Research article

Evaluation of *in vitro* antioxidant potential of *Anisomeles indica* Kuntze and exploration of its bioactive phytoconstituents

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ABSTRACT

Introduction and Aim: *Anisomeles indica* Kuntze (*A. indica*) is employed to treat a wide range of illnesses. The Present study was aimed at establishing preliminary phytochemical screening, UV-Visible spectrophotometric, FT-IR analysis, and evaluation of biological activities of *A. indica* extracts.

Materials and Methods: *A. indica* leaves were employed for the Soxhlet extraction. Furthermore, the crude extracts were utilized for phytochemical analysis and quantitative estimations of phenolics and flavonoids. UV-Vis spectrophotometric, and FT-IR analysis provided further evidence for the existence of bioactive constituents in *A. indica* extracts. The *A. indica* extracts were assessed for antioxidant potential by DPPH and metal chelation activity.

Results: The findings illustrated that *A. indica* methanol extract was found to possess the highest yield. The preliminary phytochemical screening, UV-Vis spectrophotometric, and FT-IR fingerprint analysis provided evidence for the existence of significant bioactive constituents. *A.indica* methanol extract has significant total phenol, flavonoid content, and TAC (total antioxidant capacity) among all extracts. These characteristics are attributed to substantial antioxidant activity and metal-chelating activity.

Conclusion: The findings of this study imply that *A. indica* extract possess antioxidant activity as evaluated by the potential DPPH radical scavenging and chelate metal ions. These characteristics are interconnected to the high flavonoid and phenol content, and distinctive secondary metabolites. The finding indicates that *A.indica* is abundant in active phytoconstituents, which also offer a vital source for effective therapeutic management.

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

INTRODUCTION

Medicinal plant species have been employed to treat ailments, flavor food, sustain it, and prevent illnesses, including epidemic diseases, since antiquity. The biological traits of the plants are typically derived from active substances produced during secondary metabolism (1). Various cultures use plants as medicine and the pharmaceutical industry uses them as sources of many potent drugs because they possess certain bioactive compounds (2).

Anisomeles indica Kuntze (*labiatae* family), has therapeutic potential containing substantial amounts of biologically significant phytochemicals (3-6). The *labiatae* family, also referred to as the mint family, is among the most significant families of medicinal plants. These plants typically have an aromatic fragrance and are herbs and shrubs. *A.indica* (Malabar catmint) is a woody, perennial erect shrub that grows wild throughout Southeast Asia, which includes China, India, Australia, the Philippines, Vietnam, Indonesia, Thailand, and Taiwan. It is a medicinal plant that has properties like aromatic, astringent, carminative, and tonic qualities (5). It also possesses insecticidal, analgesic, antipyretic, and antiphlogistic properties. *A. indica* leaves have been used to treat a wide range of ailments, including hypertension, inflammatory skin disease, immune system deficiencies, and liver and gastrointestinal diseases. Prior work on *A. indica* reports that it displayed radical scavenging, cyclooxygenase inhibitory activity, anti-inflammatory, and acetylcholinesterase inhibitory activity. In addition to the biological functions mentioned above *A. indica* aerial parts such as leaves are employed to treat ailments, including rheumatism, epilepsy, paralysis, convulsions, spasms, pregnancy, fever, dyspepsia, stomach issues, and intermittent fever. Additionally, *A. indica* leaves were thought to be beneficial for psoriasis, chronic rheumatism, and skin eruptions (7-13). The *A. indica* plant can be used fresh or dried as a wash for skin infections, as snakebite remedy, and as a cure for all kinds of animal poisons (14). *A.indica* leaves are chewed to relieve toothaches. Extracts of *A. indica* also possess anti-epileptic, anti-nociceptive, anxiolytic, and sedative effects. It also has antidepressant, antidiarrheal, and thrombolytic activities (12, 13). Previous findings entirely focused on the extraction of leaves and other aerial parts employing different solvents. Current study was designed to evaluate the phytochemical profiling by

UV-Visible spectrophotometric and FT-IR fingerprint studies, taking into consideration of the ethnobotanical and pharmacological attributes of the plant.

MATERIALS AND METHODS

Chemicals

Solvents viz. methanol, ethyl acetate, chloroform, and hexane were procured from SD Fine-Chem, India. Gallic acid, butylated hydroxylanisole (BHT), EDTA sodium salt, catechin, aluminum chloride, sodium nitrite, sodium carbonate, and sodium hydroxide were purchased from Sigma-Aldrich, Germany. 2, 2-diphenyl 1- picrylhydrazyl (DPPH), ferrozine, and ferric chloride were purchased from Hi-media laboratories, India. Folin-Ciocalteu reagent and molybdate reagents were purchased from Lobachemie, India. In this investigation analytical grade chemicals and solvents have been employed, with all laboratory chemical regent solutions freshly prepared when needed.

Plant collection and authentication

Pre-flowering stage *A. indica* plant leaves were harvested during monsoon (between July to September) from the field of Davangere University, Shivagangothri situated at the geographical center portion of the Karnataka state. The plant was identified and validated by Dr. Pusphalatha, Plant taxonomist at Department of Botany, Sahyadri Science College, Kuvempu University, Shivamogga, India. The voucher specimens were deposited for future use.

Preparation of crude extracts

The leaves of *A. indica* were exploited for extraction. Fresh leaves were separated, washed, shade dried at room temperature, crumbled in a blender, ground into coarse powder material, sieved, and employed for solvent extraction utilizing Soxhlet extraction apparatus. Thus, the obtained extracts were filtered, and recovered resultant filtrate was concentrated in Rota-evaporator (Evator, Medica Instruments, Mumbai, India). The resultant concentrated filtrate has been freeze-dried using Penguin Classic Plus (Lark) at 0.5 m bar (Millibar) and -60^0 C (15, 16). The resulting extracts were weighed, and the percentage of yield was quantified. The percentage of yield of different extracts, consistency, and color were recorded (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 (20^0 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.

$$\% \text{ inhibition} = \frac{\text{AControl} - \text{AExtract}}{\text{AControl}} \times 100$$

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 Fe^{2+} (ferrous ions) by the extracts of *A. indica* and standard EDTA (ethylenediamine tetra acetic acid) was estimated (27). The formation of chromogenic Fe^{2+}-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 IC_{50} value, and the percentage of inhibition of the formation of the Fe^{2+}-ferrozine complex was calculated by utilizing the below formula.

$$\% \text{ inhibition} = \frac{\text{A control} - \text{A extract}}{\text{A control}} \text{ x } 100$$

Where $A_{Control}$ is the absorbance of the control and $A_{Extract}$ 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	+	+	+	-
HNO_3 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

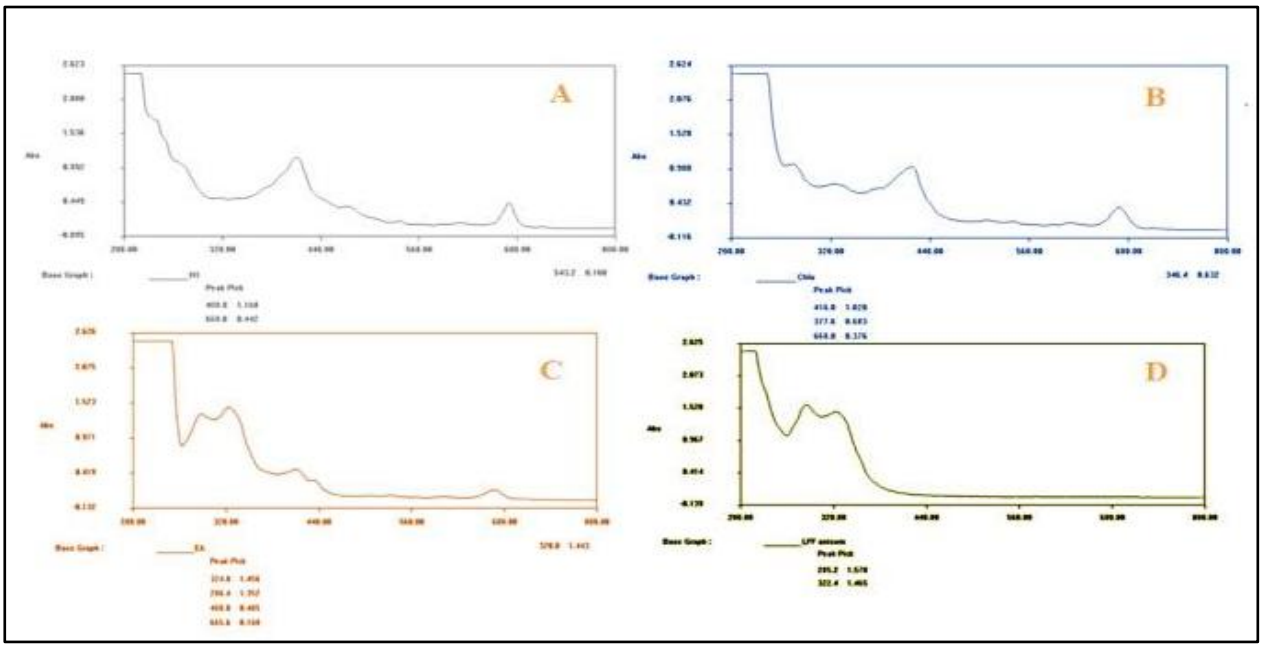

Fig. 1: UV- Visible spectrometric analysis of *A. indica* extracts, **a.** hexane extract, **b.** chloroform, **c.** ethyl acetate, **d.** methanol.

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

Presence of bioactive molecules in the *A. indica* extract was demonstrated by the UV-Visible spectrophotometric method (Fig. 1). The hexane extract 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 665.0 while *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 were identified 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 (Table 4). The occurrence of C-Cl, C-Br, C-O, C-H, O-H, C-H, and N-H was proven by FT-IR analysis, tentatively indicating the existence of active phytoconstituents such as phenols, saponins, flavonoids, tannins, terpenoids, and alkaloids (Fig. 2).

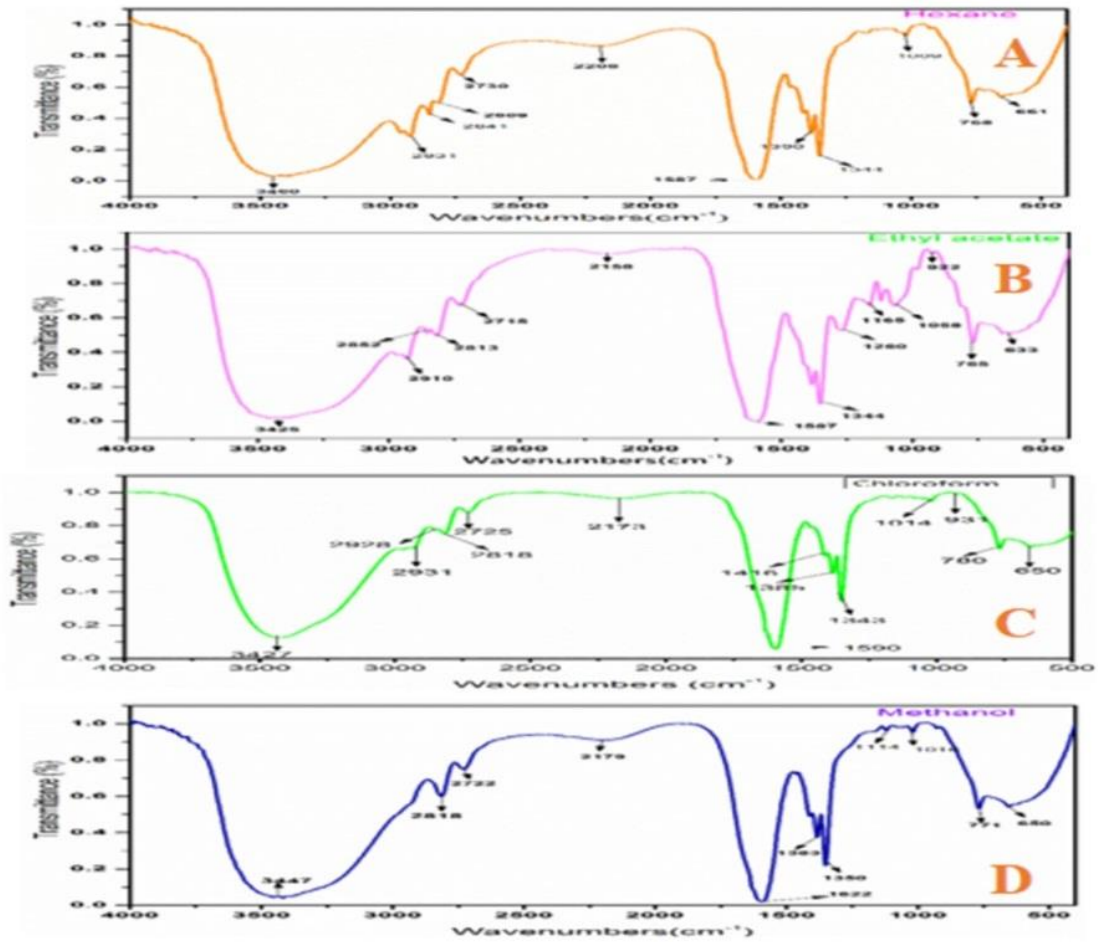

Fig. 2: FT-IR spectrum of *A. indica*, **a** hexane extract, **b** chloroform, **c** ethyl acetate, **d** methanol.

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 ethyl acetate extract and methanol extract with 16.5 ± 1.6 mg AAE/g significantly high antioxidant capacity compared to other extracts (Fig. 3, Table 5).

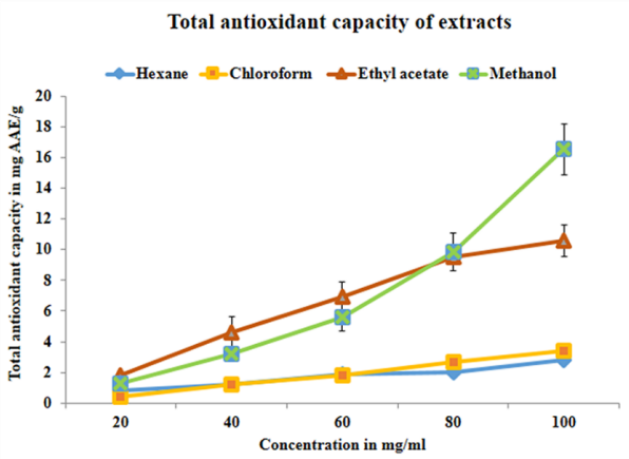

Fig. 3: Total antioxidant capacity of extracts measured in terms of mg ascorbic acid equivalents/g extract

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 scavenging potential 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 were significantly 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.

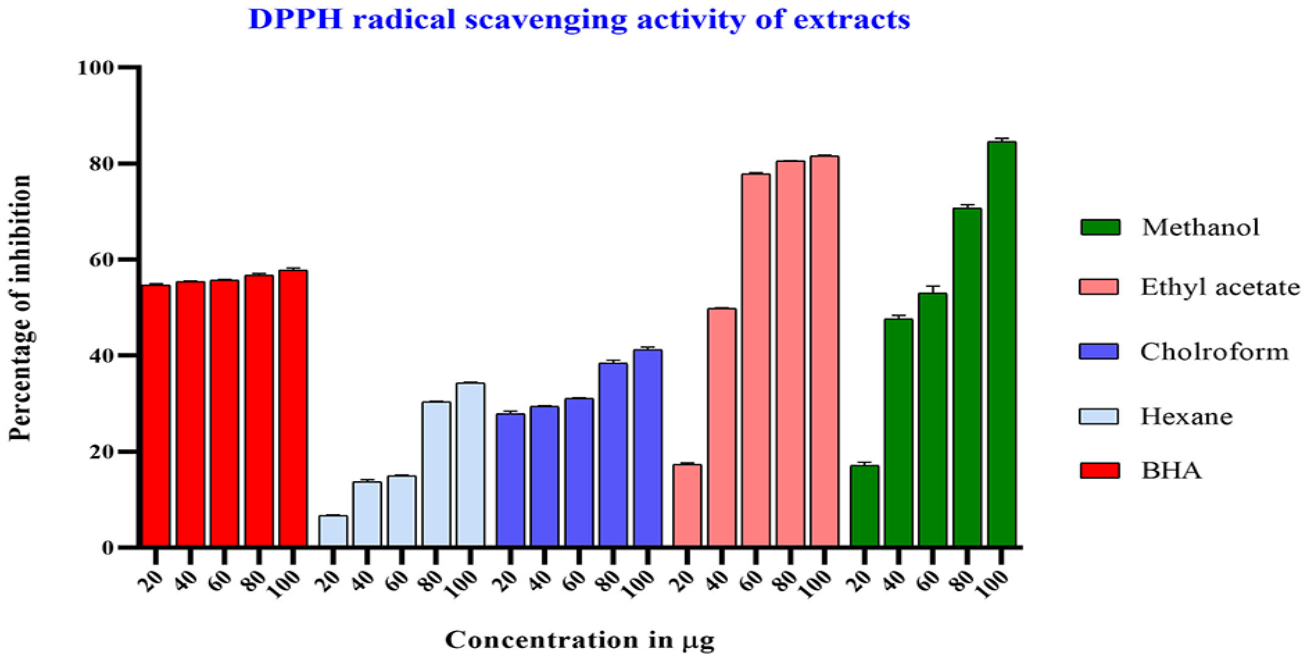

Fig. 4: Comparative analysis extracts of *A. indica* on DPPH radical scavenging capacity

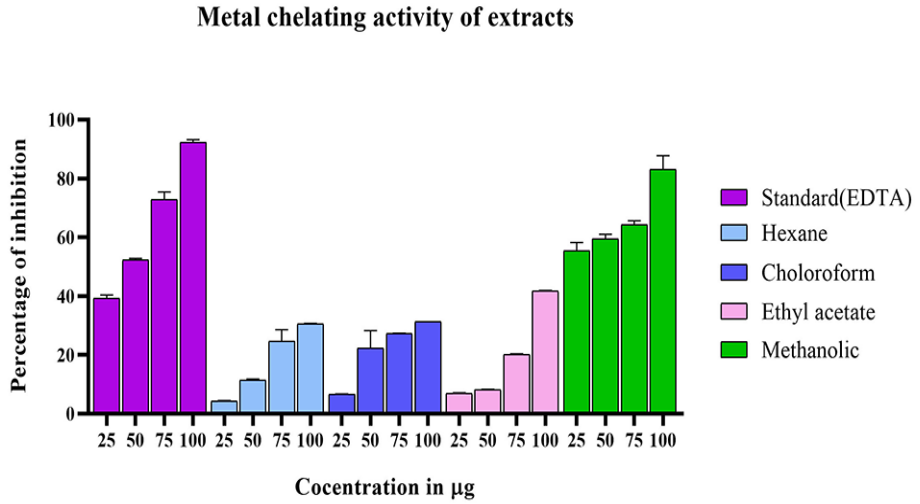

Fig. 5: Metal chelating activity of different extracts of *A. indica* and standard EDTA.

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 significant metal 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 extract respectively 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 IC_{50} values for the metal chelating activity of *A. indica* extracts were conferred in Table 6.

DISCUSSION

The outcomes of this current study imply that among all the extracts of *A. indica*, methanol solvent extraction gives the highest percent of the yield of extract. Phytochemical screening of *A. indica* extracts results in the existence of phytoconstituents such as alkaloids, tannins, phenolics, flavonoids, steroids, terpenoids, saponins, and glycosides. These preliminary phytochemical studies are supported by UV-Visible spectroscopic and FT-IR fingerprint analysis. The UV-Visible spectrophotometric technique has proven that *A. indica* extracts contain bioactive constituents. Peak values of the UV-Visible spectrum between 230-290, 300-360, and 234-676 nm were used to illustrate the existence of bioactive compounds such as flavonoids and phenolics (Fig. 1). The FT-IR spectrum was employed to identify the functional groups of the bioactive components found in *A. indica* extract based on the peak values in the IR radiation region (Fig. 2). The occurrence of C-Cl, O-H, C-Br, C-O, C-H, stretches and N-H was proven by FTIR analysis (Table 4). It has been demonstrated that FT-IT is a powerful, robust, and sensitive method for assessing molecular fingerprints of bioactive constituents. The fact that these phytochemicals were found in the hexane, chloroform, ethyl acetate, and methanol extracts of *A.indica* leaves suggested that the plant has a variety of medicinal properties, including anti-oxidative and anti-inflammatory attributes among others.

The abundance of bioactive constituents such as phenolic and flavonoid entities revealed a diverse range of biological functions and produced significant antioxidant properties. Like the results of earlier reports (28), the current study on *A. indica* extracts displayed the existence of bioactive molecules and

revealed antioxidant characteristics due to the occurrence of phenolic and flavonoids.

TPC content was evaluated for all *A. indica* extracts. Crude methanolic extracts of *A. indica* were found to have significantly high total phenolic and flavonoid content with 1.2 ± 0.1 mg GAE/g and 34.6 ± 0.3 mg CE/g dry weight extract respectively (29). The total flavonoid and phenolic amounts in crude extracts of *A. indica* were extremely high. Directly the phenolic and flavonoid compounds may contribute significantly to antioxidant activity. Methanol extract of *A. indica* was found to have a substantially higher total content of flavonoids, among all *A. indica* extracts. A strong and positive correlation coexists between flavonoid content and antioxidant activity, a phenomenon attributed to the free radical scavenging capacity of *A. indica* extracts.

Outcomes of several studies demonstrated a positive correlation between TPC and TFC in antioxidant capacity. The extremely high levels of TPC and TFC in *A. indica* extracts elicited the highest TAC of methanol extract. According to the proposed study, the *A. indica* methanol extract was found to comprise 24.3±0.6 mg AAE/g total antioxidant capacity, which was substantially higher antioxidant potential compared to other extracts. Using the DPPH (1, 1-dipheyl-2-picrylhydrazyl) radical scavenging assay method, the ability of *A. indica* extracts to scavenge free radicals was evaluated. The finding of this study revealed that the methanol crude extract of *A.indica* illustrated significant scavenging impact of DPPH radical with IC_{50} = 51.61 µg/ml compared with standard BHA (IC_{50} value 89.98) among all extracts of *A. indica* (Fig. 4). Methanol extracts had significant DPPH radical activity which is higher than standard BHA (30).

The Fe^{2+} chelation by *A. indica* extracts was anticipated. Chelating of the metal ions is the main approach employed to prevent the production of ROS (reactive oxygen species), which is interlinked to redox active metal catalysis. The most effective methanol extract of *A. indica* prevented the formation of ferrous and ferrozine complexes, suggesting that it has metal-chelating activity and captures the ferrous ion before ferrozine (29, 30). Methanol extract has a lower percentage of inhibition compared to standard EDTA with IC_{50} values for methanol, and EDTA being 29.23 µg/ml, and 23.12 µg/ml respectively, suggesting among all extracts of *A. indica*, methanol extracts had a significant metal chelating activity which is lower than standard EDTA (28).

CONCLUSION

In the proposed investigation, it was revealed that leaves of *A. indica* comprised a diverse range of secondary metabolites, some of which possess a diverse array of therapeutic potential, including anti-inflammatory and antioxidant among others. FTIR analysis *A. indica* extracts depict the structural properties. UV-Vis spectrophotometric analysis and preliminary phytochemical evaluation of hexane, chloroform, ethyl acetate, and methanol extract explore the existence of diverse active phytoconstituents.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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