International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1516-1522 www.ijprajournal.com ISSN: 2456-4494

A Review on: Extraction of Anthocynins from Blueberry Used In Ovarian Cancer by Various Extraction Methods.

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Submitted: 10-12-2022 Accepted: 20-12-2022

ABSTRACT:
Ovarian Malignant growth is the second most normal gynecologic Maligency Endometrium Disease being the most well-known reason for Death among ladies, who fostered a Gynecologist Maligency The bioactive standards contained in blueberries (Vaccinium) are different sort of anthocyanins (anthocyanidins, or phenolic aglycone, formed with sugar), chlorogenic corrosive, flavonids, alpha-linolenic corrosive, pterostilbene, resveratrol, and nutrients. After oral organization, anthocyanins can go through blood-cerebrum boundary and subsequently show up in different organs and mind. blueberries to forestall the age-related constant infections like malignant growth, diabeties, hyperlipidemia, hypertension, neurodegeneration, heftiness, and osteoporosis through its apoptosis, cell reinforcement, antiinflammation, and antiangiogenesis impacts.
Despite the fact that blueberries have recently been utilized to treat different kinds of malignant growth, the impact on OC and exact sub-atomic instrument of capability of the natural product stays obscure. Cyclooxygenase (COX)- 1 and COX-2 have been accounted for to be the biomarkers of OC. Blueberries might influence the movement of OC by influencing COX levels. To examine the issue, COX-1 and COX-2 were overexpressed or hushed in ovarian malignant growth SKOV3 cells. The impact of blueberries on SKOV3 cell not entirely settled by a MTT measure. The outcomes demonstrated that blueberries hindered the multiplication of OC cells by downregulating the degrees of COX-1 and COX-2. Blueberry (400 mg everyday) utilization diminished growth size altogether in mice with OC contrasted and the control without blueberry treatment ($P<0.05$). The outcomes propose that blueberries ought to be utilized to foster a likely non-drug treatment for OC

KEYWORDS: Anthocynins,antioxidants, stages of ovarian cancer , methods of extraction anthocynins .

INTRODUCTION

A little berry is a well-known nibble with youthful and old and has vanquished practically the whole world, it should truly be something extremely exceptional. Luckily, we can find this nutrient pressed wonder in pretty much every grocery store. In any case, where do blueberries really come from and where do they develop now?

Blueberries (Vaccinium) initially come from North America, where they are still very famous in hotcakes, muesli and numerous different dishes. From around 1909, greater products of the soil were developed in North America to make collecting simpler. Up to that point, picking blueberries had been a very difficult assignment. Developing wild blueberry hedges was hit upon.

These are currently known as developed blueberries, which have a brilliantly shaded tissue and ablue skin. This developed structure is extensively greater and furthermore better than the wild blueberry species, which is local to Europe and whose tissue is a dull blue tone. The developed blueberry is in this way, without a doubt, remotely connected with the local European blueberry.
Be that as it may, where do blueberries come from today? The Give blueberries come from the USA, Chile, Spain, Italy, Argentina and Uruguay. So berry fans can partake in this succulent delight the entire all year.

Scienticfic Classification

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kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Asterids
Order:	Ericales
Family:	Ericaceae
Genus:	Vaccinium
Section:	Vaccinium sect. Cyanococcus Rydb.

Fig no : 1 Bluberry Fruit

•Vaccinium angustifolium (lowbush blueberry):[acidic barrens, swamps and clearings, Manitoba to Labrador, south to Nova Scotia; and in the US, from Maine toward the west to Iowa and toward the south to Virginia
•Vaccinium boreale (northern blueberry): peaty barrens, Quebec and Labrador (uncommon in New Brunswick), south to New York and Massachusetts
•Vaccinium caesariense (New Jersey blueberry)
•Vaccinium corymbosum (northern highbush blueberry)[9]
•Vaccinium darrowii (evergreen blueberry)

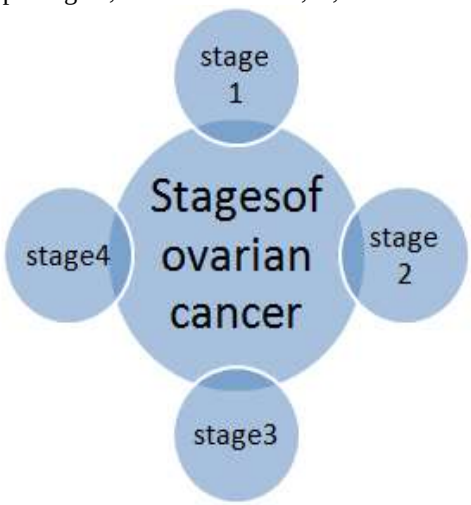
The different types of ovarian cancer is Epithelial ovarian malignant growth, which emerges from the outer layer of the ovary (the epithelium), is the most widely recognized ovarian disease. Fallopian Cylinder Malignant growth and Essential Peritoneal Disease are likewise included inside this assignment.

- ❖ Microorganism Cell ovarian malignant growth emerges from the regenerative cells of the ovaries, and is uncommon.
- ❖ Stromal cell ovarian malignant growth, which emerges from connective tissue cells, is extremely uncommon.
- ❖ Little cell carcinoma (SCCO) of the ovary is a very uncommon ovarian disease and it isn't sure if the cells in that frame of mind from ovarian epithelial cells, sex-line stromal cells or microorganism cells**.**

Sign and Symptoms of Ovarian cancer

- Bloating
- Pelvic or abdominal pain
- Difficulty eating or feeling full quickly
- Urinary symptoms (urgency or frequency)

Four stages of ovarian cancer. Each of these stages, except Stage 4, is divided into A, B, and C.



- **Stage 1**
- Stage 2
- Stage 3
- Stage 4

Stage 1 Ovarian Cancer:

- ➢ When a person has Stage 1 ovarian cancer, it means the cancer has been found in one or both ovaries. Stage 1 ovarian cancer is found in 15% of patients.
- ➢ Stage 1A: One ovary is affected by cancer.
- ➢ Stage 1B: Both ovaries are discovered to contain cancer.
- ➢ Stage 1C: One or both ovaries have cancer, and one of the following is true:

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- Cancer cells are discovered in the fluid of the peritoneal cavity, the bodily cavity that houses the majority of the organs in the abdomen, or in washings of the peritoneum (tissue lining), or o the capsule (outer covering) of the ovary has ruptured (broken open).

Stage	Relative 5-Year Survival Rate
I	90%
IA	94%
IB	92%
IC	85%

Treatment for
Stage 1 :Ovarian Cancer

A total abdominal hysterectomy, removal of the ovaries and fallopian tubes (known as a salpingo-oophorectomy), removal of the omentum (a sheet of fat that covers some abdominal organs), and biopsy of lymph nodes and other tissues in the pelvis and abdomen are typically performed on women with Stage 1 ovarian cancer. A unilateral salpingo-oophorectomy without a hysterectomy may be used to treat women of reproductive age who want to maintain their fertility and whose illness is limited to one ovary. (Omentectomy and other staging procedure steps are still carried out.) If the cancer is low. grade, there may be no more treatment; however, if the tumour is high grade, the patient.

Stage 2: Ovarian Disease

Stage 2 ovarian disease implies the malignant growth is tracked down in one or the two ovaries and has spread into different region of the pelvis. Stage 2 is a little gathering, compromising 19% of ovarian malignant growth analyze.

- Stage 2A: Disease has spread to the uterus or potentially fallopian tubes (the long slim cylinders through which eggs pass from the ovaries to the uterus).
- Stage 2B: Malignant growth has spread to other tissue inside the pelvis.
- Stage 2C: Disease is tracked down inside one or the two ovaries and has spread to the uterus or potentially fallopian tubes, or to other tissue inside the pelvis. Likewise, one of coming up next is valid:
  - cancer is tracked down outwardly surface of one or the two ovaries; or
  - the case(external covering) of the ovary has burst (torn open); or
  - cancer cells are tracked down in the liquid of the peritoneal pit (the body cavity that contains the vast majority of the organs in the midsection) or in washings of the peritoneum (tissue covering the peritoneal pit).

Stage	Relative 5-Year Survival Rate
II	70%
IIA	78%
IIB	73%
IIC	57%

Stage 2: Ovarian Malignant growth Treatment

Therapy for Stage 2 ovarian malignant growth incorporates: hysterectomy and respective salpingo-oophorectomy (expulsion of the two ovaries and fallopian tubes), debulking of however much of the growth as could reasonably be expected, and examining of lymph hubs and different tissues in the pelvis and midsection that are associated with holding onto disease. After the surgery, therapy might be one of the accompanying: 1) mix chemotherapy regardless of radiation treatment or 2) blend chemotherapy.

Stage 3: Ovarian cancer

Stage 3 ovarian malignant growth implies that the disease is tracked down in one or the two ovaries and has spread external the pelvis to different pieces of the midsection and additionally close by lymph hubs. It is additionally viewed as Stage 3 ovarian malignant growth when it has spread to the outer layer of the liver. 60% of all instances of ovarian malignant growth are analyzed when they are Stage 3.

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Stage	Relative 5-Year Survival Rate
III	39%
IIIA	59%
IIIB	52%
IIIC	39%

Stage 3: Ovarian Malignant growth Treatment

Therapy for Stage III ovarian malignant growth is equivalent to for Stage II ovarian disease: hysterectomy and respective salpingo-oophorectomy (expulsion of the two ovaries and fallopian tubes), debulking of however much of the growth as could be expected, and examining of lymph hubs and different tissues in the pelvis and midsection that are associated with holding onto disease. After medical procedure, the patient may either get mix chemotherapy potentially followed by extra medical procedure to find and eliminate any excess malignant growth.

Stage 4: Ovarian Disease

At the point when an individual is determined to have Stage 4 ovarian disease, the malignant growth has spread past the midsection to different pieces of the body, like the lungs or tissue inside the liver. Disease cells in the liquidaround the lung is additionally viewed as Stage 4 ovarian malignant growth.

Stage	Relative 5-Year Survival Rate
IV	17%

Stage 4 :Ovarian Malignant growth Treatment

Therapy for Stage 4 ovarian malignant growth will comprise of a medical procedure to eliminate however much of the cancer as could be expected, trailed by blend chemotherapy. Dive deeper into the various medicines and treatments.

Materials and strategies or methods

Materials The blueberries were bought from the Blueberry Creation Field (Guizhou, China). The blueberries were frozen upon landing in −20°C. A sum of 100 g blueberries were homogenized in a homogenizer (GYB60-65; Shanghai Donghua High Strain Homogenizer Processing plant, Shanghai, China). The blueberry juice was acquired by rotator at 10,000 × g, 40°C for 10 min (1 ml blueberry juice was delivered from 2 g blueberry).

Currently, the extraction technology of blueberry anthocyanin includes solvent extraction, enzyme extraction, and ultrasonic extraction.

Experiments on the effects of different extraction methods on the antioxidant properties of blueberry anthocyanins

Experimental materials and Equipment

The main experimental material is blueberries from the small berry garden of Jilin Agricultural University. The selected blueberry fruit (the variety is patriot) is provided by Liaoning Dandong Organic Food Co., Ltd, which has reached the fruit period of 8 weeks and is in a mature state, with a diameter of 5–10 mm, purple red, moisture content of 88%, half height clumped blueberry variety. Fresh fruits are picked and stored in a refrigerator at −20°C. During the experiment, the frozen fruits were taken out of the refrigerator at −20°C and thawed in the refrigerator at 4°C.

Experimental reagents

The preparation and the selection of experimental reagents mainly include two aspects, namely, the reagents used in the determination process and the relevant reagents used in the anthocyanin extraction process. The reagents required for the experiment are formic acid, anhydrous methanol, sodium acetate, ferric trichloride, DPPH, ABTS (Sinopharm Chemical Reagent Co., Ltd), potassium chloride, potassium hydroxide, aluminum trichloride, lead acetate (Xilong Chemical Co., Ltd), hydrochloric acid, sodium dihydrogen phosphate (Beijing Chemical Plant), ferric trichloride, potassium persulfate, ascorbic acid, trihydroxymethyl aminomethane, soybean lecithin (Sinopharm Chemical Reagent Co., Ltd), cyanidin-3-glucoside standard, and standard of mallow pigment-3-galactoside (Sigma Company).

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Experimental instruments

As with the experimental reagents, the instruments used in this experiment are also divided into three aspects: anthocyanin extraction, anthocyanin content identification, and antioxidant performance testing. The equipment and models required for the experiment include LBI206 Ultrasonic Chinese medicine processor (Jining Aobo ultrasonic electric Co., Ltd), UV-6100 Ultraviolet visible spectrophotometer, L3600D Low speed, PHSJ-3F Precision PH meter (Shanghai Yuan Analytical Instrument Co., Ltd), XDB-C18 chromatographic column, LCMS-IT/TOF High performance liquid phase ion trap/time of flight tandem mass spectrometer, LC-20AD High resolution fast liquid chromatography system (SHIMADZU Company), AL104 Electronic balance (Mettler Toledo), and XMTB Electrothermal constant temperature Water bath (Tianjin Zhonghuan Experimental Electric Furnace Co., Ltd).

Raw material pretreatment

The commercially available blueberries frozen at −20°C were thawed at room temperature, and they were stirred with a stirrer to a cloudy liquid. To make each part uniform, the blueberry juice is placed in a colloid mill at 3,000 rpm and is ground for 15 min. Then, a crude extract and an extract of blueberry were prepared separately.

Preparation of blueberry crude extract

Five solvents containing 3% trifluoroacetic acid were used, including (water, n-butanol, methanol, 70% ethanol, ethyl acetate), with a solid–liquid ratio of 1:5 (g/mL) and ultrasonic-assisted extraction at 30°C (Power 180 W) three times, each time 30 min, the material liquid ratio of the second and third times is halved. The extracts were combined and centrifuged at 4,500 rpm for 5 min, the supernatant was taken, and the solvent was concentrated under reduced pressure at 40–60°C, and the extract was freeze-dried to obtain a sample water extract. A 60% ethanol solution was used to mix uniformly at a solid–liquid ratio of 1:20 (m/V), and the mixture was extracted in a constant temperature water bath at 50°C for 90 min.

Preparation of blueberry extract

Using methanol containing 0.3% trifluoroacetic acid as a solvent, the aforementioned extraction steps are followed to obtain an extract-like extract, dissolve with 500 mL of purified water, filter, and take 300 mL of the filtrate onto a LS-305 macroporous resin column with a 0.3% trifluoroacetic acid aqueous solution (approximately 400 mL). Impurities such as sugar and acid are removed by rinsing and then eluted with 0.3% trifluoroacetic acid methanol solution (approximately 100 mL). The eluate was collected and concentrated under reduced pressure at 40°C to extract, dissolve with purified water and make up 150 mL. 25 mL of this sample solution was taken, concentrated under reduced pressure at 40°C to extract, and freeze-dried to obtain blueberry total extract (RP). The remaining 125 mL of the sample was sequentially extracted with ethyl acetate and n-butanol. The sample was extracted using ethyl acetate extraction three times, the first time with an equal volume and the next two times with a half volume, and combined extracts were concentrated under reduced pressure at 40°C to remove the solvent and freeze-dried to obtain blueberry ethyl acetate extract (ARP). The sample was again extracted with n-butanol four times, the first time with an equal volume and the next three times with a half volume, and the extract was concentrated under reduced pressure at 60°C to remove the solvent and freeze-dried to obtain blueberry n-butanol extract (BRP). The solvent was concentrated under reduced pressure at 50°C, and blueberry water extract was obtained after freeze-drying.

Choose anthocyanin extraction method

On the basis of the results of the previous research, blueberry anthocyanins were prepared. The extraction methods differ according to the uses of the anthocyanins extracted. To study the effect of different extraction methods on the antioxidant properties of blueberry anthocyanins, the contents, concentrations, and the corresponding changes in antioxidative properties of anthocyanins obtained by solvent extraction, enzymatic extraction, and ultrasonic extraction were compared.

Solvent extraction method

Anthocyanins are less stable in neutral and weakly alkaline solutions, and so the extraction process is usually performed under acidic conditions. Steps of extraction method are as follows:

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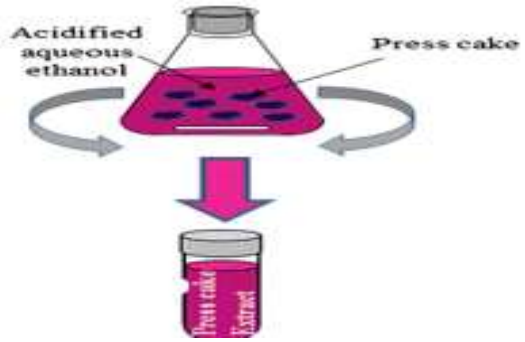
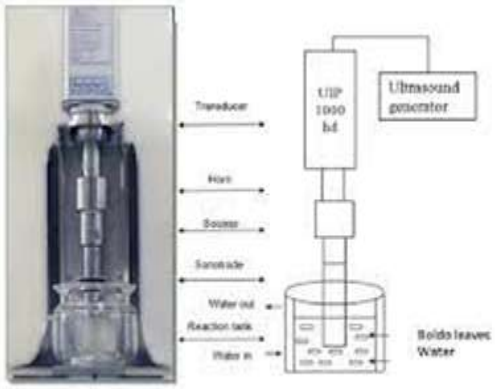


Figure No: 2 Solvent Extraction Method

Configure the solvent buffer pH 1.0 buffer: 0.2 mol/L KCl–0.2 mol/L HCl, and the pH 4.5 buffer is 1 mol/L NaAc–1 mol/L HCl. Weigh blueberries accurately, add 15 times the amount (v/w) of acidic methanol with a concentration of 80% after mechanical crushing, extract at 40°C, and determine the proportion of the solvent to collect the supernatant. Using acidic ethanol as the extractant and optimizing each factor by response surface analysis, the best extraction parameters were obtained: ethanol extract volume fraction, 60.65%; material–liquid ratio, 1:20.65(g/mL); extraction time, 122.53 min; pH, 3.0; the extraction temperature, 50°C, and the extraction was performed twice to determine the content of anthocyanins in the frozen blueberries, which was about 3.264 mg/g.

Enzymatic extraction Citric acid and sodium citrate were mixed at a certain ratio to adjust the PH. 5 g of blueberry was crushed, and 5 mg/g pectinase was used for enzymolysis at 45°C, pH 4.5, and the duration of enzymatic hydrolysis with the material–liquid ratio of 1 g:8 mL was 60 min and 90 min, and after centrifugation at 2,000 rpm in 20 min, the blueberry anthocyanin extract was collected

Ultrasonic extraction method5 g of blue poisonous fruit was weighed and 60% acidified methanol with a ratio of 1:10 (g/mL) was used as the extractant and sonicated at 50°C for 50 min to obtain blueberry anthocyanin extract . The actual quantity of extracted blueberry anthocyanin was 3.927 mg/g.



Ultrasonicator UIP1000hd for boldo leaves' extraction in batch operation [Petigny et al. 2013]

Fig No:3Ultrasonic extraction method

Extraction and purification

Three different anthocyanin extraction methods were used for purification. The purification and separation method used was centrifugation. Generally, the temperature increases during centrifugation, which has a great impact on heat-sensitive substances. Therefore, this experiment uses a refrigerated centrifuge, which can ensure that the sample is centrifuged at low temperature, so that the substance does not lose its activity . The effect of centrifugation is affected by factors such as solution viscosity, centrifugation time, and centrifugation speed. The change of the absorbance value of the solution before and after centrifugation is used as an index for analysis, and the conditions close to or greater than the absorbance value of the solution before centrifugation are preferred002E

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