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Original Article

PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITY OF *ACTINIOPTERIS RADIATA*

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ABSTRACT

Objective: The medicinal plants have been using to treat ailments since ancient times. The recent advances in science and technology impel humans to evaluate medicinal plants therapeutic efficiency and isolation of bioactive compounds in pure forms before their use in development of new drugs and their derivatives. But even now, abundant medicinal plants unevaluated scientifically. The current study was aimed to explore phytochemical constituents, antioxidant and hepatoprotective activities of *Actiniopteris radiata* root parts.

Methods: Standard procedures have been used to perform phytochemical analysis. Antioxidant activity was carried using *In vitro* methods on superoxide, hydroxyl, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals. Hepatoprotective activity was studied by paracetamol-induced liver toxicity on WISTAR albino rats. The parameters assessed were Aspartate aminotransferase (SGOT/AST), Alanine aminotransferase (SGPT/ALT), alkaline phosphatase (ALP) and total bilirubin levels.

Results: The tested extracts (hexane, ethyl acetate, and hydro-alcoholic) possess biologically active compounds such as sterols, terpenoids, glycosides, phenolics, alkaloids, flavonoids. The hydro-alcoholic extract has more phenolic contents (24.28±0.3) and flavonoid contents (22.68±0.6). The extracts showed dose dependent activity on tested free radicals and extracts showed more percentage inhibition at 320µg. The hydro-alcoholic extract showed more percentage inhibition i.e. 71.00±2.08 on DPPH free radical, 79.67±1.20 on hydroxyl free radical and 80.33±1.20 on superoxide free radical. As antioxidant activity of hexane and ethyl acetate extracts was less and they also showed less percentage protection on liver toxicity, hydro-alcoholic extract showed more percentage protection on biomedical enzyme levels of liver toxicity at high concentration i.e., 400 mg/kg b.w. The percentage protection on the enhancement of AST (SGOT), ALT (SGPT), ALP, and total bilirubin levels were 82.24%, 82.14%, 84.18%, and 82.85% are significant ($P<0.01$) as Liv52 shown percentage protection on the enhancement of Aspartate aminotransferase (SGOT), alanine aminotransferase (SGPT), Alkaline phosphatase (ALP) and total bilirubin levels were 93.58%, 92.83%, 94.67% and 93.57%.

Conclusion: The current study was aimed to explore phytochemical constituents, antioxidant and hepatoprotective activities of *Actiniopteris radiata* root parts extracts. The outcome of the current research results provides scientific evidence of the traditional usage of *Actiniopteris radiata*.

Keywords: Phytochemicals, Analysis, Oxidants, Antioxidant, Liver, Hepatotoxicity, Paracetamol, Hepatoprotection, *Actiniopteris radiata,* Roots, Percentage protection

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INTRODUCTION

The body metabolisms are very important to lead healthier life in current days [1]. The current lifestyle is significantly affecting, quality of mental and health conditions of the humans around the world. The developments in technology, communication, industrialization made our lives easy and busy but threatening the physical, and mental growth of the people. According to the World health organization (WHO), an unhealthy lifestyle is due to the presence of malnutrition, unhealthy diet, misusage of drugs, smoking, alcohol consumption and, stress [2]. All these can lead to metabolic diseases like cardiovascular problems, obesity, hypertension, skeletal disorders, and body organs' dysfunction [3]. Liver is one of the essential organs in the body, which plays a significant role in bodily functions and interlinked to other body organs' functions [4, 5]. The main functions of it include protein synthesis, hormonal metabolisms, cholesterol homeostasis, oxidation, and detoxification [3]. The recent studies on mortalities around the world, Liver diseases are one of the main reasons of deaths [6]. As it always functions, automatically has been exposed to the number of pathogens, pollutants, chemicals and different toxic substances and those are automatic leads to dysfunction of liver [7-9].

Human beings have been using natural medicines from plants, animals, minerals since back to the history of human life. Medicinal plants are relevant sources for new drug development, as they have been using for food, shelter, fuel, and clothing and the traditional medicines Ayurveda, Unani, Siddha, traditional Chinese, Iranian, Korean medication are based on medicinal plants [10]. The remarkable development around the world, including medicine makes human life easy and increased the life span of humans [11]. In spite of growth, it made pollution, people lazy, busy, and materialized for emerging of new diseases, including liver diseases [12]. The developments of modern medicines for new diseases sometimes fail to provide treatment and even more causes different side effects [13, 14]. As a result, scientific research has been going on to identify new bioactive molecules to treat various diseases, including liver toxicity [15]. A lot of medicinal plants are available as sources of bioactive compounds to the health of humans and are useful in the development of pharmaceutical products [16]. Therefore, the search for new bioactive molecules from different medicinal plants will always be helpful to improve a healthy life. In the latest decades, various medicinal plants have been evaluated scientifically about their therapeutic values and identified broad-spectrum biological active metabolites from them to treatment diseases [17]. In this point of view, the current research work has carried on one of the unexplored traditional medicinal plant i.e. *Actiniopteris radiata.*

Actiniopteris radiata is a shrub plant belongs to the family Pteridaceae and grows around tropical regions like Africa, India, Nepal, Srilanka, Madagascar. The plant possesses different traditional values such as astringent, anti-inflammatory agent, asthma, diarrhea, dysentery and, etc and our previous studies also reported about antibacterial activity of *A. radiata* root extracts [18, 19]. But a lesser amount of research was carried on *A. radiata.*

Therefore, the current research work was carried out on phytochemical screening, evaluation of antioxidant activity and, hepatoprotective activity on paracetamol-induced liver toxicity.

MATERIALS AND METHODS

Chemicals and drugs

The analytical grade solutions were used in the current study. The diagnostic kits for enzymes estimation have purchased from Span diagnostics Ltd., Gujarat, India. The liver toxic inducing drug paracetamol and standard drug Liv52 were purchased from a local market, Narasaraopet, Guntur, India.

Preparation of extracts

The *Actiniopteris radiata* plant was collected at Palnadu region, Andhra Pradesh, India. *A. radiata* has been authenticated (IND/AP/EG/YGDC-04685) by Dr. P. Prayaga Murthy, Taxonomist, Govt. Degree College, Yeleswaram, East Godavari. The collected plant material was washed to remove debris and were dried under shade. Finally, dried root part was separated and were made into coarse powder. The coarse powder was used for extraction with solvents hexane, ethyl acetate, and hydro-alcoholic (70% ethanol) successively using maceration process. The prepared extracts were stored in a desiccator for further use.

Phytochemical analysis

Standard phytochemical procedures were used to explore the phytochemical profile of *A. radiata* root extracts [4, 20-22].

In vitro anti-oxidant activity

Anti-oxidant activity of *A. radiata* root extracts was evaluated using free radicals (superoxide, hydroxyl, and DPPH) [23].

Superoxide radical scavenging activity

Superoxide scavenging activity of the selected plant extracts was evaluated as per standard method. It based on the absorption of light at 560 nm induction of superoxide free radical generation by riboflavin and corresponding to a reduction by nitroblue tetrazolium [24].

Hydroxyl radical scavenging activity

The scavenging activity of selected plant extracts on hydroxyl radical was measured as per the established method. It was studied by the competition between deoxyribose and the extract's antioxidant molecules for hydroxyl radicals generated from the Fe+2/EDTA/ H2O2 system [25].

DPPH radical scavenging activity

The DPPH radical scavenging activity was measured as per previous established method [23]. This method based on the measure of color absorbance of alcoholic DPPH solution (Blue color) after the addition of antioxidant solution (Extract/Compound) (yellow color).

Calculation of percentage inhibition

The percentage inhibition of superoxide production by the extract has calculated using the formula:

$$\text{Inhibitory ratio} = (\text{Ao} - \text{A1}) \times 100/\text{Ao}$$

Ao: Absorbance of control; A1: Absorbance of plant extract or/and Ascorbic acid.

IC50 calculation form percentage inhibition

The optical density obtained with each concentration of the extract/ascorbic acid has plotted taking concentration on X-axis and percentage inhibition on Y-axis. The graph has extrapolated to find the 50% inhibition concentration of extract/ascorbic acid.

Selection of animals

The Wistar albino rats (170-220 gm) were used to evaluate the hepatoprotective activity. The animals were obtained from M/s. Mahavir Enterprises, Hyderabad and maintained at controlled environmental conditions before the experiment and also at the time of the experiment (22±2 °C, 60±5% humidity). The animals were fed up with standard laboratory diet and water. The animal study was approved by the institutional ethical committee, approved CPCSEA, Govt of India (Reg No: 1987/PO/Re/S/17/CPCSEA). Before the study, the prepared extracts were tested for their toxicity as per The Organization for Economic Co-operation and Development (OECD) guidelines 423 [26].

Hepatoprotective activity

The extracts of *A. radiata* (Hexane extracts (HE), Ethyl acetate extract (EAE), Hydro-alcoholic extracts (HAE)) at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg b.w. were tested for their hepatoprotective activity against paracetamol-induced liver toxicity. The animals were divided into XII groups (n=6), group I as control, group II and negative control, group III as a positive group. Group I and II have treated with normal saline (2 ml/Kg b. w) and, group III was treated with Liv52 (25 mg/Kg b.w.) for seven days. The groups IV, V, VI were treated with HE at 100 mg/kg, 200 mg/kg and 400 mg/kg b.w. doses. The groups VII, VIII, IX were treated with EAE at 100 mg/kg, 200 mg/kg and 400 mg/kg b.w. doses. The groups X, XI, XII were treated with HAE at 100 mg/kg, 200 mg/kg and 400 mg/kg b.w. doses. The paracetamol was dosed to all groups except group I on the 5th day of the experiment. On the 7th day of the experiment, after 2 h of last dose treatment, the blood samples were collected from animals through retro-orbital plexus. The collected samples were centrifuged without any delay at 2400rpm/15 min. The separated serum was used to evaluate the liver function parameters such as Aspartate aminotransferase (SGOT), alanine aminotransferase (SGPT), Alkaline phosphatase (ALP) and total bilirubin levels using an auto analyzer [4, 27].

RESULTS AND DISCUSSION

The phytochemical screening of *A. radiata* extracts was carried out using standard procedures. The three extracts have a different chemical profile (table 1). The three extracts gave positive results for presence of Phytosterols, Terpenoids, Glycosides, Flavonoids, Alkaloids, and gave negative results for Amino acids, Quinones. The EAE, HAE gave positive results for presence of saponins, tannins, phenols. The HAE gave positive for Carbohydrates, but HE, EAE gave negative results. The HE gave positive for presence of but negative in EAE and HAE. The total alkaloid and flavonoid contents were estimated for the *A. radiata* extracts (table 2). The HAE extract have more alkaloid and flavonoid contents than HE and EAE extracts.

Table 1: Phytoconstituents in different extracts of *Actiniopteris radiata*

Name of the phytochemicals	Extracts of *A. radiata*		
	Hexane	Ethyl acetate	Hydro-alcoholic
Phytosterols	+	+	+
Terpenoids	+	+	+
Glycosides	+	+	+
Saponins	-	+	+
Flavonoids	+	+	+
Tannins	-	+	+
Carbohydrates	-	-	+
Alkaloids	+	+	+
Amino acids	-	-	-
Oils	+	-	-
Quinones	-	-	-
Phenols	+	+	+

+=Present,–=Absent

Table 2: Phenolic and flavanoid contents (mg/gm) of *Actiniopteris radiata* extracts

Name of the extract	Total Phenolic content (GAE)	Total flavanoid content (mg/gm)
Hexane	3.02±0.6	6.32±0.52
Ethyl acetate	18.1±0.2	21.05±0.38
Hydro-alcoholic	24.28±0.3	22.68±0.6

n=3; mean±SEM

The *Actiniopteris radiata* extracts were tested for their antioxidant activity on DPPH, hydroxyl, superoxide free radicals and were found to possess dose-dependent activity and their results are comparable with standard drug ascorbic acid (table 3). HE has less activity compared to EAE and HAE. The IC50 value of HE was not detectable on hydroxyl free radical, but the EAE, HAE extracts possess almost the same activity on hydroxyl and superoxide free radicals (table 3, table 4). The HAE has more activity on tested free radicals as it has more alkaloid and flavonoid contents.

Table 3: IC 50 values of *Actiniopteris radiata* extracts on different free radicals

Name of the plant/compound	Name of the extract	IC 50 value in µg on different free radicals		
		DPPH	Hydroxyl	Superoxide
Actiniopteris radiata	Hexane	225	ND	281
	Ethyl Acetate	256	155	140
	Hydro-Alcoholic	145	128	125
Ascorbic acid		95	103	72

ND: Not detected

Table 4: Percentage inhibition of *Actiniopteris radiata* extracts on DPPH, hydroxyl and superoxide free radical

Name of the extract/compound	Name of the free radical	Concentration				
		20 (µg)	40 (µg)	80 (µg)	160 (µg)	320 (µg)
Hexane	DPPH	7.33±0.33	15.00±1.53	20.67±0.88	39.67±0.88	65.33±1.76
Ethyl Acetate		6.33±0.88	12.00±0.58	23.33±1.45	40.00±1.15	56.00±1.53
Hydro-Alcoholic		10.67±1.20	23.33±0.88	38.67±1.45	52.33±0.67	71.00±2.08
Ascorbic acid		16.33±0.67	30.67±1.20	46.33±0.67	66.00±1.15	82.33±0.67
Hexane	Hydroxyl	4.00±0.58	9.00±0.58	18.33±0.88	34.33±1.20	45.67±1.76
Ethyl Acetate		8.33±0.88	17.00±1.15	28.00±1.15	51.00±1.73	70.67±1.45
Hydro-Alcoholic		13.00±0.58	24.00±1.53	38.00±0.58	57.67±0.88	79.67±1.20
Ascorbic acid		14.67±0.88	29.33±0.33	44.67±0.33	62.67±0.33	78.00±0.58
Hexane	Superoxide	6.00±0.58	11.33±0.88	19.67±0.88	36.00±1.15	55.33±1.76
Ethyl Acetate		10.33±1.45	18.67±1.20	33.67±1.20	55.67±1.45	69.67±1.20
Hydro-Alcoholic		12.33±0.88	23.67±1.20	39.33±0.88	59.00±0.58	80.33±1.20
Ascorbic acid		18.33±0.33	35.33±0.33	54.33±0.88	69.33±0.33	85.67±0.67

n=3; mean±SEM.

The extracts of *A. radiata* have found to be safe on their toxicity study, all extracts did not show any mortality and physiological and psychological conditions at 2000 mg/kg b.w. dose on animals under 24-72h observation. After the safety confirmation, the extracts were evaluated for their hepatoprotective activity using paracetamol-induced liver toxicity at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg b.w. The extract at those concentrations showed as antioxidant activity, dose-dependent hepatoprotective activity. The extracts effectively reduced the elevated liver functioning parameters such as AST (SGOT), ALT (SGPT), ALP, and total bilirubin levels ($P<0.05$). Group I has treated with vehicle showed no significant changes, Group II has treated paracetamol, there is a significant change in levels of biomarker enzymes, group III was administered with paracetamol (200 mg/kg b.w., s. c.) and Liv 52 (25 mg/kg per day, p. o.) have significant changes in biomarker enzymes levels compared to group II rats enzymes levels and the percentage protection to be had by Liv 52 against changes in AST (SGOT), ALT (SGPT), ALP and total bilirubin levels were 93.58%, 92.83%, 94.67% and 93.57% respectively. The percentage protection produced by HE in Groups IV, V and VI at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg b.w. on varied AST (SGOT), ALT (SGPT), ALP and total bilirubin levels were 11.59%, 11.81%, 13.08% and 13.02%, 27.71%, 27.00%, 26.27% and 26.49%, 49.87%, 50.63%, 47.89%, and 47.63% respectively. The percentage protection produced by the EAE at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg b.w. on varied of AST(SGOT), ALT (SGPT), ALP and total bilirubin levels were 23.30%, 23.21%, 23.63%, and 24.81%, 41.56%, 41.07%, 41.93% and 44.56%, 75.82%, 72.43%, 73.95% and 74.27% respectively. The percentage protection produced by the HAE at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg b.w. on AST(SGOT), ALT (SGPT), ALP and total bilirubin levels were 32.62%, 31.22%, 30.33%, and 31.55%, 53.02%, 54.12%, 53.48%, and 53.14%, 82.24%, 82.14%, 84.18%, and 82.85% respectively. The results were shown in table 5, table 6, and fig. 1.

The identification of new drugs is always difficult and exciting research from natural products [28]. As new diseases emerging and current drugs became awry to cure diseases, causing side effects around the world [29]. Liver toxicity is one of the major side effects in humans due to different drug usage on long term or improper usage of medicines [30]. As above said, liver toxicity major mortality causing disease around the world and Paracetamol is one of the common usage drugs in current days, but on its over usage will affect liver function and cause different illnesses like yellowish skin, blood clotting problems, and confusion [31]. These conditions are insisting the researchers discover new molecules from natural resourcesas pharmaceutical products [32].

Table 5: The effect of *Actiniopteris aadiata* extracts on liver enzymes in the paracetamol-induced liver toxicity

Name of the drug	AST (U/l)	ALT (U/l)	ALP (U/l)	T. bil (mg/dl)
Control	84.33±1.45	51.50±0.99	215.00±1.46	0.25±0.01
Paracetamol	216.67±1.65	170.00±1.93	531.00±2.78	1.35±0.02
Liv 52 25 mg//kg b.w.	92.83±1.08	60.00±1.06	231.83±2.01	0.34±0.01
HE 100 mg/kg b.w.	201.33±1.58	156±1.15	489.67±1.61	1.21±0.01
HE 200 mg/kg b.w.	180±1.57	138±0.97	448±1.81	1.07±0.01
HE 400 mg/kg b.w.	150.67±2.22	110±1.63	379.67±1.8	0.84±0.01
EAE 100 mg/kg b.w.	185.83±1.35	142.5±1.12	456.33±1.89	1.08±0.01
EAE 200 mg/kg b.w.	161.67±1.05	121.33±1.36	398.5±1.65	0.87±0.01
EAE 400 mg/kg b.w.	116.33±1.73	84.17±1.08	297.33±1.20	0.55±0.01
HAE 100 mg/kg b.w.	173.5±1.38	133±1.46	435.17±1.51	1.01±0.02
HAE 200 mg/kg b.w.	146.5±1.48	105.83±1.01	362±1.79	0.78±0.01
HAE 400 mg/kg b.w.	107.83±1.19	72.67±1.15	265±1.41	0.45±0.01

n=6 and mean±SD; HE-Hexane extract, EAE-Ethyl acetate extract, HAE-Hydro-alcoholic extract

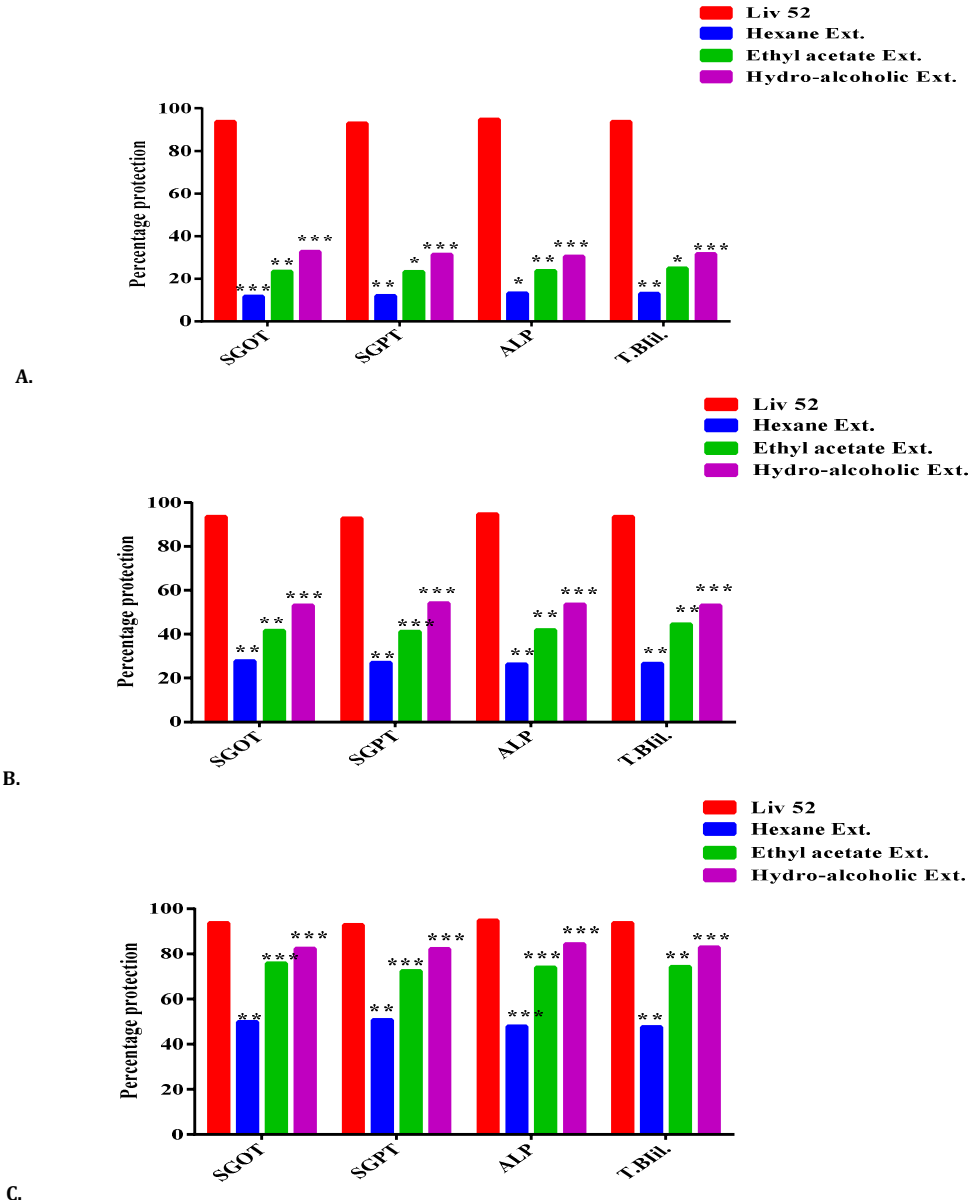

Fig. 1: Percentage protection produced by different extracts of *A. radiata* at a dose of 100 mg/kg (A), 200 mg/kg (B) and 400 mg/kg, results were analysed by using Two-way ANOVA followed by Dunnet's multiple comparision test. All gropus were compared with Liv 52 group. ***p<0.001; **p<0.01; *p<0.05; ns= Non significance.■Liv 52; ■hexane extract; ■ethyl acetate extract; ■hydro-alcoholic extract

Table 6: Percentage (%) protection of *Actiniopteris radiata* extracts on enzymes levels at different doses on paracetamol-induced liver toxicity

Name of the enzyme	Amount of the extract									
	100 mg/kg b.w.			200 mg/kg b.w.			400 mg/kg b.w.			
	Name of the extracts			Name of the extracts			Name of the extracts			Liv 52
	HE	EAE	HAE	HE	EAE	HAE	HE	EAE	HAE	
AST(U/l)	11.59	23.30	32.62	27.71	41.56	53.02	49.87	75.82	82.24	93.58
ALT(U/l)	11.81	23.21	31.22	27.00	41.07	54.15	50.63	72.43	82.14	92.83
ALP(U/l)	13.08	23.63	30.33	26.27	41.93	53.48	47.89	73.95	84.18	94.67
T. Bil (mg/dL)	13.02	24.81	31.55	26.49	44.56	53.14	47.63	74.27	82.85	93.57

HE-Hexane extract, EAE-Ethyl acetate extract, HAE-Hydro-alcoholic extract. All groups were compared with paracetamol group. Values in the parenthesis indicate percent protection in individual biochemical parameters from their elevated values caused by the hepatoprotection. The percentage of the protection is calculated as 100 ×(values of CCl_4-values of sample)/(values of CCl_4 control-values of vehicle).

Medicinal plants are evidence for having different bioactive compounds in them, and they have been using to treat different ailments in traditional medicine [33-35]. But, still, a lot of medicinal plants are unexplored about their medicinal values. Many pharmaceutical products have been reporting from natural resources, including medicinal plants in recent years [36, 37]. So, the current work was carried and identified that the root parts of *A. radiata* have different phytochemical components in them. The nature of bioactive compounds in different extracts will depend on the type of extraction solvent used. Because, our results showed the presence of different chemical constituents in three different extracts and HAE extract have more alkaloid and flavanoid contents. Different types of alkaloids and flavanoids have been reported from medicinal plants about their beneficial roles against many diseases [38, 39].

In our study, the extracts showed dose-dependent antioxidant and hepatoprotective activities. In both activities, HAE has shown more effectiveness against free radicals' reduction and maintaining the liver function against paracetamol toxicity compared to HE and EAE. The formation of free radicals (FRs) will be more effective in several diseases through liver toxicity. The developed FRs are unbalanced molecules, during their stabilization alter the physiological function of organs, including liver functions, by reacts with stable molecules and finally lead to oxidative stress (OS) [27, 40, 41]. The OS can cause unusual diseases like lipid peroxidation, DNA damage, atherosclorosis, cancers, neurodegenerative, inflammatory bowel diseases and accelerated aging [42]. The results of the current study provide scientific evidence for *A. radiata* extracts' antioxidant, hepatoprotective activities and presence of different bioactive compounds. The phenolic compounds, alkaloids, terpenoids may be responsible for hepatoprotective activity and the HAE extract possess more phenolic and alkaloid contents and it showed more antioxidant and hepatoprotective activities [43-45]. As earlier reports on different medicinal plants about their biological activities such as anti-inflammatory, anti-diabetic, hepatoprotective, antioxidant, anti-cholinesterase, the current research will be beneficial to identify the new bioactive compounds [46-50]. The further research was going on our laboratory to isolate individual bioactive molecules from *A. radiata* extracts and other medicinal plants.

CONCLUSION

The current study was aimed to explore, therapeutic activity of *A. radiata* root parts. Results of the phytochemical analysis reveals that extracts possesses presence of different phytochemical compounds and substantiate that phytochemical profile of extracts depends upon the type of extraction solvents used for extraction. The *A. radiata* root extracts also showed concentration dependent antioxidant and hepatoprotective activities as standard drugs ascorbic acid and Liv 52 and these results provide scientific evidence to therapeutic potential of *A. radiata* root. Further research is helpful and going on evaluation of various biological activities and isolation of bioactive compounds from *A. radiata.*

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Nil

AUTHORS CONTRIBUTIONS

K. Gouri Sankar: Study Design and Execution, Results Compilation, Manuscript Preparation.

B. Sri Venkateswarlu: Study Guidance, Manuscript correction.

CONFLICT OF INTERESTS

Declared none

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