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ESTIMATING THE YIN-YANG NATURE OF WESTERN HERBS: A POTENTIAL TOOL BASED ON ANTIOXIDATION- OXIDATION THEORY

Marilena Gilca[1], Laura Gaman[1], Daniela Lixandru[1], Irina Stoian[1, 2]

[1]Biochemistry Dept., Faculty of General Medicine, "Carol Davila" University of Medicine and Pharmacy, B-dul " Eroilor Sanitari" nr.8, sector 6, code 76241, Bucharest, Romania.[2]R&D IRIST LABMED SRL, Str. Miraslau, nr. 24, sector 3, code 031235, Bucharest, Romania.
Email: marilenagilca@gmail.com

Abstract

Background : One of the biggest obstacles to progress in traditional Chinese medicine (TCM) development in Western countries is the difficulty of applying the traditional concepts to the Western medicinal plants, which are not traditionally described in ancient literature. During recent years, new advances in the field of understanding Yin/Yang aspects from a modern bioscientific point of view have led to the conclusion that antioxidation-oxidation concepts might mirror a Yin-Yang relationship.
Methods: This study was intended to integrate the Yin-Yang theory of the traditional Chinese medicine with modern antioxidation-oxidation theory, and to propose a biochemical tool based on redox parameters (e.g. antioxidant capacity, chemiluminescence-CL signal inducing capacity), usable for the classification of Western medicinal plants from Yin/Yang perspective. Trolox equivalent antioxidant capacity (TEAC) of six vegetal aqueous extracts (*Symphitum officinalae* (radix)- SYM, *Inula helenium* (radix)- INU, *Calendula officinalis* (flores)- CAL, *Angelica arhanghelica* (folium)- ANG(F), *Angelica arhanghelica* (radix)- ANG(R), *Ecbalium Elaterium* (fruits)- ECB) and luminol-enhanced chemiluminescence of PMNL on addition of these vegetal extracts were measured. Percentages from the maximal or minimal values obtained were calculated for each extract (TEAC%, PMNL stimulation%, PMNL inhibition%, relative speed of action% (RSA%%)), specific Yin-Yang significance was assigned to each relative parameter. In the end, an integration of all the relative values was done, in order to find a global ”Yin” or a ”Yang” trait of each vegetal extract.
Results: TEAC decreased in the following order: SYM > INU > CAL >ANG(F) > ANG(R > ECB. Three vegetal extracts (SYM > INU > ECB) decreased the luminol-enhanced chemiluminescence of PMNL, two (ANG(R) > ANG(F)) increased it, while one (CAL) had a dual effect. After the integration of the percentages, CAL was found to have a global ”Yang” trait, while the rest of the plants had a global ”Yin” trait.
Conclusions: TEAC% and PMNL inhibition% appears to correlate with the Yin properties of herbs, while PMNL stimulation% and RSA% might correlate with Yang aspects within the formal TCM classification system, and may be useful criteria in describing the Western herbs from a TCM point of view.

Key words: antioxidant, TEAC, herbs, chemiluminescence, Yin, Yang, TCM

Abbreviations: ANG(F)- *Angelica arhanghelica*, fam. Apiaceae (folium)**;** ANG(R)- *Angelica arhanghelica*, fam. Apiaceae (radix) **;** CAL- *Calendula officinalis*, fam. Asteraceae (flores)**;** CL- chemiluminescence**;** ECB- *Ecbalium Elaterium*, fam.Cucurbitaceae (fruits)**;** INU- *Inula helenium*, fam. Asteraceae (radix)**;** ORAC- oxygen radical absorbance capacity**;** PMNL- polimorphonuclear leukocytes**;** ROS- reactive oxygen species; SYM- *Symphitum officinale*, fam. Boraginaceae (radix)**;** TEAC: Trolox equivalent antioxidant capacity.

Introduction

One of the major obstacles to progress in TCM development in Western countries is the difficulty of applying the traditional concepts to the Western medicinal plants, which are not traditionally described in ancient medicine. The integration of traditional terms into Western phytotherapy can be fraught with difficulties of understanding and errors, therefore any attempt in this area should be carefully analysed and approved.
Yin-Yang balance is the core of TCM theory. Yin and Yang are qualitative terms that describe the duality of nature through several pairs of complementary aspects (Table 1).

Tabel 1: Complementary Yin-Yang qualities

Yang	Yin
Proactive	counteractive
Function	structure
aggressive (”spear”)	protective (”shield”)
Light	dark
Hot	cold
Fire	water
Dry	moist
Fast	slow

According to TCM theory, herbs are prescribed for attainment of Yin-Yang balance (Xinrong, 2003). They are different in nature (Yin or Yang), temperature (cold or hot), strength and speed of action. The dual Yin-Yang concept is more and more used by the scientists also, in order to describe the balance/disbalance of various biological or biochemical processes, such as immunoregulation, autoimmunity, inflammation (Mills et al., 2004;

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Zhang, 2007), gene expression (Xu et al, 2013), cause and development of cancer (Wang et al, 2004). During recent years, new advances in the field of Yin- Yang biochemical correspondences have led to the conclusion that Yin might refer to antioxidation and Yang to oxidation (Ou et al, 2003; Szeto et al, 2004; Ko et al, 2006). The majority of the available studies are focused only on the antioxidant properties of the herbs in relation with their Yin-Yang nature, and none on the pro-oxidative potential of medicinal plants.

The present study has the purpose to address this Yin-Yang integration problem, and to develop a potential biochemical tool for estimation of the Yin-Yang nature of Western herbs using two opposite redox parameters (Trolox equivalents antioxidant capacity) "TEAC" and herbal-induced chemiluminescence signal of PMNLs). Free radical generation, which is associated with an ultraweak photon emission (biochemiluminescence), might be described as a Yang process (Yang is related with light, heat, while Yin with dark, coldness). Human photon recording techniques (e.g. chemiluminometry) have been proposed as a potential tool for estimating the oxidative state of organs in relation with Yin-Yang concepts (van Wijk et al, 2010) and represent a technique that allow a better understanding of the complex dynamical biosystems (van Wijk, 2013). ATP-generation through oxidative phosphorylation generates unavoidably reactive oxygen species (ROS) as by products, and this is associated with a weak emission of light (Yang aspect).

Due to the fact that Yang tonifying action involves enhancement of organ functions, which are ATP consuming processes, also cellular production of ATP might be a Yang biomarker (Ko et al, 2007). ATP is known as a macroergic compound, having a high phosphoryl transfer potential (Yang aspect). Yang tonic herbs were found to invariably induce an enhancement of ATP-generation capacity, by an increased mitochondrial electron transport and/or an increased activities of complexes I and III, which are particularly responsible for production of ROS (Ko et al, 2004; Ko et al, 2006; Ko et al, 2007; Wong et al, 2011). On the contrary, Yin tonic herbs had either no effect on ATP generation, or even suppressed this processes in both *ex vivo* and cell-based *in situ* assays (Ko et al, 2006). The cell-based assay of ATP generation capacity was already proposed as a pharmacological test for Yang tonic herbs (Wong et al, 2011).

Taking into account all these aspects, we have considered chemiluminometry (PMNL luminol chemiluminescence assay) and spectrophotometry (TEAC assay) as suitable techniques for studying and estimating Yin-Yang characteristics of five Western herbs, which have a centuries-old tradition as medicinal plants in Europe: *Symphitum officinale*, fam. Boraginaceae (radix)- SYM, *Inula helenium*, fam. Asteraceae (radix)- INU, *Calendula officinalis*, fam. Asteraceae (flores)- CAL, *Angelica arhanghelica*, fam. Apiaceae- radix ANG(R) and folium ANG(F), *Ecbalium Elaterium*, fam.Cucurbitaceae (fruits)- ECB. SYM has an antiinflammatory action, and it is traditionally used for the treatment of painful muscle and joint complaints (Staiger, 2012; Culpeper, 1995). INU exhibits diaphoretic, expectorant, alterative, and stimulant.activities and represents a herbal remedy mainly against respiratory, skin and female genital diseases (Grieve, 1971). CAL is used especially in the treatment of inflammations, gastrointestinal ulcers and dysmenorrhea (Arora, 2013; Culpeper, 1995). ANG exhibits carminative, digestive stimulant, diaphoretic, stomachic, and expectorant properties, being a good remedy for respiratory and digestive diseases (Grieve, 1971; Culpeper, 1995). ECB is a powerful cathartic, and diuretic used in dropsy and edema of renal origin (Griev, 1971; Culpeper, 1995).

We propose a methodology of herbal analysis, based on oxidation-antioxidation theory, which combined with TCM concepts, might offer some keys for a better understanding of the therapeutic potential of these herbs.

Materials and methods

Reagents

All reagents were of pure analytical quality and were purchased from Merck, Darmstadt, Germany (excepting Trolox, luminol and Ficoll 400, which were obtained from Sigma Aldrich Chemie, Steinheim, Germany). Ultrapure water was used throughout.

Samples

Blood and plasma samples were obtained from 7 healthy young non-smoker subjects (women, 5; men, 2; age 20-27 years). None had received any medications 6 months before the study. 10 ml of blood samples were taken into EDTA containing vacutainer tubes from a peripheral vein after 12 hours of fasting. The study protocol was approved by the Local Ethical Commission and written informed consent was obtained from each study participant.

Vegetal aqueous extracts

We used extracts prepared by the traditional method of water extraction. The dried plant material was crushed, macerated and eluted 1:10 with ultrapure water to obtain the hidrophilic constituents. The aqueous extracts was divided in aliquots of 1ml, labeled, and stored at -20°C until testing. This aqueous extract was used in the experiment after it was diluted 1:100 in ultrapure water. In case of SYM, an additional 1:5 dilution was performed.

Trolox equivalent antioxidant capacity (TEAC) of vegetal extracts

Total antioxidant activity was determined based on the 6-hydroxy-2,5,7,8-tetramethyl- chroman-2-carboxylic acid (Trolox) equivalent antioxidant capacity (TEAC) assay developed by Miller and Rice-Evans with minor modifications (Miller et al, 1996; Re et al, 1999). The TEAC assay measures the relative abilities of antioxidants to scavenge the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cation (ABTSx+) in comparison with the antioxidant potency of standard amounts of Trolox, a water soluble vitamin E analogue. The ABTS monocation radical was generated from the interaction between ABTS and potassium persulphate, and it was reduced in the presence of vegetal hydrogen-donating antioxidants. Vegetal extracts (300µl) were mixed with 1700 µl $ABTS^{\bullet+}$ in PBS 5 mM (phosphate buffered saline, pH 7.4) and incubated for exactly 1 min at 30 °C. Optical density (absorbance) was read at 734 nm against 5 mM phosphate-buffered saline (pH 7.4). The percentage inhibition of absorbance was calculated. The assay was calibrated against a Trolox standard curve (0.5-2.5mM Trolox), and the results were expressed as mM (mmol/l) Trolox.

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Isolation of peripheral polimorphonuclear leukocytes and measurement of luminol-enhanced chemiluminescence

PMNL were isolated from healthy blood donors by density gradient centrifugation on Ficoll 400 9% (2.5 ml Ficoll 9% solution to 5 ml blood). After centrifugation at 2000 rpm for 20 min at 20-25°C, PMNL were collected, washed twice, resuspended in 5 ml Hanks Balanced Salt Solution (HBSS) containing 0.1 mM calcium and magnesium salts (HBSS 1:10 diluted in ultrapure water) and adjusted to 2 x 10^7 cell/ml, of which 70% were neutrophils. Cell viability by Trypan Blue exclusion was ≥ 97%. PMNL were used in the assay immediately after the blood was drawn.
The ability of vegetal extracts to induce the ROS synthesis of PMNL was monitored by luminol enhanced chemiluminescence (Trush et al, 1978; Olinescu et al, 1993). The reaction mixture contained 0.1 ml resuspended PMNL in HBSS (2x10^6 cells), 0.1 ml luminol (5-amino-2,3 dihydro-1,4 phtalazinedione) (100μM) and 0.2 ml HBSS. For control, only PMNL, luminol and HBSS were used, while for the samples 100 μl vegetal aqueous extracts (1:100 diluted) were also added. Each tube was agitated for a few seconds and then placed in the thermostated chamber of the LKB 1251 photofluorometer (a temperature of 37°C was used) with a chart recorder. The chemiluminescence was recorded in millivolts (mV). The reaction was observed for 30 minutes. Both the non-stimulated controls (basal chemiluminescence- BCL) and the vegetal extract-incubated PMNL were tested. Measurements were obtained in triplicate. We have calculated three different ratios (R_{15}, R_{30}, Rmax) at 15 min, 30 min and for the extreme (maximal CL) values, as the ratio between the CL value in the presence of the vegetal aqueous extracts and BCL value (from untreated samples). Those indexes higher than 1 were named stimulatory indexes (SI), while those indexes lower than 1 were named inhibitory indexes (II). We measured also the time (T) necessary to reach the maximal CL value. Chemiluminescence of PMNL or whole blood is characterized by large inter-individual variations of both the stimulation index and kinetics even in healthy individuals (Ristola et al, 1989; Olinescu et al, 1993). Although, we preferred to use the mean value of the 7 samples for interpretation and discussions, in place of a single value obtained from a pool sample.

Statistical analysis

Results are presented as mean values with their standard errors of the means. The strength of association between pairs of variables was assessed by Pearson's correlation coefficient. A p value < 0.05 was considered statistically significant.

Results

Trolox equivalent antioxidant capacity of the vegetal aqueous extracts decreased in the following order: SYM> INU> CAL> ANG(F)> ANG(R)> ECB (Tabel 2).

Tabel 2: Trolox equivalent antioxidant capacity (TEAC) of six vegetal aqueous extracts

Vegetal aqueous extracts	Vegetal TEAC
Symphitum officinalae (radix)	413 mM Trolox
Inula helenium (radix)	56 mM Trolox
Angelica arhanghelica (folium)	34 mM Trolox
Calendula officinalis (flores)	39mM Trolox
Angelica arhanghelica (radix)	29 mM Trolox
Ecbalium Elaterium (fruits)	22 mM Trolox

The potency order of the vegetal extracts to stimulate the chemiluminescence signal of PMNL was: ANG(R) > CAL > ANG(F). The potency order of inhibition of the same processs was: ECB > SYM > INU (Tabel 3).

Tabel 3: Influence of six vegetal aqueous extracts on the chemiluminescence signal of PMNL.

Parameter	ANG(F)	CAL	ANG(R)	SYM	INU	ECB
R_{15}	3,237 ± 0,876	1,482 ± 0,866	5,355 ± 1,233	0,049 ± 0.024	0,103 ± 0,043	0,066 ± 0,006
R_{30}	3,119 ± 0,678	0,561 ± 0,299	6,175 ± 1,653	0,041 ± 0,023	0,124 ± 0,044	0,044 ± 0,012
Rmax	3,244 ± 0,765	4,508 ± 2,228	7,164 ± 1,603	0,151 ± 0,069	0,174 ± 0,086	0,117 ± 0,056
T	15min 18sec ± 3min 19sec	9min 2sec ± 5min 26sec	23min 18sec ± 5min 55sec	10min 31sec ± 6min 19sec	14min 37sec ± 9min 19sec	6min 2sec ± 5min 23sec

R_{15}, R_{30}, Rmax - ratios between the CL value in the presence of the vegetal aqueous extracts and the basal CL value (from untreated samples) at 15 min, 30 min and for the maximal CL values; T- time necessary to reach the maximal CL value. All values were expressed as mean ± standard error of the mean.

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Pearson test showed no statistical significant correlation between TEAC and CL- inducing capacity of the herbs.
Because Yin and Yang are relative, describing characteristic tendencies of one thing with reference to another (Gilca, 2013), their manifestations can not be measured as absolute values. Therefore, relative values, such as the percentages or ratios between opposite or complementary parameters (e.g. antioxidant capacity/prooxidative capacity) are probably more suitable for their description. Percentages have also the advantage of being adimensional, so that a comparison between original parameters with different units may be possible. We proposed several percentages as a potential Yin/Yang partial indicators. Due to large differences in TEAC values between *Symphitum* and the rest of the herbs, and also due to the dual significance of R (either stimulation of inhibition of PMNL), before calculating the percentages, we have also used a data logarithmic transformation, so that the values to be more uniformly distributed, and easier to be graphically represented. The logarithm transformations is commonly used for positive data in order to improve interpretability of the results. Either maximal or minimal value of the measured parameters were assigned by convention the 100% value, and the rest of the results, were calculated as percentage from this standard value.
The mathematical formula used for the calculation of percentages, and the potential traditional significance or correspondence are showed in Table 4. The percentages obtained are showed in Figure 1.

Tabel 4: Percentage parameters and their potential traditional correspondence.

Percentage parameters and mathematical formula	Potential traditional correspondence
$TEAC\% = \log_{10}TEAC_i \times 100/\log_{10}TEAC_{max}$	Yin nature (moist, protective)
$R\% = \log_{10} Ri \times 100/\log_{10}Rmax$ (if R>1) $R\% = -\log_{10} Ri \times 100/\log_{10}Rmax$ (if R<1)	Yang nature if R >1 (warm) Yin nature if R<1 (cold)
$Fastness\% = \log_{10}Ti \times 100/\log_{10}Tmin$	Yang nature (fast, active)
Slowness % = log10(1/Ti) x 100/log10(1/Tmax)	Yin nature (slow, inactive)
Relative speed of action (RSA) % = |slowness%- fasteness% |	Yin nature (if slowness% > fastness%) Yang nature (if slowness% < fastness%)

Tmin-minimal value of T; Tmax-maximal value of T

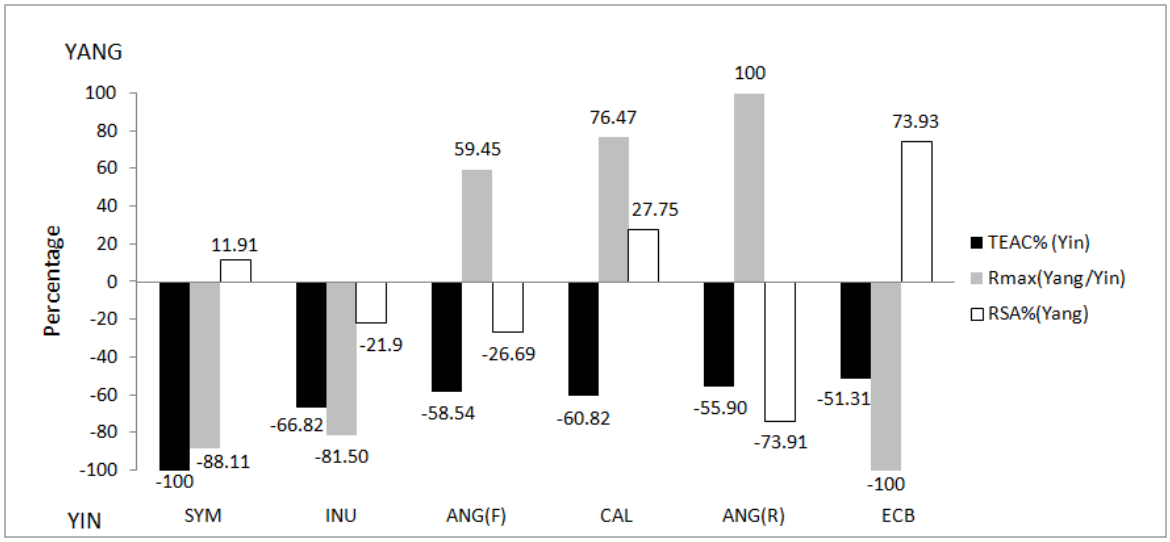


Figure 1: Yin/Yang nature of five Western medicinal plants.

TEAC- Trolox equivalent antioxidant capacity; Rmax;%- PMNL stimulation% (positive values) or PMNLs inhibition% (negative values); RSA% - relative speed of action%; *Symphitum officinale*- SYM, *Inula helenium* (radix)- INU, *Calendula officinalis* (flores)- CAL, *Angelica arhanghelica* (folium)- ANG(F), *Angelica arhanghelica* (radix)- ANG(R), *Ecballium Elaterium* (fruits)- ECB. The values represent the mean percentage values.

In the end, we integrated all the percentages, after we have assigned a negative value to those with Yin significance, and a positive value to those with Yang significance. The results were either positive, or negative (Figure 2).

The lower the negative integrated percentage values, the higher the Yin predominance of the herb (Yin strength). The higher the positive integrated percentage values, the higher the Yang predominance of the herb (Yang strength).

Discussion

Yin-Yang theory is a model that can be applied to any natural system (e.g. human body, cell, medicinal plants, etc) in order to understand better its complexity. Various teams of scientists claim that herbs having Yin nature show antioxidant properties in a higher degree than Yang active

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herbs, while the herbs having Yang nature have the capacity to increase oxidative phosphorylation, ATP synthesis, and consequently, the ROS production. Using a combined approach based on similar claims, we have tried to analyze and compare the Yin/Yang nature and Yin/Yang degree of power in the case of five Western herbs.

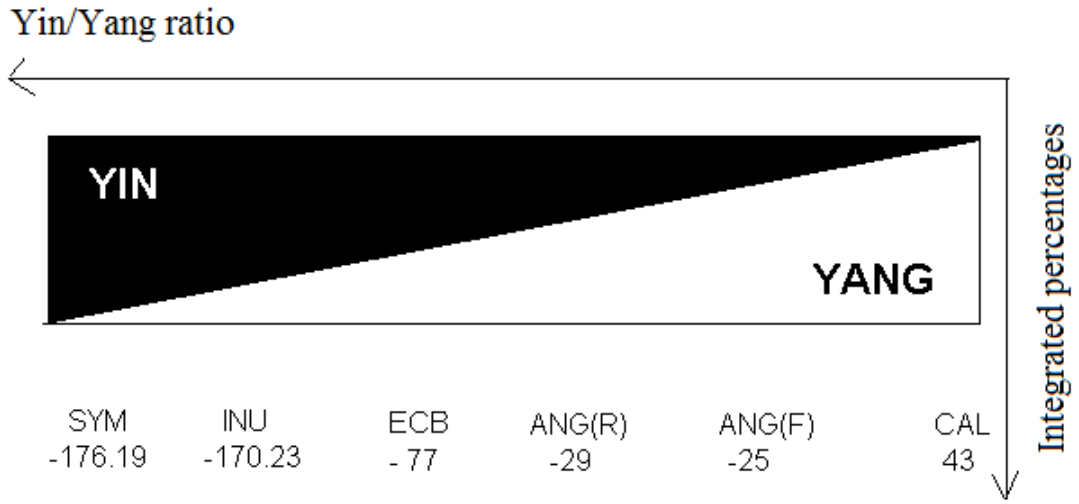


Figure 2: Yin -Yang relative nature and strength of five Western medicinal herbs.
The values represent the integrated value of all percentage type Yin-Yang indicators (IP = - %TEAC + % Rmax + % RAS). Negative values signify a stronger "Yin" trait, positive values signify a stronger "Yang" trait. SYM- *Symphitum officinale*, INU- *Inula helenium* (radix), *CAL- Calendula officinalis* (flores), ANG(F)- *Angelica arhanghelica* (folium), ANG(R)- *Angelica arhanghelica* (radix), *ECB- Ecballium Elaterium* (fruits)

The most potent Yin herb was found to be SYM. In Western phytotherapy, SYM is characterized as being cold (Yin) in the first degree, earthy and having heat clearing action (antinflammatory, antiulcerative, etc) (Culpeper, 1995). The nature of European herbs, which is described by certain Western authors (Culpeper, 1995), in terms of four qualities (cold/warm, moist/dry) might be partially correlated with the Yin-Yang nature described in TCM system. In our study SYM showed the biggest TEAC values (Yin), and it also had a significant inhibitory effect of the CL signal (cold-Yin). It is worthy to mention that SYM contains vitamin B_{12} (EMA Assesement Report, 2009), which is uncommon for herbs, and allantoin. Vitamin B_{12} is required for the red blood cell production (Blood is Yin) and allantoin stimulates the cell growth and proliferation (Yin) (Araújo et al, 2010; Ahn et al, 2013).

ANG(R) is one of the best warming remedy in Western Pharmacopea (hot in the third degree) (Culpeper, 1995). Its Chinese related specia (*Angelica sinensis*) is also described as warm (Yang aspect) and as a Blood tonifying herb (Yin aspect). Both traditional features could be recognized in our results: ANG(R) showed the strongest stimulatory effect on CL of PMNL (hot-Yang), but also this action appeared to be the slowest (slow-Yin) among the six herbs (the highest T). This might reflect the Yin-Yang relationship of *mutual transformation*: when extreme, Yang gives rise to Yin, when extreme, Yin gives rise to Yang (Liu et al, 2009). A similar situation is with ECB, which has the most potent inhibitory effect on the CL of PMNLs (Yin aspect), but also the fastest action (Yang aspect) among the six extracts (the lowest T). Due to the high accumulation of water in its fruits (the inner pressure within the fruits increases during ripening from 8.5 to 14 Bar), these explodes violently and the seeds are thrown at distances of more than 10 meters (Sengbusch, 1996-2004). The plant is called very suggestively "squirting" or "jumping" cucumber, It is also described as a drastic purgative that purge violently. It belongs to the family of cucumbers, which are cold in the second degree (Yin aspect) according with the Western herbalism (Culpeper, 1995). We estimate that in TCM, ECB would be described as both Yang invigorating and Yin nourishing herb, with a mild potency.

CAL is a well known antinflammatory and wound healing plant (ESCOP Monographs, 2003). Whilst this herb showed the weakest antioxidant activity (its TEAC is the lowest one), there was a dual effect on CL signal: stimulatory at 15 minutes (hot-Yang), and inhibitory at 30 minutes (cold-Yin). This might explain why some traditional sources consider CAL hot (hot in the second degree) (Culpeper, 1995), while other cold (Zhou et al, 2011). We suggest a more neutral nature in case of such herbs as CAL, which have a dual effect on CL. Due to the fact that nothing in nature is perfectly neutral (50% Yin and 50% Yang, or Yin and Yang in precisely the same way, at the same moment, and in the same space) (Wang, 2012), we concluded nevertheless, that CAL probably has a tendency to be warming, due to the fact that the stimulatory action is 8 times stronger than the inhibitory one. The duality of CAL was also evidentiated in other cases: immunoregulation (Jiménez-Medina et al, 2006), citoprotection-citotoxicity (Barajas-Farias LM et al, 2006).

INU is described as hot and dry in the third degree in Western phytotherapy (Culpeper, 1995). It removes the stagnancy, and it is indicated in belching, nausea, vomiting, being a potential harmonizer of the flow of Qi (Yang aspect). Flowers of *Inula helenium* are used in TCM to downbear the counterflow ascent of Stomach Qi (Wiseman et al, 1996). Unexpectedly, our results suggest a "Yin" trait of INU relative to the other herbs tested (which would not exclude a Yang predominance, when compared with other herbs non-tested). Although it is hot, INU and its compound isohelenin, showed a potent paradoxical anti-inflammatory activity (Lee et al, 2007; Mazor et al, 2000), which is related more with the "Yin" trait.

ANG(F) has similar effects with ANG(R) in terms of Yin-Yang predominance, but seems to be less potent than ANG(R) in terms of Rmax% ($p<0.05$). Our results are similar with other scientists results (Liao et al, 2008), who found a positive correlation between antioxidant properties (oxygen radical absorbance capacity- ORAC values) and Yin qualities of medicinal plants. Although, there are still opposite opinions about this correspondence. For instance, Liao et al. (2008) found a significant relationship between flavors and ORAC values, but no significant correlation

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between ORAC values and natures or functions of the herbs: bitter and/or sour herbs had the highest ORAC values, while pungent and/or sweet herbs the lowest. We did not find such a relationship between TEAC and flavors. On the contrary, SYM, which is a sweet plant, had the highest TEAC value. Szeto et al. (2007) found that more Yang tonic than Yin tonic plants had DNA protective activity against oxidant challenge, and concluded that the Yin nature may not be necessarily associated with higher antioxidative capacity. We agree with this conclusion, taking into account that antioxidative capacity is only one Yin aspect, while the Yin/Yang nature of a plant is the result of the integration of all Yin and/or Yang aspects. We suggest that antioxidant capacity might be correlated with the moist-dry pair of qualities (most Yin herbs in our study were SYM, INU and ECB, which contain an important amount of polysaccharides and mucilaginous compounds), chemiluminescence inducing capacity with the hot-cold pair, and T with fast-slow pair. Each biochemical parameter estimate only a single Yin/Yang quality.

Another important aspect is that the same herb can possess both Yin-nourishing and Yang-invigorating activities, e.g. *Cordyceps sinensis* (Siu et al, 2004; Li et al, 2009). Having a Yin-nourishing potential does not exclude the Yang invigorating capacity, which means antioxidant potential does not eliminate the stimulatory activity on the PMNL free radical generation. Therefore, it is important for the scientists to be aware of the fact that the relationship between Yin and Yang could be characterized in many different ways, being neither dualistic,or simple dialectical (Wang, 2012).

We also suggest that the herbs with higher antioxidant capacity, stronger inhibitory effect on CL signal, and which are slower in action (Yin active herbs) might be useful in the treatment of those diseases associated with oxidative stress (”excess of Yang” or ”deficiency of Yin”), while those herbs with lower antioxidant capacity, stronger stimulatory effect on the respiratory burst, and which are faster in action (Yang active herbs) might be beneficial in those pathologies associated with reductive stress (”excess of Yin” or ”deficiency of Yang”).

Limitations of the study

We underline that the absolute Yin/Yang nature of Western herbs is either difficult or impossible to be estimated, since Yin and Yang denote relative qualities. We do not claim also that only few biochemical tests are enough for solving the Yin-Yang integration problem, but these may represent a starting point. Additional redox parameters (e.g. antioxidant enzyme activities of herbal extracts) should be tested in order to confirm our hypothesis. The four qualities from Western herbalism, which trace back to Aristotle and Greek medicine, might not perfectly overlap on Yin-Yang qualities. Unfortunately, over the centuries the original meanings of both the Western and Chinese terms have been partially forgotten, so that today it is difficult to understand them, especially within the modern concepts.

Conclusions

According to our redox based method, Yin/Yang ratio of the five tested Western herbs decreases in the following order: SYM> INU> ECB>ANG(F)> ANG(R)>CAL.

The present reflections on redox properties of herbal aqueous extracts and Yin-Yang theory suggest a potential tool for estimating the Yin/Yang predominance of a herb (mainly relative to other herbs).

The work is in progress in our laboratory, in order to find better tools to identify the subtle Yin Yang nature of Western herbs.

Our paper shed some light on how the complexity of the traditional Chinese concepts should be approached from a modern bioscientific view. Further studies are necessary in order to completely solve the Yin/Yang integration problem.

Authors' contributions: MG conceived the study, carried out the chemiluminescence assays, participated substantially in interpretation of data and drafted the manuscript. LG participated in the TEAC assays and performed the mathematical calculations and statistical analysis. DL participated in chemiluminescence assays. IS carried out the TEAC assays, participated in its coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Competing interests: The authors declare that they have no competing interests.

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