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Research in clinical phytopharmacology to develop health care in developing countries: State of the art and perspectives

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Abstract

Medicinal plants are used worldwide as an alternative and/or a complementary medicine. Likewise, an interest in medicinal herbs is increasing as a precursor of pharmacological actives. Research in clinical phytopharmacology is as an alternative to develop healthcare in developing countries. The most advanced nations of the Western Hemisphere have adopted biologics and biosimilars medicine. Clinical phytopharmacology deals with all aspects of the relationship between phytomedicines and humans. The role of a clinical phytopharmacology is to develop methods and strategies that improve the quality of phytomedicine. This document is aimed primarily at decision-makers in a variety of topics in phytopharmacology research, including the development of methods and strategies that improve the quality of phytomedicine use in individual patients and patient populations. The first part of the document is related to the extraction of active principles for candidate phytomedicines selection. Following, there is preformulation of active principles for preclinical studies using polyphytotherapy alternative and combination concept. The second part of the document deals with phytopharmacy and methods to optimize production of raw materials followed by clinical evaluation. The last part of the document is concerned with phytomedicine use, problems of drugs interaction, pharmacovigilance and pharmacoeconomics. We hope that, this document will realize the great benefits that pharmacologists can bring to develop a good quality of phytomedicines

Keywords: Clinical Phytopharmacology, Polyphytotherapy, Phytomedicine, Health care

1. Introduction

Medicinal plants are used worldwide, especially in undeveloped nations. More than 80% of populations in these countries use herbal products to treat many diseases. However, the technological application to transform plants from their raw material state to medicines in commercial dosage forms such as tablets, powders of syrups, etc, remain unchanged since 1960.

List of abbreviations and definitions.

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ACT	Artemisinin -based Combination Therapy	IRB	Independent Review Board
ADR	Adverse Drug Reaction	I_{SC}	Short-circuit current
AE	Adverse Event	J_{app}	Apparent permeability
ALP	Alkaline phosphatase	K_D	Coefficient of dissociation
ALT	Alanine aminotransferase	LCMS	Liquid chromatography mass spectrometry
ARV	Antiretroviral drug	LDH	Lactate dehydrogenase
AST	Asparate aminotrasterase	LDL-C	Low Density Lipoprotein cholesterol
Bum	Bumetanide	MACEPA	Malaria Control and Evaluation Partnership in Africa
CD4+	Count of CD4 expressing helper T lymphocytes	MCH	Mean corpuscular haemoglobin
CD8+	Count of CD8 expressing cytotoxic T lymphocytes	MCHC	Mean corpuscular haemoglobin concentration
CDF	Commercial dosage form	MCV	Mean corpuscular volume
CK	Creatine phosphokinase	mL	Milliliter
CRF	Case Report Form	MRC	Medical Research Council
DDT	Dichloro Diphenyl Trichloro ethane	MS	Mass spectrometry
DMSO	Dimethylsulfoxide	NHPS	Natural health products
DTCs	Drug and Therapeutics Committees	OAPI	African Intellectual Property organization
EC_{50}	Effective concentration for 50% of maximum response	pA_2	Value of antagonist with reduced the effect of agonist of 50%
ECG	Electrocardiogram	PPAC	Polyphytotherapy alternative and combination
FDA	Food and Drug Administration (USA)	RBC	Red Blood Cells
Fr	Functional ratio	RBM	Roll Back Malaria
GABA	Gamma amino butyric acid	RDW	Red cell distribution width
GCP	Good Clinical Practice	SAE	Serious Adverse Event
GGT	Gamma Glutamyl Transpeptidase	SEM	Standard error mean
GIT	Gastrointestinal tract	SG	Specific gravity
GMAP	Global Malaria Action Plan	SLE	Systemic lupus erythematosus
Hb	Haemoglobin	T3	Triiodothyronine
HDL-C	High Density Lipoprotein - cholesterol	T3	Thyroxine
Het	Haematocrit	TSH	Thyroid Simulating Hormone
HPLC	High performance liquid chromatography	UWC	University of the Western Cape
IC_{50}	Inhibitory concentration for 50% of maximum response	VT	Vidal Duo Test
ICH	International Conference of Harmonisation	WBC	White Blood Cells
IEC	Independent Ethics Committee	WHO	World Health Organization

Nowadays, phytomedicine is preferred by many people with the limited means in less developed nations. The reasons are the following: a) the side effects are lower than the ones from allopathic medicine; b) the cost of treatment is less compared to allopathy, biologics and biosimilars medicine, and c) the development of biologics and biosimilar lead to more uncertainty with respect to the financial means of the population.

It cannot afford the aforementioned type of medical treatment due to the coast and conservation effects. Consequently, phytomedicine becomes one of the last resorts to the solution of the problem of healthcare in underdeveloped countries. The World Health Organization (WHO) and the African Organization for Intellectual Property (OAPI) support the enhancement and development of phytomedicine. In 2004, these organizations adopted guidelines to organize the homologation of phytomedicine issues of Africa's pharmacopeia. The objective of the guidelines was to establish a base for the development of clinical phytopharmacology, following the concepts of the organizations reports (WHO, 2000) and (WHO/AIPO, 2004).

Clinical phytopharmacology deals with all aspects of the relationship between drugs and humans, including development of new drugs, beneficial and adverse effects of phytomedicines, drug evaluations, phytopharmacoepidemiology, phytopharmacoeconomics, phytopharmacovigilance, safety and toxicology assessments, etc. The primary goal of clinical pharmacologists is to improve patient care, directly or indirectly, and to promote a safer and more effective use of drugs from plants.

The role of a clinical phytopharmacology is to develop methods and strategies that improve the quality of phytomedicine. A good quality of phytomedicine requires development of news or adapted strategies of research in phytomedicine evaluation, teaching, patient care, pharmaceutical industry, governments' policies (Figure 1). This work is structured in a logical order to cover the various stages of phytomedicine development from sample drug selections to the development of the desired commercial dosage form.

2. Research in clinical phytopharmacology: Functional Approach

The objective of a pharmacologist working in a clinical environment is to develop methods and strategies that improve the quality of drug use in individual patients and patient populations. His priority is research in drug evaluation, drug utilization, pharmacovigilance and pharmacoepidemiology areas. Potentially important parameters are the rational use of drugs (RUD), with their selection based on efficiency, and the adverse drug reaction (ADR) and cost. In addition, research in clinical phytopharmacology involves studies that produce new data about current drugs, including new indications and treatment of neglected patient populations (children and the elderly). It also deals with research in ADRs, pharmacogenetics and drug interactions.

Research in clinical pharmacology is an interdisciplinary subject. It is done in collaboration with pharmacists, drug analytical chemists, molecular biologists, statisticians, comp-

Figure 1: Role of Clinical Phytopharmacology.

uter specialists and clinical researchers from other medical specialties. The development of phytomedicine requires fundamental considerations, including the method of extraction and formulation of active principles, especially when the final formula contains two or more products, and preclinical studies with some conventional pharmacologic parameters to compare and selects the final formula (Fig. 2). The criteria for the phytomedicine drug selection will be based, primarily, on the pharmacological properties, that is, potency, selectivity, duration of action, safety/toxicology assessments and pharmacological properties, good aqueous solubility, crystalline, nonhygroscopic and good stability.

2.1. Extraction of active principles for candidate drug selection

Traditional healers generally use natural solvents to extract active principles in plants. Among the natural solvents, water and edible oils are often used. Some traditional medicine also uses alcohol (ethanol) from beverages like palm wine, maize, and other edible seed foods. Organic solvents are excluded in traditional medicine. In more extensive studies, the use of different extracts obtained with organic solvents induces biological effects different from those observed with natural solvents. Methanol and other organic solvents like DMSO are toxic. They are forbidden in the preparation of CDF (commercial dosage form).

To our knowledge, no phytomedicine has ever been developed in CDF with organic solvents was in human or animal health care. Those who used unauthorized solvents (methanol, petrol etc.) obtained results but they cannot produce any CDF. The ones using approved solvents (water, ethanol, comestible oil) produced good results and they produce CDF.

Some researchers try to eliminate all the organic solvents by evaporation or atomization without considering the problems of impurities and other related problems during crystallization of active principles (Maccaroni et al., 2010; Rodriguez-Hornedo and Murphy, 2004). Since, the elimination of a solvent is not complete during evaporation and atomization process. Residues of solvents are found in the final product, and some organic solvent may influence crystallization of the active principles and excipients (Doelker, 2002). Dissolution (Marjo et al., 2011; Moreno-Calvo et al., 2011; Romero et al., 1999), bioavailability and toxicity (Thakkar et al., 1977) of different drugs are directly related to their polymorphism.
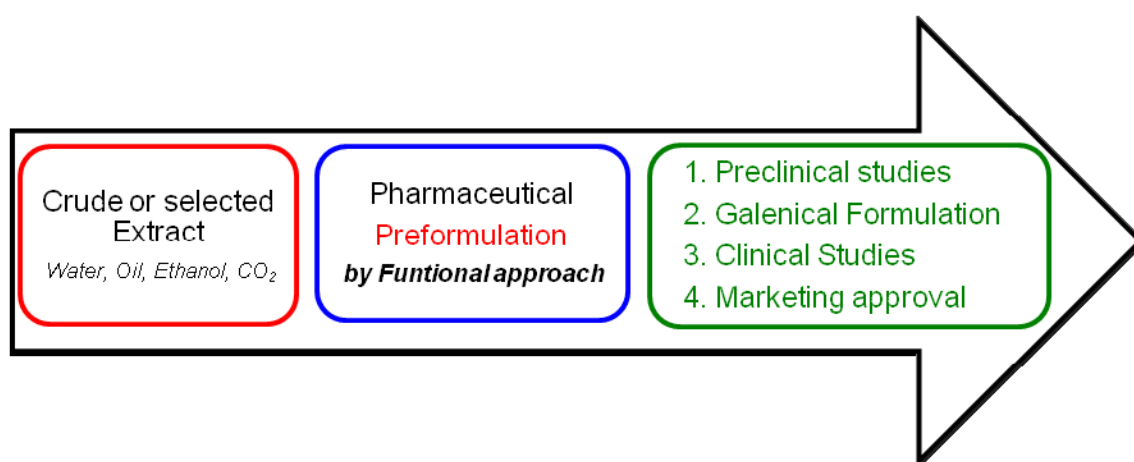

Figure 2. Development of Phytomedicines.

In 2002, Bernstein (Bernstein, 2002) pointed out that structural diversity is present in almost every facet of nature, and crystal polymorphism is one manifestation of this diversity. Polymorphism, in a chemical sense, is a solid-state phenomenon where the crystal structures of a chemical entity are different, but correspond to an identical liquid and vapour states (McCrone, 1965). Polymorphism is a common phenomenon in small organic molecules, and the occurrence of polymorphs has been extensively documented (Bernstein, 2002; Byrn et al., 1995). Specifically, in the area of pharmaceutical material differences in solubility and dissolution rate between polymorphs can have a pronounced impact on the oral bioavailability (dissolution and absorption) from the gastrointestinal tract (GI) of pharmaceuticals as exemplified by formulation investigations of tolbutamide (Kimura et al., 1999). An active principle can also induce physical form conversions during recrystallization to less stable forms. The solvents can profoundly affect the rate and extent of conversion. For example, Gu et al. (Gu et al., 2001) have studied the influence of a solvent on the rate of solvent-mediated transformations, and Mukuta et al.(Mukuta et al., 2005) have reported the role of impurities, which were found to have a profound impact on the conversion. By studying the polymorphs of sulfamerazine, Gu et al. (Gu et al., 2001) found that the rate of transformation was faster in a solvent that afforded high solubilities compared with those in which the solubility was lower. The temperature affected also the conversion.

The importance of understanding the control and robustness of polymorphs is illustrated by the Ritonavir example. Ritonavir (ABT-538) was approved by the FDA in March 1996 and marketed as a semisolid formulation. In 1998, however, batches began to fail dissolution tests, and investigations revealed that a more stable polymorph was precipitating from the formulation. As a result, Abbot had to withdraw the product from the market (Chemburkar et al., 2000). Hence, the importance of the method of extraction of active principle in the appropriate solvent in the focus to get good dissolution and stability

In conclusion, the use of traditional solvent (water, oil and ethanol) is recommended to obtain a functional extracts of a plant. However, using CO_2 was recommended (Sheth et al., 2012) for lipophile active principles during supercritical fluid extraction.

2.2. Preformulation of active principle for preclinical studies

2.2.1. Concept of preformulation of active principles

Formulation of active principle is necessary when the active formula contains two or more than two extracts of different plants. In that case, the first thing is to determine the biologic activities of each plant, and the active formula will be prepared in the basis of the common activity found between all extracts (contraction, relaxation, secretion, absorption, antimicrobial, antifungal etc.). This concept is very simple when pharmacological parameters can be determined such as effective concentration for 50% of maximum response (EC_{50} or IC_{50}) with agonists components and PA_2 with antagonists components (Daniel et al., 2001).

After classifying the extracts on the basis of their potency (evaluated by pharmacological parameters), the concentration of each plant on the final active formula is done according to the value of its functional ratio (Fr). The result is obtained by Eto's equation (1)

(Mamadou et al., 2011). The lower value (more effective extract) must be considered as a unit of preparation of a new rational final active formula.

$$F_R = {}_xEC_{50}/{}_yEC_{50} \qquad\qquad (1)$$

$_xEC_{50}$ represents the value of EC_{50} of plant x, and $_yEC_{50}$ the lowest EC_{50} found for the most effective plant extract.

Functional approach can be used to determine pharmacological parameters such as coefficient of dissociation (K_D) or fluxes across biologic barriers (J_{app}) and PA_2.

2.2.2. Determination of pharmacological parameters for preformulation

2.2.2.1. Evaluation of agonist and antagonist

Quantitative information about affinity of the antagonist and the nature of its interaction with the receptor can be derived from Arunlakshana & Schild (Arunlakshana and Schild, 1959; Schild, 1947), in which concentration-effect curves are repeated after increasing concentrations of the antagonist to determine the rightward shift (Fig. 3) of the concentration-effect curves to the agonist. The reactions are assumed to be represented by:
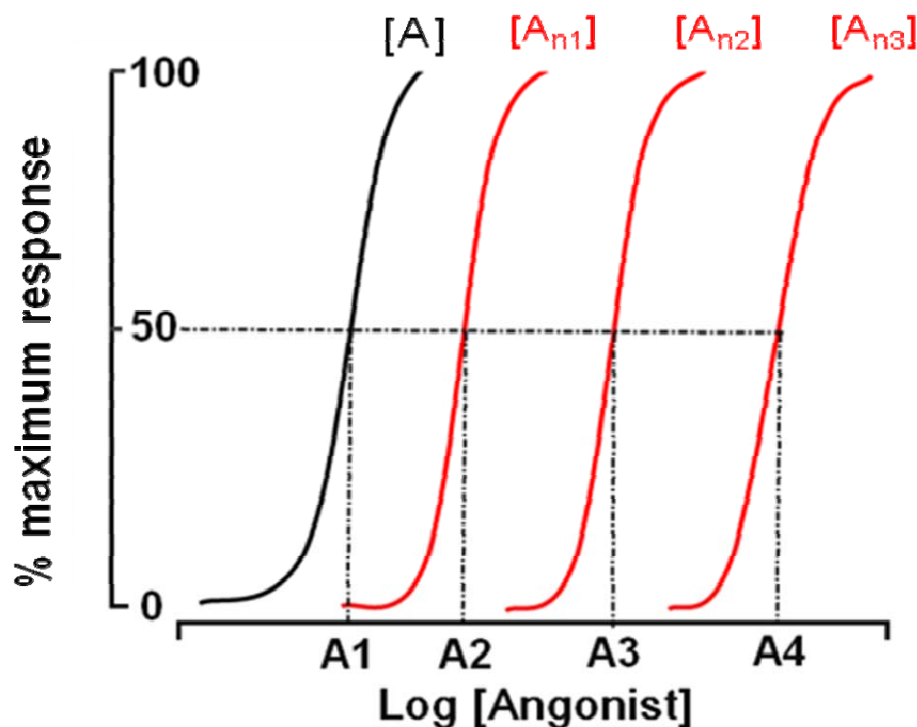
[A] + [R] $\leftrightarrow$ [AR] $\rightarrow$ Biologic effect
[A$_n$] + [R] $\leftrightarrow$ [A$_n$R] $\rightarrow$ inhibition or reduction of biologic effect

Figure 3. Consequences for agonist concentration-response of adding increasing concentrations of a competitive antagonist (A$_{n1}$, A$_{n2}$, A$_{n3}$), competing 1:1 with an agonist for a receptor.
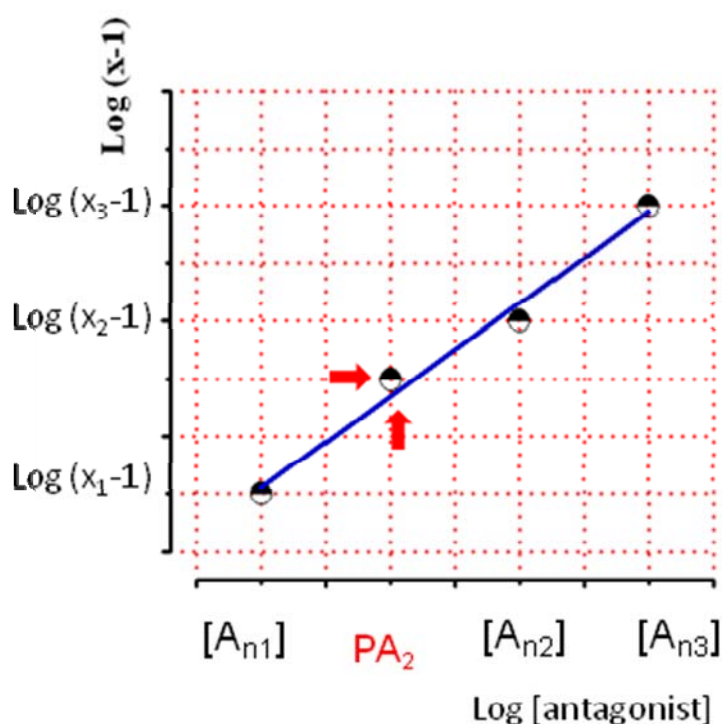
Figure 4. A Schild plot showing various Log(x-1) of agonist (A2, A3, A4), required to attain 50% maximum response with an increase in concentrations of antagonist. These values are plotted against the logarithm of the antagonist concentration (An$_1$, An$_2$, An$_3$). The pA$_2$ value gives a negative logarithm value of the antagonist concentration. When x=2, log(x-1) = 0. This characterizes the interaction of the antagonist with the receptor.

Agonist was represented by [A], antagonist [A$_n$], receptor [R], ligand-receptor [AR] and [A$_n$R]. Dose ratio (*x*) of 50% of maximum response of agonist in a presence of different antagonist (Fig. 4) concentrations was determined as:

$$x_1 = A_2/A_1, \qquad x_2 = A_3/A_1, \qquad x_3 = A_3/A_1$$

2.2.2.2. Evaluation of K$_D$

The coefficient of dissociation is always determined with radioactivity or fluorescence lifetimes. We propose a method to evaluate this pharmacological parameter by a functional approach (Eto, 1995).

The coefficient of dissociation (K$_D$) can be determined by the law of mass action.

$$[AR] / ([A] + [B]) = 1/K_D \qquad (2)$$

Where, [A] represents the free ligand concentration of active principle A, [R] the free receptor concentration, and [AR] the concentration of the receptor-ligand complex. Occupation of receptors (ρ) is linked to K$_D$ by the following equation:

$$[AR] / [R_T] = \rho = [A] / ([A] + K_D) \qquad (3)$$

Where, [R$_T$] is total receptor concentration. In dose-related profiles, E$_{max}$ is the maximum biologic response corresponding to 100% of the effect of the active principle, a condition

which reflects the stimulation of all the receptors including spare receptors. E_A is the biologic effect obtained with active principle concentration [A], and E_{Amax} the maximal value of E_A close to E_{max}. Consequently, the intrinsic activity α of an active principle is drawn from the following equation:

$$\rho\alpha = E_A / E_{Amax} = [A] / ([A] + K_D) \qquad (4)$$

In addition, [AR] / [R_T] represent the fraction of saturation of the receptors (ψ). It can be considered as E_A/E_{Amax}. With two concentrations of peptide [A] and [B], the equation (4) becomes:

$$\psi_A = \alpha ([A] / ([A] + K_D)) \qquad (a)$$
$$\psi_B = \alpha ([B] / ([B] + K_D)) \qquad (b)$$

The resolution of the above system of equations (a) and (b) gives the following relation:

$$K_D = [A][B] (\psi_A-\psi_B) / ([A]\psi_B - [B]\psi_A) \qquad (5)$$

Equation (5) is used when E_{max} represents 100% of the biologic response (Fig. 5B). In some cases, the maximum response induced by the active principle, E_{Amax} is lower than E_{max}. This is due to the existence of the spare receptors (Fig. 5A). In this case, equation (5) becomes equation (6).

$$K_D = [[A][B] (\psi_A-\psi_B) / ([A]\psi_B - [B]\psi_A)] \text{ x } (E_{max} / E_{Amax}) \qquad (6)$$

If the concentration of [A] is smaller than that of [B], with [A] / [B] ≤ 0.001, then equation (6) becomes:

$$K_D = [A] [(\psi_B / \psi_A) - 1] \text{ x } (E_{max} / E_{Amax}) \qquad (7)$$

[A] and [B] will be chosen as the lowest and the highest concentrations of a plants extracts within the linear part of concentration-response profile respectively. The concept of utilization of this type of equations can also be used when plants extracts were replaced by peptides and hormones (Eto, 1995).

2.2.2.3. Evaluation of apparent permeability

Transport of an active principle through intestinal barrier can be evaluated using the Ussing's chambers technique. This evaluation is possible if the active principle can induce biologic response such as electrogenic absorption or secretion.

Many plants extracts can induce intestinal fluid and transport of electrolytes. These are plants with berberine contents (*Berberis vulgaris, hydrastis Canadensis* etc.) and *cassia siamea* (Deachapunya et al., 2005). The concept is to measure the time-course effect of an extract on a variation of a short-circuit current (Isc), when added in serosal side and mucosal side in the focus to realize the dose-effect curve, as shown in Fig. 6 and 7.

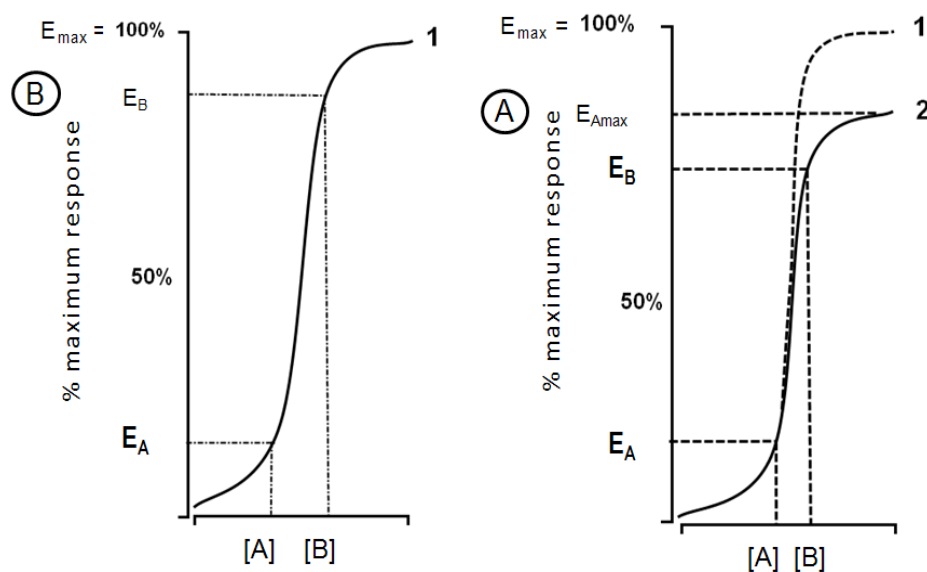The mucosal to serosal fluxes (J_{ms}) can be determined by following equation:

Figure 5: Plots of response to agonists acting at the same receptor: At left, a powerful full agonist (1) was not able to provoke 100% of response (E_{max} in plot 1), but caused a partial response (E_{Amax}, plot 2) due to spare receptors. At right, full agonist induces 100% of response (E_{max}).

$$J_{ms} = [A]/\Delta t.S \tag{8}$$

Where S represents the sheets (surface) of the tissue and $\Delta t = (t_2 - t_1)$ (see Fig. 6). The apparent permeability (fluxes) can be determined by this equation:

$$J_{app} = J_{ms} / [B] = ([A] / [B]) \times 1/ (\Delta t.S) \tag{9}$$

In general the ratio of [B]/[A] is close to 1000. The determination of pharmacologic parameters allows the determination of the functional ratio (Fr) which is the crucial value in preformulation of phytodrug candidate.
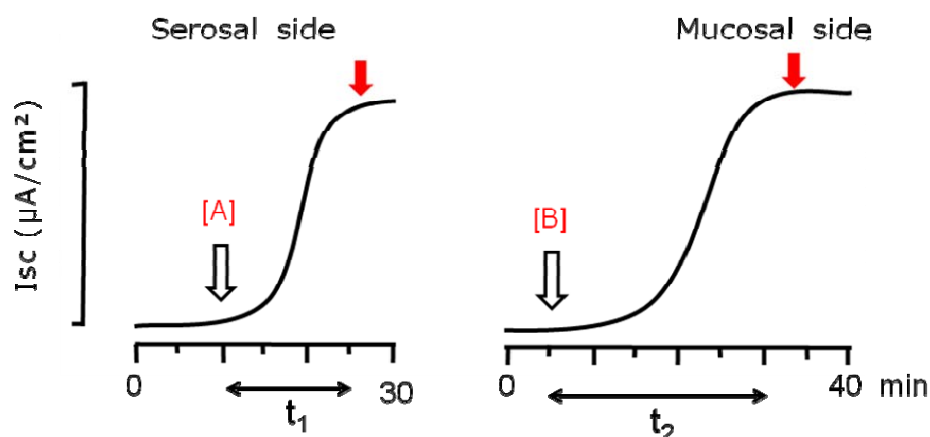
Figure 6. Typical recording of the effect of the drug on variation of Isc. In this example, the effect is the same although the concentration is different in serosal side [A] and mucosal side [B]. The time in minutes (min) was determined from addition of drug and to the maximum response on the both sides.
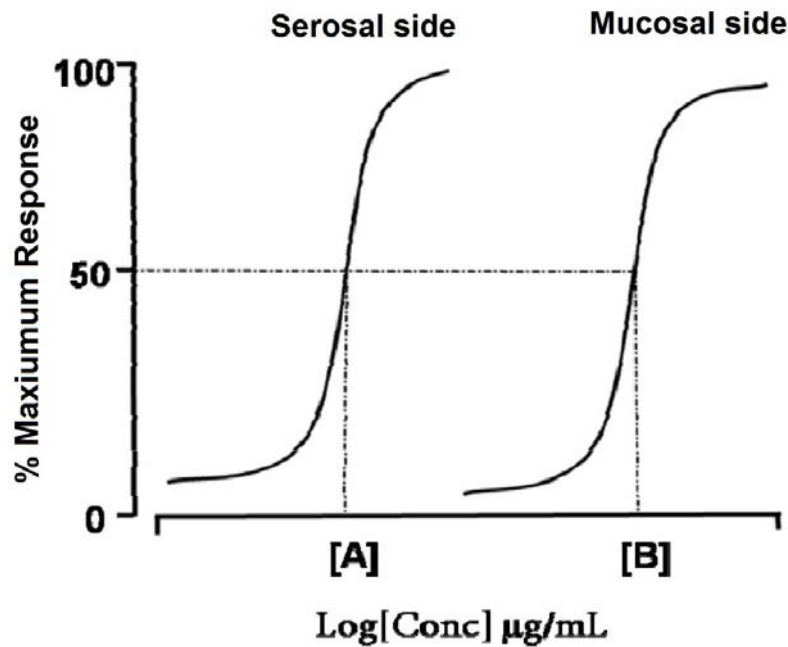
Figure 7. Dose-effect response of the same drug in serosal and mucosal side. The concentration, which induces 50% of response is different to serosal side [A] and mucosal side [B].

2.2.3. Polyphytotherapy Alternative and Combination concept (PPAC)

Polyphytotherapy alternative and combination (PPAC), is the possibility to use many plant extracts in the same formulation. It is also the possibility to alternate or combine/replace one or more plants in the formula according to the desired objective. PPAC offers phytopharmacologists a wide range of possibilities to formulate a number of combinations of plant and mushroom extracts. Thus, in the case of infectious diarrhoea, the pharmacologists can make a specific formulation to any given infection.

For example, the possibility of combining *k* plants in one preformulation among *n* plants without repetition (k < n). The equation of the combination is:

$$C_{n,k} = n! / k! (n-k)! \tag{10}$$

If *n* is the number of total anti-dyspepsia plants, is 65 (from Tab. 3), and *k* the number of the plants in one preformulation, is 3, the possibility of obtaining z different preformulations of phytomedicine sample without repetition is:

$$Z = C_{65,3} = 65! / 3! \, 62! = 43680$$

The possibilities of arrangement of numerous preformulations containing *K* plants in one preformulation among *n* total plants are given by the equation:

$$A_{n,k} = n! / (n-k)! \tag{11}$$

The possibilities of arrangement of three plants in one preformulation among 65 of total plants against constipation used in Congo basin forest (Table 4) are:

$$A_{65,3} = n! / (n-k)! = 65! / 62! = 262\,080 \tag{12}$$

Thus, PPAC offers the possibility to combine and alternate different plant extracts and offer a tremendous choice of the best formula phytomedicines. This possibility may help to formulate phytomedicines without risking the development treatment resistance.

2.2.3.1. Preformulation of antispasmodic phytomedicine

2.2.3.1.1. Libéraline: Anti-Dyspepsia phytomedicine

Traditional practitioners in Morocco commonly use the traditional infusion of fifteen plants against infantile excessive crying. We assessed the antispasmodic effect of each plant with this preparation. We then utilized PPAC to propose a rational formulation by functional approach. Illustration of the determination of the preformulation of antispasmodic phytomedicine (Liberaline[®]) is shown in Fig. 8 and Fig. 9, Table 1 and Table 2.

After determination of IC_{50} of relaxation of each plant, equation (1) was used to determine the functional ratio using as unit the lowest value of IC_{50}. In Table 1 this unit was given by *Lippia citriodora Lam.* with IC_{50} of 70.2 ± 1.1 µg /mL. When formulation was *Artemisia, Lavandula, Mentha, Rosmarinus, Lippia, Zygophyllum,* Liberaline was obtained with IC_{50} of 27.5 ± 1.1 µg/mL. A very effective phytomedicine when compared with prescribed drugs (Table 2). This result illustrates the possibility to use the functional approach and the PPAC.

The PPAC can be used to develop phytomedicines against dyspepsia and other intestinal bowel syndromes (IBS) with plants used by traditional healers in the Congo basin forest to treat dyspepsia. The possibilities (Z) to combine 6 plants in one preformulation without repetition like Liberaline with plants among those in Table 3 are:

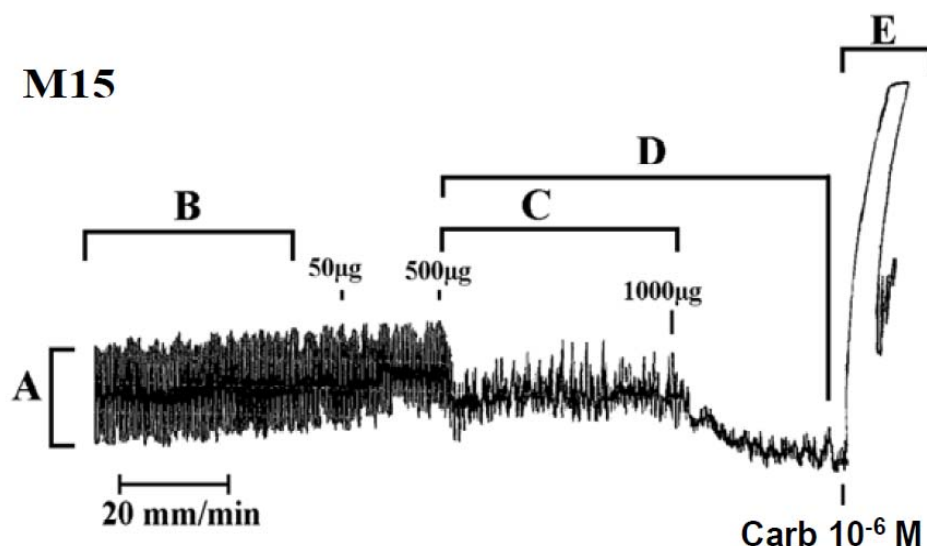$$z = C_{65,6} = 65! / 6! \, 59! = 80\,598\,880$$

Figure 8. A typical recording of the effect of traditional preparation of mixture of 15 plants (M15) on rat jejunum. Amplitude of spontaneous contraction is indicated by A and B waves of the spontaneous contraction of intestine. C shows the decrease of contraction's frequencies after addition of 500 µg/mL of M15. Relaxation of intestine is represented in D, whereas E is the contraction induced by 10^{-6} M of Carbamylcholine (Mamadou et al., 2011).
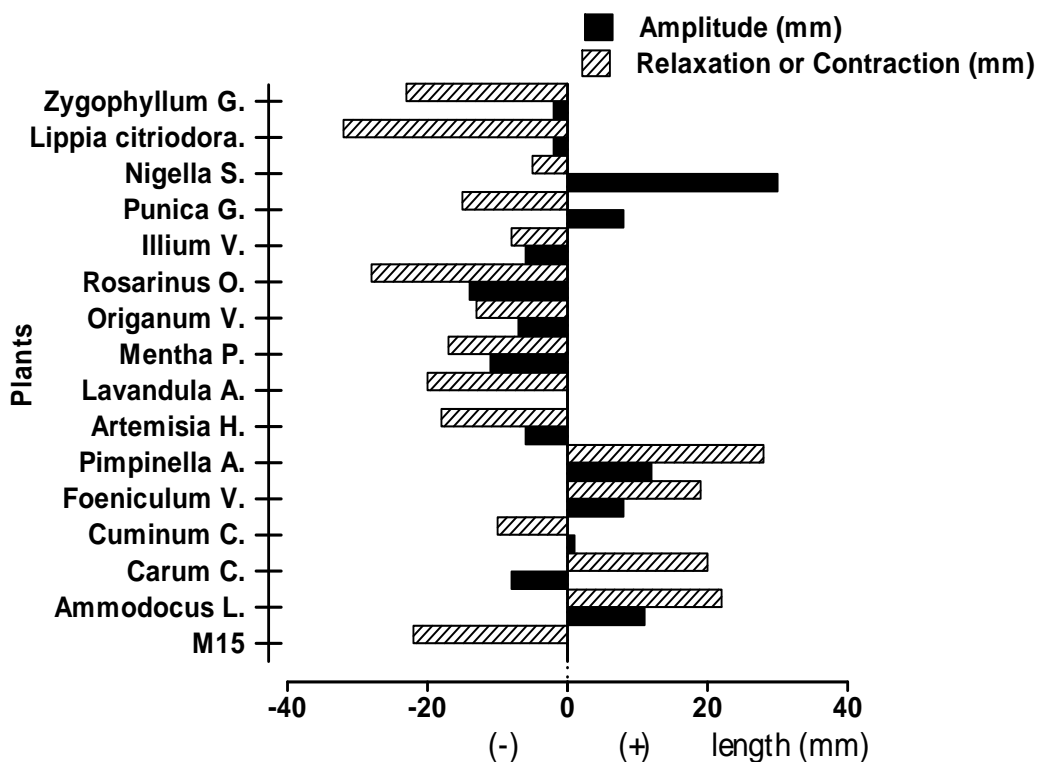
Figure 9**.** Assessment of amplitude of spontaneous contraction, relaxation or contraction of each plant composing traditional preparation (M15). All the values represented the maximum effect.

Table 1. Functional ratio of Liberaline® according to the relaxation effect.

Name	Abbrev	IC_{50} relaxation (µg/mL)	Functional ratio (F_r)
Cuminum cyminum L.	Cum	484.1 ± 1.0	6.89
Artemisia berba alba	Art	137.0 ± 1.1	1.95
Lavandula angustifolia	Lav	111.0 ± 1.2	1.58
Mentha pulegium L	Men	149.7 ± 1.1	2.13
Origanum vulgare L	Orig	204.3 ± 1.0	2.91
Rosarimus officinalis L	Ros	93.7 ± 1.2	1.33
Illicium verum	illv	603.3 ± 1.0	8.59
Punica granatum L	Pun	201.5 ± 1.2	2.87
Nigella sativa L	Nig	737.1 ± 1.1	10.49
Lippia citriodora Lam	Lip	70.2 ± 1.1	1
Zygophyllum gaetulum Emb	Zyg	103.5 ± 1.2	1.47
Artemisia, Lavandula, Mentha, Rosmarinus, Lippia, Zygophyllum	Liberaline®	27.5 ± 1.1	0.39

Values of IC_{50} are expressed as geometric mean with 95 % confidence intervals (Mamadou et al., 2011).

Table 2. Comparison of Liberaline® with antispasmodic medicines

Products		Relaxation
Name	Commercial name	IC_{50} (µg/mL)
Phloroglucinol	Spasfon®	1252.0 ± 0.2
Trimebutine	Débridat®	91.8 ± 0.1
Pinaverium bromide	Dicetel®	826.8 ± 0.3
Loperamide	Diaretyl®	51.8 ± 0.1
Liberaline®	Liberaline®	27.5 ± 1.1

Values of IC_{50} are expressed as geometric mean with 95 % confidence intervals (Mamadou et al., 2011).

2.2.3.2. Preformulation of anti-diarrhoea and anti-constipation phytomedicine

The functional approach is important in the development of phytomedicine which antagonize physiological function. The main example is, when we need to develop phytomedicines against secretary diarrhoea or constipation. Both diseases were the consequence of imbalance between intestinal absorption and secretion of water (Desjeux et al., 1994). Diarrhoea is due to increase of secretion or decrease reabsorption of intestinal fluid, whereas constipation is the lack of water in the intestinal bowel. In spite of the notion of eliminating intestinal infection (bacteria, amoeba, etc), the enterosystemic water cycle (Desjeux et al., 1980; Desjeux et al., 1994; Desjeux et al., 1977) must be taken into account in the development of an anti-secretory diarrhoea or anti-constipation phytomedicine.

The transport of ions regulates the movement of water between the environment and the body, and within the body. The balance between secretion and absorption is a dynamic process represented by the enterosystemic water cycle (Fig. 10A). The small intestine is the site where the quantity of water and electrolytes are transported. It is very important. However, from the clinical point of view, the colon is the site of water and electrolyte salvage. It is important for the regulation of stools volume.

The functional approach strategy in secretory diarrhoea is to develop a phytomedicine, which causes the reduction of intestinal secretion and increase of intestinal reabsorption of fluid (Fig. 10B). In contrast, a phytomedicine against constipation might increase intestineal secretion and reduction of reabsorption of fluid (Fig. 10C). Since, the movement of wat-
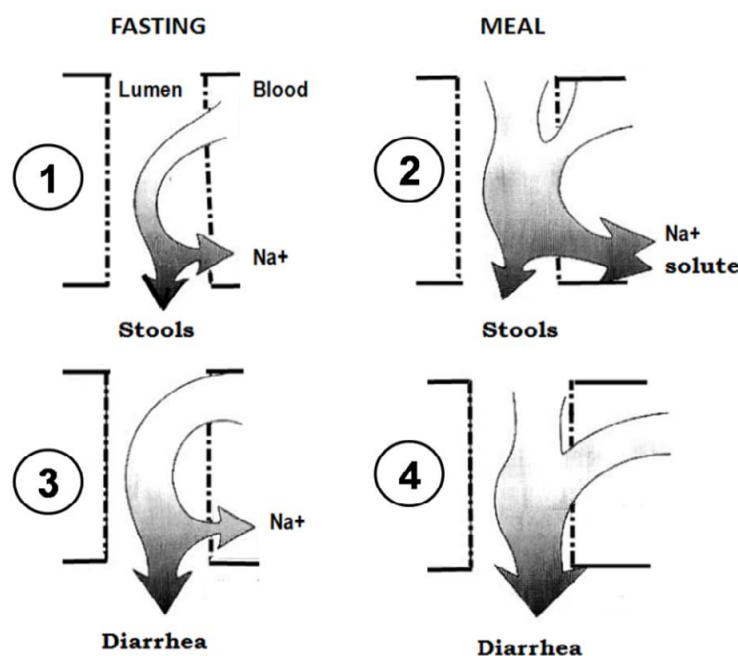

Figure 10A. The enterosystemic water cycle. During fasting (1), water that enters the lumen is reabsorbed following Na$^+$, absorption from lumen to blood. Hence, very little water is lost in stools. During a meal (2), most of the water enters the intestinal lumen as a consequence of digestive secretions (saliva, gastric, biliopanc-reatic, intestinal). Water is absorbed following Na$^+$ reabsorption, mainly through the solute-Na$^+$ co-transport system; again, little water is lost in stools. In this system, diarrhoea is a consequence of imbalance between absorption and secretion due to increased secretion (3) or decreased reabsorption (4). From Desjeux JF. With his permission.
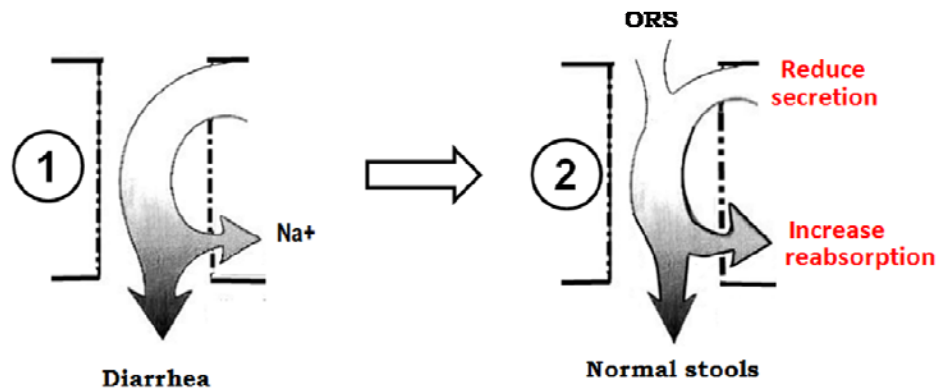
Figure 10B. Antisecretory strategy by functional approach is to reduce water secretion and increase fluid reabsorption (2), in addition to the use of an oral rehydratation solution (ORS) for children.

fluids (water) through intestinal epithelia is linked to the transport of ions (particularly Na^+ and Cl^-). The mechanisms of ion transport by the intestinal epithelial cells have been studied extensively (Desjeux et al., 1980; Donowitz, 1987). It is generally accepted that electrolyte absorptive (from lumen to blood) and secretory systems are present in two separate epithelial cell types; absorption is predominant in villous cells and secretion in crypt cells. Thus, many transported electrolytes and also water enter an intestinal or enterosystemic cycle (Desjeux et al., 1977).
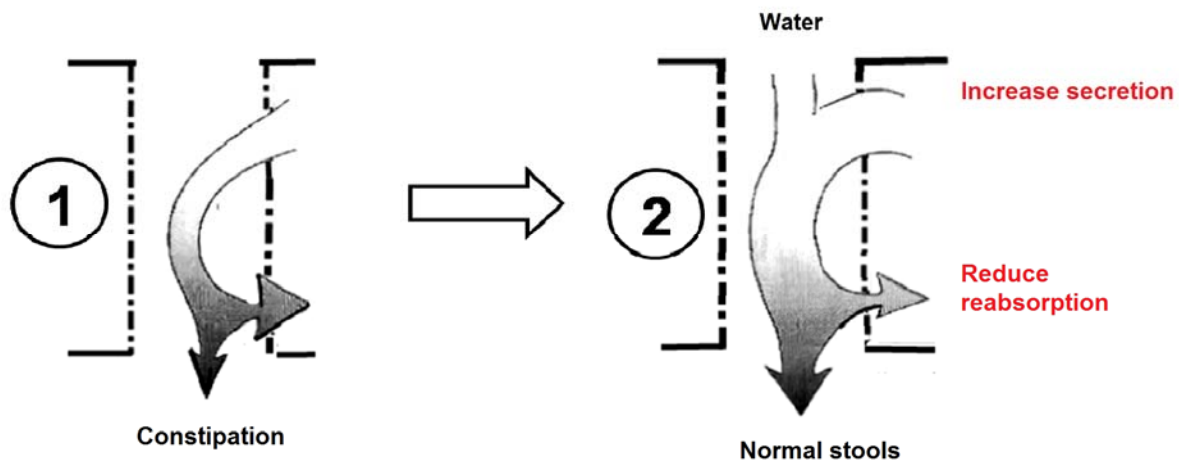

Figure 10C: Laxative strategy by functional approach is to increase intestinal secretion (1) and reduce fluid reabsorption (2), in addition to the consumption of water. It is highly recommended.

The most common cause of secretory diarrhoea is the result of physiologic manifestation of toxins, bacteria, virus, parasites and antigens. It is therefore, very important to elimiate the infection using plant extract with antimicrobial, antiprotozoal, antiviral and antifungal depending on the origin of infection. The anti infectious plant extract must be associated with antisecretory plant extract and the extract of plant which increase water and electrolytes reabsorption (Fig. 11 and Fig. 12).

In agreement with the strategy of the functional approach, more than two different extracts of plant can be used to formulate an anti-diarrheic phytomedicine. Table 3-6 showed different plants used in the Congo basin forest to treat infections of the gastrointestinal tract,

Table 3. Plants used in Congo basin forest to treat dyspepsia.

Anti dyspepsia plants (n=65)
Acanthus montanus, Afrostyrax lepidophyllus, Amaranthus gracilis, Anthium arnoldianum, Anthostema aubryanum, Brazzeia klainei, Caloncoba welwitchii, Canthium multiflorum, Ceiba pentandra, Cleistopholis patens, Cogniauxia podoleana, Copaifera religiosa, Costus fissiligulatus, Costus lucanusianus, Cylicodiscus gabunensis, Dinophora spenneroïdes, Duboscia macrocarpa, Elaeis guineensis, Entandrophragma angolense, Entandrophragma palustre, Entrandrophragma utile, Erythrococca welwitschiana, Gagnophyllum giganteum, Garcinia kola, Garcinia mannii, Geophila repens, Guarea cedrata, Hannoa klaineana, Harungana madagascarlensis, Hua gabonii, Hugonia platys-epala, Klainedoxa gabonensis, Macaranga spinosa, Maesopsis eminii, Monordica charantia, Nauclea diderrichii, Oncoba spinosa, Oxyanthus speciosus, Palisota ambigua, Palisota cheweinfurthii, Panda oleaosa, Pseudospondias microcarpa, Randia walkeri, Rinorea longicuspis, Scaphopetalum amoenum, Scaphopetalum blackii, Scaphopetalum macranthum, Scaphopetalum zenkeri, Solanum nigrum, Spilanthes acmella, Strombosia glaucescens, Strombosia grandifolia, Strombosia tetandra, Strombosia zenkeri, Tabernanthe iboga, Tetracera alnifolia, Tetracera potatoria, Thomandersia laurifolia, Trichoscypha abut, Tridesmostemom omphalocarpoïdes, Vernonia conferta, Vitex doniana, Whitefieldia brazzae, Xylopia aethiopica, Xylopia hypolambra

constipation, diarrhoea and dysentery. Among the anti-diarrhoea plants used in Africa, the mechanisms of action were overwhelmingly known, including those which increase water and electrolyte reabsorption (*Alchornea cordifolia, Adansonia digitata frut, Euphorbia hirta, Treculi africana Decne, Pentaclethra macrophylla*) and the ones reducing intestinal fluid accumulation (*Irvingia gabonensis, Hermannia incana, Xysmalobium undulatum*).

2.2.3.2.1. AES-K anti diarrhoea phytomedicine

The combination of three extracts of plant is necessary to prepare AES-K, one of the best phytomedicines anti-acute diarrhoea. The product is usually used against protozoa such like *Entamoeba histolytica*, bacteria such as *Shigella dysenteriae*, and other infestations with intestinal bacteria and typhoid. The product contains two plants with antiprotozoal and antibacterial properties (*Euphorbia hirta and Alchornea cordifolia*). It also contains unidentified antisecretory plants. The following Fig.13 shows the effect of the plant on the reduction of in-
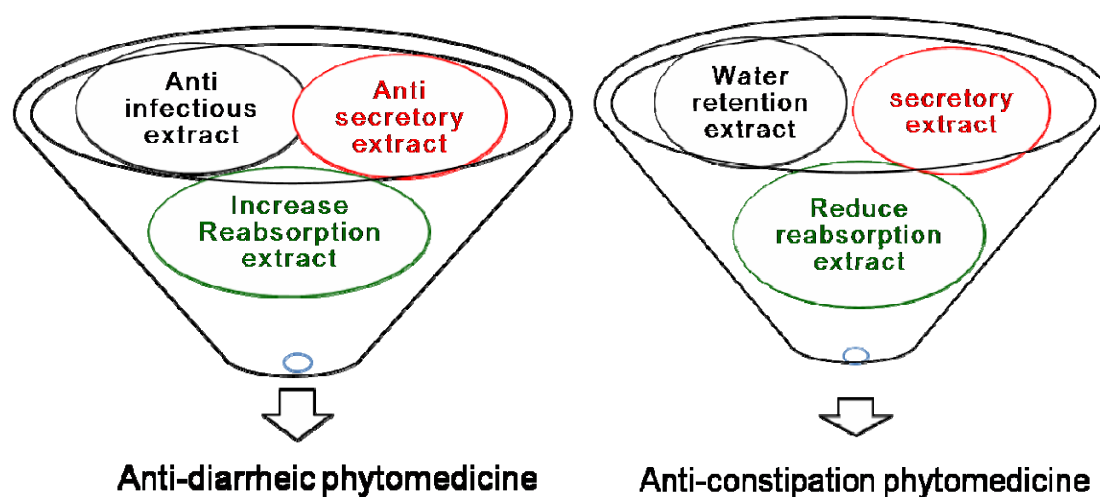

Figure 11. Functional approach for formulation of anti-diarrhoea and anti-constipation phytomedicines. Anti-diarrhoea contains three different extracts, including an anti-infectious, an antisecretory and an increased reabsorption extract. Anticonstipation contains water retention and secretory extract
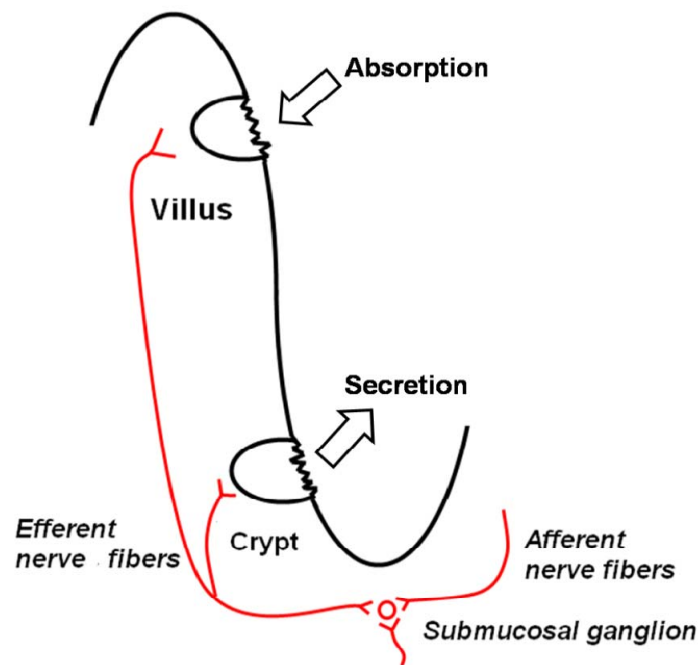
Figure 12. Cellular localization: It is generally accepted that electrolyte absorptive (from lumen to blood) and secretory systems are present in two separate epithelial cell types; absorption is predominant in villous cells and secretion in crypt cells (From Desjeux JF, with his permission).
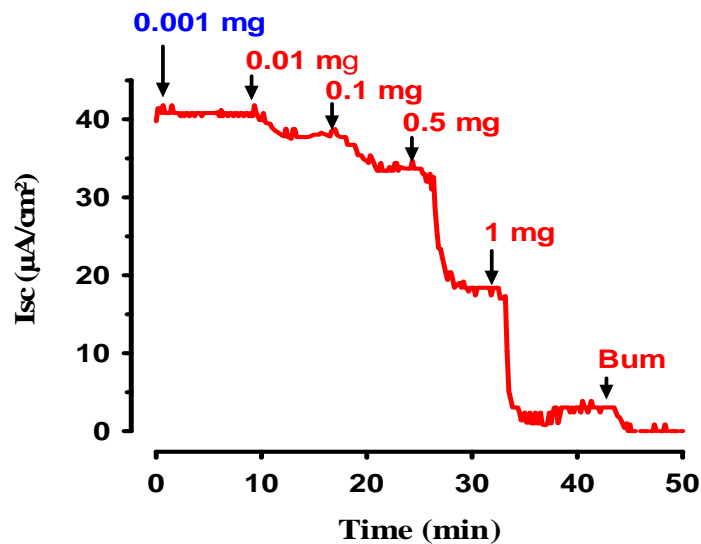

Figure 13. Typical recoding of the inhibitory effect of plants obtained from AES-K under a short-circuit current (Isc), using Ussing chambers technique. The extract of the plant induced reduction in Isc dose-dependent with the minimum concentration at 10 μg /mL and the maximum concentration with 1mg /mL. With 1 mg/mL, the response of tissue to extract is similar to the one obtained with 5.10^{-4}M of Bumetanide. Isc represents the sum of the net ion fluxes, transported across the epithelium in the absence of an electrochemical gradient (mainly Na^+, Cl^- and HCO_3^-). This value reflected the hydro-electrical permeability. The diminution of Isc indicated the reduction of secretion of fluid across the rat intestine. When the Ringer solution was replaced by a modified solution, the effect of the plant on Isc was completely abolished. Sodium was replaced by choline or chloride by isethionate and sulphate. This could be interpreted as a reduction in electrogenic secretion with stimulation of neutral NaCl absorption.

Table 4. Plants used in Congo basin forest to treat infection of gastrointestinal tract.

Antibacterial, antiviral, antiprotozoal, antifungus.	Targets infections	References
Psydium guajava, Alchornea cordifolia	*Bacillus spp, Clostridium, Entamoeba histolytica, Escherichia coli, Giardia spp, Human rotavirus, Pseudomonas, Salmonella spp, Salmonella typhimurium, Shigella flexneri, Staphylococcus aureus, Vibrio cholerae, VIH$_{1,2}$*	(Grover and Bala, 1993), (Wei et al., 2000), (Abdelrahim et al., 2002), (Goncalves et al., 2008), (Ojewole et al., 2008), (Tavares et al., 2012), (Fernandes et al., 2012), (Goncalves et al., 2005), (Tona et al., 2000), (Sriwilaijaroen et al., 2012), (Mao et al., 2010), (Birdi et al., 2011), (Alves et al., 2009), (Agbor et al., 2004), (Adeyemi et al., 2008), (Tona et al., 2000) (Ayisi and Nyadedzor, 2003),
Antibacterial, antiprotozoal (n= 35)		
Mangifera indica, Adansonia digitata, Annona muricata, Detarium microcarpum, Erythrina senegalensis, Euphorbia hirta, Holarrhena floribunda, Indigofera tinctoria, Irvingia gabonensis, Lannea acida, Pachylobus buttneri, Pterocarpus erinaceus,Stachytarpheta jamaïcensis, Ziziphus mucronat, Piptadeniastum Africana, Harungana madagascariensis Crossopteryx febrifuga, Nauclea latifolia, Maprounea africana, Pterocarpus soyauxii, Sacoglottis gabonensis, Isolona hexaloba	*Antiprotozoal, Anti helmintic, Entamoeba histolytica, Enterobacter aerogenes, Klebsiella pneumonia, Shigella dysenteriae, Streptococcus pneumonia, Trichomonas gallinae,*	(Singh et al., 2010), (Rajan et al., 2011), (Tona et al., 2000), (Galvez et al., 1993), (Assob et al., 2011), (Musuyu Muganza et al., 2012), (Iwalewa et al., 2008), (Tona et al., 2000), (Tchamadeu et al., 2011), (Nwosu et al., 2008), (Musuyu Muganza et al., 2012).
Antimicrobials (n= 6)		
Bridelia micrantha, Phyllanthus muellerianus, Pentaclethra macrophylla, Picralima nitida, Senna alata	*Staphylococus aureus, Escherichia coli, Proteus vulgaris, Pseudomonans aureginosa, Bacillus subtilis, etc.*	(Adeyemi et al., 2008), (Assob et al., 2011), (Akah et al., 1999), (Fakeye et al., 2000), (Lacmata et al., 2012), (Idu et al., 2007).

Table 5. Plants used in Congo basin forest to treat constipation.

Anti constipation plants (n=65)	References
Aframomum giganteum, Alstonia congensis, Alstonia gilletti, Amaranthus spinosus, Anchomanes difformis, Angokea gore, Anthostema aubryanum, Aucoumea klaineana, Baillonella toxisperma, Bixa orellana, Brazzeia klainei, Bridelia ferruginea, Bridelia micrantha, Camoensia maxima, Canarium schweinfurthii, Capsicum frutescens, Cassia alata, Cassia angustifolia, Cassia occidentalis, Cassia siamea, Citrullus colocynthis, Croton tchibangensis, Cynometra mannii, Cyrtogonone argentea, Dracaena fragrans, Elaeophorbia drupifera, Ficus hochstetteri, Gagnophyllum giganteum, Garcinia punctata, Guarea thompsonii, Heliotropium indicum, Ipomoea batatas, Ipomoea panic-ulata, Isolona hexaloba, Khaya ivorensis, Lagenaria vulgaris, Landolphia mannii, Landolphia owariensis, Maesopsis eminii, Mammea Africana, Maprounea membranacea, Mareya brevipes, Millettia Laurentii, Monodora myristica, Nauclea diderrichii, Neoboutonia canescens, Odyendyea gabonen-sis, Omphalocarpum pierreanum, Ongokea gore, Parinari kerstingii, Piptade-niastrum africanum, Plagiostyles africana, Premna angolensis, Psychotria gaboniae, Psychotria sp, Pycnanthus angolensis, Rauvolfia macrophylla, Rhizophora racemosa, Ricinus communis, Rinorea longicuspis, Scorodo-phloeus zenkeri, Staudtia kamerunensis, Strychnos aculeata, Tetrorchidium didymostemon, Trema guineensis	(Musuyu Muganza et al., 2012), (Deachapunya et al., 2005), (Hennebelle et al., 2009), (Jiofack et al., 2009), (Abdulrahman et al., 2004), (Appidi et al., 2010), (Schulzke et al., 2011), (Agbor et al., 2004), (Adeyemi et al., 2008), (Tal-Dia et al., 1997), (Palombo, 2006), (Badifu and Akubor, 2001), (Akah et al., 1999)

Table 6. Plants used in Congo basin forest to treat diarrhoea and dysentery.

Anti dysentery plants (n=32)	References
Adansonia digitata, Alchornea cordifolia, Allanblackia floribunda, Canna indica, Carica papaya, Combretum racemosum, Coula edulis, Dracaena mayumbensis, Euphorbia hirta, Gardenia jovis-tonnantis, Gilbertiodendron dewevrei, Harungana madagascarlensis, Irvingia gabonensis, Irvingia smithii, Maesobotrya barteri, Mitragyna ciliata, Mitragyna stipulosa, Nauclea latifolia, Parinari congensis, Parinari pygmeum, Pentaclethra macrophylla, Pterocarpus soyauxii, Raphia vinifera, Solanum nigrum, Strombosiopsis tetrandra, Symphonia globulifera, Treculia africana Decne, Treculia obovoïdea, Trichilia monadelpha, Triclisia dictyophylla, Triclisia longifolius, Vitex mandiensis	(Agbor et al., 2004), (Adeyemi et al., 2008), (Tal-Dia et al., 1997), (Palombo, 2006), (Badifu and Akubor, 2001), (Akah et al., 1999)
Anti diarrhoeal plants (n=41)	**References**
Aucoumea klaineana, Cassia occidentalis, Citrus limonum, Clerodendrum splendens, Cola nitida, Combretum platypterum, Cyathula prostata, Dacryodes edulis, Dacryodes heterotricha, Dinophora spenneroïdes, Enantia chlorantha, Erythropheum guineense, Fagara dinklagei, Fagara epreurii, Fagara laurenti, Fagara macrophylla, Fagara viridis, Geophila afzelii, Geophila sp, Heinsia crinita, Hermannia incana, Hugonia platysepala, Irvingia gabonensis, Irvingia grandifolia, Klainedoxa gabonensis, Millettia Laurentii, Panda oleosa, Pentadesma butyracea, Pseudospondias microcarpa, Rauwolfie obscura, Streptogyne crinita, Terminalia catappa, Terminalia superba, Tetracera alnifolia, Tetracera potatoria, Tetrapleura teraptera, Thomandersia butayei, Urena lobata, Xysmalobium undulatum, Zanthoxylum gilletii, Zanthoxylum tessmannii	(Musuyu Muganza et al., 2012), (Deachapunya et al., 2005), (Hennebelle et al., 2009), (Jiofack et al., 2009), (Abdulrahman et al., 2004), (Appidi et al., 2010), (Schulzke et al., 2011), (Agbor et al., 2004), (Adeyemi et al., 2008), (Tal-Dia et al., 1997), (Palombo, 2006), (Badifu and Akubor, 2001), (Akah et al., 1999)

reduction of intestinal secretion of fluid as shown the reduction of short-circuit current (Isc).

2.2.3.3. Preformulation of HIV / AIDS phytomedicine

Polyphytotherapy alternative and combination (PPAC) may use to develop phytome-dicine against AIDS (Fig. 14). The acquired immunodeficiency syndrome (AIDS) is a result of human immunodeficiency virus (HIV) infection which subsequently leads to significant suppression of immune functions. The functional approach for AIDS disease is, to develop antiviral drugs, and immunorestorative therapy, if necessary to manage opportunistic diseases with herbal medicine. The possibility to use phytotherapy and allopathy at the same time is always mentioned by medical practitioners and traditional healers in Africa. Although several studies mentioned the interaction between allopathic and phytomedicine drugs (Brown et al., 2008; Lee et al., 2006; Molto et al., 2011; Muller et al., 2012), general adverse effects due to undesired interaction can easily be eliminated. Utilization of herbal medicine depends on the state of AIDS. Based on our experiment, the combination of allopathic antiretrovirals and immunorestorative phytotherapy is advised.

2.2.3.3.1. Fagaricine (an immunorestorative phytomedicine)

Fagaricine is an immunorestorative phytomedicine prescribed in a few countries in Africa (Mokondjimobe et al., 2012). Clinical studies supported the effect of Fagaricine on
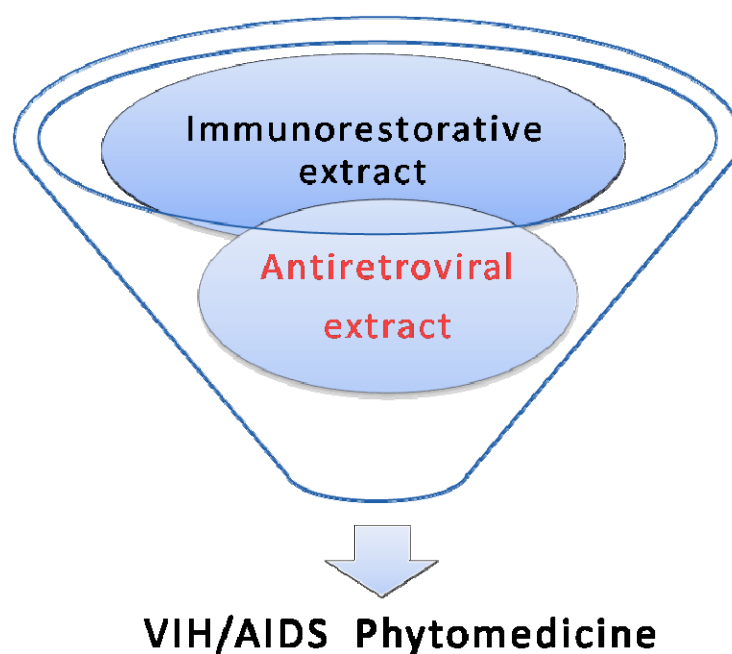
Figure 14**.** Functional approach of formulation of HIV/AIDS phytomedicine. The best formulation of phytome-dicine must contain at least an immunorestorative extract and antiretroviral extract.

Table 7. Antiretroviral and Immunorestorative herbal medicine used in Africa.

Immunorestorative herbal medicine (n=11)	References
Allium Sativum, Aloe vera, Alternanthera pungens, Azadirachta indica, Canova (herbal preparation), Hypoxis hemerocallidea, Morinda lucida, Scilla natalensis, Sorghum bicolour, Zingiber officinale, Dracaena fragrans, Fagara hertzii	(Peltzer et al., 2011), (Awodele et al., 2012), (Onifadee et al., 2011), (Pretorius et al., 2009), (Smit et al., 2009), (Djohan et al., 2009), (Mbah et al., 2007), (Nworu et al., 2012), (Moshi et al., 2012)
Antiretroviral herbal medicine (n=28)	References
Aucoumea klaineana, Cassia occidentalis, Citrus limonum, Clerodendrum splendens, Cola nitida, Combretum platypterum, Cyathula prostata, Dacryodes Acacia tortilis, Aspilia pluriseta, Azadirachta indica, Barleria eranthemoides, Bersama abyssinica, Bersama engleriana, Bulbine alooides, Cassia abbreviate, Cassia sieberiana, Combretum adenogonium, Combretum molle, Combretum paniculatum, Crinum macowani, Dodonaea angustifolia, Elaeodedron schlechteranum, Ficus cycamorus, Hypoxis sobolifera, Indigofera colutea, Lannea schweinfurthii, Leonotis leonurus, Peltophorum africanum, Plumbago zeylanica, Rumex bequaertii, Schumanniophyton magnificum, Terminalia mollis, Tithonia diversifolia, Tulbaghia violacea, Ximenia americana.	(Asres et al., 2001), (Asres and Bucar, 2005), (Bessong et al., 2005), (Cos et al., 2002), (Udeinya et al., 2004), (Houghton et al., 1994), (Klos et al., 2009), (Leteane et al., 2012), (Maregesi et al., 2010), (Mbaveng et al., 2011), (Theo et 2009)
Reduced adverse allopathic drug events (n=6)	References
Allium Sativum, Bidens pilosa, Eucalyptus globules, Lippia javanica, Moringa oleifera, Peltoforum africanum	(Mudzviti et al., 2012)

Figure 15: A) Variation of bodyweight after 24 weeks of Fagaricine treatment. B) Time-course effect of Fagaricine on CD4 restoration from a baseline categorization of CD4 before treatment. (Mokondjimobe et al., 2012). ***p<0.0001

bodyweight gain (Fig. 15A) and reduction in VT index. The capacity of Fagaricine to restore CD4 counts (Fig.15B) is effective when the immune system contains, at least 50 cells /µL of CD4 counts and somewhat superior of that of some AVRs (Fig. 16).

Different traditional medicines used against HIV/AIDS are represented in Table 7-8. According to the possibilities that PPAC offers, broadly based treatments can be prepared using African plants. For example, the possibilities to preformulate ARV phytomedicines with four plants among those of Table 8 are:

$$z = C_{146.4} = 146!/4!142! = 18\ 163\ 860 \text{ preformulations}$$

Figure 16. Regression between treatment effect on CD4 count and clinical benefit from EuroSIDA clinical endpoint trials and Fagaricine (F-532) treatment. Each data point is the comparison of two treatment groups in a randomized clinical trial. The x-axis is the difference between the treatment arms in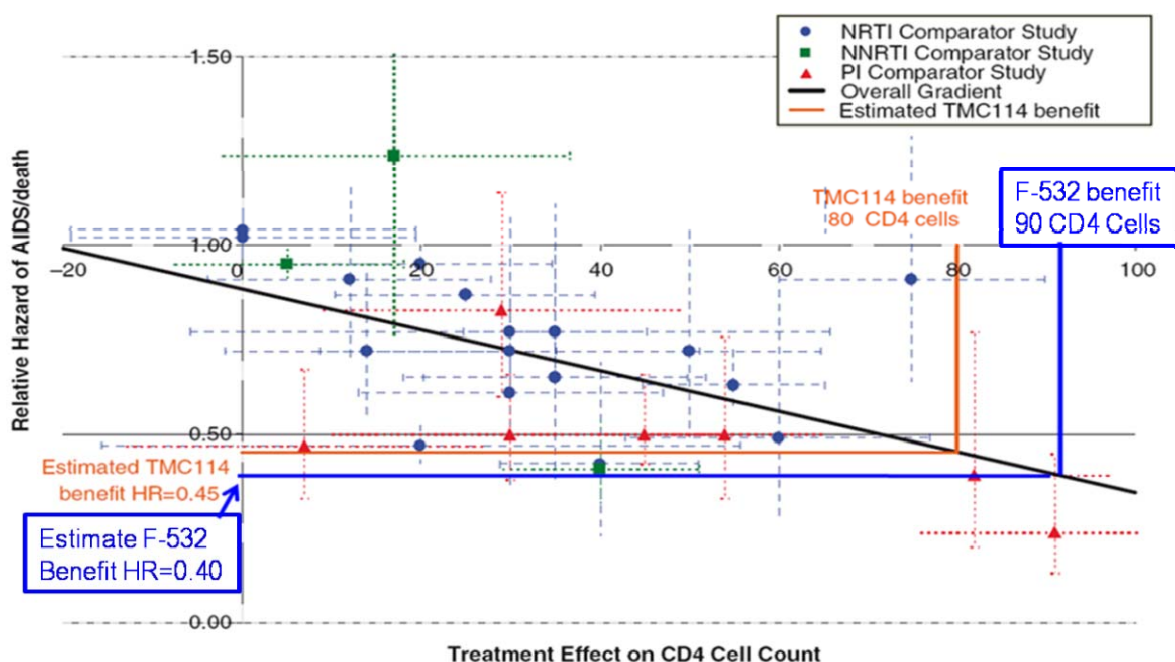 CD4 counts at weeks 16-24. For the y-axis, relative hazard of progression to AIDS/death (HR) is shown on a scale of 1.25 - 0, where a hazard ratio of 1 corresponds to no clinical benefit for the treatment arm relative to the control arm, and 0.5 corresponds to a 50% lower hazard of clinical progression for the treatment arm. The HR=0.40 (after 16 weeks of treatment) of F-532 was obtained by interpolation. NRTI is Nucleoside reverse transcriptase inhibitor. NNRTI is non nucleoside reverse transcriptase inhibitors and PI protease inhibitor (Mokondjimobe et al., 2012).

2.2.3.4. Preformulation of anti malaria phytomedicine

Malaria remains a major problem of morbidity and mortality worldwide, with social and economic impacts on the development. A vast majority of underdeveloped countries are concerned with this problem, especially in sub-Saharan countries in Africa. A lot of programs for eradication of malaria have been conducted by several local and international organisations with no real effect. Malaria continues to kill over the world. Almost a million of people die each year, among them, 8 million children.

Historical program of malaria

At end of the 19e century, Ronald Ross discovered that the vector of malaria is anopheles, setting the first mathematical model of malaria transmission (Ross, 1905; 1911).

1955: the vote of eradication of malaria by the World Health Organization (WHO) and the intensively used of dichlorodiphenyltrichloroethane (DDT).

1970: Garki project in Nigeria (Dietz et al., 1974), to faith against malaria in Africa, with the objective to control it in the Africa context.

1988: The beginning program of the Roll Back Malaria (RBM) Partnership by WHO and several other organizations and institutions. The paradigm of malaria control and elimination

Table 8. Plants used as treatment of HIV/AIDS and related opportunistic infections in Africa

Plants for treatment of opportunistic infections	References
Abrus precatorius, Abrus precatorius, Acacia erioloba, Acacia erubescens, Acacia hockii, Acacia nigrescens, Acacia nilotica, Adansonia digitata, Adenia gummifera, Agauria salicifolia, Ageratum conyzoides, Albizia amara, Albizia anthelmintica, Allium porrum, Allium sativum, Allophyllus africanus, Aloe spp, Aloe vera, Aloe zebrine, Annona senegalensis, Annona stenophylla, Antidesma venosum, Argemone Mexicana, Azadirachta indica, Bersama abyssinica, Bidens pilosa, Burkea africana, Cajanus cajan, Canthium zanzibarica, Capparis erythrocarpos, Capparis tomentosa, Carica papaya, Carissa edulis, Cassia abbreviate, Cassia mimosoides, Cathium burtti, Chenopodium Ambrosioides, Chenopodium opulifolium, Citrus aurantium, Citrus limon, Clematis hirsute, Cocos nucifera, Combretum collinum, Combretum glutinosum, Combretum spp, Commiphora africana, Conyza floribunda, Corchorus olitorius, Croton lechleri, Cucumis culeatus, Cussonia arborea, Cymbopogon citrates, Cyphostemma hildebrandtii, Cyphostemma adenocaule, Dalbergia melanoxylon, Dichrostachys cinerea, Diospyros melanoxy-lon, Diospyros mespiliformis, Diospyros spp, Dracaena steudneri, Elaeodendron buchananii, Entada abyssinica, Entada leptostachya, Eriosema psoraleoides, Erythrina abyssinica, Euclea divinorum, Euphorbia heterophyla, Ficus exasperate, Ficus sycomorus, Ficus thonningii, Garcinia buchananii, Garcinia livingstonei, Grewia avellana, Grewia bicolor, Grewia falcistipula, Grewia flava, Grewia occidentalis, Guibourtia tessmannii, Guineense cordatum, Gynandropsis gynandra, Harungana madagascariensis, Hibiscus fuscus, Hypoxis hemerocallidea, Ipomoea kituensis, Ipomoea sinensis, Jatropha curcas, Kigelia Africana, Lannea schimperi, Lannea schweinfurthii, Lannea stuhlmannii, Lannea zastrowiana, Launaea cornuta, Lophira alata, Mangifera indica, Maytenus senegalensis, Melia azedarach, Moringa stenopetala, Myrica salicifolia, Ocimum gratissimum, Ozoroa insignis, Parinari curatellifolia, Peltophorum africanum, Phyllanthus reticulates, Phytolacca dodecandra, Plectranthus barbatus, Plectran-thus comosus, Pseudospondias microcarpa, Psidium guajava, Psorospermum febrifugum, Pteridium aquilinum, Pterocarpus erinaceus, Rapanea melanophloeus, Rauvolfia vomitoria, Rhoicissus tridentate, Rhus natalensis, Rhus tenuinervis, Rhus vulgaris, Ricinus communis, Rumex usambarensis, Sansevieria trifasciatai, Sapium ellipticum, Sarcocephalus latifolius, Schrebera alata, Sclerocarya birrea, Securidaca longipedunculata, Securidaca longipedunculata, Senecio syringifolius, Senna occidentalis, Sutherlandia frutescens, Syzygium cordatum, Syzygium guineense, Tarenna graveolens, Terminalia mollis, Terminalia sericea, Thevetia peruviana, Thurnbergia alata, Tithonia diversifolia, Trema orientalis, Vagueria infausta, Vangueria infausta, Vernonia adoensis, Vernonia amygdalina, Vitex fischeri, Warburgia salutaris, Ximenia Amer-icana, Zehneria scabra	(Chinsembu and Hedimbi, 2010), (Lamorde et al., 2010), (Lubinga et al., 2012), (Minocha et al., 2011), (Muller and Kanfer, 2011), (Nagata et al., 2011), (Theo et al., 2009), (Kisangau et al., 2007)

Table 9. Plants used in Congo basin forest to treat malaria alone

Antimalarial plants (n = 29)	References
Acmella caulirhiza, Aspilia africana, Baillonella toxisperma, Buchholzia coriacea, Carapa procera, Chlorophora excelsa, Cissus sp, Costus afer, Cylicodiscus gabunense, Elaeis guineensis, Funtumia elastic, Garcinia cola, Irvingia gabonensis, Microdesmis puberula, Milletia sanagana, Morinda morindoïdes, Musanga cecropioides, Omphalocarpum elatum, Piptadeniastrum africanum, Polyalthia oliveri, Polyalthia suaveolens, Quassia africana, Senna hirsute, Solanum anguivi, Solanum torvum, Strychnos camptoneura, Trichilia rubescens, Uapaca sp.Vernonia amygdalina	(Zirihi et al., 2005), (Betti, 2004), (Betti, 2002), (CE-FAO 1999), (Nkeoua and Boundzanga, 1999), (Jiofack et al., 2009)

Table 10. Plants used in Congo basin forest to treat malaria and fever.

Antimalarial plants (n = 29)	References
Acanthospermum hispidum, Acolanthus lamborayi, Aframomum daniellii, Aframomum meleguetta, Ageratum conyzoides, Alchornea cordifolia, Alstonia boonei, Alstonia congensis, Amaranthus viridus, Anogeius leiocarpus, Anona muricata, Anonidium mannii, Anthocleista schweinfurthii, Anthocleista vogellii, Azadirachta indica, Biaxa arellana, Bidens bipinata, Bidens pilosa, Bombax flammeum, Bridelia micrantha, Brucea antidysanterica, Cadaba farinose, Canna indica, Carica papaya, Cassia alata, Cassia hirsute, Cassia occidentalis, Cataeva adansonii, Catharanthus roseus, Cedrela odorata, Celtis brieyi, Celtis cf. tessmannii, Centella asiatica, Chenopodium ambrosioïdes, Chretia cymosa, Cinchona ledgeriana, Cinchona succiruba, Cinchona calisaya, Cissus quadrangulari, Citrus limonum, Cleistopholis patens, Cleome ciliata, Clerodendron scandens, Cocos nucifera, Combretum glutinosum, Combretum latialatum, Combretum micranthum, Combretum platysterum, Combretum spinesis, Commelina benghalensis, Costus dubius, Crossopteryx febrifuga, Cylicodiscus gabunensis, Cymbopogon citrates, Dedonaea viscose, Distemonanthus benthamianus, Disthmonanthus benthamianus, Emilia coccinea, Enantia chlorantha, Entandrophragma angolense, Eremomastax speciosa, Eucalyptus globules, Eucalyptus grandis, Eucalyptus robusta, Eucalyptus saligna, Euphorbia hirta, Fagara macrophylla, Ficus exasperate, Ficus thonningii, Guibourtia tessmannii, Guiera senegalensis, Harrrisonia abyssinica, Harungana madagascariensis, Hibiscus asper, Hibiscus tiliaceus, Holarrhena floribunda, Hydrangea sp., Isolona campanulata, Justicia flava, Justicia Insularis, Khaya grandifolia, Khaya ivorensis, Lagera alata, Lantana camara, Lecaniodiscus cupanoïdes, Leea guineensis, Lophira alata, Macaranga spinosa, Mammea Africana, Mangifera caesia, Mangifera indica, Manihot esculenta, Manniophyton fulvum, Markhamia gellatiana, Markhamia sessilis, Mentha sylvestris, Milicia excels, Milletia laurentii, Mitacarpus scaber, Momordica cissoids, Momordica haranta, Momordica balsaminata, Monodora myristica, Morinda confusa, Morinda lucida, Morinda morindoïdes, Musa paradisiaca, Musa sapientum, Mytragina ciliata, Mytragina stipulosa, Napoleona vagelli, Nauclea diderrichii, Nauclea pobeguinii, Nymphea lotus, Olax subscorpioides, Oxyanthus unilocularis, Palisota hirsuta, Passiflora foetida, Pennisetum purpureum, Pentaclethra macrophylla, Peperomia pellucida, Picralima nitida, Piper umbellatum, Piptadensiastrum africanum, Pleiocarpa mutica, Pollia condensata, Polycarpa glabrifolia, Psidium guajava, Psorosperuns febrifugum, Psychotria gabonica, Pterocarpus soyauxii, Quassia Africana, Rauvolfia macrophylla, Rauvolfia obscura, Rauvolfia vomitoria, Ricinus cumunis, Rumex abyssinicus, Schumanniophytum magnificum, Sida acusa, Sida rhombifolia, Sida urens, Spathodea campanulata, Spathodea campanulata, Spilanthes acmella, Spondias monbin, Strophanthus sp., Strychnos camptoneura, Tabernaemontana crassa, Tabernaemontana penduliflora, Terminalia ivorensis, Terminalia macroptera, Tetrapleura tetraptera, Thomandersia hensi, Thonningia sanguinea, Trama guineensis, Trichilia emetia, Trichilia gilletii, Tricoscypha ferruginea, Triplotaxis stellulifera, Uvaria chamae, Uvaria klaineana, Uvaria mocoli, Uvaria versicolor, Uvariastrum pierreanum, Uvariopsis congolana, Vernonia Thomsoniana, Vernonia conferta, Voacanga Africana, Voacanga obtusa, Xylopia aethiopica	(Addae-Kyereme et al., 2001), (Kemgne et al., 2011), (Tepongning et al., 2011), (Bickii et al., 2006), (Zirihi et al., 2005), (Udeinya et al., 2004), (Mesia et al., 2010), (Betti, 2004), (Betti, 2002), (CE-FAO, 1999), (Nkeoua and Boundzanga, 1999), (Jiofack et al., 2009), (Addae-Kyereme et al., 2001), (Abiodun et al., 2011), (Kemgne et al., 2011), (Boyom et al., 2011), (Tepongning et al., 2011), (Bickii et al., 2006), (Zirihi et al., 2005), (CE-FAO, 1999), (Nkeoua and Boundzanga, 1999)

has been extended to encompass an ultimate goal of malaria eradication (Fenner et al., 1988). **2002**: WHO recommended the use of artemisinin-based combination therapy (ACT).

2005: Launched of Malaria Control and Evaluation Partnership in Africa (MACEPA).

2006: Launched of a future without malaria by WHO and its recommendation to used ACTs in first line malaria therapy and the time of its use in fixed doses.
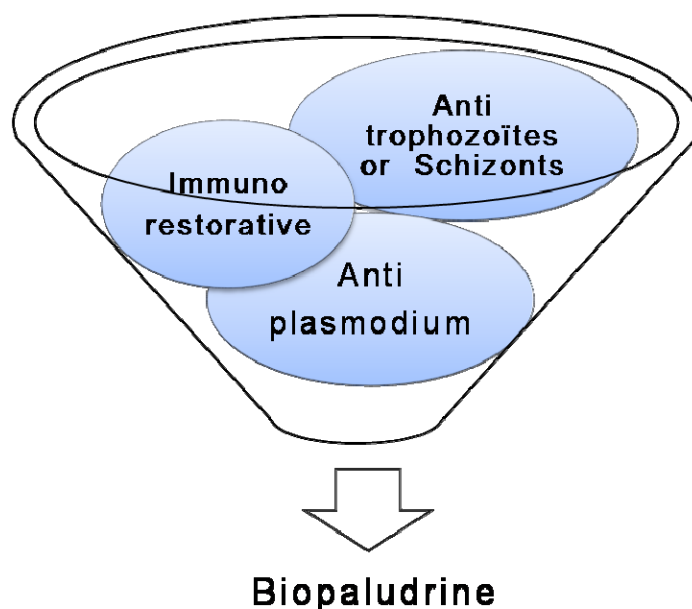
Figure 17. Composition of Biopaludrine with four plants: immunorestorative extract, and three anti-malaria plants (anti-plasmodium, anti-trophozoïdes, anti-schizonts).

2008: Launched of the Global Malaria Action Plan (GMAP) by the Roll Back Malaria partnership against a background of greatly increased investment in research and development for malaria control technologies. Since 1999 there has been extraordinary increases in funding for malaria control, and by national and global financing mechanisms since 2002 (Partnership, 2008).

According to this histological program, we have underlined that malaria continues to kill people. The objective for its eradication remains uncertain in spite of big slogans and mobilization by international partnership. We sincerely hope that the next slogan by WHO will be Polyphytotherapy Alternative and Combination (PPAC) to eradicated malaria. The use of PPAC for malaria eradication is possible, considering the large variety of plants used by traditional healers to cure malaria in worldwide.

As it is well know that, the main problem of the malaria is the resistance of plasmodium to allopathic medicines, like flavoquin, chloroquin and amodiaquine. These are ineffective to those medicines. The recommendation of WHO to use ACTs in multitherapy is the result of the multiple resistance of plasmodium to chemical drugs (malaria parasite life cycle is resume in Fig. 18). This strategy is close to the one we recommend in terms of polytherapy alternative and combination (PPAC). We have taken into consideration the use of multi-plants with others depending of their availability, or the possibility to combine existing plants with the objective of increasing the strength of the drug inside the tritherapy or quatritherapy.

For example, if we consider plants used to treat malaria and fiver in the Congo basin forest (Table 10), the possibilities to get preformulation of candidates phytomedicine in tritherapy or quatritherapy form without repetition and ordination are:

Table 11. Plants used in Congo basin forest to treat or manage diabetes.

Antidiabetic plants (n = 59)	References
Albizia zygia, Allium cepa, Allium sativum, Aloe vera, Alstonia boonei, Artabotrys aurantiacus, Bosqueiopsie giletii, Buchholzia macrophylla, Capparis tomentosa, Cassia occidentalis, Catharanthus roseus, Ceiba pentandra, Ceiba pentandra, Celtis zenkeri, Celtis zenkeri, Cissus Quadrangularis, Citrus limonia, Cola lateritia, Cola urceolata, Cola urceolata, Combretum afzelii, Costus afer, Dioscorea bulbifera, Eucalyptus globulus, Ficus exasperata, Ficus mucuso, Ficus mucuso, Garcinia cambogia, Hibiscus sabdariffa, Irvingia gabonensis, Irvingia gabonensis, Khaya anthotheca, Macaranga spinosa, Mangifera indica, Momordica charantia, Morinda lucida, Morinda lucida ,Morinda morindoïdes, Myrianthus arboreus, Ocimum gratissimum, Ouratea affinis, Paullinia pinnata, Pentadiplandra brazzea-na, Persea americana, Phyllanthus amarus, Pterocarpus soyauxii, Pycnocoma chevalieri, Rauvolfia vomitoria, Rauwolfie obscura, Ricinodendron heudelotii, Rinorea oblongifolia, Rinorea oblongifolia, riplochiton scleroxylon, Secamone spp, Staudtia kamerunensis, Staudtia kamerunensis, Terminalia superba, Triplochiton scleroxylon, Zingiber officinale	(Gurrola-Diaz et al., 2010), (Ngondi et al., 2009) , (Oben et al., 2008a), (Oben et al., 2008b), (Huang et al., 2008a), (Huang et al., 2008b), (Shih et al., 2008), (Oluyemi et al., 2007), (Ngondi et al., 2009), (Augusti, 1973), (Gray and Flatt, 1998), (Aderibigbe et al., 1999), (Akhani et al., 2004), (Jiofack et al., 2009)
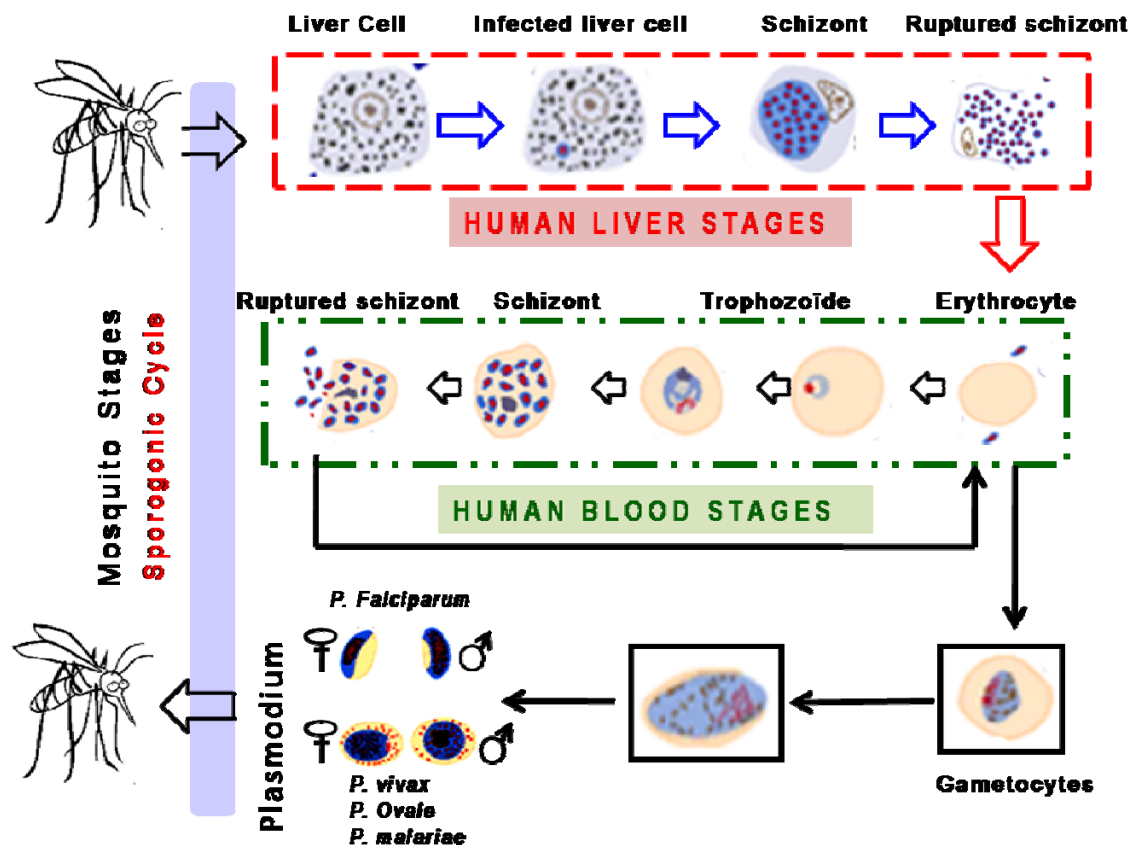
Figure 18. The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female anopheles mosquito inoculates sporozoites into the human host. Sporozoites infect liver cells and mature into schizonts, which rupture and release merozoites. Hypnozoites can persist in the liver and cause relapses by invading the bloodstream weeks, or even years later. After this initial replication in the liver (exo-erythrocytic schizogony), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony). Merozoites infect red blood cells. The ring stage trophozoites mature into schizonts, which rupture release merozoites. Some parasites differentiate into sexual erythrocytic stages (gametocytes). Blood stage parasites are responsible for the clinical manifestations of the disease. P is plasmodium.

1) Tritherapy

$$C_{170.3} = 170!/3!167! = 804\ 440 \text{ preformulations}$$

2) Quatritherapy

$$C_{170.4} = 170!/4!166! = 33\ 585\ 370 \text{ preformulations}$$

According to PPAC and Table 10, the possibility to preformulate more than 33 million of drugs in quatritherapy to flight against malaria is possible. Based on this concept,

we demonstrated that underdeveloped countries can eradicate malaria *per saecula seaculorum,* (for ever), if they need and at lower cost.

2.2.3.4.1. Biopaludrine: antimalarial phytomedicine

Malaria disease occurred generally when the defence of body is weak. I Africa we found that peoples suffering often from malaria had immune depression. We administrated Fagaricine (an immunorestorative) orally to 20 immunodepressives patients with constant malaria every month. During six months of treatment, none of the patients was infected by plasmodium. This observation allowed us to develop Biopaludrine®, an anti-malaria phytomedicine with four plants (Fig.17).

2.2.3.5. Preformulation of anti metabolic syndrome phytomedicine

Polyphytotherapy is particularly recommended to develop anti-type-2 diabetes and metabolic syndrome, although some plants exhibits insulin-like activity (Benhaddou-Andaloussi et al., 2011). Most of the commercial phytomedicine used to treat type-2, diabetes or metabolic syndrome inhibits intestinal absorption of sugar and sometime both sugar and fatty acids.

Metabolic syndrome phytomedicine contents generally three categories of plants extracts: anti-type-2 diabetes, dyslipidemia and antihypertensive (Fig. 19). Sometime one extract can contents two or three different properties such as antidiabetic, anti-hyperlipidemia and anti-hypertensive. Phytosucrophage or Biodiabetine (commercial name) is the bitherapy used as single dose dosage containing extract of *Nigella sativa* and *Olea europaea*. Phytosucrophage inhibits glucose and fatty acid absorption in intestine (Fig. 21). *Olea europaea* was consider as an antihypertensive component of Phytosucrophage. The combination of *Nigella Sativa* and *Olea europaea* is effective in treatment of metabolic syndrome.

2.2.3.5.1. Boscisucrophage: anti metabolic syndrome phytomedicine

Boscisucrophage or B-2MAY is an anti-metabolic syndrome medicine from African pharmacopeia. When pre-formulated according to the functional ratio concept and PPAC, we obtain a mixture of plant extracts which induces the reduction of intestinal absorption of sugar and fatty acids. Fig. 20 shows the typical recording of B-2MAY on short-circuit current (Isc) and Fig.21, the possible mechanism of action of B-2MAY.
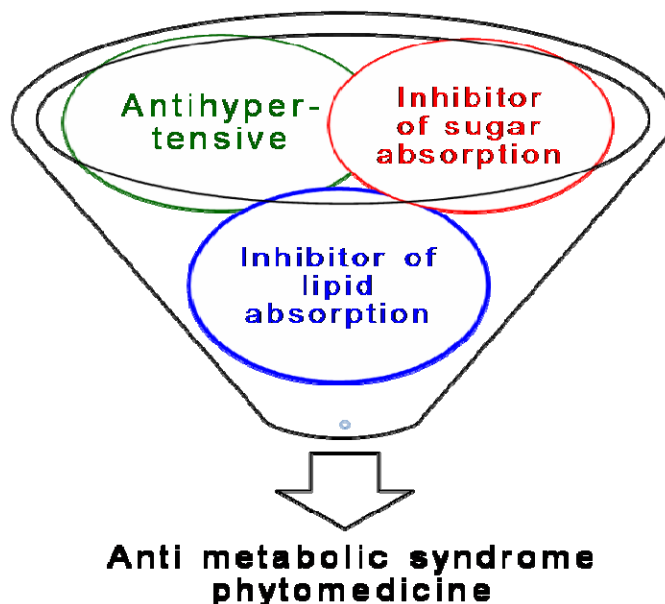
Figure 19. Anti-metabolic syndrome phytomedicine strategy. Metabolic syndrome phytomedicine must contain extracts with different biological effects (inhibitor of intestinal sugar and lipid absorption, an antihypertensive).
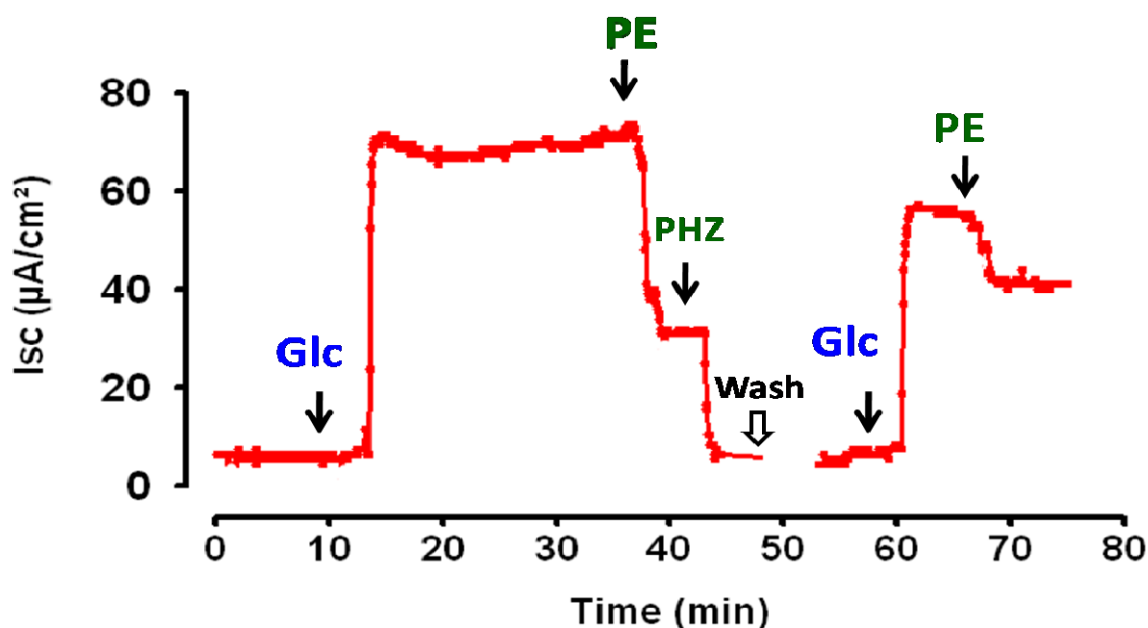
Figure 20. Typical recording of effect of B-2MAY on a short-circuit current (Isc) using Ussing chamber technique and Biodacqsoft program (TBC- Biomécatronics, France). The graphic shows that, when the glucose (20 mM) was introduced in mucosal side of rat jejunum, Isc increased to reflect the increase of glucose absorption. When B-2MAY was introduced in mucosal side after maximum response of glucose (Glc), the reduction of Isc was observed. This reduction of Isc indicated inhibition of glucose absorption. But this reduction of Isc was lower compared to that of 5.10^{-4} M of phloridzine (PHZ). The renewal of the medium after washing the tissue with a ringer solution (Wash) did not suppress the effect of plant extract, suggestion that, the effect of B-2MAY can be completely reversed by a renewal of mucosal medium.
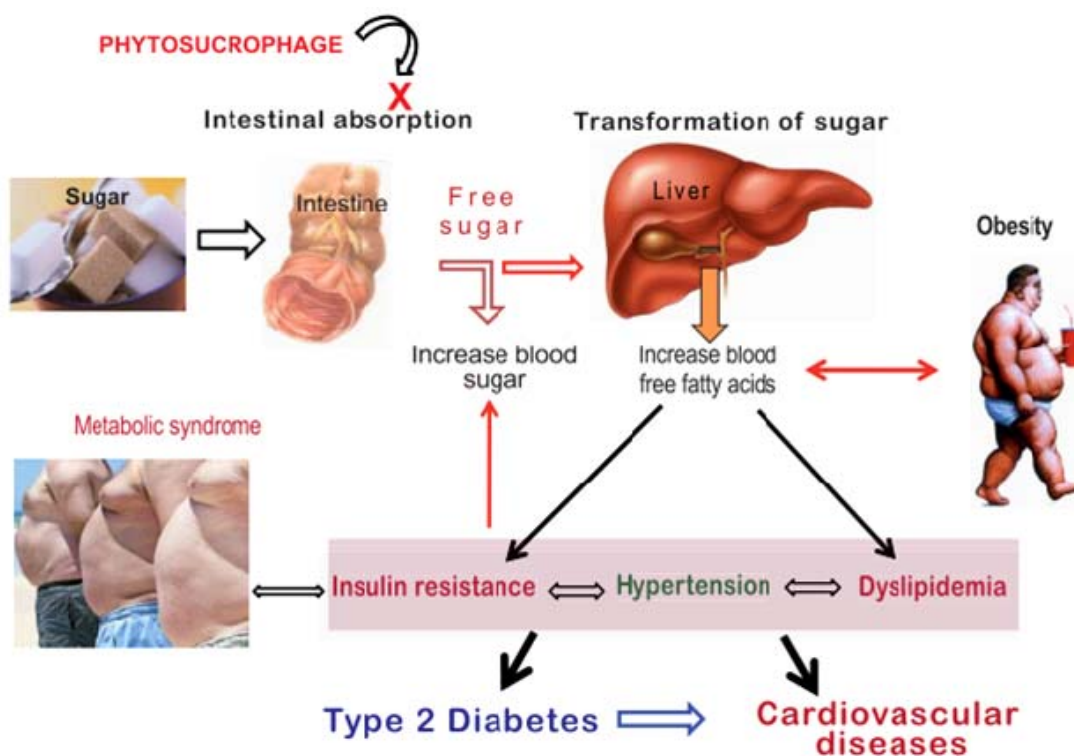
Figure 21. Role of sugar absorption and his impact on metabolic syndrome and obesity. Excessive absorption of sugar caused increases in concentration in the blood. Free sugar can also be transformed in the liver to free fatty acids which can cause obesity, dyslipidemia and insulin resistance. Imbalance of hypertension, dyslipide-mia and insulin resistance can also induce type-2 diabetes, cardiovascular diseases and metabolic syndrome. Inhibition of sugar absorption during meal by Phytosucrophage or B-2MAY regulates blood free sugar and contributed to reduce his impact on metabolic syndrome, type-2 diabetes, obesity and cardiovascular diseases.

2.2.4. Formulation of commercial dosage form for clinical evaluation

Preformulation is a very important step in the development of phytomedicine. In general, this step is combined with preclinical studies, such as pharmacodynamic and toxicology. The permanent feedback between preformulation studies and preclinical studies (Fig. 22) is necessary until we come out with an ideal preformulation phytomedicine candidate with the following characteristics:

- High potency and selective (close to that of a reference chemical drug),
- No/ very low toxicity (High margin of safety),
- High-permeability coefficient (determined by a functional approach throughout GI tract)
- High solubility in water or natural media (oil, ethanol),
- Stability.

These characteristics are important for formulation of commercial dosage form, base on biopharmaceutical criteria such as dissolution, solubility, stability in intestinal luminal fluids and bioavailability.
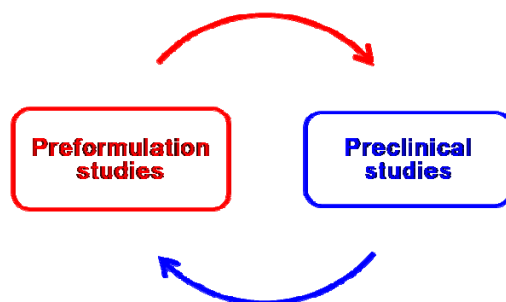
Figure 22: Feedback between preformulation and preclinical studies.

This strategy is more valid when pharmacodynamics studies can be conducted *in vitro* (in controlled area). Sometimes the development of phytomedicine became complex when prodrugs exist. In that case, evaluation of preformulation was done *in vivo,* and it can take some time due to the complexity of assessment of formulations. The best example is *Euphorbia Hirta* an anti-dysentery phytomedicine. *In vitro* studies, the aqueous extract exhibit less strength than amoebicide, vis-à-vis methanol or organic solvents. In addition, the concentration used *in vitro* is more than 200-fold than that used *in vivo* in treatment of dysentery in human therapy. This observation suggests that *Euphorbia Hirta* is the pro-drug *in vitro,* and it was transformed into active drugs in luminal fluid probably by intestinal metabolism. This suggestion was confirmed in our laboratory (personal communication) when the aqueous extract of plant was incubated in a rat intestine before the introduction in medium containing amoeba. The result obtained was completely different, and the extract became more active at the lower concentrations.

2.2.4.1. Traditional practitioner method

Administering medications orally has been, and is, still, the most convenient and popular route for patient therapeutics. Besides of being the most convenient route, especially for phytomedicines, it is not the simplest one. Since, barriers of the gastrointestinal (GI) tract, in many cases, are difficult to circumvent. In general, drug absorption from the GI tract requires that the drug be brought into solution in the GI fluids, and be capable of crossing the intestinal membrane into the systemic circulation. It has, therefore, been suggested that the drug must be in its molecular form before it can be absorbed. Hence, the rate of dissolution of the drug in the GI lumen can be a rate-limiting step in the absorption of drugs given orally.

The first problem to be solved by a pharmacologist is to determine the dosage and posology. What quantity of extract should I used as a dosage? When the origin of phytomedicine is traditional, it's easy to determine the dosage and posology following the questionnaire (question paper bellow) of ethnobotanic therapeutic benefit survey. A good questionnnaire must contain all information and the time of its preparation.

- Portion of plant (stem bark, leaves, roots, etc.),
- Directions (Form of utilization): powder (if yes, density?), decoction, tisane, infusion (hot or normal temperature), others precisions.

- ▪ Frequency (daily? how much per day? and others precisions),
- ▪ Period (morning? midday? afternoon? fed or fasting period?),
- ▪ Therapeutic indication with more precisions,
- ▪ Mode of detailed preparation (the plant with or without mixtures, number of mixed plants, the method and order of introduction of each plant with a mixed preparation and conservation,
- ▪ Expected time of therapeutic effect (minutes, hours, days, moths),

In our laboratory, we usually take a daily dosage used by traditional healers as a reference to a calculated modern dosage form. When the traditional preparation was in powder, the extraction of active principle was done in water dilution. Then the aqueous extract was filtered, concentrated and dried out using an atomizer (spray-dryer). As the traditional mixture became liquid, it was filtered and dried out by atomization. By this procedure we obtained directly the daily dosage of phytomedicine sample, at an average of 10 measures.

2.2.4.2. Comparative method

The comparative method of determining of the dosage form is empirical. It is based on a simple comparison with the existing medicine prescribed in clinic, same effect as possible and same mechanism of action. I always recommend this method when pharmacologic parameters are available such as EC_{50}, IC_{50}, and PA_2. The method was also called ''ratio equivalent''.

For example, if $[A]C_d$ represents the commercial dosage unit of reference chemical drugs (A) and $[B]C_d$ the unknown commercial dosage unit of new phytomedicine candidate (B). The effective concentration for 50% of maximum response of A is $[A]EC_{50}$, whereas that of an unknown phytomedicine is $[B]EC_{50}$. The equation of equivalent ratio is:

$$[B]C_d/[A]C_d = [B]EC_{50}/[A]EC_{50} \qquad (12)$$

If the apparent coefficient of permeability of [B] is close or equivalent of that of [A], then the commercial dosage form of new phytomedicine candidate is:

$$[B]C_d = (\,[B]EC_{50} / [A]EC_{50}\,) \times [A]C_d \qquad (13)$$

But, if the apparent coefficient of permeability of [B] ($J_{app}[B]$) is different to that of [A] ($J_{app}[A]$), with $J_{app}[B] < J_{app}[A]$, then, the equation 13 became:

$$[B]C_d = [\,(\,[B]EC_{50} / [A]EC_{50}\,) \times [A]C_d\,] \times (\,J_{app}[B] / J_{app}[A]\,) \qquad (14)$$

As an example, the determination of commercial dosage form of Liberaline was calculated as follows:

We postulated that Loperamide is an antispasmodic, mixed with musculotropic and neurotropic effect close to that of Liberaline, as shown Fig. 23. The commercial dosage form of Loperamide is 2 mg. We calculated EC_{50} in the same condition of experiment with Libera-
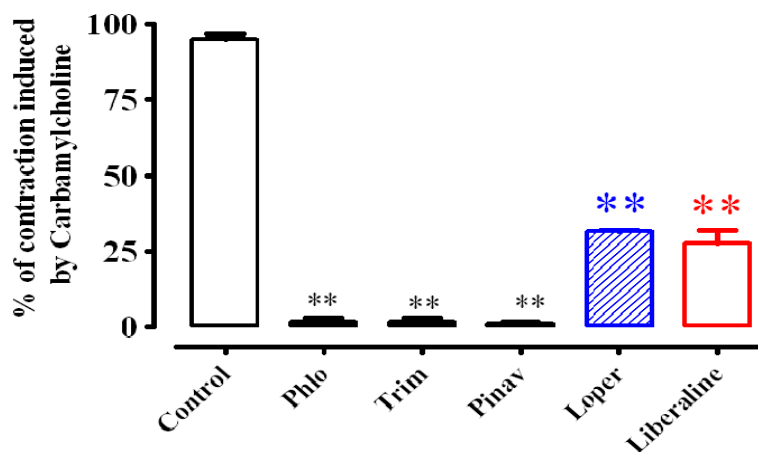
Figure 23. The effect of different medicine used in clinics to the contraction induced by 10-6 M of Carbamyl-choline. Note that the effect of Liberaline is close to that of Loperamide (Loper). Musculotropic medicines such as Phloroglucinol (Phlo), Trimebutine (Trim) and Pinaverium bromide (Pinav), did not affect the effect of Carb-amylcholine, whereas Loperamide (Loper) and Liberaline which are a mixture of antispasmodic, reduced the effect of Carbamylcholine.

aline and found EC_{50} of Loperamide of 51.8 ± 0.1 µg/mL, whereas that of Liberaline was 27.5 ± 1.1 µg/mL (see tab.2).

If we consider that the J_{app} of Liberaline is close to that of Loperamide, then comer-cial dosage unit of Liberaline is calculated as:

$$[B]C_d = (27.5 / 51.8) \times 2 = 1.06 \text{ mg}$$

2.2.4.3. Allopathy concept

During preformulation of medicine, compounds need to be evaluated in animals for acute and chronic toxicity for 7-day and 28-day single and multiple ascending doses (SADs and MADs) (Kramer et al., 2007). After toxicology studies, commercial dosage was determi-ne during clinical trials phase I and II to a suitable form to ensure systemic exposure.

2.3. Phytopharmacy and pharmaceutical production

Phytopharmaceutics is the study of how the physicochemical properties of the candid-ate phytomedicine, the formulation/delivery system, and the route of administering the drug affect the rate and extent of drug absorption. Many phytomedicines used in clinics have a traditional origin. Working with traditional healers is very important because the appropriate information generated with their collaboration can extremely be useful as a guide to the cand-idate phytodrug selection process, and for future dosage. From a personal experience and inheritance as a descendant of a family of traditional healers, I worked with, listened to and selection of the optimal compound for a pharmaceutical development.

2.3.1. Standardization and optimization of raw material

Modern methods used in development of chemical drugs (allopathy) are necessary but no sufficient in development of phytomedicine. In allopathy, the drug is generally one

active principle whereas in phytotherapy active principle is generally the mixture of several compounds, so that the concept of standardization of the extract by dosage of one compound became illusory. Only the biological dosage is close to the reality. We mentioned some methods used by African traditional healers to standardize their preparations.

2.3.1.1. Ethnomedicine concept

The Fang ethnic group (inhabitants of Cameroun, Congo, Equatorial Guinea and Gabon) was known to be experts in the use of plants in medicine and in occult sciences. Fang people considered plant as living beings. According to Fang tradition, "when a plant is sad or ill, it can provide the best medicine". Fang traditional healers can determine the best period to take medication from plants. For example, some anti-cough and anti-abdominal pain medicines were taken from bark trees during rainy seasons only. The medicines were kept dried before their use at dry seasons. Some medications were forbidden to be dried under the sun. In Cameroon, men from the village of Mekomo-Ambam, usually used Annonaceae bark as aphrodisiac. Only male tree was taken because the female bark tree was inactive. When we compared UV spectrum in our laboratory of male and female bark extract, we found the difference, suggesting that the male extract contains some compounds which were not present in the female tree.

In conclusion, it is important to take in account adverse information of the traditional healers in development of phytomedicine.

2.3.1.2. Chronobiology concept

Chronobiology must be taken into account during harvesting raw materials from some plants. Many traditional healers take raw material (from plants) only in early morning hours or at nightfall. An elderly uncle of mine usually takes certain roots of plant to treat prostate inflammation only at the moonless night. His wife provides anti-abortion medicateons in full moon night. The UV spectra of aqueous extract of plant used to stop sudden epidemic death of chickens and ducks was different when the bark was taken from trees at night time and during the day. In conclusion, Chronobiology can be a limiting factor during the harvesting raw materials from certain plants.

2.3.1.3. Amelioration of yield of extraction of active principle

The best example of improvement of the yield of raw materials is Fagaricine, an immunorestorative phytomedicine from *Fagara Hertzii*. The process of extraction of active principle is shown in Fig. 24.

The harvesting of raw material from plant is the limiting factor of the production of phytomedicine. Some developing countries are banning the industrial use of forest plants to produce phytomedicine without the authorization in focus to preserve the nature. Thus, cultivated medicinal plants become an absolute necessity, and make it compulsory for producer to get a good yield of active principles.

According to Fig.24, the active principle is obtained during the step of maceration. During this period, several operations can be done to improve the yield of extraction, includ-
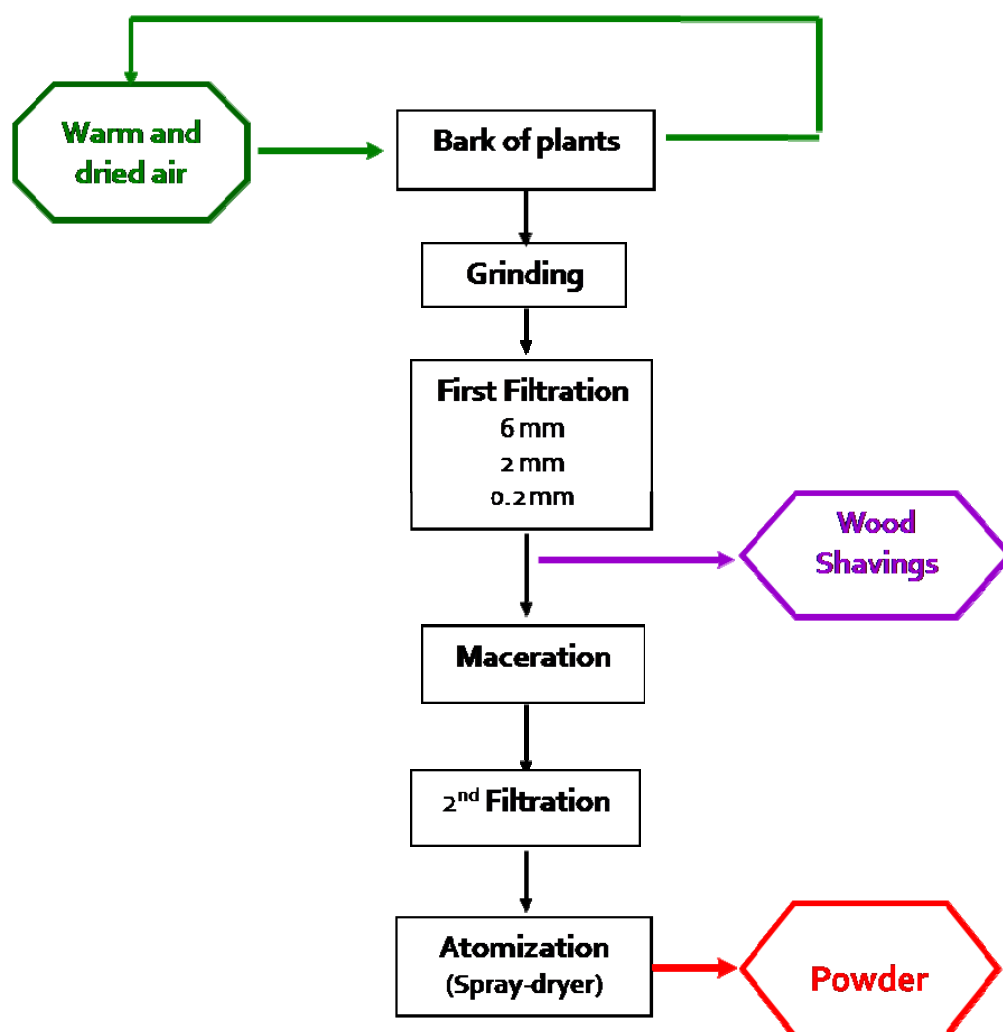
Figure 24. Process of extraction of active principle of Fagaricine

ing a few modifications of the temperature, ultrasonic extraction, renewal of the solvent, agitation, addition of salt etc.

2.3.1.4. *Optimization of extraction of active principle*

The first step of optimization of plant extraction was done through variation of three parameters: temperature, the ratio raw material/solvent, and the duration contact (Fig. 25).

The computerization of the model can be done to simulate the equation of the optimization process. It is important in that case, to determine the range of variation of each parameter, for example:

- Temperature [20°C - 70°C],
- Ratio raw material/solvent [0.5% - 9,9%]
- Time of soaking [10 min - 120 min]

After determining the range of variation of the simulation parameter, the composite plan can be applied using codes values as follow: -1.68, -1, 0, 1, 1.68, which will help find the real values after computerization (Fig.26).
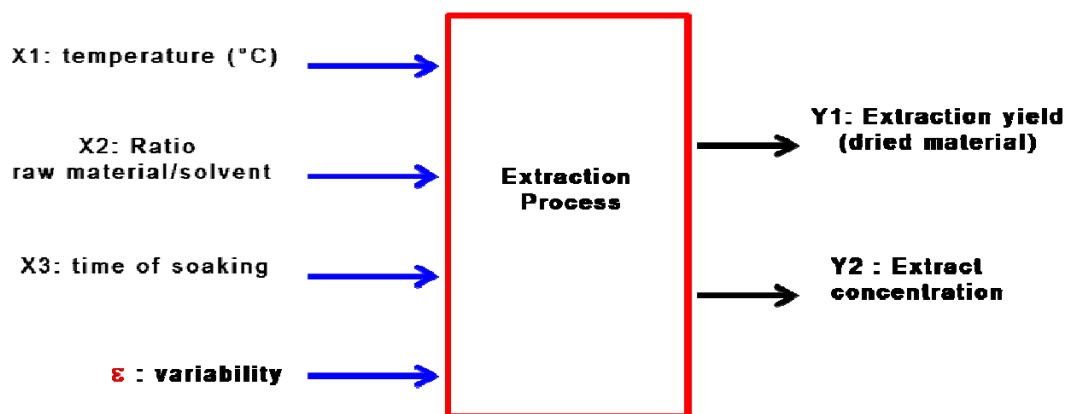
Figure 25: Method of extraction with the objective to simulate the equation of Optimization.
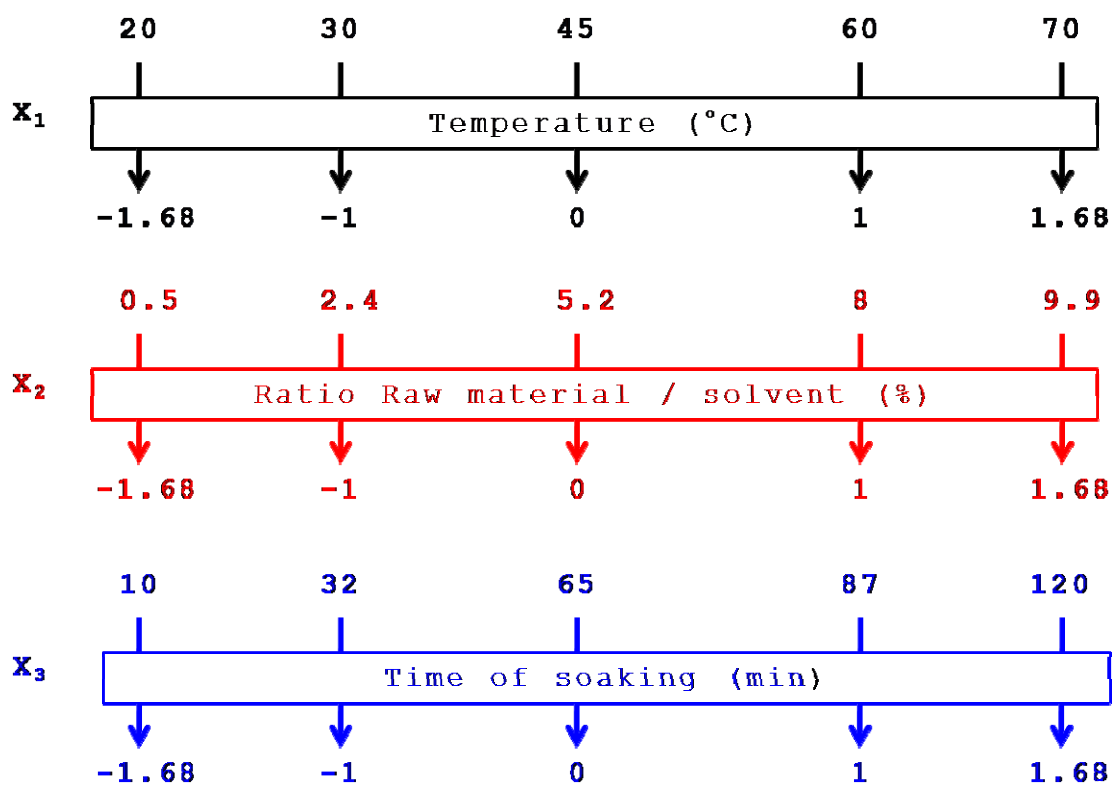
Figure 26. Values of parameters of extraction to take in account for the study.

The table 12 shows the example of optimization of Fagaricine from bark of *Fagara Hertzii* using water as solvent. The optimization result of the extraction of Fagaricine from bark of *Fagara Hertzii* proved that the variation of temperature, ratio of raw material/water and time of soaking can highly modulate the variation of yield of extract (Fagaricine).

Table 12. Result of optimization of the extraction of Fagaricine from bark of *Fagara hertzii*.

Test	Temperature	Raw M/Sol Ratio	Time of soaking	Yield of dried material (%)	Yield of Fagaricine (%)
	X1	X2	X3	Y1	Y2
Test 01	30	2.4	32	4.17	3.34
Test 02	60	2.4	32	5.42	5.41
Test 03	30	8	32	2.58	7.94
Test 04	60	8	32	4.38	16.4
Test 05	30	2.4	87	5	4.04
Test 06	60	2.4	87	5.61	6.43
Test 07	30	8	87	3.13	9.45
Test 08	60	8	87	6.13	19.22
Test 09	45	5.2	65	2.69	10
Test 10	45	5.2	65	2.88	10.53
Test 11	45	5.2	65	3.08	9.35
Test 12	20	5.2	65	3.08	5.77
Test 13	70	5.2	65	7.12	17.02
Test 14	45	0.5	65	10.01	2.01
Test 15	45	9.9	65	5.38	12.85
Test 16	45	5.2	10	2.67	7.07
Test 17	45	5.2	120	4.42	11.22

Variation of temperature, ratio of raw material/water, time of soaking can highly modulate variation of yield of extract (Fagaricine). The values represented in red colours in the tab.19 are the repetitive same tests which give the variability of the process, and the values represented in bleu colours are the added tests that help to simulate the nonlinear parameters.

Table 13. Three extraction of Fagaricine with the same raw material.

Extraction at 60°C, Raw material/Solvent Ratio = 8, Time = 87 min	Yield of extraction (%)
First extraction	19.22
Second extraction	5.49
Third extraction	1.63
Total	26.34

The values represented in red colour in the table12 are repetitive from the same tests. They show the process variability. The values represented in blue colour are from the added tests, which helped to simulate the non-linear parameters.

Temperature, contact time of the raw material with solvent (soaking), ratio of raw material and solvent mixture are not the only factors which can modulate the yield of extraction. We found that there are other parameters, including the use of stirring rods during extraction (shaking), ultrasonic system and multiple extraction of the same preparation can optimize the yield of extraction of active principle from plant (table 13).

The Fig. 27 shows the effect of stirring of the preparation, compared with simple maceration on the extraction of Fagaricine. We noted that stirring of the mixture can accelerate the extraction of active principle.

In conclusion, several factors can influence optimization of the yield of extraction of active principle from plants. A number of these factors are illustrated, including temperature, stirring, ratio raw material-solvent, time of maceration. Although the economical relevance of optimization of extraction is important, it's also necessary to preserve nature.

Table 14. Advantages and disadvantages of Clinical study designs (Strom, 2006).

Study Design	Advantages	Yield of extraction (%)
Randomized clinical trial	Most convincing design, Only design which controls for unknown or unmeasurable confounders	Most expensive Artificial Logistically most difficult Ethical objections
Cohort study	Can study multiple outcomes. Can study uncommon exposures. Selection bias less likely unbiased exposure data Incidence data available	Possibly biased outcome data More expensive If done prospectively, may take years to complete
Case-control study	Can study multiple exposures Can study uncommon diseases Logistically easier and faster Less expensive	Control selection problematic Possibly biased exposure data
Study Design	Most convincing design Only design which controls for unknown or unmeasurable confounders	Most expensive Artificial Logistically most difficult Ethical objections
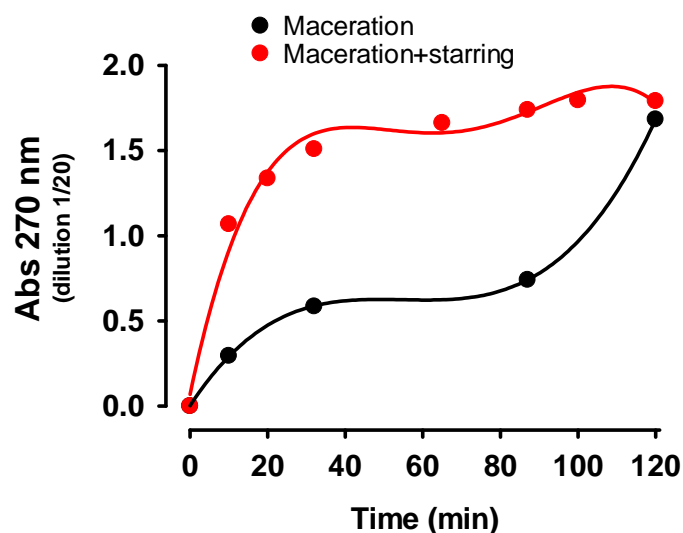
Figure 27: Effect of stirring of the preparation, compared to a simple maceration on extraction of Fagaricine. We noted that stirring of the mixture can accelerate the extraction of active principle of Fagaricine.

2.3.2. Clinical drug evaluation

Before utilisation of phytodrug in human, the candidate of drug needs to be tested on animals for exposure (toxicity). In phytomedicine, development the regulatory authority usually need at least acute (7 days toxicity with single dose) and subchronic toxicity which is 28 or 35 day single and multiple ascending doses (SADs and MADs) (Kramer et al., 2007).

Since the introduction of the EU Clinical Trial Directive 2001/20/EC in 2001, there is now a requirement for all EU countries, including the United Kingdom when it came into force in May 2004, to make a submission to the local regulatory authorities for permission to conduct the trials in human volunteers. Developing countries particularly African countries insist on this permission.

In contrast of allopathic in which clinical trials must be realised in four (I-IV) phase, the number of clinical studies was reduced to two for phytomedicine (phase III and IV), in accordance with national regulation authorities of various countries. In Africa, where phyto-drug is traditional medicine ameliorates (MTA) of category I, and plant is widely used by the population, there is no necessary for a clinical study. Only Good Manufacture Practice (GMP) recommendation is enough.

The U.S. Food and Drug Administration (FDA) has recently recommended that drug developers conduct phase 0 trial studies, a designation for exploratory, first-in-human micro-dosing studies. These are conducted prior to phase I studies and intended to speed up the development of promising drugs or imaging agents by establishing sooner on whether the drug or agent behaves in human, as it was anticipated from preclinical studies (FDA), 2006). Phase 0 trial studies involve the administration of single, sub-therapeutic dose of the new drug candidate to a small number of human subjects (close to 15) to gather preliminary data on the pharmacokinetics and pharmacodynamics. A phase 0 trial study gives no data on safety or efficacy, but drug developers can carry out these studies to classify the drug candidates and decide the one to proceed with (to take forward). The potential advantages of phase 0 trial studies are to aid candidate drug selection by getting an insight into the human. It is also to help to establish the likely pharmacological dose and the first for the subsequent phase I study. It may also identify early failures and save the company costs of further development.

During the development of phytomedicine, the phase 0 was recommended for other raisons, compared with those stated by the FDA. The first raison is the early selection of phytomedicine candidate or one of its components. The second reason is that phase 0 can replace phase III trial when the phytomedicine is in category I or II according the reference guidelines of harmonization procedure of homologation of phytomedicine in OAPI countries (WHO/AIPO, 2004). Phase 0 is always done in collaboration with traditional healers. When the reputable traditional preparation is identified as phytomedicine candidate, a few patients are selected following a medical appointment before receiving a treatment, in a customary procedure, by a traditional healer. After getting the treatment, another medical appointment takes place to see the benefits or positives results of the treatment. Those results can be used as the clinical evaluation of phytomedicine of category I and II.

In general, only phase III trial is recommended by national authority of regulation for the approval of phytomedicine.

During phase III trial, the candidate phytodrug should, ideally, be with the intended commercial formulation. A large amount of patients (at least one hundred) are involved in the trial sample, to statistically confirm the efficacy and safety of the medication. Some patients will be given phytodrug others may receive another medication (market leader), identically the same in characteristics and label, but with a worldwide commercialized name. Doctors and patients alike in the case study must be aware. They will or will not know whether they are prescribing or getting the right drug from the test. By switching the medication in a controlled manner (blind trials or otherwise), objectivity and statistical assessment of the tre-

atment under investigation are assured. Most regulatory authorities need sufficient data to demonstrate that the new product can be licensed as safe, effective, and of acceptable quality.

Other methods can be used to demonstrate that the new phytomedicine can be licensed as safe, effective, and of acceptable quality, including the cohort study and case-control reports. But case series and case reports were not recommended. The advantages and disadvantages of those methods are listed in table 14.

Case–control studies are studies that compare cases with a disease to controls without the disease, looking for differences in a previous use of treatment. As an example, one could select cases of young men with opportunistic AIDS disease and compare the disease with controls without the opportunistic AIDS disease, and looks for dissimilarities before and after the use of Fagaricine. Similar studies have been conducted, generally proving a strong association between the use of Fagaricine and the absent of opportunistic AIDS disease for people living with HIV.

Cohort studies are studies that identify subsets of a defined population and follow them over the time, looking for dissimilarities in results. Cohort studies are, generally, used to compare treated patients to untreated patients, although they can also be used to compare one treatment to another. For example, one could compare patients with AIDS, who use Fagaricine alone, to others who used Fagaricine and ARVs. The study looks for differences in frequency of opportunistic diseases or CD4 count decrease. When such studies were performed, they, in fact, confirmed the relationship between treatments with Fagaricine and the absent of opportunistic diseases, which had been observed using the analyses of case–control studies. Cohort studies can be performed either prospectively, that is, simultaneously with the events under study; or retrospectively, after the outcome under study had already occurred, by recreating the events in the past using medical records, questionnaires, or interviews. The major difference between cohort and case–control studies is the basis upon which patients are recruited into the study (Fig 28). The recruitment of patients for the case–control studies is b-
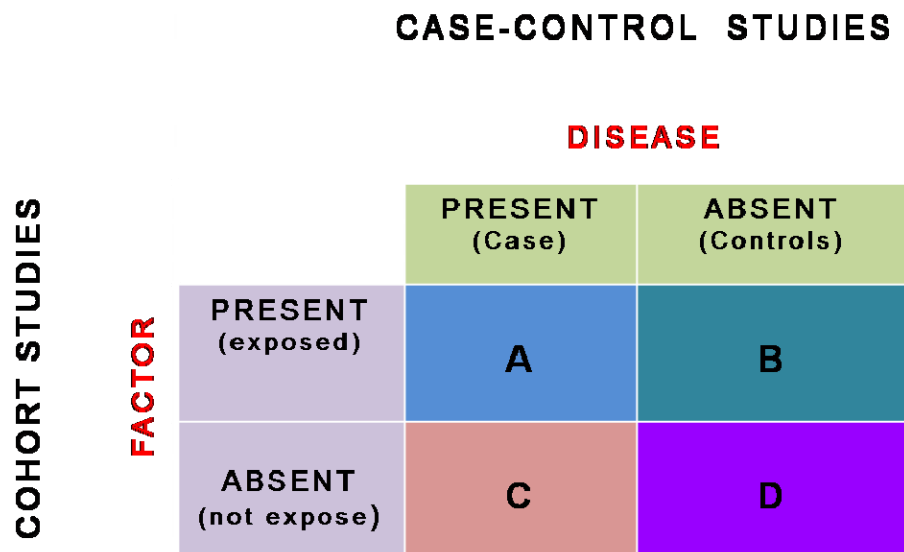

Figure 28: Cohort and case–control studies provide similar information, but approach data collection from opposite directions. Adapted from BL, Strom (Strom, 2006).

ased on the presence or absence of a disease and their previous use of medications. They are then subjected to the study. Patients are recruited into cohort studies based on the presence or absence of the use of medications. Their subsequent disease course is studied thereafter.

Randomized clinical trials are experimental studies in which the investigator controls the therapy that is to be received by each participant. Generally, an investigator uses that control to randomly allocate patients between or among the study groups, performing a randomized clinical trial. The major strength of this approach is random assignment, which is the only way to make it likely that the study groups are comparable in potential confounding variables that are either unknown or unmeasurable. For this reason, associations demonstrated in randomized clinical trials are more likely to be causal associations than those demonstrated using one of the other study designs.

2.3.2.1. Randomized trials of phytomedicine procedures

2.3.2.1.1. Study design and outcomes

The approach to double-blind, randomized and placebo-controlled trials of phytomedicine are as follows: 1) Comprehensive set of pre-clinical studies, with very detailed information about the animal toxicity of the candidate phytomedicine under investigation. 2) Phase 0 study, would typically include about 15-20 healthy volunteers over a three-month time period. It is for the assessment of the safety and efficacy of the phytodrug in a traditional form. 3) Phase III study would characteristically include at least 100 patients possibly in a multi-centre permutation to assess further the safety and efficacy of the phytomedicine. The comparison is with the standard therapy care shown for the medical condition. 4) Phase IV trial involves a comprehensive post-marketing analysis of the phytomedicine.

2.3.2.1.2. Study population

2.3.2.1.2.1. Inclusion criteria

To be enrolled in the study, the following criteria must be fulfilled: 1) Be an adult male or female, from age 18 to 45. 2) Have a body weight within 25 % of the appropriate range. 3) Have no significant disease or clinically significant abnormal laboratory values during screening. 4) Have an ECG (12-lead) with no significant abnormalities. 5) Be under no regular medical treatment. 6) Be able to communicate effectively with the study personnel. 7) Be able to understand the information of the nature of the study, and to, willingly, accept to go through the study procedures by signing a written consent of same. The consent must be read, filled out and signed by the participant before any study-related procedures are conducted.

2.3.2.1.2.2. Exclusion criteria

Any of the following is regarded as a criterion for exclusion from the study: 1) Hypersensitivity or idiosyncratic reaction to any drug or herbal products. 2) Any disease or condition which might compromise the haematopoietic, renal, endocrine, pulmonary, central nervous, cardiovascular, immunological, dermatological, gastrointestinal or any other body system. 3) History of allergies, conditions – asthma, urticaria, and eczema. 4) History of a presence

of dyspepsia, gastric ulcer or duodenal ulcer. 5) History of autoimmune disorders e.g. systemic lupus erythematosus, haemolytic anaemia. 6) History of psychiatric disorders. 7) Intake of any medication within 14 days before the start of the study. 8) Subjects who are scheduled to undergo hospitalization surgery during the study period. 9) Recent history (less than two years) of alcoholism (alcohol abuse) or unlikely to refrain from excessive alcohol consumption during the study period. 10) Those who smoke more than 10 cigarettes per day and cannot refrain from smoking during the study period. 12) A presence of clinically significant abnormal laboratory results during screening. 13) Pregnancy or breastfeeding. 14) Females of childbearing age potential not using medically accepted contraceptive measures, as judged by the examiner. 15) The use of any recreational drugs or a history of drug addiction. 16) Participation in a clinical study of any investigational product one month prior to appointment 1 or during the study.

2.3.2.1.2.3. Justification for inclusion and exclusion criteria

The criteria is set to minimize the risk, assure the participant who will pursue the set of objectives, and provide equal opportunity for inclusion, without excluding other subjects who might be constant users of the herbal medication. As a first safety study of plant medicine, children are excluded from same. Their inclusion, however, may be considered in subsequent studies.

2.3.2.1.3. Criteria for discontinuation

Subjects may be discontinued from the study treatment and assessments at any time at the discretion of the examiner. Specific reasons for discontinuing a subject from the study are the following:

- Withdrawal of informed consent.
- Development of exclusion criteria, pregnancy or other safety reasons during the study
- Protocol non-compliance.
- Incorrect enrolment or randomization of the subject.

2.3.2.1.4. Storage and Accountability

All study drugs must be kept in a secure place, under adequate storage conditions, protected from moisture and light. Record of dispensing and returns will be maintained by the trial centre. The subject must return all unused study medication for each treatment period to the trial centre. Preferably no other medicine should be taken by the subjects. The use of any incidental medication (e.g. mild analgesics, oral contraceptives, etc.) must be recorded. The patient will record the use of study medication in a diary on a daily basis, and the adherence to the prescribed treatment will be checked at every clinic visit.

2.3.2.1.5. Study measurements and endpoints

In the Fagaricine study, for example, the following endpoints will be measured (recorded).

Primary safety endpoints: 1) Adverse events (type and frequency). 2) Physical examination parameters (body mass, height, etc.). 3) Vital signs: blood pressure, heart rate, respiratory rate, body temperature. 4) ECG. 5) Urine dipsticks: leucocytes, nitrate, urobilinogen, protein, pH, blood, specific gravity, ketones, bilirubin and glucose. 6) Laboratory tests: haematology (RBC, HB, MCHC, MCH, MCV, RDW, Het, Hb, WBC, differential countlymphocytes monocytes, neutrophils, basophilic, $CD4^+$, $CD8^+$), biochemistry (serum bilirubin total and conjugated, AST, ALT, ALP, GGT, LDH, CK, Protein, albumin, globulin, cholesterol - total, LDL-C, HDL-C), glucose urea, creatinine, sodium, potassium, chloride, calcium, magnesium.

Secondary safety endpoints: Plasma levels of active constituent levels of phytomedicine, if is possible.

2.3.2.1.6. Measurements at each visit

Visit 1 or Screening Visit: The subjects will be examined within 14 days prior to the first investigational product to assess their eligibility to participate. Each subject will consent in writing to the screening process before the start of the examination. The consent form will also include the study information leaflet. The examinations and investigations will include: 1) Medical history, including history of past use of medications, demographics (date of birth, sex, race) and alcohol and tobacco consumption patterns. 2) Physical examination, including assessment of general appearance, cardiovascular, lungs, mouth, throat and abdomen and measurement of height and body weight. The examination will be made in accordance with the normal clinical routines at the trial centre. 3) Vital signs: systolic and diastolic blood pressure in supine (after 5 minutes rest) and standing positions (1 minute), pulse respiratory rate and oral temperature. 4) ECG standard 12-lead. 5) Laboratory tests: Haematology, serum biochemistry and urine analysis. 6) Urine β-HCG pregnancy test in non-hysteretomized volunteers. Fertile females must have a negative pregnancy test. 7) Baseline plasma levels of actives constituents of phytomedicine, if is possible.

Visit 2 or day 1-Randomization and first drug issue: Volunteers will be seen at the trial centre, and the examiner will confirm that volunteers are healthy and still eligible for inclusion in the study: 1) the pre-dose safety assessments and eligibility checks will be recorded on the relevant pages in the individual Case Report Form (CRFs). 2) The randomized subject will be issued with 1 month's supply of trial medication, informed about how to take the medication and given the next appointment date. 3) Subject should start the first dose at the recommended time (am: 06:00 – 10:00) on the day of the drug issue or start the next day. 4) The randomized subject will also be issued with a diary and instructed as to how to complete it.

Visit 3 to 5 treatment and assessment: For these visits, the examinations and investigations will include: 1) Collection of returned trial medication and diary cards and debriefing on information in the diary card. 2) Physical examination (as for visit1). 3) Vital signs: systolic and diastolic blood pressure in supine (after 5 minutes rest) and standing positions (1 minute), pulse, respiratory rate and oral temperature. 4) ECG standard 12-lead. 5) Laboratory tests, mainly haematology, serum biochemistry and urine analysis. 6) Urine β-HCG pregnancy test in non-hysteretomized volunteers at (visit 3 and 5 only). Fertile females must

have a negative pregnancy test. 7) Baseline plasma levels of phytomedicine actives. 8) Except for visit 5, new issue of trial medication and diary card. 9) Note: For any subject in which any of the above tests indicate levels outside the normal range, a follow-up visit (visit6) will be scheduled for an appropriate time (1 or 3 months post visit 5). All tests will be repeated at this visit.

2.3.2.1.7. Specific on measurements

Adverse events: An adverse event (AE) is the development of an undesirable medical condition – e.g. symptoms or abnormal results of an investigation – or the deterioration of a pre-existing medical condition (not relevant in this study). AE's will be collected by means of a standard question "have you had any health problems since the previous visit? AE's will be recorded at every visit. Spontaneously reported AE's and/or observed AE's and the subject's response to this question will be recorded on the AE form with information about seriousness, action taken, date of onset and recovery, maximum intensity and outcome. The subjects will be asked to assess the intensity of the reported AE according to the following scale:

- Mild: awareness of sign or symptom, but easily tolerated.
- Moderate: sufficient discomfort to cause interference with normal activities.
- Severe: incapacitating, with inability to perform normal activities.

A serious AE is an adverse event occurring during any phase of the study and at any dose of the investigating product or placebo, which fulfils one or more of the following criteria:

1) Results in death. 2) Immediately life-threatening. 3) Requires in-subject hospitalizetion. 4) Results in persistent or significant disability or incapacity.

The causality of Serious AE's (i.e. the relationship to the study treatment) will be assessed by the examiners, who upon completion of the relevant Case Report Form, must answer 'yes' or 'no' to the question one considers there is a reasonable possibility that the event may have been caused by the study medication. The following factors should be taken into consideration when deciding if there is a "reasonable possibility" that an AE might have been caused by the investigating product.

- Time-course of events and exposure to the suspicious drug. Did the AE occur in a reasonable temporary relationship to administering the suspicious drug?
- Dechallenge experience. Did the AE solve or improve the stopping or reduction of the dose of the suspicious drug?
- Rechallenge experience. Did the AE reoccur if the suspicious drug was reintroduced after having stopped from taking it?
- Laboratory tests – has a specific laboratory investigation confirmed the relationnship?
- No alternative cause. The AE cannot be reasonably explained by the aetiology, such as an underlying disease (not previously present), other drugs or environmental factors.

There would not be a "reasonable possibility" of causality if none of the above criteria apply or where there is evidence of exposure and a reasonable time course, but any dechallenge is negative or there is another more likely cause of the AE.

In the study, the Adverse Events will be noted from the interview at the time of the visits and from the daily diary input.

ECG: Standard 12-lead ECGs will be performed at the screening and during visits 3 to 5 (and post-study follow-up visits). Volunteers will only be randomized if the ECG is normal, or any abnormalities that are noted are considered by the examiner to be clinically irrelevant.

Laboratory Investigations: Blood and urine will be collected for clinical pathology tests at the screening visits 3 to 5, and, if required, at any post-study safety follow-up visits (or upon withdrawal). The following variables will be measured.

Haematology*:* Red cells: Erythrocytes, Haemoglobin, Haematocrit, MCV, MCH, and MCHC. White cells: Leucocytes and 5 part differential (neutrophils, lymphocytes, monocytes, eosinophils and basophils); $CD4^+$ and $CD8^+$ counts.

Clinical Chemistry*:* Serum analysis (total bilirubin, ALP, GGT, ALT, AST, LDH, CK, THS/T3/T4, cholesterol - total LDL-C, HDL-C; calcium, magnesium, phosphate. Serum electrolytes (sodium, potassium, chloride). Serum proteins (total protein, albumin, and globulin). Plasma glucose (non-fasting). Urinalysis (Multistix: SG, pH, protein, glucose, ketone, bilirubin, blood and urobilinogen). Urine (β-HCG pregnancy test, urea and creatinine).

Diary Measurements*:* A diary will be kept at home during the study. The following variables will be recorded:

- Intake of study medication (time, and if with food)
- Occurrence of any adverse effects
- Intake of any incidental medication

The subjects will record the time (mornings and evenings) of taking the study medication. The recordings are a measurement of compliance.

2.3.2.1.8. Statistical methods

Determination of ample size: In the absence of any useful specific data or adverse effects reported during the traditional use of the herbal medicine.

Statistical analysis: A single statistical analysis will be performed at the end of the study. An intention to treat (ITT) approach will be followed; that is, a safety statistical analysis will be based on data from all the patients who were selected at random, and from whom a meaningful data were collected. The data will be displayed graphically for a visual inspection. Descriptive statistics will be presented as means, SEM and 90% confidence levels of the means.

*Baseline characteristics***:** The subject disposition will be summarized. Protocol violations will be listed per subject, describing the nature of the violation. Subjects failing to complete the study (as well the times and reasons for discontinuation) will be displayed. The demographic, background and baseline data will be presented descriptively.

Analysis of Safety: Adverse Events, as reported throughout the course of the trial will be listed individually, per treatment group. The Pre-study and post-study findings of the physical examination, vital sign and laboratory variables (haematology, clinical chemistry and urinalysis), and 12-lead ECG will be listed individually and summarized. Values outside the normal range will be listed.

Analysis of active constituent levels: the levels of active constituents obtained during the treatment will be compared to that found before the start of the trial treatment. The average levels obtained in the trial medication and placebo groups will be compared and analyzed using an analysis of variance model.

2.3.2.1.9 Changes to the Clinical Study Protocol

The Medicines Control Council (MCC) and the Local Ethics Committees will be notified of any amendments to the Clinical Study Protocol, and no changes will be notified of any amendments to the Clinical Study Protocol. Likewise, no changes will be effected without approval from the Regulatory Authorities.

2.3.2.2. Ethics

2.3.2.2.1. Ethics review

The final study protocol, including the final version of the Subject information and Consent Form, must be approved in writing by an Independent Ethics committee (IEC) and the MCC before enrolment of any subject into the study. The principle investigator (Clinical Trial Manager) is responsible of informing the IEC of any serious adverse events (SAE) and amendments to the protocol as per regulatory requirement.

2.3.2.2.2. Ethical conduct of the study

The study will be performed in accordance with the ethical principles in the Declaration of Helsinki, consistent with the Good Clinical Practice and applicable to the regulatory requirements.

2.3.2.2.3. Subject information and consent

The investigator will make sure that the subject is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study. Subjects must also be notified that they are free to discontinue from the study at any time. The subjects should be given the opportunity to ask questions and give them time to consider the information provided. The subject's consent, signature and date of same must be obtained before conducting any specific study procedure. The examiner will keep the original Subject Informed Consent Form. A copy of the Form will be given to the subject.

2.3.2.2.4. Subject data protection

The subject information and Consent Form will explain that the study data will be stored in a computer database, maintained in confidentiality. Subjects in this database will be identified by their initials or enrolment code/subject number only. Authorized representatives of a regulatory authority (e.g. MCC) may require direct access to some parts of the trial site records relevant to the study, including subjects' medical history for data verification purposes. The examiner must keep a Subject Identification List of all the subjects who have signed the consent form, including their subject number, full and last names, and known residence addresses.

2.3.2.2.5. Insurance and Indemnity

All subjects participating in the study trials must be covered for any trial medication related to adverse effects through an insurance coverage to be taken out by TICIPS.
2.3.2.3. Data quality assurance

Data from the study will be collected in CRFs. Data editing will be performed at the trial centre, comparing the source and CRF entries. Data will be entered in a blind mode.

During the study an independent monitor will visit the investigating site to confirm that the facilities are acceptable, the investigating team is adhering to the protocol, and that data is being accurately recorded in the CRFs. Source data verification (a comparison of the data in the CRF with the subjects laboratory tests results and other source documents) will also be performed. Moreover, authorized representatives of the regulatory authority (e.g. MCC) may visit the centre to perform inspections, including source data verification.

A Clean File for the final database will be declared after entering all the data, and a quality check on a sample of the same has been performed. The database will be locked after the Clean File has been declared and data extracted for statistical analysis. Treatment codes will not be broken until Clean File. Study committee meetings will be held as needed prior to or during the study. The medical nursing and other staff involved in the study will receive proper education/information on how to conduct the study according to the protocol.

2.4. Drug utilization studies

There is a vital need to inform people on problems of interaction on traditionally used of herbal medicines and allopathy, especially since around 80 % of the developing world utilize these remedies for treating diseases and promoting health. Drug utilization studies and pharmacoepidemiological services are closely linked to the work of Drug and Therapeutics Committees (DTCs) and to quality assurance of drug therapy in clinic hospitals (Crooks, 1983; WHO, 2003). In addition, advances in drug development provide patients with new drugs, novel drug combinations, expensive biologic and biosimilar drugs and targeted drug therapy, adapted to the molecular characteristics of the disease (Blaschke, 2009; Engelberg et al., 2009) that may interact with herbal medicine.

2.4.1. Interaction of drugs

Herbal products can interfere with allopathic medical treatment through pharmaco-kinetic and pharmacodynamic interactions. Although pharmacokinetic interactions that alter drug absorption may cause variable and unsatisfactory drug bioavailability, a drug absorption enhancement effect of a herb may be used to ensure sufficient absorption of poorly absorb-able drugs. Drug–herb and herb–herb interactions are commonly divided into two classes, namely pharmacokinetic and pharmacodynamic. Pharmacokinetic interactions invol-ve proc-esses such as absorption, distribution, metabolism and excretion, while pharmacodyn-amic interactions influence the pharmacologic effect of the drug at the site of action. The mecha-nisms of altered pharmacokinetics of a drug by herbs include modulation of cytochro-me P450 enzymes, modulation of the action and expression of active transporters, such as P-gly-coprotein, modulation of gastrointestinal pH, as well as motility and formation of comple-xes (Williamson, 2003).

2.4.1.1. Herb–herb interactions

The hydrochloride salt of sinomenine, an alkaloid obtained from the stem of *Sinome-nium acutum* Rehder& Wilson (Menispermaceae), which has been used traditionally in China and Japan to treat various rheumatic and arthritic diseases (Chan et al., 2006), significantly increased the transport of vitamin C, rutin, luteolin and insulin across Caco-2 epithelial cell monolayers in the apical-to-basolateral direction (Lu et al., 2010). Sinomenine interact also on intestinal absorption of paeoniflorin in everted rat gut sac model.

2.4.1.2. Drug-herb interactions

Pharmacokinetic interactions between herbs and allopathic drugs may either decrease or increase the bioavailability of the co-administered drug. It is possible to utilize absorption interactions positively to enhance the absorption of drugs with low bioavailabilities. For example, *Sinomenium acutum* extract increase absorption of Cimetidine (Lu et al., 2010). Ci-metidine is a substrate of P-glycoprotein and part of the dose is actively pumped back into the lumen of the gastrointestinal tract (Sun et al., 2004).

Decoctions of *Hibiscus sabdariffa* L. (Family Malvaceae) are very popular for the preparation of homemade refreshing drinks and are also used medically for a variety of ailments. Particularly remarkable are the various scientific reports supporting diuretic and antihypertensive potentials. Co-administration of *Hibiscus sabdariffa* with hydrochloroth-iazide, a commonly prescribed diuretic drug caused a significant increase in the volume of urine excreted and resulted in a decrease in the pH of urine and the concentrations of sodium, bicarbonate, and chloride ions (Ndu et al., 2011). Concurrent use of natural health products (NHPs) with antiretroviral drugs (ARVs) is widespread among human immunodeficiency virus-infected patients. Tab. 15 and Tab.16 review the clinical pharmacokinetic and pharma-codynamic interactions between NHPs and prescribed drugs. Large NHPs are complex mixt-ures and are likely to contain organic compounds that may induce and/or inhibit drug metab-olizing enzymes and drug transporters.

In conclusion, interactions between herbal medicines and prescribed drugs can occur and may lead to serious clinical consequences. The clinical importance of herb-drug interact-

Table 14. Advantages and disadvantages of Clinical study designs (Strom, 2006).

Phytomedicine or active ingredients	Drug interacting or enzyme	References
African Phytomedicines		
Acacia nilotica, Agauria salicifolia, Allium sativum Bitter orange (Citrus aurantium), Canavinine, Carica papaya, Cyphostemma hildebrandtii, Elaeodendron buchananii, Hypoxis hemerocallidea, Phyllanthus amarus, Piperine from (Piper longum & nigrum), Saw palmetto, Sutherlandia frutescens, Tapinanthus sessilifolius Blume, Vernonia amygdalina, Vitamin C,	Atazanavir, Nevirapine, Ritonavir,Saquinavir Chlorpropamide, Warfarin, Phenytoin Chlorzoxazone, CYP3A4, Cyt.P450, Digoxin, Fluindione, Indinavir, Midazolam, Rifamipicin, P-glycoprotein, Pregnane receptor, Propranolol, Theophylline,	(Muller et al., 2012), (Brown et al., 2008), (Muller and Kanfer, 2011), (Oga et al., 2012a), (Hu et al., 2005), (Taesotikul et al., 2012), (Gurley et al., 2004), (Piscitelli et al., 2002), (Hu et al., 2005), (Izzo and Ernst, 2009), (Slain et al., 2005)
Antimalarial		
Lumefantrine, amodiaquin, artesunate	P- glycoprotein, Rhodamine-123, Verapamil	(Oga et al., 2012b)

Table 15. Advantages and disadvantages of Clinical study designs (Strom, 2006).

European Phytomedicine	Drug candidates or enzymes for interaction	References
Allium sativum, Echinacea purpurea, Ginkgo biloba, Hypericum perforatum, Piper methysticum, Panax ginseng, Rheum palmatum	Alcohol, Alprazolam, Amitriptyline, Antiepileptics, Aspirin, Caffeine Atorvastatin, Buspirone, Chlorpropamide, Talinolol Chlorzoxazone, Ciclosporin, Darunavir, Debrisoquine, Digoxin, Diuretics, Eletriptan, Etoposide Erythromycin, Etravirine, Indinavir, Fexofenadine, Fluindione, Gliclazide, Ibuprofen, Imatinib, Irinotecan, Ivabradine, Levodopa, Loperamide, Mephenytoin, Methadone, Midazolam, Nefazodone, Nevirapine, Nifedipine, Omeprazole, Thiazide, Oral contraceptive, Paroxetine, Phenelzine, Phenprocoumon, Tibolone, Prednisone, Quazepam, Risperidone, Ritonavir, Rofecoxib, Saquinavir, Sertraline, Simvastatin, Tacrolimus, Theophylline, Tolbutamide, Trazodone, Tryptophan, Venlafaxine, Verapamil, Voriconazole, Warfarin, Phenytoin,	(Hu et al., 2005), (Piscitelli et al., 2002), (Izzo and Ernst, 2009), (Gorski et al., 2004), (Molto et al., 2012), (Molto et al., 2011), (Molto et al., 2010), (Izzo and Ernst, 2009), (Bossaer and Odle, 2012), (Izzo and Ernst, 2009), (Borrelli and Izzo, 2009), (Izzo and Ernst, 2009), (Izzo and Ernst, 2009), (Chi et al., 2012)

tions depends on many factors associated with the particular herb, drug and patient. Herbs should be appropriately marked with a label to alert consumers of the potential interactions when used concomitantly with drugs, and to consult with their private doctors and other medical professionals.

2.4.4. Modulation of the effect of phytomedicine

Traditional practitioners also know the importance of herb-herb interaction, particularly when herbal medicines are medicated to children and weak patients. I remember of my grandmother, a famous traditional paediatrician and obstetrician, who always treated children and babies with additional adjuvant to reduce the effect of phytomedicine. Fang traditional healers consider medical herbs as natural force transporters, and that this force must be regulated according to the state of health of the patient. Patients in a state of exhaustion and babies cannot take the same treatment like patients in good state of health. Conversely, drug eenhancement is well known by traditional healers. A good example is the use of an aphrodisiac preparation and phytodrug against impotence. A homemade tonic beverage like *Alstoniagilletti, Carpolobia alba* can be transformed into an aphrodisiac when taken with seeds of *Aframomum maleguetta*. A large quantity of other plant extracts used as simple aphrodisiac can be transformed into phytomedicine against impotence when used with *Aframomum maleguetta* such as *Lippia adoensis* and *Lygodium smithianum*. In addition, we usually suppress palpitations induced by a very strong aphrodisiac like *pausinystalia yohim-ba* in combination with herbs from *asteraceae* family.

2.4.5. Pharmacovigilance

Pharmacovigilance allow getting solid documentation during the marketing phase of the live of phytomedicine, particularly on adverse drug reaction (ADR) effect, positive or negative interaction with others drugs. Spontaneous ADR reporting is carried out in order to detect unknown potential drug toxicity. The method consists of collecting individual case reports of clinical suspicions of ADRs. Data mining in ADR research is the search for structures and patterns in large ADR data bases. Pharmacovigilance can also help to discover other biological actions of phytodrugs in chronic utilisation or in long lasting use.

2.4.6. Pharmacoeconomics

Pharmacoeconomics is to evaluate the clinical, economic and humanistic aspects of pharmaceutical products, services and programmes, as well as other healthcare interventions. The objective is to provide healthcare decision-makers, providers and patients with valuable information for optimal results, and for the allocation of healthcare resources. Pharmacoeconomics incorporates health economics, clinical evaluations, risk analysis, technology assessment and health-related quality-life, epidemiology, decision sciences and health services research in the examination of drugs (Birkett et al., 2010). It is necessary to come to an objective of quantitative evaluation of "benefit" or "effectiveness" to put into cost-effectiveness models that health economists have developed with phytomedicine. Pharmacoeconomics applied to phytomedicine is important particularly when there is increasing consumptions of medical herbs and herbal products globally, both in developing and developed countries (Bodeker, 1995; Bodeker et al., 2006).

5. Conclusion

Research in clinical phytopharmacology, an elaboration of new phytomedicine, needs the development of methods and strategies that improve the quality of phytodrug use in pati-

ents. This research has always been translational because it takes into account new data in applied physiology to execute the early phase of drug studies in humans, and is actively engaged in design and improvement of clinical trials. It needs other drug experts, actors and health professionals from all over the world.

References

Bhuyan BJ, Mugesh G. (2012). Antioxidant activity of peptide-based angiotensin converting enzyme inhibitors. *Organic and Biomolecular Chemistry* 10, 2237-2247.

Food and Drug Administration (FDA),(FDA), F.a.D.A., 2006. Guidance for Industry, Investigators, and Reviewers - Exploratory IND Studies.

Abdelrahim, S.I., Almagboul, A.Z., Omer, M.E. and Elegami, A., 2002. Antimicrobial activity of *Psidium guajava* L. *Fitoterapia* 73, 713-715.

Abdulrahman, F., Inyang, I.S., Abbah, J., Binda, L., Amos, S. and Gamaniel, K., 2004. Effect of aqueous leaf extract of *Irvingia gabonensis* on gastrointestinal tract in rodents. *Indian Journal of Experimental Biology* 42, 787-791.

Abiodun O, Gbotosho G, Ajaiyeoba E, Happi T, Falade M, Wittlin S, Sowunmi A, Brun R, Oduola A. 2011. In vitro antiplasmodial activity and toxicity assessment of some plants from Nigerian ethnomedicine. *Pharmaceutical Biology* 49, 9-14.

Addae-Kyereme J, Croft SL, Kendrick H, Wright CW. 2001. Antiplasmodial activities some Ghanaian plants traditionally used for fever/malaria treatment and of some alkaloids isolated from *Pleiocarpa mutica*; in vivo antimalarial activity of pleiocarpine. *Journal of Ethnopharmacology* 76, 99-103.

Aderibigbe AO, Emudianughe TS, Lawal BA. 1999. Antihyperglycaemic effect of *Mangifera indica* in rat. *Phytotherapy Research* 13, 504-507.

Adeyemi IA, Omonigbehin AE, Stella S, Oluwatosin O, Jumoke S. 2008. Antibacterial activity of extracts of *Alchornea cordifolia* (Schum and Thonn) Mull.Arg., *Boerhavia diffusa* (L) and *Bridellia micranthaI* (Hoscht) Baill. used in traditional medicine in Nigeria on Helicobacter pylori and four diarrhoeagenic bacterial pathogens. *African Journal of Biotechnology* 7, 3761-3764.

Agbor GA, Leopold T, Jeanne NY. 2004. The antidiarrhoeal activity of *Alchornea cordifolia* leaf extract. *Phytotherapy Research* 18, 873-876.

Akah PA, Aguwa CN, Agu RU. 1999. Studies on the antidiarrhoeal properties of *Pentaclethra macrophylla* leaf extracts. *Phytotherapy Research* 13, 292-295.

Akhani SP, Vishwakarma SL, Goyal RK. 2004. Anti-diabetic activity of *Zingiber officinale* in streptozotocin-induced type I diabetic rats. *Journal of Pharmacy and Pharmacology* 56, 101-105.

Alves PM, Queiroz LM, Pereira JV, Pereira Mdo S. 2009. [In vitro antimicrobial, antiadherent and antifungal activity of Brazilian medicinal plants on oral biofilm microorganisms and strains of the genus Candida]. *Revista da Sociedade Brasileira de Medicina Tropical* 42, 222-224.

Appidi JR, Yakubu MT, Grierson DS, Afolayan AJ. 2010. Antidiarrheal activity of aqueous extract of *Hermannia incana* Cav. leaves in Wistar rats. *Methods and Findings in Experimental and Clinical Pharmacology* 32, 27-30.

Arunlakshana O, Schild HO. 1959. Some quantitative uses of drug antagonists. *British Journal of Pharmacology and Chemotherapy* 14, 48-58.

Asres K. Bucar F. 2005. Anti-HIV activity against immunodeficiency virus type 1 (HIV-I) and type II (HIV-II) of compounds isolated from the stem bark of *Combretum molle. Ethiopian Medical Journal* 43, 15-20.

Asres K, Bucar F, Kartnig T, Witvrouw M, Pannecouque C, De Clercq E. 2001. Antiviral activity against human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) of ethnobotanically selected Ethiopian medicinal plants. *Phytotherapy Research* 15, 62-69.

Assob JC, Kamga HL, Nsagha DS, Njunda AL, Nde PF, Asongalem EA, Njouendou AJ, Sandjon B, Penlap VB. 2011. Antimicrobial and toxicological activities of five medicinal plant species from Cameroon traditional medicine. *BMC Complementary and Alternative Medicine* 11, 70.

Augusti KT, 1973. Studies on the effects of a hypoglycemic principle from *Allium Cepa* Linn. *Indian Journal of Medical Research* 61, 1066-1071.

Awodele O, Olayemi SO, Adeyemo TA, Sanya TA, Dolapo DC. 2012. Use of Complementary Medicine Amongst Patients on Antiretroviral Drugs in an HIV Treatment Centre in Lagos, Nigeria. *Current Drug Safety* 7, 120-125.

Ayisi NK. Nyadedzor C. 2003. Comparative in vitro effects of AZT and extracts of *Ocimum gratissimum, Ficus polita, Clausena anisata, Alchornea cordifolia*, and *Elaeophorbia drupifera* against HIV-1 and HIV-2 infections. *Antiviral Research* 58, 25-33.

Badifu G.I. and Akubor, P.I., 2001. Influence of pH and sodium chloride on selected functional and physical properties of African breadfruit (*Treculia africana* Decne) kernel flour. *Plant Foods and Human Nutrition* 56, 105-115.

Benhaddou-Andaloussi, A., Martineau, L., Vuong, T., Meddah, B., Madiraju, P., Settaf, A. and Haddad, P.S., 2011. The in vivo antidiabetic activity of *Nigella sativa* is mediated through activateion of the AMPK pathway and increased muscle Glut4 content. *Evidence Based Complementary and Alternative Medicine* 2011, 538671.

Bernstein, J., 2002. Polymorphism in Molecular Crystals. IUCr monographs on Crystallography 14. Clarendon Press, Oxford.

Bessong, P.O., Obi, C.L., Andreola, M.L., Rojas, L.B., Pouysegu, L., Igumbor, E., Meyer, J.J., Quideau, S. and Litvak, S., 2005. Evaluation of selected South African medicinal plants for inhibitory properties against human immunodeficiency virus type 1 reverse transcriptase and integrase. *Journal of Ethnopharmacology* 99, 83-91.

Betti, J., 2002. Medicinal plants sold in Yaoundé Markets, Cameroun. *African Study Monographs* 23, 47-64.

Betti, J., 2004. An ethnobotanical study of medicinal plants among the Baka pygmies in the dja biosphere reserve, Cameroun. *African Study Monographs* 25, 1-27.

Bickii, J., Tchouya, G.R., Tchouankeu, J.C. and Tsamo, E., 2006. The antiplasmodial agents of the stem bark of Entandrophragma angolense (Meliaceae). *African Journal of Traditional and Complementary and Alternative Medicine* 4, 135-139.

Birdi, T.J., Daswani, P.G., Brijesh, S. and Tetali, P., 2011. In vitro antigiardial and antirotaviral activity of Psidium guajava L. leaves. Indian J Pharmacol 43, 616-617.

Birkett, D., Brosen, K., Cascorbi, I., Gustafsson, L.L., Maxwell, S., Rago, L., Rawlins, M., Reidenberg, M., Sjoqvist, F., Smith, T., Thuerman, P., Walubo, A. and Orme, M., 2010. Clinical pharmacology in research, teaching and health care: Considerations by IUPHAR, the International Union of Basic and Clinical Pharmacology. *Basic and Clinical Pharmacology and Toxicology* 107, 531-559.

Blaschke, T.F., 2009. Global challenges for clinical pharmacology in the developing world. *Clinical Pharmacology and Therapeutics* 85, 579-581.

Bodeker, G., 1995. Traditional health systems: policy, biodiversity, and global interdependence. *Journal of Alternative and Complementary Medicine* 1, 231-243.

Bodeker, G., Carter, G., Burford, G. and Dvorak-Little, M., 2006. HIV/AIDS: Traditional systems of health care in the management of a global epidemic. *Journal of Alternative and Complementary Medicine* 12, 563-576.

Borrelli, F. and Izzo, A.A., 2009. Herb-drug interactions with St John's wort (Hypericum perforatum): an update on clinical observations. *AAPS Journal* 11, 710-727.

Bossaer, J.B. and Odle, B.L., 2012. Probable etoposide interaction with Echinacea. *Journal of Dietary Supplements* 9, 90-95.

Boyom, F.F., Ngouana, V., Kemgne, E.A., Zollo, P.H., Menut, C., Bessiere, J.M., Gut, J. and Rosenthal, P.J., 2011. Antiplasmodial volatile extracts from *Cleistopholis patens* Engler & Diels

and *Uvariastrum pierreanum* Engl. (Engl. & Diels) (Annonaceae) growing in Cameroon. *Parasitology Research* 108, 1211-1217.

Brown, L., Heyneke, O., Brown, D., van Wyk, J.P. and Hamman, J.H., 2008. Impact of traditional medicinal plant extracts on antiretroviral drug absorption. *Journal of Ethnopharmacology* 119, 588-592.

Byrn, S., Pfeiffer, R., Ganey, M., Hoiberg, C. and Poochikian, G., 1995. Pharmaceutical solids: a strategic approach to regulatory considerations. *Pharmaceutical Research* 12, 945-954.

CE-FAO, 1999. Données statistiques des produits forestiers non-ligneux du Cameroun. In: FAO (Ed.), FAO, Yaoundé.

Chan, K., Liu, Z.Q., Jiang, Z.H., Zhou, H., Wong, Y.F., Xu, H.X. and Liu, L., 2006. The effects of sinomenine on intestinal absorption of paeoniflorin by the everted rat gut sac model. *Journal of Ethnopharmacology* 103, 425-432.

Chemburkar, S.R., Bauer, J. and Deming, K., 2000. Dealing with the impact of ritonavir polymorphs on the late stages of bulk drug process development. Org Proc Res Dev 4, 413–417.

Chi, Y.C., Juang, S.H., Chui, W.K., Hou, Y.C. and Chao, P.D., 2012. Acute and Chronic Administrations of Rheum palmatum Reduced the Bioavailability of Phenytoin in Rats: A New Herb-Drug Interaction. *Evidence Based Complementary and Alternative Medicine* 2012, 701205.

Chinsembu, K.C. and Hedimbi, M., 2010. An ethnobotanical survey of plants used to manage HIV/AIDS opportunistic infections in Katima Mulilo, Caprivi region, Namibia. *Journal of Ethnobiology and Ethnomedicine* 6, 25.

Cos, P., Hermans, N., De, B.T., Apers, S., Sindambiwe, J.B., Witvrouw, M., De, C.E., Vanden, B.D., Pieters, L. and Vlietinck, A.J., 2002. Antiviral activity of Rwandan medicinal plants against human immunodeficiency virus type-1 (HIV-1). *Phytomedicine* 9, 62-68.

Crooks, J., 1983. Drug epidemiology and clinical pharmacology: their contribution to patient care. *British Journal of Clinical Pharmacology* 16, 351-357.

Daniel, E.E., Kwan, C.Y. and Janssen, L., 2001. Pharmacological techniques for the in vitro study of intestinal smooth muscles. *Journal of Pharmacological and Toxicological Methods* 45, 141-158.

Deachapunya, C., Poonyachoti, S., Thongsaard, W. and Krishnamra, N., 2005. Barakol extracted from *Cassia siamea* stimulates chloride secretion in rat colon. *Journal of Pharmacology and Experimental Therapeutics* 314, 732-737.

Desjeux, J.F., Heyman, M. and Ben Mansour, A., 1980. Water and ion movement in intestine. In: Viral enteritis, INSERM, Paris, pp. 39.

Desjeux JF, Nath SK and Taminiau J. 1994. Organic substrate and electrolyte solutions for oral rehydration in diarrhea. *Annual Reviews in Nutrition* 14, 321-342.

Desjeux JF, Tannenbaum C, Tai YH and Curran PF. 1977. Effects of sugars and amino acids on sodium movement across small intestine. *Am J Dis Child* 131, 331-340.

Dietz K, Molineaux L and Thomas A. 1974. A malaria model tested in the African savannah. Bull World Health Organ 50, 347-357.

Djohan Y, Camara C, Monde A, Koffi G, Niamke G, Dere L, Tiahou G, Djessou P and Se-ss D, 2009. [Interest of antioxidants in the care of the patients infected by the HIV: the expe-rience of long term administration of Alternanthera pungens herb tea]. *Ann Biol Clin* (Paris) 67, 563-568.

Doelker E. 2002. [Crystalline modifications and polymorphism changes during drug manufacture]. *Ann Pharm Fr* 60, 161-176.

Donowitz M. 1987. Small intestinal and colonic linked sodium chloride absorption. New understandding of distribution and regulation. *Gastroenterology* 93, 640-643.

Engelberg AB, Kesselheim AS and Avorn J. 2009. Balancing innovation, access, and profits—market exclusivity for biologics. *New England Journal of Medicine* 361, 1917-1919.

Eto B. 1995. Evaluation of intestinal antisecretory peptides coefficient of dissociation: a functional approach. *Methods and Findings in Experimental and Clinical Pharmacology* 17, 685-691.

Fakeye TO, Itiola OA and Odelola HA. 2000. Evaluation of the antimicrobial property of the stem bark of *Picralima nitida* (Apocynaceae). *Phytotherapy Research* 14, 368-370.

Fenner, F., Henderson, D.A., Arita, I., Jezek, Z. and Ladnyi, I.D., 1988. Smallpox and its eradication. World Health Organization, Geneva.

Fernandes, T.G., de Mesquita, A.R., Randau, K.P., Franchitti, A.A. and Ximenes, E.A., 2012. In vitro synergistic effect of Psidium guineense (Swartz) in combination with antimicrobial agents against methicillin-resistant Staphylococcus aureus strains. *ScientificWorldJournal* 2012, 158237.

Galvez, J., Zarzuelo, A., Crespo, M.E., Lorente, M.D., Ocete, M.A. and Jimenez, J., 1993. Anti-diarrhoeic activity of Euphorbia hirta extract and isolation of an active flavonoid constituent. *Planta Med* 59, 333-336.

Goncalves, F.A., Andrade Neto, M., Bezerra, J.N., Macrae, A., Sousa, O.V., Fonteles-Filho, A.A. and Vieira, R.H., 2008. Antibacterial activity of GUAVA, Psidium guajava Linnaeus, leaf extracts on diarrhea-causing enteric bacteria isolated from Seabob shrimp, Xiphopenaeus kroyeri (Heller). *Rev Inst Med Trop Sao Paulo* 50, 11-15.

Goncalves, J.L., Lopes, R.C., Oliveira, D.B., Costa, S.S., Miranda, M.M., Romanos, M.T., Santos, N.S. and Wigg, M.D., 2005. In vitro anti-rotavirus activity of some medicinal plants used in Brazil against diarrhea. *J Ethnopharmacol* 99, 403-407.

Gorski, J.C., Huang, S.M., Pinto, A., Hamman, M.A., Hilligoss, J.K., Zaheer, N.A., Desai, M., Miller, M. and Hall, S.D., 2004. The effect of echinacea (*Echinacea purpurea* root) on cytochrome P450 activity in vivo. *Clin Pharmacol Ther* 75, 89-100.

Gray, A.M. and Flatt, P.R., 1998. Antihyperglycemic actions of Eucalyptus globulus (Eucalyptus) are associated with pancreatic and extra-pancreatic effects in mice. J Nutr 128, 2319-2323.

Grover, I.S. and Bala, S., 1993. Studies on antimutagenic effects of guava (Psidium guajava) in *Salmonella typhimurium*. *Mutat Res* 300, 1-3.

Gu, C.H., Young, V., Jr. and Grant, D.J., 2001. Polymorph screening: influence of solvents on the rate of solvent-mediated polymorphic transformation. *J Pharm Sci* 90, 1878-1890.

Gurley, B.J., Gardner, S.F., Hubbard, M.A., Williams, D.K., Gentry, W.B., Carrier, J., Khan, I.A., Edwards, D.J. and Shah, A., 2004. In vivo assessment of botanical supplementation on human cytochrome P450 phenotypes: Citrus aurantium, Echinacea purpurea, milk thistle, and saw palmetto. *Clin Pharmacol Ther* 76, 428-440.

Gurrola-Diaz, C.M., Garcia-Lopez, P.M., Sanchez-Enriquez, S., Troyo-Sanroman, R., Andrade- Gonzalez, I. and Gomez-Leyva, J.F., 2010. Effects of Hibiscus sabdariffa extract powder and preventive treatment (diet) on the lipid profiles of patients with metabolic syndrome (MeSy). *Phytomedicine* 17, 500-505.

Hennebelle, T., Weniger, B., Joseph, H., Sahpaz, S. and Bailleul, F., 2009. Senna alata. *Fitoterapia* 80, 385-393.

Houghton, P.J., Woldemariam, T.Z., Khan, A.I., Burke, A. and Mahmood, N., 1994. Antiviral activity of natural and semi-synthetic chromone alkaloids. *Antiviral Res* 25, 235-244.

Hu, Z., Yang, X., Ho, P.C., Chan, S.Y., Heng, P.W., Chan, E., Duan, W., Koh, H.L. and Zhou, S., 2005. Herb-drug interactions: a literature review. *Drugs* 65, 1239-1282.

Huang, H.L., Hong, Y.W., Wong, Y.H., Chen, Y.N., Chyuan, J.H., Huang, C.J. and Chao, P.M., 2008a. Bitter melon (*Momordica charantia* L.) inhibits adipocyte hypertrophy and down regulates lipogenic gene expression in adipose tissue of diet-induced obese rats. Br J Nutr 99, 230-239.

Huang, L., Ikejiri, A., Shimizu, Y., Adachi, T., Goto, Y., Toyama, J., Tanaka, H., Akashi, R., Uchida, K., Miyata, H. and Haga, T., 2008b. Immunoadjuvant activity of crude lectin extracted from *Momordica charantia* seed. *J Vet Med Sci* 70, 533-535.

Idu, M., Omonigho, S.E. and Igeleke, C.L., 2007. Preliminary investigation on the Phytochemistry and antimicrobial activity of Senna alata L. flower. *Pak J Biol Sci* 10, 806-809.

Iwalewa, E.O., Omisore, N.O., Adewunmi, C.O., Gbolade, A.A., Ademowo, O.G., Nneji, C., Agboola, O.I. and Daniyan, O.M., 2008. Anti-protozoan activities of Harungana madagascariensis stem bark extract on trichomonads and malaria. *J Ethnopharmacol* 117, 507-511.

Izzo, A.A. and Ernst, E., 2009. Interactions between herbal medicines and prescribed drugs: an updated systematic review. Drugs 69, 1777-1798.

Jiofack, T., Ayissi, I., Fokunang, C., Guedje, N. and Kemeuze, V., 2009. Ethnobotany and phytomedicine of the upper Nyong valley forest in Cameroon. *African Journal of Pharmacy and Pharmacology* 3, 144-150.

Kemgne, E.A., Mbacham, W.F., Boyom, F.F., Zollo, P.H., Tsamo, E. and Rosenthal, P.J., 2011. Invitro sensitivity of Plasmodium falciparum field isolates to extracts from Cameroonian Annonaceae plants. *Parasitol Res* 110, 109-117.

Kimura, K., Hirayama, F. and Uekama, K., 1999. Characterization of tolbutamide polymorphs (Burger's forms II and IV) and polymorphic transition behavior. *J Pharm Sci* 88, 385-391.

Kisangau, D.P., Lyaruu, H.V., Hosea, K.M. and Joseph, C.C., 2007. Use of traditional medicines in the management of HIV/AIDS opportunistic infections in Tanzania: a case in the Bukoba rural district. *J Ethnobiol Ethnomed* 3, 29.

Klos, M., van de Venter, M., Milne, P.J., Traore, H.N., Meyer, D. and Oosthuizen, V., 2009. In vitro anti-HIV activity of five selected South African medicinal plant extracts. *J Ethnopharmacol* 124, 182-188.

Kramer, J.A., Sagartz, J.E. and Morris, D.L., 2007. The application of discovery toxicology and pathology towards the design of safer pharmaceutical lead candidates. *Nat Rev Drug Discov* 6, 636-649.

Lacmata, S.T., Kuete, V., Dzoyem, J.P., Tankeo, S.B., Teke, G.N., Kuiate, J.R. and Pages, J.M., 2012. Antibacterial Activities of Selected Cameroonian Plants and Their Synergistic Effects with Antibiotics against Bacteria Expressing MDR Phenotypes. *Evid Based Complement Alternat Med* 2012, 623723.

Lamorde, M., Tabuti, J.R., Obua, C., Kukunda-Byobona, C., Lanyero, H., Byakika-Kibwika, P., Bbosa, G.S., Lubega, A., Ogwal-Okeng, J., Ryan, M., Waako, P.J. and Merry, C., 2010. Medicinal plants used by traditional medicine practitioners for the treatment of HIV/AIDS and related conditions in Uganda. *J Ethnopharmacol* 130, 43-53.

Lee, L.S., Andrade, A.S. and Flexner, C., 2006. Interactions between natural health products and antiretroviral drugs: pharmacokinetic and pharmacodynamic effects. *Clin Infect Dis* 43, 1052-1059.

Leteane, M.M., Ngwenya, B.N., Muzila, M., Namushe, A., Mwinga, J., Musonda, R., Moyo, S., Mengestu, Y.B., Abegaz, B.M. and Andrae-Marobela, K., 2012. Old plants newly discovered: *Cassia sieberiana* D.C. and *Cassia abbreviata* Oliv. Oliv. root extracts inhibit in vitro HIV-1c replication in peripheral blood mononuclear cells (PBMCs) by different modes of action. *J Ethnopharmacol* 141, 48-56.

Lu, Z., Chen, W., Viljoen, A. and Hamman, J.H., 2010. Effect of sinomenine on the in vitro intestinal epithelial transport of selected compounds. *Phytother Res* 24, 211-218.

Lubinga, S.J., Kintu, A., Atuhaire, J. and Asiimwe, S., 2012. Concomitant herbal medicine and Antiretroviral Therapy (ART) use among HIV patients in Western Uganda: A cross-sectional analysis of magnitude and patterns of use, associated factors and impact on ART adherence. AIDS Care.

Maccaroni, E., Malpezzi, L., Panzeri, W. and Masciocchi, N., 2010. Thermal and X-ray powder diffraction structural characterization of two benfluorex hydrochloride polymorphs. *J Pharm Biomed Anal* 53, 1-6.

Mamadou, G., Meddah, B., Limas-Nzouzi, N., Ait El Haj, A., Bipolo, S., Mokondjimobé, E., Mahraoui, L., Faouzi, A.M., Ducroc, R., Cherrah, Y. and Eto, B., 2011. Antispasmodic phytomed-

icine, from traditional utilization to rational formulation: functional approach. *Phytopharmacology* 1, 20-35.

Mao, Q.C., Zhou, Y.C., Li, R.M., Hu, Y.P., Liu, S.W. and Li, X.J., 2010. [Inhibition of HIV-1 mediated cell-cell fusion by saponin fraction from Psidium guajava leaf]. *Zhong Yao Cai* 33, 1751-1754.

Maregesi, S., Van Miert, S., Pannecouque, C., Feiz Haddad, M.H., Hermans, N., Wright, C.W., Vlietinck, A.J., Apers, S. and Pieters, L., 2010. Screening of Tanzanian medicinal plants against Plasmodium falciparum and human immunodeficiency virus. *Planta Med* 76, 195-201.

Marjo, C.E., Bhadbhade, M., Hook, J.M. and Rich, A.M., 2011. Polymorphism and a metastable solvate of duloxetine hydrochloride. *Mol Pharm* 8, 2454-2464.

Mbah, A.U., Udeinya, I.J., Shu, E.N., Chijioke, C.P., Nubila, T., Udeinya, F., Muobuike, A., Mmuobieri, A. and Obioma, M.S., 2007. Fractionated neem leaf extract is safe and increases CD4+ cell levels in HIV/AIDS patients. *Am J Ther* 14, 369-374.

Mbaveng, A.T., Kuete, V., Mapunya, B.M., Beng, V.P., Nkengfack, A.E., Meyer, J.J. and Lall, N., 2011. Evaluation of four Cameroonian medicinal plants for anticancer, antigonorrheal and antireverse transcriptase activities. *Environ Toxicol Pharmacol 32*, 162-167.

McCrone, W.C., 1965. Fusion Methods in Chemical Microscopy. Interscience Publishers Inc, New York.

Mesia, K., Cimanga, R.K., Dhooghe, L., Cos, P., Apers, S., Totte, J., Tona, G.L., Pieters, L., Vlietinck, A.J. and Maes, L., 2010. Antimalarial activity and toxicity evaluation of a quantified *Nauclea pobeguinii* extract. *J Ethnopharmacol* 131, 10-16.

Minocha, M., Mandava, N.K., Kwatra, D., Pal, D., Folk, W.R., Earla, R. and Mitra, A.K., 2011. Effect of short term and chronic administration of Sutherlandia frutescens on pharmacokinetics of nevirapine in rats. *Int J Pharm* 413, 44-50.

Mokondjimobe, E., Miantezila Basilua, J., Barkha, S., Dzeufiet, P.-D., Chenal, H., Otsudi'andjeka, J.-B., Bipolo, S., Besse, M., Mamadou, G., Limas-Nzouzi, N., Kamtchouing, P., Meddah, B., Okpwae Okpwae, J., Schobiltgen, F. and Eto, B., 2012. Fagaricine, a new Immunorestorative phytomedicine from Zanthoxylum heitzii: Preclinical and multicenter cohort clinical studies based on HIV-infected patients in six countries. *Phytopharmacology* 2, 26-45.

Molto, J., Valle, M., Miranda, C., Cedeno, S., Negredo, E., Barbanoj, M.J. and Clotet, B., 2010. Herb-drug interaction between Echinacea purpurea and darunavir-ritonavir in HIV-infected patients. *Antimicrob Agents Chemother* 55, 326-330.

Molto, J., Valle, M., Miranda, C., Cedeno, S., Negredo, E., Barbanoj, M.J. and Clotet, B., 2011. Herb-drug interaction between Echinacea purpurea and darunavir-ritonavir in HIV-infected patients. *Antimicrob Agents Chemother* 55, 326-330.

Molto, J., Valle, M., Miranda, C., Cedeno, S., Negredo, E. and Clotet, B., 2012. Herb-Drug Interaction between Echinacea purpurea and Etravirine in HIV-Infected Patients. *Antimicrob Agents Chemother* 56, 5328-5331.

Moreno-Calvo, E., Munto, M., Wurst, K., Ventosa, N., Masciocchi, N. and Veciana, J., 2011. Polymorphs and solvates of nicardipine hydrochloride. Selective stabilization of different diastereomeric racemates. *Mol Pharm* 8, 395-404.

Moshi, M.J., Otieno, D.F. and Weisheit, A., 2012. Ethnomedicine of the Kagera Region, north western Tanzania. Part 3: plants used in traditional medicine in Kikuku village, Muleba District. *J Ethnobiol Ethnomed* 8, 14.

Mudzviti, T., Maponga, C.C., Khoza, S., Ma, Q. and Morse, G.D., 2012. The impact of herbal drug use on adverse drug reaction profiles of patients on antiretroviral therapy in zimbabwe. *AIDS Res Treat* 2012, 434171.

Mukuta, T., Lee, A.Y. and Kawakami, T., 2005. Influence of impurities on the solution-mediated phase transformation of an active pharmaceutical ingredient. Cryst Growth Des 5, 1429-1436.

Muller, A.C. and Kanfer, I., 2011. Potential pharmacokinetic interactions between antiretrovirals and medicinal plants used as complementary and African traditional medicines. *Biopharm Drug Dispos* 32, 458-470.

Muller, A.C., Patnala, S., Kis, O., Bendayan, R. and Kanfer, I., 2012. Interactions between phytochemical components of Sutherlandia frutescens and the antiretroviral, atazanavir in vitro: implications for absorption and metabolism. *J Pharm Pharm Sci* 15, 221-233.

Musuyu Muganza, D., Fruth, B.I., Nzunzu Lami, J., Mesia, G.K., Kambu, O.K., Tona, G.L., Cimanga Kanyanga, R., Cos, P., Maes, L., Apers, S. and Pieters, L., 2012. In vitro antiprotozoal and cytotoxic activity of 33 ethonopharmacologically selected medicinal plants from Democratic Republic of Congo. *J Ethnopharmacol* 141, 301-308.

Nagata, J.M., Jew, A.R., Kimeu, J.M., Salmen, C.R., Bukusi, E.A. and Cohen, C.R., 2011. Medical pluralism on Mfangano Island: use of medicinal plants among persons living with HIV/AIDS in Suba District, Kenya. *J Ethnopharmacol* 135, 501-509.

Ndu, O.O., Nworu, C.S., Ehiemere, C.O., Ndukwe, N.C. and Ochiogu, I.S., 2011. Herb-drug interaction between the extract of Hibiscus sabdariffa L. and hydrochlorothiazide in experimental animals. *J Med Food* 14, 640-644.

Ngondi, J.L., Etoundi, B.C., Nyangono, C.B., Mbofung, C.M. and Oben, J.E., 2009. IGOB131, a novel seed extract of the West African plant Irvingia gabonensis, significantly reduces body weight and improves metabolic parameters in overweight humans in a randomized double blind placebo controlled investigation. *Lipids Health Dis* 8, 7.

Nkeoua, G. and Boundzanga, G.C., 1999. Données sur les produits forestiers non ligneux en Republique du Congo. . In: CEE-FAO (Ed.), FAO, Brazzaville.

Nworu, C.S., Akah, P.A., Okoye, F.B., Onwuakagba, C.J., Okorafor, U.O. and Esimone, C.O., 2012. Supplementation with aqueous leaf extract of Morinda lucida enhances immunorestoration and upregulates the expression of cytokines and immunostimulatory markers. *Immunol Invest* 41, 799-819.

Nwosu, C.O., Eneme, T.A., Onyeyili, P.A. and Ogugbuaja, V.O., 2008. Toxicity and anthelmintic efficacy of crude aqueous of extract of the bark of Sacoglottis gabonensis. *Fitoterapia* 79, 101-105.

Oben, J.E., Ngondi, J.L. and Blum, K., 2008a. Inhibition of Irvingia gabonensis seed extract (OB131) on adipogenesis as mediated via down regulation of the PPARgamma and leptin genes and upregulation of the adiponectin gene. *Lipids Health Dis* 7, 44.

Oben, J.E., Ngondi, J.L., Momo, C.N., Agbor, G.A. and Sobgui, C.S., 2008b. The use of a *Cissus quadrangularis/Irvingia gabonensis* combination in the management of weight loss: a double blind placebo-controlled study. *Lipids Health Dis* 7, 12.

Oga, E.F., Sekine, S., Shitara, Y. and Horie, T., 2012a. P-glycoprotein mediated efflux in Caco-2 cell monolayers: The influence of herbals on digoxin transport. *J Ethnopharmacol* 144, 612-617.

Oga, E.F., Sekine, S., Shitara, Y. and Horie, T., 2012b. Potential P-glycoprotein-mediated drug-drug interactions of antimalarial agents in Caco-2 cells. *Am J Trop Med Hyg* 87, 64-69.

Ojewole, J.A., Awe, E.O. and Chiwororo, W.D., 2008. Antidiarrhoeal activity of Psidium guajava Linn. (Myrtaceae) leaf aqueous extract in rodents. *J Smooth Muscle Res* 44, 195-207.

Oluyemi, K.A., Omotuyi, I.O., Jimoh, O.R., Adesanya, O.A., Saalu, C.L. and Josiah, S.J., 2007. Erythropoietic and anti-obesity effects of Garcinia cambogia (bitter kola) in Wistar rats. *Biotechnol Appl Biochem* 46, 69-72.

Onifadee, A.A., Jewel, A.P. and Okesina, A.B., 2011. Virologic and immunologic outcome of treatment of HIV infection with a herbal concoction, alpha-Zam, among clients seeking herbal remedy in Nigeria. *Afr J Tradit Complement Altern Med* 8, 37-44.

Palombo, E.A., 2006. Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: modes of action and effects on intestinal function. *Phytother Res* 20, 717-724.

Partnership, R.B.M., 2008. The Global Malaria Action Plan. For a Malaria Free World. RBM, Geneva.

Peltzer, K., Preez, N.F., Ramlagan, S., Fomundam, H., Anderson, J. and Chanetsa, L., 2011. Antiretrovirals and the use of traditional, complementary and alternative medicine by HIV patients in Kwazulu-Natal, South Africa: a longitudinal study. Afr J Tradit Complement Altern Med 8, 337-345.

Piscitelli, S.C., Burstein, A.H., Welden, N., Gallicano, K.D. and Falloon, J., 2002. The effect of garlic supplements on the pharmacokinetics of saquinavir. *Clin Infect Dis* 34, 234-238.

Pretorius, E., Smit, E., Oberholzer, H.M., Steyn, E., Briedenhann, S. and Franz, R.C., 2009. Investigating the ultrastructure of platelets of HIV patients treated with the immuno-regulator, Canova: a qualitative scanning electron microscopy study. *Histol Histopathol* 24, 399-405.

Rajan, S., Thirunalasundari, T. and Jeeva, S., 2011. Anti-enteric bacterial activity and phytochemical analysis of the seed kernel extract of *Mangifera indica* Linnaeus against *Shigella dysenteriae* (Shiga, corrig.) Castellani and Chalmers. *Asian Pac J Trop Med* 4, 294-300.

Rodriguez-Hornedo, N. and Murphy, D., 2004. Surfactant-facilitated crystallization of dehydrate carbamazepine during dissolution of anhydrous polymorph. *J Pharm Sci* 93, 449-460.

Romero, S., Escalera, B. and Bustamante, P., 1999. Solubility behavior of polymorphs I and II of mefenamic acid in solvent mixtures. *Int J Pharm* 178, 193-202.

Ross, R., 1905. The Logical Basis of the Sanitary Policy of Mosquito Reduction. *Science* 22, 689-699.

Ross, R. (Ed.), 1911. The prevention of malaria. John Murray, London. Schild, H.O., 1947. pA, a new scale for the measurement of drug antagonism. *Br J Pharmacol Chemother* 2, 189-206.

Schulzke, J.D., Andres, S., Amasheh, M., Fromm, A. and Gunzel, D., 2011. Anti-diarrheal mechanism of the traditional remedy Uzara via reduction of active chloride secretion. *PLoS One* 6, e18107.

Sheth, P., Sandhu, H., Singhal, D., Malick, W., Shah, N. and Kislalioglu, M.S., 2012. Nanoparticles in the pharmaceutical industry and the use of supercritical fluid technologies for nanoparticle production. *Curr Drug Deliv* 9, 269-284.

Shih, C.C., Lin, C.H. and Lin, W.L., 2008. Effects of Momordica charantia on insulin resistance and visceral obesity in mice on high-fat diet. *Diabetes Res Clin Pract* 81, 134-143

Singh, M., Khatoon, S., Singh, S., Kumar, V., Rawat, A.K. and Mehrotra, S., 2010. Antimicrobial screening of ethnobotanically important stem bark of medicinal plants. *Pharmacognosy Res* 2, 254-257.

Slain, D., Amsden, J.R., Khakoo, R.A., Fisher, M.A., Lalka, D. and Hobbs, G.R., 2005. Effect of high-dose vitamin C on the steady-state pharmacokinetics of the protease inhibitor indinavir in healthy volunteers. *Pharmacotherapy* 25, 165-170.

Smit, E., Oberholzer, H.M. and Pretorius, E., 2009. A review of immunomodulators with reference to Canova. *Homeopathy* 98, 169-176.

Sriwilaijaroen, N., Fukumoto, S., Kumagai, K., Hiramatsu, H., Odagiri, T., Tashiro, M. and Suzuki, Y., 2012. Antiviral effects of *Psidium guajava* Linn. (guava) tea on the growth of clinical isolated H1N1 viruses: its role in viral hemagglutination and neuraminidase inhibition. *Antiviral Res* 94, 139-146.

Strom, B., 2006. Study Designs Available for Pharmacoepidemiology Studies. In: B.L.S.a.S.E. KIMMEL (Ed.), Text book of pharmacoepidemiology, John Wiley & Sons, Ltd, Philadelphia.

Sun, J., He, Z.G., Cheng, G., Wang, S.J., Hao, X.H. and Zou, M.J., 2004. Multidrug resistance Pglycoprotein: crucial significance in drug disposition and interaction. *Med Sci Monit* 10, RA5-14.

Taesotikul, T., Nakajima, M., Tassaneeyakul, W. and Yokoi, T., 2012. Effects of Phyllanthus amarus on the pharmacokinetics of midazolam and cytochrome P450 activities in rats. *Xenobiotica* 42, 641-648.

Tal-Dia, A., Toure, K., Sarr, O., Sarr, M., Cisse, M.F., Garnier, P. and Wone, I., 1997. [A baobab solution for the prevention and treatment of acute dehydration in infantile diarrhea]. *Dakar Med* 42, 68-73.

Tavares, L.S., Rettore, J.V., Freitas, R.M., Porto, W.F., Duque, A.P., Singulani, J.D., Silva, O.N., Detoni, M.D., Vasconcelos, E.G., Dias, S.C., Franco, O.L. and Santos, M.D., 2012. Antimicrobial activity of recombinant Pg-AMP1, a glycine-rich peptide from guava seeds. *Peptides* 37, 294-300.

Tchamadeu, M.C., Dzeufiet, P.D., Nana, P., Kouambou Nouga, C.C., Ngueguim Tsofack, F., Allard, J., Blaes, N., Siagat, R., Zapfack, L., Girolami, J.P., Tack, I., Kamtchouing, P. and Dimo, T., 2011. Acute and sub-chronic oral toxicity studies of an aqueous stem bark extract of *Pterocarpus soyauxii* Taub (Papilionaceae) in rodents. *J Ethnopharmacol* 133, 329-335.

Tepongning, R.N., Lucantoni, L., Nasuti, C.C., Dori, G.U., Yerbanga, S.R., Lupidi, G., Marini, C., Rossi, G., Esposito, F. and Habluetzel, A., 2011. Potential of a Khaya ivorensis –*Alstonia boonei* extract combination as antimalarial prophylactic remedy. *J Ethnopharmacol* 137, 743-751.

Thakkar, A.L., Hirsch, C.A. and Page, J.G., 1977. Solid dispersion approach for overcoming bioavailability problems due to polymorphism of nabilone, a cannabinoid derivative. *J Pharm Pharmacol* 29, 783-784.

Theo, A., Masebe, T., Suzuki, Y., Kikuchi, H., Wada, S., Obi, C.L., Bessong, P.O., Usuzawa, M., Oshima, Y. and Hattori, T., 2009. Peltophorum africanum, a traditional South African medicinal plant, contains an anti HIV-1 constituent, betulinic acid. Tohoku *J Exp Med* 217, 93-99.

Tona, L., Kambu, K., Ngimbi, N., Mesia, K., Penge, O., Lusakibanza, M., Cimanga, K., De Bruyne, T., Apers, S., Totte, J., Pieters, L. and Vlietinck, A.J., 2000. Antiamoebic and spasmolytic activities of extracts from some antidiarrhoeal traditional preparations used in Kinshasa, Congo. *Phytomedicine* 7, 31-38.

Udeinya, I.J., Mbah, A.U., Chijioke, C.P. and Shu, E.N., 2004. An antimalarial extract from neem leaves is antiretroviral. *Trans R Soc Trop Med Hyg* 98, 435-437.

Wei, L., Li, Z. and Chen, B., 2000. [Clinical study on treatment of infantile rotaviral enteritis with *Psidium guajava* L]. *Zhongguo Zhong Xi Yi Jie He Za Zhi* 20, 893-895.

WHO, 2000. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. WHO/EDM/TRM/2000.1.

WHO, 2003. Introduction to Drug Utilization Research. WHO, Geneva.

WHO/AIPO, 2004. Référentiel pour l'harmonisation des procédures d'homologation des medicaments issus de la pharmacopée traditionnelle dans les pays membres de l'OAPI., African intellectual property organization, Yaoundé Cameroun, pp. 1-35.

Williamson, E.M., 2003. Drug interactions between herbal and prescription medicines. *Drug Safety* 26, 1075-1092.

Zirihi, G.N., Mambu, L., Guede-Guina, F., Bodo, B. and Grellier, P., 2005. In vitro antiplasmodial activity and cytotoxicity of 33 West African plants used for treatment of malaria. *Journal of Ethnopharmacology* 98, 281-285.