ANTIOXIDANT ACTIVITY AND RADICAL SCAVENGING EFFECT OF *MUTINGIA CALABURA* AND *GLYCYRHIZZA GLABRA* AND EVALUATION OF ITS CYTOTOXIC CAPACITY ON L929 (MURINE FIBROBLAST CELL LINE)

Arthi Rangasamy*[1], Vinoth kumar[2], Rajesh Raghupathi[3], Geetharamani[4]

[1] Research scholar, Department of Biotechnology, CMS College of Science and Commerce, Coimbatore, India.

[2]Associate Professor &Director, School of Biological Sciences, CMS College of Science and Commerce, Coimbatore, India.

[3] CEO, RND Softech Private Limited, Alumni - Harvard Business School.

[4] Professor and Dean, Dr.NGP college of Arts and Science, Coimbatore, India.

ABSTRACT

Mutingia calabura and *Glycyrhizza glabra* are widely cultivated for its valuable medicinal activity in the tropical region. The present study evaluated the antioxidant and cytotoxic effects of ethanolic plant extract of *Mutingia calabura* and *Glycyrhizza glabra* in combination. Antioxidant effects were assessed utilizing photometric assay in vitro, 2, 2-diphenylpicrylhydrazine as well as ferric, which reduces antioxidant strength, superoxide dismutase. The cytotoxicity assay was used to test cytotoxic activity for L929 (murine fibroblast cell line) cell lines. Our results show a plantation has promising anti-cancer action. The ethanolic plant extracts were able to extract compounds responsible for the antioxidant activity.

Keywords: MTT assay, Antioxidant assay, L929 cell line, *Mutingia calabura*, and *Glycyrhizza glabra.*

INTRODUCTION

Large numbers of herbal drugs have a suggested prophylactic effect besides their use for therapy of diseases. Solvent extracts of plants were made into plant produced ointments and oils in ancient times for the treatment of skin diseases. Plant parts like root, leaf, bark contains a great source of new bioactive compounds, which, due to their intrinsic biological properties, may be used in medicine as well as in other human health-promoting areas and play an important role in general medical practice for the treatment of various vascular, skin, and immune system. (Oresajo *et al.,* 2012)

During 20th century, 80% of all medicines used to treat human illness were obtained from the leaves, barks and roots of medicinal plants. Crude plant extracts were percolated alcohol and the doctors used to prescribe tablespoons of the fluid extract to be taken for a period. It is noteworthy to say that about 70% of the drugs used today are models of natural products. From 1981 to 2010, 700 natural product derived New Chemical Entities were approved for various therapies and treatments. (Allemann *et al.,* 2018)

Traditional medical knowledge has provided useful lead compounds for various skin therapy, as exemplified within the discovery of the vinca alkaloids (vincristine and vinblastine), taxols (paclitaxel and docetaxel), camptothecin and etoposide. (Lai *et al.,* 2017)

A study strongly insists that at prolonged use of chemicals may contribute to the development of tumours in people. But because health care products contain various combinations of chemicals, it is nearly impossible to point out a particular cause and effect for any specific chemical on its own. Still, many of those chemicals are considered hormone disruptors. (Costa *et al.,* 2017)

Hormone disruptors will affect by mimicking estrogen and other hormones, by blocking them which leads to hormonal imbalance of our body. (Lin *et al.,* 2017)

MATERIALS AND METHODS

Plant collection and extraction

The plants were purchased from a licensed botanist, Coimbatore, India, with their particular parts. In the study, their fresh and dry parts were used according to the requirement. Water was used to wash the organic materials. They are cut by Mortal pestle and then grounded. Ethanol extracts were prepared using the soxhelet apparatus for 16 hours. (Ayyanar *et al.,* 2012)

Cell lines

L929 (murine fibroblast cell line) cell lines were obtained from Cell Sciences National Centre, Pune. The cells were grown in Minimal Essential Medium enhanced with 10% FBS, streptomycin (100 µg/mL), and penicillin (100 U/mL) in a humidified atmosphere of 50 µg/mL CO_2 at 37 °C. (Erviana *et al.,* 2016)

Maintenance of cell lines

The L929 cell lines acquired from ATCC was immediately placed in a 37 °C water bath. It was continuously disturbed until a medium thawed. This is then centrifuged at RT for 10 minutes, about 150 to 200 g. The cells are cleaned to extract excess DMSO in freshwater and supernatant is discarded. (Insanu *et al.,* 2014)

MTT assay for cytotoxicity

Cells were preserved with DMEM medium, combined by 10 percent Fetal Bovine Serum, of 5 percent CO_2 for about 37ºC. Then KB cells are plated at such a concentration for approx. 1.2X 104 cells / well in the 96 well flat bottom tissue culture plate then allowed also to be attached overnight at 37ºC.A medium was instead discarded, but batteries are incubated to twenty-four h for specific sample concentrations (25, 50, 75,100, or 125 µg/mL). The medium was discarded, followed by the incubation, and 100 µL fresh medium was added with 10 µL of MTT (5 mg/mL). A medium was discarded during four h, then added 100 µL with DMSO for dissolve formazan crystals. In such a microtitre plate scan, an absorbance is then read around 570 nm. Cyclophosphamide has been utilized as just a positive control. Sample 1(*Mutingia calabura*), sample 2 (*Glycyrhizza glabra*), sample 3 (equal quantity of plants 1 and 2). (Zuhra *et al.,* 2018)

The formula used to measure cell survival was:

% Viability = (Test OD/ Control OD) X 100 Cytotoxicity % = 100 – % Viability

Antioxidant activity assay DPPH radical scavenging assay

The solution of 0.135 mM DPPH was formulated for ethanol as well as 1.0 ml of that was combined to 1.0 ml of the varying concentrations (0.02 – 0.1 mg) of an ethanol extract.

The reaction mixture is vortexed thoroughly and kept inside the dark at room temperature 27°C for 30 min. An absorbance was spectrophotometrically calculated around 517 nm. BHT was used as a benchmark. The following equation determined an ability to scavenge DPPH radical: DPPH radical scavenging activity (percent)= [(Abscontrol − Abssample)]/(Abscontrol)] 100 where Abscontrol is the absorption of DPPH radical+ ethanol, and Abssample is the absorption of DPPH radical+ or standard sample extract. (Najihudin *et al.,* 2017)

ABTS radical scavenging assay

Stock formulations were ABTS+ solution of 7mM as well as a persulfate solution for 2.4mM of potassium. Plant extracts was added with 1 ml of the ABTS solution and incubated for 7 minutes. Absorption was read at 734 nm using spectrophotometer (Beckman, DU 7400, and the USA).An extract's ABTS+ scavenging potential was comparison with the natural antioxidant (BHT), as well as the percentage inhibition was determined accordingly by ABTS radical scavenging action (percent): (Preethi *et al.,* 2020)

$[(Abs_{control} - Abs_{sample})]/ (Abs_{control})] \times 100$ where $Abs_{control}$ is the absorbance of ABTS radical + ethanol; Abs_{sample} is the absorbance of ABTS fundamental + sample extract /standard.

Ferric reducing/antioxidant power (FRAP) assay

The antioxidant capacity of extracts was estimated consistent with the tactic described previously by Pulido et al. (2020). We read absorbance at 593 nm. That value was represented with such extraction of mmol Fe (II)/g. (Septiani *et al.,* 2012)

Estimation of Superoxide Dismutase (SOD)

Superoxide dismutase activity was measured using Sun et al. (2018) process. In this process, the xanthine-xanthine oxidase system has been used to produce the superoxide flux, and use of nitro blue tetrazolium (NBT) as a superoxide output indicator. A degree for inhibition of an enzyme response unit providing 50 percent inhibition of NBT reductions is then calculated for SOD action. Results are expressed as U/mL. (Calderon *et al.,* 2011)

MTT Assay

The effect of anti-cancer from the combination of equal quantities of plant extract *Mutingia calabura* and *Glycyrhizza glabra* on L929 (murine fibroblast cell line) the assay was tested using micro-culture method. Plant extract concentrations were used to study the effective doses by calculating them from the dose-response curve. Findings of a mace extract's cytotoxicity evaluation toward the L929 cell line are seen in Figure 1. From the above figures, we can quickly determine the percentage of cell viability for five different concentrations of 5, 25, 50, 75, and 100 µg/mL plant extract. The image clearly indicates significant inhibition of L929 cell proliferation in a dose-dependent approach after 24 hours of treatment. Cell viability has been observed with the plant extract at higher concentrations. Hence, the half-maximal inhibitory concentration of plant extract was at the level of 75 µg/ml. (Mohamed *et al.,* 2015)

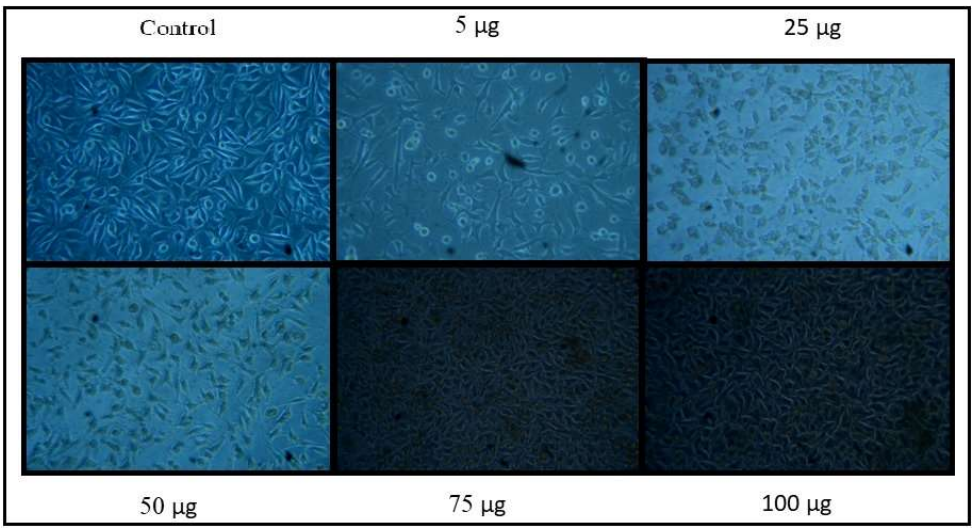

Figure 1: MTT cytotoxicity assay of extract *mutingia calabura* and *Glycyrhizza glabra* on L929 (murine fibroblast cell line)

DPPH analysis is the accurate method for testing antioxidant activity. Because of its spare-electron delocalization across the whole molecule, this is a stable radical. The donation of H+ to the DPPH radicals made a corresponding change from violet color to straw within the solution. DPPH scavenging often induced the proportionate reduction from its 517 nm absorbance. (Aruna *et al.,* 2013)

Antioxidant activity of the ethanolic plant extract was correlated with total phenolic content, and it has been further noticed which extraction radical scavenging results are directly proportional to a phenolic content of the extract.Thus the plant's ethanol extraction showed significant radical scavenging as when the concentration rose. Subsequently, DPPH scavenging continued to increase with extraction concentration. One of the underlying mechanisms of calculating antioxidant behavior was the proton-radical scavenging activity. This assay determines the scavenging of stable radical species DPPH by antioxidants compounds present in the plant extracts. These results revealed a higher rate for DPPH scavenging action of ethanolic extract, possibly due to high phenolic compounds content. (Paur *et al.,* 2011)

Acta Biomed 2024; Vol. 95, N. 1: ISSN: 0392-4203 | eISSN: 2531-6745

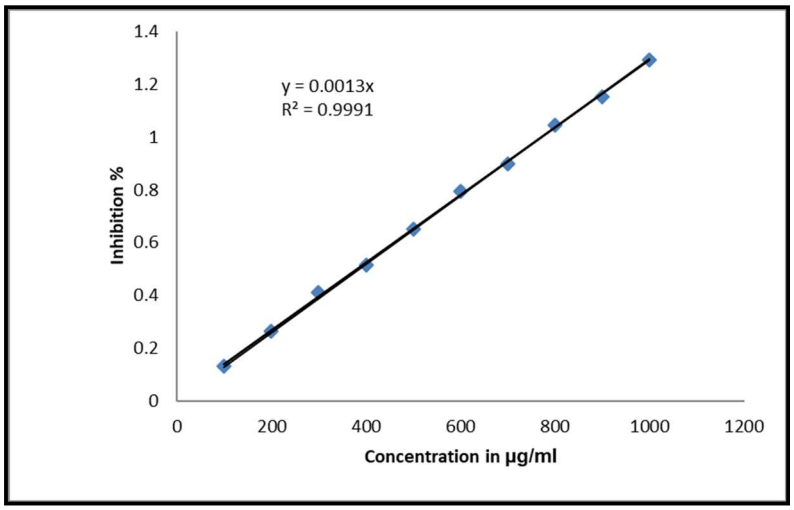

Figure 2 – IC_{50} value of DPPH radical scavenging activity

An EC_{50} cost was calculated by a scavenging process plotted towards different extraction concentrations, which was characterized as the effective antioxidant concentration required to reduce an initial DPPH radical concentration through 50%. The lowest EC_{50} shows that extracts' best capacity for the act for scavengers to DPPH radicals. Out of all excerpts, the ethanolic extract of pulp and seed showed the lowest EC_{50}, with R2 0.9.(Zainul *et al.,* 2014)

A power reduction test was typically utilized to determine the antioxidant's capacity for donating the electron. In this assay, the power of extracts to scale back Fe3+ to Fe2+.A presence for antioxidants throughout an excerpt resulted in a ferric cyanide complex (Fe3+) being decreased with the ferrous cyanide (Fe2+) form. To this assay, Fe3+ is reduced to Fe2+ by changing response from green and blue into different shades, relying upon a compounds' reducing power.It has been found whether a reduced ability for reference compounds (Ascorbic acid) becomes above all other extracts tested. This was claimed whether a substances' reduction power is likely due to their hydrogen-donating ability. Ethanolic extracts of plants, therefore, contain a high amount of reductones. Therefore ethanolic plant extract can serve donors for electrons and might react to free radicals to turn them into stable products and instead end free radical chain reactions. (Bradford *et al.,* 2016)

ABTS is indeed a protonated radical, does have a specific absorbance limit of 734 nm, which reduces with proton radicals being scavenged. A percentage inhibition reduced for most cases when time passed by because antioxidants of an aromatic plant scavenge the cation radical ABTS.(Kujala *et al.,* 2020)

The extracts from *Mutingia calabura* and *Glycyrhizza glabra* were fast and effective scavengers of ABTS+ extreme. Make a comparison that behavior with those of BHT, a typical antioxidant used for this analysis. An inhibition rate is 94.55 percent.The high correlation between ABTS+ radical extract scavenging activity and total phenolic content (R2=0.9) suggests that phenolic substances will directly contribute to an antioxidant action of such extracts.(Sadasivam *et al.,* 2017)

CONCLUSION

Antioxidant potential of *Mutingia calabura* and *Glycyrhizza glabra* extract demonstrated the highest reducing power in the ethanolic excerpt. It is concluded that the antioxidant activity of *Mutingia calabura* and *Glycyrhizza glabra* extracts were directly proportional to the phenolic contents. Amazingly, all L929 cell lines were active in the extraction. Medicinal properties of *Mutingia calabura* and *Glycyrhizza glabra* have some active principles that produce specific biologic activity, causing interference in some essential biological pathways.

REFERENCES

1. Oresajo, C., Pillai, S., Manco, M., Yatskayer, M., and Mcdaniel, D. (2012). Antioxidants and the skin : Understanding formulation and ef- ficacy, 25, 252–259.
2. Allemann,I. B., and Baumann, L. A n t i - oxidants Used in Skin Care Formulations.2018;1–8.
3. Lai-Cheong, J. E., & McGrath, J. A. Structure and function of skin, hair and nails. Medicine (United Kingdom). 2017; 45(6): 347–351. https://doi.org/10.1016/j.mpmed.2017.03.004.
4. Costa, R., and Santos, L. Delivery systems for cosmetics - From manufacturing to the skin of natural antioxidants. Powder Technology. 2017; 322: 402–416. https://doi.org/10.1016/j.powtec.2017.07.086.
5. Lin, J. T., Chen, Y. C., Chang, Y. Z., Chen, T. Y., and Yang, D. J. Effective compounds in the fruit of: Muntingia calabura Linn. cultivated in Taiwan evaluated with scavenging free radi- cals and suppressing LDL oxidation. Food and Function. 2017; 8(4): 1504–1511. https://doi.org/10.1039/c7fo00059f.
6. Ayyanar, M., and Subash-Babu, P. (2012). Syzygium cumini (L.) Skeels: A review of its phytochemical constituents and tradi- tional uses. Asian Pacific Journal of Tropi- cal Biomedicine, 2(3), 240–246. https://doi.org/10.1016/S2221-1691(12)60050-1.
7. Erviana, L., Malik, A., and Najib, A. Free An- tiradical Activity Test of Ethanol Extract of Basil Leaf (Ocimumbasilicum L.) Using the DPPH Method. Indonesian Fitofarmaka Journal. 2016; 3 (2): 164–168.
8. Insanu, M., Kusmardiyani, S. dan Hartati, R. Recent Studies on Phytochemicals and Pharmacological Effects of Eleutherine Americana Merr. Procedia Chemistry. 2014;13: 221–228. https://doi.org/10.1016/j.proche.2014.12.032.
9. Zuhra, D. Antioxidant Activity of Flavonoid Compounds from Katuk Leaves (Sauropus androgynus (L) Merr.). Journal of Sumatra Biol- ogy. . 2018; 3 (1): 10–13.
10. Najihudin, A. Antioxidant Activity of Skin Extracts and Fractions of BatangTrengguli (Cassia fistula L) with DPPH Method. IJPST. 2017; 4 (2).
11. Preethi, K. et. al. In vitro antioxidant activity of extracts from fruits of Muntingia calabura Linn. from India. Pharmacognosy Journal. 2020;2(14): 11–18. https://doi.org/10.1016/S0975-3575(10)80065-3.

12. Septiani, S. et. al. Formulation Of Antioxidant Gel Masks from the Ethanol Extract Of Melinjo Seeds (Gnetun gnemon Linn.). Students eJournals. 2012; 1 (1); 1- 25.
13. Calderon-Montano. A Review On The Di- etary Flavonoid Kaempferol. Medical Chemistry. 2011; 11:298-344. http://doi.org/10.2174/13895571179
14. Mohamed Azmathulla Khan Y, Subhas Chandrappa Mundasada, Dinesha Ramadas; Antioxidant Activity; Root, Leaves and Fruits Aqueous Extracts of Muntingia Calabura, Journal of Innovations in Pharmaceuticals and Biological Sciences, 2015; 2 (4); 363-368.
15. Aruna Sindhe M, Yadav D, Bodke, Chandrashekar A; Antioxidant and in vivo antihyperglycemic activity of Muntingia calabura leaves extracts, Der Pharmacia Lettre, 2013; 5 (3); 427-435.
16. Paur I, Carlsen MH, Halvorsen BL et al.; Antioxidants in Herbs and Spices: Roles in Oxidative Stress and Redox Signaling. In: Benzie IFF, Wachtel-Galor S, editors. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition. Boca Raton (FL): CRC Press; 2011.
17. Zainul Amiruddin Zakaria, Mohd Hijaz Mohd Sani, Manraj Singh Cheema, Arifah Abdul Kader, Teh Lay Kek and Mohd Zaki Salleh; Anti nociceptive activity of methanolic extract of Muntingia calabura leaves: further elucidation of the possible mechanisms, BMC Complementary and Alternative Medicine, 2014; 14:63.
18. Bradford MM; A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein. Analytical Biochemistry 2016; 72; 248-54.
19. Kujala TS, loponen JM, Klika KD and Pihaja K; Phenolics and betacyanins in red beetroot (Beta Vulgaris) root: description and effect of cold storage on the content of total phenolics and three individual compounds. Journal of Agricultural Food Chemistry, 2020; 48; 5338-5342.
20. Sadasivam S, Manickam A; In. Biochemical Methods. New Age Internationals, 2017; 184-186.