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GC-MS ANALYSIS AND BIOLOGICAL ACTIVITY OF CYCLIC COMPOUNDS OF DICHLOROMETHANE EXTRACT FROM *SENECIO HOGGARIENSIS*

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ABSTRACT
In the present work, the qualitative phytochemical study of extract dichloromethane of the aerial parts from *Senecio hoggariensis* (*Asteraceae*) was investigated by chromatographic analysis (GC/MS) and the evaluation of biological activity of this extract by four test of antioxidant activity and the antibacterial activity was studied by diffusion in the solid medium against five Gram positive and negative bacteria, but before this evaluation The Total Phenolic Content (TPC) was evaluated according to the Folin-Ciocaltue assay and gallic acid used as standard. The Flavonoids contents (TFC) were evaluated by using the Aluminum chloride method. The GC-MS analysis in within the limits of the experimental conditions applied, by interpreting the mass spectra obtained and identifying them by the procedure of comparison with mass spectra for the reference compounds, we have proposed ten formulas for different chemical compounds, most of them are cyclic compounds, in particular: ketones, alcohols, esters, ethers and carboxylic acids. From the results of the antioxidant activity we notice that the total phenolic content is much greater than the total content of flavonoid and this is logical because flavonoids are one of the most important main classes of phenolic compounds, and it also indicates that extract contain other phenolic compounds with different chemical structures from the structure of flavonoids, such as: phenolic acids..etc. The extract showed a powerful antioxidant activity. While the results of the antibacterial activity showed that the dichloromethane extract had a weak sensitivity against B. subtilis type. While he did not show any sensitivity to the rest of the bacterial species.

KEY WORDS : *Senecio hoggariensis*, cyclic compounds, extract dichloromethane, GC-MS analysis, antioxidant activity & antibacterial activity

INTRODUCTION

The genus *Senecio* L. (Compositae, Senecioneae) contains about 1250 kinds and is one of the major genera of flowering plants[i, ii]. It is almost universal, but it is widespread in Mediterranean climatic zones, for example, South Africa, Chile, and the Mediterranean basin. There are a few in Australia and Central America. It is absent in the West Indies[ii]. Some types of this kind are used in traditional medicine for its therapeutic properties[iii, iv] and its beneficial effects in the treatment of infections (inflammation of the intestine, liver), stomach pain, cough, eczema, and heal the wounds[v]. In Africa, *Seneciohoggariensis* is distributed in Chad, Egypt, and Niger. In Algeria, it is found widespread in the region of Hoggar and Tassili[vi]. Previous phytochemical investigations of *S. hoggariensis*revealed the separation of flavonoids of the type of flavone (Quercetine-3-glucoside), as well as a few traces of flavonols (isorhamnetin-3-rutinoside), (isorhamnetin-3-monosulphate)[vii]. From the standpoint of biological action, various parts of plants have been declared for their fundamental biological actions e.g., antioxidants, cytotoxicity[viii], antidiabetic[ix], and antibacterial[x]. Free radicals are atoms or particles that have one or more electrons in their outer orbit and react very quickly, which leads to the destruction of many cells in the human body. Therefore, the antioxidant is produced to reduce the activity of these roots, but the oxidative stress that results in several diseases increases the production of free radicals, which requires the support of external antioxidants[xi]. Certain medicinal plants that have proven to have antioxidant activity, because of their richness in biologically active compounds, are the solution to surmount the lack of antioxidant defense produced by the body[xii]. The appearance of numerous diseases resistant to various present-day antibiotics has led to problems in the global health system. From this perspective, scientists began to search for novel antibiotics, such as natural products from plant and animal sources[xiii]. In light of this, we conducted this research which aims to determine the components of the dichloromethane extraction of *S. hoggariensis* using a gas chromatography-mass spectrometric (GC-MS) analysis technique and test its antioxidant capacity with the determination of its content of phenolic and flavonoid, as well as to evaluate its antibacterial effectiveness.

MATERIALS AND METHODS

Chemical materials and reagents

Gallicacid, sodium carbonate, Folin-Ciocalteu reagent, quercetin, aluminum chloride, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ammonium persulphate, ABTS, phosphate buffer, potassium ferricyanide, trichloroacetic acid (TCA), ferric chloride, ammonium molybdate, ascorbic acid (VC), BHA and all solvents were obtained from Sigma-Aldrich and Biochem. Each chemicals and solvents used in the research were analytical grades.

Plant sample collecting

The aerial part of *S.hoggariensis* was harvested from the city of Tamanrasset, 2000 km south of Algeria, in late May 2017. The plant was classified by Prof. Dr. Ammar Eidoud, professor at the Faculty of Natural and Life Sciences, KasdiMerbah University of Ouargla.

Preparation of dichloromethane extract

After drying and grinding the aerial part of the *S.hoggariensis* plant, 100 g of the resulting powder was immersed in a mixture of EtOH/H_2O (70/30: v/v) for 24 hours at laboratory temperature three times in a row. Hereafter filtration, the solvent was evaporated on a rotary evaporator and a crude extract was got. The latter has been subjected to a liquid-liquid

extraction process using various organic solvents, such as petroleum ether, dichloromethane, ethyl acetate, and butanol, respectively.

Separation by gas chromatography - mass spectrometry

We analyzed the dichloromethane extract of *S. hoggariensis* plant using a gas chromatographer (Clarus600T) connected to a mass spectrometer (RTx-5ms) device with a length of 30 m and an inner diameter of 0.25 mm and covered with a thin metallic coating of 0.25 µm thickness, where the flow velocity of the carrier gas (helium) for the column is 1ml/min, the injected quantity is 1µl in split mode, the injection temperature is 300°C. The oven temperature was programmed from 270°C to 45°C. The mass spectrometry had the following characteristics: ionization potential 70eV, ionization source temperature 280°C.

Determination of the total phenoliccontent

The total phenolic content of the dichloromethane extract based on the Folin-Ciocalteu method according to the gallic acid reference curve[xiv],where we took 0.1ml extract and added 0.5ml of diluted Folin-Ciocalteau reagent (10%) and after 5 minutes, we add to the mixture 2ml of sodium carbonate (20%) and leave it for half an hour in the dark At room temperature, we measure the absorbance at 760nm using a UV-visible spectrophotometer. We also applied the same method to gallic acid as a reference, with the experiment repeated three times for each sample[xv]. The obtained results were expressed in milligrams of gallic acid equivalent per gram of dry weight of the extract[xiv].

Determination of the total flavonoid content

The flavonoid content of the dichloromethane extract was determined by chromatographic method of aluminum chloride ($AlCl_3$) and using quercetin as a reference flavonoids[xvi], where We took 1.5ml of each extract and added 1.5ml of aluminum chloride dissolved in ethanol (2%). We keep the previous mixture for 30 minutes in the dark and at room temperature, then measure the absorbance at wavelength 430nm by a UV-visible spectrophotometer.

We also applied the same experimental steps to quercetin, repeating the experiment three times for each sample[xvii]. The obtained results were expressed in milligrams of quercetin equivalent per gram of dry weight of the extract[xvi].

Biological potency test

Study of antioxidant activity

After the extraction process that we conducted on the studied plant, we obtained the four organic phases mentioned previously, where we took the dichloromethane extract and estimated its antioxidant activity by applying four chemical methods : DPPH test, ABTS test, FRAP test and Molybdate phosphate test (PM).

DPPH test

DPPH is a stable free radical with a violet color that changes to yellow after reduction either by gaining hydrogen or an electron from the antioxidant to become a stable molecule[xviii-xx].

We tested the ability of dichloromethane extracts to capture DPPH root by following the method of Benaissa et al. [xxi], with some modifications. We took 1.5 ml of the sample and added to it 1.5 ml of DPPH (0.003%) dissolved in ethanol, we shaken the mixture and kept it in the dark and at room temperature for half an hour, then we read the absorbance by a UV-visible spectrometer at the wavelength nm 517 with the test repeated three times.

We also estimated the efficacy of VC and BHA as reference controls in the same way as the studied extract.
To calculate the percentage of DPPH root inhibition, we use the following relationship[xxii]:

$$I\% = (\frac{A_{control} - A_{sample}}{A_{control}})x100$$

whereas:
I%: percentage of root inhibition of DPPH
$A_{control}$: absorbance in the absence of tested samples.
A_{sample}: absorbance in the presence of the tested samples.

ABTS test
This test is based on the ability of antioxidants to inhibit the cationic radical $ABTS^{+}$ which is blue-green in color resulting from the oxidation of ABTS with other compounds such as potassium phosphate (KH_2PO_4)[xxiii].
The inhibitory ability of the dichloromethane extract to inhibit the cationic radical $ABTS^{\cdot+}$ was determined according to the method adopted by Vidyalakshmi et al[xxiv]. We prepared the cationic radical $ABTS^{\cdot+}$ first by mixing 1mM of $(NH_4)_2S_2O_8$ with 2.5mM of ABTS dissolve them in phosphate-buffered saline (100mM phosphate buffer, pH=7.4, containing 150mM NaCl). The resulting mixture was placed in a water bath at a temperature of 68°C for half an hour. After heating, a concentrated solution of blue-green color containing ABTS radicals was obtained, which was diluted with phosphate-buffered saline until an absorbance reading of 0.65±0.02 at 734nm was obtained.
We took 20µl of each concentration and added to it 980µl of the blue-green solution, kept the resulting mixture in the dark and in a water bath at a temperature of 37°C for 10 minutes, then read the absorbance by a UV-visible spectrometer at the wavelength 734nm with the experiment being repeated twice for each sample. We also estimated the efficacy of VC and BHA as reference controls in the same way as the extract was treated.
The percentage of ABTS root inhibition is calculated according to the relationship used in the DPPH test.

FRAP test
The iron reactivity test is used to measure the antioxidant activity of the antioxidants returned (electron donor) in the tested sample as a direct, simple, fast and inexpensive method, as it works to return Fe^{+3} toFe^{+2}[xxv-xxvii].
We studied the iron reductive capacity by dichloromethane extract of *S. hoggariensis* according to the method of Keshaw et al[xxviii], where we took 1ml of the sample and added to it 2.5ml of phosphate buffer solution (0.2M, pH=6.6) and 2.5ml of $K_3Fe(CN)_6$ solution (1%), we put the resulting mixture in a water bath at 50°C for 20 minutes, and in order to stop the reaction, we add 2.5ml of TCA (10%) solution to it, then take 2.5ml of the resulting mixture and add 2.5ml to it of distilled water and 0.5ml of $FeCl_3$ solution (0.1%) and then we read the absorbance by UV-visible spectrometer at wavelength 700nm with the test repeated three times.
We also tested the reversibility of BHA as a reference control in the same way as before, and used VC as a standard.
The reductive capacity of the extract was expressed by calculating the amount of AEAC, which represents the equivalent antioxidant capacity of ascorbic acid according to the following relationship:

$$AEAC = \frac{K}{K'}$$

whereas:

K: is the slope of the extracted curve.

K': slope of the standard curve for VC.

Molybdate phosphate test (PM)

The antioxidant activity is determined using the phosphomolybdenum method, where this test is based on the reduction of Mo(VI) to Mo(V) by antioxidant compounds and the formation of a green complex (Phosphate/Mo(V)) in an acidic medium[xxix, xxx].

The antioxidant capacity of the studied extract was evaluated according to the method adopted by Rohan et al[xxxi], where we took 0.3ml of the sample and added 3ml of molybdate reagent to it (0.6mM of sulfur acid, 28mM of sodium phosphate and 4mM of molybdate ammonium) and then incubated the resulting mixture in a water bath at a temperature of 95°C for 90 minutes. After the incubation period has elapsed, we cool the tested samples to room temperature and then measure the absorbance by UV-Vis spectrometer at wavelength 695nm with the test repeated three times.

We also tested the reversibility of BHA as a reference control in the same way as before, and used VC as a standard.

The reflux capacity of molybdate was also determined by the dichloromethane extract by calculating the AEAC value applied in the FRAP test.

Study of antibacterial activity

The antibacterial activity of the dichloromethane extract was estimated according to the method of diffusion in a gelatinous medium, which is used to test the sensitivity of the bacterial strain to antibiotics[xxxii].

We conducted this study of dichloromethane extract on 5 reference bacterial strains (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Enterococcus faecalis*, *Bacillus subtilis*) obtained from the pedagogical laboratory of the Faculty of Nature and Life Sciences at Kasdi Merbah University – Ouargla.

In this experiment, we prepared a series of dilute concentrations using DMSO solvent from the mother concentration of dichloromethane extract, which is estimated at 200mg/ml.We have also prepared a medium suitable for the growth of all bacterial strains by melting Muller Hinton medium and pouring it into Petri dishes with a thickness of 3mm near the Bunsen burner, leaving it until it hardens and then keeping it in the oven until use.

To prepare the suspension, a colony or colonies of activated bacteria were taken for 24 hours and placed in a test tube containing 10ml of sterile physiological water, then shaken the tube well to obtain a homogeneous suspension used after 15 minutes of preparation in the culture of Petri dishes. After the previously prepared Petri dishes had dried, we dipped the sterile cotton swab in the bacterial suspension and wiped the entire gelatinous surface.

We cut small discs with a diameter of 6mm from filter paper (Whatman3) and put them in the oven at a temperature of 120°C for 20 minutes in order to sterilize them, after that we saturate the discs with 10µl solutions of increasing concentration of the extract and place them on the gel surface in a circular motion while maintaining at regular intervals between the discs, the plates are then placed upside down in the oven at a temperature of 37°C for 24 hours, then we read the results by measuring the diameter of the damping circle using the canal tube with the test repeated 3 times.

RESULTS AND DISCUSSION

Separation by gas chromatography - mass spectrometry

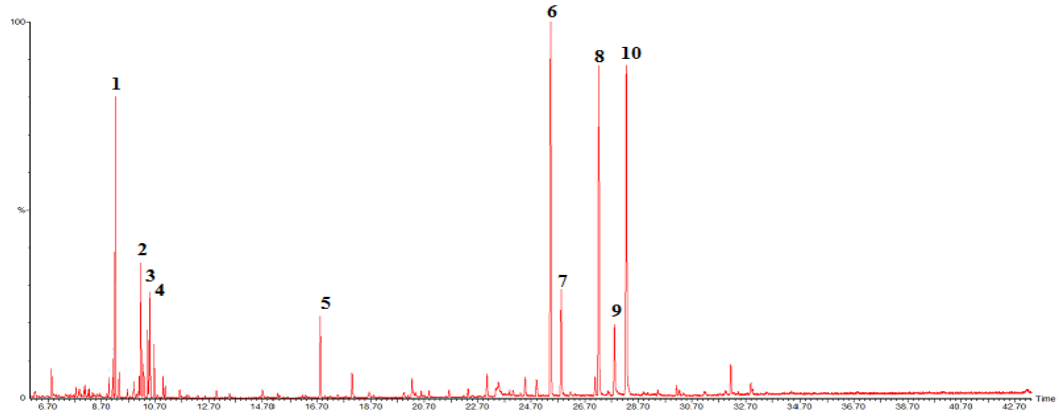
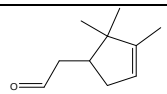
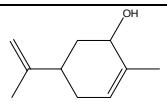
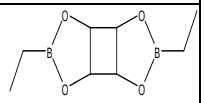
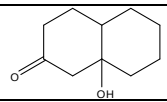
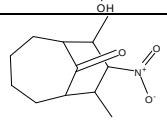
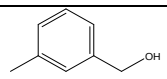
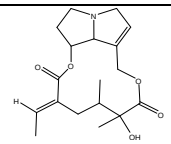
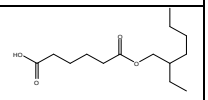
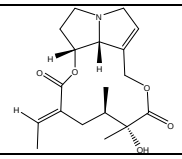


Fig 1: Chromatogram obtained from the GC-MS with the dichlomethane extract of *S. Hoggariensis*

Table 1: Chromatographic separation (GC-MS) results of the dichloromethane extract of *S. hoggariensis.*

N°	Tr (min)	**Scientific name** and molecular formula	**Masse spectra of reference compound**	**Masse spectra of compound**	**Chemical structure**
1	**9.219**	2-(2,2,3-trimethylcyclopent-3-en-1-yl)acet Aldehyde ($C_{10}H_{16}O$)	111(Pb),71,126 95,93,152(Pm)	111(Pb),71,126 95,93,152(Pm)	
2	**10.17**	2-methyl-5-prop-1-en-2-ylcyclohex-2-en-1-ol	71(Pb),111,100 119,93,91,152(Pm)	71(Pb),111,100 119,93,91,152(Pm)	
3	**10.43**	4,9-diethyl-3,5,8,10-tetraoxa-4,9-diboratricyclo[5.3.0.02,6] decane	98(Pb),70,69, 126 111,55, 168	98(Pb),70,69,126 111,55, 168	
4	**10.51**	bicyclo[4,4,0]decan-1-ol-3-one	98(Pb),70,69, 126 111,55, 168(Pm)	98(Pb),70,69, 126 111,55,168(Pm)	
5	**16.82**	7,9-dimethyl-8-nitrobicyclo [4,3,1]decan-10-one	111(Pb),178,57 109,140,107	111(Pb),178,81,10 9 140, 107	
6	**25.42**	3,5,1,7-[1,2,3,4]butane tetraylnaphtale ne-1,6(2h)-dicarboxylicacid	231(Pb),91,246,77 213,67	231(Pb),91,246,77 213,67	/
7	**25.81**	m-tolylmethanol	69(Pb),91,107,123 185,81,122(Pm)	69(Pb),91,107,123 185,81,122(Pm)	

8	**27.21**	Sencionine	136(Pb),120,94,119 138,121,80,335(Pm)	136(Pb),120,94,119 138,121,80,335(Pm)	
9	**27.80**	Mono(2-Ethylhexyl) Adipate	129(Pb),57,56,70,71 112	129(Pb),57,56,70,71 112	
10	**28.24**	Intergerrimine	119(Pb),118,93,136 94,95,121,335(Pm)	119(Pb),118,93,136 94,95,121,335(Pm)	

Through the results of the analytical study by gas chromatography connected by mass spectrometry of Dichloromethane extract listed in the table, it was found that there are 10 chemical compounds; nine of them are cyclic and one is aliphatic. By interpreting the mass spectra obtained and comparing it with the mass spectra of the reference materials, we suggest the possibility of the presence of chemical compounds classified into 5 groups, which are ketones, alcohols, Esters, ethers and carboxylic acids and the most abundant ones (represented in the largest proportions) are :
2-(2,2,3-trimethylcyclo pent-3-en-1-yl)acetaldehyde, 2-methyl-5-prop-1-en-2-ylcyclohex-2-en-1-ol4,9-diethyl-3,5,8,10-tetraoxa-4,9- diboratricyclo[5.3.0.02,6]decane, bicyclo[4,4,0]decan-1-ol-3-one,7,9-dimethyl-8- nitrobicyclo[4,3,1]decan-10-one, 3,5,1,7-[1,2,3,4]butane tetraylnaphtale ne-1,6(2h)-dicarboxylic acid, m-tolylmethanol, Sencionine, Mono(2-Ethylhexyl) Adipate, Intergerrimine.
Another study on a plant of the same genus showed the presence of 8 compounds in the aqueous extract of the leaves of the brasiliensisplant, it belongs to two chemical groups, alkaloids and ketones, the most important of which are: Intergerrimine, (4aR-8aS)-octahydroisoquinolin-3(2H)-one, Sencionine.Senecio is one of the most important pyrrolizidine alkaloids in plants of the genus (SenecionineIntegerrimine). The two compounds are heterocyclic organic compounds, mostly produced by plants as defense chemicals. against herbivores[xvi]. this is alkaloids possess mutagenic, teratogenic, carcinogenic and embryotoxic properties and therefore pose a serious threat to human health. Despite all this, there are still some plant species of the genus in use in traditional medicine.

Determination of the total phenolic and flavonoidcontent

Estimate the total phenol content in the dichloromethane extract is (28.40mg GAE/g Ex) While the total flavonoids content is (28.43 mg GAE/g Ex), By comparing the two results, we notice that The total phenolic content is much greater than the total content of flavonoid and this is logical because flavonoids are one of the most important main classes of phenolic compounds, and it also indicates that extracts contain other phenolic compounds with different chemical structures from the structure of flavonoids, such as: phenolic acids, tannins, Elk, Marinette, etc.

Biological potency test

Study of antioxidant activity

The antioxidant activity in the test DPPH and the test ABTS is determined by calculating the value of IC_{50}; whenever the lower value of IC_{50} agree the greater antioxidant activity.The antioxidant activity of the dichloromethane extract in the test DPPH was estimated to be 0.288 mg/ml much lower than the antioxidant activity of reference componds BHA and VC,

which estimed : 0.0068mg/ml and 0.0074mg/ml respectively.Same result for the test ABTS where we recorded the following values : DCM (0.879 mg/ml), BHA (0.035 mg/ml),VC (0.053 mg/ml).

As for the estimation of the antioxidant activity by the FRAP test, we note that for the extract and the reference substance, the reducing power ($AEAC$) increases with increasing concentrations ; where it was estimated in the dichloromethane extract of 216 mMgreater than that recorded in the reference substance and which was estimated of 213 Mm, while the test molybdate phosphate test (PM)we registered the following values : DCM (1.693 Mm), BHA (3.458 mM); it is clear that the antioxidant activity of the dichloromethane extract less than reference substance for this test.

Study of antibacterial activity

The results of measuring the antibacterial activity of S. hoggariensis antibiotics of the extracted phases of the plant against five bacterial strains (GM), gentamicin (AN), amikacin (CH), chloramphenicol (CIP), (ciprofloxacin) They are shown in the following table(2):

Table 2: results of tests for the antibacterial activity of dichloromethane extract of plant *S. hoggariensis*

Bacterialstrains	**Diameter of the inhibition circle**				
	12.5 mg/ml	**25 mg/ml**	**50 mg/ml**	**100 mg/ml**	**200 mg/ml**
***Enterococcus faecalis* (WDCH 0009) (Gram (+))**	-	-	7±0	7±0	7±0
***Pseudomonas aeruginosa* (ATCC9027) (Gram (-))**	-	-	-	-	-
***Staphylococcus aureus* (ATCC43300) (Gram (+))**	-	-	-	-	7.166±0.288
***Salmonella typhimurium* (ATCC14028) (Gram (-))**	-	-	-	-	7±0.5
***Bacillus subtilis* (ATCC6633) (Gram (+))**	-	-	-	8.25±4.168	8.833±0.288

(-) …….resistance

Through the results recorded in the table 1, we note that the effectiveness of the extract dichloromethane was evident on the bacteria strain *B. subtilis*. Where their sensitivity was weak and the diameter of the inhibition circle reached 8.25mm and 8.833mm at the two concentrations 100mg/ml and 200mg/ml respectively, while it did not show any sensitivity towards the rest of the concentrations, as for the four strains: the other, which are : *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus, and Salmonella typhimurium* She did not show any sensitivity to the dichloromethane extract.

CONFLICT OF INTEREST

No, conflict of interest among all authors

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CONCLUSION

The phytochemical constituent of dichloromethane extract of *S. hoggariensis*(ariel part) was identified by gas chromatography-mass spectroscopy (GC-MS) techniques. Qualitative analysis of the plant sample revealed the presence 5 groups, which are ketones, alcohols, esters, ethers and carboxylic acids and the most abundant ones (represented in the largest proportions) are :

2-(2,2,3-trimethylcyclo pent-3-en-1-yl)acetaldehyde, 2-methyl-5-prop-1-en-2-ylcyclohex-2-en-1-ol4,9-diethyl-3,5,8,10-tetraoxa-4,9- diboratricyclo[5.3.0.02,6]decane, bicyclo[4,4,0]decan-1-ol-3-one,7,9-dimethyl-8- nitrobicyclo[4,3,1]decan-10-one, 3,5,1,7-[1,2,3,4]butane tetraylnaphtale ne-1,6(2h)-dicarboxylic acid, m-tolylmethanol, Sencionine, Mono(2-Ethylhexyl) Adipate and Intergerrimine in general, nine cyclic compounds and one aliphatic compound.

Regarding the biological activity, it was found that dichloromethane extract has weak antioxidant activity, likewise for antibacterial activity his sensitivity was weak against the bacteria *Bacillus subtilis* while it did not show any sensitivity towards against the four strains: *Enterococcus faecalis, Pseudomonas aeruginosa*, *Staphylococcus aureus, and Salmonella typhimurium*

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