International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 23s, 2025
https://www.theaspd.com/ijes.php

GC-MS Profiling And Anti-Microbicidal Activities Of Urtica Dioica L. And Xanthium Strumarium L. Extract

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Abstract
Urtica dioica L., a perennial herb found in South Asia, is utilised for the treatment of many ailments owing to its antioxidant and antibacterial characteristics. Xanthium strumarium L., originating from North America, Europe, and Asia, possesses anti-inflammatory, antioxidant, and antibacterial characteristics. Furthermore, it has been examined for its potential in cancer therapy, and certain substances have demonstrated cytotoxic properties. Additional research is required in order to comprehensively comprehend their therapeutic uses. The objective of this work was to identify 23 metabolites with distinct chemical properties from the aqueous extracts obtained from the leaves of Urtica dioica. The microbicidal activity of AEUD and AEXS was assessed against gram positive Staphylococcus aureus (A) and gram negative Escherichia coli (B), demonstrating noteworthy efficacy. The results demonstrated that both extracts shown substantial microbicidal activity for these causative strains. AEED was known to be much efficacious than AEAXS in combating the microorganisms in both extracts. The results indicate that AEAD and ACEXS may serve as viable substitutes for synthetic antibiotics in the management of microbial illnesses. Additional research is required in order to comprehensively comprehend their therapeutic uses.
Keywords: *GC-MS, Urtica dioica L, Xanthium strumarium L, Staphylococcus aureus and Escherichia coli*

1. INTRODUCTION

Urtica dioica L., commonly referred as "stinging nettle," is a long lasting herb that is native to South Asian countries and Indian subcontinent. This is extensively employed for the treatment of conditions such as nephritic syndrome, haematuria like condition, liver diseases causing jaundice, menses irregularities like menorrhagia, joint condition like arthritis, and diseases like rheumatism. These plant possesses useful chemical substances such as flavonoids, phytosterols, tannins, saponins, proteins, and few amino acids. The compound exhibits a range of pharmacological properties, such as antibacterial, antioxidant, analgesic, anti-inflammatory, antiviral, immunomodulatory, hepatoprotective, anti-colitis, and anticancer actions. [1,2]

Xanthium strumarium L., also called cocklebur, an indigenous species of plant kingdom found in North America, Asia and Europe. For millennia, traditional medicine has utilised it because of its anti-inflammatory, antioxidant, and antibacterial characteristics. These chemicals exhibit efficacy in the management of health illnesses like asthma, arthritis and skin infections. Furthermore, research has been conducted to explore the possible application of this substance in cancer therapy, and certain molecules have demonstrated cytotoxic properties against cancerous cells. Additional investigation is required to comprehensively comprehend its mechanisms of action and possible therapeutic uses.[3, 4]

In present study, the microbicidal activity was assessed by using disc diffusion agar and performing Kirby-Bauer method on it, a widely recognized methodology in the field of microbiology. This technique entails placing paper discs saturated with antibiotics onto a bacterial lawn on an agar plate. The antibiotics permeate the agar, generating a concentration gradient that hinders the development of bacteria. The zone of inhibition is determined using the diameter of clear zone surrounding each disc is subsequently measured and then assess the efficacy of the antibiotic against the microorganisms being tested.[5,6]

2. METHODOLOGY

2.1 Causative bacteria

Gram-negative Escherichia coli (MTCC-452) and gram-positive Staphylococcus aureus (MTCC-96), are virulent bacteria used for this study. Microbes cultures taken from Department of Microbiology, Aakar Biotechnology , Lucknow (U.P.).

2.2 Preparation of extract

Initially, 100 g of pulverized drug were placed in a Soxhlet apparatus and defatted using 250 ml of Petroleum ether (40-60°C) in a Soxhlet apparatus. The extraction process continued until the solvent in the siphon was clear. To ensure complete defatting, a drop of the extracted material was tested on filter paper; the absence of an oily spot confirmed that defatting was complete. Following defatting, the extract was filtered, and the solvent was removed using a rotary vacuum evaporator at 50°C. The concentrated extract was then obtained under reduced pressure with a rotary evaporator to produce ethanolic extracts of Xanthium strumarium and Urtica dioica.[7]

2.3 Metabolic profiling of extract of Urtica dioica L. and Xanthium strumarium L. by GC-MS analysis

A sample was taken and then suspended in a solution of methoxylamine hydrochloride and GC-grade pyridine, then agitated at 37°C for 2 hours prior to the addition of 70 µl of MSTFA. The lipid content was evaluated via GC-MS employing a Thermo Trace GC Ultra in conjunction with Thermo Fisher DSQ II mass spectrometers. Metabolites were chromatographically separated using a 30 m x 0.25 mm Thermo TR50 column. The data was processed by using Xcalibur software. The gas chromatography oven temperature was sustained at 70°C for 5 minutes, thereafter elevated to 290°C. The sample was injected in split mode with helium as a carrier gas. The mass selective detector operated in electron impact mode with an electron energy of 70 eV. The GC-MS profile was examined with Replib, and NIST mass spectrum library, correlating the chromatogram with commercially accessible standards. A mass spectral deconvolution algorithm (Automated Mass Spectral Deconvolution and Identification System, AMDIS32) was used for processing multiple data sets [8]. The metabolite concentrations were determined based on the percentage of peak area.

Analytical Condition

Table 2.1 Instrument - GC-2010

Column Oven Temp.	120.0 °C
Injection Temp.	260.00 °C
Injection Mode	Split
Injection Volume	2 µl
Flow Control Mode	Linear Velocity
Pressure	99.3 kPa
Total Flow	16.3 mL/min
Column Flow	1.21 mL/min
Linear Velocity	41.3 cm/sec
Purge Flow	3.0 mL/min
Split Ratio	10.0
High Pressure Injection	OFF
Carrier Gas Saver	OFF
Carrier Gas	Helium
Splitter Hold	OFF
Size of GC-Vial	2 ml
Size of Capillary Column	Length- 30 meter and Inner diameter – 0.25 mm

Table 2.2 Oven Temperature Prog

Rate	Temperature (°C)	Hold Time (min)
-	120.0	2.00
10.00	300.0	20.00

Table 2.3 GC Prog (GCMS-QP2010 Ultra)

Ion Source Temp	220.00 °C
Interface Temp.	270.00 °C
Solvent Cut Time	3.50 min
Detector Gain Mode	Relative
Detector Gain	+0.00 kV
Threshold	1000

Table 2.4 MS Table

Start Time	4.30 min
End Time	39.98 min
ACQ Mode	Scan
Event Time	0.20sec
Scan Speed	3333
Start m/z	40.00
End m/z	650.00

2.4 Preparation of Discs

Discs of filter paper with a diameter of 6mm are made and autoclaved in a sterile and dry Petri dish.They are soaked in plant extracts for 6 hours, then shade dried. The concentrations are recorded as 0.1 grams per disc. The discs are then spread on cultured Petri plates, and those immersed in Butanol, Benzene, and distilled water are used as controls.[9]

2.5 Anti-microbial activity assay

The microbicidal activity tested by using this Kirby-Bauer technique. This method involves placing paper discs impregnated with antibiotics onto a lawn culture of bacteria on plate with agar. The antibiotics spreads into this agar, maintain a gradient of concentration that inhibits bacterial growth. The size of this zone of clearance called inhibition zone surrounding each disc is then used to know the effectiveness of the antibiotic against the tested bacteria. MHA plates were inoculated with Bacterial cultures of Escherichia coli and Staphylococcus aureus, and discs containing different concentrations were placed. 10% of the sample was diluted to achieve the required amount. A vehicle control was loaded with solvent alone, while a Ciprofloxacin disc (10µg) was used as a positive control. The plates were incubated at 37°C for 24 hours, and clear zones around the discs were measured and recorded.[10]

3. RESULTS ANALYSIS AND CONCLUSION

3.1 Metabolic profiling of extract of Urtica dioica L. and Xanthium strumarium L. by GC-MS analysis

Twenty three chemically different variety of metabolites comprises of fatty acids, sterols, phenolic compounds, terpenes, steroid derivatives, and α-tocopherol identified from extract of Urtica dioica L. (Table 1; Figure 1) while twenty seven chemically diverse metabolites Xanthium strumarium L. (Table 2; Figure 2) using GC-MS. Mass spectra obtained at 70 eV, with a scanning period of 0.5 seconds, and fragments vary in size from 45 to

450 Da. The eluted component is identified in the mass spectrometer. The spectra of the unidentified constituent is analyzed against the spectra of known components in the NIST collection, resulting in the identification of its name and molecular weight.

3.2 Effect of extract of Urtica dioica L. and Xanthium strumarium L. on zone inhibition assay for Anti-microbial activity

In this experiment, different concentrations of both extract were tested for a number of microbial strains which includes gram positive Staphylococcus aureus and gram negative Escherichia coli. The results showed that both Urtica dioica L. extract and Xanthium strumarium L. extract exhibited significant microbicidal activity against (A) S. aureus and (B) E.coli (Figure 3 and 4). Also, zone inhibition effect of different concentrations of extract Urtica dioica against (A) S. aureus and (B) E.coli (Figure 5 and 6). The findings suggest that Urtica dioica and Xanthium strumarium extract have that potential so can be used like natural alternatives in place of synthetic antibiotics for the treatment of microbial infections.

4. CONCLUSIONS

The study made use of gas chromatography-mass spectrometry (GC-MS) to find out 23 metabolites with distinct chemical characteristics from Urtica dioica L. and Xanthium strumarium L. The eluted component was identified using the mass detector and compared it with the spectrum of known components contained in NIST library. The microbicidal activity of Urtica dioica and Xanthium strumarium extract was assessed for Staphylococcus aureus and Escherichia coli, demonstrating noteworthy efficacy. Urtica dioica extract demonstrated more efficacy than Xanthium strumarium extract against the bacterial strains in both extracts. These findings indicate that Urtica dioica extract and Xanthium strumarium extract have the potential to serve as natural substitutes for synthetic antibiotics in the treatment of microbiological diseases.

5. REFERENCES

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Table 1: Metabolic profiling of Aqueous extract of leaves of Urtica dioica L. (AEUD) by GC-MS analysis

Peak	R. Time	Area	Area%	Name
1	13.588	140718	1.52	11,14-Eicosadienoic acid
2	14.998	314291	3.39	1,2-Benzenedicarboxylic Acid, Diethyl Ester
3	15.880	49847	0.54	1-(4-Isopropylphenyl)-2-Methylpropyl Aceta

4	16.107	98473	1.06	2-Penten-1-Ol, 5-(2,3-Dimethyltricyclo[2.2.1.0(
5	18.563	336085	3.63	Hexadecanoic acid, methyl ester
6	20.197	1347442	14.54	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
7	20.253	1377899	14.87	9-Octadecenoic acid, methyl ester, (E)-
8	20.315	100081	1.08	Cyclopropanenonanoic acid, 2-[(2-butylcyclopropyl)methyl
9	20.487	244743	2.64	Methyl stearate
10	21.064	120243	1.30	Methyl 10-trans,12-cis-octadecadienoate
11	21.610	73942	0.80	Palmitoyl chloride
12	22.013	48324	0.52	Myristic acid glycidyl ester
13	22.897	148571	1.60	1-cis-Vaccenoylglycerol
14	23.098	155415	1.68	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester
15	23.452	981151	10.59	Petroselinic acid, TMS derivative
16	24.815	1266642	13.67	1-Cyclohexyldimethylsilyloxybutane
17	24.997	338926	3.66	Petroselinic acid, TMS derivative
18	28.156	154110	1.66	Stigmast-5-en-3-ol, oleate
19	29.464	211865	2.29	4h-1-Benzopyran-4-One, 2-(3,4-Dihydroxyphen
20	30.534	1290984	13.93	Methyl 8-(2-Octanoylcyclopropyl)Octanoa
21	30.932	192281	2.07	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)
22	33.629	132132	1.43	Tris(2,4-di-tert-butylphenyl) phosphate
23	35.052	143154	1.54	1-Naphthalenepropanol, .Alpha.-Ethenylde

.

Table 2: Metabolic profiling of Aqueous extract of leaves of Xanthium strumarium L. (AEXS) by GC-MS analysis

Peak	R. Time	Area	Area%	Name
1	6.494	57981	0.68	Dodecane
2	10.360	46578	0.55	Di-trimethylsilyl peroxide
3	13.592	127293	1.50	1,9-Nonanediol
4	14.945	55526	0.65	1,2-Benzenedicarboxylic Acid, Diethyl Ester
5	14.997	368772	4.33	1,2-Benzenedicarboxylic Acid, Diethyl Ester
6	15.880	57294	0.67	1-(4-Isopropylphenyl)-2-Methylpropyl Aceta
7	17.785	142587	1.68	10(E),12(Z)-Conjugated linoleic acid
8	17.854	138770	1.63	Oleyl alcohol, trifluoroacetate

9	18.479	178009	2.09	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
10	18.562	410159	4.82	Hexadecanoic acid, methyl ester
11	19.021	160330	1.88	Hexadecanoic Acid
12	20.196	1246286	14.64	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
13	20.254	1288485	15.14	9-Octadecenoic acid, methyl ester, (E)-
14	20.311	104773	1.23	6-Octadecenoic acid, methyl ester, (Z)-
15	20.486	237395	2.79	Methyl stearate
16	21.061	120742	1.42	Methyl 10-trans,12-cis-octadecadienoate
17	22.012	79460	0.93	Myristic acid glycidyl ester
18	23.060	111660	1.31	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl este
19	23.098	128844	1.51	Oleoyl chloride
20	23.452	771680	9.07	Octadecanoic Acid, 3-Oxo-, Methyl Ester
21	23.683	38766	0.46	Glycidyl palmitate
22	24.795	850412	9.99	25-Hydroxycholesterol, 2TMS derivative
23	24.997	521428	6.13	Glycerol monostearate, 2TMS derivative
24	28.152	135749	1.60	Stigmast-5-en-3-ol, oleate
25	30.548	714084	8.39	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-
26	30.927	150885	1.77	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)
27	33.622	266072	3.13	Tris(2,4-di-tert-butylphenyl) phosphate

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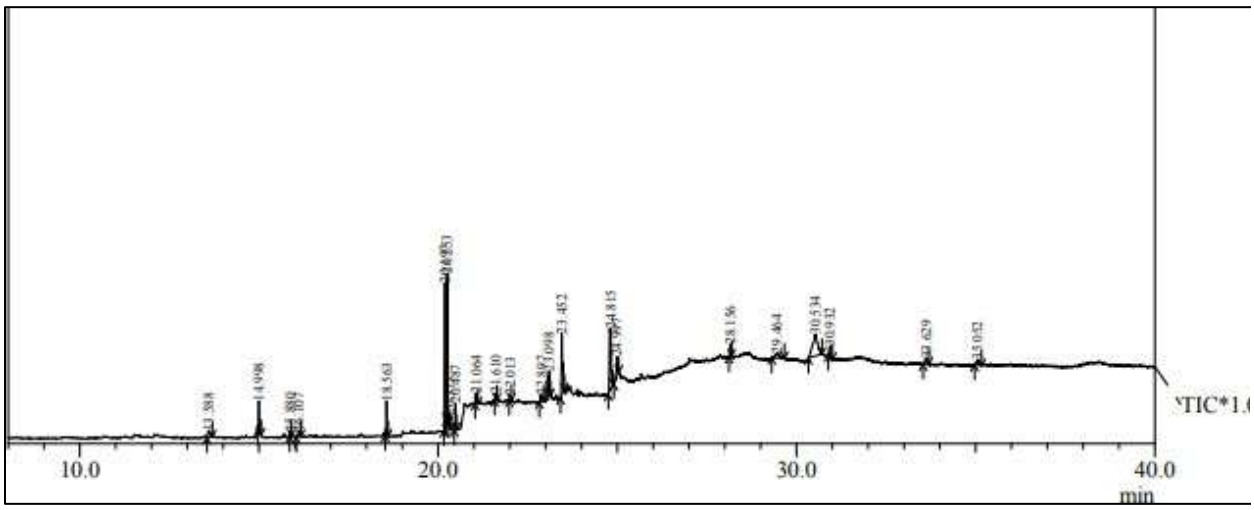

Figure 1: Metabolic profiling of Aqueous extract of leaves of Urtica dioica L. (AEUD) by GC-MS analysis

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 23s, 2025
https://www.theaspd.com/ijes.php

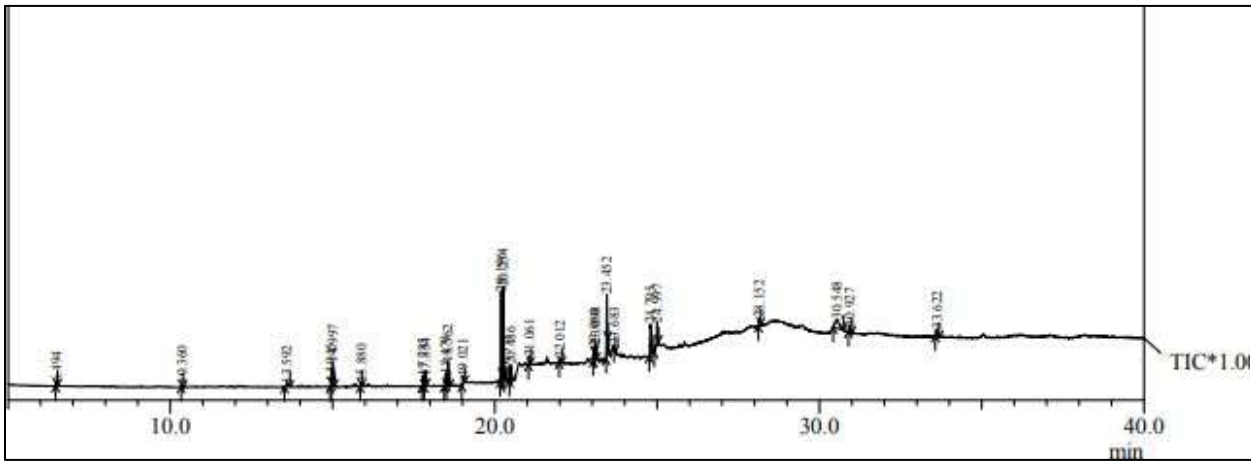

Figure 2: Metabolic profiling of Aqueous extract of leaves of Xanthium strumarium L. (AEXS) by GC-MS analysis

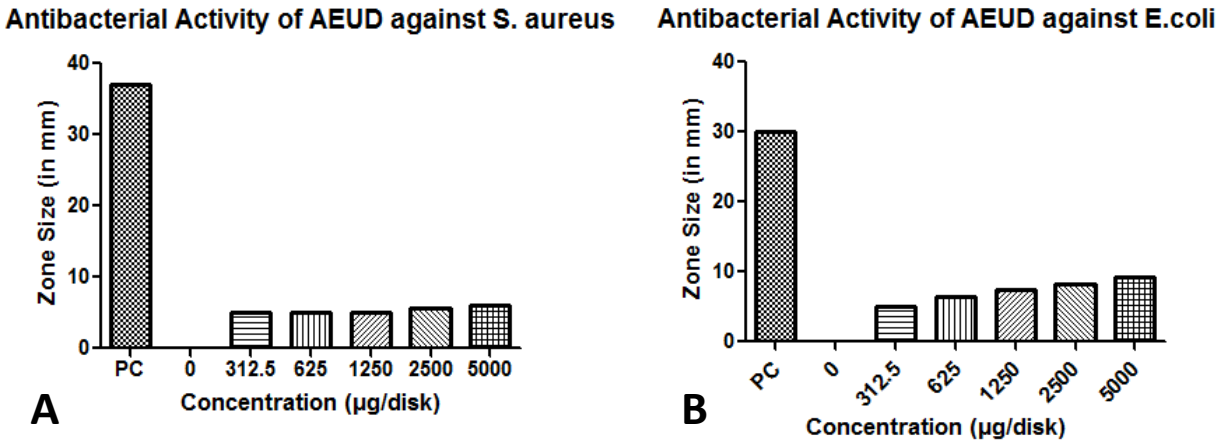

Figure 3: Antibacterial Activity of AEUD against (A) S. aureus and (B) E.coli

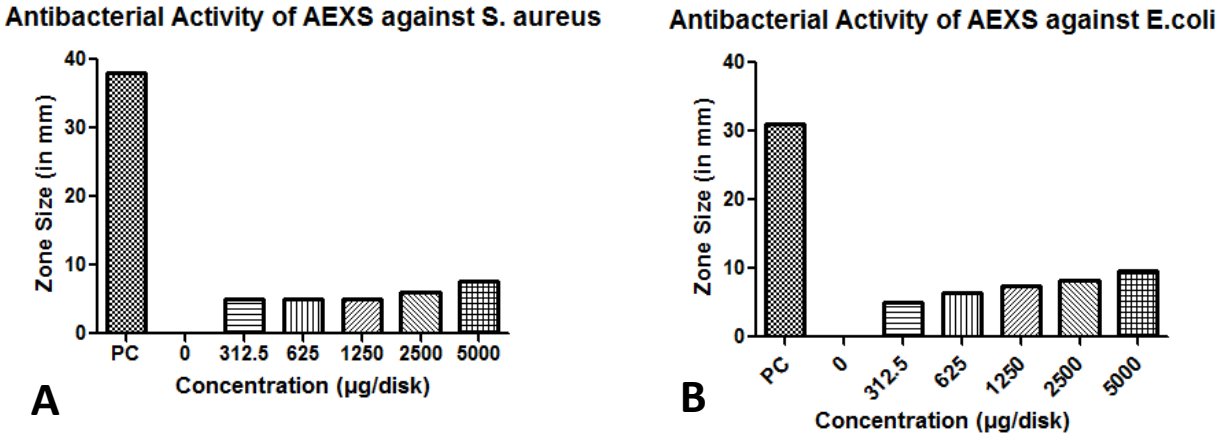

Figure 4: Antibacterial Activity of AEXS against (A) S. aureus and (B) E.coli

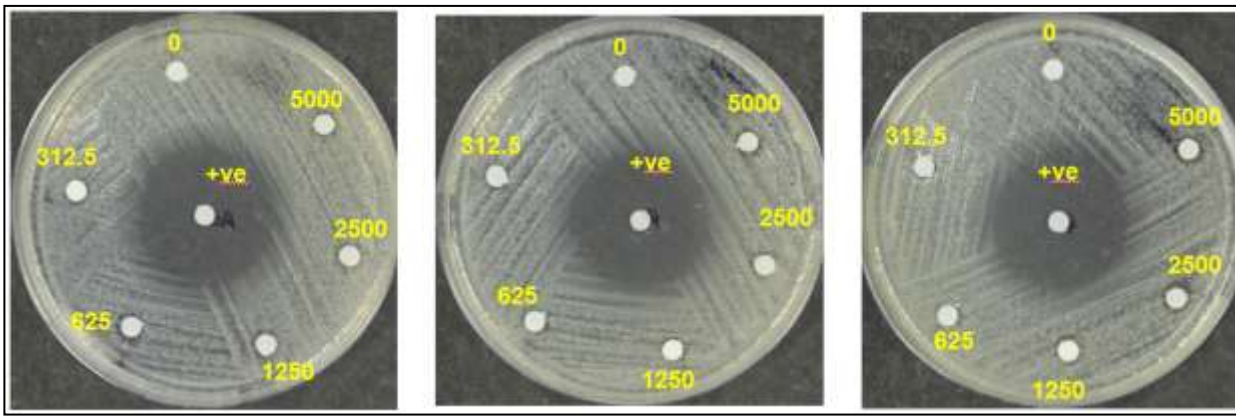

Figure 5: Zone inhibition assay of AEUD against E.coli

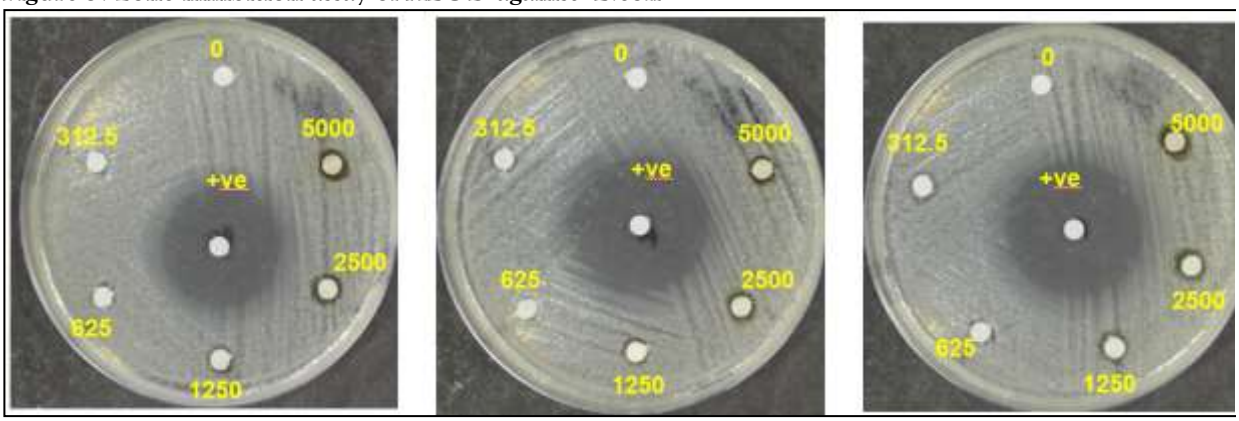

Figure 6: Zone inhibition assay of AEXS against E.coli