Pharmacognostic evaluation, chemical characterization, and antioxidant and anti-arthritic efficacy of *Ardisia solanacea* leaves

Lingarkar Silpavathi[*1], Mukesh Kumar Das[2], Prafulla Kumar Sahu[3], Sukumar Purohit[3], Manoj Pani[4], Debajyoti Das[1],

[1]School of Pharmaceutical Sciences, Siksha "O" Anusandhan Deemed to be University, Bhubaneshwar-751003, Odisha, India.

[2]IndQuench Life Science Innovations (OPC) Pvt. Ltd, Plot, No: 31/761, Devika Bihar, Dasabatia, Tamando (P), Khurda(Dist), Bhubaneswar, Odisha 751028, India

[3]School of Pharmacy, Centurion University of Technology and Management, Odisha.

[4]Indira Gandhi Institute of Pharmaceutical Sciences, IRC Village, Bhubaneshwar.

Email ID: shilpa_pharma@rediffmail.com, das_mukesh@rediffmail.com, kunasahu1@rediffmail.com, Sukumar.purohit@cutm.ac.in, drmkbigips@gmail.com, debajyotids@gmail.com

Running Title: Pharmacognostic and biochemical activity of *Ardisia solanacea*

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*Address for correspondence

Lingarkar Silpavathi

School of Pharmaceutical Sciences, Siksha "O" Anusandhan Deemed to be University, Bhubaneshwar-751003, Odisha, India.Email.i.d.: shilpa_pharma@rediffmail.com, shilpapharma70@gmail.com, Phone- +91- 9550267858

Abstract

Background: Traditional medicine is the mainstay of care for the majority of Indians who suffer from serious illnesses. The well-known Indian herb *Ardisia solanacea* (Poir.) Roxb (family: Myrsinaceae) exhibits intriguing pharmacological properties, such as immunomodulatory and cytotoxic actions.

Objective: To investigate the pharmacognostic, physical-chemical, GC-MS characterisation, and biological activity of leaves from *Ardisia solanacea* (AS) plant.

Materials and methods: The present study included an organoleptic study, macroscopy, microscopy, powder microscopy, histochemistry, physicochemical parameters, fluorescence analysis, total phenolic content, total flavonoid content, and GC-MS characterization to assess the plant's pharmacognostic potential. Following verification, the plant's leaf extract was successfully tested for in vitro anti-inflammatory and antioxidant properties.

Result: The leaves are thick, lanceolate, whole, thin at the tip, and dark green in colour, according to an organoleptic and macroscopic investigation. It was investigated whether the sample included starch, calcium oxalate, mucilage, lipids, tannins, proteins, and lignin. Following the extraction of the leaves, the presence of alkaloids, flavonoids, terpenoids, steroids, tannins, saponins, gums, and mucilage was analysed by GC-MS. In order to define the standards of the plant, study of the leaf contents discovered by powder microscopy, physicochemical characteristics, and fluorescence analysis provides accurate data. Significant antioxidant and anti-arthritic activity was shown by the leaf extract.

Conclusion: This study found that AS possesses clear pharmacognostic traits that will serve as a solid foundation for this plant's identification, authenticity, purity, and formulation of quality standards. Invitro study of the leaf extract has been shown to have remarkable anti-arthritic and antioxidant activities.

Keywords: *Ardisia solanacea*, microscopy, GC-MS, anti-arthritic, antioxidant.

Abbrevations:

RA- Rheumatoid Arthritis

AS- *Ardisia solanacea*

DPPH- α-diphenyl-β-picrylhydrazyl

ABTS- 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonicacid)

FRAP- Ferric reducing ability of plasma

UV- Ultra violet chamber

TPC-total phenolic content

TFC- total flavonoid content

HRBC- Human red blood cells

ASE- AS ethyl acetate extract

ASM- AS methanolic extract

ASW-AS water extract

AA- ascorbic acid

1. Introduction

Ethnobotanical knowledge is one of several encyclopedia-like approaches to traditional medicine. Because of its no-side-effect policy, traditional medicine is used by a large portion of India's population to treat a variety of life-threatening diseases. (Scartezzini and Speroni 2000). Plant-based pharmaceuticals have been successfully explored for the treatment of many diseases, including cancer (Rao et al. 2008), diabetes (Kumar et al. 2021), antioxidant (Scartezzini and Speroni 2000), antimicrobial (Thirumurugan et al. 2010), analgesic (Rathinavel et al. 2021), antipyretic (Ballabh and Chaurasia 2007), rheumatoid arthritis (Silpavathi et al. 2021), etc.

Rheumatoid arthritis (RA) is a severe autoimmune disease that causes joint inflammation (painful swelling). The immune system of our bodies attacks healthy cells, causing severe damage. Nonsteroidal anti-inflammatory drugs (NSAIDs) and disease-modifying anti-rheumatic drugs (DMARDs) are the first-line treatments for RA (Van Vollenhoven 2009). However, long-term use of these medications has serious side effects such as decreased bone density, gastritis, and other infections. Many plant extracts have been successfully tested for their potent anti-arthritic properties in order to address this serious concern (Soeken et al. 2003).

In accordance with the preceding statement, one of our recent studies demonstrated the use of AS leaf extract for the treatment of adjuvant-induced arthritic rats (Silpavathi et al. 2021). AS is a well-known Indian herb with numerous pharmacological properties such as immunomodulatory, cytotoxic, antimicrobial, and so on (Silpavathi et al. 2021). As a result, the current study is concentrating on the detailed pharmacognostic evaluation, invitro antioxidant and anti-arthritic potential of AS leaves. The chemical profiling of the phytoconstituents present in the leaf extract was also examined using GC-MS.

2. Materials and Methods

2.1.Collection and authentication of plant material

The leaves of *Ardisia solanacea* (Poir.) Roxb were collected in the Singhasini forests of Berhampur in February 2019. The plant was identified, authenticated, and certified by the Principal Scientist, Regional Plant Resource Center, Bhubaneswar, Orissa, and a voucher number (02/LSV/09/02/19) was provided.

2.2.Reagents and Chemicals

All of the chemicals and solvents employed in the experiment were of analytical quality and were purchased from Merck India Ltd. in Mumbai. DPPH and ABTS, were purchased from Merck India Ltd for antioxidant activity. The histology dyes were bought from Himedia.

2.3.Macroscopy and histology

Sense organs were used to look at the leaf and figure out its colour, odour, and taste. Standard methods were used to examine the macroscopic characteristics of the leaves, including leaf type, shape, apex, margin, lamina, base venation, and texture (Evans 2009). The microscopic study was done on potato-sandwich specimens that were cut into pieces with a sharp blade. The thin slide was stained using a 0.1% w/v solution of safranin dye in distilled water. With Nikon Lab Photo's two microscope units, photos with different levels of magnification were taken. Researchers looked at cross-sections of leaves and lamina, as well as venation patterns, crystals, and different types of stomata. A histochemical study also showed that the leaf sample contained starch, calcium oxalate, mucilage, lipids, tannins, proteins, and lignin. The sample was stained with potassium iodide stain to see if it had starch. Ferric chloride staining showed that the leaf sample contained tannin. Lignin was found by using a phenolic stain called phloroglucinol.

2.4.Physico-chemical analysis

A physicochemical analysis of the plant species was performed, including the determination of foreign organic matter, moisture content, ash value, and extractive values. After collecting the leaf material, the foreign matter such as sand, soil, dust, and twigs was visually removed. Following the previously published article, the moisture content and ash value (total ash, acid insoluble ash, and water soluble ash) were determined (Purohit et al. 2021). The extractive value of the leaf sample was determined in greater detail. Two separate experiments were set up, each with 5 g of sample macerated separately in 100 ml each of ethanol and water for 24 hours. Following that, 25 ml of each solvent-containing extract was placed in separate tarred petri plates and evaporated at 105 °C until the solvent was completely dried. The extractive value was also determined.

2.5.Fluorescence Analysis

The fluorescence nature of the sample was investigated by applying 1-2 drops of freshly prepared different reagents to a small amount of dry leaf powder on a grease-free, clean microscopic slide and placing it inside the UV chamber to observe the colour changes (Chase Jr and Pratt 1949).

2.6.Extraction of the Ardisia solanacea leaf powder

The leaf sample was extracted using a cold maceration process. In two different maceration jars, 50 g of leaf powder was mixed separately with methanol and water (water with 2% chloroform) in a 1:10 ratio for 72 h and 24 h, respectively. The extraction solvents were removed every other day, and fresh solvent was added to the maceration jars. The extracts were dried using a rotary evaporator after the extraction process was completed. Various extracts were also obtained and stored at 4 °C for future use.

2.7.Preliminary Phytochemical screening

According to the previously published book, different extracts were dissolved in respective solvents and qualitatively analysed for the presence of alkaloids, flavonoids, terpenoids, steroids, tannins, saponins, gums, and mucilage (Khandelwal 2008).

2.8.GC-MS analysis

GC-MS analysis was used to determine the chemical composition of various extracts obtained from the extraction. The GC-MS analysis was carried out with the help of a Thermo Trace 1300 GC and a Thermo TSQ 8000 Triple Quadrupole MS. A 5% Phenyl Polysilphenylene- siloxane column (BP 5MS (30mX 0.25mm, 0.25µm)) was used for the analysis. The initial temperature was kept at 50^{o}C and ramped up to 250^{o}C at 10^{o}C/min rate. The ion source temperature, injection temperature and MS transfer line temperature was kept at 230^{o}C, 250^{o}C and 240^{o}C respectively. For the analysis, a split-less injection mode was used. With a flow rate of 1.0 ml/min, helium was used as the carrier gas. The experiment was carried out with the help of Xcalibur 2.2SP1 and the Foundation 2.0SP1 software. This analysis discovered compounds with masses ranging from 30 to 650 g/mol. The NIST 2.0 library was used to interpret GC-MS peaks and their mass-to-charge ratio.

2.9.Total phenolic content and total flavonoid content

The total phenolic content (TPC) and total flavonoid content (TFC) of various extracts were determined using the procedure outlined in the previous article (Purohit et al. 2021). TPC and TFC values are expressed as gallic acid equivalent per 100 g of extracts (GAE/100 g) and quercetin equivalent per 100 g of extracts (QUE/100 g), respectively.

2.10. In vitro antioxidant activity

The obtained extracts were tested for antiradical activity in vitro using the DPPH, ABTS, and FRAP assays. The DPPH and ABTS analyses were carried out in accordance with the published literature (Moges et al. 2021). The ferric reducing method was also used to determine the sample's total antioxidant potential. In a nutshell, the FRAP reagent was made by combining acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ in a 10:1:1 (v/v/v) ratio. Each well received 3.4 µL of reagent and 100 µL of sample solution, which were thoroughly mixed. After 30 minutes, the absorbance was measured at 593 nm. Trolox standard curves with various concentrations were created (Benzie and Strain 1999). The results were expressed as µmol trolox equivalent/g dry weight. For each extract, all analyses were carried out in triplicate.

2.11. In vitro anti arthritic activity

2.11.1. Albumin denaturation

A 5 mL reaction mixture was prepared with 0.2 mL of egg albumin, 2.8 mL of pH 6.4 phosphate-buffer saline, and 2 mL of plant extract at 25, 50, 100, 200, 400, 800, and 1000 µg/mL concentrations. The control solution contained the same amount of egg albumin and buffer saline as well as 2 mL of distilled water. After 15 minutes at 37 °C, the reaction mixture was heated for 5 minutes at 70 °C. The absorbance of the reaction mixture was measured at 660 nm. As a comparison, distilled water was used. The percentage inhibition was calculated using Equation 1 to determine how well the extracts protected against protein denature (Qasim et al. 2020).

$$Percentage\ inhibition = 100 \times \frac{[\,Absorbance\ of\ test\ sample - 1\,]}{\text{Absorbance of control}} \quad \text{.....(1)}$$

2.11.2. HRBC test: (Hypotonicity induced heamolysis)

This assay required approximately 10 mL of blood from a healthy human volunteer, and the volunteer provided informed consent for blood collection. Following that, blood was mixed with

an equal amount of Alsever's solution before centrifugation at 3000 rpm. Packed cells at the bottom were collected, and a 10% suspension of the collected cells was made with normal saline. Hypotonic saline (2 mL), phosphate buffer (1 mL), and extracts (0.5 mL) at various concentrations (50, 100, 200, 400, 800, and 1600 µg/mL) were served, as well as 0.5 mL of 10% blood suspension as the test solution. As controls, 2 mL of distilled water, 1 mL of phosphate buffer, and 0.5 mL of a 10% RBC suspension were used. The concentrations of the standard solution are the same as those of the test solution: 0.5 mL of 10% RBC suspension, 2 mL of hypotonic saline, 1 mL of phosphate buffer, and 0.5 mL of piroxicam. All solutions were incubated for 30 minutes at 37 °C before being centrifuged at 3000 rpm. At 560 nm, spectrophotometric absorbance was measured. Equation 2 was used to calculate the percentage of membrane stabilization (Qasim et al. 2020).

$$\%\ of\ Haemolysis = \frac{OD\ test\ X100}{\text{OD Control}} \qquad \text{.....(2)}$$

2.12. Statistical analysis

All of the TPC, TFC, antioxidant, and anti-arthritic activity experiments were done in triplicate over three separate experiments. The results are presented as a mean ± standard deviation. The significance level of the anti-arthritic study was calculated using a student's t-test, and the tests were considered significant at $P< 0.001$, $P< 0.01$ and $P< 0.05$.

3. Results and Discussion

3.4. Macroscopy and microscopy

Leaves are simple, alternate, and partly spiral. The leaf lamina is elliptic-obovate in shape, and the apex is acute to acuminate. The leaf margin is entire or obscurely crenate toward the apex, where the midrib of the leaves is flat above.

Using a microscope and different pertinent colours, AS leaves were examined at the microscopic level. A professional camera was used to capture the microscopic images, and the outcomes are shown in Fig 2. The leaf has planoconvex midrib and dorsiventral symmetry. While the abaxial midrib was thick and had a shallow median furrow, the adaxial midrib was flat. The midrib was 750 µm thick in both the vertical and horizontal axes (Fig 2 a). The midrib's epidermal layer was made up of tiny, square-shaped cells with a thick cuticle. The axial portion of the ground tissue had tiny air chambers that were split by fine partition filaments. The ground tissue was parenchymatous. There are three or four layers of thick-walled, deeply pigmented, compact parenchyma cells in the adaxial portion. The midrib's circulatory system was a bit intricate. It was made up of thick horizontal segments of vascular strands that were situated on either side of the abaxial bowl-shaped vascular strands, as well as a broad, shallow abaxial arc of vascular bundles (Fig 2b). The six or seven distinct bundles that made up the abaxial arc of the vascular threads were spaced apart by a tiny gap. Each bundle was collateral, with an upper and lower xylem cluster separated by a thin layer of phloem. Xylem components with polygonal shapes, thin walls, and dense packing were discovered. The vascular components of the lateral bundles were compactly organised, slightly broader, and had thicker walls. Phloem develops in the top portion of the lateral bundles, where the tiny, darkly coloured cells are present. The midrib's lateral bundles were collateral as well, and a long row of cells made up of several upper xylem elements could be seen there. On the lower end of the xylem strands, phloem develops.

The lamina had a smooth surface and was consistently thick (Fig 2c). The lamina's epidermal cells had thick-walled, square, or elongated cells. There is little distinction in the palisade mesophyll. The thick-walled, slightly rectangular spongy mesophyll cells were separated into tiny air gaps by partition filaments (Fig 2c). Circular and wide calcium oxalate druses, quite noticeable, contained epidermal cells. The druses had a spiky surface and were spherical (Fig 2d). The lamina has sclereids with long, wide, thick walls. The lumen of the cell was broad and smooth. (Fig 2e). Surface view revealed robust, anticlinal walls on the lamina's epidermal cells. The cells were tightly packed and polygonal. The majority of the cells have thick masses of tannin. (Fig 2f).

A dense reticulate venation system was visible on the leaf. There were distinct venous islets of various sizes and forms. The lateral veins that lined the vein islets were substantial. Vein terminations that originate from lateral veins and end within the vein islets can be found there (Fig 2g). Simple and unbranched vein terminations were seen. Either straight or curved described them. In the leaf lamina, large calcium oxalate druses were rather common. (Fig 2h). It was discovered that the druses had round, small bodies. Their distribution is dispersed. Due of the crystals' birefringent nature, the druses look dazzling white when viewed in polarised light. (Fig 2i).

3.5.Histological study of AS leaves

The AS leaves were histologically examined to determine the presence of starch, mucilage, protein, lipids, and other substances. The findings are shown in Fig. 3, where starch grains were discovered in the mesophyll cells of the lamina (Fig. 3a). Druse-type crystals were frequently found in the ground parenchyma of the midrib of AS leaves. They appear bright white against a coloured background (Fig. 3b). Similarly, violet-colored mucilage was detected by applying

0.02% toluidine blue to the ground parenchyma cells of the midrib (Fig. 3c). Nile blue staining confirmed the presence of lipid in AS leaves. Phospholipids stained blue, and the lipids were found in a thick continuous layer on the epidermis (Fig. 3d). Tannins are an important secondary plant metabolite with antioxidant properties. When the leaves are stained with ferric chloride for 3-5 minutes, the presence of tannins in AS is visible as red dots. They are found in the parenchyma cells of the ground tissue. If unhydrous tannin derivatives, namely phlobapenes, are present, they stain red or brown (Fig. 2e). Proteins from AS leaves were immersed in MBB (Mercuric Bromophenol Blue) for 15 minutes, and when the sections were examined under a microscope, protein was found accumulating in the parenchyma cells and was blue in colour (Fig. 3f). Lignins are phenolic polymers found in the cell walls of xylem vessels, fibres, sclereids, and tracheids. In addition to UV and polarised light, the stain phloroglucinol can be used to detect lignins. Lignified cells turned into deep red colour (Fig. 3g).

3.6.Physico-chemical evaluation

The phytochemical analysis results are summarised in Table 1. It was discovered that the acid insoluble ash (5.0%) was lower than the total ash (9.0%). It was discovered that the water-soluble ash content was 10.5%. The current study's ash content results confirmed the absence of any physiological debris or sand. Ash analysis is an important parameter for determining the presence of macro- and microelements in a sample. Moisture content determination is an important quality control parameter for any crude drug. A higher moisture content in the plant sample promotes microbial growth, further contaminating the sample. AS leaves were found to have a moisture content of 2.5%. The nature of the metabolites present in crude extract determines its solubility. As a result, the alcohol and acid-soluble extractive values were

calculated, and no significant difference was found in either case. This implies that AS leaves have a higher concentration of polar compounds.

3.7.Fluorescence analysis

The fluorescence analysis of powdered AS leaves with various reagents was performed, and the results are shown in Table 2. Fluorescence analysis is a step-by-step process for standardising crude drugs for pharmacognostic studies. This study could help with the identification and authentication of raw drugs. This work can also be used as a reference information set for identifying a specific plant species. This research can also distinguish between substitutes and adulterants, improving the quality and efficacy of natural products.

3.8.GC-MS analysis of different extracts of AS leaves

GC-MS analysis was performed on different extracts obtained from the cold maceration process, such as ethyl acetate and methanol, for phytochemical analysis. Table 3 shows the results of the GC-MS analysis. GC-MS analysis revealed that AS leaf extracts contain a variety of polyphenols and fatty acids. Polyphenolic compounds discovered in ethyl acetate extracts included 1,2 butanediol, 1 phenyl, 1,2,4-benzenetriol, 1,3,5-benzenetriol, phenol, 3,5-bis(1,1-dimethylethyl), and megastigmatrienone. 4H-pyran-4-one, 2,3-dihydro3,5-dihydroxy-6-methyl, pentanoic acid, 5-hydroxy, 2,4-ditbutyl phenyl ester, benzoic acid, glyceraldehyde, and other compounds were found in AS methanol extracts. Aside from the various phenolic acids found in AS leaves, several fatty acids were discovered, including n-hexadecanoic acid and n-octadecanoic acid. According to the GC-MS analysis, AS leaf extracts are a natural source of polyphenols and fatty acids. Polyphenols are important secondary plant metabolites that benefit human health. Polyphenols are powerful antioxidants as well as anti-diabetic, anti-arthritic, anti-cancer, and

anti-inflammatory agents. Fatty acids, particularly unsaturated fatty acids, exhibit promising anti-radical activity while also aiding in the prevention of oxidative stress.

3.9.Total Phenolic content and total flavonoid content

The GC-MS analysis results encouraged us to confirm the total phenolic and total flavonoid content of different extracts further. Table 4 shows the total phenolic and total flavonoid content of the AS extracts. The aqueous extract of AS leaves contained the most phenolic compounds, followed by the methanol and ethyl acetate extracts. Because most phenolic acids are polar in nature, they dissolve easily in water. This current result also shows that the aqueous extract has the highest TPC. The methanolic extract, on the other hand, contained the most flavonoids, followed by the aqueous and ethyl acetate extracts. Methanol is a versatile solvent that can dissolve polar as well as nonpolar compounds. This is due to the strength of the many hydrogen bonds that form between the oxygen atoms of one alcohol molecule and the hydroxyl group of another alcohol atom. Flavonoids derived from plants are both polar and non-polar in nature. Non-polar flavonoids like isoflavones, flavones, and flavonols dissolve easily in methanol. The current study's findings also suggested that methanol could be a viable option for flavonoid extraction from AS leaves.

3.10. Invitro anti-oxidant study

The antioxidant activity of various AS leaf extracts was tested in vitro as described in the materials and methods section, and the results are shown in Fig. 4. The DPPH assay was first performed (Fig. 4a), and the aqueous extract demonstrated the highest antioxidant scavenging activity (60.91±0.41%) among its counterparts. Interestingly, the aqueous AS extract at 50 µg/ml concentration showed better antioxidant activity than commercial ascorbic acid. This finding confirmed the potent antioxidant activity of AS's aqueous leaf extract. However, no significant

difference was found between methanol and ethyl acetate extracts. In the ABTS assay, all of the extracts demonstrated concentration-dependent activity (Fig. 4b). The scavenging activity of AS aqueous extract (36.44±0.22%) was the highest, followed by methanolic (31.7±0.19%) and ethyl acetate extract (18.01±0.17%). After receiving an electron from the antioxidant molecule, DPPH and ABTS radicals are reduced and thus inhibited. The FRAP assay (Fig. 4c) yielded significantly lower results than the DPPH and ABTS assays; however, the antioxidant potential was found to follow the same order as the other two methods (aqueous extract > methanolic extract > ethyl acetate extract).

All values are given as radical scavenging percentages ± standard deviation.

Where, ASE: AS ethyl acetate extract; ASM: AS methanolic extract; ASW: AS water extract; and AA: ascorbic acid.

3.11. Invitro anti-arthritic activity

3.11.1. HRBC membrane stabilisation assay

The HRBC (Human Red Blood Cell) membrane stabilisation assay was followed by the invitro anti-arthritic activity, and the findings are shown in Table 5. When compared to other extracts like ethyl acetate and water, the methanol extract showed much better outcomes (anti-arthritic activity). Our prior research showed a similar trend, with methanol and water extract greatly decreasing paw edoema in albino arthritic rats (Silpavathi et al. 2021).

3.11.2. Albumin (egg) denaturation test

All of the extracts, as well as Diclofenac sodium, demonstrated concentration-dependent activity in the protein denaturation test. When compared to the standard drug, the water extract demonstrated significant anti-inflammatory activity (Diclofenac sodium). AS water extract has

shown significant anti-inflammatory activity, which can be used in herbal medicine. The findings are consistent with our previous report on in vivo studies (Silpavathi et al. 2021).

4. Conclusion

Diagnostic characteristics for AS leaf and powder have been established. Phytochemicals such as flavonoids, phenols, tannins, glycosides, saponins, and alkaloids have been found in substantial amounts in the leaf. This study reports for the first time on a variety of morpho-anatomical and physicochemical studies that could be utilised as a diagnostic tool in identifying and authenticating this plant, as well as in writing a monograph about it. Leaf extract has demonstrated significant anti-arthritic and antioxidant effects in in vitro experiments.

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Conflict of Interest

Authors declare no conflict of interest.

Fig 1.

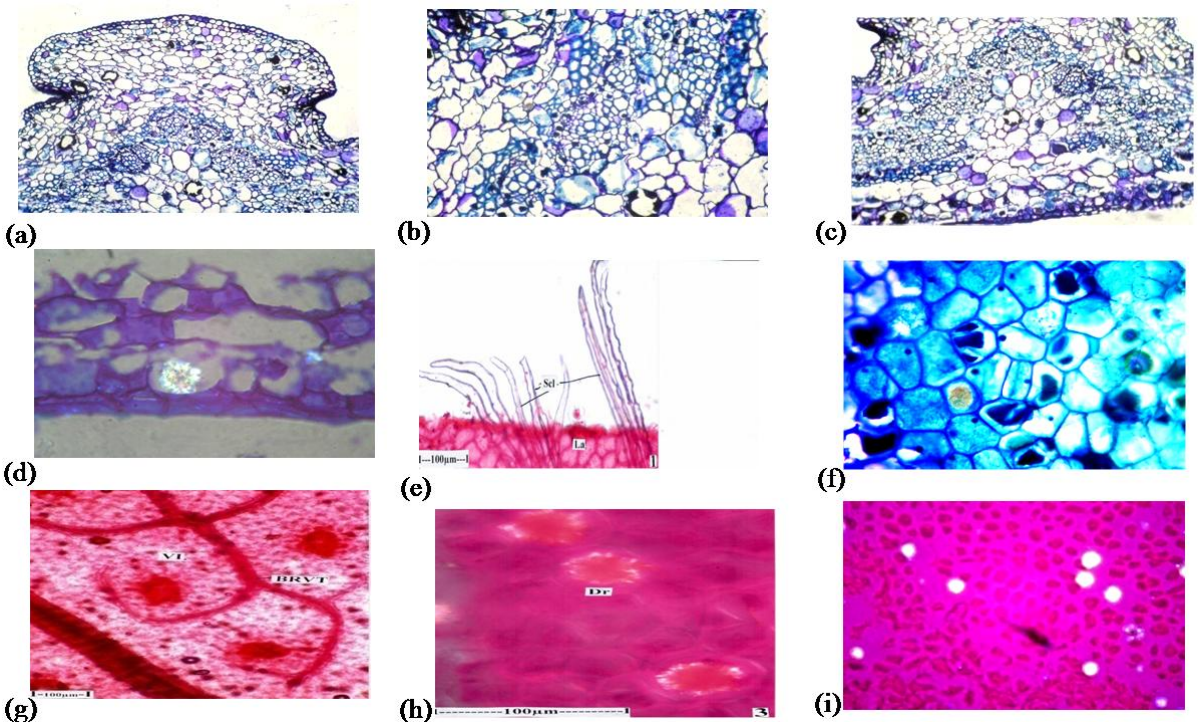

Figure 2.

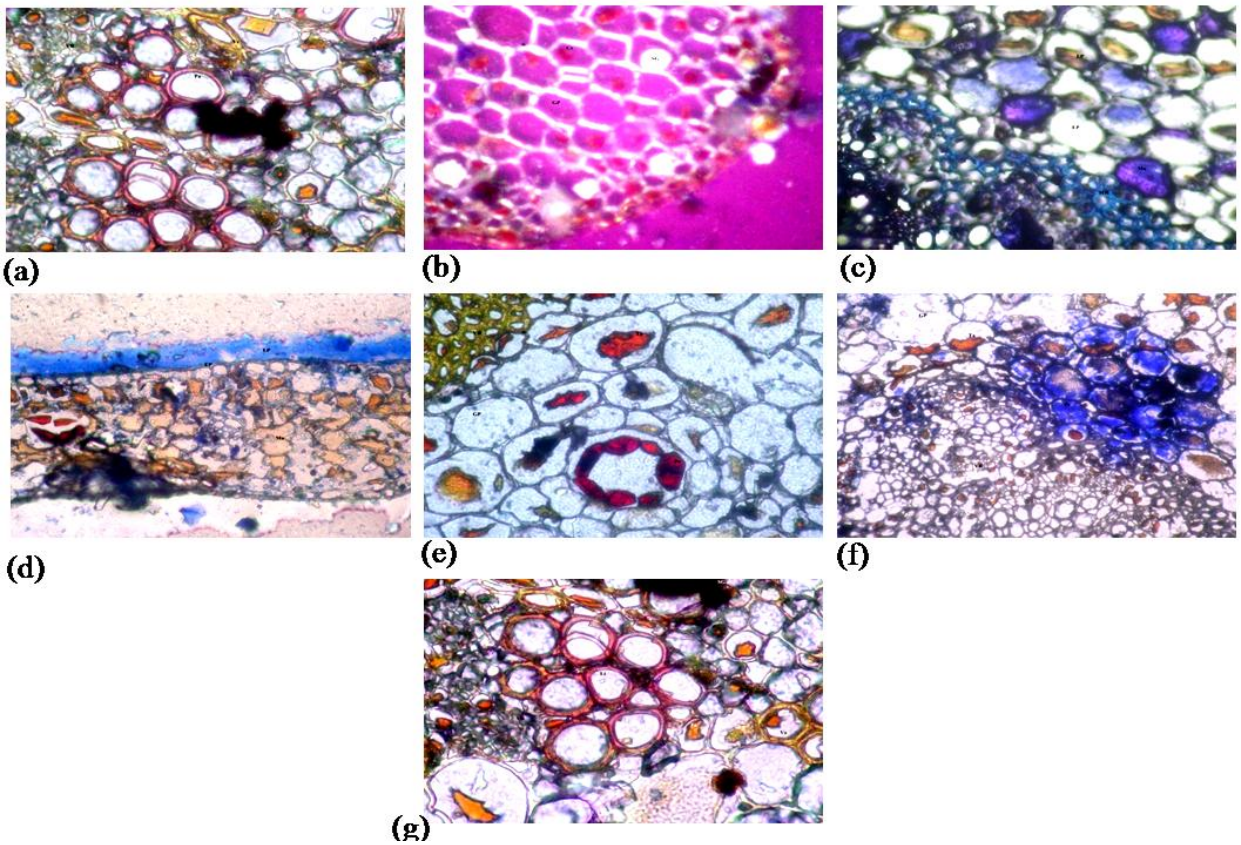

Figure 3.

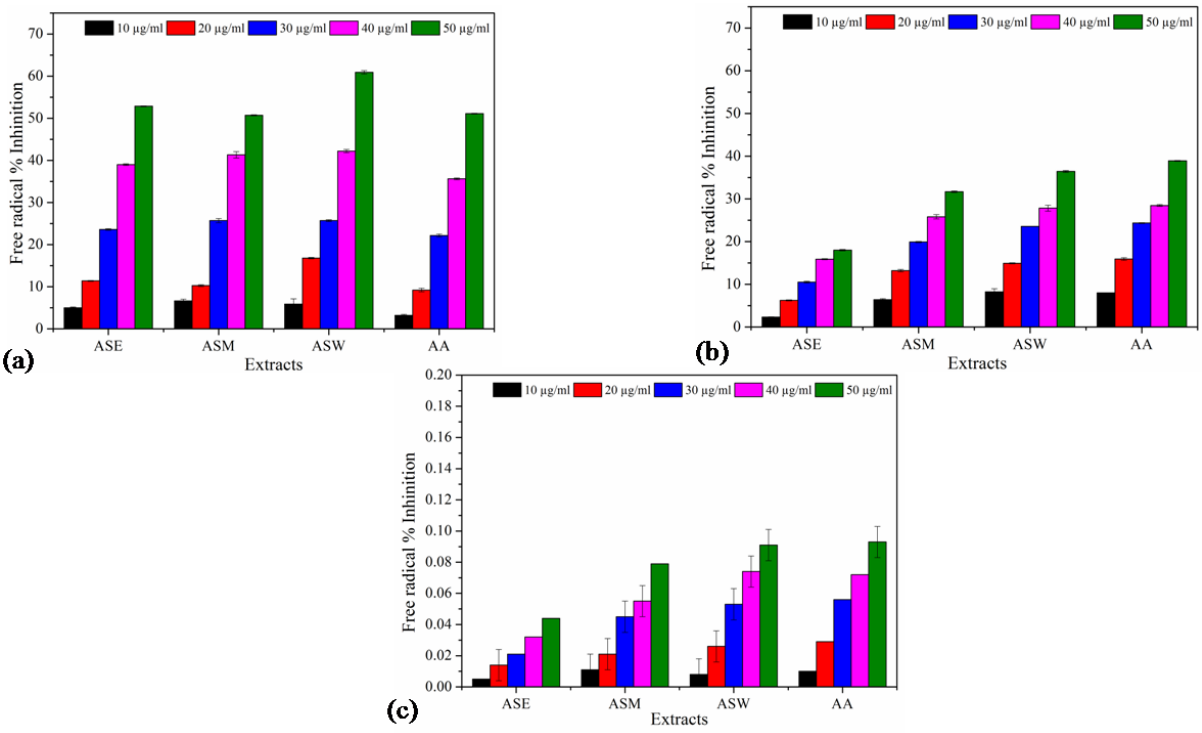

Figure 4.

Table 1. Physico-chemical evaluation of AS leaves

Parameters	% W/W
Total ash	9.0
Water soluble ash	10.5
Acid insoluble ash	5.0
Alcohol soluble extractive	4.0
Water soluble extractive	3.0
Moisture content	2.5
Foreign organic matter	<1%

Values are presented in dry weight percentage

Table 2. Fluorescence analysis of powder sample

Reagents	Day light	UV 254nm	UV 365nm
Powder as such	Green	Light Green	Dark green
Powder+ 1N HCl	Light green	Brownish yellow	Green
Powder+ 1N HNO_3	Light yellowish green	Light yellow	Light yellow
Powder+ 1N H_2SO_4	Green	Brownish green	Dark green
Powder+ 1N NaOH	Brownish green	Brown	Green
Powder+ Methanol	Dark green	Brownish green	Light green
Powder+ Ethyl acetate	Green	Light green	Light green
Powder+ dil NH_3	Yellowish green	Golden yellow	Brown
Powder+ Acetic acid	Green	Light green	Dark green

Table 3. GC-MS analysis of different extracts of *A. solanacea* leaves

Peak No	Compound Name	RT	% Area	Peak height	Molecular Formula	Molecular Weight	Nature of Compound
	ASE Extract						
1	Hydrazine, ethyl-	4.24	5.22	5376118.56	$C_2H_7NO_2$	60.09	
2	1,2Butanediol,1phenyl	14.08	1.13	714758.64	$C_{10}H_{14}O_2$	166.22	Phenolic compound
3	1,2-Ethanediol, monoacetate	16.69	6.00	3182700.37	$C_4H_8O_3$	104.105	
4	1,2-Ethanediol, monoacetate	16.99	4.76	3143982.91	$C_4H_8O_3$	104.105	
5	1-Nitro-2-propanone	20.20	1.89	1481072.00	$C_3H_5NO_3$	103.07	
6	1,2,4-Benzenetriol	24.12	1.03	720152	$C_6H_6O_3$	126.11	Phenolic

				.61			compound
7	1,3,5-Benzenetriol	25.95	1.94	1240134.07	$C_6H_6O_3$	126.1	Phenolic compound
8	Pyrazole-5-carboxylic acid, 3-methyl	26.12	1.44	1271230.58	C5H6N2O2	126.113	
9	Phenol, 3,5-bis(1,1-dimethylethyl)	26.29	1.93	1844841.34	C14H22O	206.324	Phenolic compound
10	Phenol, 2,4-bis(1,1-dimethylethyl)	27.01	2.31	3045082.59	C14H22O	206.3	Phenolic compound
11	Megastigmatrienone	29.76	1.21	1273846.82	C13H18O	190.281	Phenolic compound
12	2-Bromopropionic acid, pentadecyl ester	33.31	1.06	1250616.42	C18H35BrO2	363.4	Ester
13	1-Dodecanol, 3,7,11-trimethyl	33.45	1.08	1351209.68	C15H32O	228.414	
14	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	34.36	1.14	2280174.35	C20H40O	296.531	
15	E-11-(13-Methyl) Tetradecen-1-ol	35.25	1.96	1894952.04	C15H30O	226.3	
16	n-Hexadecanoic acid	36.36	9.39	7098060.21	C16H32O2	256.424	Fatty acid
17	n-Hexadecanoic acid	37.08	15.08	20060100.54	C16H32O2	256.424	Fatty acid
18	Oxirane, decyl	39.70	5.17	4382261.30	C12H24O	184.318	
19	cis11Hexadecenal	40.32	9.21	9349700.55	C16H30O	238.409	

20	Octadecanoic acid	40.74	1.62	2661729.69	C18H36O2	284.477	
21	Benzene, [(cyclohexyloxy)methyl]	43.06	2.40	1137287.89	C13H18O	190.281	
22	Palmitoyl chloride	46.63	1.16	851294.42	C16H31ClO	274.87	
23	Cyclododecanone,2-methyl	53.38	1.56	1363907.45	C13H24O	196.329	
	ASM Extract						
1	Acetic acid	3.99	33.72	10372770.38	C2H4O2	60.052	
2	Glyceraldehyde	5.12	1.33	180229.78	C3H6O3	90.077	Aldehyde
3	2-Pentanone, 4-hydroxy4-methyl-	7.40	1.58	612718.35	C6H12O2	116.158	Ketone
4	4H-Pyran-4-one, 2,3-dihydro3,5-dihydroxy6-methyl	17.41	5.84	1060518.60	C6H8O4	144.125	Phenolic Compound
5	Cyclohexasiloxane, dodecamethyl	21.72	1.03	289843.81	C12H36O6Si6	444.924	
6	Trisiloxane,1,1,1,5,5,5-hexamethyl3,3-bis[(trimethylsilyl)oxy]	26.12	1.18	582540.11	C12H36O4Si5	384.839	
7	Pentanoic acid, 5-hydroxy, 2,4-ditbutyl phenyl esters	26.99	2.19	988375.39	C19H30O3	306.4	
8	1,1,1,5,7,7,7-	33.45	1.21	451477	C13H40O	444.967	

	Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane			.84	5Si6		
9	Decanoic acid, 2-methyl	36.12	2.58	1336299.96	C11H22O2	186.29	
10	Benzoic acid, 2(Dimethylamino)ethyl ester	36.67	2.32	1354584.91	C11H15NO2	193.242	
11	n-Hexadecanoicacid	36.97	8.91	3523176.56	C16H32O2	256.424	Fatty acid
12	6-Octadecenoic acid, methyl ester, (Z)	39.46	1.90	923746.94	C19H36O2	296.488	
13	13Tetradecenal	40.27	5.21	1476115.92	C14H26O	210.356	
14	Octadecanoic acid	40.71	2.71	978789.04	C18H36O2	284.477	
15	9,9-Dimethoxybicyclo[3.3.1]nona2,4-dione	42.65	2.75	433388.13	C11H16O4	212.24	
16	Heptasiloxane, hexadecamethyl	43.54	3.56	1075632.28	C16H48O6Si7	533.147	
17	9,12,15-Octadecatrienoicacid,2-[(Trimethylsilyl)oxy]	46.64	1.40	275354.27	C27H52O4Si2	496.9	
18	1Monolinoleoylglyceroltrimethylsilyl	49.69	1.03	299787.06	C27H54O4Si2	498.9	

	ether					
19	Heptasiloxane, hexadecamethyl	49.94	3.14	696121.90	C16H48O6Si7	533.147
20	9Octadecenamide, (Z)	53.37	3.30	690016.00	C18H35NO	281.477
21	Benzoic acid, 3-methyl-2-trimethylsilyloxy,Trimethylsilyl ester	58.37	2.06	371736.52	C14H24O3Si2	296.51

Table 4. Total phenolic content and total flavonoid content

Extracts	Total Phenolic content	Total Flavonoid content
ASE	22.77 ± 1.07	145.2 ± 3.23
ASM	43.57 ± 0.32	320.8 ± 2.22
ASW	103.56 ± 2.21	278 ± 1.15

The values of Total Phenolic content and Total Flavonoid content are presented as mean ± SD of mg/100g dry crude extract.

Table 5. HRBC membrane stabilization method

	1600µg/ml	**800µg/ml**	**400µg/ml**	**200µg/ml**	**100µg/ml**	**50µg/ml**
ASE	55.94±0.35***	51.62±0.21**	44.80±0.35**	37.97±0.23**	28.59±0.23**	21.65±0.66**
ASM	82.48±0.66***	71.37±0.87**	63.69±2.06**	54.21±1.41**	43.37±1.37**	35.72±1.72**
ASW	81.96±0.97***	75.11±0.53***	58.81±0.96**	39.50±0.57**	25.51±1.09**	14.73±0.54***
DICL	88.55±0.07**	82.58±0.31**	63.81±1.77**	48.33±0.83**	34.13±2.27**	22.66±1.06**

O	****	****	****	****	****	****

Values are expressed as mean ± SD, ***P< 0.001, **P< 0.01, *P< 0.05 considered as significant.

Table 6 Invitro albumin denaturation test

	1000µg/ml	**800µg/ml**	**400µg/ml**	**200µg/ml**	**100µg/ml**	**50µg/ml**	**25µg/ml**
ASE	117.08±3.24***	92.83±0.83***	79.75±0.93***	58.67±1.05***	36.15±1.00***	13±1.04**	8.2±0.61***
ASM	156.88±17.75**	148.03±15.63**	114.23±13.07**	105.55±18.22**	93.57±20.56**	75.11±12.20**	65.15±8.55**
ASW	193.39±5.27***	175.73±6.11**	90.01±7.26**	77.31±3.01***	64.40±0.99***	56.41±0.70***	49.23±0.57***
Diclo	186.18±1.98***	149.39±3.16**	82.81±3.53***	72.80±1.74*	64.32±0.55**	57.33±1.00***	49.18±1.15***

Values are expressed as mean ± SD, ***P< 0.001, **P< 0.01, *P< 0.05 considered as significant.

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