Eco. Env. & Cons. 28 (October Suppl. Issue) : 2022; pp. (S448-S454)
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ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2022.v28i06s.074

Evaluation *In vitro* Antioxidant and Anti-cancer Activity of *Rauvolfia tetraphylla* L. on Breast Cancer Cell Lines

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(Received 5 April, 2022; Accepted 22 July, 2022)

ABSTRACT

Rauvolfia tetraphylla L. is a significantly endangered woody shrub that belongs to the family Apocynaceae and it has enormous medicinal properties. Although the plant *Rauvolfia tetraphylla* is used to treat cancer in the traditional medicine of some regions, its cytotoxic activity has not been subjected to rigorous investigation. Extracts were obtained from various organic solvents such as Hexane, Chloroform, Ethyl acetate and Methanol by the sequential method.The antioxidant activity was accomplished on various crude extracts by 2-diphenyl-1-picrylhydrazyl (DPPH) assay of *R. tetraphylla* and showed maximum radical scavenging activity in methanol extract of fruit (IC_{50} = 60.37 µg/ml) followed by methanol extract of the leaf (IC_{50} = 62.61µg/ml). Screening of *in vitro* cytotoxicity was performed on all the crude extracts by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on Breast Cancer cell line (MDAMB231). Among the four-leaf extracts, maximum cytotoxicity activity was obtained by methanol 73.20 µg/mL and the IC_{50} value 64.29 µg/mL. Fruit extracts showed maximum cytotoxicity in 65.30 µg/mL methanol and IC_{50} values were 74.84µg/mL.

Key words : *Rauvolfia tetraphylla, Callus induction, Phytochemical analysis, Antioxidant and cytotoxicity*

Introduction

Cancer is one of the dreadful non-communicable diseases (NCDs) that can affect any part of the body. According to World Health Organization (2019), In India, 63% of total deaths are accounted for non-communicable diseases and out of which cancer is the leading cause. It arises from transforming normal cells into tumor cells in a multistage process. There are various types of cancers are found among which lung, liver, stomach and prostate cancer are mostly affects men whereas in women affects breast, lung, thyroid and cervix cancer. (Bray *et al*., 2018). Researchers found that the five leading sites of the disease were breast, lung, mouth, cervix, uteri, and tongue. Breast cancer is one the most common form

of cancer diseases in women and it causes death for women. Over 1.3 million peoples were diagnosed throughout the world, of which 45,000 women died from breast cancer per year (Garcia *et al*., 2007). Therefore, there is a need for new strategies to prevent and cure this type of cancer.

Breast cancer is metastatic cancer, and it can commonly transfer to organs such as the liver, bone, brain, and lung. Initial treatment of the cancer disease can lead to a good diagnosis and an increase in high survival rate (DeSantis *et al*., 2015). The use of alternative drugs to suppress the tumor growth has dramatically increased; to overcome the restrictions in treating breast cancer such as metastasis leads to illness and death thereby increase the response of immune system and diminish the inflammation which is affected by cancer. (Syed Najmuddin *et al*., 2016). Anti-cancer activities derived from medicinal plants have been greatly increased where their bioactive compounds are utilized for their effective cytotoxic potential (Govind and Madhuri, 2006). Natural compounds with anti-cancer activity can bring about cell death through several mechanisms of action, including regulating the cell cycle and the triggering of apoptotic and non-apoptotic pathways. The apoptotic pathway has received more attention in research (Gali-Muhtasib *et al*., 2015).

Herbal medicine has become a safe, non-toxic, and easily available source of cancer-treating compounds. Herbs are believed to neutralize the effect of diseases in a body because of various characteristics they possess (Cheng, 1995). *Rauvolfia tetraphylla* is one of the important traditional medicines throughout the world which is traditionally used to cure several disorders. In developing and developed countries, herbal medicines have been increased to treat various diseases (Eldeen *et al*., 2016). The plant contains a vital role in the ancient system of Indian medicine, which is widely used in South Indian tribes (Rohela *et al*., 2013). According to Latha and Agastian (2015), the leaves of *R. tetraphylla* are used for treating eczema in Tamil Nadu, India. Ranganathan*et al*., (2012) reported that leaves and fruit of *R. tetraphylla* are used against snakebite in Thiruvannamalai district, Tamil Nadu. Mahalakshmi *et al*. (2019) analysed the leaves and root of *R. tetraphylla* to treat stomach pain in Odisha, India. Thus the leaves and fruit of *R. tetraphylla* are claimed to have medicinal values such as antimicrobial, antioxidant, anti-inflammatory, anti-cancer, cardioprotective, antihypertensive, insecticidal and antiparasitic activities based on the ethnobotanical practices (Behera *et al*., 2016; Mahalakshmi *et al*., 2019). However, this plant has not been studied for anti-cancer activity in human breast cancer and there are few studies were carried out for antioxidant activity. Thus, we have attempted to use this herbal plant to check the efficacy against human breast cancer cell lines. The present study aims to determine the antioxidant and anti-cancer activities of *Rauvolfia tetraphylla* leaf and fruit extract on MDA-MB-231 human breast cancer lines.

Materials and Methodology

Collection and processing of planting material

Rauvolfia tetraphylla were collected from Top slip, Western Ghats (Tamilnadu, India). The collected plant material (leaves and fruit) were washed thoroughly in tap water and dried in shade followed by grinding to a fine powder. The powdered sample were soaked in the various organic solvent for about 5 to 6 days then the extract is subjected to filtration and concentrated at room temperature. Extraction of plant material was carried out using sequential method. Successive extraction was done using solvents of increasing polarity namely Hexane, Chloroform, Ethyl acetate and Methanol. Semi solid extracts were kept in tight screw capped bottle and same were used for all the tests.

Evaluation of DPPH Scavenging Activity

According to a method reported by Hatano *et al*. (1988) the free radical scavenging activity of various crude extracts of *R. tetraphylla* leaf and fruit were analyzed using DPPH (2-diphenyl-1-picrylhydrazyl). The 0.1 mM of DPPH dissolved in methanol and various concentrations of extracts such as 10, 20, 30, 40 and 50 µg/ml tested. 1 mL of DPPH with various concentrations of extracts was added with 3 mL of methanol and incubated at room temperature for 30 min at dark. Ascorbic acid is used as a standard. Absorbance was measured at 517 nm using the Labman UV spectrometer. Decrease in absorbance of the reaction mixture indicates higher free radical scavenging activity. These measurements were performed in duplicate, and the percentage of inhibition (Pi) was calculated using the following equation:

$$\text{Percentage of inhibition} = \text{Ab-As}/\text{Ab} \times 100$$

Ab is the absorbance of the control, and *As* is the

absorbance of extract. The IC_{50} values were calculated using linear regression analysis and used to indicate antioxidant capacity. The data obtained in this study were expressed as mean ± standard deviation.

Evaluation of Anticancer Activity

Cell and culture condition

MDA-MB-231—Human breast Cancer cell line was obtained from Department of Biochemistry, Biotechnology and Bioiformatics, Avinashilingam Institute for Home Science and Higher Education Foe Women, Coimbatore. The cells were cultured in a horizontal laminar flow hood and incubated in a NUAIR cell incubator at 37°C in an atmosphere of 5% carbon dioxide and 95% air. The cells were provided with growth media of 90% Minimal Essential Medium (Eagle), fetal bovine serum as well as 5% streptomycin-penicillin. Medium were refreshed atleast three times a week and the cells were sub-cultured. The cells were re-suspended in 5mL of growth media. The cell concentration was determined by counting the cells in the hemocytometer. The live cells are clear from were counted and dead cells were left.

MTTAssay

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method, as described by Mosmann (1983), was used to detect living cells quantitatively. Approximately 2×10^4 cells/well were seeded onto 96 well plates, 100 µL of MEM medium was added and incubated at 37°C for 24 hr. Then the medium was discarded and fresh medium was added with various concentrations (10, 20, 30, 40 and 50 µg/ml) of leaf and fruit extracts of *R. tetraphylla*. The reaction mixture was incubated at 37°C in a carbon dioxide incubator for 24 hours. After respective incubation period the medium was discarded and 100 µL of fresh medium was added with 10 µL of MTT (5 kg/ml). After 2hrs of incubation, the medium was discarded, and 100 µL of DMSO was added to dissolve the formazan crystals. Then, the absorbance was noted using a spectrophotometer at 570 nm. The results were calculated using the following formula to identify the viability of the treated cells.

Viable cells (%) = Optical Density of sample/ Control OD × 100

IC_{50} values were calculated as the concentrations that show 50% inhibition of proliferation on any tested cell line.

Data Analysis and Interpretation

Free radicals play a significant role in developing tissue damage and pathological events. To investigate the ability of various crude extracts to scavenge free radicals. The study of *in-vitro* antioxidant activity was carried out using hexane, chloroform, ethyl acetate and methanol extracts of *R. tetraphylla* leaf and fruit were evaluated using DPPH antioxidant assay as shown in Table 2 & 3. The antioxidant activity was indicated by changing color from purple to yellow and OD values were observed under UV spectroscopy with the absorbance of 517 nm. Ascorbic acid is used as a standard as in Table 1. Antioxidant activity of *R. tetraphylla* showed maximum antioxidant activity in methanol extract of fruit (IC_{50} = 60.37 µg/ml) followed by methanol extract of leaf (IC_{50} = 62.61µg/ml) (Fig. 1&2). Based upon the values, the scavenging activity of all the crude extracts was relatively lower concentration than standard

Table 1.

Concentration (µg/mL)	Ascorbic acid	IC_{50}
20	28.91 ± 3.95	46.28
40	42.64 ± 4.07	
60	69.13 ± 5.6	
80	75.14 ± 2.71	
100	81.14 ± 0.86	

Values represent mean ± standard deviation.

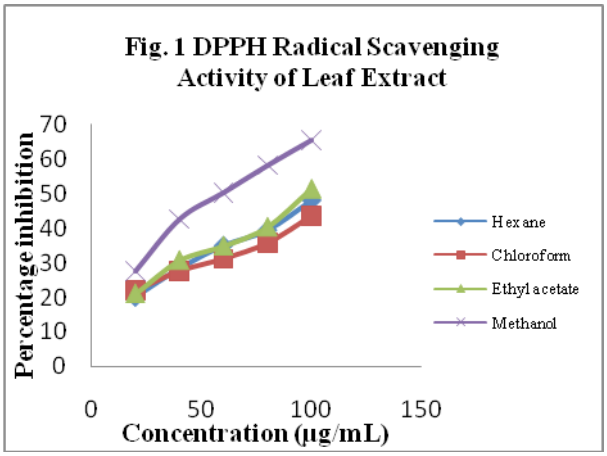
Table 2.

Concentration (µg/mL) Leaf	20	40	60	80	100	IC_{50}
Hexane extract	19.99 ± 0.68	28.26±0.08	34.83±0.07	39.33±0.18	47.93 ± 2.91	107.57
Chloroform extract	21.90 ± 0.58	27.51 ± 0.73	31.07 ± 2.14	35.65 ± 0.96	43.45 ± 1.02	130.56
Ethyl acetate extract	21.03 ± 2.38	30.67 ± 1.71	34.78 ± 0.57	40.29 ± 2.96	51.28 ± 1.4	101.02
Methanol extract	27.41 ± 0.30	42.54 ± 1.26	50.26 ± 0.18	58.19 ± 0.56	65.55 ± 0.92	62.61

Values represent mean ± standard deviation.

ascorbic acid (IC_{50} = 46.28 µg/ml), which is used as a positive control. It can be because extracts are a mixture of several molecules compared to that pure antioxidant. As alkaloids and flavonoids are present in both the extracts of leaf and fruit, they might be subsidizing towards the antioxidant property reported by Behera *et al*. (2016), which illustrates the potential of antioxidant activity.

Likewise, Vinay *et al*., (2016) analyzed four crude extracts such as methanol extract of fruit and n-hexane, dichloromethane and methanol extracts of the leaf of *R. tetraphylla* were investigated for *in vitro* antioxidant activity at different concentrations (5, 50 and 100ìg) and showed that the leaf n-hexane and methanol extracts were as considerably active. Therefore, antioxidant metabolism is essential in determining the ability of plants to survive oxidative stress. Nair *et al*., (2012) determined the antioxidant activity of methanolic crude extract obtained from *R. tetraphylla* by metal chelating, DPPH scavenging, superoxide scavenging, total antioxidant and reducing power assay. Archana and Jeyamanikandan (2015) revealed the antioxidant activity methanol extract obtained from *R. tetraphylla* leaf against DPPH assay. Prasad *et al*., (2013) evaluated radical scavenging activity of various extracts such as methanol, hexane and chloroform from the leaf of *R. tetraphylla* against DPPH scavenging and reducing power assay. Maheshu *et al*., (2010) analysed the methanol extract obtained from leaves of *R. tetraphylla* exhibited antioxidant activity against DPPH radical scavenging activity, ABTS scavenging and reducing power assays. Helan and Vignesh



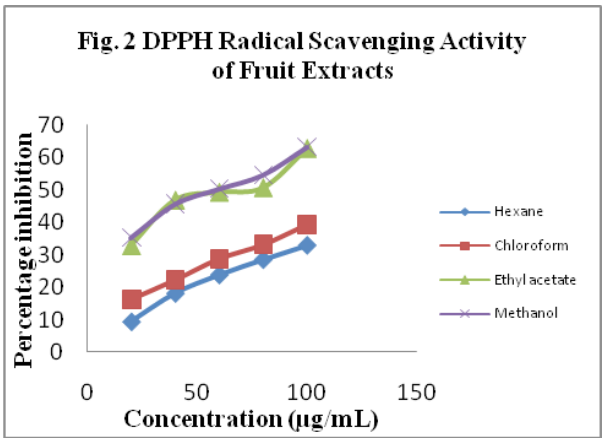


Table 3.

Concentration (µg/mL) Fruit	20	40	60	80	100	IC_{50}
Hexane extract	9.26 ± 0.02	18.01 ± 1.24	23.72 ±0.96	28.44 ± 0.96	32.85 ± 0.96	155.62
Chloroform extract	16.26 ± 0.92	22.23 ± 0.90	28.76 ±1.00	33.14 ± 3.61	39.33 ± 0.64	137.32
Ethyl acetate extract	32.97 ± 0.40	47.00 ± 0.60	49.43 ± 0.008	50.84 ± 0.25	62.90 ± 0.10	64.27
Methanol extract	35.30 ± 1.70	45.69 ± 2.60	50.40 ± 0.65	54.70 ± 0.88	63.28 ± 0.44	60.37

Values represent mean ± standard deviation.

Table 4.

S. No.	Concentration (µg/ml) Leaf	Hexane extract	Chloroform extract	Ethyl-acetate extract	Methanol extract
1	20	12.15 ± 0.003	14.78 ± 0.003	11.56 ± 0.002	14.78 ± 0.003
2	40	36.60 ± 0.006	37.62 ± 0.003	35.43 ± 0.004	39.97 ± 0.003
3	60	46.70 ± 0.005	44.80 ± 0.003	42.75 ± 0.004	48.75 ± 0.006
4	80	55.78 ± 0.003	57.68 ± 0.008	56.51 ± 0.002	58.71 ± 0.003
5	100	67.34 ± 0.009	66.91 ± 0.002	69.25 ± 0.007	73.20 ± 0.004
6	IC_{50}	69.692391	69.072084	70.105672	64.297241

Values represent mean ± standard deviation of three replicates per treatment.

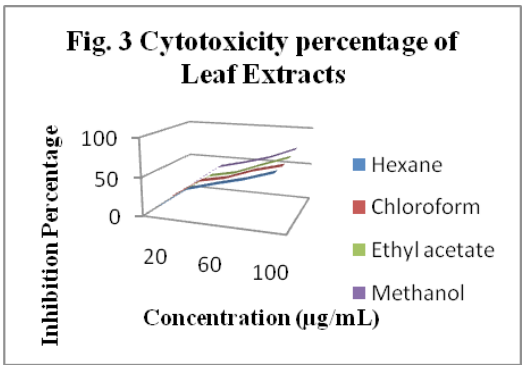
(2016) evaluated the scavenging activity of ethanol extract of leaf of *R. Canescens* against DPPH assay. Oza and Solanki (2016) analysed the methanol extract of *R. tetraphylla* leaf, fruit, and root to exhibit antioxidant activity against DPPH scavenging activity. The activity observed in the antioxidant assay was dose-dependent. Vinay *et al.*, (2019) examined silver nanoparticles from *R. tetraphylla* leaves extract to analyse the antioxidant activity and it has shown the IC_{50} (inhibitory concentration 50%) value of 82.13 µg/mL against the DPPH free radical scavenging activity.

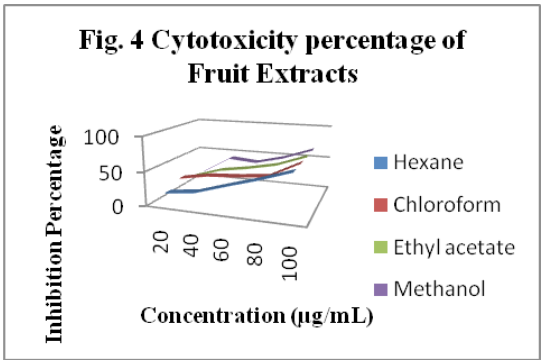
Many chronic health problems such as cardiovascular disease, inflammatory disease, cataracts, the ageing process, cancer are caused by free radicals. Antioxidants help to prevent free radicals and scavenge them to manage those diseases. DPPH free radical scavenging activity is one of the easiest, stable and more rapid methods to identify the antioxidant activity against plant extracts. As a result, all the crude extracts that showed antioxidant activity were obtained with increased concentration.

Evaluation of Anticancer activity

The *in vitro* cytotoxicity was carried out with various crude extracts such as Hexane extract, chloroform extract (CHLO), ethyl acetate (ETOAC), methanol extract (MEOH) of *R. tetraphylla* using MTT assay in Breast Cancer Cell line (MDA-MD-231) as in Tables 4&5. Among the four-leaf extracts, methanol and ethyl acetate showed maximum cytotoxicity activity of 73.20 and 69.25 µg/ml were obtained and IC_{50} values 64.29 and 70.10 µg/ml, respectively. The fruit extract showed maximum cytotoxicity in methanol and ethyl acetate of 65.30 and 64.42 µg/ml and IC_{50} values 74.84 and 77.86 µg/ml, respectively (Fig. 3 & 4).

Reserpine has been substantiated for the anti-cancer activity against breast cancer cell lines





(Abdelfatah and Efferth, 2015). Mostly, the natural compounds retain their anti-cancer activity by restrictive cancer cell metabolism. Metabolic activity is also considered important for cancer characteristics. The uncontrolled state of cancer cells makes them consume more nutrients than normal cells (Keenam and Chi, 2015). A natural compound as a mixture of conservative anti-cancer agents and metabolic activity could also increase therapeutic activity. The methodical toxicity evaluation of an active compound is obligatory for therapeutic application. This analysis will give the preventive measure about the extent of medicine before human exposure (Hunter, 2008; Saleem *et al.*, 2017).

Five new monoterpenoidindole alkaloids such as rauvotetraphyllines F–H (**1**, **3**, **4**), 17-*epi*-

Table 5.

S. No.	Concentration (µg/ml) Fruit	Hexane extract	Chloroform extract	Ethyl acetate extract	Methanol extract
1	20	17.27 ± 0.008	27.08 ± 0.004	18.15 ± 0.009	16.10 ± 0.019
2	40	22.54 ± 0.016	34.55 ± 0.001	31.91 ± 0.007	41.70 ± 0.007
3	60	36.31 ± 0.15	37.62 ± 0.0007	38.94 ± 0.008	38.21 ± 0.010
4	80	49.48 ± 0.018	42.16 ± 0.016	48.02 ± 0.007	49.34 ± 0.004
5	100	64.42 ± 0.004	63.98 ± 0.010	64.42 ± 0.011	65.30 ± 0.015
6	IC_{50}	79.789144	81.911548	77.869477	74.844369

Values represent mean ± standard deviation of three replicates per treatment.

rauvotetraphylline F (**2**) and 21-*epi*-rauvotetraphylline H (**5**) isolated from the aerial parts of *R. tetraphylla* were screened for *in vitro* cytotoxic activity against five Human cell lines namely, HL-60, SMMC-7721, A-549, MCF-7, and SW-480 by MTT methodwith an IC50 value of >40 μM (Gao *et al*., 2015). Kakad and Dhembare (2014) analysed the anti-cancer activity of *R. tetraphylla* leaf extract against chick embryo fibroblast cell line. The viability of the cell line is 50.54%. Beheral *et al*., (2016) revealed cytotoxic activity against brine shrimp lethality assay of *R. tetraphylla* leaf and fruit of hexane, chloroform, acetone and methanol extracts. Chloroform extract of leaf and acetone extract of fruit showed active cytotoxic potential.

Our study provides detailed biological activities of *R. tetraphylla* leaf and fruit extracts that can be used for numerous biomedical production and cancer research; however, further research is needed to explore the toxicity and downside effects of *R. tetraphylla* leaf and fruit extracts as a possible curative remedy for various human diseases.

Conclusion

R. tetraphylla is an important medicinal plant; various extracts such as hexane, chloroform, ethyl acetate and methanol were used for analysis. It is a renowned medicinal herb for its pharmacological activities, such as antioxidants and anti-cancer characteristics. Our results concluded that *Rauvolfia tetraphylla* is responsible for potential anti-cancer activity. Researchers have spent a tremendous amount of time and resources to find the importance of medicinal plants. Every plant contains special compounds which help in various pharmacological purposes. The work carried out was a basic approach to find out the biological activity of *Rauvolfia tetraphylla*. Further work is needed to purify and isolate an individual group of bioactive components from the plant extracts and carry out the exact potential of pharmaceutical studies.

Data Availability

The data used to support the findings of this study are incorporated within the article and can be liberally available to authors with suitable reference in their research work.

Conflict of Interest

The authors declare that there is no conflict of interest regarding this research study.

Acknowledgement

Our sincere thanks to Avinashilingam Institute for Home Science and Higher Education for Women for providing moral support to complete our project. Many thanks to Tamil Nadu forest department for collecting this plant. I would like to express my gratitude to the Department of Botany, Zoology, Biochemistry and Biotechnology for their support in completing the project. My special thanks to my supervisor for their extraordinary support in this thesis process.

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