European Journal of Food Science and Technology
Vol.5, No.3, pp.36-49, July 2017
Published by European Centre for Research Training and Development UK (www.eajournals.org)

IDENTIFICATION AND CHARACTERIZATION OF THE PHENOLIC PROFILE OF A HYDROALCOHOLIC EXTRACT OF PUNICA GRANATUM

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ABSTRACT: *More than half the world's population use or used at some point a herbal because of its potential healing. The pomegranate, as well as another 70 medicinal plants are listed as species of interest to the public health system in order to generate new effective and safe medicines. The objective of this study was to evaluate a hydroalcoholic extract of Punica granatum about their biochemical and biophysical characteristics. The extract was serially diluted and exposed to different conditions. The values of wavelengths, absorbance, pH and conductivity were observed. The analysis values, it can be concluded that the extract in question has the characteristic of stability in the different conditions and that has possibly flavonoids, tannins and anthocyanins in its composition.*

KEYWORDS: Characterization, Biochemistry, Biophysics, *Punica Granatum*, Flavonoids, Tannins, Anthocyanins.

INTRODUCTION

The potential of medicinal plants is assigned to the same substances such as alkaloids, saponins, tannins, glycosides, flavonoids and essential oils. These components account for effects such as analgesics, antiseptics, diuretics, expectorants, soothing, digestive, cicatrising, emollients, antidiarrheals, among others (SANTOS & TORRES, 2012).

According to health ministry data, the use of herbal medicines has been widespread in medicine today considering that 85% of the population used any plant at any given time to relief of symptoms of pain or discomfort (BRASIL, 2006). The fact that these plants yielding drugs in short time and with more affordable the population can be one of the reasons why the use of naturally occurring drugs have grown vertiginous way. (BRASIL, 2006).

ISSN 2056-5798(Print), ISSN 2056-5801(online)

Intended to support and encourage research in the herbal industry in order to develop safe and effective derived from herbal medicines, the health ministry announced in 2009 a listing that has 71 species of medicinal plants of interest to the single system health, RENISUS (National List of Medicinal Plants of Interest to SUS) (PORTAL DA SAÚDE, 2009). The *Costus spicatus* (cane swamp), for example, belongs to list and is able to help in cases of hypoglycaemia (NASCIMENTO, 2015).

The pomegranate is composed of 50% of its total weight per seed. Natural middle eastern, your tree is a thorny and slightly branched species, grows in arid regions and production of the fruit takes place from September February (MARTINS, 1995). The *Punica granatum* has many problems such as use for prophylaxis of tooth loss, gastrointestinal disorders and pain relief headset among others (NAVARRO *et al*., 1996).

Chen *et al*. (2015) pomegranate fruits is inserted in a group that contains a large amount ellagic acid, a polyphenol capable of inhibiting the proliferation of cancer cells and induce apoptosis. Usta *et al*. (2013) states that this same component and other ellagitannins in pomegranate this has the capacity to eliminate free radicals pose potential hypolipidemic drugs, anti-inflammatory, anticancer and induces vasodilation. *Punica granatum L*. var. granatum (Pomegranate), is an herbaceous plant found in Iran, Studies show that the alcoholic extract of *Punica granatum* fruit is able to exert a similar effect to antibiotics such as gentamicin and chloramphenicol having bearing potential cytotoxic front of microorganisms (PEIXOTO *et al.*, 2014).

In a study was aimed to document detailed ethnopharmacological knowledge of medicinal plants against livestock infections of an unexplored remote region of Pakistan. Semistructured questionnaires were used for data collection. Total 43 plants belonging to 26 families were found to be used in ethnoveterinary practices. Seeds (29%) were found to be the most frequent plant part used followed by leaves (22%). Ethnoveterinary recipes were mostly prepared in the form of decoction and powdering. Informant consensus factor (Fic) results revealed high consensus for gastrointestinal (0.81), mastitis (0.82), and dermatological infections (0.80). Curcuma longa ranked first with highest fidelity level (FL) value (66%) followed by Trachyspermum ammi that ranked second (58%). Preference ranking (PR) results showed that *Zingiber officinale*, *Punica granatum*, *Triticum aestivum*, *Gossypium hirsutum*, and *Withania coagulans* were the most preferred species for the treatment of diarrhea. Direct matrix ranking (DMR) results showed that *Morus alba*, *Melia azedarach*, *Withania coagulans*, *Cassia fistula*, *Azadirachta indica*, and *Tamarix aphylla* were the multipurpose species of the region. It was reported that is interesting to invite the attention of pharmacologists and chemists for further exploration of plants having high Fic, FL, and PR values. Conservation strategies should be adopted for the protection of multipurpose plant species. The objective of this study is to characterize biochemical and an alcoholic extract of the dried peel of the fruit of *Punica granatum*.

MATERIALS AND METHODS

The alcoholic extract of the dried bark of *Punica granatum* produced by EMBRAPA made available by the same so that the analysis could have been performed.

ISSN 2056-5798(Print), ISSN 2056-5801(online)

The alcoholic extract was characterized biochemical and biophysically. The procedure was performed by reading in UV spectrophotometer (UV-2450, SHIMADZU).
The extract was initially subjected to a serial dilution, so that it complies with the law of Lambert and Berr. This law directs that the value of the wavelength related to the absorbance scan is maintained at the lowest absorbance values and / or equal to 1. (COUTO *et al.*,1998).

Dilution 1 - 1 mL of concentrated extract (1,67g / mL) in 10 mL of distilled water (0,167g / mL).

Dilution 2 - 100µL of the 1st dilution (0,167g / mL) in 10 mL of distilled water (0,0167g / ml).

Dilution 3 - 10mL of the 2nd dilution (0,0167g / mL) in 10 mL of distilled water (0,00167g / mL).

Dilution 4 - 20 mL of the 3rd dilution (0,00167g / mL) in 100mL distilled water (0,0000167g / mL).

Dilution 5 - 120 mL of the 4th dilution (0,0000167g / mL) in 60 mL of distilled water (0,0000002783g / mL).

The first step consisted in the dilution and sample reading ultraviolet spectrophotometer (UV-2450, Shimadzu) to a wave length of 200 800 nm. Measurements were made with the aid of acrylic cuvettes.
The second step of the characterization was performed the day after the first step because it is the same procedure of that step, and the extract dilution is performed in different physical conditions and measurements are recorded after the 24-hour period.
To perform the second step, the tubes were identified according to the conditions described in Table 1.

Table 1 - Conditions for step 2 of the biochemical characterization.

1	Open tube	4	Vacuum
2	Closed tube.	5	Vacuum and in protected from light.
3	closed tube and protected from light	6	Closed tube after heating.

Source: the author

We used two groups of four 15mL conical tubes and vacuum tubes 4 under the conditions described in Table 1, where a group of six tubes were subjected to ambient temperature and another group of six tubes kept under refrigeration, totaling 12 tubes. Tests were performed in duplicate.

Statistical Tests
The results were statistically analyzed with the help of Graph Pad InStat3 software. The analyzes were performed according to the Tukey-Kramer test, the ANOVA in conjunction with the changes referring to the absorbance and wavelength between steps 1 and 2.

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RESULTS

The biochemical characterization analyzes of the alcoholic extract of Punica granatum showed the segintes results:

Representation Statement Characterization of Graphic (step1):

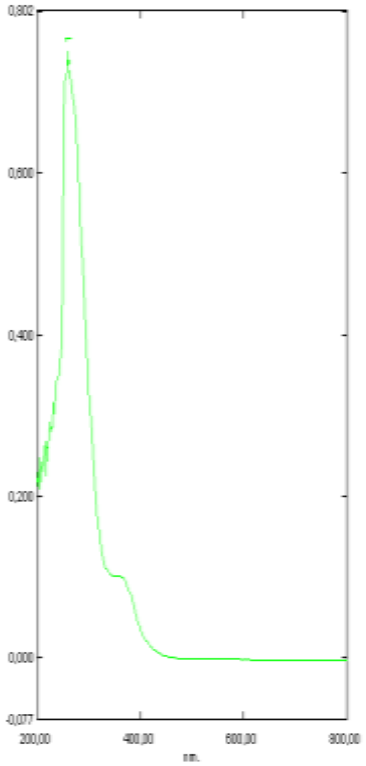

Figure 1 – Representation Statement Characterization of Graphic (step1). Source: the author

Table 2 - Sweep step 1

Wavelength (nm)	Absorbance
250	0.79

Source: the author

Note: peaks of approximately 258nm and 352 / 354nm, although not detected by the device are present.

ISSN 2056-5798(Print), ISSN 2056-5801(online)

Graphic representation of the characterization of the extract (phase 2)

Ambient Temperature

Tube 1 (Open / Ambient Temperature)

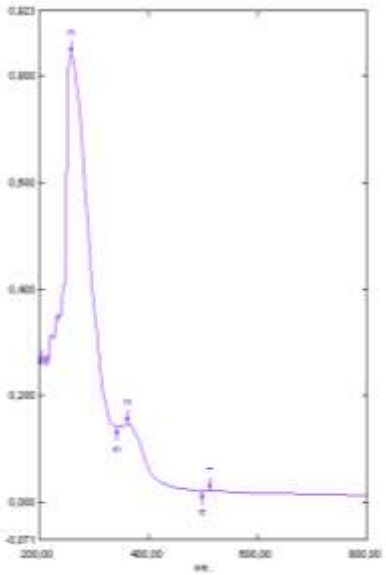

Figure 2 - Tube 1 (Open / Ambient Temperature). Source: the author

Table 3 - Tube Sweep 1 (Open/ Ambient Temperature)

Wavelength (nm)	Absorbance
512	0. 021
362	0. 146
258	0. 840
496	0. 019
342	0. 141

Source: the author

Tube 2 (Closed / Ambient Temperature)

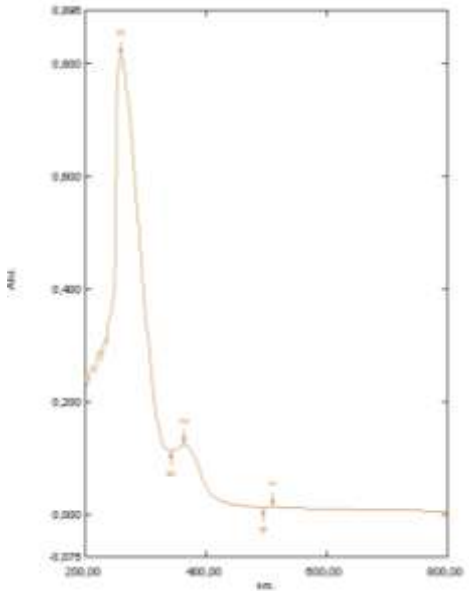

Figure 3 - Tube 2 (Closed / Ambient Temperature). Source: the author

Table 4 - Tube Sweep 2 (Closed / Ambient Temperature)

Wavelength (nm)	Absorbance
510	0. 013
364	0. 125
258	0. 815
494	0. 012
342	0. 113

Source: the author

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Tube 3 (Protect from light / Ambient temperature).

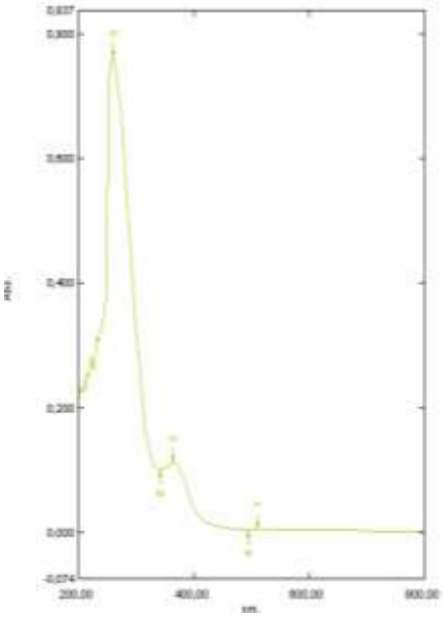

Figure 4 -Tube 3 (Protect from light / Ambient temperature). Source: the author

Table 5 - Sweep Tube 3 (Protect from light / room temperature)

Wavelength (nm)	Absorbance
510	0. 005
364	0. 113
260	0. 761
494	0. 003
342	0. 100

Source: the author

Tube 4 (Vacuum / Ambient Temperature)

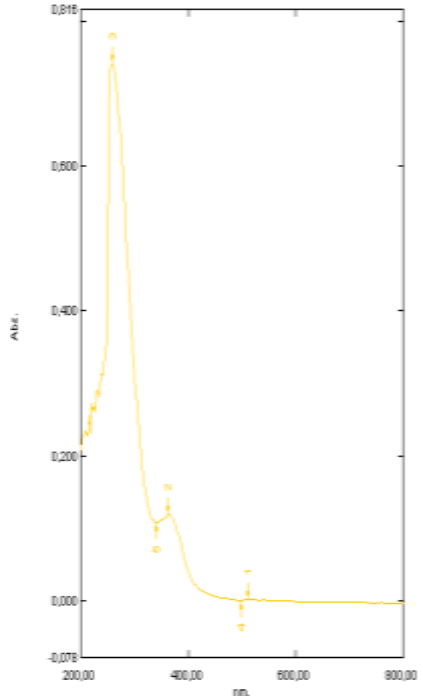

Figure 5 -Tube 4 (Vacuum / Ambient Temperature). Source: the author

Table 6 - (Sweep Tube 4 (Vacuum / Ambient Temperature)

Wavelength (nm)	Absorbance
510	0. 002
362	0. 119
258	0. 742
498	0. 000
340	0. 108

Source: the author

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Tube 5 (vacuum / protect from light / Ambient Temperature)

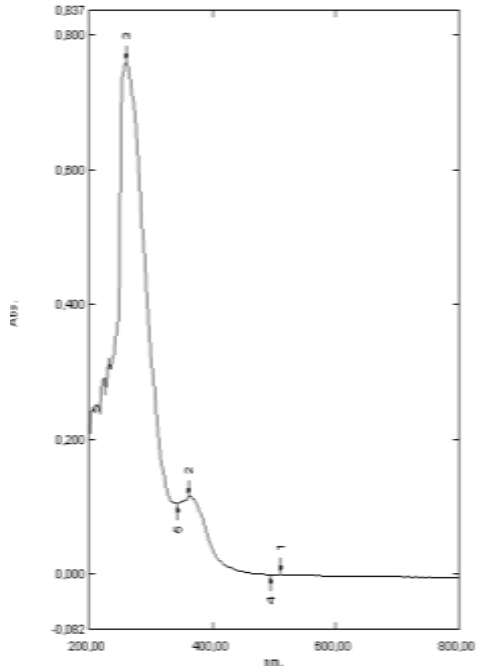

Figure 6 - Tube 5 (vacuum / protect from light / Ambient Temperature).
Source: the author

Table 7 - Sweep tube 5 (A vacuum /protect from light / Ambient Temperature)

Wavelength (nm)	Absorbance
362	0. 116
260	0. 760
344	0. 105

Source: the author

Tube 6 (Closed after heating / Ambient Temperature)

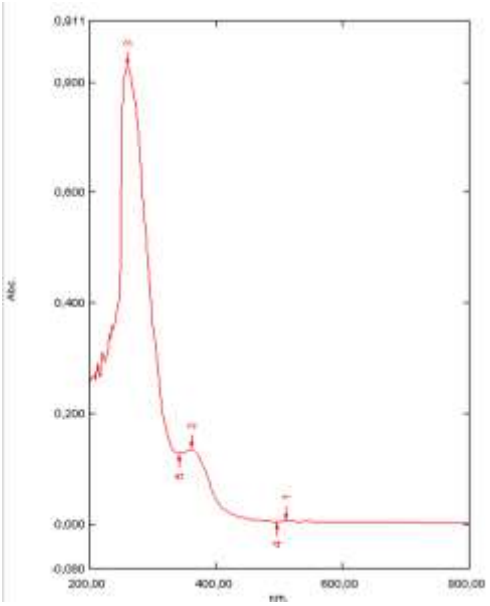

Figure 7 -Tube 6 (Closed after heating / Ambient Temperature).
Source: the author

Table 8 - Tube Sweep 6 (Closed after heating / Ambient Temperature)

Wavelength (nm)	Absorbance
510	0. 006
362	0. 136
260	0. 828
496	0. 005
342	0. 129

Source: the author

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Under refrigeration

Tube 1 (Open / under refrigeration)

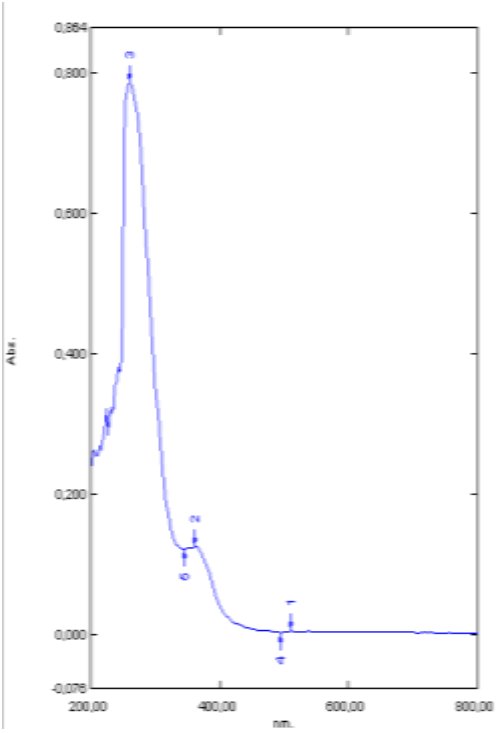

Figure 8 -Tube 1 (Open / under refrigeration). Source: the author

Table 9 - Sweep tube 1 (Open / under refrigeration)

Wavelength (nm)	Absorbance
510	0. 005
362	0. 126
260	0. 786
494	0. 003
346	0. 122

Source: the author

Tube 2 (closed/ Under refrigeration

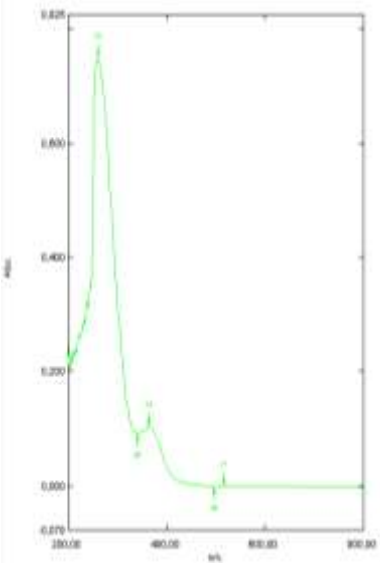

Figure 9 - Tube 2 (closed/ Under refrigeration. Source: the author

Table 10 - Sweep tube 2 (Closed / under refrigeration)

Wavelength (nm)	Absorbance
364	0. 105
260	0. 750
340	0. 092

Source: the author

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Tube 3 (Protect from light/ Under refrigeration)

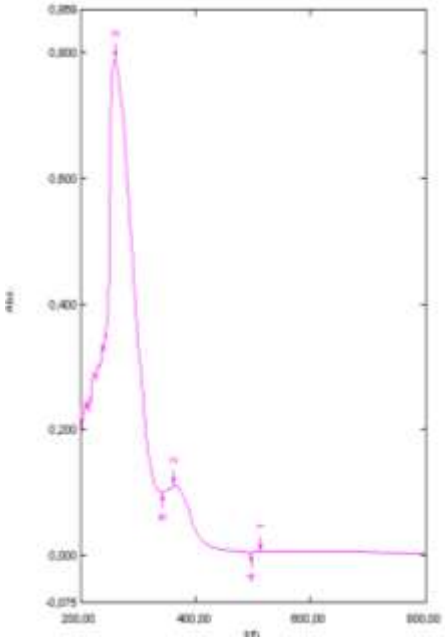


Figure 10 - Tube 3 (Protect from light/ under refrigeration). Source: the author

Table 11 - Sweep tube 3 (Protect from light / under refrigeration)

Wavelength (nm)	Absorbance
512	0. 007
362	0. 112
260	0. 791
496	0. 005
342	0. 101

Source: the author

Tube 4 (Vacuum/ under refrigeration)

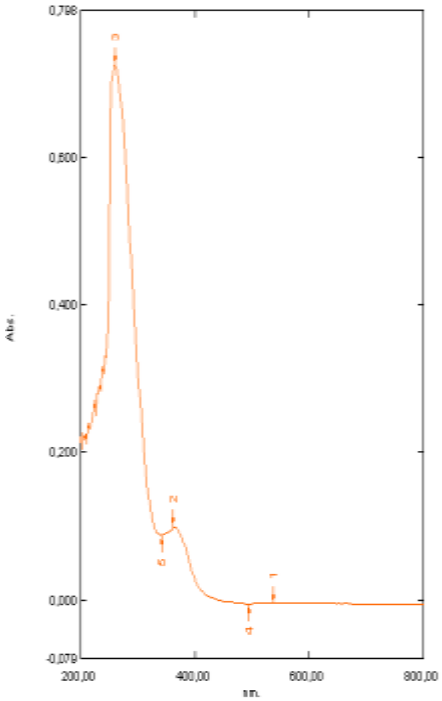


Figure 11 - Tube 4 (Vacuum/ under refrigeration). Source: the author

Table 12 - Sweep tube 4 (Vacuum / under refrigeration)

Wavelength (nm)	Absorbance
362	0. 100
260	0. 725
342	0. 008

Source: the author

ISSN 2056-5798(Print), ISSN 2056-5801(online)

Tube 5 (Vacuum / protect from light / under refrigeration)

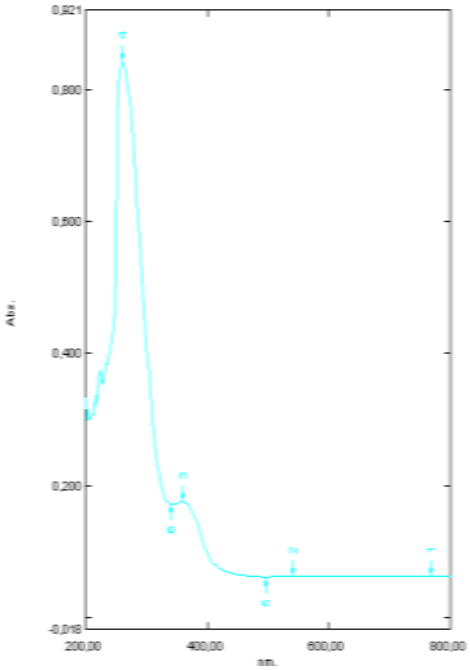


Figure 12 - Tube 5 (Vacuum / protect from light / under refrigeration). Source: the author

Table 1 - Sweep tube 5 (Vacuum/ protect from light/ under refrigeration)

Wavelength (nm)	Absorbance
768	0. 063
540	0. 063
360	0. 176
260	0. 843
496	0. 061
340	0. 171

Source: the author

Tube 6 (Closed after heating / under refrigeration)

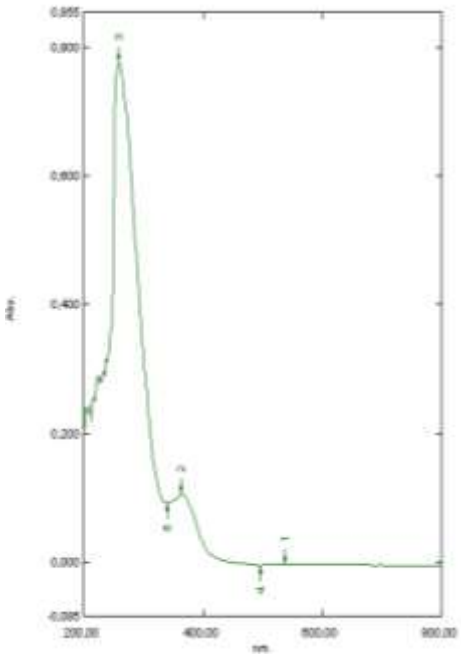


Figure 13 - Tube 6 (Closed after heating / under refrigeration). Source: the author

Table 14 - Tube Sweep 6 (Closed after heating / under refrigeration)

Wavelength (nm)	Absorbance
364	0. 105
258	0. 776
340	0. 091

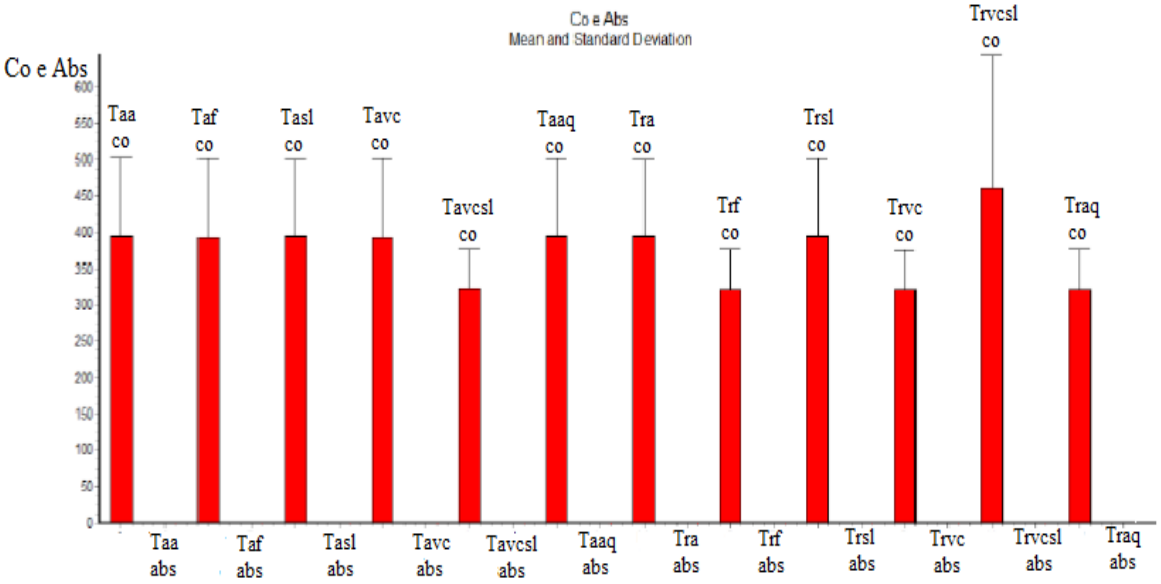
Source: the author

ISSN 2056-5798(Print), ISSN 2056-5801(online)

The wavelengths identified in the course of the experiment were 260, 342, 362 and 510nm.

Upon scanning the extract of the dried bark of *Punica granatum* UV spectrophotometer, considered differences were no significant both for the average wavelengths, and for the absorbance values.

Representation Statement Characterization of Graphic as the absorbance and wavelength (Statistical Analysis)



(abs) and wavelength (co). Taa- open tube / room temperature Taf- closed tube / room temperature Tasl- tube in the dark / ambient temperature, vacuum tube Tavc- / temperature environment, Tavcsl- vacuum tube and the dark / ambient temperature, Taaq- tube subjected to heating / room temperature treat- open tube / subjected to cooling, Trf- / closed subjected to cooling tube, tube under Trsl- / light subjected to refrigeration, vacuum tube Trvc- / subjected to cooling, Trvcsl- vacuum tube and protected from light / subjected to cooling, Traq- tube subjected to heating / cooling undergone.

DISCUSSION

Statistical analysis was able to identify the absence considered significant changes in mean values between the wavelength and the absorbance and which indicates that conditions which differentiated the extract was subjected in step 2 provide no degradation of bioactive compounds thereof, giving it the characteristic of stability.

In the spectrophotometric scan were detected wavelengths of 260, 342, 362 and 510nm in most representative in the extract. The literature states that the wavelengths between 240 to 280nm and 300 are characteristic of the presence of flavonoids (BOBIN, 1994) and the wavelength of 510 nm is characteristic of condensed tannins (ROCHA, 2011) or that this characterizes the wavelength of maximum absorbance value for cyanidin-3-glucoside (ABE, 2007).

ISSN 2056-5798(Print), ISSN 2056-5801(online)

Then it can be seen that in the statement in question, the analytical methods employed were able to show only the presence of flavonoids and tannins or a type of anthocyanin in small proportions, given that the length of 510nm was observed absorbance values very small. It is interesting to consider that Sineh *et al*. (2014); suggested that the ethalonic extract of *Punica granatum L.* var. spinosa altered cell morphology, decreased cell viability, suppressed cell proliferation and induced cell death in a time- and dose-dependent manner in WEHI-164 cells (mouse fibrosarcoma cell line), when compared to a chemotherapeutic anticancer drug, Toxol (Vesper Pharmaceuticals), with increased nucleosome production from apoptotic cells. Induction of apoptosis by the plant extract was proved by the decrease of pro-Caspase-3 and Bcl2 proteins and quantitatively confirmed by Immunoblotting analysis.

Condensed tannins also known as proanthocyanidins, are secondary metabolites which have characteristics as an input for astringent taste of food and protein precipitation (QUEIROZ *et al*., 2002). Since flavonoids have the characteristic inhibit the action of lipoxygenase and cyclooxygenase, two enzymes that are closely related to the inflammatory process. Anthocyanins are one type of flavonoid widely present in nature, and this is attributed to the blue color, violet and red fruit (ABE, 2007), moreover, anthocyanins are considered one of the most significant natural antioxidants (SANTIAGO *et al*., 2011). The high levels of polyphenols, can be one of the sources of the natural antioxidants. The absence of other bioactive compounds, according to the literature, are present in the composition *Punica granatum* may be related to the fact that seasonal factors like soil composition, humidity, temperature may interfere with the identification thereof, as they influence the level of phenolic compounds plants. (MONTEIRO *et al*., 2006; SANTOS *et al.*, 2006) or even can relate to the fact that the extract has been obtained from the processing of the bark factor that phenolic compounds are present throughout the but plant in different proportions (JARDINI *et al*., 2010).

CONCLUSION

With the analysis of this study, it can be seen that the hydroalcoholic extract of *Punica granatum* has the characteristic of stability through the different conditions and submitted it contains phenolic compounds such as tannins, flavonoids and anthocyanins. Such components may be responsible by the healing properties of the extract and of the vegetal species in study.

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