Scientific Bulletin. Series F. Biotechnologies, Vol. XX, 2016
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

GASTROPROTECTIVE POTENTIAL OF FAGUS SYLVATICA LEAVES EXTRACTS ON STRESS-INDUCED ULCER MODEL ON RATS

Lucia PIRVU[1], Ioana NICU[1], Stelian SCHIOPU[2], Dragomir COPREAN[2]

[1]Institute of Chemical-Pharmaceutical Research & Development (ICCF), 112 Vitan Road, District 3, 031299, Bucharest, Romania
[2]Ovidius University, Faculty of Natural and Agricultural Sciences, 124 Mamaia Avenue, 900527, Constanta, Romania

Corresponding author email: lucia.pirvu@yahoo.com

Abstract

Romanian folk medicine recommends Fagus sylvatica L. *bark and leaves derived products (infusions, decoctions and raw powders) for various skins, respiratory and digestive ailments. The presented work was based on this data, and it aimed to evaluate gastroprotective potential of one standardized product prepared from beech leaves collected in July, by testing it on stress-induced ulcer model on rats. Beech leaves derived product (FA) has been designed as a combination of two polar extracts: aqueous extract as source of polysaccharides compounds and ethanolic (defatted) extract as source of polyphenols compounds resulting in a final standardized product (powder) with exactly 2% (w/w) total flavones content expressed as rutin equivalents. The obtained results, the total length (mm) of superficial, medium and deep gastric lesions of exposed groups versus control group indicated that while the pre-treatment with chemical reference product Ranitidine, an inhibitor of histamine receptor (RH2), assured gastric protection percentages of 59%, 54% and 89%, the pre-treatment with beech leaves derived product (FA) demonstrated gastric protection percentages of 38%, 62% and 96% (n=6; p<0.05) on superficial, medium and deep lesions. Therefore, our results confirm gastroprotective potential of beech leaves derived products (precisely aqueous and ethanolic defatted extracts) suggesting potential use for the development of new phytomedicines targeted at the digestive system.*

Key words*: Fagus sylvatica L. folium, gastroprotective activity.*

INTRODUCTION

Romanian folk medicine (the information comes from the northern part of Romania) recommends the consumption of a few beech leaves (*Fagus sylvatica* L.) collected from spring to early summer, over 3 to 5 days, as an effective treatment of epigastric pain occurring in the spring season.

Japanese researchers (Tsutomu et al., Patent Jp. 05,139,972) have indeed proved the effectiveness of extracts from beech leaves against *Helicobacter pylori* gram-negative bacteria, the main cause for gastric lesions and subsequent chronic progression up to tumour process initiation as it is known (Konturek et al., 2006). Concerning the chemical nature of the active compounds, the same studies (Tsutomu et al., Patent Jp. 05,139,972) related anti-*Helicobacter* activity of beech leaves extracts with the presence of some epi(gallo)-catechin compounds similar to those found in green tea (*Camellia sinensis* L.) products.

On the other hand, very recent metabolomic studies (Cadahía et al., 2015) on leaves of *Fagus sylvatica* L. have revealed very complex chemical composition abounding in polyphenols compounds including numerous flavonoids derivates described with gastroproptective activity (de Lira Mota et al., 2009) such as naringenin, quercetin, cyanidol, apigenin, myricetin and luteolin derivates. Our previous studies (Pirvu et al., 2013) on beech leaves plant pieces indicated the dynamic of caffeoylquinic acid, kaempferol, apigenin, quercetin and catechin derivates along the vegetation time as well as flavonoids abundance in spring and early summer time; there were estimated total flavones content measuring from 12 to 9 mg (expressed as rutin equivalents) *per* 1 gram fresh material collected in May and, respectively, July period (±5%, w/w).

Concerning the pharmacological data, beech leaves extracts have been proved with antimicrobial (Pirvu et al., 2014) and anti-tumor (Frederich et al., 2009) properties.

Our previous studies (Pirvu et al., 2013) have also revealed high antioxidant potency of beech leaves polar extracts *versus* augmented pro-oxidant properties of non-polar extracts (dichloromethane fraction from whole ethanolic extract).

In view of these, this work was aimed to evaluate gastroprotective potential of one product prepared from beech leaves collected in July, by testing it on rats with stress-induced gastric lesions. The test product has been designed as a combination of two polar extracts: aqueous extract as source of polysaccharide compounds and ethanolic (defatted) extract as source of polyphenol compounds resulting in a final standardized product relating to total flavones content (as key compounds with gastroprotective potency).

The final interest is to better valorise Romanian folk medicine data and our previous results which indicated high antioxidant properties of beech leaves polar extracts.

MATERIALS AND METHODS

Plant material description: *Fagus sylvatica* L. *folium* (*Fagaceae* family) vegetal material was purchased from Romanian Carpathian Mountains, the Northern region called Bucovina. Taxonomic identification has been fulfilled by the team of botanists at the *National Institute of Chemical-Pharmaceutical Research and Development* (ICCF), Bucharest, Romania. Voucher specimens (FSPA20-25) are deposited in ICCF *Plant Material Storing Room*. Beech leaves vegetal material has been harvested early July, shade dried then minced as a fine plant powder.

Extracts' preparation: Technological studies started from the idea of a final product combining beech leaves polar extracts (our previous studies indicated the pro-oxidant potency of the non-polar compounds/fraction), polysaccharides and polyphenols compounds respectively, with potential citoprotective and antioxidant properties, thus assuring the chemical condition of a potential gastroprotective product.

This way, three charges of one hundred and fifty (150) grams beech leaves powder each were extracted with 1500 mL of distilled water at boiling temperature under continuous agitation system. The resulting aqueous extracts were (separately) concentrated at low pressure (*Büchi* Rotary Evaporator) and then atomized (BŰCHI Mini Spray Dryer B-290, *Switzerland*). Three brown powder products were obtained (codified F) further estimated with 50-55% total polysaccharides content (gravimetrical estimation), 0.91% total flavones content (rutin equivalents) and 1.18% total phenols content (gallic acid equivalents), (mean values, w/w).

The vegetable waste resulted after the first (hot water) extraction was further extracted with 900 mL ethanol (96%, v/v) and the resulting ethanolic extracts were concentrated at the residue. The three residues (corresponding to the three laboratory charges) were (separately) dissolved into 100 mL of distilled water then extracted with dichloromethane solvent (3x200 mL each operation, over night) in order to remove the non-polar compounds. The resulting aqueous fractions were also atomized. Three red-brown powder products have been obtained (codified A) further estimated with 5.10% total flavones content (rutin equivalents) and 12.40% total phenols content (gallic acid equivalents), (mean values, w/w).

F and A products were then combined in order to obtain the final standardized product (PA); practically, to a fixed amount of F product has been added the necessary amount of A product in order to obtain the exactly 2g% total flavones content (rutin equivalents, w/w).

Chemicals, reagents and references: Chemicals (aluminium chloride, sodium acetate, sodium carbonate), reagents (Folin-Ciocalteau, Natural Product and PEG4000 identification reagents - NP/PEG) and solvents (methanol, ethanol, dichloromethane, toluene, formic acid, acetic acid, ethyl acetate) as well as the *reference products* rutin (min. 95%), hyperoside (>97%), quercitin-3-O-xyloside (>97%), apigenin (>97%), cosmosiin (97%), vitexin (>96%), isovitexin (>98%), vitexin-2"O-rhamnoside (>98%), apiin (>99%), chlorogenic acid (>95%), caffeic acid (99%) were purchased of *Fluka* and *Sigma-Aldrich* Co (Bucharest, Romania).

An internal standard consisting in green tea (*Camellia sinensis* L. *folium*) 70% (v/v) ethanol extract has used too.

Qualitative analytical determination: Studies were performed according to *Plant Drug Analysis* (Wagner H. and Bladt S., 1996) and *High-Performance Thin - Layer Chromatography for the Analysis of Medicinal Plant* (Reich E. and Schibli A., 2008) methodologies, standard settings for polyphenols (system A) and catechins (system B) assessment: ethyl acetate - acetic acid - formic acid – water/100:12:12:26 (system A) and toluene – formic acid – acetone/9:9:2 (system B); plates (10x10) of Silica gel 60F254 – HPTLC (Merck, Darmstadt, Germany*);* reference compounds, *Sigma-Aldrich* polyphenols, were prepared as 10^{-3}M solutions in ethanol 70% (v/v).

Test products, beech leaves derived products F and A respectively, were prepared as 2g% (w/v) solutions in ethanol 70% (v/v) then filtered in vacuum system (filter paper blue). Volumes measuring from 1 to 5 µL test vegetal and test reference samples were loaded as 8 mm band length in the 10 x 10 cm Silica gel 60F HPTLC plate using Hamilton syringe and Linomat 5 instrument (CAMAG, Muttentz, Switzerland). The loaded plates were kept in TLC twin developing chamber at 18-19°C with respective mobile phase (system A and system B) up to 90 mm. The dried plates were exposed at 254 nm, then immersed into identification reagents (NP/PEG) and studied at 366 nm in order to asses the polyphenols content (yellow, orange, red, green, blue-green, or blue spots on black, non-fluorescent plate); catechins compounds were studied by the exposure at 254 nm since they appear as black spots on green fluorescent plate. Spots' assignments have been done by using reference compounds and literature data.

Estimation of Total Flavones content: Total flavones content was measured using *Romanian Pharmacopoeias* (1993) method. Briefly, 5.000 g of each, F, A and FA beech leaves derived products (powders) were twice (heat assisted) extracted with 50 mL of 50% (v/v) ethanol solvent and the resulting ethanolic extracts completed at 100 mL final volume with 50% (v/v) ethanol solution thus obtaining the *test solutions*. Three aliquots of 50 to 100 µL *test solution* (F, A and FA) were treated with 600 µL of 2.5% $AlCl_3$ and 1000 µL of 10% CH_3COONa then accurately finished at 5000 µL with (50%, v/v) ethanol. Mixtures were incubated at room temperature for 30 minutes and the absorbance of reactions at maximum absorption wavelength (386 nm) measured. Total flavones content was estimated by using rutin (ref.) standard calibration curve and the results were expressed as g total flavones (rutin equiv.) *per* 100 g vegetal product (r^2=0.9998).

Estimation of Total Phenols Content: Total phenols content was measured using *Folin-Ciocalteau* and *Romanian Pharmacopoeias* (1993) method. Briefly, three aliquots of 25 to 50 µL *solution test* (F, A and FA) were treated with 200 µL of *Folin-Ciocalteau* reagent and accurately finished at 5000 µL volumetric flasks with 5% (w/v) Na_2CO_3. Flasks were mixed and left in the dark place at room temperature for 5 min then the absorbance at 750 nm was measured. Total phenols content was estimated by using gallic acid (ref.) standard calibration curve (r^2=0.9997) and the results were expressed as g total phenols (gallic acid equivalents) *per* 100 g vegetal product.

Stress-induced rat ulcer model experiment description: Pharmacological *in vivo* studies were carried out on *Wistar* Albino rats, male, of 180 - 200 g purchased from the animal house of the Faculty of Natural and Agricultural Sciences, Constanta, Romania. The animals were maintained in a controlled environment at 22±2°C and 55±10% humidity with 12h light–dark cycle and fed with standard pellet food and water *ad libitum*. The stress-induced rat ulcer model experiment was developed as follows: animals were fasted over night; the next morning, control group animals were immobilized and immersed into cold water on dorsal position for four hours (preliminary studies indicated four hours as being the properly time necessary to achieve various type of gastric lesions and no mortality cases); in the case of treated groups, one hour before the stress experiment the animals received *per oral* (*p.o.*) the respective doses of test products (beech leaves derived product PA and chemical reference Ranitidine). At the end, control animals and treated animals were anesthetised and euthanized, the stomachs excised and washed, then the total length of each type of gastric lesions (superficial, medium and deep gastric lesions) were measured; the obtained values (mm) were compared, thus resulting the final gastric protection results expressed in percentages (GP%).

Statistical analysis: The total length (mm) of each type of superficial, medium and deep gastric lesions, were calculated as mean ± SD, n=6; differences were significantly different (Student's *t* test) if $p<0.05$; results were expressed as percentages (GP%).

RESULTS AND DISCUSSIONS

Analytical screening results

Figure 1 shows qualitative (HP-TLC) aspects referring to polyphenols content (System A setting study) of the two test products, aqueous extract enriched in polysaccharides compounds (F) and ethanolic defatted extract enriched in polyphenols compounds (A), obtained through processing *Fagus sylvatica* L. *folium* raw material collected early July by comparing with several reference products mixtures (ref.), polyphenols compounds.

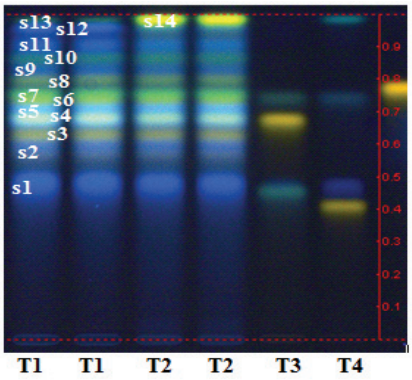

Figure 1. (HP) TLC aspects of beech leaves polar extracts comparatively to reference compounds (ref.). Tracks 1, beech leaves aqueous extract (F product, duplicate sample); Tracks 2, beech leaves ethanol (defatted) extract (A product, duplicate sample); Track 3, vitexin-2"O-rhamnoside, hyperoside, vitexin and caffeic acid mixture (ref.); Track 4, rutin, chlorogenic acid, cosmosiin and kaempferol mixture (ref.)

As shown in Figure 1, the two beech leaves polar extracts, aqueous extract (Tracks 1, duplicate sample) and ethanolic defatted extract (Tracks 2, duplicate sample) indicated similar polyphenol content with the mention that, differing on the aqueous extract, ethanolic extract favoured the extraction of important amounts of quercetin aglycone (the yellow fluorescent spot, s14, at the FRONT region). Also, numerous caffeoylquinic acids were revealed: caffeic (s12), chlorogenic (s1), neochlorohenic (s2) and isochlorogenic (s5, s9, s11) acids respectively. Quantities of apigenin (s7, s8, s10), quercetin (s3, s6) and kampferol (s4) glycosides along with apigenin (s13) and quercetin (s14) aglycones have also been revealed.

Aimed to observe the catechins presence, system B setting study (Figure 2**)** and comparison with the internal standard consisting in green tea (*Camellia sinensis* L. *folium*) 70% (v/v) ethanolic extract indicated the occurrence of at least two catechin derivates, epicatechin (*Rf*~0.68) and epigallocatechin (*Rf*~0.52), in both polar extracts (F product and A product).

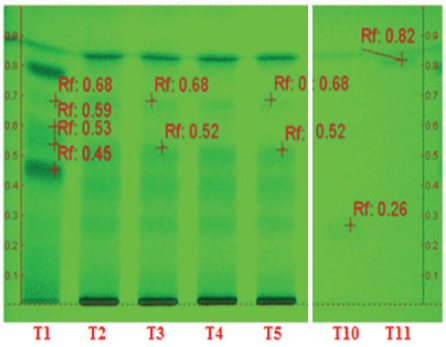

Figure 2. (HP)TLC aspects of beech leaves aqueous extract (F product) and beech leaves ethanolic extract (A product) face to green tea 70% (v/v) ethanolic extract and two caffeoylquinic acids (reference products). Track 1, green tea ethanolic extract (the internal standard); Tracks 2 - 3, beech leaves aqueous extract (F product); Tracks 4 - 5, beech leaves ethanolic extract (A product); Track 10, chlorogenic acid (ref.); Track 11, caffeic acid (ref.)

Also, green tea ethanolic extract confirmed the occurrence of the four catechin derivates, as the literature data reports [8]: epicatechin (*Rf*~0.68), epigallocatechin (*Rf*~0.53), epicatechin gallate (*Rf*~0.59) and epigaleocatechin gallate (*Rf*~0.45).

Therefore, chemical qualitative analyses indicated two similar, but not identical, beech leaves polar extracts, differing as of quercetin aglycone, found in ethanolic extract (A) only.

These extracts were further quantitatively analysed thus resulting the algorithm of the final standardized product (FA) with exactly 2g% (w/w) total flavones content expressed as rutin equivalents (see *Extract's preparation*).

***In vivo* pharmacological results**

The main purpose of pharmacological studies was the evaluation of gastro-protective potential of beech leaves derived product (FA) on gastric lesions obtained *via* stress-induced ulcer model on rats.

Concerning the stress-induced rat ulcer model experiment *in vivo*, it is well known that immobilization on dorsal position associated with low temperature both lead to the decrease of microcirculation in gastric tissue.

Exposing the vulnerable gastric mucosa to augmented quantities of gastric acid (induced by stress state) results in various gastric lesions, from superficial to medium and deep lesions, explaining stress-induced rat ulcer model on rats.

The three animals' groups were as follows:

- *Group 1* (control group, C) - fasted animals were stressed through immobilization and immersion into cold water on dorsal position, then euthanized and the total length (mm) of each gastric lesion (superficial, medium and deep lesion) measured;
- *Group 2* (group treated with chemical reference Ranitidine, R) - one hour before the stress experiment, fasted animals received (*p.o.*) the human corresponding dose of Ranitidine (27 mg/kg body), then they underwent the stress experiment;
- *Group 3* (group treated with beech leaves derived product, FA) - similarly, one hour before the stress experiment, fasted animals received (*p.o.*) the vegetal test product (FA) in dose of 500 mg/kg body (the respective dose was selected based on previous exploratory studies (Pirvu et al., 2015) and literature data as well (Eswaran et al., 2010; El-Shenawy, 2009) then they underwent the stress experiment.

At the end, the treated animals were anesthetized and euthanized, and the total length (mm) of each type of gastric lesion was measured.

Thus, by comparing the total length (mm) of each, superficial (mucosal irritations), medium (haemorrhagic) and deep (necrotic) lesions of the treated groups (R and FA) with the total length (mm) of superficial, medium and deep lesions of the control group (C) gastro-protective activity (GP%) has been estimated.

Results are summarized in Figure 3.

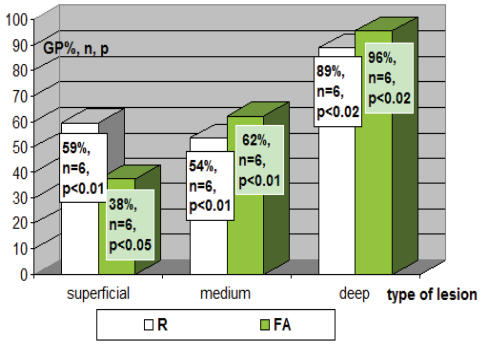

Figure 3. Gastroprotective activity of *Fagus sylvatica* L. *folium* derived product (FA) compared to chemical reference Ranitidine (R)

As shows in Figure 3, the pre-treatment with chemical reference product/Ranitidine (R) assured gastric protection percentages (GP%) of 59% ($p<0.01$), 54% ($p<0.01$) and 89% ($p<0.02$) on superficial, medium and deep gastric lesions ($n=6$); the pre-treatment with beech leaves derived product (FA) indicated gastric protection percentages of 38% ($p<0.05$), 62% ($p<0.01$) and 96% ($p<0.02$) on the same type of mucosal lesions ($n=6$). Therefore, the comparison with chemical reference, Ranitidine (a well known inhibitor of histamine receptor/RH2 and subsequently gastric acid synthesis controller) suggests gastric mucosa protective properties of polar extracts isolated from beech leaves plant part. Besides, the presence of a fine *veil* of vegetal product crossing the entire gastric mucosa has been noticed, the most probable due by tanning properties of catechin derivates; these observations could explain the short time of treatment recommended by Romanian folk medicine practice.

Together, gastroprotective potential of beech leaves derived products is sustained by present data (stress-induced ulcer model on rats) suggesting gastric mucosa protection against acid secretion but also through previous studies demonstrating high antioxidant potency of beech leaves polar extracts (Pirvu et al., 2013) as well as by means of Japanese researchers' experiments demonstrating their efficacy in controlling *H. pylori* infection (Tsutomu et al., Patent Jp. 05,139,972).

Other tree leaves extracts demonstrated with gastroprotective activity are those from *Cinnamomum tamala* T. Nees & Eberm species (*Lauraceae* family) (Eswaran et al., 2010),

traditionally used in Indian System of Medicine in order to improve numerous digestive system ailments; it was proved that leaves extract of *C. tamala* in doses of 50, 100 and 200 mg/kg body weight, *p.o.*, administered as preventive treatment (twice a day over a period of 5 days) in rats with gastric lesions induced *via* ethanol, cold-restraint and pylorus-ligation models results in a significant gastric lesion index reduction compared to ulcerated rats, in all studied models. It was concluded that the free radical scavenging activity of this tree leaves mainly sustained gastroprotective activity.

Similarly, studies upon the tropical tree *Eugenia jambolana* Lam. (*Myrtaceae* family) (El-Shenawy, 2009), aiming to evaluate potential benefits on indomethacin-induced (25 mg/kg, *p.o*) ulcer on rats, indicated that the acute gastric mucosal lesions were significantly reduced when ethanol extract of seeds, pericarp and leaves (250 and 500 mg/kg, *p.o.*) were administered.

Studies (Fernandes et al., 2010) on the ethanolic extracts of leaves from *Parkia platycephala* Benth. (*Leguminosae - Mimosoideae* family) found in Brazil indicated a protective effect (on rodents) in absolute ethanol, ethanol-HCl, ischemia-reperfusion lesion models (66%, 48% and 52% gastroprotective percentage) but not in indomethacin-induced ulcer. The results also suggested antioxidant activity as the most probable mechanism for gastric protection.

Also, studies (Speroni et al., 2011) on *Laurus nobilis* L. plant species (*Lauraceae* family), a tree found in the Mediterranean area and Europe, indicated that leaves extracts (obtained with different solvents and methods) significantly reduced animal gastric damages, chloroform and methanol crude extracts providing the most important gastroprotective effects. Similarly, the results obtained were in good agreement with antioxidant capacity also suggesting a relationship between biological effects of leaves extracts from *L. nobilis* and their scavenging activity.

CONCLUSIONS

Despite the numerous potential therapeutic benefits (based on its valuable chemical content), the main commercial applications of *Fagus sylvatica* L. leaves plant part are several external products (cosmetics) assumed with high antioxidant activity; beech leaves derived products for internal use (food supplements, dietary supplements or traditionally medicines) are quite missing.

Based on Romanian folk medicine data, our studies have revealed gastroprotective potential of beech leaves derived products, precisely the capacity of the beech leaves polar extracts to offer rat gastric mucosa protection against acid secretion stimulated *via* stress-induced ulcer model, suggesting their compliance for the development of some internal use natural medicines targeted at the digestive system.

These results should be evaluated in the context of our former results (Pirvu et al., 2013) which indicated high antioxidant potency of beech leaves polar extracts and Japanese researchers' experiments (Tsutomu et al., Patent Jp. 05,139,972) demonstrating their efficacy in controlling *Helicobacter pylori* infection but also in the context of literature data indicating potential toxic effect of different beech tree products (Husgafvel et al., 2014) and augmented pro-oxidant effects of the non-polar compounds found in dichloromethane fraction from 70% ethanol extracts (Pirvu et al., 2013).

Therefore, extensive studies on beech tree plant parts and subsequent beech extracts should to be performed to develop novel and safe phyto-medicines targeted at the digestive system.

REFERENCES

Cadahia E., Fernandez de Simon B., Aranda I., Sanz M., Sanchez-Gomez D., Pinto E., 2015. Non-targeted metabolomic profile of Fagus sylvatica L. leaves using liquid chromatography with mass spectrometry and gas chromatography with mass spectrometry. Phytochem Anal, 26(2):171-182.

El-Shenawy S. M., 2009. Evaluation of some pharmacological activities of ethanol extracts of seeds, pericarp and leaves of Eugenia Jambolana in rats. Inflammopharmacology, 17(2):85-92.

Eswaran M. B., Surendran S., Vijayakumar M., Ojha S.K., Rawat A.K., Rao Ch. V., 2010. Gastroprotective activity of Cinnamomum tamala leaves on experimental gastric ulcers in rats. J Ethnopharmacol, 128(2):537-540.

Fernandes H. B., Silva F.V., Passos F. F., Bezerra R. D., Chaves M. H., Oliveira F. A., Oliveira R. C., 2010. Gastroprotective effect of the ethanolic extract of Parkia platycephala Benth. leaves against acute gastric lesion models in rodents. Biol. Res., 43(4):451-457.

Frederich M., Marcowycz A., Cieckiewicz E., Megalizzi V., Angenot L., Kiss R., 2009. In vitro anticancer potential of tree extracts from the Walloon Region forest. Planta Med, 5(15):1634-1637.

Husgafvel Pursiainen K., 2014. Occupational Cancers, Sisko Anttila and Paolo Boffetta Publishing House, Springer, 158.

Konturek P.C., Konturek S.J., Brzozowski T., 2006. Gastric cancer and Helicobacter pylori infection. J Physiol Pharmacol, 57(3):51-65.

de Lira Mota K. S., Nunes Dias G. E., Ferreira Pinto M. E., Luiz-Ferreira A., Souza-Brito A. R. M., Hiruma-Lima C.A., Barbosa-Filho J. M., Maria Batista L., 2009. Flavonoids with Gastroprotective Activity. Molecules, 14, 979-1012.

Pirvu L., Grigore A., Bubueanu C., Draghici E., 2013. Comparative analytical and antioxidant activity studies on a series of *Fagus sylvatica* L. leaves extracts. JPC. Journal of Planar Chromatography, 26(3):237-242.

Pirvu L., Hlevca C., Nicu I., Bubueanu C., 2014. Comparative Analytical, Antioxidant and Antimicrobial Activity Studies on a Series of Vegetal Extracts Prepared from Eight Plant Species Growing in Romania. JPC. Journal of Planar Chromatography, 27(5):346-356.

Pirvu L., Panteli M., Bubueanu C., Coprean D., 2015. *Centaurea cyanus* L. Polysaccharides and Polyphenols Cooperation in Achieving Strong Rat Gastric Ulcer Protection. Open Chemistry, 13(1): 910-921.

Reich E., Schibli A., 2008. HPTLC for the Analysis of Medicinal Plants, Thieme Publishing House, N.Y.-Stuttgart.

Romanian Pharmacopoeia, 1993. Xth Edition, Medicala Publishing House, Bucharest, 334-335, 1063-1064.

Speroni E., Cervellati R., Dall'Acqua S., Guerra M. C., Greco E., Govoni P., Innocenti G., 2011. Gastroprotective effect and antioxidant properties of different Laurus nobilis L. leaf extracts. J Med Food, 14(5):499-504.

Tsutomu O., Hidehisa T., Masaru F. Patent no. Jp. 05,139,972.

Wagner H., Bladt S., 1996. Plant Drug Analysis, Second Ed., Springer Publishing House.