Biological Forum – An International Journal **15(2): 123-129(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Phytochemical, Antioxidant and Antiviral Potential of *Euphorbia milii*

U. Tiwari[1], Rashmi Parihar[2] and Sumit Kumar Dubey[2*]
[1]Department of Botany, Government E.R. Rao
P.G. Science College, Bilaspur (Chhattisgarh), India.
[2]Department of Microbiology, Government E.R. Rao
P.G. Science College, Bilaspur (Chhattisgarh), India.

(Corresponding author: Sumit Kumar Dubey)*
(Received: 22 December 2022; Revised: 27 January 2023; Accepted: 31 January 2023; Published: 07 February 2023)
(Published by Research Trend)

ABSTRACT: *Euphorbia milii* has been widely known as a remedy for skin infections, warts, cancer cells, hepatic disorders, fungal infections, nociceptive conditions, and viral infections. The secondary metabolites viz., alkaloids, anthraquinone, anthocyanin, betacyanin, glycosides, flavonoids, phlobatannins, saponin, steroids, tannins, terpenoids, reducing sugars, and amino acids were examined. The maximum efficacy of phytoconstituents extraction was noted with ethanol (91.66 %). The hot water exhibited maximum free radical scavenging activity (IC_{50}) of 6.12 µg/ml. The percentage scavenging activity (PSA) of hot water extract was calculated at 74.37%. The number of plaques in the titer plate was used as a measure of the antiviral strength of *E. milii* extracts. The ethanol extract of *E. milii* exhibited 22 plaques in the titer plate which showed maximum antiviral strength among tested solvent extracts of *E. milii*. Cyclobarbital was screened from the literature as a major bioactive constituent of *E. milii*. Later, the ZINC and Swiss-ADME profile of cyclobarbital was assessed and it showed acceptable druggability. The present course of investigation represented notable medicinal applications of *E. milii* extract as an antiviral agent that would further be extended to molecular characterization and *in silico* antiviral action modeling to get depth insight. The major challenge associated with the antiviral potential of bioactive agents is to cope with a viral mutation that is directly linked with target protein or receptor molecules. Hence consistent research and development of drug modification are required to remediate target-based bioactive agents.

Keywords: *Euphorbia milii*, secondary metabolite, phytoconstituents, scavenging activity, antiviral and medicinal application.

INTRODUCTION

The phytochemicals offer protection and healing deeds to the body without harmful side effects (Safarzadeh *et al.,* 2014). The intermingling of different groups of phytochemicals results in the pharmacotherapeutic action of medicinal flora (Kaur *et al.,* 2011). Genus *Euphorbia* is widely distributed over the tropical province of India and China (Rauf *et al*., 2014). The *Euphorbia* species are conventionally involved in folk medication systems to cure various ailments i.e., help to get rid of intestinal parasites and reduce moles, and skin-related issues (Bani *et al*., 2007). Phytochemical analysis of different *Euphorbia* species revealed that the biologically active secondary metabolites *viz.,* aesculetin, daphnetin, daucosterol, diterpenes and hyfroxybenzoic acid, kaempferol-3-glucuronide, sitosterol, and vitexicarpin (Chaman *et al.,* 2021; Zheng *et al.,* 2009). *Euphorbia milii* (alternative names; a crown of thorns and Christ plant) has been documented as a skin infections remediator, active against warts, inhibits cancer cells, cures hepatic disorder, acts as an antifungal, molluscicidal activity, and antinociceptive (Rauf *et al.,* 2014; Qaisar *et al.,* 2012; Delgado *et al.,* 2003). *E. milii* has been reported to consist of amyrin acetate, cycloartenol, flavonoids, lectin, lupeol, and triterpenes (Tonoli *et al.,* 2012). Several *Euphorbia* genera have widely been acceptable as folklore medication in Indian ayurveda to treat viral infections (Shaker *et al.,* 2022; Diop *et al*., 2018). The *Euphorbia* sp. and *E. milii*-derived phytochemicals have potential against SARS-CoV-2 (Cayona and Creencia 2022; Chaman *et al.,* 2021). Various literature has been published on secondary metabolites and medicinal applications of *E. milii* (Chaman *et al.,* 2021; Rauf *et al.,* 2014) *i.e.,* Haleshappa *et al*. (2020) revealed that *E. milii* has a significant level of flavonoids and Ben-Shabat *et al*. (2020) have reported that flavonoids and its derivative have notable antiviral potential. Hussain (2021) examined the cytotoxic and antiviral potency of *E. milii* against Peste des petits-ruminants virus and

found its methanol extract as virucidal at a significant level of $p < 0.05$. *E. milii* has expressed noteworthy antioxidant activity (Sundriyal *et al.,* 2021; Chohan *et al*., 2020; Rauf *et al*., 2014). Thereby, the present study was conducted to explore the phytochemical diversity, antioxidant potential, and antiviral efficacy of *E. milii*.

MATERIALS AND METHODS

A healthy plant of *E. milii* was collected from the Bilaspur district and brought to the laboratory (Fig. 1a). The pure culture of *E. Coli* was procured from the Department of Biotechnology, D.L.S. P.G. College, Bilaspur (C.G.). The viral particles were isolated from the nasal cavity using a sterile swab and inoculated into Dulbecco's Phosphate-Buffered Saline (DPBS). The viral inoculated DPBS was filtered through a 0.02 µm pore-sized membrane and then DPBS-based viral filtrate was used for further experimental work. The experiments were carried out in session 2021-22. All the chemicals used were of analytical grade. The instruments/apparatus were calibrated before use.

The clean plant materials were dried under shade for 7 days and then kept Dried Plant Materials (DPMs) inside a hot air oven at 45°C for 1 hour. The DPMs were subjected to maceration. The DPM was ground and filtered with a 60-mesh size net. The fine coarse powder of DPMs (Fig. 1b) was soaked in menstrum i.e., methanol, ethanol, and hot water, for five days to extract bioactive agents. The liquid extract of DPMs was strained using a sieve. The remaining solid residue (marc) was pressed to recover the occluded liquid. The sieved DPMs were filtered to clarify and then subjected to phytochemical analysis (Fig. 1c).

Fig. 1a. *Euphorbia milii* **Fig. 1b.** Powder of the whole plant **Fig. 1c.** Solvent extracts.

A. Phytochemical analysis

Phytochemical analysis of *E. milii* DPMs was carried out using the standard method with slight medication as per the literature including Sofora & Trease and Evans (Sofora, 1993; Trease and Evans 1989).

(i) Alkaloids. A 0.5 g of extract was mixed with 2.0 mL H_2SO_4 (2%) and warmed for 2 min in a water bath. The extract was then filtered and Dragendorff s reagent was dropwise added to the filtrate. The Orange-Red precipitation exhibited the presence of alkaloids in the extract.

(ii)Anthraquinone. A 0.5 g of extract was mixed with HCl (10%) and boiled in a water bath and let to meet room temperature. The chloroform was mixed in the reaction mixture in an equal ratio (1:1) and then a few drops of ammonia were added. The development of a rose-pink color upon heating indicated the anthraquinones in the extract.

(iii) Anthocyanin and Betacyanin. A 0.5 g of extract was mixed NaOH (2.0 N) and kept in a boiling water bath for 5.0 min. The appearance of bluish-green color exhibited the presence of anthocyanin and betacyanin in the extract.

(iv) Glycosides. A 0.5 g of extract was digested with HCl and then neutralized with alkaline NaOH. Fehling s solutions A and B were mixed with the extract and then the appearance of red precipitate exhibited the glycosides in the extract.

(v) Flavonoids. A 0.2 g of extract was mixed with 0.8 ml of 10% NaOH and then yellow color appeared. The reaction mixture turned yellow to colorless upon the addition of 10 drops of HCl (2M) which confirmed the presence of flavonoids content in the extract.

(vi) Phlobatannins. A 0.5 g of extract was mixed with 2.0 M of HCl and boiled. The appearance of a red precipitate indicated a positive result for phlobatannins.

(vi) Saponins. A 0.5 g of extract was mixed with some distilled water and boiled. The appearance of frothing upon boiling indicated saponins in the extract.

(viii) Steroids. A 0.5 g of extract mixed with 2.0 ml of acetic anhydride. Color change from violet to blue upon the addition of 2.0 mL of H_2SO_4 indicated a positive result for steroids.

(ix) Tannins. A 0.5 g of extracts were mixed in warm water for 2.0 min and filtered. The filtrate was added with little ferric chloride ($FeCl_3$). The development of dark-green color showed the presence of tannins in the extract.

(x) Terpenoids. A 0.5 g of extract was mixed with chloroform (2.0 ml) and H_2SO_4 (3.0 ml) gently to make a layer. The appearance of reddish-brown color at the interface indicated a positive result for terpenoids.

(xi) Reducing sugars. The extracts were shaken with distilled water and filtered. A few drops of Fehling s solutions A and B were added and boiled for a few minutes. The formation of an orange-red precipitate confirmed the presence of reducing sugar.

(xii) Amino acids. A 0.2% ninhydrin solution was added to the 0.5 g of extract and the development of blue color, upon heating indicated the presence of amino acids in the extract.

B. Efficacy of phytoconstituents extraction of solven

The efficacy of phytoconstituents extraction of solvent was calculated by the following formula:

$$\text{Efficacy of phytoconstituents extraction of solvent (\%)} = \frac{\text{Total no. phytoconstituents found in the extract}}{\text{Total no. phytoconstituents analyzed}} \times 100$$

C. Free radical scavenging assay

1,1-diphenyl-2-picrylhydrazyl radical (DPPH) assay was used to assess the antioxidant potency of DPM as mentioned by Koleva *et al*., (2002); Chohan *et al.* (2020). Methanolic DPPH was used to calculate the scavenging capacity of the selected DPMs. The spectrophotometric absorbance of diphenylpicryl hydrazine (yellow-colored) was measured at 517 nm (Kedare and Singh 2011). L -ascorbic acid was used to make a standard curve. IC_{50} (Half maximal Inhibitory Concentration) was calculated using linear regression. The absorbance value is inversely proportional to radical scavenging activity. Percentage scavenging activity (PSA) was calculated by the following formula (Kumara *et al*., 2018):

$$\text{PSA} = \frac{\text{Abs of control} - \text{Abs of test fraction}}{\text{Abs of control}} \times 100$$

IC_{50} value was calculated using the following formula (Ibrahim, 2017):

IC_{50} = (0.5 - b)/a (derived from standard curve equation was 'y = ax + b')

where x represents the IC_{50}

D. Plaque assay for antiviral strength of E. milii extract

The host cell *Escherichia coli* was grown in nutrient broth till the log phase and inoculated into Nutrient Agar Media. A 1.0 ml of fresh DPBS-based viral filtrate was mixed with 1.0 ml of *E. milii* extract and then aseptically inoculated in nutrient agar media (pre-inoculated with *E. coli*). The inoculated agar plates were incubated at 37°C till the plaque appeared. The plaques were counted. A control plate consisted of only 1.0 ml of viral suspension (in DPBS) without *E. milii* extract. The Viral titer was calculated through the following formula:

$$\text{Viral Titer (plaque forming unit /ml)} = \frac{\text{No. of Plaques}}{(D \times V)}$$

D = Dilution factor

V = Volume of diluted virus

E. Correlation between literature findings and natural product databases

Several natural product databases (NPDs) e.g., NPASS, NP Atlas, SuperNatural II, ZINC, Swiss-ADME, and so on, are available for preliminary screening of potent bioactive isolates for pharmacological applications before proceeding for further modern wet-lab analysis i.e., molecular characterization using Gas Chromatography-Mass Spectrometry (GC-MS), High-Performance Liquid Chromatography (HPLC), Nuclear Magnetic Resonance (NMR), Electron Microscope (EM), Fourier Transform Infra-Red Spectroscopy (FTIRS), X-Ray Diffraction (XRD) and so on (Sorokina and Steinbeck 2020; Irwin *et al.,* 2020; Van Santen *et al.,* 2019; Zeng *et al.,* 2018; Banerjee *et al.,* 2015). The present work was correlated with Chohan *et al.* (2020) for NPDs-omics-based prediction.

F. Statistical analysis

The statistical calculations and graphs were processed using Microsoft Office 2021. All the experiments were carried out in triplicates to minimize error.

RESULTS AND DISCUSSION

The present research work was conducted to examine the phytochemical diversity, antioxidant potential, and antiviral efficacy of *E. milii*. Three extracts of *E. milii viz*., hexane, ethanol, and hot water (Fig. 1c) were evaluated for phytochemical assessment *i.e.,* alkaloids, anthraquinone, anthocyanin, betacyanin, glycosides, flavonoids, phlobatannins, saponin, steroids, tannins, terpenoids, reducing sugars and amino acids. The result showed that the alkaloids, flavonoids, and reducing sugars were found common in all extracts of *E. milii* while Saponin content was not detected. Additionally, the hexane extract of *E. milii* consisted of phlobatannins, steroids, and terpenoids while the ethanol extract was comprised of alkaloids, anthraquinone, anthocyanin, betacyanin, glycosides, flavonoids, phlobatannins, steroids, tannins, terpenoids, reducing sugars and amino acids content. The phytochemical assessment of hot water extract exhibited the presence of alkaloids, anthocyanin, betacyaninm, glycosides, tannins, reducing sugars, and amino acids. The maximum phytochemical diversity was noted in ethanol extract and the least in hexane extract (Table 1). The efficacy of phytoconstituents extraction has achieved a maximum of 91.66 ± 2.37 % with ethanol (Fig. 2). This may be due to the capability of ethanol to dissolve both polar and non-polar phytoconstituent. Water and hexane have polar and non-polar nature respectively. Ben-Shabat *et al.* (2020) reported that flavonoids have an antiviral efficacy that could potentially be used as a drug. In the present study, a solvent extract of *E. milii* was observed with significant flavonoid content. Therefore, it is a positive indication to offer the effective utilization of *E. milii* extract as an antiviral bioactive agent. Apart from flavonoids, alkaloids, glycosides, terpenoids, and tannins have also been reported for antiviral properties against HSV (herpes simplex virus), HCV (hepatitis C virus), PEDV (Porcine epidemic diarrhea virus), HIV-1 (human immunodeficiency virus) (Corlay *et al*., 2014; Dao *et al.,* 2011; Esimone *et al*., 2010; Fang *et al.,* 2015; Kang *et al.,* 2015; Moghaddam *et al*., 2014; Lin *et al.,* 2015; Wu *et al.,* 2012).

The DPPH assay of *E. milii* extracts revealed that the hot water has maximum Free radical scavenging activity (IC_{50}) of 6.12 ±0.12 µg/ml while the minimum IC_{50} with hexane was found 3.94 (µ/ml) (Fig. 3). Chohan *et al.* (2020) reported that methanol extract of

E. milii exhibited the highest antioxidant activity of 6.41 ± 0.99 µg/ml. The percentage scavenging activity (PSA) of hexane, ethanol, and hot water extract of *E. milii* was recorded 52.59 ±1.73%, 61.24 ±2.05, and 74.37 ± 2.19 % respectively. The viral infection in cells has been reported to induce a level of free radicals, therefore the antioxidants nature of plants or herbs might play a crucial role to saturate free radicals (a Reactive Oxygen Species) and boost immunity toward the alleviation of viral infection (Hejrati *et al.,* 2021; Peterhans, 1997). The extracts of *E. milii* exhibited a significant free radical scavenging activity (IC_{50}) in the present work.

A total of 79 plaques were observed in the control titer plate while the hexane, ethanol, and hot water extract of *E. milii* exhibited 58, 22, and 47 plaques in the titer plate respectively (Fig. 4a and 4b). The ethanol extract of *E. milii* indicated a maximum antiviral potency against viral particles because of the appearance of less plaque formation. The plaque formation was defined as the measure of the antiviral potency of the *E. milii* extract i.e., the less number of plaque represented maximum antiviral efficacy. However, a single report has been published on the antiviral and cytotoxic potential of *E. milii* in which they found methanol and chloroform extract as significant (Chaman *et al*., 2021).

The correlation between the present work and relevant literature findings revealed that Chohan *et al.* (2020) reported that the GC–MS profiling of *E. milii* extract exhibited the cyclobarbital and benzodioxole derivatives as major components. Afterward, based on the aforementioned literature claim, the cyclobarbital and benzodioxole derivatives were mined in NPDs. The brief study of NPDs revealed that cyclobarbital and benzodioxole derivatives have noteworthy biological activity i.e., biological target, activity type (EC50 and IC50) and activity value, and physiochemical information i.e., molecular structure (2D and 3D) and properties along with a source of natural products with authentic reference. The findings revealed that the cyclobarbital information was available only in ZINC and Swiss-ADME databases but not in NPASS, NP Atlas, and SuperNatural II. Further, the biological database (ZINC and Swiss-ADME) profile of cyclobarbital is briefly summarized in Table 2. Maitra *et al.* (2019) reported that tea leaves derived-Cyclobarbital was act as antiproliferative agent. Reeves *et al.* (2017) claimed that anti-proliferative therapy could potentially be used to suppress viral infection-based severity i.e., HIV. Hence, It was noted that among plenty of plant-derived bioactive agents a few of them qualified for pharmacological preparations. Additionally, the NPDs are still demanding upgradation. The dry lab (NPDs-omics-based prediction) helps a researcher to predict the pharmacological potential of identified bioactive agents before proceeding with expensive molecular characterization. Afterward, the researcher could go further for assessment of the correlation of targeted potential bioactive candidates towards pharmaceutical draggability.

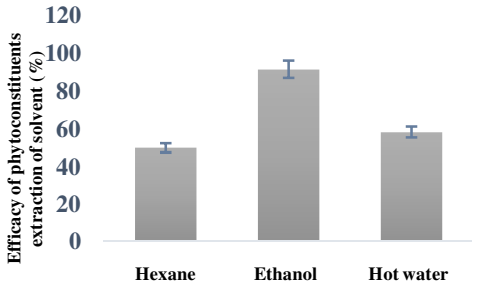


Fig. 2. Efficacy of phytoconstituents extraction of solvent (%).

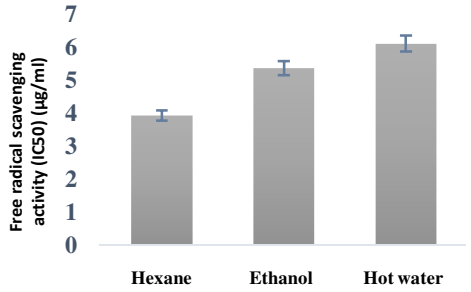


Fig. 3. Free radical scavenging activity (IC_{50}) (µg/ml).

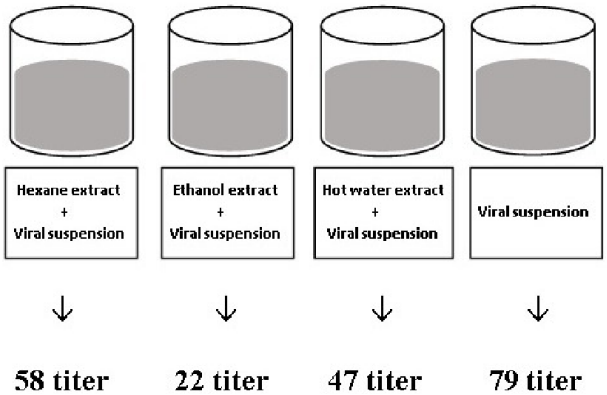


Fig. 4a. Plaque in titer plate of ethanol extract.

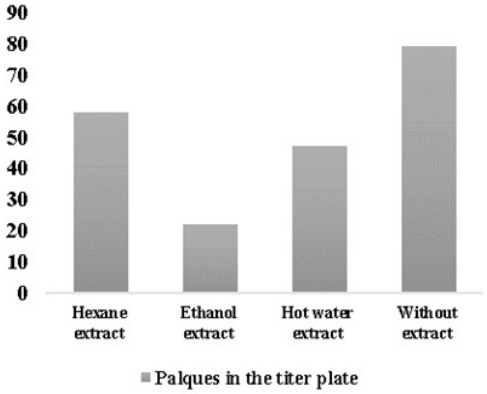


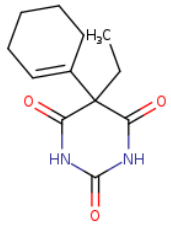
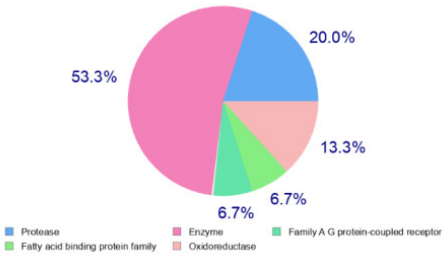
Fig. 4b. No. of Palques in the titer plate.

Table 1: Qualitative Phytochemical assessment of hexane, ethanol, and hot water extract of *E. milii*.

Phytochemical assessment	Hexane	Ethanol	Hot water
Alkaloids	+	+	+
Anthraquinone	-	+	-
Anthocyanin and betacyanin	-	+	+
Glycosides	-	+	+
Flavonoids	+	+	+
Phlobatannins	+	+	-
Saponin	-	-	-
Steroids	+	+	-
Tannins	-	+	+
Terpenoids	+	+	-
Reducing sugars	+	+	+
Amino acids	-	+	+

Positive (+); Negative (-)

Table 2: Biological database (ZINC and Swiss-ADME) profile of cyclobarbital.

Biological and physiochemical feature with special reference to pharmacological potential	Cyclobarbital
Structure (Adopted from Swiss-ADME)	
SMILES	CCC1(C2=CCCCC2)C(=O)NC(=O)NC1=O
Formula	$C_{12}H_{16}N_2O_3$
Molecular weight	236.27 g/mol (Acceptable)
TPSA	75.27 Å² (good permeability to cell membranes)
Water Solubility	Soluble
GI absorption	High
Lipinski rule (Drug-likliness)	Yes (No violation of Lipinski rule)
Bioavailability Score	0.55 (Good)
SwissTarget Prediction (Predicted by Swiss-ADME)	

CONCLUSIONS

E. milii has rich medicinal properties that could potentially be used for Ayurvedic preparations. The current research investigation revealed that the ethanol extract of *E. milii* has rich phytochemical diversity and antiviral efficacy while the water extract of *E. milii* has significant antioxidant potential. The depth of molecular characterization and *in silico* pharmaceutical modeling of *E. milii* could help to explore its antiviral potential. The future scope of the existing research would further be the characterization of bioactive agents of *E. milii* using advanced biotechnological tools and *in silico* studies for diverse medicinal applications.

Acknowledgment. The authors of the present research manuscript would like to deliver a vote of thanks to the Principal, Government E. Raghavendra Rao Postgraduate Science College, Bilaspur (C.G.) for research lab facility and D.L.S. P.G. College, Bilaspur (C.G.) for supplying bacterial culture of *Escherichia coli* for the same.
Conflict of Interest. None.

REFERENCES

Banerjee, P., Erehman, J., Gohlke, B.O.., Wilhelm, T., Preissner, R. and Dunkel, M. (2015). Super Natural II a database of natural products. *Nucleic Acids Res*., *43*, D935–D939.

Bani, S., Kaul, A., Khan, B., Gupta, V. K., Satti, N. K., Suri, K. A. and Qazi, G. N. (2007). Anti-arthritic activity of

a biopolymeric fraction from *Euphorbia tirucalli*. *J. Ethnopharmacol*, *110* (1), 92–98.

Ben-Shabat, S., Yarmolinsky, L., Porat, D. and Dahan, A. (2020). Antiviral effect of phytochemicals from medicinal plants: Applications and drug delivery strategies. *Drug Deliv. and Transl. Res*., *10*, 354–367.

Cayona, R. and Creencia, E. (2022). Phytochemicals of *Euphorbia hirta* L. and Their Inhibitory Potential against SARS-CoV-2 main Protease. *Frontiers in Molecular Biosciences*, 8.

Chaman, S., Khan, F. Z., Khokhar, R., Maab, H., Qamar, S., Zahid, S., Ahmad, M. and Hussain, K. (2021). Cytotoxic and antiviral potentials of *Euphorbia milii* var. splendens leaf against Peste des petits ruminant virus. *Tropical Journal of Pharmaceutical Research*, *18*(7), 1507–1511.

Chang, F.R., Yen, C.T., Ei-Shazly, M., Lin, W.H., Yen, M.H., Lin, K-H. and Wu, Y-C. (2012). Anti-human coronavirus (anti-HCoV) triterpenoids from the leaves of *Euphorbia neriifolia*. *Nat Prod Commun*, *7*(11), 1415-1417.

Chohan, T. A., Sarfraz, M., Rehman, K., Muhammad, T., Ghori, M. U., Khan, K. M., Afzal, I., Akash, M. S., Alamgeer, Malik, A. and Chohan, T. A. (2020). Phytochemical profiling, antioxidant and antiproliferation potential of *Euphorbia milii* var.: Experimental analysis and in-silico validation. *Saudi Journal of Biological Sciences*, *27*(11), 3025–3034.

Corlay, N., Delang, L., Valenciennes, E.G., Neyts, J., Clerc, P., Smadja, J., Guéritte, F., Leyssen, P. and Litaudona, M. (2014). Tigliane diterpenes from *Croton mauritianus* as inhibitors of Chikungunya virus replication. *Fitoterapia*, *97*, 87-91.

Dao, T. T., Nguyen, P. H., Lee, H. S., Kim, E., Park, J., Lim, S. I. and Oh, W. K. (2011). Chalcones as novel influenza A (H1N1) neuraminidase inhibitors from *Glycyrrhiza inflate*. *Bioorg. Med. Chem. Lett*., *21*, 294-298.

Delgado, I., De-Carvalho, R., De-Oliveira, A., Kuriyama, S., Oliveira-Filho, E., Souza, C. and Paumgartten, F.J.R. (2003). Absence of tumor promoting activity of Euphorbia milii latex on the mouse back skin. *Toxicol. Lett*., *145*(2), 175–180.

Diop, E. H. A., Queiroz, E. F., Kicka, R. S., Diop, S., Soldati, T. and Wolfender, J. L. (2018) Survey on medicinal plants traditionally used in senegal for the treatment of tuberculosis (TB) and assessment of their antimycobacterial activity. *J Ethnopharmacol*, *216*, 71–78.

Esimone, C. O., Gero E., Nworu, C. S., Hoffmann, D., Uberla, K. and Proksch, P. (2010). Dammarenolic acid, as ecodammaranetriterpenoid from *Aglaia* sp. shows potent anti-retroviral activity *in vitro*. *Phytomed*, *17*, 540-547.

Fang, C. Y., Chen, S. J., Wu, H. N., Ping, Y. H., Lin, C. Y., Shiuan, D., Chen, C. L., Lee, Y. R. and Huang, K. J. (2015). Honokiol, a lignanbiphenol derived from the magnolia tree, inhibits dengue virus type 2 infection. *Viruses*, *7*, 4894-4910.

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*, 77-82.

Harborne, J. B. (1973). *Phytochemical Methods*. 11th Ed., D.E. and Hall Ltd., *London*, pp: 135-203.

Hejrati, A., Nurzadeh, M. and Roham, M. (2021). Association of coronavirus pathogencity with the level of antioxidants and immune system. *J Family Med Prim Care*, *10*(2), 609-614.

Ibrahim, O. (2017). Re: How to calculate IC50 from antioxidant activities data on Excel ?, Retrieved from: https://www.researchgate.net/post/How_to_calculate_IC50_from_antioxidant_activities_data_on_Excel/588a6df9ed99e1dba14e42a8/citation/download.

Irwin, J. J., Tang, K. G., Young, J., Dandarchuluun, C., Wong, B.R., Khurelbaatar, M., Moroz, Y. S., Mayfield, J. and Sayle, R.A. (2020). ZINC20—A Free Ultralarge-Scale Chemical Database for Ligand Discovery. *J. Chem. Inf. Model*, *60*, 6065–6073.

Kang, K. B., Ming, G., Kim, G. J., Ha, T. K. Q., Choi, H., Oh, W. K. and Sung, S. H. (2015). Cyclopeptide alkaloids from the roots of *Ziziphus jujuba*. *Phytochem*, *43*, 264-267.

Kaur, R., Kapoor, K. and Kaur, H. (2011). Plants as a source of anticancer agents. *J. Nat. Prod. Plant Resour.*, *1*(1), 119–124.

Kedare, S. B. and Singh, R.P. (2011). Genesis and development of DPPH method of antioxidant assay. *J Food Sci Technol*., *48*(4): 412-22.

Koleva, I. I., Van Beek, T. A., Linssen, J. P., Groot, A. and Evstatieva, L. N. (2002). Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochem. Anal.: Int. J. Plant Chem. Biochem. Techn.*, *13*(1), 8–17.

Kumara, P., Sunil, K. and Arun, K.B. (2018). Determination of DPPH Free Radical Scavenging Activity by RP-HPLC, Rapid Sensitive Method for the Screening of Berry Fruit Juice Freeze Dried Extract. *Nat Prod Chem Res.*, *6*, 341.

Lin, L. T., Chung, C.Y., Hsu, W. C., Chang, S. P., Hung, T. C., Shields, J., Russell, R.S., Lin, C .C., Li, C. F., Yen, M. H., Tyrrell, D. L. J., Lin, C. C. and Richardson, C. D. (2015). Saikosaponin b2 is a naturally occurring terpenoid that efficiently inhibits hepatitis C virus entry. *Journal of Hepatology*, *62*(3), 541–548.

Maitra, S., De, A., Das, B., Roy, S. N., Chakraborty, R., Samanta, A. and Bhattacharya, S. (2019). Seasonal Variation of Phyto-Constituents of Tea Leaves affects Antiproliferative Potential. *J. Am. Coll. Nutr*., *38,* 415–423.

Moghaddam, E., Teoh, B. T., Sam, S. S., Lani, R., Hassandarvish, P., Chik, Z., Yueh, A., Abubakar, S. and Zandi, K. (2014). Baicalin, a metabolite of baicalein with antiviral activity against dengue virus. *Sci. Rep*., *4*, 5452.

Peterhans, E. (1997). Oxidants and antioxidants in viral diseases: disease mechanisms and metabolic regulation. *J Nutr*., *127*(5 Suppl), 962S-965S.

Qaisar, M., Naeemuddin Gilani, S. and Farooq, S. (2012). Preliminary comparative phytochemical screening of *euphorbia* species. *Am.-Eurasian J. Agric. Environ. Sci., 12*(8), 1056–1060.

Qaisar, S. and Khawaja, K. F. (2012). Cloud computing: network/security threats and countermeasures. *Interdis. J. Contemp. Res. Bus.*, *3*, 1323–1329.

Rauf, A., Khan, A., Uddin, N., Akram, M., Arfan, M., Uddin, G. and Qaisar, M. (2014). Preliminary phytochemical screening, antimicrobial and antioxidant activities of

Euphorbia milli, *Pakistan J. Pharm. Sci*., *27*(4).

Reeves, D. B., Duke, E. R., Hughes, S. M., Prlic, M., Hladik, F. and Schiffer J. T. (2017). Anti-proliferative therapy for HIV cure: a compound interest approach. *Sci Rep.*, *7,* 4011.

Safarzadeh, E., Shotorbani, S. S. and Baradaran, B. (2014). Herbal medicine as inducers of apoptosis in cancer treatment. *Adv. Pharm. Bull*., *4* (1), 421.

Shaker, K. H., Zohair, M. M., Hassan, A. Z. Sweelam H. M. and Ashour, W. E. (2022). LC–MS/MS and GC–MS based phytochemical perspectives and antimicrobial effects of endophytic fungus *Chaetomium ovatoascomatis* isolated from *Euphorbia milii*. *Arch Microbiol.*, *204*, 661.

Sofora, A. (1993). Medicinal Plants and Traditional Medicine in Africa. John Willey and Sons, *New York*, USA., pp.150-153.

Sorokina, M. and Steinbeck, C. (2020). Review on natural products databases: Where to find data in 2020. *J. Cheminform., 12*, 1–51.

Sundriyal, S., Dheer, P. S., Thapliyal, P., Arora, A., Sharma, A., Sinha, V. B., Sharma, M. D. and Rautela, I. (2021). Comparative antimicrobial activity and antioxidant profiling of *Euphorbia hirta*, *Euphorbia milli* and *Euphorbia pulcherrima*. *Annals of Agri Bio Research*, *26*(1), 1-6.

Tonoli, G., Teixeira, E., Corrêa, A., Marconcini, J., Caixeta, L., Pereira-da-Silva, M. and Mattoso, L. H. C. (2012). Cellulose micro/nanofibres from *Eucalyptus* kraft pulp: preparation and properties. *Carbohydr. Polym., 89*(1), 80–88.

Trease, G. E. and Evans, W. C. (1989). Pharmacognosy. 11th Ed., Macmillan Publishers, *London*, UK.

Van Santen, J. A., Jacob, G., Singh, A. L., Aniebok, V., Balunas, M. J., Bunsko, D., Neto, F. C., Castaño-Espriu, L., Chang, C., Clark, T.N ., Cleary Little, J. L., Delgadillo, D. A., Dorrestein, P. C., Duncan, K. R., Egan, J. M., Galey, M. M., Haeckl, F. P. J., Hua, A., Hughes, A. H., Iskakova, D., Khadilkar, A., Lee, J. H., Lee, S., LeGrow, N., Liu, D. Y., Macho, J. M., McCaughey, C. S., Medema, M. H., Neupane, R. P., O'Donnell, T. J., Paula, J. S., Sanchez, L. M., Shaikh, A. F., Soldatou, S., Terlouw, B. R., Tran, T. A., Valentine, M., van der Hooft, J. J. J., Vo, D. A., Wang, M., Wilson, D., Zink, K. E. and Linington, R. G. (2019). The natural products atlas: An open access knowledge base for microbial natural products discovery. *ACS Cent. Sci.*, *5*, 1824–1833.

Wu, S. F., Lin, C. K., Chuang, Y. S., Chang, F. R., Tseng, C. K., Wu, Y. C. and Lee, J. C. (2012). Anti-hepatitis C virus activity of 3-hydroxy caruilignan C from *Swietenia macrophylla* stems. *J. Viral. Hepat*., *19*, 364-370.

Zeng, X., Zhang, P., He, W., Qin, C., Chen, S., Tao, L., Wang, Y., Tan, Y., Gao, D., Wang, B., Chen, Z., Chen, W., Jiang, Y. Y. and Chen, Y. Z. (2018). NPASS: Natural product activity and species source database for natural product research, discovery and tool development. *Nucleic Acids Res*., *46,* D1217–D1222.

Zheng, F., Luo, Y., Wei, X. and Wang, B. (2009). Non-terpenoid constituents from the seeds of *Euphorbia lathyris*. *J. Trop. Subtrop. Bot*., *17* (3), 298–301.

How to cite this article: U. Tiwari, Rashmi Parihar and Sumit Kumar Dubey (2023). Phytochemical, Antioxidant, and Antiviral Potential of *Euphorbia milii*. *Biological Forum – An International Journal, 15*(2): 123-129.