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Research Article

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Biological activity of atomized extract of *Syzygium cumini* (L.) Skeel (*Myrtaceae*) edible fruits

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ABSTRACT

Syzygium cumini (L.) Skeels is a native tree of China and the Antilles, cultivated in many countries, including Brazil, which the fruit is popularly known as jambolan or "widow earring". Biological activitiessuch as antidiabetic (hypoglycemic agent), antidiarrheal, anti-inflammatory, antimicrobial and antioxidant are described in literature for this specie. Even though some studies have described the phytochemical profile of S. cumini, there are few studies on the biological activities evaluation of the atomized extract by spray drying.This study aims to assess cell viability by cytotoxicity assay and to evaluate the biological activities leishmanicide, antioxidant and antimicrobial of atomized aqueous extract of the Syzygium cumini (L.) Skells fruit. The atomized extract showed antioxidant activity, and showed no cell death at concentrations of 1µM, 10 µM and 100 µM, nor leishmanicideactivity at the concentrations tested. Regarding the antimicrobial activity, it was observed that the extract presents higher sensitivity to the microorganism S. flexneri. The results of the biological activity assay demonstrated that the drying process by spray dryingis a feasible and promising possibility to obtain intermediate products.

Keywords: *Syzygium cumini; Spray dryer*; biological activity; cytotoxicity; antioxidant

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INTRODUCTION

Plant species have been used in the treatment and cure of diseases due to efficiency and quality in their prophylactic and curative properties. The benefits of its use has been propagated, resulting in higher consumption of natural products and derivatives.In addition, it has aroused great interest of the scientific community on plant species in the form of medicines, since most of these drugs do not have scientific evidence about pharmacological effects. Despite phytomedicines having natural chemical constituents, they are classified as drugs and must be submitted to judicious quality control [1].

According to Judd et al. [2], the *Myrtaceae* family, is subdivided into two subfamilies: *Myrtoideae* (presenting opposite leaves and fleshy fruits type berry) and *Leptospermoideae* (with opposite or alternate leaves and fruits type capsules or nucules).

In Brazil, all native representatives belong to the subfamily *Myrtoideae*, including *Syzygium* gender [3]. The species belonging to this gender have a wide variety of ethnopharmacological applications, such as antidiabetic activity,

anti-inflammatory, antimicrobial, antioxidant, cardiotonic, cytotoxic and antitumoral, reinforcing the efficacy of the species belonging to this gender in popular medicine. From this gender, phenolic and terpene compounds were isolated [4-9].

This study examined the fruit of the *Syzygium cumini* (L.) Skeels specie, popularly known in Brazil as jambolan or "widow earring". New studies focused on this plant species can be used to demystify and disseminate accurate information on the benefits and use of these fruits. The *in natura* consumption of jambolan is conducted mostly by the people collecting the fruits directly from the trees during the harvest period.The interest in fruits is based on the attractive visual presentation and the pleasant taste when fruits are ripe, however, most of these fruits are wasted in the environment. The *Syzygium cumini* (L.) Skeels fruits could be better used, with less waste and more information about the benefits of this fruit.

Bark, fruit, seeds and leaves of this plant have a long history in popular medicine, being used in many cultures fordiabetes mellitus treatment, administered as aqueous extract or decoction, ethanolic extract or raw juice [10-11]. The seeds showed hypoglycaemic and antioxidant activity, while the decoction of the bark showed antidiarrheal and antidysenteric action. Additional studies have demonstrated sedative and anticonvulsant action and a depressant effect on the central nervous system (CNS) [7].

The fruits are rich in vitamin C, glycosides, gallic acid, tannins, anthocyanins, including cyanidin, petunidin, malvidin-glucoside and carotenoid such as trans-lutein and trans-β-carotene, isoquercetin and mirecitin [12; 13].

Converting a raw vegetable material into a product suitable for pharmaceutical industry and subsequent application for therapeutic purposes is a complex task, requiring the use of technologies with specific and standardized processes, for instance, the drying of plant extracts [14]. Drying of medicinal plants extracts aims to reduce the moisture content, allowing conservation of its physico-chemical properties for a longer period of time [15].

A wide range of techniques is being used in the process of drying plant extracts, in which the most common are lyophilization [16-17], spouted bed [18-20], fluidized bed [21-22] and *spray drying*[23 -33], where *spray drying* technique is frequently used in phytomedicine industry due to its stability and possibility of controlling the organoleptic characteristics of the final product [34].

Considering the relevance in popular medicine of natural products, when associated with *spray drying* atomization, it may represent a technological breakthrough in the phytomedicine development, enhancing stability and organoleptic aspects of the active components, therefore, valuing the natural products. Thereby, this study proposed the obtainment and drying of the aqueous extract of the*S. cumini*fruitby *spray drying*atomization, in order to produce intermediate products with improved physical characteristics for biological activity evaluation of the final product.

EXPERIMENTAL SECTION

Bioassays

Cytotoxicity assay

Macrophagecell linesJ774 were cultured and then added to different concentrations of the compounds evaluated. In control wells, cells with culture medium only or cells in the presence of the diluent used (DMSO, Sigma) were cultured.Cell viability was determined by reduction assay 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazoline bromide (MTT) after 48 hours of incubation [35], and compared to the pattern of microbial death obtained from control cultures.

Toxicity on promastigotes assay

Approximately $1x10^6$ *Leishmania amazonensis* promastigotes were plated in RPMI medium supplemented with urine. Fifty microliters of promastigotes suspended in RPMI and 50 µL of the substances at different concentrations were added to the plates. Then, 20 µL of MTT at 5 mg/mL of concentration were added, and the plates were incubated at $37^{O}C$ in a humid atmosphere containing 5% CO_2 for 2 hours. Posteriorly, 120 µL of isopropanol were added and the plates were incubated at room temperature for one hour and then, microplate reading at 550 nm was performed.

Antimicrobial activity

The atomized extract of *Syzygium cumini* fruit (SCFEAT) was solubilized in distilled water at 200 mg/ml of concentration and stored at 4 °C.

To determine the Minimum Inhibitory Concentration (MIC), the following microbiological specimens were used: *Staphylococcus aureus* (CCD-S009), *Staphylococcus epidermidis* (CCCD-S010), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), *Shigella flexneri*(CCCD-S006), *Salmonella enterica* (CCCD-S004), *Pseudomonas aeruginosa* (ATCC 27853) and *Candida albicans* (CCCD-CC001).

The SCFEAT extract was pipetted on microplates and solubilized in Brain Heart Infusion (BHI) culture medium at decreasing concentrations. Then, microbiological specimens were added at concentrations of approximately 1.5 $x10^6$ bacteria and 1.5 $x10^4$ yeast in each well.To ensure the viability of microbiological specimens, three rows in the microplate were used: positive control (PC), negative control (NC) and culture medium/microbiological specimens, in order to ensure the sterility of the microplate.

2,3,5-Triphenyltetrazolium chloride (TTC) was added (20 µL) to all wells at 0.02% of concentration. The red stained wells were considered as positive microbiological growth, while the wells with no staining after addition of the solution were considered as negative.

In order to determine the Minimum Bactericidal Concentration (MBC), aliquots that presented concentration above and equal to the Minimum Inhibitory Concentration (MIC) wereremoved from the microplates, and then plated in Petri plates containing Mueller Hinton Agar culture.

Antioxidant activity evaluation

To evaluate the antioxidante capacity of the samples, Folin-Ciocalteu reagente and 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) were used. SCFEAT extract (30 mg) was solubilized in 4 mL of DMSO and further diluted to a stock solution of 3000 ppm.

The Folin-Ciocalteu method used500 µL of the reagent pre-diluted at 1:9 v/v, 2.0 mL of the sample or standard solution and 500 µL of Na_2CO_3 (75 g/L), and the final volume was adjusted to 5.0 mL with ultrapure water. The solution was allowed to stand for 30 minutes before spectrophotometric measurements at 770 nm using glass cuvette with 1.0 cm of optical path.

The analytical reference signal was obtained from a solution containing ultrapure water, gallic acid (AG) and the standard solutions Trolox and BHT. The antioxidant capacity was calculated through analytical curves, and the results were expressed in equivalents (mg/g) of the respective standards.

The method using the ABTS •+ radical was carried out by the addition of 220 µL of stock solution (2 mmol/L), 1.0 mL of the sample diluted or standard solution and final volume adjusted to 5.0 mL with ultrapure water. Spectrophotometric measurements at 734 nm using glass cuvette with 1.0 cm of optical path. For the analytical curve, gallic acid (AG), Trolox and BHT were used as reference and, based on the data obtained, the antioxidant capacity of the sample was estimated.

The values of the extracted samples and fractions were converted to gallic acid, Trolox and BHT equivalents by applying the equation of the calibration curves.

The obtained data were presented as mean ± standard error of the mean (M ± SEM) and statistically analyzed by ANOVA followed by Dunnett's test with a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Toxicity

Cytotoxicity of a compound can be investigated by different parameters, such as cell membrane integrity, metabolism and organelle function.

In this study we assessed the cytotoxicity of atomized extract of *S. cumini* fruits (SCFEAT) in cultured cells using the MTT reduction as cell viability parameter.

Cells of the J774 macrophage cell line were used at concentrations of1 µM, 10 µM and 100 µM, as illustrated in Figure 1.

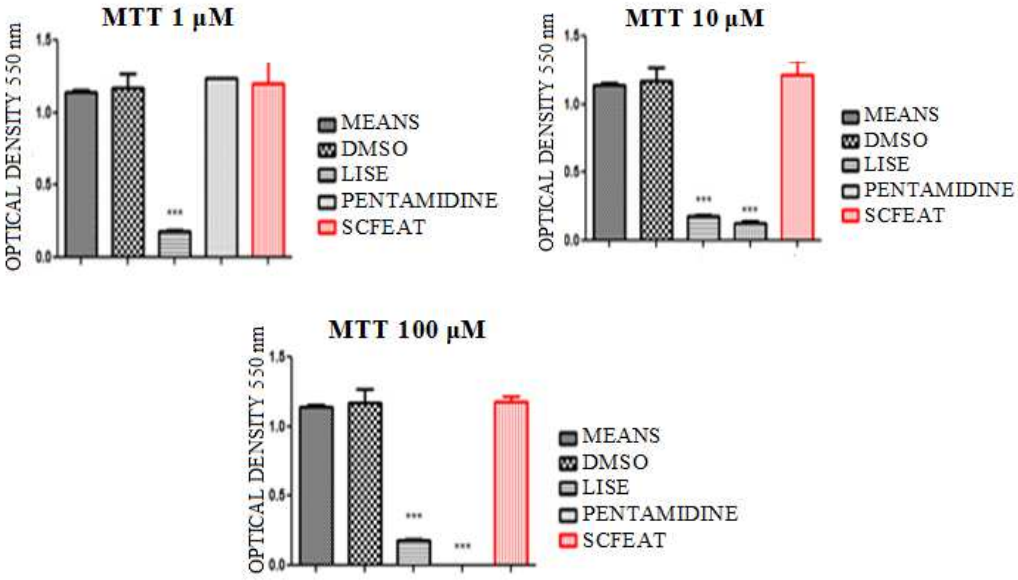

Figure 1 – Atomized extract effect of *S. cumini* fruits on cell viability at: 1 µM, 10 µM and 100 µM

The data represent an average ± EPM of triplicates of a representative experiment. The maximum effect values were considered significant when p <0.05 relative to DMSO 0.1% group; *** represents a relevant cytotoxicity towards J774 macrophage.

Results showed that SCFEAT had no cytotoxicity at the concentrations tested. Souza [18] reported that the ethanol extract of the leaves of a specie from *Myrtaceae* family presented cytotoxicity at concentration of 1250 µM, using the same line of cell culture. Based on the results of this study, the incorporation of the atomized extract of *S. cumini* in the pharmaceutical industry is a possibility, since no cytotoxicity against J744 macrophage cell line in the concentrations studied was observed.

Toxicity on promastigotes assay

From cell viability assay (MTT) results, and since the sampleshowed no cytotoxicity, the atomized extract of *S. cumini*fruits was tested for leishmanicidal activity. Thus, the viability assay with evolutionary form of *L. amazonensis* promastigote was carried out. As illustrated in Figure 2, the atomized extract of *S. cumini*fruits showed no leishamanicidal activity on any of the concentrations tested.

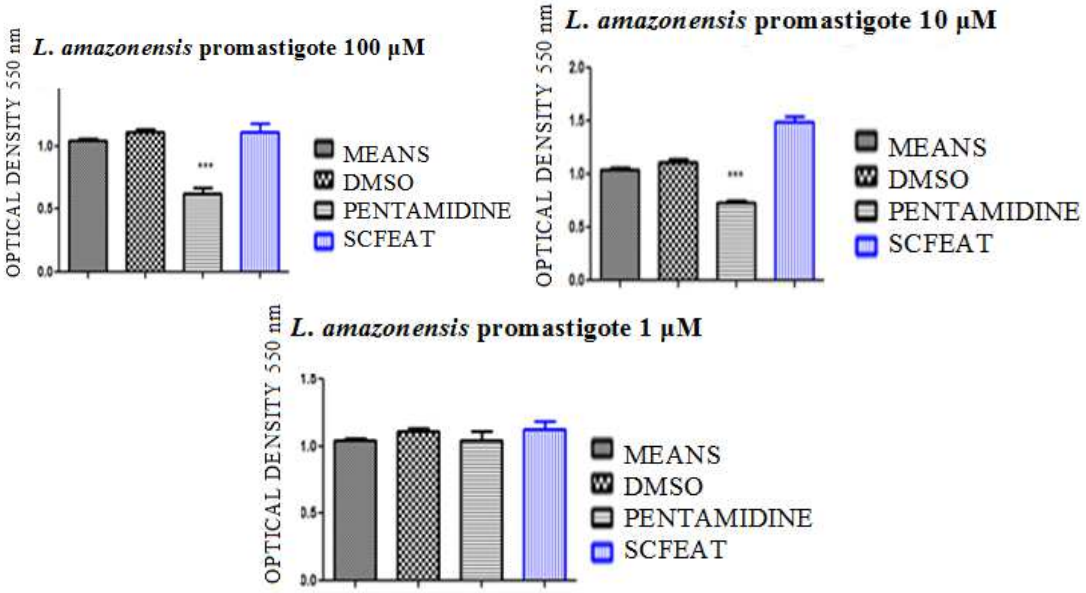

Figure 2 –Atomized extract effect of S. cumini fruits on viability of L. amazonensispromastigote at: 100 µM, 10 µM and1 µM

*These results represent the average ±EPM of triplicates of a representative experiment; The maximum effect values were considered significant when p <0.05 relative to DMSO 0.1% group; *** represents a significant cytotoxicity against L. amazonensis promastigotes.*

Antimicrobial activity of atomized extract of *Syzygium cumini* (L.) Skeel fruits

The overuse of traditional antibiotics and the increasing microbial resistance has led clinical microbiologists to seek for natural products with potential antimicrobial activity. The results of determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of SCFEAT obtained are shown in Table 1.

Table 1. Determination of minimum inhibitory, bactericidal and fungicidal concentration for the atomized extract (SCFEAT)of*Syzygium cumini* (L.) Skeels fruits

Microorganism	MIC (µg/mL)	MBC (µg/mL)	MFC (µg/mL)
GRAM-POSITIVE			
S. aureus	4150	10000	-
S. epidermidis	8300	8300	-
GRAM-NEGATIVE			
E. coli	4150	8300	
K. pneumoniae	4150	8300	
P. aeruginosa	8300	8300	
S. entérica	8300	8300	
S. flexneri	500	4150	
FUNGUS			
C. albicans	8300		8300

Table 1 shows the results of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of the atomized extract of the *Syzygium cumini* fruits, against the microorganisms *S. aureus, S. epidermidis, E. coli, K. pneumoniae, S. entérica, S. flexneri, P. aeruginosa, K. Pneumoniae* and *C. albicans.*

Considering the concentrations determined to evaluate the antimicrobial activity from 500 to 10000 µg/mL, using the microorganisms *S. aureus, S. epidermidis, E. coli, K. pneumoniae, S. enterica, S. flexneri, P. aeruginosa, K. Pneumoniae* and *C. albicans,* the highest activity in determining the MIC was obtained for the microorganism *S. flexneri,* which was more sensitive to the atomized extract. The other microorganisms showed higher MIC values. Through the determination of minimum bactericidal and fungicidal concentrations it was possible to identify *S. flexneri* as the microorganism with the highest sensitivity to the atomized extract.

The results obtained in this work are consistent with the literature, which demonstrates less effectiveness of the extracts of *Syzygium cumini* (L.) Skeels against gram-negative bacteria such as *P. aeruginosa* when compared to gram-positive bacteria such as *S. aureus* and *S. epidermidis* [36].

The divergences regarding to antimicrobial activity of *S. cumini* studies found in this work may be related to variation in the chemical compostition of the plant caused by extractive processes or environmental factors such as type of soil, cultivation conditions and harvest period[37].

Antioxidant capacity determination

Quantification of total phenolic content

Total phenolic content determination was performed by Folin-Ciocalteu method (FC) using three standards: gallic acid (hydrophilic characteristics), trolox (intermediate characteristics) and BHT (lipophilic characteristics), and the results were expressed in equivalents (mg/g). The equations of the curves obtained for the three standards are shown in Table 2 and Figure 3

Table 2 – Equations of the curves obtained by Folin-Ciocalteu method

FC					
Standard	**Linear plot (mg/L)**	**A = a C + b**			***N***
		a	**b**	**r**	
AG	0.25 – 20.00	0.0330	0.0060	0.9990	5
Trolox	0.50– 40.00	0.0070	0.0161	0.9996	5
BHT	0.50 – 30.00	0.0070	0.0007	0.9949	5

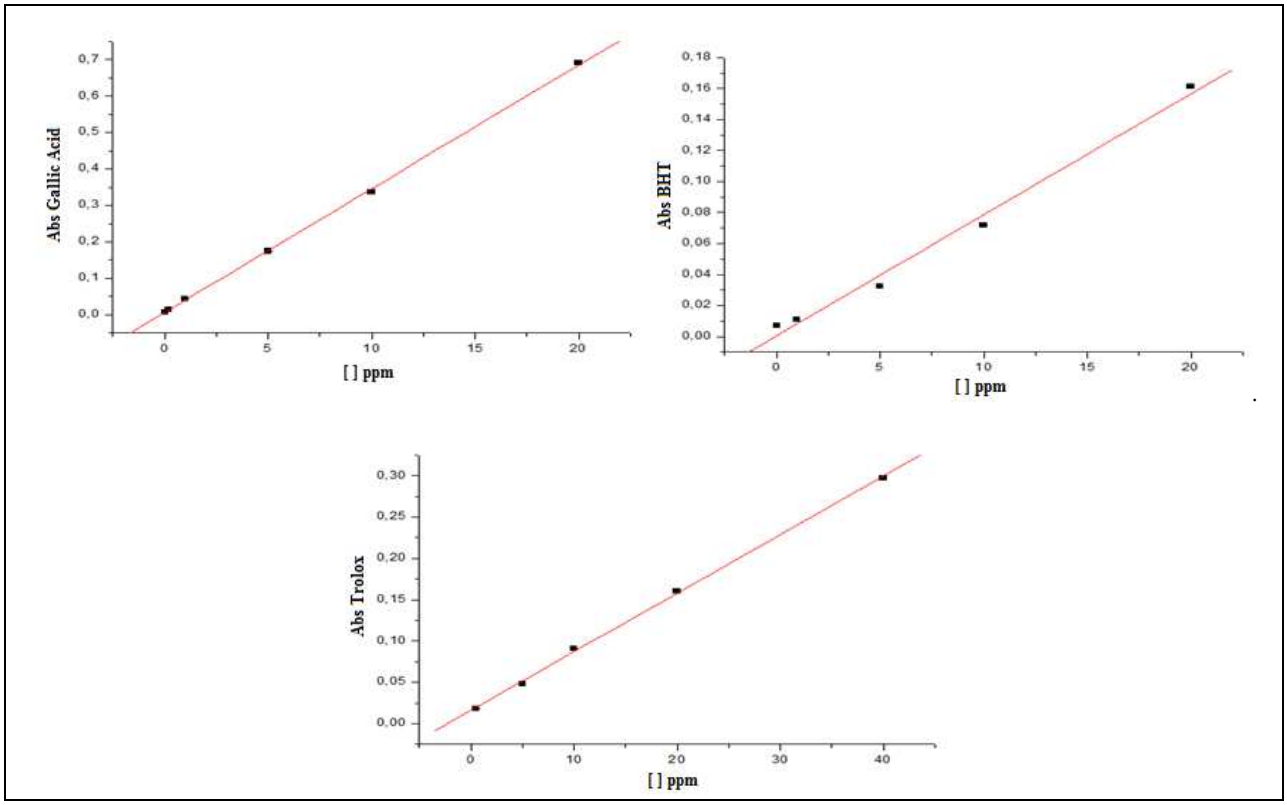

Figure 3. Analytical curve for spectrophotometric determination of total phenolics (Folin–Ciocalteu method)

From the absorbance values measured at 770 nm and using the equation obtained by the calibration curve of the standards used in the experiment, the total phenolic content found in the atomized extract were calculated, and the values were expressed in mg/g, as shown in the table 3.

Table 3 – Total phenolic content in mg/g in the atomized extract of *Syzygium cumini* (L.) Skeels

Sample *S. cumini*	Concentration (mg/g)		
	Gallic Acid	BHT	Trolox
Atomized Extract	2.3 ± 0.10	11.3 ± 0.50	5.4 ± 0.25

The results showed were significative for the extract relative to the three standards used, as shown in Table 3. According to Ali [38], phenolic compounds content in the aqueous extract corresponding to 5.42 mg AG/g and 6.66 mg AG/g was found for two varieties of *S. cumini*, named Rajamun and Kaatha, respectively

The drying process in spray dryer may be responsible for the loss of phenolic compounds, as reported by Fernandes et al. [39], in which the antioxidant activity of the atomized extract of *Psidium guajava L*. was evaluated and approximately 23% of the phenolic compounds was lost in the drying process.The studies developed by Couto et al. [14] and Souza [18] reinforce that the loss of phenolic compounds may be associated to polyphenol degradation caused by oxidative condensation and decomposition of thermally labile compounds, stages present in the atomization process.

Antioxidant activity by ABTS assay

By applying the ABTS assay for evaluating the antioxidant activity, it was possible to obtain the equations of the curves for the three standards, shown in Table 4 and Figure 4, respectively.

Table 4 – Equations of the curves obtained in the ABTS assay

ABTS					
Standard	Linear Plot (mg/mL)	%I = aC + b			N
		a	b	r	
AG	0.2 - 14	38.617	4.172	0.9990	5
Trolox	1.4 - 10	4.072	2.721	0.9999	5
BHT	2 - 18	4.872	5.173	0.9991	5

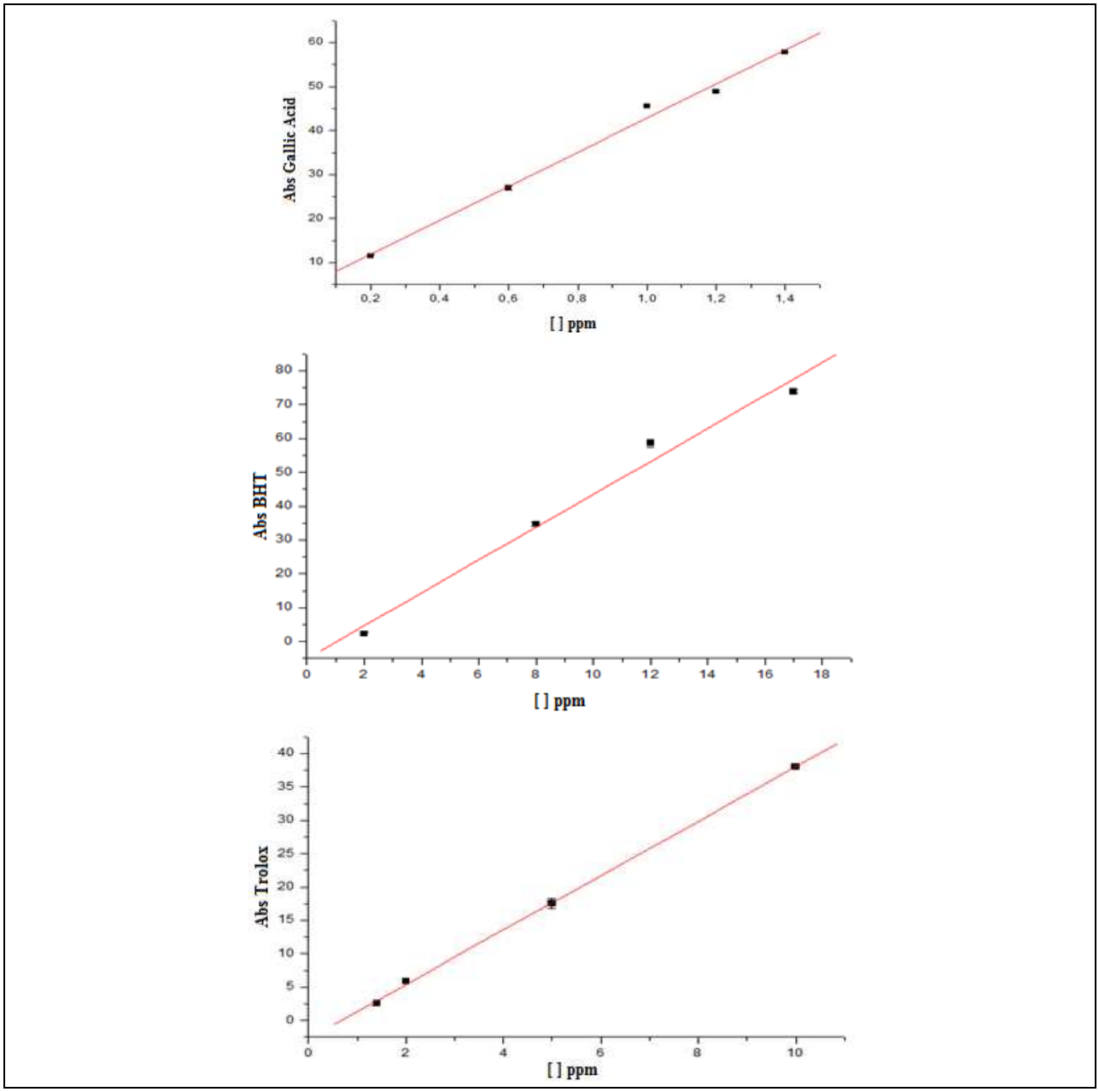

Figure 4 - Analytical calibration curve for spectrophotometric determination of antioxidant capacity (ABTS $^{+}$assay)

Table 5 – Antioxidant capacity by ABTS•+ radical scavenging assay (mg E(standard/g) in atomized extracts *Syzygium cumini* (L.) Skeels - SCFEAT

Sample*S. cumini*	Concentration (mg E(standard/g)		
	Gallic Acid	BHT	Trolox
Atomized Extract	1.6 ± 0.02	9.6 ± 0.05	17.2 ± 0.06

Vasco et al. [40] found in passion fruits of *Passiflora mollissima L.* specie, about 27.5 mg Trolox/g of sample. In the same study, other fruits such as plum, strawberry, mango and guava were analyzed, with approximately 8.75, 5, 1.25 and 10 mg Trolox/g of sample, respectively. However, these last results showed lower values in the antioxidant capacity by the ABTS method, regarding the results observed in this study.

Georgetti et al. [25] evaluated the effects of spray drying technique regarding the chemical and biological properties of soymilk associated to several types of additives, such as colloidal silicon dioxide, starch and maltodextrin, and it was observed that colloidal silicon dioxide was responsible for reducing the concentration of polyphenols and antioxidant activity compared to the other adjuvants used.

Studies on antioxidant activity of atomized extract of *S. cumini* are still scarce. Despite the lack of studies that serve as comparative parameters, it was possible to identify thatthe methods applied showed satisfactoriness and specificity, and also proved that the *S. cumini* fruits, popularly known as jambolan or "widow earring", are presented as accessible sources of natural antioxidants.

CONCLUSION

The biological activity study of *S. cumini* fruits proved to be promising in the scientific area, since there are still few publications on the subject. This study revealed that*S. cumini* fruit has antimicrobial activity against *S. flexneri* and antioxidant activity, therefore this result encourages the continuity of other studies, as well as comparative activity with other extractive processes.

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