Indian Journal of Natural Products and Resources
Vol. 17(3), September 2026, pp. 446-458
DOI: 10.56042/ijnpr.v17i3.24351

An *in vitro* study of the antioxidant, metal chelating, anti-inflammatory, and antiproliferative properties of *Acalypha indica* L. – An efficacious weedy medicinal plant

Mohd Yasin Bhat[1,2]*, Mir Zahoor Gul[1,3], Amarender[1] and Gulzar Ahmed Rather[4]

[1]Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Gachibowli, Hyderabad 500046, Telangana, India

[2]Department of Botany, Government Degree College Baramulla (Autonomous), Baramulla 193101, Jammu and Kashmir, India

[3]Department of Biochemistry, University College of Science, Osmania University, Hyderabad 500007, Telangana, India

[4]AMR and Nanotherapeutics Laboratory, Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institutes of Medical and Technical Sciences (SIMATS), Chennai 600077, Tamil Nadu, India

Received 01 October 2025; revised received 06 August 2026; accepted 14 August 2026

Acalypha indica L., an Indian medicinal plant with remarkable ethno-medicinal significance, possesses promising antioxidant, anti-inflammatory, and anticancer features. Extracts obtained from the aerial parts of this plant by utilising four different solvents exhibited a great deal of variation in the total phenolics and flavonoid content, in addition to considerable differences in the *in vitro* experimental systems in terms of the free radical scavenging ability. The methanolic extract showed the highest metal-chelating ability, with 85.82±0.38% and 72.51±3.27% for Fe^{2+} and Cu^{2+} ions, respectively, at the highest extract concentrations used. The methanolic extract also exhibited the greatest Nitric oxide (NO) inhibitory potential in Lipopolysaccharide-induced macrophages, demonstrating its anti-inflammatory potential. The ethyl acetate extract was potent in inhibiting MCF-7, HeLa, K562, and AGS cancer cells, with IC_{50} values of 60.05±1.84, 49.04±1.60, 19.46±4.70, and 29.39±2.90 µg/mL, respectively. Overall, these results provide foundational validation of the plant's therapeutic benefits in traditional systems and also recommend that this weedy plant can be a good candidate for the discovery and development of bioactive molecules in future.

Keywords: *Acalypha indica*, Antioxidant, Antiproliferative, Macrophages, Metal ion chelation

IPC code; Int. cl. (2021.01)– A61K 36/00, A61P 29/00, A61P 35/00, A61P 39/00

Introduction

Due to their proven pharmacological utility, medicinal plants and their derived natural products have attained a significant position in the research domain all over the world. The World Health Organisation (WHO) has emphasised time and again the key role of traditional medicine, a large part of which is based on medicinal herbs, in the large populace of developing countries due to its easy accessibility as well as affordability[1]. Local communities all over the world use different medicinal plants on a day-to-day basis for common ailments such as digestive issues, constipation, respiratory problems, skin diseases, fever, diarrhoea, joint pain management, wound healing, headache, etc[2,3]. With the ethnobotanical clues as well as random approaches, many compounds have been isolated from medicinal plants during the twentieth century that form an integral component of drug development, especially as antitumor and antibacterial agents. It is pertinent to note that even in the advent of the 21st century, about 11% of 252 drugs regarded as essential for public healthcare are exclusively derived from medicinal plants[4]. Recently, even in the backdrop of the COVID-19 pandemic, researchers showed keen interest in formulations from medicinal plants owing to their reported immune-boosting activity in other viral infections[5].

A large number of diseases are either caused by or aggravated by free radical entities, especially reactive Oxygen species (ROS), since they cause extensive oxidative destruction of lipids, proteins, and nucleic acids. Medicinal plants continue to remain a top priority natural base of phenolics, flavonoids, vitamins, and other numerous secondary metabolites, which express considerable antioxidant activity. These antioxidant molecules have the potential to nullify the onslaught of these harmful free radicals and, therefore, are very essential for the prevention of consequent diseases like cancer, rheumatoid arthritis, neurodegenerative diseases, etc[6]. A plethora of medicinal plants have been known to

*Correspondent author
Email: bhatyaseen1@gmail.com, dryasinbot@gmail.com

have immense significance for anti-inflammatory activity and tremendous prospects to contribute to the development of therapeutic approaches to counter diseases linked with inflammation[7]. Some medicinal plant products are currently in use for the treatment and management of cancer. A vast number of plant-derived extracts and compounds have shown promise as anticancer agents in *in vitro* systems and in animal models, but are in the process of being determined to show the requisite efficacy in the treatment of cancer in humans[8].

Acalypha indica L., commonly referred to as Indian Acalypha, Indian nettle, or Indian Copper leaf, belongs to the family Euphorbiaceae and is abundantly distributed throughout the African and Asian continents. In traditional systems, especially in rural regions, the plant, particularly its leaves, is used for the treatment of many health disorders like pneumonia, asthma, skin injuries, abdominal pains, rheumatism, etc[9]. In the Indian context, in Ayurveda and Siddha systems of medicine, it is used as a laxative, emetic, expectorant, anti-irritant, etc[10,11]. A substantial number of studies have been previously undertaken to establish and confirm various parts of this plant for antioxidant, anti-inflammatory[9,11], antihyperglycemic[10], hepatoprotective[12], wound healing[13], anti-fungal[14], anti-obesity[15], and analgesic[11] properties. Due to its promising pharmacological significance, several nanoformulations of extracts of this plant have been tested for several biological activities for improvement in efficiency, especially antibacterial, wound healing, antioxidant, cancer cell cytotoxicity etc[16,17]. Although the plant grows in abundance like a weed and is used either entirely or leaves and roots are consumed separately for medicinal benefits, the most common practice is to use its leaves for the preparation of decoctions, juices, pastes, infusions, etc[9,13,18]. Therefore, in this study, we aimed to further determine and establish the antioxidant activity of polar and non-polar extracts of aerial parts of *A. indica* by a number of assays. Further, anti-inflammatory activity was tested in macrophages in addition to the antiproliferative activity of these extracts against a few selected cancerous cell lines. Although studies of such biological activities have been reported for this plant from time to time, this is an exhaustive study with a multitude of *in vitro* assays across varied biochemical facets, in addition to the use of cancer cells of diverse origins and natures to determine bioactivity *vis-à-vis* the non-polar to very polar extracts.

Materials and Methods

Chemicals and reagents

The chemicals and solvents of analytical grade were obtained from HiMedia and Merck, India. Standard grade chemicals were procured from Sigma Aldrich Chemicals Co. (St. Louis, USA). Media for cell culture (RPMI-1640 and DMEM), fetal bovine serum (FBS), and the antibiotics were purchased from Gibco (Invitrogen, USA).

Plant material collection and extraction process

A. indica plants were collected from within the campus of the University of Hyderabad (Telangana, India) (Fig. 1). The plant material was subsequently identified and affirmed by the Botanical Survey of India, Deccan Regional Centre, Hyderabad, with reference no. BSI/DRC/2012-13/Tech/588, and a voucher specimen was kept for reference. The above-ground aerial plant material was shade-dried at the usual room temperature for a number of days and ground to a coarse powder utilising an electric blender. Extracts were prepared from 100 g powder by Soxhlet extraction apparatus using solvents of increasing polarity sequentially, i.e., n-Hexane, ethyl acetate, methanol, and water. For the purification of extracts, they were passed through Whatman No.1 filter paper. A rotary flash evaporator (Buchi, Germany) was used to dry these extracts. The resultant yield of every extract was noted. The resultant extracts were labelled as AIH (Hexane), AIE (Ethyl acetate), AIM (Methanol), and AIW (water). The extracts were stored in the dark at -20°C in air-tight amber vials for future use. Consequently, stock solutions of the extracts in Dimethyl sulfoxide (DMSO) with a concentration of 20 mg/mL were made, and working concentrations that were appropriate to different tests or experiments were used.

Quantification of total phenolics and flavonoids

The phytochemical determination of total phenolics and flavonoids of the subsequent extracts were carried out by standard methods described previously[19,20]. Results were subsequently expressed as milligram Gallic acid equivalent per gram dry weight of the material (mg GAE / g dw) and milligram Quercetin equivalent per gram dry weight of the material (mg QE/ g dw) for total phenolics and flavonoids, respectively.

In vitro antioxidant activity studies

Phosphomolybdenum method

The total antioxidant activity (TAA) of the resultant extracts was derived by using the

Fig. 1 — (a) *Acaypha indica* L. plant, (b) collected material sample of the aerial part of the plant.

Phosphomolybdenum assay with reference standard as Ascorbic Acid[19]. Briefly, aliquots of 100 µL of extracts or standard reference were mixed with 1 mL prepared solution of reagent (0.6 M Sulfuric acid, 28 mM Sodium Phosphate, and 4 mM of Ammonium molybdate) in 1.5 mL microcentrifuge tubes. These tubes, after being capped, were subsequently incubated for one and a half hours at 90°C in a thermal dry bath. Later on, they were removed and waited for some minutes to cool down at room temperature. The reading of absorbance was taken at 695 nm against blank samples on a UV-Visible spectrophotometer (Shimadzu, Japan) and calculated as mg Ascorbic Acid equivalent(AAE) per gram dry weight (dw) of the material.

Metal ion chelation ability

Ferrous (Fe^{2+}) chelation ability

The extracts of the plant were set to analysis with respect to their capability to contend with Ferrozine for chelating free iron (II) (ferrous, Fe^{2+}) ions in any solution, as implied by a decline in absorbance as per the defined method[21]. Concisely, to a solution of 100 µL of 2 mM $FeCl_2.4H_2O$, extracts in the concentration ranging from 20-200 µg/mL were added. This reaction was initiated by the introduction of 200 µL of 5 mM Ferrozine, followed by vigorous shaking of the mixtures. The solutions were left undisturbed for ten minutes at room temperature. The spectrophotometric absorbance was acquired at 562 nm against the blank sample solution prepared with $FeCl_2$ and distilled water. For this essay, Ethylenediaminetetraacetic acid (EDTA), a recognised metal ion chelator, was used as a positive control. The calculations were performed using the given equation:

$$\text{Ferrous ion chelating activity (\%)} = (ABc - ABs)/ABc \text{ X } 100$$

Where AB*c* refers to the absorbance of the negative control reaction minus any extract, and ABs implies the absorbance of the sample added with the extract and positive control, EDTA.

Chelation of Cuprous (II) ions (Cu^{2+})

The Cu^{2+} chelation potential of the extracts was evaluated according to the method defined with some alterations[22]. Precisely, 60 µL of CuSO4 aqueous solution (20 mM) was put in Hexamine-HCl buffer (30 mM; pH 5.3) comprising 0.20 mM Murexide and 30 mM KCl. After keeping the mixture intact at room temperature for almost one minute, additions of 10 µL of the extracts and positive control Sodium citrate (SC) with concentrations ranging from 40 – 400 µg/mL were made to the mixtures. The final volume was prepared with methanol to 1.5 mL. These solution mixtures were briskly vortexed and incubated

yet again at room temperature for 10 minutes. Ultimately, the absorbance reading was taken with a UV-visible spectrophotometer at 485 nm and 520 nm. The ratio of the absorbances (A485/A520) specified the free Cu^{2+} content. Therefore, the intensity of the cuprous ion chelating effect was computed as:

$$\text{Cuprous chelating activity (\%)} = \left[\frac{\left(\frac{A485}{A520}\right)\text{max} - \left(\frac{A485}{A520}\right)\text{sample}}{\left(\frac{A485}{A520}\right)\text{max} - \left(\frac{A485}{A520}\right)\text{min}}\right] X100$$

Where $\left(\frac{A485}{A520}\right)$ max designates the ratio of absorbance in mixtures without samples, $\left(\frac{A485}{A520}\right)$ sample is the ratio of absorbance in mixtures with samples and $\left(\frac{A485}{A520}\right)$ min is the absorbance ratio of mixtures without any sample and $CuSO_4$.

Free radical scavenging assays

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay

DPPH radical scavenging activity of the extracts was performed by the method described earlier[19]. Ascorbic acid (AA) was used as a positive standard for this experiment, while methanol (95 %) was taken as a blank reference.

2, 2'- Azino-bis – (3-ethylbenzothiazoline-6-Sulfonic acid) ABTS scavenging assay

This cation radical decolourisation assay *in vitro* was done as per the described method with slight modifications[23]. Briefly, for the generation of the radical cation of $ABTS^{\bullet+}$,7 mM solution of ABTS in water was prepared and then mixed with a 2.45 mM Potassium persulphate solution. The solution mixture so formed was stored in a dark milieu at room temperature for 12-16 hours for a blue colour appearance. This solution of ABTS was diluted correspondingly by ethanol so that an absorbance value of 0.700±0.02 at 734 nm wavelength is obtained. In a 96-well plate, 10 µL of plant extracts with a concentration from 10 to 100 µg/mL was loaded in each well, possessing 190 µL of already prepared ABTS solution. After gentle shaking, the whole plate containing wells with reaction mixtures was further incubated in dark conditions for 15 minutes. Ascorbic acid (AA) was used as a positive control. The absorbance reading was taken spectrophotometrically at a wavelength set at 734 nm on a microplate reader (InfiniteProTecan, Switzerland). The calculations were done as:

$$\text{ABTS scavenging activity (\%)} = \left[\frac{\text{ABc} - \text{ABs}}{\text{ABc}}\right] X\ 100$$

where ABc and ABs refer to the absorbance value of the control and sample solution, respectively.

Hydroxyl (OH) radical scavenging assay

These assays were done following the method described earlier[20]. Ascorbic acid was taken as a reference standard for this free radical scavenging assay.

Lipid peroxidation inhibition assay

The *in vitro* effect of extracts on lipid peroxidation was experimented in goat liver homogenate by the Thiobarbituric acid reactive substances (TBARS) method with minor modifications[24]. This assay typically detects lipid peroxidation by quantification of its oxidation products, mostly malondialdehyde (MDA), which react with thiobarbituric acid (TBA) under high temperature and acidic pH to form a pink chromogen. Goat liver (5 g) tissue purchased fresh from the local market was cut into coarse pieces, followed by homogenization in 10 mM KCl-Tris-HCl buffer (pH 7.4). The uniform homogenate was subsequently subjected to centrifugation at 800 g at 4°C for 15 minutes, and the lipid-rich supernatant was collected separately. With the supernatant so obtained, the extracts at several concentrations (20-200 µg/mL) were mixed, and the tubes were incubated at 37°C for 30 minutes in the presence and absence (blank) of Fenton's reagent (50 µL of 10 mM $FeCl_3$; 10 µL of 2.5 mM H_2O_2) in phosphate buffer (0.2 M, pH 7.4). The volume was adjusted to 1 mL. Further, 2 mL of ice-cold HCl (0.25 N) containing 15% Trichloroacetic acid (TCA), 0.5% thiobarbituric acid (TBA), and 0.5% Butylated hydroxy toluene (BHT) was added to the tubes, followed by heating them at 100°C for about 10 minutes. The reaction mixture was allowed to cool on an ice bath for about 5 minutes. Finally, the mixture was centrifuged at 1000 g for 10 minutes. The lipid peroxidation process and its inhibition by samples were quantified by measurement of the pink chromogen spectrophotometrically at 532 nm. Ascorbic acid was taken as a positive control for this assay. A diminution in the development of pink colour in the mixtures incubated with extracts is indicative of the ability to impede the peroxidation of lipids.

Evaluation of anti-inflammatory and antiproliferative activity

Cell lines and culture

RAW 264.7 murine macrophages, K562 (Chronic myeloid leukaemia), HeLa (Cervical carcinoma), AGS (Human Gastric adenocarcinoma), and MCF-7 (breast carcinoma) cell lines were acquired from the National Centre for Cell Science (NCCS), Pune, India. These cell lines were accordingly cultured in recommended media, DMEM and RPMI-1640, subsequently augmented with 10% FBS, antibiotics, penicillin (100 Units/mL), and streptomycin (100 µg/mL). The cells were grown in a humidified 5% CO_2 air incubator at a temperature of 37°C. Negative control for the experiments with cells was characterised by a non-treated medium including vehicle solvent as cell culture grade Dimethyl Sulfoxide DMSO (0.1%).

Determination of Nitric Oxide (NO) in macrophages (Anti-inflammatory activity)

The quantification of Nitrite, a stable NO oxidation product, in RAW 264.7 murine macrophage cells was performed at non-toxic concentrations of the extracts[25]. Briefly, cells were seeded into 96-well plates with a density of 1×10^5 cells/mL. After an incubation time of 24 hours, they were washed with a fresh medium, followed by treatment with several non-toxic concentrations of the extracts (AIH, AIM, AIW) and N-Nitro-L-Arginine methyl ester hydrochloride (L-NAME) as a positive control. Thereafter, the cells were stimulated by Lipopolysaccharide (LPS) (1 µg/mL). Again, after an incubation time of 24 hours, 100 µL of culture supernatant was mixed with the same volume (100 µL) of Griess reagent (1% sulphanilamide and 0.1% naphthylethylenediamine in 5% phosphoric acid), followed by keeping the reaction mixtures at room temperature for 10 minutes. The sodium nitrite standard curve was used for quantification after the absorbance value was taken at 540 nm on a microplate reader.

Antiproliferative activity

The cytotoxic effect of extracts of *A. indica* was evaluated *in vitro* using four different cancer cell types (K562, HeLa, AGS, and MCF-7) by the well-known 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay[20]. Briefly, cells after being plated at a density of 5×10^3 cells in each of the wells in 96-well culture plates were incubated for 24 hours. Fresh medium (200 µL) with the extracts at increasing concentration values (5, 25,50, 100, 200, 250 µg/mL) was added to the wells and further incubated for the next 48 hours. Thereafter, the medium from every well was removed cautiously and substituted with new media added with 20 µL of 5 mg/mL of MTT dissolved in Phosphate-buffered saline (PBS, pH 7.4). The cells were kept for further incubation at 37°C for 4 hours. Eventually, 100 µL Dimethyl Sulfoxide (DMSO) was put in each of the wells to dissolve the crystals of formazon that were formed. The absorbance reading was taken at 540 nm on a microplate reader (InfinitePro, Tecan Switzerland). Curcumin was considered a positive control for K562 cells, and Doxorubicin for other cell types. The percentage inhibition of cancer cells in this activity was calculated by means of the formula:

$$\text{Cell inhibition (\%)} = \left[1 - \left(\frac{\text{ABt}}{\text{AB0}}\right)\right] \text{X}100$$

Where ABt and AB0 are the absorbances of treated and untreated control wells, respectively.

Statistical analysis

The results of the independent triplicate experiments were expressed as mean ± standard deviation (SD) values. The IC_{50} values represent the concentration of extracts or standards required for 50% scavenging of radicals, chelation of metal ions, and inhibition of cancer cells. The statistical analysis, along with calculations and graphical representations, was accomplished by GraphPad Prism Version 6 software. Statistical differences among the experimental results were assessed using proper tests (one-way ANOVA, Tukey's, and Student's t-tests). Overall, p <0.05 was considered statistically significant.

Results

Phytochemical determination

The total phenolic content of the extracts was highest in AIM (2.59 ± 0.29^a mg GAE/g dw), followed by AIW (1.18±0.11^b GAE /g dw). The quantification value of the same was determined to be 0.95±0.06^bGAE /g dw in AIE, and it was found to be lowest in AIH with the value of 0.23±0.04^c GAE /g dw. Likewise, the flavonoid content decreased in the order AIW > AIM> AIE with values of 5.33±0.56^a, 3.24±0.40^b, 2.16±0.14^c QE /g dw, and could not be observed in AIH within a detectable range. Each value is represented as mean ± SD (n = 3) and different superscript letters in each imply statistically significant difference among them (p <0.05).

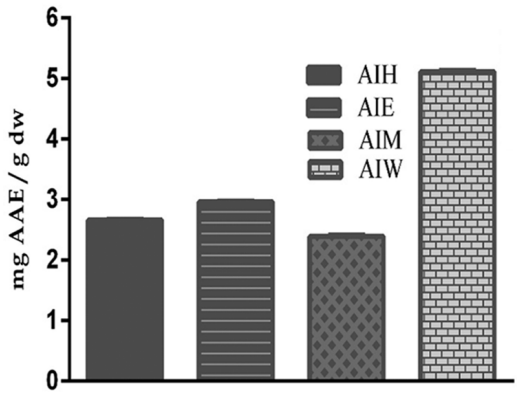

Fig. 2 — Total antioxidant activity of *Acalypha indica*'s four extracts by the Phosphomolybdenum method in terms of mg Ascorbic Acid equivalent per g dry weight of the plant material. Each value is represented as a mean ± Standard deviation (n=3).

Antioxidant activity of extracts

Phosphomolybdenum assay

The phosphomolybdate method is a very simple but useful indicator of the antioxidant capacity of extracts and pure compounds, which involves the reduction of Mo (VI) to Mo (V) by such substances, forming a greenish complex solution at acidic pH with an absorption peak at 695 nm. Among all the extracts, AIW showed the maximum total antioxidant capacity of 5.11±0.02 mg AAE/g dw of the material. The antioxidant ability of the other three extracts was lower. The antioxidant activity of AIH, AIE, and AIM was noted to be 2.66±0.01, 2.96±0.01, and 2.39±0.02 mg AAE/g dw, respectively (p <0.05) (Fig. 2).

Metal ions chelation activity

Chelation of Ferrous (Fe^{2+}) ions

The ability of the four extracts of the plant for chelation of ferrous (Fe^{2+}) ions is shown in (Fig. 3a). Ferrozine in the reaction mixture forms a colored complex with Fe^{2+}, which is inhibited quantitatively in the presence of metal chelators acting as antioxidants. AIM showed better Ferrous chelation ability than AIE, while AIH and AIW were among the least efficacious extracts that were used. By the maximum concentration of 200 µg/mL used in this experiment, AIM significantly inhibited 85.82±0.38% of ions with an IC_{50} value of 63.62±1.53 µg/mL (p <0.01). AIH and AIW extracts almost showed a saturation of chelation activity at 120 µg/mL concentration without much improvement at succeeding concentrations. It is very obvious from this assay that the extracts successfully obstructed the complex formation of ferrous ions and Ferrozine, specifying that they bind ferrous (Fe^{2+}) ions before Ferrozine in a concentration-dependent pattern (Table 1).

Cuprous ions (Cu^{2+}) chelating ability

The cuprous ions chelating activity of the extracts and the positive control Sodium citrate (SC) conformed to a concentration-dependent pattern from 40 - 400 µg/mL as presented in Fig. 3b. Although a Cu^{2+} chelating activity of SC was greater than these extracts, AIM and AIE chelated 72.51±3.27% and 55.54±2.20% of Cu^{2+} ions respectively at the maximal used concentration of 400 µg/mL (p<0.01). Among the extracts, AIW showed the lowest Cu^{2+} ion chelation of only 29.72±1.56% at 400 µg/mL. The IC_{50} values of the concerned samples are in the decreasing order of AIH>AIE> AIM>SC (Table 1).

Free radical scavenging activity

1,1-Diphenyl-2-Picryl-hydrazyl (DPPH) assay

DPPH spectrophotometric assay is one of the commonly used procedures for the assessment of antioxidant activity of extracts obtained from medicinal plants because of its simplicity and convenience. The method is based on the lessening of the purple colour of DPPH free radical in solution by antioxidants. A positive correspondence was witnessed between the increasing concentration of all the extracts and their DPPH radical scavenging ability, albeit the differences in activity among them were prominent. The resultant IC_{50} values of the extracts in comparison to Ascorbic acid are described (Table 1). In this assay, AIW showed the maximum free radical scavenging activity, inhibiting 91.76±2.41%, followed by AIM, which inhibited 82.11±0.76% at the highest used concentration of 400 µg/mL. Extracts of AIH and AIE, on the other hand, were moderately effective among the extracts for scavenging DPPH free radicals, inhibiting only 54.98±1.20% and 59.65± 3.29% of DPPH free radicals at this concentration (p <0.05) (Fig. 3c).

2, 2'-Azinobis-3-ethyl-benzothiazoline-6-Sulfonic acid (ABTS) scavenging assay

This is an electron-transfer-based assay, in which the ABTS cation with dark blue colour changes into a colourless solution upon reduction by antioxidant substances. As is very apparent from Fig. 3d, AIH is the least scavenging of this radical among the extracts, exhibiting inhibition of just 16.55±0.87% at 100 µg/mL, while AIW showed the maximum inhibition of 95.29±4.16% at this concentration.

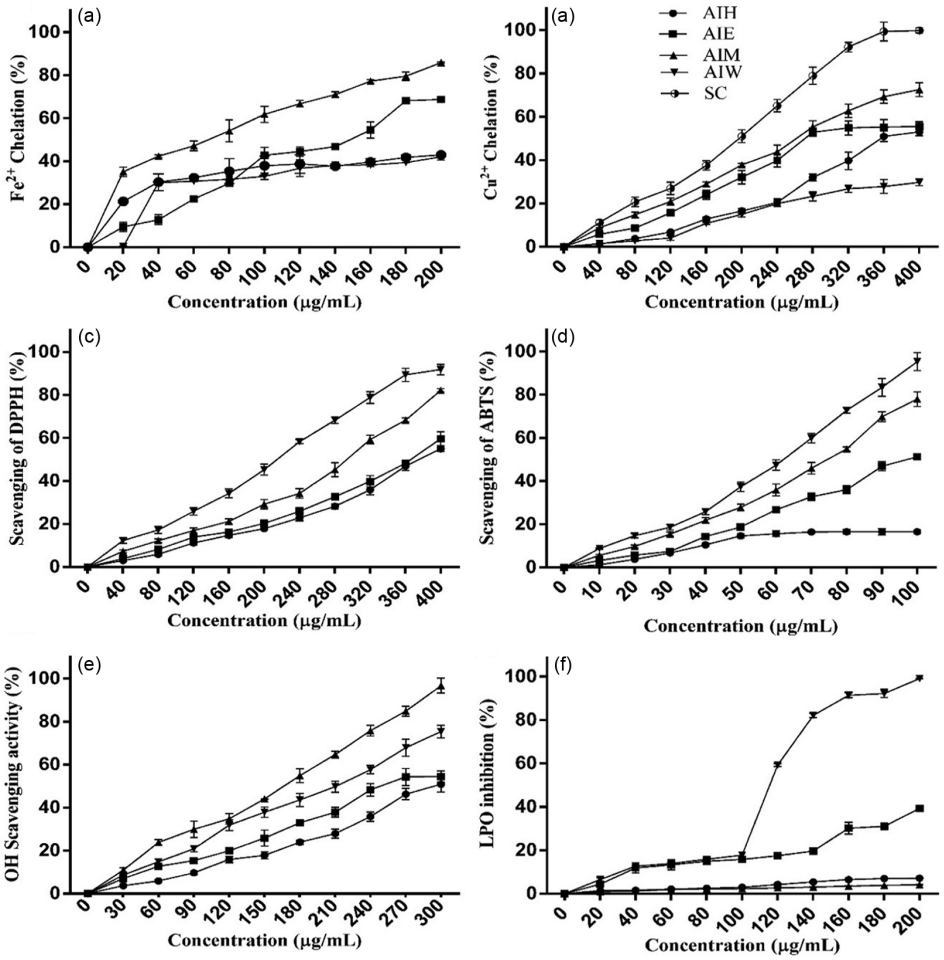

Fig. 3 — Antioxidant ability of the *A. indica* extracts at increasing concentrations by different *in vitro* assays. (a) Chelation of Ferrous (Fe^{2+}) ions, (b) Chelation of Cuprous (Cu^{2+}) ions, (c) Scavenging of DPPH free radicals, (d) ABTS radicals scavenging, (e) Hydroxyl radical scavenging, (f) Lipid peroxidation inhibition. Each value represents a mean ± Standard deviation (n=3).

Table 1 — IC_{50} values of four extracts of *A. indica* and positive controls (standards) for metal ion chelation and free radical scavenging ability

Scavenging/ chelation assay	Extracts of *A. indica* (µg/mL)				Standard compounds
	AIH	AIE	AIM	AIW	
Ferrous ion (II)	ND	149.64±7.11	63.62±1.53	ND	7.11±0.26$^{\#}$
Cuprous ion (II)	353.49±4.48	264.75±8.50	253.26±6.06	ND	196.21±10.05$^{\beta}$
DPPH$^{\bullet}$	384.04±2.33	372.75±12.90	309.54±4.67	220.85±3.84	4.88±0.25$^{\alpha}$
ABTS$^{\bullet}$	ND	97.67±1.18	76.20±3.13	63.32±0.96	4.02±0.25$^{\alpha}$
OH$^{\bullet}$	295.15±11.37	248.50±7.21	164.02±2.78	211.27±7.02	58.80±1.10$^{\alpha}$
Lipid peroxidation	ND	ND	ND	113.24±5.51	55.53±2.08$^{\alpha}$

Values are expressed in µg/mL. Results are expressed as mean (n=3±SD); ND implies Not determined. Symbols: #, β, and α imply EDTA, Sodium citrate, and Ascorbic acid, respectively

Likewise, AIE and AIM were somewhat moderately capable in the inhibition of ABTS free radicals, exhibiting the values 51.19±1.28 and 77.93±3.43% inhibition, respectively. The IC_{50} values, therefore, are in order ofAIE>AIM> AIW>AA (Table 1).

Scavenging of hydroxyl (OH•) radicals

The antioxidant potential of the extracts was also determined by the hydroxyl radical scavenging activity generated in the reaction system. All four extracts demonstrated a concentration-dependent

pattern of increments in hydroxyl radical scavenging activity from 30 – 300 µg/mL concentration. AIM exhibited the highest inhibition of 96.65±3.50% at the utmost used extract concentration of 300 µg/ mL, followed by AIW with 75.26±2.89% at the same concentration, significantly in comparison to the negative control (p <0.05). The IC_{50} value is lowest in AIM among the extracts, signifying its redox potential regarding these free radicals, while it is highest in the case of AIH, which is least effective (Fig. 3e, Table 1).

Inhibition of lipid peroxidation activity

The results from this experiment indicated that, of all the extracts, the addition of only AIW extract efficiently obstructed the magnitude of lipid peroxidation in liver homogenates at the concentrations used in this study (p <0.01). The lipid peroxidation was inhibited moderately up to 100 µg/mL and thereafter showed an abrupt increase culminating with the highest inhibition of 99.00±0.14% at 200 µg/mL extract concentration. In contrast, AIE inhibited only 39.26±0.77% of the process at the maximum concentration. AIH and AIM were insignificant in terms of inhibition of lipid peroxidation, exhibiting the values of 7.18±0.11 and 4.15±0.07% inhibition even at the utmost concentration. It is pertinent to note that the IC_{50} value for AIW is 113.24±5.51 µg/mL, demonstrating its efficacy in terms of lipid peroxidation inhibition potential (Fig. 3f, Table 1).

Nitric oxide (NO) inhibition in macrophages (Anti-inflammatory assay)

LPS is a constituent of the gram-negative bacterial cell wall, which evokes a prominent pro-inflammatory response in macrophages, including NO synthesis that converts to comparatively stable nitrite in the culture medium. The suppression of NO production by extracts or compounds in LPS-induced cells is a good indicator of their anti-inflammatory activity. In our study, there was a significant increase in NO production quantified as nitrite in macrophages by LPS treatment (29.14±2.12 µM) in comparison to the untreated group (2.88±0.16 µM) (p <0.01). However, the extracts of *A. indica* substantially reduced the NO levels in LPS-induced murine macrophages in a dose-dependent pattern, which indicated their anti-inflammatory activity at these concentrations (Fig. 4).

Antiproliferative activity of extracts

The MTT assay for four cancer cell lines of varied origin demonstrated a considerable difference in the

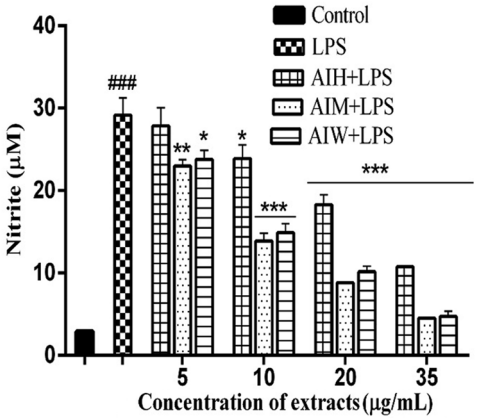

Fig. 4 — Anti-inflammatory potential of *A. indica* extracts on LPS-induced RAW 264.7 macrophages as demonstrated by NO inhibition. Results are expressed as mean ± SD, n=3.$^{###}$ represents p<0.001 with respect to the untreated control cell group,while as* p<0.05, **p<0.01and ***p<0.001 of the extracts-treated cell groups versus the LPS-induced group. Statistical significance was deduced by one-way ANOVA followed by a post hoc test.

antiproliferative action of different extracts of the plant. However, a common observation was that AIE was the most efficacious among all extracts concerning this activity. The utmost drop in cell viability percentage was observed at 250 µg/mL of the extracts after an incubation period of 48 hours in comparison to the control samples of untreated cells (p <0.01). There was a concentration-dependent death of cancer cells after the stipulated period of incubation with extracts. AIE showed maximal cytotoxicity towards all cancer cell types, with the inhibition of 95.30±0.33%, 97.17±5.46%, 90.80± 2.58%, 98.25±6.13% for K562, HeLa, AGS, and MCF-7 cells, respectively. In the case of K562 cells, the AIM was the least efficient extract, causing only 44.19±1.88% of maximum cell death. Likewise, in the case of MCF-7, HeLa, and AGS cells, AIW was the extract with the least activity, exhibiting only 51.86±0.66%, 37.34±2.69%, and 54.91±1.87% of cell death at the highest concentration of 250 µg/mL, respectively. The corresponding IC_{50} values for each cell line with all the extracts and standard positive controls are presented in Table 2.

Discussion

Plants have been the foundation of healthcare in human civilisations from primeval times. To acclimatise to a large number of biotic and abiotic stresses, they produce many secondary molecules that have biological activities[1,8]. A great array of medicinal plant secondary metabolites has long been

Table 2 — Cancer cell inhibition percentage and IC_{50} values for extracts of *A. indica* on four cancer cell lines

Cell type	Extract and standards	IC_{50} value (µg/mL)	Inhibition at highest concentration (%)
K562	AIH	180.57±0.57	57.50±0.25
	AIE	19.46±4.70	95.30±0.33
	AIM	ND	44.19±1.88
	AIW	176.7±1.12	69.10±2.66
	Curcumin	14.09±0.70	93.74±3.21
HeLa	AIH	98.63±6.46	85.64±5.35
	AIE	49.04±1.60	97.17±5.46
	AIM	175.89±10.87	75.30±3.19
	AIW	ND	37.34±2.69
	Doxorubicin	1.40±0.07	91.90±4.85
AGS	AIH	62.62±3.21	93.75±1.10
	AIE	29.39±2.90	90.80±2.58
	AIM	229.09±5.57	67.66±2.06
	AIW	ND	54.91±1.87
	Doxorubicin	0.64±0.04	94.61±3.21
MCF-7	AIH	191.61±10.69	71.63±6.15
	AIE	60.05±1.84	98.25±6.13
	AIM	220.89±7.02	72.84±2.78
	AIW	241.01±6.45	51.86±0.66
Doxorubicin		0.82±0.05	94.58±3.21

Results are expressed as mean ± SD. (n=3). ND refers to a value not determined

recognised as significant entities for the treatment of many human ailments. An excess generation of free radical species, more especially the reactive oxygen species (ROS), reactive nitrogen species (RNS), etc., in the human body due to exogenous or endogenous factors, causes oxidative stress, which, if left uncontrolled, can be detrimental, causing substantial damage to lipids, proteins, and nucleic acids. Such a cellular milieu has often been linked with chronic degenerative ailments like cancer, diabetes, Coronary artery disease, inflammatory disorders, etc. Plant-based products are finding renewed interest nowadays in therapeutics, mostly due to their antioxidant nature and free radical quenching potential[26]. The lack of efficacy, unaffordable cost, and numerous unanticipated side effects of chemotherapeutic anticancer drugs have paved the way for the pursuit of more efficient, inexpensive, and comparatively safer molecules from medicinal herbs, which will have the ability to prevent, slow, or reverse the progression of cancer[8,19]. Such a rationale also applies to chronic inflammation, where there are endeavours to find better therapeutic drugs from medicinal herbs to replace the use of NSAIDs (Non-steroidal anti-inflammatory drugs), the latter having many side complications like gastric disorders, renal failure, cardiovascular issues, etc[27]. Taking into consideration the various health uses of *A. indica* plant in the Indian traditional system, this study is an attempt to establish and validate its antioxidant, anti-inflammatory and anticancer properties.

A. indica L. possesses a great miscellany of useful compounds such as phenolics, phenolic acids, flavonoids, anthocyanins, tannins, saponins, terpenoids, alkaloids, anthraquinones, and coumarins according to earlier literature, for which it has been the centre of attention due to their antioxidant, anti-inflammatory, and anticancer properties[9]. In this study, the polar extracts AIM and AIW were shown to possess a comparatively greater quantity of phenolics and flavonoids, which could be the prominent contributors to the resultant antioxidant activity. The amount of phenolics and flavonoids, as well as the molecular composition in any extract, depends upon the nature of the solvent, among other factors. Previously, certain phenolic compounds or derivatives such as Gallic acid, Caffeic acid, catechol, Geranin, Corilagin, chebulagic acid, Ellagic acid, Syringic acid, and flavonoids or derivatives like catechin, kaempferol, Quercetin, Naringin, Naringenin, Chrysin, Hesperetin, Rutin, and Quercetin 3-O-β-D-Glucoside, etc. have been identified from this plant[18,28,29]. The phenolic compounds, due to their high degree of reactivity and structural chemistry, allow them

to perform free radical scavenging, reduction, metal chelation, and neutralisation of singlet Oxygen and therefore act as potent antioxidants[30]. Since there is a great diversity of molecules ranging from polar to non-polar (volatile compounds) in plant extracts with complexity in structures, modes of action, and physical and chemical characteristics, it is well comprehensible that no single test can alone help in defining the magnitude of its antioxidant properties. So, several assays were considered in this study for this purpose.

DPPH and $ABTS^{+}$scavenging assays detect the presence of antioxidant molecules like flavonoids and polyphenols, while the Phosphomolybdenum antioxidant method detects some phenolics, Ascorbic acid, carotenoids, and α-tocopherol[23,31]. A good degree of overall antioxidant property of the studied extracts of this plant is a substantiation of the presence of redox and free radical neutralisation properties of these molecules in the extracts. The discrepancies in antioxidant or free radical scavenging activities detected for different extracts of *A. indica* in various *in vitro* systems may probably be the result of distinctive and varied mechanisms of reactions in the different tests[32]. Flavonoids and other phenolic compounds are often associated with free radical scavenging in systems, as well as a decline in lipid peroxidation. The hydroxyl free radicals are short-lived, known to be implicated in the direct reaction with DNA, causing its breakdown, resulting in harmful mutations and even cancer[19]. The hydroxyl radicals are exceedingly reactive and can wreak oxidative havoc within the cells with the lipids, proteins, and DNA. The extracts in comparative analysis to the standard positive control in the Fenton reaction system demonstrated copious hydroxyl radical scavenging ability in a concentration-dependent manner and therefore are protective against a number of their adverse effects. The methanolic and aqueous extracts of *A. indica* exhibited abundant lipid peroxidation inhibition, which is a great index of their antioxidant activity. Superfluous amounts of free metal ions in biological systems have been known to be one of the causes for the generation of free radicals. Transition metal ions, particularly iron and copper, abruptly react with peroxides to form alkoxy radicals. It is thus quite understandable that antioxidants that bind these metal ions have a considerable role in the impedance of the vicious oxidation process[33,34].

Polyphenols have been known to possess anticancer effects owing to their significant contribution as antioxidant and anti-inflammatory mediators[34]. The anti-inflammatory nature of the extracts was deduced based on Nitric oxide inhibition in LPS-stimulated macrophages, which is a routinely cell-based method for the assessment of the anti-inflammatory nature of medicinal plant extracts[25]. The ethyl acetate extract was excluded from this study because it caused considerable cell death at the concentrations used. Though Nitric oxide is considered a known mediator and regulator of various biological processes, such as neurotransmission, smooth muscle relaxation, etc., nevertheless, its overproduction leading to oxidative stress is linked with the pathophysiology of chronic inflammation[35]. The extracts of *A. indica* at non-toxic concentrations significantly reduced the Nitric oxide amounts in LPS-stimulated macrophages, specifying their anti-inflammatory effects ($p<0.05$). Whether this reduction is due to Nitric oxide scavenging or down-regulation of the inducible Nitric oxide synthase (iNOS) gene or whether both these are contributory factors, warrants further research. Previously, certain important biologically active phytochemicals have been reported to be identified from this plant, such as Acalyphine, Acalyphamide, Aurantiamide, Tectoquinone, Quinine, Flindersin, Repandusinic acid, Ellagic acid, catechol, Chebulagic acid, Syringic acid, Geranin, Glucogallin, Sitosterol, Stigmasterol, and Quebrachitol etc. Some of these molecular species have been known to possess anti-inflammatory activity[18,29,36,37]. Anti-inflammatory action of the extracts of *A. indica* has been reported as the ability to lessen carrageenan-induced paw oedema in rats by declining neutrophil infiltration, penetration, and vascular dilation[9]. Nevertheless, the specific anti-inflammatory molecules and mechanistic studies in cells and *in vivo* are very limited for this plant.

The extracts were also evaluated for their antiproliferative potential against certain cancer cells of multiple natures and varied origins. Despite a common observation that the ethyl acetate extract of *A. indica* was the most proficient among all of them, the degree of its inhibition of different cancer cells varied with concentrations used, which is apparent from the differences in IC_{50} values. This phenomenon could be due to the variation in the metabolism of the cell and the degree of susceptibility of each cell type to the phytochemicals present in the extract. The National Cancer Institute (NCI) has designated the IC_{50} values of less than 30 μg/mL as criteria for effective cytotoxicity of the extracts[20]. From this study, it is noted that the ethyl acetate extract exhibited such values for K562 and AGS cell lines and, therefore, should be considered for further validation in *in vivo* models and so on. It is

pertinent to mention that the cytotoxic effects of silver and gold nanoparticles of the *A. indica* leaf extract against MDA-MB-231 (human breast cancer cells) by initiation of apoptosis have been reported earlier[17]. Recently, some of the anticancer compounds have been identified or isolated from the extracts of *A. indica*, such as Quercetin and Rutin, which showed tremendous efficacy against breast cancer cells with regard to initiation of apoptosis[38,39]. Solvent polarity is one of the determinants of the differences in phytochemical composition of the plant extracts with ethyl acetate being moderately polar yielding molecular scaffolds with wider chemical diversity possessing remarkable bioactive potential as against the more polar solvents like methanol and water, favouring antioxidant rich polyphenols and flavonoids[40].

Conclusion

This study substantiated that *A. indica* extracts possess great antioxidant capacity and free radical scavenging activity, as corroborated by several *in vitro* assays. The different extracts also showed indications of anti-inflammatory activity in murine macrophage cells and also anticancer properties against certain cancer cell lines. This study provides a very preliminary empirical basis for the claimed traditional and community-level health benefits of this plant. The anti-inflammatory studies need further evaluation in terms of impact on inflammatory cytokines, gene expression of inflammatory markers like Inducible Nitric Oxide Synthase (iNOS), Cyclo-oxygenase-2 (COX-2), and involvement of pathways like Mitogen activated Protein Kinase (MAPK), Nuclear factor Kappa B (NF-κB), etc. Further in-depth studies are, of course, also warranted to ascertain the cell death mechanism, such as apoptosis, cell cycle arrest, autophagy, etc. Also, the response of non-cancerous cell lines needs to be analysed for evaluation of bioefficacy and safety. Subsequently, this basic study can lay the foundation for undertaking *in vivo* studies and the pursuit of isolating different pharmacologically useful compounds for these biological activities from this plant.

Conflict of interest

The authors declare that there are no conflicts of interest.

AI use disclosure

Authors hereby declare that no Artificial Intelligence tool has been used at any stage for the preparation, writing, analysis, interpretation, or any other purpose in this manuscript.

Acknowledgement

The author MYB is thankful to University Grants Commission (UGC) for the provision of Senior Research Fellowship (SRF) under sanction order No. UH/FO/F&S/UGC/2015/2703, Dated 21-07-2015. The author MZG acknowledges the financial support in the form of Research Associateship from Council of Scientific and Industrial Research (CSIR) vide sanction file No. 09/132(0883)/2019-EMR-1 Dated 04-2019. The authors also thank Prof. Irfan A. Ghazi, (faculty at Department of Plant Sciences, University of Hyderabad) for providing requisite laboratory facilities and scientific inputs to carry out this work.

References

1 Morales F, Padilla S and Falconí F, Medicinal plants used in traditional herbal medicine in the province of Chimborazo, Ecuador, *Afr J Tradit Complement Altern Med*, 2016, **14**(1), 10–15, doi: 10.21010/ajtcam.v14i1.2.

2 Mir T A, Jan M and Khare R K, Ethnomedicinal practices and conservation status of medicinal plants in the Bandipora district of Kashmir Himalaya, *J Herbs, Spices Med Plants*, 2022, **28**(2), 125–142, doi: 10.1080/10496475.2021.2014012.

3 Mbuni Y M, Wang S, Mwangi B N, Mbari N J, Musili P M, *et al*., Medicinal plants and their traditional uses in local communities around Cherangani hills, western Kenya, *Plants*, 2020, **9**(3), 331, doi: 10.3390/plants9030331.

4 Veeresham C, Natural products derived from plants as a source of drugs, *J Adv Pharm Technol Res*, 2012, **3**(4), 200, doi: 10.4103/2231-4040.104709.

5 Adhikari B, Marasini B P, Rayamajhee B, Bhattarai B R, Lamichhane G, *et al*., Potential roles of medicinal plants for the treatment of viral diseases focusing on COVID-19: a review, *Phytother Res*, 2021, **35**(3), 1298–1312, doi: 10.1002/ptr.6893.

6 Zahin M, Ahmad I, Shireen I, Husain F M and Aqil F, Broad spectrum antioxidant properties of 20 indian medicinal plants, *J Herbs, Spices Med Plants*, 2016, **22**(2), 118–129, doi: 10.1080/10496475.2015.1057888.

7 Akhtar M A, Anti-inflammatory medicinal plants of Bangladesh - a pharmacological evaluation, *Front Pharmacol*, 2022, **13**, 809324, doi: 10.3389/fphar. 2022.809324.

8 Desai A, Qazi G, Ganju R, El-Tamer M, Singh J, *et al.*, Medicinal plants and cancer chemoprevention, *Curr Drug Metab*, 2008, **9**(7), 581–591, doi: 10.2174/138920008785821657.

9 Sahukari R, Punabaka J, Bhasha S, Ganjikunta V S, Ramudu S K, *et al.*, Phytochemical profile, free radical scavenging and anti-inflammatory properties of *Acalypha indica* root extract: evidence from *in vitro* and *in vivo* studies, *Molecules*, 2021, **26**(20), 6251, doi: 10.3390/molecules26206251.

10 Priya C L and Bhaskara Rao K V, Postprandial antihyperglycemic and antioxidant activities of *Acalypha*

indica Linn stem extract: an *in-vivo* study, *Phcog Mag*, 2016, **12**(47), 475, doi: 10.4103/0973-1296.191461.

11 Rahman M A, Bachar S C and Rahmatullah M, Analgesic and antiinflammatory activity of methanolic extract of *Acalypha indica* Linn, *Pak J Pharm Sci*, 2010, **23**(3), 256-258.

12 Dwijayanti A, Frethernety A, Hardiany N S and Purwaningsih E H, Hepatoprotective effects of *Acalypha indica* and *Centella asiatica* in rat's liver against hypoxia, *Procedia Chem*, 2015, **14**, 11–14, doi: 10.1016/j. proche.2015.03.003.

13 Ganeshkumar M, Ponrasu T, Krithika R, Iyappan K, Gayathri V S, *et al.*, Topical application of *Acalypha indica* accelerates rat cutaneous wound healing by up-regulating the expression of type I and III collagen, *J Ethnopharmacol*, 2012, **142**(1), 14–22, doi: 10.1016/j.jep.2012.04.005.

14 Abirami S, Raj B E, Soundarya T, Kannan M, Sugapriya D, *et al*., Exploring antifungal activities of acetone extract of selected Indian medicinal plants against human dermal fungal pathogens, *Saudi J Biol Sci*, 2021, **28**(4), 2180–2187, doi: 10.1016/j.sjbs.2021.01.046.

15 Naik R, Nemani H, Pothani S, Pothana S, Satyavani M, *et al*., Obesity-alleviating capabilities of *Acalypha indica*, *Pergularia demia* and *Tinospora cardifolia* leaves methanolic extracts in WNIN/GR-Ob rats, *J Nutr Intermed Metab*, 2019, **16**, 100090, doi: 10.1016/j.jnim.2019.02.001.

16 Boomi P, Ganesan R, Poorani G P, Jegatheeswaran S, Balakumar, *et al*., Phyto-engineered gold nanoparticles (aunps) with potential antibacterial, antioxidant, and wound healing activities under *in vitro* and *in vivo* conditions, *Int J Nanomed*, 2020, **15**, 7553–7568, doi: 10.2147/IJN.S257499.

17 Krishnaraj C, Muthukumaran P, Ramachandran R, Balakumaran M D and Kalaichelvan P T, *Acalypha indica* Linn: Biogenic synthesis of silver and gold nanoparticles and their cytotoxic effects against MDA-MB-231, human breast cancer cells, *Biotechnol Rep*, 2014, **4**, 42–49, doi: 10.1016/j.btre.2014.08.002.

18 Zahidin N S, Saidin S, Zulkifli R M, Muhamad I I, Ya'akob H, *et al*., Review of *Acalypha indica* L. (Euphorbiaceae) as traditional medicinal plant and its therapeutic potential, *J Ethnopharmacol*, 2017, **207**, 146–173, doi: 10.1016/ j.jep.2017.06.019.

19 Gul M Z, Bhakshu L M, Ahmad F, Kondapi A K, Qureshi I A, *et al*., Evaluation of *Abelmoschus moschatus* extracts for antioxidant, free radical scavenging, antimicrobial and antiproliferative activities using *in vitro* assays, *BMC Complement Altern Med*, 2011, **11**(1), 64, doi: 10.1186/1472-6882-11-64.

20 Gul M Z, Ahmad F, Kondapi A K, Qureshi I A and Ghazi I A, Antioxidant and antiproliferative activities of *Abrus precatorius* leaf extracts - an *in vitro* study, *BMC Complement Altern Med*, 2013, **13**(1), 53, doi: 10.1186/1472-6882-13-53.

21 Dinis T C, Madeira V M and Almeida L M, Action of phenolic derivatives (Acetaminophen, Salicylate, and 5-Aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers, *Arch Biochem Biophys*, 1994, **315**(1), 161–169, doi: 10.1006/abbi.1994.1485.

22 Cai H, Xie Z, Liu G, Sun X, Peng G, *et al*., Isolation, identification and activities of natural antioxidants from *Callicarpa* kwangtungensis Chun, *Plos One*, 2014, **9**(3), e93000, doi: 10.1371/journal.pone.0093000.

23 Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, *et al.*, Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Radic Biol Med*, 1999, **26**(9–10), 1231–1237, doi: 10.1016/S0891-5849(98)00315-3.

24 Ohkawa H, Ohishi N and Yagi K, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, *Anal Biochem*, 1979, **52**(2), 351-358, doi: 10.1016/0003-2697(79)90738-3.

25 Bhat M Y, Gul M Z, Khan N, Qureshi I A and Ghazi I A, Cytoprotective activity and anti-inflammatory properties of *Artemisia nilagirica* (Clarke) extracts - a study with macrophages, *J Biol Active Prod Nat*, 2017, **7**(3), 228-244, doi: 10.1080/22311866.2017.1334588.

26 Shaikh R, Pund M, Dawane A and Iliyas S, Evaluation of anticancer, antioxidant, and possible anti-inflammatory properties of selected medicinal plants used in Indian traditional medication, *J Tradit Complement Med*, 2014, **4**(4), 253–257, doi: 10.4103/2225-4110.128904.

27 Vonkeman H E and Van De Laar M A F J, Nonsteroidal anti-inflammatory drugs: adverse effects and their prevention, *Semin Arthritis Rheum*, 2010, **39**(4), 294–312, doi: 10.1016/j.semarthrit.2008.08.001.

28 Ravi S, Shanmugam B, Subbaiah G V, Prasad S H and Reddy K S, Identification of food preservative, stress relief compounds by GC–MS and HR-LC/Q-TOF/MS; evaluation of antioxidant activity of *Acalypha indica* leaves methanolic extract (*in vitro*) and polyphenolic fraction (*in vivo*), *J Food Sci Technol*, 2017, **54**(6), 1585–1596, doi: 10.1007/s13197-017-2590-z.

29 Pradoo A, Sriapha C, Trakulsrichai S, Tongpoo A, Kheiawsawang M, *et al*., Clinical characteristics of *Acalypha indica* poisoning, *Int J Gen Med*, 2020, **13**, 539–545, doi: 10.2147/IJGM.S263199.

30 Mathew S, Abraham T E and Zakaria Z A, Reactivity of phenolic compounds towards free radicals under *in vitro* conditions, *J Food Sci Technol*, 2015, **52**(9), 5790–5798, doi: 10.1007/s13197-014-1704-0.

31 Prieto P, Pineda M and Aguilar M, Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E, *Anal Biochem*, 1999, **269**(2), 337–341, doi: 10.1006/abio.1999.4019.

32 Christodoulou M C, Orellana Palacios J C, Hesami G, Jafarzadeh S, Lorenzo J M, *et al.*, Spectrophotometric methods for measurement of antioxidant activity in food and pharmaceuticals, *Antioxidants*, 2022, **11**(11), 2213, doi: 10.3390/antiox11112213.

33 Jomova K and Valko M, Advances in metal-induced oxidative stress and human disease, *Toxicology*, 2011, **283**(2–3), 65–87, doi: 10.1016/j.tox.2011.03.001.

34 Diaz P, Jeong S C, Lee S, Khoo C and Koyyalamudi S R, Antioxidant and anti-inflammatory activities of selected medicinal plants and fungi containing phenolic and flavonoid compounds, *Chin Med*, 2012, **7**(1), 26, doi: 10.1186/1749-8546-7-26.

35 Forstermann U and Sessa W C, Nitric oxide synthases: regulation and function, *Eur Heart J*, 2011, **33**(7), 829-837, doi: 10.1093/eurheartj/ehr304.

36 Morgan L V, Petry F, Scatolin M, De Oliveira P V, Alves B O, *et al*., Investigation of the anti-inflammatory

effects of stigmasterol in mice: insight into its mechanism of action, *Behav Pharmacol*, 2021, **32**(8), 640–651, doi: 10.1097/FBP.0000000000000658.

37 Loizou S, Lekakis I, Chrousos G P and Moutsatsou P, β-Sitosterol exhibits anti-inflammatory activity in human aortic endothelial cells, *Mol Nutr Food Res*, 2010, **54**(4), 551–558, doi: 10.1002/mnfr.200900012.

38 Chekuri S, Sirigiripeta S R, Thupakula S, Vyshnava S S, Ayesha S, *et al.*, Rutin isolated from *Acalypha indica* L: A comprehensive analysis of its antibacterial and anticancer activities, *Biochem Biophys Res Commun*, 2025, **765**, 151833, doi: 10.1016/j.bbrc.2025.151833.

39 Chekuri S, Vyshnava S S, Somisetti S L, Cheniya S B, Gandu C, *et al.*, Isolation and anticancer activity of Quercetin from *Acalypha indica* L. against breast cancer cell lines MCF-7 and MDA-MB-231, *3 Biotech*, 2023, **13**(8), 289, doi: 10.1007/s13205-023-03705-w.

40 Agunloye M O, Owo D U, Onaadepo O, Ugwu F N and Ogunyemi O M, Phytochemical characterization of ethanolic and ethyl acetate extracts of avocado *Persea americana* leaves by FT-IR and GC-MS reveals potential bioactive compounds, *Sci Rep*, 2025, **15**, 27035, doi: 10.1038/s41598-025-12150-z.