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STUDY OF THE CHANGE OF THE ELECTRICAL CONDUCTIVITY OF A LYOPHILIZED AQUEOUS EXTRACT OF *SYZYGIUM JAMBOLANUM* UNDER DIFFERENT TREATMENT CONDITIONS.

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ABSTRACT

The leaves and the fruits from *Syzygium jambolanum* DC. (*Myrtaceae*), a plant known in Brazil as sweet olive or 'jambolan', have been used by native people to treat infectious diseases, diabetes, and stomachache. This study aimed to determine the conductivity in different samples of a freeze-dried aqueous extract of *Syzygium jambolanum* in different periods of time. The readings of the electrical voltages of the samples subjected to different treatment conditions were carried out in a pH meter. Depending on the performed statistical analysis could be noticed a significant difference ($p < 0.005$) among all samples analyzed groups. From the analysis of the results we can speculate that the aqueous extract of *Syzygium jambolanum* study has in its constitution phyto molecules which exhibit significant volatility as a function of time with antioxidant potential is sensitive to the presence of oxygen and exposure to light.

KEY-WORDS: *Syzygium jambolanum*, Anthocyanin, Flavonoids, Antioxidant, Plant extract.

INTRODUCTION

For thousands of years the use of medicinal plants has played an important role throughout the world in the treatment and prevention of human disease and remains a source for discovering new drugs. Brazil has the greatest biodiversity on the planet, has a valuable traditional knowledge associated with the use of medicinal plants and has the potential to develop research that results in technologies and effective therapies. It is believed that about two thousand Brazilian plants are used as natural medicines by the people. WHO has supported the use of medicinal plants in the health field, taking into account that 80% of the world population use plants or their preparations. The use of medicinal plants should be encouraged through research demonstrating therapeutic efficacy in humans. [1, 2]

Phytotherapeutic agents are medicines playing an important role in care against pain, inflammation, disorders and other nuisances, extending the safe and effective treatment alternatives. Indicated for the symptomatic relief of low-gravity conditions and for short periods of time, such drugs can be produced from fresh or dried plants and their derivatives and have several different dosage forms such as syrups, solutions, tablets, ointments , gels and creams. According to Brazilian law, phytoterapic is the product obtained exclusively from plant active raw materials. [3, 4]

Great biodiversity is a highlight of Brazilian flora. In contrast, the therapeutic potentialities of most species used in folk medicine remain unknown. The National List of Medicinal Plants of Interest to the SUS (Renisus) is a list which contains medicinal plants that have the potential to generate products of interest to SUS. The purpose of the list is to guide studies and research that could support the development of herbal ratio available for use by the population, with safety and efficacy for the treatment of certain disease. Currently, the list contains 71 plants of interest to SUS. Among some species appear to *Cynara scolymus*, *Schinus terebentthifolius*, *Syzygium jambolanum* and *Uncaria tomentosa*, used by popular wisdom and confirmed scientifically for various diseases.[4]

The *Syzygium* genus is one of the *Myrtaceae* family genuses which is native to the tropics, particularly in tropical America and Australia. The genus consists of about 1100 species. Plants of this family are known to be rich in volatile oils that are reported to their use in

medicaments. *Syzygium jambolanum* has synonymously *Eugenia jambolana* Lam., *Myrtus cumini* Linn., *Syzygium jambolana* DC., *Syzygium cumini* (L) Skeels, *Eugenia cumini* (Linn.) Druce. The tree is known to grow in the Indian subcontinent and many other adjacent regions of South Asia such as India, Bangladesh, Nepal, Pakistan, Sri Lanka and Indonesia. The fruit has been used for long for various medical purposes and is currently widely used to treat chronic diarrhea and other enteric disorders. In addition, it has been used for a wide variety of ailments, including cough, diabetes, dysentery, inflammation and ringworm. The bark is acrid, digestive, anthelmintic and used for the treatment of sore throat, bronchitis, asthma, dysentery and ulcers. The seed extract is used to treat skin problems such as rashes and genitourinary tract ulcers (infected by *Candida albicans*) cold, cough and fever. The use of the plant is recommended in ayurvedic medicine for the treatment of diabetes mellitus. Parts of the jambolan were also reported for its antioxidant, anti-inflammatory, neuropsycho-pharmacological, anti-microbial, anti-HIV, antileishmanial and antifungal, nitric oxide scavenging, free radical scavenging, anti-diarrheal, gastroprotective and antiulcerogenic and radioprotective activities. Jambolan is rich in compounds containing anthocyanins, ellagic acid, isoquercetin, kaempferol and myrecetin. It was reported that the seeds are rich in flavonoid, a known antioxidant that is responsible for scavenge free radicals. The leaves are rich in acylated glycosides, quercetin, myricetin, myricitin, myricetin 3–O–4–acetyl–L–rhamnopyranoside, triterpenoids, esterase, galloyl carboxylase and tannin. The flowers are rich in kaempferol, quercetin, myricetin, isoquercetin (quercetin–3–glucoside), myricetin–3–L–arabinoside, quercetin–3–D–galactoside, dihydromyricetin, oleanolic acid, acetyl oleanolic acid, eugenol–triterpenoid A and eugenol–triterpenoid B. The fruits are rich in raffinose, glucose, fructose, citric acid, mallic acid, gallic acid, anthocyanins, delphinidin–3–gentiobioside, malvidin–3–laminaribioside, petunidin–3–gentiobioside, cyanidin diglycoside, petunidin and malvidin. The color of the fruits might be due to the presence of anthocyanins. One type of jambolan found in Brazil has malvidin–3–glucoside and petunidin–3–glucoside. [5]

Syzygium jambos has been used as a traditional medicine for the treatment of inflammatory diseases in Bangladesh. Hossain et al. [6] suggested that the phenolic and flavonoid compounds are responsible for acute anti-inflammatory and antioxidant activities of *S. jambos*.

Nunes et al. [7] reported the antioxidant activity of Malay apple fruit (*Syzygium malaccense)* grown in Brazil. Their study highlighted the potential of Malay apple fruit as a good source of antioxidant compounds with potential benefits to human health.

Ajiboye et al. [8], highlighted that is evident from the data generated from their study that aqueous extract of *Syzygium aromaticum* seeds enhanced membrane permeability and oxidative stress in *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. From this study we observed the bactericidal activity related to phyto molecules present in *Syzygium aromaticum* seeds.

In a study, Sanches et al. [9] demonstrated that *Syzygium cumini* leaf improved peripheral insulin sensitivity via stimulating/β-cell modulating insulin release, which was associated with improvements in metabolic outcomes in monosodium L-glutamate (MSG) -induced obese rats.

Krishnasamy et al. [10] reported in their studies with streptozotocin and nicotinamide-induced diabetic rats treated with *Syzygium densiflorum* extract, the regeneration of β-cells in Islets of Langerhans, confirming the antidiabetic, antihyperlipidemic and antioxidant activities of *S. densiflorum* fruits.

Ethnopharmacological studies have particularly addressed antidiabetic and metabolic-related effects of extracts prepared from its different parts, especially seed, and pulp-fruit, however there is a lack of studies on phytochemical profile and biological properties of its leaf. As there is considerable interest in bioactive compounds to treat metabolic syndrome and its clustered risk factors, we sought to characterize the pH and conductivity of a lyophilized aqueous extract of *Syzygium jambolanum.*

MATERIALS AND METHODS

Aerial parts of a jambolan tree were obtained from the campus of the Foundation State University Center of the West Zone (UEZO), in January 2013 at 07:00. Leaves were washed in water and allowed to soak in distilled water for 10 minutes, then were perforated. 400g leaves were used for 1L of water at 100° C to produce an extract by effusion. The extract was lyophilized (Lyophilizer Liotop, Model: L202 - Liobras) and stored at -28° C in a freezer Vertical Electrolux FE18 145 Liters.

The aqueous extract of jambolan was prepared with 10mg of lyophilized extract diluted in 10 mL of distilled water. After serial dilutions spectrophotometric reading was possible from 3.12% of the stock solution. 10mL of this solution was added to six tubes were subjected to six different conditions and stored at room temperature. The conditions were as follows: 1 exposed to light and closed; 2 exposed to light and open; 3 sheltered from the light and closed; 4 heated to 100°C; 5 vacuum tube, 6 vacuum tube and sheltered from light. The readings of the electrical voltages of these samples were performed using a pH meter (MS Tecnopon, model mPA210) after 24 and 48 hours to determine the conductivity. This procedure was performed in triplicate. The figures relating to the average of the measurements of eletrovoltagem (mV) for the measurement at the time the statement was prepared and after 24 and 48 hours under different conditions of conditioning, were treated from

the analysis performed in a statistical program (GraphPad InStat). The experiment was carried out in the Laboratory Chemical and Biological Analysis (LAQB) of the UEZO.

RESULTS

Statistical analysis designed to compare the different types of treatments considering two groups as a function of time, the treatment group at 24 hours and the treatment group in 48 hours.

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                    Unpaired t test
Do the means of 24h and 48h differ significantly?

P value
The two-tailed P value is 0.0004, considered extremely significant.

t = 5.207 with 10 degrees of freedom.
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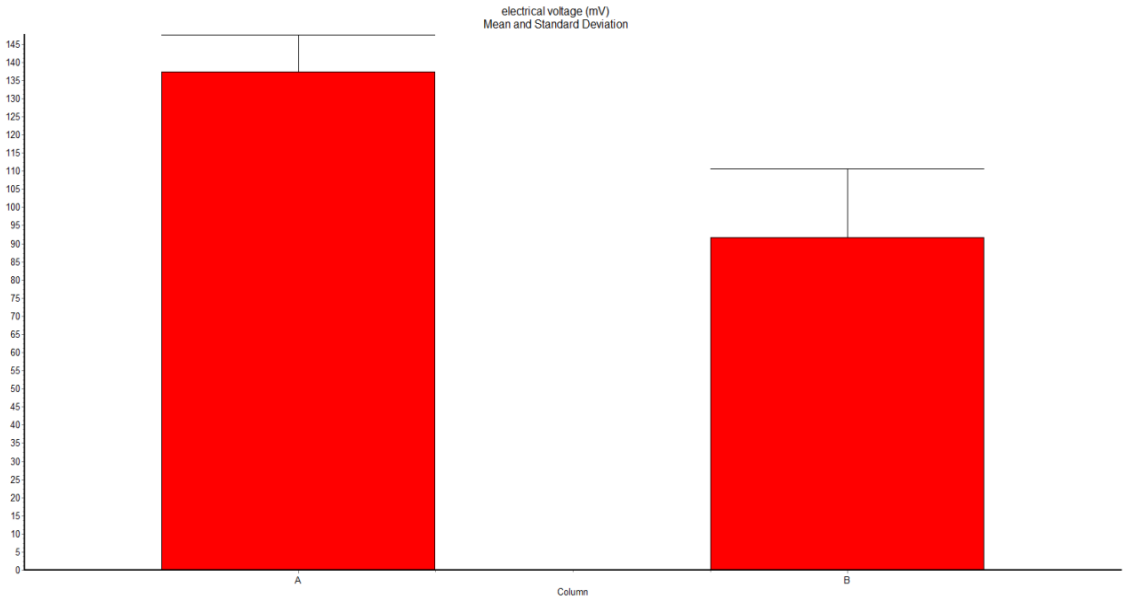

Graphic 1- Comparison of the variation of electrical voltage between 24 and 48 hours under the conditions of different treatments of samples of an aqueous extract of *Syzygium jambolanum*.

The graphic above the x-axis represents the variation in time related to the treatment time of the samples [24 (A) and 48 (B) hours] while the y axis represents the variation of electric voltage in millivolts (mV).

From the analysis of the results it can be inferred that there was a variation which was considered highly significant (p = 0.0004) when comparing the electrical voltage values (mV) on the different treatment conditions in the end of 24 hours (A) with the conditions treatment at the end of 48 hours (B).

When comparing all treatment conditions within 24 hours and within 48 hours can be observed from the statistical analysis, a highly significant difference (p <0.0001) among all treatment conditions.

```
                One-way Analysis of Variance (ANOVA)

The P value is < 0.0001, considered extremely significant.
Variation among column means is significantly greater than expected
by chance.


Tukey-Kramer Multiple Comparisons Test
If the value of q is greater than 5.099 then the P value is less
than 0.05.
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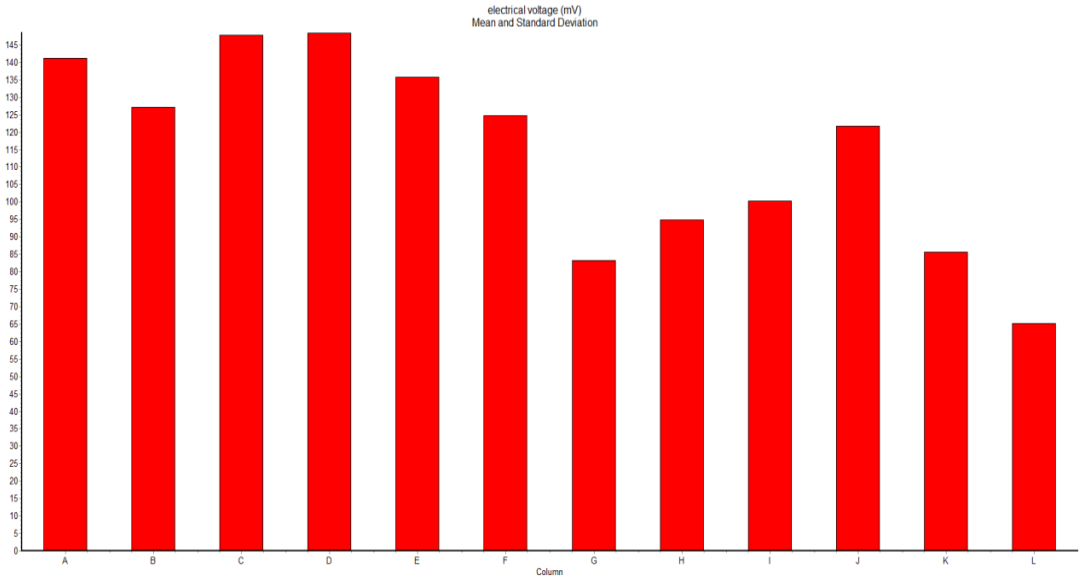

Graphic 2- Comparison of the variation of electrical voltage between the different treatments on samples of an aqueous extract of *Syzygium jambolanum* at 24 and 48 hours.

In Graphic 2, the x-axis represents the treatment time equal to 24 hours and 48 hours, respectively, while in the y-axis represents measured values related to the eletric voltage (mV).
In the X-axis the conditions were as follows:
1-) Analysis performed the end of 24 hours
A- exposed to light extract in sealed tube;
B- exposed to light extract in the open pipe;
C- housed extract light in a closed tube;
D- extract heated to 100 °C,
E- extract to vacuum;
F- vacuum pipe extract and sheltered from the light.

2-) Analysis performed at the end of 48 hours
G- exposed to light extract in sealed tube;
H- exposed to light extract in the open pipe;
I- housed extract light in a closed tube;
J. extract heated to 100 °C;
K- extract to vacuum;
L- vacuum pipe extract and sheltered from the light.
Based on the analysis of the obtained results we can observe statistically significant differences between all forms of treatment in 24 hours and 48 hours respectively.

DISCUSSION

Pharmacognosy is a science of study of natural products as a source of new drug leads and effective drug development. Rational and economic search for novel lead structures could maximize the speed of drug discovery by using powerful high technology methods. China and India have a long history in the therapeutic application of botanical drugs in traditional medicine. Traditional Chinese Medicine (TCM) and Ayurveda are considered as two of the most ancient systems of medicine, with history of more than two millennia. Medicinal plants are the principal medicinal materials used in both these systems. Great biodiversity is a highlight of Brazilian flora. In contrast, the therapeutic potentialities of most species used in folk medicine remain unknown. For centuries, herbs and plants have been used for medicinal purposes and as food as well. Large variety of active phytochemicals such as carotenoids, flavonoids, polyphenolics, terpenoids, sulfides, lignans and plant sterols has been identified in different types of herbs. These phytochemicals have different mechanisms of action. Traditional medicinal plants have been used as an alternative medicine in many parts of the world, including Brazil. Traditional medicinal plants from Brazil are potential source of alternative medicine for the local community and scientific research. Plants are a natural source of various products with diverse biological activities offering treatment for several diseases. Plant extract is a complex mixture of compounds, which can have antioxidant, antibiotic, antiviral, anticancer, antiparasitic, antifungal, hypoglycemic, anti-hypertensive and insecticide properties. The extraction of these extracts requires the use of organic solvents, which not only complicates the formulations but also makes it difficult to directly use the extracts for humans. To overcome these problems, recent research has been focused on developing new ways to formulate the plant extracts and delivering them safely with enhanced therapeutic efficacy. [11] Major obstacles associated with the use of plant extracts for clinical applications include their complex composition, toxicity risks and extract instability.

From the analyzes obtained for the comparison of the experimental procedures performed on 24 hour incubation with those achieved in 48 hours we suggest that the phytocompounds present in the aqueous extract of *Syzygium jambolanum* are unstable since the variation in results was extremely significant to comparing the data obtained with 24 hour incubation with those incubated over 48 hours.

In a study by Rodrigues et al [12] where samples of an aqueous extract of *Syzygium jambolanum* were incubated under different conditions of treatment over 24 hours was suggested by them that changes in the electrical voltage values of the samples of the aqueous extract of jambolan would be related to the presence of molecules derived from flavonoids with antioxidant properties, which can be anthocyanin molecules presented in the samples of said plant extract studied.

Additionally, it was observed that the treatments carried out over 48 hours that the aqueous extract heating enabled a higher activity of phyto molecules present in that statement, a fact that expresses the thermal resistance of phyto molecules associated with a greater possible effect of resonance of these molecules. Comparing the heated tubes and were incubated during 24 hours with 48 hours of incubation can be observed instability of these molecules over time, allowing us to infer that possibly the synthesis of phyto molecules present in the extract under study is ongoing the plant.

Different results were reported by Peixoto et al [13] who observed greater stability in the behavior of eletrovoltage for hydroalcoholic extract of *Punica granatum*. Compared with our results for an aqueous extract of *Syzygium jambolanum* we suggest that the organic solvent used for the extraction of phyto molecules for *Punica granatum* extract could have changed the electrochemical behavior of these phyto molecules.

When analyzing the results under different treatment conditions after 48 hours we noted that the complex phyto responds more the light and the presence of oxygen, this could be explained that despite the possible disintegration that such complex phyto may suffer over time certain groups would be best exposed exhibiting greater resonance effect with the exposure light beyond greater interaction with oxygen displaying an antioxidant behavior, according described by Samadder et al [14] report in their studies that an ethanolic extract of *Syzygium jambolanum* had anti-oxidant as well as anti-hyperglycemic activities in diabetic mice, and potentially useful in management of hyperglycemia. Analyzing the tubes vacuum where the extract was sheltered from light, in addition to decreased activity over time in 24 hours and 48 hours, we can also highlight the lower activity of the aqueous extract expressed in mV, indicating in this way that the electrochemical activity of the phyto constituent molecules of the extract under study is sensitive to the presence of oxygen and the presence of light, enabling us to speculate that there are chemical phyto constitution extract the presence of molecules derived from flavonoids with antioxidant properties, which can be anthocyanin molecules.

CONCLUSION

From the analysis of the results we can speculate that the aqueous extract of *Syzygium jambolanum* study has in its constitution phyto molecules which exhibit significant volatility as a function of time with antioxidant potential is sensitive to the presence of oxygen and exposure to light.

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