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The potential of traditional medicinal plants against targets associated with Covid-19

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ABSTRACT

Millions of people have been negatively affected by the Covid-19 (SARS-CoV-2) pandemic worldwide. Multiple stages characterize Covid-19 infection, initial stages include common symptoms that are milder in nature (fever, dry coughs, fatigue) with later stages being characterized by more severe symptoms such as pneumonia, acute respiratory stress disorder (ARDS), inflammation, coagulation, potentially multiple organ failure and death. This study aimed to evaluate the ethanolic extracts of twenty-three medicinal plants for their cytotoxic effects and their anticoagulant and anti-inflammatory activities. Furthermore, bioactive extracts were evaluated for potential interactions with Cytochrome P450 enzymes, which play a role in drug metabolism. Ten plants, namely, *Aspalathus linearis, Bulbine frutescens, Clausena anisata, Capparis tomentosa, Foeniculum vulgare, Myrsine africana, Podocarpus latifolius, Polygala virgata, Rhoicissus tridentata* and *Tylosema esculentum* exhibited no cytotoxicity on human lung fibroblast cells (MRC-5) and were subsequently tested for anticoagulant activity. Four plants, namely, *F. vulgare, M. africana, P. latifolius* and *R. tridentata* showed anticoagulant activity and were further tested for drug-herb interactions. Interactions with CYP 1A2, CYP 2C9, CYP 2C19, CYP 2D6 and CYP 3A4 showed that 10 μg/ml of *M. africana* and *R. tridentata* increased the activity of all enzymes. *F. vulgare* exhibited a significant increase in activity of all CYP P450 enzymes tested except for CYP 2C9, while *P. latifolius* showed significant inhibition of both CYP 2C9 and CYP 2C19 and significant increases in CYP 1A2, CYP 2D6 and CYP 3A4 activity. The given herb-drug interactions suggest caution and further investigation as both inhibition and induction of CYP P450 enzymes may lead to adverse effects in drug interactions through bioaccumulation of the drug to toxic amounts or increased clearance. During the evaluation of anti-inflammatory activity of *F. vulgare, M. africana, P. latifolius* and *R. tridentata* ethanolic extracts, it was found that *F. vulgare* inhibited the production of IL-8, IL-12p70 and IL-6 at 100 μg/ml. The extract of *M. africana* inhibited IL-12p70, IL-8, TNF-α and IL-6 cytokines at 100 μg/ml. Both *P. latifolius* and *R. tridentata* showed inhibition of IL-12p70, IL-6 and IL-8 at 100 μg/ml. It can be concluded that *F. vulgare, M. africana, P. latifolius* and *R. tridentata* have been identified as possible leads against the selected targets associated with Covid-19 infection and should be investigated further for pre-clinical studies.

1. Introduction

In December 2019, the world was first introduced to the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) which was reported to have originated from a seafood market in Wuhan, China (Yuki et al., 2020). This new virus rapidly spread worldwide having dire implications on daily life, as of July 2023 there have been over 700 million cases of Covid-19 infection reported accompanied by just under 7 million reported deaths (WHO, 2023). South Africa (SA), like many of the other countries was severely hampered by the global pandemic. The South Africa Coronavirus website created by the

Abbreviations: ARDS, acute respiratory distress syndrome; CBA, cytometric bead array; CYP P450, cytochrome P450; DMEM, Dulbecco's modified eagle medium; DMSO, dimethyl sulfoxide; EtOH, ethanol; FDA, food and drug administration; GGACK, glu-gly-arg-chloromethyl ketone; HeLa, human cervical cancer cells; HepG2, human hepatoma cells; IC_{50}, fifty percent inhibitory concentration; IL, interleukin; MG63, human osteosarcoma cells; MRC-5, human lung fibroblast cells; NaCl, sodium chloride; $NADP^+$, nicotinamide adenine dinucleotide phosphate; PBS, phosphate buffer saline; PEG, polyethylene glycol, PRU, herbarium accession number; LPS, lipopolysaccharide; SA, South Africa; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TH-1/2, T-helper cells; TNF-α, tumor necrosis factor alpha; U937, pro-monocytic, human histiocytic lymphoma cells; WHO, World Health Organization

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national government of South Africa reports over 4 million cases of Covid-19 infection of which there have been over 100 000 deaths (Coronavirus, 2023).

The most common clinical symptoms associated with Covid-19 infection are a dry cough and fever, although a wide range of additional symptoms can manifest including fatigue, headaches, nausea, vomiting and abdominal discomfort (Chen et al., 2020; Wang et al., 2020). Other less common symptoms that were reported include the loss of both taste (dysgeusia) and smell (anosmia) while more severe symptoms include progression to pneumonia, acute respiratory distress syndrome (ARDS) and potential organ failure (Cao, 2020; Gallo Marin et al., 2021).

Of additional concern is the potential development of what is known as 'long Covid' or the post-acute symptoms of Covid-19, that may be present as late as 4 weeks post infection and is said to present as persistent neurological, respiratory, or cardiovascular symptoms that could potentially last for months (Montani et al., 2022).

Due to the sudden and unexpected onset of the Covid-19 pandemic and the severity of infections, the focus of treatment was based on the initial repurposing of available treatments on the market (Stasi et al., 2020). The treatment of the initial stages of Covid-19 infection are generally supportive and symptomatic with the most common means of action being isolation or quarantine (Felsenstein et al., 2020).

From the research undertaken, which focused on the use of existing drugs, there were two main options that were considered as potential candidates. These two treatments act as both immune modulators and used primarily as an in-hospital treatment, namely corticosteroids and monoclonal antibodies (Baraniuk, 2021).

The preferred corticosteroid, dexamethasone, is readily available and after several studies, emerged as the most promising intervention in the treatment of severe symptoms of Covid-19 by means of reduction of inflammation associated with Covid-19 infection (Baruniuk, 2021). It has been estimated that 1 million lives have been spared due to the use of dexamethasone in conjunction with standard treatment methods (NHS England, 2021).

Monoclonal antibodies have shown success in helping the body's immune response to the virus. Tocilizumab is an example of a monoclonal antibody which is used to treat rheumatoid arthritis but also used as the treatment of Covid-19 in conjunction with dexamethasone or similar drugs for the treatment of infected patients (NHS England, 2021). The preliminary data from the Recovery study showed that Tocilizumab could save one life in every twenty-five patients that receive the drug, while Sarilumab, another monoclonal antibody was found to improve survival and reliance on organ support in the international Remap-Cap trial (Derde et al., 2021).

The most effective means of combatting Covid-19 infection has been shown to be through the development of vaccines. The Pfizer-BioNTech Covid-19 vaccine (Comirnaty®), an mRNA-based vaccine was the first to be approved for public use by the Food and Drug Administration (FDA), after which many more followed (Haghpanah et al., 2021). Currently the World Health Organization (WHO) lists 199 vaccines in pre-clinical development and 183 vaccines in clinical development (WHO, 2023).

Across the world there is a strong reliance on traditional medicine with as much as 80 % of the global population relying on herbal remedies as their primary source of healthcare (Hamilton, 2004). Medicinal plants are considered a global source of potential new drugs and there are many recorded cases of medicinal plants being used in the treatment of viral infections (Li et al., 2021; Zhou et al., 2016; Zhao et al., 2014; Rajbhandari et al., 2001; Abad et al., 2000).

Thus, the aim of the current study was to evaluate the potential activity of medicinal plants selected based on the previously mentioned traditional use and biological activity against viral infections and symptoms associated with Covid-19 such as coagulation and inflammation (Adhikari et al., 2021; Jalali et al., 2021).

2. Materials and methods

2.1. Materials and reagents

PrestoBlue® viability reagent was bought from Life Technologies (Johannesburg, South Africa). The MRC-5 cell line and fetal bovine serum (FBS) were purchased from Separations (Pty) Ltd. (Randburg, Johannesburg, RSA). Cell culture reagents, including Dulbecco's Modified Eagle Medium (DMEM), trypsin-EDTA (0.25 %), phosphate buffer saline (PBS), and antimicrobials, such as penicillin, streptomycin and amphotericin B, were supplied by ThermoFisher Scientific (Johannesburg, South Africa). Sterile cell culture flasks and multi-well plates were purchased from Lasec South Africa (Pty) Ltd (Midrand, South Africa). The Vivid® CYP450 Screening kits were purchased from Life Technologies (Carlsbad, CA, USA). The BD™ Cytometric Bead Array (CBA) Human Inflammatory Cytokine kit was purchased from BD Biosciences (San Diego, CA, USA). All other chemicals and reagents were of analytical grade and were acquired from Sigma Chemicals Co. (St. Louis, MO, USA).

2.2. Plant collection and extraction

The plant material was collected from the Manie van der Schijff Botanical Garden (25.75194°S 28.22889°E), or experimental farm (25.7494° S, 28.2544° E) of the University of Pretoria (Pretoria, South Africa) with herbarium voucher specimens deposited at the Schweickerdt Herbarium (PRU). A few of the plants used were purchased from Edakeni Muthi Futhi™ (both the PRU number and information on where plant material was sourced are depicted in Table 1). Briefly, the collected plant material was dried at room temperature (25 °C) and the leaves ground into a homogenous powder using an IKA grinder (IKA Labortechnik, Germany). The ground material was then extracted using ethanol at a ratio of approximately 1:4 wt (kg) by volume (L) by preparing a maceration which was placed on a shaker for 72 h (Labcon, Lab Design Engineering, Maraisburg, South Africa). The extracted plant material was then filtered through a Whatman No. 1 filter paper (Merck Chemicals (Pty) Ltd Wadeville, South Africa) using a vacuum filter (Merck Chemicals (Pty) Ltd Wadeville, South Africa). This process was repeated multiple times until the filtrate was clear. The solvent was then evaporated in a BÜCHI Rotary evaporator (Labotec (Pty) Ltd. Halfway House, South Africa) under reduced pressure at a temperature of 34 °C. The extracts were then dried completely at room temperature under a fume hood and thereafter stored at 4 °C until further use.

2.3. Cytotoxicity assay

The cytotoxicity assay was performed on 23 selected plants according to the method described by Lall et al. (2013) with slight modifications. Briefly, human lung fibroblast cells (MRC-5) were seeded in sterile 96-well microtiter plates at a working concentration of 1×10^5 cells/ml (10 000 cells/well) after which the plates were incubated at 37 °C at 5 % CO_2 for 24 h. A media control without cells served as the 0 % cell viability, whereas a media control with cells was used as the 100 % cell viability. Dimethyl sulfoxide (DMSO) at a concentration of 0.5 % was used as the vehicle control, DMSO as a toxic inducer (20 %–0,625 %) and Actinomycin D at a concentration ranging from 3.9×10^{-4} to 0.05 mg/ml The ethanolic plant extracts were dissolved in 100 % DMSO and added to the plate and serially diluted to a concentration range of 3.125- 400 μg/ml followed by the plates being incubated over a 72-hour period. Next, 20 μl of PrestoBlue reagent was added as a visual indicator of cell viability (blue to pink color change) and the plates further incubated for 2 h to allow

Table 1
The cytotoxic activity of Medicinal plants used in this study.

Plant species	Plant parts used	Accession number (PRU)	Cytotoxicity against MRC-5 Cells (μg/ml)
***Afrocarpus falcatus* (Thunb.) C.N.Page**	leaves	96,409	85.99 ± 0.89
***Artemisia afra* Jacq. ex Willd.**	aerial parts	[a]	72.65 ± 3.62
***Aspalathus linearis* (Burm.f.) R. Dahlgren.**	leaves	122,176	> 400
***Bulbine frutescens* (L.) Willd.**	leaves	122,179	> 400
***Capparis tomentosa* Lam.**	aerial parts	[a]	> 400
Clausena anisata (Willd.) Hook.f. ex Benth. var. anisate	leaves	96,689	338.40 ± 0.40
***Foeniculum vulgare* Mill.**	aerial parts	[a]	> 400
***Gunnera perpensa* L.**	leaves	120,010	100.20 ± 0.80
***Heteropyxis canescens* Oliv.**	leaves	128 232	110.95 ± 5.75
***Heteropyxis dehniae* Suess.**	leaves	128,230	102.36 ± 2.85
***Heteropyxis natalensis* Harvey**	leaves	128,231	100.52 ± 11.98
***Myrothamnus flabellifolius* Welw.**	aerial parts	[a]	231.43±15.01
***Myrsine africana* L.**	leaves	MA-S-2013-1	> 400
***Plectranthus ecklonii* Benth.**	leaves	122,337	258.05 ± 11.15
***Podocarpus henkelli* Stapf. ex Dallim. & Jacks.**	leaves	96,408	323.05 ± 37.05
***Podocarpus latifolius* (Thunb.) R.Br. ex Mirb.**	leaves	96,410	> 400
***Podocarpus madagascariensis* Baker.**	leaves	122,336	42.08 ± 0.88
***Polygala virgata* Thunb.**	aerial parts	[a]	> 400
***Rapanea melanophloeos* (L.) Mez.**	aerial parts	119,005	392.97 ± 24.12
***Rhoicissus tridentata* (L.f.) Willd. & R.B. Drumm.**	leaves	[a]	> 400
***Siphonchilus aethiopicus* (Schweif.) B.L. Burt**	aerial parts	[a]	> 400
***Tetradenia riparia* (Hochst.) Codd**	aerial parts	[a]	155.65 ± 29.35
***Tylosema esculentum* (Burch.) A. Schreib.**	leaves	13,879	> 400
Actinomycin D [b]	–	–	0.0046 ± 0.0017
DMSO [c]	–	–	1.14 ± 0.02[c]

[a] Plant material purchased from the Edakeni MuthiFuthi Trust in KwaZulu Natal, South Africa,.
[b] Positive control,.
[c] Toxic inducer.
* Value given as a percentage volume by volume (%v/v).

for the color change to occur. The fluorescence was recorded at an excitation wavelength of 560 nm and an emission wavelength of 590 nm using a VICTOR® Nivo™ Multimode Microplate Reader (PerkinElmer South Africa (Pty) Ltd.). The percentage cell viability was determined for each extract making use of the following formula.

$$\%\ cell\ viability = \left[\frac{(A_{treatment} - A_{blank})}{(A_{control} - A_{blank})}\right] \times 100\ \%$$

where (A = Fluorescence, A blank = fluorescence of media without cells control, A control = fluorescence of DMSO solvent control and A treatment = absorbance of treatment). Statistical analysis was performed using GraphPad Prism Version 4.0 (San Diego, California, USA).

2.4. Factor Xa inhibition assay

Plant samples that showed no cytotoxicity (> 400 μg/ml) were selected for anticoagulant testing, namely, *A. linearis, B. frutescens, C. anisata, C. tomentosa, F. vulgare, M. africana, P. latifolius, P. virgata, R. tridentata* and *T. esculentum*. To determine the anticoagulant effect of the samples a Factor Xa inhibition assay was conducted according to the method used by Chu et al. (2000). Briefly, 160 μl of Tris–HCl buffer solution (0.05 M Tris, 0.15 M NaCl and 0.1 % PEG-8000) at a pH of 7.4 was added to wells A1-C3 of a 96 well microtiter plate (Chu et al., 2000). Next, 10 μl of bovine FXa enzyme (0.425 nM) was added to the wells, followed by 10 μl of 100 % ethanol (EtOH). An additional 10 μl of 100 % EtOH was added to the final row of the plate as the 0 % activity (no substrate). The plates were incubated at 37 °C for 5 min before 20 μl of FXa chromogenic substrate (1.61 μM) was added to initiate the reaction. A kinetic read was performed using a BIO-TEK Power-Wave XS plate reader (Weltevreden Park, South Africa) at a wavelength of 405 nm.

The plant samples were tested at a concentration range of 1.6 μg/ml-1000 μg/ml. The extracts were dissolved in 100 % EtOH at 20 mg/ml before being further diluted at 20 times the desired concentration in the 96-well plate. The assay was repeated at the desired concentration range for each sample. For the positive drug and solvent controls, 10 μl of Glu-Gly-Arg-chloromethyl ketone (GGACK at 0.5 μg/ml) and 100 % EtOH were used respectively.

2.5. Cytochrome P450 inhibition assay

A Cytochrome P450 (CYP P450) inhibition assay was conducted to determine the inhibitory effect of the *F. vulgare, M. africana, P. latifolius* and *R. tridentata* plant extracts against various CYP P450 cytochromes namely, CYP 1A2, CYP 2C9, CYP 2C19, CYP 2D6 and CYP 3A4, which are necessary for the metabolism of medically important drugs. The assay was conducted by making use of the Vivid® CYP P450 screening kits (Life technologies, Johannesburg, SA). The assay was performed according to the manufacturer's specifications.

Briefly, CYP P450 BACULOSOMES Plus Reagent, Vivid Regeneration System and Vivid $NADP^+$were thawed and the Vivid substrate and fluorescent standards were reconstituted using acetonitrile and DMSO. A 1x Vivid CYP P450 buffer solution was prepared using distilled water (dH_2O). A standard curve was prepared at a concentration range of 7.8–500 nM through serial dilution and a negative control of 0 nM (1x reaction buffer only) was also included.

The test samples were prepared using 100 % DMSO at 2.5x the threshold testing concentration of 10 μg/ml (Picking et al., 2018). DMSO (0,0125 %) was used as a vehicle control. In a black-bottomed 96 well microtiter plate, 40 μl of the sample solutions were added to the wells with 50 μl Master Pre-mix and incubated at room temperature Following incubation, the CYP specific substrate (EOMCC for CYP1A2, CYP2C19, CYP2D6, BOMF for CYP2C9 and BOMCC for CYP3A4) and $NADP^+$ were added and the fluorescence was measured using a kinetic assay read making use of a VICTOR® Nivo™ Multimode Microplate Reader at an emission wavelength of 415 nm or 485 nm depending on which standard (blue or green) was used.

2.6. Human inflammatory cytokine assay

2.6.1. Cell supernatant collection

F. vulgare, M. africana, P. latifolius and *R. tridentata* were then used to stimulate human lung fibroblast (MRC-5) cells for supernatant to

be tested for anti-inflammatory activity. MRC-5 cells were plated in a 96 well microtiter plate at 1 × 10^5cells/ml (10 000 cells/well). The cells were incubated at 37 °C in 5 % CO_2 for 24 h. After incubation, *F. vulgare, M. africana, P. latifolius* and *R. tridentata* were reconstituted in 100 % DMSO and then added to the plate at 200 μg/ml and 100 μg/ml ($^1/_2$ and $^1/_4$ of the established non-cytotoxic threshold IC_{50} value of 400 μg/ml) then incubated for 18 h before adding 50 μl of lipopolysaccharide (LPS) at 10 ng/ml to all the wells. The plate was then incubated for a further 4 hours.

After incubation 150 μl of the supernatant was collected from the wells and stored in a freezer at −20 °C until required. The remaining 50 μl in the wells were used to measure the viability of the cells by adding 5 μl of PrestoBlue reagent and reading at an excitation of 560 nm and an emission wavelength of 590 nm using a VICTOR® Nivo™ Multimode Microplate Reader (PerkinElmer South Africa (Pty) Ltd.).

2.6.2. CBA human inflammatory cytokine assay

The concentration of cytokines (IL-8, IL-1β, IL-6, IL-10, TNF-α and IL-12p70) expressed under the influence of *F. vulgare, M. africana, P. latifolius* and *R. tridentata* were determined using an Accuri C6 BD flow cytometer (Becton Dickinson Holdings Pte Ltd, Singapore) making use of the BD Cytometric Bead Array (CBA) human inflammatory cytokines kit (BD Biosciences, San Diego, USA). The assay was performed according to the manufacturer's instructions.

Briefly, 50 μl of mixed capture bead solution were incubated with 50 μl of the collected cell supernatant or standards and the PE-labelled detection antibody. The mixture was incubated at room temperature and away from light for 3 h. The bead mixture was then washed using the provided wash buffer, the beads were then centrifuged for 5 min before the supernatant was aspirated and wash buffer added to the bead pellet for resuspension before being analyzed.

The data obtained following the flow cytometry analysis was analyzed using the FCAP array software V3.01 (BD biosciences, San Diego, USA). The concentration of the cytokines present was extrapolated from the standard curve generated by the kit standards. The results were presented as a percentage increase in expression and calculated using the following formula:

$$\%\ Increase = \left(\frac{Sample}{Treated}\right) \times 100$$

Where sample is the concentration of the selected extract (pg/ml) and treated represents the concentration of the treated control (MRC-5 cells and LPS).

2.7. Statistical analysis

All assays were performed in triplicate with three independent experiments (n = 3). The IC_{50} from the assays were calculated using non-linear regression analysis of the sigmoidal dose-response curves with constraints set at 100 (top) and 0 (bottom) using GraphPad Prism Version 4.0 software. One-way ANOVA using comparison to the positive drug (+) controls or respective DMSO solvent controls, followed by Dunnett's or Tukey's multiple comparison test (as indicated in the results section) using GraphPad Prism Version 4.0 software, with $^*p < 0.05$ and $^{**}p < 0.01$ indicating statistical significance, unless otherwise stated.

3. Results and discussion

3.1. Cytotoxicity assay

Twenty-three ethanolic plant extracts were selected to be screened for cytotoxicity on human lung fibroblast cells (MRC-5), 14 were shown to have little to no toxicity (Table 1). Of these fourteen samples, a further ten met the threshold levels for non-cytotoxicity (> 400 μg/ml), the threshold values were described according to Kuete and Efferth (2015) as follows: IC_{50}> 400 μg/ml (no cytotoxicity), 400 μg/ml > IC_{50} 200 μg/ml (mild cytotoxicity) and IC_{50}< 200 μg/ml (cytotoxicity).The non-toxic samples included *A. linearis, B. frutescens, C. anisata, C. tomentosa, F. vulgare, M. africana, P. latifolius, P. virgata, R. tridentata* and *T. esculentum*. These plant extracts were selected to undergo further evaluation for their potential activity as an anticoagulant using the Factor Xa inhibition assay.

In a study performed by Van Loggenberg et al. (2022), four aqueous extracts of *Artemisia afra* from different locations were tested for their cytotoxicity against MRC-5 cells. Three of the extracts were shown to be highly toxic with IC_{50} values of 16 μg/ml, 56 μg/ml, and 91 μg/ml, respectively, while one extract showed no toxicity with an IC_{50} of greater than 400 μg/ml, showing the importance of locality in the activity of plant secondary metabolites and those associated with their biological activity. The ethanolic extract of the aerial parts of *A. afra* exhibited an IC_{50} value of 72.65 ± 3.62 μg/ml in the present study which is comparable to the result seen in the one location by previous researchers using the same cell line (Van Loggenberg et al., 2022). The cytotoxic activity exhibited by *A. afra* could be attributed to the compound artemisinin, a compound found commonly throughout the genus which has been reported in literature to possess a variety of anti-cancer and cytotoxic activity (Adewumi et al., 2020).

No previous literature was found for the cytotoxicity of *Aspalathus linearis* against the MRC-5 cell line, however, studies have shown that two compounds isolated from *A. linearis,* namely, Phenylpyruvicacid-2-O-beta-d-glucoside and Aspalathin, were shown to have anti-apoptotic effects and that *A. linearis* protected lung fibroblasts exposed to toxins (Maarman and Lecour, 2022; Pringle et al., 2018). The results shown in the present study correlated with the available information as *A. linearis* showed no toxicity when tested against the MRC-5 cells with an IC_{50} above the highest tested concentration (> 400 μg/ml).

Bulbine frutescens has previously been shown to be cytotoxic to Chang liver cells with an IC_{50} value of 62.50 μg/ml (Van Huyssteen et al., 2011) however, this result differed drastically from that recorded in the current study against the MRC-5 cell line as *B. frutescens* showed no cytotoxicity (> 400 μg/ml). Isofuranonaphthoquinone, a compound that was derived from *B. frutescens* has been shown to have cytotoxicity against the cancerous Jurkat T cell line at 25 μg/ml suggesting potential activity, however given the lack of toxicity exhibited in the current study, against the MRC-5 cell line suggested the difference is potentially due to the differences between the cell lines (Tambama et al., 2014).

When previously tested for cytotoxicity against MRC-5 cells, *Capparis spinosa* a member of the *Capparis* genus showed no toxicity (IC_{50} of greater than 30 μg/ml) when tested for anti-plasmodial activity against *Plasmodium falciparum* (Abdel-Sattar et al., 2010). *Capparis tomentosa* tested in the current study showed no cytotoxicity with an IC_{50} of greater than 400 μg/ml suggesting intra-specific variation within the genus.

When tested against the adherent human dermal fibroblast (MRHF) cell line, essential oils of C. *anisata* were shown to have low cytotoxicity with an IC $_{50}$ at 383 μg/ml for the oil extracted by hydro distillation and no toxicity with an IC_{50} greater than 400 μg/ml for the oil extracted by solvent free microwave extraction (Lawal, 2020). Additional studies performed by Makirita et al. (2016), however, observed notable toxicity in *C. anisata* leaf chloroform (IC_{50} of 3.5761 μg/ml) and twig ethyl acetate (IC_{50} of 6.1276 μg/ml) extracts respectively (Makirita et al., 2016). The results observed in the present study are shown to be similar to that of the essential oil extracted by hydro distillation with the ethanolic extract of *C. anisata* resulting in an IC_{50} of 338.40 μg/ml.

Foeniculum vulgare extracts have been shown to exhibit no cytotoxicity when previously tested against the MRC-5 cell line (Pacifico et al., 2018). This corroborated the results in the current study as no cytotoxic activity was observed (IC_{50} > 400 μg/ml). The cytotoxic analysis of *G. perpensa* against human embryotic kidney cells (HEK293) and human hepatoma cells (HepG2) yielded values of 279.43 μg/ml and 222.33 μg/ml respectively (Maroyi, 2016). When tested against MRC-5 cells, *G. perpensa* showed moderate toxicity at an IC_{50} of 100.20 μg/ml, thus suggesting that *G. perpensa* possesses greater cytotoxic activity when exposed to lung fibroblast cells as compared to human kidney cell exposure.

Previous cytotoxic testing conducted on *H. natalensis* suggested that an increase in the concentration of the extract led to an increase in the toxicity observed in pro-monocytic human myeloid leukemia (U937) cells. When tested against various cell lines (U937 cells, Vero and Hep-G2 cells), *H. natalensis* showed an IC_{50} of 35.56 μg/ml, 147 μg/ml, and 33.66 μg/ml, respectively (Henley-Smith et al., 2018). These given results corresponded to those of *H. natalensis, H. dehniae* and *H. canescens* which appear to have similar phytochemical constituents as their IC_{50} values are similar (100.52 μg/ml, 102.36 μg/ml, and 110.96 μg/ml).

Myrothamnus flabellifolius tea extract was observed to have proliferative effect when tested on Chan liver cells at the highest testing concentration of 500 μg/ml (Chukwuma et al., 2019). These results differ to those obtained in the present study as the ethanolic extract of *M. flabellifolius* exhibited some cytotoxicity with an IC_{50} of 231.43 μg/ml. *Maesa balansae* Mez. a member of Myrsincaceae family showed high levels of toxicity when tested against MRC-5 cells with a reported IC_{50} of > 32 μg/ml (Foubert et al., 2008). *Myrsine africana,* another member within the Myrsincaceae family, however, exhibited no cytotoxicity when tested against MRC-5 cells in the current study.

Members of the Podocarpaceae family have exhibited cytotoxic activity against a large variety of human cell lines including human cervical cancer cells (HeLa) and human osteosarcoma cells (MG63) at concentrations of over 30 μmol/L (Abdillahi et al., 2010). When tested against MRC-5 cells, *P. ecklonii* (258.05 μg/ml), *P. henkelii* (323.05 μg/ml) and *P. latifolius* (> 400 μg/ml) all showed mild to no cytotoxic activity. *Afrocarpus falcatus* (IC_{50} of 85.99 μg/ml), formerly *Podocarpus falcatus,* and *P. madagascariensis* (IC_{50} of 42.08 μg/ml) however, were shown to be toxic in the current study.

In a study performed by Çaliş et al. (2023), xanthones isolated from two members of the Polygala genus, namely, *Polygala azizsancarii* and *P. peshmenii* were tested for cytotoxicity against human neuroblastoma cells (SKNAS), both xanthones showed no cytotoxic activity at the highest tested concentration of 150 μg/ml (Çaliş et al., 2023). The cytotoxic activity of *P. virgata* in this present study is shown to be similar to related species in the genus as the ethanolic extract of *P. virgata* exhibited no cytotoxicity (> 400 μg/ml). In a study performed by Gibango et al. (2020) the leaf ethanolic extract of *R. melanophloeos* was shown to have low cytotoxicity when tested against HepG2 liver cells with a reported IC_{50} of 319.5 μg/ml (Gibango et al., 2020). The results in the present study are similar to the previous studies with the ethanolic extract of *R. melanophloeos* exhibiting an IC_{50} of 392.97 μg/ml suggesting minimal cytotoxicity.

When tested against Vero cells *Rhoicissus tridentata* subsp. *Cuneifolia* was observed to be toxic with an IC_{50} of 88.5 μg/ml (Tshikilange et al., 2016). Additionally, a study by Opoku et al. (2000) found that 25 μg of the aqueous extract *R. tridentata* subsp. *cuneifolia* showed the greatest cytotoxic activity with 96.27 % inhibition while the methanolic extract showed 87.01 % inhibition. However, less cytotoxic activity was observed in the methanolic extract of *R. tridentata* at 55.21 % inhibition (Opoku et al., 2000). The present study showed no toxicity (> 400 μg/ml) by the ethanolic extract of *R. tridentata* when tested against MRC-5 cells. The results of the current study differ from those previous, however these differences may be attributed to the differences in cells tested and the solvent used in extract preparation.

Ethanolic extracts of S. *aethiopicus* have previously been shown to have cytotoxic activity against HepG2 liver cells with an IC_{50} of 113.5 μg/ml and no toxicity against Caco-2 (human epithelial colorectal adenocarcinoma cells) with an IC_{50} of 876 μg/ml (Erasmus et al., 2019). When tested in the current study the ethanolic extract of *S. aethiopicus* displayed no cytotoxicity (> 400 μg/ml).

A study performed by Chepng'etich et al. (2018) determined that a methanolic and dichloromethane extract of T. *riparia* showed promising antiproliferative activity in various cancer cell lines, DU145- Prostrate cancer cell lines (IC_{50} of 10.83 μg/ml), HCC- Breast cancer cell lines (IC_{50} of 53.62 μg/ml), and Hela-Cervical cancer cells (IC_{50} of 10.83 μg/ml). When tested again the non-cancerous Vero cell line T. riparia exhibited cytotoxicity with an IC_{50} of 85.43 μg/ml (Chepng'etich et al., 2018). The previously reported results support the results found in the present study as *T. riparia* was observed to be cytotoxic with an IC_{50} of 155.65 μg/ml. The methanolic and ethyl acetate extracts of the husk of *T. esculentum* were previously reported to show no cytotoxicity (> 400 μg/ml) when tested against Caco-2, HeLa and Vero cell lines (Mazimba et al., 2011). These results support those of the present study with the ethanolic extract of *T. esculentum* showing no cytotoxicity against the MRC-5 cell line.

3.2. Factor Xa inhibition assay

An initial broad concentration range of 1.6 μg/ml–1000 μg/ml was used to test the 10 selected extracts in order to obtain the best sigmoidal dose response curves (sufficient data points above and below the IC_{50}). Of the 10 samples that were selected for subsequent FXa inhibition, four samples, namely, *F. vulgare, M. africana, P. latifolius* and *R. tridentata* exhibited anticoagulant activity.

Previous studies of Factor Xa inhibition by Pawlaczyk et al. (2011) have shown that for herbal extracts an IC50 of 500 μg/ml were considered to be slightly inhibitory. As such, it can be determined that slight to moderate levels of FXa inhibition are observed by the samples selected in this study.

A one-way ANOVA analysis followed by Tukey's multiple comparison test was performed to determine the significance observed for *M. africana, P. latifolius* and *R. tridentata* and the positive control (GGACK) to one another other. When compared to each other *P. latifolius* and *R. tridentata* did not exhibit any statistically significant difference, however both showed significantly greater activity when compared to *M. africana*, which showed the greatest statistically significant difference in activity to GGACK.

Myrsine africana, P. latifolius and *R. tridentata* all showed activity within the initial range of 12.5 μg/ml-1000 μg/ml with *M. africana* (469.45 ± 43.55 μg/ml), *P. latifolius* (193.45 ± 10.35 μg/ml) and *R. tridentata* (139.10 ± 4.70 μg/ml) all showing IC_{50} values below the 500 μg/ml threshold (Fig. 1), suggesting that they exhibit anticoagulant activity. *Foeniculum vulgare* showed to have an IC_{50} above 1000 μg/ml.

Lee (2015) showed that the essential oils extracted from the fruit of *F. vulgare* strongly inhibited collagen induced human platelet aggregation, an alternative technique used in the determination of anticoagulation activity. Anethole, a compound isolated from *F. vulgare* has been shown to exhibit anticoagulant activity via the inhibition of platelet aggregation and clot retraction (Tognolini et al., 2007). However, this observed anti-coagulant activity reported could be due to the inhibition of the arachidonate cascade as opposed to FXa inhibition directly.

There are several examples of anticoagulant activity found for species within the Myrsinaceae family (Biswas et al., 2019). *Myrsine africana* has traditionally been used as a 'blood purifier' suggesting that this plant could have blood thinning activity, however, despite its noted potential for anticoagulant activity, *M. africana* has not been

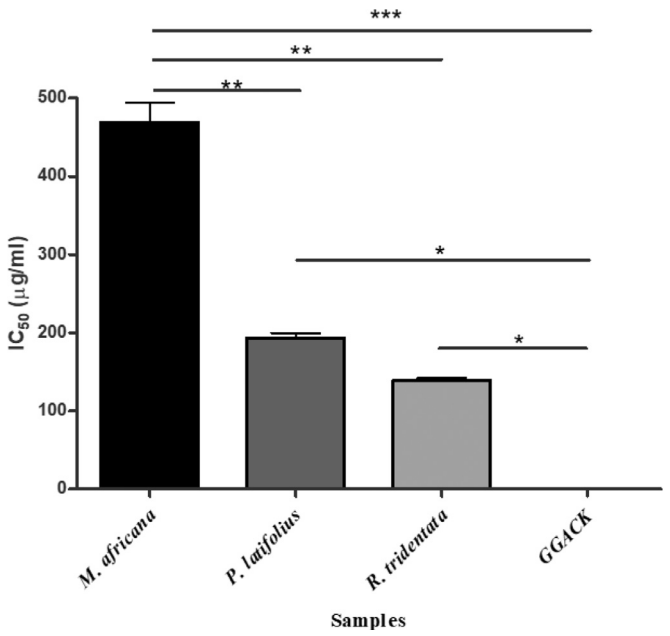

Fig. 1. The Anticoagulatory activity of ethanolic extracts of selected medicinal plants. *** ($p < 0.001$), **($p < 001$), *($p < 0.1$) represent a statistically significant difference when compared to the positive control (GGACK).

previously studied for its anticoagulant effect (Azam et al., 2011; Laraib et al., 2021).

Podocarpus flavone compounds isolated from the *Podocarpus* genus have been shown in previous molecular docking studies to have thrombolytic activity (Al Noman et al., 2015). Phenolic compounds such as resveratrol that are found within the grape family, Vitaceae, are known to have anticoagulant activity (Olas, 2022), however, the *Rhoicissus* genus and *R. tridentata* specifically, have not been studied for its anticoagulant activity.

3.3. Cytochrome P450 inhibition assay

The extracts of *F. vulgare, M. africana, P. latifolius* and *R. tridentata* were tested at a concentration of 10 μg/ml which was determined to be the maximum threshold at which activity should be observed (Picking et al., 2018). The CYP P450 inhibition assay was conducted using the Vivid™ CYP 1A2 blue screening kit and an increase in activity of all the samples compared to the 100 % activity (enzyme) control was observed (Fig. 2). All selected extracts (*F. vulgare, M. africana, P. latifolius* and *R. tridentata*) presented with a 50 % or greater increase in CYP 1A2 activity. An ANOVA analysis was performed followed by Dunnet's test comparing the significance of the results to the 100 % activity (enzyme) control. All samples recorded a significant difference ($p < 0.01$) in the percentage activity of CYP 1A2 when compared to the 100 % control.

Drug-herb interactions in particular are known to be a complex mixture of chemical constituents and metabolites that may vary in both content and concentration depending on the preparation of the herb (Hakkola et al., 2020).

Cytochrome P450 1A2 (CYP 1A2) along with CYP 2D6 and CYP 3A4 are shown to be responsible for 65–80 % of all CYP- mediated drug metabolism (Ingelman-Sundberg, 2004; Picking et al., 2018). In previous studies CYP 1A2 interaction with the ethanolic extract of *F. vulgare,* an herb commonly used by pregnant women (Langhammer and Nilsen, 2014), it was observed that fennel showed inhibition of CYP 1A2 at lower concentrations (IC_{50} = 115 ± 11 μg/ml) suggesting that it decreases the efficacy of CYP 1A2 in metabolizing its targeted compounds, potentially lowering the bioavailability of important drugs. Taherkani et al. (2020) showed that various essential oils (thymol, frenchone, trans-athenole and methyl-chavicol) isolated from *F. vulgare* were shown to be inhibitors of CYP 1A2.

When tested using the Vivid® CYP P450 screening kit, however, *F. vulgare* showed a noticeable increase in enzyme activity when compared to the 100 % control. The increased activity observed by CYP 1A2 highlights a potential risk in co-administration with other drugs as induction of CYP P450 enzymes have been shown cause an accelerated or increased clearance of the co-administered drug resulting in a decreased therapeutic effect (Amaeze et al., 2021).

As with the Factor Xa inhibition assay, no previous CYP P450 inhibition studies have been conducted on *M. africana.* Other species found within the Myrsinaceae family, however, have undergone CYP P450 inhibition testing with methanolic extracts of *Labisia pumila* (Kacip Fatimah), a popular Malaysian herb, showing no inhibition when tested against CYP 1A2 within a concentration range of 0.4 μg/ml-100 μg/ml although further in vivo prediction models indicate likely interaction with CYP 1A2, suggesting it may lead to an increase in the enzyme's metabolic activity (Manda et al., 2014). These results are consistent with what was found with *M. africana* in the current study as it was observed to increase CYP 1A2 enzyme activity.

Gunez and Dahl (2008) noted that there is an observed variation in CYP 1A2 activity amongst individuals and that the increased activity may however, be beneficial in some instances and harmful in others due to such allelic variation within the population, suggesting that further testing and analysis on the mechanism of action of CYP 1A2 may be required to determine the overall effect of the samples on activity (Gunez and Dahl, 2008).

When tested against the CYP 2C9 enzyme, *F. vulgare* and *R. tridentata* both reported an increase in the percentage activity of the CYP 2C9 (3 % and 11 %), however this increase was not statistically significant suggesting no affect in CYP 2C9 activity by the samples. *Myrsine africana* increased CYP 2C9 activity significantly (44 %) while inhibition of CYP 2C9 enzyme activity was observed for *P. latifolius* (31 % decrease) when compared to the 100 % activity control. Inhibition of the CYP enzymes resulted in the decrease of hepatic and intestinal enzyme activity leading to a build-up in drug accumulation and further toxicity (Amaeze et al., 2021).

Previous studies have shown that various essential oils extracted from *F. vulgare* exhibited no inhibition of the CYP 2C9 enzyme, supporting the results observed in this study, (Taherkhani et al., 2020). Compounds isolated from the genus *Podocarpus* have been tested for their CYP P450 inhibitory activity, with one of the compounds (4β-carboxy-19-nor-totarol) isolated, shown to inhibit CYP 2C9 activity (Deresa et al., 2022). Therefore, it is likely that large concentrations of that compound could be present in the ethanolic extract of *P. latifolius,* resulting in the levels of inhibition of CYP 2C9 observed in the current study.

The methanolic extracts of *Labisia pumila,* a member of the Myrsinaceae family to which *M. africana* belongs, a dose dependent inhibition of CYP 2C9 was shown, however, in a study conducted by Manda et al. (2014) it was determined that there was no time-dependent inhibition observed with recombinant CYP 2C9 enzymes. In the current study, there was a noticeable increase in the CYP 2C9 enzyme activity by the ethanolic extract of *M. africana* suggesting a potential negative impact on possible co-administered drugs. There have been no previous studies investigating the effect of *R. tridentata* on CYP 2C9 activity. In the current study an increase in the activity of CYP 2C9 was observed though it was statistically insignificant, again suggesting that it will have no effect on the activity of CYP 2C9 and its ability to metabolize drugs.

CYP 2C9 is largely responsible for the metabolism of oral hypoglycemic agents. It has been reported by Holstein et al. (2005) that allelic variation presented in CYP 2C9 enzymes within individuals may potentially cause a decrease in the enzyme activity (Holstein et al., 2005). Individuals presenting with this phenotype may benefit from a slight increase in activity seen by *F. vulgare* and *R. tridentata.*

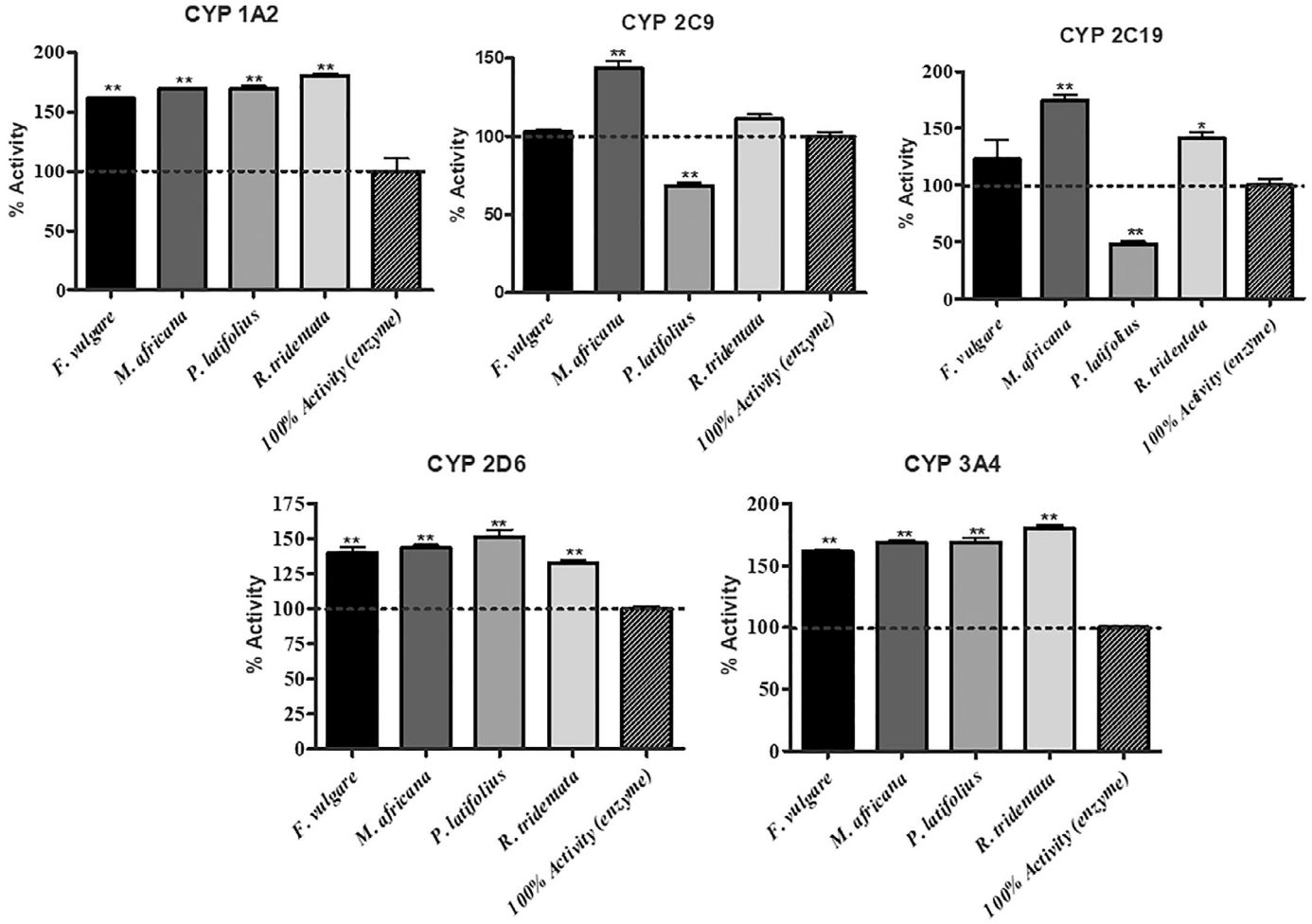

Fig. 2. The percentage activity of selected ethanolic extracts on CYP P450 enzymes at a testing concentration of 10 μg/ml. *** ($p < 0.001$), **($p < 001$), *($p < 0.1$) represent the statistical differences observed by Dunnett's test between the above samples and the 100 % activity control.

When tested for CYP 2C19 inhibitory activity, *M. africana* and *R. tridentata* instead showed a significant increase in enzyme activity (75 % and 44 %, $p < 0.01$) suggesting that a decrease in drug bioavailability should be expected if the samples are introduced into the body. *Foeniculum vulgare* increased the activity of CYP 2C19 by 22 %, however there was no statistical significance reported, as such it should have no effect on the activity of the enzyme. In the current study, *P. latifolius* exhibited significant inhibition of CYP 2C19 activity with a 52 % decrease when compared to the 100 % activity control ($p < 0.001$).

In previous studies, tuber extracts of *R. tridentata* were previously shown to be a strong dose dependent inhibitor of CYP 2C19, however a concentration of 10 μg/ml was stated to be too low to observe inhibition (Uiso, 2010). This study reported an increase in CYP 2C19 enzyme activity by the ethanolic leaf extract of *R. tridentata*, suggesting variation in the activity due to the selected plant parts for use. Manda et al. (2014) showed that *R. tridentata* did not show any time dependent inhibition of CYP 2C19, corroborating the results observed in the current study. Deresa et al. (2022) observed via *in silico* SwissADME predictions that compounds derived from *A. falcatus*, a member of the Podocarpaceae family, exhibited no inhibition of CYP 2C19 activity. This differs from the results observed by *P. latifolius* as it exhibited 52 % inhibition of the enzyme, thus implying an increased risk of interaction between *P. latifolius* and CYP 2C19.

CYP 2C19 is an enzyme that is clinically important due to its metabolism of many important drugs such as diazepam among others (Mai et al., 2000). A study performed by Wang et al. (2004) determined the ability of St John's wort, an important traditional plant extract, as an inducer of CYP 2C19. They determined that the use of St John's wort will significantly alter the bioavailability of important clinical drugs metabolized by CYP2 C19 that taken in conjunction with it (Wang et al., 2004). Thus, *F. vulgare, M. africana* and *R. tridentata* need to be further evaluated for their potential effects on drug regimens that involve metabolism by the CYP 2C19 enzyme.

For the selected extracts tested for CYP 2D6 activity, all showed a significant ($p < 0.01$) increase in activity when compared to the 100 % activity control (Fig. 2), *F. vulgare, P. latifolius, M. africana* and *R. tridentata* increased enzyme activity by 40 %, 43 %, 51 % and 32 % respectively. This is a significant observation as CYP 2D6 alone is responsible for the metabolism of up to 25 % of commonly used drugs (Owen et al., 2009). The use of these samples in conjunction with drugs that are known to be metabolized by CYP 2D6 should be carefully evaluated. In some instances, the increase in activity may again prove to be beneficial as some ethnicities have been observed to have an overall lower CYP 2D6 enzyme activity to allelic variation and polymorphisms (Bertilsson et al., 2002).

When tested for the inhibition of the CYP 3A4 enzyme it was determined that a CH_2Cl_2–MeOH extract of *P. falcatus*, a member of the *Podocarpus* genus, showed no inhibition of the enzyme (Deresa et al., 2022). This was further supported in the current study as the ethanolic extract of *P. latifolius* showed a significant increase in CYP 3A4 activity (69 %) at the tested concentration. Previous studies on the seeds of *F. vulgare* reported more than a 30 % inhibition of CYP 3A4, in this study an increase (61 %) in activity was observed

suggesting a potential difference in phytochemistry between leaves and seeds (Subehan et al., 2006). Both *M. africana* (69 %) and *R. tridentata* (80 %) exhibited a significant increase in enzyme activity suggesting that they may both have an interaction on important drugs and thus should be considered carefully when it comes to their potential use.

3.4. Human inflammatory cytokine assay

Investigation of the anti-inflammatory activity using the CBA human inflammatory cytokine assay with LPS-stimulated MRC-5 cells, there was no detectable production of IL-1β and IL-10. The results were statistically analyzed by means of an ANOVA, followed by a Dunnett's test in comparison to the stimulated cell control (MRC-5 cells treated with LPS). The results are represented in Fig. 3, where mean values below the dotted line represent a percentage decrease in production (% decrease in comparison to LPS-treated cells) and mean values above the dotted line represent percentage increase in production (% increase in comparison to LPS-treated cells).

A study performed by Moll-Bernardes et al. (2021) determined IL-12p70 to be a key biomarker for severe cases of Covid-19 infection suggesting a need to decrease the pro-inflammatory cytokines activity (Moll-Bernardes et al., 2021). *Myrsine africana* (200 μg/ml) and *P. latifolius* (100 μg/ml) significantly inhibited ($p < 0.05$) the production of IL-12p70 by 12 % and 22 % respectively, this highlighted the potential for anti-inflammatory treatment of these samples as Metronidazole, used in the treatment of Covid-19 infection has also been shown to decrease the activity of IL-12 albeit at a much greater capacity lowering the inflammatory activity of infection (Rizzo et al., 2010; Gharebaghi et al., 2020). A study performed by Moll-Bernardes et al. (2021) determined IL-12p70 to be a key biomarker for severe cases of Covid-19 infection suggesting a need to decrease the pro-inflammatory cytokines activity (Moll-Bernardes et al., 2021).

Alternatively, *P. latifolius* at a higher concentration of 200 μg/ml exhibited a significant increase in the percentage cytokine production (24 %; $p < 0.05$), this result suggests a potential pro-inflammatory response by the sample when tested at higher concentrations. Dosing is often an important factor when it comes to optimizing treatment as many drugs may possess dose dependent effects (Setty and Sigal,

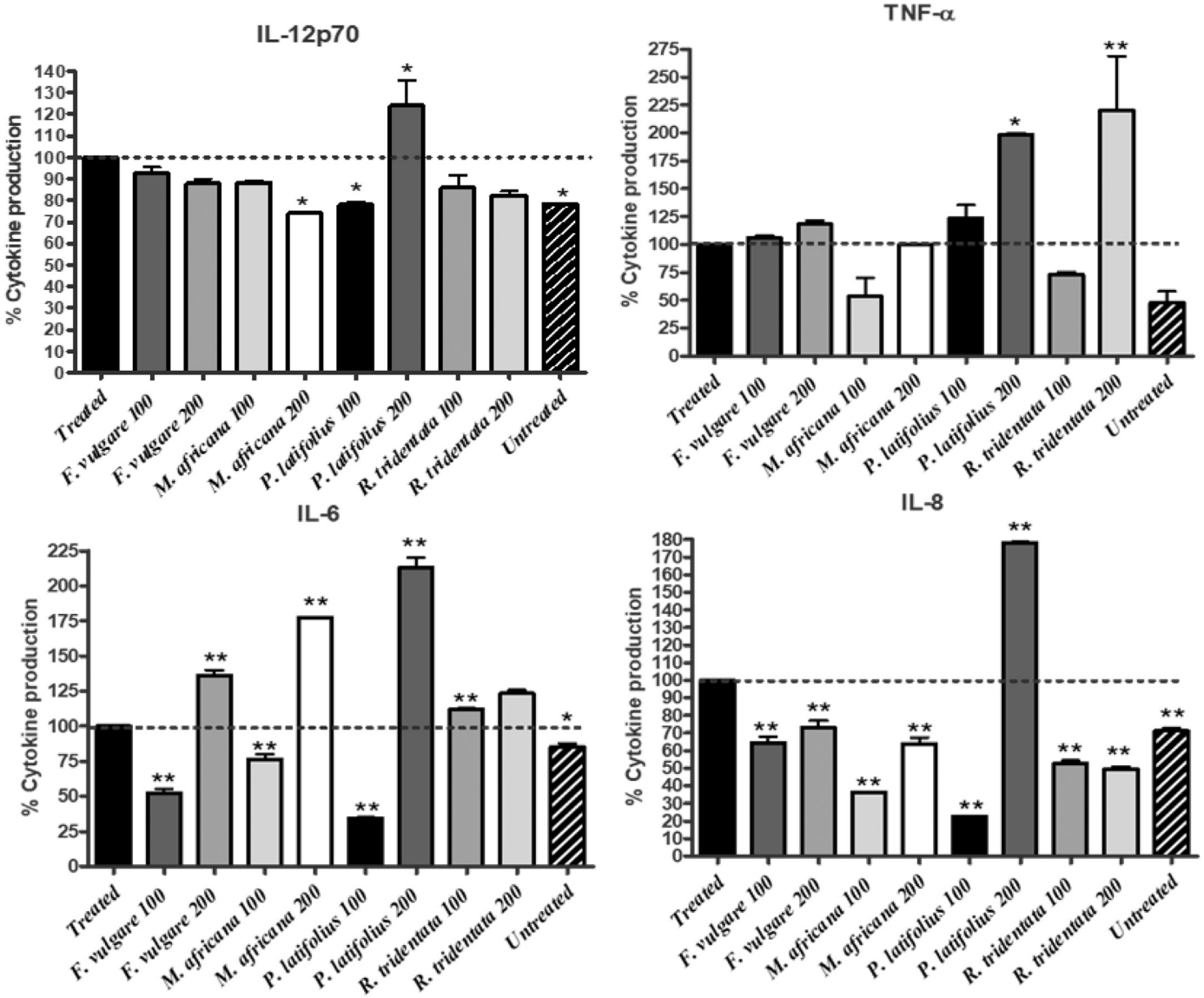

Fig. 3. The percentage production of human inflammatory cytokines by the selected ethanolic extracts at 100 ug/ml and 200 μg/ml. *($p < 0.05$), **($p < 0.01$). The untreated control consists of MRC-5 cells cultured in normal *conditions;* the treated control were cells treated with 10 μg/ml of LPS.

2005). Harpagoside, a compound derived from *Harpagophytum procumbens* has been shown to have dose dependent anti-inflammatory activity with activity reported at concentration of 100 μg/ml the same concentration at which activity was observed in the current study (Fiebich et al., 2001). Additionally, all other selected ethanolic extracts, tested at both concentrations of 100 μg/ml and 200 μg/ml were observed to inhibit IL-12p70 expression, however, these results were observed to have no significance when compared to the 100 % treated control.

Although the LPS- induced cells showed a 52 % increase in cytokine production when compared to the untreated cell control, it was determined to be statistically insignificant, this result could be due to the higher amount of deviation observed in the untreated cell samples. Both the ethanolic extracts of *M. africana* and *R. tridentata* showed inhibition of TNF-α at the 100 μg/ml concentration, these results, however, showed no statistical difference to the 100 % control suggesting that the inhibition observed is not due to the effect of the extracts. Furthermore, a significant increase in TNF-α production was observed for *P. latifolius* (98 %) and *R. tridentata* (120 %) at a concentration of 200 μg/ml ($p < 0.001$).

The results in this study suggested that the higher testing concentration of the samples may cause an increased inflammatory response (Idriss and Naismith, 2000). This view is further corroborated as none of the samples tested at 100 μg/ml were shown to stimulate TNF-α production suggesting potential to be used as a TNF-α antagonist. TNF-α is one of the early effectors in triggering an immune response to infection, through binding to the TNF-α. Receptor (TNFR1) can play a role in cell apoptosis and the prevention of infection (Jang et al., 2021). When overstimulated, increased levels TNF-α led to increased inflammation of the area, as such TNF-α antagonists are often used as treatment in viral infections (Mohd Zawawi et al., 2023).

Inhibition of IL-6 activity was observed in *F. vulgare, M. africana* and *P latifolius* and at 100 μg/ml ($p < 0.01$) with 48 %, 24 % and 66 % inhibition respectively, there was no significant difference in activity of IL-6 by *R. tridentata* at 100 μg/ml. All four samples when tested at 200 μg/ml exhibited a significant increase in the activity of IL-6, a pro-inflammatory cytokine suggesting a dose dependent response with higher concentrations eliciting an increase in pro-inflammatory cytokine production as compared to the lower concentrations of samples, examples of this dose dependent response have been reported in other medicinal plants for various biological activities (Ekor, 2014). At 200 μg/ml *F. vulgare* increased IL-6 activity by 36 %, *M. africana* by 77 %, *P. latifolius* by 113 % and *R. tridentata* by 23 %. The results indicated that the selected samples possessed anti-inflammatory activity at 100 μg/ml, this coupled with their non-cytotoxic activity when tested against MRC-5 cells (IC_{50}> 400 μg/ml) suggests the potential for the samples to be therapeutic.

All samples exhibited a strong significant difference ($p < 0.01$) in percentage cytokine production when compared to the treated control (100 % treated control) when quantifying their effects on IL-8 production. Notably only *P. latifolius* (78 %) exhibited an increase in the production of IL-8 when tested at 200 μg/ml. The remaining samples showed varying levels of inhibition, where *F. vulgare* exhibited inhibition at both 100 μg/ml (36 %) and 200 μg/ml (27 %), *M. africana* showed 64 % inhibition at 100 μg/ml and 36 % inhibition at 200 μg/ml against IL-8. *Rhoicissus tridentata* showed similar inhibition levels at both concentrations tested (47 % at 100 μg/ml and 51 % at 200 μg/ml) while *P. latifolius* exhibited a 77 % inhibition of IL-8 production at 100 μg/ml.

Of the multiple pro-inflammatory cytokines associated with Covid-19 infection, IL-8 was observed in high levels in patients with severe Covid-19 (Zhang et al., 2020). The expression of IL-8 due to Covid-19 infection was observed to be due to a significant induction and increase in the activity of AP-1 proteins which regulate the transcription of a wide variety of genes involved in many types of cellular functions (Bai et al., 2018, Zhu et al., 2021). As such the observed decrease in activity of IL-8 could be caused by a decrease in induction of the AP-1 proteins.

Previous studies on the anti-inflammatory activity of *F. vulgare* have shown a significant dose response in the treatment of LPS-induced bronchoalveolar lavage fluid (BALF) of mice (Lee et al., 2015). Treatment of the LPS-treated BALF with 125 μg/ml - 500 μg/ml of *F. vulgare* essential oil showed inhibition of the pro-inflammatory cytokines IL-6 and TNF-α in a strongly dose dependent manner with 500 μg/ml of *F. vulgare* reducing IL-6 expression by 0.58 $\pm$ 0.11 ng/ml, p = 0.017and TNF-α by 4.29 $\pm$ 0.29 ng/ml, ($p < 0.001$) respectively (Lee et al., 2015).

It is important to note that complete inhibition of the inflammatory cytokines produced by Th-1 (T-helper 1) cells such as IL-P12p70, IL-10 and TNF-α may have negative downstream impacts as they play a key role in cellular immunity. The inhibition of these cytokines could have even further downstream effects on Th-2 cell mediated immunity which regulate the other aspects of the humoral immune response and could lead to immunosuppression (Dong and Flavell, 2001). Therefore, samples concentrations that inhibit cytokine production at more moderate levels should be favored.

In this study *F. vulgare* was evaluated for its anti-inflammatory activity against 6 pro-inflammatory cytokines. The ethanolic extract of *F. vulgare* at a testing concentration of 100 μg/ml, exhibited a slight, non-significant decrease in the expression of both IL-12p70 and TNF-α. Significant levels of inhibition were observed in the expression of IL-6 and IL-8 when tested at 100 μg/ml. When tested against IL-6 at 200 μg/ml, *F. vulgare* increased the concentration of the pro-inflammatory cytokine. Thus, in this study as observed in the available literature, *F. vulgare* does show inhibition of inflammatory cytokines, however the levels of inhibition were not observed to be dose dependent as greater inhibition was shown at lower concentrations (100 μg/ml) and an increase in inflammatory cytokines observed at the higher concentration (200 μg/ml).

When tested against the human inflammatory cytokines, *M. africana* exhibited a notable increase in the expression of IL-6 at 200 μg/ml and exhibited no significant change in expression of TNF-α at the same concentration, however, at 100 μg/ml, IL-6 expression was observed to be lower although this inhibition was shown to be statistically insignificant. When tested against the remaining cytokines (IL-12p70, TNF-α, IL-6 and IL-8), inhibition was observed at both testing concentrations. Thus, *M. africana* shows overall effective anti-inflammatory activity and that it is important to note that at a concentration of 100 μg/ml shows the most consistent inhibition of cytokine production. Although there is no available research on the anti-inflammatory activity of *M africana* specific to human inflammatory cytokines, hexane and ethyl acetate fractions of the aerial parts of *M. africana* have been shown to moderate activity of reactive oxygen species with an observed inhibition of 40.90 % and 44.80 % respectively (Ahmad et al., 2011). Reactive oxygen species play a role as signaling molecules and mediators in inflammatory response and although the mechanism of action differs to that of human inflammatory cytokines, the result shows precedent for anti-inflammatory activity.

Sciadonic acid, a compound extracted from *Podocarpus nagi* a member of the Podocarpus genus has been shown to inhibit the macrophage expression of several pro-inflammatory factors including TNF-α and IL-6 at 14 % and 34 % respectively (Chen et al., 2012). A compound found within *Podocarpus macrophyllus* (Thunb.) Sweet, Nagilactone B, has been shown to possess anti-inflammatory activity (Kim et al., 2021). Kim et al. (2021) observed that Nagilactone B strongly inhibited the production of IL-6 (99.3 %) and TNF-α (28.1 %) in LPS-stimulated RAW 264.7 cells.

When the cells treated with *P. latifolius* were tested against human inflammatory cytokines it was observed that the higher tested concentrations (200 μg/ml) significantly promoted

inflammation as the cytokine expression was noticeably increased. At the lower testing concentration (100 μg/ml). Inhibitory activity was observed for IL-12p70, IL-6 and IL-8, suggesting that *P. latifolius* exhibits effective anti-inflammatory activity at lower concentrations but at increasing concentrations starts to induce an inflammatory response, suggesting that the constituents of *P. latifolius* are only effective at lower concentrations, at higher concentrations they lead to inflammation.

When tested against the human inflammatory cytokines, *R. tridentata* showed an inhibition of IL-12p70 and IL-8 at both testing concentrations while TNF-α was inhibited at the lower concentration of 100 μg/ml. These results further highlighted the anti-inflammatory potential of *R. tridentata.*

4. Conclusion

Since its advent in December 2019, Covid-19 has had a lasting negative effect on millions of people worldwide. The study investigated southern African medicinal plants, which have traditionally been used to treat respiratory disorders and Covid-19 associated symptoms including inflammation and clotting for their activity against targets that are associated with Covid-19 infection. *Foeniculum vulgare, Myrsine africana, Podocarpus latifolius* and *Rhoicissus tridentata* exhibited no cytotoxicity when tested against human lung fibroblast (MRC-5) cells with all four samples further exhibiting anticoagulant activity when tested for FXa inhibition. Potential anti-inflammatory activity due to inhibition of human inflammatory cytokines was also observed by the samples at a concentration of 100 μg/ml. There are, however, some limitations to the use of these species when taking into consideration their interactions with CYP 1A2, CYP 2D6 and CYP 3A4 which were induced and CYP 2C9 and CYP 2C19 which were inhibited, suggesting possible adverse reactions in potential drug herb interactions involving these species.

It can be concluded that *F. vulgare, M. africana, P. latifolius* and *R. tridentata* should be further investigated against Covid-19 related symptoms.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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