MS.ID-IJPBS-2671
International Journal of Pharmacy and Biological Sciences-IJPBS™ (2023) 13 (1): 145-149
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | OA Journal | MCI Approved | Index Copernicus

In vitro Assessment of Cytotoxicity and Antioxidant Activities of Sida Ovata Forssk Leaves Extract

Prashanth Kumar Nandru[1], Baswaraju Macha[1], Narsimha Reddy Yellu*

[1*] Pharmacology Division, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, Telangana-506009, India.

[1]Medicinal Chemistry Division, Jayamukhi college of Pharmacy, Kakatiya University, Narsampet, Telangana-506332, India.

Received: 10 Oct 2022 / Accepted: 8 Nov 2022/ Published online: 01 Jan 2023
***Corresponding Author Email:** ynrku@yahoo.co.in

Abstract

Sida cordata is plant of *malvaecea*e, native to India. The present study was aimed to focus on antioxidant and cytotoxic properties of methanolic and acetone extract of Sida cordata. The antioxidant activity was evaluated by DPPH free radical scavenging method, estimating total phenolic as well as flavonoid content and cytotoxic properties was evaluated by using brine shrimp lethality bioassay. For free radical scavenging IC_{50} 190µg/ml compared to ascorbic acid IC_{50} 10 µg/ml. The total phenolic content ranged from 145.45 mg/g to 130mg/gm of dry weight of extract, expressed as gallic acid equivalents. The cytotoxicity was reported in terms of lethality concentration LC_{50} for crude extract was 263.02 µg/ml. Sida cordata can be regarded as promising candidates for natural plant sources of antioxidants as well as cytotoxic compound with significant value and can be harnessed and purified into useful therapeutic drugs.

Keywords

Brine shrimp Lethality, DPPH Scavenging, Flavonoids Content, Phenolic Content.

*****

1.0. INTRODUCTION:

Drug development using natural products has been extensively explored by researchers, and the use of these naturally derived products is frequent in cancer research. Plants play a vital role in the medicinal system for the treatment of disease through their herbal compounds, either in the solitary or allied form [Tiwari et al. 2018 & Kumar and Sharma 2018]. In the Indian and global traditional therapeutic systems, plant-derived substances have been used for the treatment of numerous diseases for a long time [Shamim et al. 2016].

Extraction from different portions of plants along with their characterization of functional components and antioxidant, antimicrobial, and anticancer activities are always pursued by scientists. The other important topic that requires extensive work is establishing modern scientific evidence in support of the traditional therapeutic system [Kondhare and Lade 2017]. Researchers from all around the world are working on whether antioxidants retrieved from plants are cancer suppressors or enhancers and targeting the possibility of antioxidant activity in cancer treatment [Hawk et al. 2016]. Due to its geographical advantages, the state of Jharkhand in India is rich in plant diversity. Forty percent of the total area of Jharkhand is forest that is comprised of more than 160 plant species having medicinal value.

These plants are commonly and widely used by local tribes; therefore, it is necessary to explore these plants extensively using efficient scientific facilities [Sharma et al. 2016]. Therefore, the present study, which deals with the successive extraction and cytotoxicity assays of extracts from the leaves of the above-mentioned plants, contributes significantly to the validation of antioxidant and anticancer activities.

2.0. MATERIALS AND METHODS:

2.1. MTT Assay method: (Sendiero et al., 1998) Materials Cell culture: A549 or MCF-7. Cell number for subculture: one million cells for flask (30 ml capacity). Cell loading into plate: 1000-2000 cells per well (96-well plate). Drug solutions: 100 µl of 1 µg/ml to 100 µg/ml.

Measurement of cell viability and proliferation forms the basis for numerous in vitro assays of a cell population's response to external factors. The reduction of tetrazolium salts is now widely accepted as a reliable way to examine cell proliferation. The yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) is reduced by metabolically active cells, aspart by the action of dehydrogenase enzymes and to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazan can be solubilized and quantified spectrophotometrically. The formazan product has a low aqueous solubility and is present as purple crystals. Dissolving the resulting formazan with proper detergents such as DMSO permits the convenient quantification of product formation. The intensity of the product color is measured at 550 - 590 nm and it is directly proportional to the number of living cells in the culture. The MTT assay developed by T. Mossman (1983) is still among one of the most versatile and popular assays. Procedure 1. The adherent cells were trypsinized according to protocol and were resuspended in fresh medium after centrifugation. Cell suspension was mixed thoroughly by pipetting several times to get a uniform single cell suspension. 2. Different dilutions of drug solutions were made with final DMSO concentration in the well to be less than 1%. 3. 100 µl of cell suspension was transferred aseptically to each well of a 96 well plate and to it 100 µl of drug solution (in quadruplicate) in media was added. 4. The plate was then incubated at 370C for 72 hours in CO2 incubator. 5. After 72 hours of incubation, 20 µl of MTT was added to each well. The plate was again, incubated for 2 hours. 6. 80 µl of lysis buffer was added to each well and the plate was wrapped in aluminum foil to prevent the oxidation of the dye and then the plate was placed on a shaker for overnight. 7. The absorbances were recorded on the ELISA reader at 562 nm wavelength. The absorbance of the test was compared with that of DMSO control to get the % inhibition.

Table 1: Cumulative percentage inhibition of different extracts on MCF7 cells

Concentration (µg/ml)	PERCENTAGE INHIBITION		
	5-FLUOROURACIL	Acetone extract	Methanolic extract
20	40.65	12.64	4.6
40	54.2	24.64	11.22
60	69.71	38.97	23.54
80	86.31	47.72	37.67
100	96.48	59.71	56.51

2.2. Antioxidant activity:

In-vitro antioxidant methods Recently there are numerous methods that have been developed to evaluate antioxidant activities of compounds and of complex mixtures such as plant extracts Despite the existence of these various methods just one procedure cannot identify all possible mechanisms characterizing an antioxidant activity. Therefore, the aim of this study is to evaluate the anti-oxidative activity of methanol and acetone extract of leaves of "Sida ovata Forssk" using the following methods:

- DPPH radical scavenging activity.
- Total phenolic content

DPPH radical scavenging assay: Reagents: 0.1 Mm DPPH (1, I-Diphenyl-2-picryl-hydrazyl). Principle: DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The color changes from purple to yellow after reduction which can be quantified by its decrease of absorbance at wavelength 517 nm. Radical scavenging activity increased with an increasing percentage of free radical inhibition. The degree of discoloration indicates the free radical scavenging potentials of the sample/antioxidant by their hydrogen donating ability. The electrons become paired off and the solution loses colour stoichiometrically depending on the number of electrons taken up (Blios et al., 1958). Procedure: 1 ml of 0.1 mM solution of DPPH in methanol was added to 2.5 ml of the test extract in methanol (10-

100 µg/ml). The reaction mixture was then allowed to stand at room temperature in a dark chamber for 30 min. After 30 min absorbance was measured at 517 nm using UV-Visible spectrophotometer (Blios et al., 1958). Ascorbic acid was used as a standard.

The scavenging activity of DPPH radical (%) was calculated from the following equation:

% scavenging activity = Absorbance of control−Absorbance of sample x 100.
Absorbance of control

Table 2: Cumulative percentage inhibition of Ascorbic acid, Acetone and Methanol Extracts of DPPH Radical Scavenging Activity

Concentration (µg/ml)	PERCENTAGE OF INHIBITION		
	Ascorbic acid	Acetone	Methanol
20	28.91	12.49	15.65
40	44.62	18.6	24.61
60	60.24	27.56	39.42
80	75.61	45.34	48.95
100	88.91	62.65	60.75

2.3. Estimation of Total Phenolic Content:

Reagents: Folin-Ciocalteu reagent and 20% of Na203.

Principle: Total phenolic content was determined using the Folin-Ciocalteu colorimetric method.

Procedure: The total phenolic content of the extract was determined using the Folin Ciocal teu colorimetric method. The extract (10-100 µg/ml) or standard solution of gallic acid (10-100 µg/ml) was added to 25 ml volumetric flask containing 9 ml of distilled water. A reagent blank was prepared using distilled water instead of sample. I ml of FolinCiocalteus phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% aqueous sodium carbonate was added to the mixture. The solution was diluted to 25 ml with Double distilled H20 and mixed. After incubation for 90 min at room temperature the absorption against prepared reagent blank was determined at 760 nm using UV-Visible spectrophotometer. Quantification was done with respect to the standard gallic acid and expressed as gallic acid equivalents (GAE) in mg per gram of extract (Marinovaet al., 2005).

Total Phenolic Content (TPC) = GAE × V × D/ W

V = volume of the extract solution in mL

W = weight of the plant sample in grams

D = dilution factor

Table 3: Indicates Absorbance values of Gallic Acid

S.No	Concentration (µg/ml)	Absorbance
1	20	0.237
2	40	0.498
3	60	0.612
4	80	0.798
5	100	0.912

Table 4: Total Phenolic Content of Acetone and Methanol extracts

Sample solution(µg/ml)	Wt of extract per ml(gm)	Absorbance	GAE conc(µg/ml)	GAE conc(mg/ml)	mg GAE/gm
Acetone (1000)	0.001	0.369	58.49	0.0584	58.4
Methanol (1000)	0.001	0.294	21.39	0.0213	21.3

3.0. RESULTS AND DISCUSSIONS:

Medicinal plants are valuable for the survival of both human and animal population globally. They are rich source of phytochemicals which can be used to treat various ailments of mankind. In India, natural products derived from medicinal plants have been used traditionally for the treatment of various disorders including cancer. But the use of these medicinal plants needs proper validation and documentation for utilizing it as a drug for treating various pathophysiological conditions. The Extractions prepared by soxhlation method and % yield was found to be 52% and 64% for methanol and acetone respectively. In this study antioxidant and cytotoxicity studies are performed on Sida ovata Forssk plant leaves extract in two solvents (Methanol and Acetone). The antioxidant activity of the plant extract was then characterized using the DPPH

radical scavenging method. Antioxidant activity using DPPH was found to increase according to concentration. All the extracts of acetone and methanol extracts exhibited antioxidant activity with an IC_{50} value of 60.56 µg/ml and 69.75 µg/ml when compared to the Standard Ascorbic Acid with an IC_{50} value of the 47.20 µg/ml. when compared with methanol extract, acetone extract showed slightly increased radical scavenging activity. Plants containing phenols has antioxidant property .Total Phenolic Content (TPC) assay was conducted and the value of total phenolic content of methanol and acetone extracts was found to be 21.3 mg GAE/g and 58.4 mg GAE/g .values are expressed in gallic acid equivalents. The cytotoxic activity of the methanol and acetone extracts of Sida ovata Forssk on MCF-7 cells from human breast cancer and A549 cells from human lung cancer was investigated using MTT assay . The results showed decreased cell viability and cell growth inhibition in a dose dependent manner. The IC50 value of standard 5-Fluorouracil on both MCF7 and A549 cell lines 37.08, 51.32 µg/ml respectively. The IC50 values of Methanol extract was found to be 95.75, 98.36 µg/ml and Acetone extracts IC50 values are 82.63, 87.16 µg/ml respectively. By comparing two extracts with standard drug they showed less cytotoxic activity.

4.0. CONCLUSIONS:

The current investigation demonstrates that *S.ovata Forssk* could inhibit the free radicals by showing radical scavenging activity. It also contains polyphenols which act as an antioxidant. When comparison was made between methanol and acetone extracts, the acetone extracts showed slightly more antioxidant activity. Further cytotoxic activity performed by MTT assay by methanol and acetone extract. The acetone extracts showed slightly more antioxidant activity.

ACKNOWLEDGEMENT:

Authors thank the Principal, University College of Pharmaceutical Sciences, Kakatiya University, Warangal for providing necessary facilities. Authors PN and MB express gratitude Narsimha reddy Yellu for guidance.

5.0. References:

Akbarsha. M. A and Murugaian P, "Aspects of the male reproductive toxicity/male antifertility property of andrographolide in albino rats: effect on the testis and the cauda epididymidal spermatozoa," *Phytotherapy Research*, 14 (2000) 432–435.

Ansil PN, Wills PJ, Varun R, Cytotoxic and apoptotic activities of Amorphophallus campanulatus (Roxb.) Bl. tuber extracts against human colon carcinoma cell line HCT-15. *Saudi J Biol Sci*, 21(2014):524–531.

Atabani AE, Silitonga AS, Badruddin IA, A comprehensive review on biodiesel as an alternative energy resource and its characteristics, *Renew Sustain Energy Rev* 16(2012):2070–2093.

Bernardo MA, Silva ML, Santos, Effect of cinnamon tea on postprandial glucose concentration. *J Diabetes Res*,015:913651.
https://doi.org/10.1155/2015/913651.

Bhaskar V.H, Ajay S.S, Antimicrobial activity of Calotropis procera seeds. *Asian J Chem* 21(2002):5788.

Brand-Williams W, Cuvelier ME, Berset CLWT, Use of a free radical method to evaluate antioxidant activity, *LWT-Food Sci Technol* 28(1995):25–30.

Bukar A, Uba A, Oyeyi T , Antimicrobial profle of Moringa oleifera Lam. extracts against some food–borne microorganisms, *Bayero J Pure Appl Sci* , 3(2010):43–48.

Chang LC, Gerhäuser C, Song L, Activity-guided isolation of constituents of Tephrosia purpurea with the potential to induce the phase II enzyme, quinine reductase. J Nat Prod 60(2017):869–873.

Ding Y, Nguyen HT, Kim SI, The regulation of infammatory cytokine secretion in macrophage cell line by the chemical constituents of Rhus sylvestris, Bioorg Med Chem Lett, 19(2013):3607–3610.

Gahlaut A, Chhillar AK, Evaluation of antibacterial potential of plant extracts using resazurin based microtiter dilution assay, *Int J Pharm Pharm Sci* 5(2013):372–376.

Galib MB, Mashru M, Jagtap C, Therapeutic potentials of metals in ancient India: a review through CharakaSamhita. J Ayurveda Integr Med 2(2011):55.

Guo JR, Chen QQ, Lam CWK, Efects of karanjin on cell cycle arrest and apoptosis in human A549, HepG2 and HL-60 cancer cells. Biol Res 48(2015):40.

Hawk MA, McCallister C, Schafer ZT, Antioxidant activity during tumor progression: a necessity for the survival of cancer cells, *Cancers* 8(2016):92. https://doi.org/10.1007/s13205-017-1013-1.

Kondhare D, Lade H, Phytochemical profle, aldose reductase inhibitory, and antioxidant activities of indian traditional medicinal

Coccinia grandis (L.) fruit extract. 3 *Biotech* 7(2013):378.

Kumar G, Ghosh M, Pandey DM, Method development for optimized green synthesis of gold nanoparticles from Millettia pinnata and their activity in Non-small cell lung cancer cell lines. *IET Nanobiotechnol*, 13(2019):626–633.

Kumar R, Sharma M, Herbal nanomedicine interactions to enhance pharmacokinetics, pharmaco-dynamics, and therapeutic index for better bioavailability and biocompatibility of herbal formulations. *J Mater NanoSci,* 5(2018):35–58.

Li S, Chen G, Zhang C, Research progress of natural antioxidants in foods for the treatment of diseases, *Food Science Hum Wellness*, 3(2014):110–116.

Mako GA, Memon AH, Mughal UR, Antibacterial efects of leaves and root extract of Calotropis procera Linn, Pak *J Agri Engg Vet Sci*, 28(2011):141–149.

Muqarrabun LMR, Ahmat N, Ruzaina SAS, Medicinal uses, phytochemistry and pharmacology of Pongamia pinnata (L.) Pierre: *a review. J Ethnopharmacol* 150(2013):395–420.

Nikhil K, Sharan S, Chakraborty A, Role of isothiocyanate conjugate of pterostilbene on the inhibition of MCF-7 cell proliferation and tumor growth in Ehrlich ascitic cell induced tumor bearing mice, *Exp Cell Res,* 320(2014):311–328.

Panat NA, Maurya DK, Ghaskadbi SS, Troxerutin, a plant favonoid, protects cells against oxidative stress-induced cell death through radical scavenging mechanism, *Food Chem*, 194(2016):32–45.

Perez C, Pauli M, Bazerque P, An antibiotic assay by the agar well difusion method. *Acta Biol Med Exp*,15(1990):113–115.

https ://doi.org/10.1126/scitranslmed.3007653.