Defence Life Science Journal, Vol. 9, No. 2, April 2024, pp. 117-124, DOI : 10.14429/dlsj.9.18846

Anticancer Activity of *Hedychium coronarium* Rhizome Solvent Extracts on Colon Cancer Cells

P. Vishnu Mohan Reddy[#], Pulala Raghuveer Yadav[$] and K. Venkata Ratnam[#,*]

[#]*Department of Botany, Rayalaseema University, Kurnool–518 007, India*
[$]*Department of Biotechnology, Indian Institute of Technology Hyderabad, Kandi–502 284, India*
[*]*E-mail: drvenkatapkd@gmail.com*

ABSTRACT

Hedychium coronarium J. Koenig. is a rhizomatous herb of family Zingiberaceae. It is well known as white ginger lilly or butterfly lilly. The Rhizome has been used to treat rheumatism as a tonic and avoid bad breath. The current study aims to evaluate the anticancer, antibacterial, and antioxidant activity of petroleum ether (HCPE) and ethanol (HCAE) extracts of *Hedychium coronarium* rhizome using *in vitro* methods. The extracts were screened for anticancer activity against colon cancer cell lines and antioxidant capacity by phosphomolybdenum, DPPH, and hydroxyl radical scavenging approaches. The results revealed that HCPE and HCAE extracts have many phenolics and flavonoids. Ethanol extract showed a higher total antioxidant capacity than petroleum ether extract. Petroleum ether extract strongly reduced DPPH purple color, while ethanol extract significantly inhibited hydroxyl radicals. Cytotoxic study results revealed that the extracts strongly suppressed HCT-116 cell lines in a concentration-dependent fashion. ELISA and TUNEL assay results showed that petroleum ether extracts enhanced caspase-3-mediated DNA breaking in colon cancer cell lines. In conclusion, *H. coronarium* can be a plant-based antibacterial/antioxidant and anticancer component source. Further molecular studies are needed to isolate and characterize bioactive principles and to confirm their therapeutic value through *in vivo* models.

Keywords: Anticancer; HCT-116 cells; *Hedychium coronarium*; Rhizome

1. INTRODUCTION

Cancer is one of the leading causes of death in several countries. According to 2019 estimations done by the World Health Organization, Cancer is the first or second leading cause of death below the age of 70 worldwide. There are 277 types of cancers, of which colon/colorectal cancer causes mortality of about 9.4 %[1]. Chemotherapy is the most common method used to treat all kinds of cancers. The drugs used in chemotherapy to treat colon cancer cause side effects such as fatigue, hair loss, vomiting, diarrhea, nerve damage, etc. Demand for drugs with potential anticancer activity and low side effects have been increasing daily.

Components of about 1,70,000 are toxic among the 3,26,000 newly discovered substances[2]. Traditional medicine plants have a rich source of phyto-constituents with potential therapeutic value.

Hedychium coronarium (family Zingiberaceae) is a perennial rhizomatous herbaceous species widely cultivated in tropical and subtropical regions of India. Rhizomes cure infected nostrils, fever, tumors, and tonsillitis. Pharmacological studies on rhizomes and flower extracts of *H. coronarium* reported potent antioxidant, anti-inflammatory, analgesic, CNS depressant, antibacterial, and antifungal activities. Phytochemical constituents isolated from *H. coronarium* are diterpenes, flavonoids, phenolic components, saponins, triterpenoids, and steroids[3]. Diterpenes isolated from *H. coronarium* rhizomes were reported as potent chemotherapeutic drugs against Cancer. In this connection, we selected *Hedychium coronarium* rhizomes to screen the anticancer potential of petroleum ether and ethanol extracts on colon cancer cell lines.

Received : 15 February 2023, Revised : 28 August 2023
Accepted : 12 September 2023, Online published : 17 May 2024

2. METHODOLOGY

2.1 Plant Material

H. coronarium rhizomes were collected from the Nallamala forest, Eastern Ghats of Andhra Pradesh. The specimens were authenticated by using local and regional floras[4-5]. The specimen was deposited (887) (Fig. 1) in the Department of Botany, Rayalaseema University, Kurnool, Andhra Pradesh, India.

2.2 Preparation of Extracts

The rhizomes collected were cleaned manually, rinsed with tap water, and dried under shade. One hundred grams of powdered plant material was loaded onto Soxhlet (Borosil) and successively extracted with petroleum ether (HCPE) and ethanol/alcohol (HCAE). The solvent was separated from extracts using the distilled unit (Borosil). The extracts were subjected to phytochemical, antioxidant, and anticancer assays using *in vitro* methods.

2.3 Phytochemical Analysis

2.3.1 Preliminary Phytochemical Screening

Figure 1. *Hedychium coronarium* flowering twig.

The concentrated HCPE and HCAE extracts obtained from *H. coronarium* were subjected to qualitative phytochemical analysis using standard methods[6-9].

2.3.2 Estimation of Phenolic Content (PC)

The P.C. of HCPE and HCAE was quantified by standard FCA reagent method[10]. About 10 or 20 µL of extracts were combined with 100 µL of FCA reagent and allowed 5 - 8 min for incubation at room temperature. Then, 0.58 mL of distilled water and 300 µL of Na_2CO_3 (20%) solution were added to reach the final reaction volume of 2 mL. The reaction solution was incubated for 2 hours. The intensity of the color reaction has been measured in terms of optical density (O.D.), which was read at 765 nm. The gallic acid standard curve estimated the phenol content (P.C.) of water extracts. The quantity has been expressed in mg GAE/g dry weight.

2.3.3 Estimation of Flavonoid Content (FC)

The F.C. of HCPE and HCAE were determined by using the standardized method[11]. The reaction mixture consisting aliquots (50 and 100 µL) of the extracts and the 0.1 mL sodium nitrite (5 %), 0.1 mL aluminium chloride (10%) was incubated for 5 min at 25 ºC. Then, the aliquots were neutralized by treating with 1 M sodium hydroxide and allowed for the reaction for 15 min at 25 ºC. The resultant solutions' optical density (O.D.) values were measured at λ 510 nm. The flavonoid content (F.C.) was estimated using Quercetin as a standard component and was represented in mg QE/g dry weight. Ascorbic acid (Vitamin C) was used as a standard and positive control.

2.4 ANTIOXIDANT STUDIES

2.4.1 Ammonium Molybdate Dependent Antioxidant Activity

Ten microliters of HCPE and HCAE were treated with 1mL of phosphor molybdenum reagent. Then, the reaction mixtures were incubated in the dry bath at 95 ºC for 1½ h. The O.D. values of each sample were measured at 695 λ against blank in the UV-Vis spectrophotometer[12]. The antioxidant capacity (TAC) of the test extracts were calculated from the standard curve; the results were represented in mg ASE/g dry weight.

2.4.2 DPPH Reducing Activity

Different concentrations of HCPE and HCAE extracts combined with one mL of ethanolic-DPPH solution (4 mg/L). The solutions were incubated for 30 min. in the dark chamber at ambient temperature. After 30 min., the absorbance of test samples were measured at 517 nm in UV-spectrophotometer[13]. The following formula was used to calculate the percentage inhibition of the DPPH scavenging capacity of selected medicinal plant extracts.

% Scavenging of free radical = Control OD – Test OD X 100/ Control OD

2.4.3 OH^- Radical Scavenging Assay

The hydroxyl radical inhibitory activity of the HCPE and HCAE was determined by the standard method[14]. Briefly, different concentrations of the HCPE and HCAE extracts were mixed with 2-deoxy-ribose, H_2O_2, ascorbic acid, and EDTA, then the solutions were kept at 37 ºC for 1 hour. After that, 0.5% trichloro acetic acid, 25 mM NaOH, and TCA (10 %) were added and incubated the reaction for 20 min at 100 ºC. After attaining the ambient temperature, the O.D. values of each sample were taken at 532 nm against the control. The positive effect of the result has been compared to the standard natural antioxidant, i.e., Ascorbic acid. The percentage inhibition of hydroxyl radical scavenging capacity of selected medicinal plant extracts was calculated using the following formula.

% Inhibition of free radical = Control OD – Test OD X 100/ Control OD

2.5 ANTIBACTERIAL STUDIES

2.5.1 Microorganisms Used

To test the antibacterial efficiency of the extracts the following microorganisms are used in the present study i.e. *Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli*, and *Bacillus cereus*, to test the extracts. The microbes were procured from IMTECH, Chandigarh, India.

2.5.2 Antimicrobial Activity

The antibacterial efficacy of HCPE and HCAE was studied using the disc diffusion method[15]. Paper discs saturated with different concentrations of plant extracts were placed on the surface of agar media plates seeded with 0.1 mL of overnight microbial culture. The discs treated with concern solvents used for extraction served as negative controls. The antibacterial activity of tested plant extracts was assessed by measuring bacterial-free zones formed around the discs. The bacterial free zones diameters were calculated and tabulated.

2.5.3 Determination of MIC

The MIC values of each extract against tested pathogens were assessed by using the broth dilution method[16]. Different concentrations of the extracts, starting from 62.5 mg to 625 mg/ml were tested in this method. 100 µL of the microbial culture was added to each tube. Tubes were incubated at 37 ºC for 18 h in an incubator, and then O.D. values were measured with calorimetry at 620 nm. The extract showing no visible growth was considered as its MIC value. DMSO served as the negative control.

2.6 CELL CULTURE STUDIES

2.6.1 Cell Culture

Human colon cancer cell lines (HCT-116) purchased from NCCS, Pune, were cultured in DMEM- High Glucose medium (Himedia) containing fetal bovine serum (Himedia) in a CO_2 incubator (Healforce, China).

2.6.2 MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) Cell Viability Study

HCT-116 cell (200µL) culture was cultured in a micro titer plate at 2 X 10^4 cells/ml density without the test sample. After 24 hrs of culture, the cells were treated with HCPE and HCAE at five different concentrations ranging from 25 to 400 µg/mL and standard drug as control.

The test plates were kept in incubator for incubation at 37 °C for 24 hrs in a 5 % CO_2. After that, the plates were removed from the incubator, separated spent media and added tetrazolium salt reagent (0.5 mg/mL). Further, the cells were incubated at 37°C for 3 hrs. After that, the tetrazolium salt reagent was discorded and cells were solubilized in 100 µL of DMSO. The plates were gently stirred in a shaker to enhance dissolution. After that, the O.D. values of each sample were measured using a micro titer plate reader (Biotech) at 570 nm and 630 nm. Cell viability was calculated according to standard methods[17-18].

2.6.3 Measurement of Caspase-3 Activity by ELISA

The cells were cultured (5.3 x 10^4 cells) in 2 mL of culture medium in six-well plates. The cells were treated with a predetermined IC50 value of the HCPE and HCAE for 24 hrs; the attached cells were rinsed with cold PBS, then suspended in the same buffer, and subjected to one freeza/thaw cycle at ambient temperature. The supernatant was separated by using centrifugation and stored at -80 ºC. The supernatant was subjected to ELISA to quantify caspase -3 activity (RayBio® Human Caspase-3 ELISA Kit).

2.6.4 Determination of DNA fragmentation by TUNEL assay using Flow Cytometry analysis

To detect the effect of HCPE and HCAE on DNA fragmentation was assessed using a TUNEL assay. Cells (1-2 × 10^6 well) were cultured in six-well plates with or without *H. coronarium* extracts at 0, 25, 50, 75, or 100 µg/mL for 24 h. After treatment, cells were subjected to determination of DNA fragmentation following previous reports[19-20]. The quantity of DNA breakup in selected cells treated with HCPE, HCAE, and cisplatin was quantified by a Flow cytometer (BD FACS Calibur).

3. STATISTICAL ANALYSIS

Triplicates were maintained for each experiment, and the results were mentioned as mean and standard deviation.

3.1 Results

3.1.1 Yield of the Extracts

H. coronarium rhizomes were collected from Gundlabrahmeswaram forests of Eastern Ghats, Andhra Pradesh, and successfully extracted with petroleum ether and ethanol. Petroleum ether extract (HCPE) yielded a yellow colour oily extract with a characteristic odour (4.14 %), and ethanol (HCAE) produced a brownish-yellow colour extract (7 %), respectively.

3.1.2 Phytochemistry

3.1.2.1 Qualitative Phytochemical Studies

Preliminary phytochemical analysis of HCPE and HCAE extracts was carried out using standard methods and revealed that alkaloids, coumarins, flavonoids, and were present in the test two extracts. Volatile oils and fatty acids were noticed in HCPE (Table 1).

Table 1. Preliminary phytochemical analysis of *Hedychium coronarium* rhizome extracts

Type of compound	HCPE	HCAE
Alkaloids	+	+
Coumarins	+	+
Fatty acids	+	-
Flavonoids	+	+
Steroids	+	-
Terpenoids	-	+
Phenols	-	+
Volatile oils	+	-

HCPE: *Hedychium coronarium* petroleum ether extract, HCAE: *Hedychium coronarium* ethanol extract, +: Present, -: Absent

3.1.2.2 Total Phenolic and Flavonoid Content

The phenolic components quantity of the *H. coronarium* rhizome extracts was evaluated using the standard curve of gallic acid, showing the range of 2.51 ±1.00 to 24.44±2.14 mg GAE/g dwt., while TFC was quantified using the standard linear graph of Quercetin. The TFC of the *H. coronarium* rhizome extracts varied from 1.01±0.08 to 6.18±0.77 mg QE/g dry weight. (Fig. 2**)**.

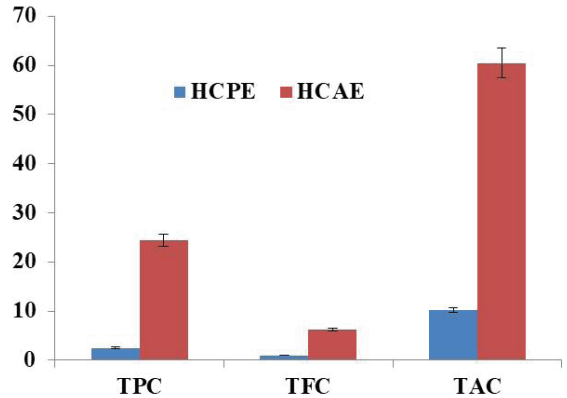

Figure 2. Total phenolic, flavonoids, and total antioxidant capacity of *H. coronarium* rhizome extracts.

3.1.3 Antioxidant Studies

3.1.3.1 Total Antioxidant Capacity Assay

Ammonium molybdate reducing capacity of the extracts obtained from *H. coronarium* rhizome was depicted

Table 2. DPPH scavenging capacity of *Hedychium coronarium* rhizome extracts

HCPE (µg/mL)	% Inhibition	HCAE (µg/mL)	% Inhibition	Gallic acid (µg/mL)	% Inhibition
50	8.70±3.37	70	12.10±1.20	1	20.01±0.2
100	16.96±2.26	140	27.80±0.27	2	32.12±0.1
150	22.49±0.73	210	36.68±1.40	4	51.11±1.21
200	40.22±4.80	280	50.08±0.19	6	64.04±0.1
250	50.12±2.11	350	64.89±1.62	8	75.00±1.0
300	55.02±0.1	420	71.70±1.43	10	82.22±1.1
350	52.45±0.21	490	74.34±1.04	12	90.01±1.01

Table 3. Hydroxyl radical scavenging capacity of *Hedychium coronarium* rhizome extracts

HCPE (µg/mL)	% Inhibition	HCAE (µg/mL)	% Inhibition	Gallic acid (µg/mL)	% Inhibition
5	11.23±2.61	5	23.23±0.43	5	08.01±0.4
10	32.23±0.76	10	28.92±3.26	10	24.01±0.2
15	53.46±1.84	12.5	52.12±0.43	15	39.21±1.1
20	71.46±0.54	15	61.23±1.08	20	50.11±0.21
25	80.07±2.93	20	66.76±1.30	25	60.04±0.1
30	83.69±1.08	25	83.38±0.65	30	74.00±1.02
35	88.61±0.00	30	85.46±0.18	35	80.22±1.21
		35	89.0±0.10		

Table 4. Antibacterial activity of *Hedychium coronarium* rhizome extracts

Organisms	Zone of inhibition mm^{-1}						
	Pet. Ether extract			Ethanol extract			Standards µg/mL
	mg/mL		MIC µg/mL	mg/mL		MIC µg/mL	
	10	25		10	25		
Bacillus cereus MTCC1429	8.66±0.57	10.66±0.57	312±0.15	7.66±0.57	10±1.0	312±0.15	18.66±1.16^{A}
Staphylococcus aureus MTCC737	10.61±0.57	12.66±0.57	312±0.15	9±1.0	11±1.0	312±0.15	18.66±1.16^{A}
Escherichia coli MTCC1687	9.33±0.57	11.33±1.15	312±0.15	8±0.0	9.66±0.57	625±1.22	36.33±1.52^{T}
Pseudomonas aeruginosa MTCC1688	11.33±1.15	16.66±1.15	156±0.0	8.66±0.57	9.66±0.57	625±1.22	36.33±1.52^{T}

Pet. Ether: Petroleum ether, MIC: Minimum inhibition concentration; A: Ampicillin, T: Tetracycline

(Fig. 2**)**. The results revealed that ethanol extract showed higher antioxidant levels (60.44 mg ASE/g dwt.) than that of petroleum ether extract (10.25 mg ASE/g dwt.).

3.1.3.2 DPPH Reducing Assay

Table 2 explains the DPPH radical quenching activity of *H. coronarium* rhizome extracts. All the test extracts reduced the DPPH purple colour in concentration dependent manner. Petroleum ether extract strongly reduced DPPH purple colour by expressing the lowest IC_{50} value (250 µg/mL) and 74 % as maximum inhibition at 490 µg/mL for HCAE extract. Gallic acid was used as a standard component.

3.1.3.3 Hydroxyl Radical Scavenging Assay

Table 3 demonstrates the hydroxyl radical scavenging potentiality of *H. coronarium* rhizome extracts. All extracts exhibited concentration-dependent activity. HCPE and HCAE extracts strongly inhibited hydroxyl radicals generated through the Fenton reaction. HCAE extract expressed the lowest IC_{50} value (12.5 µg/mL) and maximum inhibition as 89 % at (35 µg/mL) concentration.

3.1.4 Antibacterial Studies

H. coronarium rhizomes were collected from Gundlabrahmeswaram (GBM) forests of the Giddalur division, shade-dried, powdered and extracted with petroleum ether, and ethanol. The extracts were diluted and were tested against 4 bacterial strains.

The antibacterial activity results of *H. coronarium* rhizome were tabulated (Table. 4) among the test extracts petroleum ether extract showed significant inhibition against *P. aeruginosa* (16.66±1.15 mm), moderate activity against *S. aureus* (12.66±0.57mm), *E. coli* (11.33 ±1.15 mm) and *B. cereus* (10.66 ±0.57 mm). Ethanol extract exhibited less activity than petroleum ether extract.

3.1.5 Cell Culture Studies

3.1.5.1 Cytotoxic Study

Cytotoxic effects of HCPE and HCAE were assessed against HCT-116 cell lines using *in vitro* studies. The extracts were tested at various concentrations from 25 µg/mL to 400 µg/mL. All the tested extracts exhibited concentration-dependent cytotoxic effects on HCT-116 cell lines, revealing that HCPE and HCAE extracts suppressed (~65 %) the proliferation of HCT-116 cell lines at 400 µg/mL (Figure 3). Based on the potential cytotoxic effect of extracts against HCT-116 cells, further studies, i.e., ELISA and TUNEL assays, are assessed with HCT-116 cells to know the mechanism of anticancer activity of the extracts.

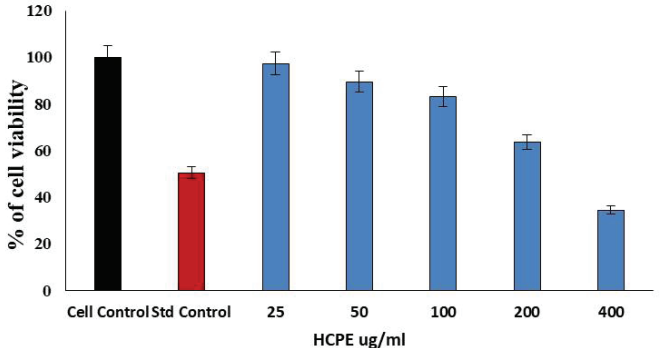

Figure 3. Cytotoxic effects of Hedychium coronarium rhizome extracts (A) Petroleum Ether extract (B): Alcohol extract in HCT-116 cell lines.

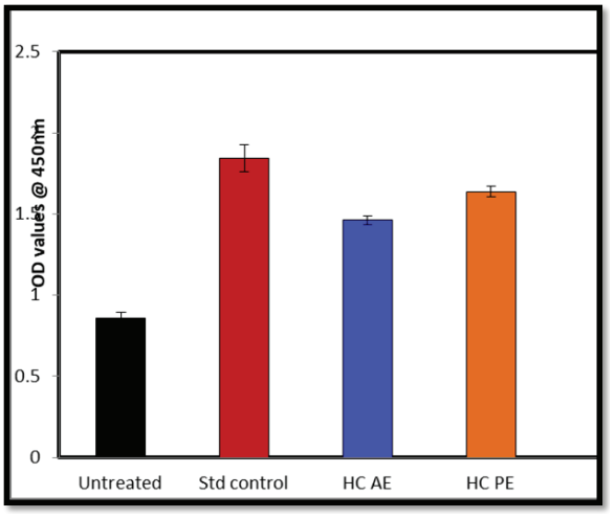

Figure 4. Caspase-3 activity of *Hedychium coronarium* rhizome extracts in HCT-116 cell lines.

3.1.5.2 Quantification of Caspase -3 by ELISA Method

Fifty percent (IC_{50}) inhibition concentration of the extracts was calculated from MTT assay and was subjected to quantitative estimation of caspase-3 in HCT 116 cell lines. Among the test extracts, HCPE exhibited the highest caspase -3 activities (142.73 pg/mL) than the HCAE extract (Fig. 4).

3.1.5.3 Effect of H. Coronarium Extracts on DNA Fragmentation in HCT-116 Cell Lines

The effect of HCPE and HCAE on DNA stands breaking in colon cancer cell lines was measured by TUNEL assay using flow cytometry analysis. The results revealed the cells treated with HCPE showed more DNA damage (85.62 %) (Fig. 5).

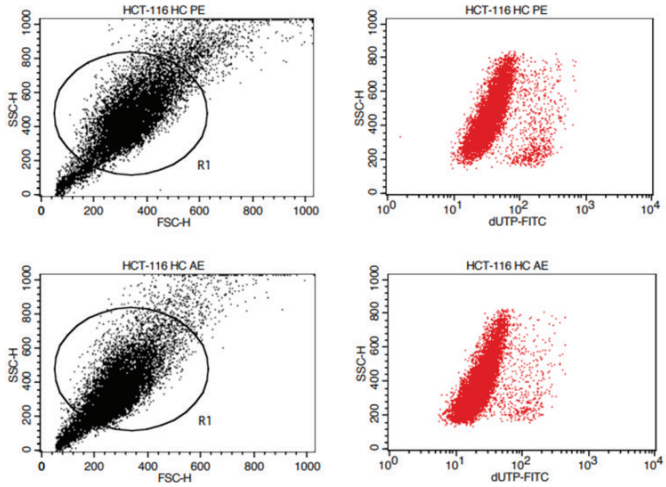

Figure 5. DNA fragmentation activity of *Hedychium coronarium* rhizome extracts in HCT-116 cell lines.

4. DISCUSSION

Hedychium coronarium is a rhizomatous herb of the family Zingiberaceae and is widely cultivated in India, Vietnam, and Southeast Asian countries. Globally, the genus *Hedychium* represents 65 species. Of these, 24 species are present in India, speculating its richness in the Indian subcontinent[21]. The chief chemical components of this species are diterpenes, i.e., coronarins A-D and iso-coronarin D, and volatile components. Phytochemical studies on this plant revealed the presence of phenolic constituents, flavonoids, tannins, saponins, etc. Pharmacological investigations on this plant indicated that it possesses potential anti-inflammatory[22], anti-nociceptive[23], anticancer[24], analgesic and CNS depressant[25], anti-urolithic[26], and antibacterial properties[27].

In the present study, we evaluated the phytochemical composition, antioxidant potential, and anti-cancer activity of *H. coronarium* rhizome extracts. Regarding quantitative estimation of phytochemical composition, Panigrahy *et al.*,[28] estimated the quantity of phenolic group components of organic solvent extracts. They reported that aqueous extract has a higher amount of phenolic content, possibly due to the variation in the climatic conditions prevalent in both atmospheric and edaphic conditions. The antioxidant capacity of *H. coronarium* rhizome extracts by phosphomolybdate method was assessed by Panigrahy[28], *et al.*, revealed that ethyl acetate extract showed higher antioxidant activity (117.56 ± 0.8) than other test five extracts.

In our study, HCAE extract demonstrated higher antioxidant capacity than petroleum ether extract. Panigrahy *et al*., [28] studied the antioxidant potential (IC_{50} values) of six extracts, i.e., hexane, dichloromethane, ethyl acetate, acetone, ethanol, and aqueous extract obtained from *H. coronarium* rhizome on eight free radical model systems. Petroleum ether extract demonstrated stronger DPPH scavenging activity (IC_{50} value 200 µg/mL) than the previous reports of Panigrahy[28], *et al*. (IC_{50} value 256.55 µg/mL). HCPE and HCAE (IC_{50} values 15 µg and 12.5 µg/mL) exhibited potential OH radical scavenging activity than gallic acid (IC_{50} value 30 µg/ mL). Effect of *H. coronarium* rhizome extracts on hydroxyl radical scavenging activity studied by Panigrahy[28], *et al*. indicated that the petroleum ether and ethanol extracts required more than 880 µg/ mL to inhibit 50 % hydroxyl radical; this may be due to the variation in the climate condition and area from where the plant material was collected.

Petroleum ether and ethanol extracts of *H. coronarium* rhizome exhibited concentration-dependent antibacterial activity against the test pathogens. Among the test pathogens, *P. aeruginosa* was strongly inhibited by petroleum ether (16mm; MIC value: 156 µg/mL) extract than the reports shown in the previous studies conducted by Asit Ray & Sangamitra Nayak[29]. Rhizomatous plants of the family Zingiberaceae are important economically and pharmacologically. Several species of this family, such as *Alpini*a, *Curcuma*, *Kaempferia*, *Zingiber*, etc., have been reported various types of pharmacological properties, including cancer. Scientific reports on the anticancer properties of gingers revealed that they showed potent tumor-reducing/ cytotoxic/antiproliferative activity against the different types of cancer cell lines through *in vitro* models[30]. Regarding *H. coronarium*, cytotoxic/anticancer activity of extracts and isolated constituents was reported against breast and cervical cancer cell lines[31-32]. This is the first report/s on anticancer activity of *H. coronarium* against HCT-116 cell lines.

Uncontrolled cell proliferation/growth is a characteristic feature of cancer. Induction of apoptosis and suppression of cancerous cell proliferation is assumed to be the best procedure for cancer therapy[33-34]. Caspase represents the cysteine proteases family, which is responsible for initiating programmed cell death by inducing DNA fragmentation, cell shrinkage, and membrane blebbing. Among the caspases, caspase -3 plays a vital role in inducing apoptosis, and it is believed to be a biomarker for programmed cell death in cancer cells[34]. In the present study, *H. coronarium* extracts strongly suppressed the growth of HCT116 cell lines in a dose-dependent way. Quantification of caspase -3 in cancerous cells is considered the best way to determine apoptosis. So, further, we quantified caspase-3 concentration in HCT-116 cell lines treated with *H. coronarium* petroleum ether and ethanol extracts using the ELISA method. The results showed that petroleum ether extract strongly suppressed the growth of selected cancerous cells by caspase-3 activity (142.73 pg/mL) than ethanol extracts.

H. coronarium, extracts were subjected to the TUNEL assay using flow cytometry analysis to detect the intracellular changes (DNA fragmentation) in colon cancer cell lines. FACS analysis showed that the extracts at tested concentrations, i.e., HCPE (299.39 µg/mL), and HCAE (228.10 µg/mL), exhibited DNA fragmentation in HCT-116 cell lines. Among the test extracts, the cells treated with petroleum ether extract showed more DNA fragmentation (85.62 %) than control cells and other extracts. The extracts are less potent than the standard cisplatin (91.31 %).

5. CONCLUSION

In the present investigation, *H. coronarium,* extracts exhibited a good amount of phytochemical composition. The extracts showed a good amount of phosphomolybdenum dependent antioxidant and strong DPPH and hydroxyl radical scavenging activities. Further, the extracts strongly suppressed the growth of the colon cancer cell lines. HCPE strongly enhanced caspase-3 activity and DNA breaking in HCT116 cell lines. The present research observations suggest that *H. coronarium,* is a rich source of natural antioxidant/ antibacterial and anticancer components. Further molecular studies are to be needed to isolate and characterize active principles and to validate their therapeutic properties in *in vivo* models.

REFERENCES

1. Sung, Hyuna.; Ferlay, Jacques.; Siegel, M.E. Rebecca L.; & Laversanne, Mathieu. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J. Clin.,* 2021, **71**, 209 –249. doi: 10.3322/caac.21660.
2. Lichota, A. & Gwozdzinski, K. Anticancer Activity of Natural Compounds from Plant and Marine Environment. *Int. J. Mol. Sci.,* 2018, **19** (11), 3533. doi: 10.3390/ijms19113533.
3. Lombardi, V.R.; Carrera, I. & Cacabelos, R. In Vitro Screening for Cytotoxic Activity of Herbal Extracts. *Evidence Based Complementory Altern. Med*., 2017, 2675631. doi: 10.1155/2017/2675631.
4. Venkata Raju, R.R. & Pullaiah, T. Flora of Kurnool. Bishen Singh Mahendra Pal Singh, Dehra Dun, India, 1995.
5. Gamble, J.S. Flora of Presidency of Madras, **1–3**, Botanical Survey of India, Calcutta, 1915- 1935.
6. Das, A.K. & Bhattacharjee, A.K. A systematic approach to phytochemical screening. *Trop. Sci.,* 1970, **12**, 54 – 58.
7. Gibbs, R.D. Chemotaxonomy of flowering plants, **1-4.** Montreal and London, 1974.
8. Amarasingham, P.P.; Bisset, N. G.; Millard, P.H. & Woods, M.C. Phytochemical survey of Malaya part III. Alkaloids and Saponins. *Econ. Taxon. Bot.,* 1964, **18**, 270 – 278. doi: 10.1007/BF02908122.
9. Harborne, J.B. Phytochemical methods. Chapman & Hall, London, 1991.
10. Singleton, V.L. & Rossi, J.A. Colorimetry of total phenolics with phosphomolybdicphosphor tungstic acid reagents. *Am. J. Enol. Vitic.*, 1965, **16** (3), 144-55. doi**:** 10.5344/ajev.1965.16.3.144.
11. Lakshman Raju, B. Phytochemical screening, quantitative estimation of total phenolics and total flavonids, antimicrobial evaluation of *Cyamopsis tetragonoloba*.

Int. J. Res. Pharm. Biomed. Sci., 2012, **3**, 1139-42.
12. Umamaheswari, M. & Chatterjee, T.K. In-vitro antioxidant activities of the fractions of *Coccinia grandis* L. leaf Extract. *African J. Tradi. Complementary Altern. Med.*, 2008, **5** (1), 61-73. PMID: 20162057.
13. Braca, A.; Sortino, C. & Politi, M. Antioxidant activity of flavonoids from *Licania licaniae flora. J. Ethnopharmacol.*, 2002, **79** (3): 379-81. doi: 10.1016/s0378-8741(01)00413-5.
14. Halliwell, B.; Gutteridge, J.M. & Aruoma, O.I. The deoxyribose method: a simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. *Anal. Biochem.*, 1987, **165** (1), 215-19. doi: 10.1016/0003-2697(87)90222-3.
15. Cruikshank, R. 11th ed. Medicinal microbiology: A guide to diagnosis and control of infection. Edinburgh and London: E and S Livingston Ltd., 1968, pp. 888.
16. National Committee for Clinical Laboratory Standards. Performance Standards for Anti-Microbial Susceptibility Testing: 11th International 91: 105-8. Supplement. NCCLS, Document M 100 - S 11, 2001.
17. Alley, M. C.; Scudiere, D. A.; Monks, A.; Czerwinski, M.; Shoemaker, R. II. & Boyd, M. R. Validation of an automated microculture tetrazolium assay (MTA) to assess growth and drug sensitivity of human tumor cell lines. *Proc. Am. Assoc. Cancer Res*., 1986, **27,** 389.
18. Enari, M.; Sakahira, H.; Yokoyama, H.; Okawa, K.; Iwamatsu, A. & Nagata, S. A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. *Nature*, 1998, **391**(6662), 43-50. doi: 10.1038/34112.
19. Li, X.; Traganos, F.; Melamed, M.R. & Darzynkiewicz, Z. Single-step procedure for labeling DNA strand breaks with fluorescein- or BODIPY-conjugated deoxynucleotides: detection of apoptosis and bromodeoxyuridine incorporation. *Cytometry*, 1995, **20**(2), 172-180. doi: 10.1002/cyto.990200210.
20. Chaithra, B.; Satish, S.; Karunakar, Hegde. & Shabaraya, A.R. Pharmacological Review on *Hedychium coronarium* Koen.: The White Ginger Lily. *Int. J. Pharma. Chem Res.*, 2017, **3** (4), 831-836.
21. Sangeeta, S.; Mohammad Ali, S.; Saha, A.; Bachar, S.C. & Islam, Md. S. Anti-inflammatory and analgesic effects of *Hedychium coronarium* Koen. *Pak. J. Pharm. Sci*, 2017, **20**(1), 42-47. PMID: 17337428.
22. Singh, Poonam & Raman, Surendra. Antinociceptive activity of ethanolic extract of the rhizomes of *Hedychium coronarium. Asian J. Sci. Tech.*, 2015, **6**(1), 980-983.
23. Endringera, Denise C.; Taveirab, Francisca S.N.; Kondratyukc, Tamara P.; Pezzutoc, John M. & Bragaa, Fernão C. Cancer chemoprevention activity of labdane diterpenes from rhizomes of *Hedychium coronarium*. *Braz. J. Pharmacogn.*, 2014, **24**(1), 408-412. doi: 10.1016/j.bjp.2014.08.002.
24. Dash R, Pritesh.; Nasrin, Mahmuda & Saha Rani, Moni. Evaluation of analgesic and neuropharmacological activities of ethanolic rhizome extract of *Hedychium coronarium*. *Int. J. Pharma Sci. Res*, 2011, **2** (4), 979-984. doi: 10.13040/IJPSR.0975-8232.2(4).979-84.
25. Chandra Shekhar, T. & Goyal, Anju. Isolation of phytoconstituents and in vitro antilithiatic by titrimetric method, antioxidant activity by 1,1- diphenyl -2-picryl hydrazyl scavenging assay method of alcoholic roots & rhizomes extract of *Hedychium coronarium* j. Koenig plant species. *Asian J. Pharm. Clin. Res*., 2015, **28** (4), 225-229.
26. Prakash, Om.; Rajput, M.; Kumar, Mahesh & Pant, A.K. Chemical Composition and Antibacterial Activity of Rhizome Oils from *Hedychium coronarium* Koenig and *Hedychium spicatum*. Buch-Ham. *J. Essen. Oil Bear. Plants.*, 2010, **13**(2), 250-259. doi: 10.1080/0972060X.2010.10643819.
27. Panigrahy, S.K.; Kumar, A. & Bhatt, R. Antioxidant potentials of successive solvent extracts from the unexplored *Hedhychium coronarium* rhizome. *J. Food Sci. Technol*., 2017, **54**(10), 3297-3306. doi: 10.1007/s13197-017-2777-3.
28. Ray, Asit. & Nayak, Sanghamitra. Phytochemical and biological activity of *Hedychium coronarium* extracts. (Lambert academic publishing), 2018, ISBN: 978-613-9-86942-8.
29. Verma, P. & Kundu, R. The Ginger Prophecy; A Review of the Underexplored Genus, *Hedychium* against Cancer. *Indian J. Pharm. Sci.*, 2020, **2**(1), 11-20. doi: 10.36468/pharmaceutical-sciences.618.
30. Donipati, P. & Sreeramulu, S.H. In vitro Anticancer Activity of *Hedychium coronarium* Against Human Breast Cancer Cell Line MCF-7. *Int. J. Adv. Res.*, 2015, **3**(10), 1497-501.
31. Ray, A.; Jena, S.; Dash, B.; Sahoo, A.; Kar, B.; Patnaik, J.; Panda, P.C.; Nayak, S. & Mahapatra, N. *Hedychium coronarium* extract arrests cell cycle progression, induces apoptosis, and impairs migration and invasion in HeLa cervical cancer cells. *Cancer Manage. Res*., 2019, **11**, 483-500. doi: 10.2147/CMAR.S190004.
32. Woo, M.; Hakem, R.; Soengas, M.S.; Duncan, G.S.; Shahinian, A.; Kagi, D.; Hakem, A.; McCurrach, M.; Khoo, W. & Kaufman, S.A. Essential contribution of caspase 3/CPP32 to apoptosis and its associated nuclear changes. *Genes Dev.*, 1998, **12**(6), 806-819. doi: 10.1101/gad.12.6.806.
33. Shi, X.; Wu, S.; Yang, Y.; Tang, L.; Wang, Y.; Dong, J.; Lu, B.; Jiang, G. & Zhao, W. AQP5 silencing suppresses p38 MAPK signaling and improves drug resistance in colon cancer cells. Tumour. *Biol.,* 2014, **35**(7), 7035-45. doi: 10.1007/s13277-014-1956-3.
34. Sui, Y.; Li, S.; Shi, P.; Wu, Y.; Li, Y.; Chen, W.; Huang, L.; Yao, H. & Lin, X. Ethyl acetate extract from *Selaginella doederleinii* Hieron inhibits the growth of human lung cancer cells A549 via caspase-dependent apoptosis pathway. *J. Ethnopharmacol.*, 2016, **190**, 261–271. doi: 10.1016/j.jep.2016.06.029.

CONTRIBUTORS

Mr P. Vishnu Mohan Reddy pursuing his doctoral research work in the department of Botany, Rayalaseema University, Kurnool.

He was involved in the conceptualisation and carried out the research work.

Dr P. Raghuveer Yadav is working as Technical Officer at IIT, Hyderabad.
His area of interest is Fluorosis prevention & control, Bio fertilizers, Wild life Conservation & Scientometrics.
He was involved in the designing of study and analyzed the data and wrote the manuscript.

Dr K. Venkata Ratnam is working as Assistant Professor, Department of Botany, Rayalaseema University, Kurnool. He is an expert in identification and screening of medicinal plant extracts for antibacterial, antifungal and cell culture studies and phytochemical analysis.
He was involved in designing the research work and prepared manuscript.