Phytochemical Investigation and Evaluation of Antioxidant and Antimicrobial Potential of *Ardisia solanacea* Leaf Extract

❖ **Kanwal Jeet[1] Versha parcha[1], Sas Bswas[2], and Sukanya Chettri[1]**

1Department of Pharmaceutical Chemistry& Chemistry DIBNS Dehradun 2 Department of forestry DIBNS Dehradun Uttrakhand, India

ABSTRACT

Introduction: *Ardisia solanacea* (Poir.) Roxb. commonly called *shoebutton,* being a useful medicinal plant constitutes various phytochemicals which are supposed to give anthelmintic, thrombolytic, antimicrobial and cytotoxic effects, rheumatic arthritis, gout, dysmenorrhea, mental fatigue, diarrhea and vertigo along with some common carminative, antacid and stimulant properties. Plant has become an invasive in sal (*Shorea robusta* Gaertn.) forest of Manduwala in Dehradun Forest Division. Keeping in view of medicinal properties associated with the plant, the present study was carried out with following objectives:

Objective: To carry out systematic analysis of phytochemicals, antioxidant activity and antimicrobial activity of various extracts of aerial parts of the plant.

Method: *Ardisia solanacea* plant was collected from the forests near Suddhowala, Dehradun district of Uttarakhand. The plant was located and subsequently identified by Dr. Sas. Biswas, HoD, Department of Forestry, Dolphin Institute Biomedical and Natural Sciences, Dehradun. After drying aerial part was extracted with solvents with increasing polarity like petroleum ether, chloroform, ethyl acetate and methanol using Soxhlet apparatus, and phytochemical investigation of all the extracts were carried out.

For antioxidant activity of these extracts, two different methods of were carried out.Total phenolic content responsible for antioxidant activity was determination by Folin-Ciocalteu method and DPPH free radical scavenging activity were performed by the standard methods. The standard was taken ascorbic acid for DPPH free radical scavenging activity.

The antimicrobial activity of the different extracts of *A. solanacea* was determined by the standard disc diffusion method . The bacterial and fungi cultures were kept at their optimum temperature and the turbidity was adjusted to 0.5MCfarland[12].

Results: The four extracts viz. petroleum ether extract, chloroform extract, ethyl acetate extract and methanolic extract were subjected to phytochemical evaluation in which petroleum ether and chloroform gave negative tests for majorly all the phytochemicals whereas ethyl acetate extract gave moderately positive test and methanol extract gave highly positive test for presence of phenolics, carbohydrates and proteins. The four extracts were subjected to the total phenolic content determination by the Folin-Ciocalteu method. At 250ppm concentration value for the PEAS Gallic acid equivalent (GAE) (µg/mg) value is found to be 69.422(µg/mg). For Ethyl acetate extract GAE value is 62.654 (µg/mg) and for methanol extract GAE value is 57.048(µg/mg).The different extracts of the plant leaves were subjected to the DPPH free radical scavenging activity with the reference standard as ascorbic acid. The highest value is found to be in methanol extract at 53.02 (µg/ml) as compared to that of ascorbic acid. The extracts were also evaluated for antimicrobial potential and highest zone of inhibition was observed in methanol extract 19 mm against *Escherichia coli* and very less effective against fungal strain.

Conclusion for integrated studies: From the study it could be concluded that methanol extract is rich in phenolics with good antioxidant and antimicrobial potential. The plant being an invasive growing gregariously and having good biomass has potential for commercial exploitation.

Key Words: *Ardisia solanacea,* Antioxidant activity,DPPH, Antimicrobial, Gallic acid

INTRODUCTION

In all over the world, men have been using the medicinal plants and animal extracts since ages, to treat and prevent various ailments [1,2]. According to USDA forest services around 40 -45% 0f modern European medicinal drugs have a basic moiety derived from different phytochemical constituents of medicinal plants. According to WHO reports, about 75-80% of population around the globe is still using raw medicinal plants for their therapeutic purposes in one way or other[1]. Several plants like Taxus bravifolia and Taxus baccata for anticancer properties and cinchona bark for healing malaria are already a primitive source of their respective drugs.

Ardisia solanacea is a plant of Ardisia genus and is very common in Himalayan region, India, China, Nepal and Bangladesh. It's 4-6m in height evergreen shrub of broad shoe like leaf found from 100-1100m above sea level. *A. solanacea (shoebutton)* being a useful medicinal plant constitutes various phytochemicals which are supposed to give anthelmintic, thrombolytic, antimicrobial and cytotoxic effects[2-5]. It is still being used by indigenous people to treat diseases like rheumatic arthritis , gout , dysmenorrhea , mental fatigue , diarrhea and vertigo along with some common carminative , antacid and stimulant properties[6] .Due to its Carminative properties it prevents the formation of gas in gastrointestinal track thereby combat flatulence[7]. *A. solanacea* has also antimicrobial properties therefore it prevents the microbial growth along with antioxidants which are good for skin and prevents

the formation of cancer cells[8,4]. The objective of this study is to evaluate the different phytochemicals and to study antoxidant and antimicrobial potential of of *A. Solanacea* extracts growing in terai region of Dehradun Uttarakhand

MATERIAL &METHODS

A Collection and identification of plant material

The *A. Solanacea* plant was collected from the forests near suddhowala, Dehradun district of Uttarakhand. The plant was identified by Dr. Das biswas, HOD department of forestry, Dolphin institute biomedical and natural sciences, Dehradun. The aerial part of the plant was kept in shade to dry for 10 days. The water content determined .The further experimental work was carried out on aerial part of the plant.

B Preparation of Extracts

Shade dried crushed leaves (200gms) of A. *solanacea* were extracted with solvents increasing polarity like petroleum ether,chloroform, ethyl acetate &methanol. The extracts so obtained was evaporated by simple distillation and obtained semi-solid concentrated extract. The extracts were completely dried and stored in tight jars and kept in desiccators.

C Tests for the Evaluation of Phytochemicals

All extracts so obtained were subjected to phytochemical investigations

1. **Tests for Alkaloid**
   - **Hager's test** – we had taken saturated solution of picric acid in a test tube, a small portion of extract containing was added and the Formation of yellow precipitates indicated the presence of alkaloid.
   - **Wagner's test**- We had taken 1.3g of iodine and 3g potassium iodide in 100ml of water then small portion of solution was taken in a test tube along with methanolic extract, appearance of reddish brown color indicated alkaloid.

2 **Test for Carbohydrate**
   - **Benedict's test**- 100g of sodium carbonate and 173g of sodium citrate (C6H7O7Na) along with 17.3g of coppersulphate pentahydrate $CuSO_4.5H_2O$ in 1000ml of water . Small portion of solution was tested with sample; upon heating we get brick red color indicated presence of reducing carbohydrate.
   - **Fehling's test**- Fehling solution 'A' ($CuSO_4$) was tested with sample of methanolic extract first, then accordingly with Fehling solution 'B' ($NaKC_4H_4O_6.4H_20$ in NaOH, brick red color upon heating indicated presence of carbohydrate.

3 **Test for Glycosides**
   - **Legal's test**- Extract was dissolved in pyridine then little amount of sodium nitroprusside was added to make it alkaline, appearance of pink to red color indicated presence of glycosides
   - **Baljet test**- Methanolic extract was subjected with sodium picrate in a round bottom flask, the appearance of yellow to orange color change indicates the presence of glycosides

4 **Test for Saponin**
   - **Saponin foam test** - Diluted about 500mg of extract in 25ml of distilled water, and mixture was shaken in attest tube for about 10 minutes. The stable formation of foam indicates the presence of Saponin.

5 **Test for Phytosterols**
   - **Liberman-Burchard's test-** We had dissolved our extract in dry chloroform in a dry test tube. Then several drops of acetic anhydride were added followed by 2 drops of concentrated H_2SO_4 and was mixed after that Spectro-photo metrically its concentration is determined

6 **Test for Protein and Amino acid**
   - **Xanthoproteic test**- 1ml of concentrated HNO_3 was mixed in 1ml of extract, heated the mixture and cooled. Then aqueous 40%w/v NaOH solution was drop wise added to make it alkaline. The colour change from yellow to orange indicated the presence of aromatic amino acid.

7 **Test for phenol**

- **Ferric chloride test-** The extract was dissolved in ethanol and a few drops of ferric chloride solution were added and bluish black colour indicated the presence of phenol.

8 **Test for Tannins**

- **Gelatin test-** For this we had taken 1% Gelatin solution in a test tube then it was made alkaline using 10% NaOH solution then 1% ethanolic extract was added and the precipitation of Gelatin confirmed the presence of tannins.

9 **Test for Flavonoid**

- **Alkaline reagent test-** The ethanolic extract was taken in a test tube, a few drops of NaOH were added , an intense yellow colour was appeared which turned colourless upon addition of a few drops of dilute acetic acid.

10 **Test for Diterpenes**

- **Copper acetate test-** The aqueous solution of extract was treated with 3-4 drops of copper acetate solution , the emerald green colour showed the presence of Diterpenes.

11 **Test for Fats and Oil**

- **Spot test-** In this test, we prepared a spot on the filter paper with the test solution upon drying that spot we got affixed oil mark on there which indicated the presence of oil.

D Total Phenolic Content

1ml of 250ppm solution of *A. Solanacea extract* was taken in a test tube along with 2ml of 10% Folin-Ciocalteu reagent aqueous solution and mixture was kept 5minutes for a successive addition of 2.5ml of 7% Na_2CO_3 aqueous solution, then the mixture was held in water bath for 30minutes at 40^0c. The absorbance of the sample was taken at 760nm wavelength from UV spectrophotometer.

Upon successive dilution of 500ppm of the Gallic acid, the readings of UV visible spectrometer were taken, and the graph for concentration of Gallic acid and absorbance was studied to get linear regression equation.

E DPPH Radical Scavenging Activity (Antioxidant Activity):

All extracts of *A. solanacea* were screened for antioxidant potential using DPPH method. 2ml of 0.2Mm methanolic DPPH solution was added to the 2ml of all extract of different concentrations then the mixture was stirred and allowed to stand for 30 minutes in a dark place[1,2] and absorbance was recorded using UV Visible spectrometer at 517nm wavelength[2] using ascorbic acid as standard. The method is adopted from Gupta et al[8-12].

F Antibacterial Activity

Antibacterial bioassay[8-12]were evaluated against gram positive bacterial strains**.** *Bacillus cereus* ,*Bacillus subtilis* & gram negative bacterial strain *Escherichia coli* &. *Staphylococcus epidermidis* by disc diffusion method. Standard inoculums (1ml/100 ml of medium) with suspension (10^5cfu/ml) were introduced onto the surface of sterile agar plates, and a sterile bent glass spreader was used for even distribution of the inoculum. The discs measuring 6 mm in diameter and 2 mm thickness were prepared from Whatman (grade no. 1) filter paper and sterilized by dry heat for 1 h. three disc of test samples were placed on three portion together with one disc with reference drug Ampicillin and disc impregnated with solvent (DMSO) as negative control. The sterile discs previously soaked in a known concentration of extracts samples (petroleum ether, choloroform and ethanol extracts in 500µg/ml, in dimethyl formamide were placed in nutrient agar medium Ciprofloxacin (20µg/disc) was used as positive control for bacteria. Plates were inverted and incubated for 24 h at 37 ± 2^0C. Diameters of zone of inhibition (mm) were determined and average diameter of test samples were calculated in triplicate sets. Zone of inhibition of test samples were compared with that produced by standard.

G Antifungal Activity

Fungal infections are most common among the human population and several therapeutic agents are also available in the market but most of them are effective as topical applications. Rare drugs are available for deep mucosal infections. All extracts were screened against fungal strain *Aspergillus niger*, Medium employed for antifungal activity was potato dextrose agar (PDA) for isolation of fungal culture and sabraud's agar medium for antifungal assay by plate diffusion method.

RESULTS AND DISCUSSION:

The four extracts viz. petroleum ether extract, chloroform extract, ethyl acetate extract and methanolic extract were subjected to phytochemical evaluation, Antioxidant and antimicrobial activity. Results are summarized in tables given below:

Table1 : Presence of Phyto constituents

Sr. no.	Phytochemical	test	PEAS	CAS	EAAS	MAS
1	Carbohydrate	Benedict's test	--	--	+	++
		Fehling test	--	--	+	++
2	Protein and Amino acid	Xanthoproteic test	--	--	+	++
3	Alkaloid	Hager's test	--	--	+	++
		Wagner test	--	--	+	++
4	Saponin	Froth test	--	--	+	++
5	Tannins	Gelatin test	--	--	+	++
6	Fats and oils	Stains test	--	--	+	++
7	Phenols	Ferric chloride test	--	--	+	++
8	Phytosterols	Libermann-Burchard's test	--	--	-	-
9	Diterpenes	Copper acetate test	--	--	--	_
10	Flavonoids	Alkaline reagent test	--	--	+	++
11	Glycosides	Legal's test	-	-	+	+
		Baljet test	-	-	+	+

Table 2: Total Phenolic Content of *A. solanacea* crude extracts and its fractions

A. Solanacea concentration (250ppm) (µg/mg)	Absorbance at 760nm	Gallic Acid Equivalent
Petroleum ether fraction PEAS	0.352	69.422
Chloroform fraction CAS	0.101	10.803
Ethyl acetate fraction EAAS	0.323	62.654
Methanolic fraction MAS	0.299	57.048

Key: Present= (+) and Negative= (-)

Table 3: Comparative Study of DPPH Radical Scavenging Activity of Different Extracts of *A. solanacea*

Concentration (Mg/ml)	Petroleum ether PEAS	Chloroform CAS	Ethylacetate EAAS	Methanol MAS	Ascorbic Acid
500	85.12	67.02	78.56	79.03	97.62
250	81.03	62.80	76.23	76.36	84.12
125	75.95	51.46	71.12	71.02	72.65
62.5	67.49	38.95	62.56	63.05	65.97
31.25	54.98	20.58	46.89	47.16	59.52
15.625	26.20	12.96	1995	20.23	56.52
7.813	14.12	11.13	12.65	12.90	54.62
3.9	10.60	7.26	6.35	6.89	49.89
1.953	8.23	6.25	3.06	3.25	42.02
IC50(µg/ml)	40.79	158.25	50.14	53.02	15.07

Table 4: Antimicobial Activity of *A. solanacea* Extracts

Test Sample	Antibacterial Activity Zone of inhibition (mm)		Antibacterial Activity Zone of inhibition (mm)		Antifungal activity Zone of inhibition (mm)
	Gram Positive		Gram Negative		
S.No	BC	BS	EC	SE	AN
PEAS	9.3±0.2	9.2±0.1	10.02±0.1	11.2±0.1	9.2±0.21
CAS	nil	2.3±0.2	nil	1.5±0.2	nil
EAAS	7.5±0.1	11.9±0.1	8.3±0.2	11±0.21	9.3±0.1
MAS	8.2±0.21	12.5±0.1	19±0.21	10.1±0.1	10.5±0.1
DMSO	-	-	-	-	-
Ciprofloxacin	37.5±0.1	40.6±0.1	33.6±0.2	30±0.1	
Ketoconazole					46.2±0.1

DMSO= Dimethyl sulpho-oxide ***PEAS=Petroleum ether Extract of A. solanacea***

CAS=Chloroform extract of A. solanacea

EAAS=Ethyl acetate Extract of A. solanacea

MAS=Methanol Extract of A. solanacea

BC = Bacillus cereus

BS= Bacillus subtilis

EC= Escherichia coli ***SE=Staphylococcusepiderds***

AN=Asperguillus niger

The different extracts of A. *solanacea* leaves were chemically analyzed for the presence of various chemical constituents. petroleum ether and chloroform gave negative tests for majorly all the phytochemicals whereas ethyl acetate extract gave moderately positive test for prscence of carbohydrates,glycosides,phenols & flavanoids while methanol extract gave highly positive test for presence of phenolics, carbohydrates and proteins.The four extract were subjected to the total phenolic content determination by the Folin-Ciocalteu method and three of them are found to have a good value of total phenolic content. At 250ppm concentration value for the PEAS Gallic acid equivalent (GAE) (µg/mg) value is found to be 69.422(µg/mg),ethyl acetate extract GAE value is 62.654 (µg/mg) and for methanol extract GAE value is 57.048(µg/mg)**.**The different extracts of the plant leaves were subjected to the DPPH free radical scavenging activity with the reference standard as ascorbic acid. The highest value is found to be in methanolic extract fraction at, 53.02 (µg/ml) as compared to ascorbic acid . The extracts were also evaluated for antimicrobial potential against *Bacillus cereus ,Bacillus subtilis , Escherichia coli & Staphylococcus epidermidis* and highest zone of inhibition was observed in methanolic extract 19 mm against E.coli.Maximum zone of inhibition (10.5 mm) was observed against *Aspergillus niger* as compared to standard drug ketoconazole 20µg/ml .

CONCLUSION

From the study it could be concluded that methanol extract is rich in phenolics with good antioxidant and antimicrobial potential. The plant being an invasive growing gregariously and having good biomass has potential for commercial exploitation.

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