ESL 3: The pharmacological assay as a tool to medicinal plants domestication

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Abstract

In Brazil studies with native medicinal plants are usually performed using non-domesticated plants and as a result the genetic variability of wild species could express different levels of active principles changing their therapeutic effect. Based on that, the aim of this study was to demonstrate that extract of different half- sib families *Cordia verbenacea* (DC), widely used as medicinal plant in Brazil, have different efficacy in the Total Growth Inhibition (TGI) of 5 different human tumor cell lines. Data were statistically analyzed using ANOVA follow by Tuckey test and a heritability estimation of the plant families was performed. The results showed that TGI are different for each plant family according with each human tumor cell line. For instance, extracts obtained from families 3,11 and 12 were more effective to inhibit the U-251 and Ht-29 cell lines compared to the other families, while extracts obtained from the family 32 was more effective against thethe PC-3 line. The heritability coefficient indicated that plant population selection could promote a genetic improvement related to its active principle and their pharmacological effect and could provide the identification of the best families according to their pharmacological efficacy. In conclusion, this study suggests that the domestication of a wild medicinal plant should be better monitored by its pharmacological effect.

Keywords: medicinal plants, pharmacological effects, domestication

Introduction

In Brazil studies with native medicinal plants are usually performed using non-domesticated plants. However, the genetic differences among the wild population make difficult a better interpretation of chemical assays. Typical genetic variability frequently occurs in wild species that, as a result, will express different levels of active principles changing the efficacy of their therapeutic effect. Indeed the molecules with pharmaceutical interest are resulted from the secondary metabolism of plants that, in turn depends on genetic and ambient factors (Khanna & Shukla, 1991; Franz, 1996; Pank, 2006). Once the intrinsic value of a medicinal plant is its therapeutic effect, concerning its active principle, pharmacological assays could be used to determine the phenotype of an individual or a studied population. *Cordia verbenacea* DC. Boraginaceae is a medicinal plant widely used in Brazil. Their effects are popularly described as anti-inflammatory, anti-arthritic, analgesic and antiulcerogenic (Lorenzi and Matos, 2008). Based on that, the aim of this study was to demonstrate that extract of different families of the *Cordia verbenacea* have different efficacy in the Total Growth Inhibition (TGI) of 5 different human tumor cell lines.

Materials and Methods

Plants extracts samples are obtained from a field experiment involving 12 half-sib families of *Cordia verbenacea*. The experimental design was a complete randomized blocks with four replications. Each parcel contained 5 plants cultivated in a 100 x 200 cm distance. After 1 year, leaves were harvested, separated from their branches and dried in an oven at 40 °C. The dried leaves are grinded to prepare a dry extract. The extract was prepared with ethanol (96º GL) in the proportion of 1:5 (dried plant: solvent) with mechanical agitation for 1 ½ h (3 x) at room temperature. The extracts were then pooled, filtered and evaporated to dryness.

Human tumor cell lines used: U251 (glioma), MCF-7 (breast), NCI-H460 (lung, non-small cells), HT-29 (colon), PC-3 (prostate) were kindly provided by National Cancer Institute (Frederick, MA, USA).

Cell culture: stock cultures were grown in medium RPMI 1640 (GIBCO) supplemented with 5 % fetal bovine serum (FBS, GIBCO) and 10 U/mL penicillin, 10 µg/mL streptomycin at 37 °C in 5 % CO_2.Antiproliferative assay: cells in 96-well plates (100 µL cells/well) were exposed to SDE (0.25, 2.5, 25 and 250 µg/mL in DMSO/RPMI) at 37 ºC, 5 % of CO_2 in air for 48 h. Doxorubicin (DOXO) was used as standard (0.025, 0.25, 2.5 and 25 µg/mL). Final dimethyl sulfoxide (DMSO) concentration did not affect cell viability (0.25 %). Before (T0 plate) and after sample addition (T1 plates), cells were fixed with 50 % trichloroacetic acid and cell growth determined by spectrophotometric quantification (540 nm) of cellular protein content using sulforhodamine B (SRB) assay (MONKS et al., 1991; SHOEMAKER, 2006). The TGI (concentration that produces total growth inhibition) was determined through non-linear regression analysis using the concentration-response curve for each cell line in the software ORIGIN 8.0® (OriginLab Corporation).

One-way ANOVA was performed to determine if the Total Growth Inhibition (TGI) were significant different ($p<0.05$) among treated groups. If so, the post hoc contrasts using the Tukey test were performed to determine the basis of the significant difference.

Results

As showed in table 1, different plant families had different magnitude of the TGI on human tumor cell lines, such as U251 (glioma), NCI-460 (lung non-small cell), PC-3 (prostate) or HT-29 (colon), but not in human tumor cell line MC-7 (breast). The effect analyzed by block was also significant; indicating that the complete randomized block experimental design choice was correct. The coefficient of variation indicates a good precision of the experiment, except concerning to the human tumor line MC-7. The heritability estimates indicates that selection in that population would promote genetic gains.

Tab. 1 Different plant families of half-sib familiesof Cordia verbenacea show differences in Total Growth Inhibition of human tumor cell lines.

Source of Variation	Freedom Degree	Mean Square				
		U251 µg/ml	MC-7 µg/ml	NCI-460 µg/ml	PC-3 µg/ml	HT-29 µg/ml
Block	3	526,4**	122,20	9547,4**	1684,4**	9088,8**
Half-sib Family	11	1209,6**	229,16	8662,1**	5930**	9371,7**
Error	33	60,7	105,90	87,9	104,6	242,9
Mean		62,4	44,3	114,2	83,3	110
Cv		12,5	23,20	8,2	12,3	14,2
h^2		0,73	0,36	0,75	0,74	0,73

The symbol (**) means different (P<0.01; ANOVA) among plant families related to Total Growth Inhibition (TGI) of U251 (glioma), MCF-7 (breast), NCI-H460 (lung, non-small cells), HT-29 (colon), PC-3 (prostate) cell lines. Cv = coefficient of variation; h^2 = heritability estimates

As shown in table 2, different families could be selected according to their effect in Total Growth Inhibition of the human tumor cell lines U-251, NCI-460, PC-3 e HT-29, but not MC-7 cell line. Plants extracts with lower TGI indicates greater concentration of the active principle or greater synergic effect. Therefore, as smaller is the mean as bigger is its TGI. Agreeing to these data it is possible to select the plant families that are more effective in each human tumor cell line tested. The statistical analysis shows (ANOVA follow by Tuckey post hoc test; p<0.05). The families 11, 3, 12 and 16 were more effective against U-251, NCI-460, PC-3 and HT-29 cell lines.The family 9 was more effective against NCI-460, PC-3 and HT-29 cell lines. The family 26 was more effective against HT-29 cell line. The family 32 was more effective against PC-3 cell line

Tab. 2 Concentration of plant extract necessary to achieve Total Growth Inhibition of different human tumor cell lines. Different letters means statistically differences (Tuckey test; p<0.05).

U-251	Half-sib family	21	32	9	24	2	33	10	26	16	12	3	11
	TGI mean (µg/ml)	123,3 a	87,2 b	71,2 bcd	68,9 bcd	67,6 bcd	66,2 bcde	58,9 bcde	48,4 cde	48,1 de	46,0 de	45,5 de	37,9 e
MCF-7	Half-sib family	33	32	9	26	10	24	11	16	3	21	2	12
	TGI mean (µg/ml)	67,1 a	56,0 a	51,4 a	51,2 a	46,7 a	44,7 a	41,7 a	37,9 a	36,3 a	31,7 a	30,5 a	30,3 a
NCI-460	Half-sib family	32	26	33	21	24	16	3	12	11	10	9	2
	TGI mean (µg/ml)	177,7 a	168,2 abc	163,9 abc	154,5 abc	150,5 abc	140,3 bcd	110,7 d	68,7 e	39,3 e	39,0 e	35,7 e	35,6 e
PC-3	Half-sib family	21	16	26	33	3	9	10	2	24	32	12	11
	TGI mean (µg/ml)	154,6 a	140,3 a	139,6 a	135,4 a	54,7 b	51,9 b	50,5 b	49,6 b	48,5 b	45,5 b	44,1 b	42,2 b
HT-29	Half-sib family	2	33	32	21	24	9	16	12	3	11	26	10
	TGI mean (µg/ml)	212,4 a	181,7 ab	169,4 abc	143,3 bc	121,5 cd	69,2 d	68,7 d	65,3 d	62,6 d	59,4 d	56,6 d	49,3 d

Conclusion

Take together, the data of this study suggest that the domestication of a wild medicinal plant should be initially monitored by its pharmacological effect instead by its chemical composition, once it is inconstant and depends on its genetic variability and ambient factors (phenotype)

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