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ASSESSMENT OF *IN-VITRO* ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF ESSENTIALOIL OBTAINED FROM *ARTEMISIA ARGYI*

Anubhav Dubey[1*], Mairaz Varis Ansari[1], Vikram Kumar Sahu[1], Amit Mishra[2]

1. Maharana Pratap College of Pharmacy Kothi, Mandhana, Kanpur - 209217, Uttar Pradesh, India.
2. Maharana Pratap College of Pharmaceutical Sciences, Kothi, Mandhana, Kanpur - 209217, Uttar Pradesh, India.

Corresponding author
Dr. Anubhav Dubey
Assistant Professor, Department of Pharmacology, Maharana Pratap College of Pharmacy, Kanpur, Uttar Pradesh, India.

Abstract
We evaluated the antibacterial activity and chemical components of *Artemisia argyi* essential oil. A clevenger device extracted essential oil from Chinese mugwort, *Artemisia argyi*, a medicinal herb. This study examines *Artemisia Argyi* essential oil's antibacterial and antioxidant effects in vitro. Gas chromatography-mass spectrometry analysis.
The essential oils were dominated by cis-chrysanthenol, 2-naphthalenemethanol, and pinocarvone. We tested the essential oil's antibacterial activity against harmful microbes using disc diffusion. *A. argyi* essential oil has excellent antibacterial action against Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus* and *E. coli*.
We further measured the essential oil's antioxidant activity using DPPH and ABTS radical scavenging tests. *A. argyi e*ssential oil showed excellent antioxidant activity (IC50 = 35.6 g/mL), suggesting its potential as a natural antioxidant.
Because of its phytochemical content, *A. argyi* essential oil may have antibacterial and antioxidant activities. Understanding the modes of action and medicinal and food uses of A. *argyi* essential oil requires further research.

Key words: *Artemisia argyi,* essential oil, antimicrobial activity, antioxidant activity.

Introduction:

People have used the traditional Chinese herb artemisia argyi for more than two thousand years. It's a fragrant perennial herb or dwarf shrub that lasts for years and never fades. According to Abad et al. (2012), there are about 500 species of Artemisia plants, which are members of the Compositae family. Polysaccharides, flavonoids, essential oils, and triterpenoids are among the bioactive compounds found in A. argyi (Zhang et al., 2013). A growing body of research (Bao, X. et al., 2013; Zeng K. et al., 2014) confirms that A. argyi regulates the immune system, fights tumors, and has anti-oxidant and

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antibacterial properties. Certain research has shown that the anti-inflammatory actions of A. argyi's active components could be mediated via the toll-like receptor 4 (TLR4)/nuclear factor-kappa B (NF-κB) signaling pathway. Health care and skin care products may benefit from A. argyi since it is a plant analogous to medicine and food (Yamamoto, N., et al., 2011) and because it has antioxidant, antibacterial, hypoglycemic, hypolipidemic, and liver protective (Batiha et al., 2020) qualities. Over time, it found its way into feed additives thanks to its own nutrients and bioactive components. Recent research (Kim et al., 2012) suggests that using A. argyi in animals' meals may enhance their digestion, absorption rate, and production performance.
Numerous studies have examined EO's ability to inhibit the growth of various microbes in laboratory settings (Miliauskas et al., 2004). However, the rise of multidrug-resistant bacteria is making infections more difficult to cure, highlighting the need for novel compounds with antimicrobial characteristics to combat these microbes (ReR et al., 1999). Over many generations of synthetic reorganizations and arrangements, a limited collection of useful molecular structures has been able to prolong the lifetimes of most antibiotics. In addition, the antibacterial characteristics of EOs have piqued the curiosity of the food, pharmaceutical, and cosmetic sectors. This is in line with the growing trend of using natural additives as a substitute for synthetic preservatives.

Materials and Methods:
Plant collection and extractions:

We obtained *Artemisia Argyi* from the herbal garden of Maharana Pratap College of Pharmacy in Kanpur. Mrs. Ruchi Sharma (Botanist) validated the plant using ACME research solutions (Ref. ACME/PA/12511). We washed the new plant material in the lab with tap water to remove contaminants, then plucked the leaves and dried them under shade at room temperature. We added 40 grams of *Artimisia Argyi* leaves and 500 ml of distilled water to a clean round-bottom flask. We placed the spherical bottom flask and its contents in an equivalent-volume heating mantle. A 1-liter round-bottom flask needs a 1- or 2-liter heating mantle. We then attached the circular-bottom flask to the clunker and condenser. Water in the round-bottom flask boiled. After fully extracting the oil from the leaves, we turned off the equipment. We put the solvent and oil in a water bath to eliminate the solvent, leaving the oil golden yellow and viscous.

Chemicals: All the chemical were of AR grade and were purchased from Vikas Sales Corporation, India.

Qualitative phytochemical screening: To confirm the existence of different bioactive components, a standard qualitative investigation of the aqueous extract was performed (Dubey et al., 2023).

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Determination of antioxidant activity:

1, 1 DPPH scavenging: We assessed DPPH radical scavenging in a sample. To put it simply, we mixed 2 mL of DPPH solution (0.1 mM) in methanol with 2 mL of each extract (0-2500 g/mL) and ascorbic acid (0-50 g/mL). We agitated the reaction mixture for 30 minutes at 250 °C in the dark. We measured the absorbance at 517 nm against a blank. We prepared the ascorbic acid positive controls in the same manner as we did the test samples. We calculated the sample's DPPH radical inhibition using the formula below:

DPPH Scavenging Activity is equal to ((Abs Control - Abs Sample)/Abs Control) 100.

We calculated the half-maximal inhibitory concentration (IC50) by plotting the concentration of standard ascorbic acid and each fraction (water) against their percentage inhibition to determine the ascorbic acid concentration required to inhibit DPPH radicals by 50%. You can calculate the IC50 using the graph equation.

ABTS (2,2'-azino-bis 3-Ethylbenzothiazoline-6- sulfonic Acid) Radical Cation Decolorization Assay:

The ABTS radical cation decolorization experiment assessed AR seed extracts' free radical quenching in water and ethanol. We produced the ABTS+ cation radical by combining 7 mM ABTS in water with 2.45 mM potassium persulfate (1:1) and letting it sit in the dark for 12-16 hours at room temperature. We diluted the ABTS+ solution with methanol to obtain 0.700 at 734 nm. We measured the absorbance 30 minutes after adding 10 l of water and ethanolic AR seed extracts (0–1000 g/ml) to 3.990 ml of diluted ABTS+ solution. ach experiment has a solvent-specific blank run. At least three variables were measured. The formula determined the blocked absorbance at 734 nm%.

$$\textbf{ABTS scavenging effect (\%)} = ((AB–AA)/ AB) \times 100$$

where AB is absorbance of ABTS radical + methanol, AA is absorbance of ABTS radical + sample extract /standard and ascorbic acid was used as standard substance.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of essential oil:

GS-MS examined AR seed extract in aqueous and ethanolic conditions. The Rtx-5MS capillary column fits snugly in the PerkinElmer Clarus 6We achieved a constant 1.0 mL/min flow rate with 99.99 percent pure helium. pure helium. We used an ionization energy approach to identify GC-MS spectral lines at 260 °C, 70 eV (electron volt), 0.2 seconds, 40 to 650 m/z fragments, 1 L injection, and 10:1 split ratio. However, the column oven ran for three minutes at 500 °C. To achieve 3000C, add 100C every minute. Plant sample components were revealed when comparing retention periods, peak areas, peak heights, and spectral line patterns to known chemicals in the NIST and Wiley-8 libraries (Dubey et al., 2023).

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Antimicrobial Activity Test:

Anti-Microbial Zone Inhibition Test (*E. coli):*

The Zone Inhibition Method (Kirby-Bauer) tested antibacterial activity. We inoculated MHA plates with 100 l of E. coli culture, adjusted to 0.5 McFarl and Unit-Unit-Approx cell density (1.5 x108 CFU/mL), and placed discs containing 10 l of different concentrations (0-5%). We serially diluted 10% of the sample to load the disk. We filled one disc per plate with solvent as the vehicle control and used a Ciprofloxacin disc (10 g) as the positive control. We incubated the E. coli plates at 37 °C for 24 hours. We measured and recorded the disc's clear zone (Chouhan et al., 2017).

Anti-Microbial Zone Inhibition Test (*S. aureus):*

The Zone Inhibition Method (Kirby-Bauer) tested antibacterial activity. We disseminated 100 liters of S. aureus (0.5 McFarland Unit—approximate cell density (1.5 x 108 CFU/mL)) bacterial culture to inoculate MHA plates, and then added 10 liters of various doses to the wells, following the instructions on the Excel sheet. We serially diluted 10% of the material to fill the well. Each plate contained a solvent-only well for the vehicle control and Ciprofloxacin wells (10 g) for the positive control. We incubated S. aureus plates at 37 °C for 24 hours (Basil Scientific Corp., India). We monitored and documented the clear zones in the well (Chouhan et al., 2017).

Anti-Fungal Zone Inhibition Test (C. *albicans):*

The Kirby-Bauer Zone Inhibition Method was used to test for antifungal activity. To inoculate SDA plates, we disseminated 100 liters of C. albicans culture, adjusted to 0.5 McFarland Unit-approximate cell density (1.5 x 108 CFU/mL), and added 10 liters of various doses to the wells specified in the excel sheet. Each plate contained a solvent-only well for the vehicle control and a 50 g Amphotericin B well for the positive control. For 24 hours, Basil Scientific Corp. India-Incubator incubated C. albicans plates at 37 °C. We measured and recorded the clear zones surrounding the well (Chouhan et al., 2017).

Minimum Inhibitory Concentration Activity (*E. coli):*

The investigation used the E. coli Minimum Inhibitory Concentration Activity: 0.5 McFall and a standard microbe dilution. We incubated 100 liters of diluted log cultures of bacteria (E. coli, MTCC452) for 24 hours in a microcentrifuge tube with 5 liters of the produced treatment dilutions (as per the Excel sheet). The Elisa Plate Reader (iMarkBiorad) took 630 nm turbidity measurements after incubation for every material in the 96-well plate. We used Ciprofloxacin (10 g) as a positive control (Chouhan et al., 2017).

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Minimum Inhibitory Concentration Activity (*S. aureus):*

The investigation used the S. aureus Minimum Inhibitory Concentration Activity of 0.5 McFall and standard microbe dilutions. For 24 hours, incubate 100 liters of diluted log cultures of S. aureus (MTCC 96) in a microcentrifuge tube with 5 liters of prepared treatment dilutions (concentrations listed in the Excel sheet). The Elisa Plate Reader (iMarkBiorad) took 630 nm turbidity measurements after incubation for every material in the 96-well plate. We used Ciprofloxacin (SRL Chem-78079) (100 g) as a positive control (Chouhan et al., 2017).

Results and discussion:

Phytochemical screening:

Once phytochemical screening identifies bioactive chemicals, it may be possible to identify drugs and medicinal formulations. Table 1 displays the results of a phytochemical study on *Artemisia Argyi* essential oil.

Table 1: Phytochemical screening of *Artemisia Argyi* essential oil

S.No	Compound	Test	Result
1.	Alkaloid test	Mayer's test	+
		Hager's test	+
2.	Saponin test	Foam Test	+
3.	Carbohydrates test	Reducing sugars	+
		Starch	+
4.	Flavonoids test	Alkaline Reagent Test	-
5.	Tannin test	Gelatin Test	-

+ = Positive- = Negative

Antioxidant activity:

DPPH and ABTS free radical scavenging activity: Table 2 Displays the results of an Antioxidant activity on *Artemisia Argyi* extracts.

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Table-2:-Antioxidant activity of *Artemisia Argyi* extract of DPPH and ABTS extracts

Herbal Extracts	**Anti-oxidant Activities**	
	DPPH (IC_{50};µg/mL)	**ABTS (IC_{50};µg/mL)**
Water extract	298.9	0.4446%
Ascorbic Acid	6.097	3.241

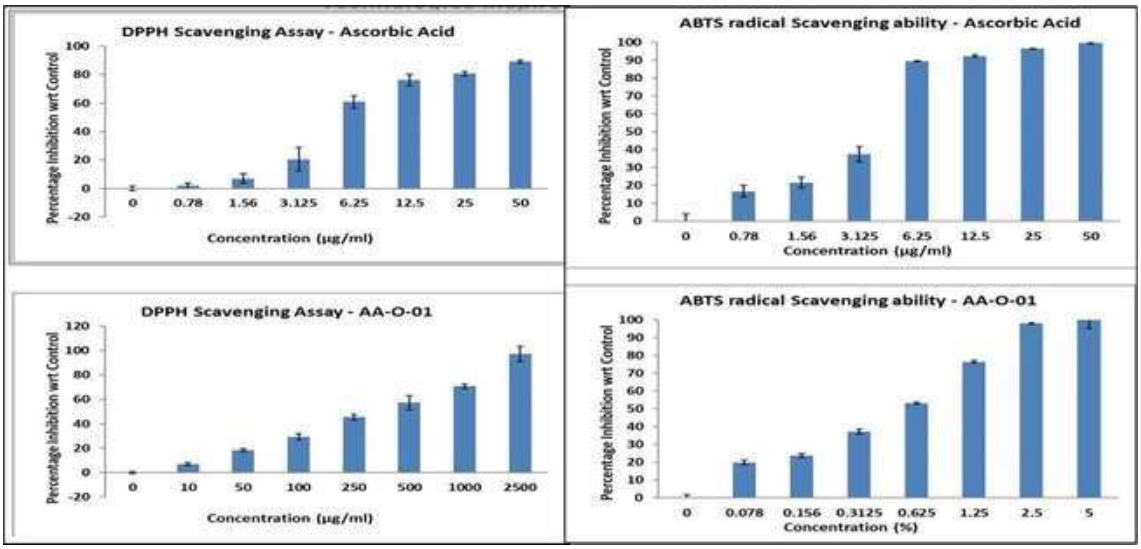

Figure 1: DPPH and ABTS free radical scavenging activity of *Artemisia Argyi* essential oil

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of essential oil:

The components of the test materials were identified (Table 3).

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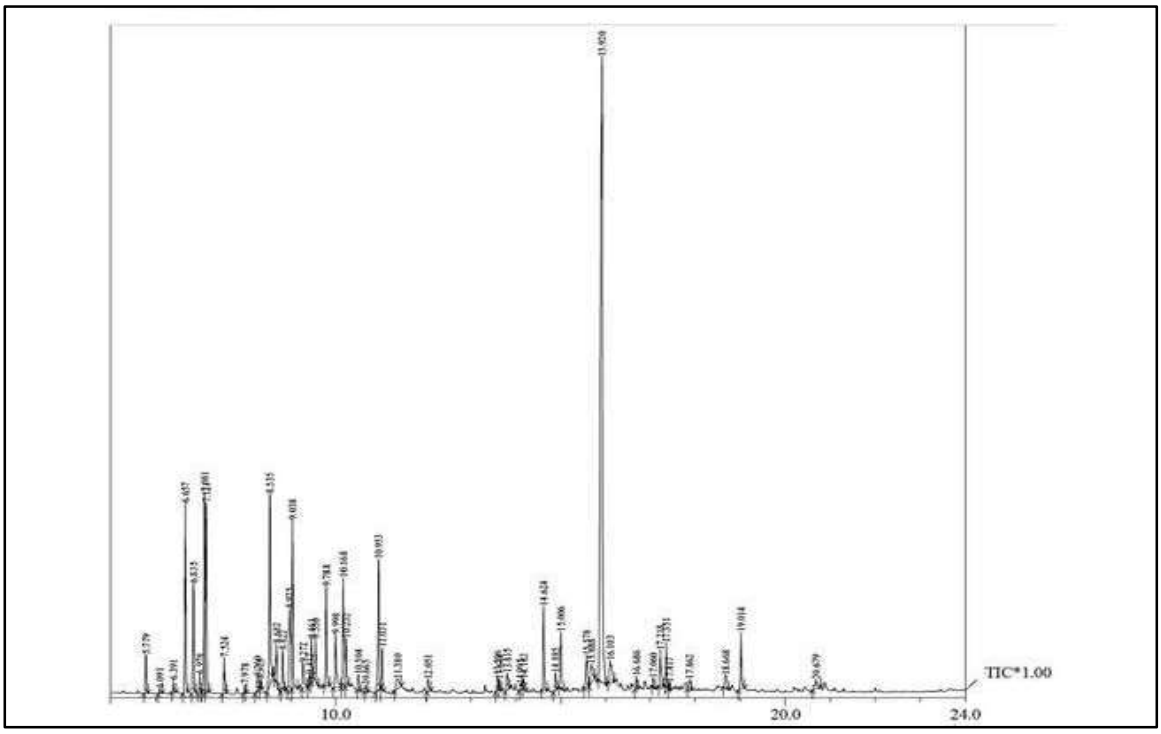

Figure-2:-Gas Chromatography-Mass Spectrometry(GC-MS)

Table-3:- Compounds of *Artemisia Argyi*

Peak	R.Time	Area	Area %	Name of Compounds
1	6.657	159892	5.12	1,3-CYCLOHEXADIENE,2-METHYL-5-(1-METHYLET
2	7.081	165341	5.29	CYCLOHEXENE,3-METHYLENE-6-(1-METHYLETHY
3	7.121	146647	4.69	2-OXABICYCLO[2.2.2]OCTANE,1,3,3-TRIMETHYL-
4	8.535	174016	5.57	Cis-Chrysanthenol
5	17.351	315150	1.01	1,3a-Ethano(1H)Inden-4-Ol,Octahydro-2,2,4,7a-Tetramethyl
6	19.014	523600	1.68	N-HexadecanoicAcid
7	14.885	143236	0.46	Bicyclo[5.2.0]Nonane,2-Methylene-4,8,8-Trimethyl-4-Vinyl
8	9.038	136208	4.36	(+)-2-Bornanone
9	9.272	287987	0.92	Pinocarvone

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10	15.920	944319	30.21	2-NAPHTHALENEMETHANOL,DECAHYDRO-.ALPHA
11	14.624	625177	2.09	1,6,10-DODECATRIEN-3-OL,3,7,11-TRIMETHYL

Antibacterial Zone Inhibition Test:

Figures 4 and 5 summarize the microbial growth inhibition by the essential oil of E. coli, which showed good antibacterial activities.

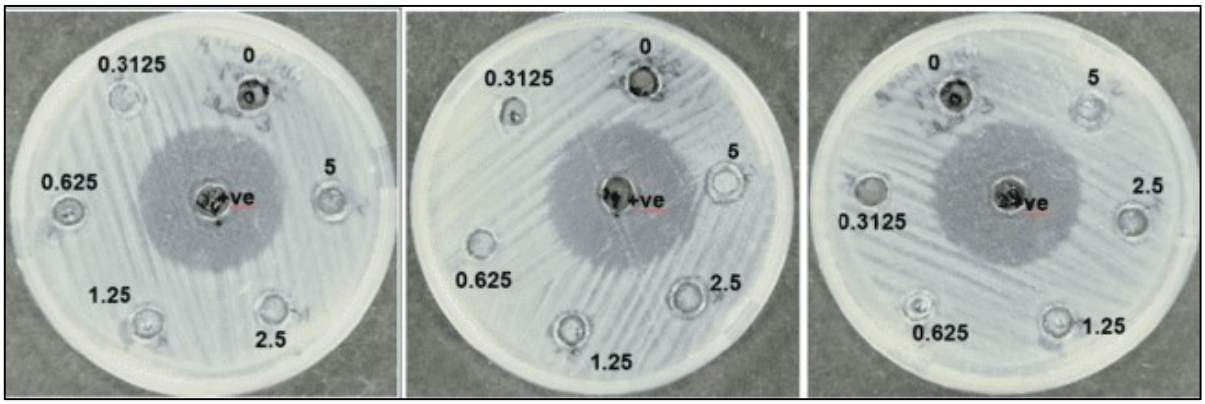

Figure-3:- Well Diffusion Method Test organism- *E.coli*

Table-4:-Anti bacterial activity of E.coli

Amount (µg/disk)	**Plate A**	**Plate B**	**Plate C**	**Average**	**SD**	**SEM**
PC	34	34	34	34	0	0
0	0	0	0	0	0	0
0.3125	0	0	0	0	0	0
0.625	0	0	0	0	0	0
1.25	7	7	7	7	0	0
2.5	8	8	8	8	0	0
5	9	8	9	8.66667	0.57735	0.33333

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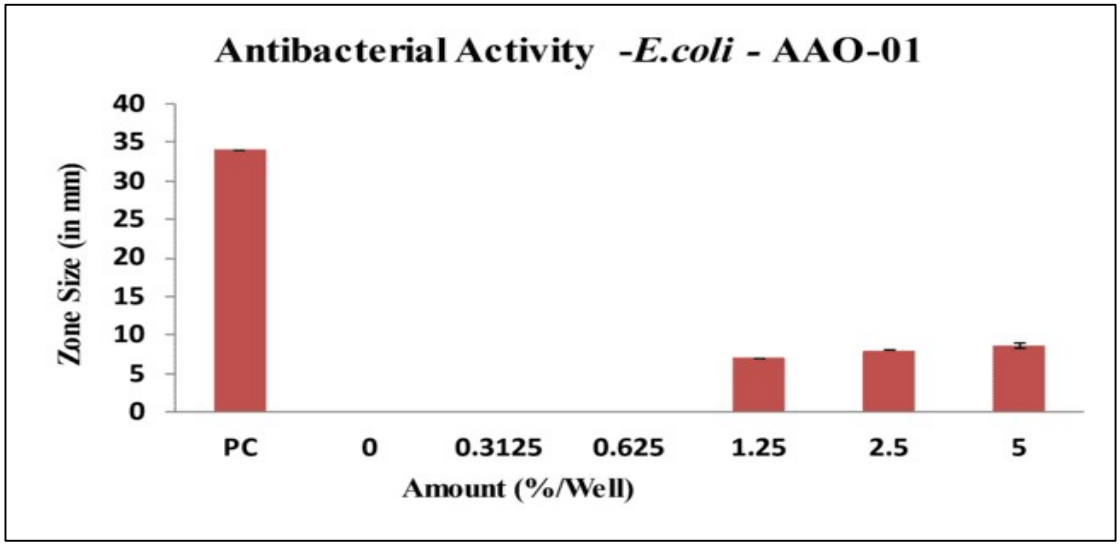

Figure-4:-Antibacterial activity of E.coli

Anti-Microbial Zone Inhibition Test (*S. aureus*):

In the experimental work, after treatment, clear zones were observed around the well-treated samples with different amounts of the sample and the positive control. DMSO was used as a negative control. The lowest zone of inhibition (9.66 mm diameter) around the well of the 50 µg sample and the highest zone of inhibition (18.66 mm diameter) around the well of the 1000 µg sample were observed against *S. aureus,* while the positive control showed the zone of inhibition (32.66 mm diameter) around the well of the positive control at the 10 µg dose.

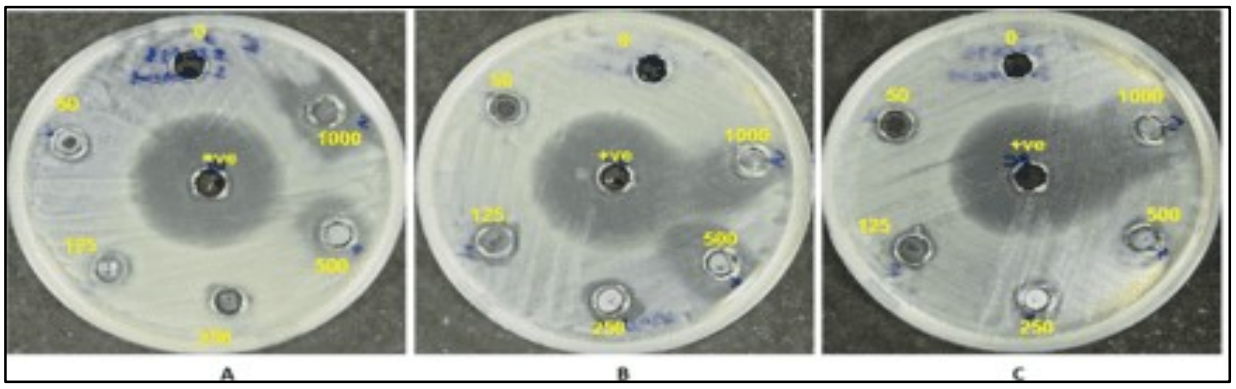

Figure-5:-Well Diffusion Method Test organism-*S.aureus*

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Table-5:- Anti microbial zone inhibition of *S.aureus*

Amount (µg/well)	Plate A	Plate B	Plate C	Average	SD	SEM
PC	32	33	33	32.6667	0.57735	0.33333
0	0	0	0	0	0	0
50	9	10	10	9.66667	0.57735	0.33333
125	10	11	10	10.3333	0.57735	0.33333
250	12	12	10	11.3333	1.1547	0.66667
500	15	18	16	16.3333	1.52753	0.88192
1000	21	18	17	18.6667	2.08167	1.20185

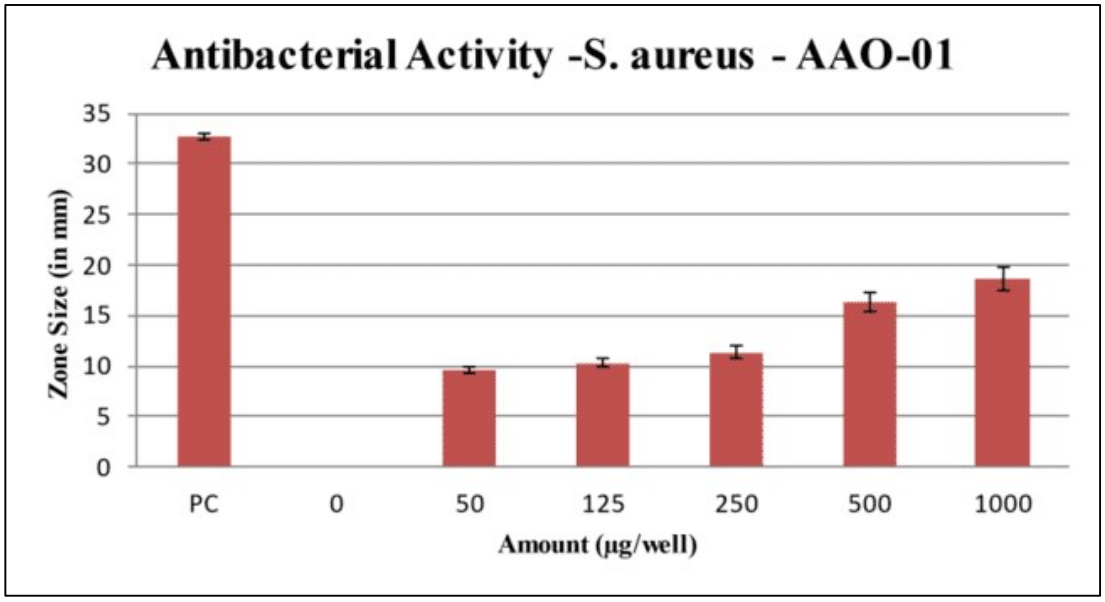

Figure-6:-Anti bacterial activity of -*S.aureus*

Well Diffusion Method Test organism: *C.* albicans

As per the results obtained from the experimental work, it was observed that the samples AAO-01 have antifungal activity against C. albicans 10.6mm diameter at 50µg/well, 11mm diameter at 125µg/well, 12.33mm diameter at 250µg/well, 12.33mm at 500µg/well, and 16mm at 1000µg/well with respect to the positive control (15.6mm diameter at the dose of 50µg/well).

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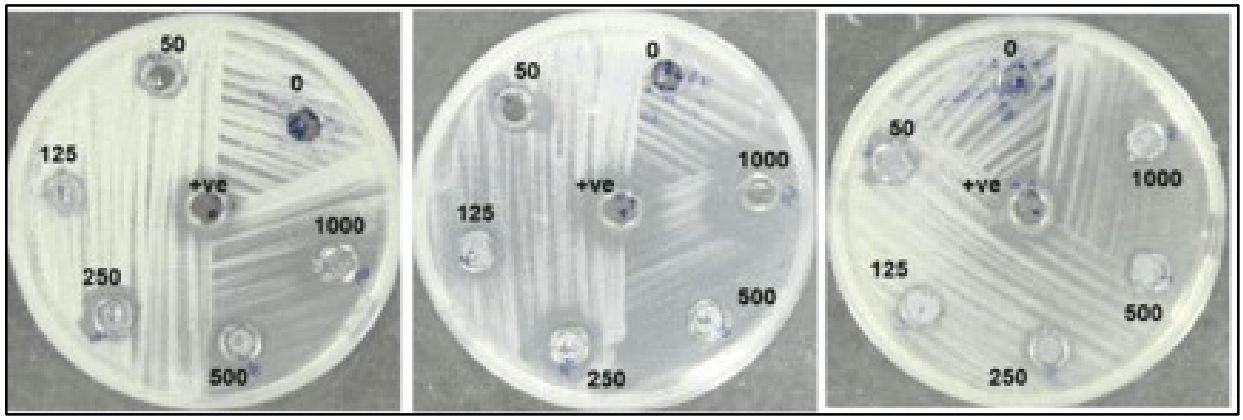

Figure-7:- Well Diffusion Method Test organism-*C.albicans*

Table-6:- Amount present per Wellin µg Dispensed Volume- 10µL Positive Control -50µg

Amount (µg/Well)	Plate A	Plate B	Plate C	Average	SD	SEM
PC	15	15	17	15.6667	1.1547	0.66667
0	0	0	0	0	0	0
50	10	12	10	10.6667	1.1547	0.66667
125	10	10	13	11	1.73205	1
250	13	10	14	12.3333	2.08167	1.20185
500	13	11	13	12.3333	1.1547	0.66667
1000	16	16	16	16	0	0

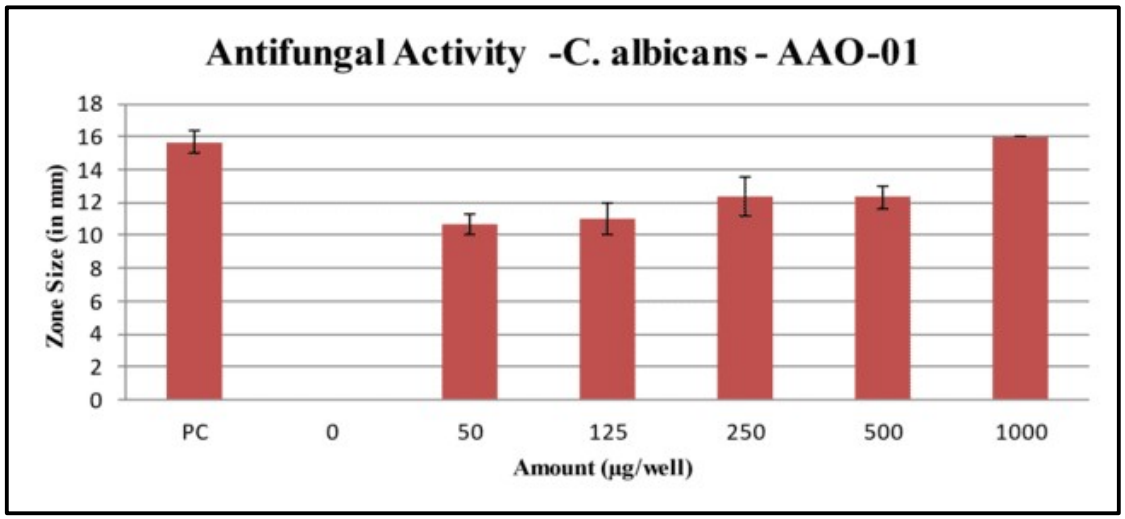

Figure-8:-Anti-fungal activity of *C.albicans*

Minimum Inhibitory Concentration Activity (*E. coli):*

Anti-microbial activity was observed in sample AA01-01 (IC_{50} = 1.024 µl/ml).

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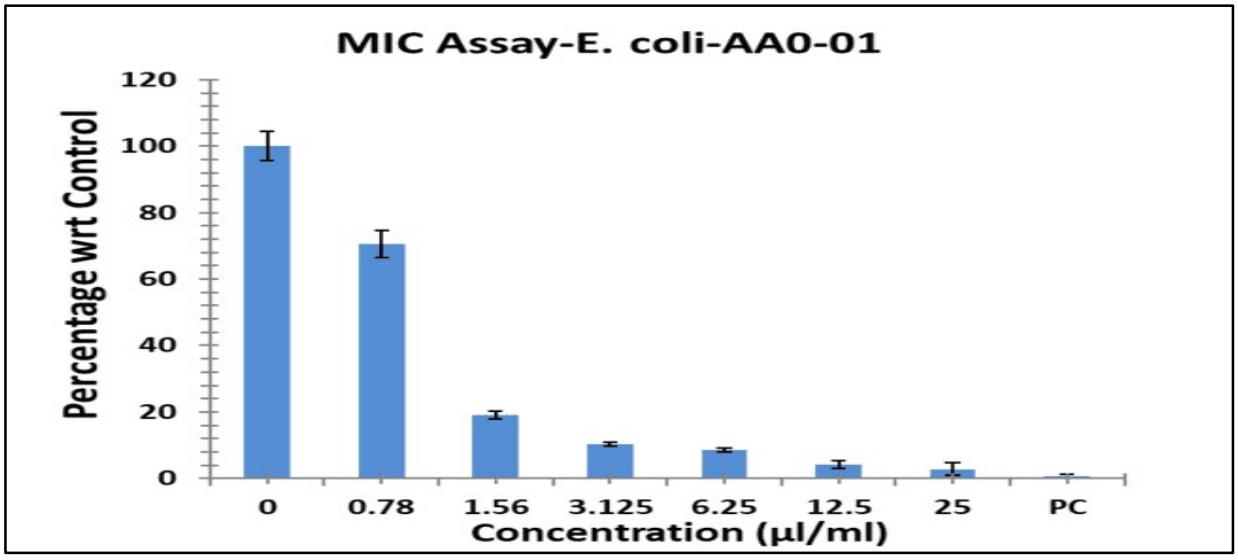

Figure-9:- MIC of *E. coli*

Table-7:- MIC of *E.Coli*

Sample Conc.	Test Replicates			
	1	**2**	**3**	**4**
0	0.449	0.43	0.511	0.485
0.78	0.482	0.515	0.433	0.475
1.56	0.248	0.263	0.271	0.266
3.125	0.19	0.186	0.177	0.183
6.25	0.174	0.173	0.183	0.172
12.5	0.199	0.201	0.183	0.183
25	0.226	0.223	0.201	0.237
PC	0.073	0.08	0.069	0.069

Minimum Inhibitory Concentration Activity (*S. aureus):*

Based on the study, it was observed that sample AAO-01 exhibited **significant anti-microbial activity** against *S. aureus* (*IC_{50}*= **0.649% and MIC = approximately** 0.09**%).**

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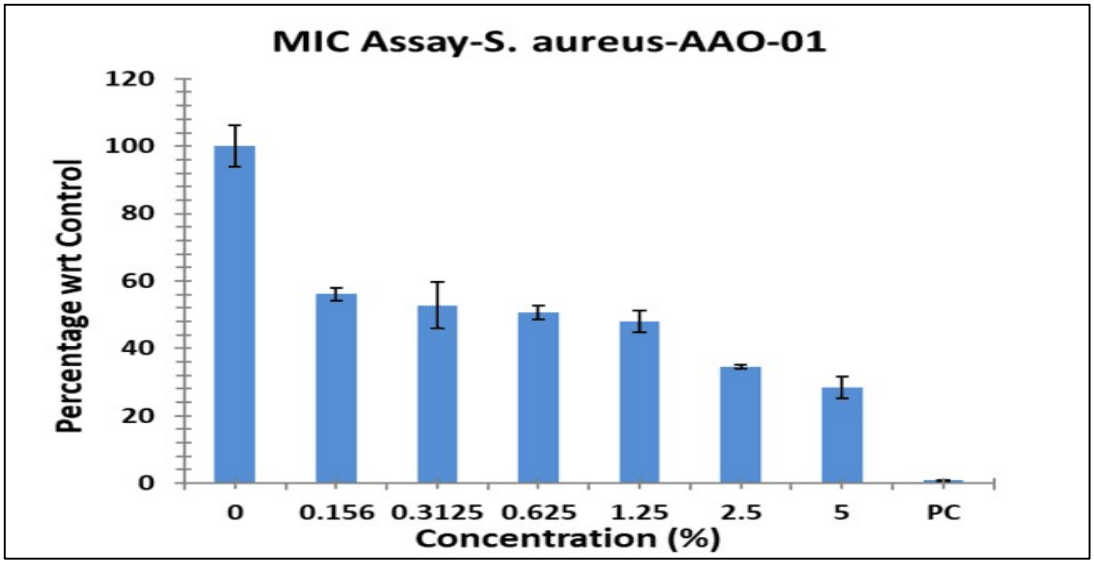

Figure-10:- MIC of S.aureus

Table-8:- MIC of S.aureus

Sample Conc.	Test Replicates			
	1	**2**	**3**	**4**
0	0.592	0.505	0.642	0.646
0.156	0.354	0.336	0.339	0.378
0.3125	0.364	0.359	0.223	0.381
0.625	0.354	0.319	0.299	0.328
1.25	0.338	0.299	0.283	0.356
2.5	0.283	0.267	0.274	0.276
5	0.308	0.292	0.231	0.277
PC	0.058	0.057	0.056	0.057

Conclusion:

Artemisia argyi essential oil has potential antioxidant and antibacterial properties. Pharmaceuticals, cosmetics, and food preservation might all benefit from its varied phytochemical content, which in turn gives it biological functions. Its full therapeutic potential and safety profile require further investigation. Researchers found that adding essential oil leaves to broth cultures infected with S. aureus and *E. coli* suppressed their development. Inhibition was more rapid against gram-negative *E. coli* bacteria than gram-positive S. aureus bacteria. Typically, the inoculum size and essential oil leaf concentration dictated the organisms' growth and survival rates. We believed the examined bacteria were sensitive to the oil because high quantities of essential oil leaves strongly suppressed their

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development. When test When tested against two types of clinically important bacteria, the essential oils that were taken from Artemisia argyi leaves had varying levels of antibacterial activity. -positive (*E. coli*) and Gram-negative (S. aureus) bacteria showed markedly reduced growth in response to the extract, according to the results of the aforementioned experiment. The current investigation also found that essential oil has antifungal effects against strains of *Candida albicans*.The essential oil from *Artemisia argyi* had a strong antibacterial effect on the bacteria that were tested. The minimum inhibitory concentration (MIC) values for Staphylococcus aureus and Escherichia coli showed this. This could lay the groundwork for its use in the clinical treatment of bacterial infections. And the antifungal activity of the volatile oil from *Artemisia argyi* shows that it significantly inhibited the test fungus, including *Candida albicans, Gillus niger, and Aspergillus flavus*. This quality suggests that *Artemisia argyi's* volatile oil may have promising applications as an antifungal against Candida albicans. *Artemisia argyi's* volatile oil, on the other hand, shows promise as a food preservative due to the widespread use of *aspergillus niger* and *aspergillus flavus*.

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Declarations:

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: The manuscript has not been published or submitted to another journal, nor is it under review.

Supplementary File

None.

Credit authorship contribution statement

Both authors participate equally.

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