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Biologically active substances with antioxidant activity isolated from the medicinal plant *Galega officinalis* L.

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Galega officinalis L. is a perennial herbaceous plant of the *Fabaceae* family, used in official medicine of Bulgaria, United Kingdom and United States for the treatment of mild forms of diabetes mellitus. Preliminary analysis shows that the plant parts contain a wide spectrum of bio-antioxidants: alkaloids, flavonoids, tannins, phenyl carboxylic acids, saponins, fatty acids, phytoestrogens and other substances. A significant part of these substances exhibit antioxidant activity. The scientific information for 13 biologically active substances with antioxidant activity isolated from *Galega officinalis L*. is presented in the review.

Keywords: Galega officinalis, antioxidant activity, diabetes mellitus

INTRODUCTION

Herbs and their plant extracts have been used for thousands of years by mankind for treatment. Plants are an inexhaustible source of diverse biologically active compounds with a potential therapeutic and/or prophylactic potential. Their detailed study and research in order to discover and reveal their main biological effects and molecular mechanisms of action are very important and lead to clarification of indications, dosage and mode of application of the medicinal plants, as well as to the discovery of new effective medical products.

Galega officinalis L. is a perennial herbaceous plant of the Fabaceae family, growing in the wetlands of Central Europe, South and West Asia, Italy, Bulgaria, Southern and Eastern Europe [1]. In folk medicine, the plant has long been used as a diaphoretic and anthelmintic means, in mild forms of diabetes and to improve lactation in nursing mothers. It is known that medication preparations improve the functioning of the heart, lower the blood pressure and help in the treatment of throat diseases and obesity [2]. In Bulgaria, the United Kingdom and the United States it is used in formal medicine to treat mild forms of diabetes [2, 3]. It is known that the above-the-ground part of the plant contains alkaloids, flavonoids (kaempferol, quercetin, etc.), tannins, phenylcarboxylic acids, saponins, fatty acids (eg, α-linolenic acid, palmitic acid and linoleic acid), phytoestrogens and vitamins [1-4]. The following biological activities are also described: hypoglycemic, lactogogic, diaphoretic and antihelmintic actions on blood clotting, anticholinesterase activity, antibacterial, anti-inflammatory and antioxidant activities [1-6]. Various extracts of *Galega officinalis L*. have been tested for antioxidant activity [6, 7] but the answer to the question: "What is this antioxidant activity due to?" remains unclear. For the research presented in our study here, the aim we set before ourselves is the following: on the basis of the available scientific information, to identify and describe the biologically active compounds with proven antioxidant activity that have been isolated so far from *Galega officinalis L.* or its extracts.

MATERIALS AND METHODS

For each of the previously described biologically active compounds isolated from the plant *Galega officinalis L* and its extracts, a research was performed of the scientific literature about the presence of antioxidant properties. As electronic sources of information, we mainly used PubMed, Google Scholar and eLIBRARY.RU. The search was conducted using combinations of the keywords, which exclusively included the chemical name of the compound (including synonymous names) and the words "antioxidant", "antiradical" and "oxygen free radicals" in Bulgarian, English and Russian.

RESULTS AND DISCUSSION

As a result of the study we carried out, we found out and identified 13 biologically active substances with antioxidant activity isolated from the medicinal plant *Galega officinalis L.* These compounds are: vasicine (peganine), vasicinone, luteolin, luteolin 5-glucoside, quercetin, rutin, isorhamnetin, kaempferol, caffeic acid, chlorogenic acid, ferulic acid, ascorbic acid and phytol.

Vasicine (peganine)

Vasicine (peganine**,** Fig 1a) is a quinazoline alkaloid that is isolated from the following plants:

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Galega officinalis L. (0.05-0.1%), *Peganum harmala* (0.1-1.0%) and *Adhatoda vasica* (0.7-3.0%) [1]. Through gas chromatographic and mass spectrometric analysis it was revealed that the total content of vasicine in the *Galega officinalis L.* plant at its various growth and development phases varied from 0.1% to 0.35% [21].

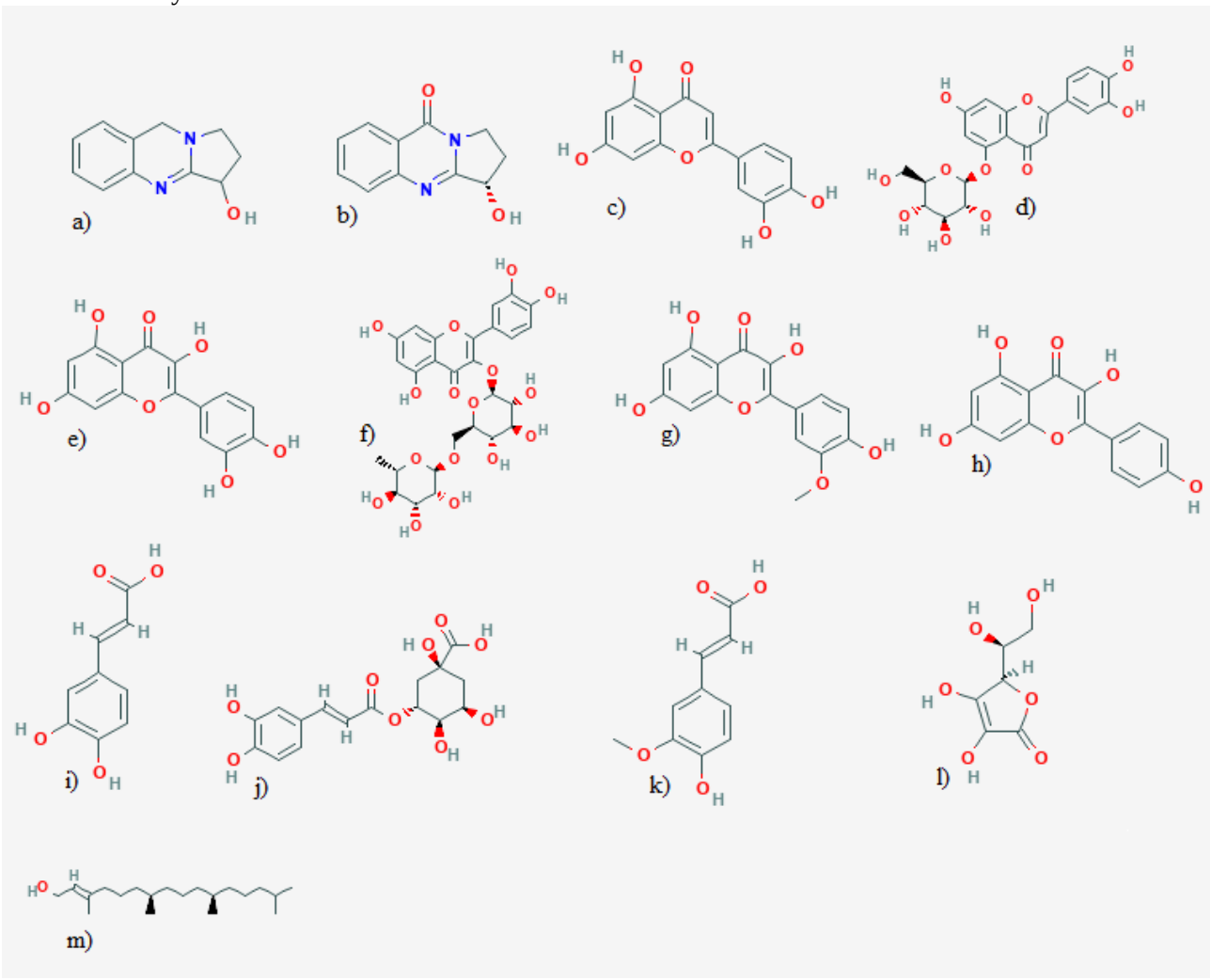

Fig. 1. 2D molecular structures of the 13 biological substances: a) vasicine (paganine)[8]; b) vasicinone [9]; c) luteolin [10]; d) luteolin 5-glucosid [11]; e) quercetin [12]; f) rutin [13]; g) isorhamnetin [14]; h) kaempferol [15]; i) caffeic acid [16]; j) chlorogenic acid [17]; k) ferulic acid [18]; l) ascorbic acid [19]; m) phytol [20].

Vasicine is a light-sensitive substance and when exposed to light, it is auto-oxidized to vasicinone [22]. Vasicine exhibits antioxidant properties in both *in vitro* and *in vivo* experiments. In *in vitro* experiments with human lung alveolar epithelial (A549) and human macrophage (THP-1) cell lines, the pretreatment with vasicine results in a reduction of $\bullet O_2^-$ and •NO radical production induced by bidi smoke concentrate, as well as to retention to a near normal (control) level of superoxide dismutase and catalase activity [23,24]. The vascine isolated from ethanolic extract of *Adhatoda vasica Nees (Acanthaceae)* and applied *in vitro* models exhibits a significant antioxidant activity as inferred by the concentration-dependent increase in the reduction of Fe^{3+} in the ferric reducing antioxidant power (FRAP) assay and significant scavenging of free radicals in DPPH radical scavenging assay [25]. The isolated alkaloid vasicine from *Justicia adhatoda* exhibits strong antioxidant activity with very low IC_{50} values in all antioxidant activity studies, such as deoxyribose degradation assay, ABTS radical cation decolouration assay and plasmid DNA protection assay [26]. *In vivo* experiments with a murine model of asthma, the administration of vasicine isolated from the leaves of *Adhatoda vasica* significantly reduced the level of lipid peroxidation products in tissue samples from rat lungs previously treated with ovalbumin and aluminum hydroxide. The use of vasicine in these animals also leads to a significant decrease in the activity of enzyme antioxidants, such as superoxide dismutase, catalase, glutathione peroxidase and reduced glutathione [27]. The following other pharmacological activities of vasicine are described in the scientific literature:

bronchodilatory, thrombopoeitic, anti-histamine, hypotensive, uterotonic activity and initiated rhythm movements of uterus [27], antimutagenic and anticarcinogenic potential [26], etc.

Vasicinone

Vasicinone (Fig.1b) is a quinazoline alkaloid and is a basic metabolite of vasicine [28]. It exerts antitussive, expectorant, bronchodilating, bronchodilatory, cardiac-stimulating, anti-anaphylactic, anti-inflammatory, antimicrobial, antiasthmatic, hepatoprotective and moderate anticancer activities [29-31]. It is also reported to have antioxidant activity in nitric oxide and ABTS radical scavenging assays [32]. In tests with A549 lung carcinoma cells, treatment with vasicinone lowers the reactive oxygen species (ROS) level and has potential free radical scavenging (DPPH, Hydroxyl) activity and ferric reducing power in cell free systems [28]. In *Galega officinalis* of the Lydia variety, the concentration of vasoninone in the above-the-ground part of the plant is 1.77mg/dm^3 [2].

Luteolin

Luteolin (Fig. 1c) is a naturally occurring flavonoid with potential antioxidant, anti-inflammatory, apoptosis-inducing and chemopreventive activities. Upon administration, luteolin scavenges free radicals, protects cells from reactive oxygen species (ROS)-induced damage [33]. The antioxidant activity of luteolin and its glycosides is associated not only with their ability to scavenge reactive oxygen and nitrogen species but also with their ability to chelate transition metals that may induce oxidative damage through the Fenton reaction, as well as inhibition of prooxidant enzymes and with the induction of antioxidant enzymes. The antioxidant activity of luteolin was studied also in *in vitro* and *in vivo* model systems [34, 35]. The amount of luteolin in methanol extract from herba *Galegae* reaches 1.7884 ± 0.0100 μg/g of dry extract [4].

Luteolin 5-glucosid

Luteolin 5-glucosid Fig.1d), isolated from the herb *Picrorhiza kurroa*, exhibits almost similar antioxidant activity as the ascorbic acid [36]. It has been found out that luteolin 5-O-glucoside inhibits lipopolysaccharide-induced nitric oxide production and tert-butyl hydroperoxide (t-BHP)-induced reactive oxygen species (ROS) generation in RAW 264.7 murine macrophage cells [37]. Luteolin 5-glucoside is isolated from the seeds of *Galega officinalis L.* [1].

Quercetin

Quercetin (Fig.1e) is a typical flavonol-type flavonoid. It possesses all the structural elements characteristic of an antioxidant: (1) an ortho-dihydroxy or catechol group in ring B, (2) and a 2, 3-double bond, and (3) the 3- and 5-OH groups with the 4-oxo group [38]. It was found to be a potent antioxidant *in vitro* and one of the most powerful scavengers of reactive oxygen species such as $\bullet O_2^-$, NO• and $ONOO^-$ [39]. It protects against free radical damage through several different pathways, exerting: direct radical scavenging action, inducible nitric oxide synthase inhibitory action, xanthine oxidase inhibitory action, membrane stabilizing action; it directly inhibits lipid peroxidation and binds chelate transition metals, such as iron and other metals that can initiate the formation of free oxygen radicals which can initiate the formation of oxygen free radicals [40]. The amount of quercetin in methanol extract from wild-grown leaves of *Galega Officinalis L* is 2,0664 ± 0,0558 μg/g of dry extract [4]. It has been identified as a biologically active substance in extracts of *Galega officinalis*, as well as of others [3].

Rutin

Rutin (Fig.1f) is a bioflavonoid compound and is a glycoside derivative of quercetin [41]. Rutin has antioxidant, anti-inflammatory, cardiovascular, neuro-defensive, anti-diabetic and anti-cancer activity. It has been found to exhibit antioxidant properties, participating in various protective mechanisms *in vitro* and *in vivo* models. It directly scavenge ROS. It increases the production of GSH and cellular oxidative defense systems are believed to be upregulated by an increased expression of numerous antioxidant enzymes, such as CAT and SOD. It also inhibits xanthine oxidase, which is involved in the generation of ROS [42].The measured amount of rutin in the leaf of *Galega officinalis L.* is 37.7 ± 3.7 mg% [43]. Rutin is also identified to be a biologically active substance in *Galega officinalis* extract by another research team [3].

Isorhamnetin

Isorhamnetin (Fig.1g) is a natural organic compound belonging to the flavonoid group and is a 3'-O-methylated metabolite of quercetin [44]. The following pharmacological properties of isoramnetine are described: antimicrobial, antioxidant, anticancer, neurological, cardiovascular, hepatoprotective, anti-inflammatory and anti-obesity effects [45]. Its amount in *Galega Officinalis L* wild-leaves extract is 4.0190 ± 0.0471 μg/g of dry extract, whereas in the methanol extract of in vitro-grown leaves it is 0.0558 ± .0.0022 μg /g of dry extract [4].

Kaempferol

Kaempferol (Fig 1h) is a natural flavonoid. It is present in many edible plants that are used for human food, as well as in many medicinal plants [46]. Its amount in wild-grown leaves of *Galega officinalis L*. extract is 0.0287 ± 0.0017 μg/g of dry extract, whereas in a methanolic extract of in vitro-grown leaves it is 1.2319 ± 0.0142 μg/g of dry extract [4]. Numerous studies have shown that kaempferol and some caempherol glycosides of kaempferol have antioxidant activity not only *in vitro* but also *in vivo*. Kaempferol has been shown to be a powerful potent scavenger for superoxide anion, hydroxyl radical and peroxinitrite at submicromolar concentrations. It also inhibits the activity of enzymes that generate ROS, such as the enzyme xanthine oxidase. It reduces the formation of the hydroxyl radical through the Fenton's reaction by chelating ferrous or cuprous ions. It may increase the expression or activity of antioxidant enzymes, such as superoxide dismutase, catalase and heme oxygenase-1. It also prevents lipid peroxidation, this activity being greater than the activity performed by its glycosides [47].

Caffeic acid

Caffeic acid (3,4-dihydroxycinnamic acid, Fig1i) is a hydrophobic phenolic compound and a phenylpropanoid derived biosynthetically from phenylalanine. It belongs to the group of hydroxycinnamates. It is isolated from a variety of plants, such as thyme, oregano, sage, strawberry, apple, coffee, potatoes and so forth [48,49]. Its amount in methanol extract of wild-grown leaves of *Galega officinalis L.*, is 2.0488 ± 0.0006 μg/g of dry extract. [4]. Caffeic acid has aslo been identified as a biologically active substance in extracts of *Galega officinalis* by yet another research team [3]. Caffeic acid exhibits a variety of biological activities, the major among which is its high antioxidant activity. It is described that it can react with free radicals more effectively than Trolox®, a water-soluble vitamin E analog [48]. It also performs anticancer, anti-AIDS and anti-inflammatory activities [50].

Chlorogenic acid

Chlorogenic acid (Fig 1j) is a caffeic acid ester and one of the stereoisomers of the quinic acid. It is widespread in nature, the largest amount being contained in coffee beans, sunflower seeds, the leaves of bilberries and white poplar leaves, as well as chicory roots [51]. Its amount in the wild-grown leaves extract of *Galega Officinalis L.*, established through using the LC-ESI-MS/MS method, is 2.1316 ± 0.1168 μg/g of dry extract, whereas in a methane extract from in vitro-grown leaves it is 4.2206 ± 0.0075 μg/g of dry extract [4]. Chlorogenic acid has strong antioxidant, antiviral, antibacterial and antifungal properties. Hypoglycemic, hypocholesterolemic, anticancer and hepatoprotective actions have also been identified. It also exhibits prebiotic properties [51].

Ferulic acid

Ferulic acid (Fig. 1k) is a representative of phenyl propanoids [52]. In plants, ferulic acid is formed by the metabolism of the phenolic amino acids phenylalanine and tyrosine [53]. The trans-isomeric form is present in plants at 70%. Ferulic acid is found in the following plants: rice (0.9%), wheat (0.66%), barley (0.14%), citrus fruits, vegetables, fruits. In corn bran, its content reaches 3.1% of the dry matter [52]. Its amount in *Galega Officinalis L*. leaf extract of methanol has been established through the LC-ESI-MS/MS method to be 6.8120 ± 0.0149 μg/g of dry extract, whereas in methanol extract of in vitro-grown leaves it is 0.6275 ± .0.0028 μg/g of dry extract [4]. It has also been identified to be a biologically active substance in extracts from *Galega officinalis* by another research team [3]. Ferulic acid exerts a wide range of therapeutic properties, such as antiallergic, anti-aggregative, anti-tumor, antitoxic, antibacterial, anti-viral, antiatherogenic, antidiabetic, antiaging, neuroprotective, radioprotective and hepatoprotective effects. Many of these properties are due to its strong antioxidant capacity. The mechanism of the anti-oxidant action of ferulic acid is accomplished by the interaction of its hydroxyl group with free organic radicals or active forms of oxygen as a result of which a stable phenoxy radical is formed and the chain reaction is interrupted by formation of complexes with the free radicals, as well as of ferulic acid dimers (curcumins). It should be noted that in spite of the stabilization of the free radicals, ferulic acid does not exert a pro-oxidant effect and from this point of view it has an advantage over some traditionally known antioxidants, for example, the ascorbic acid [52,54].

Ascorbic acid

Ascorbic acid (AA, vitamin C) is a water-soluble ketolactone with two ionizable hydroxyl groups [55] (Fig 1l). It is a water-soluble organic compound synthesized in plants, amphibians, reptiles, birds, and in some mammals. Exceptions from the last list are the primates, the Guinea pig and the human species which have lost their ability to synthesize it in the course of evolution [56]. In plants, ascorbic acid is contained in high levels in all parts of the plants, especially in the chloroplasts, in which it reaches concentrations of up to 20 mM

[57]. In the sort of the *Galega officinalis* called Lydia, the concentration of ascorbic acid in the above-ground part of the plant is 14.0 ± 0.6 mg/100g [2]. AL-ascorbic acid is a water-soluble vitamin involved in a wide range of biochemical reactions in the cells and tissues of most living organisms. It is necessary for normal growth and development [57]. AA acts as a cofactor for at least eight enzymes involved in the biosynthesis of collagen and carnitine, the conversion of neurotransmitter dopamine to noradrenaline, the metabolism of tyrosine, amidation of peptide hormones [58], etc . Its antioxidant properties have been well documented and described. It is a powerful reducing agent and scavenger of free radicals in biological systems by working efficiently inside and outside the cells. It participates in the first line of antioxidant protection, protecting lipid membranes and proteins from oxidative damage. Vitamin C releases electrons to free radicals and reduces their reactivity. Vitamin C can donate electrons to free radicals and quench their reactivity. It has been found that vitamin C is an effective scavenger against oxygen and nitrogen oxide species, superoxide radical ion, hydrogen peroxide, the hydroxyl radical, and singlet oxygen. It has also been found to be a cofactor for enzymes involved in the regulation of photosynthesis, hormone biosynthesis and the regeneration of other antioxidants. However, in the presence of redox-active ions (iron, copper), vitamin C may act as a pro-oxidant contributing to the formation of hydroxyl radicals that can trigger dangerous radical reactions [57,59]. The occurrence of ascorbic acid of anti- or pro-oxidant properties depends on the substrate concentration and the conditions under which the oxidation reaction takes place [59].

Phytol

Phytol (3,7,11,15-tetramethylhexadec-2-en-1-ol) is diterpenes and is a member of the group of branched-chain unsaturated alcohols (Fig. 1m) [60]. As an acyclic diterpene, it is widely distributed in plants as a fragment of chlorophyll and chylophinone (vitamin K1) [61]. In the *Galega officinalis Lydia* variety, the concentration of phytol in the above-ground plant is 3.24 mg/dm3 [2]. Phytol was also identified as a biologically active substance in extracts of *Galega officinalis* by another research team [62].

In *in vitro* conditions it has been found out that phytol has a strong antioxidant effect by removing hydroxyl radicals and nitric oxide, as well as preventing the formation of thiobarbituric acid reactive substances (TBARS) [60]. In another study, it was found to exhibit more promising activity against carbon dioxide anion radical ($\bullet CO_2^-$), methoxy radical ($\bullet CH_2OH$) and 2,2-diphenyl-1-picrylhydrazyl (•DPPH) radicals)[63]. In *in vivo* experiments used to to test the antioxidant activity with the *Saccharomyces cerevisiae* test, the phytol exhibits prominent protective effects [64]. Its intraperitoneal application results in a decrease in lipid peroxidation and nitrite (NO_2^-) contents in mouse hippocampus and in increased Swiss glutathione; reduced glutathione and the activity of superoxide dismutase and catalase [64]. In *in vivo* experiments with Wistar rats, a protective role was established of phytol applied per oralno against oxidative stress damage and inflammation of the kidney tissue caused by hyperglycemia in diabetes mellitus [65].

CONCLUSIONS

The substances described above can explain the antioxidant and anti-radical action of plant extracts of Galega officinalis L.

The *Galega officinalis* L plant is a promising source of biologically active substances with a wide range of properties useful in medical practice requiring profound experimental and clinical research.

REFERENCES

1. A. T. Atanasov, PhD Thesis, Trakia Univ., Stara Zagora, 2005
2. A. E. Paliy, I. E Logvinenko, L. A. Logvinenko, O. A. Grebennikova, B. A. Vinogradov, *Proceedings of the Nikitsky Botanical Garden*, **113**, 152 (2011).
3. O. Z. Barchuk, R. M. Lysiuk, A. I. Denys, O. M. Zalaska, O. S. Smalyuh, M. I. Nester, *The Pharma Innovation Journal*, , **6**, 393 (2017).
4. F. P. Karakas, G. Sahin, A. Turker, *Turk J Biol*, **40**, 1311, 2016.
5. K. Pundarikakshudu, J. K. Patel, M. S. Bodar, S. G. Deans, *Journal of Ethnopharmacology*, **77,** 111, (2001).
6. F. P. Karakas, A. U. Turker, A. Karakas, V. Mshvildadze, *Trop J Pharm Res*,; **15**, 751 (2016).
7. Y. Kiselova, D. Ivanova, G. Galunska, T. Chervenkov, D. Gerova, I. Yankova, *Bulletin of the Medical Institute after Mehrabyan*, **1**, 78 (2006).
8. https://pubchem.ncbi.nlm.nih.gov/compound/72610
9. https://pubchem.ncbi.nlm.nih.gov/image/fl.html?cid=442935
10. https://pubchem.ncbi.nlm.nih.gov/image/fl.html?cid=5280445
11. https://pubchem.ncbi.nlm.nih.gov/image/fl.html?cid=5317471
12. https://pubchem.ncbi.nlm.nih.gov/image/fl.html?cid=5280343
13. https://pubchem.ncbi.nlm.nih.gov/image/fl.html?cid=5280805
14. https://pubchem.ncbi.nlm.nih.gov/image/fl.html?cid=5281654

15. https://pubchem.ncbi.nlm.nih.gov/image/fl.html?cid=5280863
16. https://pubchem.ncbi.nlm.nih.gov/image/fl.html?cid=689043
17. https://pubchem.ncbi.nlm.nih.gov/image/fl.html?cid=1794427
18. https://pubchem.ncbi.nlm.nih.gov/compound/445858
19. https://pubchem.ncbi.nlm.nih.gov/image/fl.html?cid=54670067
20. https://pubchem.ncbi.nlm.nih.gov/image/fl.html?cid=5280435
21. I. Laakso, P. Virkajavi, H. Airaksinen, E. Varus ,*J. Chromatogr.*, **505**, 424 (1990).
22. M. D. Subramanya, S. R. Pai, G. M. Ankad, H. V. Hegde, S. Roy, S. L. Hoti, *AYU*, **37**, 135 (2016).
23. M. Pant, S. Basu, Rachana, *Indo Global Journal of Pharmaceutical Sciences*, **5**, 99, (2015).
24. M. Pant, PhD Thesis, JIIT, Noida, India, 2016.
25. D. Shahwar, M. A. Raza, S. Tariq, M. Riasat, M. Ajaib, *Pak. J. Pharm. Sci.*, **25**, 651 (2012).
26. A. Kaur, D. Katoch, B. Singh, S. Arora, *J Global Biosci*, **5**, 3836 (2016).
27. D. Srinivasarao, I. A. Jayarraj, R. Jayraaj, M. L. Prabha, *Indian J. Allergy Asthma Immunol.*, 20, 1 (2006).
28. T. Dey, P. Dutta, P. Manna, J. Kalita, H. P. D. Boruah, A. K. Buragohain, B. Unni, *Biomol Ther* **26**, 409 (2018).
29. J. Panigrahi, S. Gantait, I. C. Patel, *3 Biotec*h, **7**,280 (2017).
30. W. Liu, Y. Wang, D. D. He, S. P. Li, Y. D. Zhu, B. Jiang, X. M. Cheng, Z. Wang, C. H. Wang, *Phytomedicine*, **22**, 1088 (2015).
31. A.K. Qazi, A. Hussain, S. Khan, M. A. Aga, A. Behl, S. Ali, S. K. Singh, S. C. Taneja, B. A. Shah, A. K. Saxena, D. M. Mondhe, A. Hamid, *Cancer Lett.*, **359**, 47 (2015).
32. https://www.medkoo.com/products/24629
33. https://pubchem.ncbi.nlm.nih.gov/compound/5280445#section=Top
34. M. Lopez-Lazaro, *Mini Rev Med Chem.* **9**, 31 (2009).
35. Y. Lin, R. Shi, X. Wang, H. M. Shen, *Curr Cancer Drug Targets*, **8**, 634 (2008).
36. K. Kant, M. Walia, V. K. Agnihotri, V. Pathania, B. Singh, *Indian J Pharm Sci.* **75**, 324 (2013).
37. H. A. Jung, A. Roy, Q. A. Abdul, H. R. Kim, H. J. Park, J. S. Choi, *Nat Prod Sci*. **23**, 183 (2017).
38. C. Chen, J. Zhou, C. Ji, *Life Sci.* **87**, 333 (2010).
39. W. Wang, C. Sun, L. Mao, P. Ma, F. Liu, J. Yang, Y. Gao, *Trends Food Sci. Technol.*, **56,** 21 (2016).
40. S.S. Baghel, N. Shrivastava, R.S. Baghel, P. Agrawal, S. Rajput, *World Journal of Pharmacy and Pharmaceutical Sciences*, **1**(1), 146, (2012).
41. K.B. Magalingam, A. Radhakrishnan, N. Haleagrahara, *Int J Mol Med*., **32**, 235 (2013).
42. A.B. Enogieru, H. William, D.C. Hiss, S. Bardien, O.E. Ekpo, *Oxidative Med. Cellular Long.*, **2018**, 1 (2018).
43. P.V. Maslennikov, G.N. Chupakhina, L.N. Skrypnik, P.V. Feduraev, V.. Seledtsov, *IKBFU's Vestnik*, **7**, 110 (2014).
44. S. Seo, K. Seo, S.H. Ki, S.M. Shin, *Biol Pharm Bull.*, **39**, 1830 (2016).
45. S. Kandakumar, V. Manju, *IJTSRD*, **1**, 2456 (2017).
46. Yu.S. Tarahovskiy, Yu.A. Kim, B.S. Abdrasilov, E. N. Abrasilov, E.N. Muzafarov, Flavonoids: biochemistry, biophysics, medicine, Synchrobook Press, Pushtino, 2013.
47. J. M. Calderón-Montaño, E. Burgos-Morón, C. Pérez-Guerrero, M. López-Lázaro, *Mini Rev Med Chem.*, **11**, 298 (2011).
48. M. Kfoury, C. Geagea, S. Ruellan, H. Greige-Gerges, S. Fourmentin, *Food Chem.*, **278**, 163 (2019).
49. I. Gülçin, Toxicology, **217**, 213 (2006).
50. F.A. Khan, A. Maalik, G. Murtaza, *J. Food Drug Analysis*, 24, 695 (2016).
51. A.P. Levitsky, O.K. Vertikova, I. O. Selivansky, *Microbiol.Biotechnol.*, **2,** 6 (2010).
52. O.V. Stasevich, E. S. Likhtarovich, *Trudy BGTU*, **4**, 200 (2014).
53. A.A. Dyakov, V.N. Perfilova, I.N. Tyurenkov, *Bull. Arrhythmol*., **39**, 45 (2005).
54. M. Srinivasan, A.. Sudheer, V.P. Menon, *J Clin Biochem Nutr*, **40,** 92 (2007).
55. J. Du, J.. Cullen, G.R. Buettner, *Biochim Biophys Acta*, **1826,** 443 (2012).
56. M.A. Vaitsiakhovich, V.A. Kuchinskaya, I.Yu. Navaselsky, P.V. Hryvusevich, V.V. Samokhina, V S. Mackievic, A.I. Sokolik, V.V. Demidchik, *J. Belarusian State Univ. Biol.*, **2**, 27 (2018).
57. F. E. Pehlivan, in: Vitamin C, A. Hamza (eds.), In Tech Open, 2017, p. 23.
58. A. Corti, A. F. Casini, A. Pompella, *Arch Biochem Biophys*., 500, 107 (2010).
59. E.B. Mentschikova, V.Z. Lankin, N.K. Zenkov, I.A. Bondar, N.F. Krugowih, V.A. Trufakin, Oxidative stress. Prooxidants and antioxidants. Slovo press, Moscow: 2006.
60. C.C.M. P. Santos, M.S. Salvadori, V.G. Mota, L.M. Costa, A.A.C. Almeida, G.A.L. Oliveira, J.P. Costa, D.P. Sousa, R.M. Freitas, R.. Almeida, *Neurosci J*, **2013,** 1 (2013).
61. V.A. Kurkin, Pharmacognosy: Textbook for the students of pharmaceutical universities, Samara State Medical University and Ofort, Samara, 2007.
62. M. Nagalievska, M. Sabadashka, H. Hachkova, N. Sybirna, *BMC Complement Altern Med*. **18**, 4 (2018).
63. B. Pejin, A. Savic, M. Sokovic, J. Glamoclija, A. Ciric, M. Nikolic, K. Radotic, M. Mojovic, *Nat Prod Res*, **28**, 372 (2014).
64. J. P. Costa, M. T. Islam, P. S. Santos, P. B. Ferreira, G. L. Oliveira, M. V. Alencar, M. F. Paz, E. L. Ferreira, C. M. Feitosa, A. M. Citó, D. P. Sousa, A. A. Melo-Cavalcante, *Curr Pharm Biotechnol*, **17**, 1278 (2016).
65. O.I. Adeyomoye, E.O. Adewoye, *Recent Adv Biol Med.*, **4**, 16 (2018).