AN INTERNATIONAL FORUM FOR THE RAPID PUBLICATION OF ORIGINAL SCIENTIFIC ARTICLES DEALING WITH SCIENCES AND RELATED INTERDISCIPLINARY AREAS

SOUTHERN JOURNAL OF SCIENCES

ESTABLISHED IN 1993

Formerly known as the Southern Brazilian Journal of Chemistry

VOLUME THIRTY-ONE, NUMBER THIRTY-FIVE

ISSN: 2764-5967 - E-ISSN: 2764-5959

JUNE– 2023

Former Printed ISSN: 0104-5431 and E-ISSN: 2674-6891

SOUTHERN JOURNAL OF SCIENCES

ISSN: 2764-5967 **E-ISSN: 2764-5959**

Volume 31 **Number 35** **2023**

Dados Internacionais de Catalogação na Publicação (CIP)

S727 Southerm Journal of Sciences [recurso eletrônico] : interdisciplinary path for scientific divulgation / Araucária Associação Científica – (Fev. 2022). – Dados eletrônicos. – Nova Prata. : Araucária Associação Científica, 2023-.

Semestral
Recurso on-line
Descrição baseada em: Vol. 30, n. 33 (JUN. 2022)
Formerly known as: Southern Brazilian Journal of Chemistry
Modo de acesso: < https://sjofsciences.com>.
E-ISSN: 2764-5959
ISSN: 2764-5967

1. Química. 2. Física. 3. Biologia. 4. Ciências Naturais. 5. Farmacologia. 6. Ciências exatas. 7. Ciências aplicadas. 8. Ciências. I. Dr. D. Scientific Consulting.

UDC 001

Bibliotecário Responsável
Ednei de Freitas Silveira
CRB 10/1262

Journal E-mail: southbchem@gmail.com

SOUTHERN JOURNAL OF SCIENCES

ISSN: 2764-5967 **E-ISSN: 2764-5959**

Volume 31 **Number 35** **2023**

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SOUTHERN JOURNAL OF SCIENCES

ISSN: 2764-5959 (Online)
ISSN: 2764-5967 (Print)

DOI: 10.48141/2764-5959
Digital preservation: Portico

Former Southern Brazilian Journal of Chemistry
Former E-ISSN 2674-6891
Former ISSN 0104-5431

Available at
https://sjofsciences.com

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UDC 001

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SOUTHERN JOURNAL OF SCIENCES

ESTABLISHED IN 1993

Original research paper

CONTENT OF ANTIOXIDANTS IN SOME MEDICINAL PLANTS SOLD IN GEORGIAN PHARMACY CHAINS

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Received 14 June 2022; received in revised form 09 September 2022; accepted 28 December 2022

ABSTRACT

Background: Antioxidant compounds are widely used in medicine to protect the organism from the impact of various stresses and strengthen the immune system. The primary source of natural antioxidants is considered plant-based products. That is precisely why scientists are interested in extracting active compounds out of plant materials and use them. However, diverse resources of medicinal plants of Georgia is poorly studied and used as a source of antioxidants. **Aim:** The research aimed to study the antioxidant composition and total antioxidant activity of some dried medicinal plants sold in the pharmacy network in Tbilisi, Georgia. **Methods:** Several types of medicinal plants (3 berry plants and 3 herbaceous plants) sold in the pharmacy chain were chosen as the research object. These are *Crataegus sp., Sorbus sp., Viburnum sp., Helichrysum sp*., *Leonurus quinquelobatus* Gilib, *Origanum vulgare* L. **Results:** As a result, it has been found that the content of ascorbic acid and carotene in the studied plants is not so high to fill the daily intake of a man with an infusion prepared from one tablespoon. As for proline and the number of total antioxidants, all the plants we have chosen are high in content, considerably increasing their medicinal value. **Discussion:** The low amount of ascorbic acid and carotine found in studied plant, this increases their medicinal value, and in combination with other antioxidants/plants, the healing effect of the raw materials has been used. **Conclusions:** The studied plants are characterized by high levels of proline and antioxidant activity, so their infusions can be safely used as a source of antioxidants both in folk medicine and for the preparation of biologically active preparations.

Keywords: *Medical plants, Antioxidant, Ascorbic acid, Proline, Carotene.*

1. INTRODUCTION

The understanding of the use of medicinal plants for treatment has been formed for millennia in fighting diseases. The man studied the actions of those substances in the bark, seeds, and other parts of plants. Modern science recognizes the active effect of plants in pharmacotherapy as effective protective agents against the impact of various stresses on the organism, as well as means for strengthening the immune system (Crozier *et al*., 2006; Petrovsk, 2012; Bakuridze *et al*., 2016).

The antioxidant system of plants includes secondary metabolites of various groups, such as vitamins (tocopherol - vitamin E, ascorbic acid - vitamin C), flavonoids (anthocyanins), terpenoids (carotenes and xanthophylls), as well as antioxidant enzymes. Antioxidants of plant origin protect the human body from the development of sclerosis, and cardiovascular diseases, lessen the risk of developing cancer, help slow down the aging process, and strengthen the immune system (Nasyrov, 1983).

The use of medicinal plants in Georgia has a long history. One can find the first information about it in “The Argonautica” of Apollonius of Rhodes. As the author tells us, the use of medicinal plants in Colchis was widely spread. Hecate, famous for her knowledge of the healing properties of plants, had set up a special garden for medicinal plants, where she grew several dozens of them (Shengelia, 2016).

DOI: 10.48141/SJS.01_ETER_pgs_01_07.pdf

The flora of Georgia includes about 4100 species, of which more than 400 are used in folk and traditional medicine (Gegechkori *et al*., 2011). However, the resources of the medicinal plants in Georgia have been poorly studied and used, especially as a source of antioxidants.

Therefore the purpose of our research was to study the antioxidant composition (quantitative content of ascorbic acid, proline, and carotene) and the total antioxidant activity of some dried medicinal plants sold in the pharmacy network in Tbilisi, as well as, based on a comparison of the results obtained, to identify species-rich in antioxidant compounds, and to determine what part of the daily dose of the studied compounds is contained in the plants under the experiment

2. MATERIALS AND METHODS

2.1. Materials

Several medicinal plants (3 berry plants and 3 herbaceous plants) sold in the pharmacy network were chosen as the research object. These were: *Crataegus sp*., *Sorbus sp*., *Viburnum sp*., *Helichrysum sp*., *Leonurus quinquelobatus* Gilib, *Origanum vulgare* L. The plant material was packaged in cardboard boxes indicating the respective genera.

2.2. Methods

2.2.1 Carotene measurement

The carotene content in the dried plant material was determined spectrophotometrically. First, 3 ml of aviation gasoline was poured into 100 mg of fragmented material, which was crushed for 2-3 minutes. Colored gasoline was poured into a 50 ml volumetric flask, and 10 ml aviation gasoline was added to the remaining mass, which was crushed for another 3-5 minutes. This procedure was repeated several times until the gasoline changed color (which indicated the complete removal of carotene from the analyzed material). The optical density of the extract obtained was measured with a spectrophotometer (SPECOL11, Carl Zeiss, Germany) in the range of 448 to 484 nm. The carotene content of the plant material was calculated in mg%-s according to Equation 1:

$$\mathbf{X} = ((0.00626\ V\ D)/a)*100 \qquad \text{(Eq. 1)}$$

where X is the content of carotene in the resulting compound mg/100 g; V is the volume of the resulting solution ml; D - testimony Spectrophotometry; a - a sample of a plant product g; 0.00626 - conversion factor mg/ml (Burova and Bazarnova, 2008).

2.2.2 Proline measurement

The spectrophotometric method studied the proline content in the dried plant material. First, it was taken 100 mg of ground material added 10 ml of 3% sulfosalicylic acid to it for extraction, and filtered it. Then, 2 mg of the extract was taken from the filtrate, and 2 ml glacial acetic acid and 2 ml of ninhydrin solution were added. The flasks were covered with glass lids and placed in a water bath for an hour. Next, the extract was cooled, 4 ml of toluene was poured and shaken. The resulting extract was transferred to a separating funnel. The lower one was poured into the two layers formed, transferred the upper one, with toluene, to the flasks, and measured its optical density with a spectrophotometer. Toluene was used as a control (Bates *et al.,* 1973).

2.2.3 Ascorbic acid measurement

The content of ascorbic acid in the dried plant material was determined by titration by dichlorophenolindophenol solution. First, 1000 mg of ground material were taken, then added quartz sand and 15 ml of 2 % HCL to it and quickly crushed it to get a mushy mass. The resulting mass was filtrated. Next, the pestle was washed with 10 ml of 2 % metaphosphoric acid, to which the filtrate was added. Finally, 1 ml of the extract was taken from the filtrate, and 25 ml of distilled water was added to it. The resulting solution was titrated by 0,011 N dichlorophenolindophenol solution to pink color. The ascorbate content in the sample was calculated according to the amount of dichlorophenolindophenol consumed (Ermakov *et al*., 1987).

2.2.4 Total antioxidant activity measurement

In the dried plant material was determined spectrophotometrically. Was took 200 mg of ground material for analysis and extracted it three times with ethyl alcohol. 96 % alcohol was used for the first extraction and the two subsequent extractions - 80 %. Each extraction was carried out in closed flasks for 12 hours in the dark. In this way, the alcohol removed all the oxidants present

in the plant. The resulting extract (30 ml) was evaporated. The evaporated precipitate was dissolved in 5 ml of H2O and 5 ml of 96 % alcohol. The resulting liquid was centrifuged and placed in weighing bottles. 0,9 ml of alcohol was poured into 0,1 ml of the solution, and 0.1, 0.2, and 0.3 ml were taken separately from this mother liquor. Was added 5 ml of the solution of 40 μMDPPH (diphenylpicrylhydrazyl) to each of them and placed them in the dark for 30 minutes. Was measured the optical density of the solution (relative to alcohol). According to the indications obtained, the percentage of inhibition was calculated by Equation 2:

% of inhibition = ((C-S)/C)×100% (Eq. 2)

where C is the initial concentration of DPPH, S is the concentration of DPPH after adding an antioxidant (Koleva *et al*., 2002).

The quantitative content and total antioxidant activity of ascorbic acid, proline, and carotene in the experimental plant material are represented by mean value of three biological replicates.

3. RESULTS AND DISCUSSION

3.1. Results

The plants under study are used for treatment in the form of infusion or tea. Following the preparation rule, one tablespoon of dried mass is poured with one glass of boiling water for extraction. We were interested in how much of each antioxidant compound we studied would correspond to this amount of plants. To get an answer to this, we weighted one tablespoon of each plant and, based on the results, calculated the content of ascorbic acid, proline, carotene, and the total content of antioxidants, that is, we determined how much of these compounds a man would receive together with one glass of infusion prepared from one tablespoon (see: Table 1).

The ascorbic acid content in the plants under study fluctuated within 33-65 % mg range. Namely, its highest content was noted in *Crataegus sp*., the lowest in *Origanum vulgare* (Fig. 1). When recalculating the results obtained for one tablespoon of raw material, we found that a man, together with one glass of infusion of *Crataegus sp*., would take an average of 5,33 mg of ascorbic acid, and in the case of *Origanum vulgare*, 0,64 mg. If the food contains more than 20 % of the daily dose of ascorbic acid, it is considered a food rich in this substance.

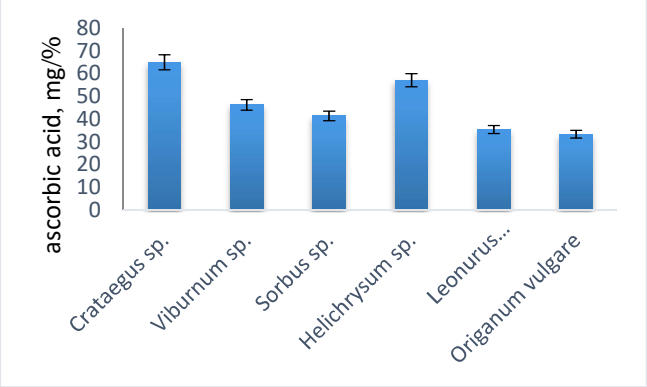

Figure 1. *The ascorbic acid content in the studied species.*

The carotene content in the studied plants ranged from 0,2 to 1,18 mg %. *Helichrysum* sp, *Leonurus quinquelobatus* were distinguished by a particularly high carotene content. In them, the content of the compound was twice as high as in other studied species. The content of carotene in*Crataegus* sp.,*Viburnum* sp., *Sorbus* sp. and*Origanum vulgare* was almost the same (Fig. 2).

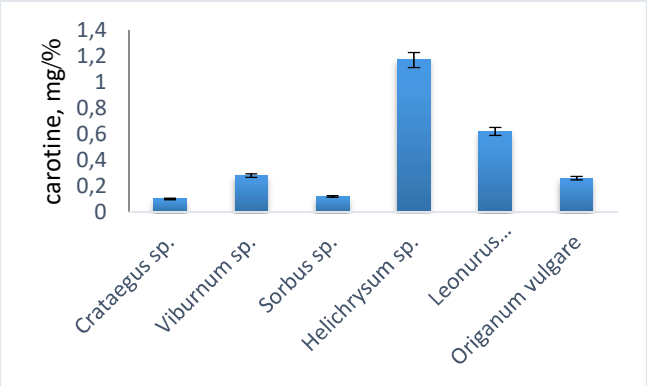

Figure 2. *The carotene content in the studied species.*

The proline content in the plants under study fluctuated within the range of 0,3-11,6 mg %, *Helichrysum* sp. stood out with a particularly high index. On the other hand, nearly the same proline content was established in raw materials of *Crataegus* sp., *Sorbus* sp., *Viburnum opulus* (Fig. 3).

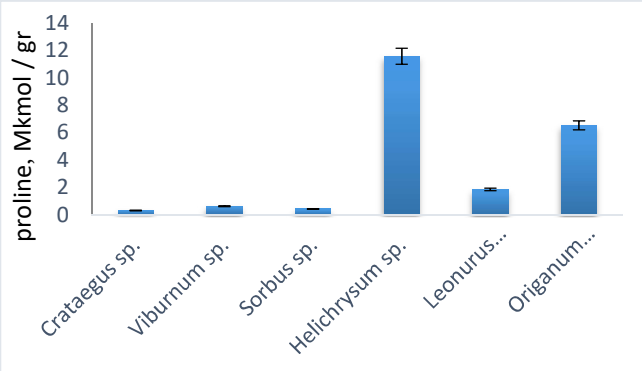

Figure 3. *The proline content in the studied species.*

Total antioxidant activity in the studied plants, with the percentage of pronounced inhibition in various plant materials, ranged from 22 to 93 %. By comparing the results obtained, it may be concluded that the researched plants are characterized by high antioxidant activity. Even though the analyzed material was dried, which means a quantitative decrease of antioxidants, *Origanum vulgar*e was distinguished with a particularly high index. The total antioxidant activity of its dried material was 93,3 % (Fig. 4).

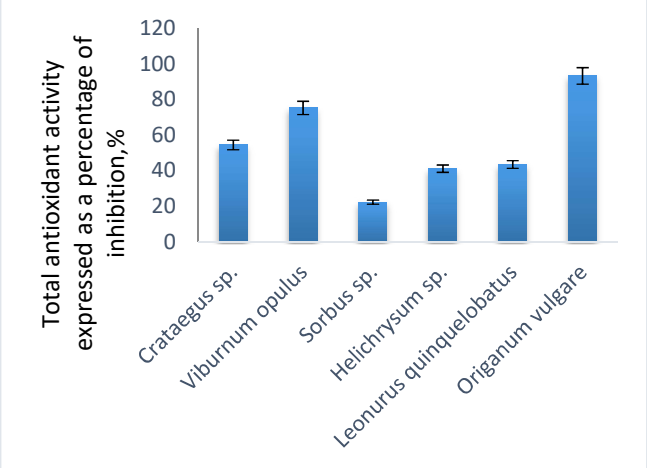

Figure 4. *Content of total antioxidant activity in the studied species*

3.2. Discussions

The recommended daily intake of vitamin C is 90-100 mg. (Carr and Frei, 1999). With this in mind, the content of ascorbic acid in the raw materials we studied is not so high that a man can replenish the daily dose with only one glass of an infusion. However, the medicinal purpose of each of the researched species does not provide for filling the lack of vitamin C in the human body. Thus, the amount of ascorbic acid found in these plants increases their medicinal value and, in combination with other antioxidants, the healing effect of the raw materials used. It should also be noted that during the drying process, many substances present in plants, including ascorbic acid, disintegrate (Frolova, 2009). Therefore, it is best to use these plants in their raw form if possible.

When calculating the carotene content in one tablespoon of raw material, it was found that a man receives an infusion prepared from one tablespoon of Helichrysum sp., having the highest carotene content of 0,028 mg of carotene. Given that the daily dose of an adult man is 5-6 mg (NCI, USA, recommend that) (Müller, 1996), the carotene content in all six plants is low. However, like ascorbic acid, carotene plays the role of an additional antioxidant when using this medicinal raw material and increases its medicinal value. It is important to not forget that during drying, particularly at light, carotene decomposes as well.

It is accepted that the daily therapeutic dose of proline for an adult man is from 500 to 1000 mg. (Kuznetsov, 1999).

Suppose the consumption of one glass of an infusion of Helichrysum sp., has the highest index among the studied plants. In that case, a body will get 3188 mg. of proline which significantly exceeds the daily human need for this compound.

Based on this, we can say that all plants we selected are characterized by a high proline content, significantly enhancing their curative effect.

The studied plants are distinguished with strong total antioxidant activity that considerably increases their medicinal value. Their infusions can be safely used as a source of antioxidants, increasing the organism's resistance to stress and prophylactic against infections.

4. CONCLUSIONS

1. The content of ascorbic acid and carotene in the studied raw materials is not so high to make up for the daily dose of a man with an infusion obtained from one tablespoon (in the studied plant material, the content of ascorbic acid and carotene ranged from 33-65 mg% to 0,2-1,18 mg%). However, the medicinal purpose of each of the studied species does not at all provide for the

replenishment of the body with vitamin C and carotene. Thus, the amount of ascorbic acid and carotene found in the researched species increases the medicinal value of these plants and, in combination with other antioxidants, the healing effect of the raw materials used.

2. All plants selected by us are distinguished by a high content of proline, which significantly increases their medicinal value (the content of proline in a tablespoon of raw materials ranged from 300 to 3000 mg with a daily intake of 500-1000 mg).

3. The studied plants are characterized by strong antioxidant activity (from 22 to 93 %), substantially increasing their medicinal value. Their infusions can be safely used as a source of antioxidants both in folk medicine and for the preparation of biologically active preparations.

5. DECLARATIONS

5.1. Study Limitations

The study is limited to the samples analyzed and the studied plants.

5.2. Acknowledgements

The authors thank the Department of plant physiology of the Institute of Botany, Ilia State University, for their attention and support in carrying out the experiments.

5.3. Funding source

The authors funded this research.

5.4. Competing Interests

The authors have declared that no competing interests exist.

5.5. Open Access

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Table 1: *Content of antioxidants in 1 tablespoon of raw material*

plant / Subs. Mg.	*Crataegus* sp.	*Sorbus* sp.	*Viburnum* sp.	*Helichrysum* sp.	*Leonurus quinquelobatus*	*Origanum vulgare*
carotene	0.0084	0.009	0.02	0.028	0.012	0.005
proline	311.14	380.66	516.94	3188	402.59	1431
ascorbic acid	5.33	3.12	3.25	1.37	0.66	0.64

SOUTHERN JOURNAL OF SCIENCES

Original research paper *ESTABLISHED IN 1993*

MODELING OF THE NITROGEN PARAMETER OF THE PARAÍBA DO SUL RIVER USING THE QUAL-UFMG METHOD

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Received 02 March 2023; received in revised form 16 April 2023; accepted 27 May 2023

ABSTRACT

Background: Due to population growth and increased use of water resources, there has been a need to implement management measures to control and monitor river parameters. **Aims:** This study aims to demonstrate the application of mathematical modeling using the QUAL-UFMG model for studying nitrogen concentration in the Paraíba do Sul River located in Volta Redonda. **Methods**: Through mathematical modeling, it is possible to identify areas of greater impact, evaluate the effectiveness of control measures, and propose a strategy to improve water quality and preserve the balance of local aquatic ecosystems. **Results**: The results obtained during the study showed that the nitrogen parameters and their derivatives are within the pre-established limits set by CONAMA. **Discussion**: During the modeling, it was observed that there is a tendency for nitrate levels to increase along the points, indicating good self-purification of the river. Organic nitrogen values increase along the points, which is due to the contribution of sewage discharge along the course of the river, as organic nitrogen and ammonia have a physiological origin in domestic sewage. **Conclusions**: Through the QUAL-UFMG model, it was possible to validate the conducted analyses and create future projections of the water quality in the Paraíba do Sul River along the studied points.

Keywords: *Mathematical modeling; Nitrogen levels; Paraíba do Sul River; Water resources; QUAL-UFMG*.

1. INTRODUCTION

Os rios são caracterizados como escoadouros naturais das áreas de drenagem adjacentes que formam bacias hídricas e possuem um sistema complexo por possuírem terra, geologia e tamanho (Toledo & Nicolella, 2002). Além de complexa, é um recurso natural esgotável e muito escasso pois cerca de 60% do consumo global de água vem de reservatórios subterrâneos não renováveis. (Resende, 2002)

Com o crescimento populacional e o aumento do uso da água em diversas áreas houve também um aumento na geração de esgotos e efluentes que vem degradando os recursos hídricos. Através disso, foram estabelecidos normas e parâmetros em diversos países com o intuito de proteger o constituinte natural valioso que é a água (Vasconcelos, 2020).

A gestão hídrica do País está disposta na Lei nº. 9.433 de 8 de janeiro de 1997, que institui a Política Nacional de Recursos Hídricos onde o permite a utilização do uso da água e a e a cobrança pelo seu uso. A cobrança pela utilização do recurso incentiva a racionalização do uso e arrecada recursos para financiamento de projetos relacionados aos recursos hídricos. Segundo a Lei nº. 9.433/97 e a resolução n° 48/2005 do CNRH além da cobrança, é também observado o volume lançado e seu regime de variação,

DOI: 10.48141/SJS.v31.n35.2023.02_GOMES_pgs_08_15.pdf

características físico-químicas, biológicas e de toxicidade detento o objetivo de uma boa qualidade e uso (Teodoro et al 2013).

A presença de diversos tipos de nutrientes na água é proveniente dos ciclos normais da natureza e o problema de contaminação está restrito a alguns micronutrientes como o nitrogênio (N) e o fósforo (P) (Resende 2002)

Um dos parâmetros que interferem na qualidade da água são as diversas formas de nitrogênio presentes na natureza. Eles estão presentes de diversas formas na natureza como nitrogênio orgânico, amônia, nitrito e nitrato. São diversas fontes desse material nos corpos hídricos sendo o principal o despejo de esgotos sanitários, efluentes industriais e fertilizantes provenientes do escoamento da água das chuvas em solos em áreas agrícolas (Theodoro et al, 2020).

Segundo a ANA (Agência Nacional de Água e Saneamento Básico), Volta Redonda pertencente ao estado do Rio de Janeiro, possui uma população de 261.403 pessoas e possui uma carga de esgoto de 13.915 sendo 8.500 remanescentes (ANA, 2022). Diante ao relatório apresentado pelo ANA, não temos o ponto de atenção ao nitrogênio, porém o nitrogênio é um parâmetro importante para que possamos monitorar a qualidade da água ao longo do tempo. Quantificar as principais formas de nitrogênio podem revelar desequilíbrios que são resultados de catástrofes naturais ou antropogênicas (SUTTI et al, 2016).

O nitrogênio possui cerca de 4x1021 g na atmosfera, solo e água sendo assim, um dos elementos mais abundantes da Terra. O ciclo do nitrogênio possui íons solúveis e espécies gasosas que são solúveis em água desta forma facilita a dispersão no ambiente (GARCIA & Cardoso 2013). Elemento fundamental na vida dos organismos e no desenvolvimento da biota aquática, porém em excesso pode ser prejudicial pois provoca a eutrofização de reservatórios e lagos com danos consideráveis com meio ambiente (GONÇALVES, 2011).

O nitrogênio pode atingir as águas fluviais principalmente através da deposição atmosférica, efluentes de esgotos, descargas fluviais e fluxo de águas subterrâneas (SUTTI et al, 2016). Parâmetros como Nitrogênio Amoniacal (NH3+, NH4+) são gerados a partir da decomposição das substâncias contidas em esgotos sanitários e são prejudiciais pois consomem o oxigênio dissolvido além de ser tóxico. O nitrato é bastante utilizado na agricultura e seu aumento na água pode causar malefícios como a doença infantil metaemoglobineína (SUTTI et al, 2016).

Com o objetivo de auxiliar o controle a gestão dos recursos hídricos são utilizadas ferramentas que possibilitam a análise e prognostico dos corpos d'agua. Os modelos matemáticos são ótimas ferramentas que permitem a simulação dos processos de autodepuração dos rios e auxilia na tomada de decisões. Esse tipo de ferramenta proporciona a simulação de condições futuras e alternativas propostas para o corpo d'agua (Ide & Ribeiro 2009).

O modelo QUAl-UFMG desenvolvido por Von Sperling (2007) é considera a modelagem unidimensional do corpo hídrico e permite a modelagem de diversos constituintes como demanda bioquímica de oxigênio, oxigênio dissolvido, nitrogênio total e suas frações (orgânico, amoniacal, nitrito e nitrato), fósforo total, fósforo orgânico e inorgânico e coliformes termotolerantes ou E.Coli. A modelagem é de fácil utilização e foram desenvolvidos em planilhas de Excel e tem como base o QUAL2-E desenvolvido pela US Envionmental Protection Agency (USEPA) (Ide & Ribeiro 2009).

O rio Paraíba do Sul pertence a três estados da região sudeste e ocupa uma área de aproximadamente 57.000 km² sendo 13.605 km² no estado de São Paulo, 20.500 km² no estado de Minas e 22.600 km² no estado do Rio de Janeiro. O curso natural do rio Paraíba do Sul, no território paulista, é ladeado pelas Serras do Mar e Mantiqueira. O rio é formado pela união do rio Paraitinga e Paraibuna e após percorrer 1.180 km e deságua no Oceano Atlântico. (Marego & Alves 2005).

1.1. Aims

Avaliar as concentrações de amônia no Rio Paraíba do Sul ao longo dos anos e demonstrar a aplicação da modelagem matemática para o estudo da concentração de nitrogênio no Rio Paraíba do Sul. A partir dos resultados, analisar se os parâmetros estão dentro dos limites pré-estabelecidos pelo órgão regulamentador.

2. MATERIAIS E MÉTODOS

2.1. Materiais

As amostras foram coletadas através de um balde coletor de aço inox, corda e foram armazenadas em frascos de 300 ml previamente preservados com ácido sulfúrico 1:1 e enviados ao laboratório através de caixas térmicas com gel para o acondicionamento com refrigeração das amostras (CETESB 2011).

2.2. Methods

As análises experimentais foram realizadas em um laboratório credenciado pelo Inmetro através da norma ABNT NBR ISO/IEC 17025:2017 e a metodologia utilizada são baseadas no o "Standard Methods" (American Public Health Association, American Water Works Association, Water Environment Federation, 2017) citadas abaixo:

Nitrato: SMWW, 23ª Edição, 2017 - Método 4500 NO_3- B

Nitrito: SMWW, 23ª Edição, 2017 - Método 4500 NO_2- B

Nitrogênio Amoniacal: SMWW, 23ª Edição, 2017 - Método 4500 NH_3- E

Nitrogênio Orgânico: SMWW, 23ª Edição, 2017, Método 4500 Norg C, NH_3 E

2.2.1. Método QUAL-UFMG

O modelo matemático QUAL-UFMG foi desenvolvido por Von Sperling (2007) e tem como o objetivo a modelagem da qualidade da água em rios. A estrutura da modelo QUAL-UFMG foi baseado na modelagem do QUAL2E e contém algumas simplificações como a não inclusão de algas e todas as suas interrelações com as demais variáveis. Além disso, o autor Von Sperling (2007) realizou também simplificações na dispersão longitudinal e a integração pelo método de Euler (Fraga 2015).

O QUAL-UFMG permite possibilita a modelagem dos seguintes parâmetros da água do rio como (Tonon 2014):

- Demanda bioquímica de oxigênio;
- Oxigênio dissolvido
- Nitrogênio total e sua fração orgânica, amoniacal, nitrito e nitrato;
- Fósforo total e suas frações orgânicas e inorgânicas
- Coliformes Termotolerantes ou E.Coli.

A modelagem é realizada através do programa Excel que foi baseado no modelo QUAL-2E e possibilita uma simulação rápida e simples. Após a entrada dos dados, os resultados podem ser visualizados facilmente através dos gráficos contidos na planilha. Os gráficos possuem os dados que são expressões pelo potencial da distância do percurso e os resultados de acordo com cada parâmetro estudado (Perin 2013).

Na modelagem foi utilizada a metodologia simplificada baseada nos principais processos de conversão como amonificação, conversão de nitrogênio orgânico em amônia, e a oxidação do nitrato em nitrito. Para a modelagem de nitrogênio foram utilizadas as equações abaixo:

Nitrogênio Orgânico, Equação 1.

$$Norg = Norg0.e^{-(Koa+Kso).t} \quad \text{(Eq. 1)}$$

Onde:

N_{org}= Nitrogênio Orgânico (mg/L);

N_{org0}= Nitrogênio Orgânico (mg/L);

K_{oa}= Coeficiente de Conversão do Nitrogênio Orgânico a Amônia;

K_{so} = coeficiente de sedimentação do N orgânico;

t = tempo (dias);

Nitrato, Equação 2.

$$Nnitra = Nnitra0.e^{Knn.t} \quad \text{(Eq. 2)}$$

Onde:

N_{nitra} = Nitrato (mg/L);

N_{nitra0} = Nitrato Inicial (mg/L);

K_{nn} = Coeficiente de Conversão do Nitrito a Nitrato;

Amônia

$$Nam = \frac{Koa.Norg + \frac{SNamon}{H}}{Kan} + C.e^{-kan.t} \quad \text{(Eq. 3)}$$

Onde:

N_{am} = Amônia (mg/L);

K_{oa} = Coeficiente de Conversão do Nitrogênio Orgânico a Amônia;

N_{org} = Nitrogênio Orgânico (mg/L);

S_{Namon} = Fluxo de Liberação de Amônia pelo Sedimento de fundo;

C = Constante da Integral;

H = Profundidade (m);

Kan = Coeficiente de Conversão da Amônia a Nitrito.

Nitrito

$$Nnitri = \frac{Kan.Nam}{Knn} + C.e^{-Knn.t} \quad \text{(Eq. 4)}$$

Através das equações acima foi possível calcular o nitrogênio total utilizando a Equação 5.

$$Ntot = Norg + Nnitra + Nam + Nnitri \quad \text{(Eq. 5)}$$

Onde: Ntot = Nitrogênio total

Para a utilização do modelo QUAL-UFMG, e desenvolvimento da modelagem para concentração de nitrogênio foram usados os coeficientes apresentados na Tabela 1 *(VON SPERLING,2007).*

Tabela 1 - Coeficiente para amônia de acordo com a QUAL-UFMG

Símbolo	Faixa de valores	Adotado
K_{nitrOD}	0 a 1	0,6
K_{oa}	0,02 a 0,40	0,4
K_{so}	0,001 a 0,1	0,1
K_{nn} (nia)	0,2 a 2	0,2
K_{an}	0,1 a 1	0,1
K_{nn} (nii)	0,2 a 2	0,2

Fonte: (*Von Sperling,2007)*

2.2.2. Local da coleta

Foram coletadas 4 amostras em pontos distintos no rio Paraíba do Sul por trimestre nos anos de 2019 até julho de 2022. Os pontos escolhidos foram estratégicos ao longo do Rio Paraíba onde temos o primeiro ponto a montante que é o ponto 1 (22°31'14.04"S, 44° 7'56.72"O), ponto central 2 (22°30'24.27"S, 44° 6'49.39"O), o primeiro ponto a jusante no ponto 3 (22°30'6.68"S, 44° 5'18.30"O) e o ponto mais distante que é o ponto 4 (22°29'55.98"S, 43°56'4.74"O). Os pontos estão identificados conforme figura 1. Nominalmente os pontos são denominados Ponte Alta, CAC, Aero e Vargem Alegre.

Figura 1 - Pontos de amostragem no mapa
Fonte: (Google Earth, 2022)

3. RESULTADOS E DISCUSSÕES

3.1. Resultados

No gráfico 1 podemos observar que os valores experimentais são similares e a curva de Nnitra possui um aumento ao longo do tempo. Observa-se que o Nam possui pontos próximo de zero e que tende a aumentar entre os pontos. O nitrogênio amoniacal é encontrado predominantemente em ambientes poluídos e o nitrato, a forma mais comum de nitrogênio, é encontrado em águas naturais não poluídas (Fraga, 2015). Desta forma, podemos observar que a modelagem mostra valores negativos para nitrogênio amoniacal e valores crescentes para o nitrato, mostrando que há uma tendência de uma boa autodepuração do rio. O nitrato caracteriza uma poluição remota pois o nitrogênio se encontra no seu último estágio de oxidação.

Conforme podemos verificar no Gráfico 2, os valores de nitrito e nitrato praticamente se mantém constante ao longo dos pontos, o nitrogênio amoniacal observamos uma diminuição nos últimos pontos.

3.2. Discussões

Conforme observamos nos gráficos 1 e 2, o valor de nitrogênio orgânico aumenta ao longo dos pontos, isso é devido a contribuição de lançamento de esgotos ao longo do curso do rio, pois o nitrogênio orgânico mais a amônia tem origem fisiológica nos esgotos domésticos.

Para aplicação do modelo da concentração de nitrogênio foram calculados a concentração inicial através da média dos resultados experimentais apresentados na tabela 3.

Comparando os resultados experimentais com os valores determinados pela norma CONAMA nº430/11 obtemos a Tabela 2.

Tabela 2 - Valores dos parâmetros de nitrogênio e limites da CONAMA 430/11

Parâmetros	Valores (ppm)	CONAMA 430/11 (ppm)
Nnitri	0,1075	1,0
Nam	0,1778	3,7
Nnitra	1,1144	10,0
Norg	0,6146	-

A modelagem foi realizada utilizando as equações descritas acima e através da média dos resultados experimentais e estão representados no gráfico 1.

Observamos que os valores estão dentro dos parâmetros estabelecidos pela CONAMA n° 430/11, e que através da modelagem demostrada no gráfico 1 observamos uma crescente do parâmetro nitrito ao longo do tempo.

4. CONCLUSÕES

De posse dos dados obtidos através da análise experimentais, e cálculos realizados para modelagem matemática, utilizando modelo QUAL-UFMG para o estudo da qualidade da água do Rio Paraíba, localizado na cidade de Volta Redonda, observou-se que haverá um aumento significativo do nitrito ao longo do tempo, o que demostra uma poluição remota visto que o nitrogênio se encontra em seu último estágio de oxidação.

Comprando os resultados experimentais com a CONAMA n° 430/11 todos os parâmetros estão dentro dos limites permitidos para consumo.

O modelo utilizado QUAL-UFMG possui diversas vantagens quando comparadas com os demais modelos, devido à facilidade de manuseio e possuir alta eficiência nos resultados obtidos, e desta forma podemos validar as análises realizadas além de criar projeções futuras.

5. DECLARAÇÕES

5.1. Limitações do estudo

O estudo é limitado as amostras analisadas nas condições especificadas.

5.2. Agradecimentos

À Companhia Siderúrgica Nacional pela infraestrutura e suporte técnico.

À Universidade de Vassouras pelo suporte técnico e apoio.

5.3. Funding source

The authors funded this research.

5.4. Competing Interests

The authors declare that they have no conflict of interest.

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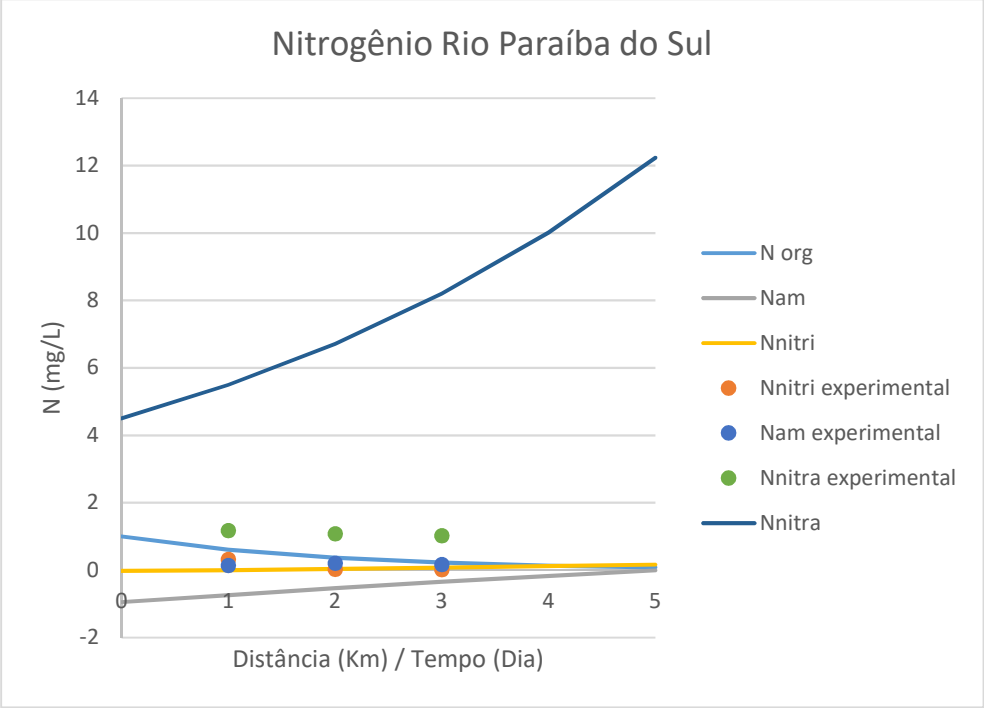

Gráfico 1 - Dados da modelagem e experimentais

Figure 2. *Example of a figure that exceeds 8.5 cm (extrapolated from the measurements of the column), so it is placed at the end of the article. Source: the author*

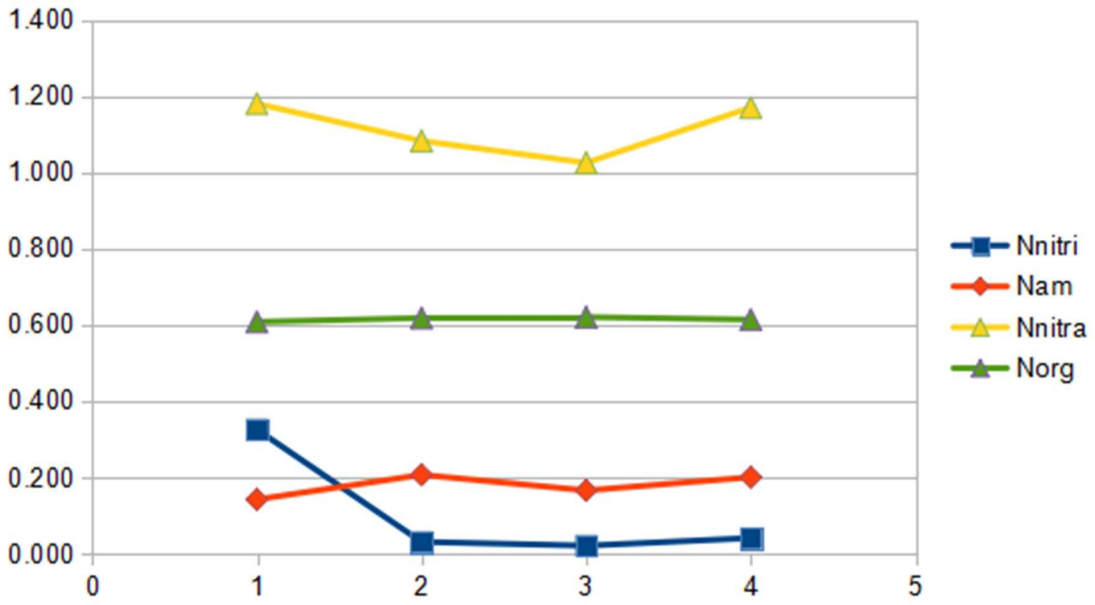

Gráfico 2 - Dados experimentais no ponto 1, 2,3 e 4

Figure 2. *Example of a figure that exceeds 8.5 cm (extrapolated from the measurements of the column), so it is placed at the end of the article. Source: the author*

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ESTABLISHED IN 1993

Original research paper

TYPE 2 DIABETES MELLITUS EFFECTS ON SEMEN PARAMETERS AND SEMINAL PLASMA

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Received 02 March 2023; received in revised form 28 April 2023; accepted 28 May 2023

ABSTRACT

Background: Diabetes Type 2 is a complex disorder described by an imbalance between insulin resistance and secretion that induce liver glucose output. It has been shown that serum insulin levels are affected by a sperm plasma membrane and acrosome. Therefore, during insulin resistance spermatogenesis changes, diabetic patients detect testicular changes. **Aims**: This research aims to know the effect of diabetic type 2 on some aspects of fertility in men by studying the characteristics of the semen and some biochemical parameters in seminal plasma. **Methods:** This study was achieved at the Center for Endocrinology and Diabetes Specialists in Maysan province from February to November 2018 and included 45 men (30 diabetic and 15 healthy in the control group) aged 30 to 59 years. The patients were divided according to age into two groups, the first (30-39) and second (40-59) years, also divided by the duration of diabetes into two groups, the first (1-5) and second (6-10) year. **Results: T**he pH of semen in the second age group (30-49 years) group and first duration (1-5 years) group were significantly decreased (P <0.05) in diabetes compared with the control group. Volume and viscosity did not have significant differences in patients compared to the control following the age and duration of diabetes. Liquefaction only in the first age (30-39 years) group significantly increased (P <0.05) compared to the control. The concentration of sperm, progressive motility, non-progressive and normal morphology decreased (P <0.05) significantly. While the sluggish, dead, and abnormal morphology significantly increased (P <0.05) in all diabetes groups compared with the control. The fructose and alkaline phosphatase values in the seminal plasma were not differing significantly in patients compared with the control. Zinc and glutathione values decreased significantly (P <0.05) compared with control in each age and duration of diabetes. **Discussion:** Insulin stimulates the Ledying cell function, defect insulin effect on spermatogenesis. Impaired sperm motility in a patient with D.M. might be attributed to many reasons, such as increased ROS level, altered mitochondria DNA, and decreased epididymal products. **Conclusion:** our measurement indicates that there is an effect of type 2 diabetes mellitus on semen parameters and seminal plasma biochemical parameters.

Keywords: *Type 2 Diabetes, Semen, Parameters*.

1. INTRODUCTION

Diabetes mellitus (D.M.) is a main affront to universal public health, and it comprises various etiology of diseases characterized by high blood glucose (Dragana *et al.,2015*). Diabetes Type I is caused by autoimmune devastation of pancreatic β cells, which produce insulin, and insulinogenic with resultant high blood glucose. It is diagnosed during childhood and requires exogenous insulin to exist (Zipitis andAkobeng,2008**).** Diabetes Type II is a complex disorder described by an imbalance between insulin resistance and insulin secretion that induce liver glucose output by preventing glycogen formation and stimulating glycogenolysis and gluconeogenesis (H.M., K., 2015).

It has been shown that serum insulin levels are affected by the sperm plasma membrane and acrosome (Silvestroni *et al*., 1992). Therefore, during insulin resistance or insulin deficiency, spermatogenesis changes and biopsies of diabetic patients detect testicular changes (Cameron *et al*.,1985). D.M. may affect male reproductive function at numerous levels due to its effects on the endocrine control of spermatogenesis or by weakening penile erection and ejaculation (Bener *et al.,*2009). Diabetes is associated with reproductive retrogradation in

DOI: 10.48141/SJS.v31.n35.2023.03_KAABY_pgs_16_25.pdf

both men and women. About 90% of diabetic persons suffer from a decline in sexual function, such as decreased libido, fertility, and impotence (Omolaoye and Du Plessis, 2018). D.M. causes an effect on the epididymis, with a negative effect on spermatozoon crossing. A varied mechanism is made as the case for the spermatozoon damage discovered in patients with diabetes, and these comprise endocrine disorders, neuropathy, and increased oxidative stress. Also, type II DM connected with hypothalamic-pituitary-gonadal (HPG) axis inhibition was a decrease in testosterone and another sex hormone (Al-Aaraji, 2016). So, the research aims to know the effect of diabetes mellitus type 2 on some aspects of fertility in men by studying the macroscopic and microscopic characteristics of the semen.

2. MATERIALS AND METHODS

2.1. Materials

The materials used in this study were listed as follows:

1- Alcohol methyl
2- Centrifuge
3- Container cup
4- EILSA
5- Glass slide and cover slips
6- Hemocytometer
7- Plain tube(10 ml)
8- Refrigerator
9- Spectrophotometers
10- ALP kit
11- Fructose kit
12- GSH kit
13- Zinc kit

2.2. Methods

2.2.1. The subjects

This study was achieved at the Center for Endocrinology and Diabetes specialist in Maysan province, including 60 men (45 diabetic and 15 healthy as the control group) aged 30 to 49 years. The number was divided by age into two groups, the first (30-39) years and second (40-49) years, also divided by the duration of diabetes into two groups, the first (1-5) years and second (6-10) years.

2.2.2 Semen collection

The ejaculates were collected after an abstinence period of (3-5days). In a sterile, no toxic, disposable container and transported to the laboratory within 30 minutes after ejaculation.

2.2.3 Semen examination

The macroscopic examination of semen included liquefaction, appearance, volume, viscosity, and pH measurement, according to WHO 2010 manual (WHO, 2010).In addition, the Microscopic examination of semen included concentration, motility [Progressive motility(A), Non-progressive motility (B), Sluggish (C), and Immotility (D)] and Sperm morphology (%) estimation according to (Tocci, and Lucchini, 2010).

2.2.4 Seminal plasma analysis

Seminal plasma preparation and Storage by Centrifugation of the semen samples for 15 minutes at 2600 rpm and rapidly recovering of the seminal supernatant plasma and placed to freeze at -20 °C to be measured biochemical parameters in seminal plasma (Vasan, 2011). The Biochemical parameters in Seminal plasma, including:

2.2.4.1 Fructose

In the presence of ATP, D- fructose transforms from Esochinase (E.K.) in fructose -6 phosphate. The fructose -6 phosphate is transformed from phosphor-Gluco-isomerase (PGI) in glucose -6 phosphate, which is transformed in 6-phosphgluconate from G6P-DH with the formation of NADPH. NADPH formed in this reaction causes an increase in absorbance at 340 nm. The principle of the kit is according to Nagasaka *et al.* (1988).

2.2.4.2 Zinc measurement

At room temperature, the zinc reacts with the chromogen present in the reagent, giving a colored complex measured by spectrophotometers with a strength proportional to the zinc concentration in the sample. The principle of the kit is according to Makino(1991).

2.2.4.3 Alkaline phosphatase (ALP) and Glutathione (GSH)

This kit uses enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay human alkaline phosphatase(ALP) and human glutathione (GSH). Add alkaline phosphatase(ALP) and glutathione (GSH) to wells that are pre-coated with glutathione (GSH) monoclonal antibody and then incubate. To wells pre-coated with alkaline phosphatase(ALP) monoclonal antibody and then incubated. After incubation, add anti-ALP antibodies labeled with biotin to unite with streptavidin-HRP, which forms the immune complex. Remove unbound enzymes after incubation and washing, then add substrates A and B. The solution will turn blue and change to yellow with the effect of acid. The shades of the solution, human alkaline phosphatase (ALP) concentration, and Human glutathione (GSH) are positively correlated.

2.3 Statistical analysis

The data obtained during this study were analyzed by one-way ANOVA using a statistical package for social science (SPSS) (Bryman and Cramer,2004). The data are presented as mean ± S.E. The difference was considered to be significant at P<0.05.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1 Semen parameters (According to age)

The pH in the second group decreased significantly (P <0.05) compared to the control group, and the control group did not differ significantly from the first group. In contrast, the first group did not differ significantly (P<0.05) compared to the second group. No significant differences (P<0.05) in the semen volume in all groups. The liquefaction in the first group was significantly increased(P <0.05) compared to the control group. It did not differ significantly from the second group, while the second group did not differ significantly (P<0.05) compared to the control group. There are no significant differences (P<0.05) in the viscosity of all groups (Table 1).

The concentration of sperm in the first and second groups decreased significantly (P <0.05) compared to the control group. However, the first and second groups did not differ significantly (P<0.05) between them. The progressive motility (A) of sperm in the first and second groups decreased significantly (P <0.05) in comparison with the control group. However, the first and second groups did not differ significantly (P<0.05) between them (Table 1). The Non-progressive motility (B) of sperm in the first and second groups decreased significantly (P <0.05) in comparison with the control group. However, the first and second groups did not differ significantly (P<0.05) between them. The Sluggish motility (C) of sperm in the second group was increased significantly (P-<0.05) in comparison with the control but did not differ significantly from the first group. At the same time, all groups increased significantly (P<0.05) compared to the control group. The Dead motility (D) of sperm in the first and second groups was increased significantly (P<0.05) in comparison with the control group. However, the first group did not differ significantly (P<0.05) in comparison with the second group (Table 1). Normal morphology of sperm in the first and second groups decreased significantly (P <0.05) compared with the control group. However, the second group did not differ significantly (P<0.05) compared to the first group. Abnormal morphology of sperm in the first and second groups was increased significantly (P <0.05) compared to the control group. Also, the first group differs significantly (P<0.05) in comparison with the second group (Table 1).

No significant variation was recorded in the value of fructose in the first and second groups compared to the control. The zinc values in the first and second groups decreased significantly (P<0.05) in comparison with the control group, while the first and second groups did not differ significantly (P<0.05) between them. No significant variation was recorded in the value of ALP in the first and second groups compared to the control. The GSH values in the first and second groups decreased significantly (P<0.05) compared to the control group. However, the first and the second group did not differ significantly (P<0.05) between them (Table 2).

3.1.2 Semen parameters (According to the duration of T2DM)

The pH in the first and second groups decreased significantly (P<0.05) compared to the control group. In contrast, the first and second groups did not differ significantly (P<0.05). The

volume no significant differences (P<0.05) in the semen volume in all groups. The liquefaction and viscosity in the first, second, and control groups did not show significant differences(P<0.05) (Table 3).

The concentration of sperm in the first and second groups decreased significantly (P<0.05) compared to the control group. However, the first and second groups did not differ significantly (P<0.05) between them. The motility (A) of sperm in the first and second groups decreased significantly(P <0.05) in comparison with the control group. However, the first and second groups did not differ significantly (P<0.05) between them. The motility (B) of sperm in the first and second groups decreased significantly (P <0.05) in comparison with the control group, but the first and second groups did not differ significantly (P<0.05) between them. The motility(C) in the second group was increased significantly (P <0.05) in comparison with the first and the control groups.

Also, the first and second groups differed significantly (P<0.05). The motility (D) of sperm in the first and second groups was increased significantly (P <0.05) in comparison with the control group. Also, the first and second groups differed significantly (P<0.05). Normal morphology of sperm in the first and second groups decreased significantly (P <0.05) compared to the control group. However, the first group did not differ significantly (P<0.05) compared to the control group. Abnormal morphology of sperm in the first and second groups was increased significantly (P <0.05 in comparison with the control group, but the first group did not differ significantly(P<0.05) compared to the second group (Table 3).

No significant variation was recorded in the value of fructose in the first and second groups, respectively, compared with the control. The zinc values in the first and second groups decreased significantly (P<0.05) compared to the control group. However, the first and second groups did not differ significantly (P<0.05) between them. No significant variation was recorded in the value of ALP in the first, second, and control groups. The GSH values in the first and second groups decreased significantly (P<0.05) compared to the control group. Also, the first and second groups did not differ significantly (P<0.05) between them (Table 4).

3.2. Discussions

3.2.1 Semen parameters (According to age)

The study recorded that pH and liquefaction in the semen of diabetes patients decreased and increased significantly. Mohammad and Ameen (2021) found no significant variation in the pH of semen values between diabetic(7.72 ± 0.21) and control(7.64 ± 0.13). The epididymis contains acetic acid, which spreads to the sperm to reduce the degree of pH (Sircar, 2008). And increased significantly in liquefaction in patients compared with control may be due to the absence of activity of fibrinolytic enzymes found in seminal fluid(Pilch and Mann,2006). The result recorded that the concentration, progressive, no progressive motility, and normal morphology are significantly decreased, while the increase in sluggish motility, dead and abnormal morphology. This result agrees with (Garcia-diez *et al.*,1991; Mohammad and Ameen,,2021), who found a decrease significantly in sperm concentration, sperm motility, and sperm morphology. Also, Bhattacharya *et al*. (2014) Found a decrease significant in percentage motility and normal morphology compared with the control. On the other hand (Ali *et al.*,1993) reported increased sperm concentration in men with insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent mellitus (NIDDM) with neuropathy, compared with control. Also, Mohammad and Ameen (2021) observed sperm concentration in males with diabetes mellitus and obesity significantly different from the control.

The germ cell apoptosis and spermatogenesis disorders in D. M. are related to local autoimmune damage. Insulin stimulates the Ledying cell function, whereas defective insulin causes defective spermatogenesis (Perrard-Sapori *et al*., 1987). Sperm cells are susceptible to oxidative stress, and sperm cell contain high concentrations of polyunsaturated fatty acid in the membrane(John Aitken *et al*., 1989). D. M. also provokes injurious blood testes barrier alteration, possibly responsible for spermatogenesis disruption (Alves *et al*., 2013). The study shows that motility was significantly lower in inpatient D. M. than in control. Impaired sperm motility in patients with D. M. might be attributed to many reasons, such as increased ROS level, altered mitochondria DNA, And decreased epididymal products (Singh *et al*., 2009; Niwas Jangir and Chand Jain,2014). D. M. leads to a marked reduction in fecundity by altering the normal

morphology of sperm cells and sperm parameters are affected in cases of D. M. Also, increased oxidative stress was hurtful to sperm morphology and is considered a major factor of low normal sperm morphology in D. M. Also increased lipid peroxidation in patients with diabetes is associated with low normal sperm morphology and increased abnormal morphology (LaVignera *et al*., 2012). Abnormal sperm would be a source of superoxide anions that bind with zinc in seminal plasma and thus decrease the zinc levels (Colagar *et al*., 2009).

The study showed a significant reduction in Zinc and GSH in diabetes compared with control, while the fructose and ALP did not reach a significant level. Our result agrees with many studies that reported the level of zinc in seminal plasma decreased significantly in patients of men diabetic in compared with control (Praveeena *et al.*, 2013; Ozturk *et al*., 2013; Ghasemi *et al*., 2016). Zn microelement is essential for male fertility, and their deficiency impedes spermatogenesis and is a reason for sperm abnormalities, and has a negative effect on serum testosterone concentration (Allouche-Fitoussi, and Breitbart, 2020). Low zinc in men may be caused prostate cancer and infertility and is linked to low libido (Mohamed, 2010). The study agrees with de Oliveira *et al*. (2016), who noted a significant reduction in GSH in diabetic rats compared to the control.

Higher levels of GSH in seminal plasma may protect against oxidative damage and progress sperm characteristics (motility and morphology) (Atig *et al*., 2012). Hyperglycemia raises O.S. by increasing ROS formation and altering the normal oxidation–reduction state. Several mechanisms contribute, such as an increased polyol pathway flux, Increased intracellular formation of advanced glycation end (AGEs) products, activation of protein kinase C, and overproduction of superoxide in the mitochondria (Brownlee, 2001). Diabetes induces alteration in the activity of the enzyme glutathione peroxidase and reductase; these enzyme found in all cell that metabolizes peroxide to water and converts glutathione disulfide back into glutathione, so alteration in their levels make cell lie down to oxidative stress and cause cell injury (Asmat *et al*., 2016).

3.2.2 Semen parameters (According to the duration of T2DM)

A study by Padrón *et al*. (1984) and Condorelli *et al*. (2018) noted a decrease significantly in sperm concentration, sperm motility, and sperm morphology compared to control with time-progressive duration diseases. Decreases in sperm concentration in diabetes mellitus compared to control may be due to low FSH and L.H., which are responsible for spermatogenesis (Maneesh *et al*., 2006). Sperm cells are susceptible to oxidative stress, and sperm cells contain high concentrations of polyunsaturated fatty acid in the membrane, increasing with progressive time duration (John Aitken *et al*., 1989).

The decreased motility observed in D. M. patients might be attributed to increased ROS levels and altered mitochondrial DNA (Niwas Jangir and Chand Jain, 2014). The author (Sikka, 1996) found a high level of reactive oxygen species in abnormal sperm compared to normal sperm. The increase in abnormal sperm may be due to increased oxidative stress and an increase in reactive oxygen species (ROS). On the other hand, decreased motility may be due to increased oxidative stress, which is accompanied by an increase in ROS, which leads to decreased mitochondrial membrane potential (MMP) that is associated with decreased motility of sperm (Condorelli *et al*., 2018; Irigoyen *et al.,* 2022). This study shows significant differences in Zinc and GSH between patients with diabetes and control and did not differ significantly in fructose, and ALP values (Adewole *et al.,* 2007) mentioned that Zinc and GSH decreased significantly in diabetes induce rats along with time-progressive diabetes. Diabetes induces a change in antioxidant agents, which play an important role in removing reactive oxygen species (ROS), and a decrease of antioxidants, exposing the cell to defects (Asmat *et al*., 2016; Amaral *et al.,*2006).

Insufficient antioxidant protection renders spermatozoa vulnerable to oxidative damage(Eskiocak *et al.,*2005). Antioxidants improve insulin sensitivity by reducing oxidative stress and insulin resistance (Udupa *et al*.,2012). Low zinc in men may be caused prostate cancer and infertility and is linked to low libido (Mohamed,2010). Zinc improves sperm quality as a membrane stabilizer and, as a component of superoxide dismutase, prevents sperm apoptosis and sperm DNA fragmentation (Omu *et al*., 2014).

4. CONCLUSIONS

It was concluded that there is an effect of type 2 diabetes mellitus on semen parameters and seminal plasma biochemical parameters.

Especially on the parameters of semen (decreased pH, concentration of sperm, mortality A and B, normal morphology) and low values of the zinc and GSH in seminal plasma.

5. DECLARATIONS

5.1. Study Limitations

The study is limited to the sample size and the analysis conducted.

5.2. Acknowledgements

We would thank Dr. Assad Yahia for assisting in the statistical analysis. Great thanks to the patients and control individuals for participation and facilitating in the completion of this study.

5.3. Funding source

The authors funded this research.

5.4. Competing Interests

There are no conflicts of interest.

6. HUMAN AND ANIMAL-RELATED STUDIES

6.1. Ethical Approval

Permission to conduct this study was issued by the health institutional Center for Endocrinology and Diabetes specialist in Maysan Province in order to facilitate mission number 332 on 27.11.2017, and a public health technician carried out the semen sampling from patients and control.

6.2. Informed Consent

A public health technician References carried out the semen sampling from patients, and control and verbal consent were taken from the participants

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Table 1: parameter of semen in diabetic mellitus type 2 and control (According to age) (mean ± S.E.)

Macroscopic examination of semen					
Parameters		**Control (30-59)year**	**First group (30-39)year**	**Second group (40-49)year**	LSD
P.H.		a 8.00±0.00	a b 7.84±0.08	b 7.80±0.60	0.17
Volume(ml)		3.53±0.21	3.13±0.37	3.20±0.38	N.S.
Liquefaction (min)		b 29.33±0.66	a 34.00±2.72	a b 30.00±0.66	4.62
Viscosity		1.00±0.03	1.05±0.05	1.08±0.09	N.S.
Microscopic examination of semen					
Concentration ($\times 10^6$/ml)		a 62.46±4.20	b 27.66±5.72	b 37.66±3.41	12.96
Motility %	Progressive(A)	a 31.33±2.46	b 5.33±1.72	b 8.00±1.52	5.54
	Non-progressive(B)	a 34.66±1.24	b 14.33±2.42	b 16.33±1.96	5.53
	Sluggish (C)	b 15.00±1.54	a 30.33±4.21	a 30.66±2.84	8.73
	Dead (D)	b 19.00±1.90	a 50.00±8.35	a 45.00±4.90	16.24
Morphology %	Normal	a 82.00±1.52	b 46.00±7.71	b 59.33±3.34	14.06
	Abnormal	b 18.00±1.52	a 54.00±6.51	c 40.66±3.34	12.30

Table 2: Biochemical parameters of semen plasma in diabetic mellitus type 2 and control (According to age) (mean ± S.E.)

Parameters	Control (30-59) year	First group (30-39) year	Second group (40-49) year	LSD
Fructose(mg/ml)	5.51±0.94	8.11±1.86	9.34±1.60	N.S.
Zinc(µg/ml)	a 162.93±9.18	b 141.86±5.38	b 132.00±3.47	18.42
ALP(IU/L)	229.70±24.82	188.48±17.77	176.91±25.42	N.S.
GSH(ng/L)	a 17.64±0.82	b 13.88±0.92	b 13.87±0.64	2.29

Table 3: Parameters of semen in diabetic type 2 and control (According to duration)

Macroscopic examination of semen					
Parameters		**Control**	**First group (1-5) year**	**Second group (6-10)year**	**LSD**
P.H.		a 8.00±0.00	b 7.76±0.07	ab 7.90±0.60	0.16
Volume (ml)		3.53±0.21	3.15±0.32	3.20±0.38	N.S.
Liquefaction (min)		29.33±0.66	34.00±2.72	30.00±0.66	N.S.
Viscosity		1.00±0.03	1.05±0.05	1.09±0.09	N.S.
Microscopic examination of semen					
Concentration (×10^6/ml)		a 62.46±4.20	b 35.05±4.53	b 28.54±4.98	13.18
Motility %	**Progressive(A)**	a 31.33±2.46	b 6.97±1.72	b 7.72±1.23	5.26
	Non-progressive (B)	a 34.66±1.24	b 15.39±1.81	b 17.74±1.23	4.56
	Sluggish (C)	c 15.00±1.54	b 22.63±2.39	a 35.90±2.76	6.53
	Dead (D)	c 19.00±2.90	a 55.00±4.35	b 38.63±2.86	10.26
Morphology %	**Normal**	a 82.00±1.52	b 60.26±3.94	b 51.81±3.83	9.78
	Abnormal	b 18.00±1.52	a 39.73±3.94	a 48.18±3.83	9.78

Table 4: Biochemical parameters of semen plasma in diabetic mellitus type 2 and control (According to duration) (mean ± S.E.)

Parameters	Control	First group (1-5)year	Second group (6-10)year	LSD
Fructose(mg/ml)	5.51±0.94	8.16±1.63	9.56±1.81	N.S.
Zinc(µg/ml)	a 162.93±9.18	b 142.52±5.38	b 140.09±5.49	19.97
ALP(IU/L)	229.70±24.82	186.75±18.08	226.60±25.90	N.S.
GSH(ng/L)	a 17.64±0.82	b 14.47±0.72	b 15.81±0.55	2.11

SOUTHERN JOURNAL OF SCIENCES

ESTABLISHED IN 1993

Review paper

NATURAL EXTRACTS AS A PROMISING SOLUTION FOR GRAM-POSITIVE ANTIBIOTIC RESISTANCE: A COMPREHENSIVE REVIEW

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Received 30 March 2023; received in revised 20 April 2023; accepted 03 June 2023

ABSTRACT

Background: Antibiotic resistance is currently one of the biggest problems in public health. Infectious diseases are the second human death cause, and the emergence of antimicrobial-resistant bacteria increases mortality and morbidity rates. There is a growing clinical need for the development of new antibiotics. In this line, WHO issued an alert about 12 bacteria with an urgent need to develop new antibiotics. **Aims:** This review aims to analyze the current knowledge of their antibacterial activity against the gram-positive pathogens listed by WHO and their extraction techniques. **Methods:** We systematically reviewed the literature in PubMed, searching publications describing the use of natural extracts as antibiotics over bacteria. The exclusion criteria consisted of limiting papers on natural extracts tested over the bacteria culture related to eleven selected bacteria, according to an alert issued by WHO in 2017, and seven plant extracts. **Results:** All the gram-positive bacteria present in the WHO alert have been treated, with different degrees of advance, with some of the plant extracts and plant-based compounds reviewed. Currently, they are in the preclinical stage. Edible herbs are more often used, as well as artemisia and wine byproducts. **Discussion:** Natural products based on plants have shown to be efficient in inhibiting bacterial growth, even in antibiotic-resistant strains. The classical extraction methods are still in use and have been improved with the available technology to improve efficiency and yield. **Conclusions:** Ongoing evidence shows that plant extracts and plant-based compounds are effective as antibacterial, with minimal effects on the host cell, a promising antibiotic source. Furthermore, they are sustainable, environmentally friendly, and renewable.

Keywords: *natural extract, bacteria, pathogen, Antibiotic-resistant, infectious diseases.*

1. INTRODUCTION

Infectious diseases are one of the most important public health problems. They are the second leading cause of death in the world. The first antibiotic was described in 1910, and opened the most fruitful period in this field. The 20th century, more precisely, the period from 1940 to 1970, is considered the golden age of antibiotics. Antibiotics are one of the most innovative medicines because of their ability to reduce morbidity and mortality caused by infections worldwide. Unfortunately, the misuse of antibiotics, especially the massive use and overuse of antibiotics, has led bacteria to develop mechanisms of resistance to antibiotics. This resistance has two main causes: through naturally occurring errors in DNA replication followed by the selection of drug-insensitive mutants that can later spread vertically in the bacterial population or

DOI: 10.48141/SJS.v31.n35.2023.04_QUINTERO_pgs_26_44.pdf

through horizontal transfer of resistance genes between bacteria. This antibiotic resistance allows microorganisms in general, and bacteria in particular, to circumvent the action of the drug used against them. This decline in the effectiveness of antibiotics in treating bacterial infections has been the subject of multiple alerts (Diallo *et al.*, 2020; Laxminarayan *et al.*, 2013; McEwen & Collignon, 2018). Their role in emerging and reemerging bacterial diseases is affecting public health. Around the world, epidemiological surveillance programs are collaborating to diagnose antimicrobial resistance worldwide (Burnham, Leeds, Nordmann, O'Grady, & Patel, 2017; Christaki, Marcou, & Tofarides, 2020; Diallo *et al.*, 2020). The pathogens that need to be urgently addressed were mainly classified into two groups. One of them, shown in Figure 1, is the antimicrobial-resistant ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter* species) (De Oliveira *et al.*, 2020). These pathogens are included in a 2017 alert from WHO that calls researchers to find effective treatments against 12 bacteria (Table 1). In this fight against AR, several approaches are being taken to discover new antibiotics. In the past, plants have been used as medicines, and currently, they are back in the spotlight as an effective tool for developing new treatments for infectious diseases using plant extracts and natural compounds (Mulat, Pandita, and Khan, 2019). In the present review, we summarized the recent findings of antibacterial activity extracts and bioactive molecules and the most commonly used techniques to obtain these extracts. We focused our search on aromatic plants, wild plants, and plants used as infusions and byproducts of the wine industry.

2. DEVELOPMENT

A systematic review was performed as described previously (QUINTERO, Cristián Andrés; VALLEJO, Mariana Guadalupe; BONTTI, Sergio; PATIÑO, Sol; PEREZ-GIRABEL, Rocío, 2022). Briefly, the literature in PubMed was explored to search for publications describing the use of natural extracts as antibiotics over bacteria, collecting and analyzing data. In order to do so, we used the following words/terms in combination: bacteria: name) AND (natural extract) AND (plant name) AND (antibacterial activity or antibiotic). The exclusion criteria consisted of limiting papers on natural extracts tested over the gram-positive bacteria culture related to eleven selected bacteria, according to an alert issued by WHO in 2017, and seven plant extracts. The search was conducted on papers published until September 2021.

3. RESULTS AND DISCUSSION:

3.1.1 History of antibiotics

Although recent history dates the beginning of the age of antibiotics to 100 years ago, there is evidence of the use of antibiotic-producing microorganisms to treat infections 2000 years ago in China, Greece, Serbia, and Egypt. Even earlier, in 1550 BC, is the oldest record of antibiotics use, being the moldy bread and medicinal soils included in the Papyrus of Ebers and Smith as medicines (Ferber & Karnath, 1999). The modern era of antibiotics has two major highlights: The first was the use of salvarsan, a synthetic arsenic-based drug, to treat *Treponema pallidum*, the causative agent of the sexually transmitted disease syphilis, by Paul Ehrlich ("PROFESSOR EHRLICH'S NEW REMEDY FOR SYPHILIS.," 1910). The second was in 1929, when Sir Alexander Fleming published his findings on the inhibitory, bactericidal, and bacteriolytic effects of Penicillium cultures on various pathogenic bacteria (Fleming, 1929). A breakthrough came in the late 1930s when Selman Waksman systematized the discovery of new antibiotics based on microorganisms. He created an efficient tool for the search of compounds with antibiotic activity based on natural products, like microbes, being the filamentous Actinomycetales as their main source of them (WAKSMAN, 1947). Their definition of an antibiotic was "a compound made by a microbe to destroy other microbes". This was the beginning of the golden age of antibiotics, which lasted from 1940 to 1970.

3.1.2 The increasing need for new antibiotics

Antibiotics can be considered one of the greatest advances in modern medicine. They have helped and saved millions of people since they became common medicine. This massive use of antibiotics, as well as the overdose of antibiotics, not only in humans but also in animals and agriculture (Laxminarayan *et al.*, 2013), is considered to be the base of Antimicrobial Resistance (AR). Increasing AR is currently a major obstacle in infection management. Global reports have drawn attention to the situation, with numbers that must call all our attention: 25.000

deaths in Europe in 2009, according to the European Center for Disease Prevention and Control, 23.000 per year in 2013 in the U.S. alone, according to U.S. Centers for Disease Control and Prevention, and the number could reach 10 million deaths per year by 2050 (Diallo *et al.*, 2020). The ongoing antibiotics affect the life cycle of bacteria. They can either directly or indirectly affect the essential processes of the bacteria, as shown in Figure 2.

The small genome size and short time span of duplications have led to the ability of bacteria to acquire AR. This resistance can be intrinsic, acquired, or adaptive. The first two cases involve modifications in their DNA by transformation, transduction, or conjugation. The adaptive response is mainly through modulation of gene expression. (Christaki *et al.*, 2020). These modifications allow bacteria to survive in the presence of antibiotics through several mechanisms, including destruction or modification of the antibiotic by the bacteria, modification of the target (as target mutations, replacement, protection, or overproduction), as well as the reduced permeability or increased efflux of the molecule (Christaki *et al.*, 2020). The emergence of multidrug-resistant bacteria or superbugs is considered an important public health problem. Currently, several health agencies and organizations worldwide are implementing surveillance programs. In 2017, the WHO issued an alert highlighting 12 bacteria in urgent need of antibiotic development due to their increasing resistance (Table 1). The alert ranked urgency on a three-tier scale, with the presence of Gram-positive bacteria into priority 2 (*Staphylococcus aureus* and *Enterococcus)* and priority 3, *Streptococcus pneumoniae.*

3.1.3 Discovery of new antibacterial agents

Regardless of the obvious need for new antibiotics, no new classes of antibiotics have been discovered in the last 40 years, and the last new antibiotics were from the 1980s. One possible reason for this is the small profit that developing new molecules means for the big pharmaceutical companies. It takes them $ 2.5 billion and up to 15 years to discover and manufacture a new antibiotic (DiMasi, Grabowski, and Hansen, 2016).

There are several approaches to discovering new molecules with antibacterial activity (see (Cook & Wright, 2022; Durand, Raoult, & Dubourg, 2019; Wohlleben, Mast, Stegmann, & Ziemert, 2016)). The following main categories are distinguished.

I-Antibodies: specifically, monoclonal antibodies are growing exponentially in the pharmaceutical industry, with various uses, with more than 100 antibodies approved by the FDA for medical treatments (Mullard, 2021). The possibility of synthesizing them by recombinant methods (Moutel, Marjou, *et al.*, 2009; Moutel, Vielemeyer, *et al.*, 2009) indicates that antibodies are an excellent target for antibiotic development.

II- Antimicrobial Peptides (AMP), small peptides (i.e., less than 100 amino acids). They were described in 1957 and are derived from blood cells (SKARNES & WATSON, 1957). They are amphipathic molecules that have hydrophobic and cationic amino acids in their structure. They are expensive and prone to hydrolysis, which limits their application.

III- Antivirulence strategy. A novel strategy is to reduce the virulence of pathogenic bacteria by inhibiting the virulence factors rather than killing the pathogen. This has the advantage of avoiding the mechanisms of antimicrobial resistance. They can be used in combination with conventional antibiotics.

IV- Bacteriophages. These microorganisms are viruses that specifically infect bacteria. They are known as molecular biology tools and were traded as antibacterial agents after their discovery, but the appearance of penicillin has eclipsed them. In recent years, they have reemerged on the scene and have been successfully used against *Salmonella* spp. and *Shigella* spp., among others (Połaska & Sokołowska, 2019).

V- Combination strategies. The use of adjuvants or the combination of different antibiotics is useful. Adjuvants are designed to inhibit the intrinsic resistance mechanisms by which bacteria evade antibiotics. In addition, combinations of different antibiotics can have a synergistic effect, i.e., they are more active than independent applications (Ejim *et al.*, 2011).

VI- Natural Products (NP). The beginnings of the antibiotic era and most of the antibiotics currently in use are derived from natural products, mainly bacteria, fungi, and plants. Bacteria and fungi produce secondary metabolites to fight for their own niche, and they have been systematically studied, purified, and used in human and veterinary medicine and also in

agriculture (Katz & Baltz, 2016). On the other hand, plants have been used in various cultures as part of traditional medicine to treat diseases. In recent decades, they have come back into focus using plant extracts, essential oils, purified bioactive molecules, or even *in vitro* production of secondary metabolites (M, A, & F, 2019). The use of extracts and essential oils, as well as the purification of the molecule(s) responsible for antibacterial activity, has the advantage of using renewable raw materials, byproducts of industrial processes, making them environmentally friendly and sustainable. The identification and isolation of the main molecules exerting antibacterial activities allowed researchers to elucidate the mechanisms of action, with possible pathways being interaction with the cell wall, cell membrane, and bacterial proteins, altering processes like bacterial adhesion, metabolite, and ion equilibria, inhibiting biofilm formation or impairing DNA synthesis (QUINTERO, Cristián Andrés; VALLEJO, Mariana Guadalupe; BONTTI, Sergio; PATIÑO, Sol; PEREZ-GIRABEL, Rocio 2022; A. Silva *et al.*, 2021).

Extracts or essential oils (EO) from cultivated or wild plants and byproducts from industries that use plants as raw materials were selected for the present work. The selection included three aromatic plants: rosemary (*Salvia rosmarinus* Spenn., Lamiaceae), syn.: *Rosmarinus officinalis* L.], thyme (*Thymus vulgaris* L., Lamiaceae), and oregano (*Origanum vulgare* L., Lamiaceae); two wild plants: jarilla (*Larrea* spp., Zygophyllaceae) and mugwort or artemisia (*Artemisia vulgaris* L., Asteraceae) one cultivated plant with industrial interest: yerba mate (*Ilex paraguariensis* A.St.-Hil., Aquifoliaceae) and a byproduct of the industry, namely wine from *Vitis vinifera* L. (Vitaceae).

Plant extracts or EO can be used for the production of leaves, tails, roots, the whole plant, the aerial parts of the plant, and also from the fruits: skin, seeds, or the whole fruit. The experimental data of *I. paraguariensis* are based on the commercial presentation that can be used infusion, called “mate”. Finally, in the case of *V. vinifera*, we included byproducts of wine and wine as raw materials (Figure 3).

3.1.4 Bacteria

The Gram-positive bacteria have been treated with some of the above-mentioned natural extracts to test their antibacterial activity. The bacteria were selected according to the WHO alert (table 1). Gram-negative bacteria were reviewed previously (QUINTERO, Cristián Andrés; VALLEJO, Mariana Guadalupe; BONTTI, Sergio; PATIÑO, Sol; PEREZ-GIRABEL, 2022).

To discover the antibacterial activity of plant-based compounds, the most common first step is using inhibition disks. The protocol starts with by imbibing of the disk with the extract or the natural compound in solution, in serial dilutions, and applied over the bacterial biofilm. The compounds diffuse in the media, and produces a halo where the bacteria can not grow, called an “inhibition zone”. The diameter of the inhibition zone is a measure of the potency of the compound. Another option, alternative or posterior, is the use of the solutions directly added to the liquid or solid culture medium, and the measurement is made in function of the growth, which is monitored by counting the colonies. The quantification of the activity is based on critical parameters, such as Minimal Inhibitory Concentration (MIC), which is defined as the lowest concentration of the antimicrobial agent that inhibits the growth of the organism. Another important parameter is the Minimal Bactericidal Concentration (MBC), which provides information on the lowest concentration at or above the MIC required to kill a microorganism. Additionally, it is usual to inform specific parameters, like MIC_{90}, which indicates the minimum concentration at which 90% of the isolates were inhibited, or IC_{50}, the inhibitory or effective concentration for 50 % of all surveyed isolates of a strain (CLSI, 2020; John E. Bennett, 2020). Table 2 summarizes the extracts and essential oils tested over each bacteria.

3.1.4.1 Staphylococcus aureus

Staphylococcus aureus is an extracellular gram-positive bacterium with spherical morphology. It is a facultative anaerobe but also grows well under aerobic conditions. *S. aureus* is associated with human infections such as facial furuncles and carbuncles, inflammation of loose connective tissue, and postoperative wound infections, being an important complication in patients with underlying pathologies. The main complication of current infections by *S. aureus* is the wide dispersion of Methicillin-Resistant strains (MRSA), which makes using beta-lactam antibiotics practically impossible. In addition, it is more frequent the appearance of strains resistant to vancomycin(John E. Bennett, 2020).

3.1.4.1.1 Artemisia

Four different species of *Artemisia* were used to inhibit the growth of *S. aureus*. In the first work, the aerial parts of *A. rupestris* L. were collected and separated into two groups: stems and flowers plus leaves, for their extraction with different solvents using ultrasound or microwave methods. The inhibition was strong, regardless of the solvent, method, or part of the plant used, except for the aqueous extract of stems, which was the less effective. The methanolic extract of flowers and leaves was the strongest inhibitor (Nokerbek, Sakipova, Chalupová, Nejezchlebová, and Hošek, 2017). Working with flavonoids isolated from *A. rupestris*, artemetin, pachypodol, chrysosplenetin, penduletin, and chrysoeriol, Lan *et al.* did not find inhibition of *S. aureus* when used alone at concentrations of 128 and 256 μg/mL. However, they found a synergism between the last three and norfloxacin up to 16-fold, chrysoeriol reduced the MIC of ciprofloxacin128-fold, and oxacillin 8-fold (Lan *et al.*, 2021). Methanolic extracts of *A. vulgaris* were able to inhibit *S. aureus*, in a study that also included three other extracts of plants used in traditional medicine, being *A. vulgaris* the most effective against *S. aureus*, with a MIC of 25 mg/mL (Manandhar, Luitel, and Dahal, 2019). Methanolic extract of *A. absinthium* L. was assayed against *S. aureus* using a reference strain (*S. aureus* ATCC 6538) and nine clinical isolates. The reference strain was the most sensitive, with a MIC of 0.625 mg/mL, and the clinical samples showed a MIC >2.5 mg/mL (Boudjelal, Smeriglio, Ginestra, Denaro, and Trombetta, 2020). Interestingly, in the same study, the authors showed no dermal toxicity nor any sign of toxicity when the extract was applied or administrated orally in mice. In 2021, Mohamed *et al.* tested the extract of *A. herba-alba* Asso and seven different compounds isolated from it against bacteria, fungi, and yeast. Both gram-positive bacteria were sensitive to the extract and six of the isolated compounds, most effective in the whole extract into the generation of inhibition zone. The lower MIC was achieved with 11-epiartapshin and benzoic acid p-(β-D-glucopyranosyloxy)-methyl ester, with 25 μg/disk (Mohamed *et al.*, 2021).

3.1.4.1.2 Oregano

Several species of *Origanum* have been used to inhibit the growth of *S. aureus*, with different degrees of success. In 2019 was published a big screening where ethanolic extracts of 67 dietary spices were tested against antibiotic-resistant bacteria like *S. aureus* and *S. enteritidis*. Among the spices, *O. vulgare* showed inhibition zones of 14 and 12 mm for *S. aureus* resistant to antibiotics or normal. In contrast, *O. majorana* L. showed inhibition zones of 17 and 15 mm for the same strains. Only for *O. majorana* was determined MIC and MBC, 1.6 mg/mL (Zhang *et al.*, 2019). In the same year, a study was published in which hydroalcoholic extracts of *O. vulgare* were assayed alone and in addition to *Hypericum perforatum L*. (Hypericaceae) extracts. While the separated extracts showed inhibition zones of 16 and 13 mm, respectively, the used in combination was 21 mm (Bahmani *et al.*, 2019). Using supercritical fluid extraction, García-Pérez *et al.* obtained an extract able to inhibit the growth of *S. aureus*, with inhibition zones of 0.3-0.4 cm, with similar results for *E.coli* (García-Pérez *et al.*, 2019). Interestingly, the EO of *O. vulgare* seems to be more effective, as was shown by Lofa *et al.* They found inhibition zones from 15 to 22 mm, working with samples of *S. aureus* isolated from the pork supply chain, with MIC of 0.01-0.02 %(V/V), similar values showed by purified thymol and carvacrol (Lofa *et al.*, 2019).

3.1.4.1.3 Rosemary and Thyme

Extracts of these two aromatic herbs have been studied individually or in comparative studies. Del Campo *et al.* used a commercial oregano extract to test their activity, which showed a MIC of 0.5 %(V/V). Interestingly, with the addition of sodium chloride 10 % (W/V), they reached a MIC of 0.13 %(V/V). The pH was also analyzed, with an optimum of pH=4,5, with a MIC of 0.06 %(V/V) (Del Campo, Amiot, and Nguyen-The, 2000). When Zaïri *et al.* compared thyme [*T. algeriensis* Boiss. & Reut. and *T. capitatus* (L.) Hoffmanns. and Link] with rosemary (*S. rosmarinus*) over ten strains of S. aureus always found a better activity of thyme, especially *T. algeriensis.* They also compared activity of the different preparation methods, following a potency order decoction > infusion > methanolic extract. The lower MIC was obtained with the decoction of both strains of thyme, with 0,25 mg/mL (Zairi *et al.*, 2018). Remarkably, the same extracts were applied over *Staphylococcus epidermis*, with the same or lower MICs. As mentioned before, Zhang *et al.* performed a big screening, including *S. rosmarinus* and *T. vulgaris,* with better inhibition zones for rosemary. Also, the MIC was better, with 0,4 against 1,6 mg/mL for thyme (Zhang *et al.*, 2019). In another comparative study, Munekata *et al.* followed the growth rate of the bacteria when they were treated with extracts of rosemary and thyme obtained by conventional or ultrasound-

assisted extraction. Even when they did not find a significant diminution of the speed, the best results were obtained with rosemary extracted conventionally with ethanol (Munekata *et al.*, 2020).

3.1.4.1.4 Wine byproducts

In the wine industry, the obtention of byproducts has great importance and diversity. One byproduct of wine production is the lees. A work published in 2013 showed the lees could inhibit the growth of *S. aureus*, requiring the previous activation: they must be irradiated with light at 400 nm. Even more, it depended on the time of irradiation: LED-light irradiation for 10 min effectively killed the bacteria with an approximate 3-log reduction, while LED-light irradiation for 20 min achieved a 5-log reduction (Tsukada, Sheng, Kamachi, and Niwano, 2016). The grape pomace was also described as a synergist of several antibiotics, as was the case for Cabernet Sauvignon grape pomace extracts and their activity in couple with antibiotics over clinical isolates of *S. aureus*, and MR *S. aureus* (Peixoto et al., 2018). The addition of grape pomace extracts augmented 30 to 75-fold the activity of antibiotics like ciprofloxacin, norfloxacin, or levofloxacin (Sanhueza *et al.*, 2017). The authors also showed synergism between grape pomace extracts and purified phenolic compounds (found in grape extracts), with an improvement of the activity from 8 to 64-fold, with a maximum for gallic acid and vanillic acid.

3.1.4.1.5. Yerba mate

Commercial brands produce yerba mate with small differences in their harvesting, drying, and grinding criteria, besides the differences in their region of origin. In this regard, Burris *et al*. performed a screening using different commercial brands of yerba mate, three from Argentina and one from Uruguay, for the extract preparation. All of them inhibited the growth of *S. aureus* ATCC 27708 and *S. aureus* SA113. The MIC was 25 μg/mL for all brands when tested over *S. aureus* ATCC 27708, >50 μg/mL for *S. aureus* SA113 for three of them, and 25 μg/mL for one brand (Burris, Davidson, Stewart, and Harte, 2011). Intriguingly, the same study showed that the MIC for *E. coli* was between 4 and 8-fold higher.

3.1.4.2 Enterococcus faecium

Enterococci are gram-positive bacteria of coccoid morphology, facultative anaerobes, and extracellular. They are an important cause of hospital-acquired infections such as septicemia and intra-abdominal sepsis, Central Nervous System infections, skin and soft tissue infections, endocarditis, and pneumonia. Of the members of the *Enterococci* family*, Enterococcus faecium* shows a greater risk for the patient since it presents a high degree of resistance to antimicrobials, including vancomycin, a glycopeptide with a good effect on the cell wall of *Enterococci* (John E. Bennett, 2020).

3.1.4.2.1 Artemisia

In two different works, *Artemisia* spp. was used to inhibit *E. faecium*, in both cases using *A. absinthium*. The first of them confronted nine plant extracts against six multidrug-resistant bacteria. The extracts were prepared with ethanol or water; the only active ones were the ethanolic extracts. The *A. absinthium* extract was just able to inhibit the growth of *E. faecium* partially, with an IC_{50} of 256 μg/mL, without reaching an inhibition greater than 90% in the maximum concentration assayed (256 μg/mL) (Khan *et al.*, 2018). However, in a later work, the water extracts did not inhibit the growth of *E. faecium* or *E. faecalis* when they were assayed in inhibition disks. Interestingly, the EO of *A. absinthium* inhibited the growth in liquid broth cultures of *E. faecium* but did not inhibit *E. faecalis* growth. Also, in both cases, none of the aqueous extracts assayed inhibited bacterial growth (Bartkiene *et al.*, 2020).

3.1.4.2.2 Oregano.

In 2009 a screening of *Enterococci* isolated from piglets was published. Over 55 enterococcal strains were isolated. They found 37 *E. faecium* and 4 *E. faecalis*. Ten selected strains were treated with *O. vulgare* extracts, which inhibited the growth of all strains (Strompfová and Lauková, 2009). In a later work, Silva and col used eight EO from herbs used in gastronomy against ten foodborne and spoilage bacteria. Among them, *E. faecium* and *E faecalis* were sensitive to oregano (*O. vulgare*) EO, reaching inhibition zones of 25 and 19 mm, respectively. Interestingly, the MIC determined was higher for *E. faecium*, 15% (V/V), while 5% (V/V) was the MIC for *E faecalis* (N. Silva, Alves, Gonçalves, Amaral, & Poeta, 2013). Another species of *Origanum*, *O. hirtum*, was used to prepare EO and hydrolates for testing their capacity to inhibit the *E. faecium* and *E. faecalis* growth, among other bacteria. The EO of *O. hirtum* showed high activity against all the tested bacteria, with a MIC and MLC of 0,125 % (V/V) for both

strains. The hydrolates, on the other hand, showed a MIC and MBC major to 50% (V/V), showing the EOs always had better antimicrobial activity than the corresponding hydrolates (Di Vito *et al.*, 2021).

3.1.4.2.3 Thyme and Rosemary

As mentioned before, Silva *et al. (2013)* tested eight EO, including thyme and rosemary. The MIC determined for thyme was 5 and 15 % (V/V) for *E. faecium* and *E. faecalis*, while for rosemary, it was above 50 % (V/V) in both cases (N. Silva et al., 2013). Thyme (*T. vulgaris*) EO showed one of the strongest effects against E. faecium and *E. faecalis*, with inhibition zones of 37 and 30 mm, respectively. On the other hand, rosemary (*S. rosmarinus)* EO was considered not inhibitory against both bacteria.

3.1.4.2.4 Wine byproducts

An interesting study was published in 2010 by Corrales and col. (Corrales *et al.*, 2010), where grape skin extracts were assayed. Riesling grapes were collected in two vineyards, one with traditional agriculture and the other with organic agriculture practices. Despite the differences in their composition, levels of quercetin and kaempferol were significantly higher in organic samples, while the content of flavonoids, catechin, epicatechin, and procyanidin B1 was higher in the conventional grape skin extracts; the antibacterial activity was similar in both extracts. Even residual pesticides in conventional farming grapes did not influence their ability to inhibit *E. faecium* or *E. faecalis* (Corrales *et al.*, 2010).

3.1.4.3 Streptococcus pneumoniae

Streptococcus pneumoniae has a spherical or slightly elliptical morphology, mostly in pairs, with a gram-positive structure. They are extracellular bacteria. *S. pneumoniae* is a facultative anaerobe with moderate growth requirements. *S. pneumoniae* primarily colonizes the upper respiratory tract of healthy individuals and can be isolated from clinical specimens of tonsillitis, pneumonia, meningitis, and otitis media in community patients. It presents an important public health problem worldwide due to its high morbidity and mortality rates, especially in children and the elderly. Over the years, *S. pneumoniae* has developed resistance mechanisms that gave rise to strains resistant to penicillin and macrolides (John E. Bennett, 2020).

3.1.4.3.1 Oregano and thyme

S. pneumoniae has been treated only with EO from *O. vulgare*, *T. daenensis* Čelak. and *T. vulgare L*. Working with *O. vulgare* and *T. daenensis,* Sharifi *et al.* found that the formation of biofilms of *S. pneumoniae* was reduced by the EOs, proportionally to their concentration. The same assay was used to successfully prove the decrease in the number of adherent bacteria and the size of aggregates. Subsequently, the expression of genes related to biofilm synthesis was analyzed by quantitative real-time RT-PCR (qPCR) and showed a significant reduction when treated with EOs. Finally, the EOs were also shown to inhibit the growth of *S. pneumoniae* at MICs between 0.625 and 10 µL/mL, with *T. daenensis* being the most effective (Sharifi, Ahmadi, and Mohammadzadeh, 2018). They also found that the EOs have a total or partial synergistic effect with ciprofloxacin and ethidium bromide. In addition, they proved by RT-PCR that the efflux pump activity was inhibited using sub-MIC concentration of the EOs (Ghafari, Sharifi, Ahmadi, and Nayeri Fasaei, 2018). Ács and col worked with seven different EO against six respiratory tract pathogens in the same year. *T. vulgare L* EO showed moderate activity against *S. pneumoniae.* Two methods tested the antibacterial activity of commercial EO: broth macrodilution test to determine MIC and MBC and vapor phase test to measure MIC produced by the volatile compounds only, showing a MIC and MBC of 0.11 and 0.22 mg/mL, respectively, for the EO, while volatile compounds MIC was 90 µL of EO/L of airspace volume (Ács *et al.*, 2018).

3.1.5 Extracts Preparation

The extracts can be obtained from the leaves, stems, bark, seeds, flowers, fruits, roots, or the whole plant, or even byproducts of the industry, which are used as raw materials for any plant. Fresh or dry vegetal material (drug) can be used, depending mainly on the stability of the components to be isolated. Usually, desiccation is required to stop enzymatic reactions that can modify the quality of the material, avoiding fungal infections as well (rust). However, for products as EOs, a fresh material is sometimes preferred due to the volatile nature of the components and their possible chemical alterations.

The next step is grinding to decrease the particle size, augment surface exposure and promote solvent penetration through cell walls that have been partially lysed in previous stages by

cutting, chopping, or pulverizing the raw material (Guglielmi, Pontecorvi, and Rotondi, 2020). Finally, the process continues with the extraction with solvent or solvent systems, including water, ethanol, methanol, and acetone, among others, in different proportions. Nowadays, Natural Deep Eutectic Solvents (NADES) are used as an innovative strategy to minimize the environmental impact of organic solvents. As extra values, NADES obtained mixtures seem to be 'ready-to-use' extracts (removal of solvent would not be necessary) and provide better biopharmaceutical properties (D. T. da Silva *et al.*, 2021).

In order to improve the extraction, temperature, time, stirring, herbal material/solvent ratio, and stability of the components are important variables to be considered. The solubility of the active ingredients, for instance, is modulated by the chosen solvent and other components in the plant matrix.

We describe the most used procedures, from traditional techniques to advanced technologies. A summary is shown in Figure 4.

3.1.5.1 Conventional techniques

3.1.5.1.1 Maceration

One of the simplest methods used for hundreds of years, maceration involves contacting the ground drug in a closed container at room temperature, with occasional stirring. It is suitable for thermolabile bioactive components. The usual time required (3-14 days) can be shortened with constant stirring (dynamic maceration). If total extraction of the components is desired, the solvent must be renewed (R. M. L. da Silva, Couto, & Bresolin, 2012).

3.1.5.1.2 Percolation

In this technique, the solvent flows through to the vegetal material contained in a cylindrical or cone-shaped vessel (percolator). After macerating at room temperature for a certain period (2-4 h) in this closed container, the liquid is slowly dropped by a tap at the bottom of the percolator (Handa SS, Khanuja, SPS, Longo G, 2018). This way, the extraction process done by initial maceration is complemented, and an enriched liquid is obtained. It is often used to prepare tinctures or fluid extracts with high concentrations of active ingredients.

3.1.5.1.3 Digestion

To increase the efficiency of the extractive process, moderate heating is applied (40-60 °C) to the vegetal material/solvent system in a short period (4-6 h), avoiding thermal decomposition of the active ingredients. Water, hydro-alcoholic solution, ethanol, or other organic solvents are used. Frequently, poorly soluble components are extracted by digestion (Hussain MK, Saquib M, 2019).

3.1.5.1.4 Infusion

Boiling water is used as an extraction solvent, added to the herbal drug, and allowed to stand for a short period (5-20 min), and then the extract is filtered and collected. As a result, highly water-soluble (polar) components are obtained and used for aromatic plants with volatile metabolites (R. M. L. da Silva *et al.*, 2012).

3.1.5.1.5 Decoction

Unlike infusion, in decoction, the herbal drug and water (at room temperature) are placed together and then boiled for a defined time (5-20 min), proceeding to filtration and collection. A higher concentration of active compounds is achieved, but due to the increased temperature (approximately 100 °C), it is applied for extracting thermo-resistant compounds, and usually, hard vegetal materials are used (e.g., roots or bark) (Azwanida, 2015; R. M. L. da Silva *et al.*, 2012).

3.1.5.1.6 Soxhlet

All previously mentioned techniques are non-continuous processes: unless an amount of fresh solvent is added and extraction is repeated, it will not be exhaustive. Soxhlet extraction is a continuous and exhausting technique. In a Soxhlet apparatus, the drug is packed into the chamber of the equipment (Soxhlet extractor), usually in a cartridge. At the same time, the organic solvent (e.g., ethanol, acetone, ethyl acetate) is contained in a glass flask and submitted to heating. A condenser is added to prevent evaporation. A side tube leads the solvent vapor from the flask to the condenser, filling the cartridge and extracting the material. A second tube, called a siphon, connects the Soxhlet extractor to the flask and evacuates the liquid extract once its maximum level is reached, returning to the flask. After extraction, waste material and enriched extract are obtained. A lower amount of solvent is required to determine the weight of the drug. Only thermo-resistant

compounds that stay unaltered in the hot solvent for long can be extracted.

3.1.5.1.7 Liquid-liquid extraction

As a derivative of the funnel separation partition, the liquid-liquid extraction is a continuous technique in which the active ingredients are partitioned between an aqueous and an organic phase according to their log P using glass equipment. Usually, this is the next step for purifying a previously obtained extract (e.g., infusion), and a succession of solvents is used as their polarities increase: hexane, dichloromethane, ethyl acetate, and butanol. As a result, a group of metabolites, from non-polar to polar compounds, are selectively obtained in each fraction, which is useful for performing bio-guided assays.

3.1.5.1.8 Hydrodistillation

In EO extraction, special techniques are performed according to the volatile nature of the components. One of the most used in industry is hydrodistillation, which comprises three types of techniques: water distillation, water, and steam distillation, and direct steam distillation. The latter is the most used for producing EO on a large scale. The steam is generated in a boiler, separated from the plant material, that stands in a perforated grid. As the steam enters the inlet to the grid, extraction is exerted at a temperature not exceeding 100°C, so no thermal alteration of the components should occur. As advantages, the amount of steam is easily regulated, and a highly purified EO is obtained (Handa SS, Khanuja, SPS, Longo G, 2018)

3.1.5.2 Advanced techniques

3.1.5.2.1 Microwave-Assisted Extraction (MAE)

Microwave radiation is applied to the solvent-soaked drug, turning the electromagnetic energy into heat. Solvent penetration into the drug is facilitated by heating, and active metabolites are extracted. Microwave radiation generates a dipole rotation in molecules of polar solvents. Heating is provided when polar molecules try to align to a magnetic field direction (at 2450 MHz), and it is generated near the surface of the material, being transferred by conduction to the rest. As a disadvantage, only dielectric absorption is generated in non-polar solvents, and small heating occurs (Hussain MK, Saquib M, 2019).

3.1.5.2.2 Ultrasonication

In ultrasound-assisted extraction, high-frequency sound energy is applied (> 20 KHz) to the vegetal material/solvent macerate to promote the disruption of the plant cell wall. This accelerates the process by reducing the extraction time. An increase in permeability produces cavitation and facilitates the release of bioactive metabolites. Nevertheless, free radicals can be generated at the high frequency used and eventually degrade the components of the sample (Hussain MK, Saquib M, 2019).

3.1.5.2.2 Ohmic heating

An alternating electric flow is applied and is forced to pass through the sample, which must have electrical conduction. Otherwise, an electrolyte is added, like NaCl, or the use of an organic solvent is advisable. The movement of ions of the sample towards the electrodes of opposite charges produces an electrical resistant heating (Joule effect), and this thermal energy produces the extraction. As an advantage, heating is more homogeneous than other techniques (e.g., MAE), and undesired effects on the sample characteristics are minimal, especially in food products (Alkanan, Altemimi, Al-Hilphy, Watson, and Pratap-Singh, 2021).

3.1.5.2.3 Supercritical Fluid Extraction (SFE)

Some substances, when subjected to high pressure and temperature beyond their critical point, behave as supercritical fluids, having properties of both gas (vaporization) and liquid (solvating characteristics). SFE offers several benefits as the replacement of organic solvents (with no solvent residues) and an increment in extraction efficiency. CO_2 is often used to produce EO due to its low cost, safety, inertness, and availability, among other characteristics. In addition, the absence of oxygen minimizes oxidative reactions during conventional extractions. Nowadays, other areas using SFE are food and nutraceuticals production. The main limiting factor to adopting this technology is the important initial capital investment (Handa SS, Khanuja, SPS, Longo G, 2018; Hussain MK, Saquib M, 2019).

3.1.5.2.4 Counter-Current Extraction (CCE)

Soaked plant material is disintegrated to produce a fine slurry. This material moves in one direction through a cylinder forced by a pump.

Solvent flow is opposite to that of the slurry; active components are partitioned between two phases, and a concentrated extract is obtained at one end of the extractor while vegetal waste material is recovered on the other side. This technique has several goals, such as using a lower amount of solvents compared to traditional ones, the extraction is executed at room temperature, which is suitable for thermolabile compounds, and heat produced during pulverization is absorbed by water in the wet preparation. Additionally, it has been reported as more efficient and effective than continuous hot extraction techniques (Handa SS, Khanuja, SPS, Longo G, 2018).

Once the extract is obtained, the following step is solvent elimination, usually employing evaporation under reduced pressure, and a completely dry sample can be obtained by lyophilization.

3.2 DISCUSSION

Microorganisms, in general, like fungi, parasites, and bacteria, can affect human health, as well as animal and plant health. They can cause several diseases with a wide range of symptoms and consequences that can even lead to death. Infectious diseases currently affect an enormous proportion of the population, which translates into very high economic costs for public health care and, even more important, being the second leading cause of death in the world.

The appearance of antibiotics in the early 20th century represented a breakthrough in modern medicine. They significantly reduced morbidity and mortality caused by infections worldwide. However, the increasing antibiotic resistance of various pathogenic bacteria made the development of new antibiotics inevitable and urgent.

Even though various strategies exist to discover and develop new molecules with antimicrobial properties, no new molecules have been approved in the last 40 years. New technologies and the restoration and improvement of old technologies lead to different strategies for the discovery of new molecules effective against bacteria. The use of natural extracts, especially plant extracts, is a promising approach to finding bioactive compounds or even an extract or EO as a therapeutic agent.

In this review, we have highlighted recent advances in the field related to gram-positive bacteria, which are included in a 2017 alert from WHO. There is a wide range of results, from wild plants to herbs used in gastronomy, to byproducts of the wine industry, including wine itself.

We have reviewed conventional and advanced techniques used for producing plant extracts, where several methods have been improved. Much remains to be done in order to improve those methods with new technologies such as nanotechnology, biotechnology, and cellular and molecular biology to improve their ability to penetrate the cell wall, their activity, their targeting, and their delivery.

Replacing classical solvents with natural deep eutectic solvents would help to reduce environmental impact. The natural extracts are active, sustainable, environmentally friendly, and renewable, in line with the latest trends in industrial development. It is necessary to continue research, including the mechanism of action, activity at the molecular level, toxicity to the host cell, and clinical phases in humans.

4. CONCLUSIONS:

Humanity is facing an alarming situation due to antimicrobial resistance and the lack of fully effective treatments for bacterial infections. This applies to human health as well as veterinary and agricultural treatments. It is a global public health problem that currently affects all countries. There is no single solution, but multidisciplinary approaches are the way to go. Equally important is rapid and accurate diagnosis to find the right treatment. Most clinics have upgraded their equipment to molecular biology techniques, according to COVID, which allows for improved diagnosis.

Current literature shows that plant extracts and natural compounds have antibacterial activity and minimal effects on the host cell. They are also sustainable, environmentally friendly, and renewable in line with global trends. The regional economy can benefit from the added value of industrial processes such as wine and infusions production and juice production using byproducts of the manufacturing process itself.

The natural extracts and their bioactive components can be used alone or in combination with traditional treatments. In this sense, they must

all be validated *in vitro* and *in vivo* and successfully pass all 5 phases to be used in humans. Most plant-based antibiotics are still in the pre-clinical stage, either in the initial phase or *in vitro*, and some have already been tested in eukaryotic cells. In some cases, they have received the patent to be used(Guglielmi *et al.*, 2020).

Of the three bacteria gram-positive, *Staphylococcus aureus* is the most studied. While the extracts used have varied in success, artemisia and wine byproducts show the best results, even when used alone or in synergy with currently used antibiotics. *S. pneumoniae* has been tested the least with natural extracts. With fewer reports, *Enterococcus faecium* has been treated with extracts, and certain compounds present in wine have been identified as responsible for antibacterial activity. Although *Streptococcus pneumoniae* has not been extensively studied, a study shows the molecular and genetic mechanism involved in the activity of the use of thyme and origano EO.

Although in ancient times, medicinal plants were used applying rudimentary extraction methods, like teas and tisanes (infusions) or poultices, the revival of therapies based on natural products in the last century encouraged the development of different extraction technologies. A study of the factors that influence the extraction processes and the physicochemical properties of the bioactive components allows the selection of optimal methods for medicinal, aromatic, or edible species. Components like polyphenols or EO, which are often mentioned in the present work, represent a challenge for extraction, not only because of their low solubility but also because of their potential decomposition over time. Even when conventional methods offer products of high quality and reduced time of operation, innovative methods are sought after by pharmaceutical and food industries since similar or higher yields of active ingredients are obtained, harmless solvents are used, and they are in line with the profile of current consumers, who prefer greener options. However, due to the variables that affect the extraction process and, therefore, the product obtained, it is necessary to standardize both the processes and the content of active components in these mixtures in order to guarantee reproducibility in biological activity tests, with potential application in antimicrobial therapy (Jha & Sit, 2022).

Taken together, it is clear that a multiple approach is needed to identify and produce new molecules with antimicrobial activity. Collaboration between academia, small biotech startups, large pharmaceutical companies, and the national governments is the best way to find effective treatments for numerous infections that currently cannot be treated.

5. DECLARATIONS

5.1. Study Limitations

The study is limited to the selected bibliography.

5.2. Acknowledgements

We thank Walter Pelaez for their invitation and permanent incentive. In addition, we thank Juan Pablo Mackern-Oberti for their critical reading.

5.3. Funding source

The work was supported by an internal grant from Universidad JA Maza.

5.4. Competing Interests

The authors declare that they have no competing interests.

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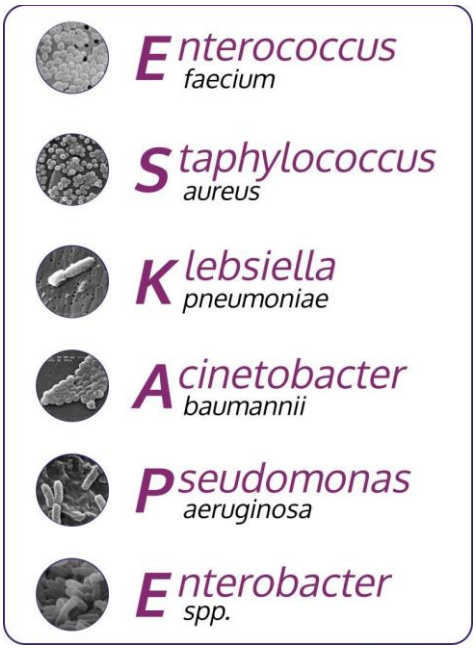

Figure 1- Antimicrobial-resistant ESKAPE pathogens. The group of pathogens responsible for several hospital infections with antimicrobial resistance in 2008.

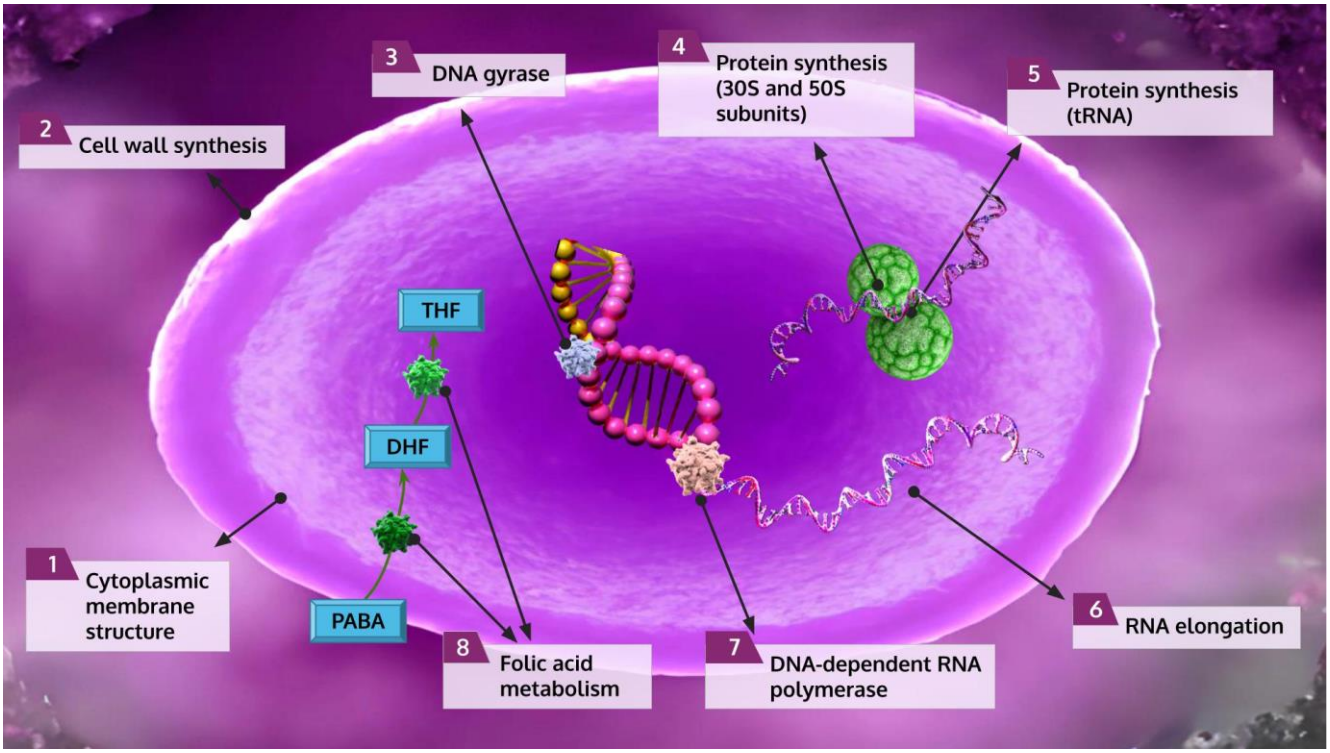

Figure 2. Action mechanisms of antibiotics

1: Changes in permeability or damage to the cytoplasmic membrane. 2: Inhibition of cell wall synthesis. 3: Inhibition of DNA gyrase. 4: Reversible inhibition of protein synthesis by subunit binding (30S or 50S). 5: Inhibition of protein synthesis by prevention of t-RNA binding to the A site. 6: Formation of a stable complex with DNA and RNA elongation prevention. 7: Inhibition of DNA-dependent RNA polymerase. 8: Inhibition of dihydropteroate synthase and dihydropteroate reductase

Figure 3. Raw material for extracts and essential oil preparation. The starting material for the extracts and EO is, in general, stems, leaves (fresh or dried), flowers and inflorescences, and for Vitis vinifera, seeds, fruit skin, whole fruit, and also byproducts of the wine industry as well as the wine itself.

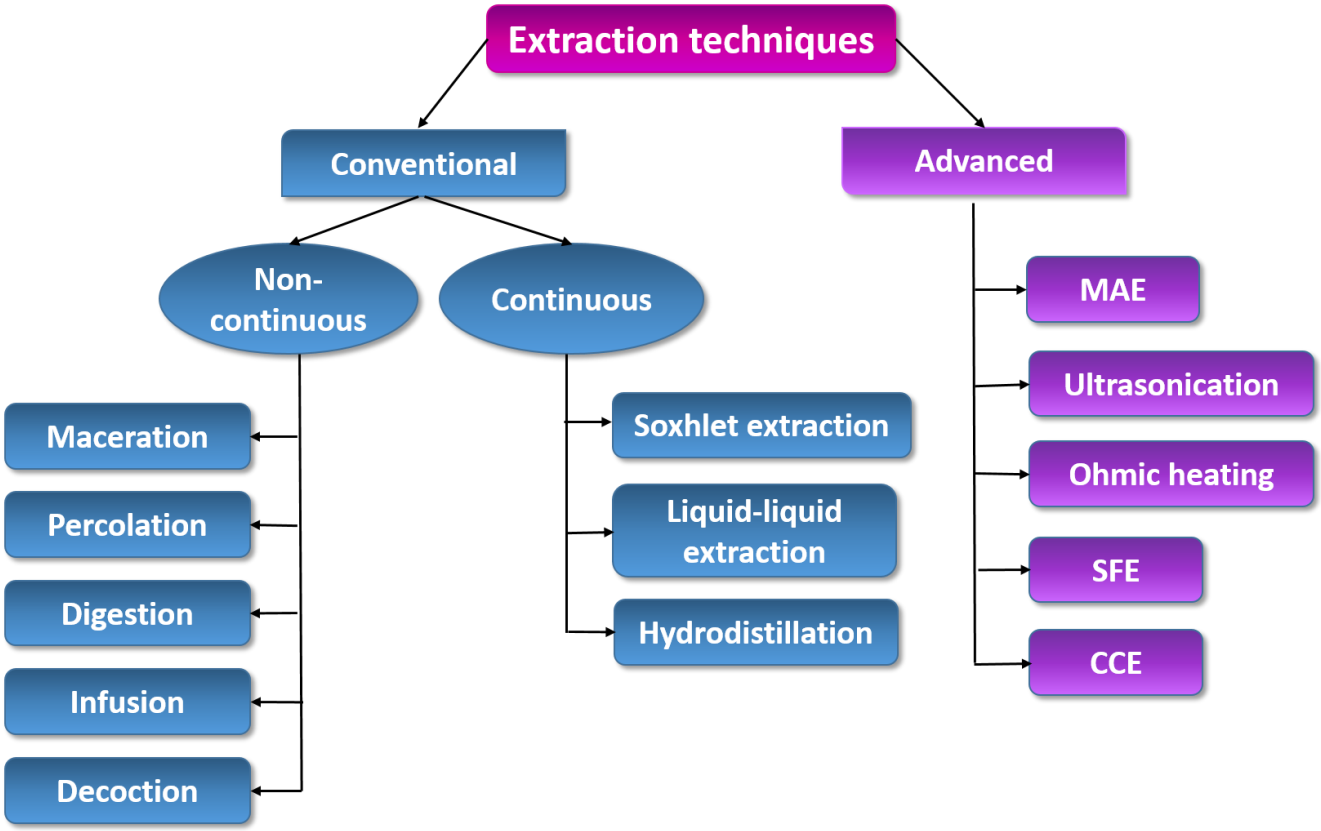

Figure 4. Schematic representation of extraction techniques. Traditional and advanced extraction techniques for isolation of bioactive metabolites from medicinal plants.

Table 1- WHO priority list of pathogens for antibiotics research

Priority	*Bacteria*	Resistance	Gram
Priority 1: CRITICAL	*Acinetobacter baumannii*	carbapenem-resistant	-
	Pseudomonas aeruginosa,	carbapenem-resistant	-
	*Enterobacteriaceae**	carbapenem-resistant, 3rd generation cephalosporin-resistant	-
Priority 2: HIGH	*Enterococcus faecium*	vancomycin-resistant	+
	Staphylococcus aureus	methicillin-resistant, vancomycin intermediate and resistant	+
	Helicobacter pylori	clarithromycin-resistant	-
	Campylobacter	fluoroquinolone-resistant	-
	Salmonella spp.	fluoroquinolone-resistant	-
	Neisseria gonorrhoeae	3rd generation cephalosporin-resistant, fluoroquinolone-resistant	-
Priority 3: MEDIUM	*Streptococcus pneumoniae*	penicillin-non-susceptible	+
	Haemophilus influenzae	ampicillin-resistant	-
	Shigella spp.	fluoroquinolone-resistant	-

*Enterobacteriaceae include: *Klebsiella pneumonia*, *Escherichia coli*, *Enterobacter* spp., *Serratia* spp., *Proteus* spp., and *Providencia* spp, *Morganella* spp

Table 2. Extracts and EOs used for the bacterial treatment. References: 1: artemisa, 2: jarilla, 3: oregano, 4: rosemary, 5: thyme, 6: wine, 7: yerba mate. +: used, -: not used

Bacterial strain	Extract-Essential Oil						
	1	2	3	4	5	6	7
Staphylococcus aureus	+	-	+	+	+	+	+
Enterococcus faecium	+	-	+	+	+	+	-
Streptococcus pneumoniae	-	-	+	-	+	-	-

SOUTHERN JOURNAL OF SCIENCES

ESTABLISHED IN 1993

Original research paper

EFFECT OF MECHANICAL ACTIVATION ON THE POTASSIUM AVAILABILITY OF PHONOLITE ROCK

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*** In Memoriam*

Received 05 December 2022; received in revised form 05 May 2023; accepted 29 May 2023

ABSTRACT

Background: Researchers are carried out to assess rocks containing potassium as an alternative source of fertilizers. These studies are important in reducing the external dependence of Brazil on this commodity. Phonolite is a rock of volcanic origin that has in its mineralogical composition the predominance of feldspar and has been potential to use as an alternative potassium source. The studied rock has 6% total-K but is unavailable to plants in natural rock. **Aim:** This project evaluated the effect of mechanical activation, wet and dry, on K availability for extractors different and its K leaching curves of phonolite from Poços de Caldas, MG, Brazil. **Methods:** Phonolite rock was subjected to mechanical activation for 30 and 60 min by wet and dry processes. Particle size distribution, XRD, and FTIR analyses characterized phonolite activated. These results were compared to the behavior of the rock with no activation. K availability (total; water-soluble; exchangeable, non-exchangeable, and structural) was determined in samples activated and no activation. K leaching curves were obtained by successive extractions with 0.01 mol L^{-1} citric acid and Mehlich-1 solutions to 1812 h and analyzed by FAAS. **Results**: The samples mechanical activation promoted a reduction in the intensity of the diffraction peaks. In the sample dry mechanical activation for 60 min, K released increased by 15% in relation to the no activation sample. K leaching curves were observed with similar behavior for the extractors and higher K availability after 235 h of total contact time. **Discussion:** Mechanical activation promoted a decrease in structural K and an increase in non-exchangeable K, released into the soil solution in the medium term. Since the mineralogical composition was not changed, the process action is more efficient in creating the structural defects suggested. K leaching curves corroborate these results, with a continuous and slow K released for a longer contact time. **Conclusions:** It was concluded that the phonolite activated by dry mechanical activation for 60 min increased K-released content compared with the wet process and ratified the possibility of the activated phonolite rock as a slow-release fertilizer.

Keywords: *phonolite, alternative fertilizer, mechanical activation, potassium exchangeable, leaching curve*.

1. INTRODUCTION

The direct use of rocks in agriculture is a natural fertilization technique called stonemeal (rocks for crops) or remineralization. This technique consists of gradually releasing nutrients from the stone powder into the ground through chemical weathering (Straaten, 2007). The advantages of the stonemeal are the geological diversity of rocks with potential for application in Brazilian agriculture which contributes to lower transport costs and helps streamline family agriculture and agro-ecological production (Plata *et al.*, 2021; Ramos *et al.*, 2014; Straaten, 2006; Theodoro and Leonardos, 2006). In addition, the use of rocks for crops is considered a global strategy for sustainable land

DOI: 10.48141/SJS.05_TEIXEIRA_pgs_45_60.pdf

use and lower production cost than synthetic fertilizers, as these new commodities consist of crushed or ground minerals that release nutrients slowly (Jena, 2021; Kleiv and Thornhill, 2007; Plata *et al.*, 2021; Ramos *et al.*, 2014; Straaten, 2006).

Several articles report application experiences with rocks in nature or associated with other rocks as alternative sources for agriculture, as well as concomitant use with organic or biological materials that may favor the availability of potassium nutrients (Bhatti *et al.*, 2011; Jena, 2021; Manning, 2010; Sanz Sconivo and Rowell, 1988; Silva *et al.*, 2013; Straaten, 2007). The use of rock dust as an alternative source for agriculture can also be applied in organic and conventional farming systems (Bhatti *et al.*, 2011; Guelfi-Silva *et al.*, 2013; Manning, 2010; Silva *et al.*, 2013), provided that the physical and chemical characteristics of such materials are known (Teixeira *et al.*, 2011; Teixeira *et al.*, 2012). However, for a mineral to be considered an alternative source for application in agriculture, it is not enough that it has a high content of nutrients; it is necessary that these nutrients are available for the plants. Thus, mineralogy is one of the most important factors in the selection of rocks that can release nutrients (Bhatti *et al.*, 2011; Guelfi-Silva *et al.*, 2013; Jena, 2021; Manning, 2010; Nascimento and Loureiro, 2004; Silva *et al.*, 2013; Teixeira *et al.*, 2011; Teixeira *et al.*, 2012). For example, feldspars potassium, mica, vermiculite, and smectite are minerals primarily related to the presence and K availability in the soil; however, rocks containing nepheline mineral are more effective in the release K than rocks that have only potash feldspar (Kleiv and Thornhill, 2007; Manning, 2010; Nascimento and Loureiro, 2004; Sanz Sconivo and Rowell, 1988; Straaten, 2007).

Phonolite is a rock of volcanic origin that has the predominance of potassium feldspar, plagioclase feldspar, and feldspathoids in its mineralogical composition. The high presence of alkaline oxides makes phonolite a melting rock widely used by the ceramic industries (Andrade *et al.*, 2005). Additionally, the application of this rock has been evaluated as an alternative potassium source, especially in acidic soils, once the release of the nutrient in potassium-rich silicates involves a surface reaction that increases with decreasing pH (Kleiv and Thornhill, 2007; Manning, 2007; Straaten, 2007; Teixeira *et al.*, 2011).

Mechanical activation is considered a branch of the mechanochemical science that features a wide variety of potential applications (d'Azevedo *et al.*, 2006; Baláž and Dutková, 2009; Baláž *et al.*, 2008; Erdemoğlu and Baláž, 2012; Kleiv and Thornhill, 2007; Pourghahramania and Akhgar, 2015; Sabah *et al.*, 2013; Sandvik *et al.*, 2011; Silva, 2009; Silva *et al.*, 2012; Temuujin *et al.*, 2003; Vdovic *et al.*, 2010). The literature contains several examples demonstrating that mechanical activation can accelerate the reaction rate and the leaching of the activated minerals, allowing reactions to occur in shorter times and at lower temperatures (d'Azevedo *et al.*, 2006; Baláž and Dutková, 2009; Baláž *et al.*, 2008; Kleiv and Thornhill, 2007; Pourghahramania and Akhgar, 2015; Temuujin *et al.*, 2003). For example, studies of mechanically activated rock by high-intensity milling for the production of ultrafine fertilizer powder have shown that mechanical activation can be used to increase the release of potassium from materials containing potassium feldspar (Kleiv and Thornhill, 2007). The increased reactivity of the products obtained by grinding was attributed to the increase of the specific surface area and structural disorder. Furthermore, adding a small amount of water in the grinding makes it possible to obtain products with higher specific surface area, greater structural disorder, and greater reactivity (Kleiv and Thornhill, 2007).

To determine the efficiency of potassium release in mineral sources, several extractors are used to facilitate the mineral weathering and simulate the acidic environment produced by the plant in the rhizosphere region (near the root). Ion exchange resins usually perform these studies, dilute salt solutions, and low molecular weight organic acids such as citric and oxalic acids, which facilitate mineral wear by forming metal-organic acid complexes (Castilhos and Meurer, 2001; Silva, 2009). Considering its availability to plants, soil, and rocks, potassium can be classified into four categories: water-soluble, exchangeable, non-exchangeable, and structural. These categories follow a decreasing order of potassium availability (Villa *et al.*, 2004). The water-soluble K corresponds to the amount of K^+ extracted by a specific volume of water and represents the content of this ion adsorbed by the colloidal particles of the soil. Exchangeable K, by definition, is one that is free to exchange with cations of saline solution added to the soils and corresponds to the ionic form that is electrostatically bound to minerals that make up the solid part of the soil or rock. The non-exchangeable K is the K^+ of reserve or

replacement of soils, for example, K^+ retained in the interlayer of some expandable 2:1 clay mineral. Finally, the structural K is the K^+ strongly bound to the crystalline structure of the minerals (Castilhos and Meurer, 2001; Nascimento and Loureiro, 2004; Silva, 2009; Straaten, 2007; Villa *et al.*, 2004).

In this context, this study aimed to verify the effect of mechanical activation on K^+ availability of phonolite rock from the Poços de Caldas Plateau, MG, Brazil. The different forms of potassium (total; water-soluble; exchangeable, non-exchangeable, and structural) were determined for the unmilled rock and for samples subjected to mechanical activation for 30 and 60 minutes, both by wet and dry processes. We also obtained K leaching curves for samples with mechanical activation.

2. MATERIALS AND METHODS

2.1. Pre-preparation of the rock

The phonolite rock in a study is from Poços de Caldas Plateau, Minas Gerais, Brazil, and has 6% of K^+ total and is mainly composed of alkali feldspar and feldspathoids, i.e., microcline and orthoclase, sanidine and nepheline (Andrade *et al.*, 2005; Teixeira *et al.*, 2011; Teixeira *et al.*, 2012). In addition, in preliminary studies on K availability, the results obtained with the phonolite rock corroborate with the results found in the literature for different silicate rocks rich in potassium (Araújo and Sampaio, 2010; Silva *et al.*, 2012; Teixeira *et al.*, 2012; Teixeira *et al.*, 2015).

The phonolite samples preparation was performed by the following steps: comminution, screening, homogenization, and quartering. First, the comminution was performed by the jaw and roller crusher to allow fragmentation of the sample up to particle size below 4.7 mm, with little production of thin materials. Next, the sample homogenization and quartering were carried out by employing a conical pile followed by a longitudinal pile to obtain a uniform distribution of the rock constituents (Sampaio *et al.*, 2007). Thus, after the preparation steps, the samples have the appropriate mass and particle size for the tests, i.e., samples of 1 to 20 kg with a particle size of less than 4.7 mm (Teixeira *et al.*, 2011; Teixeira *et al.*, 2012).

2.2. Mechanical Activation Tests

The mechanical activation assays were performed in a stainless-steel cylindrical mill with dimensions of 150 x 297 mm (diameter x length) and a body grinder of 10 bars with dimensions of 500 x 293 mm (diameter x length). The rock samples were submitted to mechanical activation, wet and dry, for 30 to 60 minutes, with a mill rotation speed of 70 rpm. In dry mechanical activation, 1 kg of rock sample was used, while in the wet mechanical activation tests, a paste containing about 1 kg of rock and 1 L of water was used. The mass of the bars used in milling was 9 kg, so the ratio of the mass of the sample to the mass of the bars was 1:9.

After mechanical activation, the samples were homogenized and quartering by means of a Jones-type splitter for obtaining aliquots with appropriate mass for conducting the characterization, K availability tests, and K leaching curves.

2.3. Samples Characterization

The homogenized unmilled phonolite and the activated samples were characterized in terms of particle size distribution and phase analysis. The unmilled phonolite was submitted to chemical and mineralogical characterization by techniques of X-ray Fluorescence (XRF), X-ray Diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR) and Scanning Electron Microscopy (SEM-EDS). XRD and FTIR characterized the active samples.

The wet particle size distribution of the prepared samples was performed with a 250 g sample and in a vibratory sifter with sieves according to Tyler series from 3.3 to 0.038 mm. The fractions obtained from the particle size distribution assays were dried in an oven at a temperature of 70 °C and weighed the sample fractions to obtain the passing percentage.

XRF analysis was performed in a PANalytical, Axios model with a rhodium tube (4kw) and a wavelength dispersion detector (WDS). The sample was fused with lithium tetraborate, and the results were expressed as oxides and standard to 100%. Humidity and fire assay were realized by gravimetric analyses at 100 and 1000 °C, respectively.

XRD analysis were performed in a Bruker-D4 Endeavor equipment, with 0.02°

goniometer step in 2θ and 1.0 second of count time and radiation Co-Kα (λ = 1.789 Å; 35 kV/40 mA), with angular range (2θ) between 4 to 80º. X-ray diffraction for samples is shown as a function of Cu-Kα radiation (λ = 1.5418 Å) to facilitate comparison with the data normally found in the literature. In addition, interpretations of XRD patterns were made by comparison with the standards of the Web ICSD database.

FTIR analysis was performed in a Magna 760 Nicolet equipment, and wave numbers range from 4000 at 400 cm-1 to 4 cm^{-1} of resolution. The spectrums were obtained using anhydrous KBr discs. Assignments of observed bands in infrared spectra were performed by trial-and-error and based on literature data.

SEM-EDS analysis were performed in a FEI Quanta 400, Bruker Quantax, in high vacuum mode was used to identify the morphology and essential components of the rock sample. Particles were placed on proper support and covered with gold (Au), using the voltaic arc method and vacuum injection, producing a gold layer of 20 nm. Samples images were obtained using a secondary electron (SE) and backscattered electron detector (BSED).

2.4. Potassium Availability

Total K, water-soluble K, exchangeable K, non-exchangeable K, and structural K were determined by procedures described in the literature (Castilhos and Meurer, 2001; Mancuso *et al.*, 2014; Melo *et al.*, 2005; Song and Huang, 1988; Villa *et al.*, 2004). For total K determination, the samples were submitted to digestion with HCl, HNO_3, and HF acids, followed by the solubilization of salts formed with diluted HNO_3 (Castilhos and Meurer, 2001; Mancuso *et al.*, 2014; Melo *et al.*, 2005; Song and Huang, 1988). The exchangeable K corresponds to the difference between water-soluble K and K-NH_4OAc. Wherein water-soluble K corresponds to K solubilized in an aqueous medium and K-NH_4OAc corresponds to K extracted with 1 mol L^{-1} acetate ammonium solution and 1:10, after stirring for 4 h on a shaking table at 300 rpm (Castilhos and Meurer, 2001; Mancuso *et al.*, 2014; Melo *et al.*, 2005; Villa *et al.*, 2004). The non-exchangeable K was determined by the difference between K-NH_4OAc and K-HNO_3, which K^+ defines extracted with 1 mol L^{-1} HNO_3 boiling for 10 minutes (Castilhos and Meurer, 2001; Mancuso *et al.*, 2014; Melo *et al.*, 2005; Song and Huang, 1988; Villa *et al.*, 2004). Moreover, K-HNO_3 may also be represented by K released, which corresponds to the sum of water-soluble K and exchangeable K, and non-exchangeable K. The structural K is the difference between total K and K-HNO_3 (Castilhos and Meurer, 2001; Melo *et al.*, 2005; Song and Huang, 1988). The K^+ contents in the solutions were determined by flame atomic absorption spectrometry (FAAS).

Atomic absorption analyses were performed on a Varian spectrometer, SpectrAA-55B model. Operating conditions for potassium determination were a C_2H_2/air flame and a potassium hollow cathode lamp, with a wavelength 766.5 nm and a slit of 1.0 nm. Analytical solutions were prepared using ultrapure water obtained with a Milli-Q system, and all reagents used were analytical grade (PA).

2.5. Potassium leaching curve

Sequential extractions obtained samples for K leaching curves with 0.01 mol L^{-1} citric acid and with the Mehlich-1 solution, which is formed by mixing 0.0125 mol L^{-1} H_2SO_4 and 0.05 mol L^{-1} HCl. In addition, K leaching was also evaluated in an aqueous medium with ultrapure water (Milli-Q), following the procedures used in the literature (Castilhos and Meurer, 2001; Melo *et al.*, 2005; Song and Huang, 1988; Villa *et al.*, 2004). Such solutions are commonly used to evaluate the K availability (Araújo and Sampaio, 2010; Castilhos and Meurer, 2001; Silva, 2009; Silva *et al.*, 2012; Silva *et al.*, 2013; Teixeira *et al.*, 2012). Furthermore, citric acid is one of the acids produced by plants in the rhizosphere region (Castilhos and Meurer, 2001; Silva, 2009).

Successive extractions were carried out up to 1812 h. The rock and the extraction solution were mixed with a 1:10 weight ratio in plastic film-capped flasks, and these were stirred on an oscillating table at 150 rpm at room temperature. In the first extraction, the mixture (rock + extractor) was agitated for 2 h and then rested for a further 2 h for the deposition of the solid materials so that the total contact time of the mixture was 4 h. The supernatant solution was filtered at 0.45 microns membrane, and the K^+ present in the solution was determined by atomic absorption spectroscopy.

To the remaining material in the flask was added a new extraction solution. Then the

mixture was agitated for a variable time followed by 2 hours of rest, filtered, and analyzed for K^+ content. This procedure was repeated until 11 extractions (4; 22; 23; 22; 95; 69; 169; 167; 288; 377, and 576 contact time), resulting in 1812 hours total extraction.

K released and the contact time accumulated correspond to the sum of the data obtained for each leaching stage.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Characterization of phonolite

The granulometric analysis of phonolite (Figure 1) showed the wide distribution of the particle size since about 8% of the particles are smaller than 0.3 mm, and less 2% of the particles are smaller than 0.038 mm. Besides, about 50% of the particles have a granulometry greater than 2.4 mm, and about 20% of the particles have granulometry greater than 1.0 mm.

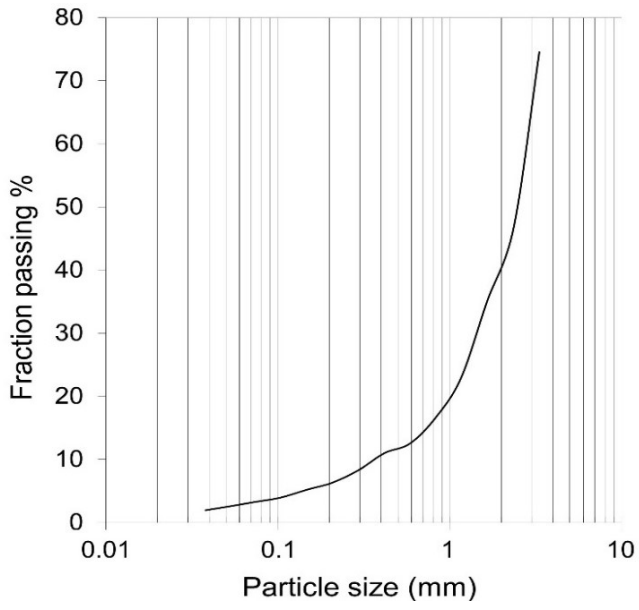

Figure 1. *Granulometric analysis of phonolite. Source: the author.*

The rock chemical composition (Table 1) where indicated that it has an appreciable K content, about 7.1% K_2O.

It was also verified that the phonolite has a high potential as a flux material with 15.4% K_2O+Na_2O, and according to the chemical classification of igneous rock (Sah, 2005), this phonolite is classified as an intermediate rock since it has 55% SiO_2 content. Besides, it has nutrients necessary for plants, such as silicon, iron, calcium, manganese, and phosphorus, that favor its application as an alternative fertilizer. Highlight that Na_2O content, about 8%, which can cause the accumulation of this element in poorly drained soils and to the formation of saline-sodic soils (Gheyi *et al.*, 2016).

Table 1. *Chemical composition of phonolite. Source: the author.*

Composition	% in weight
SiO_2	55.6
Al_2O_3	21.6
Na_2O	8.3
K_2O	7.1
Fe_2O_3	4.1
CaO	1.6
MgO	0.29
MnO	0.20
Sulfite	0.17
TiO_2	0.15
Chlorine	0.15
ZrO_2	0.14
P_2O_5	0.07
Nb_2O_5	0.04
CuO	0.03
ZnO	0.02
Rb_2O	0.01
Humidity (100 °C)	0.15
Fire assay (1000 °C)	1.40

By XRD (Figure 2A) was confirmed that the main mineralogical composition of this rock is alkaline feldspars and feldspathoids (Andrade *et al.*, 2005). The minerals found in phonolite are microcline and orthoclase, sanidine, and nepheline. These results corroborate the XRF since the rock is rich in Si, Al, Na, and K.

Analcime ($Na,K[AlSi_2O_6].H_2O$) chemical composition from phonolite show K^+ and Na^+ miscibility which in natural analcime

($Na[AlSi_2O_6].H_2O$) is 15-20% K^+ (Seryotkin and Bakakin, 2008).

FTIR spectra (Figure 2B) suggested that the main vibrations are related to the minerals of feldspar group. The vibration band at wavelength range between 3650 and 3300 cm^{-1} correspond to the functional group OH stretch (Coates, 2000). Peak at 1635 cm^{-1} indicates O-H deformation (Pekov *et al.*, 2007). The absorption band between 1200 to 900 cm^{-1} are related to Si-O-(Si,Al) asymmetric stretch (d'Azevedo *et al.*, 2006; Wu *et al.*, 2008) and corroborate with the presence of silicon and aluminum oxides determined by XRF. Peaks of Si-O-Si symmetrical stretch and Al-O stretch octahedrally coordinated were shown at 765 and 721cm^{-1} (Prud'homme *et al.*, 2011; Santos *et al.*, 2006). The peak at 583 cm^{-1} indicates O-(Si,Al)-O deformations, microcline feldspar characteristic (Suresh *et al.*, 2011). The peak at 540 cm^{-1} indicates O-Si-O deformation (Prud'homme *et al.*, 2011). Peaks at 464 and 430 cm^{-1} correspond (Si,Al)-O deformation (Wu *et al.*, 2008).

SEM-EDS, Figure 2C, allowed identifying chemical homogeneity and irregular particle morphology with dimensions between 20 and 200 μm. Besides, chemical elements as Si, Al, O, Na, and K were identified by SEM-EDS in the chemical composition of representative particles that, according to the XRD, correspond the alkaline feldspar presence as the microcline, orthoclase, sanidine, and nepheline.

3.1.2 Characterization of samples mechanically activated

Granulometric distribution to the phonolite rock samples mechanically activated (Figure 3) showed a similar fraction passing curve to the samples activated for 30 and 60 min by dry mechanical activation. The wet mechanical activation, the sample activated for 60 min had a greater fine particle, approximately 85% of the particles had dimensions less than 0.1 mm, and 100% of the particles had dimensions less than 0.2 mm. However, samples mechanically activated for 30 min wet and 60 min dry had around 50% of the particles less than 0.2 mm, and to dry mechanical activation by 30 min had particle sizes less 0.3 mm. Besides, in this activation process, 100% of the particles had dimensions less than 1.0 mm, that is larger than the particles obtained in wet activation by 60 min.

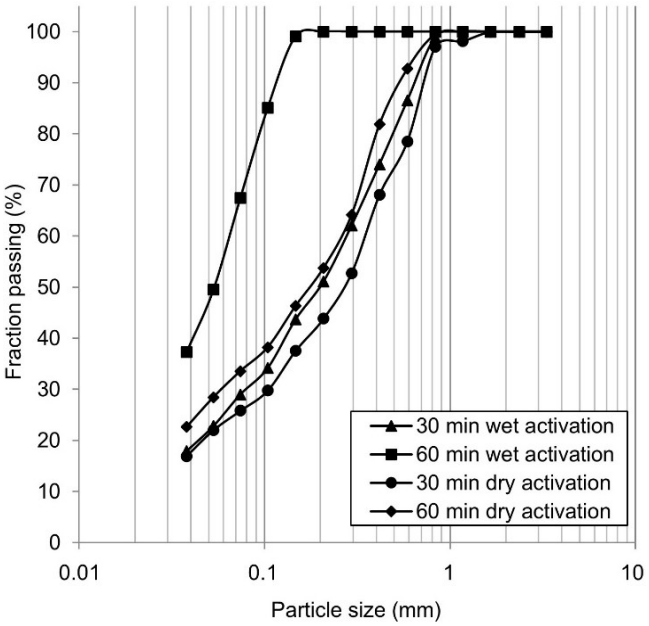

Figure 3. *Particle size distributions of phonolite after mechanical activation. Source: the author.*

XRD for samples submitted to different mechanical activation conditions (Figure 4A) showed that the water in the mechanical activation process did not change the mineral composition; however, a reduction in the intensity of the diffraction peaks was observed around 27.5° for the sample with 60 min of wet activation.

FTIR spectra of the samples after wet and dry mechanical activation (Figure 4B) showed the same behavior as unmilled rock, corroborating of the XRD results, since indicated maintenance of the mineralogical composition.

3.1.3 Potassium availability

Results to total K, water-soluble K, exchangeable K, non-exchangeable K and structural K obtained to the activated to the samples (Table 2) are shown, between brackets, as percent at the total K of each one of the samples rocks. The analysis shows that the water-soluble K in an aqueous medium is less than 0.1% of the total K contained in the rock, regardless of the time and mechanical activation mode. Regarding the exchangeable K, it was verified that mechanical activation increases the amount of potassium compared to the sample without mechanical activation. Among the results, the mechanical activation to dry for 30 min showed a lower exchangeable K content than the content obtained in the other samples

with mechanical activation. However, a low influence of time and the mechanical activation mode was observed since the percentage of exchangeable K in all activated samples had relatively low values, between 0.12 and 0.22%. These results corroborate with the data of XRD, once it was observed that the grinding mode did not interfere in the mineralogical composition of the rock. Regarding the results of non-exchangeable K, it was observed that the samples with mechanical activation for 60 min have a higher content than those activated for 30 min. However, samples activated for 60 min have a lower content of structural K than the samples activated for 30 min. In addition, samples with dry mechanical activation showed a higher amount of released K than wet activated samples. In the samples with dry mechanical activation, a higher increase of non-exchangeable K is observed, corresponding to K that is bound electrostatically to the minerals and is slowly released in the soil solution (Nascimento and Loureiro, 2004; Silva *et al.*, 2013; Straaten, 2007).

Results of K released, which corresponds to the sum of water-soluble K with exchangeable K and non-exchangeable K, reveal that the mechanically activated samples showed an increase in nutrient release compared to the non-activated samples (Figure 5). Since in the dry mechanical activation for 60 min, the released K increased by 15% in relation to the sample without mechanical activation.

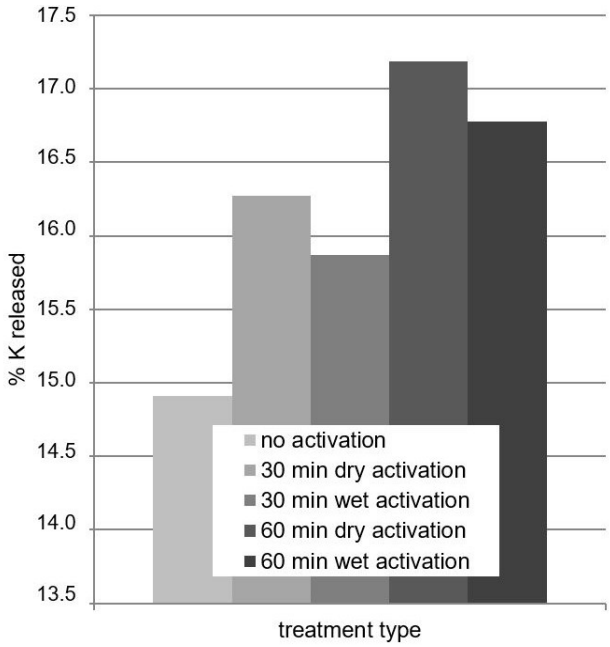

Figure 5. *K released percentage (relation to total K) of the phonolite mechanically activated. Source: the author.*

3.1.4 Potassium leaching curve

The K leaching curves of the mechanical activation samples (Figure 6) showed an increase of the K released over time by extraction with 0.01 mol L^{-1} citric acid and Melich-1 solutions.

Samples after wet or dry mechanical activation had similar K leaching curves. K released with the water extractor was very low, 62 mg kg^{-1} after 235 hours of total contact time. While for other extractors, an initial stage with a higher K leached was identified. K leaching with Mehlich-1 solution was about 6000 mg kg^{-1}, whereas the extraction with 0.01 mol L^{-1} citric acid was almost 3500 mg kg^{-1} after 235 hours of contact time. After 1812 hours, about 10000 and 7000 mg kg^{-1} of K leaching was with Mehlich-1 and 0.01 mol L^{-1} citric acid solutions, respectively, and corresponded 16 and 11% of total K in the sample.

For other forms of K in the mechanically activated samples, levels K leaching was 60 times greater than the exchangeable K after 1812 hours of sequential extraction with a citric acid solution. Therefore, evident that the extraction with 0.01 mol L^{-1} citric acid solution leaches the water-soluble K and exchangeable K together a significant part of non-exchangeable K. For sequential extraction with Mehlich-1 solution was observed a K leaching 80 times greater than the exchangeable K after 1812 hours of extraction. This K leaching corresponds to 95% of the K released, the total between the water-soluble K and exchangeable K, and the non-exchangeable K.

3.2 Discussion

Characterization of the phonolite confirmed its potential as an alternative source of potassium fertilizer since it has about 7.1% K_2O, and the main constituent minerals of the rock are from the class of alkaline feldspars (Andrade et al., 2005). According to the literature, rocks with K_2O levels above 6.0% and composed of minerals such as alkaline feldspars and micas can be used as an alternative source of potassium by means of the stonemeal method (Felix, 2014; Shirale et al., 2019). On the other hand, the high Na_2O content of the rock reinforces the importance of in-depth study of the

rock before its application in agriculture since the accumulation of this element in soils can cause the formation of saline-sodic soils (Gheyi et al., 2016) and reduce the benefits of rock-extracted K^+ ion for crops in poorly drained soils.

Granulometric distribution behavior to the phonolite rock samples is considered common in cases of dry milling and can be explained by the agglomeration of structurally modified particles during milling (Kleiv and Thornhill, 2007). In wet mechanical activation, the obtaining of a material with a greater quantity of fine particles can be explained by the fact that water acts as a lubricant, which hinders the agglomeration of the particles. This reinforces the role of water in reducing the forces of attraction between tiny particles (Kleiv and Thornhill, 2007). From an agronomic point of view, the application of a material with very fine particles implies the need for special equipment, in addition to the loss of material by the wind.

The wet milling for 60 min produced finer particles indicating more intense changes in the activated rock particles that can be attributed to a greater reduction in the crystallinity of the minerals of this rock (Baláž et al., 2008; Kleiv and Thornhill, 2007; Pourghahramania and Akhgar, 2015; Temuujin et al., 2003).

Regarding the released K, it is reasonable to assume that dry activation causes a larger defect in the material, and in contrast, the energy supplied in wet mechanical activation is probably partially dissipated by water.

The increase of non-exchangeable K in samples with dry activation can be explained by the fact of that the mechanical activation causes damage to the structure of minerals, particularly in regions near the surface of the particles, making K more accessible to the extractor (Crusciol and Soratto, 2013). Similar results have been reported in the literature for high-intensity mechanical activation, and the K availability has been shown to be related to increased surface area and structural disorder (Kleiv and Thornhill, 2007). It should be noted that, in our study, a low-intensity mechanical activation (mill speed of 70 rpm) was performed, while in the literature, the high-intensity mechanical activation (with speeds of 500-900 rpm) is usually performed (Kleiv and Thornhill, 2007).

The released K increase by 15% in the sample after dry mechanical activation compared to the non-activated sample suggests that the phonolite rock should present an improvement in the K^+ release efficiency when applied to the soil. Studies have shown that finely ground phonolite rock promoted an increase in the production of long-term crops like KCl. In addition, the rock has the advantage of having a residual effect. However, the results were not so good in the case of short-term crops (Crusciol and Soratto, 2013; Franco *et al.*, 2013; Mancuso *et al.*, 2014; Soratto and Crusciol, 2013). Therefore, our results are an incentive to carry out new research on the mechanical activation of phonolite rock as an alternative source of potassium for short-term crops.

K leaching curve showed that the potassium forms weakly bound to the crystalline mineral structure were released at the first leach stage. Subsequently, the leaching process slowly since the potassium was strongly bound to the crystalline mineral structure (Castilhos and Meurer, 2001; Silva *et al.*, 2013; Plata *et al.*, 2021). The results suggest that regardless of the mechanical activation mode, the phonolite can be considered as a source of K slow-release, i.e., the phonolite rock refers to a material of the low aqueous solubilized however had K release continuously (Silva, 2009). The K leaching 80 times greater than the exchangeable K after 1812 hours of sequential extraction with Mehlich-1 solution confirms the potassium reserve of the mechanically activated rock can be continuously made available to plants (Melo *et al.*, 2005).

Thus, leaching curve results suggest that K released increases progressively at extractions with the longer contact time, and the agriculture application of the mechanically activated phonolite rock must present a residual effect, where the structural K would be slowly made available to the plants. This effect concords with the results of the application of the finely ground phonolite rock at successive crops of soybeans, wheat, and corn that showed a similar or superior efficiency of KCl in some cases (Soratto and Crusciol, 2013). Other studies have also confirmed the efficiency of phonolite rock finely ground at long-term cultivation (Franco *et al.*, 2013; Mancuso *et al.*, 2014). However, short-term crops were observed to have low efficiency when compared to KCl application (Soratto and Crusciol, 2013) which can be explained by the low-release water-soluble K.

It is important to resalt that citric acid solution is commonly found in the rhizosphere region, and it is widely used in sequential K leaching studies to simulate the conditions of the natural environment of plants (Castilhos and Meurer, 2001; Silva, 2009). The extraction solutions operate in the K leaching by the ion exchange reaction with H^+, and through the

complex formation of groups OH and COOH with solution cations. These processes favor the decomposition of the minerals structure (Crusciol and Soratto, 2013). The 1812 hours of sequential leaching corresponds to approximately 2.5 months, and according to literature (Melo *et al.*, 2005), two months is considered a satisfactory time to simulate the growth of the annual crop conditions. This way, results shown to K leaching curve indicate that the mechanical activation of phonolite rock may be a good alternative to improve the K release for long-term cultures.

4. CONCLUSIONS

After mechanical activation, results of phonolite rock from Poços de Caldas, Brazil, reveal the low influence of the time and process mode, dry or wet, at the change of the rock mineralogical composition at water-soluble K and exchangeable K content. However, activated samples showed a significant increase in non-exchangeable K content, corresponding to K^+ electrostatically bound to the rock-forming minerals. Activated samples by the dry mechanical process for 60 min showed the highest K released and the lowest structural K content; that is it, this activation mode is more efficient in creating structural defects compared with wet activation. Besides, the results obtained at the K leaching curves confirm the possibility of phonolite rock application as a slow-release fertilizer. It should be noted that the mechanical activation improved K release potential since it increased non-exchangeable K and reduced structural K content with 95% of the K released after 1812 hours of Mehlich-1 solution extraction. The slow and continuous K release favors the mechanically activated rock application at soils of circular and intensive agriculture, as it prevents the rapid loss of K released to aquatic systems.

5. DECLARATIONS

5.1. Study Limitations

The study is limited to the sample size.

5.2. Acknowledgements

Our thanks to the Mineral Analysis Coordination and Mineral Processing Coordination at CETEM/MCTI by use of the structural lab and XRF, XRD, SEM-EDS, and FAAS analysis. Thanks to the Laboratory Analysis Multi-user of Chemistry Institute at UFRJ for supporting FTIR analysis. Thanks to Curimbaba Mining for providing the phonolite rock sample for this research.

5.3. Funding source

This study had one doctorate supporting grants that was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES).

5.4. Competing Interests

The authors declare that they have no competing interests.

5.5. Open Access

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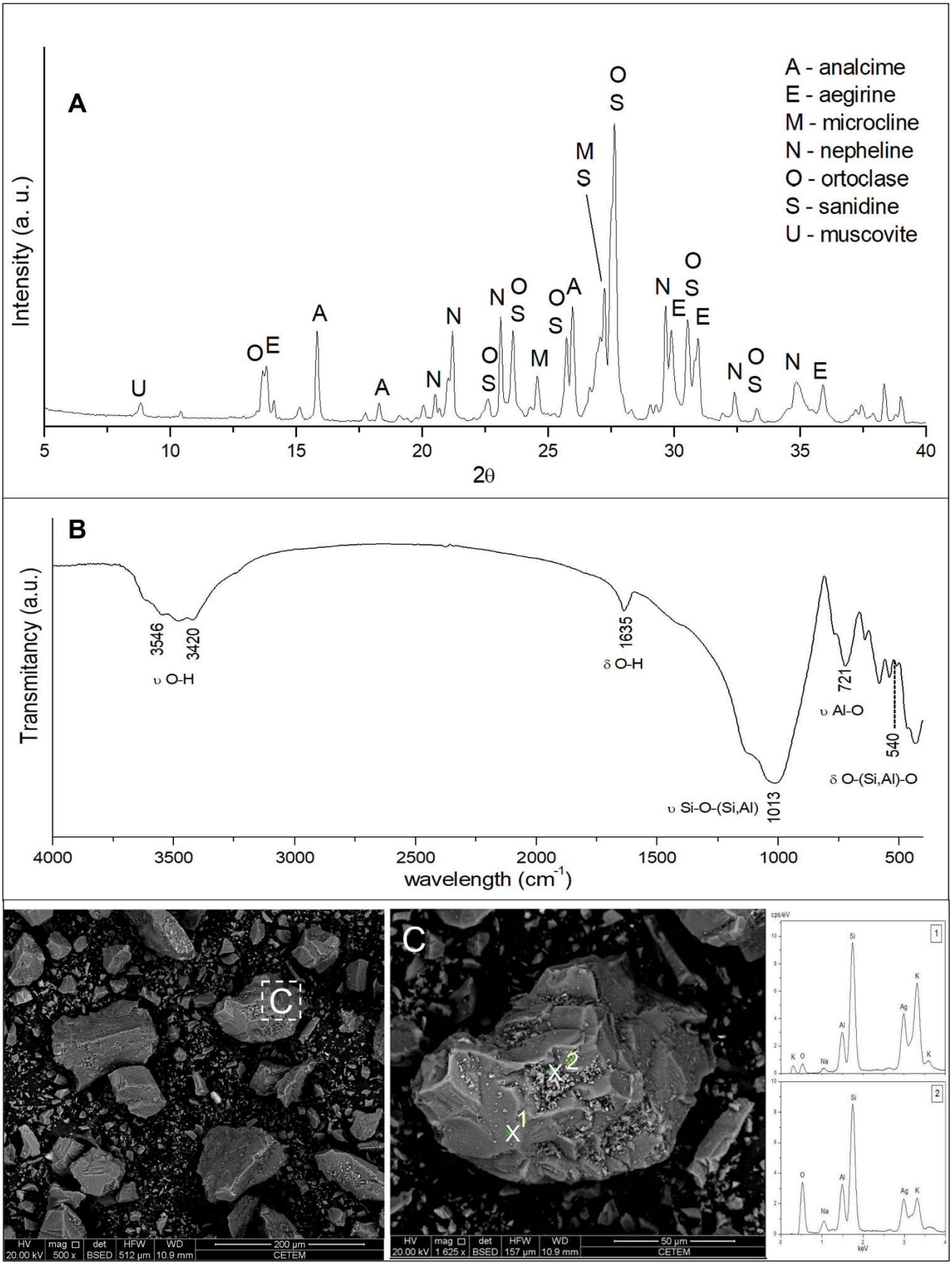

Figure 2. *Characterization of phonolite. (A) X-ray diffraction, Cu-Kα radiation (λ = 1.5418 Å). (B) Spectra FTIR, range 4000 at 400 cm^{-1}. (C) SEM-EDS images. Source: the author.*

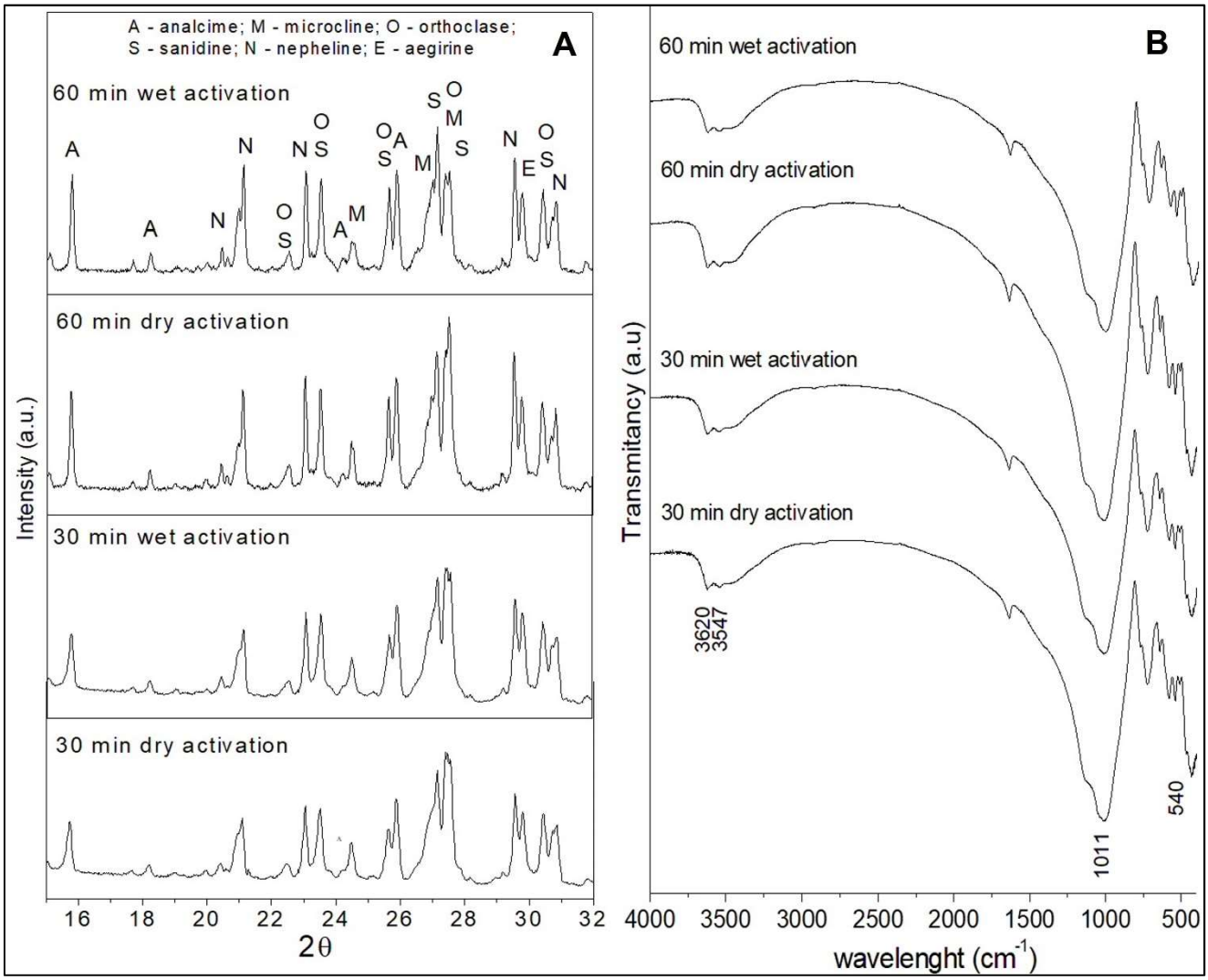

Figure 4. *Characterization of* the mechanically activated phonolite. *(A) X-ray diffraction, Cu-Kα radiation (λ = 1.5418 Á). (B) Spectra FTIR, range 4000 at 400 cm^{-1}. Source: the author*

Table 2. *K forms in phonolite before and after mechanical activation. Source: the author*

Treatments	**K_t**	**K_{sol}**	**K_{exc}**	**K_{non}**	**K_{rel}**	**K_{str}**
	---------- mg kg^{-1} ----------					
	---------- (%) ----------					
No activation	64000.00 (100.00)	17.97 (0.03)	47.65 (0.07)	9477.33 (14.84)	9542.95 (14.91)	54457.06 (85.09)
30 min wet activation	63100.00 (100.00)	29.95 (0.05)	139.24 (0.22)	9846.01 (15.60)	10015.20 (15.87)	53084.81 (84.13)
60 min wet activation	64950.00 (100.00)	16.96 (0.03)	129.84 (0.20)	10753.17 (16.56)	10899.97 (16.78)	54050.03 (83.22)
30 min dry activation	65933.00 (100.00)	21.44 (0.04)	77.25 (0.12)	10626.34 (16.12)	10725.03 (16.27)	55208.30 (83.73)
60 min dry activation	63680.00 (100.00)	27.63 (0.04)	113.84 (0.18)	10806.71 (16.97)	10947.58 (17.19)	52731.82 (82.81)

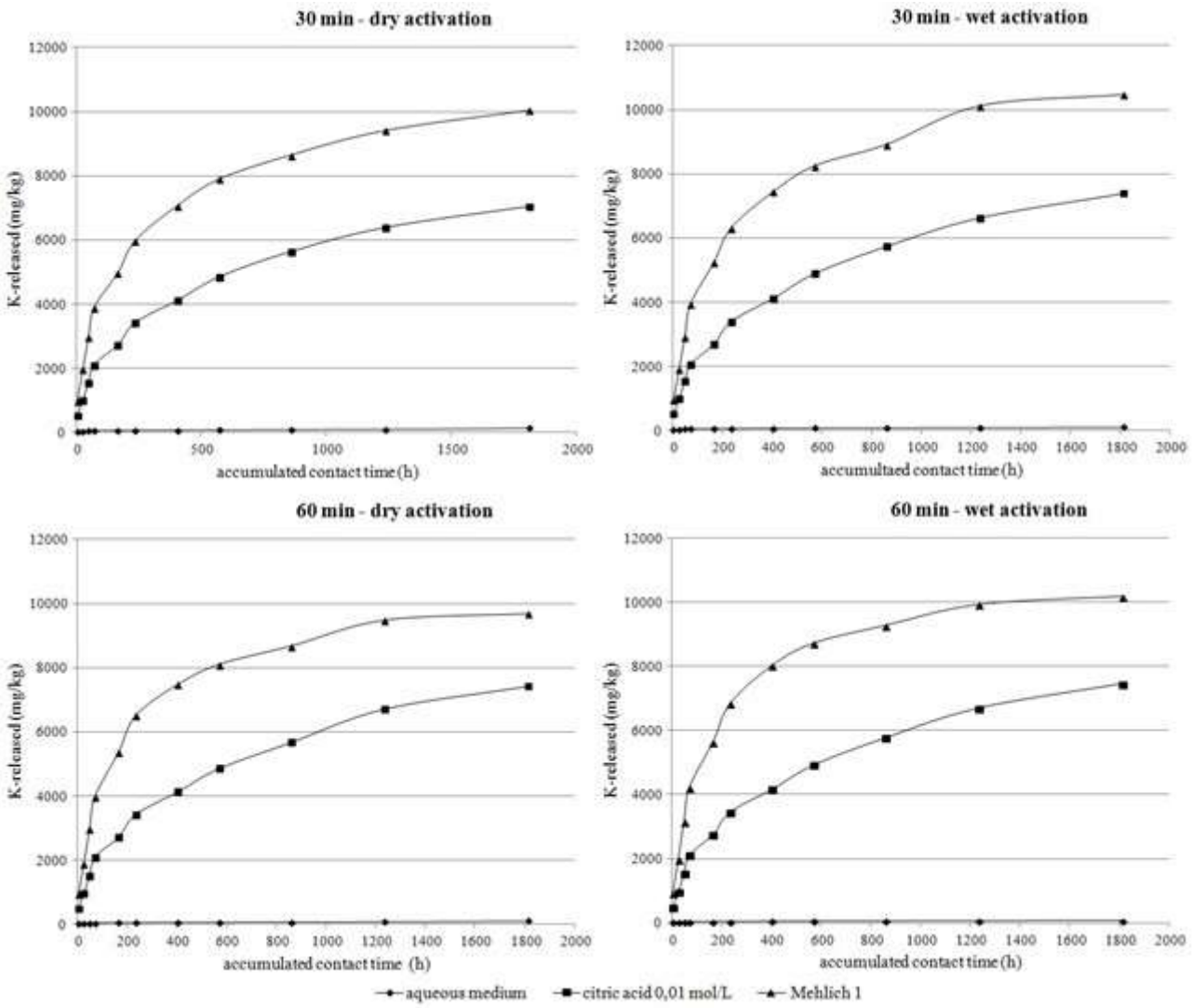

Figure 6. *Cumulative K^+ leaching curve as a function of contact time with the extraction solution, for the samples after mechanical activation wet and dry for 30 to 60 min. Source: the author.*

SOUTHERN JOURNAL OF SCIENCES

ESTABLISHED IN 1993

Original research paper

PHYSICOCHEMICAL CHARACTERISTICS OF SUGARCANE JUICES SOLD AT THREE DIFFERENT POINTS IN CUIABÁ - MT

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Received 30 April 2023; received in revised form 20 May 2022; accepted 03 June 2023

ABSTRACT

Background: Sugarcane juice is an excellent substrate for the growth of a large and diverse microbiota, and poor quality of it can affect the health of consumers. This study aimed to evaluate the acidity parameters of the juice extracted from minimally processed sugarcane at three different points in Cuiabá - MT. **Methods:** In the juice samples, the following parameters were analyzed: Brix (soluble solids), pH, acetic (volatile) acidity, and sulfuric (total) acidity. **Results**: All the Brix values were above 18. The pH values were between 5 and 6. The total and volatile acidity was below 0.8. **Discussion:** The Brix values found in this study are similar to other studies (standard deviation of 1.7). The variety of sugarcane significantly influences the Brix and its sampling period. Inappropriate handling of sugarcane promotes the microbiological decrease of the final product. The pH values were satisfactory, but the changes in temperature in one of the juices (sudden change from 24 to 22.7ºC) caused its pH to differ from the value found for the others. The total and volatile acidity content is related to the storage time of the juice, which can be contaminated by bacteria and microorganisms, affecting the characteristics of the product. When the acidity value is more significant than 0.8, there was a change in these characteristics, which did not happen in this work. **Conclusions:** The Brix values indicate an adequate maturation of the analyzed juices. The pH values are in the ideal range for sugarcane juice. The volatile and total acidity is in the ideal range, indicating quality standards for sugarcane juice.

Keywords: *Garapa. Sugarcane juice quality. Physicochemical analysis*.

1. INTRODUCTION

Sugarcane juice is a beverage obtained from crushing sugarcane through mills, and generally, its consumption is made by adding ice and citrus fruits. *Garapa*, as it is also known, is an energy drink with about 18.2% sucrose. However, it also highlights the minerals magnesium and calcium (12.5mg/100g and 9.1g/100g, respectively) and the presence of ascorbic acid (2.8mg/100g) (Rodrigues *et al.*, 2019; Santos *et al.*, 2021).

In many cities in Brazil, the sale of sugarcane juice in establishments and municipal fairs is a tradition, as is the case in the city of Cuiabá - MT.

The juice sold on public roads has some advantages, such as lower price and convenience; however, it brings together negative aspects regarding hygienic-sanitary issues (Hamerski, 2009; Andrade, 2014).

The organic acids present in sugarcane juice, in addition to citric acid, include aconitic, malic, oxalic, glycolic, succinic, and fumaric acids, among others. The composition of sugarcane varies according to the variety, stage of maturation, soil, and climatic and agricultural conditions; however, with a short shelf life due to its rapid microbiological and biochemical deterioration (Blake; Clarke; Richards, 1987).

According to Gallo (1989) and Kaufmann (2021), as it contains nutrients, high water activity, and pH between 5.0 and 5.5, sugarcane juice is an excellent substrate for growing a large and diverse microbiota. In this way, inadequate procedures in handling a food product, from the hygienic-

DOI: 10.48141/SJS.v31.n35.2023.06_GALIASSI_pgs_61_67.pdf

sanitary point of view, compromise human health, especially when it comes to this food, which is a favorable environment for microbial development.

Considering the factors mentioned above, this study aimed to evaluate the acidity parameters of the juice extracted from minimally processed sugarcane collected at three different points in Cuiabá - MT.

2. MATERIALS AND METHODS

2.1. Materials

- 1% phenolphthalein indicator solution.
- 20 mL pipette;
- 250 mL beaker;
- 50 mL beaker;
- 500 mL bottle;
- Automatic burette;
- Cotton;
- Digital refractometer Atago Master-53M;
- Magnetic capsule;
- Magnetic stirrer Marconi MA 085;
- pH meter Marconi MA-522;
- Plastic stick.
- Standardized 0.1N NaOH solution;
- Stemless funnel, 100 mm diameter;
- Stemless funnel;
- Thin absorbent tissue paper.

2.2. Methods

In the juice samples, the following parameters were analyzed: Brix (soluble solids), pH, acetic (volatile) acidity, and sulfuric (total) acidity, carried out in the premises of the Water Monitoring Laboratory - IFMT - Campus Bela Vista.

2.2.1. Brix

The Brix analysis was performed for the filtered juice and the decanted juice.

The refractometer prisms were cleaned with distilled water (reagent water type IV, from ASTM D1193) and rinsed with adsorbent paper. About 50 mL of the juice was filtered on cotton, discarding the first 10 mL of the filtrate. With the aid of a plastic stick, a few drops of the filtrate were placed on the prism, and the reading was carried out with the corrected Brix (Jaywant; Singh & Arif, 2022; de Aquino *et al*., 2018).

2.2.2. pH

The pH value was measured for the filtered juice and for the decanted juice.

The electrode was placed in the sample until it covered the glass bulb, and the pH was read (Instituto Adolfo Lutz, 2008).

2.2.3. Total acidity (sulfuric acidity)

About 100 mL of the sample was filtered with cotton over the Becker. Then, 20 mL of the sample was pipetted and placed in a 250 mL Erlenmeyer flask, adding another 50 mL of distilled water and 7 drops of phenolphthalein. The contents of the Erlenmeyer flask were titrated with the standardized 0.1N NaOH.

The sulfuric acidity (mgH_2SO_4/L of juice) was calculated using Equation 1 (Instituto Adolfo Lutz, 2008).

$$A = \frac{n * f_{NaOH} * N * 1000}{V} \quad (1)$$

where:

A: Acidity (mgAcid/L of juice);

n: volume of NaOH solution used in the titration (mL);

f_{NaOH} : NaOH correction factor;

N: normality of the NaOH solution;

V: sample volume (mL).

2.2.4. Volatile acidity (Acetic acidity)

About 100 mL of the sample was filtered with cotton. 10 mL of the filtrate was transferred to a 250 mL Erlenmeyer flask, and 100 mL of previously neutralized distilled water was added. 4 drops of phenolphthalein indicator solution were added to the solution, and then it was titrated with 0.1N NaOH solution until the indicator turned from colorless to red. The volume spent was noted (Brereton, 2003).

The acetic acidity of the juice was calculated using Equation 1, and the result is given in mgCH_3COOH/L of juice

2.2.5. Acquisition of juices

For this work, three juices were analyzed. The juices (Figure 1) were acquired in October 2022, at 3 different points in the city of Cuiabá, Mato Grosso, Brazil, and the georeferencing of the juice collection point 1, 2, and 3 can be seen in Figures 2, 3, and 4, respectively.

Figure 1. Sugarcane juices

3. RESULTS AND DISCUSSION

3.1 Results

The results of the physicochemical analyzes are shown in Table 1. Brix and pH were measured for filtered and decanted juices. Sulfuric and acetic acidity was measured just for filtered juices.

Table 1. *Physicochemical analysis results*

Variable	Juices			
	Type	**1**	**2**	**3**
ºBrix	Filtered	21	23.5	23
	Decanted	22	24.5	24
pH	Filtered	5.51	5.11	5.44
	Decanted	5.78	4.74	5.39
Total acidity (g H_2SO_4/L of juice)	Filtered	0,166	0,307	0,173
Volatile acidity (g CH_3COOH/ L of juice)	Filtered	0,218	0,396	0,208

3.2 Discussions

Analyzing the Brix values found in this study, it is noted that they are similar to those found by Soares (2017), Kunitake (2012), Andrade (2014), and Rodrigues *et a*l. (2019), with a standard deviation of 1.7.

According to Soares (2017), Brix values above 18 indicate an adequate maturation of the sugarcane. Thus, there is uniformity in the maturation of the analyzed juices. The author emphasizes that the variety of sugarcane significantly influences the Brix and its sampling period.

The filtered pH results are within the range found by Kunitake (2012), Andrade (2014), and Rodrigues *et a*l. (2019). Gallo (1989) and Kaufmann (2021) says that the pH of the sugarcane must be between 5.0 and 5.5. Other authors (Prati; Camargo, 2008; Rodrigues *et al*., 2019) found pH values between 5 and 6.17 and stated that these are satisfactory. Analyzing the pH results for the filtered juice, these are in the cited ranges, but analyzing the results for the decanted juice, juice 2 are not in the ranges.

It has already been mentioned that the quality of the sugarcane juice depends on the variety, stage of maturation, soil, and climatic and agricultural conditions of the sugarcane. Also, slightly acidic pH levels (between 5 and 6) favor microbial growth. Inappropriate handling of sugarcane, such as storage failures, inappropriate handling, storage, environmental and personal hygiene conditions, and failures in cleaning mills and other equipment, promotes the microbiological decrease of the final product (Galvão *et al*., 2019).

According to Santos (2021), the greatest contamination of sugarcane juice occurs during the milling stages and during allocation in containers for sale. As can be seen in Figure 5, the place for grinding juice 2 is in a street fair, without minimal hygiene. Still, there was a change in temperature in the filtration (24 ºC) and decanted juice (22,7 ºC). Sudden temperature changes are a relevant factor in the chemical alteration of sugarcane juice (Santos, 2021).

Figure 5. Juice 2 grinding

Analyzing the volatile and total acidity of the juices, it is noticed that they are close to the

values found by Soares (2017). The total acidity results are close to those of Tasso Júnior *et al.* (2010). Ripoli and Ripoli (2004) and Santos (2021) estimate acidity values below 0.8 as ideal quality standards for sugarcane juice. All values found are within this range.

Figure 6. Volatile (acetic) Acidity

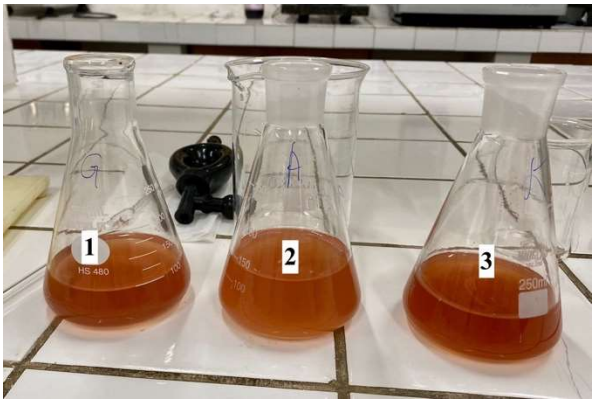

Figure 7. Total (sulfuric) Acidity

Tasso Júnior *et al.* (2010) relate the increase in acidity and dextran levels in sugarcane juice to storage time due to contamination by bacteria and microorganisms. The juice 2 was purchased 2 days after grinding, which also explains the higher acidity. It is observed that the juices with greater acidity are those where the hygiene of the place was more precarious. Also, the total acidity content is directly related to soil fertility, with the highest acidity levels found in more fertile soils (Tasso Júnior et al., 2010), which may suggest greater fertility in the soil of sugarcane juice 2.

In future studies, it is interesting to analyze the hygiene conditions of the places where the sugarcane juice was extracted and the soil where the sugarcane was cultivated, in order to, through the history of the juice, be able to better understand the physicochemical patterns and propose better justifications to the results generated.

4. CONCLUSIONS

From the analysis, it was possible to observe that the samples are within the standards commercialized in Brazil. All the Brix values were above 18, indicating an adequate maturation of the analyzed juices. The pH contents were between 5 and 6, ideal for sugarcane juice. The acetic and sulfuric acidity was always below 0.8, as indicated by the literature for good acidity.

5. DECLARATIONS

5.1. Open Access

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Figure 2. Georeferencing of the juice collection point 1 (Rua Miranda Reis, 784, Poção, Cuiabá-MT, Zip Code 78015-615)

Figure 3. Georeferencing of the juice collection point 2 (Av. Domingas Alves da Costa, 316 - São João Del Rei, Cuiabá - MT, Zip Code: 78093-080)

Figure 4. Georeferencing of the juice collection point 3 (Rua A 1, 196 - Jardim Nossa Sra. Aparecida, Cuiabá – MT, Zip Code: 78090-654)

SOUTHERN JOURNAL OF SCIENCES

Original research paper

ESTABLISHED IN 1993

COMPARATIVE STUDY OF ALCOHOLIC EXTRACTION OF COMPOUNDS FROM ARAUCARIA ANGUSTIFOLIA: MICROWAVE-ASSISTED EXTRACTION VS. MACERATION EXTRACTION

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Received 06 February 2023; received in revised form 16 May 2022; accepted 03 June 2023

ABSTRACT

Background: The *Araucaria angustifolia* is a prominent tree species indigenous to the southern regions of Brazil. The tree can be a source of different compounds of interest if properly used. **Aims:** This research aims to compare the alcoholic extraction of compounds from Araucaria angustifolia using two different methods, microwave-assisted extraction and maceration extraction. **Methods:** Alcahoolic extractions of compounds from the sawdust of Araucaria angustifolia were performed using microwave-assisted extraction and maceration extraction. The color was observed by eye inspection. The taste was observed by putting the samples into the mouth and tasting it. The olfactive test was conducted at hot and cold temperatures. **Results:** it was obtained red color solution, both from the bark and from the branch. The intensity of the color changed with the extraction method and time of maceration. The solutions tasted like "green wood". **Discussion:** MAE and Maceration extraction can provide extracts from the bark and branch of the araucaria tree. There is the possibility that the “green wood” taste of the MAE extract can be changed with the thermal treatment of the wood. **Conclusions:** microwave-assisted extraction can perform the faster extraction of compounds from Araucaria angustifolia than maceration extraction.

Keywords: *Araucaria angustifolia, Microwave-assisted extraction*, *Maceration extraction*.

1. INTRODUCTION

Araucaria angustifolia (Figure 1), commonly known as the Paraná pine or Brazilian pine, is a prominent tree species indigenous to the southern regions of Brazil, Paraguay, and Argentina (Fritzsons *et al.*, 2018; Peralta *et al.*, 2016). This evergreen conifer holds immense ecological and economic significance due to its distinct characteristics and diverse applications. With its characteristic symmetrical growth pattern and umbrella-shaped crown, Araucaria angustifolia stands as a prominent component of the region's biodiversity and contributes to ecosystem stability. Araucaria angustifolia produces edible seeds, known as *pinhão*, and bioactive compounds, among other products (Castrillon *et al.*, 2023; Silva *et al.*, 2016; Branco and Rodrigues, 2016). Conservation efforts are supported by law (Brazil, 2006), and sustainable management practices are vital to preserve the genetic diversity of Araucaria angustifolia and ensure the continued provision of its ecosystem services.

Figure 1. Araucaria angustifolia.

Microwave-assisted extraction (MAE) is a powerful technique to extract bioactive compounds from various plant sources efficiently. This method employs microwave energy to enhance the extraction process, resulting in higher yields and shorter extraction times than conventional methods. MAE works by subjecting the plant material to microwave radiation, which induces internal heat generation and facilitates the release of target compounds. MAE offers numerous advantages, including improved extraction efficiency, reduced solvent consumption, and controlled extraction parameters. Several studies have demonstrated the effectiveness of MAE in extracting bioactive compounds from plants. For example, Alchera *et al.* (2022) investigated the extraction of polyphenols from blackcurrant using MAE and observed higher extraction efficiency than conventional methods. Additionally, Drinić *et al.* (2021) explored the extraction of essential oil, hydrolat, and residual water extract from *Sideritis raeseri* using MAE and Hydro-distillation, it was reported enhanced extraction yields. These studies, along with others (e.g., Hamid Nour *et al.*, 2021; Hiew *et al.*, 2022; Quiroz *et al.*, 2019), highlight the potential of MAE as a valuable technique for extracting bioactive compounds from various plant sources.

Maceration extraction is a traditional and widely used method for extracting bioactive compounds from plant materials. This technique involves immersing the plant material in a suitable solvent for an extended period, typically ranging from several days to weeks, allowing for the gradual release of target compounds. Maceration extraction offers several advantages, including simplicity, low cost, and the ability to extract a broad range of compounds. Numerous studies have utilized maceration extraction to extract bioactive compounds from various plants. For instance, Yang *et al.* (2015) investigated the antidiabetic effects of flavonoids from Sophora flavescens EtOAc extract in type 2 diabetic KK-ay mice. Additionally, Hasni *et al.* (2021) compared the maceration and ultrasound-assisted extraction methods to obtain phenolic-rich extracts from *Eucalyptus marginata* L., it was found that the ultrasound-assisted extraction method was more efficient than the maceration method. These examples, along with others (e.g., Jurinjak *et al.*, 2022; Koraqi *et al.*, 2023; Suksaeree *et al.*, 2021), demonstrate that traditional maceration extraction is an effective method to obtaining bioactive compounds from different plant sources.

This research aims to compare, visually and by taste, the alcohol extraction of compounds from Araucaria angustifolia using two different methods: Microwave-Assisted Extraction (MAE) and Maceration Extraction. The study aims to evaluate the efficiency and effectiveness of these two extraction techniques in obtaining bioactive compounds from Araucaria angustifolia. By employing MAE, which utilizes microwave energy to enhance the extraction process, it is expected to achieve higher extraction yields and reduced extraction times compared to traditional maceration extraction. The comparison will provide valuable insights into the advantages and limitations of each technique in extracting bioactive compounds from Araucaria angustifolia.

2. MATERIALS AND METHODS

2.1. Materials

- Samples of bark and branches;
- Cheap, low-quality vodka (ethanol source);
- Sawmill for making sawdust;
- Microwave oven;
- Glassware;
- Refrigerator;
- Thermometer;
- Reagent water type IV.

2.2. Methods

2.2.1. Conversion of wood samples into sawdust

To increase the surface area, the wood samples were converted into sawdust. Figure 2 shows a cut from the samples.

Figure 2. Cut from bark and branch samples.

2.2.2. Microwave-assisted extraction (MAE)

About 100g of the bark sawdust and 100g of the branch sawdust were submerged in becker glasses with enough vodka to cover them.

The samples were taken separately into the microwave for MAE. Once it achieved hot temperature (near boiling point), it was placed inside a freezer to reduce the temperature to nearly 0 °C. The heating and cold cycles were performed three times for each sample.

Later the samples were filtered and stored for further observation.

2.2.3. Maceration extraction

About 100g of the bark sawdust and 100g of the branch sawdust were washed with reagent water type IV and later submerged in becker glasses with enough vodka to cover them.

The samples were stored for about 20 days in a closed container at room temperature (near 20 °C), protected from light.

Later the samples were filtered and stored for further observation.

2.2.4. Color, taste, and olfaction observations

The color was observed by eye inspection.

The taste was observed by putting the samples into the mouth and tasting it.

The olfactive test was conducted at hot and cold temperatures.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Results of the conversion of wood samples into sawdust

The surface area was increased, and the sawdust had different colors depending on the section of the tree. Figure 3 illustrates the red bark sawdust. The branch samples had a yellow wood color.

Figure 3. Red bark sawdust.

3.1.2. Results of the Microwave-assisted extraction (MAE)

The samples extracted from the bark were deep red, as in Figure 4. Looking at it against a small led light (Figure 5) it was possible to note the red color (Figure 6).

Figure 4. Bark sawdust Microwave-assisted extract.

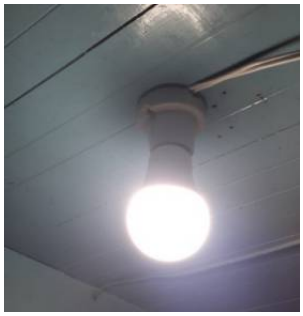

Figure 5. Light bulb.

Figure 6. Red color observed against a light bulb.

The samples from the branch were also red, but not so dark.

3.1.2. Results of the Maceration extraction

After about 20 days, the color of the samples was also red. Figure 7 shows the initial phase of the maceration.

Figure 7. Whasing the samples with reagent water type IV.

3.1.3. Results of the Color, taste, and olfaction observations

a) Regardless of the method, all samples achieved a red color. However, the tone was not the same;

b) The taste of the blank sample was bad (pure vodka). It had a strong ketone taste, confirming its low quality. The taste of the MAE extracts reminded “green wood”; even so, it appears better than the blank sample result. Tasting it too much may cause side effects that may compromise the objectivity of the tasting methodology. No teste test was performed in the maceration extraction method.

c) Hot olfaction test results point to the presence of alcohol vapors. Cold olfaction test results were inconclusive.

3.2. Discussions

Both methods, MAE and Maceration extraction, can provide extracts from the bark and branch of the araucaria tree. The difference relies on the extraction time. For the MAE, in a few hours, less than a day, all the extraction was complete. For the maceration, the time was much longer.

A possible reason for the vodka taste being less worse in the MAE extract than in the original sample is the evaporation of the ketones. There is the possibility that the “green wood” taste of the MAE extract can be changed with the thermal treatment of the wood. This fact must be studied further in the future, as it can create new

applications for the wood and bark of the Araucaria angustifolia.

The red color was a fascinating surprise; after about six months of the extraction, the color was still stable, creating the possibility for use as a natural dye for the beverage industry if the taste issue can be improved. The color looks golden red when observed in flasks that are not so thick, as in Figure 8.

Figure 7. Different ton colors of samples in flasks that are not so thick.

Due to the limitations in the analytical methods of this research, some samples were shared with other colleagues with better analytical and testing procedures.

4. CONCLUSIONS

The microwave-assisted extraction can perform the faster extraction of compounds from Araucaria angustifolia than maceration extraction. The tasting result from the method, as it was applied, does not allow consideration for using this wood to promote flavors for beverages, and more studies must be performed before its total disregard.

5. DECLARATIONS

5.1. Study Limitations

The study is limited to the sample size and the methods used in the research.

5.2. Acknowledgements

The author is grateful to XXXX for assisting in the conversion of the samples into sawdust.

5.3. Funding source

The author funded this research.

5.4. Competing Interests

The author declares that no conflict of interest exists in this publication.

5.5. Open Access

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Accepted 19 December 2022 (reprint in 2023)

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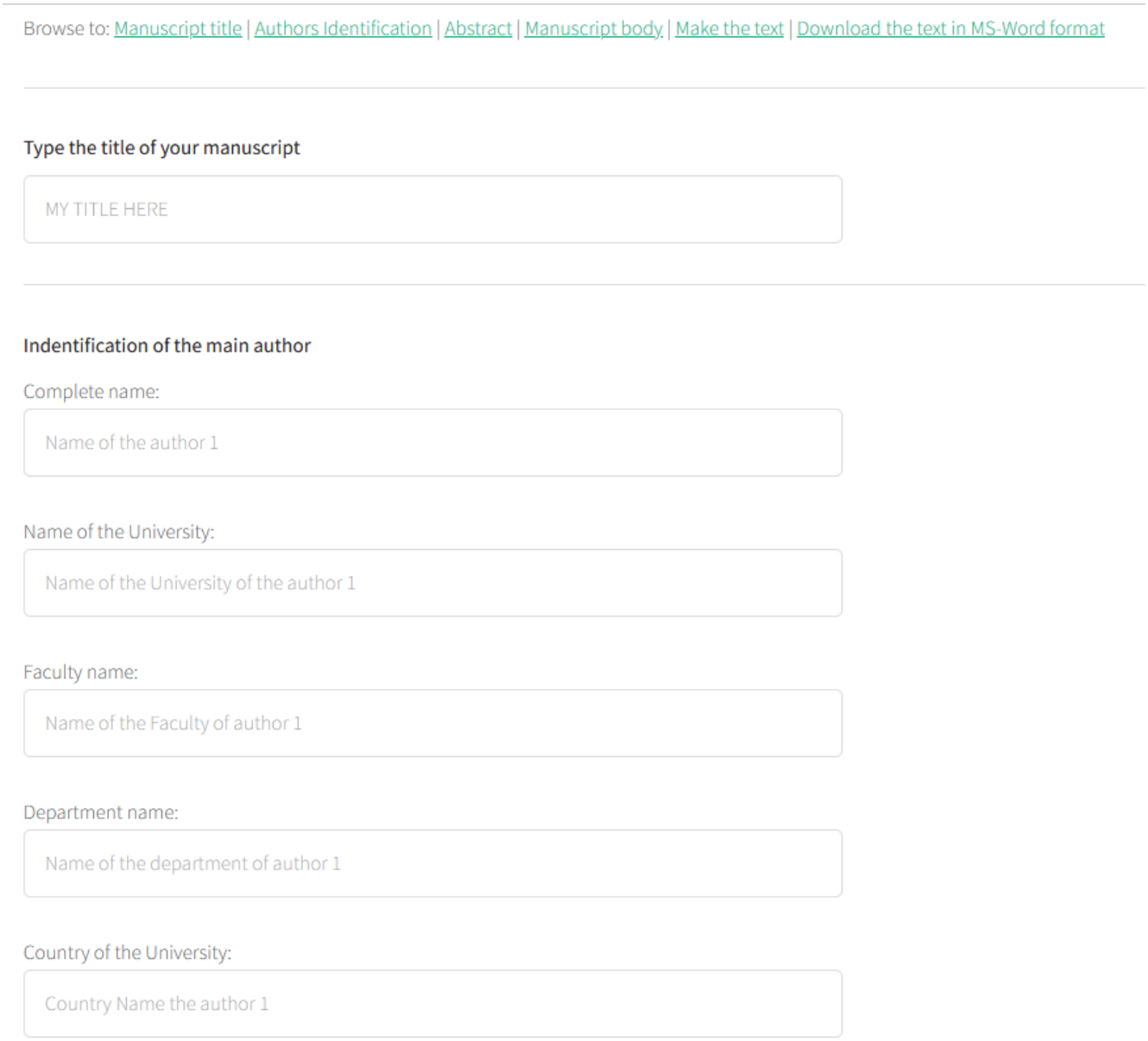

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