



## Morphological, agronomical and pharmacological characterization of *Hyptis pectinata* (L.) Poit germplasm

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**ABSTRACT:** Several species of the genus *Hyptis* have presented medicinal characteristics and are frequently indicated to treat gastro intestinal infections, gramps and pain, as well as for skin infections. Three harvests were carried out using six *H. pectinata* accessions, and the following characteristics were evaluated: plant height, canopy diameter, leaf length (L) and width (W), L/W relation and dry weight of leaves and stem. The accession SAM006 have presented highest leaf dry matter yield when the three harvests were summed. The aqueous leaf extracts of the same six *H. pectinata* accessions were tested for anti-edematogenic effect using the carrageenin-induced rat paw edema at doses of 200, 400 and 600 mg/kg. The extracts administered orally had a significant anti-edematogenic effect. The dose of 200 mg/kg of accessions SAM004 and SAM005 had a stronger effect reducing the edema by 19.6% and 15.4%, respectively. Similar results were obtained using higher dosages of the aqueous extract of the accessions SAM002, SAM003 and SAM006. It can be concluded that the accessions SAM004, SAM005 and SAM006 are promising genotypes for a plant breeding program that aims the development of a cultivar with highest leaf yielding and anti-edematogenic effect.

**Keywords:** Lamiaceae, *Hyptis pectinata*, medicinal plant, accession, leaf yield, carrageenin.

### INTRODUCTION

The genus *Hyptis* of the Lamiaceae family comprises several species such as *H. pectinata*, *H. mutabilis* and *H. fruticosa*, found along the Coastal Lands of the Northeastern areas of Brazil. They are herbaceous plants with opposing crossed leaves and entire blades. The flowers are small, clustered into axillary inflorescences, hermaphrodite, pentamer, strongly zygomorphous, and bilabiate (Joly, 1998).

*Hyptis pectinata* (L.) Poit (Lamiaceae) popularly known as "sambacaita" or "canudinho" in Sergipe and Alagoas states, is largely used in the traditional medicine for the treatment of inflammations and bacterial infections. Previous studies have shown that its aqueous extract has presented anti-edematogenic and anti-nociceptive activities (Bispo et al., 2001). Gathering plants from wild populations is the only form to obtain plant material, which has social and economic importance because it is one of the most sold plants on the free markets of Sergipe and Alagoas states.

Monocultures of sugar cane and coconut tree, together with urban development have caused a progressive loss of germplasm of many plants, including *Hyptis pectinata*. To overcome this problem, a Germplasm

Bank of *H. pectinata* has been created at the Universidade Federal de Sergipe (UFS), Brazil. Seeds of *H. pectinata* from different places and populations were collected for cultivation trials. Plants grown from the seeds were used for morphological, agronomical and pharmacological studies.

In breeding programs, one of the most important steps is the selection of promising genotypes that can be used in crossing works. The selection of these genotypes can be reached by characterizations of existent wild-type germplasm using agronomical and pharmacological properties (Blank et al., 2004). The present work was carried out to characterize *Hyptis pectinata* accessions by morphological, agronomical and pharmacological characteristics for posterior use in breeding programs.

### MATERIAL AND METHODS

#### Morphological and agronomical characterization

##### Seedling production

The seedlings were obtained in a greenhouse using seedbeds with 72 cells and volume of 121.2 cm<sup>3</sup> of substrate. They received two daily irrigations. The

substrate mixture utilized was soil, bovine manure and coconut dust on rate of 1:1:1. The mixture was chosen in a prior essay. Fourteen days after sowing the plant emergence happened, and after sixty days the seedlings were grown in field.

### Field conduction

The experiment was carried out at Research Station "Campus Rural da UFS", located in the city of São Cristóvão, Sergipe State, Brazil. Agronomic characterization was carried out for six *H. pectinata* accessions. It was utilized an experimental design of randomly blocks with three replications. One line of 12 plants composes the plots using a distance of 1.00 m between lines and 0.50 m between plants. In planting occasion 6 l/m<sup>2</sup> of bovine manure was used. Three harvests were obtained. The first harvest was carried out three months after the planting in June 06<sup>th</sup> of 2002, the second in August 08<sup>th</sup> of 2002, and the third in October 10<sup>th</sup> of 2002. The leaves were dried at 40°C in an oven with air circulation (model MA-037/18).

During harvesting the following parameters were evaluated:

- Plant height (cm): the plant height values were obtained using a measuring tape. For the statistical analysis were used the average values per plot;
- Canopy diameter (cm): the canopy diameter was obtained using a measuring tape. For the statistical analysis were used the average values per plot;
- Length (L) and width (W) of leaves (cm): it was carried out gathering randomly in plots five leaves totally expanded of each plant. The width and length of leaves were obtained by the measuring tape. For the statistical analysis was used the average values per plot;
- L/W relation: this parameter was obtained of a ratio of average width and average length of sampled leaves in plot;
- Dry weight of leaves and stems (g/plant): the plants were cut about 20 cm of soil between 2 p.m. and 4 p.m. The leaves were dried in an oven with air circulation at 40°C.

### Pharmacological characterization

**Table 1.** Geographical coordinates of the *Hyptis pectinata* accessions harvested in Sergipe State and kept in the Germplasm Bank of the Universidade Federal de Sergipe.

Accession	Origin of seeds (localization)	Geographical coordinates			Voucher numbers
		Latitude (S)	Longitude (W)	Altitude (m)	
SAM001	São Cristóvão	10° 55' 25.6"	37° 11' 56.4"	24	7455
SAM002	Neópolis	10° 18' 20.7"	36° 39' 7.2"	120	7454
SAM003	Santana do São Francisco	10° 16' 55.3"	36° 38' 33.8"	80	7453
SAM004	Malhada dos Bois	10° 21' 36.0"	36° 54' 27.6"	130	7452
SAM005	São Francisco	10° 19' 1.4"	36° 53' 36.8"	55	7451
SAM006	Propriá	10° 14' 26.3"	36° 51' 11.1"	25	7456

Abbreviations: s - south; w - west; m - meter

### Plant material

Leaves of six genotypes of *Hyptis pectinata* (L.) Poit (Lamiaceae) had been harvested (Table 1) in the flowering stage of plants cultivated at the Research Station "Campus Rural da UFS". Voucher herbarium specimens of each accession were deposited at the Herbarium of the Universidade Federal de Sergipe and the geographic coordinates determined using an Etrex model GPS (Garmin Corporation) (Table 1).

### Preparation of the aqueous extract

The leaves of *Hyptis pectinata* were dried at 40°C in an oven with air circulation (model MA-037/18) and posterior grounded in a Wiley type mill (model TE 340) until a finely granulated powder was obtained. The extract was obtained from this powder adding distilled water 1:10 (w/v) at 100°C, and kept in infusion for 30 minutes, followed by filtration. The filtrates were lyophilized and stored at 5°C. The w/w yields of the extracts starting from the dry plant material were 18.3, 17.0, 20.9, 17.5, 18.2 and 18.5% for the accessions SAM001, SAM002, SAM003, SAM004, SAM005 and SAM006, respectively. The extracts were resuspended in distilled water at the desired concentrations.

### Animals

Wistar rats of both sexes (160 - 220 g) were used for the anti-edematogenic studies. The animals were distributed into groups of eight and kept in plastic cages at a temperature of 25-28°C, with free access to food and water. Animals were fasted for 16 h before the experiment but had free access to water. The experiments with the animals were conducted in accordance with the Comissão de Experimentação em Pesquisas Animais da Universidade Federal de Sergipe (CEPA/UFS).

### Carrageenin-induced edema

A volume of 0.1 ml of 1% carrageenin (CG, an edematogenic agent) diluted in 0.9% saline was injected into the sub plantar region of the right hind paw of the

rat (Winter et al., 1962). Paw volume was measured immediately after CG injection (time 0) and at intervals of 1, 2, 3 and 4 h using a plethysmometer (model 7150, Ugo Basile, Varese, Italy). The aqueous extracts at different concentrations (200, 400 and 600 mg/kg) and indomethacin (10 mg/kg) were administered p.o. 1 h before CG injection.

### Statistical analysis

The results of the morphological and agronomical characterization were analyzed by the one-way ANOVA. Means were separated by least significant difference at  $P < 0.05$  (Tukey test). The results of the pharmacological characterization were reported as means  $\pm$  S.E.M. and analyzed by Student's *t*-test. A value of  $P < 0.05$  was considered significant.

## RESULTS

### Morphological and agronomical characterization

For the first harvest there was a statistical difference among the accessions for the following parameters: plant height, canopy diameter, leaf length and dry matter of leaves and stems (Table 2). The accession SAM006 presented superior plant height in comparison to the other genotypes. For dry matter of leaves and stems the genotypes SAM003 and SAM006 presented the highest values (Table 2).

For the second harvest the genotypes SAM006 and SAM003 presented superior values in comparison to the other genotypes for plant height and canopy diameter. However, for dry matter of leaves and stems only the genotype SAM006 was superior (Table 2). The genotypes presented a variation for plant height between 32 and 89

**Table 2.** Data of plant height (cm), canopy diameter (cm), leaf length (L) (cm), leaf width (W) (cm), L/W relation of leaves and dry weight (g/plant) of leaves and stem of six *H. pectinata* accessions.

Accession	Plant height	Canopy diameter	Leaf length	Leaf width	L/W	Dry weight	
						Leaves	Stem
First harvest							
SAM001	73.93 b	34.20 b	1.83 b	1.57 a	1.19 a	8.60 ab	3.95 b
SAM002	83.78 b	31.21 bc	2.30 ab	1.77 a	1.30 a	10.06 ab	6.18 b
SAM003	81.06 b	40.05 ab	1.97 b	1.43 a	1.38 a	20.43 a	16.65 a
SAM004	90.79 b	22.23 c	2.33 ab	1.80 a	1.30 a	3.02 b	1.02 b
SAM005	100.16 b	34.97 b	2.67 a	2.07 a	1.29 a	7.60 ab	3.58 b
SAM006	152.98 a	49.44 a	2.00 b	1.47 a	1.38 a	18.85 a	17.45 a
CV (%)	18.392	9.939	10.577	14.183	9.632	41.321	35.397
Second harvest							
SAM001	49.33 b	41.86 b	5.20 ab	4.07 ab	1.28 a	3.65 b	3.65 b
SAM002	61.60 b	43.42 b	4.90 ab	3.77 ab	1.32 a	5.82 b	5.49 b
SAM003	80.00 a	60.67 a	4.33 b	3.47 b	1.25 a	6.52 b	5.31 b
SAM004	32.06 c	23.07 c	5.47 a	4.57 ab	1.20 a	4.27 b	3.39 b
SAM005	46.64 bc	37.44 b	5.93 a	4.77 a	1.25 a	9.89 b	12.62 b
SAM006	89.35 a	65.39 a	5.50 a	4.03 ab	1.37 a	23.33 a	39.55 a
CV (%)	9.936	9.019	7.050	9.847	5.517	28.646	32.997
Third harvest							
SAM001	117.49 b	28.71 a	2.00 a	1.83 a	1.15 a	8.53 ab	11.14 c
SAM002	120.72 b	46.32 a	2.40 a	2.27 a	1.12 a	10.03 ab	15.59 c
SAM003	118.17 b	32.86 a	2.43 a	2.33 a	1.08 a	13.00 ab	19.33 bc
SAM004	110.26 b	23.75 a	2.50 a	2.53 a	1.01 a	4.54 b	9.43 c
SAM005	152.91 a	34.45 a	2.57 a	2.43 a	1.08 a	23.76 ab	28.76 b
SAM006	145.56 a	39.89 a	2.33 a	2.67 a	1.01 a	28.50 a	55.56 a
CV (%)	4.597	31.668	13.760	24.099	22.933	53.363	18.627
Sum of the three harvests							
SAM001	---	---	---	---	---	20.77 bc	18.75 d
SAM002	---	---	---	---	---	25.92 bc	27.25 cd
SAM003	---	---	---	---	---	39.95 b	41.29 bc
SAM004	---	---	---	---	---	11.83 c	13.83 d
SAM005	---	---	---	---	---	41.25 b	44.96 b
SAM006	---	---	---	---	---	70.68 a	112.55 a
CV(%)	---	---	---	---	---	27.787	12.518

Tukey test ( $P < 0,05$ ).

cm. The canopy diameters varied between 23 and 65 cm. For dry matter of leaves variation between 3 and 23 g/plant was observed.

For the third harvest the highest means of plant height were observed for genotypes SAM005 and SAM006. The highest values of dry matter of leaves and stems were observed for genotype SAM006 (Table 2). The sum of the three harvests shows that accession SAM006 produced more leaves and stems in comparison to the other genotypes (Table 2).

### Pharmacological characterization

The anti-edematogenic assay of the aqueous extract of *H. pectinata* has shown differences among the studied accessions. Dose 200 mg/kg of accession SAM001 has shown an inhibitory action at the first hour (30.4%) while accession SAM004 only at the third hour (21.0%). For accession SAM005 the inhibitory action was significant at the second hour (25.2%). The accessions SAM002 and SAM003 showed insignificant differences for anti-edematogenic activity at the in the first three hours of the experiment (Table 3). Different results have been obtained by Bispo et al. (2001), in which the anti-edematogenic activity occurred in the 600 mg/kg dose (34.0%).

Dose 400 mg/kg of the accessions SAM002 (1h), SAM003 (2h) and SAM004 (1 and 2h) presented significant inhibitory effect. For accession SAM006 the inhibitory effect was significant for the first phase (1h) (Table 3). For genotypes SAM004 and SAM005 when the dose was rise from 400 to 600 mg/kg, there was a reduction on the inhibition of the edema (Table 3). The accessions SAM002, SAM003 and SAM006 showed a dose dependent effect.

Dose 600 mg/kg of the accessions SAM001 and SAM003 has presented best results of inhibition on the edema in paw, being significant in all intervals of the time evaluated (Table 3), while the accession SAM002 showed significant inhibitory only at the second and third phase (Table 3), which is similar to previous findings (Bispo et al., 2001).

## DISCUSSION

### Morphological and agronomical characterization

In this work plant height do not present a direct relation with dry matter of leaves (Table 2). Suchorska and Osinsk (2001), evaluating five genotypes of basil, observed the genetic variation in plant characteristics. The German's accessions presented smallest plants and the low number of inflorescences. The Egyptian presented tallest plants and major number of inflorescence. It was observed that the plant with higher height resulted in a low weight of dry matter of aerial part. This can probably be due to the interaction among the canopy diameter,

node distance, leaf size and number of leaves per plant.

The data on Table 2 permit to infer about the great variability found in this species, because it is a native and non-domesticated plant. These results together with the pharmacological characteristics of those studied genotypes can be able to allow the selection of promised genotypes for a breeding program. Great variability among genotypes was observed in a work carried out by Blank et al. (2004) who characterized genotypes of *Ocimum* sp.

Researches with the identification of genotypes as those indicated in this work should contribute for strategies of variability conservation and to underline management practice for species such as *Hyptis pectinata* that has not yet been domesticated and that present an economic and medicinal potential for the Northeastern region in Brazil. However, qualitative research on chemical composition and medicinal activity of the different genotypes is essential for genotype selection in future breeding programs using these species.

For the plant height and dry leaf matter yield, the most promising genotype was SAM006. It seems that the best biomass achieved does not always leads to the best medicinal effect of the plant species.

### Pharmacological characterization

The injection of carrageenin into the rat paw induces liberation of histamins and serotoninins at the first hour and later liberation of kinins (2h) and prostaglandins (3h) occur, which are responsible for the formation of inflammatory exudates (Di Rosa, 1974).

The anti-edematogenic effect of accession SAM001 (200 mg/kg) was significant only at the first hour, which suggests that the extract contained chemical substances with anti-histaminic effect. However, when the dosage was increased to 600 mg/kg the anti-edematogenic effect was more explicit, which suggest that the chemical compounds are in minor quantities in the aqueous extract and were insufficient to promote the effect at the minor dosages. We suggest that at the dose 600 mg/kg the aqueous extract interfere on the action of the different chemical mediators, from histamins to prostaglandins.

The anti-edematogenic effect of accession SAM002 was significant for the dosages 200 and 400 mg/kg (1h), which indicate that the chemical compounds of this genotype are related with anti-histaminic substances. In higher concentration (600 mg/kg) the compounds present at the aqueous extract are more effective to inhibit kinins and prostaglandins.

The dose 400 mg/kg of the aqueous extract of accession SAM003 probably possess higher proportion of chemical constituents, which inhibits the action of the kinins (2h). When higher dose (600 mg/kg) was used an increased anti-edematogenic effect was observed which was effective in al the phases, indicating an action on the mediators as histamins, kinins and prostaglandins.

**Table 3.** Effect of aqueous extracts (200, 400 and 600 mg/kg) of *H. pectinata* accessions in carrageenin-induced in rat paw edema.

Treatment (mg/kg)	Mean $\pm$ S.E.M. (ml)				% inhibition of edema
	1 h	2 h	3 h	4 h	
200 mg/kg					
Control	0.456 $\pm$ 0.050	0.714 $\pm$ 0.042	0.737 $\pm$ 0.045	0.597 $\pm$ 0.035	-
Indomethacin 10	0.246 $\pm$ 0.065*	0.330 $\pm$ 0.070**	0.386 $\pm$ 0.074**	0.300 $\pm$ 0.078**	49.1
% inhibition	61.9	65.1	53.7	56.6	
SAM001	0.296 $\pm$ 0.058	0.565 $\pm$ 0.065	0.700 $\pm$ 0.057	0.691 $\pm$ 0.059	10.1
% inhibition	35.1	20.8	5.1	0	
SAM002	0.318 $\pm$ 0.022*	0.634 $\pm$ 0.030	0.709 $\pm$ 0.031	0.610 $\pm$ 0.023	9.4
% inhibition	30.4	11.2	3.9	0	
SAM003	0.391 $\pm$ 0.040	0.660 $\pm$ 0.039	0.700 $\pm$ 0.027	0.633 $\pm$ 0.025	4.8
% inhibition	14.2	7.5	5.1	0	
SAM004	0.324 $\pm$ 0.055	0.609 $\pm$ 0.050	0.583 $\pm$ 0.031*	0.500 $\pm$ 0.034	19.6
% inhibition	29.0	14.7	21.0	16.3	
SAM005	0.330 $\pm$ 0.068	0.534 $\pm$ 0.039**	0.666 $\pm$ 0.062	0.590 $\pm$ 0.051	15.4
% inhibition	27.7	25.2	9.7	1.2	
SAM006	0.410 $\pm$ 0.056	0.624 $\pm$ 0.058	0.804 $\pm$ 0.037	0.733 $\pm$ 0.039	0
% inhibition	10.1	12.6	0	0	
400 mg/kg					
Control	0.456 $\pm$ 0.050	0.714 $\pm$ 0.042	0.737 $\pm$ 0.045	0.597 $\pm$ 0.035	-
Indomethacin 10	0.174 $\pm$ 0.068**	0.249 $\pm$ 0.071**	0.341 $\pm$ 0.076**	0.260 $\pm$ 0.077**	59.1
% inhibition	61.9	65.1	53.7	56.6	
SAM001	0.351 $\pm$ 0.059	0.628 $\pm$ 0.057	0.783 $\pm$ 0.056	0.710 $\pm$ 0.054	1.3
% inhibition	23.0	12.1	0	0	
SAM002	0.278 $\pm$ 0.026**	0.594 $\pm$ 0.057	0.699 $\pm$ 0.041	0.621 $\pm$ 0.042	12.5
% inhibition	39.2	16.8	5.3	0	
SAM003	0.368 $\pm$ 0.057	0.543 $\pm$ 0.036**	0.611 $\pm$ 0.046	0.523 $\pm$ 0.046	18.4
% inhibition	19.5	24.0	17.1	12.5	
SAM004	0.319 $\pm$ 0.020*	0.568 $\pm$ 0.024**	0.644 $\pm$ 0.028	0.586 $\pm$ 0.028	15.5
% inhibition	30.1	20.5	12.7	1.9	
SAM005	0.358 $\pm$ 0.053	0.581 $\pm$ 0.047	0.689 $\pm$ 0.045	0.616 $\pm$ 0.038	10.4
% inhibition	21.6	18.6	6.6	0	
SAM006	0.289 $\pm$ 0.045*	0.614 $\pm$ 0.024	0.756 $\pm$ 0.028	0.679 $\pm$ 0.022	6.7
% inhibition	36.7	14.0	0	0	
600 mg/kg					
Control	0.434 $\pm$ 0.027	0.684 $\pm$ 0.044	0.755 $\pm$ 0.043	0.607 $\pm$ 0.033	-
Indomethacin 10	0.246 $\pm$ 0.065*	0.330 $\pm$ 0.070**	0.386 $\pm$ 0.074**	0.300 $\pm$ 0.078**	49.1
% inhibition	43.2	51.7	48.8	50.6	
SAM001	0.269 $\pm$ 0.036**	0.438 $\pm$ 0.048**	0.574 $\pm$ 0.033**	0.491 $\pm$ 0.036*	28.6
% inhibition	38.0	36.0	24.0	19.0	
SAM002	0.339 $\pm$ 0.050	0.454 $\pm$ 0.029**	0.608 $\pm$ 0.021*	0.588 $\pm$ 0.017	19.8
% inhibition	21.9	33.6	19.5	3.2	
SAM003	0.361 $\pm$ 0.043*	0.436 $\pm$ 0.051**	0.561 $\pm$ 0.042**	0.529 $\pm$ 0.039	23.9
% inhibition	16.7	36.2	25.7	12.9	
SAM004	0.348 $\pm$ 0.053	0.686 $\pm$ 0.081	0.814 $\pm$ 0.078	0.756 $\pm$ 0.085	0
% inhibition	19.9	0	0	0	
SAM005	0.325 $\pm$ 0.056	0.598 $\pm$ 0.045	0.651 $\pm$ 0.030	0.618 $\pm$ 0.042	11.6
% inhibition	25.1	12.6	13.7	0	
SAM006	0.369 $\pm$ 0.040	0.561 $\pm$ 0.066	0.580 $\pm$ 0.061*	0.546 $\pm$ 0.057	17.1
% inhibition	15.0	17.9	23.2	10.0	

Statistical significance: \*p &lt; 0.05, \*\*p &lt; 0.01 vs control

The anti-edematogenic effect of the accession SAM004 (200 mg/kg) is probably due to major proportion of chemical constituents that inhibit the action of the histamins, serotoninins and kinins. The anti-edematogenic effect at dose 600 mg/kg was nearly to null. Its not improbable the existence of a group of chemical constituents with synergistic or antagonistic effect.

The anti-edematogenic effect of the accession SAM005 was effective only by the dose 200 mg/kg. The activity was restricted to the phase of liberation of kinins (2h).

The chemical constituents of the aqueous extract of the accession SAM006, which are responsible for the anti-edematogenic effect, are probably in minor concentration because of the more explicit response by the doses 400 and 600 mg/kg. The minor dose probably presents chemical compounds that cause anti-histaminic effect. By the major dose its probable that the substances of the aqueous extract acts more effective on the inhibition of prostaglandins.

Higher dosages of aqueous extract of some *H. pectinata* accessions cause minor anti-edematogenic effect than minor dosages of other accessions. These results are not according to the principles of pharmacy action, where the effect of a drug is proportionally to its dosage, but the aqueous extract of *H. pectinata* may contain various chemical constituents with synergistic or antagonistic effect.

For this reason we suggest that the aqueous extract of *H. pectinata* possess anti-edematogenic properties in the carrageenin-induced edema in paw model. This fact suggests that the extract can inhibit different aspects and chemical mediators of the inflammation, since carrageenin-induced edema in paw is a classical model of acute inflammation used for the non-steroid anti-inflammatory drug tests evolving chemical mediators like histamins, serotoninins, bradikininins and prostaglandins (Di Rosa, 1974; Vinger et al., 1987). A substance with anti-inflammatory action is the one which causes the inhibition of the carrageenin action, as it happens with the aqueous extract of *H. pectinata*.

It is supposed that differences in pharmacological effects among the accessions can be due to the genetic variability existing within the genotypes, because all the accessions were cultivated at the same local and soil. These results have shown that accessions SAM004 and SAM005 have higher anti-inflammatory effect, since they promoted a stronger inhibition on the rat paw edema at the lowest dose (200 mg/kg). Similar results were obtained using higher dosages of the aqueous extract of the accessions SAM002 and SAM003.

## CONCLUSION

It can be concluded that the accessions SAM004, SAM005 and SAM006 are promising genotypes for a plant breeding program aiming to develop a new cultivar

with high leaf yield and anti-edematogenic effect. These genotypes probably present a set of genes that can be used in a composition of a new *H. pectinata* cultivar.

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