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PHYTOCHEMICAL, ANTI-OXIDANT AND ANTHELMINTIC ACTIVITIES OF AERIAL PARTS OF *APOROSA LINDLEYANA LINN*

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ABSTRACT

The present study was carried out to investigate the phytochemical constituents, *in vitro* antioxidant potential and anthelmintic activities of *Aporosa lindleyana Linn*. The dried powdered leaves of *Aporosalindleyana Linn* were extracted using petroleum ether, chloroform, ethyl acetate and methanol using a soxhlet extractor and preliminary phytochemical screening was performed using standard protocols. All the extract was evaluated for their potential antioxidant activities using test such as DPPH, hydroxyl radical and superoxide anion radical scavenging abilities. Anthelmintic activity of extract in adult Indian earthworm model were screened. Preliminary screening revealed the presence of bioactive compounds especially phenolics, tannins and flavonoids in all extracts. The paralytic (12.34±0.612) and death time (29.20±0.912) of methanolic extract was found to be significant ($P<0.05$) when compared with paralytic (2.52±0.682) and death time (5.40±0.514) of standard albendazole at 100mg/ml concentration. The results of the present study indicate that the aerial parts of *Aporosa lindleyana Linn.* exhibited strong anti-oxidant activity and possess significant anthelmintic activity and thus it is a good source of antioxidant and anthelmintic constituents.

KEYWORDS

Antioxidant, Anthelmintic, *Aporosalindleyana Linn* and Albendazole.

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INTRODUCTON

Drugs obtained from natural sources are becoming popular in recent years as an alternative therapy. As plants contains a lot of antioxidants have the ability to control the oxidative stress caused by sunbeams and oxygen, they can act as a source of new compounds with antioxidant activity. Plants contain a wide variety of free radical scavenging molecules, such as phenolic compounds[1], nitrogen compounds, vitamins, terpenoids (including carotenoids) and some other endogenous metabolites, which are rich

in antioxidant activity[2]. Natural antioxidant are potentialinhibitor of reactive oxygen species (ROS) generation, direct or indirect scavenging of free radicals and alteration of intracellular redox potential[3].

The use of natural antioxidants has gained much attention from consumers because they are considered safer than synthetic antioxidants. Recently there has been a worldwide trend towards the use and ingestion of natural antioxidants present in different parts of plants due to their phytochemical constituents[4,5]. The intake of natural antioxidants has been associated with the reduced risks of cancer, cardiovascular disease, diabetes and other diseases associated with ageing[6,7].

Helminthic infestations are nowadays being act as a cause of chronic ill health and sluggishness in the children. More than half of the world population suffers from worm infestations of one or other. Various alternative and traditional systems of treatments report the efficacy of several natural products eliminating helminthes[8]. As per WHO only synthetic drugs are frequently used in the treatment of human beings but these synthetic drugs are out of reach of millions of people and have a lot of side effects[9].

The systematic evaluation of plants used in the traditional healing system may afford more assuring data about their therapeutic value and may be helpful to face the demand of novel drugs to resist the infections and diseases[10]. With this view, the plant *Aporosa lindleyana Linn* was selected for our research.

Aporosa lindleyana Linn belonging to the family Euphorbiaceae, is a well-known tree in the Indian subcontinent for its range of uses. It is an endemic species usually found in selected sacred groves of Kasaragod district in Kerala. Various parts are widely used in folk medicine as anti-inflammatory, anthelmintics, antidiabetic, anti-dysenteric and woundhealing[11]. However, no scientific report is available in the literature regarding anti-oxidant and anthelmintic activities of the *Aporosa lindleyana Linn*. Thus, in the light of knowledge that *Aporosalindleyana Linn* is having wide folklore uses, we intend to evaluate the antioxidant and anthelmintic activities of the various extracts of *Aporosa lindleyana Linn* using *in vitro* models.

MATERIALS AND METHODS

Plant Material

Plant material of *Aporosalindleyana* leaves were collected from surroundings fields of Pathanamthitta, Kerala with the help of local farmer of the area during the month of February and authenticated by Raju Thomas, Head of the department of Botany, Baselious College, Kottayam.The plant was identified, confirmed and authenticated by comparing with an authentic specimen (Ref NO: -UCP/MGU/RIMSR/herb2).

Chemicals and instruments

DPPH were purchased from Sigma – Aldrich, USA. Remaining chemicals were analytical grade purchased from Himedia Labs., Pvt. Ltd, Mumbai, India. Measure the absorbance by Shimadzu U-V spectrophotometer.

Extraction

The aerial parts of *Aporosalindleyana Linn* were collected, shade dried, powdered mechanically and sieved through No. 20 mesh sieve. The powdered material of the whole plant was extracted by soxhlation with the solvents in the rising order of polarity, such as petroleum ether (PEA, 60º-80ºC) and then consecutively with chloroform (CEA), ethyl acetate (EAEA) and methanol (MEA). The percentage yield of the various extracts are listed in Table No.1.

Phytochemical screening of the extracts

Chemical tests were carried out for the all the extract of *Aporosal indleyana Linn* for the presence of phytochemical constituents like phenols, tannins, saponins, flavonoids, terpenoids, alkaloids, glycosides and steroids[12].

Antioxidant activity

DPPH radical scavenging activity[13]

DPPH (1-diphenyl-2-picrylhydrazyl) assay is performed to determine the free radical scavenging ability[13]. 1ml (0.1mM) of DPPH solution prepared in methanol was added to 3ml of test or standard (Gallic acid) solution at different concentration (0.25-64µg/ml). The mixture was incubated in dark at 30ºC for 30 min and the absorbance measured at

517nm. Percentage inhibition calculated on the basis of absorbance. A control reaction was carried out.

Hydroxyl radical scavenging activity[14]

Hydroxyl radical scavenging activity of the extract is determined by its ability to scavenge hydroxyl radicals produced by the EDTA-Fe^{3+}-H_2O_2-ascorbic acidsystem by Fenton reaction[14]. The reaction mixture amounts to a final volume of 1.0 ml which contains 100 µl of 2-deoxy2-ribose (28mM) in phosphate buffer solution (20mM, pH 7.4), 500µl of the extracts at various concentrations (10-160µg/ml) in buffer solution, 200µl of 1.04mM EDTA and 200µM $FeCl_3$ (1:1v/v), 100µl of H_2O_2 (1.0mM) and 100µl of ascorbic acid (1.0mM). Test samples were incubated at 37ºC for 1 h. The free radical damage inflicted on the substrate; deoxyribose was assessed with the thiobarbituric acid test. The positive control used for this assay was quercetin (10-160µg/ml). The percentage inhibition of the extracts and standard was calculated.

Superoxide radical scavenging activity[15]

The superoxide radicals are generated in a phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system by oxidation of NADH and assayed by the reduction of nitroblue tetrazolium (NBT)[15]. In this experiment, the superoxide radicals were generated in 3 ml of Tris–HCl buffer (16mM, pH 8.0) containing 78mM NADH, 50mM NBT, 10mM PMS and extracts to be tested at different concentrations (10-160µg/ml). The color reaction between superoxide radicals and NBT was detected at 560nm and the percentage inhibition calculated. Ascorbic acid (10-160 µg/ml) was used as positive control.

Calculation of 50% inhibitory concentration (IC_{50})

The concentration (µg/ml) of the extract required to scavenge 50% of the radicals was calculated by using the percentage scavenging activities at five different concentrations of the extracts. Percentage inhibition (I%) was calculated using the formula:

$$I\% = \frac{Ac - At}{Ac} x100$$

Where A_c is the absorbance of the control and A_t is the absorbance of the test sample.

Anthelmintic activity

Adult Indian earthworms, *Pheretimapostuma* resembled the intestinal roundworm parasites of human beings both anatomically and physiologically[16,17] and hence were used to study the anthelmintic activity. Indian adult earthworm 6-8cm length and 0.1-0.2cm in width were used for the in vitro anthelmintic bioassay of petroleum ether, chloroform, ethyl acetate and methanol extracts. The worms were divided into the respective group containing six earthworms in each group. All the prototypes were dissolved in minimum quantity of 2% v/v Tween 80 and then the volume was adjusted to 10ml with normal saline for making the concentration of 25, 50 and 100µg/ml. All the prototypes and standard drug solution were freshly prepared before commencement of the experiments. All the earthworms were washed in normal saline solution before they were released into 10ml of respective formulation as follows, - vehicle (2% v/v Tween 80 in normal saline), standard albendazole (25, 50 and 100mg/ml) and prototypes (25, 50 and 100mg/ml) the anthelmintic activity was determined.

Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body color. They were observed for their spontaneous motility. Observations were made for time taken to paralysis and death of individual worms.

Statistical analysis

All the experiments were carried out in triplicate and results expressed as mean ± SEM. Significant differences among means of samples were evaluated by one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Phytochemical screening of the extract

Phytochemical analysis showed the presence of tannins, phenolics, flavonoids, terpenoids and steroids in the extract (Table No.2).

DPPH radical scavenging activity

DPPH radical scavenging of various extracts of the leaves of *Aporosa lindleyana Linn* was investigated

and results were shown (Table No.3). The methanolic Extract showed the highest activity compare with other extracts. However, the scavenging activity of standard was greater than all the extracts of *Aporosalindleyana*. The highest activity was shown by MEA (IC_{50} = 2.52.±0.112) and the order of decreasing scavenging ability is MEA>EAEA (15.02±0.098) > CEA (28.33±064). All extracts showed significant (P<0.05) scavenging ability when compare with standard gallic acid (IC_{50} = 1.84±0.098).

Hydroxyl radical scavenging activity

The extracts and the standard (quercetin) inhibited the formation of hydroxyl radical in a dose dependent manner (Table No.5) the MEA (IC_{50} = 53.48±0.096) showed the maximum quenching ability followed by EAEA (IC_{50} = 73.99±0.128) and CEA (IC_{50} = 115.45±0.132). The *in vitro* radical scavenging ability of the extracts were found to be significant (p<0.05) when compared with the standard quercetin (IC_{50} = 28.12±0.092).

Superoxide radical scavenging activity

The superoxide radical scavenging ability was found to increase with increase in concentration of the extract. The MEA (IC_{50} = 40.11±0.126) was found to be an efficient scavenger of superoxide anion radical generated from PMS-NADH system *in vitro* and the activity was significant (P<0.05) when compared to that of standard ascorbic acid (IC_{50} = 33.94±0.132). The scavenging effects of extracts on the superoxide anion radical decreased in order MEA>EAEA (IC_{50} = 69.13±0.094) >CEA (IC_{50} = 103.77±0.142) (Table No.4).

Anthelmintic activity

The extracts exhibited more potent activity at a higher concentration (100mg/ml) against *Pheretimaposthuma* (earthworm). When observed the response of worms in case of paralysis and death, there was significant variations among the results produced by the different extracts at different concentrations (25, 50 and 100mg/ml) (Table No.6).

All the extracts exhibited anthelmintic activity in dose dependent manner varying from loss of motility (paralysis) to loss of response to external stimuli, which eventually advanced into death. MEA, EAEA and CEA exhibited significant anthelmintic activity in dose dependent manner when compared with reference standard albendazole. The methanolic Extract showed less time to cause paralysis(12.34±0.612) and death (29.20±0.912) of the earthworms and thus it was found to be more potent than other extracts (MEA>EAEA>CEA>PEA) at 100mg/ml concentration.

Discussions

Phytochemical screening

Various bioactive components such as phenolics, tannins, flavonoids, terpenoids and steroids were prominently revealed during the preliminary phytochemical screening. Phenolics, tannins and flavonoids were present in all the extracts whereas glycosides were absent in all the extracts. Alkaloids, terpenoids and proteins were absent in petroleum ether extract. Steroids were absent in methanol extract.

Antioxidant assay

Over production of oxidants in certain condition can cause imbalance leading to oxidative damage to large biomolecules such as lipids, DNA and proteins. Many synthetic drugs protect against oxidative damage but they have adverse side effects. Data from both scientific reports and laboratory studies show that the plant contain a large variety of substance called “plant chemicals” or “phytochemicals” that possess antioxidant activity[18,19]. Studies have attributed that antioxidant properties are due to the presence of phenols and flavanoids[20]. Thus, the presence of these components would have contributed to significant antioxidant activity of plant extracts. Antioxidant of phenolic compounds is based on their ability to donate hydrogen atom to free radicals[21]. The scavenging activity of a stable radical is considered a valid and easy assay to evaluate scavenging activity of natural compounds[22].

DPPH is a relatively stable free radical. The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH. From the present result it may be postulated that *Aporosalindleyana Linn* reduces the radical to the corresponding hydrazine when it reacts with the

hydrogen donors in the antioxidant principles. In the present study, the methanolic Extracts exhibited high DPPH radical scavenging activity compared to other extracts. Superoxide anion is oxygen centered radical with selective reactivity. This species is produced by a number of enzyme systems in auto-oxidation reactions and by non-enzymatic electron transfers that univalently reduce molecular oxygen. It can also reduce certain iron complexes such as cytochrome[23]. The present study showed potent superoxide radical scavenging activity for *Aporosa lindleyana Linn*. Increased absorbance of the reaction mixture indicated increased reducing power[24]. Methanol extract showed potent superoxide radical scavenging activity with IC_{50} value compared to standard ascorbic acid.

Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity[25]. Hydroxyl radical is one of the potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and cause damage to cell[26]. The present study shows that the extracts had significant scavenging effects on hydroxyl radical, which increased with the increase in concentration from 10-160µg/ml.

Anthelmintic activity

Helminthic infections of the gastrointestinal tract of human beings and animals have been acknowledged to have adverse effects on the health standards with a consequent lowering of resistance to other diseases. Now a days resistance to the available synthetic drugs is a major problem. Therefore, in recent years, a search for plant derived drugs is the primary choice of researchers, as they are believed to have lesser side effects and are more compatible with the physiological flora[27,28]. Phytochemical analysis of the crude extract revealed the presence of tannins, phenolics, flavonoids and alkaloids which are known to exhibit anthelmintic property[29]. Tannins and Phenolics are known to interfere with the energy generation in helminth parasites by uncoupling oxidative phosphorylation[30] and also bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite, leading to death. Based on these we can assume that tannins, phenolic compounds and flavonoids present in the leaf extract of *Aporosalindleyana Linn* may be responsible for the anthelmintic activity.

Table No.1: Percentage yield of various extracts

S.No	Extracts	% Yield (w/w)
1	PEA	8.9
2	CEA	2.4
3	EAEA	6.3
4	MEA	5.9

PEA: Pet Ether Extract of *Aporosalindleyana Linn*; **CEA:** Chloroform Extract of *Aporosalindleyana Linn* **EAEA:** Ethyl Acetate Extract of *Aporosalindleyana Linn*; **MEA:** Methanolic Extract of *Aporosalindleyana Linn.*

Table No.2: Phyto-chemical screening of various extracts

S.No	Phytochemicals	PEA	CEA	EAEA	MEA
1	Tannins and phenolics	+	+	+	+
2	Saponins	+	-	-	+
3	Flavonoids	+	+	+	+
4	Terpenoids	-	+	+	+
5	Alkaloids	-	+	+	+
6	Glycosides	-	-	-	-
7	Steroids	+	+	+	-
8	Proteins	-	+	+	+

PEA: Pet Ether Extract of *Aporosalindleyana Linn*; **CEA:** Chloroform Extract of *Aporosalindleyana Linn,* **EAEA:** Ethyl Acetate Extract of *Aporosalindleyana Linn*; **MEA:** Methanolic Extract of *Aporosalindleyana Linn.*

Table No.3: DPPH Radical scavenging activity of *Aporosa lindleyana Linn*

S.No	Conc µg/ml	Percentage Inhibition (%)				
		PEA	CEA	EAEA	MEA	Gallic Acid
1	0.25	-	-	-	6.32±0.439	08.48±0.416
2	0.5	-	-	-	17.95±0.248	19.68±0.964
3	1	-	-	05.82±0.212	33.68±0.256	31.63±0.713
4	2	-	-	09.16±0.261	46.34±0.165	53.33±0.334
5	4	05.46±0.156	10.18±0.314	18.34±0.156	60.22±0.138	69.44±0.220
6	8	16.62±0.178	23.44±0.342	36.83±0.552	77.97±0.102	86.04±0.650
7	16	21.64±0.441	38.69±0.452	51.74±0.339	84.36±0.419	92.44±0.450
8	32	26.88±0.214	53.81±0.072	66.56±0.283	89.35±0.166	98.19±0.221
9	64	32.16±0.283	58.92±0.154	85.92±0.543	92.64±0.183	100
10	IC_{50}µg/ml	#	28.33±064*	15.02±0.098*	02.52.±0.112*	01.84±0.098*

PEA: Pet Ether Extract of *Aporosalindleyana Linn*; **CEA:** Chloroform Extract of *Aporosalindleyana Linn,* **EAEA:** Ethyl Acetate Extract of *Aporosalindleyana Linn*; **MEA:** Methanolic Extract of *Aporosalindleyana Linn.* All values determined were mean ± SEM; $n = 3$. *P < 0.05 when compared with standard.

Table No.4: Hydroxyl radical scavenging activity of *Aporosa lindleyana Linn*

S.No	Conc µg/ml	Percentage Inhibition (%)				
		PEA	CEA	EAEA	MEA	Quercetin
1	10	05.38± 0.197	07.79±0.144	12.36±0.462	15.39±0.334	19.21±0.791
2	20	11.61±0.258	194.36±0.312	23.84±0.804	30.21±0.521	39.55±0.648
3	40	18.56±0.094	33.89±0.852	38.90±0.709	44.56±0.496	55.10±0.337
4	80	24.63±0.118	47.35±0.216	51.96±0.118	60.70±0.335	68.47±0.125
5	160	32.33±0.166	53.66±0.164	63.52±0.219	77.93±0.667	84.38±0.194
6	IC_{50}µg/ml	#	115.45±0.132*	73.99±0.128*	53.48±0.096*	28.12±0.092*

PEA: Pet Ether Extract of *Aporosalindleyana Linn*; **CEA:** Chloroform Extract of *Aporosalindleyana Linn,* **EAEA:** Ethyl Acetate Extract of *Aporosalindleyana Linn*; **MEA:** Methanolic Extract of *Aporosalindleyana Linn.* All values determined were mean ± SEM; $n = 3$. *P < 0.05 when compared with standard.

Table No.5: Super oxide radical scavenging activity of *Aporosa lindleyana Linn*

S.No	Conc µg/ml	Percentage Inhibition (%)				
		PEA	CEA	EAEA	MEA	Ascorbic acid
1	10	05.21± 0.136	09.63±0.923	11.64±0.813	16.33±0.136	25.34±0.089
2	20	14.27±0.482	19.81±0.165	23.88±0.966	31.98±0.065	42.88±0.163
3	40	24.36±0.942	32.64±0.771	40.18±0.692	49.96±0.129	53.09±0.292
4	80	33.18±0.772	47.65±0.398	53.66±0.251	63.81±0.308	68.22±0.186
5	160	40.95±0.420	58.92±0.224	65.19±0.238	79.38±0.140	83.56±0.402
6	IC_{50}µg/ml	#	103.77±0.142*	69.13±0.094*	40.11±0.126*	33.94±0.132*

PEA: Pet Ether Extract of *Aporosalindleyana Linn*; **CEA:** Chloroform Extract of *Aporosalindleyana Linn*, **EAEA:** Ethyl Acetate Extract of *Aporosalindleyana Linn*; **MEA:** Methanolic Extract of *Aporosalindleyana Linn*. All values determined were mean ± SEM; $n = 3$. *P < 0.05 when compared with standard.

Table No.6: Anthelmintic activities of *Aporosa lindleyana Linn* leaves

S.No	Test Sample	Concentration (mg/ml)	Time Taken for Paralysis (Minutes)	Time Taken for Death (Minutes)
1	Control (0.1% Tween in normal saline)	--	--	--
2	PEA	25	178.38±0.536*	--
		50	87.48±0.921*	--
		100	51.26±0.774*	188.12±0.336*
3	CEA	25	78.42±0.078*	169.34±0.104*
		50	56.22±0.260*	99.46±0.294*
		100	36.32±0.256*	77.40±0.216*
4	EAEA	25	40.16±0.294*	74.31±0.412*
		50	27.36±0.524*	49.58±0.362*
		100	15.39±0.493*	29.39±0.824*
5	MEA	25	33.54±0.461*	69.33±0.104*
		50	19.10±0.217*	42.45±0.453*
		100	12.34±0.612*	29.20±0.912*
6	Albendazole	25	11.31±0.206*	18.26±0.816*
		50	5.57±0.441*	12.53±0.316*
		100	2.52±0.682*	5.40±0.514*

PEA: Pet Ether Extract of *Aporosalindleyana Linn*; **CEA:** Chloroform Extract of *Aporosalindleyana Linn*; **EAEA:** Ethyl Acetate Extract of *Aporosalindleyana Linn*; **MEA:** Methanolic Exract of *Aporosalindleyana Linn*. All values determined were mean ± SEM; $n = 6$. *P < 0.05 when compared with standard.

CONCLUSION

The present study reveals that the leaf extract of *Aporosa lindleyana Linn* has significant antioxidant and anthelmintic activity. Further investigations on the isolation of active compounds present in the *Aporosa lindleyana Linn* extracts and in vivo studies are essential to identify a potential chemical entity for clinical use.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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