Journal of science faculty at Sirte University
JSFSU
2021
Peer-review Periodical Journal

جامعة سرت
Sirte University
1989

ISSN INTERNATIONAL STANDARD SERIAL NUMBER

eISSN: 2789-858X

SCIENTIFIC JOURNAL FOR THE FACULTY OF SCIENCE – SIRTE UNIVERSITY

SJFSSJ

DOI: 10.37375/issn.2789-858X - Indexed by Crossref, USA

VOLUME 3 ISSUE 2 OCTOBER 2023

Bi-annual, Peer- Reviewed, Indexed, and Open Accessed e-Journal

Legal Deposit Number@NationaL Library (Benghazi): 990/2021

1.02/2022

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Scientific Journal for the Faculty of Science-Sirte University Vol. 3, No. 2 (2023) 88-95

Scientific Journal for the Faculty of Science-Sirte University

Journal home page: http://journal.su.edu.ly/index.php/JSFSU/index

DOI: 10.37375/issn.2789-858X

In vitro phytochemical screening, Antioxidant and Anti-platelet Activities of Libyan *Arbutus pavarii* Extract

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DOI: https://doi.org/10.37375/sjfssu.v3i2.1660

ARTICLE INFO:

Received: 31 August 2023

Accepted: 20 October 2023

Published: 26 October 2023

Keywords: *Arbutus pavarii*, Antioxidant, Antiplatelet

A B S T R A C T

This study was conducted to investigate the active compound like alkaloid and phenolic compound in leaves and fruit extracts of *Arbutus pavarii* compound and to determine the potential effect of methanol extract of *Arbutus pavarii* to improve antioxidant properties activity in a dose-dependent manner with good IC50 values. The antioxidant effect of *Arbutus pavarii* was investigated by scavenger effect using 1, 1-diphenyl-2 picrylhydrazyl (DPPH). The researchers observe the possibility of *Arbutus pavarii* extract to prevent human platelets aggregation. The effect of *Arbutus pavarii extract* related to its active compounds including polyphenols which able to reduce the scavenge of the hydroxyl radicals in human body leading prevent dysfunction pathway, the mechanism underlying such effects related to a reduce the vascular formation of Reactive oxygen species (ROS). The present findings indicated that *Arbutus pavarii* has antioxidant effects and the ability to prevent human platelets aggregation. These results could be indicator to improve the verity cells dysfunction in human body, then improve the function of cardiovascular and also to reduce pathological arterial wall. Further suggest that *Arbutus pavarii* might be an interesting candidate as a good food therapeutic.

1 Introduction

Arbutus pavarii Pampan (Family Ericaceae) is a deciduous or average shrub, tree reaching up to 3 meters distributed in the West area of Libya (El-Gabel El-Akhdar). The bark has faint, smooth, and reddish, peeling. The fruit takes around 8 months to ripen, and they are spherical and warty, and turn from yellow to orange to scarlet as the autumn progresses. The strawberry fruits are edible directly as fruits or can be made into jam, but the taste is somewhat insipid (Elshatshat, 2009). Several epidemiological studies have indicated that regular intake of beverages rich in polyphenols is associated with a reduced risk of several diseases including cardiovascular diseases (Di Castelnuovo *et al*., 2002; Kuriyama *et al*., 2006). The beneficial effect has been attributed at least partly to their high polyphenol such as procyanidins and anthocyanins (Corder *et al*., 2006 ; Auger *et al*., 2010;). Moreover, the effect of anthocyanins on oxidative stress may involve its ability to inhibit Reactive Oxygenase Species (ROS) formation possibly also by regulating the expression of several pro- and antioxidant enzymes. Moreover, this effect may be attributed at least to indirect protection by activating endogenous defense systems and by modulating cellular signaling processes.

Polyphenols might protect the cardiovascular system by a variety of actions, including their ability to improve the lipid profile by their antioxidant properties, which will prevent the degradation of nitric oxide (NO) by superoxide anions (Frankel *et al*., 1993; Ndiaye *et al*., 2005; Sarr *et al*., 2006). The antioxidant properties of the phenolic compounds were reported as a scavenger of the

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1, 1-diphenyl-2 picrylhydrazyl (DPPH) (Baratto *et al*., 2003; Benhammou *et al*., 2007; Gardeli *et al*., 2007). It's also shown that the presence of Gallic acid and its derivative, like 1, 2, 3, 4, 6-pentagalloyl glucose (ply) putative role agents lipid peroxidation induced by H_2O_2 in K562 cell line (Abdelwahed *et al*., 2007). Several epidemiological studies and randomized control trials have indicated an inverse relationship between the dietary intakes of beverages rich in polyphenols on platelet activity. The primary objective of this study is to investigate the active compound in extract of *Arbutus pavarii*, and the effect of polyphenol from *Arbutus pavarii* as a potent antioxidant.

The second objective is to investigate in-vitro the extracts of *Arbutus pavarii* as antiplatelets effect lading to reduce human platelets aggregation.

2 Materials and Methods

2.1 Plant material Preparation

The leaves and fruits of *Arbutus pavarii* were collected from western area in Libya and air-dried at room temperature and reduced to fine powder by milling then stored in a dry place in airtight container.

2.2 Preliminary Phytochemical Screening

The leaves and fruits of *Arbutus pavarii* was prepared for evaluation of phytochemical constituent as following (sofowora, 1993; Trease and Evans, 2002)

2.3 Plant Material Extraction

The dried plant powder (1000 g) and 500 g of fruit was extracted by using cold maceration in methanol for 72 h. Then extraction was repeated three times, and methanol extract was collected and concentrated by rotary evaporator at 60 °C, under reduced pressure. The extracts were weighted and stored in a refrigerator at 4 °C (sofowora, 1993).

Test of alkaloids

Few mgs of each extract (leaves and fruits) was separately stirred with 1% HCl on a water bath for 5 min and filtered. These filtrates were treated with few drops of Dragendorff's reagent (Potassium bismuth iodide solution), Mayer's reagent (Potassium mercuric iodide solution), and Wagner's reagent (Potassium iodide and iodine). A formation of precipitate indicates the presence of alkaloids (Trease and Evans, 2002).

Test of Tannins

Each extract(leaves and fruits) (0.5mg) was separately stirred with distilled water (10 mL) and then filtered. A few drops of 5% ferric chloride were then added. Black or blue-green coloration or precipitate was taken as a positive result for the presence of tannins (Trease and Evans, 2002)

Test for Saponins

Each of plant extracts(leaves and fruits) (0.5) was separately shaken with distilled water (10 mL) in a test tube then heated in warm water bath for 5 min. The formation of a froth shows the presence of saponins (sofowora, 1993).

Test of Coumarins

One drop of extracts was dissolved in NaOH, then spotted on Wattman's filter paper and detected under UV light (366nm), blue fluorescence spots indicated the presence of coumarins (sofowora, 1993).

Test of Flavonoids

The extract (leaves and fruits)was treated with magnesium turnings and one drops of concentrated HCL. The formation of red or pink color showed the presence of flavonoid (sofowora, 1993).

Test of Anthraquinone Glycoside (Borntrager's Test)

The extract (leaves and fruits) solution was adding 5% H_2SO_4 (1 mL). The mixture was boiled in a water bath for 10 min and then filtered. Filtrate was then shaken with equal volume of chloroform and kept to stand for 5 min. Then the lower layer of chloroform was shaken with half of its volume with dilute ammonia. The formation of rose pink to red color of the ammoniacal layer as indication of anthraquinone glycosides (sofowora, 1993).

The MTT Assay

The MTT assay is a colorimetric assay for assessing cell metabolic activity. Cell viability was performed using HeLa cell line. Cells were treated with different concentrations of plants extracts including (0.1, 0.3, 1.0 mg/ml).100 µL/well in a 96-well plate. MTT Reagent was adding to each well at a 1:10 ratio, after incubation for 2-4 hours or overnight at 37°C. Cells well are mentoring under the microscope for the presence of a purple precipitate. 100 µL of Detergent Solution for every 10 µl of MTT Reagent added to each well (100 µl

/well for a 96-well plate or 250 µl /well for a 24-well plate). Gently mix the solution by pipetting. Cover the plate to protect it from light and incubate in the dark for 2-4 hours at room temperature and measure the absorbance in each well at 570 nm in a microtiter plate reader. (Mosmann, 1983).

DPPH(1-diphenyl-2-picrylhydrazyl) radical scavenging effect

The antioxidant properties of *Arbutus pavarii* was evaluated by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity. Methanol extract of solutions of the test samples at various concentrations ((20-100µg/mL).) was added to a solution of DPPH in methanol (0.001% w/v) in was taken into the cuvettes then *Arbutus pavarii* extracts were added followed by serial dilutions (1 mg to 500 mg) to every cuvette so that the final volume was 1 mL and after 30 min, the absorbance was read at 515 nm using a spectrophotometer. Ascorbic acid was used as a reference standard and was dissolved in methanol to make the stock solution with a concentration of 1 mg/mL. Control sample was prepared containing the same volume without any extract and reference ascorbic acid. Blank was prepared with 99% methanol.

$$\text{percentage of scavenging} = \frac{\text{Absorbance of the control} - \text{Absorbance of the test}}{sample\ x100}$$

Absorbance of the control

The inhibition curve was plotted for duplicate experiments and represented as % of mean inhibition ± standard deviation. IC50 value was determined from the graph obtained by using standard ascorbic acid following the formula "y=mx+c" from the slope of the graph. The IC50 represents the concentration where 50% inhibition of the DPPH radical is obtained.

seeds) may be used as a source of antioxidants for pharmacological and food preparations which is very well evidenced by the present work. The antioxidant activities in terms of IC50 of C. maxima extracts were summarized in *Arbutus pavarii* .

Platelet aggregation

Platelet aggregation well measured by Born's method (Born *et al*., 1971) using a dual channel automatic optical aggrego meter (560, Chrono-log Co.). Human platelet-rich plasma (PRP) was incubated at 37°C for 2 min in the aggrego meter with stirring at 1,000 rpm and exposed to various concentrations (0.1, 0.3, or 1 µg /ml) of plants for 3 min. After incubation, platelet aggregation was induced by addition of collagen (20 µg/ml) And use aspirin to compare with the extract as an anticoagulant. The resulting aggregation was measured the change in light.

3 Results

3.1 Preliminary phytochemical screening

We performed phytochemical screening using different chemicals and solvent to investigate different active compound in experimental plant extract. our results indicate the presence of coumarin, flavonoid, saponins and, these results indicator to interesting plant for antioxidant properties (table1).

Table (1): Preliminary phytochemical screening

Extracts	IC50 µg/ml
Leaves	4.2±0.22
Fruit	4.3±0.24
Ascorbic Acid	51.3±0.005

The cytotoxicity activity of methanolic extract was detected in HELA (cervical carcinoma cell line). Different concentration of plant extract was added (12.5 to 100.0 mg/ml up to 72 hours. Our results showed no cytotoxicity was detected even at concentrations up to 100 µg/mL (Figure1).

Cytotoxic assay

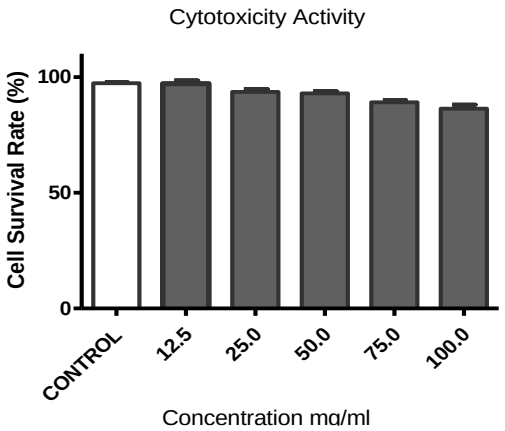

Figure (1): Cytotoxicity assay following treatment of HELA (cervical carcinoma cell line)

DPPH free radical scavenging activity assay

we calculate the percentage of antioxidant activity versus extract concentrations (IC50) values, which calculated from the linear regression of the percentage of antioxidant activity versus extracts concentrations. Both leaves and fruits extracts as shown in table (2).

Table (2): antioxidant activity of leaves and fruit

Photochemical	Crud Methanolic
Alkaloids	- Ve
Coumarin	++ Ve
Glycoside	- Ve
Falvonoid	+ Ve
Saponins	+ Ve
Tannin	+ Ve

Our results indicate that the *Arbutus pavarii* extract showing high antioxidant activities, these results may have related to the therapeutic effects. The percentage of inhibition shown respectfully concentration dependent effect in both the extract for leaves and fruits- (Figure2 and Figure3)

Antioxidant Activity

In our study, we investigated the possibility that the free radical scavenging action of *Arbutus pavarii* extract. Bot leaves and fruit of methanolic extract have potential antioxidant reduce inducing free radical, suggests that *Arbutus pavarii* has antioxidant activities that could be candidate for future therapeutic

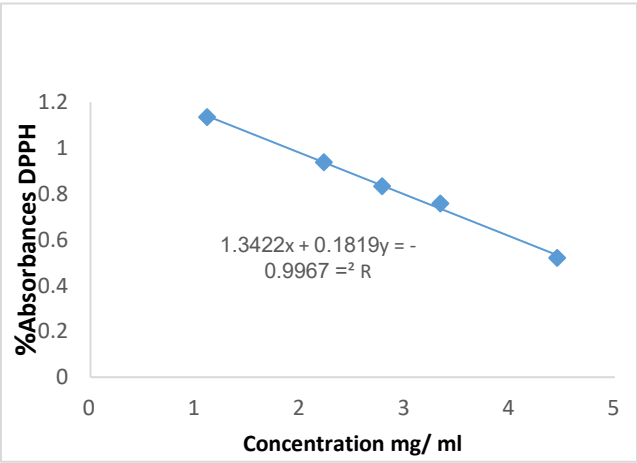

Figure (2): DPPH free radical scavenging activity of Arbutus pavarii leaves methanol extract

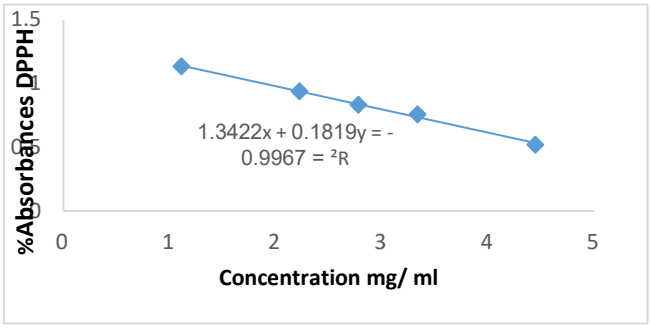

Figure (3): DPPH free radical scavenging activity of Arbutus pavarii fruit methanol extract

Effect of *Arbutus pavarii* on platelets aggregation induced by collagen.

Human platelets were pre-incubated with various concentrations of *Arbutus pavarii* (0.1, 0.3, or 1 mg/ml), and then exposed to collagen (20 µg/ml). As shown in Figure (4) strongly inhibited the platelet aggregation induced by collagen at 1 mg/ml mg/ml. The inhibitory effect on collagen -induced aggregation was more pronounced and shown dose-dependent manner. In addition, the inhibitory effect of on extract-induced (leaves and fruits) platelet aggregation was comparable to aspirin (1.0 ± 0.5 and 1.0 ± 0.7 mg/ml, respectively).

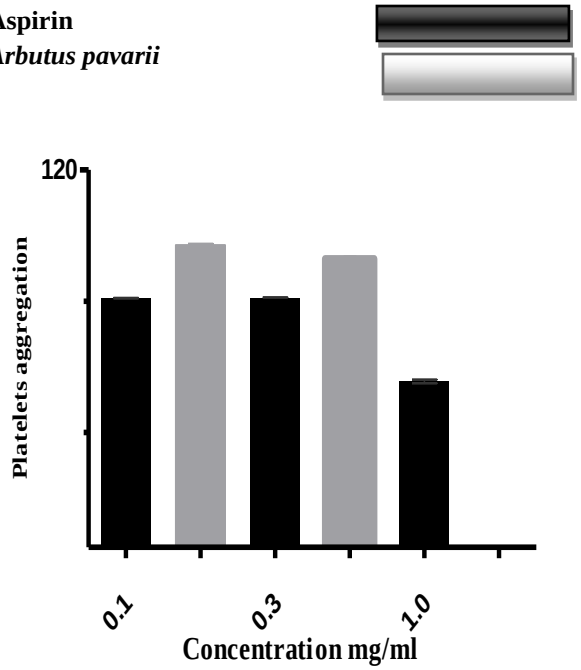

Figure (4): Effect of *Arbutus pavarii* on platelet aggregation by ADP.

Representative platelet aggregation profile in collagen treated platelets in absence or presence of aspirin (1 mg/ml), *Arbutus pavarii* (1 mg/ml) (A) and cumulative result in dose dependent manner (B). All data represent means ± S.D. (n=3). *$p < 0.05$, compared with untreated control

4 Discussion

In primary results the extract of *Arbutus pavarii* shows no potent cytotoxic activity on HELA (cervical carcinoma cell line). In second part the result shown that the *Arbutus pavarii* plant rich in active compound like alkaloids, flavonoid, coumarin, sponins ,and glycoside (Ehsani-Moghaddam *et al*., 2006),these results were compatible with previous studies indicate that *Arbutus pavarii* rich in such active compound (Navindra *et al*., 2006) in third part , we do the antioxidant properties , and our results indicate that the plant has antioxidant effect, these results was improvement by the scavenger of DPPH reagent and IC50 value which indicate dependent effect with increase the concentration of plant extract, these results were compatible with other results from different studies (Navindra P. Seeram et al., 2006) moreover theses effect may have related to the content of polyphenols, further (support an important role of polyphenols is important in preventing the cardiovascular diseases, inflammatory diseases and cancer. Polyphenols are well known not only to have antioxidant properties due to their structure but also to have the ability to modulate the activity of different kinds of enzymes and cell receptors.

As several compounds with antioxidant properties have demonstrated a capacity to reduce the harmful effect caused by reactive species in biological systems through the capture and inactivation of these reactive and potentially damaging substances. Polyphenols, constitute of group of natural products, represent a secondary metabolite of plants and that have a great variability of chemical structures. One of the fundamental structural element is the presence of hydroxyl groups which can be found in free state or bound to other forms such as; ether, ester or heteroside. The polyphenols are abundant in vegetables, and are important part of human and animal nutrition. We can find polyphenols in large quantity in fruits an Flavonoids flavonoids are further divided into many subclasses: anthocyanins, flavan- 3-ols (as monomers forming catechins and as polymers forming proanthocyanidins (condensed tannins), flavones, flavanones, flavonols, isoflavones, neoflavonoids and chalcones. They consist of a C6-C3-C6 backbone. Anthocyanidins have a basic structure, known as flavyliumcation, which contains positive charge in the C-ring, and by this positive charge it is distinguished from other flavonoids. In nature, highly unstable anthocyanidins are protected from degradation by glycosylation (anthocyanidins with sugar group(s) are called anthocyanins) generally with a number of sugars, in particular glucose, sophorose, rut nose, galactose, arabinose and xylose at different positions of the C-ring. Anthocyanins are water soluble pigments, providing the distinctive and vibrant palette of colors (red, blue and purple) found in fruits, vegetables, flowers, and other plant tissues or products. Anthocyanins are widely distributed in human diet including certain varieties of cereals and certain vegetables (cabbage, beans, onions and radishes), there are six main anthocyanidins distributed throughout the plant kingdom: cyanidin, malvidin, delphinidin, peonidin, petunidin and pelargonidin. Among them cyanidin is the most common anthocyanidin in food. Food content Phenolic acids are further divided into benzoic acid and cinnamic acid and consisting of a C1-C6 and C3-C6 backbones, respectively. They contain protocatechuic acid, vanillic acid, gallic acid and syringic acid as examples of phenolic acids in the former; and pcoumaric acid, caffeic acid, chlorogenic acid, cryptochlorogenic acid, neochlorogenicacid, ferulic acid and sinapic acid in the latter.29 Stilbenes are diphenylethene barely present in human diet, a main member is resveratrol. Lignans consists of two phenylpropane units. This group of polyphenol is further metabolized by the intestinal microflora into enterodiol and enterolactone. In general the polyphenols are well known to protect the agents a variety of diseases actions, including their ability to improve the lipid profile; dilate blood vessels by stimulating the endothelial formation of NO, and also in some blood vessels EDHF, and by their antioxidant properties, which will prevent the degradation of NO by superoxide anions (Ndiaye *et al*., 2003; Frankel et *al.*, 1993; Madeira *et al.*, 2009) .In addition, polyphenol-rich sources plants extract have been shown to reduce systolic blood pressure and to improve endothelial dysfunction in several experimental models of hypertension

(Maderia *et al*., 2009; Lee *et al*., 2011)

.Oxidative stress is a major event occurring during biological and pathological processes. Molecular oxygen (O2) is fundamental for the surviving of all aerobic organisms. Aerobic energy metabolism relies on

oxidative phosphorylation in mitochondria. However, during this process, partially reduced and highly reactive O2 metabolites, such as superoxide anions (O2-), hydrogen peroxide (H2O2) and hydroxyl radical (•OH), can be found within the cells. These chemically reactive molecules containing oxygen are called reactive oxygen species. The generation of reactive oxygen species in a biological environment exposes most living organisms to the so called 'oxygen paradox': oxygen is necessary for life and reactive oxygen species have

important roles in redox homeostasis and cell signaling but they are also potentially hazardous since reactive oxygen species may easily become a source of cell stress and tissue injury, like in most acute and chronic inflammatory processes and infections. However, most living organisms have mechanisms and strategies to overcome the excess generation of reactive oxygen species and oxidative stress, as well as to 'make use' of reactive oxygen species under physiological conditions.

Arterial thrombosis is the formation of a blood clot, thrombus, as a result of the

activation of platelets and the coagulation cascade, obstructing the flow of blood

through the circulatory system. Activation of platelets is critical to the development of platelet-rich arterial thrombosis in part because activated platelets adhering to vascular cells generate lipid peroxides and More over previous experimental animals and humans indicate the ability of polyphenol from grape-, tea-, berry-, and plant-derived products such as tea to reduce platelets30 aggregation theses effect may also have related at least to reduced risk of CVD associated with an improvement of endothelial dysfunction involving an increased formation of NO and/or NO bioavailability. Several in vivo studies have also reported vasoprotective effects by various natural products in animal models, our results indicate that strawberry tree extract are able to reduce platelets aggregation induced by collagen. Improvement from different studies also indicate the major phenolic compounds present in the strawberry tree extract are pelargonidin- 3-O-glucoside, coumaroyl- O-glucoside, and trans-cinnamoyl- O-glucoside. previous that strawberry tree is rich in phenolic compound The major groups of phenolic compounds present anthocyanins, flavonols, flavanols, ellagitannins, Gallo tannins, proanthocyanidins, and phenolic acids . our finding also indicates that the plant extract of Arbutus pavarii.

The fact that polyphenols are abundant in our diet makes it necessary to determine the nature and distribution of different kinds of polyphenols, so that we can identify which sources and key compounds are likely to provide an optimal protection especially in the cardiovascular system. Thrombosis is the formation of a blood clot, which is known as thrombus, inside a blood vessel, obstructing the flow of blood through the circulatory system. Pathologically, thrombosis may occur if the haemostatic stimulus is unregulated, either at 31 the level of stimulatory or inhibitory pathways. The balance between stimulatory and inhibitory pathways is also well established for platelets. Platelets are activated by various endogenous factors and a platelet- rich thrombus is formed subsequently in the lumen of the injured vessel. our observed indicate that *Arbutus pavarii* extract are able to inhibit the human platelets aggregation induced by collagen, studies indicate that the consumption of polyphenol-rich foods Ginkgo biloba extract may related to reduce the risk of acute coronary disease this effect may related to the ability of polyphenols to inhibition platelet aggregation and thus it could be potentially inhibited collagen, Induced Platelet aggregation.

5 Conclusion

Our preliminary results suggest that the *Arbutus pavarii* extract, revealed the presence of Coumarin, Falvonoid, Saponins, and Tannin.

It was inactive against HELA (cervical carcinoma cell line) and are inactive against HELA cells (cervical carcinoma cell line). had significant scavenging effects on the DPPH radical. *In vitro* the extract of *Arbutus pavarii* showed an antiplatelet effect. Our observations on polyphenols in general and on *Arbutus pavarii*, provide evidences suggesting that natural products might be of interest for improving liver diseases. In accordance with that, there are numerous reports that support the important role of polyphenols in preventing cardiovascular diseases, inflammatory diseases, and cancer. In addition to the antioxidant properties of polyphenols, their ability to modulate the activity of different kinds of enzymes and cell receptors are also of interest. The fact that polyphenols are abundant in our diet makes it necessary to determine the nature and distribution of different kinds of polyphenols, so that we can identify which sources and key compounds are likely to provide the greatest protection against different diseases. Further works need to detect the exact active

molecule of polyphenols and their metabolites plus more details about their bioavailability before we can proceed to introduce them in human research.

Overall further work needs to identify the pathophysiological process of portal hypertension and its complications like hepatopulmonary syndrome. Furthermore, it will be interesting to test both VSL#3 and PRBJ to study other organs affected by portal hypertension like lungs and kidneys. Our studies support that *Arbutus pavarii* rich in polyphenols are of potential interest for future work.

Recommendations

1-*Arbutus pavarii*, provide evidences suggesting that natural products might be of interest for future work.

2- Polyphenols from plant in preventing cardiovascular diseases, inflammatory diseases.

3- Polyphenols, are able to modulate the activity of different kinds of enzymes.

4- Polyphenols are abundant in our diet makes it necessary for our health.

5- Further work needs to identify the mechanism of action using animals models.

6- Investigate vivo the antiplatelet and antithrombotic activities of *Arbutus pavarii*

and if so to elucidate the underlying molecular mechanism.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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