

# *Aloe arborescens* and American cranberry (*Vaccinium macrocarpon*) extracts inhibit tumor-induced cutaneous angiogenesis in mice

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## Abstract

Research in cancer control indicates the importance of adjuvant and complementary therapies. Medicinal plants are a rich source of substances that might be used for this purpose. *Aloe vera* (*Aloe arborescens*, *Aloe barbadensis* Mill) plants are succulents belonging to the Lillacea family. *Aloe* leaves contain many physiologically active substances with indirect and direct anti-tumor activity etc. Cranberries are a group of evergreen plants in the subgenus *Oxycoccus* of the genus *Vaccinium*. Anticancer properties of cranberries were also described, hypothetically connected to their potential antiangiogenic activity. The aim of the present study was to evaluate the *in vivo* effect of three commercially available preparations of the aforementioned plants on the neovascular reaction observed after intradermal injection of syngeneic sarcoma or xenogeneic (human) lung and kidney cancer cells. We have shown that, additionally to their immunostimulatory properties, *Aloe* and cranberry preparations behave as tumor angiogenesis inhibitors. We also present a tumor-induced cutaneous angiogenesis (TIA) test as a valuable method for *in vivo* quantitative evaluation of activity of various anti-angiogenic compounds. As the extension of these studies it would be prudent to compare antiangiogenic potential of the assessed preparations with antiangiogenic compounds with clinically confirmed anticancer efficiency. It would facilitate reasoning about potential efficiency of these plant extracts, particularly in immunocompromised cancer patients, complementary to their standard therapies.

**Key words:** *Aloe arborescens*, cranberry, mice, tumors, angiogenesis.

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## Introduction

*Aloe vera* (*Aloe arborescens*, *Aloe barbadensis* Mill) plants are succulents belonging to the Lillacea family, perennial herbs probably native to North Africa and naturalized in most warmer areas of the world. Now they are cultivated in many countries, including Poland. *Aloe* leaves contain many physiologically active substances with immunomodulatory, antimicrobial, anti-inflammatory and wound-healing properties, belonging to glycoproteins, anthraquinones, polysaccharides and low-molecular-weight species [1]. Some of these compounds also presented in-

direct and direct anti-tumor activity [2-7]. It was reported in experiments on mice implanted with sarcoma cells that *Aloe* polysaccharides between 400 and 5 KDa molecular size exhibited the most potent antitumor activity *in vivo* and *in vitro* [8]. Whether anticancer properties might be partly dependent on tumor angiogenesis inhibition is not clarified since *Aloe* contains both angiogenic and anti-angiogenic factors [9-12].

Cranberries are a group of evergreen plants in the subgenus *Oxycoccus* of the genus *Vaccinium*. Traditionally the cranberry was used by the Native Americans as a food source and medicinally to treat wounds, urinary disorders,

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diarrhea, and diabetes. Cranberries contain a lot of active substances with immunotropic, antioxidant and anti-inflammatory properties. Anticancer properties of cranberries were also described. They contain 3 classes of flavonoids (flavonols, anthocyanins, and proanthocyanidins), catechins, hydroxycinnamic and other phenolic acids, and triterpenoids. Characterization of an active subfraction of proanthocyanidins revealed the presence of dimers and oligomers of catechin-epicatechin, monomeric catechins, and quercetin glycosides. The major anthocyanins found in cranberry are galactosides and arabinosides of cyanidin and peonidin. The fruits and their preparations (juices, extracts) exhibit high antioxidant properties due to substantial flavonoid and phenolic acids content. It has been demonstrated that cranberries inhibit oxidative and inflammatory damage to the vascular endothelium, oxidative processes including oxidation of low-density lipoproteins, and oxidative damage to rat neurons during simulated ischemia [13-18].

It was suggested that anti-tumor effect of cranberries administration might be partly connected with their antiangiogenic activity. Ability of anthocyanins, proanthocyanidins, quercetin and triterpene acids to inhibit angiogenesis as well as tumor progression and metastasis was reported [19-25].

The aim of the present study was to evaluate the *in vivo* effect of three commercially available preparations – *Aloe* extract Biostymina, *Aloe* + formula Bioaron C, and cranberry extract Żurawit – on the neovascular reaction observed in Balb/c mice skin 3 days after intradermal injection of syngeneic L-1 sarcoma or xenogeneic (human) lung and kidney cancer cells.

## Material and methods

### Drugs

Biostymina (*Aloe aborescens* extractum fluidum, Phytopharm Kłęka SA) amp. 1 ml. Each ampoule contains an aqueous extract (1 : 4) from fresh leaves.

Bioaron C syrup, Phytopharm, Kłęka SA (100 ml contains: Extractum *Aloe arborescens recens* fluidum – 38.4 g, Vitaminum C – 1.02 g, *Aroniae melanocarpae* succus – 23.3 g).

Żurawit 25 : 1 (Herbapol Lublin) caps. 0.5 ml. Each capsule contains 220 mg of *Vaccinium macrocarpon* (American cranberry) extract obtained from 5500 mg of cranberries.

### Mice

The study was performed on 20-22 g of body mass female, 8-10-weeks old inbred Balb/c mice delivered from the Polish Academy of Sciences. For all performed experiments, animals were handled according to the Polish law

on the protection of animals and NIH (National Institutes of Health) standards. Mice were housed 4-5 per cage and maintained under conventional conditions (room temperature 22.5-23.0°C, relative humidity 50-70%, 12 h day/night cycle) with free access to standard rodent diet and water.

All experiments were accepted by the local Ethical Committee.

### Sarcoma L-1 (syngeneic tumor)

L-1 Sarcoma cells from *in vitro* culture stock were delivered from the Warsaw Oncology Center collection thanks to dr Henryk Skurzak and passaged *in vivo* on syngeneic Balb/c mice. Briefly, sarcoma cells were grafted (10<sup>6</sup>/0.1 ml) subcutaneously into mouse sub-scapular region. After 14 days the tumor was excised, cut to smaller pieces, rubbed through stainless sieve and suspended in 5 ml of PBS. The suspension was left for 10 min at room temperature. After sedimentation the supernatant was collected and centrifuged for 10 min at 300 g. Obtained sarcoma cells were washed once with PBS for 10 min, then centrifuged at 300 g, and resuspended in Parker medium in concentration of 4 × 10<sup>6</sup>/ml. Viability of tumor cells suspension as assessed by trypan blue exclusion test was about 90%.

### Human lung cancer

Pulmonary tumor (squamous-cell carcinoma) was obtained from the Surgery Department of the Institute of Tuberculosis and Lung Diseases thanks to professor Tadeusz Orłowski, and was prepared as previously described [26]. Briefly, tumor tissue was sliced in a sterile ice-cold PBS, then treated with an enzyme cocktail containing collagenase 0.1 mg/ml and DNA-se 0.004 mg/ml, at room temperature, and stirred for 45 min. Then, the obtained suspension was filtered through a sieve, washed twice in PBS (10 min 300 × g) and suspended in Parker culture medium at a concentration of 5 × 10<sup>6</sup> cells per ml. Viability of tumor cell suspensions as assessed by trypan blue exclusion test was about 85%.

### Human kidney cancer

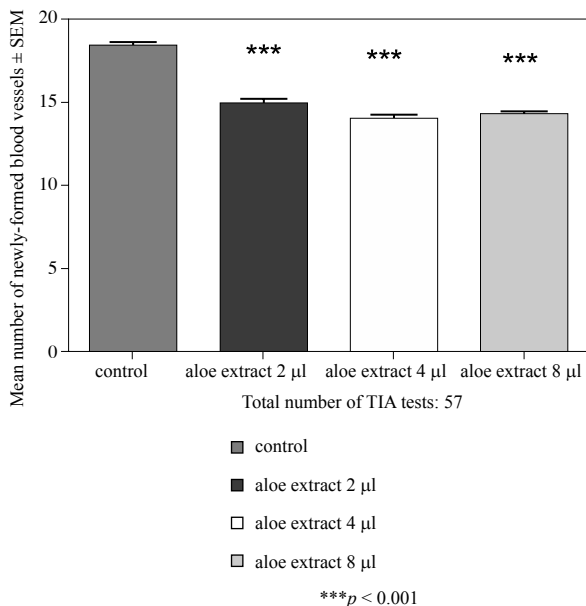
Kidney tumor (Carcinoma clarozellulare) was obtained surgically from the Department of Urology, Postgraduate Medical Center. 2.5 g of tissue were suspended in 5 ml of phosphate-buffered saline (PBS), homogenized with an ultrasonic disrupter VirSonic (Virtis) for 2 minutes, at frequency 22.5 KHz and stored at –70°C, in 1 ml aliquots.

### Tumor-induced angiogenesis test (TIA)

Multiple 0.05 ml samples of homogenate or cell suspension were injected intradermally into partly shaved, narcotised Balb/c mice (3-4 mice per group, 3-6 injections per mouse). In order to facilitate the localisation of injection sites later on, the suspension was coloured with 0.1%

**Table 1.** Statistical analysis of the results presented in Figure 1

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant?	P < 0.05?
control vs. aloe extract 2 µl	3.400	13.81	yes	***
control vs. aloe extract 4 µl	4.400	17.51	yes	***
control vs. aloe extract 8 µl	4.100	16.65	yes	***
aloe extract 2 µl vs. aloe extract 4 µl	1.000	3.858	yes	*
aloe extract 2 µl vs. aloe extract 8 µl	0.7000	2.752	no	NS
aloe extract 4 µl vs. aloe extract 8 µl	-0.3000	1.157	no	ns

**Fig. 1.** Results of experiments performed with Biostymina and human lung cancer cells. Mice were given the drug for 3 days after cells grafting

of trypan blue. On the day of grafting and on the following two days mice were fed by Eppendorf pipette with tested substances, or water as a control. In the experiment with Bioaron C, another control group of mice was fed with placebo-syrup containing aroniae and vitamin C only. After 72 hours mice were sacrificed with lethal dose of Morbital. All newly formed blood vessels were identified and counted in dissection microscope, on the inner skin surface, at magnification of 6×, in 1/3 central area of microscopic field. Identification was based on the fact that new blood vessels are thin, directed to the point of cells injection, with ramifications, and some of them are tortuous.

All experiments were performed in anaesthesia: 3.6% chloral hydrate, 0.1 ml per 10 g of body mass (Sigma); ketamine 100 mg/kg and xylazine 10 mg/kg (BIOWET, Puławy, Poland).

Statistical evaluation of the results was performed by unpaired *t* test and one-way ANOVA, and the signifi-

cance of differences between the groups was verified with a Tukey's Multiple Comparison Test (GraphPadPrism).

## Results

The effect of the aloe extract (biostymina) on the development of cutaneous angiogenesis reaction after grafting of human pulmonary squamous cancer cells is presented in Fig. 1 and Table 1. All doses of the remedy highly significantly diminished number of newly-formed blood cells in comparison to the control ( $p < 0.001$ ).

The results of experiments on the effect of Bioaron C on cutaneous angiogenesis induced by grafting of human kidney cancer homogenate are presented graphically in Fig. 2 and their statistical analysis in Table 2. Bioaron C highly significantly diminished the number of newly-formed blood vessels in comparison to the control ( $p < 0.001$ ) and to placebo ( $p < 0.01$ ). No inhibitory effect was observed for placebo (syrup without aloe extract) in comparison to the control (water).

The effect of the cranberry extract (Żurawit) on the development of cutaneous angiogenesis reaction after grafting of L-1 sarcoma cells is presented in Fig. 3 and in Table 3. Feeding of recipient mice with this remedy, for 3 days after tumor cells grafting, highly significantly diminished the number of newly-formed blood vessels in comparison to the control ( $p < 0.0001$ ).

## Discussion

Research in cancer control indicates the importance of complementary therapies. Medicinal plants are a rich source of substances which might be used for this purpose. Among them *Aloe vera* and its active compounds are of special interest. It was reported that in mice, Aloe and its compounds are effective radioprotective agents, what can be useful in increasing the tolerance dose of radiation in cancer patients [27]. In rats, oral administration of *Aloe vera* and honey reduced Walker tumor growth by decreasing cell proliferation and increasing apoptosis in tumors [28]. Other authors demonstrated beneficial effects of Aloe emodin, hydroxyanthraquinone compound, on proliferation and differentiation of highly metastatic B16-F10 melanoma

**Table 2.** Statistical analysis of the results presented in Figure 2

Tukey's Multiple Comparison Test	Mean Diff	q	Significant?	P < 0.05?
control vs. aronia + vit. C	0.9000	3.104	No	NS
control vs. Bioaron C	2.500	8.825	Yes	***
aronia + vit. C vs. Bioaron C	1.600	5.417	Yes	**

murine cells [29]. Chen *et al.* reported that *in vitro* emodin, aloe-emodin and rhein induced DNA damage followed by the inhibition of DNA repair-associated gene expression in SCC-4 human tongue cancer cells [30]. Three anthraquinones (aloesin, aloe-emodin and barbaloin) and N-terminal octapeptide derived from verectin, a biologically active 14 kDa glycoprotein extracted from *Aloe vera* leaves, exerted chemopreventive effect through modulating antioxidant and detoxification enzyme activity levels in various human cancer cell lines *in vitro*. *In vivo*, these compounds exhibited significant prolongation of the life span of tumor-transplanted animals (Ehrlich ascites carcinoma) [31].

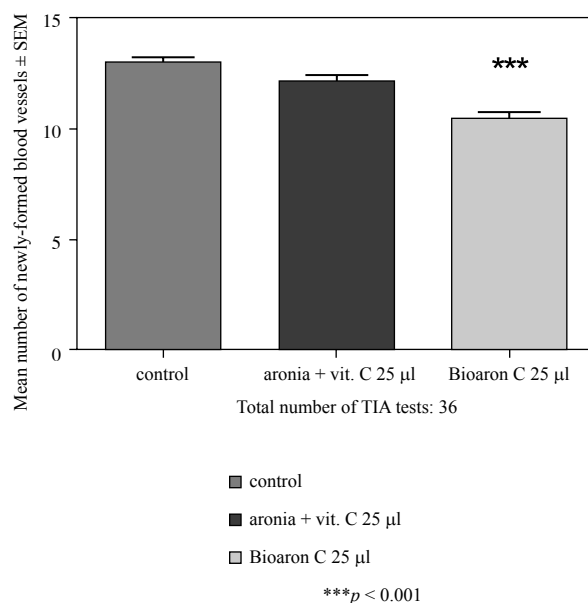
Recently, it has been reported that in human healthy volunteers, a combination of vitamin C and *Aloe vera* juice significantly increased, *in vitro* and *ex vivo*, NK cells cytotoxicity against K562 cancer cell line [32].

Our earlier studies have revealed a stimulatory effect of Biostymina, water soluble extract of the leaves of triennial plants *Aloe arborescens* Mill., and Bioaron C, herbal syrup containing Biostymina, *Aronia melanocarpa* water extract, and ascorbic acid, on cellular and humoral immunity in mice [33, 34]. Other authors performed clinical evaluation of the efficacy and safety of Bioaron C in children with recurrent bacterial and viral infections of the upper respiratory tract [35]. Fourteen days' preventive application of Bioaron C in these children was associated with the reduction of infection incidence in this group. The immunomodulatory effect of the drug depended on the baseline condition and was manifested by the effect on T cell immunity and the phagocytic activity of the neutrophils.

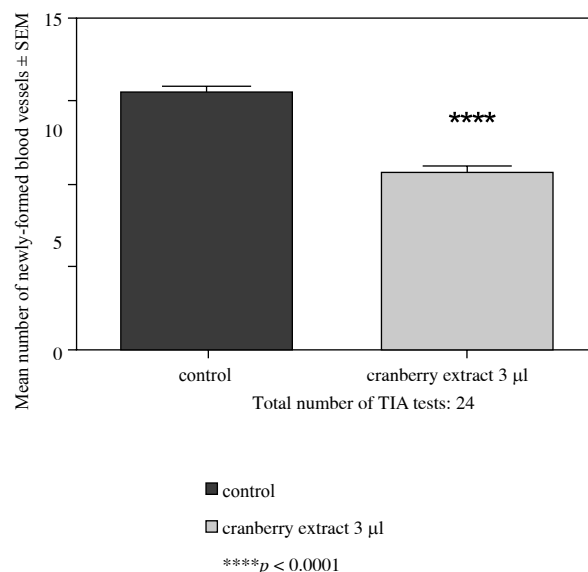
Recently, other authors have reported anti-viral activity of these herbal drugs [36]. In this paper we present evidence that, additionally to their immunostimulatory properties, Biostymina and Bioaron C behave as tumor angiogenesis inhibitors what would be of great value in

**Table 3.** Statistical analysis of the results presented in Figure 3

Unpaired t test	
p value < 0.0001	
p value summary	****
Are means significantly different? (p < 0.05)	Yes
One- or two-tailed p value?	Two-tailed
t, df	t = 8.256, df = 22



**Fig. 2.** Results of experiments performed with Bioaron C and human kidney cancer homogenate. Mice were given the drug for 3 days after tumor grafting



**Fig. 3.** Results of experiments performed with Żurawit and syngeneic for Balb/c mice tumor L-1 sarcoma. Mice were given the remedy for 3 days after tumor cells grafting

additional therapy of patients with tumors, especially immunocompromised after standard treatment.

In this paper we also present the results of our preliminary studies on the effect of the cranberry extract Żurawit on the early neovascular reaction to syngeneic tumor cells transplantation. The cranberry extract administered to recipient mice for three days significantly diminished the number of newly-formed blood vessels in the place of intradermal tumor cells injection.

It was repeatedly reported that compounds found in cranberries showed a high anticancer activity with different mechanisms of action. The total polyphenol extract inhibited proliferation of colon (HT-29, HCT-116, SW480, SW620), oral (CAL27, KB) and prostate cancer cell lines (RWPE-1, RWPE-2, 22Rv1) [37]. Pentacyclic triterpenoid ursolic acid found in the peel of cranberry fruit inhibited growth of several cancer cell lines [24, 38]. It has also been demonstrated that ursolic acid inhibited HT-29 cancer cell line growth more effectively than the cranberry proanthocyanidin fraction did [39]. Additional triterpene hydroxycinnamates (identified by HPLC and NMR as *cis*-(1) and *trans*-(2) isomers of 3-O-p-hydroxycinnamoyl ursolic acid) isolated from whole cranberry fruit have shown a higher antiproliferative activity in MCF-7 breast, ME180 cervical and PC3 prostate tumor cell lines than quercetin or cyanidin-3-galactoside [38].

Resveratrol, epigallocatechin gallate, and quercetin (a major flavonoid in cranberry fruit) are capable to induce apoptosis in cancer cells [39-44].

A large group of anticancer properties of cranberries is associated with their antiangiogenic activity. Researchers repeatedly proven that anthocyanins, proanthocyanidins, ursolic acid, quercetin and triterpene acids have an antiangiogenic activity, and inhibit tumor progression and metastasis.

A cranberry extract in human keratinocytes inhibited the expression of VEGF induced by hydrogen peroxide or TNF- $\alpha$ . Antiangiogenic activity of the cranberry extract was also observed in studies of the tumor endothelial cell line (EOMA) originating from a developing tumor hemangioma in children. The cranberry extract inhibited transcription of MCP-1, factor produced by macrophages, which stimulates angiogenesis. In mice with melanoma, ursolic acid reduced the levels of VEGF, nitric oxide and proinflammatory cytokines and increased the levels of tissue inhibitor of metalloproteinase (TIMP1) and interleukin 2 (IL-2) in serum [19-25, 45-47].

Our studies confirm the anti-angiogenic effect of the cranberry extract. Additionally, we present tumor-induced cutaneous angiogenesis (TIA) test as a valuable method, which may be used in further studies for *in vivo* quantitative evaluation of activity of various anti-angiogenic compounds present in cranberries and their extracts.

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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