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Research Article

PHYTOCHEMICAL SCREENING WITH LC-HRMS PROFILING AND *IN VITRO* BIOLOGICAL ACTIVITIES OF *ARGYREIA CUNEATA* (L.) *AND ARGYREIA SETOSA* (L.).

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ABSTRACT

Objective: The present study was designed for phytochemical screening and biological activities of *Argyreia cuneata* (L.) and *Argyreia setosa* (L.) medicinal plants.

Methods: The mature leaves of *A. cuneata* (L.) and *A. setosa* (L.) were extracted with methanol (Ac-Me, As-Me) and ethyl acetate (Ac-EA, As-EA) solvent followed by qualitative and quantitative phytochemical analysis. Antimicrobial activity was evaluated by agar well diffusion method, whereas the activities such as antioxidant, antidiabetic, and anti-inflammatory were determined by *in vitro* methods. Liquid chromatography-high-resolution mass spectroscopy (LC-HRMS) was used to recognize the bioactive components.

Results: The highest phenolic content (0.840±0.130 mg GAE/ml extract) reported from As-Me and for flavonoids 0.128±0.012 mg QE/ml from Ac-EA. The Ac-Me exhibited higher inhibition zone against all the bacteria used for study. In antioxidant activity, Ac-Me and Ac-EA report highest 2,2 dipheny l,1 picryl hydrazine (IC50=0.580±0.012 mg/ml) and nitric oxide radical scavenging potential (IC50=0.772±0.059 mg/ml), respectively. For antidiabetic activity, As-Me showed highest α-amylase inhibition activity as well as glucose adsorption. In anti-inflammatory activity, Ac-EA exhibits highest (IC50=0.529±0.009 mg/ml) protein denaturation inhibition and Ac-Me showed highest (91.56±1.96%) HRBC hemolysis inhibition. The LC-HRMS analysis of methanolic extract reports the majority of phenolic compounds.

Conclusion: The study showed that the plants *A. cuneata* (L.) and *A. setosa* (L.) are well exploited and can be used for the source of potent natural bioactive components. This study also may thereby provide an insight in screening of crude drug.

Keywords: *Argyreia cuneata*, *Argyreia setosa,* Liquid Chromatography–High-Resolution Mass Spectroscopy, Biological activities, DPPH.

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INTRODUCTION

Phytochemical analysis has great interest to identity the health benefits in drug and functional food development. The health effects of phytochemicals can identify by many *in vitro* screening methods. Due to high cost and low productivity in these methods, there is scope for improvement. Hence by utilizing molecular, chemical, or pharmacological information, researchers have proposed *in silico* methods as alternative [1,2]. The medicinal plants are the great source of essential nutrients and bioactive phytochemicals which are important in promoting health as well as preventing diseases. The vital nutrients, that is, macro- and micronutrients in plants address as crucial requirement for human health since long ago. Recently, the phytochemicals emerged as key molecules of various cellular signaling pathways that known to be secondary metabolites. These compounds are generally non-nutritive chemicals produced through several chemical pathways in plants. The large number of phytochemicals can be beneficial to the cellular functions. It also shows the effects on heath as phytochemical rich food and strongly indicates their role in curing diseases.

The plant genus *Argyreia* (L) belongs to family convolvulaceae that has been used in different system of traditional medication for the treatment of diseases and ailments of human beings. The ergot alkaloid lysergic acid amide (LSA) is a secondary plant constituent in a number of plants, but it is mainly present in considerable amounts in Convolvulaceae, like *Argyreia nervosa*. The LSA level in correlation with several vegetative adverse effects in human serum and urine analyzed [3]. The anti-diabetic potential and antibacterial activity of silver nanoparticles synthesized from *A. nervosa* have been evaluated. The preliminary phytochemical analysis of several species from genus *Argyreia* is done [4,5]. *Argyreia speciosa* possessed strong antioxidant activity, remarkable anti-diabetogenic effect comparable to glibenclamide, a well-known liver protecting herbal. The methanolic extract of *Argyreia argentea* shows potent analgesic and anti-inflammatory activity in animal model. The extract could be a new and potential source of anti-inflammatory and analgesic drug [6,7]. Three resin glycosides (two are isomers) are isolated from *Argyreia acuta*. N-Formylloline is isolated from roots of Argyreia mollis and this is the first identification of a 1-aminopyrrolizidine alkaloid (loline alkaloid) in a species of the Convolvulaceae [8,9].

A. nervosa contains the presence of some major phytochemicals such as alkaloids, glycosides, tannins, and flavonoids. The Butanolic extract of *A. speciosa* showed potent antiulcer property *in vitro* and *in vivo* study. The antiulcer potential of n-butanol fraction of methanolic extract of *A. nervosa* may be due to the synergistic effect of Quercetin and Kaempferol [10-12]. The ethanolic extract of *A. speciosa* possesses immunomodulatory activity. The compounds such as hexadecanyl p-hydroxycinnamate and scopoletin isolated from A. speciosa roots showed the antifungal activity. A. speciosa possesses significant dose-dependent gastroprotective activity, probably due to its free radical scavenging activity [13-15]. The literature survey revealed that the chemical diversity of bioactive components and pharmacological actions of many species for genus *Argyreia* remains unaddressed by rigorous scientific research to define efficacy and safety. Hence, our study focusing on to reports the active phytochemicals from the *A. cuneata* (L.) and *A. setosa* (L.) plants which show the biological activities.

METHODS

Collection of sample and preparation of extract

The A. cuneata (L.) and A. setosa (L.) plant samples were collected from western hat region covered under Junnar Tahsil of Pune

district, Maharashtra, India. Location lies in between 19°19'45.7"N 73°53'30.3"E and 19°22'38.5"N 73°48'35.9"E. The plant samples were authenticated by Dr. M. M. Lekhak, Department of Botany, Shivaji University, Kolhapur (India) with the voucher specimen no.KVS01&KVS02. The leaves of plants were washed thoroughly, and then shed dried for 15 days at room temperature. Fine powder of shed dried leaves was prepared using a mechanical grinder. Ten grams of powder were mixed in 150 ml solvent. For complete extraction, the mixture was kept overnight in shaking incubator. The suspension was, then, filtered and crude extract was obtained. The concentrated crude extract was obtained by evaporating maximum solvent using rotavapor. The viscous extract was, then, taken on Petri plates and evaporated completely. Dried leaf extract was weighed and re-dissolved in a solvent [16].

Phytochemical screening of extract

The phytochemical tests for secondary metabolites such as alkaloids, anthraquinones, proteins and amino acids, saponins, flavonoids, phenols, terpenoids, sterols, tannins, and glycosides were performed to know the existence in plant extracts tests [17-19].

Test for alkaloids

Alkaloids were detected using Meyer's test. Plant extracts + Mayer's reagent (Potassium Mercuric Iodide), formation of a yellow colored precipitate indicates the presence of alkaloids.

Test for anthraquinone

Add 0.5 ml of plant extract + 1 ml of 10% ammonium solution in aliquots, shake vigorously for 1 min. Red color indicates the presence of anthraquinone.

Test for proteins and amino acids

Ninhydrin test – add few drops of Ninhydrin reagent in 1 ml of plant extract and then keep in boiling water bath for 30 s to 1 min. Purple color to mixture indicates the presence of proteins and amino acids.

Test for saponin

Foam test – take 1 ml of plant extract in test tube and shake for 15 min. Formation of foam layer indicates the presence of saponin.

Test for flavonoids

Aluminium chloride test – take adequate amount of plant extract and add equal amount of 1% aluminium chloride solution into it. The light-yellow color indicates the presence of flavonoid.

Test for phenols

Ferric chloride test – add 1 ml of plant extract in 2 ml of 5% or 1 N aqueous ferric chloride. The deep blue or black color to mixture indicates presence of phenols.

Test for terpenoids

H_2SO_4 Test – add 0.4 ml of chloroform + 1 ml of plant extract in aliquots. Evaporate mixture on boiling water bath then cool and add 0.6 ml conc. H_2SO_4 to it. Boil mixture for 30 s. Gray color formation indicates the presence of terpenoids.

Salkowski's test – take 1 ml plant extract +2 ml of chloroform in test tube then add 3 ml of conc. H_2SO_4 from the side of the test tube. Reddish-brown color at the interface indicates the presence of terpenoids.

Test for sterols

Salkowski's test – take 1 ml mixture plant extract + 2 ml of chloroform in test tube, add 1 ml of conc. H_2SO_4 from the side of the test tube. Red color formation to chloroform layer and greenish-yellow fluorescent to acid layer indicate the presence of sterols.

Test for tannins

Ferric chloride test – take 1 ml of plant extract + 1ml distilled water, then add 2–4 drops of 1 N ferric chloride solution. The formation of black color indicates presence of tannins.

Test for glycosides

Leibermann's test – to the mixture of 1 ml of acetic acid and 1 ml of chloroform, add 1 ml of plant extract. After cooling, this mixture adds drop wise conc. H_2SO_4. The formation of green color indicates presence of glycosides.

Salkowski's test - add 2 ml of conc. H_2SO_4 drop wise to the plant extract. The reddish-brown color to mixture indicates presence of glycosides.

Determination of total phenolic content

To access the total phenolic content in plant extracts, Folin-Ciocalteu method described by Raghavendra *et al*. [20] was used. 0.5 ml extract with diluted concentrations mixed with 0.5 ml of Folin-Ciocalteu reagent (1:10) and 2 ml of sodium carbonate (2%) in separate tubes. Then mixture tubes were incubated at room temperature for 30 min. Absorbance of reaction mixture determined spectrophotometrically at 765 nm. Gallic acid used as a standard. Gallic acid (0–1000 µg/ml) standard curve plot and total phenolic content in different extracts estimated as mg Gallic acid equivalent (GAE) from the graph.

Determination of total flavonoid content

Determination of total flavonoid content in plant extracts was done by the method described by Aryal *et al*. [21]. The reaction mixtures were prepared using 1 ml of extract or Quercetin (25–200 µg/ml) solution with 0.2 ml 10% (w/v) $AlCl_3$ solution in methanol, 0.2 ml (1M) potassium acetate, and 5.6 ml Distilled water. The mixtures, then, incubated at room temp for 30 min. Absorbance was recorded at 415 nm. Quercetin used as a standard. Quercetin (0–200 µg/ml) standard curve has been plot using different concentrations and total flavonoid content in different extracts expressed as mg Quercetin equivalent from the graph.

Determination of Total protein content by Lowry's method

The total protein content in plant extracts was estimated by Lowry *et al*. [22]. The blue color developed after reaction of protein was read at 660 nm with a spectrophotometer. The concentration of protein was calculated using BSA as a standard. The total protein content was expressed in mg/ml of sample extract.

Determination of total reducing sugar content by DNSA method

The estimation of total reducing sugar content was done by DNSA method. The orange color developed after reaction of glucose with DNSA was read at 530 nm. The total reducing sugar concentration was calculated using glucose as a standard. The total content of reducing sugar expressed in mg/ml of extract [23].

Antimicrobial activity

For antimicrobial screening of A. cuneata (L.) and A. setosa (L.), leaf extract agar well diffusion method was used. Bacterial cultures (24 h old) were used. Using a sterile glass spreader, a100 µl of test bacterial suspensions were spread over the nutrient agar plates and using the 1 ml micro pipette's tip wells were made in plates. A100 µl of A. cuneata (L.) and A. setosa (L.) leaf extracts (1 mg/ml) were added to the well. For the reference standard, 100 µl of antibiotic (Ampicillin) was added and for the control 100 µl of DMSO was added to the wells. Plates were left to ensure the complete diffusion of added samples. After that, plates were kept in incubator at 37°C for 24 h. Inhibition zone was measured after incubation. No any inhibition zone was observed for DMSO as negative control. The ampicillin showed average inhibition zone 2.5 cm for all the test organisms. The % RIZD (Relative Inhibition Zone Diameter) was determined by the formula given below:

$$\%RIZD = \frac{(IZD\ sample - IZD\ negative\ control)}{IZD\ antibiotic\ control} \times 100$$

Antioxidant activity

Determination of DPPH radical scavenging activity

The stable DPPH (2,2 diphenyl,1 picryl hydrazine) free radical scavenging activity of extract evaluated using method described by Nguemfo *et al*. [24]. 1mlextracts (100–1000 μg/ml) added to 3.0ml of a solvent DPPH solution (0.004% in methanol). Then incubate the tubes in dark for 30 min, the absorbance recorded at 517 nm in a spectrophotometer, and using solvent as blank. Ascorbic acid was used as control. The percentage inhibition of the DPPH radical by sample calculated according to the formula:

% Inhibition = [A – B]/A × 100

Where A= absorbance of the DPPH control at *t* = 0 min and B = absorbance in presence of extract at a particular concentration at t = 30 min. The IC50 = conc. of sample required to scavenge 50 % DPPH radicals [24].

Determination of nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity was accessed by method described by Gangwar *et al*. [25] with minor modification. In reaction mixture, a various conc. (100–1000 μg/ml) of extract added in 10 mm Sodium Nitroprusside prepared in 0.5 M phosphate buffer (pH 7.4) with final volume of 3 ml. After incubation for 150 min at 25˚C, 1.0 ml Griess reagent [(0.33% sulfanilic acid in 20% glacial acetic acid)+ 1 ml of naphthylethylenediamine dichloride (0.1% w/v)] was added. The absorbance was measured spectrophotometrically at 546 nm. Ascorbic acid was used as a positive control. The amount of nitric oxide radical inhibition is calculated by equation given below:

% inhibition of NO radical= $(A_0 - A_1)/A_0 \times 100$

Where, A_0= absorbance before reaction and A_1= absorbance after reaction.

Anti-diabetic activity

Assay of α-Amylase inhibition

In vitro amylase inhibition was studied by method of Banerjee *et al*. [26] with some modifications. A 500 µL of the extract (100–1000 μg/ml) mixed with 500 µl of 0.1M phosphate buffer pH 6.9 containing α-amylase enzyme (fungal diastase [0.5%]). After 20-min incubation at 37°C, 500 µl of 1% starch solution in 0.1M phosphate buffer pH-6.9 (containing 6.7 mM sodium chloride) added and then incubated at 37°C for 10 min. For the control, same procedure was performed, where 500 µl buffer was added instead of enzyme. Reaction was arrested by adding 1ml of dinitrosalicylic acid reagent in reaction mixture and kept it in boiling water bath for 10 min then cooled and absorbance was measured at 540 nm using spectrophotometer. The % α-amylase enzyme inhibition was calculated using following formula;

Inhibition (%) = [A(control) – A (extract)/A(control)] × 100

Where, A = Absorbance at 540 nm

Determination of glucose adsorption capacity

Glucose adsorption capacity of the samples was determined by the method described by Das and Gayathri [27], Gauhar *et al*. [28], and Bhutkar *et al*. [29] with modifications. The plant extracts (1%) were added to 25 ml of glucose solution (50–100 mmol/l). The mixtures were incubated in a shaker water bath at 37°C for 6 h. After incubation, mixtures were centrifuged at 4800 rpm for 20 min. Supernatant was collected and glucose content determined by glucose oxidase peroxidase method. The concentration of bound glucose was determined using formula:

Glucose bound = [G1 - G6/weight of sample] × V

Where, G1 = glucose concentration at time t = 0, G6 = glucose concentration at time t = 6 h.

V = volume of sample

***In vitro* Anti-inflammatory activity**

BSA denaturation inhibition assay

Protein denaturation assay was performed by method describe by Osman *et al*. [30] with modifications. The reaction mixture consisted of the 100 µl test extracts (100–1000 µg/ml) and 100 µl of 5% aqueous solution of bovine serum albumin (BSA); 3 ml of phosphate buffer saline (PBS, pH-6.4). pH was adjusted by adding a small volume of glacial acetic acid. The mixtures were incubated at 37°C for 20 min and then heated to 70°C for 10 min. The mixture was allowed to cool for 10 min after which turbidity was measured at 660 nm. The blank comprised the sample and distilled water. Distilled water was used as the negative control. The positive control was diclofenac sodium. A % inhibition of BSA protein denaturation was calculated formula:

% inhibition = $(A_c - A_t)/A_c \times 100$

Where, A_c= absorbance of control and A_t= absorbance of test.

Egg albumin denaturation inhibition

Egg albumin protein denaturation method was performed by method described by Chippada *et al*. [31] and Dharmdeva *et al*. [32]. 0.4 ml of egg albumin (from fresh hen's egg), 5.6 ml of phosphate buffered saline (PBS, pH 6.4), and 4 ml of plant extract (100–1000 µg/ml) mixed in aliquots. Double-distilled water used as control. Then, tubes were incubated at (37°C ±2) for 15 min and then heated at 70°C for 5 min. The solutions were cooled and absorbance measured at 660 nm using inner medium (vehicle) as blank. Diclofenac sodium (100–1000 µg/ml) used as positive control. The % inhibition of egg albumin protein denaturation was calculated by given formula:

% inhibition = $(A_c - A_t)/A_c \times 100$

Where, A_c= absorbance of control and A_t= absorbance of test.

Membrane lysis assay

Preparation of erythrocyte suspension

Erythrocyte cell suspension was prepared by method described by Gunathilake *et al*. [33]. Healthy human blood collected in heparinized centrifuge tube and centrifuged at 3000 rpm for 5 min, then washed three times 1:1 normal saline (0.9% NaCl). After the centrifugation, blood sample reconstituted as suspension by 10% (v/v) isotonic buffer solution (10 mM sodium phosphate buffer pH 7.4) (For 1L isotonic buffer solution volume 0.2 g NaH_2PO_4 + 1.15g Na_2HPO_4 + 9 g NaCl) [33].

Heat-induced hemolysis

The mixture of 0.05 ml of blood cell suspension and 0.05 ml of leaves extracts (25, 50, 75, 100 µg/ml) were prepared with 2.95 ml phosphate buffer (pH-7.4). Mixtures were incubated at 54ºC for 20 min in a shaking water bath then centrifuged (2500 rpm for 3 min). After centrifugation, supernatant was collected and absorbance measured spectrophotometrically at 540 nm. Phosphate buffer solution instead of extract was used as a control.

The level of hemolysis was calculated as follows:

% inhibition of hemolysis = $(A_c - A_t)/A_c \times 100$

Where, A_c= absorbance of control and A_t= absorbance of test.

Identification of phytochemicals/metabolites using liquid chromatography-high-resolution mass spectroscopy (LC-HRMS)

Identification of bioactive compounds from the crude methanolic extract of *A. cuneata* (L.) and *A. setosa* (L.) was done by Liquid Chromatography-

Table 1: Qualitative phytochemical analysis of *A. cuneata* (L.) and *A. setosa* (L.) leaves extracts

Constituents	Name of the test	*A. cuneata* (L.)		*A. setosa* (L.)	
		Ac-Me	Ac-EA	As-Me	As-EA
Alkaloids	Mayer's test	+	+	+	+
Anthraquinones	Test for anthraquinone	+	−	−	−
Proteins and amino acids	Ninhydrin test	+	+	+	+
Saponins	Foam test	+	−	+	−
Flavonoids	Aluminum Chloride test	+	+	+	+
Phenols	Ferric chloride test	+	+	+	+
Terpenoids	i. H_2SO_4 test	+	+	+	+
	ii. Salkowski's test	+	+	+	+
Sterols	Test for sterol	+	−	+	−
Tannins	Ferric chloride test	+	−	+	−
Glycosides	i) Leibermann's test	+	+	+	+
	ii) Salkowski's test	+	+	+	+

(+) Present, (−) Absent

Table 2: Analysis of total content of Phenolic, Flavonoid, Protein, and Reducing sugar in *A. cuneata* (L.) and *A. setosa* (L.) leaves extracts. (Results are presented as Mean±SD)

Sample (1 mg/ml)	Total Phenolic content (Gallic acid equivalent in mg/ml)	Total Flavonoid content (Quercetin equivalent in mg/ml)	Total Protein content (BSA equivalent in mg/ml)	Total Reducing sugar content (Glucose equivalent in mg/ml)
Ac-Me	0.780±0.065	0.107±0.015	0.011±0.002	0.088±0.012
Ac-EA	0.600±0.097	0.128±0.012	0.016±0.005	0.074±0.015
As-Me	0.840±0.130	0.100±0.020	0.030±0.009	0.107±0.024
As-EA	0.680±0.097	0.125±0.017	0.025±0.005	0.092±0.021

Table 3: Zone of inhibition and %RZID of *A. cuneata* (L.) and *A. setosa* (L.) plant leaves extract against bacteria

Sample (1 mg/ml)	Zone of inhibition in mm			
	E. coli	*B. cereus*	*S. aureus*	*P. vulgaris*
Ac-Me	22	19	17	20
Ac-EA	20	19	15	14
As-Me	23	20	18	18
As-EA	21	17	13	15
	%RIZD			
Ac-Me	88	76	68	80
Ac-EA	80	76	60	56
As-Me	92	80	72	72
As-EA	84	68	52	60

High-Resolution Mass Spectroscopy with electrospray ionization. LC-HRMS phytochemical profiling was performed on an Agilent 6540 UHD UHPLC system, diode array detector, and electrospray ionization quadrupole time-of-flight mass spectrometer (ESI-QTOF-MS). An Agilent zorbax SB-C18 (150 × 0.5 mm, 5 µ) column was used. The following gradient uses: 0.1% formic acid in water (A), 0.1% formic acid in methanol (B). Injection volume was 10 µL and flow-rate was 0.5 mL/min. ESI-Q-TOF-MS analysis was done in positive ionization mode with mass range 100–1700 m/z [34,35].

RESULTS AND DISCUSSION

The *A. cuneata* (L.) and *A. setosa* (L.) plant leaves crude extracts were prepared with methanol (Ac-Me = *A. cuneata* methanolic extract and As-Me = *A. setosa* methanolic extract) and ethyl acetate (Ac-EA= *A. cuneata* ethyl acetate extract and As-EA= *A. setosa* ethyl acetate extract). The highest yield of extract was measured, that is, Ac-Me = 0.318±0.025/10 g dry weight. The presence of various phytocompounds was detected qualitatively. For to test the presence, primary metabolites such as carbohydrates, proteins, and amino acids and secondary metabolites such as phenolics, tannins, flavonoids, saponins, terpenoids, glycosides, and anthraquinones the standard procedures were followed. Table 1 represents results of the phytochemical analysis.

In a quantitative analysis, the total phenolic, flavonoid, protein, and reducing sugar content was evaluated. The *A. cuneata* (L.) and *A. setosa* (L.) plant leaves extracts showed a total phenolic content ranges in between 0.600±0.097 mg/ml and 0.840±0.130 mg/ml GAE, total flavonoid content in between 0.100±0.020 mg/ml and 0.128±0.012 mg/ml quercetin equivalent. The total protein content and reducing sugar content calculated using Lowry's method and DNSA method, respectively. The value ranges in between 0.011±0.002 mg/ml to 0.030±0.009 mg/ml using BSA as a standard for protein and 0.074±0.015mg/ml to 0.107±0.024 mg/ml using glucose as a standard for reducing sugar. Table 2 showed the results of quantitative phytochemical analysis.

The antimicrobial activity of *A. cuneata* (L.) and *A. setosa* (L.) plant leaves extract was determined by agar well diffusion method. The percent relative inhibition zone diameter was calculated using ampicillin as reference standard shows an inhibition zone diameter of 2.5 cm. The DMSO used as negative control and does not show any inhibition zone. The *A. cuneata* (L.) and *A. setosa* (L.) plant leaves extracts showed the significant zone of inhibition against the all studied bacteria. However, the methanolic extract of *A. setosa* (L.) showed the higher zone of inhibition against *E. coli* and *B. cereus*. Table 3 shows the results of zone of inhibition and % RIZD (Relative Inhibition Zone Diameter).

To evaluate free radical scavenging potential of *A. cuneata* (L.) and *A. setosa* (L.) plant leaves extracts, DPPH and Nitric oxide radical scavenging methods were used. The *A. cuneata* (L.) and *A. setosa* (L.) plant leaves extracts showed increasing antioxidant activity with increasing concentration along with the standard ascorbic acid. The IC 50 for DPPH free radical scavenging was determined, that is, Ac-Me=0.580±0.012 mg/ml, Ac-EA=0.580±0.004 mg/ml, As-Me=0.708±0.006 mg/ml, As-EA=0.729±0.008 mg/ml with standard ascorbic acid, and AA=0.524±0.005 mg/ml (Fig. 1). The IC 50 for Nitric oxide radical

Table 4: List of some prominent bioactive components detected by LC-HRMS/MS in methanolic extract of *A. cuneata* (Ac-Me) and *A. setosa* (As-Me)

Name of compound	RT	Mass	M/Z	Molecular Formula	Ac-Me	As-Me
Pirbuterol	0.66	240.1477	241.1549	$C_{12}H_{20}N_2O_3$	+	–
Fraxin	5.37	370.0902	393.0795	$C_{16}H_{18}O_{10}$	+	–
Kawain	6.15	230.0951	213.0919	$C_{14}H_{14}O_3$	–	+
Cichorine	6.23	176.0708	193.0740	$C_{10}H_{11}NO_3$	+	–
(R)-(+)-Citronellal	6.42	154.1357	137.1324	$C_{10}H_{18}O$	+	+
Baicalin	6.46	446.0852	446.1085	$C_{21}H_{18}O_{11}$	+	–
Myricitrin	6.57	464.0956	465.1032	$C_{21}H_{20}O_{12}$	+	+
Tentoxin	6.67	414.2257	415.2329	$C_{22}H_{30}N_4O_4$	+	–
Carthamone	6.76	448.1008	448.1240	$C_{21}H_{20}O_{11}$	+	–
(+)-Pisatin	7.00	314.0792	297.0760	$C_{17}H_{14}O_6$	–	+
Quercetin 3-O-(6-O-malonyl-β-D-glucoside)	7.05	550.0964	551.1039	$C_{24}H_{22}O_{15}$	+	–
Lauric acid	7.12	200.1776	218.2115	$C_{12}H_{24}O_2$	+	+
(±)-Taxifolin	7.20	304.0586	287.0554	$C_{15}H_{12}O_7$	+	+
Hymenoxon	8.11	282.1466	283.1539	$C_{15}H_{22}O_5$	+	–
Propafenone	9.67	341.1991	324.1956	$C_{21}H_{27}NO_3$	+	–
Zingerone	9.78	194.0950	195.1024	$C_{11}H_{14}O_3$	–	+
Mupirocin	10.41	500.2981	523.2875	$C_{26}H_{44}O_9$	–	+
Digoxin	10.95	780.4286	780.4523	$C_{41}H_{64}O_{14}$	+	–
Ricinoleic acid	11.36	298.2511	298.2744	$C_{18}H_{34}O_3$	+	+
Populin	11.68	390.1318	373.1284	$C_{20}H_{22}O_8$	+	–
Sphinganine	11.70	303.1473	302.3059	$C_{18}H_{39}NO_2$	+	+
Terminaline	12.66	363.3135	364.3210	$C_{23}H_{41}NO_2$	–	+
Phytanic Acid	12.78	312.3030	330.3368	$C_{20}H_{40}O_2$	–	+
Embelin	12.85	294.1834	277.1801	$C_{17}H_{26}O_4$	+	+
Cortol	13.42	368.2569	351.2536	$C_{21}H_{36}O_5$	+	+
Sugetriol	14.00	252.1728	235.1695	$C_{15}H_{24}O_3$	+	+
Phytol	14.06	296.3082	314.3421	$C_{20}H_{40}O$	–	+
Icacine	14.56	405.2152	388.2121	$C_{22}H_{31}NO_6$	+	–
Graphinone	14.99	296.1624	279.1592	$C_{16}H_{24}O_5$	+	+
Misoprostol	17.12	382.2720	365.2695	$C_{22}H_{38}O_5$	+	+
Lupeol	18.99	426.3872	409.3841	$C_{30}H_{50}O$	–	+
Lutein	20.53	568.4285	551.4250	$C_{40}H_{56}O_2$	+	–
Typhasterol	23.58	448.3561	431.3529	$C_{28}H_{48}O_4$	+	+

(+) Detected, (–) Not-detected

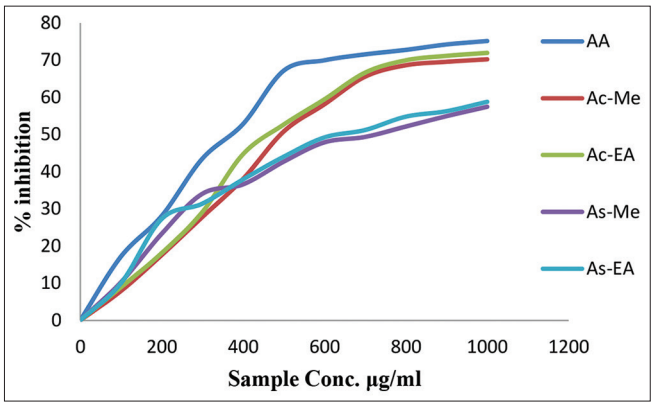

Fig. 1: Comparison of DPPH free radical scavenging activity between ascorbic acid and plant extracts. IC50 value (in mg/ml) for each sample was obtained from graph at 50% inhibition

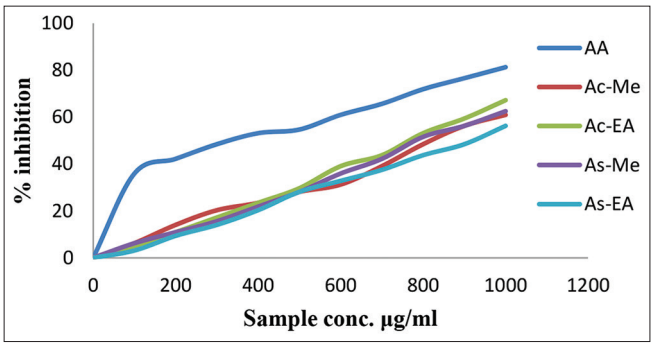

Fig. 2: Nitric oxide radical scavenging activity between ascorbic acid and plant extracts. IC50 value (in mg/ml) was derived from graph at 50% inhibition

scavenging was calculated such as Ac-Me=0.838±0.149 mg/ml, Ac-EA=0.772±0.059mg/ml,As-Me=0.783±0.166mg/ml,As-EA=0.919±0.047mg/ml with standard ascorbic acid, and AA=0.526±0.027 mg/ml (Fig. 2)

To investigate antidiabetic activity of plant extract, alpha amylase inhibition assay and glucose adsorption method were performed. The IC 50 for alpha amylase inhibition was determined, that is, Ac-Me=0.860±0.081 mg/ml, Ac-EA=0.920±0.033 mg/ml, As-Me=0.783±0.037 mg/ml, and As-EA=0.954±0.056 mg/ml with standard Acarbose=0.642±0.038 mg/ml (Fig. 3). The *A. cuneata* (L.) and *A.setosa* (L.) plant leaves extracts showed the glucose adsorption at lower concentration (Fig. 4).

To determine anti-inflammatory activity of plant extracts, the protein denaturation assay and membrane lysis assay were performed. To cause inflammation, the protein denaturation is one reason. Hence, inhibition of protein denaturation is one of the ways to determine the anti-inflammatory potential of sample. The IC 50 for BSA protein denaturation inhibition was determined, that is, Ac-Me=0.541±0.019 mg/ml, Ac-EA=0.529±0.009 mg/ml, As-Me=0.564±0.039 mg/ml, and As-EA=0.553±0.019 mg/ml with standard Diclofinac sodium=0.514±0.009 mg/ml (Fig. 5). The IC 50 for Egg albumin protein denaturation inhibition was determined, that is, Ac-Me=0.927±0.071 mg/ml, Ac-EA=0.748±0.067 mg/ml, As-Me=0.841±0.075 mg/ml, and As-EA=0.694±0.062 mg/ml with standard Diclofinac sodium=0.482±0.006 mg/ml (Fig. 6). Any cause to hemolysis leads to the inflammation and inhibition of hemolysis may have one way to evaluate the anti-inflammatory potential. The inhibition of

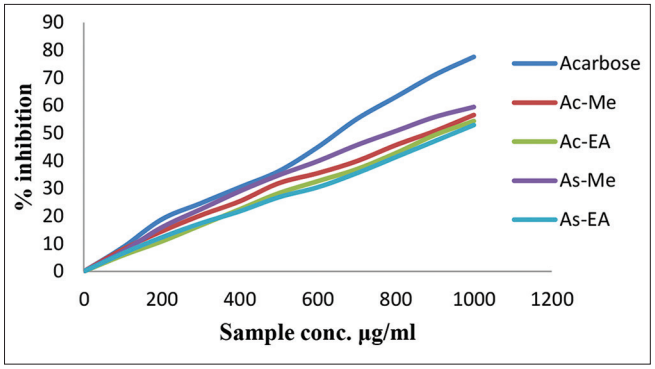

Fig. 3: Amylase inhibition potential of reference standard Acarbose and plant extracts. IC50 value (in mg/ml) was obtained from graph at 50% inhibition

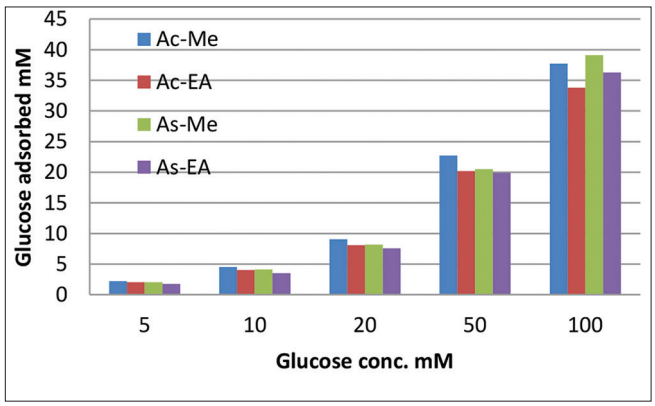

Fig. 4: Glucose adsorption showed by plant extracts at low concentration

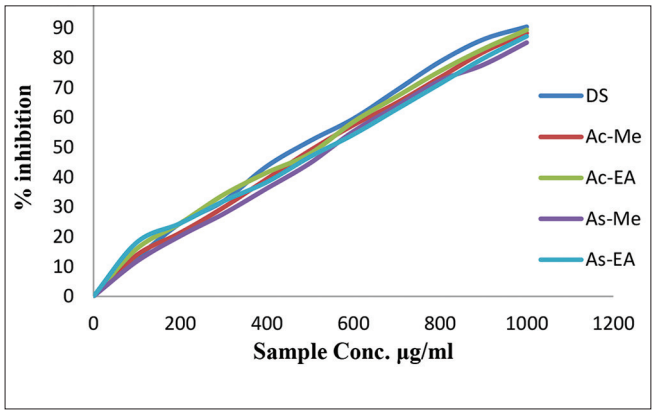

Fig. 5: Protein (BSA) denaturation inhibition of reference standard and plant extracts. IC50 value (in mg/ml) was derived from graph at 50% inhibition of protein denaturation

hemolysis of *A. cuneata* (L.) and *A. setosa* (L.) plant leaves extracts was determined. A % inhibition was determine (100 µg/ml sample conc.), that is, Ac-Me=91.56±1.96, Ac-EA=85.54±2.95, As-Me=86.74±0.98, and As-EA=84.33±2.95. The *A. cuneata* (L.) and *A. setosa* (L.) plant leaves extracts also exhibited an inhibition at lower concentration (Fig. 7).

The phytochemicals detected using the LC-HRMS/MS method in a methanol extract of leaves of *A. Cuneata* (L.) and *A. setosa* (L.) are presented in Table 4.

LC-HRMS analysis revealed presence of compounds such as embelin, citronellal, tentoxin, baicalin, propafenone, lupeol, ricinoleic acid,

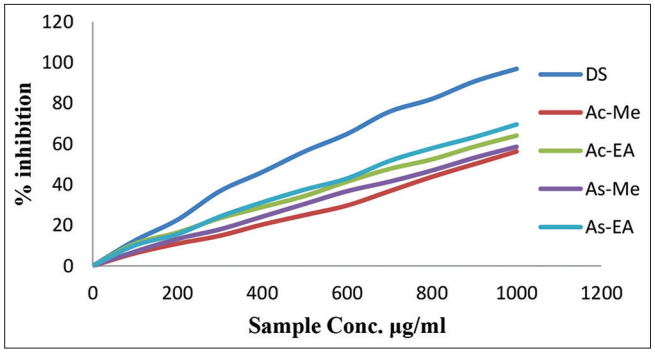

Fig. 6: Protein (Egg Albumin) denaturation of reference standard and plant extracts. IC50 value (in mg/ml) was derived from graph at 50% inhibition of protein denaturation

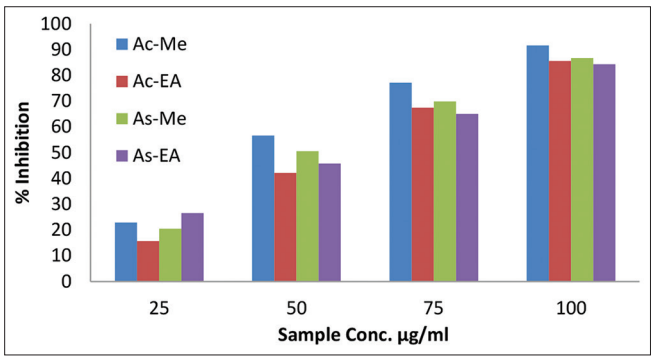

Fig. 7: Inhibition of heat induced hemolysis of plant extracts for anti-inflammatory activity

mupirocin, and zingerone were known to have various biological activities such as antimicrobial, anti-inflammatory, anxiolytic, antimalarial, and antioxidant [36-41].

CONCLUSION

The present study showed that plant leaves extracts of *A. cuneata* (L.) and *A. setosa* (L.) have a good range of phytochemicals. The presence of remarkable biological activities, that is, antimicrobial, antioxidant, antidiabetic, and anti-inflammatory in *A. cuneata* (L.) and *A. setosa* (L.) plant leaves extracts can be directly correlated to higher phenolic content. The LC-HRMS profile has provided valuable information regarding the bioactive components. Further studies may help in identifying and purifying specific compounds for potential medicinal use.

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AUTHORS' CONTRIBUTIONS

All authors contributed equally.

CONFLICTS OF INTEREST

Authors declared no conflicts of interest.

AUTHORS FUNDING

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