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Antimycobacterial, Antioxidative, Anti-Inflammatory, Cytotoxic, Anti-Biofilm and Synergistic Interaction Effects of Five Medicinal Plants Species Used for Tuberculosis Infections in Sekhukhune, Limpopo, South Africa.

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Abstract

The extraordinary occurrence of contagion and the greater than before rate of multi-drug resilient and extensively-drug resilient stresses of *Mycobacterium* species complex to the challenging of tuberculosis (TB) control has remained degraded by the Covid-19 pandemic. This outcomes in an urgent want to progress in new managements for breathing infections, and herbal might be a foundation of such cures. In this study accomplishments of roots of *Elephantorrhiza elephantina,* leaves of *Aloe marlothii, Eucalyptus camaldulensis, Euphorbia tirucalli and Schotia brachpetala* were assessed in contradiction of microorganisms connected to those instigating breathing ailments, namely *Mycobacterium tuberculosis*, *Mycobacterium bovis*, Mycobacterium africanum and *Mycobacterium smegmatis*. The herbal extracts had MIC values ranging from 0.02 to 1.3 mg/ml against all *Mycobacterium*. All plants had IC_{50} above 0.1 mg/ml which means they are non-cytotoxic against Vero kidney cells. The outcomes sustenance the native usage of these herbal in the management of TB and it is recommended that these plants might devise healing worth in the cure of TB.

Keywords: Antibacterial, Cytotoxicity, Antibiofilm, Synergy. Multi-drug resistant strain

Background

The TB control quantity were deteriorate by also introductory charges of by what means the extraordinary corona virus disease 2019 (COVID-19) pandemic might disturb TB healthiness facilities, management and avoidance determinations. Nevertheless, the significant to reminder this worldwide TB management struggles remained not on pathway smooth beforehand the beginning of the COVID-19 pandemic, and the statistical gap amongst the projected total of individuals with TB internationally and the statistics testified to public health authorities rests widespread [9].Noticeably extra responsiveness is dedicated to control of *Mycobacterium tuberculosis* in individuals, than *M. bovis* in its various multitudes [25].

Herbal and other regular products are an significant foundation of novel remedies [15] and in the previous donkey's years, approximately 50% of remedies accepted by the FDA in the United States of America have remained by-products of ordinary harvests [18].The suggestion for organic means from regular

yields is indisputable ,as is the prosperous restraint of breathing infections abstracts from ethnobotanically particular herbal [17].Consequently, ordinary therapies, exclusively those resulting from ethno medicinal plants, are quiet existence used international in the treatment and controlling of respiratory infections. Ordinary crops performance an fundamental part in the treatment and controlling of respiratory infections [16]. Now assured circumstances, statistics on the effectiveness of ethnobotanicals in contradiction of pathogens of respiratory infections are available [21]. In graceful of the improvement of resistance in those transmissible diseases with current treatments, one approach active in traditional herbal treatment to overwhelmed this sensation is the mixture of herbal therapies. To this result, exactly authors have tried the mixture of antibiotics with herbal abstract [25] whereas others have intensive on herbal abstract mixtures to accomplish a supplementary powerful anti-microbial activities [26]. Therapeutic plants signifies one of the greatest vital fields of old-style medicine totally over the world and can suggestion confidence for the expansion of alternative medicines for the management of tuberculosis. To stimulate appropriate usage of medicinal plants and regulate their prospective as a foundation of innovative remedies, it is indispensable to exemine therapeutic plants which have traditional stories status in more strengthened technique [27,28]. Herbal ingredients like iridoids, terpenes, citronellol, nerol and geraniol have revealed bioactivities [22]. Hence, choosing plants centred on ethnobotanical information might improve the prospect of innovation kinds with bioactivities. In line for extraordinary burden of multiple respiratory pathogens, multifactorial of treatment resilient abilities, around is an international appropriate imperative to determine and advance novel respiratory agents [24] ,that will respond to the needs of younger and elderly animals with poor immune system. Actually, the development of multi-drug resilient strains of pathogens results in the innovation of novel compound frameworks a precise critical pharmaceuticals significance [23].This is a modern determination directed at validating the therapeutic plants practise as conventional prescriptions aimed at respiratory infections, and wherever promising, to illuminate their antibacterial properties. These information recommend an prospect for developing fresh treatments since herbal and supplementary accepted yields to enhance the respiratory health in animals which in turn could benefits the human health systems.

2 METHODS AND MATERIALS

2.1 Plant collection and storage

The roots of *Elephantorrhiza elephantina*, leaves of *Aloe marlithii*, *Eucalyptus camaldulensis*, and *Euphorbia tirucalli*, and S*chotia brachpetala* were collected from Sekhukhune, Limpopo, South Africa under the consent of traditional leader with the traditional healer. Specimens taken to herbarium and plants marked with tickets confirmed the distinctiveness of the plants.

2.2 Plant material and extraction

Five hundred and fifty grams each of the five individual herbal were desiccated in the shade for 14 days, crushed into a bristly fine particles by means of a blender, and extracted through 100% acetone. They were filtered, rigorous on a rotary evaporator, and dried on a water bath. They remained then stored in a cool dry apartment until necessary for use.

2.3 Chemicals

Chemicals used in the assay were obtained from Highveld Biological, Johannesburg, South Africa and Doxorubicin was acquired from Pfizer, South Africa All organic solvents were of analytical grade and obtained from Sigma-Aldrich St. Louis, MO, USA. Müller Hinton agar and broth were from Sigma-Aldrich, India.

Table 1: Five plant species use in this study.

Name of plant	Common names	Family	Parts used	Traditional uses	
Elephantorrhiza elephantina	Elandsbean(Eng.),Elandsbontjie(Afr.),intolwane(isiXhosa/Zulu);Mositasane (Sepedi/Tswana)	Fabales	Roots	It is popular for the management of skin infections and acne [59].	**PRU0130632**
Aloe marlothii	Kgokgopha ya go ema(Sepedi)	Asphodelaceae	Leaves	It is used to treat various respiratory and urinary tract infection [58].	**PRU0130665**
Eucalyptus camadulensis	mopilikom(Sepedi);	Myrtaceae	Leaves	It can be medicinally used as a cure for skin cancer [60,61].	**PRU0130638**
Euphorbia tirucali	Motlhoko (Sepedi) pencil plant,rubber-hedge euphorbia(Eng.);kraalmelkbos (Afr.)	Euphorbiaceae	Leaves	In traditional medicine it is viewed as a cure for sexual ineffectiveness and an remedy for snakebite[58].	**PRU0130664**
schotia brachpetala Sond.	molope (Sepedi),	Fabaceae	Ground bark boiled in water	Bark and root utilized to clean blood, to cure nervous, heart conditions, diarrhoea and facial sores [60,61].	**PRU234371**

-: not reported, Eng: English, Afr: Afrikaans

2.4 Mycobacterium strains used in this investigation

The antimicrobial activities of the herbal abstracts were confirmed against gram-negative bacteria (Mycobacterium africanum ATCC 27853), gram-positive bacteria *Mycobacterium africanum* ATCC 29213 and (*Mycobacterium tuberculosis* ATCC 25177) *Mycobacterium smegmatis* and (*M. bovis* ATCC 27290) . Mycobacterium cultures were developed instantaneous in Mueller Hinton broth (Sigma Aldrich, SA) and accustomed to McFarland standard 1 .The Mycobacterium strains remained continuously preserved in nutrient agar at 4 °C .

2.5 Antimycobacterial activities

The Antimycobacterial activities of the five carefully chosen plants were considered by the determination of the minimum inhibitory concentration (MIC) of each plant autonomously and in combination using the micro-broth dilution assay described by Eloff [30] and Modifications of combinations.

2.6 Cytotoxicity evaluation of the plant extracts against Vero African green monkey kidney cells

The cytotoxicity assay was conducted using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay as described [33,34].The comparative safety of each abstract can be measured by means of the selectivity index.

2.7. Anti-inflammatory activity :Soybean lipoxygenase inhibition assay

The assay was performed according to a previously described procedure [37] with slight modifications.

2.8.1 Antibiofilm development

The technique of [36] was deployed to examine the plant extracts potential to inhibit development of microbial cell mass and attachment. The biomass was measured with the modified crystal violet staining technique of [35].

2.8.2 Inhibition of pre-formed biofilm

The capability of herbal abstracts to inhibit supplementary establishment and or obliteration of cell mass was also examined. The biofilm biomass was measured by means of the improved crystal violet staining assay [35].

2.8.3 Crystal violet staining assay

The technique of [36] was used for this assay, a modification of [35] .In brief, sterile distilled water was used to wash microtitre plates, air dehydrated and also oven dehydrated for 45 minutes in an oven set at 60 ^{0}C. A 100 µl of 1% crystal violet was used to stain the wells of the plate, incubated for 15 min and later, the plates were washed three times with sterile distilled water to get rid of unreactive stain. At this level, biofilm was observed as purple ring by the side of the wells. The measureable valuation of biofilm development was determined by adding 125 µl of ethanol, this is to remove the stain in the wells. A 100 µl aliquot of the ethanol was withdrawn to a new sterile plate and the absorbance was measured using a microplate reader at 590 nm. The average absorbance was determined for each sample, and their respective percentage inhibition of biofilm calculated using the formula below [36]:

$$\text{Percentage (\%) inhibition} = \frac{\text{ODNegative control} - \text{ODExperimental} \times 100}{\text{ODNegative control}}$$

2.9 Statistical analysis

Investigational outcomes were articulated as mean ± standard error of mean (SEM) of at minimum three repeats. IC50 standards for cytotoxicity experiments were resulting from a non-linear regression model (curve fit) founded on a sigmoidal dose response curve (variable) and computed using Graphpad Instat 6.0 software was used to analyse the data.

3.0 Results and discussion

Table 2:MIC of Plants

Individual and Synergy	***Mycobacterium tuberculosis***	***Mycobacterium africanum***	***Mycobacterium smegmatis***	***Mycobacterium* bovis**
A	0.63	0.16	0.08	0.63
A+B	0.16	0.02	0.16	0.02
B	1.3	0.16	0.16	1.3
C+B	0.02	0.02	0.02	0.02

C	0.313	0.63	0.63	0.313
C+D	0.02	0.02	0.02	0.02
D	0.63	0.63	1.3	0.63
E+D	0.04	0.02	0.04	0.02
E	0.035	0.16	0.63	0.04
E+C	0.02	0.02	0.02	0.02
E+B	0.02	0.02	0.02	0.02
E+C+B	0.02	0.02	0.02	0.02
E+C+D	0.03	0.04	0.02	0.02
C + A + D	0.02	0.02	0.04	0.02
B+ C + D+E	0.02	0.04	0.04	0.04
Streptomycin	0.16	0.02	0.16	0.02
Rifampicin	0.02	0.01	0.02	0.01
Isoniazid	0.63	0.04	0.63	0.04

A: *Elephantorrize elephantina*,B: *Aloe marlothi* ,C: *Eucalyptus camadulensis* ,*D: Euphorbia tirucali* ,E: *Schotia brachpetala*

3.1 Anti-mycobacterial MIC of the crude extracts

The herbal types examined were designated established on their traditional practice to pleasure tuberculosis and breathing infections in southern Africa. There were a widespread variant in activities of the abstracts in contrast to both fast-growing and pathogenic Mycobacterium species with MIC ranging from 0.02 to 1.3 µg/mL. The MIC of the extracts was determined for their antimycobacterial activities using Isoniazid, Streptomycin, Rifampicin, as an indicators for *Mycobacterium species* strains viability in 96-well microplates. The MIC of acetone extracts of plants, ranged from 0.02–1.3 µg/mL for all *Mycobacterium species* and 0.01-0.63 µg/mL, respectively for indicators . The exploration exhibited that leaves of *Schotia brachpetala* extract was the most active against both *M .smegmatis* and *M. bovis* strains (MIC 0.63-and 0.04 µg/mL). The mean MIC results of crude extracts of most plant showed significantly lower antimycobacterial activities in comparison to *rifampicin* for all strains (Table 2). Although some extracts were found less active than *Streptomycin* and *Isoniazid*, extracts of leaves of *A.marlothii* and *Schotia brachpetala* displayed remarkable antimycobacterial activities. Nevertheless, reference strain of *M. smegmatis* and *M. bovis* strains were reasonably the greatest vulnerable strains to the abstracts of the investigation acetone with MIC ≤ 50 µg/mL. Taking into account the MIC cut off for plant extracts proposed by [39,40], the activities documented for the abstract of all could be measured as noteworthy and for the other abstracts measured as reasonable to inactive.

Natural herbs stay to show a pronounced imperative part of medicine finding and improvement of extremely dynamic antimycobacterial metabolites and they can be castoff as clean combinations or as basic ingredients [42]. In various African countries and around the word countless curative floras have existed which are cast-off in the management of bacterial contagions with TB. It is our curiosity to data the anti-TB action of the herbal subsequently these herbal are described after societies to pleasure TB and other respiratory diseases especially during this time of the covid-19 pandemic. During ethnobotanical survey these herbal ensured remained described to have anti-TB activities. In these present study, the basic abstracts of leaves of *Schotia brachpetala* presented favourable antimycobacterial activities against all strains. Although the antibacterial activities of *Schotia brachpetala* had remained described against other

numerous pathogenic bacteria [44], no information was found throughout a literature exploration against mycobacterium strains apart from ethnobotanical information on these plants. Consequently, this exploration would be the most comprehensive account on their anti-mycobacterial accomplishments. The basic abstracts of leaves of *E. camaldulensis* showed the antimycobacterial activities against both *M. smegmatis* and *M. bovis* strains. This information come to an agreement with the aforementioned statement of hexane, chloroform, methanol extracts of E. camaldulensis [45,46].In the current effort establishes antimycobacterial possessions of these herbal having curative worth in the management of TB. Nonetheless, it is usually predictable that the more antimycobacterial activity of abstracts be influenced by on their lipophilic nature and exhibited improved activities [43,47].

Everything the established herbal abstracts had MIC values ranging from 0.02 to 1.3 mg/ml against *Mycobacterium tuberculosis* and *Mycobacterium africanum*. The antibacterial activities of the five plant extracts in given in (Tables 2), results showed that all plants extracts were active against the *Mycobacterium tuberculosis* and *Mycobacterium africanum* . In another study by [60] it was found that water extracts prepared from *E. elephantina* (stem rhizome) and *Elephantorrhiza burkei* (stem rhizome) had MIC values of 0.10 mg/ml against *Mycobacterium tuberculosis* which is improved activity associated to that of the root abstracts in the current study (Table 2).

Table 3 : Fractional inhibitory concentration indexes of the 1:1 combinations of the selected plants

Synergy		*Mycobacterium tuberculosis*	*Mycobacterium africanum*	***M.smeg***	**M.bovis**
1	A+B	0.337	0.25	3	0.047
2	E+D	2,0667	0.157	0.6608	0.532
3	E+C	1.064	0.1567	0.0635	0.5639
4	C+B	0.0793	0.1567	0.1567	0.0793
5	E+B	1.0154	0.25	0.1567	0.5154
6	C+D	0.0956	0.0635	0.0471	0.0956

A: *Elephantorrize elephantina*, B: Aloe marlothi ,C: *Eucalyptus camadulensis* , *D: Euphorbia tirucali* ,E: *Schotia brachpetala*

3.2. Synergistic effect of antimycobacterial

The combination of different plants of acetone extracts combined in a 1:1:1 combination potent activities were observed when *Aloe marlothii* or *Euphorbia tirucali* was combined with *Eucalyptus camaldulensis* and *Schotia brachpetala* (combination E+C+B and E+C+D) against *M. smegmatis* and *M.bovis* showed excellent activity with MIC value of 0.02mg/mL, followed by the C + A + D extracts of the same plants (MIC 0.02 and 0.04 mg/ml),and last B+ C + D+E with MIC value (0.04 mg/ml) was also prove to be more wastefully according to experience healer. A+B , E+D, E+C, C+B, E+B and C + D activity was ranging from MIC 0.02 mg/ml was shown with the different combination of extracts of the selected plants while the lowest activity was obtained at MIC 0.16 mg/ml . Synergistic effects were exhibited against ***M.smeg*** with a 0.0635, 0.157, 0.1567, 0.1567 and 0.0471FIC index values for the E+C, C+B, E+B and C + D combinations, respectively. Synergistic effects were exhibited against ***M. bovis*** with a 0.047 , 0.07930 and 0.0471 FIC index values for the A+B, C+B and C + D combinations, respectively. Meanwhile, the antagonistic effects were never observed against any mycobacterial tested (Table 4).

Aloe marlothii or Euphorbia tirucali was combined with *Eucalyptus camaldulensis* and *Schotia brachpetala* (combination E+C+B and E+C+D) against *Mycobacterium tuberculosis* and *Mycobacterium africanum* (0.02,0.03 and 0.04 mg/mL). Meanwhile, the combination with Eucalyptus *camaldulensis*, *Elephantorrize elephantina*, and *Euphorbia tirucali* (C + A + D) exhibited potent activities against *Mycobacterium tuberculosis* *Mycobacterium africanum* (0.02 mg/mL) (Table 2). When all the selected plants were combined (combination A + B+ C + D) the efficacy against all the against *Mycobacterium tuberculosis* and (0.02 mg/mL) was enhanced with average MIC values lower than the MIC values of the plants independently except *Mycobacterium africanum* with 0.04 mg/mL. Streptomycin was used as positive control and its MIC values ranged from 0.02 to 0.04 mg/mL. Using ANOVA test (one way ANOVA), the mean difference between the MIC values of some of the acetone extracts combination (A+D, E+D, E+C, C+B, E+B and C+D against all tested pathogens was statistically significant ($p < 0.05$).The Fractional Inhibitory Concentration (FIC) values were calculated as outlined above for the 1:1 combinations to establish any synergistic or antagonistic interactions. Synergistic effects were exhibited against *Mycobacterium africanum* with a 0.25, 0.157, 0.1567, 0.1567, 0.25 and 0.0635 FIC index values for the A+B , E+D, E+C, C+B, E+B and C + D combinations, respectively. Synergistic effects were exhibited against *Mycobacterium tuberculosis* with a 0.337, 0.0793 and 0.0956 FIC index values for the A+B , C+B and C + D combinations, respectively. Meanwhile, the antagonistic effects were not observed against pathogen tested (Table 4).

3.3 Cytotoxicity

Table 4: The cytotoxicity (presented as Inhibitory Concentration 50%, IC50) and selectivity index results of extracts

	IC50(mg/ml)	**Selective Indexs**			
Plant Species	**Cytotoxicity**	***Mycobacterium tuberculosis***	***Mycobacterium africanum***	***M.smeg***	**M.bovis**
Elephantorrize elephantina	**0.4164**	0.6609	2.6025	5.205	0.6609
Aloe marlothii	**0.053**	0.0407	0.331	0.331	0.0407
Eucalyptus camadulensis	**0.0365**	0.1166	0.0579	0.0579	0.1166
Euphorbia tirucali	***0.08369***	0.1328	0.1328	0.0644	0.1328
Schotia brachpetala	**0.348**	17.4	2.175	0.552	8.7
Doxorubicin	**0.005**	-	-	-	-

Therapeutic plant abstracts future for practise in medical uses necessity not partake a main result on the host cell or interfere with its ordinary physiological pathway. The extracts should be selectively toxic to the under attack bacteria or interfere directly with a precise response pathway, and this must be done without distressing the host cell or the regular physiological pathway [39,57]. The cytotoxicity of the acetone extracts of the selected plants ranged from < 36.5 to 416.40 µg/mL. Therefore, analysis of the results for the present study were interpreted as follows: highly toxic for IC50 values < 30 µg/mL, moderately toxic 30 µg/mL < IC50 ≤ 10 µg/mL and non-toxic IC50 > 10 µg/mL. Altogether extract was nontoxic to the Vero cells with the lowest LC50 (<36.5 µg/mL) that is within the cut-off point (Table 3). Compared to doxorubicin, no obvious cytotoxic activity was detected from the five plants extracts tested (Table 3). The LC50 values varied between 36.5 µg/mL and 416.40 µg/mL. Compared to doxorubicin

(LC50 of 4.51 µg/mL), all the plant extracts screened could be considered as relatively safe. It necessity be taken into thoughtfulness that there are restrictions when comparing the results between *in vitro* and *in vivo* studies. The restrictions are subjective by diverse circumstances contained by the two systems [48]. It was noteworthy to observe that *Elephantorrize elephantina* extract had the highest IC50 value (416.10 µg/mL) out of all the extracts tested against the Vero cells which means it was relatively non cytotoxic.The plant extracts in the present study had low selectivity index values ranging from 0.0407 to 17.4 (Table 3). Many plant extracts had SI values below 10 but it is promising that many had SI values above 1 which means that their biological activity was higher than their cellular toxicity. Consequently, the sophisticated the discrimination index is for a basic extract, the more likely it is that the activity is not due to a general metabolic toxin [53]. Nonetheless it is also imperative to note that cytotoxicity in *vitro* is not continually establish in vivo, since some complexes may be exposed to metabolic alteration within the natural system subsequent in less toxic products [50] , and the converse may also be true where more toxic chemicals are twisted as a result of metabolic activity. [46-50] Although the removal of lethal mechanisms by management of the abstract may produce supplementary appropriate antibacterial abstracts [54], the medley of an abstract with a high discernment index escalations the possible that a beneficial herbal medicine can be produced.

3.4 Anti-inflammatory activity

Table 5: Anti-inflammatory activity

Plants Extract	**IC_{50} (µg/ml)**
Elephantorrize elephantina	22.88
Aloe marlothi	156.8
Eucalyptus camadulensis	586.2
Euphorbia tirucali	37.9
schotia brachpetala	69.61
Curcumin	65,74

The herbal extracts inhibited Reactive oxygen species in a quantity dependant manner. The inhibition was higher in *schotia brachpetala ,Eucalyptus camaldulensis, Aloe marlothi*. Curcumin was castoff as a optimistic control only three plants had better anti-inflammatory potential than curcumin at the highest concentration tested. Effective activities were detected in *Eucalyptus camaldulensis* and *schotia brachpetala* even at the lowest concentration established. It is now thought that a slight stability amongst pro- and anti-inflammatory machineries in the body is mandatory to endorse retrieval and preserve function. Inflammation is essential in primary phases as the form protection system against external abuses such as infection or injury. Nevertheless, protracted and unrestrained inflammation can lead to undesirable effects such as cell loss and tissue modifying which may further contribute to adverse physiological effects such as deteriorated infection development and pain [11-13].

3.5.1 Prevention of cell attachment: antibiofilm activity/ antiadhesion

In this investigation, the activities of the herbal abstracts were confirmed against the biofilms of the *Staphylococcus epidermidis* microorganisms class. The result of acetone crude abstracts on the attachment and inhibition of biofilm formation is given in Table 5.The plant extracts had varying degrees of activity on the prevention of attachment. All the plants in this study inhibited attachment of *Staphylococcus epidermidis* by over 50% indicating a good anti-attachment property. The study by [60] showed that

schotia brachpetala seeds extract have a selective inhibitory action against *S. aureus and Staphylococcus epidermidis* biofim strains as well as a potential role as a new antibiofilm agent. In a recent study by [60] also showed that *schotia brachpetala* seeds are rich in polyphenols, with caffeoyltyramine and cannabisin A, B, and C being the main components of the polyphenolic fraction [59]. The influence of 0.5 mg/ml *schotia brachpetala* seeds abstract on the biofilm development by *Staphylococcus aureus*, which has been revealed to form tough biofilms [58], has been also investigated. The results in this study showed that *Eucalyptus camaldulensis* has the ability to inhibit the formation of biofilm in *Staphylococcus epidermidis* strains is in agreement [59]. The extracts showing the presence of flavonoids and sterols from the Leaves and fruits of *Eucalyptus camaldulensis*, which have been reported to bind to and inhibit matrix formation [57].Nowadays, there is no biofilm targeting therapy in the market so the best strategy is to avoid the training instead of trying to eliminate them after their graduation [57].The study show that the synergy of the medicinal plants provide by the farmers and traditional healers are a promising therapy.Biofilm results indicated that *Aloe marlothii* that were active biofilms at inhibition of 61.6% after 72h which indicate its ability to inhibited preformed biofim [57]. The brine shrimp lethality assay results revealed that *Aloe marlothii* were non-toxic to the brine shrimps [48].

Table 6: Individual and Synergy Inhibition of attachment and pre-formed biofilms

Individual and Synergy	**ATTACHMENT**	**PRE-FORM**
A	73	56.832
E+C	124.687	97,4783
B	91,9308	59,861
C+B	130,564	89,861
C	67.93669	43,669
E+B	143.987	97,936
D	86,6823	65,936
C+D	142.456	95,936
E	67	62,321
E+C+B	156.789	123.345
A+C+D	167.45	130.987

A: *Elephantorrize elephantina*,B: *Aloe marlothii*,C: *Eucalyptus camadulensis, D: Euphorbia tirucali*, E: *Schotia brachpetala*

3.5.2 Inhibition of development of pre-formed biofilms

The activities of all plant extracts on further biofilm development in 24 and 48 h preformed biofilms are presented in (Table 6). Of the five plant extracts evaluated, four plant extracts reduced *Staphylococcus epidermidis* biofilm biomass at 24 h post-development (Table 6). All of these Five had percentage inhibition values above 50%. After 48 h pre-formed biofilm, *Eucaluptys camaldulensis* extracts had poor antibiofilm activity less (50%) with 43% while all of them prevented biofilm development by over 50% while the remaining extracts showed enhancement of biofilm. Wholly of the herbal extracts was able to destroy the biofilm of *Staphylococcus epidermidis*.

4.0 Conclusion

The plant species *Elephantorrize elephantina* , *Aloe marlothii* ,*Eucalyptus camadulensis*, *Euphorbia tirucali*, and *schotia brachpetala* have complexes with antiinfametory activities, allusive of their significance in the ethnoveterinary that may build up curative in a disease condition. Future directions should be considered combining the plants best combination and different drugs from the market with the objective of increasing innovative and nontoxic healing agents for the management of human and animal infections caused by Mycobacterium species and possible testing against corona as is the threat in the respiratory disease management.

5.0 Future prospects

The situation can in future be further complicated by the potential spill over from wild animals to livestock with corona virus pandemic worsening matter . Many Bapedi farmer use simple statistic to predict the livestock TB by observing the nearest human TB cases and relate to animals or expect outbreak of bovine TB.

References

1. WHO. Available from: http://www.who.int/en/ [cited 2017 28.08.17].
2. Gralton J. The role of particle size in aerosolised pathogen transmission: a review. J Infect .2011, 62:1-13.
3. WHO **Global Tuberculosis Report 2020**
4. World Health Organisation, “Multidrug resistant tuberculosis (MDR- TB)”, Update, 2015.
5. Choi YK, Goyal SM, Joo HS. Retrospective analysis of etiologic agents associated with respiratory diseases in pigs. Can Vet J. 2003,44(9):735–7.
6. Abuzeid N, Kalsum S, Koshy RJ, Larsson M, Glader M, Andersson H, Lerm M.2014. Antimycobacterial activity of selected medicinal plants traditionally used in Sudan to treat infectious diseases. J. Ethnopharmacol. 157, 134–139.
7. Alvin A, Miller KI, Neilan BA. Exploring the potential of endophytes frommedicinal plants as sources of antimycobacterial compounds. Microbiol. Res. 2014,169 (7), 483–495.
8. Boligon AA, Piana M, Kubic TF, Mario DN, Dalmolin TV, Bonez PC,Athayde, ML. HPLC analysis and antimicrobial, antimycobacterial andantiviral activities of Tabernaemontana catharinensis A. DC. J. Appl. Biomed. 2014. 13(1), 7–18.
9. UcCachón AH, Borges-Argáez R, Said-Fernández S, Vargas-Villarreal J,González-Salazar F, Méndez-González M, Molina-Salinas GM.Naphthoquinones isolated from Diospyros anisandra exhibit potent activityagainst pan-resistant first-line drugs Mycobacterium tuberculosis strains. Pulm.Pharmacol.Ther. 2014.27 (1),114–120.
10. Green E, Samie A, Obi CL, Besson PO, Ndip RN. Inhibitory propertiesof selected South African medicinal plants against Mycobacterium tuberculosis.J. Ethnopharmacol. 2010. 130 (1), 151–157.
11. McGaw LJ, Lall N, Meyer JJM, Eloff JN. The potential of South African plants against Mycobacterium infections. J. Ethnopharmacol. 2008. 119 (3), 482–500.
12. Kumar P, Singh A, Sharma U, Singh D, Dobhal MP, Singh S. Antimycobacterial activity of plumericin and isoplumericin against MDRMycobacterium tuberculosis. Pulm. Pharmacol. Ther. 2013. 26 (3), 332–335.

13. Koul A, Arnoult E, Lounis N, Guillemont J, Andries K. The challenge ofnew drug discovery for tuberculosis. Nature. 2011. 469 (7331), 483–490.
14. Zumla A, Nahid P, Cole ST. Advances in the development of new tuberculosis drugs and treatment regimens. Nat. Rev. Drug Discov. 2013. 12 (5),388–404.
15. Dawoud MEA, Mawgoud YA, Gouda Dawoud TM. Synergistic interactions between plant extracts, some antibiotics and/or their impact upon antibiotic- resistant bacterial isolates". Afr J Biotechnol. 2013;12(24):3835–46.
16. . Ncube B, Finnie JF, Van Staden J. In vitro antimicrobial synergism within plant extract combinations from three South African medicinal bulbs. J Ethnopharmacol. 2012.139(1):81–9.
17. Tijjani, "Antibiotic- Plant Synergy as a New Strategy for Combating Drug Resistant Bacteria", Microbial pathogens and strategies for combating thm: science,technology and education, 2013. 834–837.
18. Parekh J, Chanda S. In vitro antibacterial activity of the crude methanol extract of Woodfordi Fruticosa kurz. Flower (lythraceae). Braz J Microbiol. 2007,38:204–7.
19. Chung PY, Navaratnam P, Chung LY. Synergistic antimicrobial activity between pentacyclic triterpenoids and antibiotics against Staphylococcus aureus strains. Ann Clin Microbiol Antimicrob. 2011.10:25–30.
20. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med. 1998.64:711–3.
21. van Vuuren SF, Viljoen AM. In vitro evidence of phyto-synergy for plant part combinations of Croton gratissimus (Euphorbiaceae) used in African traditional healing. JEthnopharmacol. 2008.119:700–4.
22. Mosmann T.Rapid colorimetric assay for cellular growth and survival - application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983. 65(1-2):55-63.
23. Djordjevic D, Wiedmann M, Mclandsborough L. Microtiter plate assay for assessment of Listeria Monocytogenes biofilm formation. Appl Environ Microbiol. 2002.68(6):2950–8.
24. Sandasi M,Leonard C,Viljoen A.The effect of five common essential oil components on Listeria Monocytogenes biofilms. Food Microbiol.2008.19(11):1070–5.
25. Pinto MDC, Tejeda A, Duque AL, Macias P, Determination of lipoxygenase activity in plant extracts using a modified ferrous oxidation-xylenol orange assay. Journal of Agricultural Food and Chemistry 55, 5956-5959,2007.
26. McGaw LJ, Lall N, Meyer JJM, Eloff JN. The potential of South African plants against Mycobacterium infections. Journal of Ethnopharmacology.2008b.119, 482–500.
27. Kuete V. Potential of Cameroonian plants and derived products against microbial infections: a review. Planta Medica.2010.76, 1479–1491.
28. Eloff JN. Quantification the bioactivity of plant extracts during screening and bioassay guided fractionation. Phytomedicine.2004.11, 370–371.
29. Guzman JD, Gupta A, Evangelopoulos D, Basavannacharya C, Pabon LC,Plazas EA. Anti-tubercular Screening of Natural Products from Colombian Plants: 3-Methoxynordomesticine, an Inhibitor of MurE Ligase of Mycobacterium tuberculosis. J Antimicrob Chemother .2010.65:2101–2107.
30. Lawal TO, Adeniyi B, Wan AB, Franzblau SG, Mahady GB: In Vitro Susceptibility of Mycobacterium tuberculosis to Extracts of Uvaria Afzelli Scott Elliot and Tetracera Alnifolia Willd. Afr J Biomed Res. 2011. 14:17–21.

31. Mann A, Ibrah-im K, Oyewale AO, Amupitan JO, Okogun JI. Antimycobacterial activity of Some Medicinal Plants in Niger State, Nigeria. African J Infect Dis 2009.3:44–48.
32. Lawal TO, Adeniyi BA, Idowu OS, Moody JO: In vitro activities of Eucalyptus camaldulensis Dehnh and Eucalyptus torelliana F. Muell against Nontuberculous Mycobacteria Species. Afr J Microbiol Res. 2011. 5:3652–3657.
33. Lawal TO, Adeniyi BA, Adegoke AO, Franzblau SG, Mahady GB. In vitro Susceptibility of Mycobacterium tuberculosis to extracts of Eucalyptus camaldulensis and Eucalyptus torelliana and Isolated Compounds. Pharm Biol .2012. 50:92–98.
34. Coelho TS, Cantos JB, Bispo MLF, Gonçalves RSB, Lima CHS, da Silva PEA.In vitro anti-mycobacterial activity of (E) - N' (monosubstitutedbenzylidene) isonicotinohydrazide derivatives against isoniazid- resistant strains. Infect Dis Rep 2012, 4:e13.
35. Mcgaw LJ, elgorashi EE, Eloff JN. 2014. Cytotoxicity of African
36. Medicinal Plants Against Normal Animal and Human Cells. *In:* KUETE, V. (ed.) Toxicological Survey of African Medicinal Plants. Elsevier.
37. Rivetti C, Allen TEH, Brown JB, Butler E, Carmichael PL, Colbourne JK. Vision of a near future: Bridging the human health-environment divide. Toward an integrated strategy to understand mechanisms across species for chemical safety assessment. Toxicol. In Vitro. 2020. 62:9. doi: 10.1016/j.tiv.2019.104692.
38. Mena-rejon G, Caamal-fuentes E, Cantillo-ciau Z, Cedillorivera R, Flores-guido J, Moo-puc R. In vitro cytotoxic activity of nine plants used in Mayan traditional medicine. Journal of Ethnopharmacology. 2009.121**,** 462-465.
39. WHO, WHO Global Task Force on TB Impact Measurement. World Heal Organ(2019)https://www.who.int/tb/areas-of-work/monitoring-evaluation/impact_measurement_taskforce/en/
40. Eloff JN,Famakin JO,Katerere DRP. Combretum woodii (Combretaceae) leaf extracts have high activity against Gram-negative and Gram-positive bacteria. African Journal of Biotechnology.2005. 4,1161–1166.
41. Fennell CW, Lindsey KL,McGaw LJ, Sparg SG, Stafford GI, Elgorashi EE, Grace OM,Van Staden J. Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. Journal of Ethnopharmacology. 2004. 94, 205–217.
42. York T, De Wet H, Van Vuuren SF. Plants used for treating respiratory infections in rural Maputaland, KwaZulu-Natal, South Africa. Journal of Ethnopharmacology.2011.135, 696–710.
43. Van Wyk B.-E, Van Oudtshoorn B, Gericke N. Medicinal plants of South Africa. Pretoria, Briza.2009.
44. Van Wyk B.-E. The potential of South African plants in the development of new medicinal products. South African Journal of Botany. 2011.77: 812-829.
45. Cicchese JM, Evans S, Hult C, Joslyn LR, Wessler T, Millar JA. Dynamic balance of pro- and anti-inflammatory signals controls disease and limits pathology. Immunol Rev. 2018,285(1):147–67. https://doi.org/10.1111/imr.12671 PMID: 30129209.
46. Keane MP, Strieter RM. The importance of balanced pro-inflammatory and anti-inflammatory mechanisms in diffuse lung disease. Respir Res. 2001, 3(1):5. https://doi.org/10.1186/rr177 PMID:11806840.
47. Musolino C, Allegra A, Innao V, Allegra AG, Pioggia G, Gangemi S. Inflammatory and anti-Inflammatory equilibrium, proliferative and antiproliferative balance: the role of cytokines in multiple

myeloma. Mediators Inflamm. 2017; 2017:1852517. Epub 2017/11/02. https://doi.org/10.1155/2017/1852517 PMID: 29089667; PubMed Central PMCID: PMC5635476.
48. Mantovani A,Allavena P, Sica A, Balkwill F. Cancerrelated inflammation, Nature, 454: (7203) 436–444,2008.
49. Khunoana ET, Madikizela B, Erhabor J, Nkadimeng SM, Arnot LF, Van Wyk I, McGaw LJ. A survey of plants used to treat livestock diseases in the Mnisi community, Mpumalanga, South Africa, and investigation of their antimicrobial activity.South Afr. J. Bot.(2019)
50. Bavington C, Page C. Stopping bacterial adhesion: a novel approach to treating infections. Respiration. 2005.72(4):335–44
51. Tiwari M, Donelli G, Tiwari V. Strategies for combating bacterial biofilms: a focus on anti-biofilm agents and their mechanisms of action au - Roy, Ranita. Virulence. 2018.9(1):522–54.
52. Frassinetti S,Moccia E,Caltavuturo L, Gabriele M, Longo V, Bellani L. Nutraceutical potential of hemp (Cannabis sativa L.) seeds and sprouts. Food Chemistry.2018. 262, 56–66.
53. Frassinettia S,Gabrielea M,Mocciaa E,Longoa V, Di Gioiab D. Antimicrobial and antibiofilm activity of *Cannabis sativa* L. seeds extract against *Staphylococcus aureus* and growth effects on probiotic *Lactobacillus*spp. LWT - Food Science and Technology 124. 2020.109149.
54. Nostro A, Guerrini A, Marino A, Tacchini M, Di Giulio M, Grandini A. In vitro activity of plant extracts against biofilm-producing food-related bacteria. International Journal of Food Microbiology.2016. 238, 33–39.
55. Abidi SH, Ahmed K, Sherwani SK, Bibi NU, Kazmi S,Kazmi SU. Detection of Mycobacterium smegmatis biofilm and its control by natural agents. International Journal of Current Microbiology and Applied Sciences. 2014. 3, 801–812.
56. Slee A, Brooker MIH, Duffy SM, West JG. Centre for Plant Biodiversity Research; River Red Gum. *Eucalyptus camaldulensis var. obtusa*.2006.
57. Selim SA, Adam ME,Hassan SM, Albalawi AR. Chemical composition, antimicrobial and antibiofilm activity of the essential oil and methanol extract of the Mediterranean cypress (*Cupressus sempervirens* L.). BMC Complement and Alternative Medicine. 2014.14: 179.
58. Shirinda H, Leonard C, Candy G, Van Vuuren S. Antimicrobial activity and toxicity profile of selected southern African medicinal plants against neglected gut pathogens. S Afr J Sci. 2019.115(11/12.https://doi.org/10.17159/ sajs.2019/6199.
59. Sandasi M, Leonard C, Van Vuuren S, Viljoen A. Peppermint (*Mentha Piperita*) inhibits microbial biofilms In Vitro. S Afr J Bot. 2011.77(1):80–5.
60. De La Fuente-Núñez C, Reffuveille F, Fernández L, Rew H. Bacterial biofilm development As a multicellular adaptation: antibiotic resistance and new therapeutic strategies. Curr Opin Microbiol. 2013.16(5):580–9.
61. Jamal M, Ahmad W, Andleeb S, Jalil F, Imran M, Ma N, Hussain T, Ali M, Rafiq M, Ma K. Bacterial biofilm and associated infections. J Chin Med Assoc. 2018.81(1):7–11.