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# GC-MS profiling and assessment of antioxidant and antibacterial properties of oil extracts of sichuan pepper

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**Abstract**
Sichuan pepper is a popular food additive and traditional herbal medicine. It has a slight lemony flavor and causes a tingly numbness in the mouth. It is one of the few spices which is an integral part of the traditional Nepali, Northeast Indian, Chinese, Tibetan, and Bhutanese cuisines. The spice is believed to have many medicinal values like stimulates circulation, reduces pain, improves immunity, aids in appetite, protects the stomach, so forth. This study was aimed to evaluate the effectiveness of the Sichuan Pepper oil extract against *E. coli and Staphylococcus aureus*. Essential oil extraction from Sichuan Pepper was done by using the Clevenger Apparatus. The essential oil exhibited significant DPPH radical scavenging abilities with an inhibitory percentage of 90.01% using 80 µl/ml of the oil extract. The essential oil also exhibited significant antimicrobial activity. The zone of inhibition obtained was 14 mm and 26 mm for *S. aureus* in 100% and 50% extract, respectively, whereas for *E. coli*, it was 20mm and 30mm in 100% and 50% extract, respectively. The minimum inhibitory concentration (MIC) of the essential oil of Sichuan pepper against both the organisms was found to be at $10^{-3}$µl/ml. Identification of compounds by GC-MS led to the determination of the presence of Sulfoximine in the essential oils of Sichuan Pepper. Sulfoximine has been found to have anti-cancer activity; therefore, its presence in it can be a major path-breaking study. The antimicrobial properties of Sichuan pepper which have been used by people for centuries without any signs of toxicity can be used in the traditional herbal medicines, which play a very important role in primary care systems in the developing world and are becoming increasingly popular in the developed world.

**Keywords:** sichuan pepper, antioxidant, antimicrobial, gas chromatography-mass spectrometry (GC-MS), sulfoximine

## Introduction

The Sichuan pepper is a Chinese spice that comes from China's southwestern Sichuan province and is used in Sichuan cuisine. Bhutan Lemon Pepper is the name given to the wild species of this spice. Farmers in Bhutan's west collect the uncommon Bhutan Lemon Pepper, which delivers on its name with a powerful flavor and aroma, as well as a tingling sensation. Scientifically known as *Zanthoxylum armatum*, they are generally used as a popular spice in cooking and medicine, with a long history in China, Bhutan, and Japan for both medical and commercial purposes [1-2]. More than 140 chemical compounds have been isolated and identified from *Z. armatum* as a result of increased research, including alkaloids, terpenoids, flavonoids, free fatty acids, and a small number of inorganic elements [1-2]. In addition, recent research has indicated that *Z. armatum* possesses a wide range of pharmacological actions, including analgesic, anti-inflammatory, antifungal, and antibacterial effects, antioxidant and anti-tumor effects, digestive and circulatory system benefits, and other benefits [2]. *Z. armatum* pericarps are currently designated in the pharmacopoeia as an essential traditional Chinese medicine, and their essential oils are used to assess the quality of *Z. armatum* pericarps. Zanthoxylum plants are widely referred to as "toothache trees" in Western folk medicine due to an aesthetic or irritating characteristics, which make them effective in the relief of acute and chronic pain [2]. It is derived from the seeds of at least two tiny tree species belonging to the worldwide genus Zanthoxylum. Several cultivars of *Z. armatum* have been cultivated so far as part of the procedure. Because the pericarps of Z. armatum cultivars are vivid red, they are frequently referred to as "Honghua Jiao" in Chinese. Since 1977, *Z. armatum* has been listed in the People's Republic of China Pharmacopoeia, and over 30 prescriptions including *Z. armatum* have been used to treat stomach discomfort, toothache, dyspepsia, vomiting, diarrhea, ascariasis, eczema, and other conditions [2-3].

Though there has been tremendous research done yet, it has been unclear on the common people's part as to the effects of the essential oil of the spice.

Therefore, in order to investigate that, the experiment has been designed with the following objectives: i) To measure the antioxidant and antimicrobial activities of the essential oil of Sichuan pepper. ii) To find the presence of the active phytochemicals by the GC-MS analysis. Based on this result, the activity of the essential oils of this spice may be determined, and also, the compounds present in it may be defined.

The work will be thoroughly helpful for further studies to find out the relevant compounds responsible for the activities

## Material and Methods

### Sample Collection

Sichuan Pepper was collected from the Tsirang district in Bhutan in January 2018, and it was authenticated (Acc. No.18466 dated. 15.05.2018 Ref. No. Herb/ Bot./ GU/2018/79) at Gauhati University, Assam, India.

**Extraction**

The air-dried Sichuan Pepper was reduced to a fine powder (40 size meshes) at the laboratory of Assam downtown University, Guwahati, Assam. One hundred grams of the powder was subjected to microwave-assisted hydro distillation process (Clevenger apparatus) to extract the volatile oil.

**DPPH Radical Scavenging Assay**

Essential oil extract of Sichuan Pepper was tested for the scavenging effect on DPPH radical. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517nm. Ascorbic acid was used as a reference.

**Reagent preparation**

DPPH solution (0.1 M) was prepared by dissolving 0.39 mg of DPPH in a volumetric flask, dissolved in methanol, and the final volume was made 100 ml. Thus, prepared purple-coloured DPPH free radical solution was stored at −20°C for further use.

**Working procedure**

With DMSO, different volumes (2 – 20ml) of oil extract were prepared up to 40ml, and 2.96 ml DPPH (0.1 mM) solution was added. For 20 minutes, the reaction mixture was incubated at room temperature in the dark. The absorbance of the combination was measured at 517 nm after 20 minutes. As a control, 3ml of DPPH was used. The oil extract's percent radical scavenging activity was estimated using the following formula:

$$\%RSA = \frac{AbsControl - AbsSample}{AbsControl} \times 100$$

Where RSA is the Radical Scavenging Activity;
*Abs control* is the absorbance of DPPH radical + ethanol;
*Abs sample* is the absorbance of DPPH radical + oil extract.

**Antimicrobial Evaluation**

Bacteria selected to assess the antibacterial activities of the test samples were *Staphylococcus aureus* and *Escherichia coli*. The bacterial samples were obtained from the Down Town Hospital, Guwahati, Assam.

**Disc diffusion method**

The diameter of the inhibition zone generated around the well was measured to determine antibacterial activity[4]. The test microorganisms were infected using the spread plate method on Muller Hinton Agar plates. Filter paper discs with a diameter of 5mm were soaked in 100l, 200l, 500l, and 1000l of the plant extract and placed on the agar plates that had already been prepared. Each disc was pressed down to establish total contact with the agar surface and uniformly dispersed so that they were no more than 24 mm apart in the middle.

After that, the agar plates were incubated at 37 degrees Celsius. Each plate was evaluated after 16 to 18 hours of incubation. The outcome was a consistently round zone of inhibition with a confluent lawn of growth. The diameters of the zones of full inhibition, as well as the diameter of the disc where DMSO was employed as a control, were measured.

The minimum inhibitory concentration of the oil extract was determined against the bacterial sample using the standard protocol.

**GC-MS Analysis**

The essential oil of *Z. armatum* were analyzed by Gas Chromatography and Mass Spectrometry (GC-MS). Gas Chromatography and Mass Spectrometry [PerkinElmer Clarus680GC/600MS] system equipped with a quantitative analysis by SIM mode detector, Column 60.0m x 250µm and the 0.25µm film thickness were used. The oven was programmed from an initial temperature 70 ºC (hold for 3 min) to the final temperature 300 ºC at the rate of 10 minutes. The final temperature hold up time was 06 minutes. Helium at the rate of 1 ml/min was used as the carrier gas in constant flow mode.

(Oven: Initial temp 70°C for 3 min, ramp 6°C/min to 200°C, hold 3 min, ramp 6°C/min to 300°C, hold 10 min, Inj A auto=280°C, Volume=0 µL, Split=0:1, Carrier Gas=He, Solvent Delay=10.00 min, Transfer Temp=200°C, Source Temp=180°C, Scan: 40 to 600Da,). NIST version 2 library search was used for analysis

**Result and Discussions**

**DPPH radical scavenging activity**

In the present study, essential oil extract of Sichuan pepper has been found to be effective scavengers against DPPH radical. Oil extract at various concentrations was evaluated against DPPH using the standard Ascorbic acid. An inhibition of 90.01% was observed against DPPH when compared to Ascorbic acid, which showed inhibition of 93.06% at a concentration of 80µl/ml. The presence of polyphenols, flavonoids, and phenolic chemicals in various extracts of *Z. armatum* may be responsible for their radical scavenging activity, and phenols account for the majority of plant antioxidant activity [5].

These biological and pharmacological features are mainly linked to their ability to bind proteins and their ability to scavenge free radicals[5]. Antioxidants are extremely important chemicals that have the potential to protect the body from harm caused by oxidative stress generated by free radicals. Because their hydroxyl groups donate hydrogen, plant polyphenols act as reducing agents and antioxidants [5-6].

The methanolic DPPH solution is reduced due to the generation of nonradicals when antioxidant compounds containing hydrogen-donating groups, such as flavonoids and phenols, are present [7]. Apart from their antioxidant characteristics, flavonoids and other phenolics also have antibacterial, antiviral, and anticancer capabilities [8].

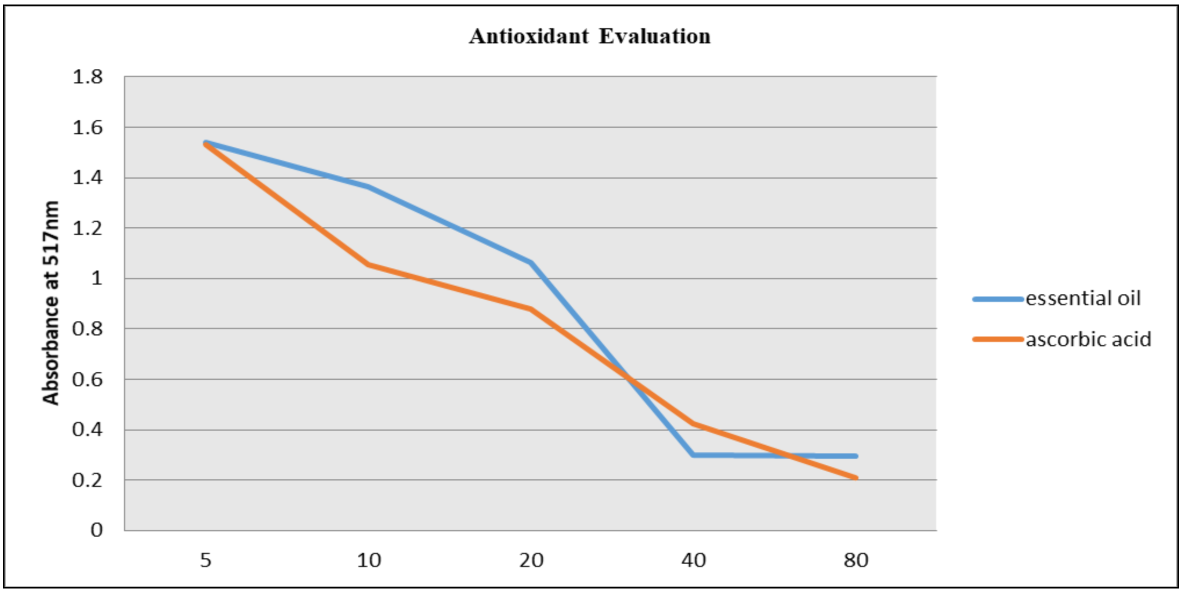


**Fig 1:** Graph showing the absorbance of essential oil and ascorbic acid at 517nm

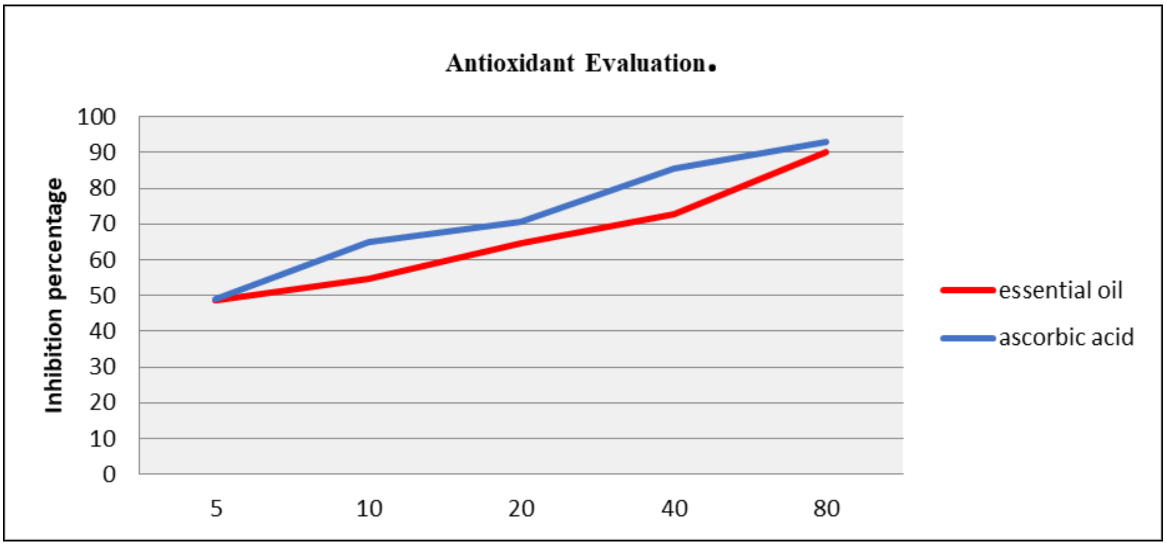


**Fig 2:** Graph showing the percentage inhibition of essential oil and ascorbic acid

**Antibacterial Activity**

In terms of the zone of inhibition produced by the tested sample against E. coli and S. aureus, our experiment demonstrates that essential oils derived from *Z. armatum* have good antibacterial activity, as shown in Table 1. The antibacterial activity of the essential oil of Sichuan pepper was determined. It was found that at a concentration of 100% the zone of inhibition was 14 mm and at 50%, it was 26 mm against *S. aureus*. Whereas for *E. coli* it was 20mm and 30mm in 100% and 50% extract respectively. The minimum inhibitory concentration (MIC) of the essential oil of Sichuan pepper was found to be at $10^{-3}$µl/ml. The reason why the inhibition was less in 100% oil extract was because of its volatile nature which evaporated the extract very fast ultimately leading to lesser inhibition compared to the 50% oil extract. The essential oils of *Z. armatum's* leaves and seeds have a high level of total antioxidant activity, as well as substantial antibacterial and antifungal activity [9-10]. The antibacterial activity of ethanol and methanol extracts of *Z. armatum* against *Escherichia coli* and *Enterococcus faecalis* is broad. As a result, it could aid in the development of new chemical classes of antibiotics or medications that might work as selecting agents for human health protection, as well as providing life instruments for the research of bacterial infections or infection [11-12].

**Table 1:** Antimicrobial evaluation of essential oil of Sichuan pepper

| Test organism | Media | Inhibition zone in mm | |
|---|---|---|---|
| | | Essential oil of Sichuan pepper | |
| | | 1000µl/ml (100%) | 500µl/ml (50%) |
| *Escherichia coli* | MHA | 20mm | 30mm |
| *Staphylococcus aureus* | MHA | 14mm | 26mm |

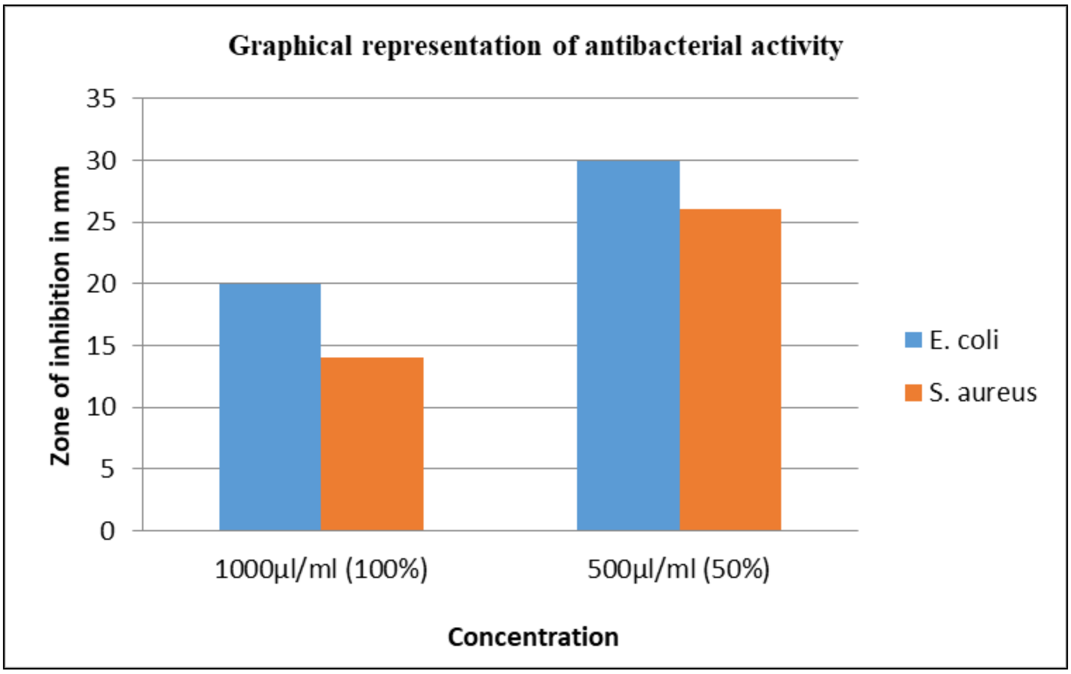


**Fig 3:** Graphical representation of the antibacterial activity

**GC-MS Analysis.**

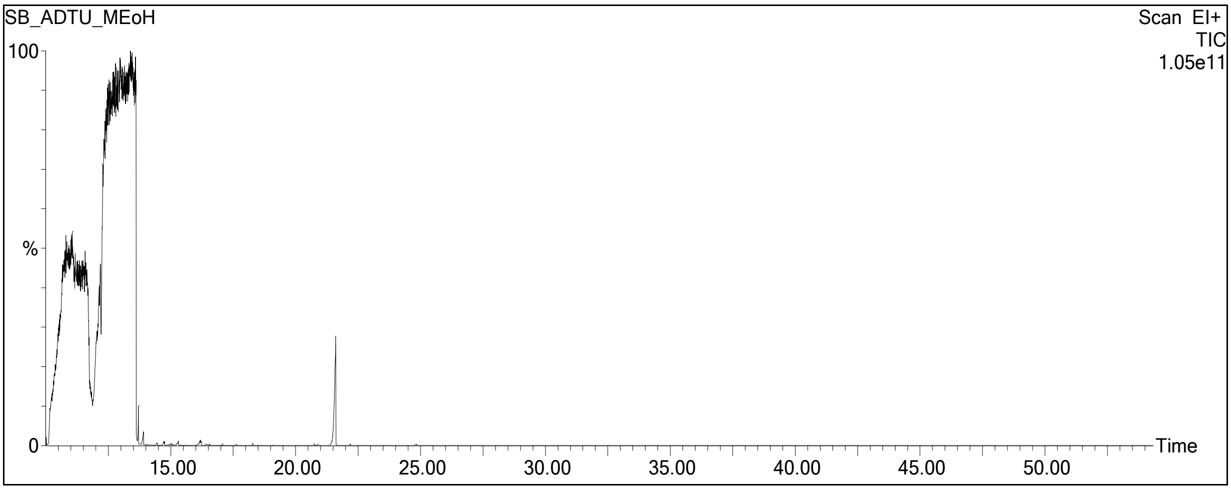


**Fig 4:** Chromatogram of essential oil of *Z. armatum*

A lot of work has been publicized on essential oils of family Rutaceae but little work on essential oils of this plant has been reported[13]. GC-MS of the oil extract shows the various peaks in Fig.4. GC-MS was performed using PerkinElmer Clarus 680GC/600MS. From the various peaks we selected four of them which showed the highest peak. Those were selected from retention time 12.96, 13.39, 13.59 and 24.84. In a previous study on the Components of Essential Oil of *Zanthoxylum armatum* by GC-MS it was found that Hydrocarbon fraction (17.35%) of the oil was much lower and oxygenated compounds comprised fairly high portion of essential oil (39.21%). Percentages of monoterpenes and sesquiterpenes found were 47.33% and 10.83% respectively [13].

In this study we found that compounds like Thiophene,2-ethyl-5-propyl, 1-Pentyn-3-ol,4-Methyl, 5-Methyl-2-hexene,c&t, 1-heptene, 2,6-dimethyl, oxirane, (1-methylbutyl), 1-pentene, 4-methyl, 1-pentene, 4-methyl, 1-pentene, 3,4-dimethyl, 4-pentenal, 2-methyl, 1-hexene, 2,5-dimethyl, 1-hexene , chloroacetic acid, hexyl ester, 5-methyl-1-hexyn-3-ol, 3-heptene, (e), 1-hexyne, 5-methyl, hexane, 1-chloro, hexane, 1-chloro, dimethallyl ether, 1,1-cyclopropanedicarbonitrile, 2-butyl-2-methyl-, presented in Fig 5.

**Table 2:** GC-MS chromatogram showing list of identified compounds.

| Hit | compound name | M.W | CAS | Formula |
|---|---|---|---|---|
| 1 | 4-methyl-1-phentyn-3-ol, | 96 | 565-68-4 | C6H10O |
| 2 | 5-methyl-2-hexene, c&t | 98 | 3404-62-4 | C7H14 |
| 3 | 1-heptene, 2,6-dimethyl- | 126 | 3074-78-0 | C9H18 |
| 4 | oxirane, (1-methylbutyl)- | 114 | 153229-39-3 | C7H14O |
| 5 | 1-pentene, 4 methyl- | 84 | 691-37-2 | C6H12 |

| | | | | |
|---|---|---|---|---|
| 6 | 1-pentene, 3,4-dimethyl- | 98 | 7385-78-6 | $C_7H_{14}$ |
| 7 | 4-pentenal, 2-methyl- | 98 | 5187-71-3 | $C_6H_{10}O$ |
| 8 | 1-hexene, 2,5-dimethyl- | 112 | 6975-92-4 | $C_8H_{16}$ |
| 9 | 1-hexene | 84 | 592-41-6 | $C_6H_{12}$ |
| 10 | chloroacetic acid, hexyl ester | 178 | 5927-57-1 | $C_8H_{15}ClO_2$ |
| 11 | 5-methyl-1-hexyn-3-ol | 112 | 61996-79-0 | $C_7H_{12}O$ |
| 12 | 3-heptene, €- | 98 | 14686-14-7 | $C_7H_{14}$ |
| 13 | 1-hexyne, 5-methyl- | 96 | 2203-80-7 | $C_7H_{12}$ |
| 14 | hexane, 1-chloro- | 120 | 544-10-5 | $C_6H_{13}Cl$ |
| 15 | dimethallyl ether | 126 | 628-56-8 | $C_8H_{14}O$ |
| 16 | 1,1-cyclopropanedicarbonitrile, 2-butyl-2-methyl- | 162 | 16738-89-9 | $C_{10}H_{14}N_2$ |

With much work coming in light regarding the composition of essential oil of *Z. armatum*, it is noted that the chemical compound presented in this work is completely different from the previous work [13-18]. This shows the compound has not been explored much which shows the promising future in discovery of antimicrobials.

## Conclusion

The Sichuan pepper has many medicinal properties and it has been seen curing certain diseases such as toothache, anti-inflammation, treatment of cough etc. Humans are the natural host of many bacterial species, which cause infections. *S. aureus* and *E. coli* are infrequent resident flora, but they are found for a wide variety of bacterial infection. The result of phytochemicals analysis for Alkaloid test, Carbohydrate test, Protein test, Glycoside test, Flavonoid’s test, Steroid’s test, Phenolic compound test shows positive result while Tannin test, Saponin test, terpenoids test shows negative result.

The antioxidant activity was performed by DPPH method. The IC50 value was found to be 4µg/ml for methanolic extract which was compared to control i.e., ascorbic acid 3µg/ml. The result for antimicrobial activity i.e., zone of inhibition, performed by well diffusion method on MHA Agar was found to be 4mm (500µg/ml), 10mm(1000µg/ml) and 3mm (500µg/ml), 8mm (1000µg/ml) for *Staphylococcus aureus* and *Escherichia coli* respectively which is comparatively less than volatile oil extract of same sample. GC-MS of the oil extract was performed using Perkin Elmer Clarus 680 GC/600MS. The study of various retention times was done and it was concluded that Sulfoximine may be the reason for antibacterial activity as per the literature review.

## Acknowledgements

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