In Vitro Analysis of the Herbal Compound Essiac®

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Abstract. *Background: Despite the recommendation of the Task Force on Alternative Therapies of the Canadian Breast Cancer Research Initiative, little research has been published on the widely used herbal compound Essiac®. We aimed to address this deficiency by conducting a series of assays to determine some of the purported activities of Essiac® in vitro. Materials and Methods: The activity of Essiac® was measured using established assays to assess anti-oxidant, fibrinolytic, anti-microbial, anti-inflammatory, immune modulation, cell-specific cytotoxicity, and impact on cytochrome P450 (CYP450) enzyme pathways. Results: Essiac® exhibited significant antioxidant activity in the ABTS assay. A 20-fold dilution of Essiac® also exhibited significant immuno-modulatory effects, specifically through stimulation of granulocyte phagocytosis, increases in CD8+ cell activation, and moderately inhibiting inflammatory pathways. Essiac® exhibited significant cell-specific cytotoxicity towards ovarian epithelial carcinoma cells (A2780). Importantly, a 20-fold dilution of Essiac® showed significant inhibition of several CYP450 enzymes, most notably CYP1A2 (37%) and CYP2C19 (24%). Essiac® demonstrated dose-dependent inhibition of clot fibrinolysis. Conclusion: In vitro analysis of Essiac® indicates significant antioxidant and immuno-modulatory properties, as well as neoplastic cell specific cytotoxicity consistent with the historical properties ascribed to this compound. Importantly, significant CYP450 and fibrinolysis inhibition were also observed. This is the first comprehensive investigation of the in vitro effects of Essiac®.*

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The use of complementary therapies amongst women with breast cancer in Canada ranges between 20% and 80% (1). Essiac® is a widely-used by breast cancer patients herbal compound; 15%-35% of the patients in the province of Ontario, Canada, use this compound (2, 3). A recent survey of Complementary and Alternative Medicine (CAM) on Essiac® use by women with breast cancer showed that 7.4% of the women surveyed have actually used it (4). Despite the concerns and recommendations of the Task Force on Alternative Therapies of the Canadian Breast Cancer Research Initiative, little research on Essiac® has been published. A herbal compound mix of the four herbs *Ulmus rubra*, *Arctium lappa*, *Rheum palmatum* and *Rumex acetosella*, Essiac® and similar products have been reported to possess many therapeutic properties, such as anti-inflammatory, immunomodulatory and antineoplastic effects, in line with those exhibited by some of its constituent herbs (5). Two recent studies have supported the proposed antineoplastic properties of Essiac®. One study demonstrated that Essiac® has cell specific cytotoxicity towards both estrogen-positive breast adenocarcinoma (MCF-7) and estrogen receptor alpha-negative adenocarcinoma (MDA-MB-468) cell lines (6). Another study using an androgen-sensitive prostate carcinoma cell line (LNCaP) also demonstrated neoplastic cytotoxicity when cells were exposed to Essiac® (7). The aim of the present study was to comprehensively assess the reported properties of Essiac® using an *in vitro* model, and then to extend the previous research in an *in vivo* model (8).

Materials and Methods

Antioxidant activity. Essiac® (Essiac International Canada, Ottawa, Canada) was centrifuged at 1,500 relative centrifugal force (rcf) for 5 minutes. Working solutions were made from the stock by appropriate dilution in 0.1 M phosphate buffer (pH 7.4). A solution of stable free radical cation (ABTS+*) was made from the oxidation of ABTS (2,2'-azino-*bis*(3-ethylbenzthiazoline-6-sulphonic acid)) with potassium persulphate, and its absorbance at 734 nm was adjusted to 0.700±0.05. Ten μl of the sample dilutions

were added to 1 ml of ABTS+* solution and decreases of absorbance were recorded after 1 minute. A concentration corresponding to 50% antioxidant activity is denoted as AC_{50}, which was obtained from a plot of antioxidant activity *versus* sample concentration. The antioxidant activity was standardised to Trolox, a water-soluble analogue of vitamin E. Trolox equivalent antioxidant activity (TEAA) of a sample is defined as the amount of Trolox equivalent to one unit of test sample when both have 50% antioxidant activity.

Fibrinolytic activity. Essiac® extract was dried at 50˚C under reduced pressure using a rotary evaporator. Water was added to the residual solid and the mixture was dissolved by sonication. Serial dilutions of this stock solution were made up in borate buffer (pH 9.0). All samples were tested in quadruplicate. Pooled plasma euglobulins from four healthy volunteers were precipitated with a 0.001% acetic acid solution and resuspended in borate buffer (pH 9.0). Ten concentrations of Essiac® (range 30 μg/mL to 14.25 mg/mL) were added to the euglobulin fraction and placed in a microtitre plate. The samples were then clotted with the addition of 0.025 M calcium chloride. The plate was scanned every 25 minutes for 20 hours at an absorbance of 405 nm. Lysis times were calculated and the fibrinolytic activity of Essiac® relative to control was determined.

Platelet aggregation. Essiac® was concentrated to 80 μg/μL in water and was used as the stock solution from which dilutions were made in sterile saline. Three agonists: i) Collagen (final concentration=2.5 μg/mL), ii) adenosine diphosphate (ADP) (final concentration=5 μM), and iii) platelet activating factor (PAF) (final concentration=0.2 μM) were used to assess platelet aggregation. Platelet rich plasma (PRP) and platelet poor plasma (PPP) were prepared from four healthy volunteers in tubes containing 0.106 M sodium citrate. PRP was prepared by centrifuging the citrated specimen at 200 xg for 10 minutes. PPP was prepared by centrifuging the remaining citrated specimen at 1800 xg for 10 minutes. The more turbid PRP was stirred in a cuvette, and the transmittance of infrared light through the sample, relative to a PPP blank, was measured and recorded. Measurements were made using a Chrono-log model CH680 Aggregometer (Chrono-log Corporation, Havertown, USA). Transmittance was plotted and the area under the aggregation curve between 0 and 6 minutes was calculated (final results expressed in millivolt seconds (mV.s)). All samples were tested in duplicate, using all agonists. Control samples treated only with saline were run before and after each set of samples to monitor platelet function. Ginger (1 μg/μL; Nature's Sunshine Products Inc., Provo, USA) provided a positive control.

Antimicrobial activity. Essiac® extract was dried at 50˚C under reduced pressure using a rotary evaporator. Water was added to the residual solid and the mixture was dissolved by sonication. Further dilutions were prepared using 0.85% NaCl. Essiac® extract was diluted in 0.85% NaCl (Oxoid Australia Pty Ltd., Thebarton, Australia) and tested at doubling dilutions from 0.125-16 mg/mL in Mueller Hinton Agar (Oxoid Australia Pty Ltd., Thebarton, Australia). Mueller Hinton agar (Oxoid) with 0.85% NaCl was used as a positive growth control and agar plates with the broad-spectrum antibiotic erythromycin (ICN Biomedical Inc. Costa Mesa, USA) at concentrations of 0.62-32 μg/mL were used for quality control. Inoculum was prepared in 0.85% NaCl to a concentration of approximately 10^7 CFU/mL for bacteria and 10^6 CFU/mL for *Candida albicans* using a CrystalSpec Nephelometer (Becton Dickinson, Franklin Lakes, USA). Plates were inoculated with 1-2 μL spots containing approximately 10^4 CFU of each bacteria and 10^3 CFU of *C. albicans*, using a multipoint replicator (MAST). Inoculated plates were then incubated aerobically at 35˚C for 20 hours. Minimum inhibitory concentrations (MIC's) for each micro-organism were determined by finding the plate with the lowest concentration of extract on which the organism would not grow.

Arachidonic acid metabolism. Essiac® extract was centrifuged at 1500 rcf for 5 minutes and the supernatant was used in cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), 5-lipoxygenase (5-LO) and phospholipase A2 (PA_2) assays. Three concentrations of Essiac® at 20%, 10% and 5% of total assay solution were tested for dose-response.

COX-1 and *COX-2* assay: The source of COX-1 was ram seminal vesicle, and the source of COX-2 was sheep placenta. Enzymes were activated with hematin cofactor (1 μM) in 0.1M Tris-HCl buffer pH 8.0, containing 5 mM EDTA and 2 mM phenol. Ten μL test and control samples were added and incubated at 37˚C for 5 minutes. The reaction was started by the addition of [1-^{14}C]-arachidonic acid substrate and the mixture was further incubated at 37˚C for 15 minutes. The enzymatic reaction was terminated by adding 40 μL of 4 M formic acid. Arachidonic acid metabolites, prostaglandin E_2 (PGE_2) and prostaglandin D_2 (PGD_2), were extracted from the mixture with chloroform and separated using thin layer chromatography (TLC). The inhibition of COX-1 activity was calculated as the percentage inhibition of the production of PGD_2 and PGE_2 by the test sample in comparison with the blank.

5-LO assay. Test samples were incubated with human recombinant 5-LO (46 μg protein) for 10 min at 24˚C before the enzyme reaction was initiated through the addition of [1-^{14}C] arachidonic acid (50 nCi). The reaction was terminated after 5 minutes by acidification. The arachidonic acid metabolite, 5(S)-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE), was extracted from the mixture with chloroform and separated using TLC. The inhibition of 5-LO activity was calculated as the percentage inhibition of the production of 5-HETE.

Phospholipase A2 assay. Porcine pancreas PA_2 (Sigma Aldrich, Sydney, Australia) with a protein concentration of 3.0 mg/mL and specific activity of 1600 units/mg protein was diluted 1:100 in PA_2 buffer before use. Ten μL of diluted enzyme solution was mixed with the appropriate amounts of test sample or control and incubated at 37˚C for 5 minutes. The reaction was initiated by the addition of 20 μL of enzyme/test sample mixture to 30 μL of radioactive labelled 1-stearoyl-2-[1-^{14}C]-arachidonyl-L3-phosphatidyl-choline (Amersham Australia, Sydney, Australia) substrate, then incubated at 37˚C for 5 minutes. The reaction was terminated by the sequential addition of (i) a 50 μL mixture of chloroform:methanol 2:1 (v/v), followed by (ii) 50 μL of chloroform, and (iii) 50 μL of 4 M KCl. The released [1-^{14}C]-arachidonic acid was separated using TLC. The radioactivity (c.p.m.) in samples was measured with liquid scintillation counting. The inhibition of PA_2 activity for the test sample was calculated as the percentage of inhibition of the formation of arachidonic acid by test sample in comparison with control (no test agent).

System controls. Controls, consisting of positive, negative and solvent controls, were performed with the test samples to verify the execution of the assays. Indomethacin was used as a positive

control for COX-1, COX-2, and 5-LO assays. Arachidonyl trifluoromethyl ketone (ATKetone) was used as a positive control for the PA_2 assay. Inactivated enzymes and assay buffers were used as negative and solvent controls (blank), respectively. Experiments were performed in triplicate.

Cell-based immunoactivity. Essiac® extract was syringe-filtered through a sterile membrane (0.2 μm) and sterile phosphate-buffered saline (PBS) was used as a solvent control. Human peripheral blood was collected into heparin or EDTA tubes preceding the experiments. Phagocytosis of *Escherichia coli* in human peripheral blood was measured using a 'Phagotest' test kit (Orpegen Pharma, Heidelberg, Germany). Phagocytosis of *E. coli* was carried out at 0˚C without phagocytic activity, and at 37˚C where phagocytosis occurred. Test samples, in concentration of 2% and 5% (v/v) and controls were added to the aliquots of human peripheral blood before the addition of *E. coli*. Results were reported as the percentage of granulocytes or monocytes which were phagocytic. Lymphocyte activation was measured according to the expression of activation marker CD69 in T-lymphocytes. BD FastImmune T-cell value bundle (Becton-Dickinson, Franklin Lakes, USA) (CD4/CD69/CD3, CD8/CD69/CD3, and γ1/γ1/CD3) was used to determine the population of activated CD4 and CD8 lymphocytes following the manufacturer's methodology. Test samples and controls were added to the aliquots of human peripheral blood and incubated at 37˚C in 5% CO_2 for 4 hours prior to flow cytometry. Results were obtained as a percentage of activated CD4 or CD8 cells (which expressed CD69). The subset population of lymphocytes in whole blood samples was determined using BD MultiTEST 4-color reagents (Becton-Dickinson) (CD3/CD8/CD45/CD4) following manufacturer's methodology. Test samples and controls were added to the aliquots of human peripheral blood and incubated at 37˚C in 5% CO_2 for 24 hours before flow cytometry. Results were reported as a percentage of CD4 or CD8 subsets in the lymphocyte population. Flow acquisition and data analysis were performed using 4-color BD FACSCalibur and Cell Quest software (version 2.3) (Becton-Dickinson). Statistical analysis was performed using GraphPad Prism Version 2.01 (GraphPad Software Inc., San Diego, USA).

Neoplastic cell specific cytotoxicity. Essiac® extract was dried under reduced atmosphere and concentrated to a dark oil (41.6 mg/ml of total extract) from which serial aqueous dilutions were made. To mimic conditions in the gut and potentially to activate compounds in the herbal mixture, the concentrated extract was enzymatically deglycosylated with commercial glucosidase and glucuronidase. Enzymatic treatment was performed in 5% ethanol in citrate buffer (pH 5.0) at 37˚C for 60 min. The reaction was stopped with rapid cooling followed by dehydration under vacuum. Enzymes were precipitated by dissolution in 70% ethanol and the supernatant was resuspended in 10% ethanol. The resuspended supernatant was used for chemical and biological testing. Essiac® and enzyme-treated Essiac® were tested against six cell lines including: HepG2 (hepatocyte carcinoma), MCF-7 (mammary gland carcinoma), A2780 (ovarian carcinoma), PC3 (prostate adenocarcinoma), A375 (malignant melanoma) and Hs27 (normal foreskin). Cells were grown in 100 μL/well of relevant media on 96-well plates and were exposed to 1:5, 1:10 and 1:20 dilutions of Essiac® liquid extract in media for 24 h at 37˚C and 5% CO_2. Water controls of the same volume were also tested. Cell viability was assessed by measuring cellular ATP (ATPlite-M assay; Perkin Elmer, Waltham, Massachusetts, USA). Final concentrations of Essiac® were 2, 4 and 8 mg/mL, respectively.

Table I. *The antioxidant activity of Essiac®.*

Samples	AC_{50}[a]	Antioxidant activity	
		% Decrease of ABTS	TEAA[b]
Trolox	2.8 μg/mL	50	-
ESSIAC	3.49 μg/mL (SEM±0.0138, N=4)	50	80.2 μg Trolox per 1 mL ESSIAC

[a]The concentration corresponding to 50% antioxidant activity; [b]Trolox equivalence antioxidant activity.

Cytochrome P450 enzymes. CYP450, 1A2 human recombinant Sf9 tissue was combined with 5 μM 3-cyano-7-ethoxycoumarin in a 1% dimethly sulfoxide (DMSO) solution and incubated for 30 min at 37˚C in 75 nM potassium phosphate buffer at pH 7.5. The amount of 3-cyano-7-ethoxycoumarin remaining after incubation was assessed using spectrofluorometric means. A greater than 50% of max stimulation or inhibition is considered significant.

Results

Antioxidant activity. Table I shows the antioxidant activity of Essiac®. The AC_{50} of Essiac® was 3.49 μL/ml, or 0.349% (v/v). The TEAA was 80.2 μg Trolox per 1 mL Essiac®. As the original Essiac® solution was found to contain 36.2±0.60 mg/mL, TEAA value of Essiac® herbal extract can also be expressed as 2.2 μg Trolox per mL Essiac® extract solid.

Fibrinolytic activity and platelet aggregation. At the concentrations tested, Essiac® produced no significant reduction in clot lysis time (Figure 1). At higher concentrations, Essiac® interfered with normal clot breakdown. No significant difference was found between results produced with the addition of Essiac® *versus* the control values for any of the three agonists (Figure 2). A comparison using one-way ANOVA (SPSS for Window, Ver 11.0, Chicago, Illinois, USA) of control *versus* the positive control (1 μg/μL ginger) demonstrated significant results for all three agonists, ADP ($F=56.3$, $p<0.001$), PAF ($F=11.2$, $p=0.002$) and collagen ($F=31.5$, $p<0.001$).

Antimicrobial activity. Essiac® extract inhibited none of the microorganisms tested at a concentration of 16 mg/mL (data not shown).

Effect on enzymes of the arachidonic acid metabolism pathway. Essiac® demonstrated a moderate inhibition of 5-LO and a slight inhibition of COX-1, COX-2 and PA_2 activities at a final concentration of 20% (v/v). The COX-1, COX-2, 5-LO and PA_2 activities were assayed in the presence of three concentrations of Essiac®. The percentage inhibitions of Essiac® tested on COX-1, COX-2, 5-LO and PA_2 are summarized in Table II.

Cell-based immunoactivity. Figure 3 shows the effect of two Essiac® concentrations (2% and 5% v/v) on the phagocytosis of *E. coli* by granulocytes and monocytes in the human peripheral blood from two donors. In comparison with the PBS control (61.57%±0.88 SEM, N=2), Essiac® at 5% v/v (75.69%±0.13 SEM, N=2) significantly enhanced the percentage of granulocytes which engulfed *E. coli* (p=0.0039). However, no significant effect on the granulocytes was observed at a concentration of 2% v/v. Moreover, no significant effect on the phagocytic activity of the monocytes was observed at 2% or 5% concentrations of Essiac®.

Activation of CD4+ and CD8+ T lymphocytes. Figure 4 depicts how the incubation of 2% Essiac® with blood samples for 4 hours results in increase (p=0.0315) in the population of activated CD4 (1.25%±0.091 SEM, N=4) compared to the PBS control (0.825%±0.122 SEM, N=4). However, this increase was not observed at 5% Essiac® (0.967%±0.029 SEM, N=4) in comparison with the PBS control (p=0.298). It appears that the marginal increase of the activated CD4 population observed with 2% Essiac® may not be caused by Essiac® itself. Moreover, in comparison with the 10 μg/ml phytohemagglutinin (PHA, 11.19%±0.230 SEM, N=2), Essiac® at both concentrations showed significantly lower activity (p<0.0001). The effect of Essiac® on the activation of CD4 requires further investigation. In the case of activated CD8 (Figure 4), Essiac® at concentrations of 2% and 5% v/v (2.553%±0.152, N=4; 2.785±0.217, N=4, respectively) more activated CD8 populations were found in comparison with the control (0.495%±0.049, N=4) (p<0.0001). Although their activation showed a highly significant reduction in activation by 10 μg/ml PHA (4.34%±0.240, N=2) (p<0.0001), both 2% and 5% Essiac® demonstrated significantly increased activity over the PBS control (p<0.0001).

Neoplastic cytotoxicity. The results are shown in Figure 5. The most sensitive cell line was A2780 with an IC_{50} of ~4 μL and the least sensitive cell line was HepG2 with an IC_{50} of ~10 μL. Essiac® contains 41.6 mg/ml of total extract. Converting the above values yields IC_{50}'s of 1.5 mg/ml and 3.8 mg/ml. Deglycolysation of the samples had no significant impact on results.

Table II. *Percentage inhibition by Essiac® of COX-1, COX-2, 5-LO and PA_2[a].*

	% Inhibition			
Essiac (%v/v)	COX-1	COX-2	5-LO	PA_2
20	44±1	49±1	55±2	35±1
10	32±1	31±0.9	41±1	16±1
5	19±1	24±0.5	16±1	12±0.5

[a]Data represent mean±S.E.M. from three experiments at each of three concentrations per test sample.

Effect on cytochrome P450 enzymes. The samples were tested against five major cytochrome P450 enzymes by MDS Pharma Services. Essiac® at 9 μg/ml had little effect and at 100 μg/mL inhibited cytochrome P450's 1A2 by 37%, 2C19 by 24%, 2C9 by 13%, 2D6 by 9% and 3A4 by 5%.

Discussion

The alleged antioxidant properties of Essiac® are considered to be derived from the known antioxidant capacities of its constituent herbs. Essiac® contains a number of known antioxidant molecules, including quercetin, genistein, biochanin A, beta-sitosterol, beta-carotene and several tannins (5). Using the previously described ABTS antioxidant assay Essiac® was determined to have a TEAC value of 900 μg/ml. As ascorbic acid has been shown to have a TEAC value of 1.26 that of Trolox® in the ABTS assay (9), this gives Essiac® a calculated vitamin C equivalent antioxidant capacity (VCEAC) value of approximately 2000 mg/100 g. In comparison with other known antioxidants, Essiac® appears to have antioxidant properties that are greater than red wine (600 mg/100 g), green tea (450 mg/100 g), black tea (250 mg/100 g) and cocoa (1128 mg/100 g) (10). The antioxidant capacity of Essiac® was also demonstrated by the work of Leonard *et al.* (11) and Cheung *et al.* (12) in an *in vitro* model.

One of the properties widely promoted in support of Essiac® is its ability to modify the immune response. Other properties, ascribed to the components of Essiac®, that may be relevant include an anti-inflammatory and anti-bacterial effect (5). The assays used to assess immunomodulatory function indicate that Essiac® can inhibit several enzymes in the arachidonic acid metabolism pathway in a dose-dependent manner (Table II). In addition, a dose-dependent enhancement of granulocyte phagocytic but not monocytic activity, was observed (Figure 3). However, no direct anti-bacterial effect was observed. An increase in macrophage phagocytic ability is consistent with results

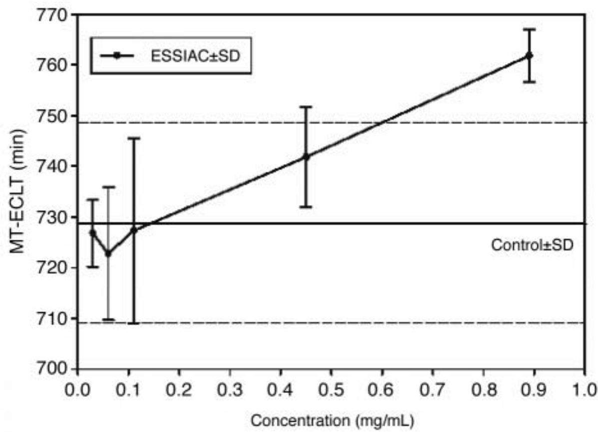

Figure 1. *The effect of Essiac® on clot lysis time.*

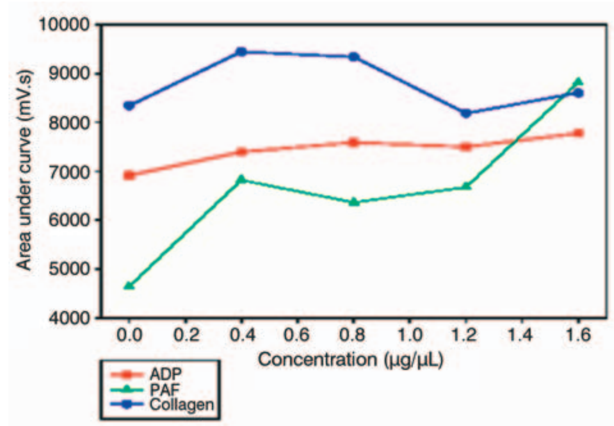

Figure 2. *The effects of Essiac® on platelet aggregation.*

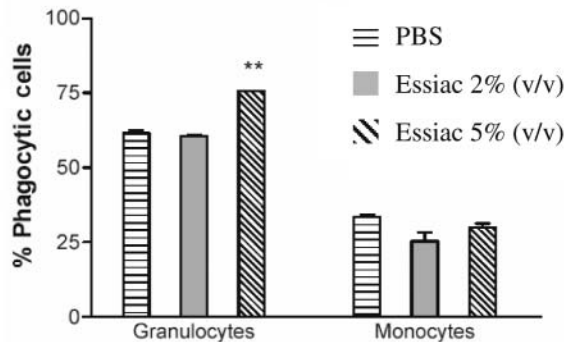

Figure 3. *The effect of Essiac® on the phagocytosis of E. coli by granulocytes and monocytes. Values represent mean percentage of E.coli cells phagocytised. Bars represent the SEM. **Significantly different from PBS and Essiac® at 2% v/v, p=0.0039.*

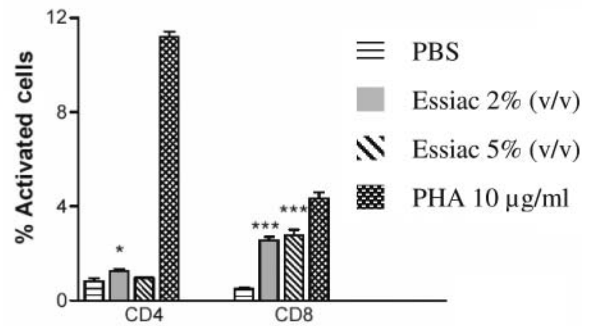

Figure 4. *The effect of Essiac® on the activation of CD4 and CD8 cells. Values represent mean percentage of Activated cells. Bars represent the SEM. *Essiac® demonstrated higher activated CD4 populations versus PBS control, p<0.0001. ***Essiac® demonstrated higher activated CD8 populations versus controls, p<0.0001.*

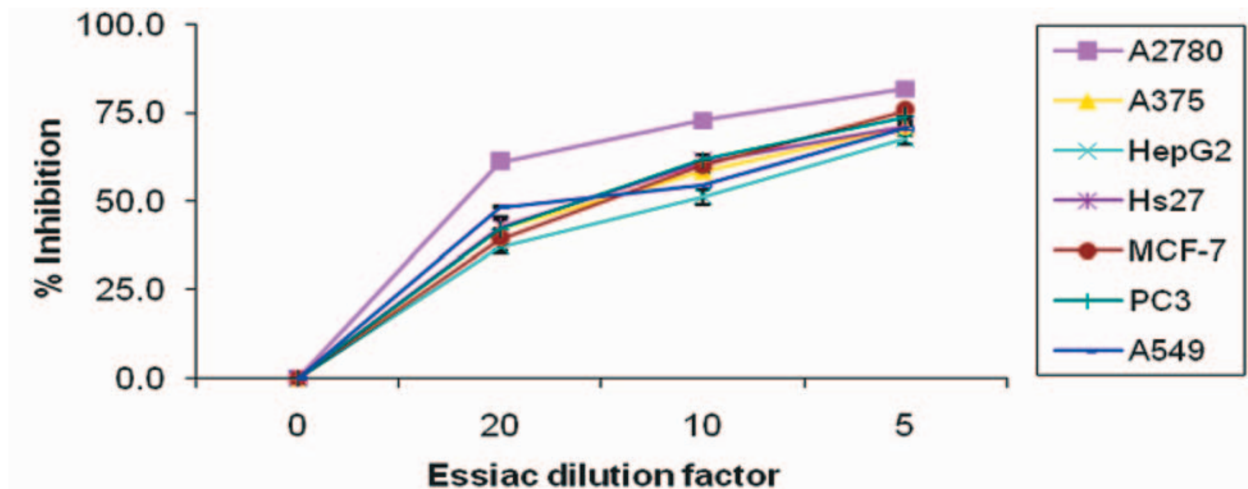

Figure 5. *Cell cytotoxicity of Essiac® % inhibition versus buffer control cell lines: A2780, ovarian carcinoma; A375, melanoma; HepG2, hepatocyte carcinoma; Hs27, human foreskin; MCF-7, mammary gland carcinoma; PC3, prostate adenocarcinoma.*

reported for Flor-Essence® (13), a herbal compound containing the four principle herbs of Essiac® and four additional ones. We found no evidence that the herbs combination in the Essiac® extract demonstrated antimicrobial properties. Previous work has found that an ethanolic extract of one of the Essiac® ingredients, *Arctium lappa*, had weak to moderate activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* (14), and an extract of *Rheum officinale* was also found to have significant activity against *Bacteroides fragilis*, an anaerobic organism (15). The discrepancy of these results is most likely due to dosage.

Treatment of isolated human T-cells from healthy donors with Essiac® significantly stimulated CD8+ cells, in a dose-dependent manner, as measured in a flow cytometric analysis of CD69 expression. Modification of suppressed T-cell function plays a significant role in cancer treatment modalities (16) and enhancement of CD8+ activity by Essiac® may provide an interesting mechanism for further investigation of the purported anticancer effects of Essiac®. Essiac® at a lower concentration had a mild stimulatory effect on CD4+ cells that was not reproducible at higher concentrations.

An investigation of the cell-specific cytotoxicity revealed that Essiac® possessed greater cytotoxicity for neoplastic cells than in normal cells, in agreement with the recent findings of others (6, 7). Several candidate molecules for this effect are present in the component herbs of Essiac®. Major classes of molecules known to possess antiproliferative properties include the anthraquinones contained in *Rheum palmatum* or *Rumex acetosella*, and the isoflavones present in *Arctium lappa* (17). Interestingly, the androgen-insensitive ovarian epithelial cell carcinoma (A2780) and prostate adenocarcinoma (PC3) cell lines showed greater sensitivity than the estrogen-positive mammary gland carcinoma (MCF-7), suggesting a non-androgen-mediated mechanism for cytotoxicity. Cytotoxicity towards PC3 cells was increased upon enzymatic deglycosylation of Essiac®, suggesting involvement of the aglycone in this effect. Treatment with beta-glucosidase does not fully represent the metabolic profile of Essiac® *in vivo*, however it does suggest some alteration of cytotoxic potential upon metabolism.

Essiac® demonstrated an inhibitory effect upon the MCF-7 tumor cells, a result which is consistent with previous findings (6, 7). There is some inconsistency in this area, however, as Kulp and colleagues have found that Essiac® actually stimulated the growth of these tumor cells (18).

Essiac® interfered with clot lysis *in vitro*, showing a dose-dependent increase in clot lysis time that was statistically significant at a 40-fold dilution of the stock solution. The Essiac® component *Ulmus* contains several tannins, members of which have been shown to inhibit the fibrinolytic enzymes t-PA, u-PA and plasmin (19). Oral administration of tannins has been found to be associated with reduced plasminogen activation and carcinogenic inhibition *in vivo* (20). Our results indicate that Essiac® at concentrations greater than that of the stock solution did not interfere with platelet aggregation, suggesting that arachidonic acid pathway inhibition is not sufficient to affect platelet function. Given the hypercoagulant state present in some oncology patients, the potential for disturbances in the haemostatic state by Essiac® warrants further investigation.

Essiac® showed variable potential to inhibit all members of the P450 family tested in the *in vitro* assay at 20-fold dilution, with greatest inhibition exhibited for the CYP1A2 isoform (data not shown), which finding should be further investigated, as patients using Essiac® report its use during chemotherapy.

Conclusion

Collectively, these results indicate that under specific *in vitro* conditions, Essiac® possesses functional effects consistent with some of the general properties ascribed to it, including both immunomodulatory effects and neoplastic cell-specific cytotoxicity. Our results indicate that Essiac® may interact with both the fibrinolytic and P450 metabolic pathways. However, the clinical significance of these observations remains undetermined.

Conflict of Interest

Essiac® International Canada supported the research through an unrestricted educational donation and was not involved in the design or conduct of the study, nor did they participate in the study. The makers and distributors of Essiac® did not review the final document nor any of the initial drafts of this publication.

References

1 Balneaves LG, Bottorff JL, Hislop TG and Herbert C: Levels of commitment: exploring complementary therapy use by women with breast cancer. J Altern Complement Med *12*: 459-466, 2006.

2 Boon H, Stewart M, Kennard MA, Gray R, Sawka C, Brown JB, McWilliam C, Gavin A, Baron RA, Aaron D and Haines-Kamka T: Use of complementary/alternative medicine by breast cancer survivors in Ontario: prevalence and perceptions. J Clin Oncol *18*: 2515-2521, 2000.

3 Gray RE GM, Fitch M, Parry N, Douglas MS and Labrecque M: Perspectives of cancer survivors interested in unconventional therapies. Journal of Psychosocial Oncology *15*: 149-171, 1997.

4 Boon HS, Olatunde F and Zick SM: Trends in complementary/alternative medicine use by breast cancer survivors: comparing survey data from 1998 and 2005. BMC Womens Health *7*: 4, 2007.

5 Tamayo C, Richardson MA, Diamond S and Skoda I: The chemistry and biological activity of herbs used in Flor-Essence herbal tonic and Essiac. Phytother Res *14*: 1-14, 2000.

6 Tai J, Cheung S, Wong S and Lowe C: *In vitro* comparison of Essiac and Flor-Essence on human tumor cell lines. Oncol Rep *11*: 471-476, 2004.
7 Ottenweller J, Putt K, Blumenthal EJ, Dhawale S and Dhawale SW: Inhibition of prostate cancer-cell proliferation by Essiac. J Altern Complement Med *10*: 687-691, 2004.
8 Leonard BJ, Kennedy DA, Cheng FC, Chang KK, Seely D and Mills E: An *in vivo* analysis of the herbal compound essiac. Anticancer Res *26*: 3057-3064, 2006.
9 Betancor-Fernandez A, Sies H, Stahl W and Polidori MC: *In vitro* antioxidant activity of 2,5,7,8-tetramethyl-2-(2'-carboxyethyl)-6-hydroxychroman (alpha-CEHC), a vitamin E metabolite. Free Radic Res *36*: 915-921, 2002.
10 Lee KW, Kim YJ, Lee HJ and Lee CY: Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. J Agric Food Chem *51*: 7292-7295, 2003.
11 Leonard SS, Keil D, Mehlman T, Proper S, Shi X and Harris GK: Essiac tea: scavenging of reactive oxygen species and effects on DNA damage. J Ethnopharmacol *103*: 288-296, 2006.
12 Cheung S, Lim KT and Tai J: Antioxidant and anti-inflammatory properties of ESSIAC and Flor-Essence. Oncol Rep *14*: 1345-1350, 2005.
13 Flora Manufacturing and Distributing Files (Unpublished Data): Academy of Agricultural Sciences of the Russian Federation Research and Manufacturing Association Research and Development Institute of Medical and Aromatic Plants, 1997.
14 Holetz FB, Pessini GL, Sanches NR, Cortez DA, Nakamura CV and Filho BP: Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Mem Inst Oswaldo Cruz *97*: 1027-1031, 2002.
15 Cyong J, Matsumoto T, Arakawa K, Kiyohara H, Yamada H and Otsuka Y: Anti-*Bacteroides fragilis* substance from rhubarb. J Ethnopharmacol *19*: 279-283, 1987.
16 Knutson KL and Disis ML: Tumor antigen-specific T helper cells in cancer immunity and immunotherapy. Cancer Immunol Immunother *54*: 721-728, 2005.
17 Yanagihara K, Ito A, Toge T and Numoto M: Antiproliferative effects of isoflavones on human cancer cell lines established from the gastrointestinal tract. Cancer Res *53*: 5815-5821, 1993.
18 Kulp KS, Montgomery JL, Nelson DO, Cutter B, Latham ER, Shattuck DL, Klotz DM and Bennett LM: Essiac and Flor-Essence herbal tonics stimulate the *in vitro* growth of human breast cancer cells. Breast Cancer Res Treat *98*: 249-259, 2006.
19 Taitzoglou IA, Tsantarliotou M, Zervos I, Kouretas D and Kokolis NA: Inhibition of human and ovine acrosomal enzymes by tannic acid *in vitro*. Reproduction *121*: 131-137, 2001.
20 Taitzoglou IA, Tsantarliotou MP, Nepka C, Zervos I, Maltezos E, Kouretas D and Kokolis NA: Dietary administration of tannic acid lowers plasminogen activator activity in the liver of C3H hepatoma bearing male mice. In Vivo *14*: 767-771, 2000.

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