SHORT COMMUNICATION

Phytochemical Characterization of *Anisomeles indica* (L.) Kuntze Extract Using GC-MS: Evaluation of Antioxidant and Antimicrobial Properties

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DOI: 10.18811/ijpen.v10i01.18

Abstract

Herbal and other plant-based medicines are effective and safe sources of medication. In this study, the methanolic extract of *Anisomeles indica* (L.) Kuntze leaves were tested to explore their chemo profiling through gas chromatography-mass spectrometry (GC-MS). The samples were also analyzed to evaluate antioxidant and antimicrobial activities. The antioxidant efficacy of the plant extract was evaluated using the FRAP, DPPH, and ABTS methodologies. The results revealed that extract had a high concentration of flavonoids and phenols and exhibited the highest antioxidant properties reflected by the inhibition of free radical DPPH (30.87 ± 0.001%). The plant extracts showed antimicrobial activities against *Pseudomonas aeruginosa* (gram-negative) and *Staphylococcus aureus* (gram-positive) bacteria with 10 and 9 mm inhibition zones, respectively. The GC-MS studies exhibited the presence of 25 compounds. The major constituents are desogestrel (100%), n-hexadecanoic acid (8.59%), 9, 12-octadecadienoic acid (Z, Z) (8.5%), gamma.-sitosterol (7.57%), and palmitic acid, TMS derivative (3.31%). The GC-MS analysis exhibited that leaves consist of bioactive compounds, such as alkaloids, terpenoids, phenolics, and fatty acids.

Keywords: *Anisomeles indica* (L.) Kuntze, Antioxidant, Antimicrobial, GC-MS, Methanolic extract.

Highlights

- *Anisomeles indica* (L.) Kuntze leaf was investigated for the biological properties.
- GC MS analysis revealed presence of 25 bioactive compounds in the herb.
- The plant extract contained high concentration of flavonoids and phenols and exhibited antioxidant properties.
- Leaf extract showed strong antimicrobial activity against bacteria *Pseudomonas aeruginosa* and *Staphylococcus aureus*.
- *A. indica* leaf extracts may be explored as a promising therapeutic approach for the treatment of various diseases.

International Journal of Plant and Environment (2024); **ISSN:** 2454-1117 (Print), 2455-202X (Online)

Introduction

Plants have many roles in medicine, encompassing the utilisation of herbal remedies, sometimes referred to as "medicinal plants." Natural remedies derived from herbs have been used in medicine for long period of time (Astutik *et al.,* 2019). Traditional medicine encompasses comprehensive knowledge, expertise, and practices derived from concepts, beliefs and experiential wisdom that are intrinsic to diverse cultures. Medication are utilised for the purpose of promoting and preserving overall well-being as well as for the treatment of both physical and mental aliment (Zidny *et al.,* 2020). Medicinal plants have the potential to play crucial roles in disease prevention. However, all herbs with active ingredients must undergo scientific study before being available for public use (Khalid *et al.,* 2022).

Humans are continuously exposed to radiations, which may cause damage to DNA, lipids, and proteins, resulting in chromosomal abnormalities. Reactive oxygen species (ROS) also known as free radicals, impair the body's antioxidant defenses. Consequently, the degradation of cell membrane and macromolecules from cell such as nucleic acid, proteins, and fats results in demised cell. The phenomenon induces cellular maturation, resulting in unregulated proliferation (Bouyahya *et al.,* 2021). Oxidative stress occurs once the cell's antioxidant response is disrupted and inadequate. Oxidative stress has the potential to induce the development of various diseases including cardiovascular disability, diabetes, liver infection, cancer, neurological infection, etc. (Engwa, 2018).

The escalating utilization of antimicrobial agents in healthcare facilities contributes to the development of microbe defense against antibiotic agents. Traditionally medicinal plant parts viz, roots, stems, leaves and flowers have been extensively employed for the treatments of numerous human aliment. The plant comprises multiple phytochemicals such as tannins,

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How to cite this article: Fasale, S.B., Nimbalkar, M.S., Mane, M.P., Waghmare, M.B., Khot, S.S. (2024). Phytochemical Characterization of *Anisomeles indica* (L.) Kuntze Extract Using GC-MS: Evaluation of Antioxidant and Antimicrobial Properties. International Journal of Plant and Environment. 10(1), 132-137.

Submitted: 02/01/2024 **Accepted:** 16/02/2024 **Published:** 30/03/2024

flavonoids, phenolic, alkaloids, terpene, and coumarins that exhibit antimicrobial properties (Gonelimali *et al.,* 2018).

Lamiaceae is a widespread aromatic plant family, comprises 236 genera and 7000 species distributed worldwide and may also be referred as the "Mint family" (Chakrabartty *et al.,* 2022). *Anisomeles indica* (L.) Kuntze (also referred as 'Indian catmint') belongs to family Lamiaceae (Sardesai and Yadav 2002). *A. indica* is native in Southern Asia. The erect perennial shrub emits a pleasant scent similar to camphor. The plant is found to grow in nearby areas of developed settlements, particularly in regions with low to moderate elevation (Ulhe and Narkhede, 2013). It has been utilized in numerous experimental studies to substantiate its antioxidant and anti-inflammatory properties (Nasrin *et al.,* 2022). It is utilized in folk medicine to treat various ailments, namely inflammatory skin diseases, liver infections, gastrointestinal infections, abdominal discomfort and immune deficiency (Govindarajan *et al.,* 2016). Several researchers conducted tests to evaluate the anticancerous, anti-anaphylactic, antibacterial, antifungal, analgesic, and antioxidant properties of *A. indica* which are highly valued in conventional medicine (Mohanraj *et al.,* 2015; Bagchi *et al.,* 2019; Lien *et al.,* 2022; Nasrin *et al.,* 2022)

Despite considerable importance of A. indica as a natural medicinal plant and its utilization in treating various diseases, comprehensive research on its antioxidant and antimicrobial properties is limited. The current research paper concerns with the biochemical analysis of *A. indica* using GC-MS analysis and study of its antioxidant properties using FRAP assay, DPPH radical scavenging activity and ABTS assay and antimicrobial properties through zone inhibition method.

Material And Methods

Collection of plant material and preparation of methanol extract.

Anisomeles indica (L.) Kuntze was collected from Masai Plateau, Kolhapur (74.088507 ºE and 16.823377 ºN) situated in the Western Ghats of India. The plant material was identified by using the flora of Kolhapur districts (Sardesai and Yadav, 2002) and deposited in the herbarium of 'The New College Kolhapur' (Maharashtra) with the accession number SBF & SSK-02. The shade-dried leaves were crushed into fine powder using a mixer grinder. The 20 gm fine powder was added to 100 mL of 99.9% methanol (AR grade) and sonicated for 30 minutes. The mixture was further kept for extraction on a rotary shaker for 24 hours. The extract was centrifuged at 5000 rpm for 10 minutes and the supernatant was filtered through Whatman filter paper no.1. The filtrate was stored at 4ºC for further analysis.

In-vitro Antioxidant Activity

FRAP assay

The ferric reducing antioxidant power (FRAP) experiment was conducted with minor changes to the method outlined by Payne *et al.,* in 2013. The reaction mixture was composed of 290 µL FRAP, which included 0.3 M acetate buffer, 5 mL of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ), and 5 mL of 20 mM $FeCl_3$, along with 10 µL of plant samples. The reaction mixtures were subjected to incubation at a temperature of 37°C for 15 minutes. The absorbance was measured 595 nm and the calibration curve was prepared using ascorbic acid. The findings were quantified in expressions of mmol of ascorbic acid per gram of dry weight.

DPPH radical scavenging activity

The antioxidant activity was assessed by utilizing the 2, 2-diphenyl-2-picrylhydrazyl (DPPH) method, following the protocol outlined by Mane *et al.,* (2022) with slight modifications. A 10 µL extract was thoroughly combined with 290 µL of a DPPH solution within an allocated well. The resulting mixtures were then incubated in darkness for 20 minutes. The measurement of absorbance was measured using a 571 nm, and ascorbic acid served as the reference compound. The proportion of DPPH scavenging action was intended using $[(OD_1\text{-}OD_2)/OD_1] \times 100$. The variables OD_1 and OD_2 represent the optical density of the control and test sample, respectively.

ABTS Assay

The evaluation of the antioxidant activity of 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was conducted using Rajurkar and Hande (2011) method, with minor modifications. For this assay, a mixture comprising 20 µL of plant solvent with amount of 1-mg/mL and 180 µL of the ABTS reagent was employed. The plates were employed in an incubator set at ambient temperature for 10 minutes. A control was established using pure ABTS, while ascorbic acid served as the reference compound. The measurement of absorbance was conducted at a wavelength of 734 nm, and the outcomes were intended using the formula $[(OD_1\text{-}OD_2)/OD_1] \times 100$. The variable OD_1 and OD_2 represent the optical density of the control and test sample, respectively.

Antimicrobial Activity through Zone Inhibition Method

The antimicrobial activity of methanolic extracts from *A. indica* leaves was assessed using the zone inhibition method against two bacterial strains: *Pseudomonas aeruginosa* (gram-negative) (MTCC 3541) and *Staphylococcus aureus* (gram-positive) (MTCC 96). Bacteriological dilutions were made using 0.5 McFarland units and the bacteria were cultured on Muller-Hinton agar (MHA) media. These dilutions were then streaked onto separate MHA plates.

In experiments involving bacterial cell lines, DMSO served as the negative control. Aseptic sterilization techniques were employed to create wells on the agar plates. The *A. indica* extracts were carefully dispensed into wells and the plates were gestated at 37°C for 24 hours. The plates were assessed for inhibitory zone around the wells. The analyses were repeated in triplicate.

GC-MS Analysis

The Agilent GC 7890 coupled to an MS 5977 B MSD and an Elite One-Fused HP 5ms capillary column. The carrier gas employed was nitrogen while the injection volume was set 1-mL with persistent flow speed of 20 mL/min, resulting in a split ratio, 20:1. During the GC -MS analysis, the temperature of the injector was retained at 200°C. The initial oven temperature was set at 50°C for 1 minute. Subsequently, the temperature was enhanced to

250 °C with a 5 °C min^{-1} rate and maintained for 1-minute. The temperature was finally raised to 280°C at a rate of 5°C min^{-1} withheld for 9 minutes MS data 40-900 amu were noted at 70 eV with a mass range of a m/z. A single quadrupole mass spectrometer detector at 150°C was used for MS analysis. The scanning frequency was 1.7 scans/ min. The elements were identified using the EI source spectral library NIST 2017.

Results And Discussion

Antioxidant Activity

The FRAP value in the leaves was 14.81 ± 0.03 mM (Table 1). Maqbool *et al.,* (2016) recorded greater FRAP value for *A. indica* leaves in chloroform 85.68± 0.183 mMol and lesser in ethyl acetate 14.44 ± 0.339 mMol.

The antioxidant molecules possess the capacity to counteract the effects of DPPH (2, 2-diphenyl-2-picrylhydrazyl) by means of electron donation which involves the transfer of hydrogen. In the current study, the percentage inhibition of DPPH scavenging activity was 30.87 ± 0.001% for leaves (Table 1). Earlier, Huang *et al.,* (2012) reported higher DPPH scavenging activity of methanolic extract *A. indica* 72.31 ± 0.34 and 88.54 ± 0.36% for BHA. Vinod *et al.,* (2014) studied the antioxidant properties of *A. malabarica* leaves, revealing that the methanol solvent displayed a higher DPPH activity of 71.26 %, whereas the hexane extracts showed a lower DPPH activity of 20.45 %. Krishna *et al.,* (2018) found that ethanolic extracts of *A. malabarica* showed 51.95 ± 0.18% antioxidant activity. In the current studies, leaves exhibited an inhibition of ABTS at a rate of 29.41 ± 0.001% (Table 1). Huang *et al.,* (2012) conducted an antioxidant investigation on *A. indica* in methanolic extracts at various concentrations. They observed 95.86 ± 0.09% activity of ABTS assay at 1.5 µg/mL concentration. Similarly Supriya and Growther (2021) choose methanol, ethyl acetate, water, hexane and chloroform as extraction solvent. They noted that methanolic extracts exhibited a significant ABTS activity of 89.21 ± 0.17% at a concentration of 100 µg/mL and the chloroform extract exhibited a lower activity of 57.90± 0.17%.

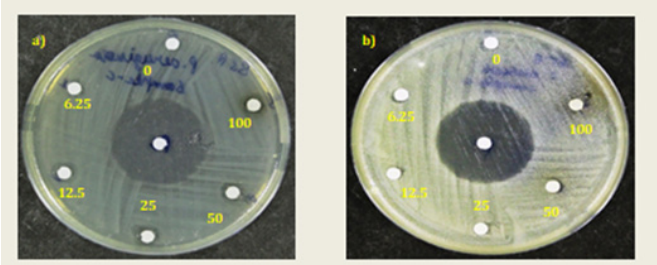

Fig. 1: Antibacterial Potential of *A. indica* methanolic leaf extracts against a) *P. aeruginosa* and b) *S. aureus*. Amount present per well in %. Dispensed volume-10 µL, Positive control-10 µg

Table 1: Antioxidant assay of methanolic extracts of *A. indica* leaf (mean ± SEM of triplicate)

Sample Name	*FRAP mM/g*	*DPPH % inhibition*	*ABTS % inhibition*
AILM	14.81 ± 0.03	30.87 ± 0.001	29.41 ± 0.001

Note: The statistical analysis was carried out using MS-excel program

Antimicrobial Activity

The zone inhibition technique was utilized to estimate the antimicrobial efficacy action of the methanolic solvent from the leaves of *A. indica*. The findings (Table 2 Fig. 1) showed significant antibacterial efficacy of 100 µg/mL concentrations against bacteria *Pseudomonas aeruginosa* (gram-negative) 10 mm and *Staphylococcus aureus* (gram-positive) 9 nm zone of inhibition. Patel and Patel (2013) observed that the methanolic extracts of *A. indica* displayed a zone of inhibition measuring 17.00 ± 1.00 and 16.00 ± 1.00 mm against bacterial strains *S. aureus* and *P. aeruginosa,* respectively. Antil *et al.,* (2019) reported comparable findings in their study on *A. indica*. The methanolic extracts were found to have inhibitory effects against *Pseudomonas aeruginosa* as indicated by a mean diameter of 11.5 ± 0.50 mm. The most potent antifungal activity was observed against *Rhizopus oryzae* with in a mean diameter of inhibition zone 11 ± 0.0 mm.

GC-MS Analysis

The methanolic extract of *A. indica* was analyzed using gas chromatography and mass spectrometry (GC-MS) resulting in the identification of total 25 components (Table 3 and Fig. 2). The table also shows the compounds' retention time, peak area, percentage, and chemical nature. The major phytochemical compounds were desogestrel (100%), 9, 12- n-hexadecanoic acid (8.59%), octadecadienoic acid (Z, Z) (8.5%), gamma-Sitosterol (7.57 %), palmitic acid, TMS derivative (3.31%), benzene, 1,4-dichloro (2.71%), alpha-Tocospiro A (2%).

During the present study, desogestrel was reported to have a higher concentration in leaves (100%). Desogestrel is known for its application in the formulation of hormonal contraceptive methods. These compounds are distinguished by its lipophilic nature, which influences the metabolism of blood lipid mixtures (Atia *et al.,* 2018). γ-Sitosterol, a sterol lipid, exhibited antihyperglycemic properties through its ability to enhance the release of insulin in response to glucose (Yahya *et al.,* 2021). Tripathi *et al.,* (2013) identified γ-sitosterol in *Girardinia heterophylla* leaves which showed efficacy against human lungs and breast adenocarcinoma cancer cells. Plant sterols have exhibited significant anticancer properties. Stigmasterol has been recognized for its anticancerous properties against a range of cancer cell lines, encompassing ovarian, lung, gastric, and breast cancers (Zhang *et al.,* 2022).

Table 2: Antimicrobial activity of methanolic extracts of *A. indica* leaf (mean ± SEM of triplicate)

Microorganism	*Control*	*Zone of inhibition (mm)*				
		6.25 µg/ml	*12.5 µg/ml*	*25 µg/ml*	*50 µg/ml*	*100 µg/ml*
P. aeruginosa	34	8 ± 0.00	8.66 ± 0.57	8.6 6± 0.57	9.33± 0.57	10±0.00
S. aureus	35.6	8±0.00	8±0.00	8.33±0.57	8.66±0.57	9±0.00

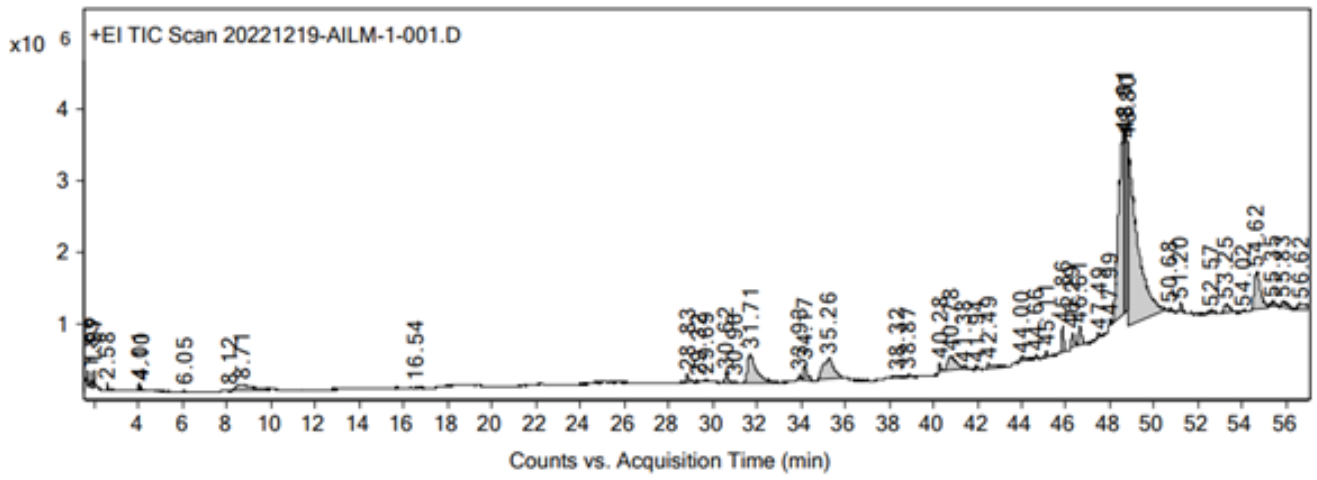

Fig. 2: Gas Chromatogram of methanolic extracts of *A. Indica* leaves

Table 3: The compounds identified through GC MS analysis in the methanolic extract of *A. indica* leaves.

Sr. No.	*Name of Compound*	*RT time (min)*	*Area %*	*Formula*	*Mol. Weight (g/mole)*
1	Trichloromethane	1.664	0.28	$C_2H_5O_2$	119.38
2	Silane, dimethoxydimethyl-	1.826	0.6	$C_3H_6Cl_3$	120.22
3	3,5-Dithiahexanol 5,5-dioxide	1.97	0.73	$C_4H_{10}O_3S_2$	130.22
4	Propane, 1,1-dimethoxy-2-methyl-	2.582	0.21	$C_6H_{14}O_2$	118.17
5	Methyl vinyl ketone	2.83	0.03	C_4H_6O	70.09
6	Propanoic acid, pentyl ester	4.006	0.12	$C_8H_{16}O_2$	144.21
7	Propane, 1,1,3,3-tetramethoxy-	4.102	0.07	$C_7H_{16}O_4$	164.20
8	alpha.-Phellandrene	6.053	0.02	$C_{10}H_{16}$	136.23
9	Heptane, 3,3,5-trimethyl-	8.003	0.04	$C_{10}H_{22}$	142.28
10	3-Ethyl-3-methylheptane	8.118	0.03	$C_{10}H_{22}$	142.28
11	Benzene, 1,4-dichloro-	8.711	2.71	$C_6H_4Cl_2$	147.00
12	Sulfurous acid, octyl 2-pentyl ester	9.964	0.04	$C_{13}H_{28}O_3S$	264.43
13	2- Butanol, 2,3-dimethyl-, acetate	16.313	0.06	$C_8H_{16}O_2$	144.21
14	Neophytadiene	29.69	0.15	C_20H_{38}	278.51
15	Hexadecanoic acid, methyl ester	30.618	0.8	$C_{17}H_{34}O$	270.45
16	n-Hexadecanoic acid	31.708	8.59	$C_{16}H_{32}O_2$	256.4
17	11,14,17-Eicosatrienoic acid, methyl ester	33.926	0.28	$C_{20}H_{34}O_2$	320.50
18	Phytol	34.165	1.09	$C_{20}H_{40}O$	296.53
19	9,12-Octadecadienoic acid (Z,Z)-	35.255	8.5	$C_{18}H_{32}O_2$	280.44
20	3-Hydroxypropyl palmitate, TMS derivative	40.285	0.56	$C_{19}H_{38}O_3$	386.7
21	Palmitic Acid, TMS derivative	40.782	3.31	$C_{19}H_{40}O_2Si$	328.60
22	Squalene	45.86	1.72	$C_{30}H_{50}$	410.73
23	alpha.-Tocospiro A	46.615	2	$C_{29}H_{50}O_4$	462.7
24	Desogestrel	48.709	100	$C_{22}H_{30}O$	310.47
25	gamma.-Sitosterol	54.618	7.57	$C_{29}H_{52}O_2$	414.70

Hexadecanoic acid has demonstrated antifungal, antimicrobial, and antioxidant properties and can lower blood cholesterol levels (Muflihunna *et al.,* 2021). Hrichi *et al.*, (2022) informed therapeutic assets of n-hexadecanoic acid that include an anti-mutagenic, anti-seborrheic, anti-eczematous, cytoprotective, anti-hypoxic and sclerosing activity. Neophytadiene is a type

of diterpenoid with potent biological characteristics such as antioxidant, antimicrobial, anti-inflammatory and antifungal activity (Kumari *et al.,* 2022). Phytol is classified as diterpenes, especially as an unsaturated acyclic alcohol. The compounds exhibit properties associated with antioxidant, cytotoxic, anti-inflammatory, antibacterial, autophagy-inducing, anxiolytic, and immune system-modulating activity (Olivia *et al.,* 2021).

Conclusion

The present study exhibited that the methanolic extracts have a decent phytochemical source. The *A. indica* leaves contain phytochemicals, namely phenolics, flavonoids, terpenoids, coumarins, tannins, saponins, and significant antioxidant and antibacterial activities. The existence of antioxidant and antimicrobial substances viz, neophytadiene, hexadecanoic acid methyl ester, phytol, 3-ethyl-3-methylheptane, and alpha.-phellandrene in *A. indica* leaves exhibits its capacity to function as a promising therapeutic option for controlling many diseases caused by microbes.

Acknowledgements

The authors express their gratitude to the Head Department of Botany, Shivaji University Kolhapur for their provision of essential infrastructural facilities to perform experimental work and valuable guidance.

Authors Contribution

Fasale S.B: Material collection and preparation of extracts. Khot S.S: Writing original draft and editing. Waghmare M.B: Review and editing. Nimbalkar M.S: Conceptualization, supervision and validation. Mane M.P: Assisted in sample collection and data interpretation.

Conflict Of Interest

The author asserts that there is no conflict of interest.

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