Optimized state of extraction of phenolic compounds of *Boscia senegalensis* (Pers.) Lam. ex Poir. and evaluation of their antihyperglycemic activities

ABSTRACT

The resurgence of diabetes and some side effects of synthetic drugs used for its treatment led us to study a plant used in traditional medicine, *Boscia senegalensis* for its treatment. This work aims to optimize the aqueous extract of *B. senegalensis* and to evaluate its antihyperglycemic activity. For this study, several phytochemical assays were performed, to obtain extracts with better concentrations and good effectiveness. The analyses allowed to see the possible correlations between the variables (time and mass of the powder) then the answers show that the effects have P values 0.05. This indicates that they are significantly different from zero to 95.0% confidence level with different optimal areas for good extraction of phenolic compounds from this plant. Several experiments are performed on normoglycemic rats on the model of oral-induced hyperglycemia. The extract is administered at a single dose of 200 mg/kg in proportion to the animal's weight. Blood sugar normally varies up to a level where the extract has its effects. The peaks are reached at 30 min showing here that the extracts significantly lower blood sugar.

Keywords: *Boscia senegalensis*, optimization, extraction, phenolic compounds, antihyperglycemia.

1. INTRODUCTION

According to the World Health Organization (WHO), diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin or the body does not properly use the insulin it produces. Insulin is a hormone that regulates the concentration of sugar in the blood [1].

The global prevalence of diabetes in 2012 was estimated at more than 371 million diabetics of all types, a significant increase of 110% from the year 2000. It affects about 4% of the world's population and is expected to increase by 5.4% in 2025 [2].

We can distinguish several types of diabetes namely insulin-dependent diabetes (DID) which most often affects children. It can occur at any age resulting in the destruction of up to 90% of insulin-secreting cells causing its deficiency [3]. Non-insulin-dependent diabetes (IDDM) typically begins after age 40 and accounts for 90% of all global cases [4].Recent research on phenolic compounds in general and flavonoids is very advanced because of their various physiological properties such as their antioxidant action [5] modulating the activity of certain enzymes, vasculoprotective [6], anti-inflammatory[7] and antidiabetic [8].

Generally, diabetes is treated with insulin, oral antidiabetics such as hypoglycemic sulfonamides, biguanides, α-glucosidases inhibitors and glinides [9].

However, in most developing African countries, where poverty prevents access to synthetic medicines, people are forced to turn to herbal medicines. More than 80% of the population uses traditional medicine to meet their primary health care needs [10].

In addition, the use of plant extracts is important for reducing the use of drugs, known to be the main trigger for the development and spread of antimicrobial resistance, a major global threat to human health [11].

The active principles of these traditional remedies are not, often elucidated. They coexist in these remedies with other compounds that, in some cases, mask their beneficial actions [12].

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Boscia senegalensis (pers.) Lam. ex Pear of the family Capparidacea is a shrub that can reach 1-5 m in height. It usually grows in clumps. The plant has yellow-brownish fruits at maturity is recommended in the treatment of hemorrhoids, female sterility, intestinal worms, colic [13]. It is one of the species used in the programme to combat desertification [14].

The species grows mainly in the regions of the Sahel, the Centre-North, the North and north of the Boucle du Mouhoun. In Chad, hydroalcoholic extract of *B. senegalensis* seeds was used as a hypoglycemic [15].

Although the work done on *B. senegalensis* is sufficiently advanced, none has been interested in the optimal conditions of antihyperglycemic activity. It is in this perspective that the present work aims to determine the optimal conditions of extraction of the active ingredients of *B. senegalensis* and to study their antihyperglycemic activity.

MATERIAL AND METHODS

2.1. Material

Plant material

The seeds of B.senegalensis are harvested in N'Djamena the capital of Chad. The identification of this species is made at the National herbarium of the Institute of Research in Livestock for Development, IRED (N'Djaména) under the number (n° 1344).

Figure 1: *Boscia senegalensis* seeds *(Togdjim 2023)*

Animal material

The animals used are rats of both sexes (Rattus norvegicus) of the wistar strain, 4 to 6 weeks with free access to water and food. These rats were acclimatized to the conditions of breeding of the Laboratory's pet shop at the Food Quality Control Center (CECOQDA) before the experiment.

2.2. Methods

Sampling and production of *Boscia senegalensis* flour

Boscia senegalensis seeds were harvested in N'Djamena. The seeds were then dried in the shade at room temperature of 37° for 2 weeks and brought to the Physicochemical Laboratory at the Food Quality Control Center (CECOQDA) in N'Djamena/ Chad for conservation. The seeds are then made into powder using the grain mill for extraction and dosing of the chemical elements present.

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Optimization plan

For extraction, an experimental design called a centered composite design was used. This plan aims to make the most of the extraction in order to have an optimal area in good conditions. The composite centered work plan was arranged as follows:

- 2k experiments where k represents the factors and 2 the levels (for this work there are 3 factors: temperature, time and mass ratio);
- 6 star experiments;
- 3 experiments in the centre.

We have 17 experiments (trials).

Determination of water content and dry matter of flour

Humidity (TH): method ISO 672 and ISO 4318:1978 [16]

5g of the sample were put in oven for 1h at 105°C Describe the complete method

The TH was obtained as follows:

$TH=M_0+ MPE - M_1 \times 100/MPE$

TH: Moisture Content M0: Mass of empty crucible; MPE: Mass of test sample; M1: Mass of empty crucible + dry matter.

Dry matter rate: Standard method ISO 1572:1987 [17]

5g were placed in the oven at a temperature of 105°C for 2 hours. The crucible was placed in a dryer for 10 to 15 minutes to cool. The crucible containing sample was weighed again. The dry matter content is obtained by:

% MS= $(M_2-M_0)/(M_1-M_0)$ x100

MS: Dry Matter; M_0: Empty Crucible Mass; M_1: Empty Crucible Mass + Dry Matter; M_2: Empty Crucible Mass + Test Case Mass.

Determination of phytochemical properties

Total phenol content: Makkar et al. method (1993) [18]

1g of flour *Boscia senegalensis* was introduced in a test tube, 20ml of ethanol 70% are added. The whole was agitated vigorously for 1h on vortex and centrifuged at 3500trs/ min for 10min at room temperature. The supernatant was collected in a tightly closed bottle and kept cool.

Aliquots of polyphenol extract (0.05 ml) are placed in test tubes and 100 ml of distilled water has been added. A volume of 0,5 ml of Folin Ciocalteu reagent prepared at 1/10 (v/v) and a volume of 0,4 ml of sodium carbonate at 7,5 % (w/v) are added successively to the test tubes. The tubes are then agitated and the absorbance was measured at 760 nm. The standard range was realized in the same way from a stock solution of gallic acid at 0.02%. The amount of total polyphenols is expressed in milligrams of gallic acid per 100g of dry product (mg/100g) from the regression equation DO = a Q + b established with the range. The calculation formula is as follows:

$$Q = \left[\frac{q \times F \times 100}{(MS \times m)} \right] \times 100$$

With F: dilution factor; m: sample mass of the test sample; q: quantity of material in a test sample, MS: dry matter of the test sample.

Flavonoid content: The colorimetric method of Mitic et al., (2014) [19]

0,5g of the extract are mixed with 0,5 ml of the reaction medium (Methanol/Water/Acetic acid 14:5:1). The solution thus prepared is mixed with 4 mL of the reagent consisting of 133 mg of aluminum chloride (AlCl3.6H2O)

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+ 400 mg of sodium acetate in 100 ml of distilled water. After 15 minutes of incubation, the absorbance is read against a white at 430 nm. The calibration was performed using a 0.2 to 1 mg/mL quercetin solution and the results expressed in mg equivalent quercetin/g dry extract.

Total reducing power: method described by Oyaizu (1986) and Duh et al., (1997) [20]

In a test tube, 1 ml of each extract with 0.5 ml of phosphate buffer solution (0.2 M, PH 6.6) and 2.5 ml of potassium hexacyanoferrate solution [K3Fe(CN) 6] to 1. Describe the complete method?

Extraction

The powder mass (5g) of the plant was tested in a flask containing a constant volume of 150 mL of distilled water. The whole was kept boiling at various temperatures in a water bath meeting the extraction protocol. After cooling the extract is centrifuged at 1500 rpm for 10 minutes. The supernatant (filtrate) was then collected on wattman filter paper and stored in a sterile, hermetically sealed bottle for use following the experiment.\

Antihyperglycemic properties

Prior to the baseline blood glucose measurement, the rats were subjected to an 18 h water fasting. After this fasting time, they were weighed. A drop of blood from the distal end of the tail was deposited on the range of a test strip mounted on a glucometer type One Touch R Ultra TM (CE Life Scan, Inc, USA) for automatic reading. The animals were divided into nineteen (19) lots of three (3) rats and all treated.

Studies of antihyperglycemic properties of aqueous extracts *B.senegalensis* in normal rats with oral hyperglycemia

-Thirty (30) minutes after administration of distilled water (10 ml/kg), the negative control lot received D-glucose solution (2.5 mg/kg);

-In the experimental groups, gavage of the D-glucose solution was done 30 min after administration of different extracts of the same plant at the dose of 2g/kg of body weight.

The products were administered by gavage using a 5 ml tube.

Blood sugar levels were taken on time: 0 mn ,30 mn ,60 mn and 120 mn.

Analysis of results

The analysis of the results was made using the software STATGRAPHICS centurion XVI, Sigma Plot 11.0. For the comparison of mean values, correlations and glycemic parameters, the ANOVA test was used.

3. RESULTS AND DISCUSSION

Determination of total phenols

Table 1 shows the experimental matrix for the determination of total phenols. The concentrations of the different extracts increase with the mass of the powder to a concentration from which the content begins to kiss.

Table 1: Experimental matrix for total phenol assay

N°	Température	Time	Mass ratio	Phenols
1	40	10	10	48,765±4,009
2	80	10	10	50,505±6,387
3	40	30	10	72,631±9,997
4	80	30	10	62,610±12,625
5	40	10	30	105,512±10,918
6	80	10	30	114,576±4,348
7	40	30	30	80,933±19,911
8	80	30	30	59,484±23,146
9	26,36	20	20	47,236±2,063

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10	93,63	20	20	67,702±0,000
11	60	3,18	20	60,019±11,843
12	60	37,32	20	93,033±1,527
13	60	20	3,18	23,369±4,044
14	60	20	37,32	23,167±17,442
15	60	20	20	69,555±1,118
16	60	20	20	51,633±0,111
17	60	20	20	49,690±4,255

Table 2 shows the analysis of variance for total phenols.

Table 2: Analysis of variance for total phenols

Source	Sum of squares	dl	Middle square	F – Ratio	P - Value
A: température	13,8538	1	13,8538	0,03	0,8761
B : Temps	10,2351	1	10,2351	0,02	0,8934
C : masse farine	1156,12	1	1156,12	2,18	0,1832
AA	295.014	1	295.014	0,56	0,4799
AB	223,39	1	223,39	0,42	0,5369
AC	2,1043	1	2,1043	0,00	0,9515
BB	1583,94	1	1583,94	2,99	0,1275
BC	1671,65	1	1671,65	3,15	0,1190
CC	548.659	1	548.659	1,04	0,3428
Erreur totale	3709,81	7	529 974		
Total	9935,93	16			

Table 2 of the analysis of variance for total phenols has no effect because the P-values 0.05, meaning, there is no indication of autocorrelation.

For the analysis of variance of different glycemia, all effects have P-values of 0.05, indicating that they are not significant at the 95.0% confidence level.

As no significance is observed for the case of total phenols, no possibility of having the response surface.

Optimal area of total phenols

Table 3 shows the combination of factor levels that maximize total phenols over the indicated region

Table 3: The optimal values of total phenols

Optimal value = 148,741

Factor	Weak	High	Optimum
Temperature	26,3641	93,6359	93,6359
Time	3,18207	36,8179	3,18207
Flour mass	3,18207	36,8179	32,5815

For the best conditions of extraction of total phenols, it takes a time t=3 min and flour mass m=32 g as the two factors that influence significantly.

Determination of flavonoids

The table below represents the experimental matrix for the determination of flavonoids. Given the different concentrations of our various extracts, we can say that they increase with the mass of the powder previously used for extraction.

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Table 4: Experimental matrix for the determination of flavonoids

N°	Temperature	Time	Mass ratio	flavonoids
1	40	10	10	1,441±0,131
2	80	10	10	1,751±0,102
3	40	30	10	3,101±0,051
4	80	30	10	2,745±0,653
5	40	10	30	7,541±1,153
6	80	10	30	8,965±0,445
7	40	30	30	9,746±0,739
8	80	30	30	12,923±0,842
9	26,36	20	20	3,415±0,151
10	93,63	20	20	6,870±0,343
11	60	3,18	20	4,207±0,610
12	60	37,32	20	14,121±0,858
13	60	20	3,18	0,397±0,113
14	60	20	37,32	17,550±1,540
15	60	20	20	2,965±0,449
16	60	20	20	4,184±0,520
17	60	20	20	4,834±0,353

Analysis of variance and optimization of flavonoids

Table 5 shows the analysis of flavonoids

Table 5: Analysis of variance for flavonoids

Source	Sum of squares	dl	Middle square	F – Ratio	P - Value
A: temperature	7,86851	1	7,86851	1,69	0,2352
B : Time	47,5793	1	47,5793	10,20	0,0152
C : flour mass	254 738	1	254 738	54,59	0,0002
AA	0,00985471	1	0,00985471	0,00	0,9646
AB	0,147669	1	0,147669	0,03	0,8638
AC	2,70058	1	2,70058	0,58	0,4716
BB	21,8514	1	21,8514	4,68	0,0672
BC	1,53869	1	1,53869	0,33	0,5838
CC	19,7878	1	19,7878	4,24	0,0784
total error	32,6626	7	4,66609		
Total	384 886	16			

Two (2) effects have P-values 0.05 indicating that they are significantly different from zero to 95.0% confidence level. For some effects with P-value 0.05, there is no indication of self-correlation.

For the analysis of variance of different glycemia, all effects have P-values of 0.05, indicating that they are not significant at the 95.0% confidence level.

Correlation with flavonoids

After dosage and analysis of flavonoids, several factors influence optimization. At this level (flavonoid), the significant correlations observed here are:

- Flavonoid correlation and powder mass, with r=0.813 and p 0.05;
- Flavonoid correlation and reducing power, r=0.722 and p 0.05.

Optimal zone of flavonoids

Table 6 shows the combination of factor levels that maximize flavonoids over the indicated region

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Table 6: Optimal values of flavonoids

optimal value = 25,989

Factor	Weak	High	Optimum
Temperature	26,3641	93,6359	93,6359
Time	3,18207	36,8179	36,8179
Flour mass	3,18207	36,8179	36,1638

For the best optimal conditions of flavonoid extraction, it takes a time t=36min and flour mass m=36g as these are the two factors that significantly influence.

Determination of reducing power

Table 7 shows the experimental matrix for the determination of reducing powers.

Table 7: Experimental matrix for the determination of reducing power

N°	Temperature	Time	Mass ratio	reducing power
1	40	10	10	201,339±18,309
2	80	10	10	248,145±15,087
3	40	30	10	363,172±30,829
4	80	30	10	338,265±32,469
5	40	10	30	546,058±63,979
6	80	10	30	985,589±85,952
7	40	30	30	828,571±8,418
8	80	30	30	754,112±57,118
9	26,36	20	20	755,348±24,285
10	93,63	20	20	604,839±26,068
11	60	3,18	20	379,487±57,567
12	60	37,32	20	847,364±16,938
13	60	20	3,18	81,916±4,683
14	60	20	37,32	902,116±45,764
15	60	20	20	674,603±67,130
16	60	20	20	764,509±2,953
17	60	20	20	799,929±58,435

Given the different concentrations of our various extracts, we can say that these capacities increase with the mass of the powder and the time of extraction.

Analysis of variance and optimization of reducing powers

Table 8 ANOVA divides the variability of reducing power into separate parts for each effect

Table 8: Analysis of variance for reducing powers

Source	Sum of squares	dl	Middle square	F – Ratio	P - Value
A: temperature	1311,73	1	1311,73	0,09	0,7754
B : Time	86974,5	1	86974,5	5,83	0,0464
C : flour mass	818227,0	1	818227,0	54,86	0,0001
AA	13934,5	1	13934,5	0,93	0,3659
AB	42881,0	1	42881,0	2,88	0,1338
AC	14721,1	1	14721,1	0,99	0,3536
BB	38882,2	1	38882,2	2,61	0,1504
BC	5045,94	1	5045,94	0,34	0,5790
CC	116492,0	1	116492,0	7,81	0,0267
total error	104399,0	7	14914,2		

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Total 1,20025 E6 16

In this case, two (2) effects have P 0.05 values, indicating that they are significantly different from zero at the 95.0% confidence level.

For the analysis of variance of different glycemia, all effects have P-values of 0.05, indicating that they are not significant at the 95.0% confidence level.

Correlation with reducing powers

Several factors influenced the optimization of reducing powers during the experiment. At this level (flavonoid), the correlations observed here are:

- Correlation of reducing powers and flavonoids, r=0.822 and p 0.05;
- Correlation of reducing powers and powder mass, r= 0.826 and p 0.05.

Optimal zone of reducing powers

Table 9 shows the combination of factor levels that maximize the reduction power over the indicated region.

Table 9: Optimum values of reducing powers

optimal value = 943,542

Factor	Weak	High	Optimum
température	26,3641	93,6359	93,6359
Temps	3,18207	36,8179	12,7891
masse farine	3,18207	36,8179	36,4772

So for the best conditions of extraction of reducing powers, it would be necessary to have a time t=12 min and the mass of flour m=36g as these are the two factors that significantly influence.

Antihyperglycemic properties

Table 10 shows the different glycemia at 0, 30 and 120 min.

Table 10: Glycemic values

N°	T (° C)	t (mn)	m/v (%)	Gly 0 min	Gly 30 min	Gly 120 min	30-0	30-120	120-0
1	40	30	30	72,5	172,5	93	137,931	85,484	28,276
2	40	10	30	56,5	142,5	99	152,212	43,939	75,221
3	40	30	10	57,5	117,5	75,5	104,348	55,629	31,304
4	80	10	30	59,5	113	83	89,916	36,145	39,496
5	60	20	20	78,5	138	96	75,796	43,750	22,293
6	93,636	20	20	57,5	158	95	174,783	66,316	65,217
7	26,364	20	20	65,5	142,5	93	117,557	53,226	41,985
8	60	20	3,182	64,5	108,5	96	68,217	13,021	48,837
9	60	20	36,818	92	158,5	97,5	72,283	62,564	5,978
10	80	10	10	71,5	170,5	71,5	138,462	138,462	0,000
11	40	10	10	57	183,5	76	221,930	141,447	33,333
12	60	20	20	61,5	172	72,5	179,675	137,241	17,886
13	60	36,818	20	74,5	179	75	140,268	138,667	0,671
14	60	3,182	20	70,5	139,5	74,5	97,872	87,248	5,674
15	60	20	20	65	159	70	144,615	127,143	7,692
16	80	30	30	68,5	147,5	88,5	115,328	66,667	29,197
17	80	30	10	58,5	131,5	59	124,786	122,881	0,855

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n°: number, t (mn): time in minute, T (°C): Temperature in degrees Celsius/v (%): mass/volume in percentage, Gly: glucose in mg/dL.

There was a significant decrease in blood sugar levels over the 120 minutes for all groups treated with the different extracts. Indeed, from 30 minutes, the difference in the glycemia of the treated groups is already significant ($p<0.05$) compared to the initial untreated control. This drop in blood sugar continues over time and differentially according to the extracts administered to reach the limit.

With respect to differences in blood sugar, blood sugar values are significant at 30-0, 30-120 and 120-0 respectively.

Correlations between blood sugar levels

It is the pairs of variables that allowed us to have the following correlations:

- Blood sugar 120-0 and blood sugar 120 (r = 0.6587 and p = 0.0040);
- Blood glucose 30-120 and blood glucose 120 (r= -0.7597 and p = 0.0004);
- Blood glucose 30-120 and blood glucose 30 (r = 0.7583 and p = 0.0004);
- Blood glucose 30-120 and blood glucose 30-0 (r= 0.6628 and p = 0.0037);
- Blood glucose 30 and blood glucose 30-120 (r = 0.783 and p = 0.0043).

For the correlations between the glycemia presented above, the P values 0.05 are found, indicating that they are significantly different from zero to 95.0% confidence level.

DISCUSSION

This work aims to determine the optimal conditions of antihyperglycemic activities of *B.senegalensis* extracts on an experimental model, normal rats. To do this, to allow us to have more details about the different extraction conditions of our plant, the centered composite plan is used. The solvent used for extraction is distilled water, capable of extracting substances with polar groups (N'Diaye et al., 2008) [21].

In the first part of our work, the results showed the presence of phenolic compounds such as total phenols, flavonoids and total reducers in plant extracts. There is an increasing increase in the levels of elucidated phenolic compounds. This is explained by the fact that the content of phenolic compounds increases with the mass of the flour until the concentration from which the content decreases. It can also be added that the volume of solvent used (150 ml) is not sufficient to extract a significant amount of phenolic compounds. With saturation reached, there would be recapture of the molecules of the complexes. However, the content of phenolic compounds practically does not vary and remains increasing.

We also note in the same part of this work that flavonoid concentrations are lower than those of total phenols. This could be explained by the fact that flavonoids are an integral part of total phenols or that phenolic compounds are more represented by total water-soluble phenols than flavonoids.

The estimated effects of flavonoids have an average of 4.121 1, 244, those of total phenols an average of 55.527 13.2653 and the reducing powers, average of 749 754 70.370, showing here the logic in our analyses of the results. It should also be noted that reducing capacity is significantly important. Reducing power is the ability of extracts to reduce iron III to iron II. The formation of the complex with iron II thus prevents the production of OH radicals, used to assess inhibitory effects (Bhujbal et al., 2009) [22].

By establishing the relationship between the contents of total phenols, flavonoids and the reducing activity of the different extracts of the studied plant, there is no correlation between total phenol content and reducing capacity

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but this relationship is weak with total phenols with. On the other hand, the reducing activity is strongly linked to the flavonoid content.

The second part of our work, the results of this study show that all extracts (administration at a single dose for all extracts to animal batches 200 mg/kg) used are of antihyperglycemic activity. Indeed, from 30 min after gavage, the various extracts slowed the increase in blood sugar by preventing it from exceeding the glycemic peak obtained with the hyperglycemic control at 30 min of gavage. Moreover, these active substances caused a hypoglycemia more or less important from 120 min of the administration as shown by the curve? the time effect of extracts on blood sugar in treated animals. Herbal extracts have been shown to have the same efficacy as antidiabetic drugs and no side effects [23].

The results of our study clearly show a very active use of glucose by peripheral tissues, explained by an increase in glucose tolerance in these tissues when animals are treated with phenolic compounds [24]. In addition, blood sugar variables correlate with P 0.05 indicating that they are significantly different from zero to 95.0% confidence. These correlations result from linear regressions. Cunha et al., (2008) had a positive correlation of insulin-secreting blood glucose drop, which corroborates our present study. We could also add that since each animal is responsible for its physiological state, it will be a little complicated for us to have the optimal area. Then correlations at this level will only be found between blood glucose differences.

CONCLUSION

Mainly in this work, the optimization of the extraction of phenolic compounds shows a richness of the plant *Boscia senegalensis* with a very high rate of polyphenols. Moreover, these phenolic compounds have correlations with powder mass and all effects have P values of 0.05, indicating that they are significant at the 95.0% confidence level.

The second part of this work, the results obtained show an important antihyperglycemic activity of flavonoids, total phenols and reducing powers of the plant. This effect results in an internalization of blood glucose in peripheral tissues, such as the liver and its storage of substance as hepatic glycogen and also an activation of insulin secretion in laboratory animals.

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