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An ethnobotanical survey and inhibitory effects on NLRP3 inflammasomes/Caspase-1 of herbal recipes' extracts traditionally used in Rwanda for asthma treatment

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Abstract:

Ethnopharmacological relevance

Respiratory diseases and asthma, in particular, are nowadays a global health problem. In Rwanda, some traditional healers claim to treat asthma with plant-based recipes, though there is no scientific proof so far.

Aim of the study

¹ Equal contribution

Our study aimed at evaluating the toxicity and the anti-inflammatory effect of plant recipes used in Rwanda against asthma in order to select potential candidates for further characterization of the active compounds.

Materials and methods

Water (aqueous) and methanol-dichloromethane (organic) extracts from selected folkloric recipes were submitted for toxicity test on THP-1 derived macrophages using CellTiter-Glo Luminescent Cell Viability Assay. The evaluation of the anti-inflammatory effect of the plant extracts was carried out using the Caspase-Glo 1 Inflammasome assay on THP-1 -derived macrophages.

Results

Most of both organic and aqueous extract showed more than 95% of cell viability up to 200 µg/ml, except for R03Cn organic extract that inhibited 25% of the cell viability. Plant extracts inhibited caspase-1 activation in THP-1 derived macrophages in a dose-dependent manner. Four extracts (R03Cn and R07Kn aqueous extracts, R10MK and R19Sz organic extracts) strongly downregulated the activation of caspase-1 (more than 70% at 50 µg/ml). In general, organic extracts exhibited better caspase-1 inhibitory effects than their aqueous counterparts.

Conclusions

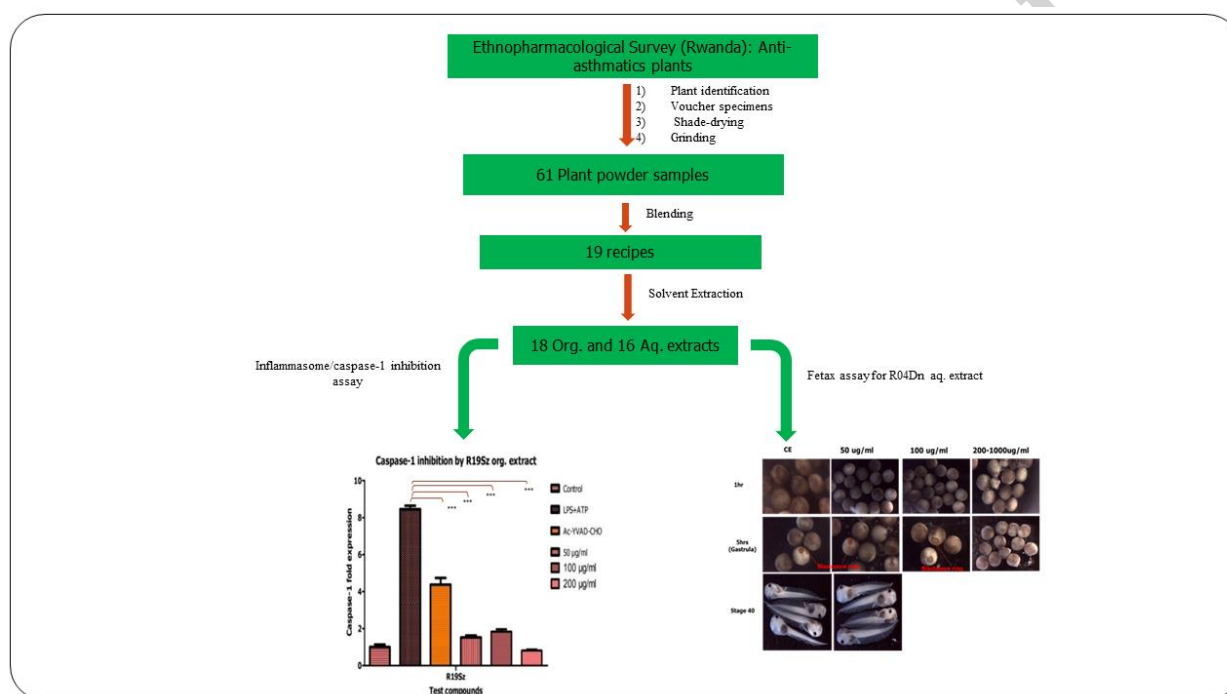
The inhibition of inflammasome/caspase-1 is one of key mechanisms of action in asthma. Some traditional recipes are active on this mechanism and are thus strong candidates for the treatment of asthma and other inflammasome-mediated diseases. Further investigations are needed to characterize active molecules.

abbreviations

FETAX, Frog Embryo Teratogenesis Assay – Xenopus; WHO, World Health Organization; COPD, Chronic Obstructive Pulmonary Disease; Th2, T helper 2; Th17, T helper 17; IL-1β, Interleukin one beta; TNF-α, Tumor necrosis factor alpha; NLRP3, NOD-Like Receptor family, Pyrin domain containing 3 protein; DMSO, Dimethyl sulfoxide; ATCC, American Type Culture Collection; PBS, Phosphate-buffered saline; RPMI, Roswell Park Memorial Institute Medium; FBS, Fetal Bovine Serum; PMA, Phorbol-12-Myristate 13-Acetate; MBS, Medium Barth's Solution; LPS, Lipopolysaccharide; ATP, Adenosine Triphosphate; AC-YVAD-CHO, N-acetyl-L-tyrosyl-L-valyl-N-[(1S)-2-carboxyl-1-formylethyl]-L-alaninamide; S, second; Min, minute; H,

hour; μl , microliter ; μg , microgram ; l, liter ; g, gram; $^{\circ}\text{C}$, degree Celsius; nM, nanomolar; ml, milliliter ; ARES , Académie de Recherche et d'Enseignement Supérieur

Graphical Abstract



Keywords: Asthma; medicinal plants; Rwanda; inflammasome/caspase-1; THP-1-derived macrophages; *Xenopus* embryos

1. Introduction

Respiratory diseases appear to be among the major categories of non-communicable diseases and its prevalence accounted, worldwide, for more than 1 billion of patients by the year 2013 (Marciniuk et al., 2013). Among these respiratory diseases asthma is heading with 334 million of patients followed by chronic obstructive pulmonary disease (210 million), allergic rhinitis (40 million), and other respiratory diseases (Global Asthma Network, 2014; World Health Organization, 2010). With the increasing adoption of modern lifestyle and the development of urbanization, which is associated with increasing exposure to pollutants, the prevalence of asthma is set to increase with an estimated additional 100 million people by 2025 (World Health Organization, 2010; Yeo et al., 2008). In Africa, the prevalence of asthma is estimated at around 50 million of patients (Musafiri et al., 2011). The disease is making a very heavy economic burden that is beyond the capacity of developing countries. Though there is no global economic study about asthma treatment costs, a study conducted in 5 countries (Canada, USA, Germany, Singapore, and Switzerland) evaluated the cost at up to 30 billion USD in 2009 (Global Asthma Network, 2014; World Health Organization, 2010).

Asthma is defined as a chronic inflammatory disease of the airways resulting from its interaction with the immune system (Besnard et al., 2012; Garcia, 2006). Due to the fact that the airways are constantly in contact with the environment, they are exposed to the noxious stimuli which involve the upset of the immune system to clear these stimuli (Pinkerton et al., 2017). Sometimes, the body defense system fails to maintain a delicate balance between immune-mediated clearance of these stimuli and avoiding inadvertent self-harm from chronic inflammation. As a result, the immune system inappropriately leads inflammation against its own cells, resulting in different chronic inflammatory diseases including respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD) (Wanderer, 2008). Several inflammatory cells and mediators have been reported to be involved in asthma. Depending on inflammatory patterns involved, there are two main subtypes of asthma, the eosinophilic (Th2-derived cytokines) and the nucleophilic (Th17-derived cytokines and chemokines) (Pinkerton et al., 2017). The nucleophilic asthma is the most severe and resistant to corticoid-based treatment and is characterized by a high proportion of neutrophils recruited by inflammatory cytokines like IL-1 β and TNF- α . It is well known that inflammasomes are involved in the upregulation of these

cytokines, particularly the IL-1 β through the activation of caspase-1 (Lin and Liu, 2017; Madouri et al., 2015) . In fact, inflammasomes are cytosol-assembled multimeric complexes largely involved in several auto-inflammatory and auto-immune diseases including asthma, atherosclerosis, Alzheimer, diabetes, etc. (Dhimolea, 2010; Fenini et al., 2017) . When assembled, inflammasomes induce the activation of caspase-1 responsible for the conversion of premature IL-1 β into its mature form. Corticosteroids are widely used as the main treatment of the disease. Besides the side effects linked to the use of corticoids, some patients showed also a resistance to the treatment, mainly those with high level of neutrophils and IL-1 β in their sputum (Madouri et al., 2015; Pinkerton et al., 2017). For these patients, specific treatment based on the use of monoclonal antibodies such as Keliximab, Gomiliximab, Anrukinzumab, etc. have been reported to alleviate the severity of the disease (Dhimolea, 2010). However, this treatment using monoclonal antibodies is quite expensive making its affordability to developing countries difficult. Considering these concerns, natural products, particularly plants, can be an important source of new drugs. Some plants extracts have been also widely studied in different models of antiasthmatic tests though few of them were evaluated at molecular level. *Aloe vera* and propolis, among others, have been reported to possess inflammasome inhibitory properties and even some active compounds were proposed as responsible for their activities (Tózsér and Benkő, 2016). In Rwanda, some traditional healers claim to possess traditional medicines for the treatment of asthma, though there is no scientific study to substantiate their efficacy or their safety. It is therefore important not only to investigate them therapeutically, but also to screen for their hazardous health effects. Therefore, the present study was aimed at gathering information on plants and/or plant parts used in the treatment of asthma in Rwanda, evaluating both their toxicity and their NLRP3 inflammasomes inhibitory properties in the hope of finding new potential drug candidates. The choice of the inflammasome method was driven by the fact that the method is placed in the upstream of the most useful *in vitro* anti-inflammatory tests such lipoxygenase, cyclooxygenase and myeloperoxidase (Akula and Odhav, 2008; Rathinam and Fitzgerald, 2016) . Thus, inhibiting inflammasome activation will lead to the inhibition of the downstream inflammatory enzymes and/or mediators.

2. Material and method

2.1 Ethnobotanical survey

The ethnobotanical survey was conducted in Southern province of Rwanda in June and December 2016. The southern province is composed of 8 districts including Kamonyi, Muhanga, Ruhango, Nyanza, Huye, Gisagara, Nyamagabe and Nyaruguru. During the survey, we conducted semi-structured interviews with 16 traditional healers by using questionnaires (supplementary material Table S.1). Respondents were selected by purposive sampling method (Tongco, 2007) on the basis of their knowledge and experience in traditional medicine. Questionnaires were used to obtain information on plant species used to treat asthma, morphological parts used, recipe preparation and processing, mode of administration, etc. Plant species mentioned were identified with reference to herbarium specimens and various literature resources by Nshutiyayesu Samuel (from the University of Rwanda) and the authentication and ID verification by Dr Olivier Lachenaud (from the Botanic Garden Meise/Belgium respectively) (Troupin, 1982). All the plant names provided are accepted scientific names as cross-checked on <http://www.theplantlist.org>. Herbal voucher specimens of the collected samples were deposited in official herbaria, in the Botanic Garden Meise/Belgium and/or in the National Herbarium of Rwanda/Rwanda.

2.2 Preparation of recipes crude extracts

Based on traditional health practioners information, 19 recipes were made from 61 medicinal plants. Since testing individually all these 61 plants would be much costly, and since plants could have activity only in combination, we set our selection criteria for further studies to be based on the activity of recipes. Through the literature, the selection of plants to be bio-prospected is generally based on random selection, ethno-directed selection or chemotaxonomy approach (Silva et al., 2013). From the ethno-directed selection, plants can also be selected, for further studies, based either on the citation frequency (Kamagaju et al., 2013) or on comparison with the literature on similar uses (Mukazayire et al., 2011). However, nothing proves that the most cited are the ones most efficient as plants can play different roles within a medicinal recipe such as masking the taste/odor, ensuring a better consistence, etc. (Ejike et al., 2013). Therefore, we based our selection criteria on the efficiency of the recipes. Plants from the most active recipes will be chosen for further studies. The 19 recipes were extracted using both organic solvent (methanol-dichloromethane; 3:1) and distilled water in a volume of 10 times the weight of the plant materials. For the preparation of organic extracts, a mixture of methanol-dichloromethane (3 :1) was used for 3 times 1 hour under constant agitation at room temperature.

The solvent was then first removed on a rotary evaporator (pressure : 260 mm Hg, temperature : 30° C) and lastly dried in a vacuum concentrator. For the aqueous extracts, plant materials were boiled for up to 10 min, filtered and kept at -20° C before being lyophilized overnight.

2.3 Preparation of test solutions

A stock solution of organic extracts was prepared in Dimethyl sulfoxide (DMSO; Sigma) at a concentration of 20 mg/ml and kept in the cold room (4 °C) before use. For the aqueous extracts, stock solutions were prepared in Phosphate-buffered saline (PBS; Life technologies) at the concentration of 50 mg/ml or 25 mg/ml. Stock solutions were further diluted to the required concentrations with complete cell culture medium. The final concentrations of DMSO and PBS were 1% and 4% respectively and these solvents had no or minor effect on the cell viability at their working concentrations.

2.4 Cell culture and differentiation

Human leukaemia THP-1 monocyte cells were purchased from American Tissue Type Culture Collection (**ATCC; CRL-9609**) and were cultured according to the VIGO-project protocol (Hirsch, 2014). Cells were cultured at a density between 10^5 and 2×10^5 cells/ml, in RPMI 1640 (Life Technologies) supplemented with 10% heat-inactivated Foetal Bovine Serum (FBS; Life technologies), 1% glutamine, and 1% of penicillin-streptomycin (Life technologies) and maintained at 37°C under 5% CO₂ in a humidified atmosphere. Cells were sub-cultured every 2 or 3 days, the density was not allowed to exceed 10^6 cells/ml. Cells were passaged at least six times before any experiment. For the determination of cell densities, the cell counting was done using a Neubauer counting chamber with trypan blue (0.4% in PBS; pH= 7.4; Life technologies) staining. THP-1 monocytes were differentiated into THP-1 macrophages with 20 nM of phorbol-12-myristate 13-acetate (PMA; Sigma) was added to a final concentration of 20 nM for 2 days. During the study, cells were used when the cell density attended 7×10^5 to 10^6 cells/ml. it was then adjusted with the complete culture medium to obtain a final density of 5×10^5 cells/ml and 100 µl of cell suspension were seeded in each well of the 96-well culture plate.

2.5 Cell viability evaluation of plant extracts

Prior to the caspase-1 activity evaluation, the effect of extracts on the cell viability was screened. In order to find out the sub-lethal dose for extracts, a dose-dependent preliminary assay for all extracts was performed for 24 hours. The CellTiter-Glo Luminescent Cell Viability Assay

(Promega) was used to perform the viability of THP-1 derived macrophages under different treatment conditions by following the manufacturer's instructions. Briefly, PMA-differentiated cells were incubated in the presence or absence of plant extracts for 24 hours at 37° C in a humidified 5 % CO₂ atmosphere. From each well, PMA-containing medium was pipetted and directly replaced with fresh complete medium containing plant extracts at different concentrations. The maximum dose was 200 µg/ml and 1000 µg/ml for organic (Methanol-Dichloromethane; 3:1) and aqueous extracts respectively. For each experiment, a control made up of vehicle-treated cells and a vehicle control were prepared to determine the background and vehicle effect on the cell viability respectively. After 24 hours of incubation, cells were allowed to equilibrate at room temperature for approximately 30 min before adding the celltiter glo reagent. Meanwhile, 50 µl of the supernatant were removed, having therefore approximately 50 µl in each culture well. A proportion of 50 µl of the CellTiter-Glo reagent was then added to 50 µl of medium containing cells in a 96-well plate and, covered by an aluminium sheet (to protect from light), shaken for 2 minutes on an orbital shaker to induce cell lysis. It was then further incubated for additional 10 minutes at room temperature for the stabilization of the luminescent signal before reading the luminescence on a Berthold Technologies luminometer (XS³ LB 960) for 0.1 s/well. The cell viability (%) was calculated using the following formula:

$$\text{Viability (\%)} = [\text{RLU (treated cells)} / \text{RLU (control cells)}] \times 100$$

2.6 Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX)

FETAX testing was conducted as described by (Prati et al., 2000). Adult female frogs were stimulated with 500 units of human chorionic gonadotropin and *Xenopus* embryos were obtained by *in vitro* fertilization. 50 embryos were exposed to the aqueous R04Dn extract diluted on the Medium Barth's Solution (MBS) at the indicated concentrations. Other 50 embryos were exposed to MBS solution alone and designated MBS solution controls (negative controls). All solutions were changed every 24 h during the experiment. Following 4 days of exposure (stage 46 embryos), embryos were fixed in 4% paraformaldehyde diluted in PBS (pH 7.0). Embryos morphology and number were recorded using a light microscope.

2.7 Caspase-1 activity evaluation

The evaluation of caspase-1 activity was done according to (Juliana et al., 2012) with slight modifications. Briefly, THP-1 cells were grown for 2 days in 96-well plates to a density of 5x10⁴ cells/well in the presence of 20 nM PMA at 37° C in a humidified 5% CO₂ incubator. PMA-

supplemented medium was then discarded and macrophages thereof were pretreated with a fresh medium containing different recipes' extracts concentrations or 10 μ M AC-YVAD-CHO (control) for 1 hour. Cells were further incubated with LPS (Sigma) (at a final concentration of 1 μ g/ml) for additional 3 hours and then with 5 μ M ATP (Invivogen) for 30 min. The caspase-1 activity was measured using the Caspase-Glo 1 Inflammasome Assay according to the manufacturer's instructions (Promega). The 96-well plate containing cells was removed from the incubator and 50 μ l of the supernatant in each well was transferred to the corresponding well of a new white 96-well plate. An aliquot of 50 μ l of the Caspase-Glo 1 reagent was then added to each well and gently mixed on a plate shaker at 300 rpm for 30 s. The mixture was then incubated for 1 hour at room temperature before measuring the luminescence on a Berthold Technologies luminometer.

2.8 Data presentation and analysis

For both the cell viability and caspase-1 inhibition, each test was done in triplicates and all the experiments were reproduced (all done at least twice). Data were analysed using GraphPad Prism 5 and the results are expressed in mean $\pm$ standard deviation. The p-values less than 0.05 were considered to be statistically significant.

3. Results

3.1 Information on plant species as reported by traditional health practitioners

More than 87% of our informants had an experience of more than 20 years. Moreover, 81% of the total interviewees inherited the knowledge and skills in traditional medicine from their parents, and most of them claimed to have treated more than 10 patients each. The respondents are aged between 40-70 years, dominated by men (56%). Most of the interviewees have only attended primary school level (50%), and 56.25% of all respondents reported that their main income generation activity is subsistence farming (Fig. 1).

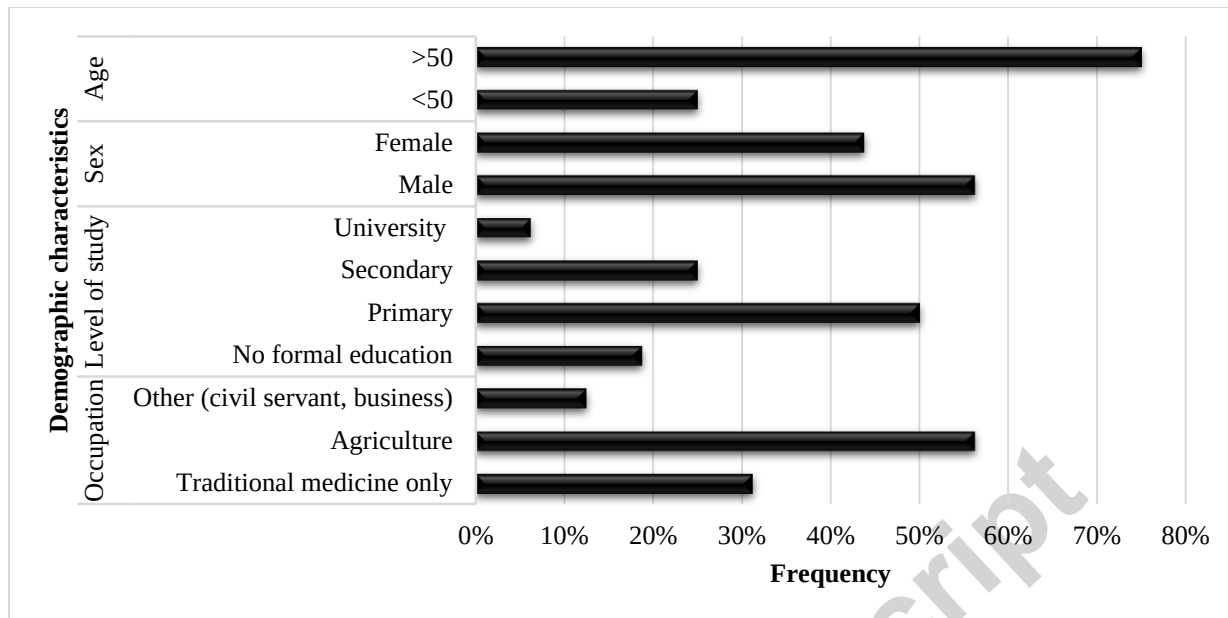


Fig. 1: Social demographic characteristics of respondents. The survey was conducted in the Southern province of Rwanda and informants were chosen by purposive sampling method based on their knowledge and experience in the traditional medicine.

This study identified a total number of 61 different plant species used for asthma treatment (Table 1), among which 43 (70.5 %) are indigenous, while 18 (29.5 %) are of exotic origin. These species belong to 36 families, mostly dominated by Asteraceae and Fabaceae (10 % each) (data not shown). The most frequently used species include *Ocimum gratissimum* (23.5 %), followed by *Maesa lanceolata*, *Eucalyptus globulus* subsp. *maidenii* and *Hibiscus noldeae* (17.6 % each). Leaves are mostly used in 19 drug recipes (51 %) (Table 2). Decoction is the main mode of preparation of the recipes (77 %), and treatment is mostly done via oral route (97 %) (data not shown). Plant species are used in association in all inventoried recipes. In some cases, natural products such as honey were also reported to be used for asthma treatment (reported in 40 % of the recipes). In addition, eggs, goat fat and mineral salt were also reported among products used in drug recipes to treat asthma. In most cases, informants have reported the prohibition of tobacco and alcohol (47.1 % and 58.8 % respectively) when the patient is under treatment. Many of the plant species used are collected from the wild (67.2 %), while the domesticated species do occupy 29.5 % of the total species, and 27.14 % of all plants were considered by the respondents as rare and hard to find.

Table 1: Medicinal plants and other non herbal ingredients as reported to be used in the treatment of asthma by respondents

Family	Species name	Kinyarwanda name	Frequency ^a	Part used	Mode of preparation	Mode of administration	Origin	Place of collection	Voucher number
Acanthaceae	<i>Acanthus polystachius</i> Delile	Igitovu	1	Leaves	Decoction	Oral	In .	S 01° 57' 51.0''; E 029° 58' 59.3''	Toma ni J.C.D. 029
Amoryllidaceae	<i>Allium cepa</i> L.	Igitunguru	1	Bulb	Salad	Oral	Ex.	-	Toma ni J.C.D. 030
Anacardiaceae	<i>Mangifera indica</i> L.	Umwembe	1	Leaves	Decoction	Oral	Ex.	S 02° 37' 15.9''; E 029° 44' 26.5''	Toma ni J.C.D. 031
Apiaceae	<i>Apium graveolens</i> L.	Seleri	1	Aerial parts	Salad	Oral	Ex.	-	Toma ni J.C.D. 032
Apocynaceae	<i>Tabernaemontana stapfiana</i> Britten	Umukamisha	1	Leaves	Decoction	Oral	In .	-	Toma ni J.C.D. 007
Asphodelaceae	<i>Aloe</i> sp.	Igikakaru bamba	1	Leaves	Decoction	Oral	Ex.	S 02° 20' 26.3''; E 029° 46' 47.1''	Toma ni J.C.D. 060
Asteraceae	<i>Anisopappus chinensis</i> subsp. <i>africanus</i> (Hook.f.) S.Ortiz & Paiva	Umuretezaho	1	Leaves	Sauna	Inhalation	In .	-	Toma ni J.C.D. 003
Asteraceae	<i>Aspilia</i> sp.	Inyagasambu	1	Aerial parts	Decoction	Oral	In .	-	Toma ni J.C.D. 033
Asteraceae	<i>Bidens pilosa</i> L.	Inyabarasanya	1	Aerial parts	Decoction	Oral	In .	S 02° 34' 00.1''; E 029° 46' 19.5''	Toma ni J.C.D. 034

Asteraceae	<i>Distephanus angulifolius</i> (DC.) H.Rob. & B.Kahn	Umuhe muto	1	Leaves	Juice	Nasal	In	S 02°37' 16.0'' E 029° 44' 26.4''	Tomanini J.C.D. 001
Asteraceae	<i>Polydora serratuloides</i> (DC.) H.Rob.	Ubukubo	1	Aerial parts	Decoction	Oral	In	S 02°34' 00.1'' E 029°46' 19.5''	Tomanini J.C.D. 010
Asteraceae	<i>Vernonia amygdalina</i> Delile	Umubirizi	2	Leaves and roots	Decoction	Oral	In	S 02° 37' 16.0''; E 029° 44' 26.4''	Tomanini J.C.D. 035
Bignoniaceae	<i>Markhamia lutea</i> (Benth.) K.Schum.	Umusave	1	Leaves	Decoction	Oral	In	S 02° 12' 15.6''; E 029° 47' 14.3''	Tomanini J.C.D. 036
Capparidaceae	<i>Capparis tomentosa</i> Lam.	Umukorokombe	2	Roots	Decoction, Sauna	Oral, inhalation	In		Tomanini J.C.D. 005
Crasulaceae	<i>Kalanchoe sp.</i>	Ikinetene te	1	Leaves	Decoction	Oral	Ex.	S 02° 34' 00.1''; E 029° 46' 19.5''	Tomanini J.C.D. 012
Cucurbitaceae	<i>Lagenaria sphaerica</i> (Sond.) Naudin	Umutanga	1	Roots	Decoction	Oral	In		Tomanini J.C.D. 037
Cucurbitaceae	<i>Momordica foetida</i> Schumacher	Umwishywa	1	Aerial parts	Decoction	Oral	In	S 02° 12' 15.6''; E 029° 47' 14.2''	Tomanini J.C.D. 038
Euphorbiaceae	<i>Euphorbia heterophylla</i> L.	Akaziraruguma	1	Aerial parts	Decoction	Oral	In	S 02° 34' 00.1'' E 029° 46' 19.5''	Tomanini J.C.D. 009
Fabaceae	<i>Acacia mearnsii</i> De Wild.	Ibarakatsi	2	Leaves and bark	Decoction, Infusion	Oral	Ex.	S 02° 37' 11.2'' E 029° 44' 01.7''	Tomanini J.C.D. 006
Fabaceae	<i>Cajanus cajan</i> (L.) Millsp.	Umukunde	2	Leaves	Decoction	Oral	Nat.	S 02° 37' 16.0''; E 029° 44' 26.4''	Tomanini J.C.D. 019
Fabaceae	<i>Eriosema montanum</i> Baker f.	Umupfuyantoki	2	Roots	Decoction, Infusion	Oral	In	S 02° 37' 13.1''; E 029° 44' 02.1''	Tomanini J.C.D. 020
Fabaceae	<i>Erythrina abyssinica</i> DC.	Umuko	2	Leaves and flowers	Decoction	Oral	In	S 02° 05' 11.9''; E 029° 44' 09.1''	Tomanini J.C.D. 039
Fabaceae	<i>Senna occidentalis</i> (L.) Link	Umukubayoka	1	Leaves	Decoction	Oral	Nat.	S 02° 37' 16.0''; E	Tomanini

								. 029° 44' 26.4''	J.C.D. 040
Faba ceae	<i>Tephrosia vogelii</i> Hoo k.f.	Umuruk u	1	Leaves	Decocti on	Oral	In	S02°34' 21.2'' E029° 45' 47.7''	Toma ni J.C.D. 015
Lami aceae	<i>Mentha × piperita</i> L.	Mante	1	Aerial parts	Decocti on	Oral	E x.		Toma ni J.C.D. 041
Lami aceae	<i>Ocimum gratissimum</i> var. <i>suave</i> (Willd.) Hook.f.	Umweny a	4	Leaves and infloresc ence	Decocti on, Infusio n	Oral	In	S02° 12' 15.6'' E029° 47' 14.2''	Toma ni J.C.D. 016
Lami aceae	<i>Tetradenia riparia</i> (Ho chst.) Codd	Umuravu mba	1	Leaves	Decocti on	Oral	In	S 02° 37' 16.0''; E 029° 44' 26.4''	Toma ni J.C.D. 042
Malv aceae	<i>Abutilon mauritianum</i> (Jacq.) Medik.	Umuhuh u muto	1	Leaves	Decocti on	Oral	In	S 02° 34' 00.1''; E 029° 46' 19.5''	Toma ni J.C.D. 026
Malv aceae	<i>Hibiscus noldeae</i> Baker f.	Ruberwa	3	Aerial parts	Decocti on, Infusio n	Oral	In	S 02° 37' 11.2''; E 029° 44' 01.7''	Toma ni J.C.D. 021
Meli aceae	<i>Cedrela odorata</i> L.	Isederi	1	Fruits	Infusio n	Oral	E x.	S 02° 37' 15.9''; E 029° 44' 26.5''	Toma ni J.C.D. 043
Myri caceae	<i>Myrica kandtiana</i> Engl .	Umusing iza	1	Leaves	Sauna	Inhal ation	In		Toma ni J.C.D. 004
Myrt aceae	<i>Eucalyptus globulus</i> su bsp. <i>maidenii</i> (F. Muell.) J.B. Kirkp.	Inturusu y'umwer u/Kinuka	3	Leaves	Decocti on	Oral	E x.		Toma ni J.C.D. 044
Myrt aceae	<i>Syzygium guineense</i> su bsp. <i>parvifolium</i> (Engl.)	Umugote	1	Leaves	Infusio n	oral	In	S 02° 37' 16.0''; E 029° 44' 26.4''	Toma ni J.C.D. 045
Onag raceae	<i>Ludwigia abyssinica</i> A .Rich.	Umuziga ngore	1	Aerial parts	Decocti on	Oral	In	S 02° 20' 26.3''; E 029° 46' 47.1''	Toma ni J.C.D. 018
Oxali dace ae	<i>Oxalis corniculata</i> L.	Ubwuny u bwa nyamanz a	1	Aerial parts	Decocti on, Infusio n	Oral	In		Toma ni J.C.D. 025
Passi flora ceae	<i>Passiflora edulis</i> Sims	Intababar a/Marak uja	1	Leaves	Decocti on	Oral	E x.		Toma ni J.C.D. 027

Phyllanthaceae	<i>Phyllanthus fraternus</i> G.L.Webster	Uruheza	1	Aerial parts	Decoction	Oral	In .	S 02° 34' 00.1''; E 029° 46' 19.5''	Toma ni J.C.D. 028
Phytolaccaceae	<i>Phytolacca dodecandra</i> L'Hér.	Umuhoko	1	Roots	Ash	Oral	In .		Toma ni J.C.D. 046
Pinaceae	<i>Pinus patula</i> Schiede ex Schltdl. & Cham.	Pinusi	2	Leaves	Decoction	Oral	Ex.		Toma ni J.C.D. 047
Poaceae	<i>Cynodon dactylon</i> (L.) Pers.	Umucaca	1	Aerial parts	Decoction	Oral	In .	S 02° 05' 11.9''; E 029° 44' 09.1''	Toma ni J.C.D. 048
Poaceae	<i>Eleusine coracana</i> (L.) Gaertn.	Uburo	1	Grains	Flour	Oral	Ex.	S 02° 47' 35.1''; E 029° 28' 35.3''	Toma ni J.C.D. 049
Poaceae	<i>Pennisetum purpureum</i> Schumach.	Urubingo	1	Leaves	Decoction	Oral	In .		Toma ni J.C.D. 050
Poaceae	<i>Zea mays</i> L.	Ikigori	1	Corn cob	Ash	Oral	Ex.		Toma ni J.C.D. 051
Polygalaceae	<i>Securidaca longipedunculata</i> Fresen.	Umunyagasozi	1	Roots	Infusion	Oral	In .	S 02° 11' 14.9''; E 029° 46' 54.7''	Toma ni J.C.D. 052
Polygonaceae	<i>Oxygonum sinuatum</i> (Hochst. & Steud ex Meisn.) Dammer	Ubuhandanzovu	1	Aerial parts	Infusion	Oral	In .	S 02° 37' 13.1''; E 029° 44' 02.1''	Toma ni J.C.D. 022
Primulaceae	<i>Lysimachia ruhmeriana</i> Vatke	Umuyobora	1	Aerial parts	Decoction	Oral	In .	S 02° 05' 23.2''; E 029° 44' 07.2''	Toma ni J.C.D. 017
Primulaceae	<i>Maesa lanceolata</i> Forsk.	Umuhanga	3	Leaves	Decoction, Sauna	Oral, inhalation	In .	S 02° 05' 10.1'' E 029° 44' 10.5''	Toma ni J.C.D. 002
Ranunculaceae	<i>Clematis hirsuta</i> Guill. & Perr.	Umunkamba	1	Roots	Decoction	Oral	In .	S 02° 05' 10.1'' E 029° 44' 10.5''	Toma ni J.C.D. 053
Rosaceae	<i>Rubus apetalus</i> Poir.	Umukeri	1	Leaves and roots	Decoction	Oral	In .		Toma ni J.C.D. 024
Rubiaceae	<i>Coffea</i> sp.	Ikawa	1	Leaves	Decoction	Oral	Ex.		Toma ni

									J.C.D. 054
Rubiaceae	<i>Rubia cordifolia</i> L.	Umukarambwe	2	Aerial parts	Decoction, Infusion	Oral	In .	S 02° 40' 56.1''; E 029° 40' 13.1''	Toma ni J.C.D. 023
Rutaceae	<i>Citrus x limon</i> (L.) Osbeck	Indimu	1	Fruits	Salad	Oral	Ex.		Toma ni J.C.D. 055
Rutaceae	<i>Clausena anisata</i> (Willd.) Hook.f. ex Benth.	Umuno	1	Leaves	Decoction	Oral	In .	S 02° 34' 00.1''; E 029° 46' 19.5''	Toma ni J.C.D. 011
Rutaceae	<i>Zanthoxylum chalybeum</i> Engl.	Intare y'irungu	1	Roots	Decoction	Oral	In .		Toma ni J.C.D. 056
Solanaceae	<i>Datura stramonium</i> L.	Rwiziririga	1	Leaves	Decoction	Oral	Nat .	S 02° 05' 02.6''; E 029° 44' 24.5''	Toma ni J.C.D. 057
Solanaceae	<i>Physalis peruviana</i> L.	Umuhuhu	1	Leaves	Decoction	Oral	Ex.	S 02° 37' 13.1'' E 029° 44' 02.1''	Toma ni J.C.D. 013
Solanaceae	<i>Solanum aculeastrum</i> Dunal	Umutoboto/umurembe	1	Fruits	Maceration	Oral	In .		Toma ni J.C.D. 008
Solanaceae	<i>Solanum dasyphyllum</i> Schumach. & Thonn.	Igitoborwa	1	Leaves	Decoction	Oral	In .	S 02° 20' 26.3''; E 029° 46' 47.1''	Toma ni J.C.D. 058
Solanaceae	<i>Solanum terminale</i> Forssk.	Umuhurankuba	1	Leaves	Decoction	Oral	In .	S 02° 40' 56.1'' E 029° 40' 13.1''	Toma ni J.C.D. 014
Tropaeolaceae	<i>Tropaeolum majus</i> L.	Kapusine	1	Aerial parts	Decoction	Oral	Ex.		Toma ni J.C.D. 059
Zingiberaceae	<i>Zingiber officinale</i> Roscoe	Tangawizi	1	Rhizome	Decoction	Oral	Ex.		
NA	Mineral salt	Igikukuru	2	NA	Maceration	Oral			
NA	Eggs	Amagi	2	NA	Maceration	Oral			
NA	Honey	Ubuki	5	NA	Maceration,	Oral			

					decoction	
NA	Goat fat	Urugimb u rw'ihene	1	NA	Decoction	Oral

^a Frequency: indicates how many times a given species was mentioned by traditional health practitioners.

Table 2: Composition, mode of preparation and administration of the recipes as reported by traditional healers

Recipe	Composition	Mode of preparation	Mode of Administration	Side effect
R01As	Fresh plants of <i>Pennisetum purpureum</i> (aerial parts) (40 %), <i>A. polystachius</i> Delile (leaves) (40 %), <i>Zingiber officinale</i> (tuber) (20 %)	Decoction	Oral, ¼ glass for adults	None
R02Cr	Equal amount of fresh plants of <i>Polydora serratuloides</i> (aerial parts), <i>Clausena anisata</i> (leaves) and <i>Kalanchoe sp.</i> (leaves)	Decoction	Adults (4 tea spoons x 3/d), children (2 tea spoons x 3/d)	None
R03Cn	Equal amount of fresh plants of: <i>Vernonia amygdalina</i> (leaves), <i>Tetradenia riparia</i> (leaves), <i>Ocimum gratissimum</i> (leaves), <i>Senna occidentalis</i> (leaves), <i>Aspilia sp.</i> (aerial parts), <i>Erythrina abyssinica</i> (leaves), <i>Lagenaria sphaerica</i> (roots) and honey	Decoction	Oral: Adults (1/2 glass x 3/day)	
R04Dn	Fresh plants of <i>Maesa lanceolata</i> (2 leaves), <i>Anisopappus chinensis</i> (4 plants), <i>Myrica kandtiana</i> (30 leaves) and <i>Capparis tomentosa</i> (10 cm of the root)	Decoction	Inhalation (once a week)	Dizziness
R05Gs	Equal amount of fresh plants of <i>Zanthoxylum chalybeum</i> (leaves), <i>Ocimum gratissimum</i> (leaves), <i>Eucalyptus globulus</i> (leaves), <i>Momordica foetida</i> (aerial parts), <i>Markhamia lutea</i> (leaves), <i>Pinus patula</i> (leaves), mineral salt (a pinch)	Decoction	Oral: Adults (1/2 glass x 3/day); children (6 spoons x 3/day)	None

R06Gd	Equal amount of fresh plants of: <i>Passiflora edulis</i> (leaves), <i>Phyllanthus fraternus</i> (aerial parts), <i>Cajanus cajan</i> (leaves), <i>Ocimum gratissimum</i> (leaves)	Decoction	Oral: children (1 tea spoon x 3/d); adults (2 tea spoons x 3/d) for adults	None
R07Kn	<i>Eucalyptus globulus</i> (1 handful of leaves), <i>Rubus apetalus</i> (1 root of 20 cm), <i>Capparis tomentosa</i> (3 roots of 15 cm), <i>Mangifera indica</i> (1 handful of leaves), <i>Tabernaemontana stapfiana</i> (3 leaves), <i>Maesa lanceolata</i> (1 handful of leaves), <i>Oxalis corniculata</i> (1 handful of aerial parts), <i>Coffea</i> sp. (1 handful of leaves), Goat fat (1 spoon) and honey (3 spoons)	Decoction	Oral: Adults (1/2 glass x 3/day); Children (3 spoons x 3/day)	None
R08Mt	Equal amount of <i>Rubia cordifolia</i> (aerial parts) and <i>Hibiscus noldeae</i> (aerial parts)	Decoction/infusion	Oral; children (1/2 glass x 3/d); adults (1 glass x 3/d)	None
R09Mg	Burning 5 dried corncobs (<i>Zea mays</i>) and 5 roots of 40 cm of <i>Phytolacca dodecandra</i> . Add a handful of <i>Eleusine coracana</i> flour and a pinch of mineral salt and 1 egg	powder	Adults (lick 1 pinch/day)	None
R10Mk	Fresh plants of <i>Solanum dasycyllum</i> (8 leaves), <i>Ludwigia abyssinica</i> (10 aerial parts) and <i>Aloe</i> sp. (5 leaves)	Decoction	Oral: Adults (1 glass/day) children (3 spoons/day)	None
R11Mw	Fresh plants of <i>Hibiscus noldeae</i> (50 %), <i>Cajanus cajan</i> (25 %), <i>Eriosema montanum</i> (25 %)	Decoction		None
R12Ny	<i>Syzygium parvifolium</i> (1 tea spoon of stem bark powder), <i>Securidaca longipedunculata</i> (1 tea spoon of root powder), <i>Distephanus angulifolius</i> (1/4 tea spoon of leaves powder), <i>Apium graveolens</i> (1 handful of fresh aerial parts), <i>Citrus x limon</i> (3 fresh fruits), <i>Tropaeolum majus</i> (1 handful of fresh aerial parts), <i>Allium cepa</i> (1 tuber) and honey (1/4 kg)	Decoction/infusion	Oral and nasal (1 tea spoon x 3/day)	None

R13Nz	2 tablespoons of <i>Physalis peruviana</i> leaves powder, 1 table spoon of <i>Solanum terminale</i> and ½ teaspoon <i>Tephrosia vogelii</i> powder	Decoction	Oral; 1tsp for children and 2 tea spoon for adults	Abortifacient , dizziness or even death at high doses.
R14oe	<i>Solanum aculeastrum</i> (2 fruits), milk (1glass) and egg (1)	Maceration	Oral: Adults (1glass x 2/day)	None
R15Pi1	Fresh plants of <i>Lysimachia ruhmeriana</i> (2 handfuls of leaves), <i>Clematis hirsuta</i> (3 roots of 20 cm), and <i>Erythrina abyssinica</i> (10 flowers)	Decoction	Oral: Adults (1 spoon x 3/day)	Unkown
R16Pi2	Fresh plants of <i>Datura stramonium</i> (6 leaves), <i>Cynodon dactylon</i> (3 aerial parts), <i>Maesa lanceolata</i> (4 leaves) and honey (3 spoons)	Decoction	Oral: Adults (2spoons x 2/day)	Dizziness
R17Rt	Fresh plants of <i>Eucalyptus globulus</i> (2 handfuls of leaves), <i>Vernonia amygdalina</i> (2 table spoon of root powder), <i>Acacia mearnsii</i> (2 handfuls of leaves), <i>Pinus patula</i> (2 handfuls of leaves) and mineral salt (1 tea spoon)	Decoction	Oral: Adults (3 spoons x 3/day) Children (1 spoon x 3/day)	Unkown
R18Sh	Equal amount of fresh aerial parts of <i>Bidens pilosa</i> , <i>Abutilon mauritianum</i> and <i>Euphorbia heterophylla</i>	Decoction	Oral: Adults (4 spoons x 3/day); Children (1spoon x 3/day)	None
R19Sz	Fresh plant materials of <i>Ocimum gratissimum</i> (1 handful of leaves), <i>Hibiscus noldeae</i> (3 handfuls of aerial parts), <i>Eriosema montanum</i> (2 handfuls of roots), <i>Acacia mearnsii</i> (1 handful of stem bark), <i>Rubia cordifolia</i> (2 handfuls of aerial parts) <i>Oxygonum sinuatum</i> (2 handfuls of aerial parts), <i>Oxalis corniculata</i> (2 handfuls of aerial parts), <i>Cedrela odorata</i> (3 handfuls of fruits)	Decoction	Oral: Adults (1 tea spoon x 3/day)	None

3.2 Cell viability

In the present study, macrophages derived from the human leukemia monocytic cell line, THP-1, were used to investigate inflammatory response towards our plant extracts. The cell viability was studied for all tested samples and was determined from the ratio of viable cells over total cells. During our study, all viability below 80 % was considered as significant. Due to the toxic effect of DMSO to the cell viability (results not shown), the test concentration was limited to 200 µg/ml (corresponding to the 1 % DMSO) while aqueous extracts were tested up to 1000 µg/ml. Up to 200 µg/ml, both aqueous and organic extracts revealed a good tolerance by the THP-1 derived macrophages (viability > 90 %) except for the R03Cn organic extract whose cell viability dropped down to 75.1 % (supplementary Table S.2). In general, organic extracts were slightly more toxic than their aqueous counterparts at the same concentration. For example, the R03Cn organic extract was significantly toxic at 200 µg/ml (75.1 %) while its aqueous counterpart revealed no effect on the cell viability at higher doses (103.7 % at 1000 µg/ml). As shown in the Table S.2, among 19 aqueous extracts tested, 4 have shown a dose-dependent reduction of cell viability that become highly significant at 600 µg/ml. These are R07Kn, R13Nz and R04Dn aqueous extracts which reduced the cell viability to 61 %, 32.5 %, 0.2 % and 80 % respectively at 1000 µg/ml.

3.3 Genotoxicity study

For the genotoxicity evaluation, one extract (aqueous extract of R04Dn) was evaluated on *Xenopus laevis* embryos. This extract (R04Dn) (see the composition in the Table 2) was the most toxic on THP-1-derived macrophages. The results of the genotoxicity assay are shown in Fig. 2. As it can be seen on the figure, after 1 hour of treatment, all the embryos treated with more than 200 µg/ml stopped their development. After 5 hours of treatment, only the embryos treated with the extract at 50 and 100 µg/ml continued their development. However, at this time a gastrula retardation was observed at 100 µg/ml and only embryos treated with the extract at 50 µg/ml continued to develop up to the larval stage (stage 46).

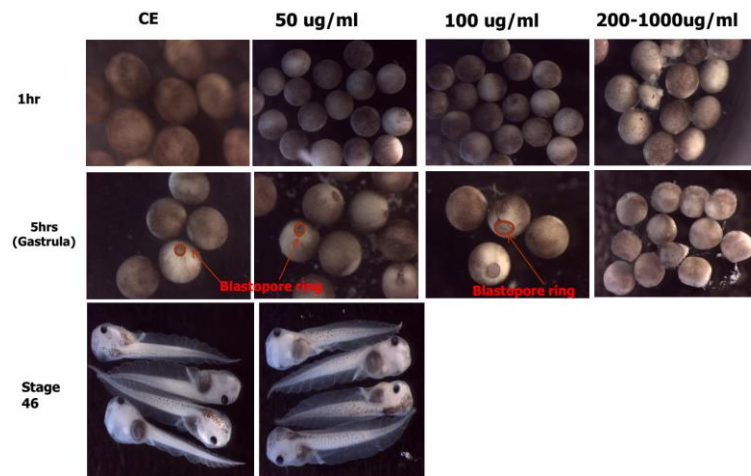


Fig. 2: R04Dn aqueous extract induces genotoxic effect. FETAX assay was performed as described in *Materials and Methods*. 50 *Xenopus* embryos were plated, at room temperature, in 10 ml of MBS solution with or without R04Dn aqueous extract at different test concentrations (50 to 1000 μ g/ml). After each treatment period, embryos were fixed in 4% (w/v) paraformaldehyde diluted in PBS (pH 7.0) and thereafter observed under a light microscope.

3.4 Inhibitory effects of the extracts on Caspase-1 activation

Table 3: Inhibition of caspase-1 activation in LPS- and ATP-activated THP-1 derived macrophages by recipes' aqueous and organic extracts.

Extract name	Caspase-1 inhibition (%)					
	50 μ g/ml		100 μ g/ml		200 μ g/ml	
	Org.	Aq.	Org.	Aq.	org.	Aq.
Control	100 $\pm$					
R01As	42.8 $\pm$ 0.8	22.9 $\pm$ 14.7	59.9 $\pm$ 7.0	2.6 $\pm$ 5.6	42.8 $\pm$ 7.5	41.7 $\pm$ 16.6
R02Cr	54.5 $\pm$ 4.1	44.1 $\pm$ 5.0	69.6 $\pm$ 1.0	31.4 $\pm$ 18.4	72.8 $\pm$ 1.2	63.6 $\pm$ 6.2
R03Cn	77.2 $\pm$ 2.7	80.3 $\pm$ 2.1	82.7 $\pm$ 2.7	81.0 $\pm$ 3.5	88.0 $\pm$ 1.8	85.4 $\pm$ 4.2
R04Dn	59.3 $\pm$ 3.2	40.4 $\pm$ 3.3	63.4 $\pm$ 13.1	36.0 $\pm$ 6.8	69.2 $\pm$ 16.8	11.5 $\pm$ 16.2
R05Gs	53.3 $\pm$ 10.9	51.9 $\pm$ 3.4	27.5 $\pm$ 4.6	63.8 $\pm$ 3.7	32.9 $\pm$ 8.3	47.8 $\pm$ 2.7
R06Gd	15.8 $\pm$ 5.9	45.0 $\pm$ 9.0	23.7 $\pm$ 7.1	53.1 $\pm$ 0.1	44.5 $\pm$ 6.2	83.5 $\pm$ 9.6
R07Kn	62.5 $\pm$ 0.2	83.1 $\pm$ 1.9	75.3 $\pm$ 4.2	75.1 $\pm$ 11.8	69.0 $\pm$ 4.6	85.4 $\pm$ 13.3
R08Mt	53.8 $\pm$ 2.2	39.1 $\pm$ 15.6	61.4 $\pm$ 1.9	38.4 $\pm$ 5.8	63.7 $\pm$ 15.6	52.9 $\pm$ 9.0
R09Mg	41.2 $\pm$ 3.7	24.7 $\pm$ 1.2	32.1 $\pm$ 5.8	23.2 $\pm$ 5.8	23.2 $\pm$ 5.0	15.6 $\pm$ 7.2
R10Mk	72.1 $\pm$ 14.9	54.2 $\pm$ 5.8	87.4 $\pm$ 3.8	59.5 $\pm$ 4.1	97.2 $\pm$ 3.0	86.1 $\pm$ 4.7
R11Mw	39.3 $\pm$ 1.3	50.4 $\pm$ 2.4	52.7 $\pm$ 1.8	51.6 $\pm$ 11.3	54.0 $\pm$ 8.9	50.2 $\pm$ 9.4
R12Ny*	-5.4 $\pm$ 7.4	-40.6 $\pm$ 6.5	-17.2 $\pm$ 5.7	-63.3 $\pm$ 3.5	-	-
						20.5 $\pm$ 11.9
R13Nz	19.9 $\pm$ 0.2	18.9 $\pm$ 2.2	71.2 $\pm$ 2.7	25.3 $\pm$ 12.5	79.4 $\pm$ 7.4	48.5 $\pm$ 2.7
R14Oe	-	32.5 $\pm$ 5.3	-	25.1 $\pm$ 5.4	-	31.8 $\pm$ 4.7
R15Pi1	24.9 $\pm$ 4.9	44.7 $\pm$ 8.2	46.1 $\pm$ 2.4	27.7 $\pm$ 3.4	39.4 $\pm$ 4.4	12.5 $\pm$ 8.4
R16Pi2	33.8 $\pm$ 4.3	-	49.8 $\pm$ 9.3	-	-9.7 $\pm$ 4.8	-
R17Rt	63.0 $\pm$ 5.7	29.6 $\pm$ 14.3	43.9 $\pm$ 4.4	62.5 $\pm$ 3.6	38.8 $\pm$ 1.6	73.7 $\pm$ 2.3
R18Sh	19.7 $\pm$ 2.4	-	37.6 $\pm$ 3.2	-	27.0 $\pm$ 14.5	-
R19Sz	93.1 $\pm$ 1.4	-	88.8 $\pm$ 1.7	-	102.6 $\pm$ 0.9	-

* The sign means that the extract induces activation of caspase-1

As shown in the Table 3, four out of 19 tested extracts showed significant caspase-1 inhibition activity in a dose-dependent manner. These are R03Cn and R07Kn aq. extracts and R10Mk and R19Sz organic extracts which, at 50 µg/ml, inhibited the caspase-1 activation at 80.3%, 83.1 %, 72.1% and 93.1 % respectively. R19Sz was far the best caspase-1 inhibitor (Fig. 3) and its composition as well as the previously reported ethnopharmacological use of its plant ingredients are given in the Table 4.

Table 4: Previously reported ethnopharmacological uses of the plants from the most active recipe, R19Sz

Plants	Previous ethnopharmacology reports
<i>Ocimum gratissimum</i>	The plant extracts and/or its main polyphenol compound (Rosmarinic acid) have been reported to have anti-asthmatic property (Costa et al., 2012; Takano et al., 2004), immunomodulatory activity (Kang et al., 2003); antioxidant (Akinmoladun et al., 2007), antinociceptive and anti-inflammatory (Rabelo et al., 2003; Tanko et al., 2008), etc.
<i>Hibiscus noldeae</i>	The plant has been reported to be traditionally used in the treatment of urogenital infections, postpartum abdominal pain, swelling of the legs cleaning of the baby, facilitation of delivery, sprains, headache, asthma, wounds, women infertility, abortion prevention (Chifundera, 2001; Telefo et al., 2011; Yemele et al., 2015).
<i>Oxygonum sinuatum</i>	Traditionally, the plant is reported to be used in the treatment of bilharzias, conjunctivitis tonsillitis, boils, fungal infections, eye infection (Matu and Van Staden, 2003) and has been used as a vegetable during famine by Zulu people (Akula and Odhav, 2008). It has been reported to have anti-inflammatory activity through inhibition of cyclooxygenase (Matu and Van Staden, 2003) and lipoxygenase activities (Akula and Odhav, 2008; Mzindle, 2017) immunomodulatory activity (Padayachee, 2012). Emodin has been isolated from the plant and reported to be responsible of its angiogenesis inhibitory activity (Crawford et al., 2011).
<i>Cedrela odorata</i>	The plant is reputed in the folk medicine for its use as an astringent, emetic, leukorrheal, diarrhea, vomiting, hemorrhage, indigestion, malaria and/or fever, anti-cancer, anti-ulcer, anti-inflammatory and anti-gastritis (Kipassa et al., 2008; Silva et al., 2006). The plant has been reported to have a high content of polyphenols and terpenoids (oleanolic and ursolic acids and luteolin) which are responsible of its antioxidant properties (Rashed, 2014). Different compounds have been isolated from the seeds such as mexicanolide (Ia) and 7-deacetyl-7-oxogedunin (II), etc. (Okorie and Taylor, 1968). The plant extract and its limonoidgedunin compound have been reported to have antimalarial activity (MacKinnon et al., 1997), hypoglycemic and genotoxic activities (Almonte-Flores et al., 2015)
<i>Rubia cordifolia</i>	<i>Rubia cordifolia</i> has been reported to possess antihyperglycemic, gastroprotective and antistress activities (Verma et al., 2016), wound healing properties (Devika, 2015). Some chemical molecules (Rubicordins A-C, Rubiadin, Alizarin, etc.) have been isolated from the plant and reported to possess cytotoxic, anti-inflammatory, antithrombotic and pro-angiogenic, hepatoprotective, antitumor, antimutagenic, immunomodulatory activities through inhibition of NF-kB (Chen et al., 2018; Gao et al., 2016; Verma et al., 2016).
<i>Acacia mearnsii</i>	The plant is traditionally used in the treatment of respiratory disease including tuberculosis, asthma, etc. (Tabuti et al., 2010). Its essential oils, where phytol and cis-verbeneol are major components, have been reported to have high anti-inflammatory activity in rats (Avoseh et al., 2015). The plant polyphenols (robinetinidol, fisetinidol, catechin and gallocatechin, etc.) and proanthocyanidins are used, in clinical, as anti-obesity, anti-diabetes (Ikarashi et al., 2018; Ogawa et al., 2013). Anti-inflammatory studies revealed that the extracts, mainly polyphenols (fisetinidol, catechin, etc.) of <i>Acacia mearnsii</i> inhibit inflammatory mediators (Xiong

<i>Uxalis orniculata</i>	et al., 2017). The plant is traditionally used in the treatment of yaws, tooth decay (Ngezahayo et al., 2015). Some studies have shown that the plant has wound healing, cardio-relaxant, anticancer, antimicrobial, anti-epileptic, gastroprotective, anti-amoebic, anti-ulcer, anti-nociceptive, anti-inflammatory, anxiolytic, nephrotoxicity, hepatoprotective, hypolipidemic, steroidogenic, antioxidant, anti-arthritis and hypoglycemic, etc. (Aruna et al., 2017; Dutta et al., 2016). Some chemicals known to have anti-inflammatory activity have been isolated from the plant including vitexin, luteolin, etc. (Borghi et al., 2013; Sharma and Kumari, 2014).
<i>Eriosema montanum</i>	The plant is traditionally used in the treatment of conjunctivitis, cough, snake bite (Cos et al., 2002b). Its defatted ethanol extract showed anti-HIV activity and anti-inflammatory activity through the inhibition of the complement system (Cos et al., 2002a, 2002b).

Unlike other recipes that repressed the activation of the caspase-1, both aqueous and organic extracts of R12Ny instead induced the overexpression of caspase-1, with much activity in the aqueous extract. The pharmacological activity seems to be dependent on the extraction solvent system used and aqueous extracts were more efficient than the organic (methanol-dichloromethane) extracts or vice-versa.

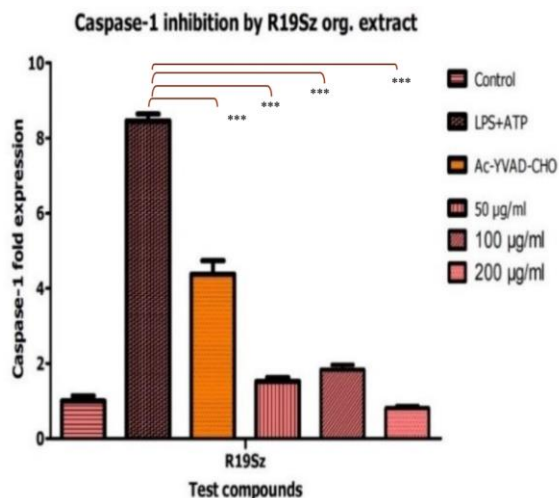


Fig. 3: Inhibition of caspase-1 activities by the most active recipe's extract (Organic R19Sz). The composition and previous ethno-pharmacological use of its plant ingredients are given in Table 4. THP-1 derived macrophages were pre-treated by R19Sz for 1h, primed by LPS (1µg/ml) for 3 h followed by induction by ATP for 30min. 50 µl of the culture supernatant were used to determine the caspase-1 activity using caspase-1 Glo inflammasome assay (Promega). Done in triplicate each, two independent experiments revealed similar strong inhibition of the caspase-1 activities. *** means that the caspase-1 activity inhibition is very significant (p < 0.0001)

4. Discussions

4.1 Social demographic characteristics of respondents

In this study, there was more male than female respondents. This is consistent with some other studies (Kayani et al., 2014; Mukazayire et al., 2011; Tugume et al., 2016). By considering education level and main sources of income, the findings reveal that interviewees have just only basic education (primary school) or illiterate, and mostly earning their income from subsistence farming or practicing traditional medicine. Some reasons are due to the fact that traditional medicine is often considered as not efficient, or lacking proper handling (preparation and processing of recipes, dosages, conservation, etc.) by educated people, who also have better livelihoods conditions. This also relates to the fact that non-educated and older people are more knowledgeable than schooled and younger people, and this constitutes a threat to the practice (Fatiha et al., 2017; Kamagaju et al., 2013; Mukazayire et al., 2011). The lack of interest by young generations is a threat to this traditional knowledge (Okello and Ssegawa, 2007).

4.2 Plant species used in traditional medicine to treat asthma

Regarding the plant species used to treat asthma, the high proportion of indigenous species (70.5 %) indicates that the native species constitute an important biological asset for the country, from which different benefits can be obtained. The fact that most of these species are collected from their natural habitat makes their conservation and more studies on their biological uses of paramount importance. The dominance of the species belonging to Asteraceae family in identified plant species has been observed in different studies, indicating the importance of this family in traditional medicine, especially in the treatment of respiratory diseases (Focho et al., 2009; Moshi et al., 2010; Sbai-Jouilil et al., 2017).

Furthermore, this study found that many different parts of the medicinal plant species are used as medicines. Commonly, leaves are used with high frequency and this finding is in line with most of other results of ethno-medicinal studies in Africa (Giday et al., 2003; Kamatenesi-Mugisha and Oryem-Origa, 2007; Togola et al., 2005). Regarding the mode of administration, the oral route is the most used as it is believed that the medicine taken orally will cleanse the body more thoroughly (De Wet et al., 2012). This confirms the results already reported by several previous studies (Kayani et al., 2014; Mukazayire et al., 2011; Ssegawa and Kasenene, 2007).

4.3 Cell viability

In order to exclude the possible interference of cytotoxicity on the expression of inflammasome/caspase-1 activity, the effects of the extracts on the viability of THP-1 derived macrophages were evaluated using the celltiter-Glo luminescent cell viability assay kit (Promega).

According to the results, organic extracts were slightly more toxic than aqueous extracts. This seems to be logical since, compared to water that is a polar solvent, the used organic solvent system dissolves a wide range of compounds, from which some can be less soluble in water and toxic, and this may be the probable reason of the noticed difference of effect on the cell viability. The most toxic recipes (R07Kn, R13Nz, R10Mk and R04Dn) have no common plant ingredients except for R07Kn and R04Dn that have *Capparis tomentosa* root as a common ingredient, suggesting that each recipe may contain at least a toxic plant. R13Nz and R10Mk contain *Tephrosia vogelii* and *Aloe species* respectively. These plants are well known to possess cytotoxicity, and Aloe emodin isolated from *Aloe vera* has been reported to be a potential anticancer candidate thanks to its cytotoxic properties against a wide range of cancer cell lines despite the lack of clinical studies (Huang et al., 2013; Sanders et al., 2017). During our ethnobotanical survey, the owner of the R04Dn recipe has said that the drug is taken by inhalation and once per week. Though the traditional healer says the drug has no toxic effect, he mentioned also that the patient may have dizziness and perspiration as side effects of the treatment when the drug is inhaled for too long. These side effects may be a result of toxicity revealed in this study.

4.4 R04Dn aqueous extract induces genotoxicity on *Xenopus* embryos

Though many people do turn to the medicinal plants for treatment, few of them are aware of their potential harmful side effects. In fact, people do think that natural means safe, but many medicinal plants have been reported to possess genotoxic properties and/or other adverse health effects (Rashed et al., 2015). In this study, it has also been demonstrated that the recipe R04Dn was genotoxic. The results obtained with FETAX showed no significant genotoxic effect at the concentration below 50 µg/ml. At higher concentrations, however, the obtained results indicate that there is a high risk of a possible abortive and/or teratogenic effect of this medicine. When comparing the cytotoxicity of the extract to its genotoxic effect, we noticed that *Xenopus* embryos are far more sensitive to the extract than are the THP-1 cells.

4.5 Inflammasome inhibition

Given the importance of the NLRP3 inflammasomes/caspase-1 in the respiratory diseases, especially in asthmatic patients, we established a screening assay specific to the activity of caspase-1 to discover extracts that target the NLRP3 inflammasomes/caspase-1 signaling. LPS and ATP treatment of THP-1 derived macrophages induces the activation of TLR4 and NLRP3 (Juliana et al., 2012). Consequently, LPS-primed and ATP-stimulated macrophages provide us with a proper test model for investigating anti-inflammatory properties of our extracts.

Previous studies have proved the capacity of natural compounds to inhibit inflammasome and/or its downstream cytokins, including plant extracts or purified compounds thereof, mainly polyphenols (Tózsér and Benkő, 2016). Some of these previously studied natural products or related compounds were mentioned in some of our recipes by traditional health practitioners (supplementary material table S.3). In fact, mangiferin and quercetine, two compounds isolated from *Mangifera indica* and *Phyllanthus fraternus* respectively, have shown inflammasome inhibitory activity (Cao et al., 2017; Domiciano et al., 2017; Makare et al., 2001). On one hand, these two plants were indicated in some of our recipes (R07Kn & R06Gd respectively) and the noticed activity of these recipes may probably be attributed to the same compounds. On the other hand, *Aloe vera* and its isolated compound aloe emodin as well as honey and/or its major components (ellagic acid, phenolic acids, flavonoids, etc.) have been reported to possess inhibitory activity on inflammasome (Budai et al., 2013; Kassim et al., 2010; Xiao et al., 2016). Since *Aloe sp.* is in the same genus as *Aloe vera*, there is a high probability that common compounds to these two species are responsible of the noticed activity from R10Mk. Aqueous extract of R07Kn showed better caspase-1 inhibitory activity than its organic counterpart, and the recipe contains an extract of *Mangifera indica*. Since mangiferin is known to be sparingly soluble in water while insoluble in a number of nonpolar solvents (Acosta et al., 2016), this may justify why aqueous extract of R07Kn was more efficient than the organic extract though we can't limit our interpretation on mangiferin since there can be other compounds involved in the activity. Very interestingly, during our study, some recipes showed stronger activity than recipes with the already studied ingredients. This means that plants in these recipes are strong candidates for the isolation of new active compounds involved in the inflammasome/caspase-1 regulation. This may probably be due to the fact that Ac-YVAD-CHO is a selective inhibitor of the caspase-1 while plant extracts act on several different targets (Pan et al., 2013). In fact, most diseases, especially genetic diseases, have a concerted action of multiple targets and/or genetic

abnormalities (Pan et al., 2013) and medicinal plants extracts, as well as drugs thereof, are known to hit multiple targets which results in better activities than single compounds (Pan et al., 2013). On the other hand, the aqueous and organic extracts of R12Ny induced the activation of caspase-1. This activity may come from lemon extract present in the recipe (Table 2) since citrus fruits are reported to contain plant lectins (Nachbar and Oppenheim, 1980). In fact, plant lectins have been reported to promote inflammatory disorders through the activation of the NLRP3 inflammasomes (Gong et al., 2017). Our further investigations aiming at isolating the potential molecules of the recipe responsible for the caspase-1 activation effects would allow to elucidate the case.

During our ethnobotanical survey, more than 90 % of the recipes were reported to be prepared using crushed fresh plant materials with an assumption that drying will diminish the treating efficacy of plants. However, for practical reasons, we used dried powders to prepare both aqueous and organic extracts. By using the organic solvent system, we had in mind that with crushed fresh plant material some water insoluble compounds (nonpolar) may diffuse in the recipe. Results from this study confirmed this traditional health practioners' assertion since for most of the recipes (55.6 %), organic extracts were better caspase-1 inhibitors than their aqueous counterparts.

Based on cytotoxicity s and results of the caspase-1 inhibitory activity, R19Sz recipe is the best candidate for further studies on individual plants that make up the recipe. As mentioned above, selection criteria to study hit molecules from medicinal plants is generally based on the citation frequency during an ethnopharmacological survey (Kamagaju et al., 2013) or by comparing results of ethnopharmacological survey to the related uses from the literature (Mukazayire et al., 2011). If this was the case, plants like *Ocimum gratissimum*, *Hibiscus noldeae*, *Maesa lanceolata* and *Eucalyptus maidenii* would be selected for further studies along with honey. Recipes containing these plants were not necessarily the most active (supplementary material Table S.3). Taking *Ocimum gratissimum* as an example, one can see that if it was the most active, R19Sz extract (94 %) would be far less active than R06Gd extract (45 %). In fact, in R19Sz, *Ocimum gratissimum* was 8-fold diluted (ocimum + 7 other plants) while it was 4-fold diluted in R06Gd (Supplementary Table S.3). Another example goes back to the R10Mk extract that showed the 4th better inhibitory activity while its plants (*Aloe sp.*, *Solanum dasyphyllum* and *Ludwigia*

abyssinica) were stated only once. To the best of our knowledge, the present study is the first to provide evidence that selection criteria should not be limited to the citation frequency during an ethnobotanical survey.

5. Conclusion

In the present study, we have been able to confirm the anti-inflammatory property of some of our traditional herbal recipes through the inhibition of inflammasome/caspase-1 activity. As our main goal was to evaluate the caspase-1 activity, our study was limited to the determination of THP-1 macrophages viability, as they are used for caspase-1 assay. THP-1 derived macrophages being less sensitive than monocytes, it is recommendable to further determine the safety of these recipes on normal monocytes. This study demonstrates the potential efficacy of some herbal extracts that are used in Rwanda in the treatment of asthma, especially in severe asthma. However, from the toxicological results, it should be noted that the consumption of herbal medicines is not without risk, and their prescription should therefore be done with caution by both the traditional health practitioner and the patient. Cytokine-targeting therapies are gaining an increasing role in the treatment of different inflammatory diseases including asthma (Dinarello, 2010; Wanderer, 2008). These preliminary results are very encouraging in this way since as stated above, the caspase-1 inhibition leads to the inhibition of the downstream cytokines cascade and proinflammatory cells recruitment. Plants in the most active recipes need to be further studied for the identification of active ingredient.

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Appendix A. Supplementary material

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Supplementary material

An ethnobotanical survey and NRLP3 inflammasomes/Caspase-1 inhibition of herbal recipes extracts traditionally used in Rwanda for asthma treatment

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Table S.1: Questionnaire for the survey on medicinal plants used for the treatment of asthma in the Southern Province of Rwanda

During our ethnopharmacological survey, we prefer to use an oral interview with the aid of a semi-structured questionnaire administered to traditional healers, herbalists, herb sellers and, if possible, to some treated patients because it allows the freedom to pursue many lines of questioning. All interviews will be recorded with participant permission

Section I: Information on the respondent

Sex: Male Female

Age:

Marital status: Single ☐ Married ☐ Widow ☐

Educational level: Naught ☐ Primary ☐ Secondary ☐ University ☐

Profession:

Location: District Sector Cell Geographical coordinates:

Practice duration (experience):

Knowledge heritage: From Parents ☐ From a friend ☐ From a third person ☐ other.....

Time of the interview:

Section II: Information on the plant material (from traditional healers)

Folk name:

Scientific name:

Type of the plant: Wild ☐ Cultivated ☐ Adventitious ☐

Plant availability:

Plant geographical coordinates:

Harvesting technique: Manual ☐ other

Harvesting condition (gathering season and/or hour):

Part used:

Quantities of plant used:

Usage: Fresh ☐ Dried ☐ After some treatment.....

If dried, method used

Recipe: Plant only ☐ Mixture with other plants and/or materials:

Method of preparation:

Dosage:

Mode of administration (oral, massage, etc):

Posology: For children Adults Old persons.....

Treatment duration (how many days):

Treated asthma: Very severe ☐ moderate asthma ☐ At early stage ☐

Conservation method: Under shadow ☐ Under ☐light other.....

Side effects:

Use caution (prohibitions during the treatment):

Toxicity (can this remedy be dangerous? Are there any counter-indications?): Yes ☐ No ☐

I don't know ☐ Other:

Treatment results: Healing ☐ Improvement ☐ Explanation:

Average number of treated persons:

Section III: Information on patients

Sex: Male ☐ Female ☐

Age:

How did you know you have asthma?

- By Medical consultation ☐ Self-medication ☐ other

Description of the ailment:

Did you go to hospital for modern treatment? Yes ☐ No ☐

- If Yes, what kind of treatment?:
- For how long?
- Treatment results: Healing ☐ Improvement ☐ other

Why did go for traditional treatment?

Asthma severity (Crisis frequency) before treatment:

Treatment duration:

Posology:

How long it takes to feel the improvement?

Asthma severity after treatment:

Comparison with modern treatment:

Side effects associated with the traditional treatment:

- During treatment:
- After treatment:

Table S.2: Effects of the recipes' aqueous and organic extracts on the cell viability (THP-1 derived macrophages).

Extract name	Cell viability (%)									
	50 µg/ml		100µg/ml		200µg/ml		600µg/ml		1000µg/ml	
	Aq.	Org.	Aq.	Org.	Aq.	Org.	Aq.	Org.	Aq.	Org.
Control	100±0.02	±100±0.05	100±0.02	±100±0.05	100±0.02	±100±0.05	100±0.02	±100±0.05	100±0.02	
R01As	99.8±0.02	99.2±0.26	99.9±0.02	99.2±0.07	99.5±0.05	98.5±0.56	97.2±0.39	-	95.2±0.19	-
R02Cr	100.0±0.1	100.2±0.02	99.9±0.03	100.1±0.03	99.9±0.02	98.7±1.00	99.4±0.06	-	98.7±0.1	-
R03Cn	101.9±1.41	99.4±0.04	100.4±0.73	98.8±0.1	104.5±1.86	75.1 ±0.4	104.0±0.24	-	104.0±0.95	-
R04Dn	99.4±0.01	100.2±0.02	99.1±0.05	100.2±0.01	96.5±0.27	98.0	50.4 ±2.29	-	0.2 ±0.1	-
R05Gs	99.9±0.01	100.1±0.02	99.8±0.05	100.2±0.06	99.8±0.02	100.2±0.05	99.7±0.04	-	99.8±0.07	-
R06Gd	99.8±0.07	100.1±0.03	99.8±0.01	100.1±0.02	99.4±0.06	100.1±0.3	97.0±0.12	-	88.8±0.46	-
R07Kn	98.1±0.85	99.4±0.04	101.5±2.43	98.4±0.21	101.6±1.31	98.4±0.03	86.8±0.57	-	61.0 ±1.2	-
R08Mt	103.8±2.31	99.6±0.04	105.2±0.61	99.6±0.04	103.3±0.78	99.6±0.28	99.1±3.94	-	99.9±0.6	-
R09Mg	99.7±0.02	99.7±0.01	99.7±0.01	99.4±0.12	99.7±0.02	98.9±0.01	99.6±0.03	-	99.8±0.05	-
R10Mk	99.9±0.06	100.2±0.06	99.9±0.08	99.8±0.22	99.1±0.1	99.4±0.08	-	-	80.5 ±0.02	-
R11Mw	99.7±1.12	99.4±0.14	98.0±3.15	98.4±0.28	102.2±2.41	93.6 ±0.43	101.0±2.43	-	96.3±1.56	-
R12Ny	99.9±0.05	99.6±0.06	99.8±0.07	99.6±0.03	99.8±0.02	99.6±0.04	99.7±0.06	-	99.3±0.06	-
R13Nz	99.0±2.76	100.1±0.06	98.6±0.22	100.0±0.07	96.4±1.02	99.2±0.17	78.6±6.39	-	32.5 ±9.1	-
R15Pi1	99.9±0.12	99.9±0.04	99.7±0.15	99.9±0.02	99.5±0.02	99.7±0.03	99.5±0.06	-	99.1±0.12	-
R16Pi2	-	99.8±0.04	-	99.7±0.03	-	99.7±0.06	-	-	-	-
R17Rt	98.9±2.19	99.7±0.01	99.9±1.63	99.7±0.07	98.8±0.8	99.7±0.00	95.3±2.08	-	92.9±0.25	-
R18Sh	-	100.2±0.13	-	100.3±0.04	-	99.9±0.01	-	-	-	-
R19Sz	-	100.1±0.01	-	100.2 ±0.01	-	99.8±0.11	-	-	-	-

Table S.3: Report on previous ethnopharmacological uses of the most cited plant species and caspase-1 inhibition activity by the corresponding recipes

Plants	Recipes	Caspase-1 inhibition (%)	Plants by recipe	Previous ethnopharmacology reports
Honey	R03Cn	77.2	8	Honey has been reported to possess antibacterial anti-inflammatory, antioxidant, antiviral properties (Bean, 2012; Owoyele et al., 2011; Yaghoobi et al., 2013). It has also been reported to be traditionally used for the treatment of skin burns, diabetic and gastric ulcers, respiratory diseases, eczema, dandruffs (Owoyele et al., 2011). Main anti-inflammatory components have been isolated including glycoproteins (MRJP-1 and MRJP-3), ellagic acid, kaempherol, quercetin, rutin, p-coumaric acid, etc (Bean, 2012; Pyrzynska and Biesaga, 2009; Xiao et al., 2016).
	R05Gs	53.3	7	
	R07Kn	83.1	9	
	R12Ny	-40.6	8	
	R16Pi2	33.8	4	
<i>Ocimum gratissimum</i>	R03Cn	77.2	8	The plant extracts and/or its main polyphenol compound (Rosmarinic acid) have been reported to have anti-asthmatic property (Costa et al., 2012; Takano et al., 2004), immunomodulatory activity (Kang et al., 2003); antioxidant (Akinmoladun et al., 2007), antinociceptive and anti-inflammatory (Rabelo et al., 2003; Tanko et al., 2008), etc.
	R05Gd	53.3	7	
	R06Gs	45.0	4	
	R19Sz	94.0	8	
<i>Hibiscus noldeae</i>	R08Mt	53.8	2	The plant has been reported to be traditionally used in the treatment of urogenital infections, postpartum abdominal pain, swelling of the legs cleaning of the baby, facilitation of delivery, sprains, headache, asthma, wounds, women infertility, abortion prevention (Chifundera, 2001; Telefo et al., 2011; Yemele et al., 2015).
	R11Mw	50.0	3	
	R19Sz	94.0	8	
<i>Maesa lanceolata</i>	R04Dn	59.0	4	The plant has been reported to possess antimicrobial, virucidal, molluscicidal, hemolytic, fungistatic and antimutagenic properties and maesanin was reported to possess strong anti-inflammatory activities (Sindambiwe et al., 1999; Taddese et al., 2003), immunomodulatory activity by stimulating phagocytosis. Its compounds (maesanin, dihyromasanin and maesanol) were reported to have antiasthmatic activity through inhibition of lipoxxygenase (Cos et al., 2002b; Schneider and Bucar, 2005). Maesasaponin has been reported to possess antimutagenic activity (Melek et al., 2015).
	R07Kn	83.1	9	
	R16Pi2	33.8	4	
<i>Eucalyptus globulus</i> subsp. <i>Maidenii</i>	R05Gs	53.3	7	Eucalyptus and/or its components (resveratrol, piceatannol, gallic acid, macrocapal G, etc) have shown anticancer, antimicrobial, anti-inflammatory activities (Tian et al., 2012).
	R07Kn	83.1	9	
	R17Rt	63.0	4	

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