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RESEARCH ARTICLE

Extraction, Chemical Composition, Antioxidant, and Antibacterial Activities of Essential Oil Using Aerial Part of *Artemisia indica*.

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Abstract

Artemisia indica is a significant medicinal plant which has crucial applications such as antioxidant and antibacterial. The main aim of this study is to evaluate antioxidant potential, antibacterial activity, and extraction of essential oil and GC-MS analysis of essential oil collected from aerial parts of *A. indica.* The ethanolic extract of this plant exhibit potent antioxidant potential which is 17.75 ± 0.38 µg/mL. The methanolic and ethanolic extract had excellent zone of inhibition which is 21 and 20 mm against *E. coli*. This zone of inhibition is nearly close to positive control (neomycin 28 mm). 39 phytoconstitunets were identified from the AID (*A. indica* collected from Dadeldhura), similarly, 37 compounds were indentified in AIK sample (*A. indica* collected from Kanchanpur). In AID sample, α- thujone is the major phytoconstituents whereas in AIK sample eucalyptol is the major phytoconstituents. 21 phytoconstituents are common in both the sample. The half maximal inhibitory concentration for AID sample is 23.15 ± 1.59 µg/mL which is very effective antioxidant potential as compare to essential oil of AIK sample. Similarly, AID sample has very significant zone of inhibition (16 mm) against *S. aureus*. This zone of inhibition of AID sample shows good antibacterial potential. Due to diverse range of biological activity of *A. inidica,* it could be be used for the development of new drugs or new medications.

Keywords: Antibacterial, Antioxidant, *Artemisia indica*, Essential oil, GC-MS,

Introduction

Plants have a variety of pharmacological properties arising due to the presence of secondary metabolites [1]. As a rich source of novel medications, medicinal plants are recognized as a priceless natural reservoir. The pharmacological effects of various medicinal plants against a range of diseases have been continuously investigated. There is a need for more research on these natural resources since medicinal plants can be broadly divided into two categories: those with therapeutic qualities confirmed by science and those that are thought to be therapeutic based on traditional knowledge but lack comprehensive scientific investigation [2].

Natural remedies made from microbes, plants, and animals have a long history in traditional medicine. According to fossil records, people have been using plants as medicine for at least 60,000 years. A wide range of physiologically active compounds with promising pharmacological potential have been produced by the evolutionary development of chemical diversity in these natural chemicals

over millions of years [3] A wide range of physiologically active compounds with promising pharmacological potential have been produced by the evolutionary development of chemical diversity in these natural chemicals over millions of years [4]. Furthermore, they help in protecting Plants from environmental stressors including illnesses and ultraviolet (UV) light [5].

The perennial herb *Artemisia indica* (Asteraceae), referred to locally as "Titepati," is found in the western Himalayas. It has long been utilized by the locals to treat hepatobilary disorders, persistent fever, and dyspepsia. There have been reports of antihelminthic, antiseptic, and antispasmodic properties in *A. indica's* leaves and flowering stems [6]. The Anthemideae tribe's large genus Artemisia has significant therapeutic plants that are presently receiving phytochemical attention due to their production of essential oils and biological and chemical variety [7]. In Nepal, the plant's juice is used to treat indigestion, diarrhoea, and Dysentery. Young leaves are added to rice to provide flavour and colour, and they are cooked and consumed with barley [8]. This plant has plenty of secondary metabolites, which may be related to its defense systems because of how little it is susceptible to insect, fungal, and bacterial infections [9]. Many secondary metabolites found in this plant may be related to its defense systems, as demonstrated by its less susceptible to bacterial, fungal, and insect attacks. Among the substances found in previous research are terpenoids, phenylpropanoids, flavonoids, coumarins, sterols, and alkaloids [10]. Artemisia species from different origin showed a dominant presence of α-thujone, β-thujone, 1, 8-cineole, germacrene-D, vulgarone-B, borneol, β-caryophyllene, caryophyllene oxide, davanone, artemisiaketone, and chrysanthenone [11].

The purpose of the current investigation was to analyse the components of the essential oil of *A. indica* together with its biological activity. To the best of our knowledge, there are not many reports the specifics the antimicrobial and antioxidant properties of *A. indica* essential oil. However, some previous studies have focused on the composition of essential oils, which differs significantly from the findings presented in this research article. The comparison of antioxidant and antibacterial properties of crude extract and essential oil have not been well studied yet. The figure of aerial parts of *A. indica* is shown in **Figure 1.**

Figure 1 Aerial parts of *Artemisia indica*

Materials and Methods

Plant Sample Collection and Extraction

The aerial parts of *A. indica* were collected in March 2020 from two different geographical region (Mahendranagar, Kanchanpur and Dadeldhura Nepal) at an approximate altitude of 229 m for Kanchanpur, 1400 m for Dadeldhura and identified by the Central Department of Botany, Tribhuvan University, and Kathmandu, Nepal. The collected aerial parts of *A. indica* was washed, cleaned, shade dried and grind into small pieces. To prepare the sample for analysis, approximately 10 grams of powdered material were dissolved in 200 mL (1:20) of five different solvents, selected based on their polarity: methanol, ethanol, ethyl acetate, dichloromethane (DCM), and hexane, from most polar to least polar. The sample underwent maceration, with the contents being shaken every 24 hours over three days to enhance extraction. Following this period, the mixture was filtered to separate the solid residues, and the resulting filtrate was subsequently dried using a rotary evaporator,

maintained at a temperature of 40-45 °C. This systematic approach ensures effective extraction of the desired components from the powdered sample. The extract was obtained using the cold maceration process and has been stored at 4 °C to ensure its stability for further analysis [12].

Evaluation of Antioxidant Activity

The antioxidant activity of plant extract and essential oil were evaluated by using standard protocol [13]. The antioxidant activity was measured using a 96-well plate reader that was modified from the colorimetric method. Each of the 96-well plates received 100 µL of plant extract and essential oil. To effectively address the hydrophobic nature of oil, we initially diluted it in methanol at several Concentrations, including 10, 25, 50, 100, and 200 µg/mL. This approach allowed us to optimize the solubility and facilitate further experimentation. At 517 nm, the initial reading was recorded. After that, 100 µL of DPPH were added to each 96-well plate. The final measurement at 517 nm was recorded using a microplate reader.

Radical scavenging capacity

$$= \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$$

Where $Abs_{control}$ = absorbance of control, Abs_{sample} = absorbance of sample

Inhibitory concentration (IC_{50}) was calculated using GraphPad Prism (version 8.0.2.263)

Evaluation of Antibacterial Activity

Antibacterial activity was assessed using the agar well diffusion method on Mueller-Hinton Agar (MHA) plates [14]. The test microorganisms, which included *Escherichia coli* (ATCC 25312), *Klebsiella pneumoniae* (ATCC 700603), *Staphylococcus aureus* (ATCC 43300), and *Shigella sonnei* (ATCC 25931), were grown in Mueller Hinton Broth (MHB) and incubated for 24 hours at 37 °C. To ensure consistent bacterial density, the broth's turbidity was adjusted to a 0.5 McFarland standard. A cork borer created wells in the agar plates, which were then filled with 50 mL of the plant extract and essential oil. Negative control well received 50% DMSO, while positive control well were filled with 50% neomycin. To carry out antimicrobial activity of essential oils, at first the solution was made in 50 % DMSO. The Petri dishes were allowed to sit for 15 minutes to ensure diffusion before being incubated at 37 °C for 18 to 24 hours. The antibacterial effectiveness of the plant extract and essential oil was assessed by measuring and observing the zones of clearance after incubation.

Extraction of Essential Oil

The Clevenger apparatus was used to perform hydrodistillation, which was used to extract the essential oil from the fresh aerial parts of *A. indica* collected from two geographical regions (Kanchanpur and Dadeldhura). A range of solvents with varying degrees of polarity, including n-hexane, dichloromethane, ethyl acetate, methanol, and water, were used to extract essential oils. In this experiment, water was used to separate the essential oil. After that, the plant material (500 grams) was chopped into tiny pieces and taken in a 1000 mL round-bottom flask. Flasks containing powder samples were filled with 600 mL of water as solvent. A Clevenger-type equipment for hydrodistillation was then added to the setup. The flask content was heated using heating mantle and allowed for boiling point for two hours. A glass vial containing the extracted essential oil was kept for later examination. During the process, anhydrous sodium sulphate was used to remove moisture, sealed, labelled, and stored in light-resistant vials at 4-6 °C for GC-MS analysis [15]. The yield percentage of essential oil can be calculated using the following formula:

$$\text{Yield percentage of essential oils} = \frac{Volume\ of\ essential\ oils\ obtained\ in\ mL}{Weight\ of\ plant\ material\ used\ (g)} \times 100\ \%$$

Gas Chromatography-Mass Spectrometry

The Department of Plant Resources, Thapathali, Kathmandu, Nepal, collaborated to analyze the extracted essential oil using GC-MS. A GC-MS-QP 2010 apparatus was used to perform gas chromatography-mass spectrometry (GC-MS) analysis under the given circumstances. Helium was used as the carrier gas in the Rtx-5MS column, which had the following measurements: 30 meters in length, 0.25 mm in inner diameter, and 0.25 mm in film thickness. With successive holding times of 2.0 and 5.0 minutes, the column's temperature fluctuated between 80 °C and 300 °C. In the meantime, the interface and ion source temperatures were kept at 250 °C and 200 °C, respectively. The identification process was conducted with a comparison using mass spectrometry (MS) with the NIST library.

Statistical Analysis

The Gen5 Microplate Reader was used for data collection and the data was analysed using Microsoft Excel. The results for antioxidant activity were presented as mean ± standard error of the mean (SEM). The inhibitory concentration (IC_{50}) values were computed using GraphPad Prism software (version 8.0.2.263). This analytical approach ensured a comprehensive evaluation of the data obtained from the

experiments. One-way ANOVA test was used for the comparison of the data. Values with $p < 0.05$ were considered statistically different.

Results and Discussion

Antioxidant Activity

The IC_{50} of methanolic extract of aerial parts of *A. indica* (sample AIK) ranges from 17.75 ± 0.38 µg/mL to 255.8 ± 0.11 µg/mL. The ethanolic extract shows more potent antioxidant potential. The IC_{50} of this extract was found to be 17.75 ± 0.38 µg/mL, and DCM and Hexane extracts had more than 500 µg/mL antioxidant potential (weak potential). Antioxidant activity is inversely related to the IC_{50} value. Plant extracts having a lower IC_{50} value is more potent towards antioxidant activity [16]. The current findings are supported by earlier research that found the methanolic extract's IC_{50} value to be comparable [17]. Other bioactive substances like carotenoids, tocopherols, and vitamin C also play a major role in antioxidant effects, even though overall phenolic and flavonoid contents frequently have a substantial correlation with antioxidant activity. Climate and environmental variables can also affect antioxidant capacity [18]. The IC_{50} of different solvent extracts of this plant is shown in **Table 1.**

Table 1 IC_{50} of different solvent extracts of the AIK sample.

Part	Plant extracts	IC_{50} (µg/mL)
Aerial parts	Methanol	57.44±0.22
	Ethanol	17.75±0.38
	Ethyl acetate	255.8±0.11
	DCM	>500
	Hexane	>500
	Quercetin	3.431±1.61

*Quercetin = positive control

Antioxidant values are significantly different from each other at $p < 0.05$.

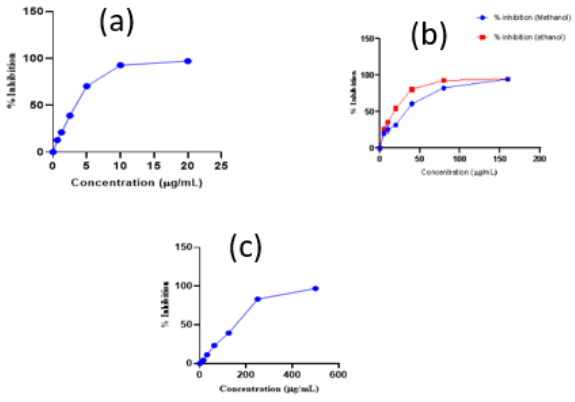

Figure 2: A plot for % inhibition against concentration for a) standard quercetin b) methanol and ethanol AIK extracts c) ethyl acetate extract

Antibacterial Activity

The antimicrobial activity in terms of the zone of inhibition of various crude extracts of the aerial part of *A. indica* (AIK sample) against *K. pneumoniae*, *E. coli*, S. *sonnei,* and *S. aureus,* are shown in **Table 2.**The methanolic extract had a strong antibacterial activity with a ZOI of 21 mm against *Escherichia coli.* This was quite close to the positive control, namely Neomycin 28 mm. Among all extracts, the DCM extract shows very weak antibacterial potential against *K. pneumoniae* (Neomycin control 28 mm). These outcomes align with earlier research on *Artemisia indica* methanolic extract, which has shown similar antibacterial activity against the same bacterial strains [19]. The presence of secondary metabolites like flavonols and rutin is probably what causes the antibacterial action. Furthermore, substances like vitamins, minerals, carotenoids, saponins, and enzymes might possibly be involved in the antibacterial actions that have been noted [20]. The antimicrobial test slides of different solvent extracts are shown in **Figure 3.**

Table 2: ZOI of different solvent extract against SS, KP, SA and E. coli (measured in mm)

Crude extract	*Sigella sonnei*	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*E. coli*
Methanol	13	17	17	21
Ethanol	14	19	19	20
DCM	18	11	14	21
Positive control	28	28	27	28
Negative control	-	-	-	-

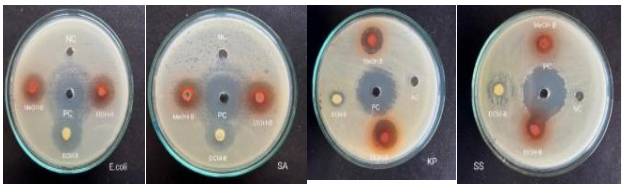

Figure 3 Antibacterial test slides of plant extract (SS = *Shigella sonne,* KP = *Klebsiella pneumonia,* SA = *Staphylococcus aureus,* E. coli = *Escherichia coli*

Chemical Composition of the Aerial Part of *A. indica*

The GC-MS was used to analyse the major phytoconstituents found in the essential oil of the aerial parts of *A. indica.* 39 major phytoconsituents were found in the AID (*A. indica* collected from Dadeldhura) sample. Likewise, 37 phytoconstituents were found in the AIK (*A. inidica* collected from Kanchanpur) sample. *α*-Thujone was present in the greatest portion, which is 15.74% out of 39 compounds in the AID sample of essential oil. 0.54 % of *trans-β*-Ocimene was the lowest percentage of phytoconstituents found in AID sample. The major phytoconstituents found in the AIK sample are eucalyptol, which is 12.50 %. The lowest portion of phytoconstituents found AIK sample is p-cymene (0.72 %) [21].The numerous pharmacological and biological processes are attributed to phytochemicals found in leaves and rhizomes. As an illustration, the essential oil of the aerial component contains α-Thujone, which has larvicidal and insecticidal properties [22]. The yield percentage of essential oil obtained from AIK sample was found to be 0.5 %. Whereas, 0.6 % was the yield of AID sample collected from Dadeldhura. The GC-MS chromatogram of AID and AIK sample are shown in **Figure S1 and S2 (supplementary materials)** The major phytoconstituents found in the essential oil of AID and AIK samples are shown in the **Table 3 and 4.** The structure of major phytoconstituents found in both AID and AIK sample are shown in **Figure 4.**

Table 3: Major phytoconstituents of essential oil obtained from aerial parts of *A. indica* (AID sample)

Peak	R. Time	Area	Area %	Name
1	13.344	1088564	0.72	*α*-Pinene
2	14.034	981627	0.65	Camphene
3	14.034	4772603	3.15	Sabinene

4	15.786	1428941	0.94	Myrcene
5	17.086	1282570	0.85	*α*-Terpinene
6	17.462	1538996	1.02	*p*-Cymene
7	17.670	3966406	2.62	Limonene
8	17.831	11678006	7.71	Eucalyptol
9	17.986	2664232	1.76	*(Z)-β*-Ocimene
10	18.497	818131	0.54	*trans-β*-Ocimene
11	19.119	4406949	2.91	Artemisia ketone
12	20,220	935965	0.62	Linalool, Methyl ether
13	21.345	960994	0.63	4,7,7-Trimethylbicyclo[3.2.0]hept-3-ene-6-one
14	21.459	23837893	15.74	*α*-Thujone
15	21.973	14727263	9.72	*β* –Thujone
16	22.176	336474	2.20	(*4E,6Z*)-Alloocimene
17	22.408	1769040	1.17	3-Caren-10-al
18	23.045	2282163	1.51	3,3a,4,5,6,6a-Hexahydro-3a,4,4-trimethyl-3,5-methylcyclopentapyrazole
19	23.420	4474393	2.95	Camphor
20	24.275	2420996	1.60	*cis*-Myrtanol
21	24.413	2081221	1.37	Isoborneol
22	24.919	4054320	2.68	Terpinen-4-ol
23	25.529	1764789	1.17	*α* –Terpineol
24	26.317	1559776	1.03	3,3a,4,5,6,6a-Hexahydro-3a,4,4-trimethyl-3,5-methylcyclopentapyrazole
25	27.580	920640	0.61	*trans*-chrysanthenyl acetate
26	29.974	5686043	3.75	Lavanduyl acetate
27	30.562	1207248	0.80	Perilla alcohol
28	34.711	920915	0.61	*β*-Cubebene
29	36.109	13579557	8.97	*trans*-Caryophyllene
30	37.547	1254721	0.83	*α*-Humelene
31	38.366	7736026	5.11	*γ*-Curcumene
32	38.493	870838	0.57	*α*-Curcumene
33	38.676	9184888	6.06	*γ*-Cadinen
34	38.827	1184879	0.78	(*E,E*)-*α*-Farnesene
35	39.166	1322310	0.87	*γ*-Amorphene
36	39.332	1376660	0.91	Bicyclogermacrene
37	40.287	3942999	2.61	*δ*-Cadinene
38	45.429	2183281	1.44	*β*-Eudesmal
39	45.511	1255979	0.83	Bulnesol

Table 4: Constituents of essential oil obtained from aerial parts of *A. indica* (AIK sample).

Peak	R. Time	Area	Area %	Name
1	13.348	733878	0.79	α-Pinene
2	14.034	1854573	2.00	Camphene
3	15.213	766902	0.83	Hept-(2*E*)-en-1-ol
4	15.790	1084670	1.17	Myrcene
5	17.446	664083	0.72	*ρ*-cymene
6	17.672	1680765	1.82	Limenone
7	17.834	11573737	12.50	Eucalyptol
8	17.989	1230891	1.33	*trans-β*-Ocimene

9	19.135	5893516	6.37	Artemisia ketone
10	19.533	995925	1.08	*γ*-terpinene
11	20.221	1502446	1.62	2,3-Diazabicyclo[2.2.1]hept-2-ene
12	21.055	727203	0.79	Terpinolene
13	21.344	1511437	1.63	*α*-Thujene
14	21.455	1536492	1.66	*α*-Thujone
15	21.979	1102879	1.19	*β*-Thujone
16	22.113	1917195	2.07	(1.alpha.,4.alpha.,4a.alpha.,7a.alpha.)-4,4a,5,7a-Tetrahydro-8,8-dimethyl-1,4-methano-1H-cyclopentadipyridazine
17	22.408	2750667	2.97	Chrysanthenone
18	22.820	702009	0.76	*trans*-3-Caren-2-ol
19	23.421	8491571	9.17	Camphor
20	24.416	4898821	5.29	Isoborneol
21	24.924	2667933	2.88	Terpinen-4-ol
22	25.371	768058	0.83	*trans*-Isocarveol
23	25.534	1383120	1.49	α-Terpineol
24	25.767	2280880	2.46	Methyl salicylate
25	26.845	894263	0.97	*trans*-Carveol
26	27.263	789001	0.85	*cis*-Carveol
27	29.982	2459630	2.66	Lavandulyl acetate
28	30.586	876402	0.95	Perilla alcohol
29	36.113	8333397	9.00	*trans*-Caryophyllene
30	37.553	759724	0.82	*α*-Humene
31	38.368	3678499	3.97	*γ*-Curcumene
32	38.679	6789691	7.34	*γ*-Amorphene
33	38.831	1172766	1.27	*α*-Zingiberene
34	39.168	791887	0.86	*α*-Murolene
35	39.334	867286	0.94	*α*-Bulnesene
36	40.292	2363768	2.55	*δ*-Cadinene
37	45.433	4059121	4.99	Bulnesol

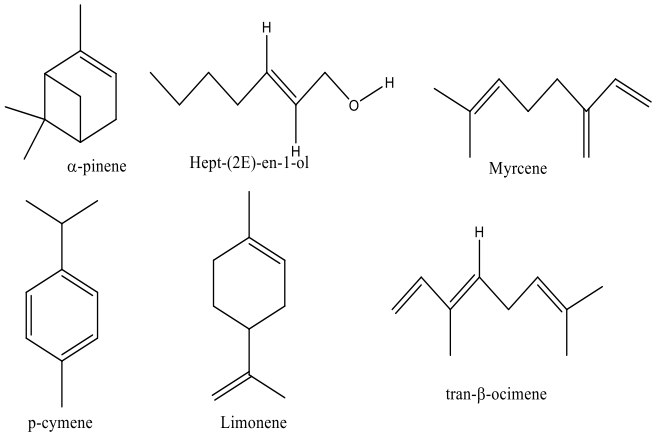

Figure 4: Major phyconstituents found in aerial parts of *A. indica* (both sample AID and AIK)

Biological Activities of Essential Oil

Antioxidant Activity

The AID sample had minimum IC_{50} values with 23.15 ± 1.59 µg/mL, whereas AIK sample exhibits higher IC_{50} values. Such variation may have been caused by differences in the number and the concentrations of secondary metabolites due to plant growth, environmental stress, and genetics. Antioxidant molecules in plants prevent various chronic diseases such as diabetes, cancer, and neurodegenerative disorders [23]. The IC_{50} of AID and AIK sample are shown in **Table 5** and the plot of % inhibition against concentration for AID and AIK sample are displayed in **Figure 5**.

Table 5: IC_{50} values of AID and AIK sample

Plant	Essential Oil	IC_{50} (µg/mL)
Artemisia indica	AID	23.15 ±1.59
	AIK	>500
	Quercetin	3.431±1.61

*Quercetin = positive control Antioxidants values are significantly different from each other at p <0.05

Antibacterial Activity

The essential oil collected from sample AID had maximum zone of inhibition which is 16 mm against *S. aureus* and sample AIK shows minimum zone of inhibition against *S. aureus* which is 10 mm. The positive control for both bacterial strains was found to be 25 mm. Significant zones of inhibition showed that the essential oil had excellent antibacterial action, especially against *Staphylococcus aureus*. A review of the literature showed that several essential oils of Artemisia and the main constituents *of A. indica* essential oil have been shown to have antibacterial properties in the past [24]. The zone of inhibition of both sample AID and AIK are shown in **Table 6**. The test slides of antimicrobial activity are shown in **Figure 6**.

Table 6: Antimicrobial activity of essential oil against *K. pneumoniae* and *S. aureus*

Essential Oil	*Klebsiella pneumoniae*	*Staphylococcus aureus*
AID	13	16
AIK	11	10
PC	25	25
NC	-	-

AID = *A. indica* collected from Dadeldhura

AIK =*A. indica* collected from kanchanpur

PC = Positive control

NC = Negative control

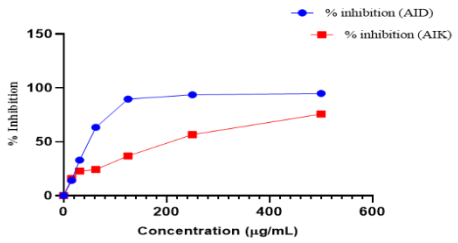

Figure 5: A plot % inhibition against concentration for AID and AIK sample

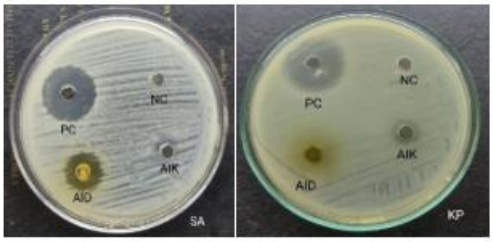

Figure 6: Antimicrobial test slides of essential oil against *K. pneumoniae* and *S. aureus*

Conclusions

The aerial part of *A. indica* collected from

Kanchanpur and Dadeldhura districts were allowed for extraction process. Among the all solvent extracts tested, the ethanolic extract exhibited the highest antioxidant potential as compared to other extract. The methanolic and ethanolic extract of AIK sample shows potent antimicrobial activity against *Escherichia coli*. The essential oils of aerial parts of *A. indica* were extracted by clevenger's apparatus and was subjected to GC-MS analysis. 39 compounds were identified in AID sample and 37 compounds were identified in AIK sample. In AID sample, α-thujone is major phytoconstituents but in AIK sample eucalyptol is major phytoconstituents. This shows phytoconstituents varies based on geographical region. In both sample 21 compounds were common. AID sample has good antioxidant potential as compare to AIK sample. Likewise, AID sample shows good antibacterial activity against *S. aureus* as compare to AIK sample. This medicinal plant has wide range of biological properties so this plant may be used for the development of new drug or medication.

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Author's Contribution Statement

D. R. Jaishi: Methodology, Investigation, Data Curation, Data analysis, Writing: original manuscript, **I. Ojha:** Data analysis, Writing: Review & Editing, **S. R. Ojha:** Statistical analysis, Writing: review & editing, **P. Pal:** Methodology, Data Curation, Formal Analysis, **B. Thalal:** Methodology, Writing: review & editing, **P. N. Chaudhary**: Writing: review & editing, Supervision.

Conflict of Interest

The authors do not have any conflict of interest throughout this research work.

Data Availability Statement

All of the data produced by this study will be provided on request.

Supplementary Materials: (as separate file)

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