Journal of Bioscience and Applied Research
https://jbaar.journals.ekb.eg/

Exploring the Phytochemical Composition of Methanolic Extract from Launaea nudicaulis: Investigating its Antioxidant, Anticancer, and Anti-Dengue Activities against *Aedes aegypti*

Abadi M. Mashlawi[1], Areshi S.[1], Naimah Asid H Alanazi[2], Gamal Mohamed [3], Abdel Daim Z. J.[1], Mohammed A. Alfattah[1], Dalia A. Mohamed[4], Eman Fayad[5] and Salama A. Salama[1 *]

1. Biology Department, College of Science, Jazan University, Jazan City/ Postal code 82817/ Saudi Arabia/ Tel. 00966540432176/. Email; amashlawi@jazanu.edu.sa, sareshi@jazanu.edu.sa, malfattah@jazanu.edu.sa, sasalama@jazanu.edu.sa, zdayem@jazanu.edu.sa
2. Department of Biology, College of Sciences, University of Ha'il, KSA. N.alenezy@uoh.edu.sa
3. Human Anatomy Department, Faculty of Medicine, Jazan University, KSA.Email: gmahmed@jazanu.edu.sa
4- Biology Department, University College of Al Khurmah, Taif University P.O. Box 11099, Taif 21944, KSA Email; Daliia@tu.edu.sa
5- Biotechnology Department College of Sciences, Taif University P.O. Box 11099, Taif 21944 KSA Email; e.esmail@tu.edu.sa
Corresponding author: **Salama A. Salama**

DOI: 10.21608/jbaar.2024.343721

Abstract

Asteraceae is considered to be one of the most medicinal significant families. One family species *Launaea nudicaulis* is globally used in traditional medicine to treat various ailments. The objective of the current investigation was to evaluate the chemicalcomposition of the methanol extract of *L. nudicaulis* and its possible biological impacts. In the present study, the GC-MS analysis identified 10 components, with fatty acid derivatives, terpenes, and alkaloids as the main compound classes. The major components are (2E,6E,10E)-3,7,11,15-Tetramethyl-2,6,10,14-hexadecatetraenyl acetate (29.20%), 2-Ethylhexanoic acid (24.57%) and thunbergol (20.53%). The antioxidant assay shows the *L. nudicaulis* extract has good DPPH radical scavenging ability with an IC_{50} valuefor the shoot extract is 32.52 mg/L., which is linked to its phytochemical composition. The cytotoxicity results demonstrate selective anticancer effects of the extract against HepG- 2 and PC3 cells compared to normal cells. The MeOH extract demonstrated an IC_{50} valueof 38.56 and 46.30 µg/ml of HePG-2 and PC3 cancer cells, respectively. Additionally, the extract's excellent position as a perfect natural insecticide is highlighted in the current work. Using methanol as a solvent, the extract shows great larvicidal potential against Aedes aegypti L. Researchers are encouraged to employ natural insecticides when the insects are still young, not when they are older. The findings validate the traditional therapeutic applications of wild plants.

Keywords: *Launaea,* Asteraceae, DPPH, GC-MS, Antitumor, MTT assay.

Received: December 10, 2023. Accepted: February 26, 2024. Published: March 22, 2024

Introduction

Plants play a vital part in several biological processes, since they contain a wide range of substances that have varied and important impacts on living beings. The complex chemical makeup of plants has been used for ages by both traditional medicine and contemporary research [1]. Phytochemicals are a noteworthy group of plant substances that have significant biological effects. The bioactive compounds consist of several molecules including alkaloids, flavonoids, terpenoids, and polyphenols. Each component contributes distinct features to the plant and has potential uses [2; 3]. Medicinal plants have been used in many traditional medical systems worldwide for their healing properties. In addition to medicines, plants play a crucial role in the manufacturing of herbal remedies, which are essential for maintaining good health and preventing illnesses [4].

Asteraceae (Compositae) is a prominent plant family in the dicotyledonous group,characterized by its extensive number of species (1,620 genera and 23,600 species) that are found worldwide (5). Comprising around 10% of the total number of blooming plants globally [6,7]. The predominant members of this family are herbaceous; however, shrubs, trees, creepers, and climbers have also been documented [8]. The classification includes species that are edible, medicinal, toxic, invasive, and endangered [9]. Several species of the *Launaea* genus exhibit antioxidant, anticancer, insecticidal, and cytotoxic properties. *Launaea nudicaulis* is commonly known as Al-Hewa in the Arabic region. It is distributed in different habitats such as sandy and alluvial soils, desert wadis, plains, Mediterranean coast, Desert, and Red Sea coast [10]. Its leaves are used in folk medicine for the treatment of children's fever, skin itches, eczema, swelling, bilious fever, ulcers, and cuts [11, 12]. They are often usedin traditional medicine to treat stomachic and skin ailments [13]. The literature review identified the existence of triterpenes, sesquiterpene lactones, steroids, flavonoids, and coumarins in the genus *Launaea*, as reported by [14; 15, and 16].

Plants are essential sources of antioxidants, which protect cells from free radicaldamage. Free radicals are reactive molecules produced by biological processes or external factors including UV radiation, pollution, and toxins. Oxidative stress from uncontrolled free radicals may cause sickness and aging. Plant-derived antioxidants neutralize free radicals, improving health [17]. Plants play a significant role in the development of anticancer therapies, offering a rich source of bioactive compounds with potential anti-cancer properties [18]. The exploration of plant-derived compounds in cancer research has led to the discovery of numerous molecules that exhibit anti-tumor effects through various mechanisms. Several plant-based compounds have demonstrated promising results in preclinical studies and are being investigated for their potential as cancer treatments [19].

The present work used GC-MS spectroscopy to analyze the chemical compositionof the methanol extract of *Launaea nudicaulis*. This study aimed to investigate the biochemical components responsible for its biological effects. The research examined the antioxidant activity of the isolated plant by using the DPPH free radical scavengingtest, in addition to assessing its *in vitro* anticancer activities.

2. Materials and Methods

2.1. Plant material and extraction process:

The shoot system of *Launaea nudicaulis* was obtained from the Faifa Mountains, Jazan Province of Saudi Arabia. The samples were collected in March during the spring season, then stored in paper bags and transported to the laboratory. The samples were pulverized, enclosed in a paper bag, and left to desiccate for one week under shade at an ambient temperature of 25 °C. Plant specimen identification was conducted, as stated by [20]. The samples were cleaned and allowed to dry naturally. 10 g of dried plant material was placed in a 250-mL conical flask

with 150 mL methanol. After that, the mixture was shaken continuously for two hours at room temperature in a water bathshaker (Memmert WB14, Schwabach, Germany). The mixture was filtered using Whatman filter papers (no. 1, 125 mm, Cat. No. 1001 125, Germany). The final plant extract concentrations were evaluated and stored at 4 °C.

2.2. *Gas chromatography-mass spectrometry analysis (GC-MS)*

The chemical composition of *L. nudicaulis* was determined using a well-established method described by [22]. The methanolic extract was analyzed using a Trace-GC-TSQ mass spectrometer (Thermo-Scientific, Austin, T.X., USA) equipped with a direct capillary column TG-5MS (30 mm × 0.25 mm × 0.25 mm film thickness) [23]. Furthermore, after comparing the mass spectrum data with the mass spectrometry lists WILEY-09 and NIST, it was feasible to determinethe chemical makeup of each plant component that was extracted.

2.3. *Antioxidant Activity*

The DPPH scavenging test was used to assess the antioxidant activity of the methanolic extracts obtained from the aerial portions of *L. nudicaulis*. This method was previously described by [24]. Methanolic extract concentrations were generated by diluting methanol to get concentrations of 5, 10, 20, 30, 40, and 50 mg mL^{-1}. A reaction mixture was prepared by rapidly stirring equal portions of newly manufactured 0.3 mM DPPH and each quantity of the methanolic extract for 30 minutes at 25°C. Furthermore, a control group was given ascorbic acid at concentrations of 1.0, 2.5, 5, 10, 15, and 20 mg mL^{-1}, using the same procedure as the other treatments. The spectrophotometer readings, obtained at a wavelength of 517 nm, were evaluated using a Milton Roy Spectronic 21D UV-Visible Spectrophotometer, which was produced in the United States. The quantity of methanolic extract required to visually evaluate the 50% decrease in DPPH color (IC_{50}) was measured.

$$\%\,Inhibition = \frac{\text{A Control} - \text{A Sample}}{\text{A Control}} x100$$

2.4. Anticancer Activity

Hepatocellular carcinoma (HePG-2) and mammary gland cancer (MCF-7) were bought from ATCC holding company for biological goods and vaccines (VACSERA), Cairo, Egypt. Doxorubicin was a frequent cancer drug. The extracted *L. nudicaulis* was tested for cytotoxicity and cell proliferation using a standard colorimetric MTT assay according to [25]. MTT (2-(4,5-dimethylthiazol2-yl)-3,5-diphenyl-2H- tetrazolium bromide) was converted from yellow to purple by live cell mitochondrial succinate dehydrogenases. RPMI-1640 medium with 10% fetal bovine serum was used to produce cell strains. Penicillin (100 units/mL) and streptomycin (100 g/mL) were introduced to a 5% CO_2 incubator. Cell lines were seeded at 1.0×10^4 cells/well on a 96-well plate and incubated at 37°C for 48 hours with 5% CO2. After 24 hours of growth, cells were treated with different test sample dosages. After 24 hours of medication treatment, MTT solution (5 mg/mL, 20 L) was added and incubated for 4 hours. Each wellreceived 100 L of DMSO to dissolve violet formazan. A plate reader measured absorbanceat 570 nm during colorimetric analysis (EXL 800, New York, NY, USA). Origin 8.0® (OriginLab Corporation, https://www.originlab.com/; Accessed March 27, 2021) generated IC50 values using nonlinear regression (sigmoid type). The cell growth inhibition % was calculated using optical density (OD) to represent the absorbance read of the control and tested sample.

$$\% \, Inhibition = \frac{\text{OD Control} - \text{OD Sample}}{\text{OD Control}} x100$$

2.5. Larvae collection and mosquito rearing:

Eggs of Aedes aegypti were collected from colonies reared at the vector control laboratory, Biology Department, College of Science, Jazan University, Saudi Arabia. The eggs were placed in trays 20×15×5 cm filled with 500 ml tap water (70–75% relative humidity; 29 ± 1°C; photoperiod 14: 10 h (light: dark). The first instar larvae were hatched the next day and fed on the cultural medium; yeast powder and dog biscuit (ratio 1:2 w/w). On the third day, the larvae molted into the second instar. The third instar larvae hatched on the fifth day and were obtained for larvicidal experiments in the present study. The life conditions were changed to be at 70-85% relative humidity, 28 ± 2 °C; photoperiod of 12: 12 h (light: dark). The larvae were fed on chicken liver and feeding was continued till the larvae transformed into pupae. Pupae were collected to be used in the pupal experimental part. Pupae were transferred to glass cups (12 cm x 9.8 cm diameter) containing 500 ml clean water and covered with a net to inhibit adult emergence. The adults were moved into the cage (20 x20x20 cm) and fed on 10% sucrose solution soaked in cotton (male adults) and blood fitted unit covered with parafilm membrane (female adults) under the same previous conditions. Females were deprived and kept in a separate netted cage of mosquitoes for adulticidal experiments [26, 27, and 28].

Bioassay procedure:

Bioassays were performed according to the World Health Organization protocol of larval, pupal, and adult susceptibility test methods [27]. The early third instar larvae, pupae, and female adult mosquitoes (2-5 days old fed) of A. aegypti were treated with various concentrations of the studied plant part species (Leaves and stems) in groups of waxed paper cups containing 300 ml of sterilized mineral water. Three replicates were carried out, for a total of 10 tested staged insects for each plant part extract concentration besides control trials. Temephos is known as a common chemical positive control insecticidal solvent used following the method of [29]. It was prepared using 1 ml of the solvent in 249 ml of water. Cumulative mortalities, pupation, and adult emergence were recorded per staged insect at 24 h after exposure [30].

2.6. Statistical Analysis

Costat (CoHort software, Monterey, CA, USA) was used to assess antioxidant activity. Assays were done three times with three replications. A one-way ANOVA was used to examine the statistical significance of sample changes.

3. Results and discussion

3.1. Gas chromatography-mass spectrometry analysis (GC-MS)

The chemical composition of the extracted *L. nudicaulis* was determined using Gas-Chromatography Mass Spectroscopy (GC-MS). Figure 1 illustrates the correlation between the relative quantity of various components identified in the extracted plant and the specific retention time at which each component was discovered. The findings shown in Table 1 validate the identification of 10 components derived from the analyzed plant material. This identification was made by comparing the mass spectra of the components with the spectroscopic databases of WILEY 09 and NIST. In general, (2E,6E,10E)-3,7,11,15- Tetramethyl-2,6,10,14-hexadecatetraenyl acetate (29.20%), 2-

Ethylhexanoic acid (24.57%) and thunbergol (20.53%) are situated as the major component, which was identified after 55.71, 8.02 and 56.66 min, respectively. Consequently, other constitutes were characterized with high percent of composition such as valproic acid (13.54%), 6- Isopropenyl-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-2(1H)-naphthalenone (3.06%) and lupeol (2.93%) with total composition percent 19.53%. The alkaloid components were seen to have a relative abundance of 17.75, which is considered extremely low. On the other hand, the components of fatty acids and fatty acid derivatives were detected withina retention time range of 4.14 to 57.04 minutes. The predominant constituents of terpeneswere determined based on their retention duration, which fell within the range of 55.06-55.66 minutes. In a previous study, chemical classes reported in *L. nudicaulis* include essential oils [11, 12] flavonoids, phenolics, alkaloids [31], sesqui-, di-, and triterpenoid/steroids in addition to sphingolipids [32].

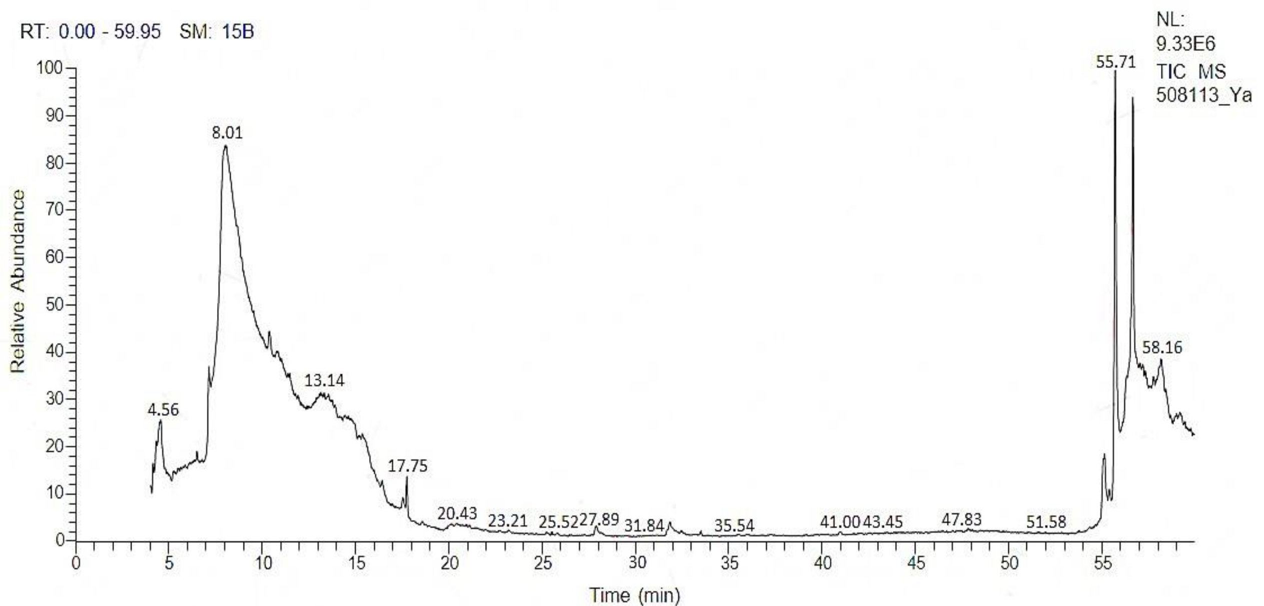

Figure 1. The chromatogram of the methanol extract from *L. nudicaulis* shoots were examined by GC-MS.

Table 1. Chemical constituents identified by GC/MS technique from ND extract.

No.	RT (min.)	Chemical name	Classification	MW	MF	Composition %
Fatty acid and Fatty acid derivatives						
1	4.14	2-Ethyl hexanoic acid, methyl ester	Fatty acid derivatives	158	$C_9H_{18}O_2$	1.35
2	7.93	Valproic acid	Fatty acid	144	$C_8H_{16}O_2$	13.54
3	8.02	2-Ethylhexanoic acid	Fatty acid	144	$C_8H_{16}O_2$	24.57
4	31.84	13,16-Octadecadiynoic acid, methyl ester	Fatty acid derivatives	290	$C_{19}H_{30}O_2$	1.73
5	57.04	9,12,15-Octadecatrienoic acid	Fatty acid derivatives	278	$C_{18}H_{30}O_2$	0.55
Alkaloid						
6	17.75	6H-[1]Benzopyrano [4,3-C]isoquinoline-6,11 (5H)-dione	Alkaloid	263	$C_{16}H_9NO_3$	2.26
Terpenes						
7	55.06	6-Isopropenyl-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-2(1H)-naphthalenone	Sesquiterpene	218	$C_{15}H_{22}O$	3.06
8	55.14	Lupeol	Triterpene	426	$C_{30}H_{50}O$	2.93
9	55.71	(2E,6E,10E)-3,7,11,15-Tetramethyl-2,6,10,14-hexadecatetraenyl acetate	Diterpene	332	$C_{22}H_{36}O_2$	29.20
10	56.66	Thunbergol	Diterpene	290	$C_{20}H_{34}O$	20.53

RT: retention time (min.), MW: Molecular Weight, MF: Molecular Formula

3.2. Antioxidant activity - DPPH assay:

The antioxidant activity of *L. nudicaulis* methanolic extract was assessed by comparing its ability to scavenge DPPH free radicals with that of ascorbic acid. The scavenging effects of plant extracts and the standard on the DPPH radical were expressed using half maximum inhibitory concentration (IC_{50}) values (Table 2). Asmaller IC_{50} value indicates a higher ability to remove DPPH radicals. Table 2 demonstrates that the shoot extract has the highest antioxidant scavenging activity, as corroborated by the findings. The IC_{50} value for the shoot extract is 32.52 mg/L. The primary factor governing the process of evaluating the antioxidant capacity of the testedextract is the prevalence of fatty acids and their derivatives (41.74%) and terpenes (55.72%) among all the isolated components. The current findings of *L. nudicaulis* align with the findings reported by [33], [34] and [35].

On the other hand, fatty acids, and lipids, which were extracted from *Reichardia tingitana*, and *Emex spinosa*, showed strong antioxidant capabilities for scavenging the free radicals in the solution [23, 35]. In this study 2- Ethylhexanoic acid, (2E,6E,10E)-3,7,11,15-Tetramethyl-2,6,10,14-hexadecatetraenyl acetate and thunbergol are the three main constituents, with 24.57, 29.20% and 20.53% acting as significant antioxidant agents, respectively [36, 37, 38,39]. The antioxidant capacity of bioactive compounds is typically determined by the ability of reactive oxygen species, such as phenolics, fatty acids, terpenes, oxygenated hydrocarbons, or carbohydrates, to scavenge or stabilize free radicals [40]. We have previously documented the chemical composition, antioxidant properties, and phytotoxic effects of essential oils derived from three species of *Launaea* plants, including *L. nudicaulis* [12]. Considering the historical background of the genus and the strong biological effects of *L. nudicaulis*, particularly its antioxidant properties, available evidence indicates that it may have potential as an antidiabetic agent [41, 42].

Table 2. Radical scavenging activity percent (%), and IC_{50} values (mg/mL) at various concentrations of the methanol extracted from *L. nudicaulis* and the standard ascorbic acidby DPPH assay.

Plant species	Concentration (mg/ml)	Scavenging activity (%)	IC_{50} (mg/ml)
L. nudicaulis	50	68.98±2.97	32.52
	40	64.06±2.75	
	30	48.70±1.98	
	20	34.28±1.47	
	10	22.64±0.97	
	5	6.22±0.21	
	$LSD_{0.05}$		
Ascorbic acid	20	69.35±2.73	11.38
	15	56.67±2.05	
	10	50.01±1.65	
	5	41.33±1.30	
	2.5	29.36±0.94	
	1	17.47±0.32	
	$LSD_{0.05}$		

Values are average (n = 3) ± standard deviation. $LSD_{0.05}$ expressed the calculated least of the smallest significance between two means as each test was run on those two means (calculated by Factorial ANOVA).

3.3. Cytotoxic Activity

The present study assessed the cytotoxic effects of the plant sample extract using an MTT test. The sample underwent *in vitro* testing against two tumor cell lines, namely HepG-2 and PC3. Doxorubicin was chosen as a benchmark medicine to compare the outcomes of the tested sample against various cancer cells. The tests used seven different concentrations of each plant extract (1.56, 3.125, 6.25, 12.50, 25, 50, and 100 μg mL^{-1}) that were generated by a serial dilution process (Table 2). The findings indicate that the methanolic extracts of the plant had dose-dependent cytotoxic activity, which was similar to that of doxorubicin, a reference standard (Table 2). In the present study, the MeOH extract demonstrated an IC_{50} value of 38.56 and 46.30 μg/ml of HePG-2 and PC3cancer cells, respectively (Table 3). Additionally, the methanol extract of *L. nudicaulis* exhibited the most potent cytotoxic activity, with inhibitory effects of 54.95% and 61.84% on HePG-2 and PC3 cells, respectively. Conversely, the extract exhibited a mere 14.06% inhibition on normal cells (WI-38), suggesting that the extract is specifically harmful to cancer cells and not normal ones (Figure 2).

Undeniably, cancer continues to be a prominent issue in public health, regardless of how it is examined [41]. Medicinal plants are becoming recognized as a valuable natural source of anticancer drugs due to their antioxidant and anti-mutagenic qualities. Additionally, their low cost, easy availability, and absence of side effects contribute to their growing popularity. Due to this rationale, medicinal plants are being regarded as a possible option for cancer treatment [43]. Our results appear in agreement with previous studies that showed *L. nudicaulis* plant extracts to have cytotoxic effects against some cancer cells [44]. Moreover, the pharmacological uses of several extracts of *L. nudicaulis* have been thoroughly investigated in many studies. These applications include antioxidant properties [12], and antibacterial activities [11].

Table 3. IC_{50} values of the prepared plant samples of *L. nudicaulis* and Zn-O NP anddoxorubicin as standard.

Tested samples	Cell lines		
	HePG-2	PC3	WI-38
Doxorubicin	5.04	7.81	> 100
L. nudicaulis	38.56	46.30	> 100

Hepatocellular carcinoma (HePG-2), Human prostate cancer (PC3), Normal cell (WI-38). IC_{50}: inhibitory concentration (µg): 1–10 (very strong), 11–20 (strong), 21–50 (moderate), 51–100 (weak), and above 100 (non-cytotoxic).

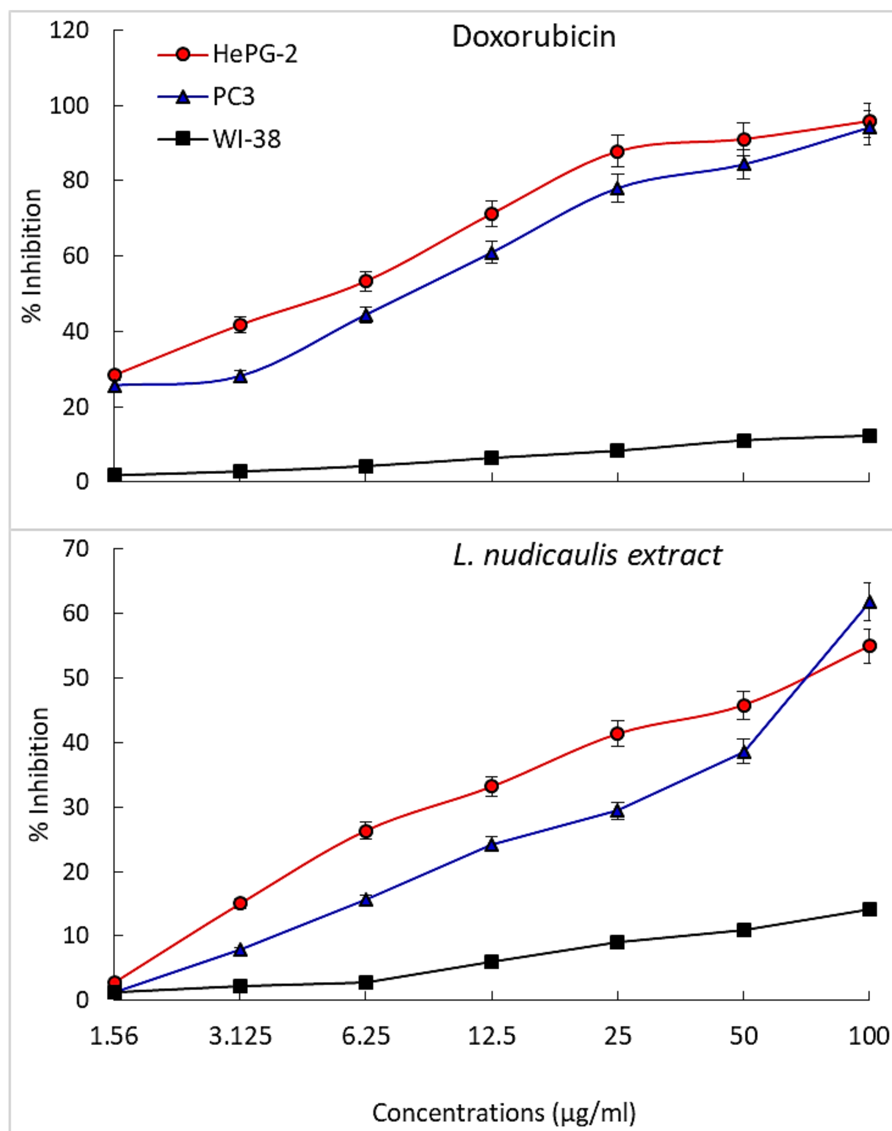

Figure 2. Cytotoxic activity of the prepared plant samples of *L. nudicaulis* against the tumor andnormal cells (WI-38) at different concentrations, and doxorubicin.

3.4. Anti-Dengue Vector Activity

Data recorded in Table (4) indicated the biological activity of the methanolic extract of L. nudicaulis against the 3rd instar larvae of Aedes aegypti.

The highest larval mortality (66.6%) was observed at the concentration of 500 ppm and the lowest mortality (13.3%) was observed at the lowest concentration 62.5 ppm, compared to 0.0% for control mosquitoes. The survivorship of pupae resulting from treated larvae was found not affected by L. nudicaulis extract, except for the two highest concentrations (500 and 250ppm), the pupal mortality recorded 15.1 and 10.5% compared to 00 for the control group. The total larval and pupal mortality was 82.2, 46.8, 30.7, and 12.4 at the concentrations 500,250,125 and 62.5ppm compared to 0.0% for the control. The percentage of adult emergence was slightly affected only at the two highest concentrations, where it recorded 84.4 and 89.5% compared to 100% for the control. The present data in agreement with [45] Methanolic extracts of L. nudicaulis and L. resedifolia at a concentration of 0.786 mg/cm2 exhibited 27.5±5 and 45±11 percent insecticidal activity against red flour beetle. L. nudicaulis was also insecticidal against Sabz Tela of cotton plants up to 100% activity. And insecticidal effects, cytotoxic effects, and antifungal properties [43].

Table (4): Biological effect of methanolic extract of *L. nudicaulis* on different stages of *Aedes aegypti*.

Conc. ppm	Larval mortality %	Pupation %	Pupal Mortality %	Malformed pupae %	Larval and pupal Mortality %	Adult Emergence %	Adult Mortality %
500	66.6	33.4	15.6	0	82.2	84.4	9
250	46.3	53.7	10.5	0	46.8	89.5	16.2
125	30.7	69.3	0	0	30.7	100	0
62.5	12.4	87.6	0	0	12.4	100	0
Contr	0	100	0	0	0	100	0

Conclusion

In conclusion, the antioxidant assay indicates that the methanol extract of L. nudicaulis exhibited a maximum percentage inhibition (68.98 mg/ml) in the DPPH assay.Furthermore, the results show selective and cytotoxic effects of the MeOH extract of *L.nudicaulis* towards HePG-2 and PC3 cancer cells with 2E,6E,10E)-3,7,11,15-Tetramethyl- 2,6,10,14-hexadecatetraenyl acetate; 2-Ethylhexanoic acid and thunbergol being a major component contributing to this observed effect. This indicates that *L. nudicaulis* is an affordable and secure therapeutic option for antioxidant and anticancer therapy. The methanolic extract *of L. nudicaulis* is regarded as the best natural insecticide against the initial stages of *Aedes aegypti* L.

Disclaimer: None

Conflict of Interest: None

Funding Disclosure: None

References

1. Süntar, I. Importance of ethnopharmacological studies in drug discovery: role of medicinal plants.Phytochemistry Reviews, 2020, 19(5), pp.1199-1209.
2. Velu, G., Palanichamy, V. and Rajan, A.P. Phytochemical and pharmacological importance of plant secondary metabolites in modern medicine. Bioorganic phase in natural food: an overview, 2018, pp.135- 156.
3. Sharma, A., Sharma, S., Kumar, A., Kumar, V. and Sharma, A.K. Plant secondary metabolites: An introduction of their chemistry and biological significance with physicochemical aspect. In Plant Secondary Metabolites: Physico-Chemical Properties and Therapeutic Applications, 2022, (pp. 1-45). Singapore: Springer Nature Singapore.
4. Shedoeva, A., Leavesley, D., Upton, Z. and Fan, C. Wound healing and the use of medicinal plants. Evidence-Based Complementary and Alternative Medicine, 2019.
5. Funk, V.A.; Bayer, R.J.; Keeley, S.; Chan, R.; Watson, L.; Gemeinholzer, B.; Schilling, E.; Panrelo, J.L.; Baldwin, B. G.; Garcia-Jacas, N.and et al. Everywhere but Antarctica: using a supertree to understand the diversity and distribution of the Compositae. In Biology Skr Edited by Friis I, Balslev H., 2005, 55: 343-373.
6. Funk V.A.; Susanna, A.; Stuessy, T.; Bayer, R. (eds) Systematics, evolution and biogeography of the Compositae. IAPT, Vienna: 2009, 171–189.
7. Bessada, S.M.; Barreira, J.C. and Oliveira, M.B.P. Asteraceae species with most prominent bioactivity and their potential applications: A review. Industrial Crops and Products, 2015, 76: 604-615.
8. Garcia, S.; Panero, J.L.; Siroky, J. Kovarik, A. Repeated reunions and splits feature the highly dynamic evolution of 5S and 35S ribosomal RNA genes (rDNA) in the Asteraceae family. BMC Plant Biology, 2010, 10: 176.
9. Heywood, V.H.; Brummitt, R.K.; Culham, A. and Seberg, O. Asteraceae. In: Flowering Plant Families of the World. New York, Firefly Books. 2007, Pp. 46-51.
10. Boulos, L. Flora of Egypt, Vol., 3. Al Hadara Publishing, Cairo, Egypt,2002.
11. Al-Mahrezi, J.A., Al-Sabahi, J.N., Akhtar, M.S., Selim, D. and Weli, A.M. Essential oil composition and antimicrobial screening of Launaea nudicaulis grown in Oman. International Journal of Pharmaceutical Sciences and Research, 2011, 2(12), p.3166.
12. Elshamy, A.I., Abd-ElGawad, A.M., El-Amier, Y.A., El Gendy, A.E.N.G. and Al-Rowaily, S.L. Interspecific variation, antioxidant and allelopathic activity of the essential oil from three Launaea species growing naturally in heterogeneous habitats in Egypt. Flavour and fragrance journal, 2019, 34(5), pp.316-328.
13. Rashid, S.; Ashraf, M.; Bibi, S. and Anjum, R. Insecticidal and Cytotoxic Activities of Launaea nudicaulis (Roxb.) and Launaea resedifolia (Linn.) Pak. J. Biol. Sci., 2000, 3(5):808–809.
14. Mansour, R.M.A.; Ahmed A.A. and Saleh N.A.M. Flavone Glucosides of some Launaea Species, Phytochemistry, 1984, 22: 2630-2631.
15. Yadav, R.A. and Chakravarti, N. New Antifungal Triterpenoid Saponin from Launaea pinnatifida Cass. Indian Journal of Chemistry (2009).
16. Moussaoui, F.; Zellagui, A.; Segueni, N.; Touil, A. and Rhouati, S. Flavonoid Constituents from Algerian Launaea resedifolia (O.K.) and Their Antimicrobial Activity. Rec. Nat. Prod. 2010,4:1 91-95
17. Das, K. and Roychoudhury, A. Reactive oxygen species (ROS) and response of antioxidants as ROS- scavengers during

environmental stress in plants. Frontiers in environmental science, 2014, 2, p.53.

18. Patel, B., Das, S., Prakash, R. and Yasir, M. Natural bioactive compound with anticancer potential.International Journal of Advances in Pharmaceutical Sciences, 2010, 1. (1)
19. Dehelean, C.A., Marcovici, I., Soica, C., Mioc, M., Coricovac, D., Iurciuc, S., Cretu, O.M. and Pinzaru, I. Plant-derived anticancer compounds as new perspectives in drug discovery and alternative therapy. Molecules, 2021, 26(4), p.1109.
20. Collenette, S. Wildflowers of Saudi Arabia; National Commission for Wildlife Conservation and Development (NCWCD): Riyadh, Saudi Arabia, 1999.Selected Fatty Acids. Journal of Oleo Science, 2016, 65(1), pp.81-89.
21. Salama, S.A.; Al-Faifi, Z.E. and El-Amier, Y.A. Chemical Composition of Reichardia tingitana Methanolic Extract and Its Potential Antioxidant, Antimicrobial, Cytotoxic and Larvicidal Activity. Plants, 2022, 11(15), pp. 2028.
22. Miguel, M.G. Antioxidant activity of medicinal and aromatic plants. A review. Flavour and Fragrance Journal, 2010, 25(5), pp. 291-312.
23. Bondock, S.; Adel, S.; Etman, H.A.; Badria, F.A., Synthesis and antitumor evaluation of some new 1, 3, 4- oxadiazole-based heterocycles European Journal of Medicinal Chemistry, 2012, 48, 192-199.
24. Govindarajan M. and Sivakumar R. Larvicidal, ovicidal, and adulticidal efficacy of Erythrina indica (Lam.) (Family: Fabaceae) against Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus (Diptera: Culicidae). Parasitol. Res. 2014, 113(2):777–791.
25. Jurnal I. D. Adulticide Efficacy of Artemisia vulgaris L. against Aedes aegypti. Parasitol. Res., 2019, Vol.20 No. 2, 123-128.
26. Panneerselvam, C., Murugan, K., Kovendan, K. and Kumar, P. M. Mosquito larvicidal, pupicidal, adulti¬cidal, and repellent activity of Artemisia nilagirica (Family: Compositae) against Anopheles stephensi and Aedes aegypti. Parasitol. Res., 2012, 111(6): 2241-2251.
27. WHO. Guidelines for Laboratory and Field Testing of Mosquito Larvicides. World Health Organization, Geneva. 2005, pp.12.
28. El-Sharkawy, E.R., Ed-Dra, A. and Abdallah, E.M. Phytochemical, antimicrobial and antioxidant properties of Launaea nudicaulis and Farsetia hamiltonii. Journal of Biological Control, 2017, 31(2), pp.102- 109.
29. Sarma R., Adhikari K., Mahanta S. and Khanikor B. (2019). Combinations of plant essential oil based terpene compounds as larvicidal and adulticidal agent against Aedes aegypti (Diptera: Culicidae). Sci. Rep., 9(1): 9471.
30. El-Sharkawy, E.R., Ed-Dra, A. and Abdallah, E.M. Phytochemical, antimicrobial and antioxidant properties of Launaea nudicaulis and Farsetia hamiltonii. Journal of Biological Control, 2017, 31(2), pp.102- 109.
31. Saleem, M., Parveen, S., Riaz, N., Tahir, M.N., Ashraf, M., Afzal, I., Ali, M.S., Malik, A. and Jabbar, A. New bioactive natural products from Launaea nudicaulis. Phytochemistry Letters, 2012, 5(4), pp.793-799.
32. Abd-ElGawad, A.M.; El-Amier, Y.A.; Assaeed, A.M.; Al-Rowaily, S.L., Interspecific variations in the habitats of Reichardia tingitana (L.) Roth leading to changes in its bioactive constituents and allelopathic activity Saudi Journal of Biological Sciences 2020, 27, 489-499.
33. Cornara, L.; La Rocca, A.; Marsili, S.; Mariotti, M., Traditional uses of plants in the Eastern Riviera (Liguria, Italy) Journal of Ethnopharmacology 2009, 125, 16-30.
34. El-Amier, Y.A., Abduljabbar, B.T., El-Zayat, M.M., Sarker, T.C. and Abd-ElGawad, A.M. Synthesis of Metal Nanoparticles via Pulicaria

undulata and an Evaluation of Their Antimicrobial, Antioxidant, and Cytotoxic Activities. Chemistry, 2023, 5(4), pp.2075-2093.

35. Ka, M.H., Choi, E.H., Chun, H.S. and Lee, K.G. Antioxidative activity of volatile extracts isolated from Angelica tenuissimae roots, peppermint leaves, pine needles, and sweet flag leaves. Journal of agricultural and food chemistry, 2005, 53(10), pp.4124-4129.
36. Wahyuni, F.S., Shaari, K., Stanslas, J., Lajis, N. and Hamidi, D. Cytotoxic compounds from the leaves of Garcinia cowa Roxb. Journal of Applied Pharmaceutical Science, 2015,5(2), pp.006-011.
37. Reddy, Y.S., Kaki, S.S., Rao, B.B., Jain, N. and Vijayalakshmi, P. Study on Synthesis, Characterization and Antiproliferative Activity of Novel Diisopropylphenyl Esters of
38. El-Afify, S.M., El-Metwaly, M.A., Abbas, M.A. and El-Amier, Y.A. In Vitro Assessment of Antioxidant and Cytotoxic Activities of Zygophyllum coccineum L. Methanolic Extract. environment, 2024, 7, p.8.
39. El-Amier, Y.A., El-Halawany, E.F., Soliman, H.M. and El Hayyany, L.Y. Primary GC-MS chemical analysis of alcoholic extract of Emex spinosa (L.) Campd. and screening of their antioxidant, antibacterial, and cytotoxic characteristics. Bulletin of the Chemical Society of Ethiopia, 2023, 37(1), pp.101- 114.
40. Sepehri-Moghadam, H., Rahbarian, R. and Sadoughi, S.D. The effect of aqueous extract of Launaea acanthodes (Boiss.) O. Kuntze on the serum level of insulin and blood glucose and histomorphological changes of pancreas in diabetic rats. Feyz Journal of Kashan University of Medical Sciences, 2015, 19(1).
41. Adinortey, M.B., Sarfo, J.K., Kwarteng, J., Adinortey, C.A., Ekloh, W., Kuatsienu, L.E. and Kwadwo Nyarko, A. The ethnopharmacological and nutraceutical relevance of Launaea taraxacifolia (Willd.) Amin ex C. Jeffrey. Evidence-Based Complementary and Alternative Medicine, 2018.
42. Tagne, R.S., Telefo, B.P., Nyemb, J.N., Yemele, D.M., Njina, S.N., Goka, S.M.C., Lienou, L.L., Kamdje, A.H.N., Moundipa, P.F. and Farooq, A.D. Anticancer and antioxidant activities of methanol extracts and fractions of some Cameroonian medicinal plants. Asian Pacific Journal of Tropical Medicine, 2014, 7, pp. S442-S447.
43. Mazher, M., Ishtiaq, M., Mushtaq, W., Maqbool, M., Zahid, N. and Husain, T. Comprehensive Review of Phytochemistry and Bioactivities of Citrullus Colocynthis (L.) Schrad. Pharm. Res, 2020,4(4), p.486.
44. Cole, M.D. Key antifungal, antibacterial and anti-insect assays—a critical review. Biochemical Systematics and Ecology, 1994,22(8), pp.837-856.
45. Samia Rashid, Mohammad Ashraf, Rubeen Anjum and Shahida Bibi. Insecticidal and Cytotoxic Activities of Launaea nudicaulis (Roxb.) and Launaea resedifolia (Linn.),2000, 3 (5): 808-809.