BIOINFORMATION
Discovery at the interface of physical and biological sciences

OPEN ACCESS GOLD

BIOMEDICAL INFORMATICS

www.bioinformation.net
Volume 17(5)

Research Article

Antioxidant, antimicrobial and cytotoxicity of n-hexane extract from *Mollugo nudicaulis* Lam.

Siva Shankar Raj[1], Chella Perumal Palanisamy[2] & Mani Panagal[1*]

[1]Department of Biotechnology, Annai College of Arts and Science, Kovilacheri, Tamil Nadu, India; email: nirmal2014@gmail.com (S.S.R), mani.panagal@yahoo.com (M.P); [2]State Key Laboratory of Biobased Material and Green Papermaking, College of Food Science and Engineering, Qilu University of Technology, Shandong Academy of Science, Jinan 250353, China; E-mail: perumalbioinfo@gmail.com
Corresponding Author*, Panagal Mani - Email: mani.panagal@yahoo.com; Mobile: +91-9976918228

Received April 25, 2021; Revised May 28, 2021; Accepted May 28, 2021, Published May 31, 2021

DOI: 10.6026/97320630017573

Declaration on official E-mail:
The corresponding author declares that official e-mail from their institution is not available for all authors

Declaration on Publication Ethics:
The authors state that they adhere with COPE guidelines on publishing ethics as described elsewhere at https://publicationethics.org/. The authors also undertake that they are not associated with any other third party (governmental or non-governmental agencies) linking with any form of unethical issues connecting to this publication. The authors also declare that they are not withholding any information that is misleading to the publisher in regard to this article.

Abstract
The antioxidant, antimicrobial and anticancer activity of n-hexane extract of *Mollugo nudicaulis* Lam. is of interest. The antioxidant activity of the extract was determined by separate methods of radical scavenging assays. Antimicrobial activity was analyzed by disc diffusion method on fungi species, gram positive and negative species. The anticancer potential of plant extract was evaluated on A2780 cell lines by MTT assay. Results exposed that, the n-hexane extract of M.nudicaulis possess comparable significant antioxidant activity with IC_{50} values of 12.79±0.82, 36.65±0.03 and 19.59±0.26 µg/mL, on DPPH, nitric oxide and hydroxyl radical scavenging assays respectively, and also possess notable reducing power of 0.84±0.04 (Reducing power assay) and 0.75±0.02 (FRAB assay) at the maximum concentration of 200 µg/mL of the n-hexane extract. Antimicrobial activity of extract exhibited maximum zone of inhibition ranged from 11.1 ± 0.3 to 14 .5± 0.3 mm on tested microorganism. The anticancer activity of plant extract found to be strong cell growth inhibitory activity with minimal IC_{50} values of 32.46±0.92 µg/mL on A2780 cell lines. Collectively this study can be concluded that, the n-hexane extract of *M.nudicaulis* might act as possible antioxidant, antimicrobial and anticancer agents.

Keywords: *M. nudicaulis*; n-hexane extract; antioxidant activity; antimicrobial activity; anticancer activity

Background:
Oxidative stress is refers to imbalance of reactive oxygen species (ROS) during the metabolic activity of the cells, which is an essential risk factor in the pathogenesis of numerous chronic diseases **[1].** ROS are well-known agents involved in the pathogenesis of sicknesses such as asthma, inflammatory arthropathies, diabetes, Parkinson's and Alzheimer's diseases, cancers as well as atherosclerosis. It is also said to be responsible for

BIOINFORMATION
Discovery at the interface of physical and biological sciences

the human aging **[2].** Antioxidant from natural product could delays or inhibits the oxidative damage to a target molecule. The main characteristic of an antioxidant is its capability to trap the free radicals **[3].** In current scenario, research and development (R&D) in pharmaceutical industries has intensive to discover the superior approach for the treatment of cancer and multidrug resistance **[4].** Cancer is one of the leading causes of death in high-income countries and it has exceeded the heart disease as the No. 1, which emphasizing the crucial requisite to change treatment and prevention the approach of this disease. Available FDA approved cancer drugs (such as alkylating agents, platinum compounds and antimetabolites) also might cause the cytotoxicity. Before existing random result of DNA mutations, it's possible that cancer could have much deeper roots that would be better targeted with natural therapies than toxicity **[5, 6].** The World Health Organization (WHO) exclusive antibiotic resistance is a major problem from 1994 due to lack of new classes of antibiotics. Hence, beneficial and or less harmful treatment is needed which is only possible in combination of natural product from the medicinal plants **[7].**

The frequent use of synthetic drug leads to cause the variety of side effects and occasionally drug resistance **[8].** Unlike synthetic drug, natural products are possessing significant activities in control and prevention of various disease and disorders without causing unwanted side effects. Hence, in the search of new natural product preparation from the medicinal plants are subjected to resolve this issue **[9].** *Mollugo nudicaulis* Lam. (*M. nudicaulis*) is one of the Indian medicinal plants, which belongs to Molluginaceae family. Traditionally, it is used in Indian phytotherapy for the treatment of wound, cough, cold, fever, inflammation, cancer, urinary and kidney infections **[10, 11].** Ethanolic extract of *M. nudicaulis* leaves possess variety of phytochemicals like alkaloids, flavonoids, terpinoids and other phenolic compounds, it is also exhibited the anti-diabetic, anti-inflammatory activities in animal model **[12].** Till to date, no date has been reported about n-hexane extract of *M. nudicaulis* and its potential biological activities. Therefore, it is of interest to analyze the antioxidant, antimicrobial and anticancer activity of n-hexane extract of *M. nudicaulis.*

Materials and Methods:

Plant collection and authentication:

The fresh whole plant of *M.nudicaulis* was collected from in the area of Keeranur, Pudukkottai District, Tamil Nadu, India and it was authenticated by Dr. G.V.S. Murthy, Botanical Survey of India, Tamilnadu Agricultural University Campus, Coimbatore, Tamil Nadu (Reference: BSI/SRC/5/23/10-11/Tech 420), then the plant materials were dried under the shade condition, powdered and stored in air tighten container at 4°C for future analysis **[13].**

Extract preparation:

The plant material of *M.nudicaulis* was extracted by the method of exhaustive extraction **[14].** Briefly, 300g of the plant material was soaked in 1500 mL of n-hexane contained flask and it was kept on the rotating shaker for 72 hrs at 25°C (average room temperature). Finally the collected extract was concentrated through rotary evaporator (RE-2A evaporator) set at 40°C. Further it was stored at 4°C for future studies.

Antioxidant assays:

The antioxidant potential of *M.nudicaulis* extract was determined through standard assays such as α, α-diphenyl-β-picrylhydrazyl (DPPH), nitric oxide, hydroxyl scavenging assays, reducing power and FRAP assays.

DPPH scavenging assay:

DPPH scavenging potential of plant extract was analyzed according to Blois(1958) method **[15].** In brief, the 100 µM of DPPH was dissolved in ethanol, 1 mL of this mixture added to 1 mL of different concentration (3.12-200 µg/ml) of isolated phytocompound were mixed vigorously and stands at room temperature for 30 min. The decreases absorbance values were observed at 517 nm against blank solution (ethanol). The DPPH scavenging capacity was calculated by reduction of radical percentage. Each test was analyzed in triplicate. Decreased absorbance values signified higher free radical scavenging activity.

Nitric oxide scavenging activity:

Nitric oxide was produced through SNP and it was analyzed by Garratt (1964) method **[16].** Briefly, the effective combination contains 10 mM of SNP, phosphate buffer (pH 7.4) and different concentration (3.12-200 µg/ml) of extract in the volume of 3mL. It was incubated at room temperature for 150 min, then 1 mL of sulfanilamide was mixed in 0.5 mL of the incubated solution and it was allowed to stands for 5 min. Finally, 1 mL of NED (0.1% w/v) was added and the mixture was incubated for another 30 min at room temperature. The pink chromophore was produced during the diazotization of nitrite ions with sulphanilamide and subsequent coupling with NED was analyzed by spectrophotometrically at 540 nm against the blank solution. Each test was performed in triplicate.

Hydroxyl scavenging activity:

Hydroxyl radical scavenging capacity of plant extract has analysed through proposed method of Elizabeth and Rao (1990) with slight

BIOINFORMATION
Discovery at the interface of physical and biological sciences

modification **[17].** Freshly prepared 1 ml of reaction mixture includes 2-deoxy-2-ribose (2.8 mM), KH_2PO_4-KOH buffer (20 mM, pH 7.4), $FeCl_3$ (100 µM), EDTA (100 µM), H_2O_2 (1.0 mM), ascorbic acid (100 µM) and different concentration (3.12-200 µg/ml) of extract was incubated at 37°C for 1 hr, followed by 0.5 ml of the reaction mixture was added to 1 ml of 2.8% TCA and 1 ml 1% aqueous TBA then it was incubated at 90°C for 15 min (for the colour development). The absorbance was calculated at 532 nm against a suitable blank solution. Each test was analysed in triplicate.

Reducing power activity:
The reducing power activity of plant extract was measured through Oyaizu (1986) proposed method **[18].** In brief, the different concentration (3.12-200 ug/mL) of the plant extract (0.5 mL) were added with 0.5 mL phosphate buffer (0.2 M, pH 6.6) and 0.5 ml potassium hexacyanoferrate (0.1%), then it was incubated at 50°C in the water bath for 20 min. Later, 0.5 ml of 10% TCA was added in the mixture. 1 mL of top section solution was added in 1 ml of distilled water, and then 0.1 mL 0.01% of $FeCl_3$ solution was added. Then the mixture allowed stands for 10 min at 25°C. The absorbance was calculated at 700 nm against the appropriate blank solution. Each test was analyzed in triplicate. Superior absorbance values specify greater reducing power.

FRAP reducing activity:
The antioxidant capacity of plant extract was measured using FRAP assay proposed by Benzie and Strain (1996) [19]. A prospective antioxidant might decrease the ferric ion (Fe3+) to the ferrous ion (Fe2+), finally it produce a blue complex of Fe2+/TPTZ, which enhanced the absorption at 593 nm. In brief, FRAP reagent was prepared by the mixture of solution contains 10 µM of TPTZ, 40 µM of HCL, 20 µM of $FeCl_3$ at 10:1:1 ratio and 0.3 M of acetate buffer. It was prepared freshly and stands at 37°C. 800 µL of reagent was mixed with 80 µL of sample solution contains various concentration of plant extract (3.12-200 µg/mL). This mixture was shancked vigorously and stands for 30 min at 37°C. The absorbance has taken at 595 nm. All the analysis was done in triplicate.

Antimicrobial activity:
Antimicrobial potential of plant extract has determined through Bauer *et al (*1966) proposed method [20]. Shortly, the sterile disc (5 mm) was dipped in various doses of plant extract (5, 10 and 15 µg/ml) and dried at 40°C. Each disc was drenched in DMSO used for negative control and chloromophenicol was used for positive control. Inocula [108 CFU] has broaden on the sterile of nutrient agar medium plate using cotton swabs after that, the plates were incubated at 35° C for 20 min. The discs were placed in the plates and it was incubated at 37° C for 24 hrs. Correspondingly antifungal activity was measured by afore mentioned method in Sabouraud Dextrose Agar (SDA) medium. The standard streptomycin (10µg/disc) was used as positive control. Each plate was incubated at 25°C for 72 hrs. The zone of inhibition was calculated in mm. Each test was performed in triplicate.

Anticancer activity:
Cell growth inhibition was determined by MTT assay [21]. Briefly, 5000 cells were seeded in each well on 96 well plates and cultured for 24 hrs, then treated with different concentration (3.12, 6.25, 12.5, 25, 50, 100, 200 µg/mL) of plant extract while cyclophosphamide was used as positive control. The cells were then incubated for 24 hrs at 37°C in 5% CO_2. At the end of incubation, the medium was removed and 10 µL of MTT was added followed by 100 µL of DMSO was added to each well to solubilize the formazan crystals. It was then left in dark place for 4 hrs at room temprature. The absorbence was measured at the wavelength of 595 nm using a mircotitre plate reader and results were analyzed in triplicate and the percentage was calculated.

Statistical analysis:
The obtained results from the assays were showed as mean ± SD. The Statistical evaluations were measured through statistical package program (SPSS 10.0, IBM, Armonk, New York, United States).

Results and Discussion:
Antioxidant activity:
Generally, the antioxidants are complicated to protect the organisms againts the ROS produced free radicals which leads to various disease and disorders in the human disease management system **[22].** Novel methods are proposed to measure the antioxidant activity of phytocompounds but the standard scavenging assays referred based on their free radical scavenging capacity **[23].**

Table 1: Antioxidant potential of plant extract

Extracts	Inhibitory concentration (IC_{50} values) in µg/ml		
	DPPH scavenging activity	Nitric oxide scavenging activity	Hydroxyl scavenging activity
n-hexane extract	12.79±0.82	36.65±0.03	19.59±0.26
Ascorbic acid	08.94±0.52	27.62±0.12	13.28±0.19

BIOINFORMATION
Discovery at the interface of physical and biological sciences

Note: In the maximum concentration of 200 µg/mL, n-hexane extract of *M.nudicalis* showed the considerable antioxidant activity when compared with ascorbic acid.

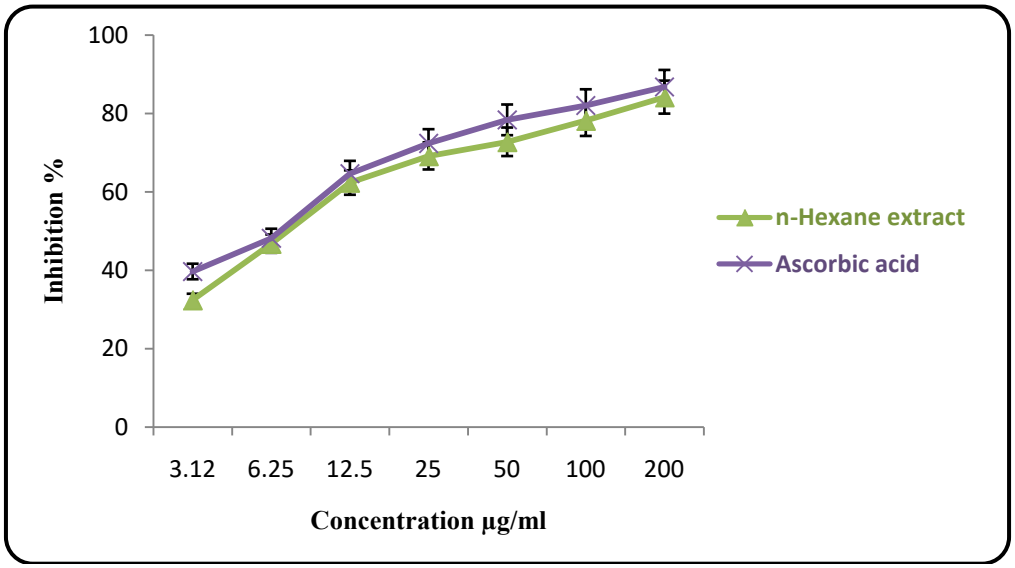


Figure 1: DPPH radical scavenging activity of n-hexane extract

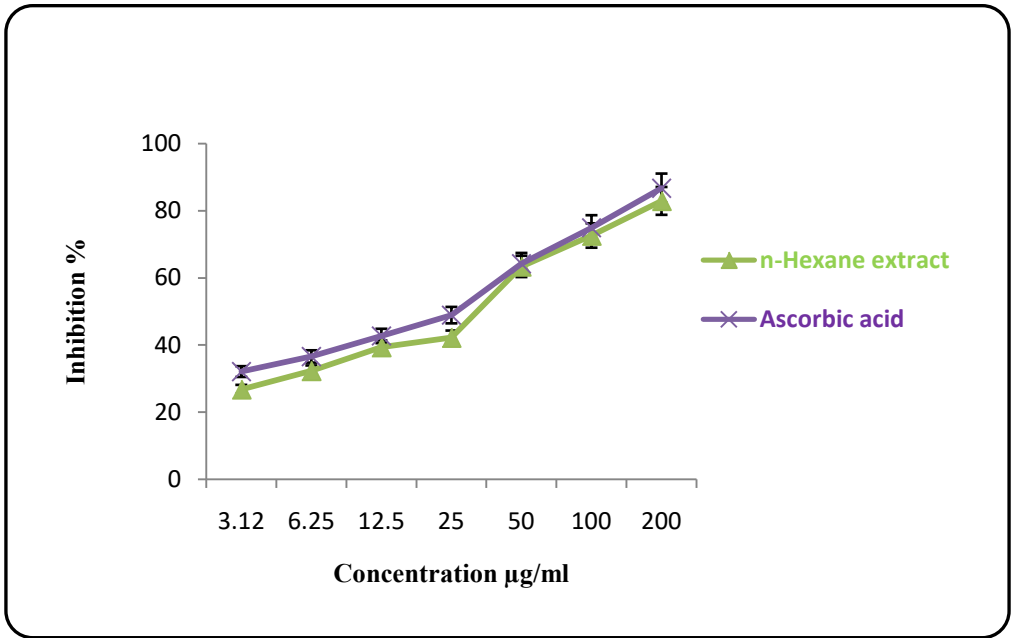


Figure 2: Nitric oxide radical scavenging activity of n-hexane extract

BIOINFORMATION
Discovery at the interface of physical and biological sciences

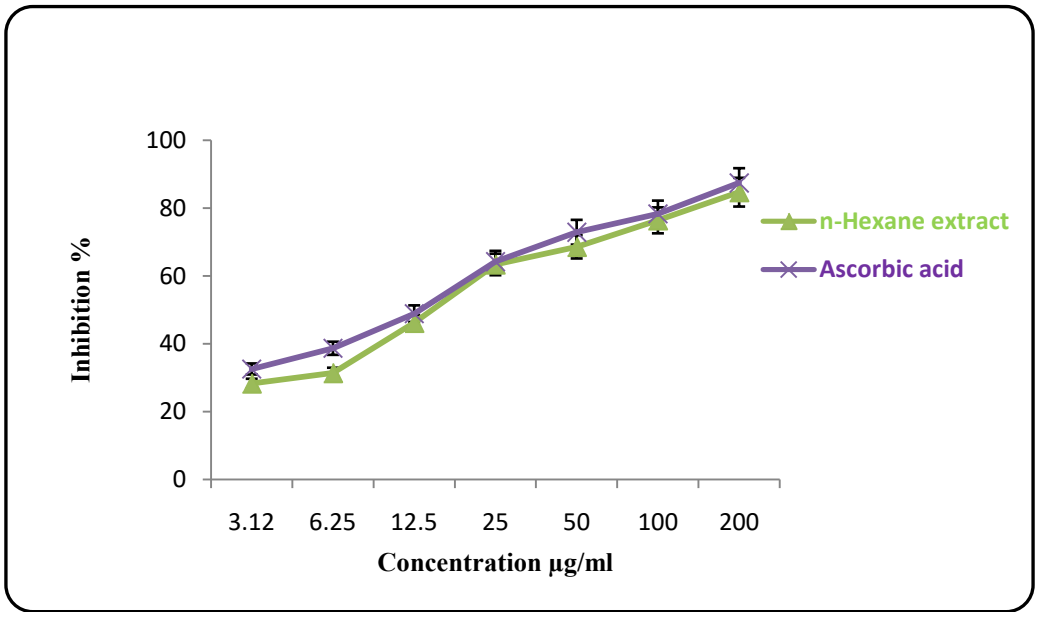


Figure 3: Hydroxyl radical scavenging activity of n-hexane extract

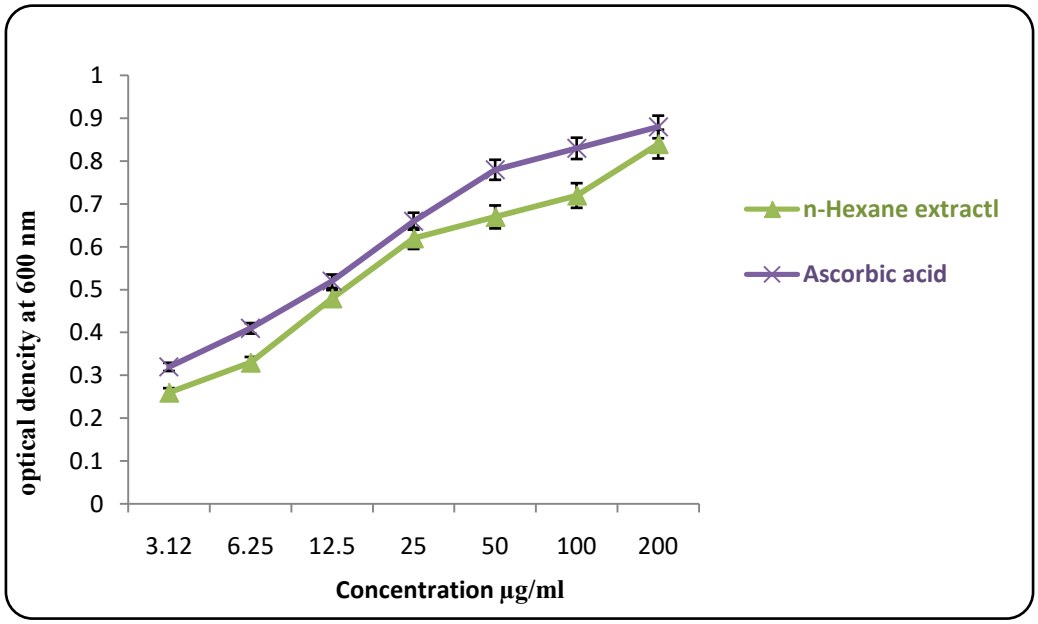


Figure 4: Reducing power activity of plant extract

BIOINFORMATION
Discovery at the interface of physical and biological sciences

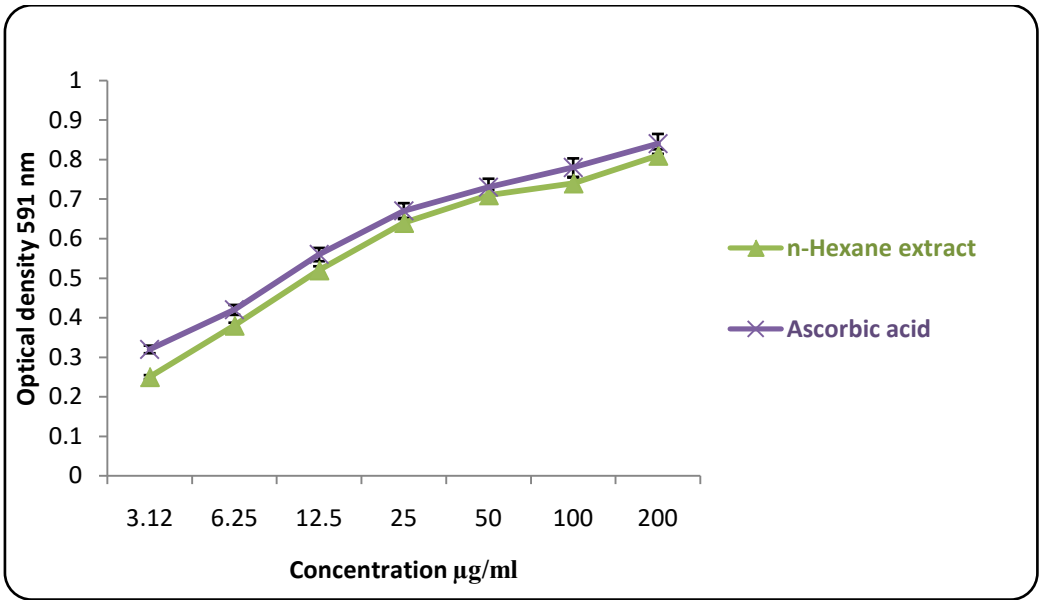


Figure 5: FRAB assay activity of plant extract

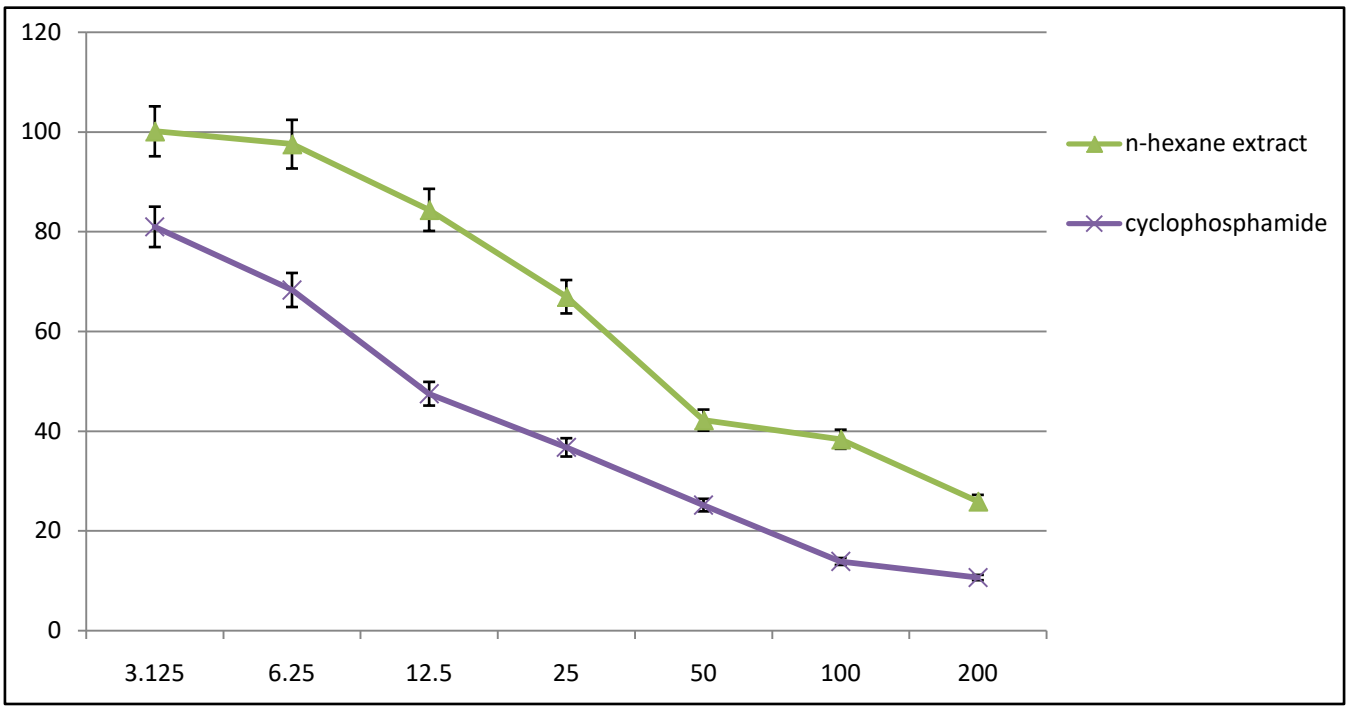


Figure 6: Cell growth inhibitory assay

BIOINFORMATION
Discovery at the interface of physical and biological sciences

DPPH scavenging activity:
It is the well-known procedure to determine the antioxidative properties of plant extracts or phytocompounds [24]. Usually the free radicals are scavenged by an antioxidant, which donates an electron, or hydrogen ion to a radical and consequently, a constant molecule is produced [25]. The dose-dependant DPPH scavenging effect of n-hexane extract expressed the comparable and significant IC_{50} values of 12.79±0.82 µg/mL when compared with ascorbic acid value of 08.94±0.52 µg/mL (**Figure 1 and Table 1**).

Nitric oxide scavenging activity:
Nitric oxide is well-known pro-inflammatory mediator, which is concerned, in variety of physiological events like, smooth muscle relaxant, inhibition of platelet aggregation and regulation of cell-mediated toxicity [26]. However, over production of nitric oxide may leads to pathogenesis of some inflammatory diseases. Therefore, nitric oxide inhibitory agent could be favorable for management of inflammatory reaction **[27].** The nitric oxide scavenging activity of n-hexane extract holds the significant IC_{50} value of 36.65±0.03 µg/mL (**Figure2 and Table 1**) when compared with ascorbic acid as the standard drug (27.62±0.12 µg/mL) which indicates that, n-hexane extract of *M. nudicaulis* might have the capable to inhibit the nitric oxide.

Hydroxyl scavenging activity:
Production of hydroxyl radicals by Fenton reaction degrades deoxy ribose causing oxidative DNA damage **[27].** The n-hexane extract and reference drug of ascorbic acid exposed their significant IC_{50} values were 19.59±0.26 µg/mL and 13.28±0.19 µg/mL respectively (**Figure 3 and Table 1).** The major ROS of hydroxyl radicals are causing lipid peroxidation and different biological damages **[28].** This assay can suggest that, n-hexane extract of *M.nudicaulis* might capable to eliminate the hydroxyl radicals from the sugar module of malondialdehyde like oxidant and barred the oxidatiion reaction.

Table 2: Antimicrobial activity

No.	Microorganism species	Inhibitory zone at 15 µg/mL	
		n-hexane extract (mm)	Antibiotic (mm)
1	S.aureus	12.2 ± 0.1	14.6 ± 0.2
2	S.aureus	11.1 ± 0.3	12.3 ± 0.3
3	E.coli	12.6 ± 0.2	14.1 ± 0.3
4	K.pneumoniae	13.4 ± 0.1	14.7 ± 0.3
5	A.niger	14 .5± 0.3	15.8 ± 0.1
6	A.terreus	11.3 ± 0.2	13.2 ± 0.2

Note: The n-hexane extract of *M.nudicaulis* showed the highest inhibition zone against tested microorganisms at the concentration of 15 µg/mL.

Reducing power activity:
Mostly, it can be used to assess the capability of an antioxidant to donate an electron **[29].** In this assay, the capacity of plant extract reducing power capacity has analyzed through transformation of Fe^{3+} to Fe^{2+} in the presence of extract and reference drug of ascorbic acid. At the concentration of 200 µg/ml, the greatest absorbance of n-hexane extract and reference drug were 0.84±0.04 and 0.88±0.03 respectively (Figure 4), which indicates that, n-hexane extract of *M.nudicaulis* has considerable reducing power activity. Hence, it could serve as electron donors and it may react with free radicals to change the constant products. It may leads to stop the free radical chain reaction.

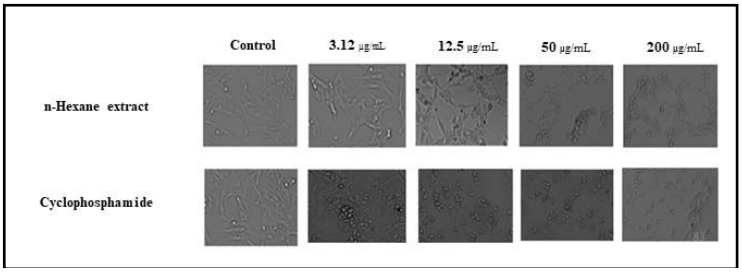


Figure 7: A2780 cell lines treated with n-hexane extract and standard drug**.**

FRAP assay:
This assay is mainly based on decreasing effects of an antioxidant responding through a ferric tripyridyltriazine complex and generates the colored ferrous tri-pyridyltriazine. The reducing power of plant extract or compound associated with action through terminating free radical chain by a hydrogen atom donation **[30].** The n-hexane extract of *M. nudicaulis* exposed highest absorbance of 0.81±0.02, while ascorbic acid has 0.84±0.05 at the concentration of 200 µg/ml (**Figure 5**) which indicates that, the plant extract might have considerable reducing power. Antioxidant activity on free radical scavenging assay on plant extract exposed the dose dependant percentage of scavenging activities which is expressed as half maximal inhibitory concentration (IC_{50}) value and it is possess strong antioxidant activity which indicates that, the plant extract might scavenge the free radicals.

Antimicrobial activity:
Antimicrobial activity of *M.nudicaulis* extract and reference drugs were determined by disc diffusion method **(Table 2**) against a variety of microorganisms, which are regularly, meets in infectious diseases. The antimicrobial activity of extract expressed the highest inhibition zone against tested microorganisms such as, *Staphylococcus aureus* (12.2 ± 0.1 mm), *Streptococcus aureus* (11.1 ± 0.3

BIOINFORMATION
Discovery at the interface of physical and biological sciences

mm), *Escherichia coli* (12.6 ± 0.2 mm), *Klebsiella pneumoniae* (13.4 ± 0.1 mm), *Aspergillus niger* (14 .5± 0.3 mm) *and Aspergillus terreus* (11.3 ± 0.2 mm). Multidrug resistance is growing rabidly by the microorganisms during recent decades. The modern researchers have urged to search the new drug sources which have improved therapeutic properties [31]. Thus, results indicated that, the n-hexane extract of *M.nudicaulis* have significant antimicrobial activity against the disease causing microorganisms and it could have the potential cidal effects on those microorganisms.

Cell growth inhibitory assay (MTT assay)

The *in vitro* anticancer activity of n-hexane extract from *M.nudicaulis* in the different concentration ranging from 3.12 to 200 µg/mL against A2780 cell lines was analyzed by cell growth inhibitory assay. Plant extract showed 86% of inhibition (Figure 6) at the highest concentration of 200 µg/mL. It possesses comparable significant cell growth inhibitory activity (Figure 7) in the ovarian cancer cell lines of A2780 with low concentration (IC_{50} value) as 32.46±0.92 µg/mL, when compared with cyclophosphamide 10.17±0.53 µg/mL. The induction of cell death occurs in a very low concentration range like other potential anticancer drug **[32].** Thus, it may be a good candidate for anticancer agent.

Conclusion:

Data shows the antioxidant potential of n-hexane extract of *M. nudicaulis* exposed the significant scavenging activity on DPPH, nitric oxide, hydroxyl scavenging, reducing power, FRAP reducing assays. Moreover, it also possesses notable antimicrobial activity on tested microorganisms when compared with referenced antibiotic. It has strong cell growth inhibitory activity on A2780 cell lines. Together this study can conclude that, n-hexane extract of *M. nudicaulis* holds antioxidant and antimicrobial and anticancer activity. In future, it might use to develop a novel drug for the hindrance and treatment of diseases which are associated with microbial infections and oxidative stress. Nevertheless further studies are needed to authenticate the current finding.

Acknowledgement:

The authors are thankful to Annai College of Atrs and Science, Kovilacheri, Tamil Nadu, India for providing facilities and encouragement to complete this research work fine fulfilment.

Conflict of interest:

The authors declare no conflict of interest.

References: (PMID missing from authors)

[1] Perumal PC *et al. Asian Pac J Trop Dis,* 2012 **2**: S952.
[2] Palanisamy CP *et al. S Afr J Bot.* 2019 **124**: 311.
[3] Poornima *et al. Biomed Res Int.* 2014 **2014:** 240243 [PMID: 25136566].
[4] Kumarasamy KK *et al. Lancet Infect Dis.* 2010 **1**: 597.
[5] Palanisamy CP *et al. J Pharm Bioallied Sci.* 2019 **11**: 155 [PMID: 31148892].
[6] Barlow R *et al. J Adv Bio Biotech.* 2016 **5**: 1.
[7] Perumal PC *et al. J Appl Pharm Sci.* 2014 **4:** 6.
[8] Perumal PC *et al. Pharmacol Res.* 2018 **10**: 296.
[9] Palanisamy CP *et al. Bangladesh J Pharm.* 2016 **11**: 545.
[10] Rajamanikandan S *et al. Asian Pac J Trop Med.* 2012 **5**: 862.
[11] Singh R *et al. Asian J Exp Sci.* 2009 **23**: 215.
[12] Ragupathy S *et al. India. J Ethnobiol Ethnomed.* 2008 **4**: 1.
[13] Rajamanikandan S *et al. Ind J Pharm Edu Res.* 2011 **45**: 310.
[14] Perumal PC and Ashafa AOT. *J Young Pharm.* 2018; **10**:173.
[15] Blois MS. *Nature* 1958 **26**:1199.
[16] Garratt DC. *Chapman and Hall: London, UK,* 1964; pp.456–458.
[17] Elizabeth K and Rao MNA. *Int J Pharm.*1990 58: 237.
[18] Oyaizu M. *Japanese J Nutr Diet.* 1986 **44:** 307.
[19] Sowmya S *et al. Anal Chem Lett.* 2016 **5**: 291.
[20] Bauer AW *et al. Am J Clin Pathol.* 1966 **45**: 493 [PMID: 5325707].
[21] Ngamwongsatit P *et al. J Microb Meth.* 2008 **73**: 211 [PMID: 18417231].
[22] Priyanga S *et al. Int J Curr Pharm Rev Res.* 2015 **6**:169.
[23] Lu JM *et al. J Cell Mol Med.* 2010 **14**: 840 [PMID: 19754673].
[24] Hartwig VA *et al. Ciênc Tecnol Aliment.* 2012 **32**: 126.
[25] Nithya P and Madhavi C. *J Taibah Univ Sci.* 2017 **11**: 40.
[26] Perumal PC *et al. Pharmacognosy Res.* 2015 7: 121[PMID: 25598646].
[27] Pacher P *et al.* Physiol Rev. 2007 **87**: 315 [PMID: 17237348].
[28] Phaniendra A *et al. Indian J Clin Biochem.* 2015 **30**: 11 [PMID: 25646037].
[29] Bora KS & Sharma A. *Pharm Biol.* 2011 **49**: 1216 [PMID: 21999109].
[30] Sowndhararajan K and Kang SC. *Saudi J Biol Sci.* 2013 **20**: 319 [PMID: 24235867].
[31] Tamokou JDD *et al. Iran J Med Sci.* 2011 36: 24 [PMID: 23365474].
[32] Tripathi SK *et al. J Mol Recognit.* 2012; 25:504–512.

BIOINFORMATION
Discovery at the interface of physical and biological sciences

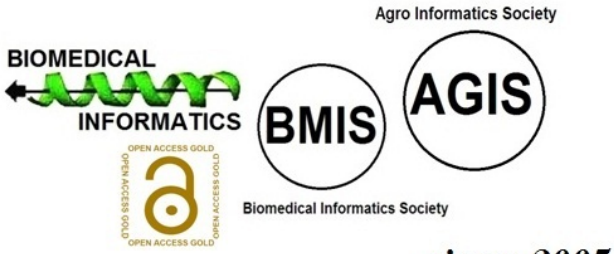
OPEN ACCESS GOLD

Edited by P Kangueane
Citation: Raj *et al.* Bioinformation 17(5): 573-582 (2021)

Articles published in BIOINFORMATION are open for relevant post publication comments and criticisms, which will be published immediately linking to the original article for FREE of cost without open access charges. Comments should be concise, coherent and critical in less than 1000 words.

BIOINFORMATION
Discovery at the interface of physical and biological sciences

OPEN ACCESS GOLD



BIOINFORMATION
Discovery at the interface of physical and biological sciences

indexed in