*ERURJ, 2023, 2, 2, 308-319*

*(Mini review)*

# Boldo phytochemical and pharmacological activities; updated Mini-review

*Mostafa B. Abouelela[1]*, Reem A. Naimy [2], Omnya A. Elshafey [2], Momen M. Mahmoud [2], Mahmoud Mohamed Elgez [2], Mariem M. Ahmed [2], Nada M. Abdallah [2], Yousab A. Boshra [2], Ahmed S. Mohamed [2], Asmaa E. Saad [2], Maha I. Abdelhay [2], Ahmed M. Ali[2], Ehab El Sayed [2], Osama E. Mohamed [2], Sara E. Mohamed [2], Ahmed Mohamed [2], Omayma Elgindi[1]*

[1]*Department of Pharmacognosy, Faculty of Pharmacy, Egyptian Russian University, Badr City, Cairo-Suez Road, Cairo 11829, Egypt.*

[2]*Pharm D Program, Faculty of Pharmacy, Egyptian Russian University, Badr City, Cairo-Suez Road, Cairo11829, Egypt.*

*Corresponding author:

Mostafa B. Abouelela, E-mail: mostafa-basheer@eru.edu.eg, Tel: +201009167097

*Received 4th April 2023, Revised 13th April 2023, Accepted 16th April 2023*

*DOI: 10.21608/erurj.2023.203801.1018*

**ABSTRACT:**

*Peumus boldus* is a plant belonging to the family Monimiaceae. It is native to a small perennial tree, dispersed globally, commonly known as boldo. It is distributed mainly in South America (Chile), and the Mediterranean region. It has several uses in traditional medicine. Boldo leaves and bark are the main parts of the plant known mainly for their richness in alkaloids. They are responsible for boldo pharmacological activities. Boldine is the most abundant detected alkaloid within boldo. Other non-alkaloids identified compounds as phenolic compounds, flavon-3-ol (mainly isorhamnetin and kaempferol) and glycosides. Moreover, essential oils; are mainly 1,8-cineole, p-cymene, Limonene, and ascaridole. These chemical compositions may be responsible for *in vivo* and *in vitro* biological activities as antioxidant activity, antimicrobial activity, antifungal activity, anti-parasitic activity, and insecticidal effect. The main aim of this review is to present an updated overview of the significance of pharmacological activities and chemical composition of boldo and its isolated alkaloid boldine.

**Keywords**

*Peumus boldus* – Alkaloids – Boldine – Phenolics – Anticancer activity – Antioxidant activity – Antimicrobial activity

## 1. Introduction

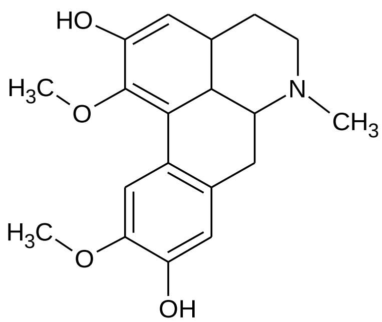
*Peumus boldus* is a plentiful and widely distributed native small perennial tree, typically from arid zones, commonly known as boldo and belongs to the family Monimiaceae (1). It is naturally developed in a range of climates from warm and semiarid to cool and rainy (1). It is one of the most widely known medicinal herbs of Chile, South America, and the Mediterranean zone (1). Several pharmacopoeias have also acknowledged it as a herbal remedy, primarily for the management of liver complaints (2). Moreover, it is recognized as a herbal remedy for gastrointestinal ailments (3) gallbladder, rheumatism, cytoprotective, anti-inflammatory, antipyretic, anti-atherosclerotic, hypoglycemic, as well as effective against the pathogenic bacteria *Helicobacter pylori* (4). Alkaloids are the main bioactive constituents isolated from boldo which may be responsible for its pharmacological activities (5). Several biological studies reported that boldo has antioxidant (1), antimicrobial activity (6), antifungal activity (7), hepatoprotective activity (8), and anticancer (9).

## 2. Bioactive metabolites of boldo:

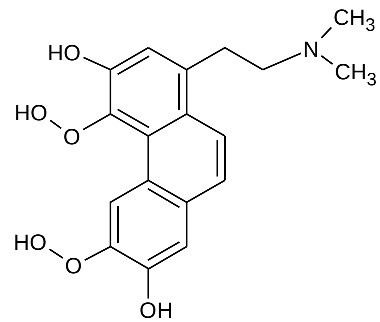
### 2.1 Alkaloids

Alkaloids are the main active constituents identified in boldo which are responsible for its main pharmacological activities. The alkaloid contents were investigated using UHPLC-MS/MS analysis in leaves, bark, root and wood. Results showed that alkaloids concentration in the wood was more abundant than in leaves (10). Mainly bark contains boldine which is responsible for its protective effect against insect and fungal attempts on the plant (10). Moreover, N-methylsecoboldine was found in a small amount in the bark (10). The main alkaloids in leaves were N-methyllaurotetanine and laurotetanine (10).

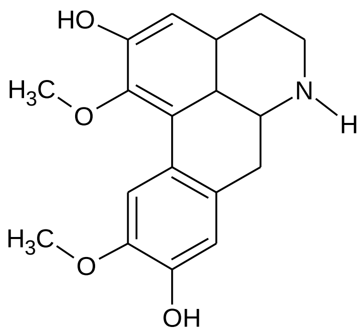
In the stem, root, and wood, where they are more prevalent than other alkaloids, laurolitsine and boldine are both present in comparable concentrations (10).



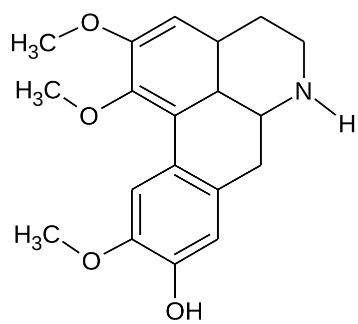
Boldine



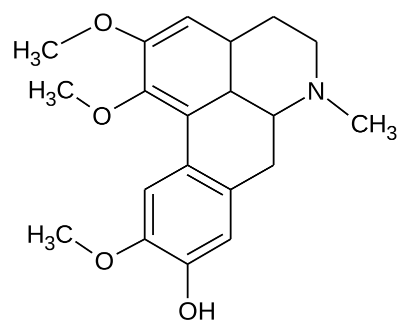
N-methylsecoboldine



Laurolitsine



Laurotetanine

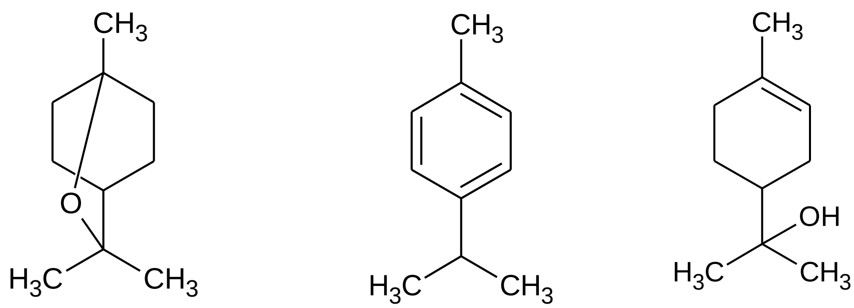


N-methyllaurotetanine

***Figure 1. Identified alkaloids from Boldo***

## 2.2 Essential oils

Complex mixtures of volatile substances called "essential oils" are derived from the secretory glands in various plant sections, such as leaves and flowers, which may have the potential to be biologically active. The volatile profile of *Peumus boldus* leaves was analyzed using GC/MS analysis (11). The results showed that the main identified oils were 1,8-cineole, p-cymene, limonene, ascaridole, β-phellandrene, pinene, terpinen-4-ol, terpineol, sabinene and terpinene (11). Meanwhile, in another study it showed the volatile profile of Chilean *peumus boldus* leaves using GC/MS analysis. The results showed that the main identified oil was Terpineol, 1.8-cineole, p-cymene, terpinen-4-ol, spathulenol, limonene dioxide, and isoaromadendrene epoxide (12).




1,8-cineole

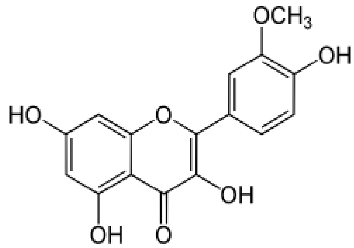
Terpineol

p-cymene

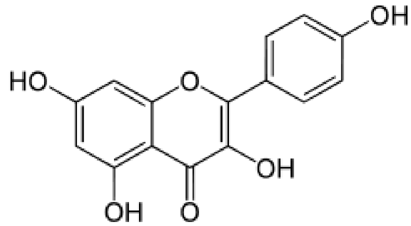
***Figure 2. Identified essential oils from Boldo***

## 2.3 Phenolic compounds

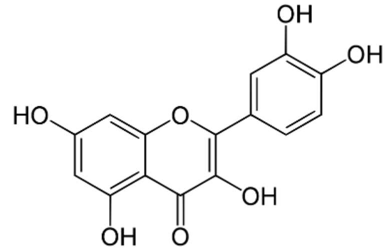
The majority of plant tissues, including those in fruits and veggies, contain phenolic compounds, which are widely dispersed phytochemicals. They are produced as secondary compounds via the phenylpropanoid and shikimic acid pathways, which have a variety of pharmacological characteristics (13). In a study, boldo leaves were investigated for phenolic contents using HPLC-DAD (5). Results showed the main non-alkaloidal phenolic were flavon-3-ol mainly isorhamnetin and kaempferol (5). In another study leaves phenolic contents were investigated using HPLC analysis. Results showed the presence of gallic acid, chlorogenic acid, caffeic acid, syringic acid, rutin, ellagic acid, ferulic acid, naringenin, daidzein, and quercetin (14). In another study, phenolic contents were investigated using HPLC analysis. Results showed the most abundant compound in aqueous extracts of fresh leaves was isoquercitrin, followed by quercitrin and ellagic acid (15). The predominant compound in extracts from the microwave-dried leaves was isoquercitrin, followed by kaempferol and quercetin. The most abundant compound in the aqueous extracts of naturally dried leaves was isoquercitrin and kaempferol followed by quercetin, caffeic acid, and luteolin. The highest amount of total quantified phenolic compound is present in the extract of fresh leaves followed by the extract of microwave-dried leaves and then the extract of naturally dried leaves (15).



Isorhamnetin



Kaempferol



Quercetin

***Figure 3. Identified phenolic compounds from Boldo***

## 3. Biological activities of Boldo

### 3.1 Antioxidant activity

The capacity to biosynthesize a variety of non-enzymatic antioxidants that can reduce ROS-induced oxidative damage is connate in plants. The methanolic extract of boldo leaves was investigated using the Trolox Equivalent Antioxidant Capacity assay. Results showed a considerable antioxidant activity (6.66 ± 0.17 mM), which may be related to 1,8-cineole and phytol compounds identified by GC-MS analysis (16). In another study antioxidant activity of essential oils and teas of commercial samples of Chilean boldo was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (17). Results showed that teas had better antioxidant activity than essential oil of boldo due to the presence of Terpineol and limonene dioxide which are the major compounds (17). These results, demonstrate that the non-volatile portion of these plants has a higher concentration of antioxidant activity (17). Meanwhile, in another report, the antioxidant activity of boldo leaves aqueous extract was evaluated against ABTS•+ radicals. It showed significant antioxidant activities with 720.56 ± 15.00 µMol eq TE/g of extract (18) this may be due to the presence of phenolic contents; Gallic acid, chlorogenic acid, rutin and catechin in aqueous extract detected using UHPLC (18).

### 3.2 Antimicrobial activity

Antimicrobial agent resistance has grown to be a serious and urgent worldwide issue, leading to using natural products as antimicrobial agents. In a study to investigate the effect of boldo on the expression of antimicrobial peptides and inflammatory markers in canine Keratinocytes. The results showed that boldo is a promising option for the management of bacterial overgrowth in Atopic and had no pro-inflammatory effects and the plant extraction may be used in the treatment of infection of cutaneous skin disease (19). Moreover, the ethanolic extract of boldo leaves was investigated using a disk diffusion sensitivity test. The results showed slight antimicrobial activity against *S. aureus*, but no antimicrobial activity against the *E. coli* strain tested (16). Another study using the microdilution technique to evaluate the antibacterial activity of boldo extract. The results showed that the extract had significant antimicrobial activity against *Escherichia coli ATCC 25922*, *Pseudomonas aeruginosa ATCC 15442, and Salmonella typhimurium ATCC 14028*; all strains of *Staphylococcus aureus* especially Gram-positive ATCC 6538, *Bacillus cereus CCMG14* and *Bacillus Subtilis ATCC 6633*. (20).

**3.3 Anti-parasitic activity**

The development of alternative control strategies that rely less on the use of synthetic drugs is being driven by rising drug resistance in livestock parasites that reside in the gastrointestinal tract as well as worries about chemical residues in animal products and the environment (21). The use of bioactive forages with antiparasitic properties as food for animals or as possible sources of the novel, natural parasiticides is a strategy that is being studied more and more (21). The anti-Leishmanial effect of boldine isolated from boldo leaves was investigated using an MTT viability assay (21). Results showed that boldine reduces this parasitic infection and had a potent activity in the treatment of leishmaniasis infection (21).

**3.4 Insecticidal effect**

New approaches to plant crop protection against predators and pathogens are required to satisfy the growing global population's desire for food while avoiding the use of chemicals that are harmful to the environment (11). The insecticidal effect of boldo essential oil was investigated against larvae of the filariasis vector *Culex quinquefasciatus* and *Musca domestica*. The results showed that the essential oil had LC50/LD50 values of 67.9 $mg \cdot L^{-1}$ and 98.5 $\mu g \cdot adult^{-1}$ respectively which indicated a considerable insecticidal activity (11). Meanwhile, in another study, the Chilean Boldo essential oil was investigated for insecticidal activity (12). Results showed that the essential oil of Chilean Boldo showed potent activity against *Chrysomya megacephala* in all of its development phases (12).

**3.5 Anticancer activity.**

Cancer is one of the leading causes of death globally. It is a significant obstacle to raising the life span in this century (22). Depending on the type and stage of cancer, the treatment differs. The majority of patients receive a mixture of therapies, like surgery, combined with chemotherapy and radiotherapy (22). However, some people now combine complementary and alternative medicine with conventional medical treatments due to the negative side effects of conventional therapy and drug resistance (22). Boldine alkaloid from boldo leaves was investigated for its anticancer activity against growth and cell death of DU-145 androgen-independent prostate cancer cell line and the cell viability was measured by MTT test and LDH release. Genomic DNA, caspase-3 activity, and expression of cleaved caspase-9, Hsp70, Bcl-2 and Bax proteins were analyzed to study the apoptotic process (22). The findings indicate that boldine can decrease cell viability and cell death caused via an intrinsic apoptotic pathway that likely includes the down-regulation of heat shock protein (22). Boldine treatment of DU-145 cells at concentrations of 60–120 M resulted in a rise in caspase-3 enzyme activity and Bax protein expression, along with a more pronounced decrease in Bcl-2 (22). Caspase-9 was also demonstrated to be visibly triggered. Moreover, the production of Hsp70 was decreased by the boldine quercetin, a well-known Hsp70 protein inhibitor (22).

### 3.6 Nephroprotective effect

The maintenance of our endocrine system, acid-base equilibrium, blood pressure, erythropoiesis, and other functions depend greatly on the kidneys (23). One of the most frequent kidney issues, nephrotoxicity happens when the body is exposed to a toxin or medication. Numerous medicinal plants showed renal tissue protection from kidney damage (23). The Nephroprotective effect of boldine isolated from boldo against cisplatin-induced rat renal injury was studied (23). Results showed its effect by addressing the expression of iNOS, oxidative stress, caspase-3, and renal damage indicators. Moreover, by reducing oxidative stress, inflammation, histopathological changes, and caspase 3 expressions, it has a promising protective impact on cisplatin nephrotoxicity (23). Another study to investigate the nephroprotective effect of boldine using the 2K1C hypertension model rat models. The results showed that boldine decreased the proteinuria/creatininuria ratio, plasma thiobarbituric acid reactive substances, and slightly decreased systolic blood pressure while having no impact on control animals. Boldine had reduced levels of Alpha-smooth muscle actin, collagen type III, macrophage infiltration, and osteopontin than untreated 2K1C rats did. In 2K1C rats, boldine stopped the rise in ACE-1 and transformed growth factor-β, indicating that boldine lessens kidney damage (24).

### 3.7 Hepatoprotective activity

The liver is one of the most important organs in the body, playing a fundamental role in the regulation of diverse processes, among which the metabolism, secretion, storage, and detoxification of endogenous and exogenous substances are prominent (14). The hepatoprotective activity of Boldo was investigated by measuring lipid peroxidation, liver antioxidant enzymes, reactive oxygen species generation, DNA damage, gene expression, and histopathological changes induced by Amistar fungicide in male rats (14). The results showed that pretreatment with boldo extract increased the antioxidant activities of Superoxide dismutases and catalase as well as elevated the antioxidant genes expression (Glutathione peroxidase and glutathione reductase). Moreover pretreated rats with boldo showed a mild dilated and congested vein was present (14).

### 3.8 Gastroprotective activity

The pathogenesis of stomach ulcers is a multifactorial disease that is still common. It is caused by a variety of factors, including a stressful lifestyle, alcohol consumption, and the use of steroidal and nonsteroidal anti-inflammatory drugs and medications that increase the secretion of gastric acid and pepsin (25). One of the biggest problems in modern medicine is the prevention or treatment of peptic ulcers (25). The gastroprotective activity of boldo was studied on gastric ulcers induced by ethanol and indomethacin in mice (25). The results showed it had considerable activity by reducing lesion area and reducing oxidative stress, inflammatory mediators in ethanol-ulcerated tissue and increased mucin-like glycoprotein amount (25).


***Figure 4: Major pharmacological activities of Boldo***

## Conclusion

In this work, the phytochemical constituents and pharmacological effects of *Peumus boldus* were reported as updated mini-review. The main active constituents identified were alkaloids. Boldine alkaloid is the main identified alkaloid which is found in the leaves, bark and roots of boldo. It may be responsible for its pharmacological activities. Furthermore, N-methylsecoboldine, N-methyllaurotetanine, laurotetanine, and laurolitsine. Essential oils identified in boldo were mainly 1,8-cineole, p-cymene, limonene, ascaridole, β-phellandrene, pinene, terpinen-4-ol, terpineol, sabinene and terpinene which may be responsible for its antimicrobial activities. Phenolic compounds were flavon-3-ol mainly isorhamnetin, kaempferol, gallic acid, chlorogenic acid, caffeic acid, syringic acid, rutin, ellagic acid, ferulic acid, naringenin, daidzein, querectin, isoquercitrin, kaempferol, caffeic acid, and luteolin. These phenolic compounds are responsible for its pharmacological anti-oxidant and anti-inflammatory activities. Boldo showed several pharmacological activities as insecticidal, and anti-parasitic activities which may be due to boldine alkaloids. Moreover, it showed anti-cancer activities which may need further studies to investigate this activity.

## Conflict of interest

The authors declare no conflicts of interest.

## References:

1. Quezada N, Asencio M, Del Valle J, Aguilera J, Gómez B. Antioxidant activity of the crude extract, alkaloid fraction, and flavonoid fraction from Boldo (Peumus boldus Molina) leaves. Journal of food science. 2004;69(5):C371-C6.

2. Speisky H, Cassels BK. Boldo and boldine: an emerging case of natural drug development. Pharmacological Research. 1994;29(1):1-12.

3. Carbajal R, Yisfalem A, Pradhan N, Baumstein D, Chaudhari A, editors. Case report: boldo (Peumus boldus) and tacrolimus interaction in a renal transplant patient. Transplantation Proceedings; 2014: Elsevier 2400-2402.

4. Teixeira CCC, de Freitas Cabral TP, de Sousa JPB, de Pádua Teixeira S, Bastos JK, de Freitas LAP. Study of quality assurance for Peumus boldus M products by botanic profiling, extraction optimization, HPLC quantification and antioxidant assay. Pharmacognosy Journal. 2016;8(3).

5. Cassels BK, Fuentes-Barros G, Castro-Saavedra S. Boldo, its secondary metabolites and their derivatives. Current Traditional Medicine. 2019;5(1):31-65.

6. Mazutti M, Mossi A, Cansian R, Corazza M, Dariva C, Oliveira JV. Chemical profile and antimicrobial activity of Boldo (Peumus boldus Molina) extracts obtained by compressed carbon dioxide extraction. Brazilian Journal of Chemical Engineering. 2008;25:427-34.

7. Passone MA, Girardi NS, Etcheverry M. Antifungal and antiaflatoxigenic activity by vapor contact of three essential oils, and effects of environmental factors on their efficacy. LWT-Food Science and Technology. 2013;53(2):434-44.

8. Mondal J, Bishayee K, Panigrahi AK, Khuda-Bukhsh AR. Low doses of ethanolic extract of Boldo (Peumus boldus) can ameliorate toxicity generated by cisplatin in normal liver cells of mice in vivo and in WRL-68 cells in vitro, but not in cancer cells in vivo or in vitro. Journal of Integrative Medicine. 2014;12(5):425-38.

9. Garbarino J, Troncoso N, Frasca G, Cardile V, Russo A. Potential anticancer activity against human epithelial cancer cells of Peumus boldus leaf extract. Natural Product Communications. 2008;3(12):1934578X0800301229.

10. Fuentes-Barros G, Castro-Saavedra S, Liberona L, Acevedo-Fuentes W, Tirapegui C, Mattar C, et al. Variation of the alkaloid content of Peumus boldus (boldo). Fitoterapia. 2018;127:179-85.

11. Pavela R, Benelli G, Petrelli R, Cappellacci L, Lupidi G, Sut S, et al. Exploring the insecticidal potential of boldo (Peumus boldus) essential oil: toxicity to pests and vectors and non-target impact on the microcrustacean Daphnia magna. Molecules. 2019;24(5):879.

12. Viana TdS, Dias RF, Vianna ACdS, Moreira RF, Aguiar VM. Evaluation of Chilean Boldo essential oil as a natural insecticide Against Chrysomya megacephala (Diptera: Calliphoridae). Journal of Medical Entomology. 2020;57(5):1364-72.

13. de la Rosa LA, Moreno-Escamilla JO, Rodrigo-García J, Alvarez-Parrilla E. Phenolic compounds. Postharvest physiology and biochemistry of fruits and vegetables: Elsevier; 2019. 253-71.

14. Refaie A, Shalby AB, Booles HF, Kassem SM, Eshak MG, Farrag ARH, et al. Hepatoprotective impact of Boldo (Peumus boldus) extract against azoxystrobin induced DNA damage, gene expression modulation, biochemical and histopathological alterations mediated-ROS generation in male rats. Egyptian Journal of Chemistry. 2022;65(8):687-98.

15. Trapp KC, Hister CAL, Laughinghouse IV HD, Boligon AA, Tedesco SB. Determination of phenolic compounds and evaluation of cytotoxicity in Plectranthus barbatus using the Allium cepa test. Caryologia. 2020;73(2):143-53.

16. Bonilla J, Sobral PJdA. Antioxidant and antimicrobial properties of ethanolic extracts of guarana, boldo, rosemary and cinnamon. Brazilian Journal of Food Technology. 2017;20.

17. de Souza WFM, Mariano XM, Isnard JL, de Souza GS, de Souza Gomes AL, de Carvalho RJT, et al. Evaluation of the volatile composition, toxicological and antioxidant potentials of the essential oils and teas of commercial Chilean boldo samples. Food Research International. 2019;124:27-33.

18. Rodríguez M, Bertolino M, Irazusta A, Irazoqui M, Medrano A, editors. In Vitro Bioaccessibility and Antioxidant Capacity of Extracts Obtained from Boldo Leaves (Peu-mus boldus) For Its Application as a Functional Ingredient. Presented at the 2nd International Electronic Conference on Foods; Vol 15, 2021: Proceedings: 1-7.

19. Santoro D, Ahrens K, Vesny R, Navarro C, Gatto H, Marsella R. Evaluation of the in vitro effect of Boldo and Meadowsweet plant extracts on the expression of antimicrobial peptides and inflammatory markers in canine keratinocytes. Research in veterinary science. 2017;115:255-62.

20. Vieitez I, Maceiras L, Jachmanián I, Alborés S. Antioxidant and antibacterial activity of different extracts from herbs obtained by maceration or supercritical technology. The Journal of Supercritical Fluids. 2018;133:58-64.

21. Salama IC, Arrais-Lima C, Arrais-Silva WW. Evaluation of boldine activity against intracellular amastigotes of Leishmania amazonensis. The Korean Journal of Parasitology. 2017;55(3):337.

22. Venera C, Rosanna A, Adriana CG, Alessandra R. Boldine Activates Intrinsic Apoptotic Pathway in DU-145 Androgen-Independent Prostate Cancer Cell Line. Journal of Analytical Oncology. 2019;8:10-7.

23. TURGUT N, GÜNGÖR H, EKİCİ M, ERDOGAN M, KARAYIGIT M, KARA H. Boldine provides protective effect against nephrotoxicity induced by cisplatin in Wistar rats: Role of oxidative stress, inflammation and caspase-3. Biocell. 2022;46(9).

24. Gómez GI, Velarde V. Boldine improves kidney damage in the goldblatt 2K1C model avoiding the increase in TGF-β. International Journal of Molecular Sciences. 2018;19(7):1864.

25. Boeing T, Mariano LNB, Dos Santos AC, Tolentino B, Vargas AC, de Souza P, et al. Gastroprotective effect of the alkaloid boldine: Involvement of non-protein sulfhydryl groups, prostanoids and reduction on oxidative stress. Chemico-Biological Interactions. 2020;327:109166.