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Evaluation of Antibacterial and Antifungal Activity of Herbs Used in Treatment of Diabetes, Malaria and Pneumonia in Kisii and Nyamira Counties Region, Kenya

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Abstract

The herbs *Carissa spinarum, Physalis minima* and *Toddalia asiatica* have traditionally been used in healing diabetes, malaria and pneumonia by the communities around the Kisii region, Kenya. However, in the available literature, there is scanty information on effectiveness of different plant parts of the herbs in healing the ailments. The objective of this study was to investigate the potential antimicrobial and antifungal activity of methanolic extract of whole plant *Physalis minima,* leaf and root of *Carissa spinarum* and *Toddalia asiatica* against gram positive bacteria *Staphylococcus aureus* (ATCC 25923), gram negative bacteria *Escherichia coli* (ATCC 25922) and fungus *Candida albicans* (ATCC14053). Antibiotic disc methicillin, cotrimoxazole, chloramphenicol, gentamicin, ampicillin, nalidixic and nitrofurantoin were used in the study. In each herb, plant part was extracted by soaking in methanol/dichloromethane in ratio 1:1 for a week, filtered, concentrated by rotary vapor and cooled. The same process was repeated three times for all samples. The study was conducted by agar well diffusion method. Methanolic root extract of *Toddalia asiatica* showed highest antibacterial activity against *Staphylococcus aureus* (ATCC 25923), root extract of *Carissa spinarum* had highest antibacterial activity against *Escherichia coli* (ATCC 25922) while root extract of *Toddalia asiatica* showed highest antifungal activity. It was concluded that root extract of *Toddalia asiatica* showed highest antibacterial activity 16.7mm against *Staphylococcus aureus* (ATCC 25923), root extract of *Carissa spinarum* had highest antibacterial activity 10 mm against *Escherichia coli* (ATCC 25922) while root extract of *Toddalia asiatica* had highest antifungal activity 18 mm against *Candida albicans*.

Keywords: Carissa spinarum, Physalis minima, Toddalia asiatica, Antibacterial. Antifungal. Evaluation

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1 Introduction

Phytochemical constituents of *Carissa spinarum* was reported having flavonoids, phenolic, tannins saponins, triterpenoids, steroids and glycosides [1]. The chemical composition of *Carissa spinarum* root was reported having Carissone, carindone, carinol, odoroside, digitoxigenin, glucose and D-digitalose while leaf had triterpene, alcohol, ursolic acid [1]. The root and leaf extracts of *Carissa spinarum* was reported to have shown biological activities such as antidiabetic, antibacterial, antiviral, anti-plasmodial, antioxidant, antiarthritic, antihelmintic and wound healing [1]. The root extract of *Carissa spinarum* is used as snakes repellent [1]. *Carissa spinarum* is relatively safe or non-toxic. The findings of this experimental in animal study indicate that ethanol extract of the roots of *Carissa spinarum* possesses anti-arthritic properties; and therefore lend pharmacological credence to the folkloric and ethnomedical uses of the plant in the treatment of arthritic conditions. The roots have been used for the treatment of rheumatism, cleaning worm infested wounds of animals and in snake bite [2]. In Chinese system of medicine the roots of *Carissa spinarum* was used for treatment of painful inflammatory, arthritic, hepatitis and rheumatoid arthritis. The herb *Carissa spinarum*, Family Apocynaceae has been used in treatment of microbial infections such as venereal, respiratory and gastrointestinal infections [3], [4]. *Carissa spinarum* roots have been used for the treatment of inflammation disorders and in snake bite [5], [6], [7]. In Chinese system of medicine the *Carissa spinarum* has been used for the treatment of chest complaints, gonorrhoea, syphilis, rabies and sickle cell anaemia [7]. Earlier studies have shown that *Carissa spinarum* extract possesses antibacterial and antioxidant activity [2],[6],[7]. *Physalis minima*, Family Solanaceae is useful in treatment of inflammations, antigonorrhoeic, enlargement of the spleen and abdominal troubles [8], [9]. Fruits and flowers are used in stomach pain and in constipation, herb paste is applied in ear disorders. Ripen fruits are used in gastric trouble. The decoction of the whole plant is consumed as a remedy for cancer, asthma, bronchitis, inflammation, enlargement of spleen, urinary disorder, abdominal troubles and headache [10]. The roots are used for treatment of diabetes [8]. *Toddalia asitica*, Family Rutaceae, has antitumor, antibacterial, antifungal, anti-malaria and antiviral activity [5], [11]. Alkaloid in crude extract has anti-inflammatory, anti-malarial and anti-leukimatic properties [11]. The coumarine derivatives have antiplasmodial, antimicrobial activity and root decoctions are drunk to treat malaria [12].

2 Materials and Methods

2.1 Materials

This subsections lists the chemicals, equipment and glass wares to be used in carrying out the various experiments.

2.2 The chemicals

All chemicals and reagents used will be of analytical grade. They include the following: Gallic acid, Foline-Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, quercetin, aluminium chloride, silica gel, sodium acetate, sodium carbonate , Ethanol , HPLC methanol, HPLC hexane, TLC plates, Conc. (H_2SO_4), Conc. HCl, C_2H_5OH, C_3H_6O, CH_2Cl_2, $CHCl_3$, NH_3, KI, Nutrient agar, Glacial acetic acid, Olive oil, $C_2H_5OO^-$, NaOH, Dragencloffs reagent, 2% H_2SO_4, , $(C_2H_5OO)_2Pb$, 1% KOH, 1M HCl , 1M H_2SO_4 , 50% HNO_3, I_2 , 5% $FeCl_3$, among other Laboratory reagents.

2.3 The equipment

Equipments used includes the following: Bruch Rota vapours R-4000, Atomic absorption spectrometry (AAS), FT-IR-8400, PH-meter, UV-spectrophotometer, GC-MS, among other Laboratory equipments.

2.4 The glass wares

Glass wares used included the following: beakers, cylinders, conical flasks, volumetric flasks, droppers, glass Columns, burets, pipettes, among other Laboratory glass wares.

2.5 Test microbes

2.5.1 Antibacterial activity:

The test organisms; gram-positive bacterium *Staphylococcus aureus* (ATCC 25923) and gram-negative bacterium *Escherichia coli* (ATCC 25922)

2.5.2 Antifungal activity:

The test organisms; fungus *Candida albicans* (ATCC14053).

2.6 Plant Materials

Herb identification and collection

Parts of *Carissa spinarum*, *Physalis minima* and *Toddalia asiatica* were collected in January 2015 from same ecological zones in Kisii region Southwest Kenya. The verification of the herbs was done by Prof. S. M. Kariuki, Botanist; Egerton University, Kenya. The voucher Specimen information of dried herb specimen were taken and kept in the Egerton University Herbarium.

2.7 Preparation of Samples

The extraction of each selected herb of interest material was done using standard procedures as described by [5], [6], [13], [14], [15]. The herb materials of each sample were collected and cleaned, dried at room temperature, crushed into powder and stored in an air tight glass container. *Toddalia asiatica* leaf powder 2.3 Kg was weighed by electrical chemical balance then soaked in 1:1 methanol in dichloromethane for five days to allow the phytochemical compounds to be extracted by the solvent. The same process of *Toddalia asiatica* leaf was repeated three times. The same procedure was also repeated for 1.45 Kg of *Toddalia asiatica* root bark, 2.4721 Kg of *Carissa spinarum* leaf, 1.2231 Kg of *Carissa spinarum* root bark and 1.144 Kg of whole plant *Physalis minima*. The extracts of each sample was filtered by clean cotton wool and whatman filter paper No.1, and then concentrated using a rotary vapor on 45°C. The concentrated extract was placed in the Laboratory to allow the solvent escape and solidify awaiting further investigations.

2.8 Agar well diffusion method

The methanolic extracts of whole plant *Physalis minima*, leaf and root bark from *Carissa spinarum* and *Toddalia asiatica* were each investigated for antibacterial and antifungal activities against gram-positive bacterium *Staphylococcus aureus* (ATCC 25923), gram-negative bacterium *Escherichia coli,* (ATCC 25922) and fungus *Candida albicans* (ATCC14053) by agar well diffusion assay according to standard procedures as described by [4], [14]. The pure solvents were used as control while the standard antibiotic disc erythromycin (Ery), methicillin (Met), cotrimoxazole (Co), chloramphenicol (Chl), ampicillin (AMP), gentamicin (G), ampicillin (A), nitrofurantoin (NF), nalidixic acid (Na), streptomycin (S), sulphamethoxazole (Sx), tetracycline (T), and lincomycin (Lin) were used as reference. All microorganisms were obtained at the Microbiology laboratory, College of Health Sciences (COHES) Jomo Kenyatta University of Agriculture and Technology, Kenya. The microorganisms were cultured and used for antimicrobial activity tests at the Microbiology laboratory of Food Science and Technology of Jomo Kenyatta University of Agriculture and Technology, Kenya.

2.9 Antibacterial activity

The test organisms; gram-positive bacterium *Staphylococcus aureus* (ATCC25923), gram-negative bacterium *Escherichia coli* (ATCC25922) and fungus *Candida albicans* (ATCC14053) were used for antibacterial and antifungal tests by agar well diffusion assay [14]. In the test tube, 20 ml nutrient agar was melted at 100°C and stabilized at 45°C for about 15 minutes. About 0.1 ml inoculums of each gram-positive bacterium *Staphylococcus aureus* (ATCC 25923) was added from culture tubes to the agar in the test tube by the use of a loop. The test tube containing the agar and the inoculums was then rolled in between the palms gently to mix the inoculums thoroughly with the agar [14]. The loop was flamed before it was used each time. The content of the test tube was poured into Petri dishes and allowed to set. The Petri dishes was then labelled with the respective organism (inoculums) and date. By means of a 6mm cork borer, four cups were bored in triplicate, well separated and equidistant from each other in the agar. The cups were labelled with the three

crude extracts and control methanol. Each cup was filled with its corresponding sample extract (mg/ml) to about three-quarters full. The same procedure for each methanolic extract of whole plant *Physalis minima*, leaf and root extract of *Carissa spinarum* and *Toddalia asiatica*, pure solvent methanol, antibiotic disc, gram-positive bacterium *Staphylococcus aureus* (ATCC 25923), gram-negative bacterium *Escherichia coli* (ATCC25922) and fungus *Candida albicans* (ATCC14053) was repeated. They were kept on a bench at room temperature for about 60 minutes (for the extracts to diffuse into the agar). The plates were then incubated aerobically at 37^0C and examined for any zone of inhibition after 24 hours. The reading was done against a dark background under reflected light. The diameters of the zones of growth of inhibition(mm) was measured with the help of Hi-Antibiotic zone scale (ranged 1 cm to 35 cm or 10 mm to 400 mm) from the underside of the covered plates for spots with inhibitions. The average of the diameters was taken [14]. The actual zones were calculated by subtracting the diameter of the cups (6mm) from the total zone of growth.

2.10 Antifungal activity test

The antifungal activity test was carried out according to standard procedures as described by [4], [14]. Twenty grams (20.0 g) of potato slices were boiled with distilled water in 100ml to prepare potato infusions. Dextrose (2 g) mixed with potato infusion agar (2 g) was added as a solidifying agent. The contents were mixed and autoclaved. In the test tube, 20 ml sterilized mixture of dextrose with potato infusion agar was poured and stabilized at 45°C for about 15 minutes. About 0.1 ml inoculums, fungus *Candida albicans* (yeast) (ATCC14053) was added from culture tubes to the dextrose with potato infusion agar in the test tube by the use of a loop. The test tube containing the dextrose with potato infusion agar and the inoculums was then rolled in between the palms gently to mix the inoculums thoroughly with the dextrose with potato infusion agar. The loop was flamed before it was used each time. The content of the test tube was poured into a Petri dish and allowed to set. The Petri dishes was then labelled with the respective organism (inoculums). By means of a 6 mm cork borer, four cups were bored in triplicate, well separated and equidistant from each other in the dextrose with potato infusion agar. The cups were labelled with the three crude extracts of *Carissa spinarum*. Each cup was filled with its corresponding sample extract (mg/ml) to about three-quarters full. The same procedure was repeated for the pure solvent (methanol), each extract of *Physalis minima*, *Toddalia asiatica* and antibiotic disc. They were kept on a bench at room temperature for about 60 minutes (for the extracts to diffuse into the agar). The extracts of *Carissa spinarum* was poured into the wells using sterile syringe. The plates were incubated for 48 hours at 37±2°C for fungal activity. The plates were observed for the zone formation around the wells. The zone of inhibition was measured by Hi-Antibiotic zone scale (ranged 1 cm to 35 cm or 10 mm to 400 mm) from the underside of the covered plates for spots with inhibitions and calculated by measuring the diameter of the inhibition zone around the well (mm) including the well diameter. The readings were taken in triplicate and the average values were determined. The same procedure was repeated for blank samples (methanol with no extract), methanolic extract of whole plant *Physalis minima*, leaf and root of *Toddalia asiatica*. The actual zones of inhibitions (mm) were calculated by subtracting the diameter of the cups (6 mm) from the total zone of inhibition measured (Table1).

3 Results and Discussions

Methanolic extract of whole plant *Physalis minima*, leaf and root of *Carissa spinarum* and *Toddalia asiatic* were investigated for antibacterial and antifungal activity by agar well diffusion method (Table 1).

All experiments were done in triplicate. The mean and standard deviation of at least three experiments was determined and results reported as mean values±standard deviation. The limit of statistical significance was set at p-level ≤ 0.05.

Table 1: Mean inhibitory zone of three herbs against *E. coli, S. aureus, C. albican*.

Herb / Antiobiotic	Concentration	Diameter zone of inhibition in mm		
	Extracts(mg/ml), antibiotic disc (µg/ml)	*Staphylococcus aureus* (ATCC 25923)	*Escherichia coli* (ATCC 25922)	*Candida albicans* (ATCC14053)
Carissa spinarum root bark	2.5	4.3	10	1
Physalis minima Whole plant	2.5	10	1	3
Toddalia asiatica root bark	2.5	16.7	8.3	18
Antibiotic disc(drugs)				
Erythromycin(Ery)	15	-	-	-
Methicillin (Met)	5	10	-	10
Cotrimoxazole(Co)	25	10	17	15
Chloramphenicol(Chl)	30	4	-	4
Ampicillin (AMP)	10	-	-	-
Gentamicin (G)	10	10	17	-
Ampicillin (A)	25	10	17	-
Nitrofurantoin(NF)	200	10	11	-
Nalidixic acid (Na)	30	4	17	15
Streptomycin (S)	25	8	17	15
Sulphamethoxazole(Sx)	200	16	17	15
Tetracycline (T)	100	15	-	-
Lincomycin (Lin)	2	7	-	-

From Table 1, results showed root extract of *Toddalia asiatica* had highest antibacterial activity of 16.7 mm at 2.5 mg/ml against gram-positive bacterium *Staphylococcus aureus* (ATCC 25923), followed by whole plant extract of *Physalis minima* 10 mm at 2.5 mg/ml and root extract of *Carissa spinarum* 4.3 mm at 2.5 mg/ml against *Staphylococcus aureus* (ATCC 25923) compare to diameter zone of inhibition (mm) of antibiotic sulphamethoxazole 16 mm at 0.2 mg/ml, tetracycline 15 mm at 0.1 mg/ml, methicillin 10 mm at 0.005 mg/ml, cotrimoxazole 10 mm at 0.025 mg/ml, gentamicin 10 mm at 0.01 mg/ml, ampicillin 10 mm at 0.025 mg/ml, nitrofurantoin 10 mm at 0.2 mg/ml, streptomycin 8 mm at 0.025 mg/ml, lincomycin 7 mm at 0.002 mg/ml, nalidixic acid 4 mm at 0.03 mg/ml and chloramphenicol 4 mm at 0.03 mg/ml. Root extract of *Carissa spinarum* recorded highest antibacterial activity of 10 mm at 2.5 mg/ml against gram-negative bacterium *Escherichia coli* (ATCC 25922), followed by root extract of *Toddalia asiatica* 8.3 mm at 2.5 mg/ml and whole plant extract of *Physalis minima* 1.0mm at 2.5 mg/ml against *Escherichia coli* (ATCC 25922) compare to diameter zone inhibition (mm) of antibiotic cotrimoxazole 17 mm at 0.025 mg/ml, gentamicin 17 mm at 0.01 mg/ml, ampicillin 17 mm at 0.025 mg/ml, nalidixic acid 17mm at 0.03 mg/ml, streptomycin 17 mm at 0.025 mg/ml, sulphamethoxazole 17 mm at 0.2 mg/ml and nitrofurantoin 11 mm at 0.2 mg/ml. The root extract of *Toddalia asiatica* extract recorded the highest antifungal activity of 18.0 mm at 2.5 mg/ml against the fungus *Candida albicans* (ATCC14053) followed by whole plant extract of *Physalis minima* 3.0 mm at 2.5 mg/ml and root extract of *Carissa spinarum* 1.0 mm 2.5 mg/ml against *Candida albicans* (ATCC14053) compare to diameter zone of inhibition (mm) of antibiotic cotrimoxazole 15 mm at 0.025 mg/ml, nalidixic acid 15 mm at 0.03 mg/ml, streptomycin 15 mm at 0.025 mg/ml, sulphamethoxazole 15 mm at 0.2 mg/ml, methicillin 10 mm at 0.01 mg/ml and chloramphenicol 4 mm at 0.03 mg/ml. The results revealed that root extract of *Toddalia asiatica* registered the highest diameter zone of inhibition against *Staphylococcus aureus* (ATCC25923) and *Candida albicans* (ATCC14053) more than all antibiotic disc used. The results of this study showed that the extracts of whole plant *Physalis minima*, root of *Carissa spinarum* and *Toddalia asiatica* exhibited diameter zone of inhibition while leaf extracts of *Carissa spinarum* and *Toddalia asiatica* displayed no diameter zone of inhibition which indicated no antibacterial and antifungal activity.

It has been reported by [16] that root extract of *Carissa spinarum* showed diameter zone of inhibition (mm) of 13.33±1.53 at 10 mg/ml against gram positive bacteria *Staphylococcus aureus (*ATCC 25923) compared to 20.66±1.53 at 0.030 mg/ml and 19.66±1.53 at 0.015 mg/ml of ampicillin and ciprofloxacin standards used. A report by [16] also showed

that the methanolic root extract of *Carissa spinarum* had diameter zone of inhibition of 11.66±0.47 mm at 10 mg/ml against gram negative bacteria *Escherichia coli* DSM 1103 compared to 19.66±2.08 at 0.030 mg/ml and 19.00±1.00 at 0.015 mg/ml of ampicillin and ciprofloxacin standards used. The leaf extract of *Carissa spinarum* was reported by [16] and showed diameter zone of inhibition (mm) of 8.33±1.53 mm at 10 mg/ml against gram positive bacteria *Staphylococcus aureus (*ATCC 25923) compared to 20.66±1.53 at 0.030 mg/ml and 19.66±1.53 at 0.015 mg/ml of ampicillin and ciprofloxacin standards used. The leaf extract of *Carissa spinarum* was reported by [16] showed diameter zone of inhibition (mm) of 2.33±0.58 at 10 mg/ml against gram negative bacteria *Escherichia coli* DSM 1103 compared to 19.66±2.08 at 0.030 mg/ml and 19.00±1.00 at 0.015 mg/ml of ampicillin and ciprofloxacin standards used. In contrast, leaf extract of *Carissa spinarum* in this study did not show any activity against gram positive bacteria *Staphylococcus aureus* (ATCC25923) and gram negative bacteria *Escherichia coli* (ATCC25922) while the bacteria species used by [16] displayed antibacterial activity. The leaf extract of *Carissa spinarum* and *Toddalia asiatica* in their crude form made up of complex composition of chemicals compared to the standard drugs which were pure compounds, therefore displaying no antibacterial activity in the tested extract. Further purification of the extracts could lead to isolation of pure compounds with increased antimicrobial activity or application of the combination therapy amongst the extracts to potentiate their activity. The root extract of *Carissa spinarum* in this study showed diameter zone of inhibition (mm) 4.3 at 2.5 mg/ml against gram positive bacteria *Staphylococcus aureus* (ATCC25923) compared to 10 mm at 0.025 mg/ml of ampicillin used in this study. The bacterial *Staphylococcus aureus* (ATCC25923) used by [16] revealed higher antibacterial activity at higher extract concentration than similar *Staphylococcus aureus* (ATCC25923) used in this study which might be attributed to four times higher extract concentration(mg/ml) used by [16] from Samunge village at Loliondo in Ngorongoro district located in northern Tanzania. The root extract of *Carissa spinarum* in this study showed diameter zone of inhibition (mm) 10 at 2.5 mg/ml against gram negative bacteria *Escherichia coli* (ATCC25922) compared to 17 mm at 0.025 mg/ml of ampicillin used in this study. The antibacterial activity of root extract of *Carissa spinarum* in this study against gram negative bacteria *Escherichia coli* (ATCC25922) was higher than that reported by [16] with a dfferent bacteria species *Escherichia coli* (DSM1103) from Samunge village at Loliondo in Ngorongoro district located in northern Tanzania.. This variation in antibacterial activity can be attributed to the differences in morphological constitutions between these bacteria. It has been reported by [5] and [6] that the difference in sensitivity between gram positive and gram negative bacteria against leaf and root extract of *Carissa spinarum* might be attributed to the differences in morphological constitutions between these bacteria, gram negative bacteria having an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to antimicrobial chemical substances. It has also been reported by [5] and [6] that the gram positive bacteria are more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier. Therefore, the cell walls of gram negative bacteria are more complex in lay out than the gram positive ones acting as a diffusional barrier and making them less susceptible to the antimicrobial agents than are gram positive bacteria (Table 1). In spite of this permeability differences, however, some of the extracts had still exerted some degree of inhibition against gram negative organisms as well (Table 1). It has been reported by [16] that screening of phytochemicals in *Carissa spinarum* revealed the presence of phenolic, flavonoids, tannins, saponins, coumarins, steroids and cardiac glycoside. Tannins have the ability to inactivate microbial adhesins, enzymes, and cell envelope, transport proteins and polysaccharides. The flavonoids have shown to exhibit antibacterial activity against *Staphylococu aureus* and *Escherichia coli.* The hydroxyl group of flavonoids attributes to antioxidant and chelating action to enhance antimicrobial activity. The present study showed leaf, root extract of *Carissa spinarum*

containing saponin exhibited antibacterial activity. Antimicrobial activity of saponin is associated with the ability of forming pore in the cell membrane and therefore giving the toxic material free access to the cell. It has been reported by [17] that leaf extract of *Physalis minima* recorded diameter zone of inhibition (mm) 3.0±1.5 against *Staphylococcus aureus* and 4.0±0.5 against *Escherichia coli*. The stem extract of *Physalis minima* recorded diameter zone of inhibition (mm) 4.0±0.5 against *Staphylococcus aureus* and 3.0±0.5 against *Escherichia coli*. The methanolic fruit extract of of*Physalis minima* recorded diameter zone of inhibition (mm) 5.0±0.5 against *Staphylococcus aureus* and 2.0±0.0 against *Escherichia coli*. The antibacterial activity (mm) of methanolic extract of *Physalis minima* reported by [17] ranged 1 mm to 5.5 mm compared to 1 mm to 10 mm registered in this study. The antibacterial activity (mm) of methanolic leaf, stem, fruit extract of *Physalis minima* reported by [18] was 3.0±1.5, 4.0±0.5 and 5.0±0.5 compared to 10 mm recorded against *Staphylococcus aureus* (ATCC25923) in this study. The antibacterial activity (mm) of methanolic leaf, stem, fruit extract of *Physalis minima* reported by [18] was 3.0±1.5, 4.0±0.5 and 5.0±0.5 compared to 1.0 mm recorded against *Escherichia coli* in this study. It has been reported by [17] that the antimicrobial activity of *Physalis minima* is mainly due to the presence of essential oils, flavonoids, triterpenoids and natural polyphenolic compounds or free hydroxyl groups in the methanolic extract which is in agreement with results of this study. The results of the present study of *Physalis minima* are directly correlated with the observations of previous work done elsewhere by [17].

4 Conclusion

It was concluded that all methanolic extract of whole plant *Physalis minima*, root extract of *Carissa spinarum* and *Toddalia asiatica* showed antibacterial and antifungal activity while methanolic leaf extract of *Carissa spinarum* and *Toddalia asiatica* displayed no diameter zone of inhibition which indicated no antibacterial and antifungal activity. The methanolic root extract of *Toddalia asiatica* registered the highest antibacterial activity against gram positive bacteria *Staphylococcus aureus (*ATCC25923) and antifungal activity against *Candida albicans* (ATCC14053) while root extract of *Carissa spinarum* recorded highest antibacterial activity against gram-negative bacterium *Escherichia coli* (ATCC 25922). The study suggests likelihood of future designing and development of potentially active antibacterial and antifungal agents from *Carissa spinarum*, *Physalis minima* and *Toddalia asiatica* for the treatment of infectious diseases. This finding provided information justifying the use of these herbs for medicinal purposes.

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6 References

[1] Fatima, A., Singh, P., P, Agarwal, P., Irchhaiya, R., Alok, S., Verma, A. (2013). Treatment of various diseases by *Carissa spinarum* L.-A promising shrub. *International journal of pharmaceutical sciences and research*,4(**7**):2489-2495.

[2] Hegde, K., Cijo, I., Joshi, A. B., (2010). Antiarthritic activity of *Carissa spinarum* root extract in freund's adjuvant induced polyarthritis in rats. *Pharmacologyonline*, 2: 713-718

[3] Ibrahim, H., Oyi, R. A., Ehinmidu, J. O., Musa, K. Y., and Bright, N. T., (2010). Antimicrobial activity of the water extracts of the leaves and fruits of *Carissa edulis* Vahl (Apocynaceae). *Journal of medicinal plants research*, 4(11): 1028-1032.

[4] Maobe, M. A. G., Gatebe, E., Gitu, L., and Rotich, H., (2012). Profile of Heavy Metals in Selected Medicinal Plants Used for the Treatment of Diabetes, Malaria and Pneumonia in Kisii Region, Southwest Kenya. *Global Journal of Pharmacology* 6 (3):245-251.

[5] Rose, B. N. and Prasad, N. K., (2013). Preliminary Phytochemical and Pharmacognostical Evaluation of *Carissa spinarum* Leaves. Asian Journal of Pharmaceutical and Technology., 3(1): 30-33.

[6] Maobe, A. G. M, Gatebe, E., Gitu, L. and Rotich, H., (2013). Antifungal activity of eight selected medicinal herbs used for the treatment of diabetes, malaria and pneumonia in Kisii region, southwest Kenya. *World Journal of Medical Sciences,* 8(1): 74-78

[7] Maobe, A. G. M, Gatebe, E., Gitu, L., and Rotich, H., (2013b). Antimicrobial activities of eight selected medicinal herbs used for the treatment of diabetes, malaria and pneumonia in Kisii region, southwest Kenya. *Global Journal of Pharmacology*, 7(1): 25-33

[8] Ya'u, J., Yaro, A. H., Abubakar, M. S., Anuka, J. A., Hussaini, I. M., (2008). Anticonvulsant Activity of *Carissa edulis* (Vahl) (Apocynaceae) Root Bark Extract. *Journal of Ethno pharmacology*, 120:255–258

[9] Kumar, K.H.N., and Chauhan, J. B., (2 016). Phytochemical screening and anticancer activity of leaf extracts of Physalis minima. *Journal of advances in natural sciences*, 3(2): 283- 287

[10] Ramar, K., Ayyadurai, V., (2016). *In vitro* callus induction studies of *Physalis minima* (L). An important medicinal plant. *International journal of pharmaceutical science and research*, 1(**1**): 01-03. ISSN: 2455-4685

[11] Joshi Anand, T., Liakhat Ali, S. K., Sudhir, M., Manjula, R. R., Jaya Sravani, P., Saraswathi Devi, L., (2014). Phyto chemical investigation and evaluation of analgesic activity of *Physalis Minima*, *World Journal of Pharmacy and Pharmaceutical Sciences*,3:471-478.

[12] Balasubramaniam , L., A., Manivannan, R., Paramaguru, R., and Vijayakumar, M., (2011). Evaluation of anti-inflammatory and antioxidant activities of stem bark of *Todalia asiatica*, Lam. using different experimental models. *Global Journal of Pharmacology*. 5(2): 67-72.

[13] EL-Kamali, H. H., (2009). Medicinal Plants in East and Central Africa: Challenges and Constraints. *Ethnobotanical Leaflets*, 13: 364-69.

[14] Kunle, O. F., Egharevba, H. O. and Ahmadu, P. O., 2012. Standardization of Herbal Medicines - A review. International Journal of Biodiversity and Conservation, 4(3): 101-112.

[15] Jagessar, R. C., Mars, A. and Gomes, G., (2008). Selective Antimicrobial Properties of *Phyllanthus acidus* Leaf Extract against *Candida albicans, Escherichia coli* and *Staphylococcus aureus* using Stokes Disc diffusion, Well Diffusion, Streak Plate and a Dilution Method. *Nature and Science*, 6(2): 24-38

[16] Mosihuzzanman M. and Choudhary, M. I., (2008). Protocols on Safety, Efficacy, Standardization and Documentation of Herbal Medicine, *Pure Applied Chemistry* 80 **(**10**)** 2195-2230

[17] Rubaka, C., Ndakidemi, P., Malebo, H., Shahada, F., (2014). Analysis of phytochemical and antibacterial activity of *Carissa spinarum* Linn Crude Extracts. *European Journal of Medicinal Plants*, 4(8): 937-945.

[18] Nathiya, M. and Dorcus, D., (2012). Preliminary phytochemical and anti-bacterial studies on *Physalis minima* Linn.. International Journal of Current Research in Biosciences and Plant Biology, 24-30 Research article ISSN 2250-1770