Document heading   doi:

In vitro modulation of oxidative burst via release of reactive oxygen species from immune cells by extracts of selected tropical medicinal herbs and food plants

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ARTICLE INFO

ABSTRACT

Objective: To evaluate in vitro immunomodulating properties and potential cytotoxicity of six tropical medicinal herbs and food plants namely Antidesma madagascariense (Euphorbiaceae) (AM), Erythroxylum macrocarpum (Erythroxylaceae) (EM), Faujasiopsis flexuosa (Asteraceae) (FF), Pittosporum senacia (Pittosporaceae) (PS), Momordica charantia (Cucurbitaceae) (MC) and Ocimum tenuiflorum (Lamiaceae) (OT). **Methods:** Initially, the crude water and methanol extracts were probed for their capacity to trigger immune cells′ NADPH oxidase and MPO−dependent activities as measured by lucigenin− and luminol−amplified chemiluminescence, respectively; as compared to receptor−dependent (serum opsonised zymosan− OPZ) or receptor−independent phorbol myristerate acetate (PMA). **Results:** Preliminary screening on whole human blood oxidative burst activity showed significant and concentration−dependent immunomodulating properties of three plants AM, FF and OT. Further investigations of the fractions on isolated human polymorphonuclear cells (PMNs) and mice monocytes using two different pathways for activation of phagocytic oxidative burst showed that ethyl acetate fraction was the most potent extract. None of the active samples had cell−death effects on human PMNs, under the assay conditions as determined by the trypan−blue exclusion assay. Since PMA and OPZ NADPH oxidase complex is activated via different transduction pathways, these results suggest that AM, FF and OT does not affect a specific transductional pathway, but rather directly inhibit a final common biochemical target such as the NADPH oxidase enzyme and/or scavenges ROS. **Conclusions:** Our findings suggest that some of these plants extracts/fractions were able to modulate significantly immune response of phagocytes and monocytes at different steps, emphasizing their potential as a source of new natural alternative immunomodulatory agents.

1. Introduction

Herbal therapy is holistic approach. It is, in fact, able to look beyond the symptoms to the underlying systemic imbalance; when skillfully applied by the trained practitioner; herbal medicine can offer very real and permanent solutions to various health problems, many of which seemingly intractable to pharmaceutical intervention[1,2].

The use of and search for drugs and dietary supplements derived from food plants have accelerated in recent years whereby ethnopharmacologists, botanists, microbiologists, and natural−product chemists are combing the floral world for phytochemicals and "leads" which could be developed for the treatment of various ailments. The World Health Organization (WHO) has estimated that 80% of the population

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of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs[3]. For instance, the use of herbs and medicinal plant products has become a mainstream phenomenon over the past two decades in many countries where herbs and phytomedicines (herbal remedies) has the fastest growing segments in retail pharmacies and supermarkets[2,5–7].

Indeed, it is clear from available literature that modern pharmacopoeia still contains at least 25% drug derived from plants and many others, which are synthetic analogues of compounds isolated from plants[5,6]. Despite the availability of different approaches for the discovery of therapeuticals, natural plant products remain as one of the best reservoirs of new structural types. Concurrently, many people in developing countries such as China, Taiwan, India, Pakistan, Mauritius, as well as Latin America have begun to turn to alternative therapies as cheap sources of complex bioactive compounds and evidence of the beneficial therapeutic effects of these medicinal herbs is seen in their continued use. The medicinal importance of natural product molecules lies not only in their pharmacological or chemotherapeutic effects but also in their role as template for the production of new drug molecules. Knowledge gained from the use of medicinal and food plants and their active ingredients has served as the foundation for much of modern pharmacology and many modern drugs have their origin in ethnopharmacology. Additionally, the development of modern chemistry has permitted the isolation of chemicals from medicinal herbs which have served as drugs or starting materials for the synthesis of many commercially important drugs used today[7–11]. Drugs such as aspirin, digrtalin, morphine, metformin and quinine amongst others were all originally isolated or synthesised from materials derived from plants[8,9]. Medicinal plants, unlike pharmacological drugs, commonly have several chemicals working together catalytically and synergistically to produce a combined effect that surpasses the total activity of the individual constituents[10,11]. The combined action of these substances increases the activity of the main medicinal constituent by speeding up or slowing down its assimilation in the body. Secondary substances from plant origins might also increase the stability of the active compound(s) or phytochemicals, minimise the rate of undesired side effects, and have an additive, potentiating, or antagonistic effect[15,16].

In Mauritius, the local population has a long-standing tradition in the use of medicinal food plants. Many indigenous and endemic plant species of Mauritius have been used in folkloric medicine to treat various ailments of man. Available reports tend to highlight that indigenous folk-medicinal plant preservation and study is vital because such plants are fully adapted to local environments and conditions as compared to introduced species. Pharmacologically active compounds and phytochemicals, isolated from endemic and indigenous herbs and used in folk medicine in Mauritius, have been the active areas

of interest. Currently, several kinds of extracts from various exotic, endemic and indigenous plants are sold as decoctions or "tisanes" in several markets across Mauritius to treat minor ailments. This native herbal folk medicinal practice forms an essential part of the heritage of the local pharmacopoeia of Mauritius. Nonetheless, even with this vast array of data, only a few medicinal food plants of Mauritius have been scientifically evaluated for their possible medicinal application; also there has been no major attempt so far to evaluate the medicinal flora of the Island of Mauritius for its potential immunomodulating properties. Therefore, six traditional medicinal plants of Mauritius have been investigated for their possible immunomodulating and cytotoxic properties *in vitro*. These extracts were screened for the inhibition of oxidative burst using luminol/lucigenin– induced chemiluminescence technique. Comparison of opsonised zymosan (OPZ) and phorbol myristerate acetate (PMA) activators on immunomodulation properties of these plants extracts was also conducted.

2. Material and methods

2.1. Reagents, chemicals, and equipments

Luminol (3–aminophthalhydrazide) was purchased from Research Organics, while lucigenin (bis–N– methylacridinium nitrate) and Hanks Balance Salts Solution (HBSS) were purchased from Sigma, Germany. Lymphocytes Separation Medium (LSM) was purchased from MP Biomedicals, Inc., Germany. Zymosan–A (*Saccharomyces cerevisiae* origin) and phorbol 12–myristate 13–acetate (PMA) were purchased from Fluka (BioChemika). Dimethylsulfoxide (DMSO), ethanol and ammonium chloride of analytical grades were purchased from Merck Chemicals, Darmstadt, Germany. The Luminometer used was Luminoskan RS.

2.2. Preparation of extracts

Unripe fruits of *Momordica charantia* (Cucurbitaceae) (MC), used in the study, were obtained from a commercial source throughout the island. Leaves of the endemic plants, *Pittosporum senacia* (Pittosporaceae) (PS), and *Faujasiopsis flexuosa* (Asteraceae) (FF) were collected from Conservation Management Areas (Maccabe Forest), situated in the upper humid region of Petrin and Forest–Side conservation area, Mauritius. Exotic plant holy basil *Ocimum tenuiflorum* (Lamiaceae) (OT) was obtained from the University of Mauritius farm. The Curator of the National Herbarium, at the Mauritius Sugar Industry Research Institute (MSIRI) confirmed the identity of the plants where the voucher specimens were also deposited for future reference.

Freshly collected plant materials were either air dried or dried in a drying cabinet at 50 ℃ for 5 to 7 d. Total of 10 g of the dried plant materials (leaves and fruits where

appropriate) of the plant species were separately crushed and ground into fine powders by using a food blender. The outer rind of MC fruit was removed before drying. The solvent was distilled off under reduced pressure to afford crude plant extract. The paste was collected in water for examination. In addition, crude methanolic extracts were tested for comparative purposes. Methanolic extracts were obtained by triple soaking in 80% methanol at room temperature for 3 d. Crude methanolic and aqueous extracts were obtained by removing the solvent under reduced pressure. The extracts were concentrated *in vacuo* using a rotary evaporator (Model Buchi rotavapor R–114, Switzerland) that ensures evaporation of bulky solutions to small volume concentrates without bumping at temperatures between 70 ℃ to 100 ℃. The resultant concentrate was weighed and the gummy material collected in the appropriate solvent for examination. The paste–like suspension was diluted in DMSO for further experiments. Crude methanolic extracts were fractionated by solvent–solvent extraction procedure into dichloromethane, ethyl acetate, *n*–butanol and aqueous fractions for two successive 24 h periods, respectively[17]. In all, 6 different extracts/fractions were tested for activity *in vitro*.

2.3. Preparation of luminol, lucegenin and opsonized zymosan

Luminol was prepared as; 1.8 mg of luminol was dissolved in 1 mL of sodium borate buffer and vortexed for 5–10 min. Then it was further diluted up in 9 mL of Hank's balance salt solution [Ca^{2+} and Mg^{2+}] (HBSS^{++}) to give 180 μg luminol/mL. 25.5 mg of lucigenin was dissolve in 10 mL of distill water. Both were stored at −20 ℃ for later use. The opsonization of zymosan was carried out as 100 mg of zymosan, phosphate buffer saline (PBS) and 5 mL fresh pooled serum from healthy human male volunteers was incubated at 37 ℃ in shaking water bath for 30 min. The cells were washed twice with PBS and the pellets were finally resuspended in 5 mL of PBS and kept frozen at −20 ℃. The mixture was thawed, diluted and added to the cell suspension immediately before use[17–19].

2.4. Isolation of human polymorphoneutrophils (PMNs)

Heparinized venous blood (20 mL) was collected aseptically from healthy adult male volunteers (25–38 years age) at Dr. Panjwani Center for Molecular Medicine and Drug Research, Pakistan, and neutrophils were isolated by density gradient centrifugation[18]. Briefly 20 mL whole blood was gently layered onto 15 mL lymphocytes separation medium (LSM) and centrifuged at 400 g for 25 min at 24 ℃. Centrifugation sediment the erythrocytes and PMNs and band MNCs was above the LSM. The lymphocytes were aspirated by using a clean Pasteur pipette and centrifuge again with equal volume of LSM for 10 min at 300 g at 24 ℃. The cells were washed again and resuspended in Hank's balance salt solution [Ca^{2+} and Mg^{2+}–free] (HBSS$^-$) and stored in ice for later use. The neutrophils separated with the erythrocytes were then

collected and washed with equal volume of HBSS^{--}. Any contaminating erythrocytes were removed by hypotonic lysis unless otherwise stated. Following isolation, the cells were resuspended in HBSS^{--}. A cell count was performed on a haemocytometer and cell count was determined using the trypan blue exclusion method and adjusting the cell concentration to 1×10^6 cells/mL.

2.5. Isolation of macrophages

Resident macrophages in Balb–c mice (30–40 g) were obtained by intra–peritoneal lavage with 10 mL of sterile phosphate buffered saline (PBS). The peritoneal cells were collected by centrifugation (290 g, 4 ℃ for 5 min), washed and then resuspended in RPMI medium. Any erythrocyte in the cell pellets were lysed by hypotonic solution (0.2% NaCl). Isotonicity was restored with 1.6% NaCl solution. The cell number was determined by counting in a hemocytometer and cell viability was tested by the trypan–blue dye exclusion technique[17,18].

2.6. Chemiluminescence assay

Following isolation, cells were resuspended in Hank's balance salt solution [Ca^{2+} and Mg^{2+}] (HBSS^{++}) at a concentration of 1×10^6 cells/mL. Luminol or lucegenin enhanced chemiluminescence assay was performed as described previously modifications[17,18]. Briefly 25 μL whole blood (1:50 dilution in sterile PBS, pH 7.4) {Experiments with whole blood were performed immediately after blood drawing}, PMNs (1×10^6) or MNCs (1×10^6), suspended in HBSS^{++}. The mixtures were incubated with 25 μL serial dilution of respective plant extracts (6.25–100 μg/mL) at 37 ℃ for 30 min in the thermostated chamber of the luminometer. To each well 25 μL OPZ or PMA, followed by 25 μL (7×10^{-5} M) luminol or lucegenin. HBSS^{++} was added to a 96 well flat bottom plate to the final volume to 200 μL. HBSS^{++} alone was run as a control. The Luminometer results were monitored as chemiluminescence relative luminescence unit (RLU) with peak and total integral values set with repeated scans at 30 s intervals and 1 sec points measuring time[18].

2.7. Cell viability test

Cell viability was determined using the Trypan blue exclusion method. PMNs (1×10^6) were incubated for 60 and 120 min with 50 or 100 μg/mL of plant extracts each in triplicate at room temperature. As the blue dye uptake is an indication of cell death, the percentage viability was calculated from the total cell counts[18–20].

2.8. Statistical analysis

Students *t*–test was performed to compare the significance

mean differences between the control and tested extracts for various chemiluminescence results. Differences were considered to be significant at levels of $P<0.05$. Percentage of dead cells obtained in the cytotoxicity assay was determined using ANOVA[18–20].

3. Results

The preliminary screening results on human whole blood phagocytes showed that only three out of the six crude water and methanolic extracts were found to possess significant inhibitory activity at the initial tested doses (25, 50 and 100 μ g/mL). The results are shown in Table 1. These are namely leaves extracts of *Antidesma madagascariense* (AM), *Faujasiopsis flexuosa* (FF), and *Ocimum tenuiflorum* (OT) and the order of activity was AM > FF > OT. Moreover, it was observed that the crude methanolic extract displayed the best activity for all the concentrations tested, to this extract was selected for further experiments on other isolated immune cells (PMNs and MNCs) *via* different pathways of oxidative burst activation.

The crude methanolic extracts of AM, FF and OT were further investigated for their effects on OPZ activated human polymorphoneutrophils (PMNs). The results are presented in Figure 1. All the extracts showed dose–dependent significant inhibition of chemiluminescence. Again AM was found to be most potent in inhibiting the reactive oxygen species (ROS) production, followed by FF and OT (inhibitory activity in the order AM>FF>OT).
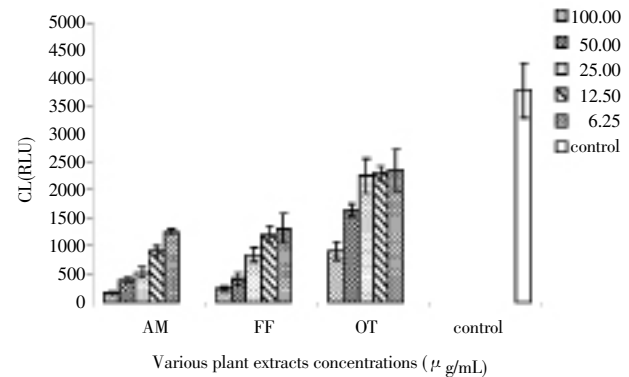

Figure 1. Inhibitory property of the methanolic extracts on serum opsonised zymosan activated PMN cells for ROS production (luminol based). Each bar represents triplicate reading ± S.D. *Values significantly higher ($P<0.05$) from the control. CL: Chemiluminescence, RLU: Relative Luminescence Unit. Note: For all botanical names refer to the key in Table 1.

Table 1
Effect of the crude methanolic and water extracts on whole blood phagocytes for ROS production.

Extract tested	Concentration (μ g/mL)	RLU		Inhibitory (%)[#]	
		CWE	CME	CWE	CME
AM[§]	100	62.23 ± 4.32*	50.36 ± 2.13*	92.9	94.2
	50	156.63 ± 13.36*	136.69 ± 12.98*	82.2	84.5
	25	225.46 ± 18.91*	214.14 ± 20.15*	74.4	75.7
FF[§]	100	106.34 ± 8.63*	99.63 ± 6.54*	87.9	88.7
	50	252.54 ± 10.32*	243.34 ± 11.54*	71.3	72.4
	25	335.67 ± 18.97*	346.09 ± 22.16*	61.9	60.7
EM	100	837.67 ± 24.54	867.36 ± 19.87	4.8	1.5
	50	675.89 ± 27.54	875.19 ± 17.52	23.2	0.5
	25	715.57 ± 27.14	798.82 ± 32.18	18.7	9.2
OT[§]	100	95.71 ± 7.14*	85.26 ± 18.32*	89.1	90.3
	50	175.69 ± 15.32*	159.37 ± 17.41*	80.0	81.9
	25	300.17 ± 14.65*	306.37 ± 22.67*	65.9	65.1
PS	100	605.52 ± 30.65	687.84 ± 24.65	31.2	21.8
	50	794.05 ± 28.65	737.19 ± 22.18	9.8	16.2
	25	801.69 ± 17.63	845.67 ± 13.21	8.9	3.9
MC	100	524.12 ± 28.15*	306.69 ± 27.32*	40.4	65.2
	50	767.09 ± 31.04	756.39 ± 19.63	12.8	14.1
	25	837.69 ± 48.32	806.37 ± 58.52	4.8	8.4
Control**		880.12 ± 83.61			

*Values significantly different ($P<0.05$) from the control. **Control is cells incubated with media without extracts. [#]Compared to control (without extracts added). RLU: Relative Luminescence Unit (mean value ± SD). CWE: Crude water extract, CME: Crude methanol extract. AM: *Antidesma madagascariense*, EM: *Erythroxylum macrocarpum*, FF: *Faujasiopsis flexuosa*, PS: *Pittosporum senacia*, MC: *Momordica charantia*, OT: *Ocimum tenuiflorum*. [§]Plants selected for further investigation due to their significant activity at all concentrations tested.

In another set of experiment luminol was substituted with lucigenin but activated by opsonised zymosan and the results are depicted in Figure 2. The most potent inhibitory activity was observed for leaves of OT followed by AM and FF (inhibitory activity in the order of OT>AM>FF). Interestingly, the highest inhibitory activity was observed for OT at all concentrations tested. At lower concentrations (12.5 and 6.25 μ g/mL) of the plant extracts, AM and FF showed no significant activity (P>0.05) as compared to the control experiments.
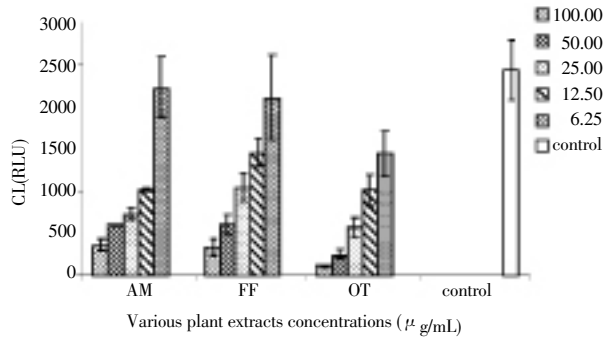

Figure 2. Inhibitory properties of the methanol extracts on serum opsonised zymosan activated PMN cells ROS production (lucigenin based).
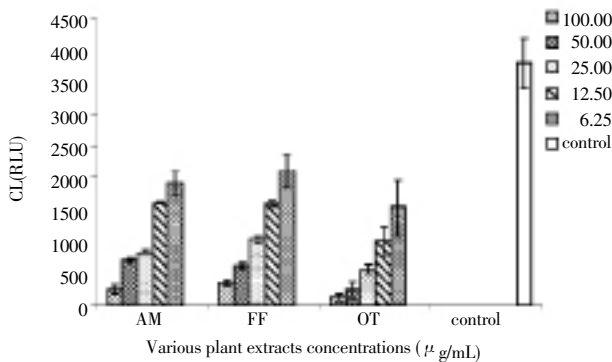

Figure 3. Inhibitory properties of the methanol extracts on phorbol myristerate acetate activated PMN cells (luminol based).
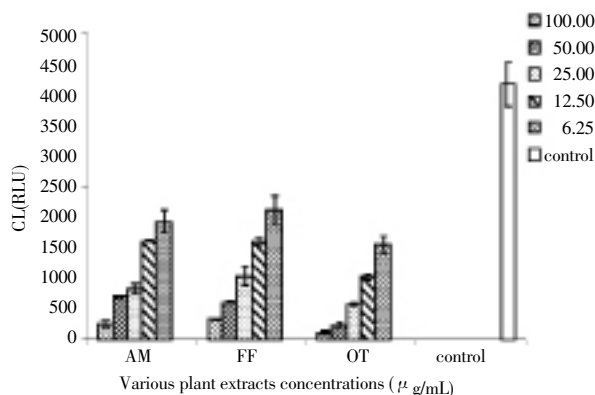

Figure 4. Inhibitory properties of the methanol extracts on serum opsonised zymosan activated macrophages (luminol based).
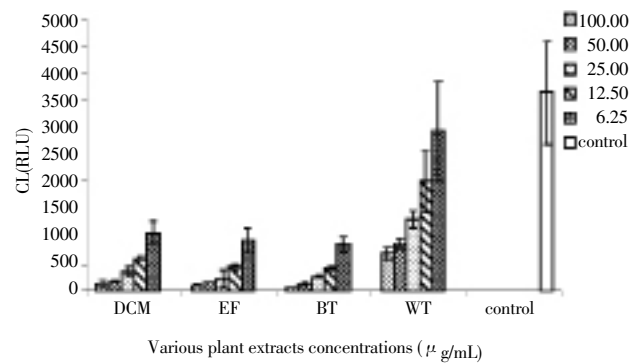

Figure 5. Inhibitory properties of *Antidesma madagascariense* fractions. Human polymorphoneutrophils were activated by serum opsonised zymosan (luminol based). CL: Chemiluminescence. RLU: Relative Luminescence Unit. DCM: Dichloromethane; ET: Ethylacetate; BT: *n*–butanol; WT: water fraction. *Each bar represents triplicate reading.
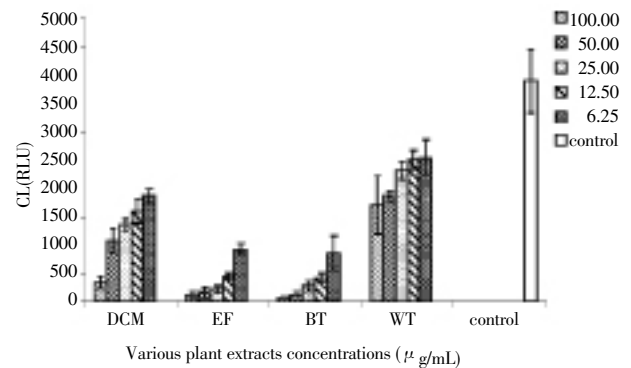

Figure 6. Inhibitory properties of *Faujasiopsis flexuosa* fractions. Human polymorphoneutrophils were activated by serum opsonised zymosan (luminol based).
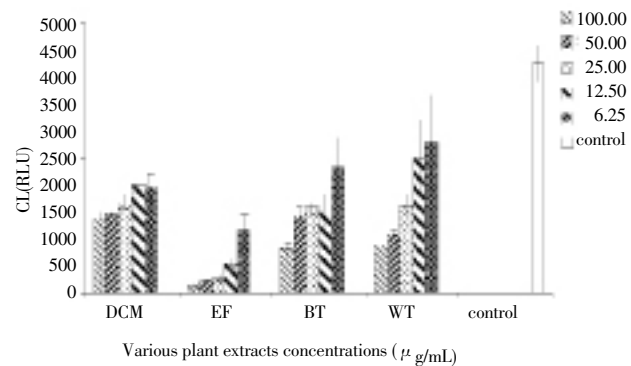

Figure 7. Inhibitory properties of Ocimum tenuiflorum fractions. Human polymorphoneutrophils were activated by serum opsonised zymosan (luminol based).

Figure 3 depicts results when the crude methanol extracts were incubated with 12–myristate 13–acetate phorbol (PMA), the protein kinase C activator and luminol as enhancer. The most potent inhibitory activity was observed for leaves of OT, followed by AM and FF.

In addition, all these three active extracts were investigated

for any possible effects on macrophages, isolated from mice (Figure 4) activated with serum opsonised zymosan and the luminol based chemiluminescence assay. All extracts showed concentration−dependent and significant inhibition (*P*<0.05) of free radical production. The order of activity was found to be OT > AM > FF.

In order to rule out the possibility of cell death which might account for false positive results, all the three active methanolic and crude water extracts were incubated for 2 h with isolated human polymorphoneutrophils. Cells were found viable (>90%) significantly after 1−2 h of incubation with all the potent extracts (AM, FF and OT) (Table 2).

The fractionated extracts of AM, FF and OT were tested in order to obtain a clear picture and to identify which fraction(s) were the most potent. The results are presented in Figures 5−7. Interestingly, the ethyl acetate fractions for all the three plants tested were the most active fraction sat all concentrations (6.25, 12.5, 25, 50 and 100 μ g/mL) tested. Comparatively, the water fraction of *Antidesma madagascariense* showed less inhibitory activity as compared to other fractions (Dichloromethane, Ethyl acetate, *n*−butanol) and no statistically significant inhibition was recorded at 6.25 μ g/mL of the water fraction as compared to the control experiments.

Table 2

Viability of human polymorphoneutrophils after 1−2 h incubation with crude water and methanol extracts.

Plants tested	Crude water extract[a]				Crude methanol extract[a]			
	After 60 min		After 120 min		After 60 min		After 120 min	
	50 μ g/mL	100 μ g/mL	50 μ g/mL	100 μ g/mL	50 μ g/mL	100 μ g/mL	50 μ g/mL	100 μ g/mL
AM	94.7 ± 2.52	98.3 ± 6.69	91.0 ± 1.20	95.7 ± 2.52	95.3 ± 0.58	96.0 ± 2.09	94.3 ± 2.08	96.0 ± 3.16
FF	98.3 ± 2.08	97.3 ± 0.58	95.3 ± 1.53	97.3 ± 0.58	97.3 ± 0.58	95.3 ± 2.08	92.0 ± 2.00	95.3 ± 2.08
OT	98.7 ± 1.15	97.7 ± 1.15	96.0 ± 1.00	98.3 ± 1.53	91.3 ± 2.31	94.7 ± 2.08	94.7 ± 0.58	94.7 ± 2.08
Control	99.0 ± 1.31	98.5 ± 0.71	99.0 ± 2.36	99.5 ± 3.71	99.0 ± 2.10	98.5 ± 1.71	99.0 ± 4.56	97.5 ± 3.71

[a]Results are expressed as means S.E.M of three observations in each group. *Values significantly different (*P*<0.05) from the control in each group. Note: For all botanical names refer to the key in Table 1.

4. Discussion

Traditional medicinal herbs and food plants of tropical countries remain one of the main sources of natural products for new therapies. In the present study, extracts/fractions of selected traditional medicinal herbs were tested for their capacity to trigger phagocyte myeloperoxidase (MPO)−dependent and MPO−independent activities as measured by lucigenin− and luminol−amplified chemiluminescence, respectively, as compared to receptor−dependent opsonised zymosan (OPZ) or receptor−independent phorbol myristerate acetate (PMA). In the present investigation, we have employed two chemiluminonogenic probes and stimulants since they have different luminescence mechanisms, and they have been widely used in differential measurements for reactive oxygen species (ROS)[18,20,21]. OPZ is a phagocytic particle that binds to receptors existing on the neutrophil cell surface, while PMA directly binds to protein kinase C (PKC) without functional modification at the receptor level[21].

It is an established fact that ROS generated by stimulated PMNs and monocytes, play an important role in host defense against invading microorganisms. Upon triggering, PMNs start to consume a large amount of oxygen, which is converted into ROS. This process is known as the respiratory or oxidative burst. Although ROS formation by neutrophils may be a physiological response which is advantageous to the host, the process is certainly also disadvantageous since it may lead to excessive tissue damage, and eventually

to inflammation, which has been implicated in many pathologies. Moreover, it has been observed that NADPH oxidase activity in PMNs is significantly higher in vascular tissue (saphenous vein and internal mammary artery) obtained from diabetic patients[22]. Therefore, medicinal food and herbs that can interfere with ROS production may be useful to prevent the tissue destruction and hence delaying the onset of many diseases. Possible beneficial effects of inhibitors of oxygen radical production in inflammatory processes have prompted investigators to search for lead compounds from plants, including culinary herbs and food plants. To this effect, in the present study, we have investigated the effects of six medicinal plant extracts for their abilities to block NADPH oxidase−dependent ROS generation by two different stimuli (OPZ and PMA) and measured as luminol− and lucigenin−enhanced chemiluminescence.

Preliminary screening of the of the six medicinal crude water and methanol extracts on whole blood cells oxidative burst showed that methanolic extracts of AM, FF and OT were the most active extracts. Further test on these crude methanol extracts on isolated PMNs with two different activators of oxidative burst; OPZ and PMA showed that they produced dose dependent significant inhibition of ROS generated. Two different stimuli were used in order to investigate the specificity of inhibition of these extracts as OPZ and PMA have been found to generate ROS *via* different pathways. The OPZ agent triggers both interferon

gamma (Fc γ R) and complement receptors (CR), activating PMNs. This leads to the generation of various ROS by these cells. The initial ROS produced by activated PMNs is superoxide anion ($O_2^{.-}$), generated by NADPH oxidase complex. Production of $O_2^{.-}$ has been specifically measured by lucigenin–enhanced chemiluminescence assay in this study. This anion is converted to H_2O_2 spontaneously or by the enzyme superoxide dismutase. Additionally, PMNs azurophilic granules contain large quantities of MPO, which catalyses the reaction of H_2O_2 with halide ions (Cl^-, Br^-, I^-) to produce halogenated species. These ROS are especially involved in the MPO, and thus both chemiluminonogenic probes have been used in this study to understand the biochemical pathways involved on ROS generation and the mechanism of action of potential fractions that inhibit this process. Interestingly, the methanolic extracts were found to attenuate lucigenin chemiluminescence, stimulated by OPZ, in a dose–dependent manner. OPZ activates membrane–binding phospholipase C (PLC) through G protein by binding to receptors expressed on the neutrophil surface. Activated PLC hydrolyses phosphatidylinositol 4,5–bisphosphate (PIP2) into inositol–1,4,5–triphosphate (IP3) and 1,2–diacylglycerol (DG). IP3 increases calcium concentration within the cell and DG activates PKC. These two pathways are responsible in the mediation of NADPH–oxidase activation. PMA on the other hand, directly binds to PKC and activates NADPH–oxidase [21,24].

Data obtained from the present study have showed that both pathways of activation of oxidative burst have been blocked by–since a significant inhibition was recorded for all the three plants tested (AM, FF and OT). In the present experimental settings for ROS detection, lucigenin must first undergo reduction, while luminol must undergo one electron oxidation to generate an unstable endoperoxide, the decomposition of which generates light by photon–emission. Luminol largely detects $HOCl^-$, which means that luminol detection is mainly dependent on the MPO/H_2O_2 system, while detection using lucigenin is MPO–independent and more specific for $O_2^{.-}$. Luminol ($C_8H_7N_3O_2$) is a versatile chemical that exhibits chemiluminescence and is able to enter the cell and thereby detects intra– as well as extracellularly produced ROS. Lucigenin ($C_{28}H_{22}N_4O_6$) on the other hand, is an aromatic compound and used in areas which include chemoluminescence that can react only with the extracellular ROS as it is practically incapable of passing the cell membrane and thereby only detects extracellular events[24]. Since PMA and OPZ activate NADPH oxidase *via* different transduction pathways, results from the present study suggest that AM, FF and OT does not affect a specific transductional pathway, but rather directly inhibits a final common biochemical target like the NADPH oxidase enzyme or scavenges ROS.

In stimulated PMNs, inhibition of chemiluminescence may be mediated by three main mechanisms: cell death, scavenging of ROS and inhibition of enzymes involved in the signal transduction pathways of the ROS generation process by these cells. The first hypothesis was initially ruled out because none of the tested samples had toxic and cell–death effects on human PMNs and monocytes, under the assay conditions as determined by the trypan–blue exclusion assay. Initially, we found that the methanol extracts were most potent and possessed significant chemiluminescence inhibition activities. It is to be noted that several reports suggest methanol as an extractive solvent tend to extract all the phytochemicals as opposed to water fraction and many of these phytochemicals isolated have been reported to block both pathways of activation of oxidative. However, throughout the study, it is observed that the different crude extracts, as well as the different fractions, exhibit different level of biological activities. Moreover, in the present study it was also important to test the water fractions, since it is the most widely used method of using these plants in many countries. Hence, the observed biological activities in the present study to some extent tend to validate the medicinal uses of these food plants. Interestingly, we have recently reported AM, OT and FF to possess important biologically active phytochemicals such as alkaloids, phenols, flavonoids and tannins which are well known for their antioxidant properties[25,26]. To this effect, it is most probable that phytochemicals in these medicinal plants might justify their observed immunomodulating properties and can be of therapeutic importance in the management of diseases.

For the first time tropical medicinal herbs and food plants of Mauritius have been studied for possible immunomodulatory properties *in vitro*. The results suggest that some of these plants were able to modulate significantly the immune response of phagocytes and monocytes at different steps, emphasizing their potential as a source of new immunomodulatory agents. However, it should be noted that a battery of *in vivo* tests as well as double–blinded randomized controlled clinical studies should be conducted on these plants to confirm whether the *in vitro* results reported here could be translated into *in vivo* activities that might support the traditional uses of these medicinal food and herbs in humans.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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