Baobab Dried Fruit Pulp – An application for Novel Foods Approval in the EU as a food ingredient.

Application written and submitted on behalf of PhytoTrade Africa by the natural products consulting company:

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PhytoTrade Africa is registered in South Africa (registration number – 2006/001433/08). The registered name is SANPROTA (ASSOCIATION INCORPORATED UNDER SECTION 21) trading as PhytoTrade Africa. SANPROTA stands for the Southern African Natural Products Trade Association.

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Producers and Manufacturers:

PhytoTrade Africa has 58 members. Producers that want to export their Baobab fruit pulp are required to be assessed for the capacity to produce the product to appropriate standards. For this purpose PhytoTrade Africa operates a Pre-Qualified Supplier (PQS) system. The system includes assessment of members' capabilities regarding:
(1) Raw material supplies – volumes and quality
(2) Traceability
(3) Collation practices
(4) Processing equipment
(5) Packaging
(6) Shipping
(7) Handling orders and logistics
(8) Conformity to PhytoTrade Africa's Fair Trade and Environmental Charters
(9) Exclusivity arrangements with selected partners and clients

Members who achieve PQS status are required to sell their relevant product to PhytoTrade Africa-approved manufacturers and commercial partners (9) above. The members either in, or intending to enter, the PQS programme for Baobab fruit pulp are listed in appendix 1. PQS-status members will only be entitled to access the EU market with Baobab fruit pulp meeting the specification as determined in this application. Further, PhytoTrade Africa collaborates with a trader and manufacturer of Baobab fruit pulp products in South Africa. This company is also listed in appendix 1. The company has HACCP standards and provides additional quality control capability as required by selected EU clients. This company will only be entitled to trade Baobab fruit pulp originating from PhytoTrade Africa's members as listed in appendix 1.

PhytoTrade Africa hereby registers the organisations and companies in Appendix 1 as our "consortium" of producers and manufacturers of Baobab fruit pulp.

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1.0 Introduction

PhytoTrade Africa wish to place on the market in the European Union, dried Baobab Fruit Pulp derived from the fruits of *Adansonia digitata* for use as a nutritional food ingredient. Approval for this product is sought under the EC regulation No. 258/97 which is concerned with the introduction of novel foods and ingredients into the EU and ensures that the novel food in question is assessed for its safety prior to its introduction to the general public. The product is falling under and is also applicable under category 2 (categories of novel foods and novel food ingredients identified in Article 1 (2)(e) of Regulation (EC) No 258/97. This describes the novel food as "Foods and food ingredients consisting of or isolated from plants and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating and breeding practices and which have a history of safe food use. Since the product is derived from plant material obtained from non - GM sources, the classification under category 4, "Scientific Classification of Novel Foods for the Assessment of Wholesomeness" which facilitates the nutritional and safety of the novel food, is applicable. According to Commission Recommendation 97/618/EC which provides guidance on presentation of data required for the safety assessment of novel foods, this product is classified as class 2 "Complex novel food from non –GM sources" and sub section 2.2 "the source of the novel foods which has no history of food use in the community (class 2.2)".

2.0 Specification of Baobab Fruit Pulp

The Baobab tree *Adansonia digitata* is a member of the Bombacaceae family which consists of around 20 genera and around 180 species (Heywood, 1993) including closely related species such as *Adansonia gregori* and *Adanosnia madagascariensis* (Shukla *et al*., 2001). Also known as the "upside down tree", on pollination by fruit bats, it produces large green or brownish fruits which are capsules and characteristically indehiscent. The capsules contain a soft whitish powdery pulp and reinform, kidney shaped seeds (Sidibe & Williams 2002).

The Baobab tree is found primarily in South Africa, Botswana, Namibia, Mozambique and Zimbabwe (Keith & Palgrave, 2000) but it is also common in America (Rashford, 1994), India, Sri Lanka, Malaysia, China, Jamaica and Holland (Sidibe & Williams, 2002). Its taxonomic classification is as follows:

Kingdom	**Plantae**
Division	**Magnoliophyta**
Class	**Magnoliopsida**
Order	**Malvales**
Family	**Bombacaceae**

Bombacaceae in L. Watson and M.J. Dallwitz (1992 onwards) *The families of flowering plants: descriptions*.

2.1 Phytochemistry of Baobab, *Adansonia digitata*

The literature has reported the isolation of a number of compounds from *Adansonia digitata*. These compounds have been found in a various parts of the plant including the seeds, roots, leaves, bark, and the fruits.

2.1.1 Seeds

JP Bianchi et al (1982) reported the extraction of several class of chemicals from the unsaponfiable matter the seeds of two species *Adansonia, A. grandieri* and *A. suarezensis*. The major components were 4-demethylsterols (23-42%), tocopherols (10-37%), and hydrocarbons (15-17%). In both species, sitosterol (81-88%) was the most predominant 4-demethylsterol, and gamma-tocopherol (68-98%) was the major compound in the tocopherol group. In the hydrocarbon fraction, squalene (40-75%) was the major component.

Gray *et al* (1986) reported the isolation of O-acetylethanolamine from the seeds of *A. digitata*. The dansyl derivative was isolated and used to identify the molecular structure. The O-acetylethanolamine is believed to have anti-inflammatory properties. Fatty acids have also been isolated from the seeds, including the identification of cyclopropenoid fatty acids (CPFA's). These compounds are more fully discussed in section 8.4.)

2.1.2 Roots

Kumar *et al* (1987) reported the isolation of a new flavonol from the roots of *A. digitata*. This flavonol glycoside was identified as fisetin-7-O-alpha-rhamnopyranoside. Y N Shukla et al (2001) reported in a review that 3,7-dihydroxy-flava-4-one-5-O-beta-D-galactopyranosyl-(1-4)-beta-D-glucopyranoside and also quercitin-7-O-beta-D-xylopyranoside had also been isolated from the roots.

2.1.3 Leaves

Garrett C Smith et al (1996) extracted a series of vitamin A precursors from the leaves of *A.digitata.* Alpha carotene was found at 1.01mg/100g, beta-carotene was found at 5.92mg/100g, and cryptoxanthin was found at 0.81mg/100g of leaves.

2.1.4 Bark

A review by Y N Shukla et al (2001) reported that friedelin, lupeol, baurenol, and betulinic acid had all been isolated from the bark of *A. digitata.* Tuani et al (1997) found several classes of compounds including flavonoids, glycosides, and tannins, but did not mention specific compounds.

2.1.5 Fruit

Al-Qawari et al (2003), states that the triterpenoids beta-sitosterol, beta-amyrin palmitate, alpha-amyrin palmitate, and ursolic acids have been found in the fruit.
A report by Airan and Desai (1954) highlighted the presence of organic acids in the fruit pulp. These included citric, tartaric, malic, succinic, and ascorbic acid. Nour et al (1980) confirmed the observations of Airan and Desai when they determined that the pulp contained ascorbic acid, tartaric acid, mainly water soluble pectins, and the elements of iron and calcium.

2.2 Specification of PhytoTrade's product – Baobab Dried Fruit Pulp

2.2.1 Introduction

A number of analyses were conducted on PhytoTrade's Baobab dried fruit pulp to establish a compositional profile of the product. Other analyses were used to support the safety of the product, in terms of potential toxic components; to indicate stability of the product and also for potential contamination by pesticides, microbes, etc. The analysis were conducted by Herbal Sciences International Ltd, either “in house” or by using external laboratories were necessary. Where available, accredited methods have been used and the methodology is provided and the accreditation included in the appendices. Where possible and relevant, each analysis has been conducted in duplicate to demonstrate consistency of the results. These analyses were conducted on different batches of the fruit pulp as outlined below. The source of each batch and any blending requirements are described in detail in appendix 2 (confidential).

Baobab dried fruit pulp – samples from each of the three batches analysed were obtained from different regions and labeled throughout this application in the following way:

Batch / Region 1 – (Labelled as H.S.I.300/3/1)
Batch / Region 2 – (Labelled as H.S.I.300/3/2)
Batch / Region 3 – (Labelled as H.S.I.300/3/3)

** When each batch was analysed in duplicate, these were written up in the application with the designation A or B. E.g., duplicate analysis of H.S.I.300/3/1 is labelled as H.S.I.300/3/1/A and H.S.I.300/3/1/B*

Region 3 is PhytoTrade’s main supplier with sources of Baobab dried fruit pulp from region 1 and 2, being included in the application. This is to show the variation of allowable limits in terms of analysis PhytoTrade would like to include for complying with novel foods approval for this product.

PhytoTrade Africa is also collaborating with other companies in order to offer baobab fruit in different product formats to industry. Because of the pectin content solutions can have undesirable viscosity and cloudiness, and can limit product applications. One product format is a depectinised extract powder. This is manufactured using standard industry processing technologies. Processing steps include (i) mixing baobab fruit pulp powder with water (ii) incubating with standard industry enzymes typically used in fruit clarification and processing (iii) separation using flotation and membrane filtration (iv) drying and concentration using standard practices including vacuum.

Since the composition of these products is similar to Baobab fruit pulp itself, we would like the above products to be included in the approval of this novel food application.

The following analysis were undertaken to provide compositional data on each sample and are described in the following section.

2.2.2 Consistency of Baobab dried fruit pulp

The table below presents the analytical data pertinent to describing the composition of the three PhytoTrade Baobab dried fruit pulp samples and their consistency within the parameters chosen.

Table I
Comparative analytical data on PhytoTrade's Baobab dried fruit pulp samples

Component	H.S.I 300/3/1/ A	H.S.I 300/3/1/ B	H.S.I 300/3/2/ A	H.S.I 300/3/2/ B	H.S.I 300/3/3/ A	H.S.I 300/3/3/ B
Moisture (loss on drying) (g/100g)	11.1	11.2	12.4	12.5	13.6	13.7
Protein (g/100g)	3.24	3.15	2.83	2.81	2.04	2.03
Fat (g/100g)	0.5	0.4	0.6	0.7	0.4	0.5
Ash (g/100g)	6.6	6.4	5.7	5.7	5.5	5.5
Total carbohydrate (g/100g)	78.6	78.9	78.5	78.3	78.5	78.3
Total dietary fibre (g/100g)	45.8	46.4	53.9	53.7	51.4	52.2
Available carbohydrate (g/100g)	32.8	32.5	24.6	24.6	27.1	26.1
Energy (kcal/100g)	149	146	115	116	120	117
Energy (kJ/100g)	631	620	488	492	510	496
Total sugars (as glucose) (g/100g)	25.3	24.3	17.0	16.9	21.2	20.9
Sodium (mg/100g)	7.74	7.42	7.92	12.2	8.64	8.83
Saturated fats (g/100g)	0.22	0.20	0.26	0.24	0.21	0.25
Monounsaturated fats (g/100g)	0.14	0.10	0.21	0.23	0.13	0.16
Polyunsaturated fats (g/100g)	0.12	0.08	0.10	0.20	0.04	0.07
Vitamin B1 (HCl) (mg/100g)	0.11	0.09	0.08	0.08	0.05	0.06
Vitamin B2 (mg/100g)	0.02	0.02	0.01	0.02	0.03	0.02

Despite the regional differences and the age of the samples, there are some consistent features that can be gleaned from the data. Moisture content varies by 2% between samples and total ash by 1%. The total fat content of these samples differs by only 0.3g, and the saturated fat content varies by no more than 0.06 g between samples. The total carbohydrate content of the fruit pulp for all samples, ranges between 78.3

to 78.9g, whilst the total sugar content ranges from 16.9 to 25.3g. Total dietary fibre content is high, ranging from 45 to nearly 54%. Vitamin B1 content shows some variation, but there is very little variation with the vitamin B2 content, 0.01 to 0.03 mg/g.

A thin layer chromatographic fingerprint of the PhytoTrade baobab dried fruit pulp samples was also undertaken (see appendix 3 for details and methodology). Here, it was successfully demonstrated that sterols are present in all three samples (Rf = 0.58) by running against a standard, β-sitosterol (Rf = 0.58) at the same time. TLC did not however, separate the individual sterols: campesterol, stigamsterol, isofucasterol, and avenasterol. All of these are reported to be present in *Adansonia digitata* (Gaydou et al, 1982). The tlc fingerprint produced showed consistency throughout all the samples analysed.

3.0 Effect of the production process applied to Baobab dried fruit pulp

PhytoTrade Africa's business focuses on the commercial development of exotic fruits and medicinal plants that are wild harvested in a sustainable manner from non-cultivated plants growing wild in selected areas of Southern Africa (www.PhytoTradeafrica.com). PhytoTrade focuses on species that are abundant and that can be collected with minimal environmental impact. Consequently, the fruits and/or seeds are the main parts of the plant that are collected rather than the roots or the bark of a particular species.

With respect to the Baobab tree, PhytoTrade have focused on the fruits of this plant rather than the leaves, roots and stem bark and the collection of the fruit and its processing are described below.

PhytoTrade Africa use a simple process to obtain the fruit pulp. The fruits are collected from the trees, and the hard shells are cracked open and the pulp is separated from the seeds and shell. This is milled, separated into course and fine lots (particle size 3-600µ) and then bagged. During the processing method, the moisture content falls from an already low value to a resulting material which is around 10 – 13% when packaged.

Product preparation:
The Baobab fruit has a hard shell (epicarp) with a velvety covering. Inside the shell is the seed (pericarp and seed) which are hard and dark coloured, and is surrounded by dry, light / cream coloured fruit pulp (mesocarp) forming lumps. Dry, slightly darker fibrous material is also contained within the fruit.

The fruit pulp / mesocarp is what is consumed traditionally, and is fundamentally equivalent to the product PhytoTrade Africa wished to place on the EU market.

The production process to attain the proposed product specification is simple and exclusively mechanical.

The processing steps are:

- Harvesting of fruits
- Cracking the hard outer shell and removing the content
- Mechanical separation of the seed, fibrous material and mesocarp
- Mesh / screen separation of unwanted fibrous and flaky material from fine, clean mesocarp powder (the baobab fruit pulp powder)
- Storage in clean food-grade packaging

Batch to batch consistency – To minimise fluctuations of the fruit pulp from different areas, within a particular region, the pulp is blended to give a consistent batch/product as described in appendix 2. Specification sheets for each of the different batches are included in the appendices (see appendix 4).

3.1 Stability of Baobab fruit pulp

To demonstrate the stability of the product PhytoTrade analysed samples from the same regions that have been stored for over a year and compared these to recently harvested material from the same areas. The results and conclusions from this are detailed in appendix 5.

4.0 History of the organism used as the source of Baobab dried fruit pulp

In this section the harvesting methods used to obtain the raw material are discussed and also includes methods of transport and storage conditions. These are achieved by using Phytotrade’s quality control system as described below:

4.1 PhytoTrade Africa’s Pre-Qualified Supplier (PQS) system as quality control

(1) Raw material supplies – volumes and quality

Members are assessed to ensure they can supply the correct volumes and quality to the selected market. PQS of baobab fruit pulp are required to have training in quality control programmes for rural harvesters. This includes a system of grading to eliminate any inappropriate quality such as material harvested too early, or too late in the season, for the supply chain. Rural packaging, storage, and transportation must meet agreed standards. The standards are based on accepted raw material supply practices for industries such as out-sourced production of chilly and peppers for paprika product for the EU market.

(2) Traceability

An internal control system capable of meeting requirements of "organic" certification is required. Individual harvesters are trained and registered, and comprise supply groups with co-ordinators or "chairs" of producer groups acting as contact and control points. Material is traceable from storage warehouse back to individual harvesters through practices such as supplying numbered food-grade plastic bags and other audit trail methodologies common in food and pharmaceutical raw material supply chains. The traceability systems are required to be formal and documented for inspection purposes.

(3) Collation practices

Warehousing and bulking-up facilities are inspected for cleanliness and appropriateness for supply into food chains. Pest control systems are required. Minimum and maximum temperatures, moisture and other relevant conditions are inspected.

(4) Processing

The suitability of processing methods and equipment is inspected and required to conform to approved mechanical processes. Approved equipment includes de-hullers, hammer mills, shakers, and sifters / mesh screens.

All processing steps must accommodate traceability requirements through batch numbers for each production run.

(5) Packaging

Only food grade packing materials are approved for use, and are monitored in the PQS system.

(6) Shipping

Only producers with efficient International export and shipping facilities are approved by the PQS system.

(7) Handling orders and logistics

PQS members are required to understand all elements of handling orders and other commercial trade logistics.

(8) Conformity to PhytoTrade Africa's Fair Trade and Environmental Charters

All members sign PhytoTrade Africa's Fair Trade and Environmental charters. PQS members are required to demonstrate fundamental adherence with the principles and practices of the charters.

(9) Exclusivity arrangements with selected partners and clients

PQS members are only allowed to supply export material to PhytoTrade Africa-approved partners and clients. Approved partners and clients are selected on the basis of, amongst other factors, their quality control systems. For example, they would be required to be registered HACCP suppliers with systems required to sell into the EU market.

Baobab's traditional role in the diet at locations outside the community is discussed in the history of use section in 6.0. Further details of the use of Baobab fruit pulp outside of the community are described in appendix 7a along with further details on the abundance of the material in Southern Africa.

5.0 Anticipated Intake of Baobab dried fruit pulp and extent of use

5.1 Introduction

PhytoTrade plan to use Baobab dried fruit pulp in such products as smoothies, cereal bars and other health food products. The anticipated intake of the pulp in such products will be around 5- 10% depending on the particular food product in question. These levels a have been determined by a number of experiments that are outlined below. Further details are available in appendix 6.

Leatherhead Food International carried out preliminary work to determine the potential applications of the pulp as an ingredient in food and drink products. This is a summary of their findings on the use of baobab fruit pulp in smoothies and fruit bars.

5.2 Smoothie Drink Products

To identify the optimum level for smoothie drinks using baobab dried fruit pulp, a series of baobab fruit pulp solutions were prepared. An evaluation of these smoothie products showed that the drinks with 6% and 8% by weight fruit pulp were by the far best.

5.3 Cereal Bar Products

A series of fruit bar formulations containing different quantities of baobab dried fruit pulp were prepared. These were produced by a dry mix process.
An assessment of the cereal bars demonstrated that 5-10% by weight fruit pulp produced acceptable fruit bars with good flavour and a chewy texture.

5.4 Anticipated levels with regard to the medicinal effects of Baobab

Since PhytoTrade seek to use Baobab dried fruit pulp as a food ingredient, literature searches were performed to explore the range of medicinal properties of the fruit and what levels of ingestion were required in order to have the described medicinal effects. These are described more fully in section 6.2. The conclusions reached with regard to the known medicinal effects of Baobab dried fruit pulp, are that in the anticipated levels of use, no medicinal effects are expected to be observed.

5.5 Conclusions

PhytoTrade plan to incorporate approximately 6-10g of baobab dried fruit pulp in 100g smoothies and 10-15g in a 100g fruit bar and similar food products. The Leatherhead Food results clearly demonstrate that these values are acceptable for the incorporation of baobab pulp at these levels. Further uses include a de-pectinated

Baobab fruit pulp and the use of the fruit pulp in other food products such as biscuits, confectionary, and other related food products.

6.0 Information from previous human exposure to Baobab dried fruit pulp or its source

This section details the history of use of Baobab in the EU and in other parts of the world. The possible ant nutritional and potential toxic factors are discussed more fully in section 8.0

6.1 Traditional Food Uses of Baobab Fruit Pulp

The pulp of the Baobab fruit is reported to have numerous uses by the indigenous people of Africa. (Lewicki, 1974). Bosch et al (2004) reported that the fruit had a soft, white, edible and nutritious flesh called monkey bread. This fruit is eaten as a sweet and used to make ice-cream (Bosch et al, 2004).

The Hausi-speaking farmers and Fulani cattle owners, who live in the savannah region of Northern Nigeria, produce an emulsion from the fruit pulp by removing the seeds and fibres, kneading in cold water, and straining this emulsion through a sieve (Nicol 1957). The resulting white or whitish yellow fluid is used to dilute thick guinea corn to a thin gruel, a traditional morning or midday meal (Bosch et al, 2004), Nicol, 1957). This emulsion was also used by the Fulanis to adulterate their milk (Bosch et 2004, Erosmele et al, 1991, Nicol, 1957).

Several authors have reported that the fruit pulp is used to make a refreshing drink (Bosch et al 2004, Carr 1955, Nicol, 1957). In Sudan, this drink is called 'gubdi' and made from cold water to preserve the vitamins (Bosch et al, 2004). Amongst the Hausi farmers, the baobab fruit juice mixture is a popular drink and is available during the hot times of the year (Nicol, 1957).

The baobab fruit pulp is reported to be used in cooking. Amongst some African people the baobab is sometimes called the 'cream of tartar' tree because the acidic nature of the fruit (Carr, 1955). The dried pulp can be used in baking as alternative for cream of tartar (Bosch et al, 2004).

Finally, Diop et al (1988) reported that the pulp was rich in calcium and this was the main reason that the baobab was largely consumed by pregnant women and children in Senegal. A study of pregnant women in Gambia, by Prentice et al (1993), reported that eating the fruit pulp in season (December to April) without the seeds once a day, contributed 30mg/day calcium to the diet.

Further surveys on the use of Baobab in Africa and any local reported toxic effects have also been undertaken by PhytoTrade themselves:

A questionnaire was administered to people attending the PhytoTrade Africa AGM on 30^{th} May 2006. The objective was to try and establish from people with direct knowledge of the uses of Baobab fruit the following (i) what format the fruit is consumed in (ii) who consumes the products (iii) the frequency of the consumption

(iv) the quantities in which it is consumed and (v) whether there are any known toxicity / safety concerns. It is acknowledged that the procedure and questionnaire do not constitute a highly scientific and authoritative study on the subject. However, the responses confirm what is learned from the literature. This includes:

- Baobab fruit is widely consumed in a variety of formats wherever the resource occurs

- The old, young, healthy, infirm and pregnant women are known to consume Baobab fruit

- The product is considered healthy

- There are no known toxicity / safety concerns with the consumption of Baobab fruit products. The only response that could be cause for concern seems to be when the product is consumed in excess, it can encourage diarrhoea. This is common with many fruits. The above questionnaires are detailed in the appendices (appendix 7a edited questionnaire on history and safe use of Baobab fruit).

A further questionnaire, with similar objectives, was administered to a different audience. This audience comprised of professional institutions and authoritative sources of independent of PhytoTrade Africa. The results of the process are to found in the appendices (appendix 7a_toxicity and use questionnaire of Baobab fruit).

PhytoTrade also under took a detailed literature search on the history of use of Baobab fruit pulp. Numerous references were found demonstrating its widespread use not only in Africa, but other continents such as Asia and Australia. The details of this are presented in appendix 7b, but the main findings are summarized here:

Further details of historical use

- Baobab fruit, also known as 'pain de singe' is sold in areas of Paris where the local population is made up of West and Central African immigrants.
- Most of the baobab fruit appears to come via a trade route with Senegal and possibly Mali.
- The Ivory Coast is also believed to another source of importation.

Use in Europe

- The Baobab fruit company is a major supplier in Europe of Baobab fruit (www.baobabfruitco.com).
- Amongst the uses of Baobab fruit in Europe is as an ingredient in the pills known as 'terra lemnia sigilatta.
- Baobab fruit supplements have been endorsed by Italian cyclists, a Formula one driver, and AC Milan football players.

Use in Africa

- Responding to a food frequency questionnaire in Ouassala, Western Mali, households reported that Baobab fruit was consumed on a regular basis.
- Countless references to its use as refreshing drink and a nutritional food.
- Used in baptismal ceremonies as an ingredient for traditional dishes.
- Pregnant women and children singled out as people most likely to consume Baobab fruit.

Use in the Rest of the World

- In Canada, Baobab is listed as a substance in cosmetics and care products regulated under the Foods and Drug act between January 1, 1987 and September 13, 2001.
- In India, *Adansonia digitata* is widespread and similarly consumed by Indians.
- In Australia, *Adansonia gregorii*, a closely related species to *A.digitata* was considered by the Australian Food Standards agency as 'not novel' and given food status in March 2005, presumably indicating its safe us in the Australian community References and further details can be found in appendix 7b.

6.2 Medicinal uses of Baobab Fruit Pulp

6.2.1 Folklore

All parts of the baobab tree are believed to have medicinal properties according to traditional folklore (Haerdi 1964, Kerhero 1974, Kokwaro 1976, Watt and Breyer-Brandwijk 1962).

Of particular interest is the use of baobab fruit and seeds to treat dysentery as reported by both Kerhero and Watt/Breyer-Brandwijk. This is covered by the work of Tal-Dia et al (see rehydration 6.3.4), where dried fruit pulp was used as an alternative to produce a rehydration fluid in children affected by acute diarrhea. The main advantage here was the fact that the baobab fruit pulp powder was available in the local area and did not have to airlifted into the region by aid agencies such as WHO.

Watt/Breyer-Brandwijk also report that baobab has febrifuge properties but do not offer any data on the amount of fruit consumed to treat this condition. Ferbrifuge is another word for anti-pyresis, and will be discussed later (see 6.3.3 Anti-inflammatory, analgesic, and anti-pyretic properties) However, whilst Baobab fruit pulp may lower elevated body temperature, normal body temperature is not affected. (Ramadan et al, 1994).

6.3 Scientific studies

In this section various scientific research work that has been undertaken on Baobab is reviewed. Where applicable it is demonstrated that at the level of intended use, Baobab dried fruit pulp is not expected to display any medicinal effects.

6.3.1 Trypanocidal properties

Atawodi et al (2003) compared the trypanocidal effects of *Adansonia digitata* with the commercial drug DIMANAL *in vitro*. The results demonstrated that the root extract showed no activity with the equivalent of 10g of dried root material, and with 20g of dried root material, there was a small effect, but this was 2 ½ times weaker than

DIMINAL at the same concentration. However, since the root of *Adansonia* and not fruit pulp was tested, these effects anti-trypanicidal are not expected to be observed in PhytoTrades dried Baobab fruit product.

6.3.2 Anti-oxidant properties

Manfredni *et al* (2002) examined the anti-oxidant properties of the fruit pulp from *Adansonia digitata* and compared this with several other fruits including orange, strawberry, kiwi, and a standard, grape seed extract.

The results demonstrated that *Adansonia digitata* fruit pulp had similar *in vitro* anti-oxidant properties to the standard grape seed extract (oligomeric proanthocyanidin) and over 100 times the anti-oxidant activity of oranges.

6.3.3 Anti-inflammatory, analgesic, and anti-pyretic properties

Ramadan et al (1994) demonstrated that the water extract of the fruit pulp of *Adansonia digitata* had similar anti-inflammatory properties to phenylbutazone in rats (see section 8.3 for more details).

Extrapolating these results in a 70kg human would give a comparable dosage of phenylbutazone as 1.05g and baobab pulp as 28g. However, the effective dose of phenylbutazone in humans is 300mg (Bird et al, 1983) and this is approximately 2/3 less than the 1.05g figure extrapolated above. Therefore, PhytoTrade's Baobab pulp should produce effects at 1/3 of 28, which is approximately 9g.

Taking into account the concentration of the fruit pulp by Ramadan and the water content of the PhytoTrade product as approximately 13% by weight, this produces a value of 12 to 18g of baobab fruit pulp that would potential be needed to observe any anti inflammatory effects in humans and this is at a much higher level than PhytoTrade plan to use in various food products.

In the same paper, the analgesic effect of baobab fruit pulp was compared to aspirin.

The 400mg/kg fruit pulp extract showed a slight analgesic effect. Extrapolating this to a 70kg human gives a comparable dosage of aspirin as 3.5g and dried baobab fruit pulp as 28g. However, the effective dose of aspirin is 325mg (BAYER website page) and this is 1/10 of the extrapolated value. Therefore the baobab pulp could produce effects at 2.8g. However, taking into account the concentration of the fruit pulp by Ramadan and the water content of the PhytoTrade product as 13% by weight provides a theoretical value of around 3.8 to 4.2g of baobab fruit pulp that would need to be ingested to observe any effects. However this is only a theoretical extrapolation and with respect to the *in vivo* analgesic effect, one cannot conclude that this analgesia will be observed in humans at the estimated levels, due to differences in both absorption and metabolism kinetics of rodents and man.

The antipyretic effect of the fruit pulp extract in the same paper (Ramadan et al, 1994) was also compared to aspirin in rats. The results showed a marked anti-pyretic effect at both 400mg/kg and 800 mg/kg concentration levels of the extract that was similar to aspirin. However, since anti-pyresis lowers elevated body temperature and does not lower normal body temperature (Avery Complete Guide to Medicines, 2001) these effects are not expected to effect rodents or humans who have normal body temperatures.

6.3.4 Rehydration Treatment

Tal-Dia et al (1997) compared the efficacy of a local solution made from the fruit of the baobab against the WHO standard solution for treatment of dehydration as a result of acute diarrhea. The “pain de singe” as this baobab solution is known locally, is made up in 1 litre of water with 5 sugar cubes and 14 spoons of powdered baobab fruit. This is approximately equivalent to 36.4g of dried Baobab fruit powder.
Although the WHO solution was superior, there was no statistical difference between these two solutions. No medicinal claims were made about the baobab fruit pulp in treating the causes of the acute diarrhea. The solution was simply used to re-hydrate the patients being treated.

6.3.5 Anti-viral properties

Hudson *et al* (2000a) demonstrated the antibiotic and antiviral properties of both *Adansonia* root and leaf extracts against a range of viruses and bacteria. Hudson *et al* (2000b) were able to show that both root and leaf extracts were effective viricidal agents against herpes at levels <62.5 ug/ml *in vitro*. Hussain *et al* (1991) had previously confirmed the antibiotic potential of *Adansonia* root extracts against completely different strains of bacteria. As can be seen all these anti-viral and anti-bacterial effects were found in extracts of *Adansonia* leaves and root, but not the fruit pulp.

6.3.6 Hepatoprotection

Al-Qawari et al (2003) has examined the hepatoprotective effects of Baobab fruit pulp in rats exposed to carbon tetrachloride. Here, the fruit pulp and seeds were soaked in cold water for 24 hours and filtered. This filtrate was collected and freeze dried.

Table II

Hepatoprotective effects of Baobab: Dose, route and length of treatment

Treatment	Dose, route and length of treatment
Saline	1ml saline orally for 5 days
Extract	1mg/kg orally 15 days
Carbon tetrachloride in saline	0.5ml/kg iv + 1ml saline for 5 days
Carbon tetrachloride + extract	1mg/kg extract orally 1 day, then extract + 0.5ml/kg carbon tetrachloride iv for 5 days, followed by extract for 15 days

Several different enzyme markers were used to measure liver damage. ALT (alanine transferase), AST (aspartate transferase), and ALP (alkaline phosphatase) are indicators of hepatic disease. Alb (albumin) is used to indicate severity of the disease.

Table III
Hepatoprotective effects of Baobab: Activities and markers

Treatment	ALT	AST	ALP	ALB
Saline	48.43 +/- 1.03	103.1 +/- 3.2	137.3 +/- 1.1	1.88 +/- 0.01
Extract	45.78 +/- 1.19	110.4 +/- 2.6	135.9 +/- 1.5	1.99 +/- 0.01
Carbon tetrachloride in saline	85.60 +/- 1.01	297.4 +/- 1.6	287.4 +/- 1.6	1.31 +/- 0.03
Carbon tetrachloride + extract	50.31 +/-1.98	126.6 +/- 1.8	149.5 +/- 1.8	1.80 +/- 0.03

These results clearly demonstrate a potential hepatoprotective effect for baobab fruit pulp provided the extract is given before CCl_4 induced liver damage is evident. Extrapolating these results to a human weighing 70kg would provide a value of around 70mg for any effects to be observed. Ingestion of Baobab fruit pulp at the levels in used in various food products could therefore potentially have hepatoprotective effects, although it is not clear how relevant these potential effects are when extrapolating from rats into humans.

6.4 Conclusion

Numerous medicinal effects and ethnic uses of Baobab fruit pulp have been discussed in the literature. However, the data from these reports do not appear to support any medicinal effects occurring when the fruit pulp is consumed at the levels PhytoTrade plan to use. These are 6-10g in a smoothie of 10-15g in a fruit bar and other similar food products. From the above information, and detailed literature searches on the safety of Baobab fruit (See appendix 8) no adverse long or short term health concerns have been reported with using specifically, dried Baobab fruit pulp. Potential toxic factors are however discussed in the safety section of this application section (see section 8).

7.0 Nutritional information on Baobab dried fruit pulp

The fruit pulp of Baobab has a range of nutritional benefits including among others high ascorbic acid content, high pectin content, linoleic acid, and several B vitamins. These are discussed in detail below:

7.1 Ascorbic Acid Content

The ascorbic acid content of baobab fruit pulp has been compared with oranges by Manfredini *et al* (2002), and shown to be at least at least three times higher (150-499 mg/100g vs 46mg/100g). The current EC RDA value is given as 60mg/day and so 10g of fruit pulp would provide at least 25% the recommended daily amount of ascorbic acid.

Table IV
Analysis results of vitamin C content in PhytoTrade's Baobab fruit pulp samples

Sample	Vitamin C content (mg/100g)
H.S.I 300/3/1/A	158
H.S.I 300/3/1/B	163
H.S.I 300/3/2/A	93
H.S.I 300/3/2/B	82.6
H.S.I 300/3/3/A	74.1
H.S.I 300/3/3/B	76.2

Duplicate analysis of the three PhytoTrade baobab dried fruit pulp samples, produced a range of vitamin C content from 74 to 163 mg/100g of fruit pulp (see appendix 9) for more details and the methodology used). These values fall in the lower range of those figures quoted by Manfredini *et al* (2002), but are still higher those found in oranges. From the figures in table IV, PhytoTrade's product contains 1.6 to 3.5 times the ascorbic acid levels of oranges.

7.2 Pectin Content

The dried fruit pulp contains a high level of pectins; indeed the value has been found to be as high as 56% for water soluble pectin content by weight (Sanghi 1978 and Nour et al 1980). These indigestible but soluble fibers are thought to be an important component of our diet.

Table V
Analysis results of pectin content in PhytoTrade Baobab Fruit Pulp samples

Sample	Pectin (g/100g fruit pulp)
H.S.I 300/3/1	23.4
H.S.I 300/3/2	33.8
H.S.I 300/3/3	30.0

Duplicate analysis of the three PhytoTrade baobab dried fruit pulp samples (see Table V above), found pectin levels in the range of 23.4 to 33.8% by weight (see appendix 10 for more details and the methodology used), which is similar to previous published reports described above. Although not as high as the figures quoted by Sanghi (1978) and Nour et al (1980) compared to oranges, PhytoTrade's baobab fruit pulp still has over 9 to 14 times more soluble fibre than for example, Florida oranges (4.4g fibre/185g fruit; nutritiondata.com).

7.3 Fatty Acid Content

Linoleic acid is an omega 6 fatty acid and is an essential fatty acid and natural sources of this fatty acid include sunflower oil and safflower oil. In safflower oil, linoleic acid constitutes 75% of the oil. Compared to oranges where no linoleic acid is detected (nutritiondata.com figures – Florida orange) there is 2.3mg/100g of oil in baobab fruit pulp (Glew et al, 1997). Linoleic acid is the precursor of gamma linolenic acid which is also an omega 6 polyunsaturated fatty acid. Levels of this fatty acid are of the order 15mg/100g fruit pulp (Glew et al, 1997).
PhytoTrade's Baobab dried fruit pulp was analysed to assess the levels of these compounds in their product:

Table VI
Analysis results of Fatty Acid content in PhytoTrade Baobab dried fruit pulp samples

Fatty Acid	H.S.I 300/3/1/A (% total fatty acid)	H.S.I 300/3/1/B (% total fatty acid)	H.S.I 300/3/2/A (% total fatty acid)	H.S.I 300/3/2/B (% total fatty acid)	H.S.I 300/3/3/A (% total fatty acid)	H.S.I 300/3/3/B (% total fatty acid)
Alpha-linolenic acid	20.1	17.5	7.6	8.1	10.4	11.4
Linoleic acid	13.3	13.5	19.9	20.4	18.5	20.0
Oleic acid	19.7	20.6	31.0	31.0	24.9	24.7

Table VI is a summary of the fatty acid profile of duplicate samples from the batches of PhytoTrade Baobab dried fruit pulp. The full range of acids analysed for are included in appendix 11. The range of alpha linolenic acid content is 7.6 to 20.1% of total fatty acids and the range of linoleic acid is much smaller, 13.3 to 20.4 % of total fatty acids. Despite the variation between the individual fatty acids in the samples, the overall percentage of essential fatty acid content, i.e. linolenic acid + linoleic acid + oleic acid, is between 51% (H.S.I 300/3/1/B) to just under 60% (H.S.I 300/3/2/B) of the total content.
In PhytoTrade products, linoleic acid content was found to 13.3 to 20.4% by weight. This is significantly higher than the figures quoted by Glew et al (1997) who stated that there baobab samples contained just 2.3% by weight. There is no obvious answer as to why there is such a large variation, although Glew et al (1997) reported that the boron trifluoride/methanol method was used produce their fatty acid derivatives, whereas in the analysis performed on PhytoTrade's product, sodium methoxide/methanol was used.

The omega n-3 fatty acid precursor, alpha linolenic acid is also present in high amounts, as is the omega n-9 fatty acid precursor, oleic acid. This suggests that dried Baobab fruit pulp provides an excellent source of these essential fatty acids.

7.4 Nutritional constituents

As described earlier, PhytoTrade's Baobab dried fruit pulp also contains a number of common nutritional components as shown in table VII below.

Table VII
Analysis results of the main nutritional components found in PhytoTrade's Baobab dried fruit pulp samples

	H.S.I 300/3/1/ A	**H.S.I 300/3/1/ B**	**H.S.I 300/3/2/ A**	**H.S.I 300/3/2/ B**	**H.S.I 300/3/3/ A**	**H.S.I 300/3/3/ B**
Moisture (loss on drying) (g/100g)	11.1	11.2	12.4	12.5	13.6	13.7
Protein (g/100g)	3.24	3.15	2.83	2.81	2.04	2.03
Fat (g/100g)	0.5	0.4	0.6	0.7	0.4	0.5
Ash (g/100g)	6.6	6.4	5.7	5.7	5.5	5.5
Total carbohydrate (g/100g)	78.6	78.9	78.5	78.3	78.5	78.3
Total dietary fibre (g/100g)	45.8	46.4	53.9	53.7	51.4	52.2
Available carbohydrate (g/100g)	32.8	32.5	24.6	24.6	27.1	26.1
Energy (kcal/100g)	149	146	115	116	120	117
Energy (kJ/100g)	631	620	488	492	510	496
Total sugars (as glucose) (g/100g)	25.3	24.3	17.0	16.9	21.2	20.9
Sodium (mg/100g)	7.74	7.42	7.92	12.2	8.64	8.83
Saturated fats (g/100g)	0.22	0.20	0.26	0.24	0.21	0.25
Monounsaturated fats (g/100g)	0.14	0.10	0.21	0.23	0.13	0.16
Polyunsaturated fats (g/100g)	0.12	0.08	0.10	0.20	0.04	0.07

The variation of these components has been described earlier in this application (see consistency of Baobab dried fruit pulp in section 2.2). Full details and methodology can be found in appendix 12.

7.5 Vitamin B Content

A range of B vitamins are also present in the fruit pulp including thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), and pyridoxine (vitamin B6). Values are presented below per 100g of fruit.

Table VIII
Comparison of B vitamin content in Baobab fruit from various sources

B Vitamins	**Baobab fruit pulp (Baobab Fruit Co.) (mg)**	**Florida oranges (nutritiondata.com) (mg)**	**EC RDA values (mg)**
Thiamine(B1)	0.038	0.12	1.40
Riboflavin(B2)	0.06	0.06	1.60
Niacin (B3)	2.16	0.38	18
Pyridoxine (B6)	2.13	0.38	2

PhytoTrade's samples compared favourably with the literature values as can be seen from the following table:

Table VIIIa
Analysis of Vitamin B levels in PhytoTrade's Baobab dried fruit samples

	H.S.I 300/3/1/ A	**H.S.I 300/3/1/ B**	**H.S.I 300/3/2/ A**	**H.S.I 300/3/2/ B**	**H.S.I 300/3/3/ A**	**H.S.I 300/3/3/ B**
Vitamin B1 (HCl) (mg/100g)	0.11	0.09	0.08	0.08	0.05	0.06
Vitamin B2 (mg/100g)	0.02	0.02	0.01	0.02	0.03	0.02

A comparison of the PhytoTrade analysis results (table VIIIa) with the those quoted by the Baobab fruit company (see table VIII) shows that all the PhytoTrade samples have higher vitamin B1 contents but lower vitamin B2 content (see appendix 12).

7.6 Trace Elements

A comparison of the data for PhytoTrade's product shows that phosphorous and calcium levels are in broad agreement with the figures of Glew et al (1997), whilst being higher compared to Kalenga Saka et al (1994). Magnesium levels for the PhytoTrade product are 15 to 40% lower than either of fruit pulps examined by Kalenga Saka et al (1994) and Glew et al (1997). There are no values for the potassium levels from the Glew et al (1997) paper; however, the PhytoTrade fruit pulp has 15 to 30% lower potassium than the Kalenga Saka et al (1994) sample. The levels of iron are comparable with Kalenga Saka et al (1994) producing a median figure of 65 mg/kg. However, the iron levels are much higher than those detected by Glew et al (1997).

There are also lower levels of sodium in PhytoTrade's product compared to both the other samples, nearly 40% lower than the sample of Glew at al (1997) and other 6 times lower than the sample of Kalenga Saka et al (1994)

Table IX
Comparison of Trace Metal Analysis Results with data from Kalenga Saka et al (1994) and Glew et al (1997)

Trace elements	Data from Kalenga Saka et al (1994) (mg/kg)	Data from Glew et al (1997) (mg/kg)	Herbal Sciences International ltd analysis data (mg/kg)
P	450	733	561-733
Ca	1156	3410	2570-3700
Mg	2090	2090	1260-1790
K	28364	-	20100-23900
Na	188	54.6	7-31
Fe	58	17	39.5-91.3

As can be seen from the above table, the variation in phosphorous and potassium levels is relatively small. The same is also true for the copper and zinc levels (see appendix 13). Sodium levels are of the order 7 to 12 mg/kg, apart from one very high reading of 31 mg/kg in sample H.S.I. 300/3/1. The remaining elements, calcium, manganese, iron and magnesium show some variation in content. H.S.I. 300/3/1 has the highest magnesium and manganese levels, H.S.I. 300/3/2 has the highest calcium levels, and H.S.I. 300/3/3 has the highest iron levels (see appendix 13 for more details and the methodology used).

7.7 Heavy Metal Analysis

PhytoTrade's product was analysed for four main heavy metals: Mercury, Lead, Cadmium and Arsenic and the analytical results were compared with the EU regulation standards for content of heavy metals in foodstuffs. In UK law, arsenic in foodstuffs is set at 1mg/kg (Arsenic in Food Law 1959, as amended). Clearly, all the PhytoTrade samples show levels of arsenic below this standard. Cadmium is regulated by EU Law under EC/466/2001 where the level of this heavy metal is set at 0.05 mg/kg wet weight. Once again, the levels are well below this safety standard. Mercury levels are governed by EU law under the regulations EC/466/2001 and recently amended as EC/78/2005. These levels are set at the lowest level of 0.5mg/kg wet weight. All fruit samples are below this standard. Lead levels in food are also governed by EC/466/2001, but this was recently amended with SANCO/15/2004 rev 1. The maximum permitted level for lead is 0.2 mg/kg in dried vine fruit, such as sultanas, raisins and currants. There does not appear to be a level specifically set for fresh or other dried fruits. The maximum 0.2 mg/kg level for lead is currently under review as detailed in SANCO/15/2004 rev 1 whilst more data is collected.

Table X
Analysis Data of Heavy Metals Content in PhytoTrade's Baobab dried fruit samples

sample	Arsenic (mg/kg)	Cadmium (mg/kg)	Lead (mg/kg)	Mercury (mg/kg)
H.S.I 300/3/1/A	< 0.10	< 0.010	0.28	< 0.004
H.S.I 300/3/1/B	< 0.10	< 0.010	0.36	< 0.004
H.S.I 300/3/2/A	0.26	0.010	0.19	< 0.004
H.S.I 300/3/1/B	0.28	0.010	0.13	<0.004
H.S.I 300/3/3/A	< 0.10	< 0.010	0.09	< 0.004
H.S.I 300/3/3/B	< 0.10	< 0.010	0.08	<0.004

The full details including the methodology are to be found in appendix 14.

7.8 Amino acid content
PhytoTrade's samples were analysed (see appendix 15) for a range of common amino acids as portrayed in table VIII below:

Table XI
Analysis of amino acid composition in PhytoTrade's Baobab dried fruit samples

Amino acid (total)	H.S.I 300/3/1/A	H.S.I 300/3/1/B	H.S.I 300/3/2/A	H.S.I 300/3/2/B	H.S.I 300/3/3/A	H.S.I 300/3/3/B
Tryptophan (g/100g)	0.05	0.05	0.04	0.04	0.03	0.03
Aspartic acid (g/100g)	0.34	0.34	0.26	0.27	0.2	0.2
Serine (g/100g)	0.34	0.34	0.27	0.33	0.23	0.28
Glutamic acid (g/100g)	0.37	0.39	0.36	0.38	0.29	0.29
glycine(g/100 g)	0.18	0.19	0.16	0.17	0.13	0.14
Histidine (g/100g)	0.07	0.07	0.06	0.07	0.06	0.07
Arginine (g/100g)	0.13	0.13	0.13	0.13	0.1	0.1

Amino acid (total)	H.S.I 300/3/1/A	H.S.I 300/3/1/B	H.S.I 300/3/2/A	H.S.I 300/3/2/B	H.S.I 300/3/3/A	H.S.I 300/3/3/B
Threonine (g/100g)	0.17	0.18	0.14	0.16	0.12	0.13
Alanine (g/100g)	0.19	0.19	0.16	0.17	0.13	0.14
Proline (g/100g)	0.22	0.25	0.2	0.21	0.17	0.16
Cystine (g/100g)	0.1	0.09	0.07	0.08	0.05	0.05
Tyrosine (g/100g)	0.11	0.11	0.08	0.08	0.07	0.08
Valine (g/100g)	0.14	0.15	0.11	0.11	0.09	0.1
Methionine (g/100g)	0.04	0.04	0.03	0.04	0.02	0.03
Lysine (g/100g)	0.16	0.17	0.12	0.12	0.1	0.1
Iso-leucine (g/100g)	0.1	0.12	0.08	0.08	0.07	0.07
Leucine (g/100g)	0.18	0.19	0.16	0.16	0.13	0.14
Phenylalanine (g/100g)	0.16	0.17	0.15	0.15	0.12	0.12

Despite the variation in age of the batches and the different geographical harvesting areas of the PhytoTrade Baobab dried fruit pulp samples, the amino acid analysis produced consistent results. The only notable exceptions were with aspartic acid and glutamic acid content where the variation across all samples was 0.2 to 0.34 g/100g and 0.29-0.39 g/100g of Baobab dried fruit pulp respectively. See appendix 17 for more details of the methodology used and the analytical results.

7.9 Microbiological Content

All three PhytoTrade samples were screened for a range of microbiological contaminants (see table XII below):

Table XII
Averaged values of PhytoTrade's Microbiological Analysis Results

Microbiological test	H.S.I 300/3/1	H.S.I 300/3/2	H.S.I 300/3/3
Total viable count (cfu/g)	3.77×10^3	250	3.20×10^4
Coliforms (presumptive) (cfu/g)	< 10	< 10	< 10
E.coli (presumptive) (cfu/g)	< 10	< 10	< 10
Staphylococcus aureus (cfu/g)	< 20	< 20	< 20
Faecal *streptococci* (cfu/g)	< 20	< 20	< 20
Yeasts (cfu/g)	< 20	< 20	< 20
Moulds (cfu/g)	3.49×10^3	570	1.19×10^4
Salmonella sp. (/25g)	Not detected	Not detected	Not detected

All samples from the three batches of PhytoTrade Baobab dried fruit pulp showed low levels of Coliforms (presumptive), *E.coli* (presumptive), *Staphylococcus aureus,* Faecal *streptococci,* and also the Yeasts, and *Salmonella sp.* were within acceptable safety limits (*E.coli* and *Salmonella* levels governed by EC/2073/2005). Total viable count levels for all three samples are within the safety limit of < 10^5 cfu's for dried fruit (Working Group Document, Communicable Diseases and Public Health **3** pp163-167 [2000]). Mould levels were also detected at relatively low levels. The methodology and details of the results can be found in appendix 16.

7.10 Pesticides Residues Content

All three baobab batches were subjected to multi-residue screen for pesticide content. The samples from the three Baobab dried fruit pulp batches were mixed together into one sample and this produced the following results (see table XIII below):

Table XIII
Results of PhytoTrade's Baobab Fruit Pulp Multi-Residue Pesticide Screen

Class of pesticide	Residue detected (mg/kg)
organophosphorous	< 0.05 mg/kg
organochlorine	< 0.02 mg/kg
organonitrogen	< 0.05 mg/kg
dicarboximides	< 0.05 mg/kg
strobilurin	< 0.05 mg/kg
triazine	< 0.05 mg/kg
pyrethoids	< 0.05 mg/kg

The multi-residue screen results have been resolved into the main classes of pesticides as shown above. For all classes of pesticides, the results were below the detection levels of this screen and within the guidelines laid down by The Pesticides Regulations 2006 (Statutory Instrument 2006 no.985).

The PhytoTrade Baobab fruit is wild harvested, so no pesticides are expected to found (see appendix 17 for more details and the methodology used). These results are in agreement with previous data on other species that PhytoTrade, "wild harvest" and which also failed to contain any pesticide residues within the limits specified.

7.11 Anti-nutrients in Baobab Dried Fruit Pulp

Liener (1980) reports that there are four main classes of anti-nutrients in foodstuffs. These are protein inhibitors, glycosides, phenols, and a miscellaneous class which includes anti-vitamins, anti-enzymes, and toxic fatty acids.

The analysis of the PhytoTrade Baobab dried fruit pulp samples has specifically looked for the presence of four such anti-nutrients. Cyanide analysis of the fruit pulp involved examining the aqueous extract and an hydrolysis extract to determine whether any cyanogenic glycosides or free cyanide were present. No cyanide was detected at the limit < 5 mg/kg (see appendix 21 determination of cyanide content in Baobab dried fruit for more details)

All the PhytoTrade Baobab dried fruit pulp samples were tested for alkaloid content by comparison with known alkaloid standards. Thin layer chromatography analysis revealed no alkaloids present at the detection limit of 10 µg/g of fruit pulp (for more details see the safety section of the application).

Finally, the PhytoTrade Baobab dried fruit pulp samples were tested for the presence of potentially toxic fatty acids. Erucic acid has been shown to have a variety of health impacts in studies, and foods containing high levels of this fatty acid are considered unfit for consumption. The EC limit for erucic acid in foods has a statutory limit of < 5% total content in oil (The Erucic Acid in Food Regulations, 1977).
The fatty acid analysis of all three samples showed levels below the detection limit of < 0.10% (see appendix 11-determination of fatty acids in Baobab dried fruit pulp by gas chromatography).

Cyclopropene fatty acids were also analysed for. These are known to be present in Baobab seed oil and can display toxic effects in animal models fed a diet, very high in these cyclopropene fatty acids (Bezard et al, 1993a, Bezard et al, 1993b, Gaydou et al, 1995, Bezard et al, 1996). This is discussed more fully in section 8.4.The PhytoTrade Baobab dried fruit pulp samples showed a maximum level of 0.26 mg/g of these compounds, well below the limit expected to show any toxic effects to humans - see appendix 18 for more details of the analysis.

In conclusion, several classes of anti-nutrients were tested for in PhytoTrade Baobab dried fruit pulp samples and all were found at very low levels.

8.0 Safety Profile and Toxicity Studies of Baobab dried fruit pulp

The following information is provided on Baobab dried fruit pulp to support its safe consumption in the community.

8.1 Introduction - Literature surveys and history of use

Baobab is widely consumed in many parts of Africa and this has been detailed in section 6.0. Also PhytoTrade have conducted extensive literature searches on any adverse effects or any potential toxicity reports of the pulp - see appendices 7a and 8. No mention was found with regard to Baobab fruit pulp in these searches, supporting the safe history of use of this product.

The following studies are also included to support the safety and lack of toxicity of Baobab dried fruit pulp in the amounts intended for use.

8.2 Skin test- Irritation

To determine whether baobab fruit pulp (from a different source from Phytotrade's product) would produce any irritant effects, Marzatico (2001), exposed 25 volunteers to a 10% dilution of the fruit pulp solution using a modified Draize test.

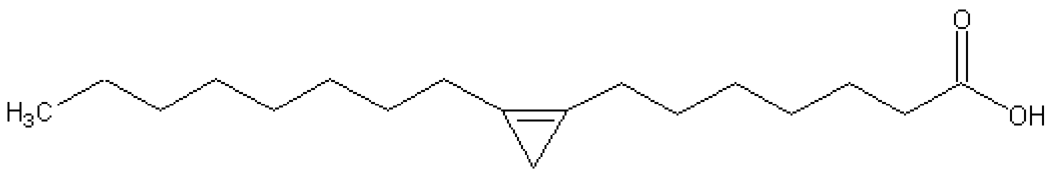
The modified Draize test involved subjecting the human volunteers to a continuous induction period with patch exchange 3 times a week until a total of 10 patches had been applied. The patches were reapplied to the same site, and only if moderate inflammation had developed, the next patch was moved to an adjacent skin site. The human volunteers were challenged on naive skin two weeks later with a 72 h patch test of a non-irritating concentration of the compound.

The results demonstrated that baobab fruit pulp was non-irritant.

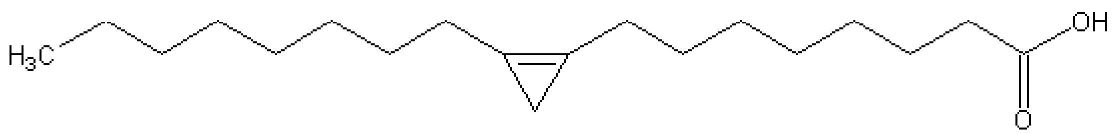
8.3 LD50 Test in rodents

Ramadan et al (1994) carried out LD_{50} studies in rodents to determine the acute toxicity of baobab fruit pulp (which was from a different source from Phytotrade's product). An aqueous extract was produced from freeze dried pulp that had previously been extracted with distilled water (80g filtered freeze dried product from 110g of fruit pulp). Increasing amounts of the aqueous extract were administered to rodents via intraperitoneal administration until 50% of the sample group had died. The results produced an LD50 value in rodents of 8000mg/kg. If this value is extrapolated to a 70kg human this would provide an LD50 value of 560g. However, as we have previously mentioned, PhytoTrade's Baobab dried fruit pulp has not been concentrated through extraction and also contains around 13% water by weight. This means that the predicted LD50 value for PhytoTrade's product would be around 1/3 to ½ of this value; giving an extrapolated LD50 value of around 746 to 840g. Since the anticipated intake of PhytoTrade's Baobab fruit pulp is around 15 g/day, this is well below the predicted LD50 value of 840g.

8.4 Cyclopropene Fatty Acids
Cyclopropene fatty acids, such as malvalic acid and sterculic acid (see diagram below), are found in the seed oil of baobab, however, there are no reports of these chemicals being present in the fruit pulp itself. There have been several studies on the effect of these compounds both *in vitro* in rat liver microsomes and *in vivo* in rats (Bezard et al, 1993a, Bezard et al, 1993b, Gaydou et al, 1995, Bezard et al, 1996). All rats were fed various diets that included 10% weight oil, of which the cyclopropenoid fatty acid content was 13.7%.



malvalic acid (8,9-methylene-8-heptadececenoic acid)



sterculic acid (9,10-methylene-9-octadecenoic acid)

All these studies reported effects that inhibited the fatty acid biosynthetic pathways, specifically affecting the desaturation pathways of lipids. This resulted in decreased membrane quality, decreased eicosanoid biosynthesis (e.g. arachidonic acid), dysfunction of adrenals resulting in decreased biosynthesis of corticosteroids, and decreased liver P450 enzyme activity.

There have been no adverse health reports in humans (Aitzetmuller, 1996), and this is believed to be due to the fact that indigenous people heat the oil during cooking. This is known to decrease the levels of cyclopropene fatty acids to very low levels. PhytoTrade's Baobab Fruit Pulp was analysed for the detection of CPFA's using GC-MS and comparison to known cyclopropene fatty acids. The exact methodology is in appendix 18 but the main results are reported below in table XIV:

Table XIV

Analysis of PhytoTrade's dried Baobab fruit pulp for cyclopropene fatty acids

Fatty Acid	**HSI300/3/1 A (mg/g)**	**HSI300/3/1 B (mg/g)**	**HSI300/3/2 A (mg/g)**	**HSI300/3/2 B (mg/g)**	**HSI300/3/3 A (mg/g)**	**HSI300/3/3 B (mg/g)**
16:0 palmitic	0.63	0.67	1.21	1.15	0.90	0.89
16:1 hexadecenoic	0.02	0.01	0.02	0.01	0.02	0.02
17:0 heptadecenoic	0.01	0.01	0.02	0.03	0.02	0.02
16:3 hexadecatrienoic			0.03	0.03	0.03	0.03
malvalic	**0.03**	**0.03**	**0.18**	**0.16**	**0.09**	**0.09**
18:0 stearic	0.05	0.06	0.19	0.18	0.10	0.11
18:1 oleic	0.43	0.49	1.57	1.46	0.84	0.83
18:2 linoleic	0.28	0.34	1.01	0.95	0.67	0.67
sterculic	**0.01**	**0.01**	**0.08**	**0.08**	**0.02**	**0.02**
18:3 α-linolenic	0.51	0.47	0.56	0.56	0.51	0.52
20:0 icosanoic	0.02	0.02	0.05	0.05	0.04	0.05
22:0	0.03	0.03	0.02	0.04	0.06	0.02
Total Fatty Acids	**2.02**	**2.14**	**4.94**	**4.70**	**3.30**	**3.27**

*NOTE: The fatty acids in **bold** are CPFA's*

The above table lists the results of the fatty acid analysis of samples from the three different batches of baobab dried fruit pulp. The levels of cyclopropene fatty acids vary between the three samples, with HSI300/3/2 showing the highest levels of malvalic acid (average 0.17 mg/g) and sterculic acid (average 0.08 mg/g).

The UK government recommends that the average person consumes no more than 35% fat in their diet (www.bbc.co.uk/health). This equates to 76g of fat for women and 100g of fat for men. The *in vivo* rat experiments (Bezard *et al* 1993a, Gaydou *et al* 1995, and Bezard *et al* 1996) consumed a diet of 10% fat made from baobab seed oil (13.7% cyclopropene fatty acids). Therefore, from these rat experiments we would expect to see the same effects when humans consumed a theoretical extrapolated value of around 10.4-13.7g of cyclopropene fatty acids.

PhytoTrade plan to use only 6-10g of baobab dried fruit pulp in a smoothie or 10-15g in a fruit bar. Assuming that the equivalent of 15g of baobab fruit pulp is consumed in one meal, this would equate to around a consumption of only 3.75 mg of cyclopropene fatty acids per meal from the analytical data outlined in table XIV above. As such, the amount of cyclopropene fatty acids in the baobab fruit pulp samples are over 3000 times below the level where toxic effects were observed in rats and are therefore unlikely to display toxic effects in humans when consumed at the anticipated intake levels.

8.5 LC50 brine shrimp assay

Tuani et al (1994) compared the activity of indigenous plants with a folkloric reputation for pesticidal activity and their LD50 values. *Adansonia* leaves and bark were extracted and these extracts were introduced to brine shrimp to determine their LC50 values.

Table XV

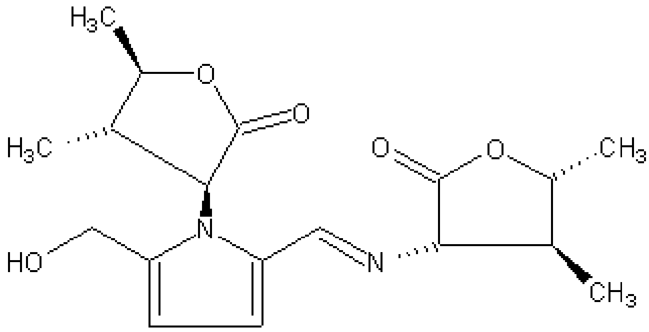
Effects of brine shrimp on different extracts of Baobab leaves and bark

Solvent extract	Plant Part	LC50 (ppm)
Adansonia ethanol extract	Leaves	110.9
	Bark	40.6
Adansonia pet. ether extract	Leaves	>1000
	Bark	-

8.6 Adansonin

Adansonin has been described as an alkaloid that has been reportedly isolated from *Adansonia digitata*. The bark of the *Adansonia digitata* tree is the only component that may contain the toxic alkaloid. Reference to this substance is not consistent, sometimes it is referred to as Adansonin and other times as Adansonine. However, in all cases the alkaloid is not listed in Beilstein or Chemical Abstracts from 1907 to 2005. The only formal reference to its existence is in the Merck index of chemicals and drugs: an encyclopedia for the chemist, pharmacist, and allied professions, 1952. There is a no supporting data on this particular alkaloid including what the structure of this molecule is or whether there are any therapeutic/toxic effects. However, another member of the *Bombacaceae* family of which *Adansonia* belongs, is known to contain the pyrrole based alkaloid, funebrine. This alkaloid was isolated from *Quararibea fenebris vischer*. It is unclear whether this group of alkaloids are would also be found in Boabab species. The issue is further confused by the work of Tuani et al (1994) who examined the bark and leaves of *Adansonia* whilst screening for bioactive phytochemicals. According to their results no alkaloids were detected, only flavonoids, general glycosides, and tannins.

Recent studies by PhytoTrade were undertaken to establish the potential presence of alkaloids in the dried fruit pulp of *Adansonia digitata*. Samples from batches HSI300/3/1, HSI300/3/2, and HSI300/3/3 were subjected to standard phytochemical methods for the detection of alkaloids using thin layer chromatography and visualization using Dragendorf's reagent (Wagner H. et al, Plant Drug analysis: a thin layer chromatography atlas, Springer Verlag, Berlin 1984. Harborne J.B. Phytochemical Methods: Chapman and Hall,1999). The results are detailed in appendix 19 but the results showed that no alkaloids were present in the "alkaloid rich fraction" at a detection limit of 5ug / 0.5g – 0.001% dry wt in any of the dried fruit samples. Considering the extensive history of use of the dried fruit pulp, if any alkaloids were present at lower levels of detection, they are probably unlikely to be a cause for concern.



Structure of Funebrine

8.7 Ochratoxin Content

Levels of Ochratoxin A in foodstuffs are regulated by EU Law under EC/123/2005, recently amended from EC/466/2001. Here, the permitted levels of this mycootoxin in food is given as 10 µg/kg for dried vine fruit. This includes currants, raisins, and sultanas. No other permitted levels could be found for other dried fruit products.

Table XVI
Analysis Data of Ochratoxin A - Content in PhytoTrade's Baobab Dried Fruit Pulp Samples

Sample	Ochratoxin A (µg/kg)
H.S.I 300/3/1/A	< 0.1
H.S.I 300/3/1/B	< 0.1
H.S.I 300/3/2/A	< 0.1
H.S.I 300/3/2/B	< 0.1
H.S.I 300/3/3/A	< 0.1
H.S.I 300/3/3/B	< 0.1

Examination of the analytical data shows that Ochratoxin A levels in the baobab fruit pulp samples were < 0.1 µg/kg, and this is well below the EC safety standard. The full details are included in appendix 20.

No other mycotoxins, such as aflatoxin, were tested for in the PhytoTrade samples since these toxins are not generally expected to be found in dried fruit pulp.

8.8 Cyanide Content

Ghani and Abejule (1986) demonstrated that baobab fruit pulp contains small amounts of cyanide in the region of 0.0049%. Consequently, PhytoTrade's product was analysed for cyanide content in different batches of the products:

Table XVII
Analysis results of cyanide content in PhytoTrade's Baobab Dried Fruit Pulp samples

Sample	Aqueous extract of fruit pulp (mg/kg)	Hydrolysis mixture of fruit pulp (mg/kg)
H.S.I 300/3/1	< 5	< 5
H.S.I 300/3/2	< 5	< 5
H.S.I 300/3/3	< 5	< 5

The results of the cyanide analysis of the PhytoTrade product, showed that all three samples did not contain cyanide at limit of detection for this particular analytical technique, i.e., less than 5mg/kg (see appendix 21 for more details and the methodology used). This equates to a figure of less than 0.00005%. A comparison with the figures of Ghani and Abejule (1986) of 0.0049% reveals that PhytoTrade's product contains negligible, if any cyanide.

8.9 Toxicity of closely related species and the botanical family

A literature search was conducted on other closely related species to Baobab, the Bombacaceae family and the closely related Malvaceae family in which it has occasionally been placed. No apparent toxicity issues were highlighted within this family and in fact a number of species belonging to this family are known to be edible indicating at least, lack of any immediate toxicity. For example, the following species are known to be eaten in different parts of the World:

- The fruits of the durian tree (*Durio zibethinus* , which are often referred to as the "King of Fruits" and are eaten all over South East Asia (Watson, L., and Dallwitz, M.J. (1992);
- Malabar Chestnut *Pachira aquatica,* the seeds of the fruits taste like chestnuts and are ground up to make bread (Purdue 2005);
- The fruits of *Ceiba pentandra* are widely used in traditional medicine in Asia and Africa (Kawanishi et al., 2002) and the seeds are edible after roasting (Duke 2005);
- *Bombax malabaricum* is widely used throughout India as folk medicine mainly as a diuretic and aphrodisiac (Gunaseka et al., 2003);
- *Quararibea cordata* known as "chupa-chupa" is an edible fruit found in Amazonia (Purdue 2005)
- The seeds of *Eriodendron anfractuosum* are eaten in India when roasted (Purdue 2005).

The family as stated previously is also closely related to the Malvaceae family (Heywood 1993) which contains other economically important species such as various Hibiscus species (used as herbal teas) and specifically *Hibiscus esculentus* (Okra) used as a common vegetable in cooking.

8.10 Conclusion

Summarising all of the above safety data on *Adansonia* fruit pulp, there is no evidence that consuming 6-10g of baobab fruit pulp in a smoothie or 10-15g in a fruit bar would have any harmful effects.

9.0 Allergenicity of Baobab dried fruit pulp

There is no publicised evidence in the literature for allergenic effects caused by direct consumption of *Adansonia digitata* fruit pulp. Indeed, a recent report by Lorenzoni-Chiesura et al (2000) recommended that species belonging to in the Malvaceae family as having the least or no allergenic potential. As further evidence to support this claim, the work of Marzatico et al (2001), demonstrated no dermatological toxicity or allergic reaction when fruit pulp solutions were exposed to humans using a modified Draize test.

PhytoTrades product is a non-genetically modified produced fruit in which the protein content is not new to the food chain. Also, there have been no reported allergic reactions with regard to this plant species despite extensive consumption particularly in both Africa and India. The genus and close families of the Baobab also have no reported allergenic effects. Also the closely related fruits of the *Adansonia gregori* a baobab tree found in Australia is commonly eaten by Aboriginals and the Food Standards Agency of Australia and New Zealand have declared this food to have a 'limited history of safe use in indigenous communities with no safety concerns identified and no harmful substances identified' (FSANZ Food Standards Code, pp 99, 5 October 2005).

Taking into account the above information and the extensive history of use of Baobab fruit pulp as outlined in section 6.0 it is unlikely that *Adansonia digitata* will provoke an allergenic response in individuals who consume this product.

10.0 Appendices

Appendix 1 **Members of PhytoTrade Africa in the Baobab fruit pulp Pre-Qualified Supplier (PQS) Programme**

Appendix 2 **(Confidential) Sourcing and blending of Baobab Dried Fruit Pulp from PhytoTrade's suppliers**

Appendix 3 **Analysis of β-sitosterol in Baobab Dried Fruit Pulp samples and generation of a fingerprint by quantitative thin layer chromatography**

Appendix 4 **Specification Sheets for the different batches/regions of Baobab Dried Fruit Pulp**

Appendix 5 **Stability Data and Conclusions**

Appendix 6 **Anticipated Intake – Leatherhead supporting information**

Appendix 7a **Toxicity and use questionnaire of Baobab fruit**
Appendix 7a **Edited questionnaire on history and safe use of Baobab fruit**

Appendix 7b **History of Use – PhytoTrade Literature search**

Appendix 8 **PhytoTrade Safety and Toxicity Literature Searches**

Appendix 9 **Determination of Vitamin C content in Baobab Dried Fruit Pulp by High Pressure Liquid Chromatography**

Appendix 10 **Determination of Pectin Content in Baobab Dried Fruit Pulp by Colourimetric Assay**

Appendix 11 **Determination of Fatty Acids in Baobab Dried Fruit Pulp by Gas Chromatography**

Appendix 12 **Determination of the Nutritional Composition of Baobab Dried Fruit Pulp**

-Moisture Content
-Protein Content
-Total Fat Content
-Ash Content
-Dietary Fibre Content
-Energy
-Sugar Content
-Sodium Content
-Fatty Acid Content
-Vitamin B Content

Appendix 13 Determination of Trace Elements in Baobab Dried Fruit Pulp by Inductively Coupled Plasma Atomic Emission Spectroscopy

Appendix 14 Determination of Heavy Metals content in Baobab Dried Fruit Pulp

Appendix 15 Determination of Amino Acid Content in Baobab Dried Fruit Pulp

Appendix 16 Determination of the Microbiological Contamination of Baobab Dried Fruit Pulp

Appendix 17 Determination of Pesticides Residues in Baobab Dried Fruit Pulp

Appendix 18 Determination of Cyclopropene Fatty Acid Content in Baobab Dried Fruit Pulp by Gas Chromatography

Appendix 19 Determination of Alkaloid Content in Baobab Dried Fruit Pulp

Appendix 20 Determination of Ochratoxin A in Baobab Dried Fruit Pulp by High Pressure Liquid Chromatography

Appendix 21 Determination of the Cyanide Content in Baobab Dried Fruit Pulp

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