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STUDY ON ANTIOXIDANT AND PHYTOCONSTITUENTS POTENTIAL OF *Anisomeles indica*

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ABSTRACT

Anisomeles indica Kuntze (A. indica) is utilized in the treatment of various ailments. The current investigation aimed to conduct preliminary phytochemical screening, UV-Visible spectrophotometric analysis, FT-IR characterization, and to evaluate the biological activities of extracts derived from A. indica. The leaves of A. indica were subjected to Soxhlet extraction. Subsequently, the crude extracts were analyzed for their phytochemical composition and quantitatively assessed for phenolic and flavonoid content. The results from UV-Vis spectrophotometric and FT-IR analyses further confirmed the presence of bioactive compounds in the extracts of A. indica. The antioxidant potential of these extracts was evaluated using DPPH assays and metal chelation tests. The results indicated that the methanol extract of A. indica exhibited the highest yield. Preliminary phytochemical screening, along with UV-Vis spectrophotometric and FT-IR fingerprinting, provided substantial evidence for the presence of significant bioactive constituents. Notably, the methanol extract of A. indica demonstrated considerable total phenolic and flavonoid content, as well as total antioxidant capacity (TAC) compared to other extracts. These properties are linked to pronounced antioxidant and metal-chelating activities. The outcomes of this study suggest that A. indica extracts possess antioxidant capabilities, as evidenced by their DPPH radical scavenging potential and ability to chelate metal ions. These attributes are associated with elevated levels of flavonoids and phenolics, as well as unique secondary metabolites. The findings underscore that A. indica is rich in active phytoconstituents, which may serve as a valuable resource for effective therapeutic interventions.

Keywords: Phytochemicals; UV-Visible spectroscopy; *Anisomeles indica* Kuntze; FTIR**;** Antioxidant; Metal chelation.

INTRODUCTION

Medicinal plants have been utilized since ancient times for the treatment of various ailments, enhancement of food flavor, preservation, and prevention of diseases, including epidemics. The therapeutic properties of these plants are primarily attributed to the active compounds generated through secondary metabolic processes. Different cultures have historically relied on plants for medicinal purposes, and the pharmaceutical sector continues to harness these natural

resources for the development of numerous effective drugs due to the presence of specific bioactive constituents. Anisomeles indica Kuntze, belonging to the Lamiaceae family, exhibits significant therapeutic potential due to its rich content of biologically active phytochemicals. The Lamiaceae family, commonly known as the mint family, is recognized as one of the most important families of medicinal plants, characterized by their aromatic properties and herbaceous or shrubby forms. A. indica, also known as Malabar catmint, is a perennial, woody shrub that thrives in the wild across Southeast Asia, including regions such as China, India, Australia, the Philippines, Vietnam, Indonesia, Thailand, and Taiwan. This plant is noted for its medicinal attributes, which include aromatic, astringent, carminative, and tonic effects. Furthermore, it exhibits insecticidal, analgesic, antipyretic, and anti-inflammatory properties. The leaves of A. indica have been traditionally employed to address a variety of health issues, such as hypertension, inflammatory skin conditions, immune deficiencies, and disorders of the liver and gastrointestinal tract. Previous studies on A. indica have demonstrated its radical scavenging abilities, cyclooxygenase inhibition, anti-inflammatory effects, and acetylcholinesterase inhibition. Beyond these biological activities, the aerial parts of A. indica, particularly the leaves, are utilized in the treatment of conditions including rheumatism, epilepsy, paralysis, convulsions, spasms, pregnancy-related issues, fever, dyspepsia, gastrointestinal disturbances, and intermittent fever. Additionally, the leaves are believed to provide relief for psoriasis, chronic rheumatism, and skin eruptions. The A. indica plant can be applied in both fresh and dried forms for treating skin infections, as a remedy for snakebites, and for various other therapeutic purposes.

MATERIALS AND METHODS

Chemicals

Solvents such as methanol, ethyl acetate, chloroform, and hexane were obtained from SD Fine-Chem, India. Gallic acid, butylated hydroxylanisole (BHT), EDTA sodium salt, catechin, aluminum chloride, sodium nitrite, sodium carbonate, and sodium hydroxide were sourced from Sigma-Aldrich, Germany. Additionally, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferrozine, and ferric chloride were acquired from Hi-media Laboratories, India. The Folin-Ciocalteu reagent and molybdate reagents were procured from Lobachemie, India. This study utilized analytical grade chemicals and solvents, with all laboratory reagent solutions prepared fresh as required.

Plant collection and authentication

Pre-flowering stage *A. indica* plant leaves were harvested during monsoon (between July to September) from the field of Glocal University, Saharanpur of the Uttar Pradesh state. The plant was identified and validated by Plant taxonomist at Department of Botany, Glocal school of Science,Glocal University, Saharanpur Uttar Pradesh , India. The voucher specimens were deposited for future use.

Preparation of crude extracts

The leaves of A. indica were utilized for extraction purposes. Freshly collected leaves were separated and thoroughly washed, then shade-dried at ambient temperature. Subsequently, they were crumbled using a blender and ground into a coarse powder, which was then sieved to obtain a uniform particle size. This powder was subjected to solvent extraction via a Soxhlet extraction apparatus. The extracts obtained were filtered, and the resulting filtrate was concentrated using a rotary evaporator (Evator, Medica Instruments, Mumbai, India). The concentrated filtrate underwent freeze-drying with a Penguin Classic Plus (Lark) at a pressure of 0.5 mbar and a temperature of -60°C. The final extracts were weighed, and the yield percentage was calculated. Additionally, the yield percentages, consistency, and color of the various extracts were documented (Table 1).

Preliminary phytoconstituents evaluation of *A. indica* extracts

The preliminary phytoconstituents analysis was done to investigate qualitative detection. Analytical results from these qualitative tests were based on the precipitate formation or color intensity (17). The standard tests outlined in the

literature were employed to validate the existence of phytoconstituents including glycosides, alkaloids, phenolic, saponins, terpenoids, and steroids.

Quantitative assessment of total flavonoid and phenol content

Total phenol

The amount of total phenolics in *A. indica* extracts were enumerated by Folin- Ciocalteau reagent (FCR) method (18, 19). The total phenolic content of *A. indica* extracts is expressed by way of Gallic acid equivalents (GAE) per gram dry weight of the extract.

Total flavonoid

Quantitative assessment of flavonoid content in *A. indica* extracts and standard quercetin of various concentrations enumerated by the aluminum chloride a colorimetric method (20, 21). The outcomes were expressed by way of catechin equivalents (CE)/g dry weight of the plant extract.

UV-VIS spectrophotometric analysis of *A. indica* extracts

A.indica extracts were employed for UV- VIS spectrophotometric analysis recorded in a single-beam UV- VIS spectrophotometer (Systronics-119) scan range from 200-800 nm with a scan speed of 400 nm/min (15, 17). The characteristics of the absorption spectrum and absorbance were monitored.

Fourier-transform infrared spectrometer (FTIR) analysis of *A. indica* extracts

A fourier-transform infrared spectrometer (FTIR) was employed to obtain spectra that were utilized to assess the structural attributes of the selected plant extract. Lyophilized extracts were utilized for the FTIR analysis because they enhance the intensity of spectral bands while limiting interference from water and other organic solvents. FTIR spectrum has been recorded on Bruker alpha Eco-ATR, Optics, (attenuated total reflectance), Germany, associated with (ZnSe) a reflection crystal. The Spectra has been recorded at ambient temperature (200 C) using OPUS software (v. 5.5, Bruker Optics, Germany) for processing and frequencies ranging from 4000 to 600 cm-1 (22).

Evaluation of total antioxidant capacity of *A. indica* extracts

A. indica extracts and standard ascorbic acid of various concentrations were utilized to evaluate their total antioxidant capacity by employing phosphor- molybdenum reagent (23, 24). The optical density (OD) was monitored at 695 nm using a spectrophotometer (ELICO, India) along with a reagent blank. Total antioxidant capacity is described by a way of ascorbic acid equivalents (AAE) per dry weight of the extract (25).

Assessment of the free radical scavenging potential of extracts of *A. indica* by the DPPH assay

The antioxidant potential of *A. indica* extracts was determined, based on the extremely stable α, α -diphenyl-β-picryl hydrazyl scavenging capability (26).

The purple stable radical DPPH solution transformed to a yellowish non-radical DPPH solution by *A. indic*a extracts, at 510 nm the change in absorbance was assessed by employing UV–Visible spectrophotometer (ELICO), by utilizing the following equation for percentage of radical inhibition evaluation.

% inhibition= AControl−AExtract/AControl ×100

Where A Control is the absorbance of the control and A Extract is the absorbance of the extracts

Metal chelating activity of *A. indica* extracts

Chelation of Fe2+ (ferrous ions) by the extracts of *A. indica* and standard EDTA (ethylenediamine tetra acetic acid) was estimated (27). The formation of chromogenic Fe2+-ferrozine complex was prevented in the presence of *A. indica* extracts, which implies that it extracts chelate iron. Utilizing a UV-Visible spectrometer, optical density (OD) was monitored at 510 nm. The outcomes were expressed by a way of IC50 value, and the percentage of inhibition of the formation of the Fe2+-ferrozine complex was calculated by utilizing the below formula. % inhibition = A control−A extract/A control x 100

Where AControl is the absorbance of the control and AExtract is the absorbance of the extract.

Statistical analysis

Each trial was assessed in triplicates, data were tabulated as mean ± SD (standard deviation). IC_{50} (The IC_{50} value is the 50% of inhibition of its activity under the assay conditions) values, from the *in vitro* data, were ascertained by regression analysis using Graph pad prism software, MS-excel program, and Origin labs software.

RESULTS

The yield of *A. indica* extracts

The various solvents employed had a different impact on the yield of extract. In contrast to *A.indica* methanol extract, hexane, chloroform, and ethyl acetate extracts displayed lower yield. The percentage of *A.indica* methanol extract obtained after freeze-drying was found to be 6 ± 0.5g/100 g dry weight of plant material. The methanol extract was found to possess the maximum yield among all extracts, implying that the methanol has substantial extractable efficacy in solvent extraction of phytoconstituents from plant matter. The percentage of yield of extracts, consistency, and color of *A.indica* extracts were represented in Table 1.

Phytochemical screening of *A. indica* extracts

Bioactive phytoconstituents' evaluation of *A. indica* extracts illustrated the existence of glycosides, alkaloids, tannins, phenolics, flavonoids, steroids, terpenoids, and saponins, based on the visual appearance and precipitation formation resulting from the addition of chemical reagents, results are depicted in Table 2.

Quantitative assessment of total flavonoid and phenol content

Total phenol

The total phenolic content of *A. indica* methanol extracts were found to be significantly high, contrary to other extracts. The outcomes of total phenol assessment in extracts of *A. indica* were depicted in Table 3. A strong positive linear interconnection is present between antioxidant activity and high phenolic content.

Total flavonoid

A. indica crude methanol extract has been found to comprise 34.6 ± 0.3 mg CE/g. The methanol extract of *A. indica* was found to be extensively high in total flavonoid quantity. A strong linear interrelationship occurs between flavonoid content and antioxidant activity, a phenomenon attributed to the scavenging of free radicals by *A. indica* extracts. The findings of the total flavonoid content of *A. indica* extracts were shown in Table 3. Outcomes were estimated utilizing a linear calibration graph of catechin.

Table 1: Percentage of the yield of extracts of *Anisomeles indica* Kuntze

***A. indica* extracts**	**Yield percentage (g/100 g of dry weight)**	**Consistency**	**Color**
Hexane	3.2 ± 0.5	Greasy	Blackish
Chloroform	2.6 ± 0.1	Sticky	Deep Greenish
Ethyl acetate	1 ± 0.5	Powder	Dark brownish
Methanol	6 ± 0.5	Sticky	Brownish

Table 2: Phytochemical screening of *A. indica* extracts

Phytochemical tests	**Hexane**	**Chloroform**	**Ethyl acetate**	**Methanol**
I. Alkaloids				
Dragendroff's test	++	++	++	-
Mayer's test	++	++	++	-
Hager's test	++	++	++	-
Tannins and Phenolics				
$FeCl_3$ test	-	-	-	+
Potassium dichromate test	+	-	+	-
Iodine test	+	+	+	-
HNO3 test	+	+	+	-

II. Flavonoids				
$FeCl_3$ test	-	-	+	++
Alkaline test	-	-	++	++
Lead acetate test	+	+	+	++
III. Steroids				
Salkowski reaction	+	+	+	+
Libermann-Burchard reaction	+	+	+	+
IV. Terpenoids				
Salkowski reaction	+	+	+	+
Libermann-Burchard reaction	+	+	+	+
V. Saponins				
Foam test	-	-	+	++
VI. Glycosides				
Killer-Killiani test	+	+	+	++
Anthraquinone glycoside test	+	+	+	-

Values are represented as mean ± SD (standard deviation) in triplicates.

++, indicates high presence; +, indicates faint presence; –, indicates the absence

Table 3: Quantitative estimation of total phenolic content and flavonoid content of *A. indica* extracts.

***A. indica* extracts**	**Total phenolic content (mg GAE/g of dry mass)**	**Total flavonoid content (mg CE/g of dry mass)**
Hexane	0.5 ± 0.1	9.3 ± 0.2
Chloroform	0.08 ± 0.01	10.1 ± 0.3
Ethyl acetate	0.55 ± 0.16	27.5 ± 0.2
Methanol	1.2 ± 0.10	34.6 ± 0.3

GAE-Gallic acid equivalents and CE-Catechin equivalents.

Values were represented as mean ± SD

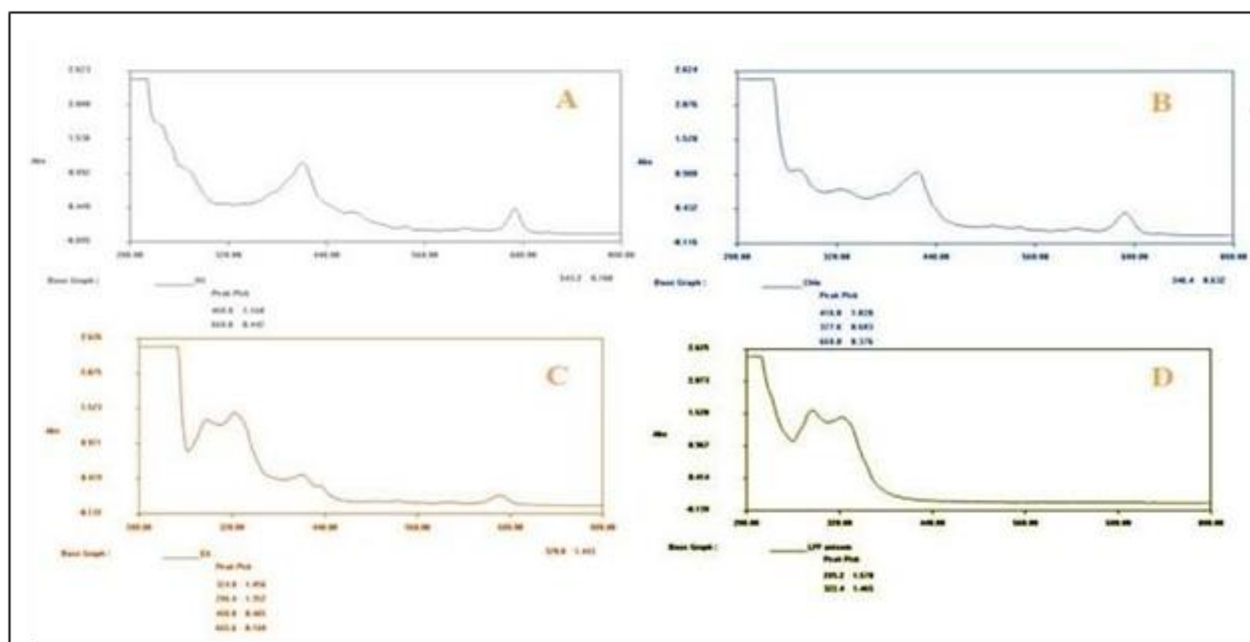

Fig. 1: UV- Visible spectrometric analysis of *A. indica* extracts, **a.** hexane extract, **b.** chloroform,**c.** ethyl acetate, **d.** methanol.

UV-Visible spectrophotometric analysis of *A. indica* extracts

Presence of bioactive molecules in the *A. indica* extractwas demonstrated by the UV-Visiblespectrophotometric method (Fig. 1). The hexaneextract of *A. indica* displays two absorption spectra at

408.8 nm and 668.0 nm. Chloroform extract has three absorption spectra notably, 416.0, 377.6, and 668.0 respectively. The ethyl acetate extract of *A. indica* has four absorption values at 324.8, 286.4, 408.8, and 665.0while *A. indica* methanolic extract shows two major absorption regions such as 285.2 and 322.4. The presence of flavonoids and phenolic compounds was illustrated by UV- visible spectrum peak values between 230-290, 300-360, and 234-676 nm shown
(Fig. 1).

Fourier transform infrared spectrometer (FTIR) of
***A. indica* extracts**

FTIR was employed to investigate the structural fingerprint information such as different types of chemical bonding, stretches, and their functional groups of phytoconstituents that occur in *A. indica*extracts. FTIR is sophisticated biophysical equipment that assists the development of the spectrum and illustrates the accurate and precise wavelengths of the electromagnetic spectrum which are absorbed in the infrared range. Distinct phytoconstituents can be distinguished because this absorbance spectrum has been quite identifying of the specific compound. To explore the bioactive components in *A.indica* extract FT-IR spectrum, extract samples have been employed for FT-IR analysis. Distinct structural groups wereidentified by their characteristic peak. FTIR results of the extract were displayed in Table 4. Between 600 to 4000 cm^{-1}, 10 imperative bands were identified (Table4). The occurrence of C-Cl, C-Br, C-O, C-H, O-H, C- H, and N-H was proven by FT-IR analysis, tentativelyindicating the existence of active phytoconstituentssuch as phenols, saponins, flavonoids, tannins, terpenoids, and alkaloids (Fig. 2).

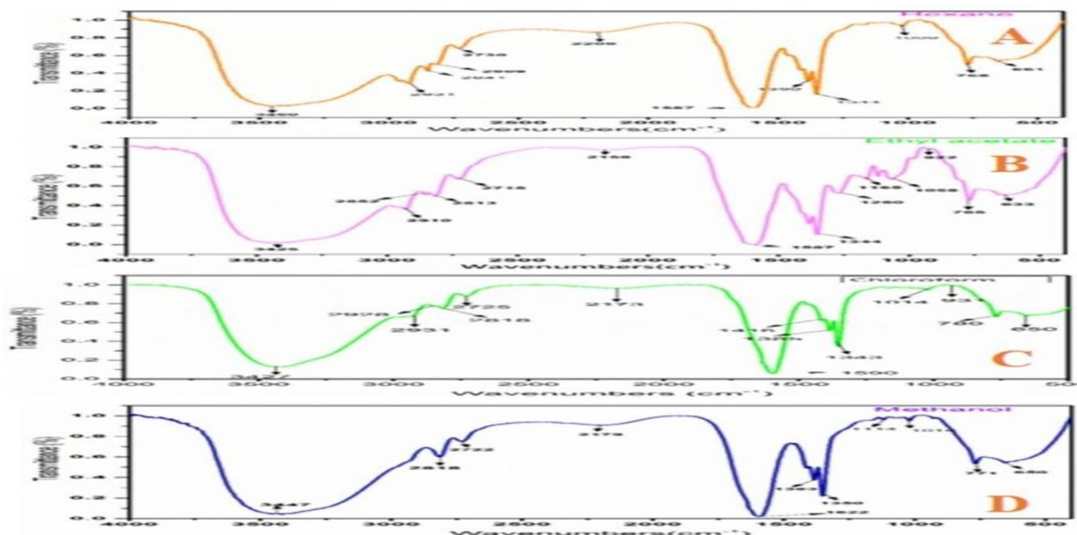

Fig. 2: FT-IR spectrum of *A. indica,* **a** hexane extract, **b** chloroform, **c** ethyl acetate, **d** methanol.

Total antioxidant capacity of extracts of *A. indica* extracts

The *A. indica* extracts have been utilized to evaluate their total antioxidant capacity assessed by employing the

phosphomolybdate method. In the current study, the total antioxidant capacity of *A.indica* extracts at maximum concentrations was found to be 2.8 ± 0.06 mg AAE/g of hexane extract, for chloroform extract was shown to be 3.4 ± 0.3mg AAE/g of chloroform extract, for ethyl acetate 10.5 ± 1.0 mg AAE/g of ethylacetate extract and methanol extract with 16.5 ± 1.6 mgAAE/g significantly high antioxidant capacity compared to other extracts (Fig. 3, Table 5).

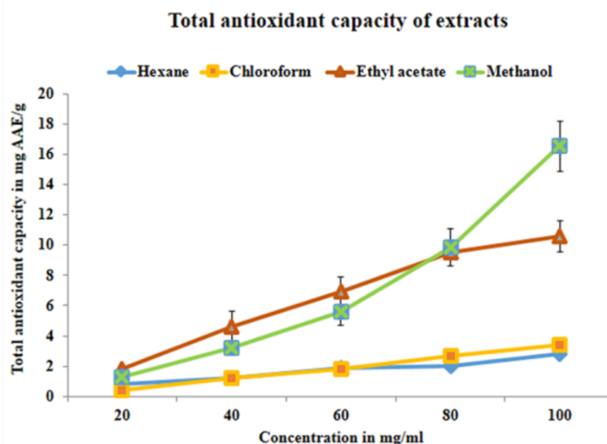

Fig. 3: Total antioxidant capacity of extracts measured interms of mg ascorbic acid equivalents/g extract

Table 4: FT-IR spectrum of *A. indica* extracts (hexane, chloroform, ethyl acetate, and methanol)

Band	Wave numbers range cm^{-1}	Band location cm^{-1}				Band interaction	Band assignments	Possible compound
		Hexane	Chloroform	Ethyl acetate	Methanol			
A	400-800	661	650	663	650	Stretch	C-Br	Akyl halides
B	700-800	759	780	765	771	Stretch	C-Cl	Akyl halides
C	800-1100	1009	931, 1014	922, 1058	1016	Stretch	C-O	Alcohol
D	1100-1200	-	-	1165	1114	Stretch	C-O	Ester
E	1100-1500	1344, 1390	1343, 1416, 1385	1260, 1344	1350, 1383	Bend	C-H	Alkanes
F	1500-1650	1587	1590	1587	1550, 1650	Bend	N-H	Secondary amines
G	2000-2800	2208, 2730	2173, 2725, 2818	2158, 2715	2179, 2722	Stretch	C-H	Alkane
H	2800-2900	2809, 2841	2813, 2852	-	2818	Stretch	C-H	Alkane
I	2900-3000	2931	2928	2910	-	Stretch	C-H	Alkane
J	3000-3800	3460	3427	3425	3447	Stretch	O-H	Alcohol, phenolics

cm^{-1}, wave number

Table 5: Total antioxidant capacity of *A. indica* extracts

Concentration	Hexane mg AAE/g of DW	Chloroform mg AAE/g of DW	Ethyl acetate mg AAE/ g of DW	Methanol mg AAE/ g of DW

20	0.8 ± 0.1	0.43 ± 0.04	1.2 ± 0. 08	1.8 ± 0.1
40	1.2 ± 0.07	1.2 ± 0.08	3.2 ± 1.0	5.3 ± 0.1
60	1.89 ± 0.1	1.8 ± 0.1	5.6 ± 0.9	7.7 ± 0.8
80	2 ± 0.2	2.7 ± 0.2	9.3 ± 0.2	9.3 ± 1.2
100	2.8 ± 0.06	3.4 ± 0.3	10.5 ± 1.0	16.5 ± 1.6

AAE-Ascorbic acid equivalents, DW-dry weight

Free radical scavenging potential of *A. indica* extracts by the DPPH assay

The impact of antioxidants on DPPH radical scavenging was attributed to their hydrogen-donating capability. The results of the DPPH radical scavengingpotential assay revealed that *A. indica* extracts have a significant free radical scavenging capability, 34.14 ± 0.1%, 41.24 ± 0.5%, 81.65 ± 0.1%, and 84.64 ± 0.6% for hexane, chloroform, ethyl acetate, and methanol extract respectively at 100 µg/ml (Fig. 4). The radical scavenging effects of *A. indica* methanol extracts weresignificantly higher than that of the standard BHA(57.84 ± 0.4% at 100 µg/ml). The outcomes of this study were tabulated in Table 5.

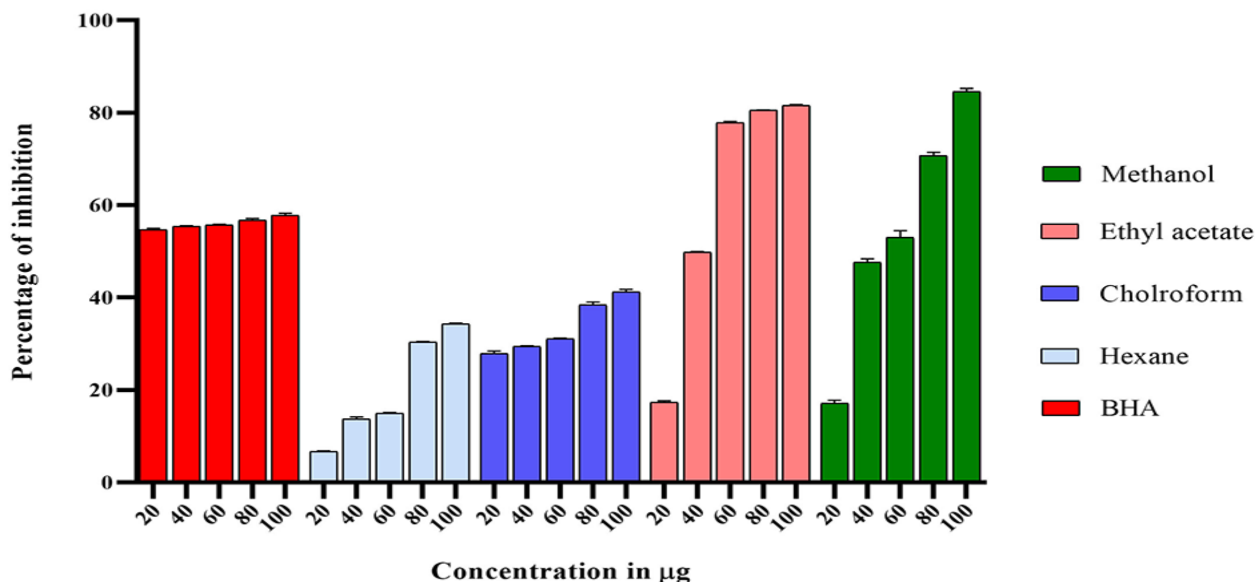

Fig. 4: Comparative analysis extracts of *A. indica* on DPPH radical scavenging capacity

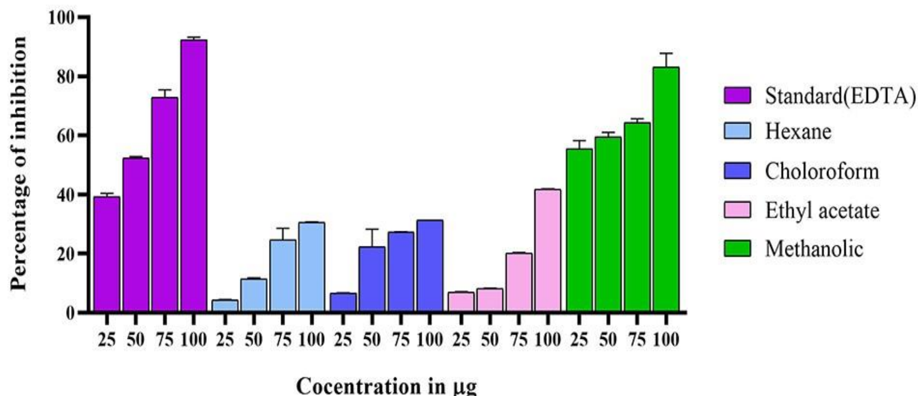

Fig. 5: Metal chelating activity of different extracts of *A. indica* and standard EDTA.

Table 6: IC_{50} values of *A. indica* extracts for DPPH radical scavenging activity and metal (Fe^{2+}) chelating activity

Sl. No	Extract	IC_{50} (µg/ml)
DPPH radical scavenging activity of extracts		
1	BHA (Standard)	89.98
2	Hexane	138.52
3	Chloroform	152.5
4	Ethyl acetate	53.51
5	Methanol	51.61
Metal chelating activity of extracts		
1	EDTA (Standard)	23.12
2	Hexane	124.35
3	Chloroform	143.60
4	Ethyl acetate	87
5	Methanol	29.23

IC_{50} (The IC_{50} value is the 50% of inhibition of its activity under the assay conditions)

Metal chelating activity of *A. indica* extracts

A. indica extracts were used to determine the metal chelating activity using standard EDTA. The impact on ferrous ions chelation by *A. indica* extracts was displayed (Fig. 5). *A. indica* extracts have a significantmetal chelating effect with the percentage of inhibition 92.33 ± 0.7%, 30.53 ± 0.1%, 31.24 ± 0.5%, 41.69 ±0.2%, and 83.11 ± 3.0% for EDTA, hexane, chloroform, ethyl acetate, and methanol extractrespectively at 100 µg/ml. The absorbance of Fe^{2+}-ferrozine complex was reduced in a dose-dependent manner. *A. indica* methanol extracts had a significant metal chelating activity which is lower than standard EDTA. The IC50 values for the metal chelating activityof *A. indica* extracts were conferred in Table 6.

CONCLUSION

In the current study, it was demonstrated that the leaves of A. indica contain a wide variety of secondary metabolites, many of which exhibit significant therapeutic properties, such as anti-inflammatory and antioxidant effects, among others. The structural characteristics of A. indica extracts were illustrated through FTIR analysis. Additionally, UV-Vis spectrophotometric analysis and initial phytochemical assessments of hexane, chloroform, ethyl acetate, and

methanol extracts were conducted to investigate the presence of various active phytoconstituents.

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