

**A PRELIMINARY STUDY of the EFFECT
of *RUBUS* sp. EXTRACTS on THE
EXCITATORY and INHIBITORY AMINO
ACIDS LEVELS in HIPPOCAMPUS and
CEREBRAL CORTEX in RATS.**

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INTRODUCTION

- ✓ Blackberries (*Rubus sp*), and are very complex in terms of genetic background, growth characteristics, and number of species.
- ✓ Blackberries are rich sources of phytochemicals which may impact positively on human health.
- ✓ However, studies to elucidate the potential therapeutic properties of blackberries have been limited.

INTRODUCTION

- ✓ Therefore, the present research was focused both on preparation and characterization of polyphenolic extracts from blackberry cultivars grown in Brazil and on investigation of their biological properties.

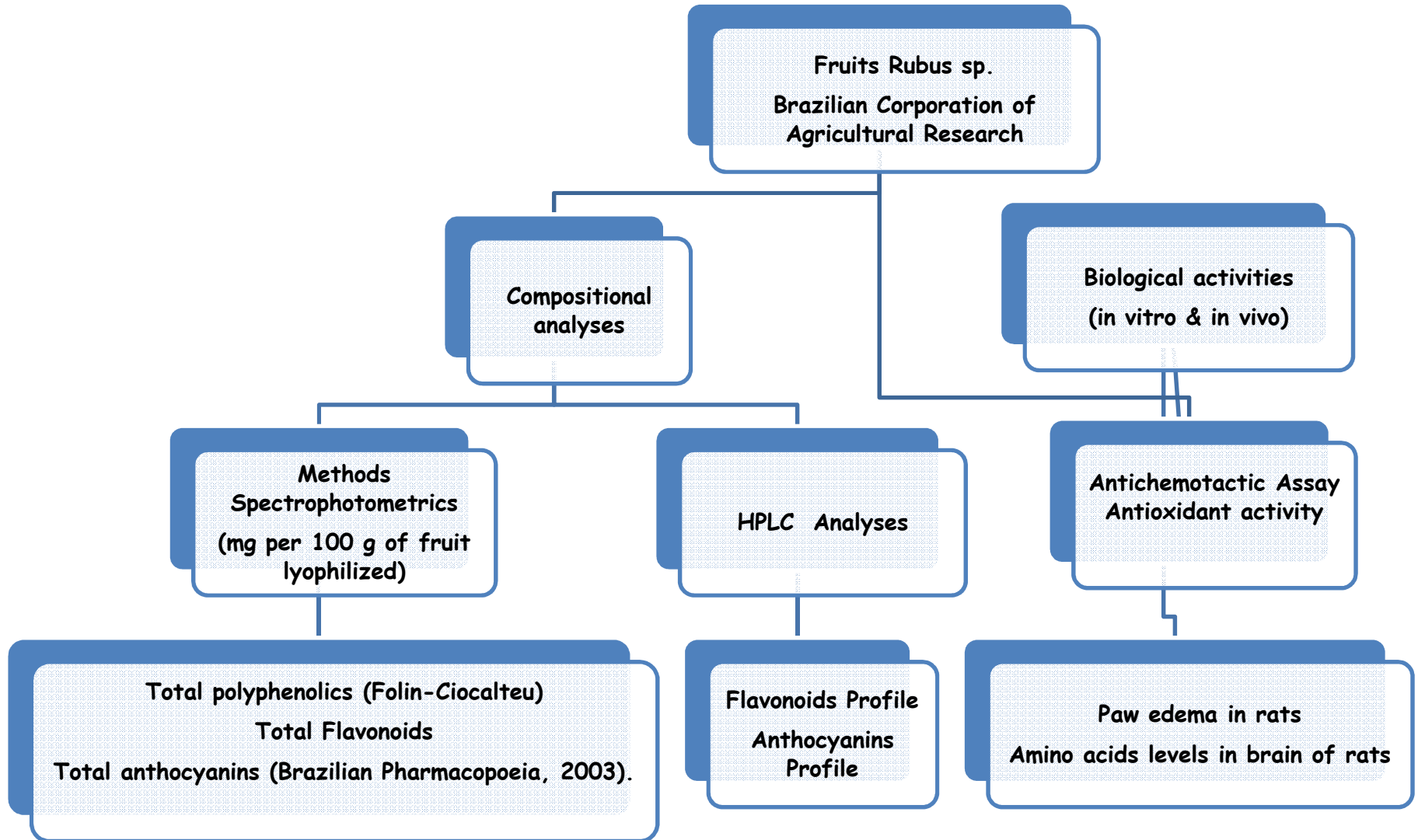
INTRODUCTION

- The total phenolic and anthocyanin content, and total antioxidant capacity were compared.
- The major types of flavonoids and anthocyanins were determined.
- Furthermore, the whole extract was fractionated and the bioactivity of anthocyanin fraction and whole extract was compared.

INTRODUCTION

- ✓ These studies can be divided into two parts:
 - Investigation of possible anti-inflammatory activity in vitro and in vivo.
 - Evaluation of the effects of extracts on the levels of excitatory and inhibitory amino acids in the brain of rodents subjected to chronic treatment (HPLC, Waters pico tag[®]) .

Material & Methods



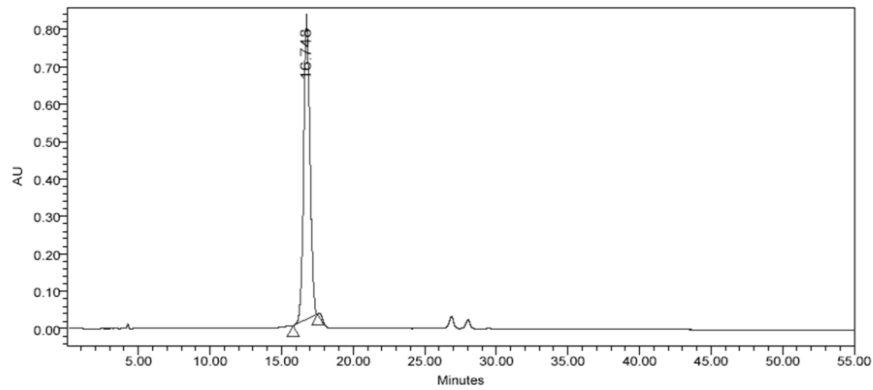
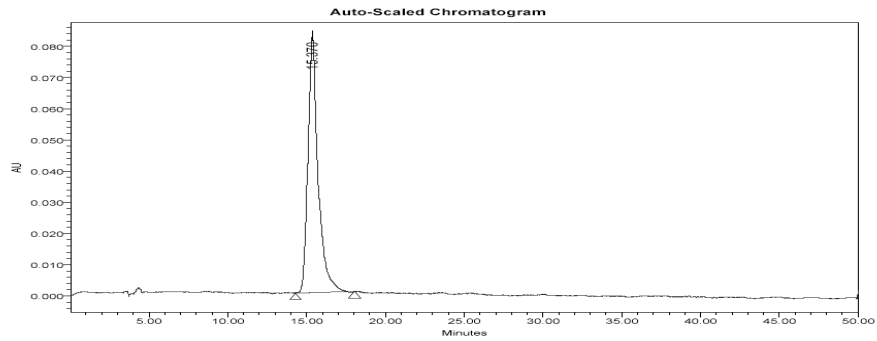
Compositional Analysis

TABLE 1.

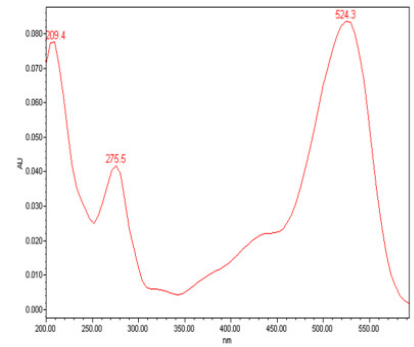
TOTAL ANTHOCYANINS, FLAVONOIDS AND POLYPHENOLS IN BLACKBERRIES (VALUES ARE AVERAGES OF TRIPPLICATE ANALYSES). TOTAL ANTHOCYANINS WERE EXPRESSED AS CYANIDIN-3-GLUCOSIDE EQUIVALENTS EXPRESSED PER 100 G OF FRUIT LYOPHILIZED. POLYPHENOLS AND FLAVONOIDS CONCENTRATION BASED UPON GALLIC ACID OR QUERCETIN, RESPECTIVELY, AS STANDARD EXPRESSED PER 100 G OF LYOPHILIZED FRUIT

Blackberries			
Cultivars	Anthocyanins	Flavonoids	Polyphenols
<u>(mg/100 g fruit lyophilized)</u>			
<i>Caingangue</i>	500 ± 1.86	67 ± 0.66	1,399 ± 0.02
<i>Tupy</i>	650 ± 3.51	40 ± 0.88	1,301 ± 0.02
<i>Guarany</i>	627 ± 4.93	96 ± 0.57	1,310 ± 0.10
<i>Cherokee</i>	524 ± 2.90	75 ± 0.58	1,206 ± 0.02
<i>Choctaw</i>	785 ± 5.20	78 ± 0.57	1,706 ± 0.05
<i>Arapaho</i>	860 ± 0.88	13 ± 0.33	1,513 ± 0.03
<i>Brazos</i>	843 ± 2.00	55 ± 0.88	1,605 ± 0.02
<i>Xavante</i>	583 ± 4.93	94 ± 0.66	1,799 ± 0.02
<i>Comanche</i>	681 ± 5.50	102 ± 0.90	2,188 ± 0.06

RESULTS



A



B

Figure 1: cyanidin 3 glucoside,
a) Retention time and UV spectra in HPLC-DAD (b).

RESULTS

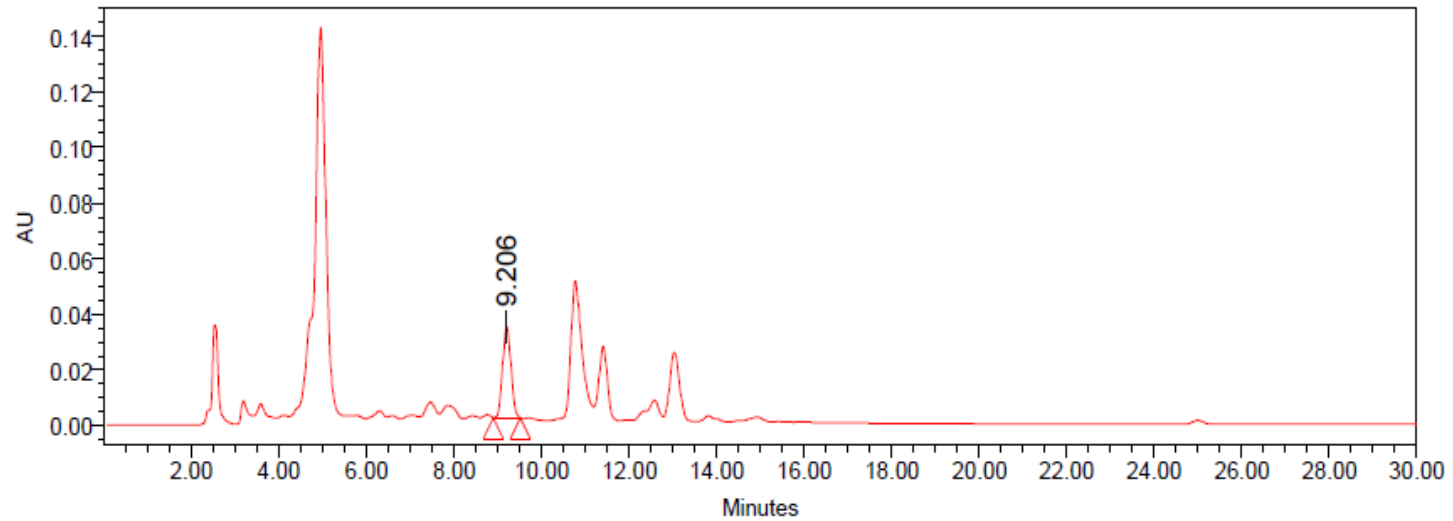


Figure 2. Flavonoids profile

RESULTS

TABLE 2.
INDIVIDUAL FLAVONOIDS IN BLACKBERRIES (VALUES ARE AVERAGES OF
TRIPPLICATE ANALYSES)

Cultivar	Rutin	Hyperoside + Isoquercitrin	Quercitrin
	<u>(mg% fruit lyophilized \pm RSD%)</u>		
<i>Caingangue</i>	1.747 \pm 0.52	6.023 \pm 2.14	1.002 \pm 0.56
<i>Tupy</i>	2.553 \pm 2.44	6.734 \pm 1.95	1.875 \pm 6.68
<i>Guarany</i>	1.771 \pm 6.07	6.983 \pm 4.82	1.513 \pm 2.56
<i>Cherokee</i>	3.876 \pm 3.14	8.287 \pm 4.58	3.997 \pm 4.58
<i>Choctaw</i>	3.943 \pm 0.87	8.958 \pm 1.33	3.932 \pm 1.33
<i>Arapho</i>	3.623 \pm 4.58	7.857 \pm 2.83	2.782 \pm 2.83
<i>Brazos</i>	3.343 \pm 4.54	5.789 \pm 2.16	2.873 \pm 2.16
<i>Xavante</i>	3.824 \pm 0.29	8.607 \pm 6.64	4.936 \pm 6.64
<i>Comanche</i>	4.082 \pm 0.86	9.836 \pm 0.26	4.829 \pm 2.47

RESULTS

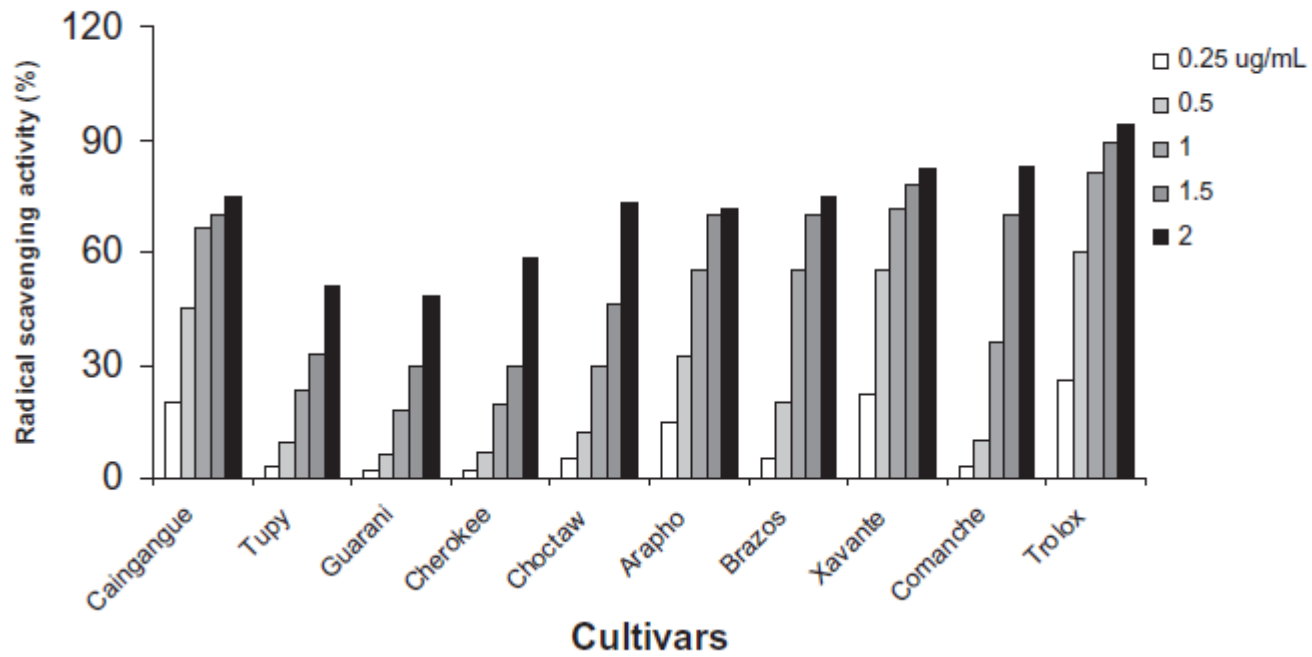


FIG. 1. RADICAL SCAVENGING ACTIVITIES

RESULTS

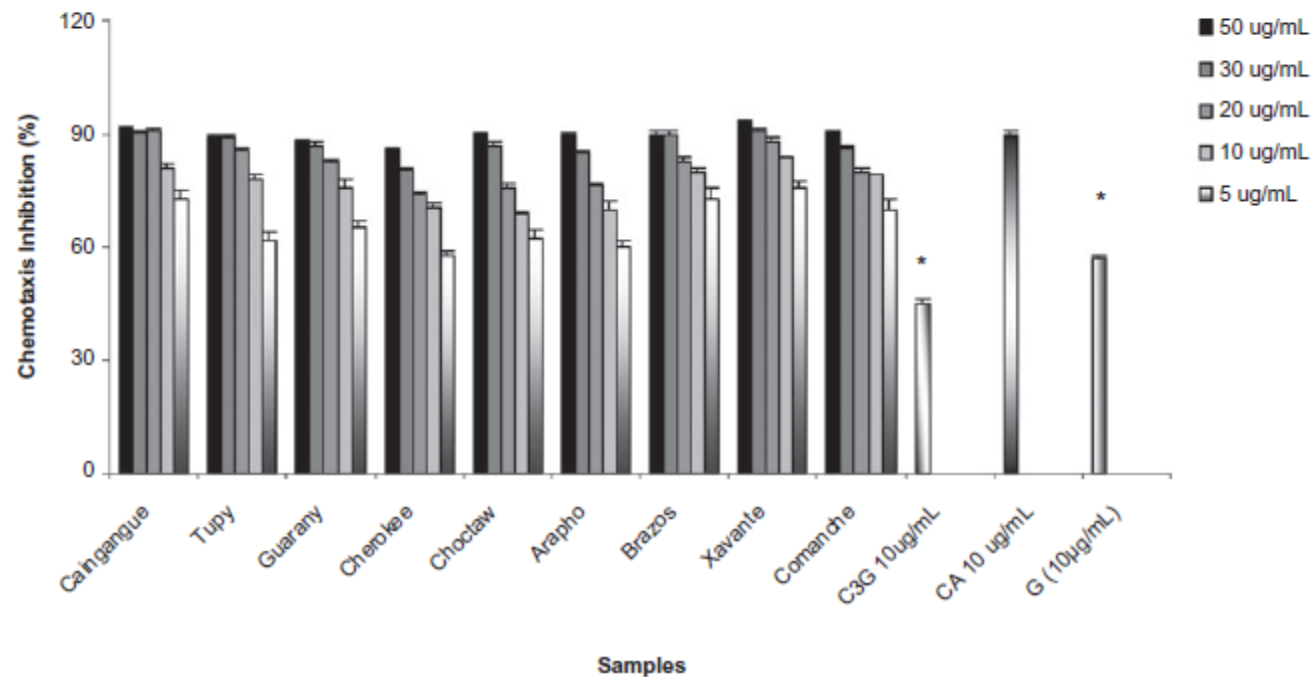


FIG. 2. EFFECTS OF EXTRACTS TESTED ON THE *IN VITRO* CHEMOTAXIS OF POLYMORPHONUCLEAR NEUTROPHILS (PMNS) TOWARDS LIPOPOLYSACCHARIDE (LPS)

Chemotaxis in the presence of test compounds is expressed as the percentage of the maximal chemotaxis to LPS in the same experiment.

Data are expressed as means \pm standard deviation of 10 separate measurements.

Asterisks indicate a significant difference in relation to total extract ($P < 0.05$)

Positive control: G, genistein; CA, cyanidin (aglycone); C3G, cyanidin-3-glucoside.

RESULTS

TABLE 3.

ANTI-EDEMATOGENIC EFFECT OF TOTAL EXTRACT (TE) OR CYANIDIN (CY) ON 1% CARRAGEENAN-INDUCED RAT PAW EDEMA EXTRACTS (DOSES INDICATED) WERE ADMINISTERED ORALLY 21 DAYS BEFORE SUBPLANTAR CARRAGEENAN INJECTION

Groups	Edema dm (cm)			
	1 h	2 h	3 h	4 h
Dose ug/kg				
NS	1.08 ± 0.13	1.28 ± 0.16	1.64 ± 0.15	1.69 ± 0.17
Indo	0.73 ± 0.23-(32%)	0.69 ± 0.22-(46%)	0.76 ± 0.22-(54%)	0.60 ± 0.13-(64%)
TE (6.50)	0.45 ± 0.11* (58%)	0.54 ± 0.21* (58%)	0.55 ± 0.17* (66%)	0.64 ± 0.13* (62%)
TE (3.20)	0.53 ± 0.15* (51%)	0.79 ± 0.18* (38%)	0.81 ± 0.11* (50%)	0.87 ± 0.09* (48%)
TE (1.50)	0.56 ± 0.15* (48%)	0.91 ± 0.28-(29%)	1.11 ± 0.20-(31%)	1.23 ± 0.13-(27%)
Cy (6.50)	0.62 ± 0.25* (44%)	0.65 ± 0.13* (47%)	0.91 ± 0.11-(38%)	1.18 ± 0.10†-(30%)

Control animals were treated with indomethacin (Indo, 5 mg/kg, p.o.) or saline (NS). The values represent the mean standard error of the mean. of the variation in the paw volume of 8–10 animals for each group, $P < 0.05$.

* Different from control.

† Different from positive control.

RESULTS

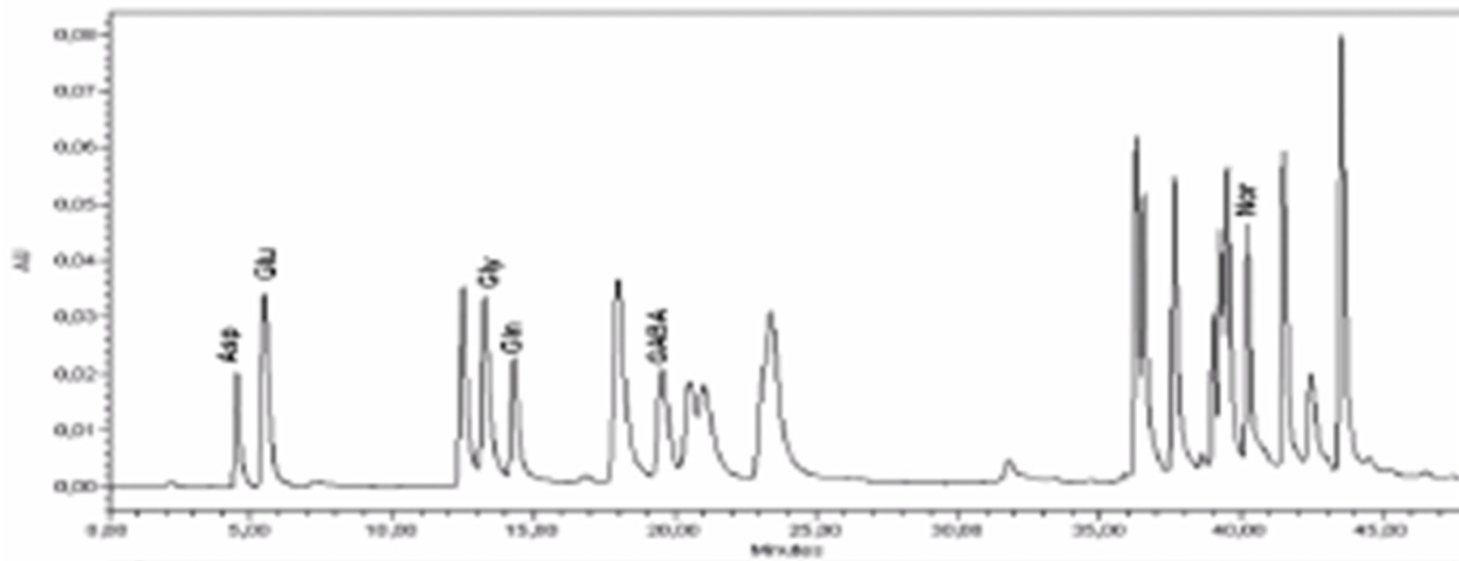
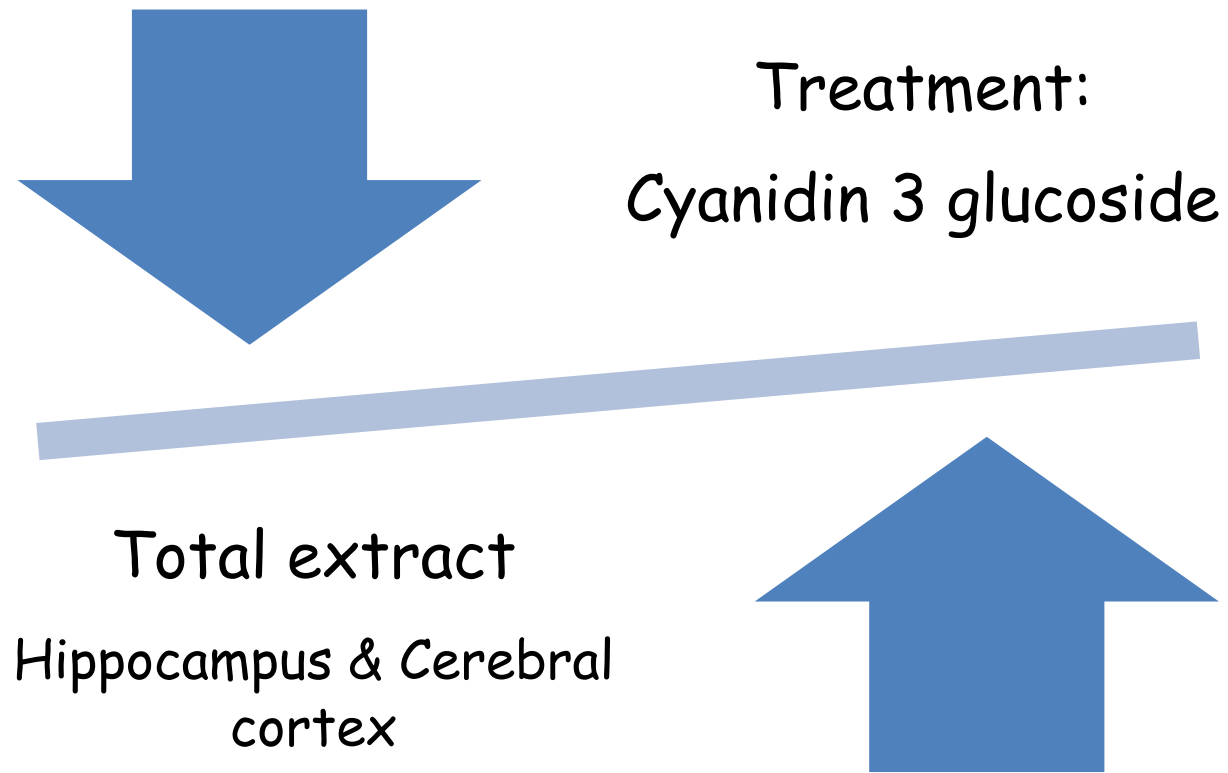


Figure: Asp = aspartato; Glu = glutamato; Gly = glicina; Gln = glutamina; GABA = ácido-gama-aminobutírico; Nor = norleucina.

RESULTS



CONCLUSION

- Extracts were prepared from blackberries grown in Brazil and were shown to possess significant antioxidant, antichemotactic and anti-inflammatory properties.
- The anthocyanin fractions were separated and were found to have antichemotactic and antiinflammatory activities.

CONCLUSION

- We found that, animals submitted to the treatment with total extract presented an increase of glutamate levels.
- However significant decrease on the glutamate levels was observed in both structures of animals treated with cyanidin 3 glucoside isolated.

CONCLUSION

- The results suggest that both extracts presents effects on the central nervous system, since the treatment caused alterations on the amino acids levels in the hippocampus and cerebral cortex in rats.
- In addition, we gain more knowledge on tissue disposition of phytochemical polyphenolics.

Thanks

for your interest in my work!

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