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Research Article

Inhibitory effects of hydroethanolic extracts from three Cameroonian medicinal plants on proteins inflammation and growth of multi-resistant strains of *Mycobacterium tuberculosis*

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Abstract

The present work aimed to determine the phytochemical components and evaluate the *in vitro* anti-inflammatory and anti-mycobacterial effects of hydroethanolic extracts of *Allium sativum* L bulbs, *Drypetes gossweileri* S. MOORE stem-barks and *Pentadiplandra brazzeana* Baill roots against several resistant strains of *Mycobacterium tuberculosis*. The phytochemical screenings of extracts were carried out according the colorimetric and precipitation tests to reveal the presence of phytochemical compounds. The anti-inflammatory effects of extracts were evaluated using *in vitro* Bovine Serum Albumin denaturation and proteinase inhibitory action assays. The inhibitory parameters of hydro-ethanol extracts were evaluated by the microdilution method against *Mycobacterium tuberculosis*. The phytochemical screening of hydro-ethanol extracts revealed the presence of phenols, polyphenols, flavonoids, alkaloids, catechic tannins, triterpens, steroids, anthocyanins and leucoanthocyanins. The anti-inflammatory activity of hydro-ethanol extracts of *D. gossweileri*, *P. brazzeana* and *A. sativum* have shown the inhibitory concentrations 50 (IC₅₀) values ranging from 356.70, 183.30 and 226.30 µg/mL for BSA denaturation and 31.92, 33.62 and 56.93 µg/mL for proteinase inhibitory action respectively. The hydroethanolic extracts of *D. gossweileri*, *P. brazzeana* and *A. sativum* exhibited moderate and weak anti-mycobacterial activities with the minimum inhibitory concentrations (MICs) ranging from 312.5 to 2500 µg/mL. *A. sativum* hydro-ethanol extract has shown the highest anti-mycobacterial activity with MIC of 312.5 µg/mL against isoniazid resistant of *M. tuberculosis* and extremely resistant drug strain of *M. tuberculosis*. These results suggest that hydro-ethanol extracts of *A. sativum*, *D. gossweileri* and *P. brazzeana* are efficient against tuberculosis caused by multi-resistant *Mycobacterium tuberculosis* strains and are able to resorb the inflammation induced during infection.

Keywords: Anti-inflammatory activity, Anti-mycobacterial effect, Hydroethanolic extracts, Medicinal plants, Phytochemical screening.

1. INTRODUCTION

The research of medicinal plants through their secondary metabolites possessing anti-inflammatory and antimicrobial properties have been on the rise due to their potential use in the therapy of various chronic and infectious diseases like tuberculosis. Epidemiology and experimental studies have implicated pro-inflammatory damage arising from the denaturation process of functional and structural proteins and membrane lipids as the primary cause of chronic and infectious diseases. Due to risk of adverse effects encountered with the use of synthetic antibiotics, medicinal

plants may offer an alternative source for antimicrobial agent with significant activity against various pathogens¹. In addition, a number of antibiotics have lost their effectiveness due to the development of resistant strains, mostly through the expression of resistance genes¹.

Inflammation is a complex process, which is considered as a primary physiologic defence mechanism that helps body to protect itself against burn, toxic chemicals, allergens, other noxious stimuli or infectious diseases². Uncontrolled and persistent inflammation can play an etiological role in many of these chronic diseases and can be a source of worsening

infectious diseases³. Although it is a defence mechanism, the complex events and mediators involved the inflammatory reaction can induce, maintain or aggravate many respiratory diseases like pneumonia or tuberculosis⁴. Tuberculosis (TB) is an infectious and inflammatory disease caused by *Mycobacterium tuberculosis* complex bacteria^{5,6,7}. It generally affects the lungs and is transmitted from a patient through exhaled droplets that diffuse into the air to a healthy person⁵. In 2009, the World Health Organization (WHO) estimated that one third of the world's population have been infected by the bacillus of Koch and 10 % of infected individuals have developed the disease⁷. In 2016, 8.5 and 8.3 million new TB cases were estimated by WHO at for the years, 2013 and 2014 respectively⁸. In 2017, 10 million new cases of tuberculosis were recorded with about 1.3 million dead⁹. Thus, these incidence rates show that *Mycobacterium tuberculosis* remains the pathogen responsible for the leading cause of death in humans compared to other microbial species⁹. This resistance is due to the inadequate used of drugs which provokes the emergence of multidrug resistant strains like Isoniazid or Rifampicin resistant strains, Multi-Drug resistant strains (MDR) and Ultra-Drug resistant strains (XDR). To solve this public health problem, the WHO suggested to look for an alternative and effective therapies⁹. Therefore, the development of potent anti-mycobacterial drugs that also possesses anti-inflammatory effects with fewer side effects is necessary from medicinal plants origin.

The used of Cameroonian medicinal plants as TB treatment is recurrent as well as an ethnobotanical survey has shown that several medicinal plants like *Allium sativum* L. (Amaryllidaceae), *Drypetes gossweileri* S. MOORE (Putranjivaceae) and *Pentadiplandra brazzeana* Baill. (Pentadiplandraceae) could be an approach of choice¹⁰.

These plants process diverse biological activities including anti-carcinogenic, anti-atherosclerotic, antithrombotic, antimicrobial, anti-inflammatory and antioxidant effects of their essential oils^{10,11,12}. In the Nkam sub-division (Littoral region of Cameroon), people often used their crude extracts deriving from maceration, decoction and infusion to treat tuberculosis¹⁰. Even if the antimycobacterial potential of the essential oil extracted from these plants have been shown against resistant strains, the present study was undertaken to evaluate the phytochemical components, *in vitro* anti-inflammatory and anti-mycobacterial efficiency of hydroethanolic extracts from three cameroonian medicinal plants against multi-resistant strains of *Mycobacterium tuberculosis*.

2. MATERIEL AND METHODS

2.1. Chemical reagents and solvents.

Glycerol and Tween 80 were purchased from Sigma-Aldrich (France). The Bovine Serum Albumin (1mM), Phosphate Buffer Saline (0.2 M; pH=7.4), Middlebrook 7H9 OADC (oleic acid, albumin, dextrose, catalase) supplement, Alamar Blue solution and PANTA (Polymixin, Amphotericin B, Naladixic acid, Trimethoprim and Azlocillin) antibiotic supplement were purchased from Becton Dickinson (USA). All other reagents and solvents were of analytical grade.

2.2. Collection and authentication of plant materials.

Three aromatics and/or medicinal plants namely *D. gossweileri* stem-barks, *P. brazzeana* roots and *A. sativum* bulbs, were collected at 6 a.m from Littoral, West and North Regions of Cameroon respectively in January 2016. The botanical identification and authentication of the plants was carried out at the National Herbarium of Cameroon

(Yaoundé) where voucher specimens are kept under the 25749/SR/Cam, 42918/SR/Cam and 25742/SR/Cam respectively.

2.3. Extraction procedure.

The *D. gossweileri* stem-barks, *P. brazzeana* roots and *A. sativum* bulbs were air-dried at room temperature and weighed. 200 g of aliquots of each pulverized and dried plant material were extracted by maceration in hydroethanolic mixture (20/80; v/v), twice for 48 hours at room temperature ($27 \pm 2^\circ\text{C}$) in enclosed flasks. Each sample was filtered through a Whatman N° 1 filter paper, and evaporated under reduced pressure with rotative evaporate to obtain hydro-ethanol extracts¹⁴. These extracts were stored at 4°C until use. The extraction yields were calculated as the ratio of the mass of hydroethanolic extract to the mass of the starting plant material. It expressed as a percentage.

2.4. Phytochemical screening of hydroethanolic extracts.

Preliminary phytochemical screening of secondary metabolites were carried out according to the methods described by Harborne, and Trease and Evans^{15,16}.

2.5. *In vitro* anti-inflammatory assay.

2.5.1. Bovine Serum Albumin (BSA) denaturation assay.

Anti-denaturation assay was conducted as described by Betote *et al.*, with slight modifications¹⁷. The reaction mixture was consisting of test hydroethanolic extracts or sodium diclofenac and 5% aqueous solution of bovine serum albumin. The mixture were incubated at 37°C for 20 min and then heated to 70°C for 15 min; after cooling the samples, the turbidity was measured at 660 nm using a spectrophotometer of the brand Thermo-Fisher-Scientific: Evolution 300 UV-VIS. The experiment was performed in triplicate. The percent inhibition of bovine serum albumin denaturation was calculated as follows, where $Abs_{control}$ is the absorbance without sample, Abs_{sample} is the absorbance of sample (hydroethanolic extracts /standard).

$$I (\%) = \frac{Abs_{control} - Abs_{extracts/sodium\ diclofenac}}{Abs_{control}} \times 100$$

2.5.2. Proteinase inhibitory action.

The test was performed according to the modified method of Oyedepo *et al.*¹³. The reaction mixture (2 mL) was containing 0.06 mg trypsin, 1 mL of 20mM Tris-HCl buffer (pH7.4) and 1mL test hydro-ethanol extracts or sodium diclofenac of different concentrations. The reaction mixture was incubated at 37°C for 5 minutes and then 1 mL of 0.8 % (W/V) casein was added. The mixture was incubated for an additional 20 min, 2 mL of 70 % perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage of inhibition of proteinase inhibitory activity was calculated.

$$I (\%) = \frac{Abs_{control} - Abs_{extracts/sodium\ diclofenac}}{Abs_{control}} \times 100$$

Where, $Abs_{control}$ is the absorbance of control tube and $Abs_{extracts/diclofenac\ sodium}$ is the absorbance of sample tube.

2.6. Anti-mycobacterial activity assay.

2.6.1. Mycobacterial cultures of *Mycobacterium tuberculosis*.

Three *Mycobacterium tuberculosis* strains isolated from patients at the tuberculosis control centre in the South Region of Cameroon and characterized at the Laboratory for Tuberculosis Research and Pharmacology (the Biotechnology Centre of the University of Yaoundé I) where they have been screened to drug resistant using Line probe assay. The testes strains included an Isoniazid resistant strain codify as AC₄₅, Rifampicin resistant strain (AC₇₉) and *M. tuberculosis* extensively drug resistant strain (XDR). These bacterial strains are maintained on slant of Middlebrook 7H11 Agar medium supplemented with 10 % Middlebrook 7H9 OADC supplement and PANTA an antibiotic supplement.

2.6.2. In vitro anti-mycobacterial assessment of extracts.

The geometric serial broth microdilution method was carried out according the Microplate Alamar Blue Assay (MABA) described previously by Collins and Franzblau and modified by Jimenez-Arellanes *et al.*^{18,19}. A stock solution was prepared by diluting the respective sample in 10 % tween 80. Stock solution was then added to Middlebrook 7H9 broth to reach final samples concentrations ranging from 5000 µg/mL to 9.765 µg/mL. Serial dilutions were inoculated with mycobacteria inocula (1.5×10^8 cells/mL prepared from the Middlebrook 7H9 Broth medium supplemented with glycerol, tween 80, and Middlebrook 7H9 OADC and PANTA supplements). Each 96 wells microtiter plates was mixed and incubated at 37 °C for 7 days. Positive controls consisted of Rifampicin and Isoniazid at 250 µg/mL and negative controls was contained no drugs and blank contained no inoculum and/or drug. The concentration of tween 80 in the assay was kept at a concentration to ensure that the effect on bacterial growth was minimal. Upon incubation periods, 40 µL of 0.02 % resazurin were added to individual wells and the plates reincubated for additional 1 day and checked daily for color change. Change in resazurin color from blue to pink indicated reduction of the indicator and thus bacterial growth. The Minimal Inhibitory Concentration (MIC) was

defined as the lowest concentration of samples at which the microorganism did not demonstrate growth^{20,21}.

2.6.3. Determination of mycobactericidal effect.

Mycobactericidal effect of hydroethanolic extracts and standard drugs on pathogens was determined by microdilution method²⁰. The Minimal Bactericidal Concentrations (MBC) of promising hydroethanolic extracts was assessed by sub-culturing MIC test microtiter plates on Middlebrook broth 7H9 + 10 % (AODC+ PANTA). The MBC was considered as highest dilution or lowest concentration at which no growth occurred in the medium. All the experiments were done in duplicate and repeated three times. The anti-mycobacterial effect was deemed bactericidal or bacteriostatic depending on the ratio: MBC/MIC. If CMB/CMI is lower than four, the anti-mycobacterial effect of plants extracts is bactericidal and bacteriostatic when CMB/CMI higher than four¹⁹.

2.7. Statistical analysis.

Each data was expressed as mean $\pm$ SD (n = 3). The analysis was done by ANOVA followed by test Dunnett's. The difference between standard and hydroethanolic extracts concentrations was considered significant at $p < 0.05$. The graphical representation of the data and IC₅₀ was performed using the Graph Pad Prism 8.0.1 software (Microsoft, USA).

3. RESULTS

3.1. Phytochemical Screening of hydroethanolic extracts.

The qualitative analysis of phytochemical compounds of *A. sativum*, *D. gossweileri* and *P. brazzeana* were carried out and hydroethanolic extracts showed the presence of various chemical constituents such phenols, polyphenols, flavonoids, alkaloids, catechic tannins, triterpens, steroids, anthocyanins and leucoanthocyanins. These results show high level of its possible medicinal value. The results are shown in (Table 1).

Table 1: Preliminary phytochemical screening

N°	Secondary metabolites	Hydroethanolic extracts		
		<i>P. brazzeana</i>	<i>D. gossweileri</i>	<i>A. sativum</i>
1	Phenols	-	+	-
2	Polyphenols	+	+	+
3	Flavonoids	+	+	+
4	Alcaloids	+	+	+
5	Cathechic tannins	+	+	+
6	Triterpens	-	+	+
8	Steroids	-	-	+
9	Anthocyanins	+	+	+
10	Leucoanthocyanins	+	+	+

Legend: (+) indicate the presence of active constituents; (-) indicates the absence of active constituents.

Unlike other classes of secondary metabolites that are ubiquitous to all three plant extracts, phenols were found exclusively in *D. gossweileri* and steroids in *A. sativum*.

3.2. In vitro Anti-inflammatory Activity.

3.2.1. Inhibition of Protein (BSA) denaturation.

The hydroethanolic extracts of *A. sativum*, *D. gossweileri* and *P. brazzeana* displayed significant activity (Figure 1). The hydroethanolic extracts of *P. brazzeana* at 250 and 500 µg/mL showed highest inhibitions 96.28 % and 98.03 % of Bovine Serum Albumin denaturation ($p < 0.05$) respectively.

While, *A. sativum* and *D. gossweileri* showed 97.60 % and 93.81 % of inhibition of protein denaturation at 500 µg/mL respectively. The hydroethanolic extracts of *P. brazzeana* has also showed a higher inhibition concentration 50 of 91.67 µg/mL than *A. sativum* (113.10 µg/mL) and *D. gossweileri* (159.50 µg/mL) extracts. These extracts are effective in inhibiting heat induced albumin denaturation compared with that of the standard Sodium diclofenac (IC₅₀ of 6.97 ± 0.98 µg/mL) (Figure 2).

Figure 1: Effect of hydroethanolic extracts of *A. sativum*, *D. gossweileri* and *P. brazzeana* on the inhibition of Bovine Serum Albumin denaturation ($p < 0.05$).

Figure 2: Comparison of IC_{50} of Bovine Serum Albumin denaturation between hydroethanolic extracts of *A. sativum*, *D. gossweileri* and *P. brazzeana* and Sodium diclofenac. Data are expressed as mean $\pm$ SD, Sodium diclofenac was used as a reference compound. Statistical differences between Sodium diclofenac and plant extracts are analyzed by the Dunnett's test ($***p < 0.001$). Statistical differences ($a > b > c$) between plant extracts are analyzed by the Dunnett's test ($p < 0.05$).

3.2.2. Proteinase Inhibitory Action.

The hydroethanolic extracts of *A. sativum*, *D. gossweileri* and *P. brazzeana* exhibited significant anti-proteinase activity (Figure 3). The best inhibition concentration 50 was observed from stem-barks extract of *D. gossweileri* (31.92

$\mu\text{g/mL}$) and *P. brazzeana* roots extract (33.62 $\mu\text{g/mL}$) in decreasing order was *A. sativum* hydroethanolic extract (56.93 $\mu\text{g/mL}$). The standard Sodium diclofenac drug (8.39 $\mu\text{g/mL}$) showed the best proteinase inhibitory action (Figure 4).

Figure 3: Effect of hydroethanolic extracts of *A. sativum*, *D. gossweileri* and *P. brazzeana* on the inhibition of proteinase inhibitory action ($p < 0.05$).

Figure 4: Comparison of IC₅₀ of proteinase inhibitory action between hydroethanolic extracts of *A. sativum*, *D. gossweileri* and *P. brazzeana* and Sodium diclofenac. Data are expressed as mean $\pm$ SD, Sodium diclofenac was used as a reference compound. Statistical differences between Sodium diclofenac and plant extracts are analyzed by the Dunnett's test (*** p <0.001). Statistical differences (a>b) between plant extracts are analyzed by the Dunnett's test (p <0.05).

3.3. Antimycobacterial Assay.

The hydroethanolic extracts of *P. brazzeana*, *D. gossweileri* and *A. sativum* were evaluated for their anti-mycobacterial effects against three resistant strains of *M. tuberculosis*. The results are presented in Table 2. The strains used possess a drug-resistant profile representing the majority of clinical isolates of tuberculous patient's drug control in the south Region of Cameroon. These results showed that the hydroethanolic extracts of *A. sativum*, *D. gossweileri* and *P. brazzeana*, exhibited moderate anti-mycobacterial activity against the resistant strains *M. tuberculosis* AC₄₅, AC₇₉ and UJ

with MICs ranging from 312.5-2500 µg/mL. Out of these extracts, *A. sativum* has exhibited very high anti-tuberculosis activities on both the Isoniazid resistant strain and Extensively drug resistant strain UJ with same MIC value of 312.5 µg/mL. While the MDR *M. tuberculosis* AC₇₉ has been shown less susceptibility to the plant extracts. A bactericidal effect ($1 < \text{MBC/MIC} \leq 2$) has been observed with all hydroethanolic extracts against Isoniazid (AC₄₅) and Rifampicin (AC₇₉) resistant strains, and XDR strain (UJ) respectively; with the exception of the hydroethanolic extract of *A. sativum* that showed a bacteriostatic effect ($\text{MBC/MIC} \leq 4$) on *M. tuberculosis* UJ.

Table 2: Plant materials used for anti-mycobacterial activity, MICs and MBCs values of their hydroethanolic extracts, against resistant strains of *Mycobacterium tuberculosis* AC₄₅, AC₇₉ and XDR.

N°	Plant species	Part used in this study	Extraction yields ^a (%)	Inhibition parameters (µg/mL)								
				AC ₄₅			AC ₇₉			XDR		
				MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
1	<i>D. gossweileri</i>	Stem-barks	13.19 %	1250	1250	1	2500	5000	2	1250	1250	1
2	<i>P. brazzeana</i>	Roots	6.42 %	2500	2500	1	2500	2500	1	625	1250	2
3	<i>A. sativum</i>	Bulbs	8.55 %	312.5	312.5	1	2500	2500	1	312.5	1250	4
4	Rifampicin (RIF)			0.98	3.91	4	1.95	n.d	n.d	15.63	n.d	n.d
5	Isoniazid (INH)			7.81	n.d	n.d	0.12	0.98	8	7.81	n.d	n.d

Legend: ^a The present extraction yields were calculated in percentages (w/w); MIC: minimal inhibitory concentration; MBC: minimal bactericidal concentration; n.d: not determined; AC₄₅: Isoniazid resistant strain of *M. tuberculosis*; AC₇₉: Rifampicin resistant strain of *M. tuberculosis* and XDR: Extremely drug resistant strain of *M. tuberculosis*.

4. DISCUSSION

The results of phytochemical screening confirmed the use of *P. brazzeana* roots, *D. gossweileri* stem-barks and *A. sativum* bulbs in traditional medicine. We found the secondary metabolites classes, anti-inflammatory and antimycobacterial activities in these plant extracts. The general qualitative analysis of hydroethanolic extracts of *P. brazzeana*, *D. gossweileri* and *A. sativum* were carried out and extracts showed the presence of various secondary metabolites such as phenols, polyphenols, flavonoids, alkaloids, catechic tannins, triterpens, steroids,

anthocyanins and leucoanthocyanins showing that these extracts have a high level of medicinal value. The study carried out by Montaut *et al.*, and Nyegue *et al.*, reported the presence of sulfur-containing compounds found in stem barks such as glucosinabin (4-hydroxybenzyl) and glucotropaeolin (benzyl glucosinolate) in *gossweileri* extracts and *P. brazzeana* roots meanwhile organosulphure compounds like allicin, an ajoeme were found in *A. sativum* hydroethanolic extract^{22,23,26}. The presence of these compounds might be responsible for the diverse of biological activities²⁴.

The extracts of *D. gossweileri*, *P. brazzeana* and *A. sativum* were assessed for its anti-inflammatory activity and the results were compared with Sodium diclofenac standard. Denaturation of proteins like Bovine Serum Albumin is a well-documented cause of inflammation. Maximum inhibition was observed from roots of *P. brazzeana* hydroethanolic extract, which has showed 98.03 % of inhibition of BSA denaturation at 500 µg/mL. Diclofenac sodium, standard anti-inflammatory drug showed 94.28 % of inhibition of protein at the concentration of 500 µg/mL. Same to the inhibition of denaturation of bovine serum albumin, *P. brazzeana* hydroethanolic extract has showed best percentage of inhibitory value (74.19 %) using the proteinase inhibitory action assay.

The secondary metabolites present in *A. sativum* hydroethanolic extracts like allicin and ajoene or di-methyl sulphide and tri-methyl sulphide inhibit the complex events and mediators involved during the inflammatory reaction by reducing the level of reactive oxygenated compounds and some inflammatory mediators like Tumour Necrosis Factors (TNF)²⁵. These extracts may also inhibit the release of lysosomal content of neutrophils at the site of inflammation. These neutrophils lysosomal constituents include bactericidal enzymes and proteinases, which upon extracellular release cause further tissue inflammation and damage. The obtained results indicated that the secondary metabolites contained in the hydroethanolic extracts of *P. brazzeana*, *D. gossweileri* and *A. sativum* were capable to resorb the inflammatory processes with a significant level of protection against the mechanisms of protein denaturation and inhibition of lytic enzymes by these bioactive compounds^{25,26}.

In this study, the results demonstrated that the plant extracts inhibited all the resistant strains with the concentrations ranging from 312.5-2500 µg/mL. The *A. sativum* and *P. brazzeana* extracts exhibited better activity than *D. gossweileri* stem barks extract. The activity of hydroethanolic extracts of *A. sativum* have showed more effects against the extensively drug resistant isolate and might be due to the activities of allicin and ajoene²⁶. For *P. brazzeana* and *D. gossweileri* ethanolic extract, many suggested that glycosinolates found these plants are the main compounds with antimicrobial and inflammatory and anticancer activities. It the difference of action mechanisms of plants^{22,23}. These plant mechanisms have been reported to have multi-factorial mechanisms due to various phytochemical components that confer their effects at simultaneously²⁶.

These results are in accordance with one of the previous studies which showed that garlic vegetable oil and hydroethanolic extracts inhibited resistant isolate and, H₃₇Rv and Rifampicin resistant strains of *M. tuberculosis* at concentrations of 0.98, 100 and 200 µg/mL respectively^{27,28}. Another study demonstrated the activity of *P. brazzeana* hydroethanolic extract against H₃₇Ra and clinical isolate of *M. tuberculosis* at the both concentration of 125 µg/mL²⁹. The anti-mycobacterial activity of the tested *D. gossweileri* stem barks extract has not been reported before and therefore our results can be evaluated as the first report about the anti-mycobacterial properties. Compare to the activity of hydroethanolic extracts, the essential oils of these plants exhibited higher activity with MIC ranging 78.17 to 312.50 µg/mL. This difference can be due to the lipophilic character of mycolic acid contained in cell wall of *M. tuberculosis* have a high affinity with lipophilic substance like essential oils. This facilitates their up-take in cells and their interaction with cell targets.

The possible explanations for this difference in results among the studies might be due to various species of plant families that differ in concentration of bioactive constituents. This difference in biological activity of ethanolic extracts also be influenced by the vegetative cycle of the plants, the place and the harvest period, the edaphic conditions such as the climate, the type of soil and that are responsible for the variability of their chemical composition^{23,30}. The present study was assumed that the hydroethanolic extracts retain their inhibitory activities due to their various bioactive constituents, against which the gene resistance development might be difficult³¹.

5. CONCLUSION

Phytochemical studies showed the presence of phenols, polyphenols, flavonoids, alkaloids, catechic tannins, triterpens, steroids, anthocyanins and leucoanthocyanins in hydroethanolic extracts of *D. gossweileri*, *P. brazzeana* and *A. sativum*. Protein denaturation and proteinase inhibitory action assay showed that hydroethanolic extracts of *D. gossweileri*, *P. brazzeana* and *A. sativum* have good anti-inflammatory activity and according to the Microplate Alamar Blue assay a moderate antimycobacterial activity. These results suggest that the hydroethanolic extracts of these plants could be a potential source of natural anti-inflammatory and antimycobacterial compounds. This founding also indicates that at the concentration of 2500 µg/mL each of these hydroethanolic extract could present anti-inflammatory and anti-mycobacterial potential in animal models infected by the multidrug resistant strain of *M. tuberculosis*.

Abbreviations

INH: Isoniazid; MABA: Microplate Alamar Blue Assay; MBC: Minimal Bactericidal Concentrations; MDR: Multi-Drug Resistant; MIC: Minimum Inhibitory Concentration; OADC: Oleic acid-Albumin-Dextrose-Catalase; RIF: Rifampicin; TB: Tuberculosis; XDR: Extensively Drug Resistant; WHO: World Health Organization.

Competing interests: The authors declare that they have no competing interests.

Author's contribution

EDFNM carried out the research work, wrote the first draft of the manuscript and revised the manuscript. PHDB assisted in the research work, carried out the determination of phytochemical analysis of plant extracts, assisted in the anti-inflammatory and anti-mycobacterial assays, revised the manuscript and submitted the manuscript. CWK assisted in the anti-mycobacterial assay and revised the manuscript. CFMB assisted in the anti-mycobacterial assay and revised the manuscript. NMA identified the project, guided the research and revised the manuscript. All authors have read and approved the final version of the manuscript.

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