

## Article

# *L. Serriola's* Protective Impact on Lung Histology Against Benzo(A)Pyrene-Induced Carcinogenesis in Mice

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**Abstract:** Natural plants are considered an important factor in human resistance to diseases. This study aimed to evaluate Protective effect of *L. serriole* on histological features of Lungs induced by a carcinogen benzo (a) pyrene. The histological study of the lungs of mice showed that there were significant changes in the normal liver structure in the group of animals that took doses of the carcinogenic substance benzo(a)pyrene, which caused damage to the lung tissue. A disease in the normal structure of the lung tissue in a normal way. The results of the histological diagnosis of the lung showed the presence of hyperemia at the concentration dose of 0.003 mg/kg. When the concentration was given at 0.0015 mg/kg, it was found that there were histological changes, infiltration of inflammatory cells, compared to the control group .The results of changes in the measurements of internal parameters of the lungs in the group that was taken with a carcinogen benzo(a)pyrene only showed a significant increase at the level of probability 0.05 in the average measurements of the internal parameters of the alveoli and bronchi, while the results of the dose of alcoholic extract of wild beer lettuce showed a concentration of 0.006 mg/kg, 0.003 mg/kg, and 0.0015 mg/kg showed a slight increase at a probability level of 0.05 compared to the control group.

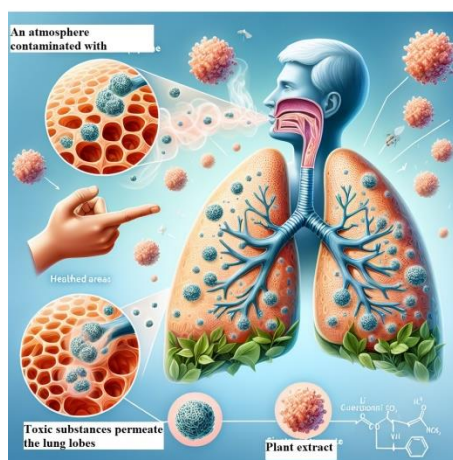
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**Keywords:** *L. serriole* , benzo (a) pyrene, Lungs tissue, Carcinogens, Histological changes



Graphical abstract of the protective role of *L. Serriole* on benzo (a) pyrene

## 1. Introduction

Medicinal plants and the effective chemicals extracted from them are considered an important factor in human resistance to diseases. Effective substances were extracted from them, which showed hopeful effects in the treatment of many diseases (El-Esawi et al., 2017; Hameed et al., 2021). Recent research and studies have tended to use the plant in Treatment of many diseases, including cancer diseases, and it has also been used experimentally in inhibiting cancerous cell lines in laboratories, as compounds such as phenolic compounds, flavonoids, and isoflavonoids have been found that have an inhibitory effect on some cancerous cell lines, and recent research has shown that phytochemicals It has anti-cancer activity by targeting a wide range of cell signaling pathways at various levels (Sessa et al., 2000; Mohammed et al., 2020). The wild lettuce plant, *Lactuca serriole* of the Asteraceae family, is rich in active compounds (Koc et al., 2015). These include phenols and flavonoids that are highly effective against cancer (Muniyandi et al., 2019), in addition to their anti-cancer activity , Antioxidant also contains the milk of the latex plant called *Lactucarium* (Ahmed et al., 2016), which is used in medicine because of its many medicinal properties such as its effectiveness as an anti-cancer Anticancer , Diuretic, Emollient, Febrifuge, Hypnotic, Narcotic, Tonic, Galactagogue, Sedative, Anxiety, Neuroses, and joint pain, as indicated by (Janbaz et al., 2013) indicated that the methanolic extract of the aerial parts of the wild lettuce plant *Lactuca serriole* has a significant effect in reducing anxiety and depression as well as symptoms, and the active compounds present in the alcoholic extract of the wild lettuce plant *Lactuca serriole* have many biological activities that include antimicrobials, anti-mitochondrial adhesion, conducted a study on the antagonistic effect of *Lactuca serriole* against scorpion poison *Buthus atlantis*, which showed that the alcoholic extract of wild lettuce plant has a significant protective ability against scorpion poison (Bouimeja et al., 2019; Abdulilah et al., 2019), anti-ulcer (Shukurlu & Goger, 2021). Benzo(a)pyrene is one of the main environmental pollutants resulting from incomplete combustion of organic materials, forest fires, volcanic eruptions, fossil fuels and cracking wood, industrial processes (Singh et al., 2016), where studies confirmed that the association between exposure to polycyclic aromatic hydrocarbons found in Urban air and lung cancer (Majumder et al., 2021), and the study aimed to study the protective effect of the alcoholic extract of wild lettuce plant *Lactuca serriole* in protecting the lung after treating mice with the carcinogen benzo(a)pyrene.

## 2. Materials and Method

### 2.1. Preparation of the plant extract

The alcoholic extract 99% was prepared according to the (Hameed *et al.*, 2020), method, by soaking 4g of wild lettuce powder in 100ml of methyl alcohol at a concentration of 99% for 24hrs in a shaking table incubator, filtering using Whatman 1 filter papers. Centrifuged for 10 min. at a speed of 2000 rpm, after that the alcohol was evaporated in the incubator for 48 hrs, then the extract was suspended in 10 ml of distilled water, and three concentrations of 0.006 mg/kg, 0.003 mg/kg, and 0.001 mg/kg were prepared. The carcinogen Benzo(a)pyrene was prepared from Guokang China. The carcinogen was dissolved in vegetable oil according to the method (Alhamdow *et al.*, 2021; Jassim & Hameed, 2020)

### 2.2. Laboratory animal collection

35 adult male mice were used from the National Center for Drug Control in Baghdad. They were placed in plastic cages for animal breeding in the animal house of the College of Education for Women / Anbar University. The mice were distributed among the cages, with 7 mice in one cage. Mice were subjected to appropriate laboratory conditions in terms of ventilation, temperature and appropriate lighting, while giving them water continuously, and feeding them with standard diet. The protective plant extract was given 30

days before giving the plant the carcinogenic substance. mice were distributed into 5 groups, in each group 7 mice, for a period of 30 days: G1 the negative control group: it was given distilled water only. G2, the positive control group: it was treated with the carcinogen Benzopyrene orally, at a concentration of 0.001 mg/kg, after dissolving it in vegetable oil, using the tool for dosing mice, as the carcinogen was used at a concentration of 0.001 mg/kg, at a rate of one dose per week. G3: It was treated with alcoholic extract of *Lactuca* plant orally, using the dosing device of gavage mice, as the alcoholic extract of the plant was used at a concentration of 0.006 mg / kg, daily. G4: It was treated with alcoholic extract of *L. serriole* plant orally, using the dosing device of gavage mice, as the alcoholic extract of the plant was used at a concentration of 0.003 mg / kg, on a daily basis. G5:- It was treated with an alcoholic extract of the *Lactuca* plant orally, using the dosing device of gavage mice, as the alcoholic extract of the plant was used at a concentration of 0.0015 mg/kg, daily.

### 2.3. Preparation of Histological Sections

The animals were liberated after the expiration of the prescribed treatment period, according to the method of McKenzie (2011), as they were anesthetized with chloroform, then the animals were dissected and the lungs were removed and placed in plastic containers containing formalin at a concentration of 10% for the purpose of biochemical and histological study, They were treated with a physiological solution for the purpose of washing them, then they were cut into small pieces of 1 cm in size and placed in formalin buffer at a concentration of 10% for 48 hrs. After that, the samples are washed with tap water. For a period of 20-30 minutes, in order to get rid of the excess fixative solution, after that, routine methods were applied according to Luna, 1968) in the preparation of slides

### 2.4. Statistical Analysis

The data were statistically analyzed according to the Complete Randomized Design (CRD) model using the statistical program Genn Stat- Tenth Edition Version and the significant difference of the means were tested using the least significant difference test L.S.D. at the probability level ( $p \leq 0.05$ )(AL-Rawi and Khalaf ALLah ).

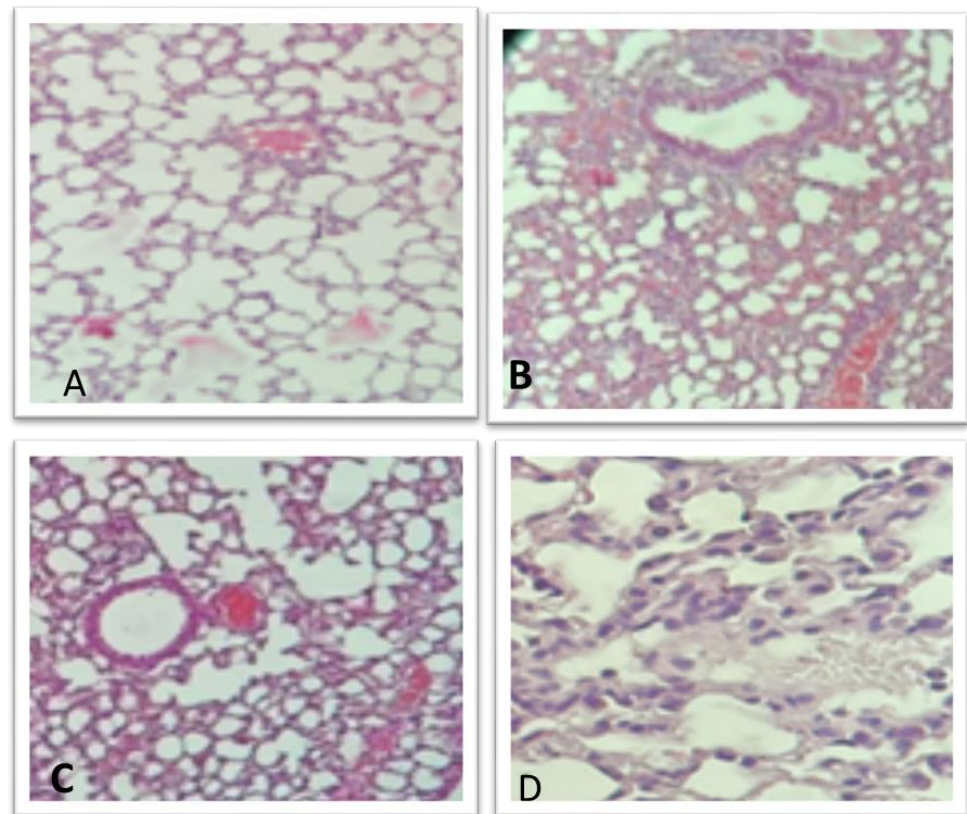
## 3. Results and Discussion

The results of the histological examination of the lung under the light microscope showed that there were histological changes in the lung tissue, according to the treatments, compared with the healthy control group. For a period of 30 days, changes appeared in the normal structure of the lung tissue. These changes were represented by congestion of blood vessels with an increase in the thickness of the lung epithelium, hyperplasia of the lining cells in the bronchioles, a large hyperplasia of smooth cells with infiltration of inflammatory cells, and the destruction of alveoli and pulmonary vesicles, as in (Figure 1A).

The histological diagnosis of the lung were shown in a group of animals that were dosed orally with alcoholic extract of wild lettuce plant *L. serriole* at a concentration of 0.006 mg/kg for 30 days in conjunction with dosing them with the carcinogen Benzo(a)pyrene at a concentration of 0.001 mg/kg in one dose per week for 30 days. days and compared with the control group, the results of the histological diagnosis of the lung tissue were represented by the structure of the lung tissue approaching the normal shape of the lung with congestion of blood vessels as in Figure 1B, While the results of the histological examination of the lung in a group of animals that were dosed orally with an alcoholic extract of wild lettuce plant *L. serriole* at a concentration of 0.003 mg/kg for a period of 30 days and in conjunction with an oral dose of the carcinogen Benzopyrene at a concentration of 0.001 mg/kg at a rate of one dose per week for a period of 30 days and compared with control group, the results showed congestion of blood vessels with infiltration of inflammatory cells as in Figure 1C. When mice were dosed orally with alcoholic extract of *L. serriole* at a concentration of 0.0015 mg/kg for a period of 30 days and simultaneously

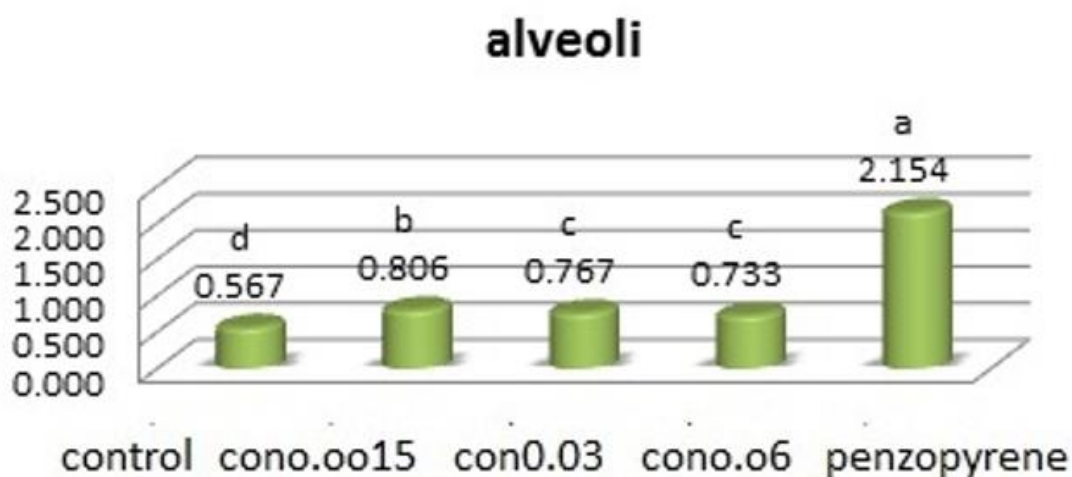
with dosed orally with the carcinogen Benzopyrene at a concentration of 0.001 mg/kg at one dose per week for a period of 30 days and compared the results with the control group, the histological diagnosis of the tissues was shown. Lung infiltration of inflammatory cells and congestion of blood vessels as in Figure 1D.

The histological study of the lung in a group of animals that were dosed only with the carcinogenic substance benzo(a)pyrene showed the presence of histological changes in the lung tissue, which is represented by the destruction of the alveoli, pulmonary vesicles, and the congestion of blood vessels, infiltration of inflammatory cells, and these results agreed with (Mohammad et al., 2017), as they indicated that benzo(a)pyrene may cause significant changes in the pulmonary tissue, including an increase in the thickness of the lung epithelium and the destruction consistent with the study (Majumder et al., 2021), which indicated that olive leaf extract, *Olea europaea* L, is rich in phenolic compounds that possess antioxidant and anti-inflammatory properties as well. They protect the lung from cancer caused by benzopyrene and associated with oxidative stress. By preserving the activity of antioxidants, therefore, it was used as a protector against cancer caused by benzopyrene, which caused deformation of the alveoli and an increase in the number of nuclei in the cells of the alveolar wall, in addition to excessive cell proliferation and thus damage to the lung structure in animals that were dosed orally with benzopyrene when compared to animals in the control group.

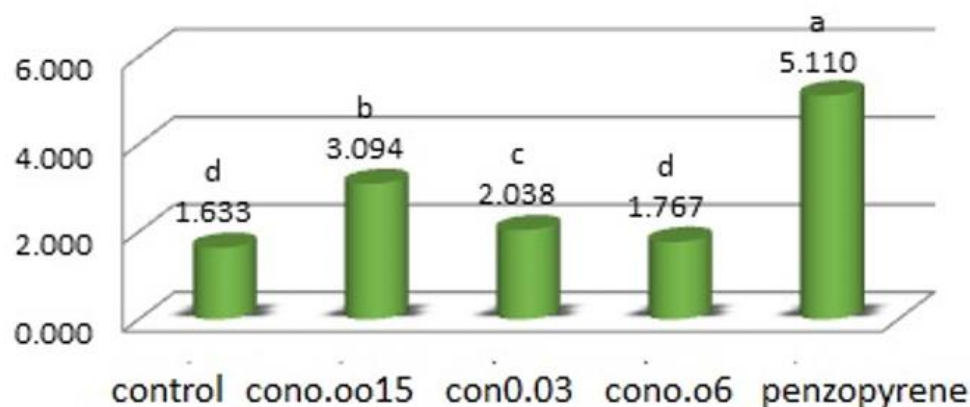


**Figure 1.** A: Shows no histological changes of lungs in control (H&E) 10x, B: Tissue section of a mouse lung from the group treated with benzopyrene (B(a)p only) showing A\_ vascular congestion B\_ smooth cell hyperplasia C\_ inflammatory cell infiltration 40x, C: Tissues section of lung from the group treated with alcoholic extract of wild lettuce plant *L. serriole* at a concentration of 0.006 mg/kg and benzopyrene B (a)p, showing the histological appearance close to normal with congestion of blood vessels H&E 10x), D: Tissue section in a mouse lung from the group treated with alcoholic extract of wild lettuce plant *L. serriole* at a concentration of 0.0015 mg/kg and benzopyrene B (a)p, showing, Congestion of blood vessels, Infiltration of inflammatory cells 10X.

There are statistically significant differences at the level of  $0.05 \geq P$  in the average measurements of the diameters of the internal features of the lung represented by the alveoli and the bronchi. *serriole* at a concentration of 0.0015 mg/kg in conjunction with dosing the carcinogen Benzo(a)pyrene, resulted in a significant increase in the average measurements of the internal parameters of the lung with an average of 0.806  $\mu\text{m}$  for the alveoli and 3.094  $\mu\text{m}$  for the trachea compared with the control group, while when the concentration was dosed at 0.003 mg/kg In conjunction with the administration of the carcinogenic substance Benzo(a)pyrene, it was found that there was a significant increase in the average measurements of the diameters of the internal features of the lung, as the average height, respectively, was 0.767  $\mu\text{m}$  for the pulmonary vesicle and 2.038  $\mu\text{m}$  for the trachea, compared with the control group, when it was dosed at a concentration of 0.006 mg/kg. In conjunction with her dose of the carcinogenic substance Benzo(a)pyrene, it was found that there was a rise in the average measurements of the diameters of the internal features of the kidney, as it reached 0.733 micrometers for the alveolus. pulmonary and 1.767 micrometers for the trachea compared to the negative control group, as well as if the animals were dosed the carcinogen only, while the results of the statistical analysis indicated that there were significant differences with statistical significance when the carcinogen Benzo(a)pyrene was dosed only, as it was found that there was a high Significantly significant in the average of the internal parameters of the lung, as it reached 2.154  $\mu\text{m}$  for the alveoli and 5.110  $\mu\text{m}$  for the bronchi, compared with the control group Figure 2 , that the measurements of the internal parameters of the lung in the groups that were previously dosed with the alcoholic extract of wild lettuce plant *L. serriole*, which showed greater protection against the damage caused by the carcinogen Benzo(a)pyrene in the measurements of the internal parameters of the lung, as the group of animals that showed It was dosed with the alcoholic extract of wild lettuce plant at a concentration of 0.006 mg/kg. The results were very close to the control group more than the other concentrations of the extract. The reason may be the effect of the alcoholic extract of wild lettuce plant *L. serriole* against the inflammation caused by benzo(a)pyrene, and also because of its ability to relieve spasm. It works to remove the spasm of the smooth muscles lining the lung and the trachea. It gave great protection to the internal features of the lung represented by the bronchi. This was confirmed by (Servi & Doğan, 2020), as it proved that the alcoholic extract of the wild lettuce plant has a spasmolytic and bronchodilator effect in addition to its effect in smooth muscle relaxation.



**Figure 2.** Measurements of alveolar diameters



**Figure 3.** Measurements of tracheal diameters

Figure 3 shows the measurements of the diameters of the internal parameters of the lung for the group that was dosed with the carcinogen Benzo(a)pyrene only. Significant changes occurred in the internal parameters of the lung, including a change in the diameters of the pulmonary vesicle and the bronchi and smooth muscle laceration. The reason may be due to the effects of benzopyrene, which caused with a significant enlargement of both the alveoli and the trachea, it also caused damage to the epithelial layer lining the lung, and thus caused weakness in the vital functions of the lung, and this is consistent with (Rubin, 2021), which proved that low-level exposure to polycyclic aromatic hydrocarbons is closely related to weakness. And decreased vital functions of the lung.

#### 4. Conclusion

The effectiveness of the alcoholic extract in preventing the significant and toxic damage that results from continuous exposure to the toxic substance benzopyrene.

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**Conflicts of Interest:** The authors declare no conflict of interest.

consistent with the study (Majumder et al., 2021), which indicated that olive leaf extract, *Olea europaea* L, is rich in phenolic compounds that possess antioxidant and anti-inflammatory properties as well. They protect the lung from cancer caused by benzopyrene and associated with oxidative stress. By preserving the activity of antioxidants, therefore, it was used as a protector against cancer caused by benzopyrene, which caused deformation of the alveoli and an increase in the number of nuclei in the cells of the alveolar wall, in addition to excessive cell proliferation and thus damage to the lung structure in animals that were dosed orally with benzopyrene when compared to animals in the control group.

## REFERENCES

1. Ahmed SI, Hayat MQ, Tahir M, Mansoor Q, Ismail M, Keck K, Bates RB. Pharmacologically active flavonoids from the anticancer, antioxidant and antimicrobial extracts of *Cassia angustifolia* Vahl. *BMC complementary and alternative medicine*. 2016 Dec;16(1):1-9.
2. Alhamdow A, Zettergren A, Kull I, Hallberg J, Andersson N, Ekström S, Berglund M, Wheelock CE, Essig YJ, Kraus AM, Georgelis A. Low-level exposure to polycyclic aromatic hydrocarbons is associated with reduced lung function among Swedish young adults. *Environmental Research*. 2021 Jun 1;197:111169.
3. Bouimeja B, Yetongnon KH, Touloun O, Berrougui H, Laaradia MA, Ouanaïmi F, Chait A, Boumezzough A. Studies on antivenom activity of *Lactuca serriola* methanolic extract against *Buthus atlantis* scorpion venom by in vivo methods. *South African Journal of Botany*. 2019 Sep 1;125:270-9.
4. Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. *Nature*. 2017 Jan 19;541(7637):321-30.
5. Dawood RW, Sayer SH, Hameed AT. Cancer protective Effects of *L. Serriole* Extract against Carcinogen Benzo (A) Pyrene in White Mice Liver: Histological Study. *HIV Nursing*. 2022 Oct 30;22(2):1978-81.
6. El-Esawi MA, Elkelish A, Elansary HO, Ali HM, Elshikh M, Witczak J, Ahmad M. Genetic transformation and hairy root induction enhance the antioxidant potential of *Lactuca serriola* L. *Oxidative medicine and cellular longevity*. 2017 Jan 1;2017.
7. Farhan HN, Hameed AT, Aobad HM. The biological activity of some *Pseudomonas* sp. isolates on growth of three plant pathogenic fungi under incubator conditions. *Advances in Environ. Biol*. 2010 Jan 1;4(1):53-7.
8. Hameed AT, Abid Al-alh NM, Jumaa AW. Antioxidant Activity and Phytominerals Study of Some Asteraceae Species Growth in Western of Iraq. *Indian Journal of Forensic Medicine & Toxicology*. 2021 Jan 1;15(1).
9. Hameed AT, Dawd SM, Al Bahadly ZK. Ecological Study and Peroxidase Activity of Some Medical Plant (Asteraceae) Growth Wildly in Anbar Governorate-Iraq. In *Journal of Physics: Conference Series* 2021 Mar 1 (Vol. 1818, No. 1, p. 012037). IOP Publishing.
10. Janbaz KH, Latif MF, Saqib F, Imran I, Zia-Ul-Haq M, De Feo V. Pharmacological effects of *Lactuca serriola* L. in experimental model of gastrointestinal, respiratory, and vascular ailments. *Evidence-based complementary and alternative medicine*. 2013 Jan 1;2013.
11. Jassim RA, Hameed AT. Molecular Characterization of Selected Genera of the (Apiaceae) Family using SSR Molecular Markers. *Indian Journal of Forensic Medicine & Toxicology*. 2020 Oct 1;14(4).
12. Koc S, Işgor BS, Işgor YG, Şomali Moghaddam N, Yildirim O. The potential medicinal value of plants from Asteraceae family with antioxidant defense enzymes as biological targets. *Pharmaceutical biology*. 2015 May 4;53(5):746-51.
13. Hamood K, Hameed AT, Azzam MR, Mohammed IH. Chemical composition and antimicrobial activities of the flavonoids *Ammi majus* L growing broadly in Western Iraq. In *AIP Conference Proceedings* 2022 Dec 2 (Vol. 2547, No. 1). AIP Publishing. <https://doi.org/10.1063/5.0112686>
14. Majumder D, Debnath R, Nath P, Libin Kumar KV, Debnath M, Tribedi P, Maiti D. Bromelain and *Olea europaea* (L.) leaf extract mediated alleviation of benzo (a) pyrene induced lung cancer through Nrf2 and NFκB pathway. *Environmental Science and Pollution Research*. 2021 Sep;28:47306-26.
15. Mohammed IH, Hameed AT, Salman HF. Phytochemical and Biological of *Anthemis nobilis* (Asteraceae family) a Native Herbs of Iraq. *Systematic Reviews in Pharmacy*. 2020 Feb 1;11(2).
16. Muniyandi K, George E, Sathyanarayanan S, George BP, Abrahamse H, Thamburaj S, Thangaraj P. Phenolics, tannins, flavonoids and anthocyanins contents influenced antioxidant and anticancer activities of *Rubus* fruits from Western Ghats, India. *Food Science and Human Wellness*. 2019 Mar 1;8(1):73-81.
17. Rosen G. Mesmerism and surgery: A strange chapter in the history of anesthesia. *Journal of the history of medicine and allied sciences*. 1946 Oct 1;1(4):527-50.
18. Rubin H. Synergistic mechanisms in carcinogenesis by polycyclic aromatic hydrocarbons and by tobacco smoke: a bio-historical perspective with updates. *Carcinogenesis*. 2001 Dec 1;22(12):1903-30.
19. Servi H, Doğan A. Chemical composition of essential oil from aerial parts of *Lactuca serriola* L. *Aurum Journal of Health Sciences*. 2020 May 5;2(2):83-90.
20. Sessa RA, Bennett MH, Lewis MJ, Mansfield JW, Beale MH. Metabolite profiling of sesquiterpene lactones from *Lactuca* species: Major latex components are novel oxalate and sulfate conjugates of lactucin and its derivatives. *Journal of Biological Chemistry*. 2000 Sep 1;275(35):26877-84.

21. Shukurlu EN, Goger F. Investigation of chemical components extracted from the aerial parts of *Lactuca serriola* L. by liquid chromatography–mass spectrometric method. *Plant & Fungal Research*. 2021 Dec;4(2):50-5.
22. Singh L, Varshney JG, Agarwal T. Polycyclic aromatic hydrocarbons' formation and occurrence in processed food. *Food chemistry*. 2016 May 15;199:768-81.
23. Teng Y, Shen Y, Luo Y, Sun X, Sun M, Fu D, Li Z, Christie P. Influence of *Rhizobium meliloti* on phytoremediation of polycyclic aromatic hydrocarbons by alfalfa in an aged contaminated soil. *Journal of hazardous materials*. 2011 Feb 28;186(2-3):1271-6.

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