Archives of Advances
in Biosciences

Original Article:

Study of the Anti-bacterial and Anti-tumor Effects of Calycosin

Ebrahim Khademi[1], Behdokht Jamali[1*]

1. Department of microbiology, Kherad Institute of Higher Education, Bushehr, Iran.

Cite this article as: Khademi E, Jamali B. Study of the Anti-bacterial and Anti-tumor Effects of Calycosin. Archives of Advances in Biosciences. 2023; 14:E41056. https://doi.org/10.22037/aab.v14i.41056

https://journals.sbmu.ac.ir/aab/article/view/41056

Article info:
Received: 31 Jan 2023
Accepted: 03 Apr 2023
Published: 23 Oct 2023

*** Corresponding author:**

Behdokht Jamali, PhD.

Address: Department of microbiology, Kherad Institute of Higher Education, Bushehr, Iran.

E-mail:
Behdokht.jamali2023@gmail.com

Abstract

Introduction: The spread of antibiotic-resistant pathogenic strains has led to growing concerns in the world. One of the solutions to overcome this global problem is to research natural compounds and find natural ones with antibacterial properties. In this research, therefore, the antibacterial effects of calycosin on some important pathogens were studied. Also, we studied the antitumor effects of calycosin on HT-29 cell line.

Materials and Methods: After preparation of calycosin, its antioxidant capacity was measured. Then, the indicated strains were prepared from the microbiology department of Tehran Medical University and were then cultured. The disk diffusion, microdilution and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium (MTT) methods were used for evaluating antibacterial activity, determining minimum inhibition concentration (MIC), studying the antitumor effects of calycosin, respectively.

Results: Calycosin showed a strong antioxidant capacity and its IC_{50} for 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging was measured to be 120 µg/ml. The study of the antibacterial effects of calycosin indicated that it was more effective on gram-positive bacteria than gram-negative bacteria. The MIC of calycosin for pathogens *E. coli*, *Shigella dysenteriae*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Bacillus cereus* was calculated to be 32, 32, 40, 26 and 28 µg/ml, respectively. To determine the synergistic effects, a concentration of 1.2 MIC of calycosin was used along with antibiotics; the results indicated the synergistic effects of antibiotics and calycosin on the studied pathogens, except for *Staphylococcus aureus*. Calycosin reduced HT-29 cell line viability at 2000µg/mL.

Conclusion: In general, it was concluded that calycosin has strong antibacterial activity on the pathogens used, especially Gram-positive bacteria *Staphylococcus aureus* and *Bacillus cereus*; besides, it has synergistic effects with antibiotics. In this field, in vivo and clinical studies are needed.

Keywords: Bacteria, Calycosin, Pathogen, Synergism

1. Introduction

Active biological compounds in medicinal plants have always been the focus of active scientists in this field. In recent years, due to the need for new therapeutic agents, this interest has been growing dramatically [1]. Medicinal plants are very useful and economical. They contain active compounds that are used in the treatment of many human diseases.

The increasing use of Iranian traditional medicine (ITM) in the treatment of various diseases has attracted the attention of researchers [2]. One of the plants that has been the focus of many studies in the scientific field is the *Astragali radix* (AR) root [3], which has a wide medicinal profile and has shown to have anti-diabetic effects [4], liver protection [5], anti-inflammatory [6], anti-cancer and neuroprotective [7] effects. The main active ingredient in AR is calycosin (CA), which is an isoflavonoid. In previous research, this compound has provided antitumor [8], antioxidant [9], antiviral [10] and anti-ischemic [11] effects. Also, CA has shown to have a neuroprotective effect. In a recent study, this compound showed antibacterial effects against *Escherichia coli* and *Klebsiella pneumoniae* pathogens [12]. In the mentioned research [12], CA showed synergistic effects with polymyxin B in inhibiting bacteria. Hence, calycosin can be considered as an antibacterial compound and its potential against a variety of pathogens can be studied.

Ubiquitous bacterial pathogens cause a variety of infections, including urinary tract infections, nosocomial infections, wound infections, brain abscesses, asthma, pneumonia, and skin diseases [13]. These infections can lead to fatal consequences if not properly treated or remain untreated. Antibiotics are used worldwide to treat a variety of bacterial infections. However, bacteria have gradually become resistant to these antibiotics. In addition, in recent years, the misuse of antibiotics has necessitated the rapid finding of antibacterial therapeutic agents [14].

The prescription of antibiotics has increased drastically in recent years, and this has led to the emergence of antibiotic-resistant pathogenic strains [15]. In addition, antibiotics have several adverse effects, including hypersensitivity, reduction of beneficial intestinal flora, immunosuppression, and allergic reactions [16]. In order to solve this problem, medicinal plants and their natural compounds have attracted the attention of researchers as an adjuvant treatment in infectious diseases.

Colon cancer has been one of the most common malignancies in recent years, with a high mortality rate in patients [17]. Despite recent advances in the treatment of this disease, colon cancer has not yet been completely cured and more research is needed in this field. The treatment of this cancer includes chemotherapy, radiation therapy, and immunotherapy, causing many side effects in patients and leading to a decrease in the quality of life [18]. It seems that finding natural compounds with antitumor properties is of great importance [19]. The antitumor effects of CA have been reported in malignancies such as osteosarcoma [20], colorectal cancer [21], and breast cancer [22]. However, there is a need to more meticulously examine the effect of this compound on colon cancer.

Therefore, given that the resistance of pathogenic bacteria to antibiotics has caused increasing concerns in the health system of countries, the aim of the current research was to evaluate the antibacterial effects of CA on *E. coli*, *Shigella dysenteriae*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Bacillus cereus*. Also, the anti-tumor effects of CA on HT-29 cell line was investigated in the present study.

2. Materials and Methods

Preparation of calycosin

CA was purchased from Sigma (CAS# 20575-57-9) and was then dissolved in DMSO to prepare concentrations of 0, 12.5, 25, 50, 75, 100 and 200 µg/ml.

Measurement of antioxidant capacity

1 mL of the indicated concentrations was combined with 1 mL of DPPH and brought to a concentration of 4000 µL using methanol. After vortexing the samples and placing them in the dark, the absorbance was read at a wavelength of 517 nm using a spectrophotometer. DPPH free radical inhibition percentage was calculated with the following formula:

$$I (\%) = (A_0 - A_s)/A_0 * 100$$

Where, A_0 and A_s were the OD of control and sample, respectively.

Antibacterial activity

E. coli (ATCC 1399), *S. dysenteriae* (ATCC1188), *S. typhimurium* (ATCC1735), *S. aureus* (ATCC 1112) and *B. cereus* (ATCC 1247) were obtained from the Microbiology Department of Tehran University of Medical Sciences. Disc diffusion method was used to study the antibacterial effects of CA. For this purpose, after culturing the bacteria in agarose broth medium and forming a colony, a colony was selected from the culture medium and placed on Mueller Hinton culture medium. Then, 50 µL of the indicated concentrations of CA were injected on the blank disks and after one day, these disks were placed in Mueller Hinton culture medium at equal intervals. Then, the plate was incubated at 37°C for three days. Ciprofloxacin was used as a control sample.

Minimum inhibition concentration (MIC)

MIC was performed based on the microdilution method [23]. A colony of each of the studied bacteria was cultured in LB medium and cultured for 24 hours at 37°C. When the cultures entered the logarithmic phase of growth, they were diluted to a concentration of 10^8 CFU/mL. Different concentrations of CA were added to the prepared bacterial dilution in a 96-well plate containing 50 microliters of MHB medium. Plates were incubated for 18 hours at 37°C, and then 50 microliters of TCC were added to observe the growth of bacteria. The well that showed no color change and concentration with the lowest value was considered as MIC of CA.

Minimum bactericidal concentration (MBC)

To determine MBC, 3 µL of each culture medium was removed from each non-growing well and placed on the surface of Mueller Hinton culture medium. After 20 h of incubation at 36 °C, MBC was defined visually as the lowest concentration at which no viable cells grew on the plates.

The synergistic activity

The studied bacterial were cultured in BHI broth medium at 37°C. Then, the disc of streptomycin, polymyxin B, ciprofloxacin, and vancomycin antibiotics (diameter 5 mm) was placed on the surface and ½ MIC concentration of CA was added to determine the synergistic effect between this compound and antibiotics. Finally, they were incubated at 37°C for one day. The zone inhibition diameter was then determined by a ruler.

Anti-tumor activity of Calycosin

To investigate the antitumor activity of CA, first, HT-29 cells were prepared and cultured in RPMI medium. Then, MTT test was used. Briefly, 5 x 10^4 HT-29 cells were cultured in 96-well plates and treated with 5, 50, 500, 1000 and 2000 µg/ml calycosin and 20 µl of MTT solution were added. Finally, after 48 h of incubation, the intensity of light absorption at 570 nm was measured.

Statistical analysis

GraphPadPrismv. 8 software was utilized for analyzing data analyzing through graphs and ANOVA. Tukey's test$^{P<0.05}$ was used for comparing significant differences.

3. Results

CA antioxidant activity

In the current study, the DPPH method was used to determine the antioxidant potential of CA, and its results are shown in Figure 1. The results indicated that by increasing the concentration of CA, the percentage of DPPH free radical inhibition increased. Also, comparing the antioxidant activity of CA in different concentrations showed that CA in low concentrations leads to greater inhibition of DPPH free radical. The IC_{50} of CA was calculated at a concentration of 120 µg/ml. These results showed that CA has strong antioxidant activity.

Antibacterial activity of CA

The results of the present study showed that increasing the concentration of calycosin from 5 to 25 µg/ml increases the antibacterial activity against *E. coli*, *S. dysenteriae*, *S. typhimurium*, *S. aureus* and *B. cereus*. The highest inhibition of all bacteria was observed at the concentration of 25 µg/ml CA. The high growth inhibition rate of *S. aureus* was observed at 25 µg/ml CA; the diameter of inhibition zone was 13 cm. However, *S. dysenteriae* and *S. typhimurium* showed low sensitivity to CA. However, other bacteria seem to be sensitive to this compound (Table 1).

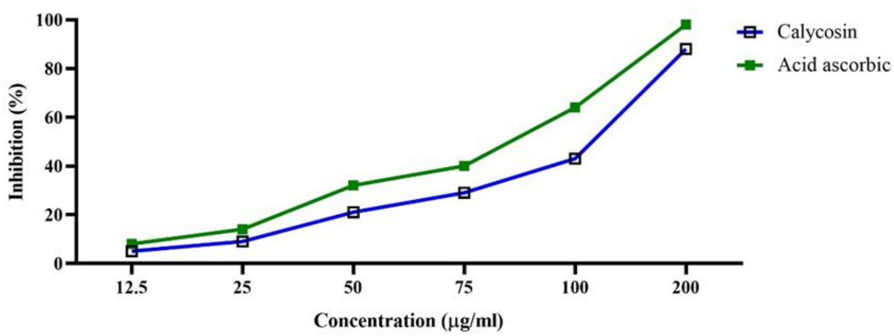

Figure 1. DPPH free radical inhibition percentage by different concentrations of calycosin and ascorbic acid as control

Table 1. Antibacterial activity of calycosin evaluated by disk diffusion method

CA (µg/mL)	*E. coli*	*S. dysenteriae*	*S. aureus*	*B. cereus*	*S. typhimurium*
5	-	-	-	-	-
10	-	-	3	3	-
15	4	-	6	5	-
20	7	3	8	9	2
25	11	7	13	12	6

Data are inhibition zone diameter (mm)

MIC and MBC

The MIC of CA against *E. coli*, *S. dysenteriae*, *S. typhimurium*, *S. aureus* and *B. cereus* was calculated to be 32, 32, 40, 26 and 28 µg/ml, respectively (Table 2). Meanwhile, the MBC of CA for *E. coli*, *S. dysenteriae*, *S. typhimurium*, *S. aureus* and *B. cereus* was calculated to be 76, 72, 67, 65 and 83 µg/ml, respectively.

The synergistic activity of CA with antibiotics

The synergistic effects between the polymyxin B and vancomycin antibiotics along with CA were observed in inhibiting the growth of *E. coli*. However, CA did not have a synergistic effect on streptomycin and ciprofloxacin antibiotics in inhibiting the growth of this pathogen (Table 3).

Table 2. The MIC of calycosin against *E. coli*, *S. dysenteriae*, *S. typhimurium*, *S. aureus* and *B. cereus*

	MIC				
	E. coli	***S. dysenteriae***	***S. aureus***	***B. cereus***	***S. typhimurium***
CA (µg/mL)	32	32	26	28	40
	MBC				
CA (µg/mL)	76	72	67	65	83

Table 3. The synergistic effects of CA on streptomycin, polymyxin B, ciprofloxacin and vancomycin against *E. coli*, *S. dysenteriae*, *S. typhimurium*, *S. aureus* and *B. cereus*

Pathogen	**Antibiotics**	**Inhibition zone diameter of antibiotics**	**Inhibition zone diameter of antibiotics +CA**
E. coli	polymyxin B	15	24
	streptomycin	12	13
	ciprofloxacin	13	14
	vancomycin	16	21
S. dysenteriae	polymyxin B	16	17
	streptomycin	14	14
	ciprofloxacin	12	16
	vancomycin	20	19
S. typhimurium	polymyxin B	12	22
	streptomycin	14	18
	ciprofloxacin	13	17
	vancomycin	16	19
S. aureus	polymyxin B	8	12
	streptomycin	13	12
	ciprofloxacin	11	12
	vancomycin	17	18
B. cereus	polymyxin B	9	19
	streptomycin	10	17
	ciprofloxacin	13	15
	vancomycin	15	22

Data are inhibition zone diameter (mm)

The synergistic effects of CA on the ciprofloxacin were observed in inhibiting the growth of *S. dysenteriae*. However, CA did not show synergistic effects on other antibiotics in reducing the growth of this pathogen.

CA showed synergistic effects on polymyxin B, streptomycin, ciprofloxacin and vancomycin in inhibiting the growth of *S. typhimurium*. The most synergistic effects of CA were seen on polymyxin B, in the sense that the inhibition zone diameter under antibiotic was 12 mm, and when it was combined with calycosin, this value increased to 22 mm (Table 3).

No synergistic effect was observed between the used antibiotics and CA in inhibiting the growth of *S. aureus*. However, CA has shown to inhibit *B. cereus* strongly when used in combination with the polymyxin B, streptomycin, or vancomycin.

Anti-tumor activity of CA

As can be seen, the viability of cells decreased with an increase in CA concentration and time. A decrease in the viability of HT-29 cells was observed at a concentration of 500 mg/ml and above in 24 hours, and with the passage of time, the rate of cell viability decreased. The lowest viability of HT-29 cell line was seen in 2000 µg/mL CA at 72 hours treatment (Figure 2). Therefore, our results showed that CA had anti-tumor effects on colon cancer cell line.

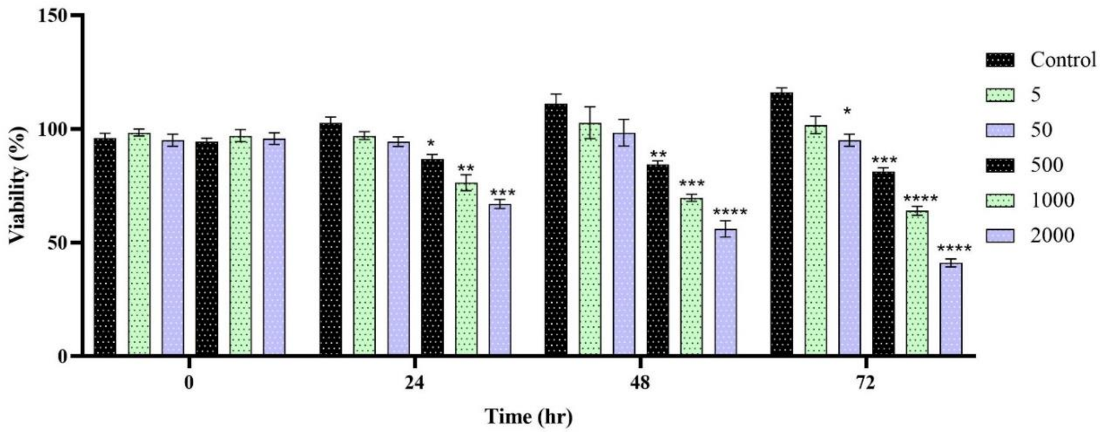

Figure 2. The viability percentage of HT-29 cell line treated with the different concentrations (µg/mL) of calycosin. *, **, *** and **** showed significant differences

4. Discussion

CA has antioxidant activity and its IC_{50} for DPPH free radical scavenging was calculated as 120 µg/ml. Also, CA inhibited *E. coli*, *S. dysenteriae*, *S. typhimurium*, *S. aureus* and *B. cereus*. Nevertheless, CA had strong effects on Gram-positive bacteria such as *S. aureus* and *B. cereus* than Gram-negative ones. Also, the synergistic effects of CA on the streptomycin, polymyxin B, ciprofloxacin and vancomycin antibiotics were also studied and confirmed against the studied bacteria other than *S. aureus*.

As mentioned, calycosin in the present study showed antioxidant effects, which is similar to the findings of other studies. For example, Wang et al., (2014) showed that CA can inhibit DPPH free radicals by its anti-oxidant capacity [24]. Similarly, Toukam et al., (2018) indicated that CA has antioxidant properties by scavenging DPPH [25]. CA is the richest isoflavone that is abundantly found in *Astragali radix*. This molecule has attracted a lot of attention due to its numerous medical functions [26]. CA is a member of the 7-hydroxyisoflavone class that has hydroxy group at the 3 and a methoxy group at the 4 positions. It acts as a metabolite and an antioxidant. This compound with its chemical properties can neutralize reactive oxygen species (ROS) and prevent cell damage [27].

Antibiotic-resistant strains of bacteria have been found in almost all human societies. This has complicated the problems of treating infectious diseases and limited the treatment options. Among the different antibiotics, Polymyxin B and Colistin are considered for this category of antibiotic-resistant infections [28]. MCR-1 is a plasmid-mediated resistance gene that harbors resistance to polymyxins. Therefore, a new strategy against MCR-1-producing resistant bacteria by targeting this resistance enzyme is needed. The results of the present study showed that when polymyxin B antibiotic is used together with calycosin, synergistic effects are observed in inhibiting the growth of *E. coli*, *S. dysenteriae*, *S. typhimurium* and *B. cereus*. This finding is very important because CA can help prevent resistance to antibiotics in these bacteria. In a study, it was shown that calycosin inhibits the growth of gram-negative bacteria resistant to polymyxin B without affecting the expression of MCR-1 protein indicating the restoration of the bactericidal effect of this antibiotic by calycosin [12]. This compound has broad pharmacological effects such as antiviral, anti-inflammatory and antioxidant effects [29]. In the present study, it was shown for the first time that this compound can inhibit the growth of *E. coli*, *S. dysenteriae*, *S. typhimurium*, *S. aureus* and *B. cereus* bacteria; this is one of the novelties of the current research. However, more studies are needed to understand the growth inhibition mechanisms of these bacteria. The hydroxyl phenol functional group is present in CA, and one of the possible reasons for inhibiting the growth of bacteria in the present study can be attributed to the presence of this functional group. Also, the presence of the hydroxy group at the 7 positions of CA can be one of the reasons for its bactericidal activity [30]. Studies have shown that removing this functional group from isoflavone leads to the loss of bactericidal activity [30]. Therefore, there is a strong correlation between the bactericidal structure-activity of calycosin compound.

One of the important results of the current research is confirming the synergistic effects of streptomycin, ciprofloxacin and vancomycin antibiotics with calycosin, which is being reported for the first time. Finding natural compounds with antibacterial properties and verifying their synergistic effects with antibiotics is of great importance, because these compounds can reduce the prescribed dose of antibiotics in a variety of diseases and, therefore, minimize the possibility of antibiotic resistance [31]. The World Health Organization has placed special emphasis on finding natural compounds to replace antibiotics; this research showed that CA has the potential to be used therapeutically in infections caused by *E. coli*, *S. dysenteriae*, *S. typhimurium*, *S. aureus* and *B. cereus* along with antibiotics. However, in vivo studies and clinical trials are needed to confirm the effects of this compound.

We also studied the antitumor effects of calycosin on HT-29 cells. The results indicated the antitumor effects of this compound. Antitumor effects of CA have been reported in some studies. The mechanism of antitumor effects of this compound was attributed to its effect on MAPK and PI3K/Akt pathways [22]. Also, downregulation of c-Met was reported as one of the inhibitory mechanisms of calycosin in glioblastomas [32]. Induction of apoptosis was suggested as the main anti-tumor mechanism of CA in colorectal cancer cell by regulation of ERβ/miR-17 signaling pathway [21]. Therefore, CA with antitumor effects in a variety of malignancies can be considered as an adjuvant cancer treatment option. However, there is a need for clinical studies in this field.

5. Conclusion

In general, it can be concluded that CA has strong potential for antibacterial effects against *E. coli*, *S. dysenteriae*, *S. typhimurium*, *S. aureus* and *B. cereus* bacteria. These antibacterial effects become stronger when combined with streptomycin, polymyxin B, ciprofloxacin, and vancomycin antibiotics leading to synergistic effects. Also, this compound showed anti-tumor effects on HT-29 colon cancer cell line. The current study confirms that calycosin has antibacterial and antitumor effects. Nevertheless, clinical studies in this field are warranted for application in clinical conditions and infections.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Author's contributions

Ebrahim Khademi wrote the draft and conducted data analysis. Behdokht Jamali was responsible for project management and revising the manuscript.

Conflict of interest

There were no conflicts of interest among authors.

Acknowledgments

We are extremely grateful to Dr. Mehrdad Hashmi for his comments and feedback during the study.

References

[1] Krishnaiah D, Sarbatly R, Nithyanandam R. A review of the antioxidant potential of medicinal plant species. Food Bioprod Process. 2011; 89(3):217-33. [DOI: 10.1016/j.fbp.2010.04.008]

[2] Wang D, Hu G, Wang J, Yan D, Wang M, Yang L, et al. Studying CNS effects of Traditional Chinese Medicine using zebrafish models. J Ethnopharmacol. 2021; 267:113383. [DOI: 10.1016/j.jep.2020.113383] [PMID]

[3] Yu D, Duan Y, Bao Y, Wei C, An L. Isoflavonoids from Astragalus mongholicus protect PC12 cells from toxicity induced by l-glutamate. J Ethnopharmacol. 2005; 98(1):89-94. [DOI: 10.1016/j.jep.2004.12.027] [PMID]

[4] Or PMY, Lam FFY, Kwan YW, Cho CH, Lau CP, Yu H, et al. Effects of Radix Astragali and Radix Rehmanniae, the components of an anti-diabetic foot ulcer herbal formula, on metabolism of model CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A4 probe substrates in pooled human liver microsomes and specific CYP isoforms. Phytomedicine. 2012; 19(6):535-44. [DOI: 10.1016/j.phymed.2011.12.005] [PMID]

[5] Zou J, Li W, Wang G, Fang S, Cai J, Wang T, et al. Hepatoprotective effects of Huangqi decoction (Astragali Radix and Glycyrrhizae Radix et

Rhizoma) on cholestatic liver injury in mice: Involvement of alleviating intestinal microbiota dysbiosis. J Ethnopharmacol. 2021; 267:113544. [DOI: 10.1016/j.jep.2020.113544]

[6] Hoo RLC, Wong JYL, Qiao CF, Xu A, Xu HX, Lam KSL. The effective fraction isolated from Radix Astragali alleviates glucose intolerance, insulin resistance and hypertriglyceridemia in db/db diabetic mice through its anti-inflammatory activity. Nutr Metab (Lond). 2010; 7(1):67. [DOI: 10.1186/1743-7075-7-67] [PMID]

[7] Gong AGW, Duan R, Wang HY, Kong XP, Dong TTX, Tsim KWK, et al. Evaluation of the Pharmaceutical Properties and Value of Astragali Radix. Medicines. 2018; 5(2). [DOI: 10.3390/medicines5020046] [PMID]

[8] Huang C, Li R, Shi W, Huang Z. Discovery of the anti-tumor mechanism of calycosin against colorectal cancer by using system pharmacology approach. Med Sci Monit. 2019; 25:5589. [DOI: 10.12659/MSM.918250] [PMID]

[9] Guo C, Tong L, Xi M, Yang H, Dong H, Wen A. Neuroprotective effect of calycosin on cerebral ischemia and reperfusion injury in rats. J Ethnopharmacol. 2012; 144(3):768-74. [DOI: 10.1016/j.jep.2012.09.056] [PMID]

[10] Ma C-H, Wang R-R, Tian R-R, Ye G, Fan M-S, Zheng Y-T, et al. Calycosin 7-O-β-D-glucopyranoside, an anti-HIV agent from the roots of Astragalus membranaceus var. mongholicus. Chem Nat Compd. 2009; 45(2):282-5. [DOI: 10.1007/s10600-009-9305-2]

[11] Fan Y, Wu D-Z, Gong Y-Q, Zhou J-Y, Hu Z-B. Effects of calycosin on the impairment of barrier function induced by hypoxia in human umbilical vein endothelial cells. Eur J Pharmacol. 2003; 481(1):33-40. [DOI: 10.1016/j.ejphar.2003.09.007] [PMID]

[12] Liu X, Sun X, Deng X, Lv X, Wang J. Calycosin enhances the bactericidal efficacy of polymyxin B by inhibiting MCR-1 in vitro. J Appl Microbiol. 2020; 129(3):532-40. [DOI: 10.1111/jam.14635] [PMID]

[13] Griffin AS, West SA, Buckling A. Cooperation and competition in pathogenic bacteria. Nature. 2004; 430(7003):1024-7. [DOI: 10.1038/nature02744] [PMID]

[14] Ogawara H. Comparison of Antibiotic Resistance Mechanisms in Antibiotic-Producing and Pathogenic Bacteria. Molecules. 2019; 24(19):3430. [DOI: 10.3390/molecules24193430] [PMID]

[15] Wright GD. Antibiotic resistance in the environment: a link to the clinic?. Curr Opin Microbiol. 2010; 13(5):589-94. [DOI: 10.1016/j.mib.2010.08.005] [PMID]

[16] Cunha BA. ANTIBIOTIC SIDE EFFECTS. Med Clin North Am. 2001; 85(1):149-85. [DOI: 10.1016/s0025-7125(05)70309-6] [PMID]

[17] Labianca R, Beretta GD, Kildani B, Milesi L, Merlin F, Mosconi S, et al. Colon cancer. Crit Rev Oncol Hematol. 2010; 74(2):106-33. [DOI: 10.1016/j.critrevonc.2010.01.010] [PMID]

[18] Birgisson H, Påhlman L, Gunnarsson U, Glimelius B. Late adverse effects of radiation therapy for rectal cancer–a systematic overview. Acta oncol. 2007; 46(4):504-16. [DOI: 10.1080/02841860701348670] [PMID]

[19] Rejhová A, Opattová A, Čumová A, Slíva D, Vodička P. Natural compounds and combination therapy in colorectal cancer treatment. Eur J Med Chem. 2018; 144:582-94. [DOI: 10.1016/j.ejmech.2017.12.039] [PMID]

[20] Tan J, Qin X, Liu B, Mo H, Wu Z, Yuan Z. Integrative findings indicate anti-tumor biotargets and molecular mechanisms of calycosin against osteosarcoma. Biomed Pharmacother. 2020; 126:110096. [DOI: 10.1016/j.biopha.2020.110096] [PMID]

[21] Chen J, Zhao X, Li X, Wu Y. Calycosin induces apoptosis by the regulation of ERβ/miR-17 signaling pathway in human colorectal cancer cells. Food Funct. 2015; 6(9):3091-7. [DOI: 10.1039/c5fo00374a] [PMID]

[22] Chen J, Hou R, Zhang X, Ye Y, Wang Y, Tian J. Calycosin suppresses breast cancer cell growth via ERβ-dependent regulation of IGF-1R, p38 MAPK and PI3K/Akt pathways. PloS one. 2014; 9(3):e91245. [DOI: 10.1371/journal.pone.0091245] [PMID]

[23] Wiegand I, Hilpert K, Hancock REW. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc. 2008; 3(2):163-75. [DOI: 10.1038/nprot.2007.521] [PMID]

[24] Wang F, Zhao S, Li F, Zhang B, Qu Y, Sun T, et al. Investigation of antioxidant interactions between Radix Astragali and Cimicifuga foetida and identification of synergistic antioxidant compounds. PLoS One. 2014; 9(1):e87221. [DOI: 10.1371/journal.pone.0087221] [PMID]

[25] Toukam PD, Tagatsing MF, Tchokouaha Yamthe

LR, Baishya G, Barua NC, Tchinda AT, et al. Novel saponin and benzofuran isoflavonoid with in vitro anti-inflammatory and free radical scavenging activities from the stem bark of Pterocarpus erinaceus (Poir). Phytochemistry Letters. 2018;28:69-75. [DOI: 10.1016/j.phytol.2018.09.006]

[26] Sun H, Yin M, Qian W, Yin H. Calycosin, a Phytoestrogen Isoflavone, Induces Apoptosis of Estrogen Receptor-Positive MG-63 Osteosarcoma Cells via the Phosphatidylinositol 3-Kinase (PI3K)/AKT/Mammalian Target of Rapamycin (mTOR) Pathway. Med Sci Monit. 2018; 24:6178-86. [DOI: 10.12659/MSM.910201] [PMID]

[27] Mukne AP, Viswanathan V, Phadatare AG. Structure pre-requisites for isoflavones as effective antibacterial agents. Pharmacogn Rev. 2011; 5(9):13-8. [DOI: 10.4103/0973-7847.79095] [PMID]

[28] Bedenić B, Cavrić G, Vranić-Ladavac M, Barišić N, Karčić N, Tot T, et al. COMPARISON OF TWO DIFFERENT METHODS FOR TIGECYCLINE SUSCEPTIBILITY TESTING IN ACINETOBACTER BAUMANNII. Acta Clin Croat. 2018; 57(4):618-23. [DOI: 10.20471/acc.2018.57.04.02] [PMID]

[29] Gao J, Liu ZJ, Chen T, Zhao D. Pharmaceutical properties of calycosin, the major bioactive isoflavonoid in the dry root extract of Radix astragali. Pharm Biol. 2014; 52(9):1217-22. [DOI: 10.3109/13880209.2013.879188] [PMID]

[30] Mahabusarakam W, Deachathai S, Phongpaichit S, Jansakul C, Taylor WC. A benzil and isoflavone derivatives from Derris scandens Benth. Phytochemistry. 2004; 65(8):1185-91. [DOI: 10.1016/j.phytochem.2004.03.006] [PMID]

[31] Hemaiswarya S, Kruthiventi AK, Doble M. Synergism between natural products and antibiotics against infectious diseases. Phytomedicine. 2008; 15(8):639-52. [DOI: 10.1016/j.phymed.2008.06.008] [PMID]

[32] Nie X, Zhou Y, Li X, Xu J, Pan X, Yin R, et al. Calycosin down-regulates c-Met to suppress development of glioblastomas. J Biosci. 2019; 44(4):96. [PMID]