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Screening of secondary metabolite products from *Salmonella typhimurium* Using GCMS and Evaluation of Its efficiency of Antibacterial Activity

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Abstract:- This research aims to analyze the active and biologically active biochemical products of Salmonella typhimurium bacteria and evaluate in vitro the antibacterial activities of the biologically active secondary metabolites. In practice, biochemical components known as bioactive substances, sometimes referred to as secondary metabolites, have been screened using GC-MS techniques. Next, the antibacterial activity of the methanolic extract of Salmonella typhimurium was experimentally evaluated in vitro. GC-MS analysis of Salmonella typhimurium revealed the presence of the following: 6-Aza-5,7,12,14-tetrathiapentacene, β-HIMACHALENOXIDE, 3-Ethyl-o-xilene, Trimethylphenylsilane, o-Mercaptoaniline, Cyclononasiloxane, octadecamethyl, 2, 6,10,14,18-Pentamethyl-2,6,10,14,18-Eicosapentaene, Methyl Stearate, Oxacyclotetradecane-2-One, 13-Methyl, L-Pyroglutamic Acid, Glycerol. Salmonella typhimurium metabolites also showed significant activity against Staphylococcus epidermidis (20.94 ± 0.29). .

Keyword:- Salmonella typhimurium, Secondary metabolites, Antibacterial, GC/MS.

Supplementary information The online version of this article (https://doi.org/xx.xxx/xxx.xx) contains supplementary material,which is available to autho-rized users.

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Introduction

Worldwide, Salmonella enterica is a leading cause of food poisoning, and among the many serovars of this bacterium, Salmonella typhimurium is among the most prevalent. Scientists and researchers have focused heavily on studying the interactions between S. Typhimurium and the animal host, particularly on the factors that enable the bacterium to directly penetrate the host's defences and manipulate infected host cells for its own benefit, because most human cases of disease are caused by foods of animal origin [1, 2]. We are quickly learning that virulence and metabolism in infected hosts are intimately related, but up until now, these studies have disregarded the crucial metabolic components that enable bacteria to proliferate in the host. For Salmonella typhimurium to grow in the intestine, cultured epithelial and macrophage-like cell lines, systemic sites during invasive salmonellosis, and long-term

asymptomatic colonisation of the host, certain metabolic factors are required. The majority of our knowledge is based on studies conducted in laboratory settings with small numbers of well-characterized strains; however, there is a lack of precision regarding the degree to which in vivo metabolism varies across serotypes [4, 5], and the majority of our results are derived from experiments performed on infected mice or rats. Verifying that our present, realistic understanding of host-parasite interactions regarding Salmonella metabolism is accurate for all serotypes and possible hosts is both important and crucial [6]. Food poisoning, which can affect both humans and animals, is directly caused by Salmonella typhimurium. In this context, it is important to remember that the infection incidence is substantially increased for every Salmonella serotype [7]. By analysing a wide variety of tiny molecular components, metabolomics offers a plausible and sensible approach to studying the pathophysiology of Salmonella typhimurium. Scientists tested five popular extraction methods—cold methanol (CM), chloroform-methanol cocktail (CMC), hot ethanol (HE), and perchloric acid (PCA)—because no universally accepted practical methodology exists for producing these metabolites.[8] The same holds true for alkaloids (AL) because of their unique and well-known ability to remove compounds from inside S. typhimurium cells. Due to the lack of initial gastrointestinal symptoms and the impossibility of effectively isolating the bacteria from stool samples, the patient may experience the septic form of Salmonella infection during the intermediate stage of the infection [9]. Whether the infection stays contained in the intestines and doesn't spread or goes straight to the circulation relies on the patient's resistance as well as the virulence and severity of the Salmonella strain. One possible antecedent to enteric fever symptoms is gastroenteritis, which typically resolves on its own before systemic illness manifests locally [10]. A wide variety of nonspecific symptoms, such as fever, myalgia, lack of appetite, headache, and constipation, can accompany enteric fever. Because enteric fevers are serious illnesses that can be deadly if not treated promptly, antibiotics must be administered without delay [11]. Salmonellosis poses a significant threat to public health due to the vast and varied animal populations. The purpose of this study is to examine the biological antibacterial activities of the secondary metabolites produced by the Salmonella typhimurium bacterium and to assess their in vitro biological activity.

Materials and Methods

Metabolite creation and identification in a perfect environment

Regularly, Salmonella typhimurium was grown aerobically at 37°C in Luria-Bertani (LB) medium. Agar spiked with xylose-lysine-deoxycholate (XLD) from Beckton Dickinson Difco and left to incubate at 37°C overnight allowed the purity of the Salmonella colonies to be confirmed. Our sample only included red colonies with black centres.

The bioactive natural chemical components of Salmonella typhimurium were studied spectroscopically using gas chromatography–mass spectrometry.

Analogue Devices 789 The GC-MS analysis was carried out using a specific instrument. A DB-5MS column manufactured by Folsom, California's J&W Scientific was utilised for the gas chromatography. The following were the measurements of this column: 30 mm with an inner diameter of 0.25 mm and a film thickness of 0.25 mm. The oven temperature was maintained constant in this trial as it was in the previous one. The helium carrier gas was introduced at a flow rate of 1 mL/min. Shortly after the gas chromatography (GC) column's effluent was heated to 250 degrees Celsius, it was transferred into the mass spectrometer's (MS) source via a transfer line [12]. To achieve ionisation, the ion source was maintained at 230 °C and a voltage of 70 eV was used.

Comparison of the antimicrobial activity of a secondary metabolite with seven different harmful microorganisms

The agar was prepared by boring five-millimeter-diameter holes with a sterilised cork-borer. Next, the wells were supplemented with 25 µl of the sample solutions that included the metabolites that Salmonella typhimurium produces. Bacillus cereus, Staphylococcus subtilis, Staphylococcus epidermidis, Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcus pyogenes, and Staphylococcus aureus were the test pathogens that were obtained using swabs and then placed onto plates of Muller Hinton agar. The control solvent was methanol.

Statistical analysis

In order to analyse the data obtained from an SPSS (Version 11.6) database, several statistical techniques were employed, including calculating the mean value and doing an analysis of variance (ANOVA).

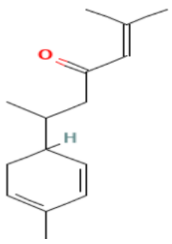
Results and Discussion

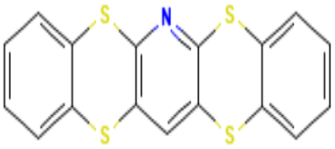
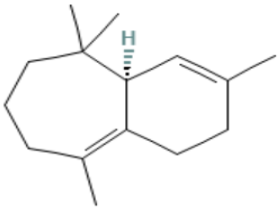
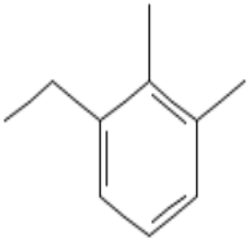
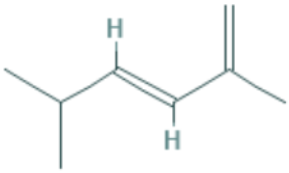
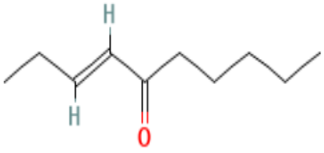
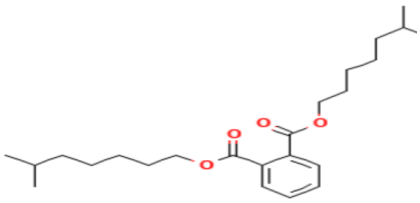
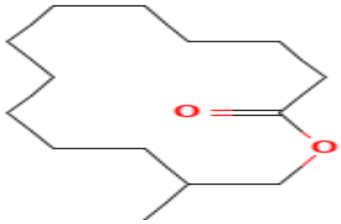
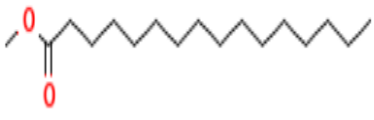
The fact that salmonella can infect 2.5 million individuals annually is indicative of how rapidly this disease is really spreading across the globe [13]. Salmonella germs are real sources of illness in both humans and animals everywhere. Although most cases of salmonellosis in humans resolve on their own, the infection can sometimes cause a severe infection known as enteric fever, which necessitates the use of antibiotics administered quickly. Livestock animals suffer substantial and ethically devastating losses when infected with salmonella. There were over eleven peaks in the GC-MS chromatogram that corresponded to the compounds that were identified. The compounds mentioned are 6-Aza-5,7,12,14-tetrathiapentacene, β-HIMACHALENOXIDE, 3-Ethyl-o-xylene, Trimethylphenylsilane, o-Mercaptoaniline, Cyclononasiloxane, octadecamethyl, 2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaen, Methyl stearate, Oxacyclotetradecan-2-one, 13-methyl, L-Pyroglutamic acid, glycerol.

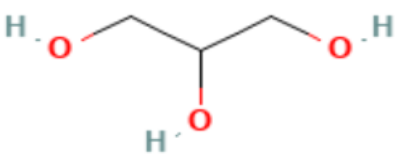
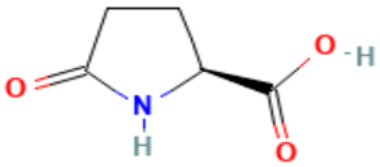
Glycerol consists of three hydroxyl groups attached to the propane atoms at positions 1, 2, and 3 in its triple bond structure. In addition to its functions as an osmolyte, solvent, detergent, geroprotector, and metabolite in humans [14], it is also found in algae, Saccharomyces cerevisiae, Escherichia coli, and mice. A triol and an alditol, that is.

Secondary metabolites generated by Salmonella typhimurium were tested for their antimicrobial effectiveness against seven different pathogenic pathogens. This investigation looked at the bioactivity of seven different pathogens using the methanolic Salmonella typhimurium extract and two common antibiotics, Amikacin and Bacteracin : *Streptococcus pyogenes* (15.73±0.25, 13.25±0.21, and 08.84± 0.19), *Pseudomonas aeruginosa* (13.71±0.21, 14.42±0.22, and 12.33±0.20), *Enterococcus faecalis* (09.57±0.19, 15.26±0.23, and 12.37±0.20). *Bacillus cereus* (10.65±0.20, 12.38±0.20, and 16.12±0.25), *Bacillus subtilis* (09.77±0.19, 07.90±0.16, and 12.14±0.20), *Staphylococcus epidermidis* (18.12±0.27, 14.62±0.22, and 20.94±0.29), *Staphylococcus aureus* (15.71±0.23, 12.98±0.20, and 20.80±0.29). There was a notable effect of Salmonella typhimurium metabolites against Staphylococcus epidermidis (20.94±0.29).

The current research set out to compare and uncover biochemical patterns in Salmonella Typhimurium using a GC-MS metabolomics technique. Metabolome analysis to clarify metabolic differences between sessile development and biofilms has received scant attention up until recently. Although MS is quite sensitive, it is not as quantitative as other methods. Metabolome changes are unavoidable due to the use of chromatography to separate metabolites. Therefore, a more complete metabolite profile should be obtained by combining NMR- and MS-based analytical methods rather than using each technique independently. Diverse bacterial species have developed resistance to antibiotics by evolving to use other molecules as survival mechanisms, such as enzymes or receptors [15, 16]. The cells keep alive and keep synthesising peptidoglycan, which means that blocking other active sites in the resistance could be an alternative method. In order to combat drug resistance in both Gram-positive and Gram-negative bacterial species, it is crucial to identify various secondary metabolites for their ability to inhibit drug-metabolizing enzymes [17]. This research will greatly contribute to the development of effective combinations of phytochemicals and antibiotics.

Compound	**Structure**	**Molecular Formula**	**Molecular Weight**
6-Aza-5,7,12,14-tetrathiapentacene		$C_{17}H_9NS_4$	355.5 g/mol

β-HIMACHALENOXIDE		$C_{15}H_{24}$	204.35 g/mol
3-Ethyl-o-xylene		$C_{10}H_{14}$	134.22 g/mol
Trimethylphenylsilane		$C_9H_{14}Si$	150.29 g/mol
o-Mercaptoaniline		C_6H_7NS	125.19 g/mol
Cyclononasiloxane, octadecamethyl		$C_{18}H_{54}O_9Si_9$	667.4 g/mol
2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene		$C_{25}H_{42}$	342.6 g/mol
Methyl stearate		$C_{19}H_{38}O_2$	298.5 g/mol
Oxacyclotetradecan-2-one, 13-methyl		$C_{14}H_{26}O_2$	226.35 g/mol

glycerol		$C_3H_8O_3$	92.09 g/mol
L-Pyroglutamic acid		$C_5H_7NO_3$	129.11 g/mol

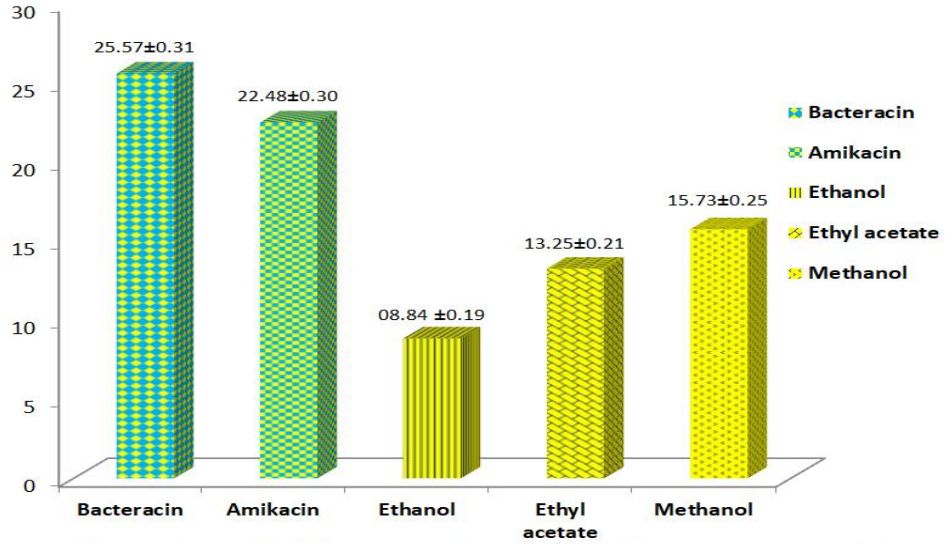


Figure 1. Zone of inhibition (mm) of various bioactive chemical compounds derived from *Salmonella typhimurium* against *Streptococcus pyogenes*

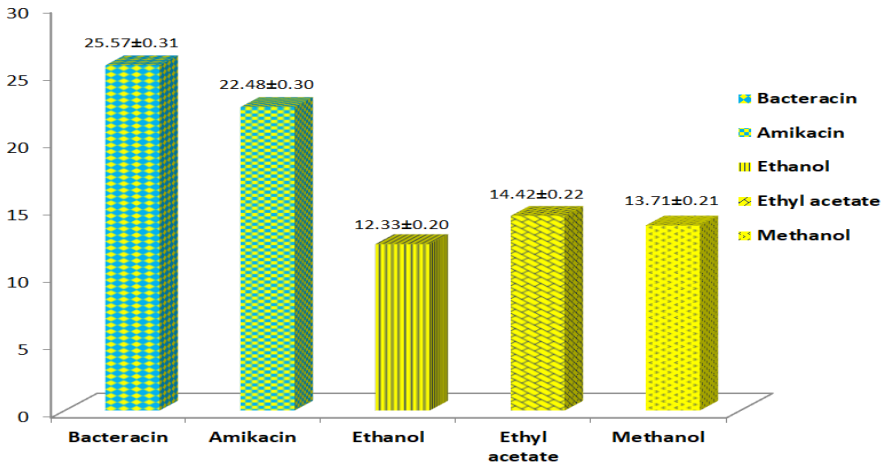


Figure 2. Zone of inhibition (mm) of various bioactive chemical compounds derived from *Salmonella typhimurium* against *Pseudomonas aeruginosa*

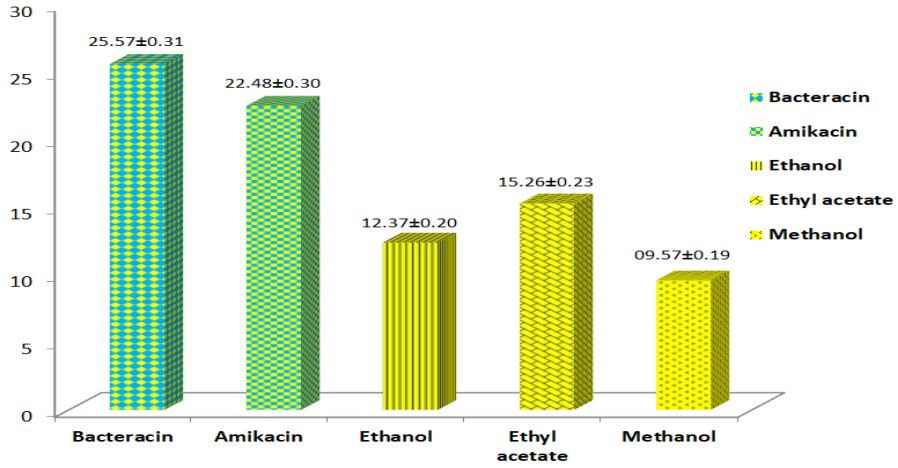


Figure 3. Zone of inhibition (mm) of various bioactive chemical compounds derived from *Salmonella typhimurium* against *Enterococcus faecalis*

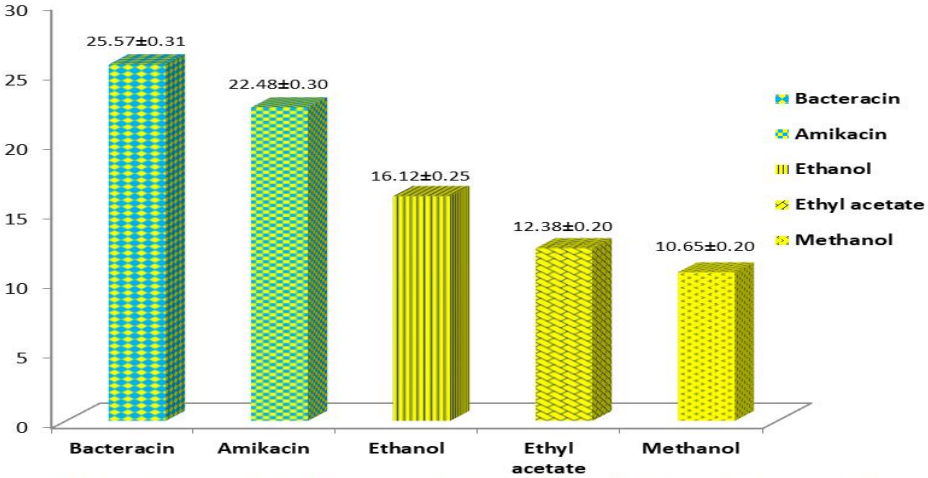


Figure 4. Zone of inhibition (mm) of various bioactive chemical compounds derived from *Salmonella typhimurium* against *Bacillus cereus*

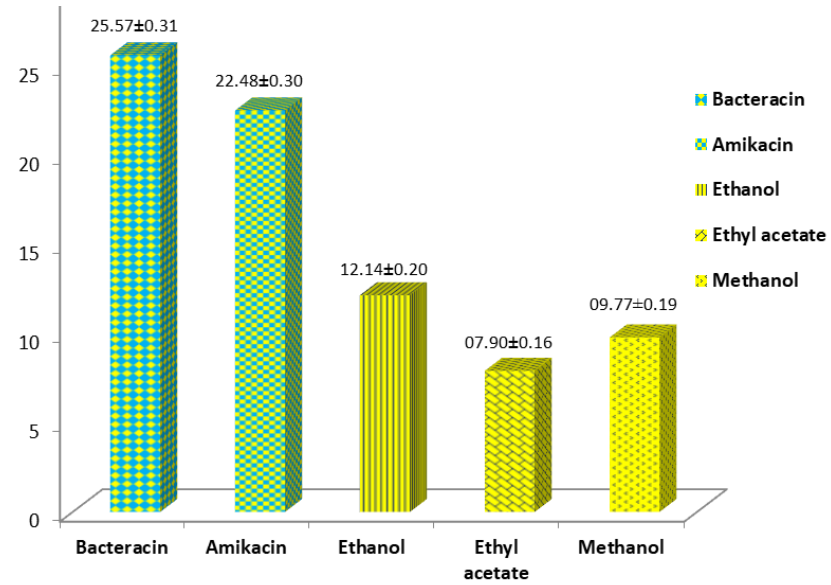


Figure 5. Zone of inhibition (mm) of various bioactive chemical compounds derived from *Salmonella typhimurium* against *Bacillus subtilis*

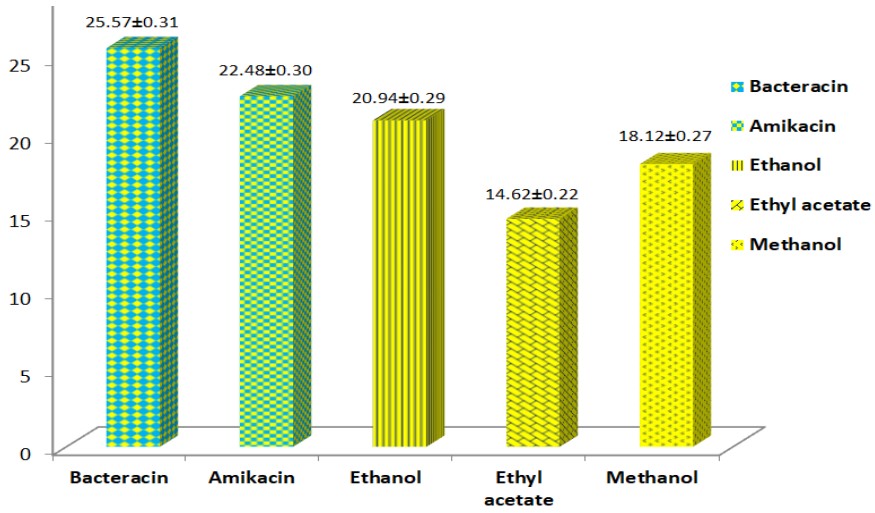


Figure 6. Zone of inhibition (mm) of various bioactive chemical compounds derived from *Salmonella typhimurium* against *Staphylococcus epidermidis*

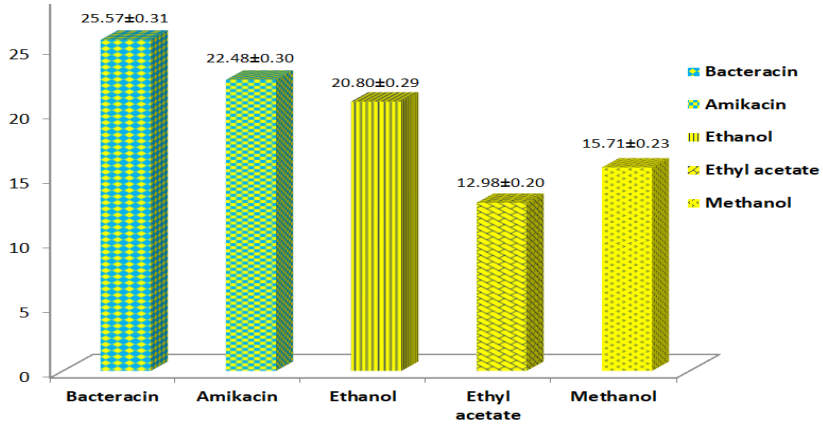


Figure 7. Zone of inhibition (mm) of various bioactive chemical compounds derived from *Salmonella typhimurium* against *Staphylococcus aureus*

Conclusion

The bioactive secondary metabolites were discovered to be effective against a wide variety of bacteria, either alone or in conjunction with traditional antibiotics. In addition, it has been observed that secondary metabolites have the ability to work in tandem with other antibiotics, enhancing their effectiveness against different types of bacteria, including multidrug-resistant ones. Since this component has received little attention, it is recommended that all potential implications of medication interactions involving secondary metabolites be determined. Finally, the investigations all point to secondary metabolites' potential importance in future medication development, either as an alternative therapy or in conjunction with traditional antibacterial medicines.

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