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Pharmacological and Phytochemical Assessment of *Anagallis arvensis* L. Leaf Extracts

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The present study elucidates anti-inflammatory potential and biochemical activity *via* 2,2′-diphenyl-1-picrylhydrazyl scavenging potential (DPPH), total antioxidant activity (TAA), ferric reducing antioxidant power (FRAP), ferrous chelating activity (FCA), total phenolic content (TPC), total flavonoid content (TFC) of aqua-methanol (AqM) and aqua-acetone (AqA) extracts of *Anagallis arvensis* L. leaf along with mineral content (AAS) and quantitative phytochemicals (FT-IR and GC-MS). AqM extract exhibited maximum anti-inflammatory activity (82.90 ± 0.91%), DPPH (65.06 ± 1.87%), TAA (46.85 ± 7.32 µg VCE/mg), FRAP (73.82 ± 1.21 µg TE/mg), TPC (104.17 ± 1.41 µg GAE/mg); while AqA showed maximum FCA (64.77 ± 1.61%) and TFC (19.12 ± 1.24 µg QE/mg). FT-IR spectra of AqM extract ranged from 1020.58 to 3853.42 cm^{-1}. The major six phytochemicals investigated through GC-MS were 9-octadecenoic acid (Z)-methylester; 2-hexadecen-1-ol-3,7,11,15-tetramethyl-[R-[R*,R*-(E)]]; methyl elaidate; 2-methoxy-4-vinylphenol; 9-octadecenamide-(Z)-; and benzoic acid 2-hydroxy-phenylmethyl ester exhibiting antioxidant and anti-inflammatory properties. The present investigation characterizes the pharmacognostic and phytochemical profile of *A. arvensis* leading towards its futuristic significance in pharmaceutical and nutraceutical industries.

Keywords: *Anagallis arvensis*, Anti-inflammation, Antioxidants, Phytochemicals.

INTRODUCTION

Reactive oxygen species (ROS) are the natural outcome of regular metabolic activities that are crucial for the cell survival, proliferation, apoptosis, growth and defence when present in appropriate quantity. But their increment facilitates the hazardous free radical formation in the body causing damage to the vital organic compounds like protein, carbohydrates, lipids, *etc.* and ultimately resulting in anti-inflammation as a defence response [1]. So, to neutralize and stabilize the perilous effects of ROS and other free radicals, antioxidants perform substantial role in human body by enzymatic and non-enzymatic metabolic reactions [2]. In plants, different classes of phytochemicals (*viz.* phenols, tannins, flavonoids, quinones, alkaloids, steroids, cardiac glycosides, saponins, terpenoids, anthocyanins) are present in the form of secondary metabolites, of which phenols and flavonoids are the chief compounds exhibiting antioxidant, anti-inflammatory and antimicrobial characteristics [3]. The secondary metabolites (defence phytochemicals) of plant origin are preferred more as antioxidants upon synthetic ones because of their low side effects, less or non-toxicity and cost effectiveness [4].

Anagallis arvensis L. of Primulaceae family, commonly called 'scarlet pimpernel' (in English) and 'jonkmari' (in Hindi), is an annual herb (creeper), having simple leaves and solitary axillary flowers (bisexual). The herb mainly flourishes on sandy wasteland and has cosmopolitan distribution in both hemispheres' temperate regions. In India, the plant species is distributed in mountainous areas of Bengal and North West India, Himalayas (at about 2700 m. from Nepal westwards to adjacent Shimla); hills of Nilgiris, Central India and Sri Lanka. The plant blooms and bears fruits from December to April. A saponin namely 'anagallin' is the principal compound of *A. arvensis* along with other phytoconstituents such as anagallin B, anagallinone B, arvenin I and II, cucurbitacin (bitter), cucurbitacin E and glycosides. A vast literature indicate its traditional and

commercial economic uses. Medicinally, this herb is rich in saponins and used to treat liver cirrhosis, urinary infections, leprosy, gallstones, renal calculi, gout, lung infections, flu, cold, cerebral infections and epilepsy. The plant also possesses antifungal and antiviral (against polio and *Herpes simplex* virus) properties. In China, the herb treats hydrophobia and snake bite [5,6].

Previously, several studies have been conducted related to the antioxidant activities of *A. arvensis*, but few studies are available focusing on the quantitative bioactive phytochemicals and anti-inflammatory propensity of the reference plant species. Different reports like antiviral activity of saponin from *A. arvensis* by Amoros *et al.* [7], antifungal and antioxidant activities by Lopez *et al.* [8], antifungal properties and cytotoxicity by Soberón *et al.* [9], antibacterial, antioxidant, antifungal, antitumor and cytotoxic activities by Shakoor *et al.* [10] and cytotoxicity, antioxidant potential and phytochemical evaluation *via* UPHLC-MS by Saleem *et al.* [11] have been documented. However, the research information concerning screening of anti-inflammatory potential *via* egg albumin denaturation assay and phytochemical composition *via* GC-MS of the reference plant species is still missing in the previous literature. Keeping that view in mind, the present study is aimed on to assess the anti-inflammatory, antioxidant and phytochemical potential of *A. arvensis*.

EXPERIMENTAL

Collection and identification: Matured fresh leaf stock was collected from the campus area (29º1′29.07″ N and 79º29′23.16″ E) of G.B. Pant University of Agriculture & Technology, Pantnagar, India. The collected voucher specimen of plant species was identified and authenticated by considering the Eflora India [12], Eflora Pantnagar [13] and finally by Dr. D.S. Rawat, Department of Biological Sciences, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture & Technology, Pantnagar, India.

Chemicals: Acetone, methanol, diclofenac sodium, disodium biphosphate, potassium chloride, sodium chloride, 2,2′-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), ammonium molybdate, sodium phosphate, sulphuric acid, ascorbic acid, ferric chloride, hydrochloric acid, 2,4,6-*tris*(2-pyridyl)-1,3,5-triazine (TPTZ), trolox, ferrozine, disodium ethylenediaminetetraacetic acid (Na_2EDTA), ferrous chloride tetrahydrate, Folin-Ciocalteu, sodium carbonate, gallic acid, ammonium chloride, quercetin, EDTA, *etc*. All these analytical grade chemicals were purchased from different commercial sources like Sigma-Aldrich (Germany), Hi-Media (India), Merck, Darmstadt (Germany) and Ranbaxy (India).

Extract preparation: The collected leaf stock was thoroughly washed by distilled water, dried in shade and grounded mechanically. The extraction (1:10 w/v) was completed by taking 20:80 v/v ratio of aqua-methanol (AqM) and aqua-acetone (AqA) and mixture was orbital shaken at 30 ºC (for 10 days) at 150 rpm. Finally the extracts were filtered through Whatmann No. 1, solvent was evaporated at 40 ºC in water bath, yielded and freeze stored at 4 ºC for experiments.

Phytochemical investigation: The different phytoconstituents (proteins, carbohydrates, phenols, tannins, flavonoids, quinones, alkaloids, steroids, cardiac glycosides, saponins and terpenoids) in both AqM and AqA extracts of *A. arvensis* were qualitatively analyzed by following the standardized methods [14,15].

Anti-inflammatory activity

Protein-denaturation assay: Protein (egg albumin) denaturation assay, based on protein (denatured) turbidity reduction with increasing concentration, was carried out according to Sakat *et al.* [16] and Ngoua-Meye-Misso *et al.* [17] methods with some modifications. The mixtures of 1 mL standard drug (diclofenac sodium) or plant extracts (20, 40, 60, 80 and 100 µL conc.), 1.9 mL phosphate buffer saline (1.44 g Na_2HPO_4, 0.2 g KCl and 8 g NaCl of 6.4 pH) and 0.1 mL hen's fresh egg-albumin were reacted at 37 ºC for 20 min followed by gradual temperature rise (up to 70 °C) for 5 min. The absorbance of different turbid reaction mixtures were noted (at 660 nm) and denaturation inhibition was calculated by the following formula:

$$\text{Denaturation inhibition (\%)} = \left(1 - \frac{A_t}{A_c}\right) \times 100$$

where, A_t and A_c are absorbance of sample (treatments) and control (standard drug) at 660 nm, respectively.

Antioxidant evaluation

2,2′-Diphenyl-1-picrylhydrazyl (DPPH•) scavenging assay: Free DPPH radical scavenging potential (%) was assayed according to Brand-Williams *et al.* [18] method with minor modifications. About 1 mL BHT (standard) or plant extracts at different concentrations (20 to 100 µL) were admixed with 3 mL DPPH solution (0.004% prepared in methanol) for 1 h. Absorbance of the resultant scavenged yellow product was measured (at 517 nm) and scavenging values were calculated by following formula:

$$\text{Scavenging activity (\%)} = \left(1 - \frac{A_t}{A_c}\right) \times 100$$

where, A_t and A_c are absorbance of sample (treatments) and control at 517 nm, respectively.

The DPPH• IC_{50} (50% inhibitory concentration) was measured by plotting scavenging activity on ordinate and plant extracts concentration on abscissa.

Phosphomolybdenum assay: Total antioxidant activity (TAA) was investigated by Prieto *et al.* [19] phosphomolybdenum method showing molybdenum(VI) reduction to molybdenum(V). The reaction mixture of 1 mL plant extracts of different concentrations (20 to 100 µL) and 3 mL reagent solution (4 mM ammonium molybdate, 28 mM sodium phosphate and 0.6 M sulphuric acid) was reacted in water bath at 95 ºC for 90 min. The absorbance at 695 nm of green coloured product was measured and values were calculated as µg ascorbic acid equivalent (AAE) per mg extract.

Ferric (Fe^{3+}) reducing antioxidant power (FRAP) assay: The ferric reducing power of the plant extract was determined by modified method of Benzie & Strain [20]. Pre-incubated at 37 ºC fresh FRAP reagent (300 mM sodium buffer, pH 3.6;

10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3$) was reacted with 1 mL plant extracts of different concentrations (20 to 100 µL) for 30 min at 37 ºC. The absorbance at 593 nm of resultant blue product (Fe^{2+}-TPTZ complex) was measured and reducing power was calculated as µg trolox equivalents (TE) per mg extract.

Ferrous chelating activity (FCA): Chelation activity was analyzed by using Hsu *et al.* [21] method based on the plant extracts potential to destabilize ferrous (Fe^{2+}) ion-ferrozine complex. About 1 mL standard Na_2EDTA or plant extracts of different concentrations (20 to 100 µL) were reacted with 0.2 mL ferrozine (5 mM) and 0.1 mL $FeCl_2 \cdot 4H_2O$ (2 mM) for 10 min by maintaining 5 mL total volume with solvents (AqM and AqA). The absorbance at 562 nm of reduced red colour product was noted and chelation was calculated following the formula:

$$\text{Chelating activity } (\%) = \left(1 - \frac{A_t}{A_c}\right) \times 100$$

where, A_t and A_c are absorbance of sample (treatments) and control at 562 nm, respectively.

The FCA IC_{50} (50% inhibitory concentration) was calculated by plotting chelation activity on ordinate and plant extracts concentration on abscissa.

Total phenolic content (TPC): Total phenol content was determined by following modified Folin-Ciocalteu procedure of Wolfe *et al.* [22]. About 0.5 mL plant extracts of different concentrations (20 to 100 µL) were admixed with 0.2 mL Folin-Ciocalteu reagent for 5 min and alkalized with 7% Na_2CO_3 saturated) were incubated for 1 h. The absorbance of blue product was measured at 765 nm was measured and values were expressed as µg gallic acid equivalents (GAE) per mg extract.

Total flavonoid content (TFC): Total flavonoid content was determined by slightly modified aluminium chloride method of Djeridane *et al.* [23]. Equal quantity of plant extracts (100 µg/mL) and $AlCl_3$ (2%) were reacted for 1 h and absorbance of yellow coloured product was measured at 420 nm. The values were expressed as µg quercetin equivalents (QE) per mg extract.

Total protein content: Total protein quantification of different plant extracts was determined by following Lowry *et al.* [24] method based on the reaction of cupric ions and peptide nitrogen followed by Folins-Ciocalteu phosphomolybdic and phosphotungstic acid reduction into heteromolybdenum (blue product). Protein was extracted by homogenization of fresh leaves of reference plant (500 mg) in chilled potassium phosphate buffer (5 mL and pH 7.0) and centrifuged at 15,000 rpm for 20 min. About 1 mL extracted protein was reacted for 10 min with 4.5 mL reagent A (0.5% $CuSO_4$ in 1% sodium potassium tartarate and 2% Na_2CO_3 in 0.1 N NaOH, 1:1 v/v) followed by 30 min incubation with reagent B (Folin-Ciocalteu and distilled water, 1:1 v/v). The absorbance of blue product at 660 nm was measured and protein values were expressed as at 660 µg bovine serum albumin (BSA) equivalent/mg extract.

Enzymatic antioxidant analysis

Superoxide dismutase (SOD) assay: Superoxide dismutase activity was assayed by photochemical method of Ries & Giannopolitis [25]. Enzyme was extracted from 1 g fresh plant leaves crushed in 4 mL chilled buffer (100 mM potassium phosphate buffer of pH 7.0 and 0.1 mM EDTA) followed by 15 min centrifugation (at 15000 rpm) at 4 ºC. About 100 µL leaf enzyme was reacted with 3 mL reagent (0.1 µM EDTA, 2 µM riboflavin, 13 mM methionine, 50 mM phosphate buffer of pH 7.8 and 75 µM NBT) and illuminated for 30 min along with control (illuminated reagent without enzyme) against blank (non-illuminated mixture) and absorbance was measured at 560 nm. SOD (One unit) needed for photoreduction of 50% NBT was calculated by following formula:

$$Z = \frac{X - A}{X} \times 100$$

where, Z = sample photoreduction %; X = absorbance of control; A = absorbance of plant enzyme; and Z/50 = total SOD unit. Finally, SOD values were expressed as unit (U) per mg fresh weight (FW).

Peroxidase (POD) assay: Peroxidase activity was performed by following slightly modified methods of Kar & Mishra [26] and Reddy *et al.* [27]. Enzyme extraction was done by homogenization of 1 g fresh mature leaves in chilled 125 µM potassium phosphate buffer of pH 6.8 (1:8 w/v) followed by 20 min centrifugation (at 12000 rpm) at 4 ºC. Reaction of 100 µL plant enzyme with 3 mL pyrogallol (1 mM) followed by adding 0.5 mL H_2O_2 (1%) was performed for measuring the absorbance at 470 nm against blank (reaction mixture excluding H_2O_2) at 10 s intervals for 1 min. Further reaction was stopped by adding 5% H_2SO_4 (1 mL) and POD values were expressed as nmol/mg protein.

Catalase (CAT) assay: Catalase activity was determined by following slightly modified procedure of Kar & Mishra [28]. Enzyme was extracted by homogenization of 1 g mature plant leaves in chilled 100 mM sodium phosphate buffer of 6.8 pH (1:8 w/v) followed by 20 min centrifugation (at 12,000 rpm) at 4 ºC. Then reaction of 0.1 mL plant enzyme with 2 mL reagent (0.1 mM EDTA, 50 mM H_2O_2 and 200 mM potassium phosphate buffer of pH 7.0) was performed for measuring the absorbance (at 240 nm) at 10 s intervals for 1 min. The CAT values were expressed as nmol/min/mg of protein.

Mineral content

Atomic absorption spectrophotometric (AAS) assay: The plant leaf powder (40 mg) was reacted in open on hot plate with 6 mL mixture of $HClO_4$ and HNO_3 (1:5 v/v) for 2 h followed by adding HNO_3 and HCl (1:1 v/v) for complete digestion for 4 h at 300 ºC resulting in colourless liquid. Completely dried and digested plant sample was dissolved in 5 mL deionized water for AAS analysis.

Quantitative phytochemical characterization

Fourier transform-infrared (FT-IR) spectroscopy: Mixture of 1 mg plant extract and 100 mg potassium bromide (1% w/w) of spectroscopic grade was pressed at 8 MPa in 1 mm transparent disk. The functional groups present in plant extract were determined by using Alpha ECO-ATR spectrometer (Bruker) within scanning frequency range of 3500 to 1000 cm^{-1}. The FT-IR experiment was performed at 21 ºC with 50% relative humidity.

Gas chromatography-mass spectrometry (GC-MS): The phytochemical characterization of AqM plant extract (5 mg/mL) of *A. arvensis* was determined by further dissolving the extract in pure methanol, which was further syringe filtered and loading sample extract in GCMS-QP2010 Ultra system armed with a gas chromatograph and a mass spectrometer assembly. Initial oven temperature was set at 60 ºC (3 min) and then isothermally risen for 21 min up to 280 ºC (15 min) at 10 ºC/min rate. Carrier helium gas flow rate was 1.21 mL per min. GC programme temperatures settings of ion source and interface were 230 and 270 ºC, respectively with solvent cut time of 3.50 min. Chromatographic conditions comprised of 73.3 kPa pressure, 260 ºC injector temperature, 60 ºC column oven temperature, 40.1 cm/s linear velocity and 10.0 injection split ratio. Mass spectrometry included mass spectra time (4 to 49.98 min) and scan range (40 to 650 *m/z*). The NIST library was referred for identifying the unknown bioactive phytochemicals of the plant extract.

Statistical analysis: The data of triplicates was represented as mean ± S.E. Analysis of variance (ANOVA) was applied with help of STPR software to investigate the anti-inflammatory and antioxidant potential and tracer minerals of plant extract. The significant difference at $p < 0.05$ of all means of different assays was calculated by Duncan's multiple range test (DMRT) in SPSS version 16.0 software. The Pearson's correlation coefficient matrix within varied parameters of plant species extract was established with help of SPSS.

RESULTS AND DISCUSSION

Phytochemical analysis: Both aqua-methanol (AqM) and aqua-acetone (AqA) extracts of *A. arvensis* L. leaf contained all the phytochemicals except quinones and cardiac glycosides, respectively. The yield in AqM (12.51 ± 1.31) was higher than AqA (11.21 ± 0.79) (Table-1).

TABLE-1
PHYTOCHEMICAL ANALYSIS AND EXTRACTION YIELD (%) OF *Anagallis arvensis* IN AqM AND AqA (MEAN ± S.E.)

Phytochemicals/ Yield	Tests	Plant extracts	
		AqM	AqA
Proteins	Xanthoproteic test	+	+
Carbohydrates	Molisch's test	+	+
Phenols	Ferric chloride test	+	+
Tannins	Lead acetate test	+	+
Flavonoids	Sulfuric acid test	+	+
Quinones	Hydrochloric acid test	-	+
Alkaloids	Mayer's test	+	+
Steroids	Liebermann-Burchard's test	+	+
Cardiac glycosides	Keller-Kiliani test	+	–
Saponins	Froth test	+	+
Terpenoids	Salkowski's test	+	+
Yield (%) (w/w)	–	12.51 ± 1.31[a]	11.21 ± 0.79[b]

(+) Presence; (–) absence; (w/w) weight by weight.
Superscripted letters denote significant difference ($p < 0.05$) between yield values.

Protein-denaturation inhibition: The principle lying behind protein-denaturation assay is the potential of plant extract to inhibit the denaturation or coagulation (heat treated turbid egg albumin) of albumin and converting the sample transparent (stabilized protein). The magnitude of turbidity is spectrophotometrically at 660 nm measured. The protein (albumin) denaturation inhibition (%) in both AqM and AqA extracts followed concentration dependent manner (Fig. 1). The protein denaturation inhibition (%) at highest concentration (100 µg/mL) was 82.90 ± 0.91 and 98.41 ± 0.49 in AqM, while 68.26 ± 1.69 and 97.04 ± 0.91 in AqA for *A. arvensis* and diclofenac sodium (standard), respectively.

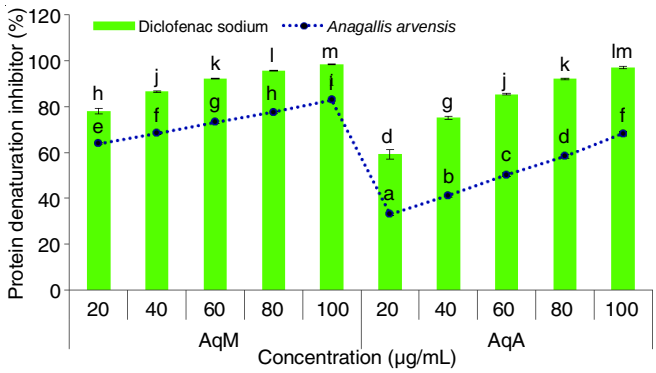

Fig. 1. Albumin denaturation inhibition (%) in AqM and AqA extracts of *A. arvensis* in different concentrations. Superscripted letters denote significant difference ($p < 0.05$)

DPPH scavenging potential: The most frequent and immediate DPPH$^{\bullet}$ scavenging assay is based on the tendency of plant antioxidants (H^+ ion donor) to alleviate the purple DPPH$^{\bullet}$ free radicals and yielding yellow scavenged product at 517 nm [29]. Radical scavenging potential in both AqM and AqA extracts of *A. arvensis* exhibited dose-dependent relationship (Fig. 2) with significant difference ($p < 0.05$). The DPPH scavenging activity (%) at 100 µg/mL concentration was 65.06 ± 1.87 and 96.75 ± 1.00 in AqM, while 50.19 ± 1.64 and 96.75 ± 1.00 in AqA for *A. arvensis* and BHT, respectively. The DPPH IC_{50} (µg/mL) was 69.86 and 1.21 in AqM, while 104.05 and 1.21 in AqA for *A. arvensis* and BHT (standard), respectively.

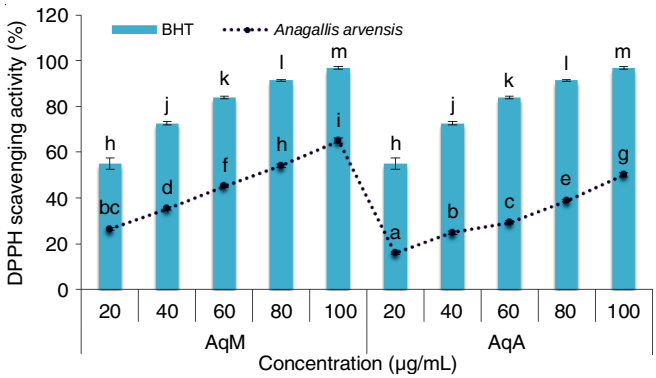

Fig. 2. DPPH scavenging activity (%) in AqM and AqA extracts of *A. arvensis* in different concentrations. Superscripted letters denote significant difference ($p < 0.05$)

Total antioxidant activity: Total antioxidant activity measured *via* phosphomolybdenum method emphasizes the plant potential to reduce molybdenum(VI) into molybdenum(V)

and yielding green product at 695 nm, the intensity of which indicates the increasing antioxidant ability of plant [19]. Showing dose-dependent relationship, the total antioxidant activity (µg VCE/mg extract) at 100 µg/mL dose was 46.85 ± 7.32 in AqM and 30.22 ± 0.25 in AqA for *A. arvensis* (Fig. 3) with significant difference ($p < 0.05$).

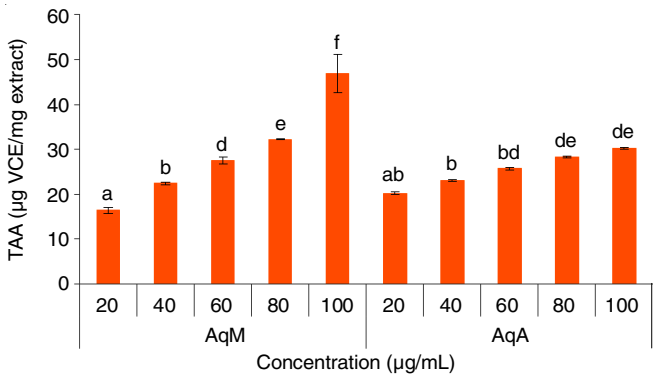

Fig. 3. Total antioxidant activity (TAC) (µg VCE/mg extract) in AqM and AqA extracts of *A. arvensis* in different concentrations. Superscripted letters denote significant difference ($p < 0.05$)

Ferric reducing antioxidant power (FRAP): The reducing power assay explains the plant antioxidants (reductants) capacity to reduce yellow Fe^{3+}-TPTZ (ferric tripyridyl triazine) into blue Fe^{2+}-TPTZ complex. The blue colour intensity at 593 nm explicates more reducing power of plant extract [30]. Iron(III) ion reducing antioxidant power (µg TE/mg extract) increased with increasing concentrations (Fig. 4) at significant level ($p < 0.05$). At highest concentration (100 µg/mL), the reducing power (µg TE/mg extract) of *A. arvensis* was 73.82 ± 1.21 and 14.26 ± 0.80 in AqM and AqA extracts, respectively.

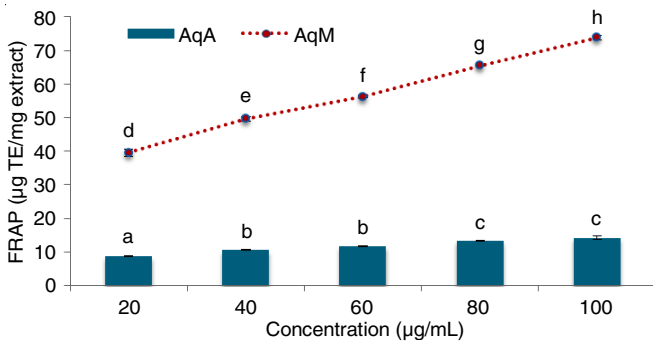

Fig. 4. Ferric reducing antioxidant power (FRAP) (µg TE/mg extract) in AqM and AqA extracts of *A. arvensis* in different concentrations. Superscripted letters denote significant difference ($p < 0.05$)

Ferrous chelation activity (FCA): Transition metal (iron) chelation includes hindrance of decomposing catalysis nature of hydroperxides by ferrous ions (chelators present in plant) and indicated by colour (blue) reduction at 593 nm [31]. Similar to DPPH scavenging activity, the ferrous chelation potential (%) of AqM and AqA extracts of *A. arvensis* showed dose dependent relationship (Fig. 5) with significant difference ($p < 0.05$). The chelation capacity (%) at maximum concentration (100 µg/mL) was 61.31 ± 1.95 and 96.36 ± 1.12 in AqM, while 64.77 ± 1.61 and 96.17 ± 1.18 in AqA for *A. arvensis* and Na_2EDTA

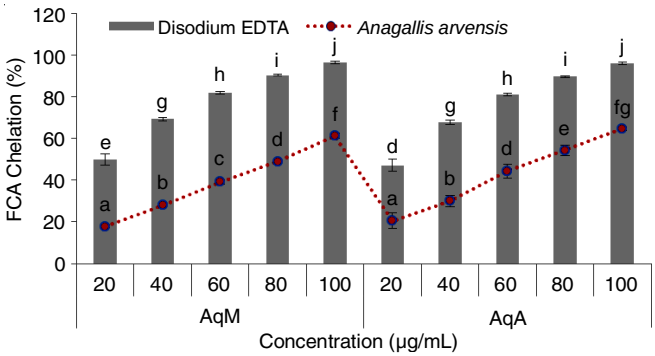

Fig. 5. Ferrous chelation activity (%) in AqM and AqA extracts of *A. arvensis* in different concentrations. Superscripted letters denote significant difference ($p < 0.05$)

(standard), respectively. The chelation IC_{50} (µg/mL) was 80.3 and 11.6 in AqM, whereas 72.75 and 15.92 in AqA for *A. arvensis* and Na_2EDTA, respectively.

Total phenol content (TPC): Phenols (low molecular weight secondary metabolites) act as chain-breaking agents for free radical chain, thus exhibit antioxidant effects [32]. With concentration supportive pattern, the total phenol content (µg GAE/mg extract) at maximum concentration (100 µg/mL) was 104.17 ± 1.41 and 68.73 ± 0.93 in AqM and AqA extracts, respectively along with more TPC in AqM extract (Fig. 6).

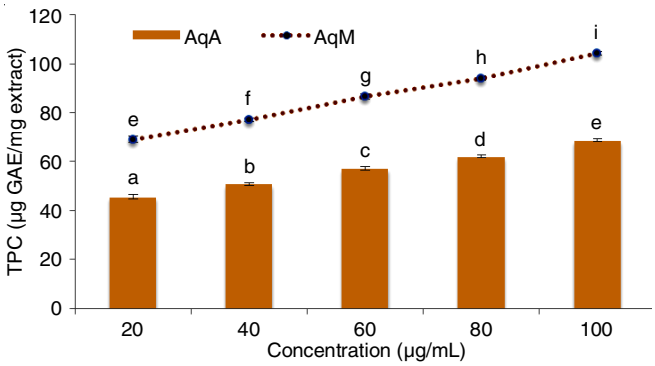

Fig. 6. Total phenol content (TPC) (µg GAE/mg extract) in AqM and AqA extracts of *A. arvensis* in different concentrations. Superscripted letters denote significant difference ($p < 0.05$)

Total flavonoid content (TFC): Flavonoids (15-carbon structure with one heterocyclic and two phenyl rings) are yellow coloured plant pigments (secondary metabolites) act as antioxidants, symbiotic nitrogen fixers, UV filters, impart colours to plant parts [33]. Total flavonoid content (µg QE/mg extract) of *A. arvensis* at 100 µg/mL dose was 7.31 ± 0.38 and 19.12 ± 1.24 in AqM and AqA extracts, respectively.

Total protein content: About 50% of daily protein requirement of human is alone accomplished by starch-based cereals (wheat, rice and maize). This human dependency on only starch based diet brings protein deficiency in developing nations that can be ameliorated by including wild edible plants in their diet [34,35]. Total protein content (mg/mL fresh weight) of *A. arvensis* leaves was 35.06 ± 0.83.

Enzymatic antioxidants: Free radicals generated daily in human body during regular metabolism denature the vital cell components (carbohydrates, lipids, proteins, *etc.*) that can

be dwindled by enzymatic antioxidants acting as defense tools [36]. In present investigation, the enzyme antioxidant activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were assessed as a supporting tool for the antioxidant capacity of *A. arvensis*. The SOD (U/mg FW), POD (nmol/mg of protein) and CAT (µmol/min/mg protein) values in *A. arvensis* with significant difference ($p < 0.05$) are represented in Fig. 7.

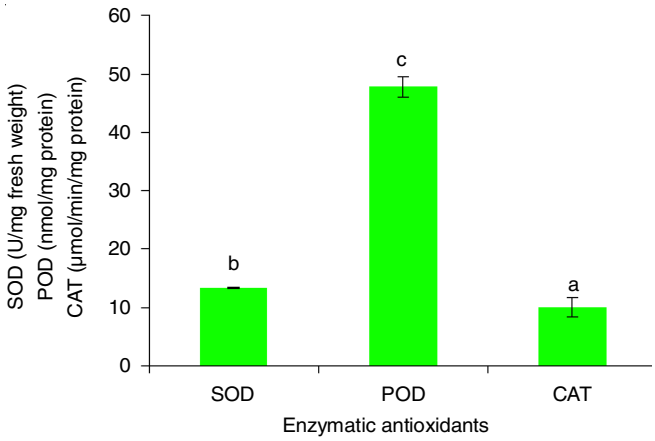

Fig. 7. Enzymatic antioxidant activity (superoxide dismutase, peroxidase and catalase) in fresh and mature leaf enzyme extracts of *A. arvensis*. Superscripted letters denote significant difference ($p < 0.05$)

Mineral content: Mineral contents (µg/mg) in present study were investigated *via* atomic absorption spectrophotometry (AAS) for supporting the anti-inflammatory and antioxidant activities of *A. arvensis* (Fig. 8). The mineral content values of the present investigation were also compared with the permissible consuming limit per day (manganese = 3 mg, copper = 0.9 mg and cobalt = 5 to 8 µg) determined by FSAI (Food Safety Authority of Ireland) [37].

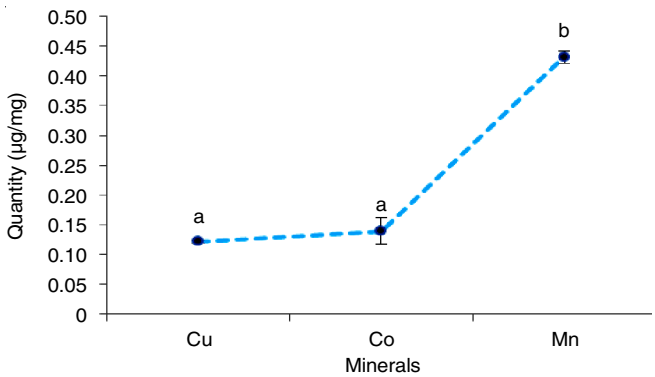

Fig. 8. Mineral estimation (µg/mg) *via* atomic absorption spectrophotometer in *A. arvensis* fresh and matured leaves. Superscripted letters denote significant difference ($p < 0.05$)

FT-IR studies: The FT-IR analysis of AqM of *A. arvensis* revealed diverse functional groups as indicators of major and minor phytocompounds by creating absorption bands spectra ranging from 3853.42 to 1020.58 cm^{-1} (Fig. 9) for *A. arvensis* (Table-2).

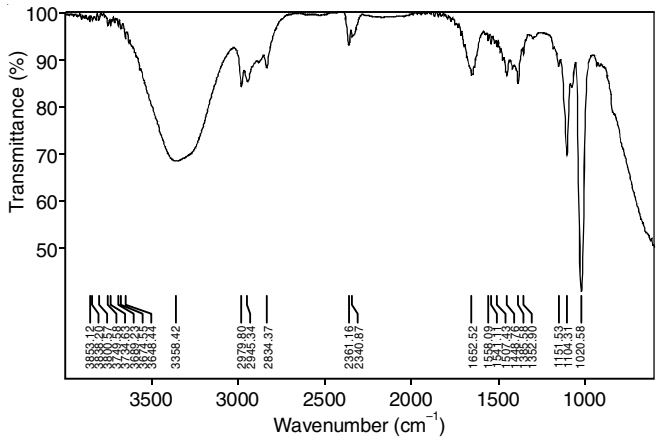

Fig. 9. FT-IR spectrophotometric profile of *A. arvensis* showing diverse functional groups peaks

The broad peak of 3358.42 cm^{-1} represents free hydroxyl (–OH: Ar–OH *str.*) groups of alcohols, organic acids, phenols and water; and amide and amine (N–H *str.*) groups of water and proteins, respectively; smaller peaks between 2979.80 to 2834.37 cm^{-1} represent symmetric and asymmetric alkanes, alkenes and alkynes (C–C, C=C and C≡C) of lipids and fatty acids; array of broad peaks between 1652.52 to 1104.31 cm^{-1} represent carbonyl groups (C–O) of esters and ketones (–C=O), aldehydes (–CHO), carboxylic acids (–COOH), nitro compounds (–NO), phosphoryl groups (P=O), aliphatic amines (C–N) and nucleic acids; and lower sharp peak of 1020.58 cm^{-1} represents aromatic compounds (di and trisubstituted) due to C=C stretching, ether and aryl hydrocarbons n(C–O–C).

The FT-IR spectrophotometric profile of *A. arvensis* may fortify the phytocompounds unveiled *via* GC-MS analysis. The functional groups under 3358.42 cm^{-1} band may constitute γ-sitosterol, 2-Methoxy-4-vinylphenol, 1-eicosanol, octadecanamide, oleic acid amide, 2,5-dimethoxy-4-ethylamphethiamine; 2979.80 to 2834.37 cm^{-1} may include glycidyl palmitate, methyl stearate, pyridine, 1,2,3,6-tetrahydro-1-methyl-4-phenyl-, 13-hexyloxacyclotridec-10-en-2-one, docosanoic acid, methyl ester, methyl palmitate, 9,12-octadecadienoic acid (Z,Z)-, methyl ester, eicosanoic acid, methyl ester; 1652.52 to 1104.31 cm^{-1} may possibly indicate methyl tetradecanoate, megastigmatrienone-4, valtrate, cyclopentadecanone and 1020.58 cm^{-1} could signify the presence of 2(4*H*)-benzofuranone, 5,6,7,7a-

TABLE-2
DOMINANT FUNCTIONAL GROUPS ALONG WITH VIBRATIONAL MODES AND PEAKS (cm^{-1}) OF *A. arvensis* ANALYZED *via* FT-IR

Functional groups	Vibrational mode	Peaks (cm^{-1})
Alcohols, organic acids, phenols, primary and secondary amides and amines	–OH, N–H	3358.42
Saturated and unsaturated hydrocarbons	C–C, C=C, C≡C	2834.37 to 2979.80
α,β-Unsaturated ketones, aldehydes, carboxylic acid, nitro compounds, aliphatic amines, esters, phosphoryls and nucleic acids	C–O, –COOH, –C=O, C–N, P=O, –NO	1104.31 to 1652.52
Aromatics, ethers and aryl hydrocarbons	n(C–O–C), C=C	1020.58

tetrahydro-4,4,7a-trimethyl-, benzoic acid, 2-hydroxy-, phenylmethyl ester, docosyl nonyl ether, 6-hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2.

GC-MS characterization: The AqM extract of *A. arvensis* revealed the presence of 59 bioactive phytocompounds of different classes namely phenols, triterpenoids, fatty acids, amides, flavonoids, alcohols, terpenes, esters, vitamins, ketones, *etc*. that have been shown in Table-3 with molecular formula, molecular weight, area (%) and retention time (min). The GC-MS chromatogram of AqM leaf extract of *A. arvensis* is shown in Fig. 10. The amount (%) of these bioactive phytocompounds ranged from 0.12% ((R-(E))-1-(2,6,6-trimethyl-2-cyclohexane-1-yl)pent-1-ene-3-one) to 11.13% (9-octadecenoic acid (Z)-, methyl ester). Area wise (%), the top leading six phytocompounds are 9-octadecenoic acid (z)-, methyl ester (11.13%); 2-hexadecen-1-ol,3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]] (11.11%); methyl elaidate (9.00%); 2-methoxy-4-vinylphenol (6.29%); 9-octa-decenamide, (Z)- (6.78%) and benzoic acid, 2-hydroxy-, phenyl-methyl ester (4.35%) contributing 48.66% composition of the total phytocompounds and strongly supporting the anti-

TABLE-3
BIOACTIVE PHYTOCOMPOUNDS REVEALED IN AqM LEAF EXTRACT OF *A. arvensis via* GC-MS ANALYSIS

Name of compound	Retention time (min)	Area (%)	m.w. (g/mol)	m.f.
2-Methoxy-4-vinylphenol	11.857	6.29	150.17	$C_9H_{10}O_2$
Pyrrolidine, 1-(1-cyclohexen-1-yl)-	13.229	0.31	151.25	$C_{10}H_{17}N$
Hydroxy-α-terpenyl acetate	13.841	0.26	212.28	$C_{12}H_{20}O_3$
12-Hydroxy-16,17-dimethylpregn-4-ene-1,20-dione	13.893	0.32	358.50	$C_{23}H_{34}O_3$
(3-E)-3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	14.002	0.51	206.32	$C_{14}H_{22}O$
(R-(E))-1-(2,6,6-trimethyl-2-cyclohexane-1-yl)pent-1-ene-3-one	14.519	0.12	206.32	$C_{14}H_{22}O$
2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-,	14.795	0.27	196.24	$C_{11}H_{16}O_3$
Benzenemethanol, α-trichloromethyl)-, acetate	14.933	1.36	267.54	$C_{10}H_9Cl_3O_2$
2,5-Dimethoxy-4-ethylamphethiamine	15.099	1.28	223.31	$C_{13}H_{21}NO_2$
Diethyl Phthalate	15.382	2.28	222.24	$C_{12}H_{14}O_4$
(3R,3aS,6S,7R)-3,6,8,8-.tetramethyloctahydro-1H-3a,7-.methanozulen-6-ol	15.808	0.20	222.37	$C_{15}H_{26}O$
Megastigmatrienone-4	15.881	0.24	190.28	$C_{13}H_{18}O$
2-Propenal, 3-phenyl-, monopentyl derivative	16.106	1.94	136.16	C_9H_8O
7-Oxabicyclo[4.1.0]heptan-3-ol,6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-	16.370	0.68	226.31	$C_{13}H_{22}O_3$
3,4,4-trimethyl-3-(3-oxo-but-1-enyl)-bicyclo[4.1.0]heptan-2-one	16.569	0.39	220.31	$C_{14}H_{20}O_2$
Pyridine, 1,2,3,6-tetrahydro-1-methyl-4-phenyl-	16.733	0.80	173.25	$C_{12}H_{15}N$
Methyl tetradecanoate	16.899	0.22	242.40	$C_{15}H_{30}O_2$
Octanal, 2-(phenylmethylene)-	17.232	2.32	216.32	$C_{15}H_{20}O$
6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2	17.581	1.08	196.24	$C_{11}H_{16}O_3$
Valtrate	17.731	0.15	422.50	$C_{22}H_{30}O_8$
2,6,10-trimethyl,14-ethylene-14-pentadecene	18.118	1.03	278.00	$C_{20}H_{38}$
2-Pentadecanone, 6,10,14-trimethyl-	18.177	0.96	268.50	$C_{18}H_{36}O$
Tonalide	18.380	1.31	258.40	$C_{18}H_{26}O$
Neophytadiene	18.567	0.40	278.50	$C_{20}H_{38}$
Benzoic acid, 2-hydroxy-, phenylmethyl ester	18.636	4.35	228.24	$C_{14}H_{12}O$
Methyl palmitate	19.021	2.77	270.50	$C_{17}H_{34}O_2$
Cyclo-l-prolyl-l-proline	19.318	2.48	194.23	$C_{16}H_{17}N_3O_2$
5h,10h-Dipyrrolo[1,2-a:1',2'-d] pyrazine-5,10-dione, octahydro-, (5AS-CIS)	19.426	1.01	194.24	$C_{10}H_{14}N_2O_2$
1-Aminocyclopentanecarboxylic acid, N-neopentyloxycarbonyl-,octadecyl ester	20.473	0.25	425.65	$C_{25}H_{47}NO_4$
Cyclopentadecanone	20.536	0.29	224.38	$C_{15}H_{28}O$
9,12-octadecadienoic acid (Z,Z)-, methyl ester	20.657	3.51	294.47	$C_{18}H_{32}O_2$
Methyl elaidate	20.718	9.00	296.50	$C_{19}H_{36}O_2$
2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-E]]	20.827	11.11	296.50	$C_{20}H_{40}O$
Methyl stearate	20.953	1.36	298.50	$C_{19}H_{38}O_2$
Cyclohexyl benzoate	21.200	0.09	204.26	$C_{13}H_{16}O_2$
Oleic acid amide	21.524	1.78	281.50	$C_{18}H_{35}NO$
3,7,11,15-tetramethylhexadec-2-en-1-yl acetate	21.735	0.38	338.57	$C_{22}H_{42}O_2$
Glycidyl palmitate	22.492	0.34	312.50	$C_{19}H_{36}O_3$
Methyl (11E)-11-eicosenoate	22.540	0.74	324.50	$C_{21}H_{40}O_2$
cis-11-Eicosenoic acid, methyl ester	22.602	0.39	324.50	$C_{21}H_{40}O_2$
13-Hexyloxacyclotridec-10-en-2-one	22.750	0.13	280.40	$C_{18}H_{32}O_2$
Eicosanoic acid, methyl ester	22.784	0.33	326.56	$C_{21}H_{42}O_2$
trans,trans-9,12-Octadecadienoic acid, propyl ester	23.139	0.55	322.53	$C_{21}H_{38}O_2$
Octadecanamide	23.464	0.36	283.50	$C_{18}H_{37}NO$
3-cyclopentylpropionic acid, 2-dimethylaminoethyl ester	24.286, 22.377	1.29, 0.64	213.32	$C_{12}H_{23}NO_2$
Cyclododecyne	24.409	0.26	164.29	$C_{12}H_{20}$
9-Octadecenal, (Z)-	24.476	1.14	266.50	$C_{18}H_{34}O$
1-Eicosanol	24.748	0.64	298.50	$C_{20}H_{42}O$

9-Octadecenoic acid (z)-, methyl ester	24.854, 20.776	11.13, 0.93	296.49	$C_{19}H_{36}O_2$
cis-10-Nonadecenoic acid, methyl ester	24.941	1.06	310.50	$C_{20}H_{38}O_2$
Docosanoic acid, methyl ester	25.209	1.09	354.61	$C_{23}H_{46}O_2$
Docosyl nonyl ether	28.040	0.54	452.80	$C_{31}H_{64}O$
15-Tetracosenoic acid, methyl ester, (Z)-	28.120	0.86	380.60	$C_{25}H_{48}O_2$
Tetracosanoic acid, methyl ester	28.442	1.29	382.66	$C_{25}H_{50}O_2$
9-Octadecenamide, (Z)-	29.033, 23.197	3.82, 2.96,	281.48	$C_{18}H_{35}NO$
Fumaric acid, 2-dimethylaminoethyl octadecyl ester	29.998	0.88	439.70	$C_{26}H_{49}NO_4$
(R)-6-Methoxy-2,8-dimethyl-2-((4R,8R)-4,8,12-trimethyltridecyl) chroman	32.601	1.21	416.68	$C_{28}H_{48}O_2$
Vitamin E	33.961	1.11	430.71	$C_{29}H_{50}O_2$
γ-sitosterol	38.043	3.21	414.70	$C_{29}H_{50}O$
Total		100.00		

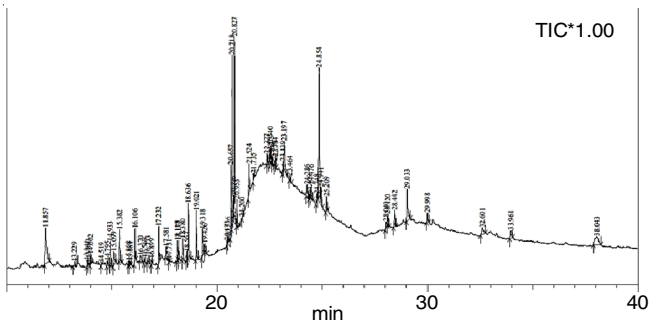

Fig. 10. GC-MC chromatogram of AqM leaf extract of *A. arvensis*

inflammatory and antioxidant potential of the reference plant species. Out of 59 phytocompounds, 26 have biological activities and medicinal properties as shown in Table-4. The above mentioned six major compounds have exhibited anti-inflammatory, antioxidant and anticancer features, which clearly strengthen the present study parameters. Other minor compounds showed several biological activities like hypolipidemic, antimicrobial, anti-androgenic, hypocholesterolenic, cytotoxic, antiviral, anti-angeogenic and antidiarrhoeal, flavouring agents, *etc*. some phytocompounds showed unique properties such as methyl tetradecanoate (0.22%) as platelet aggregation inhibitor,

TABLE-4
BIOACTIVE PHYTOCOMPOUNDS ANALYZED IN AqM LEAF EXTRACT OF *A. arvensis via* GC-MS ANALYSIS WITH BIOLOGICAL ACTIVITIES

Chemical name	Property	Biological activity	Ref.
2-Methoxy-4-vinylphenol	Phenolic compound	Anti-inflammatory, antioxidant, antimicrobial and flavouring agent.	[38,39]
γ-Sitosterol	Triterpenoid	Antimicrobial, antiviral, antioxidant, anticancer, antidiabetic, anti-inflammatory, antiangeogenic and antidiarrhoeal.	[40]
9-Octadecenoic acid (z)-, methyl ester	Oleic acid derivative	Antimicrobial, antiandrogenic, anti-inflammatory, anticancer, dermatitigenic, anemiagenic, 5α-reductase inhibitor, insectifuge and hypocholesterologenic.	[41,42]
2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	Volatile terpene	Flavouring agent and attractant pheromone in red imported fire ants (RIFA),	[43]
Methyl tetradecanoate	New aggreceride A	Platelet aggregation inhibitor	[44]
Valtrate	Irioids (a cyclopentanopyran monoterpenoid)	Antifungal, cytotoxic, antitumor, anxiolytic and anti-ovarian cancer agent.	[45,46]
2,6,10-Trimethyl,14-ethylene-14-pentadecene	Neophytadiene terpenoid	Anti-proliferative	[47-49]
Methyl palmitate	Saturated fatty acid	Antifungal, flavouring agent, antioxidant, 5α-reductase inhibitor, antibacterial, anti-androgenic, hypocholesterolenic and nematicidal.	[50-52]
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Linoleic acid ester	Antioxidant, hypocholesterolenic, antihistaminic androgenic, antieczematic, anticoronary, insect repellent, antiacne, hepatoprotective, 5α-reductase inhibitor, nematicidal and antiarthritic.	[53,54]
Eicosanoic acid, methyl ester	Arachidic acid	α-Glucosidase inhibitor	[55]
Neophytadiene	Terpenoid	Antipyretic, anti-inflammatory, antioxidant, antifungal, analgesic and antimicrobial.	[40]
Vitamin E	Vitamin	Antiageing, antioxidant, hypoglycaemic, analgesic, antitumor, anti-inflammatory, anticancer, antileukemic, vasodilator, antispasmodic, anticoronary and antibronchitic.	[54,56,57]
Cyclopentadecanone	Macrocyclic musk (ketones)	Used in fragrances, cosmetics, food and medicines.	[58]
Oleic acid amide	Fatty acid amide derivative	Treats obesity and sleeping disorders.	[59,60]
Docosanoic acid, methyl ester	Fatty acid	Used in therapeutic and diagnostic activities.	[55]
Octanal, 2-(phenylmethylene)-	Flavonoid aldehyde	Used as flavouring additive in pharmaceutical and food industries; as fragrance in cosmetics; as pesticide against insects and arachnids.	[61,62]

6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2	Loliolide derivative	Anti-inflammatory agent.	[63]
Benzoic acid, 2-hydroxy-, phenylmethyl ester	Salicylic acid benzyl ester	Used as UV-light absorber in cosmetics and floral perfumes fixative.	[64,65]
9-octadecenamide, (Z)-	Oleamide	Antioxidant, hypolipidemic, antimicrobial, endogenous sleep-inducing chemical and signaling molecule.	[66-68]
1-Eicosanol	Arachidyl alcohol	Emollient and consistency agent in cosmetics and pharmaceutical industries.	[69]
Megastigmatrienone-4	Cyclohexenone	Flavouring agent and antieczematic.	[70]
13-hexyloxacyclotridec-10-en-2-one	Fatty acid	Antitumor and antimicrobial agent.	[71,72]
2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-E]]	Phytol	Anti-inflammatory, anticancer, antimicrobial, anti-arthritic, diuretic, flavouring and cytotoxic agent used to manufacture vitamin-E.	[73]
Methyl elaidate	Unsaturated fatty acid methyl ester	Anticancer agent and apoptosis inducer.	[74]
Tonalide	Musk tetralin	Fragrance agent in cosmetics	[75]
2-Propenal, 3-phenyl-, monopentyl derivative	Cinnamaldehyde	Hypoglycaemic, vasodilator, antifungal, treats ACD (allergic contact dermatitis), antimicrobial, flavorant, food adulterant, insecticidal, antimalarial, congestion inhibitor, anticancer, tyrosinase inhibitor, hypolipidemic and hypocholesterolenic.	[76]

2(4*H*)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl- (0.27%) as attractant pheromone in red imported fire ants (RIFA), eicosanoic acid, methyl ester (0.33%) as α-glucosidase inhibitor.

Correlation analysis: In present study, the correlation matrix in Table-5 clearly indicates that all the parameters (anti-inflammatory potential, DPPH, TAA, FRAP, FCA and TPC) in both AqM and AqA solvents are correlated and supporting each other having significantly strong positive Pearson's correlation ($p < 0.05$). Even very strong positive correlation (1.00**) have been found between DPPH *vs.* anti-inflammatory, FCA *vs.* Anti-inflammatory, TPC *vs.* anti-inflammatory, DPPH *vs.* FCA, DPPH *vs.* TPC, FCA *vs.* TPC in AqM; while DPPH *vs.* TPC in AqA extracts. Thus, AqM showed more positive correlation than AqA extract.

The present study focused on a comprehended antioxidant, anti-inflammatory activities and phytochemical assessment of AqM and AqA leaf extracts of *A. arvensis*. On the whole, AqM performed better to supress free radicals and inflammation than AqA extract. It could be due to the higher solvent polarity and gas chromatographic potentiality of methanol to dissolve very minute molecules than acetone to show better antioxidant and anti-inflammatory activitives. However, ferrous chelating activity (FCA) and total flavonoids (TFC) were found in AqA extracts that may be due to the less polarity of acetone to easily dissolve macromolecules (flavonoids), which hardly break due to their higher aromatic resonance and tendency of flavonoids to chelate the metals directly correlates this aspect [77]. In addition, the correlation coefficient between FCA and TFC was established and gained $R^2 = 1.00$, which undeniably supports the metal chelation propensity of flavonoids.

Lopez *et al.* [8] reported 113.39 ± 8.82 µg/mL DPPH IC_{50} in aerial part methanol extract of *A. arvensis*; Shakoor *et al.* [10] reported 85% DPPH scavenging activity in leaf methanol extract; Yasmeen [78] reported 65.30 ± 0.3% DPPH scavenging potential in aerial part methanol extract of *A. arvensis* that is almost equal to the present study AqM; Saleem *et al.* [11] reported 7.8% yield, 231 µg/mL DPPH IC_{50}, 37.34 ± 1.06 mg GAE/g TAA, 82.97 mg TE/g extract FRAP, 15.84 ± 1.35 mg EDTAE/g metal chelation, 27.54 ± 0.92 mg GAE/g TPC and 26.15 ± 0.85 mg QE/g TFC in aerial MeOH extract of *A. arvensis*.

The varying anti-inflammatory and antioxidant values may be due to diverse experimental methods, plant samples, different solvents used and diversified methodologies and

TABLE-5
CORRELATION MATRIX OF DIFFERENT PARAMETERS IN AqM AND AqA EXTRACTS OF *Anagallis arvensis*

	Anti-inflammatory	DPPH	TAA	FRAP	FCA	TPC
AqM extract						
Anti-inflammatory	1.00					
DPPH	1.000**	1.00				
TAA	0.975**	0.975**	1.00			
FRAP	0.997**	0.997**	0.967**	1.00		
FCA	1.000**	1.000**	0.975**	0.997**	1.00	
TPC	1.000**	1.000**	0.975**	0.996**	1.000**	1.00
AqA extract						
Anti-inflammatory	1.00					
DPPH	0.992**	1.00				
TAA	0.995**	0.981**	1.00			
FRAP	0.991**	0.980**	0.998**	1.00		
FCA	0.997**	0.980**	0.997**	0.991**	1.00	
TPC	1.000**	0.989**	0.995**	0.989**	0.998**	1.00

certainly the phytocompounds, which bring discrepancies in assays. Thus, several assays together are applied to obtain the experimental accuracy. Further, the phytoconstituents analyzed by GC-MS exhibited multifarious bioactivities and medicinal properties such as antimicrobial, antifungal, anti-inflammatory, antioxidant, anticancer, antidiabetic, hypocholesterolenic, nematicidal, antibronchitic, anticoronary, hypolipidemic, hepatoprotective and so on.

Conclusion

The present work represents a coherent analysis on the anti-inflammatory and biochemical potential of *A. arvensis* leaf extract. The excessive use of the same medicine or drug in repetition leads to reduce the effectiveness of drug against any disease or microbe, which becomes resistant and causes infections and inflammation (a common reflex reaction) in the body tissues. The GC-MS analysis of *A. arvensis* in current research study leads to the future role of the plant as a remedy and natural treasury of medicinal bioactive phyto-compounds such as anti-inflammatory agents, enzymatic and non-enzymatic antioxidants, mineral nutrients, *etc*. in pharmaceutical and nutraceutical fields. Further research is required to isolate, purify and characterize the bioactive phytoconsti-tuents of the reference plant species to help in mitigating the human ailments and replace the synthetic medicines, which impair the health.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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