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An *in vitro* Antibacterial Activity Test of *Phytolaca dodecandra, Adhatoda schimperiana* and *lepidium sativum* Against Two Major Skin Bacterial Pathogens of Equine

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Abstract: The present experimental study was carried out from November 2018 to April 2019 to evaluate *in vitro* antibacterial activity of *Phytolaca dodecandra*, *Adhatoda schimperiana* and *Lepidium sativum* plant extracts against *Streptococcus equi* and *Corynebacterium pseudotuberculosis*. The selected plants used in the study were harvested from Kombolcha. Equines affected with strangles and ulcerative lymphangitis were purposively selected to obtain appropriate samples for bacterial culture. A total of 11 nasal swabs or draining abscess from lymph nodes was cultured for bacterial growth. In the present study, leaf, seed and root extracts of *P. dodecandra*, leaves of *A. schimperiana* and seeds of *L. sativum* were collected and extracted by maceration method using 70% ethanol at Aklilu Lemma Institute of Pathbiology (ALIPB). Three different concentrations of each extract with two replicates were prepared using Dimethylsulfoxide (DMSO). Preliminary screening test for the extract was made using agar well diffusion method. Muller Hinton agar (38 gm) medium was used for antimicrobial sensitivity test. A wider Zone of inhibition was demonstrated by ethanol extract of *L.sativum* leaf against *C. pseudotuberculosis* and *S. equi* that were 19.46±2.51 mm and 13.15±0.54 mm at 200mg/ml, respectively. However, there was no significant (P>0.05) difference in the zone of inhibition at this concentration. The leaf of *P.dodecandra* against *S. equi* showed second wider zone of inhibition 18.45±1.83 mm at 200mg/ml concentration, that was closely followed by *L. sativum* at 100 mg/ml and 50 mg/ml concentrations (17.67±2.99mm and 15.36±2.60mm respectively). The seed of *P.dodecandra* exhibited an inhibition zone of 12.65±0.68 against *S.equi* at 200mg/ml concentration. This study revealed that all the three selected plants exhibited antibacterial activity against different pathogenic bacterial strains. However, further large scale experimentals studies using different solvent and standard medium is highly recommended.

Key words: Antibacterial · *C. Pseudotuberculosis* · *In Vitro* · Plant Exracts · *S. Equi*

INTRODUCTION

The equine population of the world is currently reported to be about 112.5 million of which 44.3 million donkeys, 58.5 million horses and the remaining are mules [1]. More than 97% of donkeys are found in developing countries specifically kept for work. Working animals provide an essential transport resource in developing countries worldwide [2]. Ethiopia possess 2.3 million horses, 8.4 million donkeys and 0.49 million mules. According to this estimate, Ethiopia has the largest donkey population in the world, representing over 17% of the world and 37.4% of the African donkey population [3].

Equines have a prominent position in the county's agricultural and transport systems since the transportation activities are performed by Equids [4]. They are mainly used as draught and pack animals and they are also used for ploughing in some parts of the country [5]. Despite all these uses, they are suffering from several diseases such as Epizootic lymphangitis (EL), African horse sickness, Strangles, Ulcerative lympangitis, dourine, rabies, anthrax, horse mange and Babesiosis [6]. Among these, Strangle seems the common in Ethiopia. Strangle is one of the most common equine respiratory diseases caused by a bacterium *Streptococcus equi* subspecies equi. The drugs of choice for strangle is

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penicillin either as procaine penicillin or potassium (Sodium) penicillin [2].

Ulcerative lymphangitis, or pigeon fever, is another a bacterial infection of the lymphatic vessels of the skin in horses, cattle, sheep and goats. *Corynebacterium pseudotuberculosis* is the classical cause of the disease. In ulcerative lymphangitis due to *C. pseudotuberculosis*, edema and fibrosis may cause lameness and deformity of the limbs. Local treatment of ulcers is a common and effective practice for only restricted lesions. However, parenteral injections of penicillin or tetracycline are necessary in severe cases.

Antibiotic resistance has become a global problem. Antibiotics have been of value in controlling many infections, but they depend on judicious use to minimize the incidence of resistance forms [7]. The worldwide problem of antibiotic resistance impacts negatively on antibiotic therapy thus making successful empiric therapy much more difficult to achieve. The emergence of drug resistance is an evolutionary process that is based on selection for organisms that have an enhanced ability to survive and reproduce in the presence of a drug [8].

In Ethiopia, traditional medicine has played a significant role in treating health problems in both livestock and humans. Knowledge of medicinal plants of Ethiopia and their uses provides vital contribution to human and livestock health care needs throughout the country [9]. In Ethiopia, plant remedies are still the most important and sometimes the only sources of therapeutics for nearly 80% of human and more than 90% of livestock population. Estimated floras of 6500 to 7000 species of higher plants are of medically important and out of these medicinal plants 12% are endemic to Ethiopia [10].

In Ethiopia, little emphasis has been given to ethnobotanical studies over the past decades, even if there has been some attempt in investigating medicinal plants and indigenous knowledge on sustainable use and management of plant resources. Many treatment types have been tried, but it was without success [11]. Among different traditional medicinal plants, *Phytolacca dodecandra, Lepidium sativum and Adhatoda schimperiana* are listed out in most literature. *Lepidium sativum* (*L. Sativum*) is a fast growing edible herb which belongs to family Brassicaceae. The genus Lepidium comprises several species growing in moist temperate warm climate. It contains volatile essential aromatic oils, active principle and fatty oils and carbohydrate, protein, fatty acid, vitamin C and vitamin B-complex, flavonoids, isothiocynates [12]. However, limited experimental evidence is available on antibacterial activity of extracts from these three plants against *S. equi* and *C. pseudotuberculosis* which are major skin bacterial pathogens of equines and thus the current study aimed to evaluate the antibacterial effect of the extracts from *phytolaca dodecandra*, *Adhatoda schimperiana* and *Lepidium sativum* against *streptococcus equi* and *Corynebacterium pseudotuberculosis* in Equine.

MATERIALS AND METHODS

Study Area: The three plant materials were harvested from Kombolcha and nearby wereda, South Wollo, Amhara Regional State, Ethiopia. The experimental investigation was carried out in Addis Ababa University College of Veterinary Medicine and Agriculture, Bishoftu, Ethiopia. Bishoftu is located 45 Kms south east of Addia Ababa. The area is located at 9°N latitude and 40°E longitudes at an altitude of 1850 masl. It has an annual rainfall of 866 mm. The mean annual maximum and minimum temperatures are 26°C and 14°C respectively with mean relative humidity of 61.3% [13].

The Study Design: The study design was an experimental design of in vitro anti-bacterial activityof extract from selected medicinal plants, namely *A. Schimperiana* (local name sensel), *L. Sativum* (local name feto) and *P. dodecandra* (local name endod) against *S. equi* subspecies equi and *C. pseudotuberculosis*. Investigation of plants were carried out in Kombolcha and neighbouring wereda based on past studies and their traditional uses were to treat infections. Finally, the antibacterial activity of ethanol extract inhibition zone were compared with standard.

Plant Materials Used for the Study: P. dodecandra (Endod" in Amharic), the bark leaves, roots and seed and leaves *of A. schimperiana* and seeds of *L. sativum* were used to assess the antibacterial effect on the two chosen bacteria.

Sample Collection and Test Bacterial Isolation: Equines affected with strangles and ulcerative lymphangitis were purposively selected for sampling of recommended appropriate samples. Samples were collected from clinical cases of strangles and ulcerative lymphangitis in donkey or horse presented to SPANA Veterinary Clinic and Donkey Health and Welfare Project (DHWP) at CVMA (college of veterinary Medicine and agriculture) for

treatment and open wound management. Bacterial species, *S. equi subspecies equi* and *C. Pseudotuberculos* were isolated from strangles and ulcerative lymphangitis cases..These organisms were selected based on its disease burden and increasing trend of antibiotic resistance in the developing countries. Before sample collection, the animals were restrained and the skin around the lesion was disinfected using cotton wool soaked in 70% alcohol to avoid any extraneous contamination. Nasal swabs or draining lymph nodes (swabs or material obtained from draining abscesses) were cultured for bacterial growth.

The isolation and identification of the bacterium was based on cultural examination, microscopic morphology and biochemical reactions. The collected swabs were streaked on plates of blood agar enriched with 5-7% sheep blood by sterile inoculation loop. The plates were incubated at 37°C for 24-48 hr. After incubation, cultures were examined for significant growth.Gram stained smears revealed gram positive cocci which were arranged in pairs and short chains. Clinical specimen on blood agar showed small, circular, translucent, glistening colonies with Beta hemolysis. There was no growth on Mc-Conkey medium. The isolate grew well on Edward medium at 37°C. The fermentation tests revealed that the isolates were positive for maltose and negative for lactose sorbitol, trehalose and negative for oxidase and catalase [14].

Study Methodology: The study was an *in-vitro* experimental trial on selected medicinal plant against *S. equi* sub species equi and *C. pseudotuberculosis* commonly isolated from horse and donkeys affected from strangles and ulcerative lymphangitis.

Plant Collection and Pre- Extraction Preparation: Cultivated *L. sativum* seeds, *P. dodecandra* seed and leaf as well as *A. schimperiana* leaf were collected from Kombolcha in December 18, 2019. Plant samples were mainly selected based on its use traditionally by farmers for the treatment of wound and on past studies [15,16]. After harvesting, the plant were washed with tap water to remove unnecessary particles, then, all plant parts were dried under shed for two weeks and stored at room temprature until processed. The material were sieved and weighed before maceration.

Preparation of Crude Extracts for the *In-vitro* Experiment: After the parts of plant which are grindined and sieved by fine mesh 50grams (gms) of each test plants were weighted using sensitive balance and it was put in to a bottle and 250 ml of 70 % ethanol added and mixing takes place at maximum speed shaking for 72 hours on the orbital shaker to help through mixing and enough maceration of the plant parts. Then after 72 hours of shaking each samples were strained using strainer or gauze to remove the solids. Further straining were takes place using Whatmanns no. 1 filter paper (Camlab, UK) to obtain a solution free of solids. The solution was concentrated using a vacuum rotary evaporator to remove the solvent (ethanol). Note that the roots of *P. dodecandra* did not give yield (removed as solvent when concentrated by evaporator). Clean petri-dishes were prepared and weighed their initial weight using sensitive balance and labeled and the concentrated plant extract were put on these petri-dishes and the weight were recorded and then the weight of the extract were recorded by subtracting final weight from initial weight of the petri-dish. The plant extracts were then be kept in hot air dry-oven at 370C for 4 days to help evaporate the remaining solvent. The resulting concentrated plant extracts were kept at refrigerator until it will be tested for anti-microbial activity.

Preparation of Antimicrobial Wells from Plant Extract for the *In-vitro* Experiment: Three different concentrations 200 mg/ml, 100 mg/ml and 50 mg/ml of each extract were prepared using Dimethylsulfoxide (DMSO). From the first to third universal bottles, 1ml of DMSO was added and in the first tube 200 mg of plant extracts, in the second bottle 100 mg of plant extract and in the last bottle 50 mg of plant extract was added. Similar procedureS were used for all plant extracts with three different concentrations about a total of 12 universal bottles containg a mixture of plant extract and DMSO. Mixing was done by vortex mixor. For each plant extracts, on each plate, equidistant wells were made with a 6mm diameter sterilized cork borer. The labeled wells were filled with 100µL of 200, 100 and 50 mg/mL of test extracts. This was used to determine antimicrobial effects of the respective plant types on isolated and grown bacteria).

Preparation of the Test Bacteria: The plant extracts were tested against *S. equi* subspecies equi obtained from nasal swap and pus of horse and *C. pseudotuberculosis* from pus sample of horse. The bacterial isolates were maintained on nutrient agar and subcultured every three days. Each bacterial isolates were suspended in 5 ml of

saline solution and adjusted to give a concentration of bacterial cells to 0.5 McFarland turbidity standard prior to antibacterial testing.

Agar Well Diffusion Assay for Antimicrobial Sensitivity Test: The antimicrobial test was conducted using agar well diffusion method. Muller hinton agar (38 gm) medium was used for antimicrobial sensitivity test and was mixed one litre of distlled water, boiled to dissolve completely and autoclaved at 121°C for 15 minutes. The medium was later dispensed into 90mm sterile agar plates and left to set. The agar plates was incubated for 24 hours at 37°C to confirm their sterility. Then no growth occurred after 24 hours, the plates was considered sterile and was used for antimicrobial sensitivity test [17]. Then, the well isolated colonies of the same morphology were scooped using a wire loop from the nutrient agar and was mixed using sterile normal saline and was agitated with vortex mixer. The turbidity of the bacterial suspension was adjusted by comparing with 0.5 McFarland turbidity standard. The McFarland standard was prepared by dissolving 0.5 g of $BaCl_2$ in 50 ml of water to obtain a 1% solution of barium chloride (w/v). Sulphuric acid (1%) was prepared in a 100 ml volumetric flask. To prepare the 0.5 McFarland standard, 0.05 ml of the 1% $BaCl_2$ solution was mixed with 9.95 ml of H_2SO4 solution [18].

The standard and the test suspension were placed in a 10 ml sized test tubes and compared against a white background with contrasting black lines until the turbidity of the test suspension was equates the turbidity standard. Adjustement of turbidity was made by adding saline or colonies depending on the degree of turbidity for isolates.Asterile swap was dipped into the standardized suspension of bacteria and excess fluid was minimized by pressing and rotating the swap firmly against the inside of the tube above the fluid level. The swap was streaked in three directions over the entire surface of the agar with objective of obtaining uniform inoculations and a final sweep with the swap was made against the agar around the rim of the petridish. The inoculated plates were allowed standing for not more than 15 minutes and the positive control/ antibiotic disc was placed on the agar surface using a sterile forceps and for each extracts, on each plate, equidistant wells were made with a 6mm diameter sterilized cork borer. The labeled wells were filled with 100µL of 200, 100 and 50 mg/mL of test extracts. For comparison, Pencillin G and DMSO (100 µL/well) were used as a positive and negative control, respectively. Then, the plates were allowed to stand on the laboratory bench for 2 hr to allow proper diffusion of the extracts into the media. Finally, the plates were incubated at 37°C for 24 hr. After incubation, the resulting diameters of zones of inhibition, including the diameter of the well, were measured using a caliper and reported in millimeter (mm) [19].

Data Analysis: All the data obtained from the experiment were entered into Excel Microsoft Office and transferred to Stata version 14 (2015) for analysis [20]. One-way ANOVA was used to compare difference within each group and bacteria type. Results were expressed as Mean±S.D. and presented as tables and graphs. A value of $P<0.05$ was considered significant for all analysis.

RESULTS

Antibacterial Activity: The present study demonstrated that the three plant extracts used in the study exhibited a varying degree of antimicrobial activity against *S. equi* and *C. pseudotuberculosis* as presented in Table 1. For *C. pseudotuberculosis*, L. sativum at higher concentration (200mg/ml) showed a better zone of inhibition (19.46±2.51mm) as compared to other plant extracts and respective lowest concentration (50mg/ml) which exhibited (15.36±2.60mm) zone of inhibition. The highest antibacterial activity against *S. equi* was recorded with the leaf extract of P. dodecandra (18.45±1.83mm) at 200mg/ml concentration followed by the seed extracts of L. sativum (13.15±0.54mm) at 200mg/ml concentration and seed extracts of P. dodecandra (12.65±0.68mm). The antimicrobial activity in terms of zone of inhibition (in mm diameter) of ethanol extracts of those three plants of different parts at different concentrations of 200, 100 and 50 mg/ml against two pathogenic organisms, *S. equi* and *C. pseudotuberculosis* are presented in Table 1.

Seed and leaf extracts of the *P. dodecandra* and Seed extracts of *L. sativum* at 200mg/ml concentration showed a better inhibitory activity against *S. equi* whereas seed extracts of the *P. dodecandra* and *L. sativum* at 200mg/ml concentration a medium inhibitory activity. Seed extracts of the *L. sativum* at three concentrations (200mg/ml, 100mg/ml & 50mg/ml) proven a better antibacterial activity against *C. pseudotuberculosis* whereas extracts of the other medicinal plants showed no to low inhibitory activity as depicted in Table 2.

Table 1: Agar well diffusion assay results of three medicinal plants against two bacterial pathogens

			Zone of inhibition (ZI) in Millimeter (MM)	
Extracted plant	Part used	Concentration	*Streptococcus equi*	*Corynebacterium pseudotuberculosis*
P. dodecandra	Seed	200mg/ml	12.65±0.68	9±0.086
		100mg/ml	11.5±0.46	6.5±0.46
		50 mg/ml	9.65±0.39	6.85±0.33
	Leaf	200mg/ml	18.45±1.83	8.69±0.58
		100mg/ml	9.39±1.59	7.01±0.68
		50 mg/ml	7.85± 1.69	Ziz
L. sativum	Seed	200mg/ml	13.15±0.54	19.46±2.51
		100mg/ml	10.32±1.36	17.67±2.99
		50 mg/ml	7.15±0.54	15.36±2.60
A.schimperiana	Leaf	200mg/ml	9.65±2.79	Ziz
		100mg/ml	9.64±1.63	Ziz
		50 mg/ml	Ziz	Ziz
Sta.Pencillin G			≥ 24 mm	-
DMSO			Ziz	Ziz

Ziz stands for Zero inhibition zone

Table 2: The quantitation of antimicrobial activity for plant extracts measured by the agar well diffusion method

Extracted plant	Part used	Concentration	*Streptococcus equi*	*Corynebacterium pseudotuberculosis*
P. dodecandra	Seed	200mg/ml	+++	+
		100mg/ml	++	_
		50 mg/ml	+	_
	Leaf	200mg/ml	+++	+
		100mg/ml	+	_
		50 mg/ml	_	_
L. sativum	Seed	200mg/ml	+++	+++
		100mg/ml	++	+++
		50 mg/ml	_	+++
A. Schimperiana	Leaf	200mg/ml	+	_
		100mg/ml	+	_
		50 mg/ml	_	_

where: Diameter of the inhibition zone: no inhibition (-), 8-9.5 mm (+), 10-12 mm (++), > 12 mm (+++) [21].

Table 3: Analysis of variance (ANOVA) Result showing association between Mean Zone of growth inhibition with plant type, plant part, bacteria and amount of concentration

			ANOVA		
Variable	Analysis	Df	Mean of square	F	P-Value
Concentration (200mg/ml,	Between group	2	88.625	2.815	0.083
100mg/ml & 50mg/ml)	With in group	21	31.488		
Bacteria (*S. equi* and	Between group	1	42.667	1.179	0.289
C. pseudotuberculosis)	With in group	22	36.174		
Plant part (Leaf and seed)	Between group	1	181.500	6.078	0.022
	With in group	22	29.864		
Plant extract/type (*P. dodecandra,*	Between group	2	145.042	5.554	0.012
L. Sativum, A.schimperiana)	With in group	21	26.115		

There was a statistical significant variation ($P<0.05$) in zone of inhibition among plant part and plant type against both bacteria. However, no statistical difference ($P>0.05$) observed among other variables included in the study (concentration and type of bacteria) as indicated in Table 3.

DISCUSSION

This study indicated that the concentration of 200mg/ml of *L. sativum* seed was active against both organisms, which is about Zone of inhibition (13.15±0.54 mm and 19.46±2.51) mm against *S. equi* and

C. pseudotuberculosis respectively and 100 mg/ml of *L. sativum* seed extract was active against *C. pseudotuberculosis* (Zone of inhibition (17.67±2.99 mm) and *S. equi* (Zone of inhibition 10.32±1.36) and 50 mg/ml of *L. sativum* was active against *C. pseudotuberculosis* (zone of inhibition 15.36±2.60) but the same concentration of *L. sativum* did not show activity against *S. equi*. The slight difference might be attributed to the amount concentration of the extract. However, a study conducted by Endalkachew and Negesse [22] on methanol extract of *L. sativum* leave reported absence of antibacterial activity against *Streptococcus spp. and Staphaylococcus aurus,* which is 6mm for both organisms. The present study showed better activity than Endalkachew and Negesse [22] report. The difference might be from solvent, the organism difference and the part of plant used. In another study on antibacterial activity of *L. Sativum* for *Streptococcus pneumoniae* standard (ATCC63) and clinical isolate of *Streptococcus pneumoniae*, ethanol extract of *L. Sativum* was active with Mean zone of inhibition 27.38±1.98mm and 25.68±1.48 mm respectively [23], which indicates that the present study was shows lower activity.However, in another study *L. sativum* seed methanol extracts showed antibacterial activity against *S. aureus* and *E. Coli* with the inhibition zone of 15mm and 17mm in diameter, respectively [24]. This might be because of the difference in the plant parts used, type of bacteria, extraction solvent and the concentration of plant extract. In another study, The ethanol extract of *L. sativum* seed showed greater zone of inhibition against *P. aeruginosa* (18 mm) as compared to methanol extract (17 mm) and ethanol extract showed 15 mm zone of inhibition against *S. aureus* [25]. This shows the difference might be due to the extraction solvent used and the nature of tested bacteria. Generally factors like extraction technique, parts of the plant used, concentration and type of solvent used can greatly affect the activity the extract against microorganisms.

Statistical analysis has revealed that; there is a significant relation ($P<0.05$)(0.012 and 0.022) between plant extracts and plant part respectively in the inhibition of growth of *S. equi* and *C. tuberculosis* Accordingly higher Zone of inhibition was recorded in *L. sativum* (19.46±2.51) than in *A. schimperiana* (0.000±0.000) which may be due to differences in the bioactive ingredients of the plant. There was no significant association ($P>0.05$) (0.083 and 0.289) between the concentration of crude extract and bacteria with the inhibition of growth of *S. equi* and *C. Pseudotuberculosis.*

In this study, the extract from seeds and leaves of *P. dodecandra* have antibacterial activity against *S. equi* (Zone of inhibition 12.65±0.68 mm and 18.45±1.83 respectively) at a concentration of 200mg/ml which showed a strong antibacterial activity whereas the lower activity was recorded against *S. equi* at 100mg/ml showed (Zone of inhibition 11.5±0.46mm) and least activity at 50 mg/ml showed (Zone of inhibition 9.65±0.39mm). About 200mg/ml of seed and leaf of *P. dodecandra* extract was showed lower antibacterial activity against *C. pseudotuberculosis* (Zone of inhibition 9±0.086mm and 8.69±0.58mm respectively) where as other concentrations did not show activity against both organisms. No similar studies were conducted on those organisms but there was finding on other bacterias with the reports that methanol, ethanol and chloroform extracts of *P. dodecandra* were not showing any antibacterial activity against five bacterias namely *Salmonella typhumurium*, *Staphylococcus aurus*, *E.coli* and *Shigela sonnei* [16]. However, oppossing results were reported by [26], where seed of *P. dodecandra* showed an antibacterial activity against *E.coli* (9.7 ± 0.6mm), *Staphylococcus aurus*(10.3 ± 2.1mm), *Pseudomonas aeruginosa*(9.3 ± 2.5mm) and Salmonella spp (11.0 ± 1.0mm). This indicates that the present study of *P.dodecandra* against bacterias like *S. equi* was better than other bacterias as it showed higher activity.

In the current study, *A. schimperiana* showed antibacterial activity against *S. equi* at 200mg/ml and 100 mg/ml (9.65±2.79) and 9.64±1.63mm inhibition zone respectively, where as there was no any activity against *C. pseudotuberculosis*. Similar studies were not conducted on *A. schimperiana* against *S. equi* and *C. pseudotuberculosis*. In another study which was made on other bacterias, methanol extracts of *A. schimperiana* leaf had shown an antibacterial activity against *S. aureus* with zone of inhibition 20mm [27]. In general, these medicinal plants have showed promising anti-bacterial activity against *S. equi* and *C. pseudotuberculosis*. The variation of efficacy among these phyto-preparations against the bacteria could be attributed to many factors like the way of plant preparation, season of collection, stage of the plant, place of collection, way of drying, means of extraction, the solvent used, preservation or storage of the extract till evaporation.

CONCLUSION

It can be concluded that the crude ethanol extracts of *P. dodecandra* and *L. sativum* have shown

proven antibacterial activity against *S. equi* and *C. Pseudotuberculosis* with different potency. As a result, the growth of both pathogenic bacteria was completely inhibited with the exception of *A.schimperiana for C. pseudotuberculosis*. In this study, we found that *L. sativum* extracts possess antibacterial activities. The antibacterial potency of these medicinal plants is generally believed to be due to bioactive compounds found in the seeds and leaves. Therefore, *L. sativum and P. dodecandra* could be an excellent source for antibacterial agents for medical applications. This investigation thus provides a scientific basis for the use of the plant extracts in homemade remedies and their potential use in the treatment of microbial-induced ailments.

Based on the above conclusion, the following recommendations are forwarded:

- Further investigation on the fractionated components using different solvent and standard medium for the in vitro test.
- *In-vivo* studies should be conducted to identify the safety margins, toxicity, proper dose and route of administration of these plant extracts to use effectively and efficiently.

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