Antioxidant capacity of *Adansonia digitata* fruit pulp and leaves

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In the recent years, in the attempt to counteract the detrimental effects of oxidative damages is always more convincing the strategy of implementing the diet with antioxidants nutrients, especially deriving from natural sources. Etnobotanical studies have confirmed the high content of antioxidant vitamins in Adansonia digitata fruit constituents and leaves. Baobab fruit pulp can be considered a much valuable source containing levels of vitamin C ranging 2,8-3 g/kg. In view of these considerations, by means of photochemiluminescence method (PCL), the Integral Antioxidant Capacity (IAC) of aqueous/methanol extracts from Adansonia digitata fruit pulp and leaves, was assessed in comparison to those deriving from other natural sources of antioxidants, with particular consideration to the contribution from the ascorbic acid component (i.e. orange, kiwi, apple and strawberry). The results were calculated in terms of g fresh weight (FW), uncooked portion size, using Trolox as standard compound. When compared together IAC values for the examined product resulted as follows: Adansonia digitata fruit pulp≥ 90% OPC rich extract > Adansonia digitata dry leaves >> Adansonia digitata leaves glycolic extract >>> strawberry fresh fruit pulp > kiwi fresh fruit pulp > orange fresh fruit pulp > apple fresh fruit pulp and peel. Results clearly indicate the interesting antioxidant properties of the fruit, in particular the IAC value of baobab fruit pulp was 10 time more high than that of orange pulp, with value of 11.1 mmol/g (FW) and 0.3 mmol/g (FW) respectively.

INTRODUCTION

During the last decade, the concept of health promotion has become a legitimate part of health care. Epidemiological evidences links intake of ascorbic acid (AA) and other antioxidant micronutrients to health, in virtue of their capability of trapping reactive oxygen species (ROS) cause of a broad spectrum damages to biological systems [1]. In the recent years, in the attempt to counteract the oxidative stress damages, the strategy of implementing the diet with antioxidants, especially deriving from natural sources, is becoming more and more convincing. In this regards, several studies has been directed toward the evaluation of several naturally antioxidant properties of many naturally occurring botanicals and herbs, potentially useful as nutriceutical ingredients [2]. In the course of our ethnobotanical research directed to highlight the antioxidant capacity of natural antioxidant phyto-extracts, we have recently focused our attention on *Adansonia Digitata L.* (*Bombacaceae*), an african plant known as baobab tree. Leaves, bark and fruits of this tree are traditionally employed in several african regions as foodstuffs and for medicinal purposes, and for that reason baobab is also named "the small pharmacy" or "chemist tree" [3-4]. The native african populations commonly use the Baobab fruit as famine food to prepare decoctions, sauces and natural refreshing drink, due to its nutritional properties [5-7]. The pulp is therapeutically employed as febrifuge, analgesic, anti-diarrhea / anti-dysentery and for treatment of smallpox and measles [4].

Up to date, in an attempt to scientifically substantiate the traditional use of baobab by the natives, several ethnobotany studies have been conducted to characterize the bioactive constituents and the biological, pharmacological properties [8-9]. However, the major interest on baobab product relies in its ascorbic acid (AA, Vitamin C) and dietary fibres content. In particular baobab fruit pulp represents the most important natural sources of AA, while the leaves are characterized by the content on provitamin A [10]. In this regards, the baobab fruit pulp can be considered a highly valuable source containing levels of vitamin C raging from 2,8-3 g/kg, in comparison to the fruits that are generally considered the best source of ascorbic acid approximately six times more than the content of an orange. Regarding other parts of the plant, the highest level of provitamin A was detected in the young leaves, especially when they are used as dried material [11-12]. The content of provitamin A, expressed as Retinol equivalents, is between 9 to 27 mg/kg depending on the method of leaf drying. The combination of small leaves and shade drying pushes the provitamin A content up to 27 mg Retinol Equivalents per gram of dried leaf powder [13].To the best of our knowledge in the regards of antioxidant activity, previous investigations were conducted only on fresh leaves, not considering any other parts of the plant [14]. In view of these considerations, we started the present study in order to investigate, by means of a photochemiluminescence method (PCL), the Integral Antioxidant Capacity (IAC) of aqueous/methanol extracts from *Adansonia digitata* products. Fresh fruit pulp, fruit shell and dry leaves, were evaluated, together with glycolic extracts, in comparison to other fresh fruits (i.e. orange, kiwi, apple and strawberry) and an OPC (oligomeric proantocyanidins) rich vegetal extract, commonly considered rich in

antioxidants. These other products have been taken into account because of the contribution deriving theirs ascorbic acid content (Table 1), but not excluding other antioxidants such are the lipid soluble ones.

The Adansonia digitata fruit

The Baobab fruit pulp is contained in a very resistant external capsule named epicarp; the internal ripe fruit, endocarp, is split in small floury, dehydrated and powdery slices that enclose multiple seeds and filaments, the red fibers, that subdivide the pulp in segments [15]. The ripe fruit pulp appears as naturally dehydrated, powdery, whitish colored and with a slightly acidulous taste, and its separation from the shell only needs of a single mechanical process without any extraction, concentration or chemical treatment [5]. This ensure to the pulp the characteristic of a slightly processed food.

Table 1 - ascorbic acid contents in some fruits, expressed as mg of vitamin each 100 grams of product

Fruit	Latin name	mg ascorbic acid / 100 grams
Baobab	*Adansonia digitata*	150-499 [16]
Kiwifruit, yellow	*Actinidia chinensis*	52[17]-120 [16]
Orange	*Citrus sinensis*	46 [18]
Apple	*Malus sylvestris*	6 [18]
Peach	*Prunus persica*	4-13 [18-19]
Strawberry	*Fragaria x ananassa*	61 [18]

Material and method

Materials

ACW (Antioxidant Capacity of Water soluble substance) and ACL (Antioxidant Capacity of Liposoluble substance) kits (no. 400.801) were purchased from, Analytik Jena AG, Jena, Germany; Trolox ((S)-(2)-6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid)) (no. 39,192-1) was purchased from Aldrich, Sigma-Aldrich, Taufkirchen, Germany). Sample of Baobab fruit pulp, dry leaves, fruit shell grounded , fruit glycolic extract, leaves glycolic extract and fibres were purchased from Baobab Fruit Co.,Verona, Italy; 90% OPC grape seed extract was purchased from Polichimica , Bologna, Italy. Several varieties of each fruit and vegetable, depending on their availability, were purchased at local supermarkets.

Preparation of samples for PCL analysis

Preparation of the Trolox Standard solution

500 _l of Reagent 1 (Kit ACL, AnalytikJena) were added to the vial containing Trolox (Reagent 4, Kit ACL, AnalytikJena) and mixed by vortex for 20-30 seconds. The obtained stock solution was then diluted 1:100 with Reagent 1, in order to prepare the Standard solution with a concentration of 1 nMol/L.

Measurements were done using 10 and 15 µl volumes of the sample, and were repeated two times.

ACW and ACL sample preparation – General procedure

An exact quantity of *Adansonia digitata* products or fruit sample, was suspended in 1 mL methanol HPLC grade, for the measure with the ACL kit, or 1 mL water, HPLC grade, for the measure with the ACW kit, and they were mixed by vortex for 1 minute at room temperature.

The obtained solution was then filtered through HPLC filter (Chemtek Analitica, Bologna, Italy) by a syringe and diluted with Reagent 1 of ACL or ACW kit (AnalytikJena, Jena, Germany).

Results are expressed as mmol equivalents, in antioxidant activity, of Trolox for each gram of product under examination.

Samples preparation for the determination of lipid soluble antioxidant capacity Adansonia digitata fruit pulp

35,9 mg of Baobab fruit pulp were used, and the sample was prepared following the general procedure. The obtained solution was diluted 1:100 and measurements were conducted using 5, 10 and 15 µl volumes of the sample.

Adansonia digitata dry leaves

39,1 mg of Baobab dry leaves were used, and the sample was prepared following the general procedure. The obtained solution was diluted 1:100 and measurements were conducted using 5, 10 and 15 µl volumes of the sample.

Kiwi fruit pulp

218,5 mg of fresh kiwi fruit pulp were sliced and centrifuged, and the sample was prepared following the general procedure. The obtained solution was diluted 1:4 and measurements were conducted using 5 and 10 µl volumes of the sample.

Orange fruit pulp

267,8 mg of fresh orange fruit pulp were squeezed and centrifuged, and the sample was prepared following the general procedure. The obtained solution was diluted 1:10 and measurements were conducted using 10 µl volumes of the sample.

Strawberry fruit pulp

285,4 mg of fresh strawberry fruit pulp were sliced and centrifuged, and the sample was prepared following the general procedure. The obtained solution was diluted 1:10 and measurements were conducted using 10 µl volumes of the sample.

Apple fruit pulp and peel

452,9 mg of fresh apple fruit pulp and peel were sliced and centrifuged, and the sample was prepared following the general procedure. The obtained solution was diluted 1:5 and measurements were conducted using 10 µl volumes of the sample.

Vegetal extract with 90% OPC (Oligomeric proanthocyanidins)

35,8 mg of 90% OPC vegetal extract were used, and the sample was prepared following the general procedure. The obtained solution was diluted 1:1000 and measurements were done using 10 and 15 µl volumes of the sample.

Samples preparation for the determination of water soluble antioxidant capacity

Baobab fruit pulp

50 mg of Baobab fruit pulp were used, and the sample was

prepared following the general procedure. The obtained solution was diluted 1:100 and measurements were conducted using 10, 30 and 50 µl volumes of the sample.

Baobab dry leaves
38,3 mg of Baobab dry leaves were used, and the sample was prepared following the general procedure. The obtained solution was diluted 1:100 and measurements were conducted using 10, 15 and 20 µl volumes of the sample.

Kiwi fruit pulp
256,9 mg of fresh kiwi fruit pulp were sliced and centrifuged, and the sample was prepared following the general procedure. The obtained solution was diluted 1:4 and measurements were conducted using 5 and 10 µl volumes of the sample.

Orange fruit pulp
206 mg of fresh orange fruit pulp were squeezed and centrifuged, and the sample was prepared following the general procedure. The obtained solution was diluted 1:10 and measurements were conducted using 10 µl volumes of the sample.

Strawberry fruit pulp
184,8 mg of fresh strawberry fruit pulp were sliced and centrifuged, and the sample was prepared following the general procedure. The obtained solution was diluted 1:10 and measurements were conducted using 10 µl volumes of the sample.

Apple fruit pulp and peel
470 mg of fresh apple fruit pulp and peel were sliced and centrifuged, and the sample was prepared following the general procedure. The obtained solution was diluted 1:5 and measurements were conducted using 10 µl volumes of the sample.

Vegetal extract with 90% OPC (Oligomeric ProCyanthocyanidin)
35,8 mg of 90% OPC vegetal extract were used, and the sample was prepared following the general procedure. The obtained solution was diluted 1:1000 and measurements were done using 10 and 15 µl volumes of the sample.

Photochemiluminescence method (PCL)

In the PCL assay (Photochemiluminescence) the photochemical generation of free radicals is combined with the sensitive detection by using chemiluminescence. The PCL is based on the photo-induced autoxidation inhibition of luminol by antioxidants, mediated from the radical anion superoxide ($O2^{\bullet-}$) and is suitable to measure the radical scavenging properties of single antioxidants as well as more complex systems in the nanomolare range [20]. Luminol works as photosensitiser as well as oxygen radical detection reagent. The antioxidant potential is measured by means of the lag phase at different concentrations, calculated by a Trolox calibration curve and expressed as mmol equivalents in antioxidant activity of a reference compound (i.e. Trolox). The PCL method was carried out with the procedure described by Popov and Lewin [21]. and can be conducted by two different protocols ACW and ACL that consent to measure the antioxidant capacity of the water- and lipid-soluble components respectively. In the water soluble fraction antioxidants such are flavonoids, ascorbic acid, aminoacids etc. are detected, while in the lipid soluble fraction tocopherols, tocotrienols, carotenoids, etc. are measured. The most widely used methods for measuring antioxidant activity involve the generation of radical species and the presence of antioxidants determining the disappearance of these radicals. Most of the assays determine the antioxidant activity in the micromolare range needing minutes or hours. The PCL assay, which is easy and rapid to perform, presents numerous advantages: it does not requires high temperatures to generate radicals and it is more sensitive to measure, in few minutes, the scavenging activity of antioxidants against the superoxide radical which is one of the most dangerous reactive oxygen species (ROS) also occurring in human body [22].

Results and discussion

In view of the ever increasing importance of health promotion and of the benefits, related to the use of antioxidant rich preparations, we have undertaken the present work to determine the antioxidant capacity of *Adansonia Digitata* fruit and leaves in comparison with that of other, vitamin C rich, known fruits in order to conduce a comparative evaluation. All fruit products were examined in their natural form as fresh wet preparation, avoiding any further chemical-physical process. Moreover, the activity of an antioxidants plant product extract, such is a glycolic grape seeds extract 90% concentrated in OPC (oligomeric proanthocyanidins), was also considered. To this end we have introduced a new parameter termed as IAC (Integral Antioxidant Capacity) which represents the sum of the antioxidant capacity of hydrophilic and lipophilic antioxidants, calculated as mmol equivalents in activity of Trolox, determined in the best experimental conditions for each kind of plant product. In our opinion, it is important to conduce separated determination because of the different nature of the single antioxidants contained in the product under examination. Thus potency of lipophilic antioxidants cannot be properly measured in the same experimental conditions as for the hydrophilic ones. As a consequence, the true antioxidant capacity of a sample will be than better described by the sum of the two separated values. Among the different method available for the determination of antioxidant capacity, the photochemiluminescence method (PCL) has been chosen for its sensitivity and reliability. Moreover, this latter is based on the photo-induced autoxidation inhibition of the luminol by antioxidants, mediated from the radical anion superoxide ($O2^{\bullet-}$) thus it gives a measure of the protective capacity of a plant product against ROS which are, among the many, the most dangerous species of free radicals for leaving beings.

As it can be seen in the Table 2 and Figure 1, the highest water-soluble antioxidant capacity was observed for baobab products, in particular for dry leaves and fruit pulp that resulted endowed with a potent capacity, corresponding to 6-7 mmol/g of Trolox, followed by the glycolic extract from leaves (4 mmol/g). In comparison to the baobab fruit pulp: orange (0,1 mmol/g), strawberry (0,90 mmol/g), apple (0,16 mmol/g) and kiwi (0,34 mmol/g) all resulted endowed with a lower capacity (Table 2, Figure 1). Finally, it is noteworthy to note that pulp and leaves capacity was slightly higher than that of the potent, grape seeds, OPC rich extract (6.16 mmol/g).

Table 2 - Water-soluble antioxidant capacity, corresponding to the activity expressed as mmol equivalents of Trolox for each gram of tested product. The value is the mean of 3 measures ± SD

Products	Trolox s (mmol/g)
Fruit pulp	6,96 ± 0.057
Dry Leaves	6,39 ± 0,344
Fruit shell grounded	9,35 ± 1,100
Fruit glycolic extract	0,93 ± 0,053
Leaves glycolic extract	4,39 ± 0,976
Kiwi fruit Pulp	0,34 ± 0,007
Orange fresh Pulp	0,10 ± 0,009
Strawberry fresh Pulp	0,90 ± 0,004
Apple fresh Pulp	0,16 ± 0,014
90% OPC Vegetal extract	6,16 ± 0,233

Table 3 - Lipid soluble antioxidant capacity, corresponding to the activity expressed as mmol equivalents of Trolox for each gram of tested product. The value is the mean of 3 measures ± SD

Products	Trolox equivalents (mmol/g)
Fruit pulp	4,148 ± 0.706
Dry Leaves	2,35 ± 0,762
Fruit shell grounded	0,46 ± 0,066
Fruit glycolic extract	0,092 ± 0,003
Leaves glycolic extract	0,025 ± 0,002
Kiwi fruit Pulp	0,0035 ± 0,0013
Orange fresh Pulp	0,003 ± 0,0008
Strawberry fresh Pulp	0,0062 ± 0,0002
Apple fresh Pulp	0,0015 ± 0,0002
90% OPC Vegetal extract	4,093 ± 0,070

Concerning the lipid-soluble antioxidant capacity, again baobab fruit pulp resulted the most interesting among those tested. Also in this case it showed the highest capacity (4.148 mmol/g) followed by the 90% OPC rich vegetal extract (4.093 mmol/g) and dry leaves (2.35 mmol/g) (Table 3 and Fig 2). The other plant products considered were all endowed with a very limited capacity, this might be explained on the light of a low content in lipid-soluble antioxidants.

Fig. 1

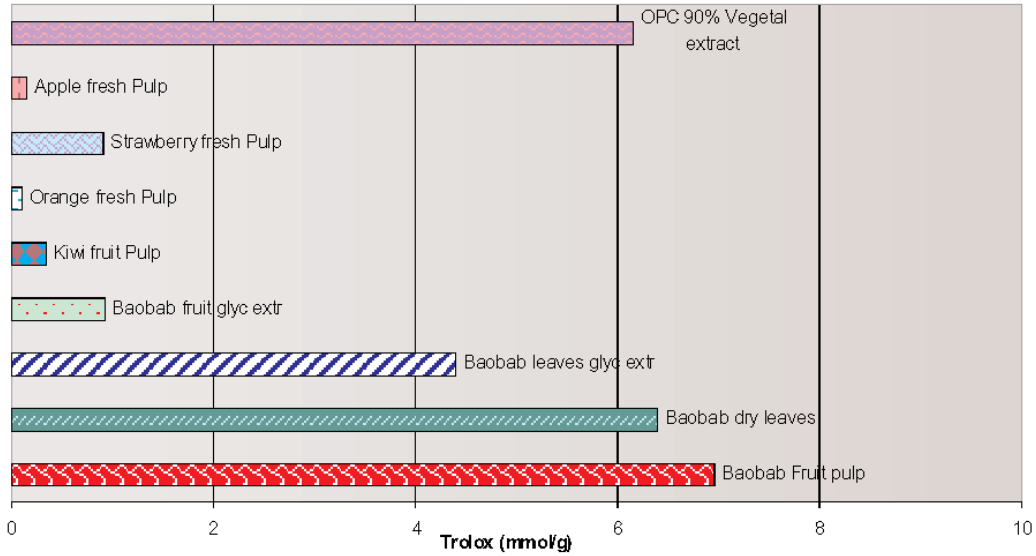
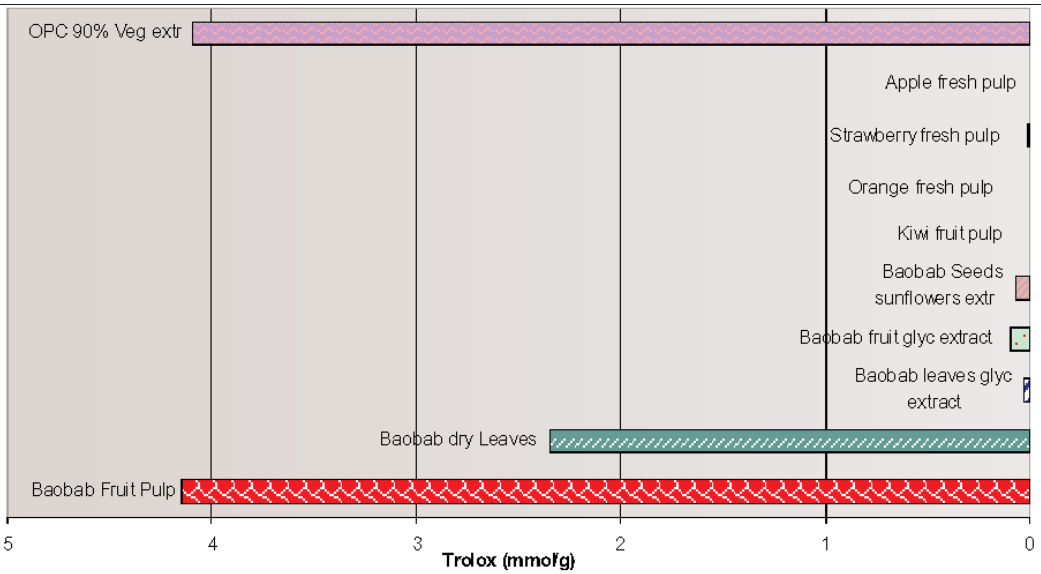


Fig. 2



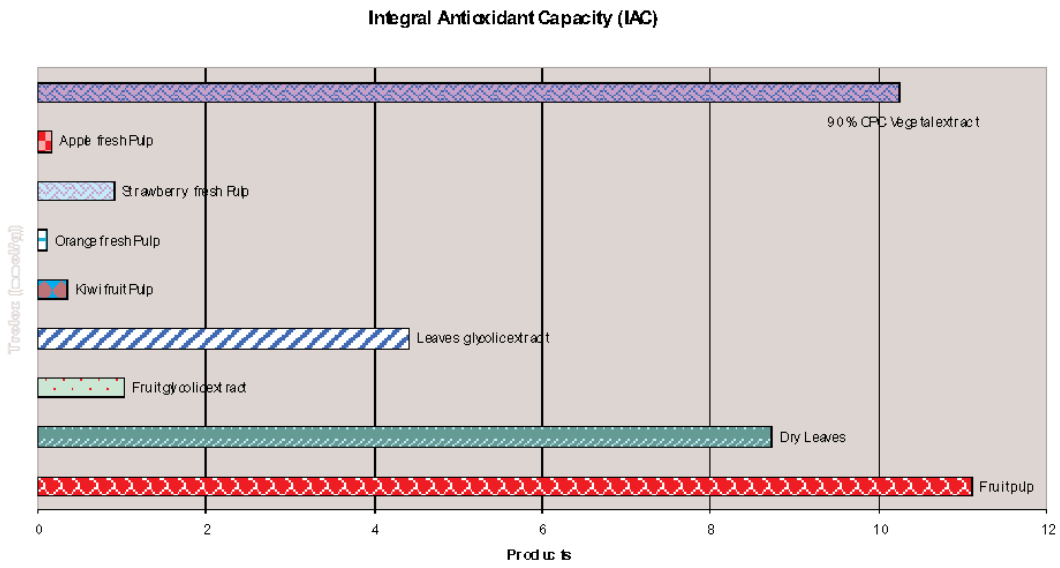
When comparing water- to lipid-soluble antioxidant capacity of plant products, it can be observed that the fruit pulp and dry leaves from Adansonia digitata and 90% OPC rich extract, showed the highest values in both cases. In all other products, the higher antioxidant capacity was observed in the water soluble component, thus suggesting in the ascorbic acid content the major contribution to the activity.

These data well compare with the known values reported in literature (Table 1), for example orange fruit that contain about six time less ascorbic acid in the respect of baobab fruit pulp, shows in our test system, a water-soluble antioxidant capacity which is about 7 times lower that that of the latter fruit.

Table 4 - Integral antioxidant capacity (IAC) corresponding to the sum of the corresponding water- and lipid-soluble antioxidants capacity.

Products	IAC
Fruit pulp	11,11
Dry Leaves	8,74
Fruit glycolic extract	1,02
Leaves glycolic extract	4,41
Kiwi fruit Pulp	0,344
Orange fresh Pulp	0,103
Strawberry fresh Pulp	0,906
Apple fresh Pulp	0,162
90% OPC Vegetal extract	10,25

Fig. 3



As it can be seen in Table 4 and Figure 3, the results of the study can be easily understood by the reading of the IAC values for the evaluated plant products. Taken toghether the data obtained clearly shows that products from *Adansonia digitata* are endowed with very interesting antioxidant capacity. In particular, best capacity was found for fruit pulp with a IAC as high as 11.11 mmol/g of Trolox. Also very interesting were dry leaves (8.74 mmol/g) and leaves glycolic exctract (4.41 mmol/g). The 90% OPC extract IAC value (10.25 mmol/g) confirmed these kind of products as effective in the protection of free radicals, being active in view of the presence of both, lipophilic and hydrophilic, kind of antioxidants. However, in the case of the IAC of *Adansonia digitata* fruit pulp and leaves it is very interesting to note that the activity was related to just a plant component, very sightly processed (drying in the case of leaves and mechanical separation in the case of fruit pulp), thus conferring to the product the as much as possible natural charachteristics. On the contrary, in the case of OPC rich extract, for example, the product was obtained by an enrichment process from the natural source. In conclusion, when compared toghether IAC values for the examined product resulted as follows: *Adansonia digitata* fruit pulp≥90% OPC rich extract > *Adansonia digitata* dry leaves >> *Adansonia digitata* leaves glycolic extract >>> strawberry fresh fruit pulp > kiwi fresh fruit pulp > orange fresh fruit pulp > apple fresh fruit pulp and peel.

Conclusions

This current study reports on the antioxidant capacity of products deriving from *Adansonia digitata*, a plant up to date only known for the high content of vitamin C of the fruit, and for the centenary use in traditional african medicine. This investigation, until all the active components of this plant will be clearly established, was conducted as an initial step to elucidate the therapeutical, nutriceutical and cosmeceutical potential of *Adansonia digitata* plant products. The analytical method used in this study, the PCL assay, was chosen because rapid, relatively simple, and reproducible, making it an attractive biomonitoring tool especially for nutrition and food technologies. In this study we have introduced a novel concept based on the Integral Antioxidant Capacity (IAC), expressed as the sum of the water and lipid antioxidant capacity referred to a common reference compounds, Trolox. This value resulted to be a useful index to describe the capacity of complex samples, such

are those of natural origin, to counteract reactive oxygen species and in particular the superoxide anion, very toxic for human health. If is confirmed that the health benefits of fruits and vegetables are mediated through their antioxidant content, in virtue of high antioxidant capacity bounded to the characteristic of a slightly processed food, it seems reasonable to consider the baobab fruit pulp as new valuable ingredient for food and/or nutriceutical application in the promotion of health.

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