Trends Phytochem. Res. 4(4) 2020 177-192

Trends in Phytochemical Research (TPR)

Journal Homepage: http://tpr.iau-shahrood.ac.ir

Original Research Article

Chemical composition, insect antifeeding, insecticidal, herbicidal, antioxidant and anti-inflammatory potential of *Ardisia solonaceae* Roxb. root extract

BAHAAR ANJUM[1], RAVENDRA KUMAR[1]✉*, RANDEEP KUMAR[1], OM PRAKASH[1], R.M. SRIVASTAVA[2], D.S. RAWAT[3] AND A.K. PANT[1]

[1]Department of Chemistry, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar-263145, U.S. Nagar, Uttarakhand, India
[2]Department of Entomology, College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar-263145, U.S. Nagar, Uttarakhand, India
[3]Department of Biological Sciences, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar-263145, U.S. Nagar, Uttarakhand, India

ABSTRACT

The objectives of this research were to investigate the qualitative and quantitative analysis of Ethyl Acetate Root Extract of *Ardisia solanacea* Roxb. (EREAS) and estimation of its biological activities. Phytochemical screening of EREAS showed the abundance of total phenolics, flavanoids, ortho-dihydric phenols, alkaloids, diterpenes and triterpenes etc. The quantitative analysis of EREAS was also carried out by GC/MS and α-amyrenone (13.3%) was found to be the major component. Antifeeding activity monitored through no choice leaf dip method against *Spilosoma oblique*. The results revealed dose and time dependent antifeeding activity, where the 100% mortality was observed signifying the intense insecticidal activity. The herbicidal activity of extracts was evaluated against the *Raphanus raphanistrum* seeds. Accordingly, EREAS showed effective herbicidal activity in terms of inhibition of seed germination, coleoptile length and the radical length. Evaluation of antioxidant activity was performed via DPPH radical scavenging, reducing power and metal chelating of Fe^{2+} activities. EREAS possessed potential antioxidant property and revealed good anti-inflammatory activity.

ARTICLE HISTORY

Received: 19 March 2020
Revised: 08 June 2020
Accepted: 05 October 2020
ePublished: 02 December 2020

KEYWORDS

α-Amyrenone
Antifeeding
Anti-inflammatory activity
Antioxidant
Ardisia solanacea
Herbicidal
Insecticidal activity

1. Introduction

Medicinal plants are sprinkled all over the world, having a large treasure of potential biological active components hidden within them. It has been reported from time immemorial that plants possess huge medicinal importance and the human race had a dependence on plant derived components and herbs for their food and health care issues (Mohammadhosseini et al., 2017; Mohammadhosseini et al., 2019). As per WHO estimates, plant drugs provide nearly 80% to the health needs of the entire world population. Medicinal herbs have been used in the traditional medicine system for several herbal preparations since prehistoric times (Mohammadhosseini, 2017; Wansi et al., 2018). Many studies have revealed that plants promote health and well-being to human beings. The utility of herbal remedies is not only cost-effective but also safe and almost independent from any serious adverse effect. The rural elders, farmers and tribal cultures have incredible knowledge about the plants being used for various purposes of health since thousands of years and are still a part of medical practices by folks of various regions of Indian sub-continents and China, Middle East and African countries, South American and other developing countries of the world (Venditti et al., 2018). India is a large hub of medicinal plants where more than 45,000 plant species have been documented and among these, several thousands have been claimed to prove medicinal properties (Mohammadhosseini, 2017). Ayurveda and other oriental medicinal systems describe how to use the plants in the treatment of a variety of ailments. To date, various secondary plant metabolites possessing established biological activities have been identified and discovered. In fact, the plant products are extensively used in various medicinal systems by different

✉Corresponding author: Ravendra Kumar
Tel: +91-9759204533 ; Fax: +91-5944-233473
E-mail address: ravichemistry.kumar@gmail.com

practitioners who require proper documentation and in some reports for further enhancement of their medicinal values (Mohammadhosseini, 2017). In the morden era, the interest in the area of natural product medicines is growing exponentially due to the increased awareness of people towards the adverse effects of synthetic drugs (Venditti et al., 2018). Family Myrsinaceae R.Br. (Order-Myrsinales) comprises of one of the largest family of flowering plants which includes about 35 genera and even up to 1000 species (Hutchinson, 1973). The genus *Ardisia* Sw. has been known to be the largest genus in the family Myrsinaceae, and even more than 500 species are common in subtropical and tropical areas globally including Africa, Asia, Australia and America (Chen and Pipoly, 1996). The genus has been recognized for a number of biological activities like anthelmintic, antimicrobial (Mohammad et al., 2015), analgesic (Shah et al., 2011), anticonvulsant, anti-inflamatory (Yang et al., 2001), antioxidant, antitumor (Newell, 2010; Ramirez-Mares et al., 2010), antiplatelet, cytotoxic, trypanocidals (Fujioka et al., 1988; Jia et al., 1994), antifungal, piscicidal and insecticidal activities (Berenbaum, 1995; Kessler and Baldwin, 2002). A large number of species of the genus *Ardisia* are used in the traditional medicine system for different ailments like to cure stomachache and it has a folkloric history in the treatment of various diseases, such as inflammation, fever, intestinal worms, rheumatism, scabies, snake-bites, cholera, and cancer treatment (Hideka and De-Mejia, 2005).

Ardisia solanacea Roxb. commonly known as Adavimayuri/shoebutton is an important shrub belonging to the family Myrsinaceae. This plant is native of Pakistan, India, Sri Lanka, China and is widely distributed in Southeast Asia (Bailey, 1925). It is used for a variety of medicinal purposes, e.g. fever, dysmenorrhea, liver disorders, diarrhea, pains, bacterial infections, rheumatic arthritis, gout, mental disorder, skin sore and vertigo (Khatun et al., 2013). Its roots have been found to possess potentially active antibacterial activity, cytotoxic, thrombolytic, antioxidant, antirheumatic, februifuge, antidiarrhoic, skin sore and vertigo, while its seed paste has anti-fungal action (Karuppusamy, 2007). The traditional uses of *A. solanacea* in various states of India are listed in Table 1. Basha et al. (2016) have reported a preliminary phytochemical analysis of petroleum ether, ethyl acetate, chloroform, methanol and aqueous root extracts of *A. solanacea* and showed the presence of flavonoids, saponin, tannins, alkaloids and glycosides. Bauerenol, α-amyrin, β-amyrin, β-sitosterol, tannin, β-carotene, tocopherol, phenols, phytic acid, lectins and ascorbic acid in hexane, benzene and aqueous leaf extracts of *A. solanacea* (Ahmad et al., 1977; Khan and Ashraf, 1991; Chandran et al., 2015). In continuation of research work on *A. solanacea* in our laboratory, qualitative and quantitative analysis and different biological activities of the ethyl acetate leaf and stem extract of *A. solanacea* Roxb. have already been reported by Anjum et al. (2019).

The present research reports a systematic study on both qualitative estimation of secondary metabolites and quantitative identification of chemical composition (GC/MS), total phenolics estimation, *in vitro* antioxidant, anti-inflammatory, insect antifeeding and insecticidal activity of the ethyl acetate root extract of *A. solanacea*.

2. Experimental

2.1. Collection of plant material

The plant material was collected in blooming phase in June and July 2018 from Kashipur (29° 12' 37.5156'' N and 78° 57' 42.5880'' E.), Tarai region of Uttarakhand, India (Fig. 1). The plant identification was done by one of the co-authors (D.S. Rawat). The voucher specimen of *Ardisia solanacea* Roxb. with Acc No. of GBPUH-984/4.4.2019 was deposited at the herbarium of Department of Biological Sciences, C.B.S.&H., G. B. Pant University of Agriculture & Technology, Pantnagar.

2.2. Preparation of extract

The fresh root material of *A. solanacea* was extracted in ethyl acetate using Soxhlet method. The obtained extract was filtered and concentrated using rotary evaporator. The mean yield of the prepared extract was found to be 0.06% (w/w) (2.38 g). It was stored at 4 °C for further chemical analysis and determination of biological activity.

2.3. Qualitative analysis

The freshly prepared Ethyl Acetate Root Extract of *A. solanacea* (EREAS) was subjected to qualitative chemical examination to identify various classes of biologically active constituents using the standard protocols reported in the literature (Shail, 2011).

2.4. Quantitative analysis

2.4.1. GC/MS analysis

The phytochemicals of the root extract of *A. solanacea* were identified by using GC/MS. The GC/MS analyses were carried out using GC/MS-QP 2010 Plus equipment fitted with Ultra Rtx-5MS column of size 30 m × 0.25 mm and film thickness 0.25 µm. The initial column temperature was programmed to 50 °C for 2 min, and gradually increased to 250 °C at a rate of 3 °C/min. And finally at the rate of 5 °C/min until reaching the final temperature of 310 °C. The temperature for injector and the detector was kept at 300 °C (split), and 310 °C, respectively. Helium (He) was the carrier gas used at a linear flow rate of 1.21 mL/min and 69.0 kPa pressure. The operation of MS detector was performed under EI condition (ionization energy of 70 eV) with an injection volume of 0.1 µL and split mode of 1:120. All compounds of extracts were identified via mass spectral database search (NIST14. lib, FFNSC2.lib, WILEY8.LIB) followed by the matching of spectra and K.I. (Kovats index) with literature data (Adams, 2007).

Table 1
Traditional uses of *A. solanacea* in various state of India.

S.N.	States	Traditional uses		References
		Plant part	Uses	
1	Andhra Pradesh	Tender leaves	Used as leafy vegetable	Reddy et al. (2007)
2	Odisha	Root	Root bark paste mixed with dried flower powder of Kanchan and goat milk (4:3:4) is administered twice a day for 15 days	Dhal et al. (2015)
			Against asthma	
		Root bark	Fever	Pattanaik et al. (2008)
3	Tamil Nadu	Leaves	Used as vegetable	Merlin-Franco and Narasimhan (2009)
		Fruits	Edible, used in fits and to treat eye pain	
		Plant	Plant used in rituals	
4	Kerala	Bark	The bark of this plant is ground with the fruits of *Piper nigrum* L. var. *nigrum*, the tuber of *Plumbago zeylanica* L, and the bark of *Zyzyphus trinervia* Roxb. the ground mixture is chewed to cure tooth ache	Devi Prasad and Shyma (2013)
5	Meghalaya	Fruits	Ripe fruits eaten raw	Singh et al. (2012)
6	Great Nicobar Island	Fruits	Raw fruits	Elanchezhian et al. (2007)

Fig. 1. Shrub/small tree of *Ardisia solanacea* Roxb. growing in its natural habitat (Photograph taken from Tarai region, Uttarakhand, India).

2.4.2. Total phenolics, flavonoid and ortho-dihydric phenols content

2.4.2.1. Total phenolic content

Total phenolic content of extract was evaluated using the Folin-Ciocalteu method as per developed protocol (Singleton and Rossi, 1965). Briefly, 1.0 mL of Folin-Ciocalteu phenol reagent (Sigma-Aldrich) was mixed with 0.5 mL of extract solutions, 1.0 mL of aqueous solution of Na_2CO_3 (7.0%) and 5.0 mL of distilled water and the mixture was blended thoroughly. Thereafter, the mixture was kept in the dark for 30 min at 25 °C and the relevant absorbance was measured at 765 nm. Evaluation of total phenolic content was assessed using the extrapolation of the calibration curve from the standard gallic acid concentrations measurement. The tests were carried out in triplicate and presented as gallic acid equivalent (GAE) in mg/g of dry weight.

2.4.2.2. Total flavonoids content

Total flavonoids content of the extract was evaluated using the method developed by Choi et al. (2006). In

brief, 1.0 mL of extract was added to 1.25 mL of distilled water and 75 µL of $NaNO_3$ (5.0%) and incubated for 5 min. Then, 150 µL of $AlCl_3$ (10.0%), 500 µL of NaOH (1.0 M) and 275 µL of distilled water were added to the reaction mixture and mixed well. The absorbance was finally taken at 510 nm. This experiment was carried out in triplicate and total flavonoid content was expressed as Catechin Equivalents (CNE) in mg/g of dry weight.

2.4.2.3. Total ortho-dihydric phenols content

Ortho-dihydric phenols content of extract was described by the method of Mahadevan and Sridhar (1986). Accordingly, to 1 mL of the extract solution, equal volume of HCl (0.5 N), 1 mL of Arnow's reagent, 2 mL of NaOH (1.0 N) and 4.5 mL of distilled water were also added. The mixture was mixed well and its absorbance was measured at 515 nm. The standard curve was prepared by employing various concentrations of catechol. Total ortho-dihydric phenols content was expressed with Catechol Equivalent (CLE) in mg/g of dry weight.

2.5. Antifeedant and insecticidal activity

2.5.1. Test insect

The antifeedant and insecticidal activity of the root extract were evaluated against Bihar hairy caterpillar (*Spilosoma obliqua* belonging to family Erebidae and order Lepidoptera). Its third instars onward stages cause main harm and damage to crops by defoliation of the leaf (polyphagous in nature) (Hussain and Begum, 1995; Gupta and Bhattacharya, 2008; Warad and Kalleshwaraswamy, 2017).

2.5.2. Collection of larvae and maintainance

Insects for test were collected from the field of soybean (Glycine max) crop from C.R.C. (Crop Research Center), G.B.P.U.A.&T., Pantnagar, Uttarakhand, India in July and August 2018. The rearing of test insects was carried in a glass jar covered with muslin cloth in the fine laboratory conditions, maintaining temperature at 27 °C and relative humidity at 75-80%. Test insects were fed with fresh leaf of soybean on every day. And finally the fourth instars larvae were kept for 12-24 hrs starvation and then employed for their antifeeding potential and insecticidal activity.

2.5.3. Antifeeding activity

The antifeeding activity of the root extract of *A. solanacea* was estimated by employing the developed standard protocol (Vattikonda and Sangam, 2016) against fourth instar larvae of *S. obliqua* [Bihar hairy caterpillar (B.H.C.)] using a disc in no choice bioassay method. The experiment was fulfilled in petri dishes and a moist sheet of filter paper was placed at the bottom of each petri plate to facilitate relative humidity and also to keep the soybean leaf fresh. The leaf (each of area 25 sq.cm) were dipped in extract of varying amounts 5%, 10%, 15%, 20% and 25%, dried in air by fanning and finally introduced in petri plates as food stuff for test insects. To each petri plate, a fourth instar larvae having starvation of 24 hrs were released. Observations were noted at the time interval of 12, 24, 36 and 48 hrs after the release of the test insect. The percent antifeeding (feeding inhibition) activity of the plant extract of *A. solanacea* was calculated by employing the formula (Eqn. 1):

Percent antifeeding= [(Leaf area consumed in control - leaf area consumed in treatment)/(Leaf area consumed in control + leaf area consumed in treatment)] ×100 (Eqn. 1)

2.5.4. Insecticidal activity

The evaluation of insecticidal bioassay of ethyl acetate extract of *A. solanacea* was conducted by using leaf dip method (Tabashnik and Cushing, 1987). Soybean leaf was first cleaned and washed with distilled water and air dried for an hour. Each soybean leaf was cut into an area of 25 sq.cm and then dipped in the solution of extract made in dimethyl sulphoxide (DMSO) and methanol to smooth the progress of uniform treatment of active ingredient for few seconds. The obtained leaf discs were kept slanting for 2-3 min on a blotting paper and placed in the tray to drain out excess solution for two hrs at room temperature. Five, third instar adult larvae, which were starved for 6 hrs, released on each petri dish in individual petri plate. At the bottom of each plate, blotting paper was placed. These petri plates were taken under observation for 72 h to watch any insecticidal action. This activity was held under fine laboratory conditions, maintaining temperature at 27 °C and relative humidity at 75-80%. The mortality (%) was calculated after 24, 48 and 72 h of the treatment using Abbott's formula (Shen and Shao, 2005). The LD_{50} values were analyzed by Probit analysis (Eloff, 1998).

2.6. Herbicidal activity

The seed germination inhibition activity of EREAS under examination was carried on *Raphanus raphanistrum* (raddish) seeds by using the method given by Sahu and Devkota (2013) with slight modification.

2.6.1. Source of *Raphanus raphanistrum* seeds and pendimethalin

Raphanus raphanistrum subsp. *sativus* (L.) Domin (Syn. *Raphanus sativus* L.) (Radish) seeds were commercially bought from the seed store of Pantnagar. In addition, pendimethalin being used as a standard herbicide was supplied by Weed Control Laboratory, C.R.C, G.B.P.U.A.&T. Pantnagar.

2.6.2. Preparation of test solutions

A stock solution of EREAS (10%) was prepared in distilled water. This stock solution was further serial

diluted to four concentrations of 1%, 2.5%, and 5% and 7.5% respectively for testing seed germination inhibition activity. Pendimethalin was taken as standard.

2.6.3. Bioassay

Raphanus raphanistrum seeds were sterilized using 5.0% sodium hypochlorite solution diluted upto 1:100 and used 15 minutes before experimentation. The petri plates were firstly covered with ordinary filter papers and then test solution was poured. Ten sterilized seeds were put in each petri plates and allowed to germinate at (25 ± 1 °C) in the incubator with around 12 hrs of photoperiod. Distilled water was used as control. The entire experiment was carried out in triplicate. After 90 hrs, the number of germinated seeds was counted for each amount and inhibition values (%) for seed germination were calculated.

2.7. *In vitro* antioxidant activity

2.7.1. DPPH free radical scavenging activity

In vitro evaluation of the free radical scavenging activity of the extracts was performed using the stable radical, 2,2-diphenyl-2-picrylhydrazyl (DPPH) assay following the method described by Kumar et al. (2019) with little modifications based on the ability of sample to neutralize DPPH radical. Under the experimental conditions, DPPH is a stable free radical that can accept hydrogen radical or an electron to convert it into a stable diamagnetic molecule. Various concentrations of plant extract (50-250 µg/mL) were mixed with 5 mL of methanol solution of DPPH (0.004%), shaken well and kept in the dark for 30 min at 25 °C for incubation. The absorbance was then measured at 517 nm. DPPH in methanol without extract was used as negative control. On the other hand, standard antioxidant butylhydroxytoluene (BHT) was used for positive control. The DPPH radical inhibition (IC%) was calculated by using the Eqn. 2.

Percent inhibition (%) of radical scavenger = IC% = $(A_0 - At)/A_0 \times 100$ (Eqn. 2)

Where A_0, A_t and IC(%) respectively imply the absorbance value of control sample, the absorbance value of test sample and the inhibitory concentration(%).

Percent inhibition was plotted against concentrations and the standard curve was drawn using standard antioxidant (BHT) to calculate the IC_{50} values for standard and different concentration of extract. A lower IC_{50} value indicated more DPPH radical scavenging activity.

2.7.2. Reducing power activity

The reducing power of the *A. solanacea* extract was determined using the method of Kumar et al. (2019). Different concentrations of extract (50-250 µg/mL) were mixed with 2.5 mL of phosphate buffer (20 mM, pH 6.6) and 2.5 mL of potassium ferricyanide $[K_3Fe(CN)_6]$ (0.5 mL, 1.0%). The mixture was then incubated at 50 °C for 20 min. A portion of trichloroacetic acid (2.5 mL, 10%) was added to the mixture, which was then centrifuged for 10 min at 650 rpm. 1 mL of the upper layer of the solution was mixed with 5 mL of distilled water and 1 mL of $FeCl_3$ (0.1%) for 10 min, and then the absorbance was measured at 700 nm, with higher absorbance indicating greater reducing power (Kumar et al., 2019). All the readings were taken as triplicate with respect to catechin which was used as a standard. The reducing power of samples was calculated using the Eqn. 3 given below:

Percent inhibition (%) of reducing power activity = $(A_0-A_t)/A_0 \times 100$ (Eqn. 3)

Where A_0 and A_t respectively show the absorbance value of control and test samples.

Percent inhibition was plotted against concentrations and the standard curve was drawn using standard antioxidant (catechin) to calculate the RP_{50} values for standard and different extract samples. The lower RP_{50} value indicated greater reducing power ability.

2.7.3. Metal chelating activity

The metal chelating activity of Fe^{2+} of plant extract was measured according to the reported method by Kumar et al. (2019). Different concentrations of tested extract samples were mixed with 0.1 mL of $FeCl_2.4H_2O$ (2 mM), 0.2 mL of ferrozine (5 mM) and 4.7 mL of methanol making volume up to 5 mL. The mixture was then mixed and shaken. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. The mixture was incubated for 30 min at 25 °C, and the absorbance of the Fe^{2+}, ferrozine was then measured at 562 nm. EDTA was used as standard antioxidant. The ability of the extract to chelate ferrous ion was calculated using the following formula (Eqn. 4):

Percent inhibition (%) of chelating ability = $(A_0- A_t)/A_0 \times 100$ (Eqn. 4)

Where A_0 and A_t respectively account for the absorbance value of control and test samples. The percent inhibition was plotted against concentrations and the standard curve was drawn using standard antioxidant (EDTA) to calculate the IC_{50} values for standard and extracts.

2.8. *In vitro* anti-inflammatory activity

The method developed by Kar et al. (2012) was adopted for the evaluation of *in vitro* anti-inflammatory activity with little modifications. Briefly, a sample was prepared consisting of 2 mL of extract at different concentrations (50-250 µg/mL) and 200 µL of fresh albumin protein (100 ppm) and 2.8 mL of phosphate buffered saline (PBS, pH 6.4). Then, the reaction mixture was made up to 5 mL and kept for incubation at 37 °C for 15 min and heated at 70 °C for 5 min. Double distilled water was taken as control. After cooling at room, the corresponding absorbance was measured at 660 nm. Diclofenac sodium was taken as positive control at the same concentration. The percentage inhibition of protein denaturation was evaluated by the Eqn. 5 given below:

Percent inhibition (%) of protein denaturation = $(A_0-$

$A_t)/A_0 \times 100$ (Eqn. 5)

Where A_0 and A_t respectively represent the absorbance value of control and test samples.

2.9. Data analysis

Experiments were performed in parallel triplicate and all data were reported as mean ± standard deviation. The mean values and standard deviation were calculated statistically. Data analyzed with two-way analysis with replication and found to be significant at $p < 0.05$. A statistical analysis was performed using the SPSS 16.0 software package. The mortality (%) was calculated using Abbott's formula and the LD_{50} values were analyzed by Probit analysis.

3. Results and Discussion

3.1. Qualitative analysis

Preliminary phytochemical analysis of EREAS showed the abundance of secondary metabolites such as alkaloids, carbohydrates, resins, diterpenes, triterpenes, fats and oils. Glycosides, phytosterols, and flavonoids were present in moderate amounts of EREAS. The plant extracts also showed mild results for saponins, phenols, tannins, proteins and amino acids. However, the current study showed negative result for ninhydrin test for protein and amino acids. The results have been summarized in Table 2.

The ethyl acetate root extract of *A. solanacea* demonstrated the presence of alkaloids, carbohydrates, steroids, diterpenes, triterpenes, tannins, fats and oils as secondary metabolites with potential biological activities. Basha et al. (2016) reported preliminary phytochemical analysis of petroleum ether, ethyl acetate, chloroform, methanol and aqueous root extract of *A. solanacea* and found the presence of flavonoids, saponin, tannins, alkaloids and glycosides. Samal (2013) reported the presence of flavonoids, glycosides and phenolic compounds in the alcoholic leaf extract of *A. solanacea*. Desai et al. (1967) reported the absence of saponins in the stems and roots extracts of *A. solanacea*. Chandran et al. (2015) have shown that the leaf aqueous extract of *A. solanacea* exhibited a small amount of tannin, β-carotene, tocopherol and ascorbic acid. The phenols, phytic acid and lectins were also recorded in aqueous extract of the plant.

3.2. Quantitative analysis

3.2.1. Chemical composition of extract by GC/MS analysis

The analysis of ethyl acetate root extracts of *A. solanacea* by GC/MS analysis showed fifty-four compounds (peaks) in the corresponding GC/MS chromatogram (Table 3 and Fig. 2), which were identified except (RT = 42.017 min) according to their retention time on Ultra Rtx-5MS column and retention indices (NIST14.lib, FFNSC2.lib, WILEY8.LIB, Adams, 2007). The extract mainly comprised of hydrocarbons, fatty acids and terpenoids. α-Amyrenone (13.3%) was found to be the major component of root extract followed by 4,6,6-trimethyl-2-(3-methylbuta-1,3-dienyl)-3-oxatricyclo [5.1.0.0(2,4)]octane (10.0%), 3-hydroxy-3,7,11,15-tetramethylhexadecanoic acid silylat (5.4%) and palmitic acid (3.0%). The other major phytoconstituents investigated from the extract were 1-naphthelenol 5,6,7,8-tetrahydro-2,5- dimethyl-8-(-1-methylethyl) (1.2%) and docosane (1.0%). However, the minor components in the extract of root contributing less than 1.0% to the total extract were β-asarone (0.6%), lithocholic acid (0.2%), τ-muurolol (0.1%) fumaric acid (0.1%), neophytadiene (0.5%), myristic acid (0.1%), estradiol, 3-deoxy (0.4%), *cis*,*cis*-linoleic acid (0.3%), *cis*-vaccenic acid (0.6%), phytol (0.2%), squalene (0.5%), β-stigmasterol (0.6%), and gorgost-5-en-3-ol, (3.beta.)-, tms derivative (0.2%). The structures of major compounds present in roots extract are being illustrated in Fig. 3. In our study, GC/MS analysis of phytochemical of root extract revealed the presence of α and β-amyrin type pentacyclic triterpenoid compounds, α-amyrenone, 4,6,6-trimethyl-2-(3-methylbuta-1,3-dienyl)-3-oxatricyclo octane, etc. The presence of these compounds might be responsible for the reported biological activities.

3.2.2. Total phenols, flavonoids and ortho-dihydric phenol content

The total phenolic contents were determined and expressed in gallic acid equivalent (mg of GAE/g of samples), while total flavonoid content was expressed as catechin equivalents (CNE) in mg/g of dry weight and total ortho-dihydric phenols content was expressed with catechol equivalent (CLE) in mg/g of dry weight. EREAS contained 323.99 ± 0.04 mg/g GAE total phenolics, 14.69 ± 0.15 mg/g CNE total flavonoid and 35.55 ± 0.11 mg/g CLE total ortho-dihydric phenol (Table 4).

A. solanacea ethyl aceate root extract exhibited significant phenolic, flavonoid and ortho-dihydric phenol contents. Amin et al. (2015) reported total phenolic content of *A. solanacea* MeOH, petroleum ether, CCl_4 and $CHCl_3$ extracts and showed total phenolic content of 58.35 µg of GAE/mg, 69.41 µg of GAE/mg, 37.41 µg of GAE/1 mg and 10.82 µg of GAE/mg of the extracts, respectively. The total phenolic content of aqueous and MeOH extracts of *A. solanacea* leaf were 0.030 ± 0.01 and 0.040 ± 0.22 mg of catechin equivalent/mg dried extract, respectively. The flavonoid content in aqueous and MeOH extract of the plant were 0.257 ± 0.02 and 0.404 ± 0.03 mg of catechin equivalent/mg dried extract, respectively. Chandran et al. (2015) also reported that total phenolic content present in *A. solancea* leaf in significant quantity (113.42 µg/g).

3.3. Antifeeding activity

The antifeeding activity screening of the ethyl acetate root extract of *A. solanacea* (Bihar Hairy Caterpillar) was assessed against *S. obliqua* (B.H.C.) using no choice/non

Table 2
Qualitative phytochemical screening of ethyl acetate root extract of *A. solanacea*.

S.No.	Phytochemical test	Name of the test	EREAS
1	Alkaloids	Mayer's test	+++
		Wagner's test	+++
		Hager's test	+++
2	Carbohydrates	Mohlisch's test	+++
		Fehling's test	+++
3	Glycosides	Borntrager's test	++
		Keller-Killiani test	++
4	Saponins	Froth test	+
		Foam test	+
5	Phytosterols	Salkowski's test	+
6	Fats and oils	Stain test	+++
7	Resin	Acetone-water test	+
8	Phenols	Ferric chloride test	+
9	Tannin	Gelatin test	+
10	Flavonoids	Alkaline reagent test	+
		Lead acetate test	+
11	Proteins and amino acids	Xanthoprotiec test	++
		Ninhydrin test	-
		Biuret test	+
12	Diterpenes	Copper acetate test	+++
13	Triterpenes	Tshugajeu test	+++

EREAS= Ethyl acetate root extract of *A. solanacea* (- = absent, + = mild, ++ = moderate, +++ = abundance)

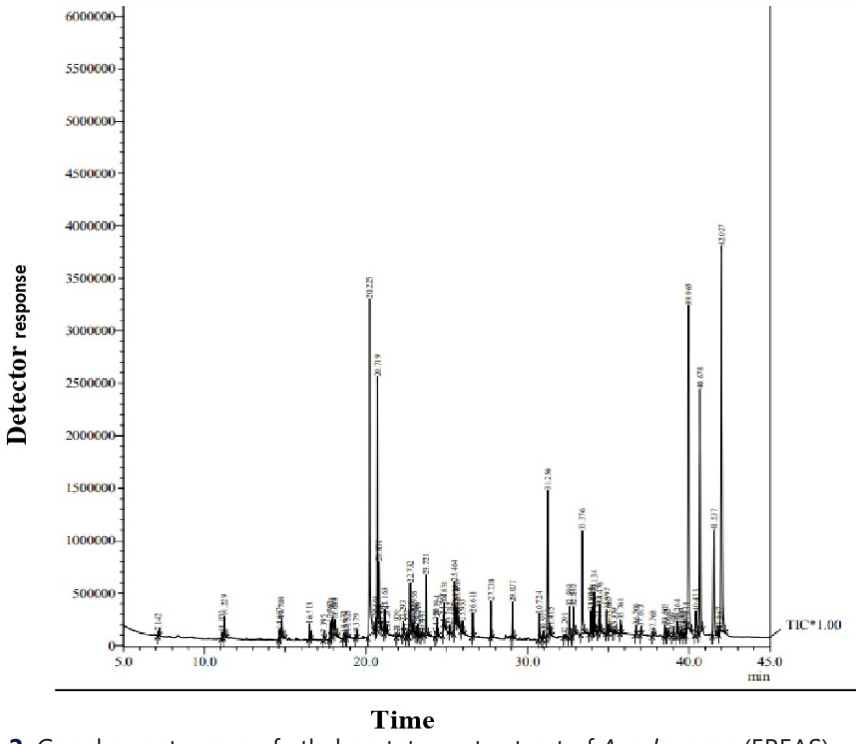

Fig. 2. Gas chromatogram of ethyl acetate root extract of *A. solanacea* (EREAS).

Table 3
Chemical composition of the ethyl acetate root extract of *A. solanacea* (EREAS).

S.No.	Name of compounds	Mol. formula	R.I.	Area % (amount)
1	undecane	$C_{11}H_{24}$	-	0.1
2	dodecane	$C_{12}H_{26}$	1115	0.7
3	undecanol	$C_{11}H_{24}O$	-	0.2
4	tetradecanol	$C_{14}H_{30}O$	-	0.3
5	hexahydro-2h-pyrido(1,2-a) pyrazin-3(4h)-one	$C_8H_{14}N_2O$	1317	0.9
6	heptadecane	$C_{17}H_{36}$	-	0.8
7	4,6,6-trimethyl-2-(3-methylbuta-1,3-dienyl)-3-oxatricyclo [5.1.0.0(2,4)]octane	C15H22O	1407	10.1
8	nonadecane	$C_{20}H_{42}$	-	0.3
9	2-bromo dodecane	$C_{12}H_{25}Br$	1446	0.1
10	1-dodecanol, tms derivative	$C_{15}H_{34}OSi$	1498	0.1
11	2,4-ditert -butylphenol	$C_{14}H_{22}O$	1555	0.4
12	β-asarone	$C_{12}H_{16}O_3$	1568	0.6
13	pentadecane	$C_{15}H_{32}$	-	0.3
14	τ-muurolol	$C_{15}H_{26}O$	1580	0.1
15	lithocholic acid, tms	$C_{30}H_{46}O_3Si_2$	-	0.2
16	undecanoic acid, 11-fluoro-, trimethylsilyl ester	$C_{14}H_{29}FO_2Si$	-	0.1
17	1-naphthalenol,5,6,7,8-tetrahydro-2,5-dimethyl-8-(1-methylethyl	$C_{15}H_{22}O$	1757	1.2
18	myristic acid, tms derivative	$C_{17}H_{36}O_2Si$	1788	0.1
19	9-octadecene, (e)	$C_{18}H_{36}$	1818	0.4
20	methyl palmitate	$C_{17}H_{34}O_2$	1878	0.3
21	estradiol, 3-deoxy	$C_{18}H_{24}O$	1949	0.4
22	9-eicosene, (e)	$C_{20}H_{40}$	2017	0.3
23	phytol, tms derivative	$C_{23}H_{48}OSi$	2086	0.2
24	palmitic acid, tms derivative	$C_{19}H_{40}O_2Si$	-	3
25	eicosane	$C_{20}H_{42}$	2000	0.8
26	methyl linolelaidate	$C_{19}H_{34}O_2$	2093	0.1
27	heptadecane	$C_{17}H_{36}$	-	0.1
28	heneicosane	$C_{21}H_{44}$	2109	0.9
29	neophytadiene	$C_{20}H_{38}$	-	0.5
30	cis-vaccenic acid	$C_{18}H_{34}O_2$	2175	0.6
31	3-hydroxy-3,7,11,15-tetramethylhexadecanoic acid, silylat	$C_{26}H_{56}O_3Si_2$	-	5.4
32	cis,cis-linoleic acid	$C_{18}H_{32}O_2$	2183	0.3
33	stearic acid, tms derivative	$C_{21}H_{44}O_2Si$	2186	0.3
34	oleic acid, (z)-, tms derivative	$C_{21}H_{42}O_2Si$	2194	0.4
35	linolsaeue,trimethylsilylester	$C_{21}H_{40}O_2Si$	2202	0.1
36	fumaric acid, bis (tms)	C10H20O4Si2	-	0.1
37	2,2,10,10- tetramethyl-5-[(tms)ox)]	$C_{14}H_{36}O_3Si_3$	-	0.1
38	epimethendiol-diotms	$C_{26}H_{48}O_2Si_2$	-	0.2
39	1-heneicosanol	$C_{21}H_{44}O$	2351	0.2
40	tetratetracontane	$C_{44}H_{90}$	-	0.5
41	hexacosane	$C_{26}H_{54}$	2606	0.3
42	mevalonic acid, 3tms	$C_{15}H_{36}O_4Si_3$	-	0.1
43	α-amyrenone	$C_{30}H_{48}O$	-	13.3

Table 3 Continued

S.No.	Name of compounds	Mol. formula	R.I.	Area %(amount)
44	β-stigmasterol	$C_{29}H_{48}O$	2739	0.6
45	stigmasta-5,22-dien-3-ol	$C_{29}H_{48}O$	-	0.4
46	β-sitosterol, tms	$C_{32}H_{58}OSi$	-	0.2
47	docosane	$C_{22}H_{46}$	2200	1
48	gorgost-5-en-3-ol, (3. beta.)- tms	$C_{33}H_{58}OSi$	2826	0.2
49	nonadecane	$C_{19}H_{40}$	-	0.1
50	isononacosane	$C_{29}H_{60}$	-	0.2
51	lupeol	$C_{30}H_{50}O$	2848	0.1
52	α-amyrin	$C_{30}H_{50}O$	2873	10.2
53	squalene	$C_{30}H_{50}$	2914	0.5
54	vitamin E	$C_{29}H_{50}O_2$	3149	0.2

preferential leaf dip method (Vattikonda and Sangam, 2016). The higher antifeeding index indicates decreased rate of feeding by the insect. The result suggests that the extract possesses significant capability to inhibit the feeding activity of the test insect at all the tested amounts and at all the time intervals and the results analyzed to be significant ($p < 0.05$) for all the replications (Table 5).

3.4. Insecticidal activity

EREAS was evaluated for its insecticidal activity against *S. obliqua* insect using leaf dip bioassay method. Five, third instar larvae of *S. obliqua* were used for different concentration of extract to test the bioactivity. EREAS was the most effective and showed excellent mortality in concentration dependent manner. The extract showed significant mortality rate of 20.00%, 46.67%, 66.67 and 73.33% at 10, 15, 20, and 25% concentrations respectively after 72 h (Table 6). χ^2-Values, regression equations and LD_{50} of ethyl acetate root extract of *A. solanacea* against Bihar hairy caterpillar (*Spilosoma obliqua*) insect after 24, 48 and 72 h of treatment have been shown in Table 7. α-Amyrenone has been reported with remarkable anti-inflammatory and anti-hypersensitivity effects (Rosilene et al., 2017). The antifeeding activity in the extract was found to be the highest at 25% and sequentially decreased upto lowest antifeeding activity at 5% in concentration dependent manner. The antifeeding activity is found to be the highest after 12 h interval of treatment application and decreased with the subsequent time intervals as represented in Table 5. Crude extracts of various plant species constituting active compounds like sesquiterpenes, diterpenoids, triterpenes, lactones, quinolene, phenolics, fatty acids, saponins, alkaloids, exhibited antifeeding, insecticidal and growth inhibitory properties (Arivoli and Tennyson, 2013). Therefore, the antifeeding activity of EREAS could be due to the major or minor constituents of the extract. Since promising insecticidal activity was found in the root extract, the actual biocomponent responsible for it should be further investigated. The mortality percentage was established to be directly proportional to the concentration of the extract (Table 6). Results of probit analysis for evaluation of the LC_{50} values, 95% confidence limits and regression equations at 24, 48 and 72 h for mortality of BHC are presented in Table 7.The LC_{50} values of the root extracts of *A. solanacea* (EREAS) at 24, 48 and 72 h after treatment are 12.02, 10.21 and 8.60 µg/cm^2, respectively. The chi-square values of the root extract at 24, 48 and 72 h after treatment were found to be 0.20, 0.55 and 1.50, as well (Table 7).

3.5. Herbicidal activity

The herbicidal activity of EREAS was assessed against the radish *Raphanus raphanistrum* seeds to evaluate the potential impacts of various extracts on different growth parameters such as percent inhibition of seed germination, percent inhibition of coleoptiles and radical growth.

3.5.1. Inhibition of seed germination

Inhibition of seed germination was estimated as the degree of herbicidal potential. Number of seeds germinated was firstly counted and then consequently the percent inhibition of the seeds germinated was calculated on day to day basis till the 100% seed germination was achieved at different concentrations ranges from 1%, 2.5%, 5%, 7.5% and 10% of extract. It was measured as 75.69%, 84.83%, 91.32%, 98.23% and 100.00% in increasing order of concentrations, respectively (Table 8). IC_{50} was calculated as 0.04 ± 0.02% (Table 9).

3.5.2. Inhibition of coleoptile growth

The percent inhibition of coleoptile growth of the seeds germinated was measured at the time when 100% seed germination was achieved at different concentrations range from 1%, 2.5%, 5%, 7.5% and 10% of extract. It was measured as 78.22%, 88.77%, 93.35%, 97.45% and 100.00% in increasing order of concentrations, respectively (Table 10). IC_{50} was calculated 0.05 ± 0.02%

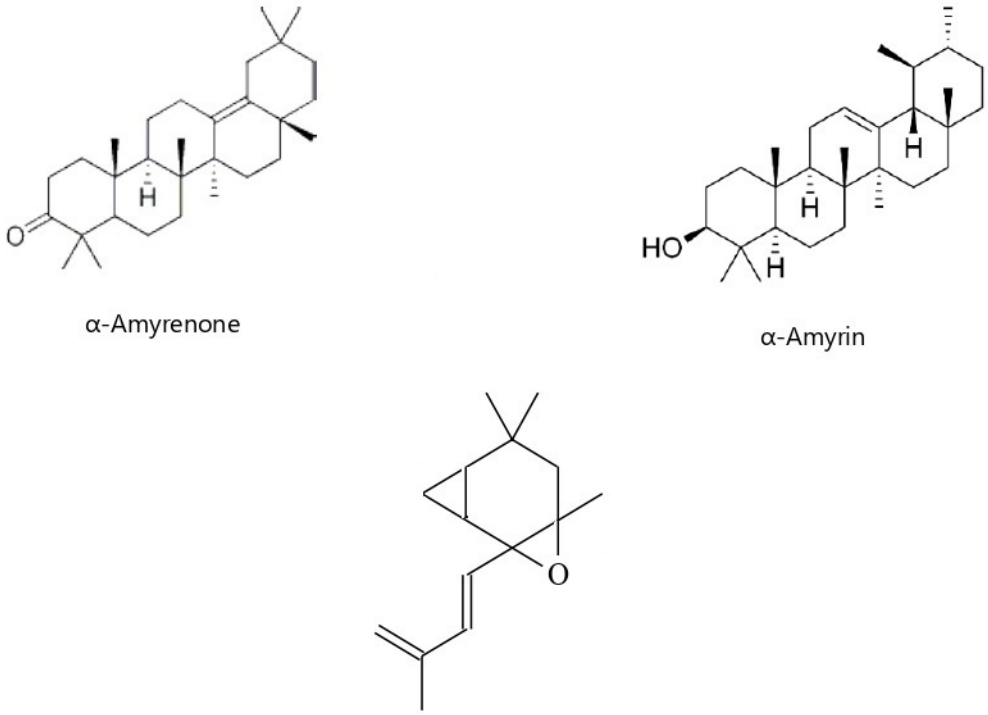

Fig. 3. Structure of major compounds present in ethyl acetate root extract of *A. solanacea*.

Table 4
Quantitative estimation of total phenolics, total flavonoids and ortho-dihydric phenol of ethyl acetate root extract of *A. solanacea* (EREAS).

Phytochemical assay	EREAS
Total phenolic content	317.93 ± 0.02 mg/gm of GAE
Total flavonoids content	14.69 ± 0.15 mg/gm of CNE
Ortho-dihydric phenol content	35.55 ± 0.11mg/gm of CLE

EREAS: Ethyl Acetate Root Extract of *A. solanacea*, **GAE**: Gallic Acid Equivalent, **CNE**:Catechin Equivalent, **CLE**: Catechol Equivalent

(Table 11).

3.5.3. Inhibition of radicle growth

The percent inhibition of radicle growth of seeds germinated was calculated at the time when 100% seed germination was achieved at different concentrations range from 1%, 2.5%, 5%, 7.5% and 10% of extract. The percent inhibition of radical growth was measured as 75.88%, 84.95%, 90.44%, 97.63% and 100.00% in increasing order of concentrations respectively in case of EREAS (Table 12). IC_{50} was calculated as EREAS (0.06 ± 0.03%) (Table 13). A literature survey revealed that till date no herbicidal activity has been reported for *A. solanacea* and any other species of *Ardisia*. In the current investigation, phytotoxic ability of the botanicals might be due to the presence of phytochemical components in the extracts (Wu et al., 1996). Lu et al. (2011) also stated that the herbicidal or phytotoxicity appeared may be due to the high phytochemical content in the botanicals, viz. phenols, flavonoids, terpenoids, alkaloids etc. or even may be due to the synergistic interaction of the major and the minor components present in the botanicals (Tiwari et al., 2006; Park et al., 2008).

3.6. *In vitro* antioxidant activity

The *in vitro* antioxidant properties of EREAS was evaluated by 2,2-diphenylpicryl hydrazyl (DPPH) radical scavenging activity, reducing power activity and chelating activity of Fe^{2+} ion compared with

Table 5
Antifeedant activity (%) of ethyl acetate root extracts *A. solanacea* (EREAS) with different concentrations.

Doses %	After 12 h		After 24 h		After 36 h		After 48 h	
	Leaf area consumed	Antifeeding activity(%)	Leaf area consumed	Antifeeding activity(%)	Leaf area consumed	Antifeeding activity(%)	Leaf area consumed	Antifeeding activity(%)
5	00 ± 00	100.00 ± 00	00 ± 00	100.00 ± 00	00 ± 00	100.00 ± 00	00 ± 00	100.00 ± 00
10	00 ± 00	100.00 ± 00	00 ± 00	100.00 ± 00	00 ± 00	100.00 ± 00	00 ± 00	100.00 ± 00
15	00 ± 00	100.00 ± 00	00 ± 00	100.00 ± 00	00 ± 00	100.00 ± 00	00 ± 00	100.00 ± 00
20	00 ± 00	100.00 ± 00	00 ± 00	100.00 ± 00	00 ± 00	100.00 ± 00	00 ± 00	100.00 ± 00
25	00 ± 00	100.00 ± 00	00 ± 00	100.00 ± 00	00 ± 00	100.00 ± 00	00 ± 00	100.00 ± 00
Control	17.35 ± 8.13	-	18.93 ± 5.84	-	20.72± 4.02	-	25.00 ± 00	-

% Antifeeding activity of ethyl acetate extract calculated by the method, reported by the earlier Vattikonda and Sangam (2016), data analyzed with two-way analysis with replication and found to be significant at $p < 0.05$.

Table 6
Mortality percentage of Bihar hairy caterpillar (*Spilosoma obliqua*) insect, treated with ethyl acetate root extract of *A. solanacea* (EREAS) by leaf dip method.

S.No.	Concent ration	No of insect used	No of insect dead			Average mortality(%)			Corrected mortality(%)
			24 h	48 h	72 h	24 h	48 h	72 h	
1	25%	5	3	4	4	60	80	80	73.33 ± 02
2	20%	5	2	4	4	40	80	80	66.67 ± 03
3	15%	5	1	3	3	20	60	60	46.67 ± 08
4	10%	5	0	1	2	0	20	40	20.00 ± 06
5	5%	5	0	1	2	0	20	40	20.00 ± 04

The % mortality was calculated after 24, 48 and 72 h of the treatment using Abbott's formula (Shen and Shao, 2005).

the standard antioxidant (Table 14). DPPH radical scavenging activities of root extracts were determined for all the tested samples at selected dose levels with different concentrations (50, 100, 150, 200 and 250 µg/mL). A continuous decrease in the absorbance of DPPH was observed in the presence of antioxidants in the

Table 7
χ^2-Values, regression equations and LD_{50} of ethyl acetate root extract of *A. solanacea* against Bihar hairy caterpillar (*Spilosoma obliqua*) insect, after 24, 48 and 72 h of treatment.

HAF	LC_{30} (µg/cm²)	LC_{50} (µg/cm²)	LC_{90} (µg/cm²)	Fiducial limit			Chi square	Reg equation
				Lower limit	Upper limit			
24	7.2	12.02	42.4	8.01	16.57		0.2	y = 2.332x + 2.485
48	6.05	10.21	36.97	6. 13	13.92		0.55	y = 2.312x + 2.674
72	5.04	8.6	32.21	4.4	11.88		1.5	y = 2.316x + 2.835

The LD50 values were analyzed by Probit analysis (Eloff, 1998).

tested sample which correlates with the free radical scavenging potential of the antioxidant. Root extract was found to possess good DPPH radical scavenging activity with an IC_{50} value of 190.78 ± 0.14 µg/mL with respect to the BHT used as standard. A good reducing power activity was achieved with the extract, having the RP_{50} value of 277.74 ± 1.09 µg/mL, in comparison to the catechin taken as standard antioxidant (RP_{50} = 177.71 ± 1.56 µg/mL). EREAS also showed strong metal chelating effect with an IC_{50} of 1.99 ± 0.09 µg/mL with respect to EDTA used as standard (IC_{50} = 2.85 ± 0.01 µg/mL). Statistical analysis reported to be significantly different ($p < 0.01$). IC_{50} values for antioxidant activity expressed as mean ± standard deviation taken in triplicates and analyzed to be significantly different ($p < 0.01$). Significant antioxidant properties of the extract might be possibly due to the significant amount of phenols, flavonoids and ortho-dihydric phenols (Liu et al., 2008; Liu et al., 2009; Lu et al., 2011). EREAS was also found to possess significant antioxidant properties since the ethyl acetate root extract of *A. solanacea* consisted of complex mixture of numerous components. Total phenolics, the major and minor components in the ethyl acetate root extract of *A. solanacea* might be responsible for the antioxidant properties. Overall, the order in which plant possesses the activity is metal chelating > DPPH radical scavenging > reducing power as lower the IC_{50} value accounts for higher antioxidant activity.

3.7. *In vitro* anti-inflammatory activity

EREAS exhibited the potential to inhibit protein denaturation at all tested concentrations (50-250 µg/mL) having percent inhibition from 35.00 ± 0.89% to 88.42 ± 0.84% (Table 15). Statistical analysis reported to be significantly different ($p < 0.01$). The IC_{50} values for extract was 1.79 ± 0.13 µg/mL and for diclofenac sodium was 2.80 ± 0.07 µg/mL. The results of the current preliminary study stated that the root extract of *A. solanacea* possessed discernible *in vitro* anti-inflammatory effect against the denaturation of albumin protein. Anti-inflammatory activity shown by other species of *Ardisia* like *A. cornudentata*, *A. crispa A. teysmanniana* and *A. tinctoria* with different extracts and active components present in the extracts have been reported to be significantly acute and chronic (Sumino et al.,2001; Yang et al., 2001; Chang et al., 2011). The anti-inflammatory activity performed by the ethyl acetate extract of *A. solanacea* might be due to the presence of major components/mixture of components in the extracts. Further definitive studies are still necessary to find out the mechanism and other components present in various ethyl acetate extracts of *A. solanacea* for its anti-inflammatory actions.

4. Concluding remarks

The present research results suggested a potential antioxidant activity for the ethyl acetate root extract of *A. solanacea* (EREAS), supporting their use in the

Table 8
Inhibition of seed germination (%) of ethyl acetate root extract of *A. solanacea*.

Extract/standard	% Inhibition of germination					
	Replicates-3	1.00%	2.50%	5.00%	7.50%	10.00%
EREAS	Mean	75.69	84.83	91.32	98.23	100
	STD	6.24	5.94	4.47	1.74	0
Pendimethalin	Mean	100	100	100	100	100
	STD	0	0	0	0	0

EREAS= Ethyl Acetate Root Extract of *A. solanacea*. Data analyzed with two-way analysis with replication and found to be significant at $p < 0.05$.

Table 9
IC_{50} values of seed germination inhibition by ethyl acetate root extract of *A. solanacea*.

Extract	IC_{50} Values			IC_{50}
	R1	R2	R3	
EREAS	0.06	0.04	0.02	0.04 ± 0.02

EREAS= Ethyl Acetate Root Extract of *A. solanacea*. Data analyzed with two-way analysis with replication and found to be significant at $p < 0.05$.

Table 10
Inhibition of coleoptile growth (%) of ethyl acetate root extract of *A. solanacea*.

Extract/standard	% Inhibition of coleoptiles growth					
	Replicates-3	1.00%	2.50%	5.00%	7.50%	10.00%
EREAS	Mean	78.22	88.77	93.35	97.45	100
	STD	7.65	6.25	3.67	2.57	0
Pendimethalin	Mean	100	100	100	100	100
	STD	0	0	0	0	0

EREAS= Ethyl Acetate Root Extract of *A. solanacea*. Data analyzed with two-way analysis with replication and found to be significant at $p < 0.05$.

Table 11
IC_{50} values of coleoptile length germination by ethyl acetate root extract of *A. solanacea*.

Extract	IC_{50} Values			IC_{50}
	R1	R2	R3	
EREAS	0.06	0.04	0.02	0.04 ± 0.02

EREAS= Ethyl Acetate Root Extract of *A. solanacea*. Data analyzed with two-way analysis with replication and found to be significant at $p < 0.05$.

Table 12
% Inhibition of radical growth of ethyl acetate extract of *A. solanacea*.

Extract/standard	% Inhibition of radical growth					
	Replicates-3	1.00%	2.50%	5.00%	7.50%	10.00%
EREAS	Mean	75.88	84.95	90.44	97.63	100
	STD	5.21	4.43	4.53	2.57	0
Pendimethalin	Mean	100	100	100	100	100
	STD	0	0	0	0	0

EREAS= Ethyl Acetate Root Extract of *A. solanacea*. Data analyzed with two-way analysis with replication and found to be significant at $p < 0.05$.

Table 13

IC_{50} values of radical germination inhibition by ethyl acetate root extract of *A. solanacea*.

Extract	IC_{50} values			IC_{50}
	R1	R2	R3	
EREAS	0.06	0.04	0.02	0.04 ± 0.03

EREAS= Ethyl Acetate Root Extract of *A. solanacea*. Data analyzed with two-way analysis with replication and found to be significant at $p < 0.05$.

Table 14

In vitro antioxidant activities of the ethyl acetate root extract of *A. solanacea* expressed in terms of their IC_{50} values

Extract/standard	DPPH radical scavenging activity (µg/mL)	Reducing power activity (µg/mL)	Metal chelating activity of Fe^{2+} (µg/mL)
EREAS	190.78 ± 0.14	277.74 ± 1.09	2.79 ± 0.01
BHT	107.48 ± 0.70	-	-
Catechin	-	177.71 ± 1.56	-
EDTA	-	-	2.85 ± 0.01

EREAS: Ethyl Acetate Root Extract of *A. solanacea*, **BHT:** Butylated Hydroxyl Toluene, **DPPH:** 2,2-Diphenyl-1- 685 picrylhydrazyl, **EDTA:** Ethylene Diammine Tetraacetate (Na salt), All the IC_{50} values expressed as mean ± standard 686 deviation taken in triplicates and analyzed to be significantly different ($p < 0.01$).

Table 15

In vitro anti-inflammatory activity in terms of IC_{50} values for ethyl acetate root extract of *A. solanacea* (EREAS).

Extract/standard	IC_{50} value
EREAS	1.79 ± 0.13
Diclofenac sodium	2.80 ± 0.07

% *In-vitro* anti-inflammatory activity of Ethyl Acetate Root Extract of *A. solanacea* (**EREAS**) versus the standard anti-inflammatory agent (diclofenac sodium), IC_{50} values plotted as mean ± standard deviation with percent inhibition at various concentrations are significantly different ($p < 0.01$).

traditional medicine and also indicating the plant to be supplemented as an important component in food, cosmetic and pharmaceutical industries. In addition, the above botanical may be utilized as Integrated Pest Management Programme (IPM) of *Spilosoma obliqua* after repeated field experiments. Because of higher antifeedant activity against lepidopterous pests, the root extracts of *A. solanacea* may have good potential for protecting pulse crops suffering heavy damage due to these pests. This plant product may be exploited for management of other lepidopterous pests in many crops including storage insect's pest. Because of its presence in local areas, proper cultivation practices may be adopted for its production and utilization in Pest Management Programme. The above botanical opens new perspectives on the application of the root extracts of *A. solanacea* as novel botanical herbicides for weed management. It can be employed in a new herbicidal formulation and offers new strategies and pathways for the biopesticide industry to create eco-friendlyalternative to chemical herbicides.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

The authors express special reverence to the Advance Instrumentation Research Facility, Jawaharlal Nehru University, New Delhi for providing GC-MS facility.

References

Adams, R.P., 2007. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. Allured Business Media Publishing Corporation, Carol Stream, Illinois, USA, 4, pp. 542-544.

Ahmad, S.A., Catalano, S., Marsili, A., Morelli, I., Scartoni, V., 1977. Chemical examination of the leaves of *Ardisia solanacea*. Planta Med. 32, 162-1646.

Amin, M.N., Banik, S., Ibrahim, M., Moghal, M.M.R., Majumder, M.M., Siddika, R., Alam, M.K., Jitu, K.M.R.M., Anonna, S.N., 2015. A study on *Ardisia solanacea* for evaluation of phytochemical and pharmacological properties. Int. J. Pharmacogn. Phytochem. 7(1), 8-15.

Anjum, B., Kumar, R., Kumar, R., Prakash, O., Srivastava, R.M., Pant, A.K., 2019. Phytochemical analysis, antioxidant, anti-inflammatory and insect antifeeding activity of *Ardisia solanacea* Roxb. extracts. JBAPN 9(5), 372-386.

Arivoli, S., Tennyson, S., 2013. Antifeeding activity, developmental indices and morphogenetic variations of plant extracts against *Spodoptera litura* (Fab) (Lepidoptera: Noctudae). J. Entomol. Zool. Stud. 1, 7-96.

Bailey, I.H., 1925. The Standard Cyclopedia of Horticulture. McMillan Corporation London, 2, pp. 674-680.

Basha, D.J., Murthy, B., Prakash, C., 2016. Pharmacognostical standardization, preliminary phytochemical investigation of root stocks of *Ardisia solanacea* Roxb. J. Chem. Pharma. Res. 8,1107-1113.

Berenbaum, M., 1995. Phototoxicity of plant secondary metabolites: insect and mammalian perspectives. Arch. Insect Biochem. Physiol. 29, 119-134.

Chandran, P.R., Manju, S., Vysakhi, M.V., Shaji, P.K., Achuthan, N.G., 2015. Nutritional and antinutritional properties of the leaf of *Ardisia solanacea* Roxb. (Myrsinaceae), a fodder additive. Int. Food Res. J. 22, 324-331.

Chang, C.P., Chang, H.S., Peng, C.F., Lee, S.J., Chen, I.S. 2011. Antitubercular resorcinol analogs and benzenoid C-glucoside from the roots of *Ardisia cornudentata*. Planta Med. 77(4), 60-65.

Chen, C., Pipoly, J.J., 1996. Myrsinaceae. Flora of China. St. Louis, U.S.A., Science Press, Beijing and Missouri Botanical Garden Press. 1, pp. 138-145.

Choi, Y., Lee, S.M., Chun, J., Lee, H.B., Lee, J., 2006. Influences of heat treatment on antioxidant activities and polyphenolic compounds of shiitake (*Lentinus edodes*) mushroom. J. Food Chem. 99, 381-387.

Dhal, N.K., Panda, S.S., Muduli, S.D., 2015. Traditional uses of medicinal plants by native people in Nawarangpur district, Odisha, India. Asian J. Plant Sci. Res. 5(2), 27-33.

Desai, P.D., Dutia, M.D., Ganculy, A.K., Govindachari, T.R., Joshi, B.S, Kamat, V.N., Prakash, D., Rane, D.F., Sathe, S.S., Vishawanathan, N., 1967. A study of medicinal plants. Indian J. Chem. 5, 523-532.

Devi Prasad, A.G., Shyma, T.B. 2013. Medicinal plants used by the tribes of Vythiri taluk, Wayanad district (Kerala state) for the treatment of human and domestic animal ailments. J. Med. Plant. Res. 7(20), 1439-1451.

Elanchezhian, R., Senthil, K.R., Beena, S.J., Suryanarayana, M.A. 2007. Ethnobotany of Shompens-a primitive tribe of Great Nicobar Island. Indian J. Tradit. Know. 6(2), 342-345.

Eloff, J. N., 1998. Which extractant should be used for the screening and isolation of antimicrobial components from plants. J. Ethnopharmacol. 60, 1-8.

Fujioka, M., Koda, S., Morimoto, Y., Biemann, K., 1988. Structure of FR-900359: a cyclic despeptide from *Ardisia crenata* Sims. J. Org. Chem. 53, 2820-2845.

Gupta, G., Bhattacharya, A.K., 2008. Assessing toxicity of post emergence herbicides to the *Spilarctia obliqua* Walker (Lepidoptera: Arctiidae). J. Pest Sci. 81, 9-15.

Hideka, K., De-Mejia, E., 2005. The genus *Ardisia*: a novel source of health-promoting compounds and phytopharmaceuticals. J. Ethnopharmacol. 9, 347-354.

Hussain, M., Begum, M., 1995. Food preference of jute hairy caterpillar (*Spilosoma obliqua* Walk.) on some varieties of jute. Bang. J. Entomol. 5, 57-59.

Hutchinson, J., 1973. The Families of Flowering Plants, Oxford University Press, London, 3, pp. 968-999.

Jia, Z., Koike, K., Nikaido, T., Ohmoto, T., Ni, M., 1994. Triterpenoid saponins from *Ardisia crenata* and their inhibitory activity on cAMP phosphodiesterase. Chem. Pharma. Bull. 42, 2309-2314.

Kar, B., Kumar, R.S., Karmakar, I., Dola, N., Bala, A., Mazumder, U.K., Hadar, P.K., 2012. Antioxidant and in-vitro anti-inflammatory activities of *Mimusops elengi* leaves. Asian Pac. J. Trop. Biomed. 2, 976-980.

Karuppusamy, S., 2007. Medicinal plants used by Paliyan tribes of Sirumalai hills of Southern India. Nat. Prod. Rad. 6, 436-442.

Kessler, A., Baldwin, I.T., 2002. Plant responses to insect herbivory: the emerging molecular analysis. Annu. Rev. Plant Biol. 53, 299-328.

Khan, M.T.J., Ashraf, M., 1991. Chemistry and antibacterial activity of the constituents of the leaves of *Ardisia solanacea*. Fitoterapia 62, 65-68.

Khatun, A., Rahman, M., Kabir, S., Akter, M.N., Chowdhury, S.A., 2013. Phytochemical and pharmacological properties of methanolic extract of *Ardisia humilis* Vahl. (Myrsinaceae). Int. J. Res. Ayurveda Pharm. 1-23.

Kumar, R., Anjum, B., Kumar, R., Prakash, O., Panday, G., Pant, A.K., Srivastava, R.M., 2019. Chemical composition, total phenolics assay and biological activities of chloroform extract from *Limnophila indica* (L.) Druce. J. Entomol. Zool. Stud. 7, 594-599.

Liu, H., Qiu, N., Ding, H., Yao, R., 2008. Polyphenols contents and antioxidant capacity of 68 Chinese herbals suitable for medical or food uses. Food Res. Int. 41, 363-370.

Liu, S.C., Lin, J.T., Wang, C.K., Chen, H.Y., Yang, D.J., 2009. Antioxidant properties of various solvent extracts from lychee (*Litchi chinenesis* Sonn.) flowers. Food Chem. 114, 577-581.

Lu, X., Wang, J., Al-Qadiri, H.M., Ross, C.F., Powers, J.R.J., Tang, Rasco, B.A., 2011. Determination of total phenolic content and antioxidant capacity of onion (*Allium cepa*) and shallot (*Allium oschaninii*) using infrared spectroscopy. Food Chem. 129, 637-644.

Mahadevan, A., Sridhar, R., 1986. In Methods of Physiological Plant Pathology. Sivakami Publication,

Chennai. 44, pp. 190-192.
Mohammadhosseini, M., 2017. Essential oils extracted using microwave-assisted hydrodistillation from aerial parts of eleven *Artemisia* species: Chemical compositions and diversities in different geographical regions of Iran. Rec. Nat. Prod. 11(2), 114-129.
Mohammadhosseini, M., 2017. The ethnobotanical, phytochemical and pharmacological properties and medicinal applications of essential oils and extracts of different *Ziziphora* species. Ind. Crop Prod. 105, 164-192.
Mohammadhosseini, M., Venditti, A., Sarker, S.D., Nahar, L., Akbarzadeh, A., 2019. The genus *Ferula*: Ethnobotany, phytochemistry and bioactivities - A review. Ind. Crop Prod. 129, 350-394.
Mohammadhosseini, M., Sarker, S.D., Akbarzadeh, A., 2017. Chemical composition of the essential oils and extracts of *Achillea* species and their biological activities: A review. J. Ethnopharmacol. 199, 257-315.
Merlin, F.F., Narasimhan, D. 2009. What is traditional knowledge. Indian J. Tradit. Know. 8(4), 645-648.
Mohammad, N.A., Shimul, B., Ibrahim, M., Moghal, M.R., 2015. A study on *Ardisia solanacea* for evaluation of phytochemical and pharmacological properties. Int. J. Pharmacog. Phytochem. Res. 7, 8-15.
Newell, A.M., Yousef, G.G., Lila, M.A., Ramírez-Mares, M.V., De-Mejia, E.G., 2010. Comparative *in vitro* bioactivities of tea extracts from six species of *Ardisia* and their effect on growth inhibition of HepG2 cells. J. Ethnopharmacol. 130, 536-544.
Park, M., Bae, J., Lee, D.S., 2008. Antibacterial activity of gingerol and gingerol isolated from ginger rhizome against periodontal bacteria. Phytother. Res. 22(11), 1446-1449.
Pattanaik, C., Reddy, C.S., Murthy, M.S. 2008. An ethnobotanical survey of medicinal plants used by the Didayi tribe of Malkangiri district of Orissa, India. Fitoterapia 79(2), 67-71.
Ramirez-Mares, M.V., Sanchez-Burgos, J.A., Hernandez-Carlos, B., 2010. Antioxidant, antimicrobial and antitopoisomerase screening of the stem bark extracts of *Ardisia compressa*. Pakistan J. Nutr. 65, 307-313.
Reddy, K.N., Pattanaik, C., Reddy, C.S., Raju, V.S., 2007. Traditional knowledge of wild food plant in Andhra Pradesh. Indian J. Tradit. Know. 6(1), 223-229.
Rosilene, G.S.F., Walter, F.S., Valdir, F.V., Adley, A.N.L., Emerson, S.L., 2017. Physicochemical characterization and biological activities of the triterpenic mixture α,β-amyrenone. Molecules 22, 298.
Sahu, A., Devkota, A., 2013. Allelopathic effects of aqueous extract of leaves of *Mikania micrantha* H.B.K. on seed germination and seedling growth of *Oryza sativa* and *Raphanus sativus*. Sci. World 11, 91-93.
Samal, P.K., 2013. Assessment of hypolipidemic effect of *Ardisia solanacea* in high fat diet induced rats. RJPPD 5, 147-150.
Shah, B.N., Seth, A.K., Maheshwari, K.M., 2011. A review on medicinal plants as a source of anti-inflammatory agents. Res. J. Med. Pl. 66, 101-115.
Shail, G.G., 2011. Phytochemical analysis of the Indian medicinal plant *Argyreia involcurata*. Int. J. Res. Pharm. Biomed. Sci. 2, 1178-1782.
Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphor molybdic phosphotungstic acid reagents. Am. J. Enol. Viticult. 16, 144-158.
Singh, B., Sinha, B.K., Phukan, S.J., Borthakur, S.K., Singh, V.N., 2012. Wild edible plants used by Garo tribes of Nokrek Biosphere Reserve in Meghalaya, India. Indian J. Tradit. Know. 11(1), 166-171.
Sumino, M., Sekine, T., Ruangrungsi, N., Ikegami, F., 2001. Ardisiphenols A-C, novel antioxidants from the fruits of *Ardisia colorata*. Chem. Pharm. Bull. 49(8), 1664-1665.
Tabashnik, B.E., Cushing, N.L., 1987. Leaf residue vs. topical bioassays for assessing insecticide resistance in the diamond back moth, *Plutella xylostella* L. FAO. Plant Prot. Bull. 35, 11-14.
Tiwari, V., Shanker, R., Srivastava, J., Vankar, P.S., 2006. Change in antioxidant activity of spices-turmeric and ginger on heat treatment. Env. Agricult. Food Chem. 66, 1313-1317.
Venditti, A., Frezza, C., Serafini, I., Pulone, S., Scardelletti, G., Sciubba, F., Bianco, A., Serafini, M., 2018. Chemical profiling of the fruits of *Styrax officinalis* L. from Monti Lucretili (Latium region, Central Italy): Chemotaxonomy and nutraceutical potential. Trends Phytochem. Res. 2(1), 1-12.
Vattikonda, S.R., Sangam, S.R., 2016. Assessment of antifeedant potential of azadirachtin, a plant product of *Azadirachta indica* against *Papilio demoleus* L. (Lepidoptera: Papilionidae) larvae. J. Entomol. Zool. Stud. 4, 956-959.
Wansi, J.D., Sewald, N., Nahar, L., Martin, C., Sarker, S.D., 2018. Bioactive essential oils from the Cameroonian rain forest: A review - Part I. Trends Phytochem. Res. 2(4), 187-234.
Warad, M., Kalleshwaraswamy, C.M., 2017. Biology of Bihar hairy caterpillar, (*Spilarctia obliqua* Walker) (Erebidae: Lepidoptera) on field bean. J. Entomol. Zool. Stud. 12, 1256-1260.
Wu, J.J., Yang, J.S., Liu, M.S., 1996. Effects of irradiation on the volatile compounds of garlic (*Allium sativum* L). J. Sci. Food Agric. 70, 506-508.
Yang, L.K., Khoo-Beattie, C., Goh, K.L., Chng, B.L., Yoganathan, K., Lai, Y.H., Butler, M.S., 2001. *Ardisia* quinones from *Ardisia teysmanniana*. Phytochemistry 58, 1235-1238.