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EVALUATION OF THE BIO PROTECTIVE VALUE OF THE LEAVES OF SIXTY VARIETIES OF TARO (*COLOCASIA ESCULENTA*) CULTIVATED IN BURKINA FASO

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ABSTRACT

Colocasia esculenta is a plant of the family of Araceae, of Asian origin, known under the name of taro. Taro is a perennial herbaceous plant whose large leaves are mainly used human consumption in several countries. It is grown in Burkina Faso mainly for its tubers. However, taro leaves are eaten sparingly in Burkina unlike other countries which have made it a staple food. Furthermore, we know that edible leaves are nutrient-rich and bio-protective in nature. The objective of this work was to determine the composition of bioactive elements in these leaves in order to detect their bioprotective role. Analysis of the bioactive constituents gave the following results: flavonoids (3.08 to 270.55 µg EQ / 100 mg fresh leaves), total phenols (46.06 to 474.02 µg Gallic Acid Equivalent (GAE) / 100 mg fresh leaves), acid ascorbic (3.37 to 154.69 µg / 100 mg fresh leaves), 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) (5.26 to 52.21 µg Ascorbic Acid Equivalent (AAE) / 100 mg fresh leaves), Ferric Reducting Assay Power (FRAP) (181.39 to 886.77 µg AAE / 100 mg leaves fresh). This study allowed us to conclude that taro leaves (Colocasia esculenta) studied were of protective interest with regard to their compositions as secondary food metabolites.

Contribution/ Originality: This study is original insofar as no other study has yet been carried out on taro leaves in Burkina Faso. Also, the bio-protective properties found in these leaves will boost their consumption and improve the health of populations. Functional foods will be formulated from these leaves and will reduce malnutrition.

1. INTRODUCTION

Consumption of the leaves has become a common practice these days due to the nutritional qualities contained in these leaves. Moreover, the Food and Agriculture Organization [1] recommended a diversification of the diet through the consumption of tubers, traditional leaves which, according to it, contain both oligonutrients and

bioactive molecules. Among the leaves consumed, we note in Burkina Faso, the leaves of cowpea, moringa, taro... However, having a mainly cereal agriculture, agricultural extension institutions and services in Burkina Faso are less interested in the tuber sector [2] and especially its leaves. Many countries have been able to achieve food security and guarantee their economic growth by improving cereal yields, but other countries like Burkina Faso still remain in poverty and undernourishment despite the efforts made in this area [3]. Efforts should then be extended to other types of culture. If studies are made on certain leaves such as cowpea and moringa, it is not the same for taro leaves (Colocasia esculenta) which is a species at risk in Burkina Faso. However, C. Esculenta has good agronomic potential if its ecological requirements are met [4]. It also has high nutritional and therapeutic values [5]. About sixty varieties of taro are cultivated in Burkina when there is little knowledge of their protective values. In this study we assess the bioprotective value of taro leaves grown in Burkina to help increase agricultural production and food diversification.

2. METHODS

In order to analyze the bioprotective properties of taro leaves, 60 varieties of the species were harvested in the experimental field of the Pr Joseph Ki ZERBO University located in Gampela (25 km east of Ouagadougou).

The leaves of the different varieties were removed using a pair of scissors and placed in plastic bags, closed and labeled. They were then crushed using a grinder (Moulinex) and put in a refrigerator to avoid their degradation.

2.1. Determination of Phenolic Compounds

Five hundred crushed leaves were homogenized in 5 ml of methanol then the homogenate was centrifuged at 5000 rpm for 10 minutes. The supernatant was collected then 5ml of methanol was added to the falcon containing the ground material and the whole was stirred for 24 hours. The mixture was then centrifuged again at 5000 rpm for 10 minutes and the supernatant collected. The supernatants are mixed and we have a concentration of 50mg/ml for the extracts. The supernatant obtained was used for the determination of total polyphenols. Total phenols were estimated by the Folin-Ciocalteu method as described by Singleton, et al. [5]. The method evaluates all of the phenolic compounds by reduction of the phosphomolybdo-tungstic reagent or Folin-Ciocalteu reagent (FCR). A volume of 25 µl of supernatant was then mixed with 105 µl of the Folin-Ciocalteu reagent (0.2 N) in a 96-well microplate and the whole was incubated for 5 min. 100 µl of an aqueous sodium carbonate solution (Na 2 CO 3) at 75 g / l were then added and mixed with the vortex, and finally incubated for 1 to 2 h. After incubation, the optical densities (OD) were read at 760 nm using a spectrophotometer. Three readings were taken per sample. A mixture of 100 µl of FCR and 130µl of Na 2 CO 3 was used for the measurement of the blank. The total phenol contents were determined using a reference curve drawn with gallic acid (0-200 mg / l). The results were expressed in µg Gallic Acid Equivalent (GAE) per 100 mg of ground taro leaves.

2.2. Determination of the Flavonoid Content

Five hundred of crushed leaves were homogenized in 5 ml of 95 ° methanol then the homogenate was centrifuged at 5000 rpm for 10 min. The supernatant was collected and then 5 ml of 95 ° methanol was added to the falcon containing the ground material and the whole was stirred for 24 hours. The mixture was then centrifuged again at 5000 rpm for 10 min and the second supernatant collected. The two supernatants are mixed and we have a concentration of 50 mg / ml for the extracts. The supernatant obtained was used for the determination of total flavonoids. The flavonoid content in our samples was determined by the Dowd method as adapted by Arvouet-Grand, et al. [6]. The method assesses all of the compounds reacting with aluminum chloride (chelating properties). A volume of 75 µl of 2% of aluminum chloride $AlCl_3$ (in analytical methanol) was mixed with an equal volume of 100 mg / ml extract in a 96-well microplate. The optical densities are read after 10 min of incubation at

the wavelength of 415 nm using a spectrophotometer and the values obtained were directly extrapolated using a standard quercetin curve (0- 200 mg / l) previously traced.

For each variety considered, the reading was repeated three times and the results obtained were expressed in microgram quercetin equivalent per 100 milligrams of ground taro leaves (µg EQ/100 mg of taro leaves).

2.3. Quantification of 2,2-Diphenyl-1-Picrylhydrazyl (DPPH)

The anti-radical activity was measured from fresh taro leaves crushed with a solution of DPPH according to Blois [7]. About 500 mg of ground leaves were homogenized in 5 ml of 95 ° methanol and the homogenate was centrifuged at 5000 rpm for 10 min. The supernatant was collected and then 5 ml of 95 ° methanol was added to the falcon containing the ground material and the whole was stirred for 24 hours. The mixture was then centrifuged again at 5000 rpm for 10 min and the second supernatant collected. The two supernatants are mixed and we have a concentration of 50 mg / ml for the extracts. 100 µl of the methanolic solution of the extracts were added to 200 µl of DPPH solution in the 96-well microplate. The mixture was left in the dark for 30 min and the absorbance was read against a blank at 517 nm. The positive control was represented by a standard antioxidant: butyl hydroxytoluene (BHT) and butyl hydroxyanisol (BHA), the absorbance of which was measured under the same conditions as the samples, at concentrations of (0-1.9 mg / ml) and (0-2.5 mg / ml).

2.4. Dosage of Ferric Reducting Assay Power (FRAP)

The FRAP method measures the ability of phenols to reduce Fe (III) ions to Fe (II). The content of the reducing power of iron was determined according to the method described by Sombié, et al. [8]. In a test tube containing 500ul of extract, 1250 µl of potassium hexacyanoferrate [K3Fe (CN)6] 1% in distilled water. The whole was heated to 50°C in a water bath for 30 minutes. A volume of 1250 µl of trichloroacetic acid (10%) was then added and the mixture centrifuged at 2000 rpm for 10 minutes. An aliquot of 625 µL of supernatant is transferred to another learndorf tube to which 625µl of distilled water and 125 µl of freshly prepared 0.1% FeCl3 in distilled water. White without sample is prepared under the same conditions, replacing the extract with distilled water.

The absorbance of the reaction medium was read at 700 nm against the blank which makes it possible to calibrate the device (spectrophotometer). Ascorbic acid was used as a control and its absorbance was measured under the same conditions as the samples. An increase in absorbance corresponds to an increase in the reducing power of the extracts tested.

2.5. Evaluation of the Ascorbic Acid Content

The content of vitamin C was determined according to the method of Mehta, et al. [9]. One hundred mg of fresh crushed leaves were homogenized in 2 ml of distilled water. The whole was centrifuged at 3000 rpm for 5 minutes. The supernatant was used for the estimation of vitamin C. To do this, a volume of 50 ml of the supernatant was mixed with 150 ml of the DCPIP solution (0.2M). The optical densities were read after 10 min of incubation at the wavelength of 515 nm using a spectrophotometer and the values obtained were directly extrapolated using a standard curve of ascorbic acid. previously traced.

3. RESULTS AND DISCUSSION

3.1. Total Phenols and Flavonoids

Total phenols and flavonoids were quantified in methanolic macerations and the results obtained are presented in Table 1. Total phenols were determined from a regression curve obtained from a solution of gallic acid. As for the quantity of flavonoids, expressed in quercetin equivalents (QE), it was determined from a regression curve (quercetin).

The total phenol contents of the methanolic maceration of the leaves vary from 46.06 to 474.02 µg EAG / 100 mg fresh leaves. The variety BF / KE / 10 presented the best content with a concentration of 474.02 µg EAG / 100mg fresh leaves and the variety CE / THA / 13, the lowest content (46.06 µg EAG / 100mg fresh leaves. These results show that taro leaves contain protective properties. Indeed, phenolic compounds are a therapeutically and economically interesting family and arouse much interest by their antioxidant potential. Sombié, et al. [8] in their studies on the leaves of cowpeas found a concentration in total phenols of 2243 µg EAG / 100mg of fresh cowpea leaves. Sombié, et al. [8] Like these cowpea leaf leaves which are widely consumed in our countries, taro leaves could also be consumed. in addition, given the content of phenolic compounds, taro leaves could be exploited in herbal medicine, in specialties for owners's vasculoprotective (flavonoids, anthocyanins, tannins), antispasmodic (phloroglucinol) [10] Prevention and treatment of cancer, inflammatory and cardiovascular diseases and as additives in food, pharmaceutical and cosmetic [11].

The flavonoid contents varied from 3.08 and 270.55 µg EQ / 100mg of fresh leaves in the methanolic extracts.

The highest value was obtained in the variety BF / KE / 19 (270.55 µg EQ / 100mg fresh leaves) and BF / CO / 10 showed the lowest content with a concentration of 3.08 µg EQ / 100mg of fresh leaves. These values are higher than those of Sombié, et al. [8] which found a content of 210 µg EQ / 100mg of fresh cowpea leaves [8]. The remarkable flavonoid content of our leaves is of great bioprotection interest ranging from beneficial biological effects for cardio-metabolic health [12] to antioxidant, anticarcinogenic [13] vasculoprotective, anti-hepatotoxic, antiallergic, anti-inflammatory [14] properties. , antiulcers [15] and even significant anti-tumors [16].

Table 1. Results of the total phenol and flavonoid content.

Varieties	Flavonoid content (µg EQ / 100mg fresh leaves)	Polyphenol content (µg EAG / 100mg fresh leaves)
CE / IND / 16	246.88	104.77
BL / SM / 13	209.48	49.72
BF / KE / 12	33.48	75.42
BF / KE / 05	34.35	87.89
BF / F / KE / 04	19.95	86.06
BL / SM / 143	57.01	145.1
CE / THA / 12	237.21	103.59
BF / CO / 03	53.41	91.23
BF / KE / 11	43.88	78.97
BL / SM / 116	57.81	122.73
BF / KE / 01	83.08	85.2
BL / SM / 115	25.88	134.24
BF / KE / 08	56.21	108.11
BL / PNG / 03	64.35	141.23
BL / HW / 05	213.35	108.65
BF / KE / 13	114.48	126.49
BL / PNG / 08	47.15	113.16
BF / CO / 10	3.08	100.26
CE / IND / 06	24.15	193.38
CE / JP / 02	49.81	158.97
BF / KE / 15	120.75	100.69
CE / THA / 05	158.21	98.32
BL / SM / 136	57.28	461.01
BF / CO / 11	104.15	72.3
BL / SM / 148	26.95	129.29
BL / SM / 135	129.75	151.01
BF / KE / 16	120.75	113.7
BF / CO / 01	40.08	134.88
BL / SM / 147	180.15	190.58
BL / SM / 132	52.48	112.62
CE / MAL / 06	87.28	249.94

Varieties	Flavonoid content (µg EQ / 100mg fresh leaves)	Polyphenol content (µg EAG / 100mg fresh leaves)
BF / CO / 05	6.4	140.87
BF / KE / 09	63.61	414.56
BF / CO / 08	73.61	128
CE / IND / 32	208.61	118.86
BF / KE / 07	117.41	192.62
BL / SM / 120	119.68	137.68
BF / KE / 06	120.35	196.71
CE / THA / 13	70.95	46.06
BF / CO / 06	73.48	296.39
CE / KND / 12	85.15	268.97
CE / IND / 14	43.41	214.45
BF / CO / 04	150.41	350.9
CE / THA / 10	154.75	455.31
BF / CO / 02	36.21	215.85
BF / KE / 17	190.95	264.99
BF / KE / 14	203.75	171.12
BF / CO / 09	127.55	56.17
BL / PNG / 10	159.08	170.58
BF / KE / 19	270.55	99.4
CE / IND / 24	182.41	214.13
BF / KE / 03	165.55	405.96
BF / KE / 10	32.81	474.02
CE / MAL / 02	71.55	413.05
CE / MAL / 12	8.41	294.67
BF / KE / 18	68.21	55.31
BL / SM / 152	89.55	132.62
BF / KE / 02	138.61	170.58
BF / CO / 07	225.21	85.63
BL / SM / 138	59.28	307.14

3.2. DPPH and FRAP

The evaluation of the antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method according to Velázquez, et al. [17] was applied to the methanolic extracts of taro leaves. The results of the antioxidant activity are recorded in Table 2.

The free radical scavenging values of DPPH range from 5.26 to 52.21 µg AAE / 100mg for the leaves. Variety CE / THA / 12 showed the highest value and the lowest activity was observed in CE / IND / 06. These values are different from those of Sombié, et al. [8] who had found a value of 11.90 µg AAE / 100mg of fresh cowpea leaves. Taro leaves have a significant capacity for trapping 2,2-diphenyl-1-picrylhydrazyl radicals. They are then likely to participate in the functioning of certain enzymes, in the transduction of cellular signals, in the immune defense against pathogenic agents, in the apoptosis of tumor cells, in the cell cycle, in the functioning of certain neurons and in particular those of the memory, ovum fertilization, gene regulation [18].

The evaluation of the antioxidant activity by FRAP according to Sombié, et al. [8] shows that in the mechanism of the antioxidant activity of phenolic compounds, the reduction of Fe (III) is often used as an indicator of electron donor. Ascorbic acid was used as the standard for the development of the calibration curve. The results of this study are shown in Table 2.

The values of flavonoid contents vary from 6.40 µg AAE / 100 mg fresh leaves for the variety BF / CO / 05 and 886.77 µg AAE / 100 mg fresh leaves for BL / PNG / 10. Our values are higher than those found by Sombié, et al. [8] in their work on the nutritional value of cowpea seeds. In fact, they had found as a capacity for iron reduction in cowpea seeds a value of 331.46 µg AAE / 100 mg of crushed seed. Hydroxyl radicals are the most highly reactive and endogenous radicals generated during aerobic metabolism. They damage the Desoxyribonucleic Acid (DNA) strand and can cause carcinogenesis, mutagenesis and cytotoxicity [19]. Biological reactions often produce free

radicals in the body which are partly associated with the etiology of cancers and other chronic diseases [19]. In addition, some varieties of taro cause good iron reduction attributes. The leaves of these varieties of taro may reduce the risk of chronic disease and cancer.

Table 2. Results of antioxidant capacity by DPPH and FRAP.

Varieties	FRAP content (µg AAE / 100mg fresh leaves)	DPPH content (µg AAE / 100mg fresh leaves)
CE / IND / 16	254.95	13.68
BL / SM / 13	197.45	18.56
BF / KE / 12	221.61	45.35
BF / KE / 05	181.39	48.70
BF / F / KE / 04	227.81	42.98
BL / SM / 143	254.07	10.54
CE / THA / 12	211.30	52.21
BF / CO / 03	250.85	23.03
BF / KE / 11	254.51	49.63
BL / SM / 116	247.09	21.28
BF / KE / 01	240.33	46.78
BL / SM / 115	283.31	6.19
BF / KE / 08	246.20	41.78
BL / PNG / 03	316.77	10.00
BL / HW / 05	208.98	6.41
BF / KE / 13	192.69	37.38
BL / PNG / 08	262.60	10.30
BF / CO / 10	265.92	37.02
CE / IND / 06	249.97	5.26
CE / JP / 02	190.70	9.55
BF / KE / 15	238.78	38.50
CE / THA / 05	247.86	9.61
BL / SM / 136	191.25	7.04
BF / CO / 11	331.17	40.87
BL / SM / 148	405.84	12.53
BL / SM / 135	308.79	14.83
BF / KE / 16	257.61	41.25
BF / CO / 01	460.68	31.96
BL / SM / 147	544.99	10.79
BL / SM / 132	521.39	14.83
CE / MAL / 06	349.01	12.65
BF / CO / 05	6.40	11.89
BF / KE / 09	399.97	34.63
BF / CO / 08	334.61	24.04
CE / IND / 32	512.64	14.20
BF / KE / 07	352.67	43.50
BL / SM / 120	569.14	14.54
BF / KE / 06	308.79	24.80
CE / THA / 13	402.08	15.59
BF / CO / 06	303.14	42.65
CE / KND / 12	530.81	11.91
CE / IND / 14	310.12	13.88
BF / CO / 04	414.71	19.11
CE / THA / 10	434.76	14.46
BF / CO / 02	339.37	30.70
BF / KE / 17	349.12	30.30
BF / KE / 14	246.86	48.69
BF / CO / 09	508.54	9.87
BL / PNG / 10	886.77	11.19
BF / KE / 19	259.27	30.76
CE / IND / 24	283.20	32.90
BF / KE / 03	415.04	34.65

BF / KE / 10	303.14	39.91
CE / MAL / 02	516.96	14.31
CE / MAL / 12	409.83	14.99
BF / KE / 18	264.70	46.64
BL / SM / 152	363.41	14.29
BF / KE / 02	300.60	26.46
BF / CO / 07	285.64	36.12
BL / SM / 138	531.14	15.65

Ascorbic Acid: The results of our analysis of the ascorbic acid parameter are shown in Table 3.

Table 3. Results of the ascorbic acid content.

Varieties	Ascorbic Acid (µg / 100mg Fresh Leaves)
CE / IND / 16	54.26
BL / SM / 13	43.90
BF / KE / 12	19.31
BF / KE / 05	21.97
BF / F / KE / 04	87.77
BL / SM / 143	55.09
CE / THA / 12	52.76
BF / CO / 03	52.49
BF / KE / 11	52.65
BL / SM / 116	66.28
BF / KE / 01	80.85
BL / SM / 115	68.44
BF / KE / 08	154.69
BL / PNG / 03	40.25
BL / HW / 05	32.77
BF / KE / 13	3.37
BL / PNG / 08	7.18
BF / CO / 10	5.18
CE / IND / 06	76.75
CE / JP / 02	133.53
BF / KE / 15	84.34
CE / THA / 05	26.12
BL / SM / 136	42.35
BF / CO / 11	51.71
BL / SM / 148	24.40
BL / SM / 135	36.53
BF / KE / 16	53.15
BF / CO / 01	34.21
BL / SM / 147	15.10
BL / SM / 132	26.73
CE / MAL / 06	6.40
BF / CO / 05	80.08
BF / KE / 09	25.57
BF / CO / 08	22.63
CE / IND / 32	7.18
BF / KE / 07	10.28
BL / SM / 120	14.93
BF / KE / 06	13.66
CE / THA / 13	24.46
BF / CO / 06	50.49
CE / KND / 12	34.43
CE / IND / 14	15.60
BF / CO / 04	21.13
CE / THA / 10	30.16
BF / CO / 02	28.06
BF / KE / 17	72.82

Varieties	Ascorbic Acid (µg / 100mg Fresh Leaves)
BF / KE / 14	24.29
BF / CO / 09	33.10
BL / PNG / 10	17.09
BF / KE / 19	25.46
CE / IND / 24	23.13
BF / KE / 03	82.23
BF / KE / 10	8.89
CE / MAL / 02	66.23
CE / MAL / 12	21.97
BF / KE / 18	70.60
BL / SM / 152	6.40
BF / KE / 02	54.87
BF / CO / 07	57.92
BL / SM / 138	27.17

The results of analyzes of the vitamin C content of the samples vary between 3.37 to 154.69 µg / 100 mg fresh leaves with an average of 40.70 µg / 100 mg. The lowest content was observed in variety BF / KE / 13. The greatest content was observed at that of BF / KE / 08. For the evaluation of the nutritional value of amaranth leaves, Lc, et al. [20] found a content of 35.56 µg / 100 mg of fresh leaves [20] a value lower than some of ours. This difference can be explained by the stage of maturity of the leaves at harvest time [21]. In Chay-Prove and Goebel [22] Found an ascorbic acid content of 10 µg / 100mg in the leaves of Colocasia esculenta in Australia, a value lower than the average value of ascorbic acid contained in our leaves [22]. This testifies to the richness of the taro leaves cultivated in Burkina in ascorbic acid. Given the importance of vitamin C as a powerful reducing and antioxidant agent capable of limiting the harmful effects of free radicals, taro leaves would be recommended for this purpose.

4. CONCLUSION

Malnutrition remains one of the major problems responsible for infant mortality in Burkina Faso. This is due not only to the quantity and quality of food ingested by the population, but also to the lack of information and popularization of the country's cultures. World Health Organization (WHO) therefore recommends diversifying the various food menus. Diversification which must be accompanied by food security. From our study, we note that the young taro leaves (Colocasia esculenta) were of bio-protective interest with regard to their composition in bioactive elements. They constituted an important source of organic substances (polyphenols, flavonoids etc.). However, studies on the possible toxicity of these leaves should be carried out in order to guarantee the safe consumption of taro leaves.

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