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Antioxidant properties of aqueous extracts of *Sarcocephalus latifolius* roots and effects on sports performance in rats

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Abstract

The objective of this study was to evaluate the antioxidant properties of *Sarcocephalus latifolius* root extracts and their effects on sports performance in rats. A total of 15 male Wistar rats were used and divided into 3 groups. The GrpEDSE and GrpED+Entr groups were each subjected to 7mL/kg distilled water by body weight, and then the GrpSL+Entr group was treated with 300mg/kg body weight of aqueous extract of *Sarcocephalus latifolius* roots. Rats belonging to GrpED+Entr, GrpSL+Entr groups were subjected to swimming for fifteen days. Twenty-four hours after the last training session, the evaluation was done. The results revealed that the studied plant has an antioxidant property with a value of 405.97±2.39 mmol EAA/mg. Then, the training induced a significant increase ($p<0.001$) in swimming performance in treated rats belonging to GrpSL+Entr groups with a running time of 321.214±22.09 min. These extracts of *Sarcocephalus latifolius* leaves represent natural antioxidants and would thus participate in the improvement of performance in athletes.

Keywords: plants, aqueous extract, swimming, wistar rats.

Introduction

Achieving athletic performance in the athlete requires work based not only on the mind but also on technique, physical qualities and a rigorous diet. The development of these physical qualities requires an increased need for oxygen (O_2) responsible for the production of free radicals (Coisne, 2007). However, during intense exercise, the production of free radicals or reactive oxygen and nitrogen species (RONS) increases and can inhibit muscle contractile function, resulting in muscle fatigue and decreased performance (Bentley et al., 2015). In order to fight against this muscle fatigue, top athletes resort to antioxidant supplementation (Higgins et al., 2020), which is a method to reduce muscle damage during physical exertion and improve performance (Higgins et al., 2020). Vitamin E, for example, appears to be one of the most widely available antioxidants (Braakhuis et Hopkins, 2015). It refers to fat-soluble compounds including α-tocopherol, tocopherols and tocotrienols (Neubauer et Yfanti, 2015). The latter two act as powerful free radical scavengers in membranes and lipoproteins. Then, they quench fatty acid peroxides and produce tocopheroxyl radicals, the resulting tocopheroxyl radicals can be reduced by an appropriate reducing agent such as ubiquinol or vitamin C to finally regenerate vitamin E which is the most prevalent in nature (Powers et al., 2014). Vitamin E, present in lipid-rich structures such as the sarcoplasmic reticulum, eliminates free radicals produced by mitochondria, reduces lipid peroxidation and membrane damage (Bentley et al., 2015), thus allowing for the improvement of sports performance. In this sense, Yi et al. (2014) showed that the consumption of 75 g of almonds containing vitamin E consumed as a single supplement before exercise over 4 weeks, resulted in improved sports performance (measured in terms of distance covered). In addition, acute almond supplementation (60 g, 2 h before exercise) was reported to improve performance in endurance exercise in trained subjects (Esquius et al., 2020). In the animal model, Lee et al. (2009) showed that the tocotrienol-rich fraction (TRF) increased hepatic and muscular glycogen and reduced exercise-induced oxidative stress, as well as blood lactate imposed on swimming rats.

In the West African context, the population prefers to use food plants that have antioxidant capacity and represent potential sources of natural antioxidants for therapeutic or industrial purposes: This is the case of the pulp and leaves of *Adansonia digitata* L., a plant used by the populations of the Kara region in Togo (Kpatcha et al., 2016a), which has antioxidant capacity and anti-fatigue and anti-stress effects (Kpatcha et al., 2016b).

In Benin, the traditional pharmacopoeia highlights the antioxidant properties of several plant species including Liliospsida, *Torilis leptophylla*, *Cleome ibercia*, *Senna Siamea* and *Sarcocephalus latifolius* (Farimani et al., 2016). Unfortunately, to our knowledge, the antioxidant properties of these and their role in improving sports performance have not been the subject of scientific publications.

Thus, in the concern to better value the medicinal plants useful to the physical activities, less expensive while securing the athletes of the phenomena compared to the phenomenon of doping, the present study aims at evaluating the antioxidant properties of *Sarcocephalus latifolius* and their effects on the sports performance.

Materials and Methods

1-Method

a) Preparation of crude extracts

The decoction method was used to perform the extraction of total chemical principles. For this purpose, 50 g of powder was dissolved in 500 mL of distilled water and boiled for 30 minutes. The mixture thus obtained after cooling underwent a series of three filters through an absorbent cotton. The filtrate received was transferred to a 1000 mL flask and subjected to evaporation at 40°C using a rotavapor ((Heidolph Laborota 4000 efficient) coupled to a water cooler (Julabo FL 300).

The following formula Rdt= (Weight of dry extract/ Initial weight of powder) x 100 was used to calculate the yield after weighing the dry residue (Houngbeme et al., 2014).

b) Evaluation of the antioxidant power of extracts

The use of the DPPH radical allowed the evaluation of the antioxidant activity of *Sarcocephalus latifolius* extracts. According to the method of Gandonou et al. (2018), the principle of the test is based on the measurement of the capacity of the substance to reduce the free radical DPPH°(2,2'-diphenyl-1-picryl hydrazyl. This reduction of the radical is monitored by UV-Vis spectrophotometry, by measuring the decrease in absorbance at 517 nm caused by the antioxidants. The DPPH (2,2 azyl) free radical in the presence of the free radical scavengers can be monitored in turn by UV-Vis spectrophotometry, measuring the decrease in absorbance at 517 nm caused by the antioxidants. In the presence of the free radical scavengers, the purple-colored DPPH (2,2 Diphenyl 1 picryl hydrazyl) is reduced to the yellow-colored 2,2 Diphenyl 1 picryl hydrazine. A stock solution of the extract is thus prepared at Cm=10-2 mg/mL of analytical grade methanol (Sigma-Aldrich). Then, 1.5 mL of the extract solution is mixed with 3 mL of the methanolic DPPH solution. The mixture is incubated for 30 minutes at room temperature and the absorbance is read at 517nm against a blank. The positive control is represented by a solution of a standard antioxidant; ascorbic acid (purity 99.5%, Sgma-Aldrich) is also prepared under the same conditions as the samples but with a variable concentration range 0.10mg/mL. The antioxidant activity of the extract was determined using the calibration curve established with ascorbic acid. Each assay is performed in duplicate. Antioxidant activity is expressed as mmol ascorbic acid equivalent per gram of extract (mmol EAA/mg).

c) Plant material

It consisted of roots of *Sarcocephalus latifolius* (F.S.S) harvested, dried and powdered in February 2022 in the city of Parakou in Benin. A dose of 300 mg/kg, which represents the most effective dose, was administered (Agbodjogbé, 2013).

d) Animal material

Male rats of Wistar strain, weighing 170 ± 10 g were obtained from the animal house of the Exercise Physiology Research Unit in Porto-Novo. The animals were housed in wire mesh cages equipped with feeders and drinkers and were fed wheat bran, corn meal and running tap water. They were also subjected to a regime of 12 hours of light, 12 hours of darkness at a temperature of 28 °± 1°C and a humidity of 60 ± 5%. Access to food and water was ad libitum.

2- Evaluation of the effect of plants on the sports performance of rats

a) Parameters that influenced the swimming protocol and choice of the tank

The swimming performance of each rat was determined using a stopwatch. Regarding the choice of the tank, the protocol of Kpatcha et al. (2016b) was used in this study. In this sense, an inverted cone-shaped circular basin with a small radius of 20 cm (base), a height of 120 cm and a large radius of 40 cm (surface) was used as a swimming pool for the rats. This basin was filled with water to a height of 100 cm and maintained at a temperature between 35 and 36°C (fig1).

Figure 1 : Circular tank

b) Rat swimming protocol and constitution of the experimental groups

Rats were randomly divided into three groups (n = 5). They were then treated with a dose of 300 mg/kg body weight per day. A gastric tube was used for oral administration for 15 days. Thus, the GrpEDSE group consisted of the rats treated with 7 mL/kg of distilled water per body weight and not subjected to training. The GrpED+Entr group was also composed of rats treated with 7 mL/kg of distilled water per body weight and then subjected to training. Finally, the GrpSL+Entr group consisted of rats treated with the aqueous extract of the roots of *Sarcocephalus latifolius* and then trained.

c) Habituation of rats to swimming

According to the protocol used by Kpatcha et al. (2016b) and then modified by us, the animals belonging to the GrpED+Entr and GrpSL+Entr groups were subjected to swimming for 15 days in batches of five in the pool. One week before the manipulations, the rats belonging to these different groups were subjected to 15 minutes of swimming per day for five days in order to avoid problems related to stress on the one hand and on the other hand to have an idea compared to their natural performance.

d) Training of the rats to swim

The protocol used by Kpatcha et al. (2016b) and modified for this study was used to train the rats. They were trained to swim without load for 15 days, six days a week, and then rested on the seventh day but with feeding. During training, they were subjected to 30 minutes of swimming on the first day of the experiment, and an increment of 10 min/day is made on the following days to reach one (01) hour on the fourth day. From the fourth to the fifteenth day, the rats were subjected to one hour of swimming per day. The administration of the plant was done one hour before the beginning of the exercise. To avoid confounding effects, the GrpEDSE group (no training) was maintained in a pool containing 3 cm of water at the same temperature. Twenty-four hours after the last training session, all rats belonging to the GrpEDSE, GrpED+Entr, GrpSL+Entr groups were subjected to swimming. Uncoordinated movements and remaining underwater for 10 seconds without surfacing were considered as the criteria for rat exhaustion. In these cases, the swimming was stopped, the swimming time was recorded in minutes for each rat, and then the average per group was calculated.

To avoid the influence of circadian variations in physical activity, the swimming exercise was performed from 9:00 am to 5:00 pm.

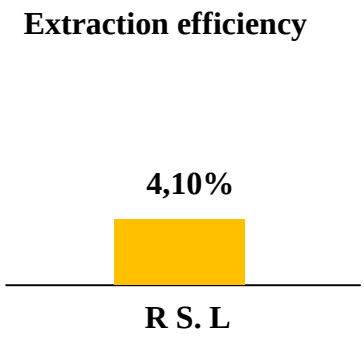
3- Statistical analysis

The Stat View software (version 5) from Abacus concepts Inc (Berkeley, CA, USA) was used to process the results of the present study. First, descriptive statistics were performed and then the mean values plus or minus the standard deviation were expressed. Then, the Kruskall Wallis test was used to compare the mean values of the running times performed by the rats. When the Kruskall Wallis test was significant, the Mann Whitney U test, a binary test, was used. The significance level for all tests, was set at $p < 0.05$.

Results

1- Yields of the aqueous extract of *Sarcocephalus latifolius* root

Figure 2 shows the yield of the extraction of *Sarcocephalus latifolius* roots. From the analysis of the figure, it can be seen that the best yield obtained by the aqueous extract of the roots of *Sarcocephalus latifolius* with a rate of 4.10 %.



RS.L = *Sarcocephalus latifolius* root

Figure 2: Extraction yield of *Sarcocephalus latifolius* root

2- Chemical constituents of *Sarcocephalus latifolius* root

a) According to the colored reactions in the tube and phytochemical analysis of the aqueous extract of *Sarcocephalus latifolius* root

The phytochemical screening showed the presence of major chemical groups. It appears from the analysis of table 1, that coumarins, phenolic compounds, anthracenic C-heterosides, alkaloids, terpenes, quinone derivatives, saponosides, steroids, mucilages, reducing compounds are present in this plant contrary to free anthracenics, cardenolides and cyanogenic derivatives which are absent.

Table 1: Results of the screening of the aqueous extract of *Sarcocephalus latifolius* root (RS.S)

Active principles		RS.S
Alkaloids		+
Polyphenolic compound	Catechetical Tannin	+
	Gallic Tannin	+
	Anthocyan	+
	Flavonoids	+
	Leuco-anthocyan	+
Coumarins		+
Anthracene C-heterosides		+
Quinone compound		+
Saponosides		+
Terpenes		+
Steroids		+
Mucilage		+
Reductive compounds		+
Free Anthracenics		-
Cardenolids		-
Cyanogenic compounds		-

b) Antioxidant activity

The calibration curve of the antioxidant activity of vitamin C is presented in the figure 3.

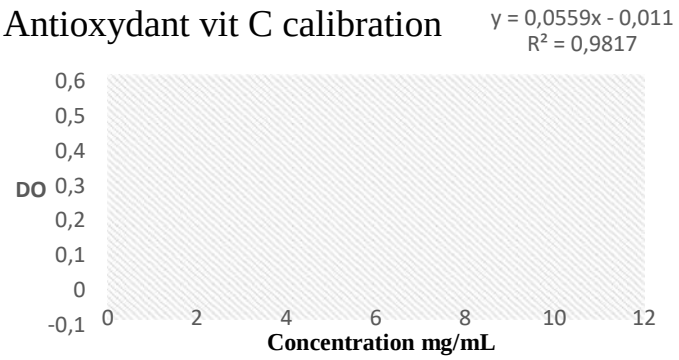


Figure 3: Calibration curve of the standard reducing ascorbic acid of the radical

The application of the regression equation to the different OD measurements of the binary extract-DPPH resulted in the values recorded in the table below (Table 2). The analysis of the table revealed that the studied plant has a significant antioxidant property with a mean value of 405.97 ± 2.39 mmol EAA/mg.

Table 2: Antioxidant activity of R.S.L

Extracts	Activity in mmol EAA/mg		m ± s
	Essai 1	Essai 2	
R.S. L	408,37	403,58	405,97 ± 2,39

RS. L = roots of *Sarcocephalus latifolius* ; m ± s : mean ± standard deviation

3- Effects of plant administration combined with training on the performance of rats

The analysis in Table 3 shows that all rats that were trained significantly improved their swimming performance as $p < 0.01$.

Table 3 : Effects of training on the performance of rats subjected to extracts

	Grp_{EDSE} n=5 (m ± s)	**$Grp_{ED+Entr}$ n=5 (m ± s)**	**$Grp_{SL+Entr}$ n=5 (m ± s)**	**p**
Weight (g)	161.98 ± 5.806	163.580 ± 9.99	166.84 ± 3.448	
Time (min)	56.308 ± 6.246	205.826 ± 21.654**	321.214 ± 22.090**	0.001

Legend: n: number of participants; m ± s: mean plus or minus standard deviation; g: gram; min: minutes; Grp_{EDSE}: group treated with distilled water and not trained; $Grp_{ED+Entr}$: group treated with distilled water and trained; $Grp_{SL+Entr}$: *Sarcocephalus latifolius* treated group then subjected to training; p: probable value; **: significance level p less than 0.01

4-Effectiveness of the plant on sports performance

Analysis of Table 4 shows that rats treated with *Sarcocephalus latifolius* root extracts and subjected to training have a significantly ($p < 0.01$) longer swimming time than those treated with distilled water and subjected to the same training load 205.826 ± 21.654 min).

Table 4: Effects of *Sarcocephalus Latifolius* roots on performance

	$Grp_{ED+Entr}$ n=5 (m ± s)	**$Grp_{SL+Entr}$ n=5 (m ± s)**	**p**
Weight (g)	163.580 ± 9.99	166.84 ± 3.448	
Time (min)	205.826 ± 21.654	321.214 ± 22.090**	0.007

Legend: n: number of participants; m ± s: mean plus or minus standard deviation; g: gram; min: minutes; $Grp_{SL+Entr}$: group treated with *Sarcocephalus latifoulius* and then trained; $Grp_{ED+Entr}$: group treated with distilled water and trained; p: probability value;**: significance level p less than 0.01

Discussion

The objective of the present study was to evaluate the antioxidant properties and then the effects of extracts from the roots of *Sarcocephalus latifolius* on athletic endurance in rats. This plant was chosen because it is used in the traditional pharmacopoeia in Benin to treat several pathologies such as malaria, dysentery, fever and hypertension. It is also used in health promotion as a powerful antioxidant (Abbah et al., 2010 ; Ngo et al., 2009 ; Amos et al., 2005). In the present study, only aspects related to antioxidant activity and sports performance were addressed.

Regarding antioxidant activity, the results of phytochemical screening revealed the presence of steroids, terpenes, polyphenols, coumarins, saponosides, mucilages, reducing compounds, alkaloids, tannins and quinone derivatives. The polyphenolic compounds mainly identified in the root extracts of *Sarcocephalus latifolius* confirm the antiradical activity. These results are similar to those obtained by Agbodjogbé et al. (2013) and Ahonsou (2011). Moreover, according to N'Guessan et al. (2009), the polyphenolic compounds observed are widely distributed in plant tissues, among which are numerous antiradical and antioxidant molecules. Therefore, the antioxidant activity of the aqueous extract of the roots of *Sarcocephalus latifolius* could be linked to a high content of phenols and flavonoids. These results are in line with those of Osama et al. (2017) who showed a high content of phenols and flavonoids after phytochemical analysis of the bark of the same plant in Sudan. On the other hand, a similar study Dhalwal et al. (2008) carried out using methanolic extracts of leaves and roots of *Sarcocephalus latifolius* showed that the total content of phenols present was 0.016 ± 0.03 and 0.036 ± 0.05 mg GAE/g DW, very low values compared to that observed by Osama et al. (2017) which is 78.21 ± 2.4 mg gaE/g DW. The reasons for this large variation could be due to the part of the plant studied that would contain a different chemical composition, the solvent used for extraction, the method used, the origin of the samples and environmental factors that are factors that were not considered in this study. It is important to note that phenolic compounds are powerful antioxidants because they allow the direct scavenging of ROS (Reactive Oxygen Species), the inhibition of enzymes and the chelation of metallic traces responsible for the production of ROS (Reactive Oxygen Species). Apart from the properties previously mentioned, flavonoids intervene in digestion, reduce cardiovascular risks by acting as free radical scavengers, preventing and repairing the damage caused by ROS (Bruneton, 2009). Also, it would be important to say that these flavonoids have antioxidant and anti-ulcer activities (Pincemail et al., 1998) because they are able to modulate the activity of certain enzymes and modify the behavior of several cellular systems.

The presence of tannins in the present study could be related to the impermeability of the outermost layers of the skin and mucous membranes, which according to Bruneton (2009) protects the underlying layers, promoting tissue regeneration. The presence also of coumarimes and polyphenolic compounds could explain the antioxidant Gandonou et al., 2018), anti-inflammatory, antimicrobial and anticoagulant properties in this study (Dosseh et al., 2014). Since the root extract of *Sarcocephalus latifolius* has antioxidant and anti-inflammatory properties, could it not have effects on sports performance? In this sense, in order to find answers to this question in the present study, the rats were subjected to swimming. Swimming was chosen because when performed in a group, it promotes more vigorous exercise than when rats are allowed to swim alone or run alone on a treadmill (Trayhurn, 2017). In addition, the weight of the rats used did not vary. Therefore, it is plausible to state that the weight of the rats did not influence the performance of the rats because studies have shown a decrease in performance following an increase in body weight on the performance of athletes, during running, swimming or vertical jumps (Al-hashem et al., 2012).

Furthermore, after 15 days of training, the performances obtained proved that training alone without extract supplementation had a positive effect on performance (increase in swimming time) on trained rats compared to the control group without training. The results also showed that the group of rats that received the *Sarcocephalus latifolius* root extract and underwent the training for two weeks (Table 4) significantly improved the swimming time. This longer swimming time than that observed in the second group suggests that the extract used in this study demonstrates a greater resistance to fatigue. These results are probably obvious as they are similar to the experimental study conducted by Kpatcha et al. (2016b) which showed that oral supplementation with *Adansonia digitata* leaf or pulp improved both endurance capacity and time to exhaustion independently of the exercise regime in rats. Other complementary studies could be envisaged in order to evaluate the blood modifications linked to the consumption of this extract in healthy subjects and sportsmen and to validate the doses to be taken by taking into account the sex, the health status (woman in pregnancy or not), and the age groups.

Conclusion

The objective of the present study was to analyze the antioxidant properties and then to evaluate the effects of the extracts of the roots of *Sarcocephalus latifolius* on the sports performance in the animal model. The results showed that the extract used in traditional medicine in the West African zone and precisely in Benin contains active principles such as alkaloids, polyphenols, flavonoids and presents antiradical and antioxidant activities allowing to be used as phytotherapy. In addition, the administration of an aqueous extract of the roots of *Sarcocephalus latifolius* at the dose of 300 mg/kg significantly improved the physical performance of rats. These results suggest that the extract of the roots of *Sarcocephalus latifolius* could be considered as a product with high antioxidant potential in the food and pharmaceutical industries. However, other studies could be conducted in order to scientifically validate its use in the sports world without any risk related to the doping phenomenon.

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