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Short Communication

Phytochemical and biological study of phenolic fraction from Geoffroea decorticans bark

[Estudio fitoquímico y biológico de la fracción fenólica de corteza de Geoffroea decorticans]

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Abstract: The stem bark of Geoffroea decorticans (Gill.ex Hook. et Arn.) Burk. (Fabaceae) was used medicinally to cure several skin affections; however, previous phytochemical and biological antecedents that supported its vulnerary property were not found yet. A purified methanolic fraction from G. decorticans bark (PFGB), was derivatized by silylation for GC/MS analysis, and complementary analyses by two-dimensional TLC and UV-Vis spectroscopy against detected standards compounds. Nine phenolic compounds were characterized as gallic acid, quercetin, kaempferol, apigenin, protocatechuic acid, taxifolin, 4-Hydroxybenzoic acid; among these two methoxy-flavonoids. PFGB showed significant antibacterial activity (MICs = 125 µg/mL) against Staphylococcus aureus (25923) and Enterococcus faecalis (29212) ATCC strains. Cytotoxic effects on human lymphocytes activated or not by LPS were not observed up to 100 µg/mL, by MTT colorimetric assay. The results obtained through the ethnopharmacological approach of this work contribute to the scientific validation of the vulnerary medicinal use of G. decorticans.

Keywords: Geoffroea decorticans bark; Phenolic fraction; Antibacterial; Cytotoxicity; Phytochemical analysis .

Resumen: La corteza de Geoffroea decorticans (Gill.ex Hook. EtArn.) Burk. (Fabaceae) se utiliza para curar diferentes afecciones de la piel, sin embargo, no encontramos antecedentes fitoquímicos y biológicos que validen esta propiedad medicinal conocida como vulneraria. Una fracción purificada de corteza de G. decorticans (PFGB), fue derivatizada por sililación y analizada por CG/EM, complementariamente se analizó por CCF-2D y espectroscopia UV-Vis, contra sustancias estándares detectadas. Se caracterizaron nueve compuestos fenólicos: ácido gálico, quercetina, kaempferol, apigenina, ácido protocatéquico, taxifolin, ácido 4-hidroxibenzoico, 2'-hidroxi-3,4,4',5-tetrametoxi-chalcone and 7,3',4',5'-tetrametoxiflavanone. PFGB evidenció actividad antibacteriana significativa (CIMs = 125 µg/mL) contra las cepas ATCC de Staphylococcus aureus (25923) y Enterococcus faecalis (29212). No se observaron efectos citotóxicos hasta 100 µg/mL sobre linfocitos humanos activados o no por LPS, a través del ensayo colorimétrico con MTT. Los resultados obtenidos del abordaje etnofarmacológico de este trabajo, contribuyen con la validación científica del uso medicinal vulnerario de G. decorticans.

Palabras clave: corteza de Geoffroea decorticans; fracción fenólica; anitibacteriano; citotoxicidad; análisis fitoquímico.

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INTRODUCTION

Geoffroea decorticans (Gill.ex Hook. et Arn.) Burk. (Fabaceae), known as "chañar", is a tree that grows in several regions of Argentina (mainly in the north and center of the country), Bolivia, Southern Peru, Chile, Western Paraguay and Uruguay (Alonso & Desmarchelier, 2015). Traditionally, chañar is used with medicinal purposes as abortifacient, anti-asthmatic, emollient, against snake bites, to treat urinary and respiratory tracts infections, and as vulnerary (treatment for several skin affections) (Barboza et al., 2009; Martinez et al., 2019).

Considering the vulnerary medicinal use of G. decorticans stem bark, widely extended in our region, as well as the phytochemical background of the plant barks, which were rich in phenolic compounds that presented wide interspecific chemical diversity, and moreover are recognized due to their multiple biological activities, the research from traditional use prospect and type of secondary metabolites, could be substantial to know the more relevant biological activities associated with the chemical characterization of its main constituents, which has not been investigated yet in the bark of this species. Accordingly, with all the above, this work researched for the first time the antibacterial activity against species that infect skin wounds, and cytotoxicity on human lymphocytes, of a purified phenolic fraction of G. decorticans stem bark (PFGB), as well as chemical characterization of its components, to validate its traditional usage, and to know the medicinal potential of phenolic phytocomplex studied.

MATERIALS AND METHODS

Chemical and Reagents

Dimethyl sulfoxide (DMSO), n-hexano, methanol and acetone, from Sintorgan Labs. (Buenos Aires, Argentina), N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA), 3-[4.5-dimethylthiazol-2-yl]-2.5-diphenyltetrazolium bromide (MTT), histopaque 1077, phytohemagglutinin (PHA), lipopolysaccharides (LPS), kaempferol, quercetin, apigenin, gallic acid and methoxyamine, from Sigma-Aldrich (Misuri, USA.); penicillin/streptomycin/amphotericin B (100X), phosphate buffered saline (PBS) and Hank's salt (HBSS), from MicroVet Labs. (Buenos Aires, Argentina); pyridine, from Cicarelli Labs. (Santa Fé, Argentina); Ciriax © i.v, from Roemmers Labs. (Buenos Aires, Argentina); Muller Hinton, from Britania Labs. (Buenos Aires, Argentina); fetal bovine serum (FBS), from Natocor Biotechnology (Córdoba, Argentina); RPMI 1640 with L- Glutamine, HEPES and phenol red, from Biological Industries (Cromwell, USA).

Plant Material

Stem bark from Geoffroea decorticans Burk. (Fabaceae), was collected in 9 de Julio Department, Chaco-Argentina (27°12'02.4"S 60°58'52.5"W). The species was taxonomically classified by the biologist Lic. Nora Muruaga from Instituto "Miguel Lillo", Tucumán, Argentina, and the herborized material was deposited in the Herbarium of the same Institute for future reference (voucher number: LIL 612255). Stem barks were cleaned, dried, and stored as powder in caramel colored, filled and tightly closed flask, at -20°C.

Preparation of the phenolic fraction from G. decorticans stem bark

Stem bark grounded powder was washed with n-hexane, dried and extracted by Soxhlet with methanol 100% (16% p/v), for 24 h. The extract was dried by using rotary evaporator at 30°C. The dried material obtained represented the total extract. Phenolic constituents were extracted by gently mixing of total extract (1 g) in acetone (25 mL) at room temperature, and centrifuging the mixture at 2000 g for 30 min to separate the soluble fraction; this procedure was repeated three times, and supernatants collected were pooled and represented the purified phenolic fraction from G. decorticans stem bark (PFGB).

Phytochemical analysis of PFGB

Silylation derivatization and analysis conditions by GC/MS

PFGB (0.4 mg) was derivatized by addition of 80 µL of 20 mg/mL methoxyamine dissolved in anhydrous pyridine. After incubation at 37°C for 90 min, 140 µL of N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) were incorporated, and then incubated at 37°C for 30 min.

GC/MS analysis of silylated sample was carried out in a ThermoElectron TraceGcUltra gas chromatograph coupled to a Polaris Q mass detector. The column used was a DB-5 column (30 m x 0.32 mm inner diameter, 0.25 µm; Agilent, California, USA). Conditions: a Helium flow rate at 1 mL/min; Injector temperature: 280°C; injection volume: 1 µL in

the splitless mode; temperature program: isothermal for 2 min at 45°C, increased at 10°C/min to 150°C, isothermal for 1 min, increased at 10 °C/min to 270°C, and isothermal for 1 min. The mass spectrometer was run in the electron ionization mode (70 eV). Mass spectra were acquired by scanning along the m/z 50-1000 range. Interpretation of MS spectra thorough the NIST MS search software (version 2.2). MS spectra of each detected component were compared with spectra of the NIST library and Mainlib databases. The search was performed with a Minimum match factor of 75 for all peaks.

Two-dimensional TLC analysis

PFGB was analyzed by two-dimensional TLC on plastic plates coated with cellulose (Merck, Germany). The mobile phase were: 1D Forestal (acetic acid- conc. hydrochloric acid- water, 30:3:10, v/v/v); 2D HOAc 15% (v/v). The developed dried plates were revealed under visible and UV light (254 or 365 nm, UV Lamp Model UV 5L-58 Mineralight Lamp) before and after spraying with either 1% methanolic 2-aminoethyl diphenylborate/Polyethylene glycol (NP/PEG) reagents (Wagner & Bladt, 1996). Results were compared with those obtained by a mixture of assumed standards (Kaempferol, Quercetin, Apigenin and Gallic acid).

UV-VIS Spectroscopic analysis

For UV-VIS spectrophotometer analysis, PFGB was dissolved in methanol (0.5 mg/mL), loaded in 1 cm OPL quartz cuvette, and scanned in the wavelength ranging from 250-600 nm using Beckman DU 650 Spectrophotometer. The characteristic peaks were recorded and compared with characteristic λmax reported on specific literature.

Bioactivity assays of PFGB

Antibacterial assays

Antibacterial activity of the PFGB was assayed against Staphylococcus aureus (ATCC 29213 and ATCC 25923), Escherichia coli (ATCC 25922) and Enterococcus faecalis (ATCC 29212), pathogenic species that cause skin and soft tissue infections (Lowy 1998; Rhoads et al., 2012). These strains were cultured on solid Mueller Hinton (MH) medium, from cultures kept at -20°C previously activated at 37°C for 2 h.

Dot-blot bioautography test: According to Sgariglia et al. (2009), briefly, 62.5-750 µg of PFGB were dot-blotting on Silica gel plates (4 x 7 cm), and ciprofloxacin was used as control. 4 mL of semi-solid MH (0.8% agar), molten at 50°C and inoculated with each strain (1 x 106 CFU/mL) were distributed on pre-blotted and labeled plates; these were incubated at 37°C, and after 24 h were sprayed with MTT solution (2.5% p/v) and incubated again for 30 min to observe the inhibition halos around blotted dots with different amounts of sample. Diameters of inhibition halos were measured with caliber in three directions.

Broth microdilution method: This assay was applied to determine the minimal inhibitory concentrations (MIC). Briefly, PFGB dilutions were prepared according to Andrews et al. (2001), and were tested between 100-1000 µg/mL against E. coli ATCC 25922 and E. faecalis ATCC 29212, and 50-1000 µg/mL against two strains of S. aureus (ATCC 29213 and ATCC 25923). Ciprofloxacin was used as positive control. Procedures were performed in according to Sgariglia et al. (2009).

Cytotoxicity assay

Cell Model: primary cultures of human lymphocytes activated or not by LPS (bacterial lipopolysaccharides). Lymphocytes isolation was carried out in according to Noroozi et al. (1998), from fresh whole blood from healthy volunteers in the age range of 25-35 years old by using Histopaque 1077. No adherent peripheral blood lymphocytes (PBL) were separated from adherent cells by aspiration, and number of viable cells was determined by Trypan blue exclusion test, on a hematological counter (Neubauer Chamber), and cultured in complete RPMI 1640 medium (10% FBS, 100 IU/mL of penicillin, 100 ng/mL of streptomycin and 0.25 µg/mL of amphotericin B) during 24 h. Havested lymphocytes were cultured in complete RPMI 1640 medium supplemented with Concanavalin A (4 µg/mL) as mitogen agent, on 5% CO_2 atmosphere at 37°C, during 24 h.

MTT colorimetric assay: PFGB concentrations to be tested (10-100 µg/mL) and aliquots of cell suspension were loaded in 24-well plate (1x105 cells/mL; final volume: 1mL/well). The plate was incubated for 24 h at 37°C and 5% CO_2; after this, contents of each well were transferred to 1.5 mL conical tubes and were centrifuged at 100 g, 5 minutes,

supernatants corresponding to PFGB treated cell cultures and controls, were replaced by a solution of MTT (0.25 mg/mL) in serum-free RPMI 1640 medium. After 3 h of incubation at 37°C and 5% CO_2, these solutions were centrifuged at 900 g, 10 min; the supernatant was removed, and cells were washed with HBSS. At the final, the wash supernatant was replaced by DMSO to dissolve formazan. Absorbances were measured in ELISA reader (Bio-rad. California, USA) at 590 nm. Complete RPMI 1640 medium was used as blank solution.

Statistical analysis

Experiments were performed in triplicate and the results were expressed as mean ± standard deviation (SD). Student's t test was applied as statistical analysis; $\varrho > 0.05$ was considered statistically significant.

RESULTS

Phytochemical analysis of PFGB

GC/MS analysis

Several peaks with distinct mass fragmentation patterns were detected by analysis of the total ion chromatogram (TIC) of silylated PFGB. Comparison of spectral data acquired with MS Libraries (NIST and Mainlib) led to the identification of 5 phenolic compounds: Taxifolin (1); 7,3',4',5'-Tetramethoxyflavanone (2); 2'-Hydroxy-3,4,4',5-tetramethoxychalcone (3); Protocatechuic acid, 3-tert-butyl(dimethyl)silyl (TBDMS) derivative (4) and 4-Hydroxybenzoic acid, 2-trimethylsilyl (TMS) derivative (5), whose fragmentation patterns are listed in Table No. 1.

Table No. 1
Compounds identified from silylated phenolic fraction of G. decorticans stem bark (PFGB) by GC/MS analysis

Peak	Rt, min	Compound	Molecular formula	MW	Reference ions[a] (%BPI)
1	09.61	Taxifolin	$C_{15}H_{12}O_7$	304	231 (100), 149 (66), 241(6)
2	13.26	7,3',4',5'-Tetramethoxyflavanone	$C_{19}H_{20}O_6$	344	149 (100), 133 (31), 148 (20), 162 (18), 121 (12), 159 (8)
3	15.95	2'-Hydroxy-3,4,4',5-tetramethoxychalcone	$C_{19}H_{20}O_6$	344	149 (100), 163 (48), 150 (16), 121 (14), 133 (14), 159 (8)
4	26.23	Protocatechuic acid, 3 TBDMS derivative	$C_{25}H_{48}O_4Si_3$	496	439 (100), 73 (67), 440 (41), 441 (19), 193 (18), 223 (8)
5	26.24	4-Hydroxybenzoic acid, 2TMS derivative	$C_{12}H_{22}O_3Si_2$	282	73 (100), 267 (71), 193 (63), 223 (45), 45 (18), 268 (16), 282, M+ (14), 75 (13), 126 (12), 194 (10)

References: Rt, retention time; MW, molecular weight; [a] Mass spectrometry libraries (NIST, 2014 and Mainlib); M+, molecular ion; BPI, base peak intensity

TLC and UV-VIS spectrum analysis

Gallic acid, quercetin, kaempferol and apigenin standards showed matching with PFGB spots at relation fronts Rf (x 100) 60/50, 38/0, 69/0 and 70/6 respectively, on chromatogram developed with 1D-Forestal/2D-HOAc 15%, sprayed with NP/PEG reagent, and observed under 365 nm UV lamp; Protocatechuic acid was observed as a blue spot at Rf 79/60, which was agreed with data reported by Tanchev and Ioncheva, 1976 and Kowalski and Wolski, 2003. Furthermore, a brown spot at Rf 78-92/0 was consistent with the presence of methoxy-flavonoids (Harborne, 1984; Mabry, 1970), which were detected also by GC/MS (Peaks 2 and 3, Table No. 1).

Characteristic λmax (nm) values (MeOH) detected by UV-Vis spectroscopy, supported besides the presence of compounds such as gallic acid (256, 294), quercetin (270), kaempferol (255, 374), apigenin (266, 368), protocatechuic (267, 336), 2'-Hydroxy-3,4,4',5-tetramethoxychalcone (317), 7,3',4',5'-Tetramethoxyflavanone (270, 295), taxifolin (290) and 4-Hydroxybenzoic acid (272) in PFGB, in agreed with spectral data reported by Mabry et al., 1970 and Harborne, 1984.

Antibacterial activity of PFGB

Inhibition halos (IH, mm) values of Ciprofloxacin on S. aureus (ATCC 29213) and E. coli (ATCC 25922) were agreed

with those reported by CLSI (Patel, 2017) for these strains, validating the experimental procedures carried out. IH were observed against all species assayed by dot-blot bioautography (Table No. 2), indicating the presence of antibacterial compounds. PFGB showed significant activity against S. aureus strains from 72.5 µg (IH 6.0), increasing the IH diameter up to 750 µg (IH 14.5); *E. faecalis* ATCC 29212 was susceptible between 125 and 750 µg (IH: 6.0-9.5); minor activity was observed against *E. coli* ATCC 25922 between 500-750 µg (IH: 8.5-9.0).

Table No. 2
Antibacterial activity of the phenolic fraction of G. decorticans stem bark (PFGB)

	IH, mm from Dot-blot bioautography[a]						MIC From Broth Microdilution[b]
PFGB [µg]	**62.5**	**93.75**	**125**	**250**	**500**	**750**	**PFGB µg/mL**
Microorganism							
S. aureus (ATCC 29213)	**6.0±0.7**	**6.5±0.5**	**8.5±0.4**	**11.0±0.8**	**/**	**/**	**250**
S. aureus (ATCC 25923)	**/**	**7.5±0.3**	**9.2±0.2**	**11.7±0.3**	**13.0±0.1**	**14.5±0.2**	**125**
E. faecalis (ATCC 29212)	**-**	**-**	**6.0±0.2**	**6.2 ±0.2**	**8.5±0.3**	**9.5±0.2**	**125**
E. coli (ATCC 25922)	**-**	**-**	**-**	**-**	**8.5±0.2**	**9.0±0.1**	**-**

References: IH: inhibition halo; (/): untested concentrations; (-): no activity was observed at the amounts tested; Inoculum [a]: 1x10^6 CFU/mL; [b]: 1x10^5 CFU/mL

Analysis of cytotoxicity

PFGB did not evidence cytotoxic effects up to 100 µg/mL, in according to the metabolic activity measured by using MTT. The cell viability obtained was greater than 75%, limit tolerated for human lymphocytes (Figure No. 1). Significant differences between non-activated and LPS-activated cells were not observed under the assayed conditions.

DISCUSSION

When considering as starting study perspective the type of secondary metabolite related to the plant part (stem bark), that was associated to the medicinal property (vulnerary), the extraction methods focused on obtaining phenolic components. This study demonstrated that PFGB is capable of inhibiting the growth of bacterial strains involved in skin infections; previous studies demonstrated that certain phenolic compounds such as apigenin, quercetin, gallic acid and protocatechuic acid, that were isolated from others species, showed antibacterial activity against S. aureus ATCC 25923 and 29213, and E. faecalis ATCC 29212 (Özçelik et al., 2011; Alves et al., 2013; Wang et al., 2019); therefore, presence of such compounds in PFGB would justified partially the activity observed. Information available about structure–activity relationships and mechanisms of antibacterial action of flavonoid compounds indicated that hydroxylation at position 5 and 7 of the A ring of kaempferol, quercetin and taxifolin are important on this activity; in addition, hydroxylation on the B and C rings increased the antimicrobial activity of these compounds (Woźnicka et al., 2013); taking into account that apigenin possess the structural characteristics detailed, could be the main responsible for the antibacterial activity observed, without avoiding the contribution of other constituents (Farhadi et al., 2018).

On the other hand, cytotoxic effects of PFGB up to 100 µg/mL were not observed; apigenin, kaempferol, quercetin and taxifolin showed cytotoxic activity on tumor and normal cell lines, when were individually evaluated (Mori et al., 1988; Matsuo et al., 2005; Li et al., 2008); therefore, our results suggested that these bioactive compounds could be safer for its application into PFGB phytocomplex.

Flavones, flavonols and methoxylated flavonoids proved to have anti-inflammatory activities, which would contribute to explain the vulnerary property of G. decorticans (Jofre et al., 2017), therefore it would be interesting to delve into the

chemical identification of the methoxyflavonoids detected in PFGB and determine its effects on inflammatory targets, since due to the lipophilic characteristics, these compounds would be more efficient crossing biological membranes, and exerting their effects at the intracellular level.

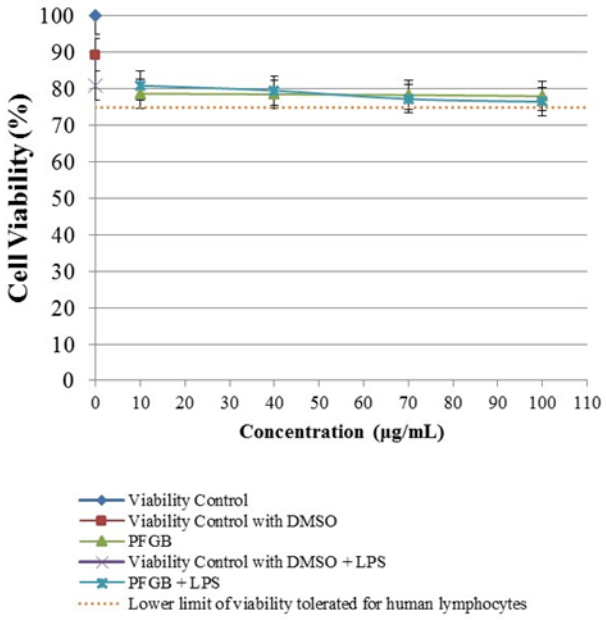

Figure No. 1
Cytotoxicity of phenolic fraction of G. decorticans stem bark (PFGB) determined by MTT colorimetric assay on non-activated and LPS-activated human lymphocytes. Cells were treated with PFGB at different concentrations (10–100 µg/mL) for 24 h. The results were expressed as % viability (p<0.05).

CONCLUSIONS

This work report, for the first time, the phytochemical composition of a phenolic fraction from G. decorticans stem bark, related to their antibacterial and cytotoxicity. G. decorticans stem bark study contributed to the scientific validation of the traditional use of this species as vulnerary, exposed potential applications of the phenolic phytocomplex obtained from this, and put forward challenges to continue advancing in the ethnopharmacological knowledge of native species of Latin America and in the discovery of bioactive metabolites.

REFERENCES

Alonso J, Desmarchelier C. 2015. Plantas medicinales autóctonas de la Argentina. Corpus, Ciudad Autónoma de Buenos Aires, Argentina.

Alves M, Ferreira I, Froufe H, Abreu R, Martins A. 2013. Pintado M. Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. **J Appl Microbiol** 115: 346-357. https://doi.org/10.1111/jam.12196

Barboza GE, Cantero JJ, Núñez C, Pacciaroni A, Ariza Espinar L. 2009. Medicinal plants: A general review and a phytochemical and ethnopharmacological screening of the native Argentine Flora. **Kurtziana** 34: 7-365.

Farhadi F, Khameneh B, Iranshahi M, Iranshahy M. 2018. Antibacterial activity of flavonoids and their structure–activity relationship: An update review. **Phytother Res** 1-28. https://doi.org/10.1002/ptr.6208

Harborne J. 1984. Phytochemical methods: A guide to modern techniques of plant analysis. Chapman and Hall Ltd, London, England.

Jofré M, Fusco M, Favier L et al. 2017. Anti-inflammatory activity and antioxidant potential of aqueous extracts from stem bark of Geoffroea decorticans. **J Pharm Pharmacol** 5: 703-707. https://doi.org/10.17265/2328-2150/2017.10.001

Kowalski R, Wolski T. 2003. TLC and HPLC analysis of the phenolic acids in Silphium perfoliatum L. Leaves,

inflorescences and rhizomes. **J Planar Chromatogr Mod TLC** 16: 230-236. **https://doi.org/10.1556/jpc.16.2003.3.12**

Li N, Liu J, Zhang J, Yu B. 2008. Comparative evaluation of cytotoxicity and antioxidative activity of 20 flavonoids. **J Agric Food Chem** 56: 3876-3883. **https://doi.org/10.1021/jf073520n**

Lowy F. 1998. Staphylococcus aureus Infections. **N Engl J Med** 339: 520-532. **https://doi.org/10.1056/NEJM199808203390806**

Mabry T, Markham K, Thomas M. 1970. **Systematic identification of flavonoids**. Springer-Verlag, New York, USA.

Martinez JL, Muñoz-Acevedo A, Rai M. 2019. **Ethnobotany local knowledge and traditions**. CRC Press, Boca Raton, USA.

Matsuo M, Sasaki N, Saga K, Kaneko T. 2005. Cytotoxicity of flavonoids toward cultured normal human cells. **Biol Pharm Bull** 28: 253-259. **https://doi.org/10.1248/bpb.28.253**

Mori A, Nishino C, Enoki N, Tawata S. 1988. Cytotoxicity of plant flavonoids against HeLa cells. **Phytochemistry** 27: 1017-1020. **https://doi.org/10.1016/0031-9422(88)80264-4**

Noroozi M, Angerson W, Lean M. 1998. Effects of flavonoids and vitamin C on oxidative DNA damage to human lymphocytes. **Am J Clin Nutr** 67: 1210-1218. **https://doi.org/10.1093/ajcn/67.6.1210**

Özçelik B, Kartal M, Orhan I. 2011. Cytotoxicity, antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolic acids. **Pharm Biol** 49: 396-402. **https://doi.org/10.3109/13880209.2010.519390**

Patel J. 2017. **Performance standards for antimicrobial susceptibility testing**. CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, USA.

Rhoads D, Wolcott R, Sun Y, Dowd S. 2012. Comparison of culture and molecular identification of bacteria in chronic wounds. **Int J Mol Sci** 13: 2535-2550. **https://doi.org/10.3390/ijms13032535**

Sgariglia M, Soberón J, Sampietro D, Quiroga E, Vattuone M. 2009. **Bioassays on microorganisms: antifungal and antibacterial activities**. In: Sampietro D, Catalan C, Vattuone M (ed). Isolation, identification and characterization of allelochemicals/natural products. Science Publishers, Enfield, UK.

Tanchev S, Ioncheva N. 1976. Products of thermal degradation of the anthocyanins cyanidin-3-glucoside, cyanidin-3-rutinoside and cyanidin-3-sophoroside. **Food / Nahrung** 20: 889-893.

Wagner H, Bladt S. 1996. **Plant drug analysis: A thin layer chromatography atlas**. Springer, Berlin, Germany.

Wang M, Firrman J, Liu L, Yam K. 2019. A review on flavonoid apigenin: dietary intake, ADME, antimicrobial effects, and interactions with human gut microbiota. **Biomed Res Int** 2019: 1-18. **https://doi.org/10.1155/2019/7010467**

Woźnicka E, Kuźniar A, Nowak D, Nykiel E, Kopacz M, Gruszecka J, Golec K. 2013. Comparative study on the antibacterial activity of some flavonoids and their sulfonic derivatives. **Acta Pol Pharm** 70: 567-571.