https://doi.org/10.33472/AFJBS.6.6.2024.6561-6572

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Anticancer, Antidiabetic, Antioxidant Properties and Phytoconstituents of Efficacy of Methanolic Extract of *Euphorbia milii* Leaves

Sanjeevkumar Giri[1], Priyanka S. Jamade[1], Balaji Pendakur[2], Sanjotha G.[3], Sudheer Manawadi[3], Sandeep V. Binorkar,[4] Nagineni Sudarshan Rao[5] and Sharangouda J. Patil[6*]

[1]Department of Pharmaceutical Chemistry, Karnataka State Women`s University, Vijayapura-586108, Karnataka, India
[2]Department of Pharmaceutical Chemistry, Sri Padmavati School of Pharmacy, Tiruchanoor, Tirupati - 517503, Andhra Pradesh, India
[3]Department of Biotechnology, Government Science College, Hassan - 573201, Karnataka, India
[4]Department of Agadatantra, Government Ayurveda College, Vazirabad, Nanded - 431601, Maharashtra, India
[5]Department of Pharmaceutics, Shri Vishnu College of Pharmacy, Vishnupur, Bhimavaram 534202, Andhra Pradesh, India
[*6]Department of Zoology, NMKRV College for Women (Autonomous), Bengaluru - 560011, Karnataka, India

***Corresponding Author Email: shajapatil@gmail.com**

Article Info

Volume 6, Issue 6, June 2024

Received: 16 March 2024

Accepted: 26 April 2024

Published: 29 June 2024

doi: 10.33472/AFJBS.6.6.2024.6561-6572

ABSTRACT:

Research on finding new therapeutic drug for antioxidant, anticancer, antidiabetic is need of hour through natural products and exploring phytoconstituents to add up in the medicinal chemistry to study in detail and expel their various other properties. The current work emphasize on ornamental plant *Euphorbia milii* leaves to determine biological properties and phytoconstituents in methanol extract. To investigate the antioxidant activity, DPPH free radical scavenging assay was carried out, for anticancer property using MTT (3-(4, 5-dimethythiazolyl 21-2 5-dphenyttetrazolum bromide) in vitro cell proliferation assay was used, for antidiabetic studies used Alpha-amylase inhibitory assay was applied and also screening phytoconstituents in *Euphorbia milii* leaves methanolic extract. The anticancer activity found potent in *in vitro* proliferation MTT assay using A375 human melanoma cell lines by showing IC_{50} value 199.45µg/ml when compared to that of Cisplatin standard. Antidiabetic studies carried out by α-amylase inhibitory assay and found potent by showing IC_{50} value 171.28 µg/ml and in antioxidant activity was assessed by DPPH and found potent by showing IC_{50} value 63.06 µg/ml. These biological properties were due to rich phytochemicals in the methanolic extract positive for glycosides, alkaloids, steroids, flavonoids, phenols and saponins. The investigation concludes that *E. milii leaves* exhibited very good anticancer, antidiabetic, and antioxidant activities which are highly appreciable and further studies by pure molecule isolation and characterization may use it as novel biological remedies against various diseases.

Keywords: Antioxidant, Antidiabetic, Anticancer, Cell lines, Cisplatin, Phytochemicals

1. Introduction

Since the beginning of human civilization, medicinal plants have been used by mankind for its therapeutic value. Nature has been a source of medicinal tressure for thousands of years and an impressive number of modern drugs have been isolated from natural source. Many these isolations were based on the uses of the agents in traditional medicine. The plant based, traditional medicine system continues to play an essential role in health care, with about 80% of worlds inhabitants relying mainly on traditional medicines or primary health care. Medicinal plants are plants containing inherent active ingredient used to cure disease or relieve pain. The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed [1,2]. Modern pharmacopoeia still contains at least 25% drugs derived from plants many others, which are synthetic analogues, built on prototype compounds isolated from plant. The medicinal properties of plant could be based on antioxidant, antimicrobial, antipyretic effects of phytochemicals in them [3, 4]. According to World Health Organization, medicinal plants could be the best source to obtained a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy [5].

Medicinal plant produces bioactive compounds used mainly for medicinal purposes. These compounds either acts on different system of animals including man, or act through interfering in the metabolism of microbes infecting them. The microbes may be pathogenic or symbiotic. In either way the in regulating host-microbe interaction in favour of the host. So, the identification of bioactive compounds in plants, their isolation, purification and characterization of active ingredients in crude extracts by various analytical method is important.

Euphorbia milii is many branched evergreen shrubs reaching to a height of 60-90 cm. It thrives on dry to moderately moist, in full sun on well-drained soil. It is sensitive to temperature below 35°F in the winter. In hot summer regions, appreciates some noon shade. Although *Euphorbia milii* Des Moul is tolerant of poor soils, especially rocky-sandy soils, and even of drought, regular applications of moderate hydration may result in improved bloom with less leaf drop. Wet soils, especially during the winter, can be lethal. It performs best in locations with adequate air movement. Indoor plants require intense light and thrive in a coarse soil-based potting mix. Propagate from cuttings of the tip. The popular name *E. milii* Des Moul relates to the notion held by some that the crown of thorns worn by Jesus Christ during his crucifixion was fashioned from this plant stem [6].

Recent research indicates that over 5% of Euphorbia species are utilised medicinally. *E. milii* Des Moul is frequently used in folk medicine to treat warts, cancer, hepatitis, and trichiasis [7]. The whole plant paste is applied to dislocated animal bones, the leaves are used to treat snake bites and ringworm, and the seeds are used as a laxative for children. *E. milii* Des Moul flower powder and whole plant ash are used orally to treat asthma at doses of 500 mg three times a day and 250-500 mg twice a day, respectively. Other medical uses for Euphorbia species are numerous and include the treatment of digestive problems, blood syndromes, genitourinary syndromes, microbial infection, scorpion stings, pregnancies/ puerperium, as well as sensory difficulties [8, 9]. The formulations are used as skin remedies to alleviate warts, itching, hair loss, dermatitis, acne, sunburn, boils, rashes and irritation, as well as for their disinfecting, antiseptic and emollient characteristics. *E. milii* Des Moul undiluted latex was discovered to irritate the eyes and skin of mammals. While several diterpene esters of ingenol are powerful skin irritants, they lack tumour promoting potential when compared to other intently linked ingenol and phorbol derivatives [10, 11]. Milli amines obtained from *E. milii* Des Moul latex were found to be highly molluscicidal (12). Haleshappa et al [13] reported for the strong phytochemicals, antioxidants and antimicrobial potential in the ethanolic extract of *E. milii* thorns.

E. milii is an ornamental plant belongs to Euphorbiaceae family. As per the literature less work were found with this plant, hence, detailed investigation of biological and phytochemical property was carried out to emphasize their usefulness in science and society for the therapeutic benefits.

2. Materials and Methods

2.1 Plant Materials

Ornamental plant of *Euphorbia milii* leaves were collected in the July 2022 from our university gardens, with the help of botanist and authenticated by Dr. Sanjeevkumar Giri, Department of Pharmaceutical Chemistry and General Chemistry, Akka Mahadevi Women's University, Vijayapura, Karnataka, India.

Fig. (1). Morphological Features of *Euphorbia milii* Ornamental Plant

2.2 Preparation of Plant Extracts

The leaves of the ornamental plant *Euphorbia milii* were collected, washed cleanly in distilled water and shade dried for complete removal of moisture. The dried leaves were chopped in to small pieces, powdered and used for Soxhlet extraction using highly polar solvent methanol for 24 hr and dried using Buchi's rotary vacuum evaporator and stored in refrigerated.

2.3 Phytoconstituents Analysis

Phytoconstituents analysis of methanol extract of the leaves was carried out in order to analyse the class of organic metabolites. The all the extracts of *Euphorbia milii* leaves were analysed by standard chemical tests as described by Sharangouda and Patil [13], Harborne [14, 15] and Fransworth [16] to determine alkaloids, carbohydrates, flavonoids, glycosides, phenols, saponins steroids, tannins, tritepenoids.

2.4 Anticancer Studies by MTT Assay using A375 Human Melanoma Cell Lines of Methanolic Extract of *Euphorbia milii* Leaves

2.4.1 Principle

The reduction of tetrazolium salts is now widely accepted as a reliable way to examine cell proliferation. The yellow tetrazolium MTT (3-(4, 5-dimethythiazolyl 21-2 5-dphenyttetrazolum bromide) is reduced by metabolically active cells in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometric means. The assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability [17, 18].

2.4.2 Procedure

The cells were trypsinized and aspirated into a 15ml centrifuge tube Cell pellet was obtained by centrifugation at 300 x g. The cell count was adjusted, using DMEM medium, such that 200µl of suspension contained approximately 10.000 cells.

To each well of the 95 well microtitre plate. 200µl of the cell suspension was added and the plate was incubated at 37°C and 5% CO atmosphere for 24 h.

After 24 hr the spent medium was aspirated. 200µ of different test concentrations (100, 200, 300, 400 and 500 pg/ml from stock) of test drugs were added to the respective wells. The plate was then incubated at 37°C and 5% CO atmosphere for 24 h.

The plate was removed from the incubator and the drug containing media was aspirated 100µ of medium containing 10% MTT reagent was then added to each well to get a final concentration of 0.5mg/ml and the plate was incubated at 37°C and 5% CO atmosphere for 3 hours.

The culture medium was removed completely without disturbing the crystals formed. Then 100µl of solubilisation solution (DMSO) was added and the plate was gently shaken in a rotatory shaker to solubilize the formed formazan 6 The absorbance was measured using a microplate reader at a wavelength of 570 nm and also at 630 nm. The percentage growth inhibition was calculated, after the subtracting the background and the blank and the concentration of test drug needed to inhibit cell growth by 50 % (IC_{50}) was generated from the dose response curve for cell line.

2.5 Antidiabetic Activity by α-amylase Inhibitory Assay of Methanolic Extract of *Euphorbia milii* Leaves

2.5.1 Principle

α-amylase activity can be measured in-vitro by hydrolysis of starch in the presence of α-amylase enzyme. This process was quantified by using DNS reagent which gives orange-red colour with starch. The reduced intensity of orange colour indicates the enzyme-induced hydrolysis of starch into monosaccharide. If the extract possesses α-amylase inhibitory activity, the intensity of the colour will be more. In other words, the intensity of colour in the test sample is directly proportional to the α-amylase inhibitory activity [20].

2.5.2 Protocol

In an Eppendorf tube, 1ml of PBS solution was mixed with 0.5 ml of different concentrations (50,100, 150, 200 and 250µg/ml) of samples with the standard solution and 200µl of 0.5mg/ml α-amylase was added followed by 200µl of 5mg/ml starch solution and incubated for 10 minutes at room temperature. Control was takes as starch with amylase and without amylase. Then the reaction mixture was stopped by adding 400µl of DNS solution heating the mixture

in boiling water bath for 5 min and cooled. The absorbance was measured at 540 nm (Labman UV Visible Spectrophotometer) [21].
The percent of enzyme inhibition was calculated using the following formula:
% of α-amylase inhibition = [(Ac − As)/Ac] × 100
where, Ac and As are the absorbance of control and sample, respectively.
Acarbose was used as standard.

2.6 Antioxidant Activity by DPPH Assay of Methanolic Extract of *Euphorbia milii* Leaves

2.6.1 Principle
The DPPH assay is popular in natural product antioxidant studies. One of the reasons is that this method is simple and sensitive. This assay is based on the theory that a hydrogen donor is an antioxidant. It measures compounds that are radical scavengers. Figure 1, below, shows the mechanism by which DPPH• accepts hydrogen from an antioxidant. DPPH• is one of the few stable and commercially available organic nitrogen radicals [22]. The antioxidant effect is proportional to the disappearance of DPPH• in test samples. Monitoring DPPH• with a UV spectrometer has become the most commonly used method because of its simplicity and accuracy. DPPH• shows a strong absorption maximum at 517 nm (purple). The color turns from purple to yellow followed by the formation of DPPH upon absorption of hydrogen from an antioxidant. This reaction is stoichiometric with respect to the number of hydrogen atoms absorbed. Therefore, the antioxidant effect can be easily evaluated by following the decrease of UV absorption at 517 nm.

2.6.2 Protocol
In an Eppendorf tube, 1.0 mL DPPH working solution (0.2 mM) was mixed with 0.5 ml of different concentrations (20, 40, 60, 80 and 100ug) directly from the given stock of test samples and the standard (20, 40, 60, 80, 100 ug/ml) solution and incubated for 30 minutes in dark at room temperature. The absorbance was measured at 517 nm (Labman UV Visible Spectrophotometer).
The percent antioxidant or radical scavenging activity was calculated using the following formula:
%Antioxidant activity = [(Ac − As)/Ac] × 100
where, Ac and As are the absorbance of control and sample, respectively.
Ascorbic acid was used as standard.

Statistical Analysis
All the experiments were carried out in triplicates and were expressed as mean difference of standard deviation ± standard error (SD ± SE). The data were statistically analyzed using Microsoft Office Excel 2007.

3. Results and Discussion

3.1 Phytoconstituent Analysis Methanolic Extract of *Euphorbia milii* Leaves
The qualitative analysis of phytoconstituents of *Euphorbia milii* leaves resulted for methanol extract positive for phenols, flavonoids, steroids, glycosides, saponins and alkaloids and negative for tannins and terpenoids (Table 1). Such work similarly found with many of the medicinal plants and reported for the active phytoconstituents in various extracts and their characterized molecules with different process of extraction in qualitative and quantitative approach [23 - 28].

Table 1: Showing results of Qualitative Phytoconstituent Analysis of *Euphorbia milii* leaves

Phytoconstituent Analysis	Results of *Euphorbia milii* Leaves
	Methanol Extract
Phenols	+
Flavonoids	+
Steroids	+
Glycosides	+
Tannin	-
Saponins	+
Alkaloids	+
Terpenoids	-

+ = Positive; - = Negative

3.2 Anticancer Studies by MTT Cytotoxicity Assay using Human Melanoma Cell Lines on Standard Cisplatin along with Methanol extract of *Euphorbia milii* Leaves

The cytotoxicity study was carried out for methanol extract of *Euphorbia milii* leaves on A375 human melanoma cell lines at different concentrations to determine the IC_{50} by MTT assay. Cytotoxicity of methanol extract of the leaves of *E. milii* against A375 cell lines and found to be 89.83%, 76.72%, 59.71%, 48.60% and 39.14% toxic at a concentration of 50, 100, 150, 200, and 250 µg/ml. The IC_{50} value of 199.45 µg/ml obtained for A375 of methanol extract shown very less effect on cell viability also found to suppress the cell proliferation with concentration dependent and it was showed good cytotoxicity compared to standard Cisplatin 27.12% in concentration of 15ug/ml. The percentage of cell viability was found to be increasing with dose dependent concentration of the tested extract and that has shown in Table 2 and Figure 2.

Giri et al.[29], reported similar findings on various extracts of *Indigophora cordifolia* aerial parts on graded concentration and found 100 % of inhibition at 500 µg/ml of cell growth of MCF-7 cell lines by MTT assay along with relative antioxidant activity. From last two decades researchers investigated many medicinal plants in India and global perspective in in vitro studies for finding novel molecules against cancers and other associated diseases [29 - 31].

However, these studies may further to work it for purification and guide to the further action for fractionation and isolation of potential anti-inflammatory and anticancer active compounds from the plant *E. milii* leaves by the spectroscopy studies.

Table 2: The Viability (%) rate and IC_{50} values of the *Euphorbia milii* Leaves extracts for A375 melanoma ell lines by MTT assay

Samples	Concentration (µg/mL)	Inhibition (%)	Viability (%)	IC_{50} value (µg/mL)
Cisplatin Standard	15	0.405±0.26	27.126	21.35
Methanol Extract	50	1.357±0.26	89.082	199.45
	100	1.172±0.23	76.72	
	150	0.918±0.23	59.71	
	200	0.75±0.30	48.6	
	250	0.611±0.30	39.14	

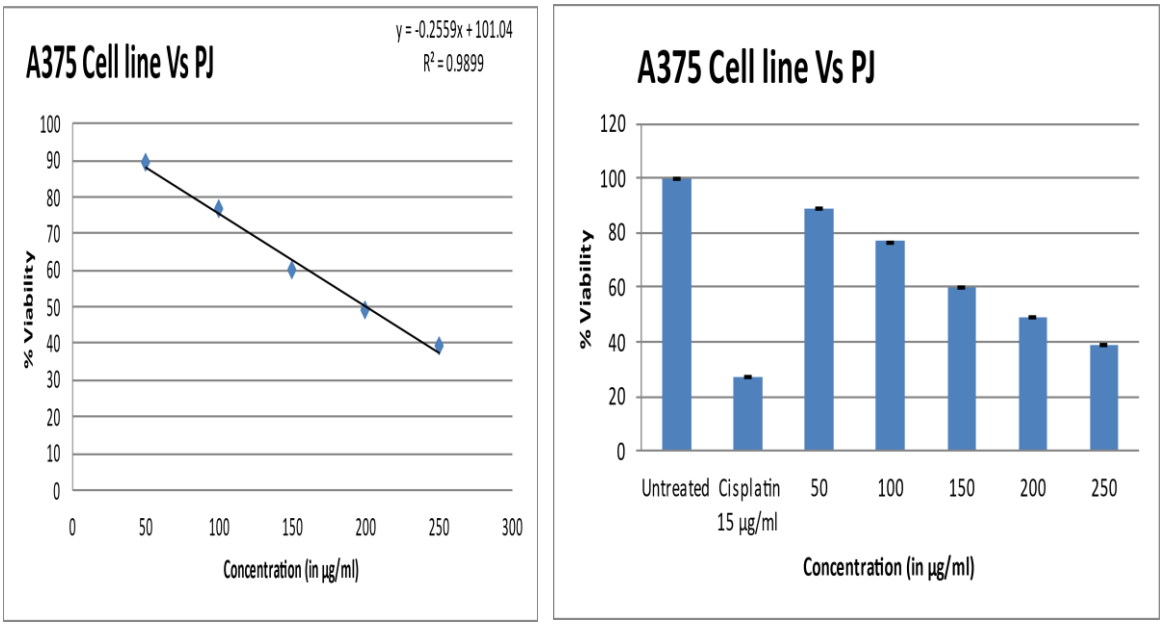

Fig. (2). Showing Results of Viability (%) and IC_{50} values with various Concentration of standard Cisplatin along with Methanol Extract of *E. milii* leaves on A375 Melanoma cell lines

3.2 Antidiabetic Studies by α-amylase Inhibitory Assay using Standard Acarbose along with Methanol extract of *Euphorbia milii* Leaves

The antidiabetic study was carried out methanol extract of *Euphorbia milii* leaves at different concentrations to determine the IC_{50} by α-amylase inhibitory assay. Hydrolysis of starch in the presence of α-amylase enzyme activity of methanol extract of the leaves of *E. milii* against DNS reagent which resulted orange red colour depends on concentration and found to be 25.16%, 34.23%, 42.88%, 55.22% and 69.23% inhibitory activity using the concentration of 50, 100, 150, 200, and 250 µg/ml. The IC_{50} value of 171.28 µg/ml obtained for methanol extract when compared to standard Acarbose concentration of 10 µg/ml exhibiting IC_{50} 83.33 µg/ml.

The percentage of inhibitory activity was found to be decreasing with dose dependent concentration of the tested extract and that has shown in Table 3 and Figure 3.

Similar studies were represented in α-amylase inhibitory effects of ethanol and hexane extracts of *Phyllanthus amarus* in the graded concentration and found maximum in the higher concentration inhibiting 80.48 % and 75.32 % in ethanol and hexane extracts [32]. Bhosale et al., [33] reported pancreatic α-amylase inhibitory activity of aqueous extracts of various plant products in in vitro studies and found Aqueous extract of leaves and rhizome of *Curcuma longa* showed highest anti-amylase potential with IC_{50} values of 0.53±0.10 and 0.96±0.29 mg/ml respectively. IC_{50} values of other extracts ranged between 1.24±0.49 to 4.50±0.38 mg/ml. The biological origin of antidiabetic medicine was become more important than the synthetic and these were available in market with more demand, and such products were possible due to medicinal plants which were extensively worked and became literature for the researchers and industry people as an alternative medicine [34, 35].

Table 3: α-amylase Inhibitory Assay of Methanolic Extract of *Euphorbia milii* Leaves

Samples	Concentration (µg/mL)	Inhibition (%)	IC_{50} value (µg/mL)
Acarbose Standard	100	0.280±0.007	83.33
Methanol Extract	50	0.924±0.001	171.28
	100	0.812±0.004	
	150	0.705±0.003	
	200	0.553±0.003	
	250	0.380±0.002	

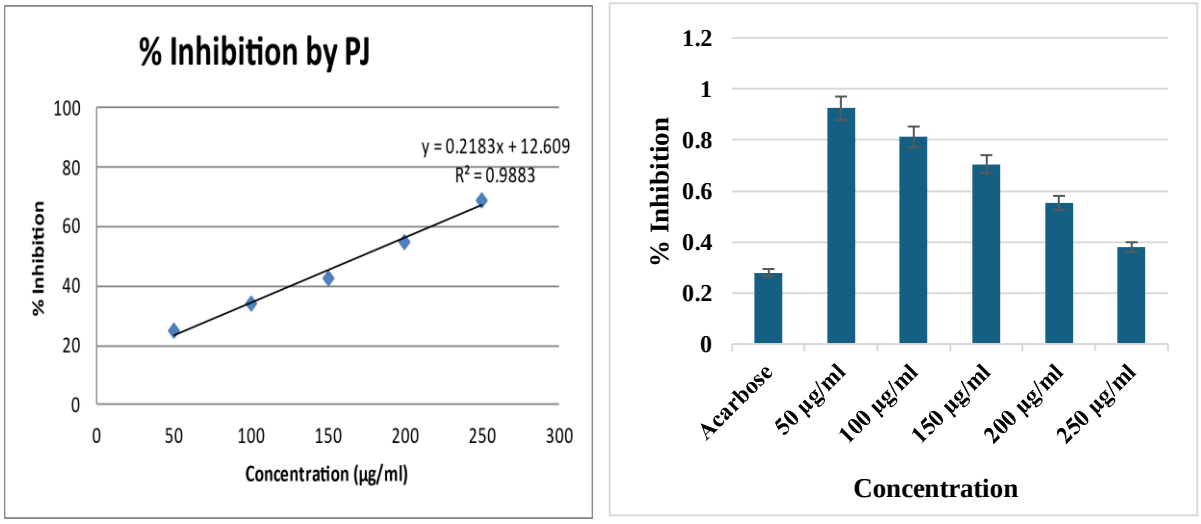

Fig. (3). Showing Results of Inhibition (%) and IC_{50} values with various Concentration of Methanol Extract of *E. milii* Leaves along with Standard Acarbose on α-amylase Inhibitory Assay

3.3 Antioxidant Activity by DPPH Free Radical Scavenging Assay of Methanol extract of *Euphorbia milii* Leaves

The methanol extract of *E. milii* leaves exhibited a significant dose dependent inhibition of DPPH free radical scavenging activity. A concentration-dependent assay was carried out with the extract and the results are presented in figure 4. The five graded concentrations were used in the study along with blank, cell control and standard control. Methanol extract showed free radical scavenging activity as 14.20%, 36.87%, 47.90%, 65.08% and 74.52 % inhibition at 20, 40, 60, 80 and 100 µg/ml concentrations respectively. On the other hand, standard gallic acid showed 52.80% inhibition. The inhibitory concentration (IC_{50}) value of the *E. milii* leaves extract exhibited 63.06 µg/mL against the DPPH and that has shown in Table 4 and Figure 4.

The methanol extract of *E. milii* leaves on free radical scavenging activity using DPPH assay showed maximum activity in comparison to Gallic acid control. The concentration dependent increase in scavenging activity of the extract may be due to the ability of hydrogen donor during oxidation reaction [36]. It was also observed that no one concentration could achieve total inhibition of the enzymes in the studied extract. The dose inhibition curve and IC_{50} value 137.89µg/mL of petroleum ether extract, showing maximum free radical scavenging activity due to the crude nature of the extract, considering it as a sign of possessing potential antioxidant property. Such free radical scavenging activity results were observed with similar findings, and also IC_{50} value higher than that of standard when the crude extract of plants for the biochemical studies were used [37, 38].

Table 4: DPPH Free Radical Scavenging Assay of Methanolic Extract of *Euphorbia milii* Leaves

Samples	Concentration (µg/mL)	Inhibition (%)	IC_{50} value (µg/mL)
Gallic Acid Standard	100	0.150±0.017	52.80
Methanol Extract	20	0.593±0.001	63.06
	40	0.436±2.660	
	60	0.360±0.013	
	80	0.241±1.813	
	100	0.176±0.002	

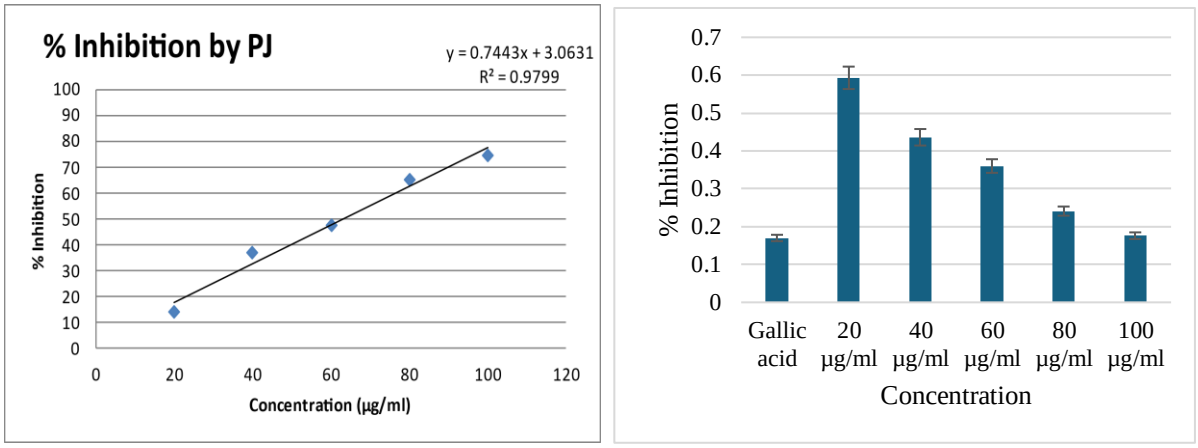

Fig. (4). Showing Results of Inhibition (%) and IC_{50} values with various Concentration of Methanol Extract of *E. milii* Leaves along with Standard Gallic acid on DPPH Free Radical Scavenging Assay

4. Conclusion

The current findings of this study revealed that methanol extract of *E. milii* leaves possess the potent biological activities such as anticancer, antidiabetic, antioxidant which are emphasized due to the richness of phytoconstituents in the extract and may one of the metabolites held responsible for these properties. It is evidently recognized the anticancer potential on A375 human cell lines by MTT assay in higher concentration of methanol extract similar to that of standard Cisplatin studies. In antidiabetic studies also resulted significantly inhibiting α-amylase enzyme in dose dependent manner along with standard Acarbose, whereas, antioxidant studies proven with free radical scavenging by DPPH due to the richness of phytoconstituents in the methanol extract and exhibited more than that of standard Gallic acid. Hence, plant is highly potential as an ethnomedicinal for various diseases which also reported with other parts of the plant previously. The ornamental plant may use it in pharmacological purpose by further research by clinical studies and characterization of novel molecules to commercial it in the ayurvedic industry for combating medical issues as a natural remedy.

5. References

1. Sofowora, A., Ogunbodede, E., and Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. African Journal of Traditional, Complementary, and Alternative Medicines, 10(5): 210–229.
2. UNESCO, Culture and Health, Orientation Texts-World Decade for Cultural Development 1988 - 1997. Paris, France: 1996. Document CLT/DEC/PRO - 1996. p. 129
3. Cowman, M. (1999). Plant products as antimicrobial agents. Clinical Microbiology Reviews, 12(4): 564-582.
4. Adesokan, A.A., Yakubu, M.T., Owoyele, B.V., Akanji, M.A., Soladoye, A.O. and Lawal, O. (2008). Effect of administration of aqueous and ethanolic extracts of *Enantia chlorantha* stem bark on brewer's yeast-induced pyresis in rats African. Journal of Biochemistry Research, 6: 33-37
5. Nascimento, F., Locatelli, J., Freitas, C. and Silva, G. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Brazilian Journal of Microbiology, 31. 10.1590/S1517-83822000000400003.

6. Sagar, S. (2021). A review on phytopharmacology of medicinal plant: *Euphorbia milii* des moul. International Research Journal of Pharmacy, 12: 67-74. 10.7897/2230-8407.1206146.
7. Pascal. O.A., Bertrand, A.E., Esaie, T., Sylvie, H.A. and Eloi, A. (2017). A review of the ethnomedical uses, phytochemistry and pharmacology of the Euphorbia genus. The Pharma Innovation Journal, 6(1): 34-39.
8. Flory, M. and Vinusha, C. (2015). Phytochemical screening and antimicrobial studies in leaf extract of *Euphorbia milii*. International Journal for Multidisciplinary Research, 1(1): 13-19.
9. Aleksandrov, M., Maksimova, V. and Gudeva, L. (2019). Review of the anticancer and cytotoxic activity of some species from genus Euphorbia. Agriculturae Conspectus Scientifcus, 84(1): 1-5.
10. Alley, M.C., Scudiere, D.A., Monks, A., Czerwinski, M., Shoemaker, R.II. and Boyd, M.R. (1986). Validation of an automated microculture tetrazolium assay (MTA) to assess growth and drug sensitivity of human tumor cell lines. Proceedings of American Association of Cancer Research, 27: 389.
11. Fonseca, K.C., Morais, N., Queiroz, M., Silva, M., Gomes, M., Costa, J., Mamede, C., Torres, F., Silva, N., Beletti, M., Canabrave, H. and Oliveria, F. (2010). Purification and biochemical characterization of Eumiliin from *Euphorbia milii* var. hislopii latex. Phytochemistry, 71(7): 708-715.
12. Bijekar, S. and Gayatri, M.C. (2014). Ethnomedicinal properties of Euphorbiaceae family- A comprehensive review. International Journal of Phytomedicine, 6(2):144-156.
13. Haleshappa, R., Keshamma, E., Girija, C.R., Thanmayi, M., Nagesh, C.G., Lubna Fahmeen, G.H., Lavanya, M. and Patil, S.J. (2020). Phytochemical study and antioxidant properties of ethanolic extracts of *Euphorbia milii*. Asian Journal of Biological Sciences, 13(1): 77-82
14. Sharangouda and Patil, S.B. (2004). Phytochemical screening and antifertility activity of various extracts of *Citrus medica* (Lemon) seeds in albino rats. Advances in Pharmacology and Toxicology, 8(2): 71-74.
15. Harborne, J.B. (1973). Phytochemical Methods: A guide to modern techniques of plant analysis, Chapman and Hall, London, UK.
16. Harborne, J.B. (1993). Phytochemistry. London: Academic Press, 89-131.
17. Farnsworth, N.R. (1966). Biological and phytochemical screening of plants. Journal of Pharmaceutical Sciences, 55(3): 225-76.
18. Giri, S., Ankali, S., Pavani, M., Binorkar, S.K., Keshamma, E., Kolgi, R.R., Lavanya, L. and Patil, S.J. (2024). Anti-inflammatory, anticancer and phytochemical potential of Indigofera cordifolia various extracts. African Journal of Biological Sciences, 6(6): 5419-5429.
19. Sylvester, P.W. (2011). Optimization of the tetrazolium dye (MTT) colorimetric assay for cellular growth and viability. Drug Design and Discovery, 716(9): 157-168.
20. Ononamadu, C.J., Alhassan, A.J., Imam, A.A., Ibrahim, A., Ihegboro, G.O., Owolarafe, A. and Sule, M.S. (2019). In vitro and in vivo anti-diabetic and anti-oxidant activities of methanolic leaf extracts of *Ocimum canum*. Caspian Journal of Internal Medicine, 10(2): 162-175.
21. Kazeem, M.I., Dansu, T.V. and Adeola, S.A. (2013). Inhibitory effect of *Azadirachta indica* A Juss leaf extract on the activities of α-amylase and α-Glucosidase. Pakistan Journal of Biological Science,16: 1358–62.
22. Blois, M.S. (1958). Antioxidant determinations by the use of a stable free radical. Nature, 181: 1199-1250.

23. Kolgi, R.R., Haleshappa, R., Sajeeda, N., Keshamma, E., Karigar, C.S. and Patil, S.J. (2021). Antioxidant studies, in vitro cytotoxic and cell viability assay of flavonoids and alkaloids of *Leucas aspera* (Wild.) Linn leaves. Asian Journal of Biological and Life Sciences, 10(1): 165-171.
24. Londonkar, R.L., Patil, S.J., Patil, S.B. (2009). Phytochemical and contraceptive property of *Sida acuta* Burm Fi. Ind. in albino rats. International Journal of Pharmatech Research, 1(4): 1260-1266.
25. Haleshappa, R., Patil, S.J., Usha, T. and Murthy, S.K.M. (2020). Phytochemicals, antioxidant profile and GCMS analysis of ethanol extract of *Simarouba glauca* seeds. Asian Journal of Biological and Life Sciences, 9(3): 379-385.
26. Haleshappa, R., Patil, S.J. and Murthy, S.K.M. (2021). Phytochemical analysis, in vitro evaluation of antioxidant and free radical scavenging activity of *Simarouba glauca* seeds. Advances in Pharmacology and Pharmacy, 9(1): 01-08.
27. Haleshappa, R., Sajeeda, N., Kolgi, R.R., Patil, S.J. and Murthy, S.K.M. (2022). Phytochemicals, anti-nutritional factors and proximate analysis of *Simarouba glauca* seeds. International Advanced Research Journal in Science, Engineering and Technology, 09(3): 218-227.
28. Sreedharan, S., Gothe, S., Aier, K., Kirankumar, S.V., Kalva, P.K. and Patil, S.J. (2020). Bioactive molecules and antimicrobial studies of *Rhus semialata* seeds. Research Journal of Medicinal Plants, 13(1): 10-17.
29. Bairagi, J.H., Haritha, G., Yadav, K., Garg, S., Rani, V., Pulipati, S., Kolgi, R.R., Pundir, R. and Patil, S.J. (2023). To study of *Artemisia nilagirica* leaves for their antithyroid, oxidative and antihyperglycemic properties. Journal of Advanced Zoology, 44(S4): 40–51.
30. Dakshayini, P.N., Roy, U.B., Kolgi, R.R., Singh, S.R., Sharma, S., Shrivastava, R. and Patil, S.J. (2023). Efficacy of phytoextracts on female reproduction and impact on diabetes mellitus. Journal of Advanced Zoology, 44(4): 1154–1160.
31. Roy, U.B., Seethalaxmi, R., Renuka Jyothi, S., Dsouza, M.R., Mahishi, P., Premalatha, S.J. and Patil, S.J. (2023). Herbal medical product for metabolic diseases: A new pharmacological approach. Pakistan Heart Journal, 56(2): 1451-1450.
32. Tamil, I.G., Dineshkumar, B., Nandhakumar, M., Senthilkumar, M. and Mitra, A. (2010). In vitro study on α-amylase inhibitory activity of an Indian medicinal plant, Phyllanthus amarus. Indian Journal of Pharmacology, 42(5): 280–282.
33. Bhosale, H., Sonsale, P. and Shaheen, U., (2018). In Vitro Studies on Alpha Amylase Inhibitory Activity of Indian Medicinal Plants. International Journal of Pharmacy and Biological Sciences, 8(3): 636-640.
34. Bagewadi, S. and Giri, S. Phytochemical profile, antimicrobial, antidiabetic and anti-inflammatory studies of *Gynandropsis pentaphylla* leaves extracts. Journal of Advanced Zoology, 44(S-6): 01:08.
35. Olayinka O.A., Alashi Adeola M. and Aluko Rotimi E. (2021). Inhibition of the in vitro activities of α-amylase and pancreatic lipase by aqueous extracts of *Amaranthus viridis, Solanum macrocarpon* and *Telfairia occidentalis* leaves. Frontiers in Nutrition, 8, doi=10.3389/fnut.2021.772903
36. Pushpa, T.C., Gupta, A.A., Shukla, N., Gupta, P.C., Kumar, S., Alice Sheba S., Sharma, M.K. and Patil, S.J. (2023). Evaluation of in vitro, anti-inflammatory and anti-oxidant activity on the aqueous and ethanolic extract of leaves of *Hygrophila balsamica*. China Petroleum Processing and Petrochemical Technology, 23(2): 2750-2764.
37. Prithiviraj, N., Louis, L.E.P., Patil, S.J., Adam, J.K. and Krishna, S.B.N. (2024). Therapeutic potential of biologically active peptides from marine organisms for

biomedical applications. In: Studies in Natural Products Chemistry, Chapter 13, 81, 467-500.

38. Patil, S.J., Renuka Jyothi S., Sadashiv S.O., Vishwantaha T., Adam, J.K. and Naidu, S.B.K. (2023). Antioxidant potential of *Zephyranthes citrina* seed extract in Saccharomyces cerevisiae's oxidative stress response system seeds. Current Concepts in Biotechnology and Pharmacy, 17(Suppl. Iss- 3A): 1129 – 1140.