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PLANT COMPOUNDS SYNERGISTIC ACTIVITY BENEFITS ON HUMAN HEALTH

Lucia Pirvu[1]*, Alice Grigore[1], Corina Bubueanu[1], Cristina Hlevca[1], Svetlana Colceru-Mihul[1]
[1]National Institute for Chemical-Pharmaceutical R&D (INCDCF), Bucharest, Romania

ABSTRACT. Although less potent than allopathic medicines, anti-inflammatory as well as antimicrobials plant derived products are often more effective in treating human illnesses mainly owing phytocompounds synergistic activity. For example, in the specific case of anti-inflammatory activity, there are data proving the synergism between polyphenols and polysaccharides fractions; similarly for antioxidant activity, especially if also combines triterpenic acids in order to increase phytocompounds solubility. In the specific case of antimicrobials, scientific results suggest phenylcarboxylic acid class boosting effect upon flavonoids sub-classes. Given that vegetal world has been always a source of human health (the practice of self-medication using herbs dating from the Palaeolithic) phytocompounds efficacy mechanisms needs to be revealed and further adopted for the design and development of novel highly effective natural or combined medicines.

Keywords: antioxidant, anti-inflammatory, phytocompounds, synergistic activity

INTRODUCTION

It is well known that a large amount of the currently active pharmaceutical ingredients, part of the nowadays health system are on basis of *isolated phytocompounds* or *whole* or *selective* vegetal extracts. Regarding the effectiveness of these plant derived medicines, although less potent than allopath drugs, plant compounds synergistic activity plays a major role in their therapeutic efficacy acting in a manner that *the effective concentration of ingredients in combination is significantly reduced or the effects of ingredients in combination are significantly increased with respect to that of each individual ingredient* (Pulok *et al.,* 2011). Briefly, it is considered that the synergism exists if the speared parts (separate compounds or whole or selective extracts) induce a biological effect more significantly than the sum of either alone.

Accordingly, studies (Shale *et al.,* 2005) on *Malva parviflora* leaves and roots extracts tested for antibacterial and anti-inflammatory activity using the disc diffusion and cyclooxygenase-1 (Cox-1) bioassays indicated that if hexane extracts isolated from both, leaves and roots were very active, the water extracts presented the least inhibitory activity. Further bioassay-guided fractionation of the root dichloromethane extract indicated that Cox-1 anti-inflammatory activity was caused by at least two compounds that acted synergistically to produce final biological effect. Similarly, cancer disease studies have confirmed high benefits of the plant compounds synergistic activity. Accordingly, starting from the fact that the reduction of the inflammation is an important anticancer therapeutic opportunity, studies (Lin *et al.,* 2007) on four anti-proliferative phytocompounds isolated from *Wedelia chinensis* ((an oriental herbal medicine assigned with the ability to modulate the androgen receptor (AR) activation of transcription from prostate-specific antigen promoter in PCa cells)) indicated that the formula that combined the four active compounds, respectively indole-3-carboxyl-aldehyde, wedelolactone, luteolin and apigenin decreased the dosage of each compound required to achieve maximal AR inhibition. These active compounds specifically inhibited the growth of AR-dependent PCa cells and as a combination formula they also synergistically suppressed growth in androgen dependent PCa cells all suggesting the synergistic effects of the active compounds in *Wedelia chinensis*. Moreover, studies indicated that plant compounds present synergism with allopath medicines. As proof, studies on plant derived antimicrobials indicated that specific compounds act as multidrug resistance modifiers by counteracting all known microbial resistance mechanisms, respectively by 1)active site modification, 2)antibiotic destruction using specific enzymes (such as beta-lactamases), 3)antibiotic influx inhibition or 4)antibiotic efflux stimulation (Shanmugam *et al.,* 2008). For instance, studies have revealed that if myricetin flavonol acts in synergism with amoxicillin/clavulanate, ampicillin/sulbactam and cefoxitin antibiotics on methicillin-susceptible *Staphylococcus aureus* (MSSA) and extended-spectrum–lactamases (ESBL) producing *K. pneumoniae* (Lin *et al.,* 2005), catechins and epigallocatechin gallates (EGCg) flavanols from green tea shown the capacity to restore the activity of the penicillin (Zhao *et al.,* 2002). Many other examples of plant compounds antimicrobial synergistic activity are available (Shanmugam *et al.,* 2008).

In support, the present work presents data revealing 1)polyphenols, in particular flavonols and 2)polysaccharides synergism transposed in high antioxidant, anti-inflammatory activity demonstrated on both, *in vitro* chemiluminescence studies and *in vivo* castor oil (*oleum ricini*) induced colitis in rats.

MATERIALS AND METHODS

Vegetal products description

The vegetal products (three laboratory charges) were prepared as follows:

-polyphenols fraction, precisely flavonols fraction was prepared by (70%) ethanol extraction of scales of *Alii cepae* L. *bulbu*s raw material. The extraction has been done at reflux temperature and continuously agitation state. The resulted suspension was precipitated (4°C) and the precipitate dried at exicator (30-35°C). A yellow powder with high content in flavonoids, further called polyphenols fraction (codified QT) resulted.

-polysaccharides fraction was prepared by water extraction of the leaves of *Althaea officinalis* L. at boiling temperature and continuously agitation state. The resulted extract was precipitated in ethanol. The resulted precipitate was dried at exicator (30-35°C). A grey powder with high content in polysaccharides, further called polyphenols fraction (codified P) resulted.

-combined vegetal product (codified QTP) has obtained by mixing QT and P fractions using a mathematical algorithm to results a final product with precisely 4% total flavones expressed as quercetin equivalents.

Chemical composition of the studied vegetal products is presented in Table 1.

Table 1

Chemical composition of the studied vegetal products

Tested product	Product code	Total flavones[a] (g%)	Total phenols[b] (g%)	Total polysaccharides[c] (g%)
Allii cepae bulbus scales polyphenols fraction	QT	20.3	18.5**	-
Althaea officinalis leaves polysaccharides fraction	P	0.4*	0.42	68.0
Combined vegetal product	QTP	4.0	-	96.0

[a]Total flavones was expressed as quercetin and rutin* equivalents.
[b]Total phenols was expressed as gallic acid equivalents. Note: **total phenols content under total flavones content is usually in the case of vegetal extracts dominated of flavonoids compounds
[c]Total polysaccharides was measured using gravimetrical method.

Chemicals

Chemicals ($AlCl_3$, CH_3COONa, H_2O_2, luminol, 0.2M TRIS-HCl pH 8.5, dimethyl sulfoxide/DMSO), reagents (*Folin-Ciocalteau*, *Natural Product*), solvents (methanol, ethanol, ethyl acetate, formic acid, acetic acid) were purchased of *Sigma-Aldrich* Co (Bucharest, Romania).

*Reference products** as rutin (min. 95%), quercetin (95%), apigenin (>97%), kaempferol (95%), cosmosiin (97%), vitexin (>96%), apiin (>97%), chlorogenic acid (>95%), caffeic acid (99%), gentisic (95%) and gallic acid (95%) were purchased of *Fluka and Sigma-Aldrich* Co (Bucharest, Romania).

Note*: Reference products were further prepared as 10^{-3}M in (70%) ethanol solution.

Analytical studies

Qualitative analysis was performed using (HP)TLC technique according Hildebert Wagner et. al and Eike Reich et. al references, respectively using general method for polyphenols screening, ethyl acetate-acetic acid-formic acid-water (100:12:12:26) system. Studies were done using CAMAG Linomat 5 instrument.

Quantitative estimations of total phenols as well as of total flavones were done by using standard *Romanian Pharmacopoeia* (*FR. X*) methods, *Folin-Ciocalteau* and $AlCl_3$ in base medium (CH_3COONa), respectively. It was used the UV/Vis Hélios γ (*Thermo Electron Corporation*) spectrophotometer instrument. Total polysaccharides were estimated using *FR.* X gravimetric method.

In vitro antioxidant activity studies

Free radical scavenging activity (antioxidant activity) was measured by Iftimie N et. al method. Studies were performed using the chemiluminometer TD 20/20, Turner Design, USA. Briefly, (three) aliquots of 50 µL tested sample (prepared in distilled water and DMSO) were mixed with 200 µL 10^{-3}M luminol (prepared in DMSO), 700 µL 0.2M – TRIS-HCl *p*H 8.6 and 50 µL 10^{-3}M H_2O_2 (prepared in bi-distilled water); must be noted that all vegetal samples have been similarly prepared by dissolving 0.2 g vegetal product into 100 mL solvent, dimethylsulfoxide (DMSO) or distilled water. In parallel, a reference sample consisting in 50 µL solvent sample was mixed with 200 µL 10^{-3}M luminol, 700 µL 0.2M – TRIS-HCl *p*H 8.6 and 50 µL 10^{-3}M H_2O_2. Five seconds after the reaction initiation, the intensity of the chemiluminescence/CL (activity units/a.u.) of each vegetal test sample was measured; similarly for reference sample. The antioxidant activity of the vegetal test samples were then calculated and expressed as percents (AA%) – see formula:

$$AA\% = \frac{\text{CL reaction intensity of the reference sample (a.u.) - CL reaction intensity of the tested sample (a.u.)}}{\text{CL reaction intensity of the reference sample (a.u.)}} \times 100$$

In vivo anti-inflammatory activity studies

In vivo anti-inflammatory activity studies were fulfilled on three groups of *Wistar* rats, male. Bowel lesions were produced using castor oil/*oleum ricini* (*Ricinus comunis* L.), a very potent bowel inflammatory agent. Five combinations of doses of *oleum ricini* have been previously tested. The obtained results indicated that a dose of 16ml *oleum ricini per* kg body *per oral* (p.o.) in the first day followed by 8ml *oleum ricini per* kg body p.o in the second day were the properly doses leading to moderate to high rat bowel lesions.

The three rat groups were as follows:

Group 1. Control group - these animals received standard food and water all period of the study (7 days);

Group 2. Exposed untreated group – these animals received, besides standard food and water, 16 ml *oleum ricini* / kg body p.o. in the first day, 8 ml *oleum ricini* / kg body p.o. in the second day after that five days the animals were observed and killed in the seventh day of experiment;

Group 3. Exposed treated group – first two days animals received 500mg combined vegetal product (QTP) / kg body p.o, next two days the same doses of combined vegetal product concomitantly with *oleum ricini* doses (16 ml *oleum ricini* / kg body p.o. on the third day and 8 ml *oleum ricini* / kg body p.o. on the fourth day) and the last three days the combined vegetal product only, as effective treatment. Animals were also fed with standard food and water and killed on the seventh day of experiment.

At the end of the experiment, *in vivo* antioxidant, anti-inflammatory activity by measuring the intestinal level of malondialdehyde (MDH), reduced glutathione (GSH), superoxid dismutase (SOD) and cathalase (CAT) of each animal of each tested group was evaluated.

Statistical analysis

Data are expressed as mean (SD). Significance of differences was assessed by Student's *t* test. Differences were considered to be significantly different if $p<0.05$.

RESULTS AND DISCUSSION

Case study: Synergistic activity of polyphenols and polysaccharides fractions isolated from *Allii cepae bulbus* L. scales and *Althaea officinalis L.* leaves vegetal raw materials evaluated in terms of antioxidant potency and rat bowel anti-inflammatory activity.

Figure 1 illustrates chemical qualitative composition (HPTLC method) of the studied vegetal fractions, *Allii cepae bulbus* L. scales polyphenols fraction (QT) and *Althaea officinalis* L. *leaves* polysaccharides fraction (P) respectively, comparatively to reference products samples (ref.).

The samples were disposed as follows:

T1: quercetin-3-O-glucorhamnoside/rutin, apigenin-8-C-glucoside/vitexin, apigenin-7-O-glucoside/ cosmosiin and gentisic acid (ref.);

T2: apigenin-7-(2-O-apiosylglucoside)/apiin, vitexin, cosmosiin and gallic acid (ref.);

T3: apiin, cosmosiin and quercetin and apigenin (ref.);

T4: rutin, chlorogenic acid, cosmosiin and kaempferol (ref.);

T5: rutin, chlorogenic acid and caffeic acid (ref.);

T6-T8*: Allium cepa* polyphenols fraction (QT) – triplicate (coresponding to the three laboratory charges)*;*

T9-T11: *Althaea off.* polysaccharides fraction (P) – triplicate (coresponding to the three laboratory charges).

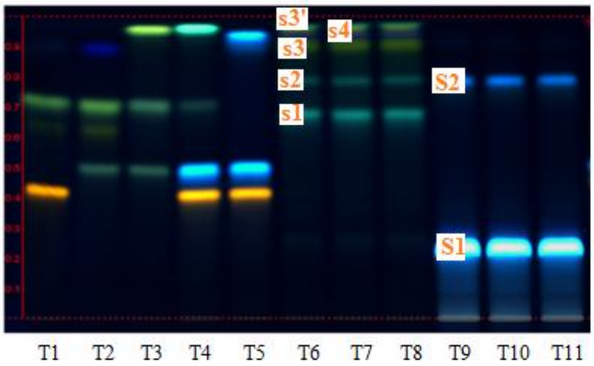

Fig. 1 HPTLC aspects of the two selective vegetal extracts, respectively polyphenols fraction prepared from *Allium cepa* L. scales raw material (T6-T8) and polysaccharides fraction prepared from *Althaea officinalis* L. leaves raw material (T9-T11) face to some reference compounds mixtures (T1-T5).

The obtained results indicated the following data: polyphenols fraction prepared from from *Allium cepa* L. scales raw material (see T6-T8 tracks) has revealed three main flavonols compounds, respectively two blue-green fluorescente (fl.) spots (s1 and s2) attributed to quercetin-4'-glycosides, also known as spiraeosides aside high quantities of quercetin aglycone visualized as two yellow fl. spots (s3 and s3') at the Front of the chromatogram plus very small amounts of caffeic acid aglycone (s4), the blue fl. spot also at the Front of the chromatogram; in the specific case of polysaccharides fraction prepared from *Althaea officinalis* L. leaves raw material (see T9-T11) there were also revealed two polyphenols compounds as two blue fl. spots (S1 and S2) both attributed to kaempferol glycosides; the attribution of these two spot was on basis of maximum absorption wavelengths (about 370nm) resulted at their treatment with $AlCl_3$ in base medium.

Figure 2 presents the antioxidant activity (AA%) of the three vegetal products, respectively the combined vegetal product (QTP) *versus* the two *selective* extracts, *Allium cepa* L. polyphenols fraction (QT) and *Althaea officinalis* L. polysaccharides fraction (P) dissolved in distilled water and dimethylsulfoxide, as well.

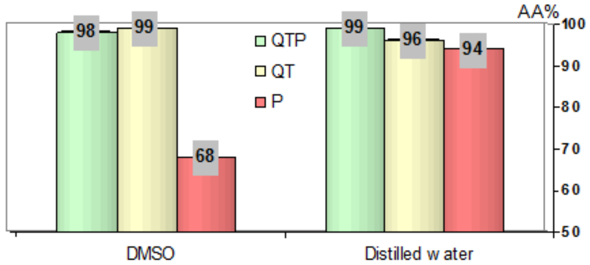

Fig. 2 Comparative antioxidant activity of the combined vegetal product (QTP) *versus* the two *selective* extracts,
Allium cepa polyphenols fraction (QT) and *Althaea officinalis* polysaccharides fraction (P)

The results indicated a strong antioxidant activity in the case of combined vegetal product (QTP) as well as the major role of the solvent environment in order to evidence the specific fraction responsible for the antioxidant activity. Thus, it can be observed that in water, the antioxidant properties of polysaccharides fraction is significantly amplified referring to that in DMSO. Nevertheless, the combined vegetal product has the same activity in DMSO and distilled water, thus indicating the dominant role of polyphenols fraction on scavenger activity of the final product. Such a behavior is highly relevant for the therapeutically activity of vegetal products, being known that DMSO represents an appropriate solvent used as model for simulation of biologic fluids. Finally, DMSO solvent also revealed the synergism between polysaccharides and polyphenols fractions on the global activity of the combined vegetal product.

Subsequently *in vivo* pharmacological studies on three groups of *Wistar* rats (male) with bowel lesions produced with castor oil (*oleum ricini*) ingestion had to reveal high anti-inflammatory activity of the combined vegetal product (QTP), thus confirming previously *in vitro* chemiluminescence results. Table 2 presents the intestinal level of malondialdehyde (MDH) and reduced glutathione (GSH) and superoxide dismutase (SOD) and cathalase (CAT) activities of the exposed untreated group animals (group 2) and exposed treated group animals (group 3) comparatively to control group animals (group 1).

Table 2

In vivo anti-inflammatory activity of the combined vegetal product (QTP) based on *Althaea officinalis L*. leaves polysaccharides fraction and *Allium cepa L*. scales polyphenols fraction

Groups	MDH (nM/g tissue)	GSH (g/mg proteins)	SOD (Units/mg proteins)	CAT (Units/mg proteins)
Group 1. X ± DS n	127,7±14,61 6	43,41±1,54 5	0,326 ± 0,001 5	0,969±0,318 6
Group 2. X ± DS n t p< ± P%	316,24±11,21 5 9,88 0,001 +147,64	49,90±4,15 6 - NS +14,95	0,275±0,014 6 2,77 0,05 -15,64	0,716±0,115 5 - NS -26,11
Group 3. X ± DS n t p< ± P%	41,71±3,55 5 5,52 0,001 -67,34	40,96±1,03 5 - SN -5,64	40,96±1,03 6 - NS -3,06	0,218±0,024 5 2,145 NS -77,50

X ± ES = (pro/anti)-inflammatory tissue marker level/activity ± standard deviation;
n = total animals; t; p< Student's *t* test; NS = ***Not Statistically Significant***;
P% = the protection percent comparatively to control group.

Being an irritable product, *oleum ricini* (castor oil) is frequently used to obtain bowel tissue reaction, peristaltic muscle stimulation or augmented inflammatory lesions respectively, function of ingested doses.

In the present study, doses of 16ml *oleum ricini* / kg body (p.o.) in the first day followed by 8ml *oleum ricini* / kg body (p.o.) in the second day produced augmented rat bowel lesions as *in vivo* (anti/pro)-inflammatory tissue markers revealed. Accordingly, it can be observed that the exposed untreated group (group 2) revealed levels of malondialdehyde (MDH) 2.5 times higher than the control group (group 1) as well as a decreased level of anti-inflammatory superoxid dismutase (SOD) enzyme level. Additionally, the exposed untreated group animals (group 2) presented diarrhea (four days consecutively) and even mortality. Differently, the group treated with combined vegetal product (group 3), two days as pre-treatment, next two days concomitantly with *oleum ricini* doses and another three days as effective treatment counteracted pro-inflammatory malondialdehyde (MDH) tissue marker rising and stimulated anti-inflammatory superoxid dismutase (SOD) activity. Moreover, comparatively to the exposed untreated group (group 2), the exposed treated group (group 3) presented a shorter period of diarrhea (two days only) and did not shown mortality.

Concluding, our studies indicated the augmented anti-inflammatory, anti-colitis activity of the vegetal product combining polysaccharides fraction from *Althaea officinalis* L. and polyphenols (in fact quercetin and spiraeosides flavonols) fraction from *Allium cepa* L. likely explained by their active compounds antioxidant properties and also synergistic activity, as *in vitro* chemiluminescence studies suggested.

Finally, must be noticed similar results when combined polysaccharides and polyphenols fractions from *Centaurea cyanus* L. aerial part tested on rats with gastric lesions obtained *via* stress-induced (immobilization and immersions in cold water) model as well as between phenylcarboxylic acids and flavonoids polyphenols classes from leaves of *Fagus sylvatica* as concerning antimicrobial potency.

In support of our results are studies (Fruet *et al.*, 2012) on cattail rhizome flour (*Typha angustifolia* L.) tested on trinitrobenzenesulphonic acid (TNBS) rat colitis model; cattail rhizome flour active compounds are saponins, flavonoids and coumarins. In addition it was also investigated the effects of cattail rhizome flour on the intestinal anti-inflammatory activity of prednisolone, a reference drug used for the treatment of human inflammatory bowel diseases (IBD). Thus, studies on several concentrations of this vegetal product indicated that dietary supplementation with 10% cattail rhizome flour produced the best effects at reducing the extension of the lesion, the colon weight ratio, adherences to adjacent organs and also diarrhoea. These effects were also related to inhibition of myeloperoxidase (MPO) and alkaline phosphatase (AP) activities and an attenuation of glutathione (GSH) depletion. More, 10% cattail rhizome flour supplementation was as effective as prednisolone but no synergistic effects between vegetal and chemical products were observed. Similarly, it was concluded that the prevention of TNBS-induced colon damage likely resulted from the antioxidant properties of the active compounds.

CONCLUSIONS

Although less potent than allopathic medicines, plant derived products are often more effective in treating human illnesses mainly owing phytocompounds synergistic activity.

In support, there are many literature data suggesting plant compounds synergism in making final antioxidant, anti-inflammatory and antimicrobial activity as well as data suggesting vegetal and chemical compounds synergistic activity. For example, our *in vivo* pharmacological studies on a vegetal product combining polysaccharides from leaves of *Althaea officinalis* L. and polyphenols, precisely quercetin and spiraeosides from scales of *Allii cepae* L. *bulbus* tested on castor oil rat colitis model indicated that the treatment (p.o.) with doses of 500 mg vegetal product *per* kg body *per* day, seven days consecutively totally counteracted the two doses of castor oil (16ml *oleum ricini per* kg body in the first day followed by 8ml *oleum ricini per* kg body in the second day) established as producing augmented inflammatory response at the level of rat bowel tissue. These effects were related to inhibition of malondialdehyde (MDH) inflammatory tissue marker production as well as the enhancement of superoxid dismutase (SOD) anti-inflammatory enzyme activity likely explained by polyphenols and polysaccharides compounds antioxidant properties and also synergistic activity, as *in vitro* chemiluminescence studies suggested.

Similar results where obtained when combined polysaccharides and polyphenols from *Centaurea cyanus* L. aerial part tested on gastric lesions obtained *via* stress-induced ulcer rat model or phenylcarboxilic acids and flavonoids polyphenols classes from leaves of *Fagus sylvatica* L. as concerning antimicrobial potency on methicillin-resistant *Staphylococcus aureus* (MRSA) standard or clinically isolated strains.

Given these and other numerous scientific data proving phytocompounds efficacy in treating different human illnesses their mechanisms needs to be better studied and further adopted for the design and development of novel highly effective natural or combined medicines.

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