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Morpho-chemical Profiling of *Anisomeles indica* (L.) Kuntze from the Western Ghats using SEM–EDX and FT-IR Analytical Study

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Abstract

The current study focuses on the characterization of *Anisomeles indica* (L.) Kuntze stem, a medicinal plant native to the Western Ghats, using Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM-EDX) and Fourier Transform Infrared Spectroscopy (FT-IR). SEM-EDX provided insights into the surface morphology and elemental composition of the stem. The EDX was employed to analyze the mass percentage, while SEM showed the presence of different elements viz., carbon, oxygen, sodium, magnesium, aluminum, silicon, phosphorus, chlorine, calcium, magnesium, and iron. The carbon was the highest component (45.95%) as compared with other minerals. FT-IR analysis was performed to identify peak values corresponding to various functional groups, including hydroxyl, alcohols, phenols, alkyl halides, alkanes, proteins, carboxylic acids, and lipids. The findings revealed the presence of essential phytochemicals and trace elements, confirming the therapeutic potential of *Anisomeles indica*. This study enhances the understanding of its structural and chemical properties, supporting its traditional medicinal applications and future pharmacological research.

***Key words*:** *Anisomeles indica,* SEM-EDX, Western Ghats, Medicinal plant

Medicinal plant products have been used for the treatment of human ailments since the dawn of human habitation. Plants and plant components that have nutritional and medicinal value are known as medicinal plants. Nutritional uses of therapeutic plants have persisted despite the development of several bioactive components for disease therapy. Around 70,000 plant species have been traditionally used in ethnomedicine worldwide, with their analgesic properties in folk medicine [1]. According to the World Health Organization (WHO), more than 80% of the world's population still relies primarily on herbal medicine or plant-based medicines to treat various diseases [2]. A bioactive compound contributes the therapy of medicinal plants, influencing drug discovery and traditional healing practices [3]. The mint family (Lamiaceae) is widely used in traditional medicine it includes 236 genera and 7000 species distributed worldwide and 45 genera and 574 species in India [4-5]. The genus *Anisomeles* R. Br. is a member of the family Lamiaceae and is usually known as 'Indian Catmint' and it is native of South East Asia [6]. The genus has been traditionally used for its anti-allergic, anti-cancer, anti-anaphylactic, antibacterial, antifungal, and analgesic effects [7]. *Anisomeles indica* (L.) Kuntze belongs to the Lamiaceae family [8]. *Anisomeles indica* is native to northern Australia, Malaysia, and Southeast Asia is a perennial or annual shrub with a height of 50 to 100 cm and a pleasant aromatic fragrance due to the presence of volatile essential oils [9]. Flowers are in various shades of blue, white, and pink from October to November [10]. The plant *Anisomeles indica* is used to treat stomach, dyspepsia, fever, urinary tract infection, convulsions, skin disorder and tetanus [11].

The pharmaceutical industry has greatly benefited from the application of SEM-EDX and FT-IR technology. SEM-EDX is an invaluable tool for comprehensive elemental analysis, as it is essential for identifying essential nutrients, trace elements, and potential contaminants such as heavy metals [12]. It aids in understanding plant physiology, environmental interactions, and the impact of pollutants. Its application extends to fields such as herbal medicine authentication, soil-plant interactions, and nanotechnology research. Additionally, SEM provides detailed imaging of plant structures, while EDX detects and quantifies the elemental composition of tissues [13]. FT-IR is a widely used analytical technique for identifying functional groups in chemical compounds. In addition to its role in quality control and industrial processes, it is extensively employed in the analysis and authentication of herbal medicines [14].

However, the available information regarding the phytochemicals that have been studied on *Anisomeles indica* is limited. Using SEM-EDX and FT-IR, the objective of this study was to investigate the methanolic extract of *Anisomeles indica* stem for the detection of phytoconstituents and bioactive components.

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MATERIALS AND METHODS

Collection of plant material and preparation of methanolic extract

Anisomeles indica (L.) Kuntze was collected from Kerli Road, Kolhapur (74.1841 °E and 16.7514 ºN) situated in the Western Ghats of India. The specimens were identified using the Flora of the Kolhapur Districts [8] and cataloged in the herbarium of The New College, Kolhapur (Maharashtra) under the accession numbers SBF and SSK-02. The shade-dried leaves were crushed into fine powder using mixer grinder. The fine powder of stem was used for SEM-EDX.

Sample preparation for scanning electron microscope

The 2 mg fine powder of the samples was placed on adhesive tape and coated with a 5 nm layer of gold. This specimen was scanned using a wide angle X-ray beams at an acceleration voltage 20.00 kV and the microphotographs were captured at magnification 500x using SEM Jeol model JSM-IT200 from Japan provided by the Rayat Shikshan Sanstha's Instrumentation Department Satara.

FT-IR analysis

The characteristics of auxochromic functional groups present in the powder of selected plants were recorded using FT-IR spectrophotometer (Bruker 4600 type A). The spectrum was loaded in the range from 400-4000 cm^{-1} with the resolution of 4 cm^{-1} and scanning speed 2 mm/sec. Peaks in the spectrum were analyzed in accordance with the literature [15].

RESULTS AND DISCUSSION

SEM-EDX analysis

Energy Dispersive X-ray (EDX) analysis of the stem exhibited that carbon and oxygen were the chief elements, accounting for 45.95 ± 0.27% and 43.69 ± 0.54% (weight %) respectively (Table 1). Therefore, energy dispersive spectroscopy was used to determine the relative abundance of the elements including carbon, oxygen, magnesium, phosphorus, chlorine, potassium calcium, manganese, iron, and zinc present in the sample (Table 1).

The major proportion of carbon and oxygen is attributed to the predominance of organic biomolecules such as cell wall components, middle lamella lignin, and secondary metabolites. The results are in consensus with the previously recorded elemental profiles reported for medicinal plants which are rich in phenolics and terpenoids [16]. Several elements that are crucial for metabolism, growth, development and proper functioning of our bodies cannot be synthesized by our bodies, and we need to obtain them form various foods sources and drugs [17]. The present study showed found that carbon had the highest weight concentration (45.95±0.27 %) amongst different elements in the extract followed by oxygen (43.69±0.54 %). The other element noticed were magnesium (0.62±0.05 %), phosphorus (0.23±0.03 %), chlorine (0.71±0.04 %), potassium (3.61±0.10 %), calcium (0.91±0.06 %), manganese (0.04±0.03 %), iron (2.49±0.86 %), zinc (0.56±0.09 %), and cobalt (1.19±0.44 %).

Potassium (3.61 ± 0.10%) was another abundant macro-mineral revealed after C and O. Potassium plays a vital role in several processes, including osmotic regulation, enzyme activation, and secondary metabolite synthesis. As potassium salts are associated with anti-inflammatory properties and diuretic effect; higher K concentration in *A. indica* stem can be attributed to its therapeutic properties [18]. Calcium (0.91 ± 0.06%), magnesium (0.62 ± 0.05%), and phosphorus (0.23 ± 0.03%) were detected in moderate concentrations. These elements are part of structural components of plant body, cell wall and chlorophyll.

Micro elements including iron (2.49 ± 0.86%), zinc (0.56 ± 0.09%), manganese (0.04 ± 0.03%), and cobalt (1.19 ± 0.44%) were detected. Presence of these micronutrients supports the ethnomedicinal use of *A. indica*, as trace elements usually increase the pharmacological activity through synergistic interactions with phytochemicals. The presence of chlorine (0.71 ± 0.04%) signifies nutritionally important mineral, confirming its medicinal relevance.

Table 1 The percentage of elements identified through EDX in *Anisomeles indica* stem

Element	Stem (%)	Atom %
Carbon (C)	45.95 ± 0.27	56.27 ± 0.33
Oxygen (O)	43.69 ± 0.54	40.17 ± 0.50
Magnesium (Mg)	0.62 ± 0.05	0.37 ± 0.03
Phosphorous (P)	0.23 ± 0.03	0.11 ± 0.01
Chlorine (Cl)	0.71 ± 0.04	0.29 ± 0.02
Potassium (K)	3.61 ± 0.10	1.36 ± 0.04
Calcium (Ca)	0.91 ± 0.06	0.33 ± 0.02
Manganese (Mn)	0.04 ± 0.03	0.01 ± 0.01
Iron (Fe)	2.49 ± 0.86	0.66 ± 0.23
Zinc (Zn)	0.56 ± 0.09	0.13 ± 0.02
Cobalt (Co)	1.19 ± 0.44	0.30 ± 0.11
Total	100	100

The notably high content of oxygen appears to play a significant role in various biochemical and physiological processes within the cells of the plant. Oxygen is essential for several cellular biochemical and physiological processes while magnesium accounts for 40% of muscle and 1% of fluid in the human body [17]. Magnesium plays an important role in the human body. It contributes to about 40% of muscle tissue about 1% of the bodily fluid and activates over 300 enzymes [19]. Chlorine is a vital element that plays an indispensable role in the neurophysiological functions of the digestive and renal system [20].

Calcium is an essential component for the bones, teeth, and cellular membranes. It also plays a crucial role in regulating blood pressure [21]. Iron is primary component of blood haemoglobin and myoglobin, and it is also present in numerous enzymes that are essential for human [22]. Cobalt is an important element for various industrial uses [23] and zinc is known to have beneficial effect on fertility, overall health and various metabolic processes such as cell growth, tissue differentiation and the treatment of aliments [24].

Hemoglobin, myoglobin and a plethora of nutrients rely on iron for their structure and function [25]. Numerous studies

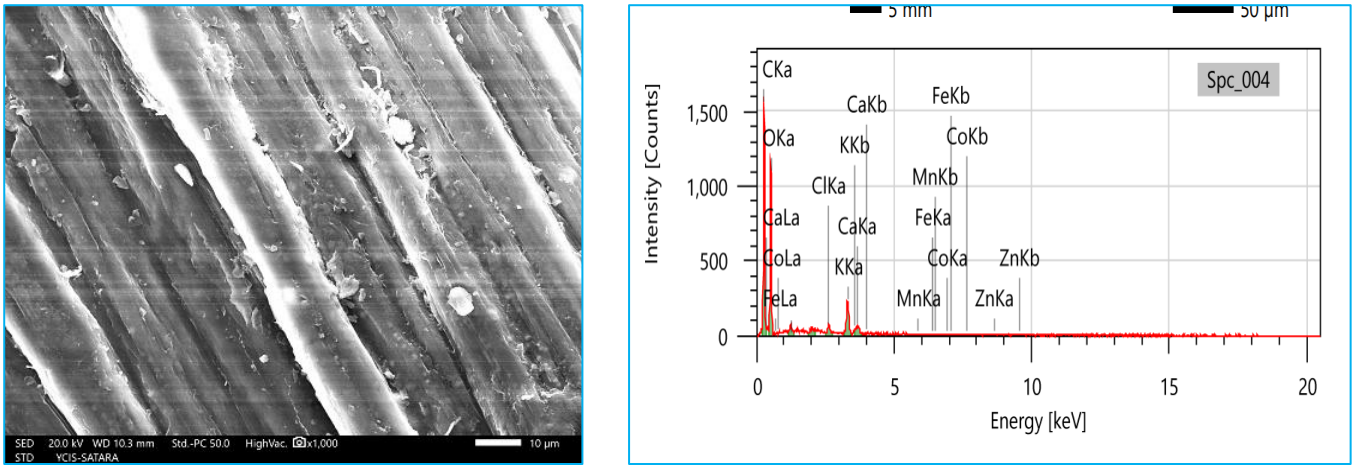
have demonstrated a relation between foods and the occurrence of cardiovascular diseases. The presences of all these elements in stem of *Anisomeles indica* its nutritive value.

FT-IR analysis

Fourier Transform Infrared (FTIR) spectroscopy provided insights into the chemical nature of compounds present in the stem extract (Table 2, Fig 2). The spectrum revealed some important characteristic absorption bands representing array of functional groups. The peak at 3727.73 cm^{-1} and 3423.03 cm^{-1} attributed to the O-H stretching vibration associated with alcohols and phenolic compounds. It suggests the presence of hydroxyl-rich molecules like polyphenols, flavonoids, and glycosides. These compounds are responsible for antioxidant, anti-inflammatory, and antimicrobial properties reported for *Anisomeles indica*.

Table 2 FTIR analysis of *Anisomeles indica* stem

Wavelength (cm^{-1})	Functional group	Compound class	Characteristic absorption	Intensity
3727.73 3423.03	O-H stretch	Alcohol	3000-4000	Strong
2924.52 2360.44	C-H stretching	Alkane	2850-3000	Strong
2337.3 2295.84	O-H stretching	Carboxylic acid	2300-2400	Weak
1604.48	N-H bend	Amines	1580-1650	Medium
1022.09	C-O stretch	Carboxylic acid	1000-1320	Strong
676.892 472.474	C-H bend	Alkynes	600-800	Strong



(a) (b)

Fig 1 Elementary analysis of *A. indica* stem: a) Shows area from where energy dispersive X-ray has been taken and b) Energy dispersive X-ray spectroscopic peak of the sample

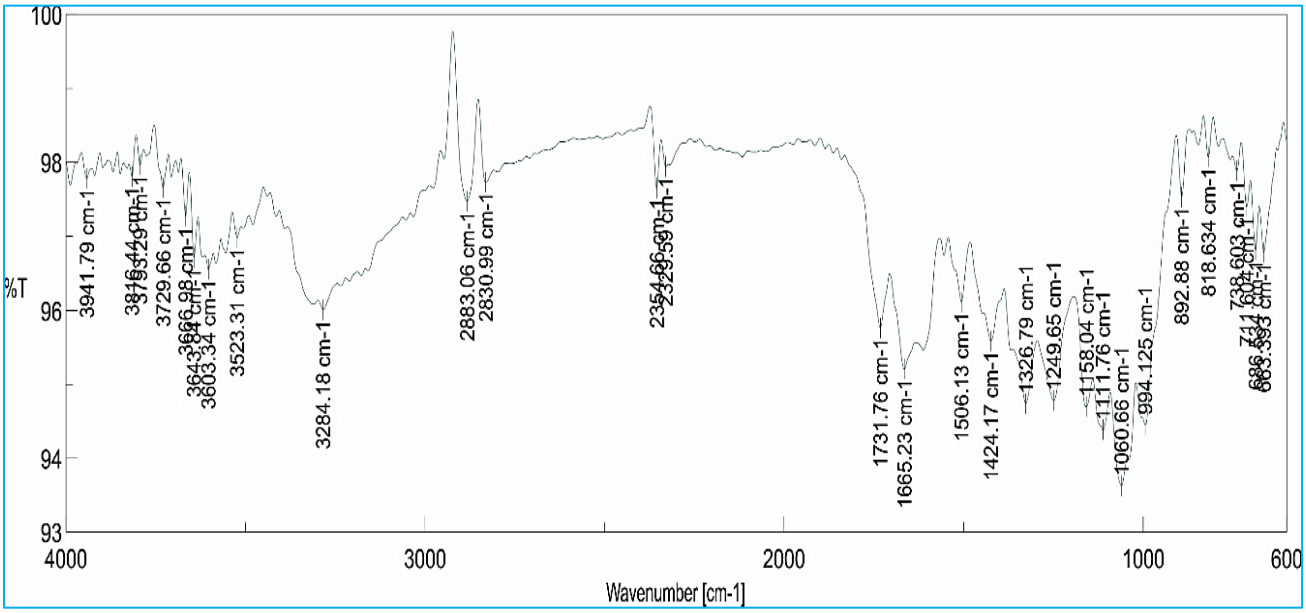


Fig 2 FTIR spectrum of *Anisomeles indica* stem

A noticeable spectral peak at 2924.52 cm^{-1} can be correlated with C-H stretching of aliphatic chains (alkanes). The peak indicates the presence of lipids, terpenoids, and other hydrocarbon structures. Terpenoids are characteristic of many members of the Lamiaceae family and are often responsible for aromatic and pharmacological properties.

The peaks observed at 2360.44 cm^{-1} and 2337.3 cm^{-1} indicates the presence of carboxylic acid with O-H stretching vibration. It suggests the presence of organic acids or ester derivatives. These compounds are responsible for antimicrobial and preservative properties. The peak at 1604.48 cm^{-1} corresponds to the N-H bending vibration in the amine compound, suggesting presence of nitrogen-containing compounds like alkaloids or proteins. Alkaloids have pharmacologically significance and contribute to analgesic or antimicrobial properties. The carboxylic acid was indicated by a peak at 1022.09 cm^{-1} with strong intensity. The peak corresponds to carboxylic acids, alcohols, or ethers, indicating the presence of polysaccharides and glycosidic compounds. These molecules usually responsible for immunomodulatory and antioxidant activities. The prevalence alkyne group was denoted by a strong intensity peak at 676.89 cm^{-1} which displays to C-H bending and supports presence of complex hydrocarbon structures such as secondary metabolites.

According to Sheeba *et al.* [26], the FTIR spectroscopy analysis of leaves of *Anisomeles malabarica* revealed the presence of alkanes, hydroxyl groups, aliphatic, nitrogen and oxygen compounds. Packialakshmi and Nilofer [27] also conducted the FTIR analysis of *Anisomeles malabarica* leaves, flower, stem and boiled leaves. They found presence of various functional groups including halogen, aldehyde, carboxylic acid and alkanes. Kavimani *et al.* [15] conducted FTIR spectroscopy for analysis of *Anisomeles malabarica* leaves. They noticed a broad-spectrum peak at 3774 cm^{-1} and 3448 cm^{-1} which was associated with the strong O-H stretching of alcohol compounds. In present investigation, peaks at 3786.54 cm^{-1} and 3296.71 cm^{-1} were observed, indicating O-H stretching vibration associated alcohol functional groups present in the leaves of *A. indica*. They also observed that the peak at 2924 cm^{-1} and 2855 cm^{-1} corresponds to the C-H stretching vibration exhibited by alkanes functional group.

CONCLUSION

FTIR spectroscopy analysis revealed the presence of several auxochromic functional groups, which contribute to the bioactivity and chemical properties of the extracts. These functional groups included aromatic group, alcohol, carbonyl group, alkane, ether, and carboxylic group. The presence of these groups suggests a complex chemical structure in the extracts potentially enhancing their pharmacological properties and interactions with biological systems. The *Anisomeles indica* plant species, showed presence of 11 essential elements necessary for metabolism, growth, development and proper functioning. Among these, carbon and oxygen were found in the highest amounts supporting energy production and cellular processes. In contrast, sodium and copper were present in smaller quantities, yet they are still crucial for specific biochemical functions including nerve signaling and enzyme activity.

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Author's contribution

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

Conflict of interest

The author asserts that there is no conflict of interest.

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