eugenol, vanillin, 1, 2, 4-triazole, and heptane were identified through the process of comparing the mass spectra of the constituents with those in the NIST library. Dodecane acid and octadecatrienoic acid were the fatty acid contents that were found, in addition to a few additional minor constituents.

Discussion

The utilization of plant extracts and phytochemicals, both of which are known to possess antibacterial characteristics, was of great relevance in the field of medicinal treatments. There have been a number of studies conducted in a variety of counties over the course of the past several years to demonstrate the effectiveness of this method. There have been numerous applications of plants because of the antibacterial properties that they possess. These properties are a result of substances that are produced by the plant's secondary metabolism. Within the scope of this investigation, we attempt to determine whether or not the solvent extract of *Glycyrrhiza glabra* possesses antibacterial properties against clinical isolates. In this study, 6 of were betalactamase and 8 of were biofilm positive isolates were observed.

Biofilm promotes bacterial persistence by resisting, antibiotic treatment and host immune responses. Biofilm causing isolates was very difficult to treat because highly resistant to antibiotics. It's a serious global threat and challenge to health care professionals. Additionally, the ethanol extract of *Glycyrrhiza glabra* exhibited inhibitory action against all of the isolates that were evaluated for its antibacterial properties. During the process of suppressing the four isolates, a concentration of 2.5 milligrams of plant extract was utilized. The results of this investigation showed that the ethanol extract of *Glycyrrhiza glabra* had a more powerful effect against *S. aureus* than it did against *K. pneumoniae. Glycyrrhiza glabra* fruit was analyzed using GC-MS chromatography, which was performed on the ethanolic extract of the fruit. the existence of phytochemical ingredients was indicated by the appearance of a number of peaks.

A comparison of the mass spectra of the constituents with those of the NIST library allowed for the identification and characterization of the phytocompounds that contribute to the therapeutic actions of the plant. Some of the compounds that were found to exhibit antimicrobial action were eugenol, vanillin, and silane compound. These three compounds were found to be the most prevalent among the compounds that were found.

Conclusion

In this scenario, the screening of plant extracts has been of considerable interest to scientists for the purpose of discovering new pharmaceuticals that are effective in the treatment of a number of ailments. Approximately twenty percent of the plants or their extracts that are found around the world have been subjected to either biological or pharmacological examinations. *Glycyrrhiza glabra* included a useful component that demonstrated antibacterial efficacy against clinical isolates that produced beta-lactamases. Because of this, it has the potential to be utilized as an antibacterial agent against a wide variety of microbes, surpassing the effectiveness of conventional antibiotics.

Table 1: Antibacterial activity of Ethanol extract of *Glycyrrhiza glabra*

S. No	Bacterial isolates	Con. of plant extract (mg) Zone of inhibition in mm				Ethanol	Ciproflaxcin
		2.5	5	7.5	10		
1.	*S.aureus 2*	11±1.6	12±0.81	15±1.6	17±1.6	-	-
2.	*S.aureus 3*	11±1.6	12±0.81	14v1.6	16±1.6	-	28
3.	*S.aureus 5*	12±1.6	14.2±1.6	16±1.6	24±0.51	-	32
4.	*K.pneumoniae*1	-	10±1.6	14±1.6	16±0.81	-	-
5.	*K.pneumoniae*5	13±0.81	15±0.16	18±1.6	21±0.81	-	-

Table 2: Antibacterial activity of chloroform extract of *Glycyrrhiza glabra*

S. No	Bacterial isolates	Con. of plant extract (mg) Zone of inhibition in mm				Ethanol	Ciproflaxcin
		2.5	5	7.5	10		
1.	*S.aureus 2*	11±1.6	12±1.6	14±1.6	15±1.6	-	-
2.	*S.aureus 3*	14±1.8	16±1.6	18±0.8	20±1.6	-	28
3.	*S.aureus 5*	-	12±1.6	13±0.8	16±1.6	-	32
4.	*K.pneumoniae* 4	12±1.2	14±1.6	16±1.6	18±1.8	-	-
5.	*K.pneumoniae* 5	10±1.6	11±1.6	13±0.8	15±1.6	-	-

Table 3: Identification of bioactive compounds from ethanol extract of *Glycyrrhiza glabra* by GCMS

S. No	Components name	Molecular formula	M. weight (g/mol)
1.	Eugenol	$C_{10}H_{12}O_2$	164.2
2.	Vanillin	$C_8H_8O_3$	52.15

3.	1, 2, 4-Triazole	$C_2H_3N_3$	69.0654
4.	Silane	SiH_4	32.12
5.	Dodecane	$C_{12}H_{26}$	170.33
6.	Octadecane	$C_{18}H_{38}$	254.494

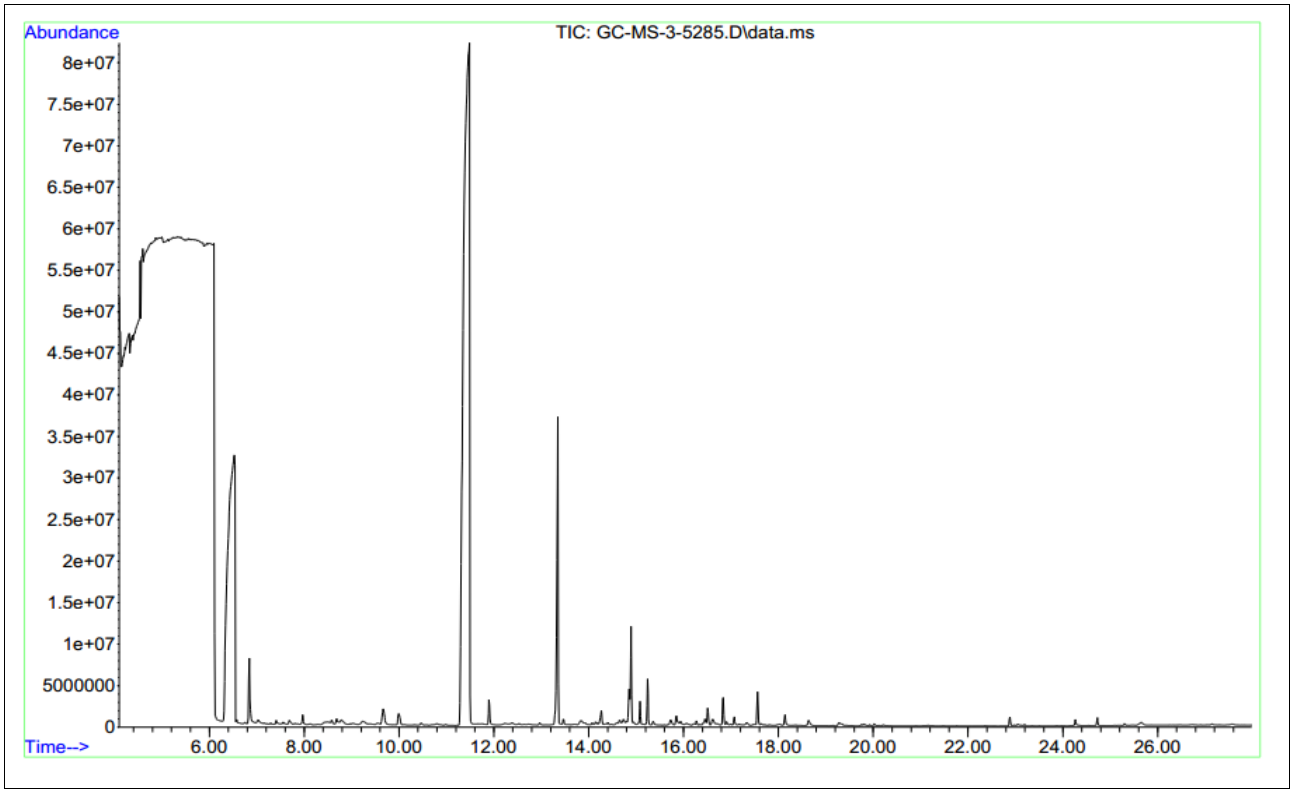

Fig 1: Spectrum analysis of *Glycyrrhiza glabra* extract

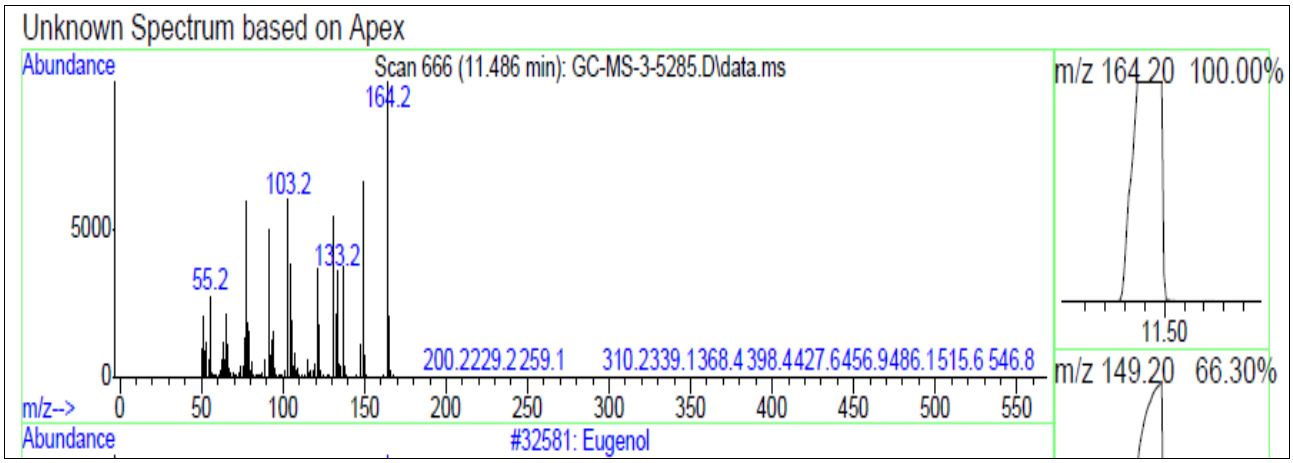

Fig 2: GCMS analysis of Eugenol

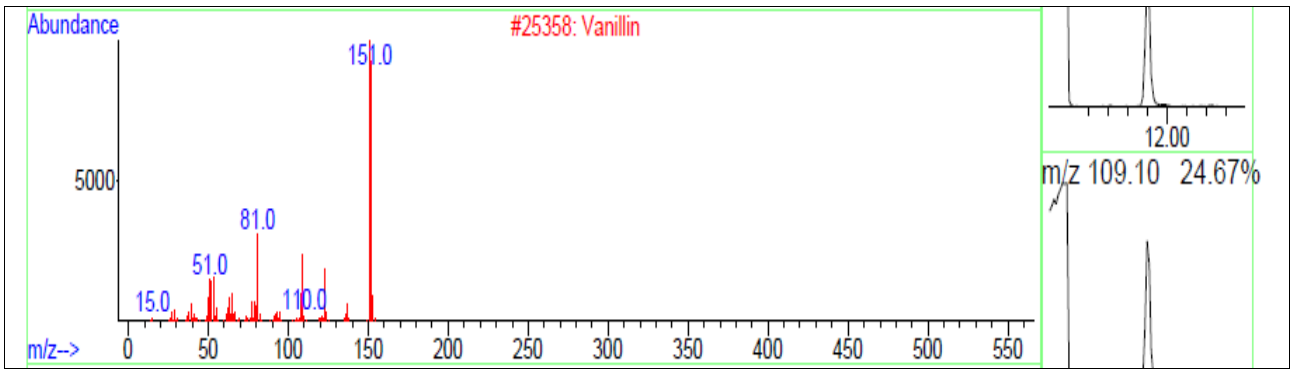

Fig 3: GCMS analysis Vanillin

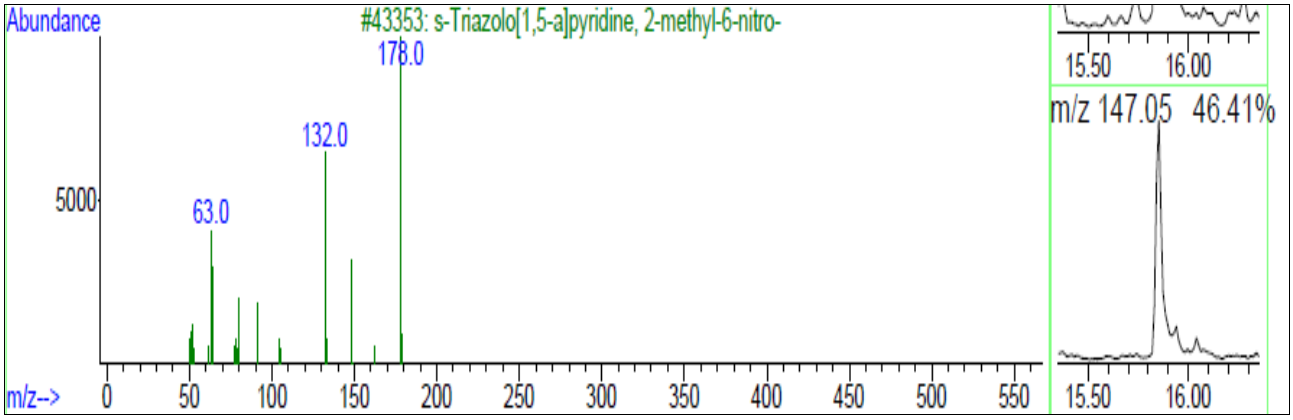

Fig 4: GCMS analysis of 1, 2, 4-Triazole

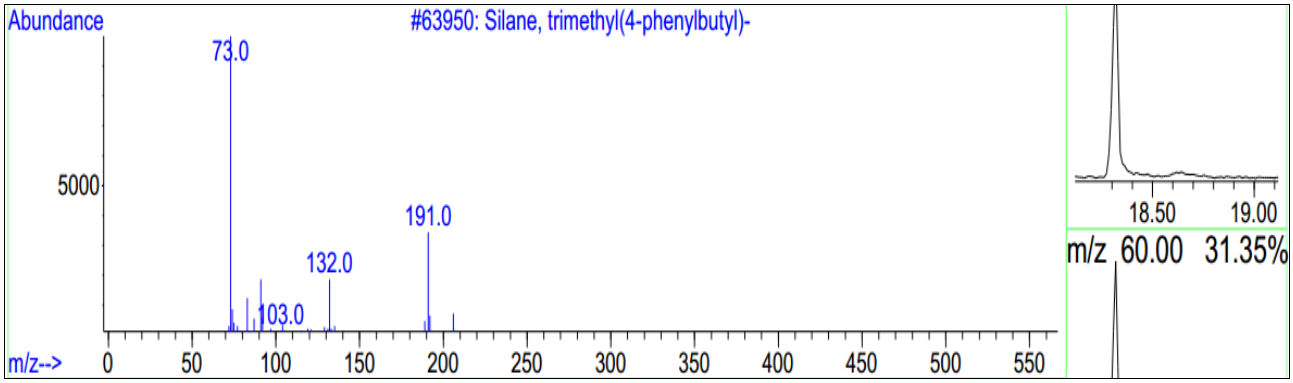

Fig 5: Silane

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