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Assessing the Efficacy and Safety of African Herbal Medicine for Treatment of Diseases in Tanzania

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Abstract: Herbal medicines play a greater role in prevention and management of both communicable and noncommunicable diseases. However, most communities misuse and abuse traditional medicines when using without revealing their safety and efficacy and sometimes heal by associating with myth. Water concoction of *Bersama abyssinica* stembark and *Ocimum gratissimum* leaves, a registered herbal medicine (Care Spiradul ®) in Tanzania was screened for active metabolites, antimicrobial activity and cytotoxicity activity for validating its use for treatment of respiratory related diseases. This herbal remidy has been used for treatment of Asthma, pneumonia, Tuberculosis, Lung cancer, COVID-19 and other respiratory diseases. The extraction of active compounds was done by decoction and infusion of stem and leaves respectively in clean and soft water (7.0pH). Ten (100g) of stem bark was soaked in IL of water boiled at 100°C for 15 followed by infusion of 10g leaves when decoction temperature reached 80°C for 20 minutes and thereafter left for cooling. The resultant concoction was filtered using muslin cloth to separate particles and phytochemical analysis was done using GC-MS and LM-MS/MS techniques. The phytochemical analysis revealed the presence five active compounds in groups of coumarin, flavonoid, glucose, acid and vitamin namely; 3-acetamido coumarin, 3-hydroxy -3-4-5 trimethoxyflavone, 3-hydroxy -3-4-5 trimethoxyflavone, B-D glucopyranose, 1,6-anhydro, citric acid and vitamin C respectively which showed a synergetic effect against respiratory diseases. This study revealed effectiveness of water extract for treatment of respiratory diseases including COVID-19, asthma, pharyngitis, tonsillitis, bronchitis, pneumonia, Tuberculosis and diarrhea. This study revealed the efficacy and safety of Care Spiradul and recommends further study on clinical trial and improvement of formulation into dry package to increase shelf life.

Keywords: Herbal Medicine, Concoction, Chronic Respiratory Diseases, Tuberculosis, Traditional Medicine

1. Introduction

Chronic diseases especially respiratory diseases (CRD) threaten health across the world where its deaths are estimated to be more than 4 billion annually and more than 500 million patients live in developing countries [11, 10]. These diseases include; asthma, bronchitis, Tuberculosis, respiratory allergies, Chronic Obstructive Pulmonary Disease (COPD) and occupational lung diseases (COLD). Although people of all ages and classes suffer from chronic diseases, severe cases are reported in developing world including Tanzania increasing economic burden [6]. These diseases are also the major cause of morbidity and mortality in children and productive group impacting household and national economy [17]. Chronic Obstructive Pulmonary Disease occurs as a result of chronic inflammation due to limitation of airflow in airways including bronchitis and bronchiolitis or lung parenchyma due to exposure to smoke or smoking behavior [3, 7]. However, inflammation on the respiratory system might have magnifying effect respiratory disease patients [39]. Although tobacco smokers are at high risk of getting infected with COPD, studies showed that non-smokers are at risk as well due to exposure to several pollutants in environment [31]. Chronic Obstructive Pulmonary Disease is also accelerated by coinfections with infectious diseases including; Tuberculosis, COVID-19, Human Immunodeficiency Virus (HIV) and other viral

diseases causing complications during management [30, 15, 16]. However, treatment of infectious diseases include pneumonia caused by *Klebsiella pneumonia*, lung infection due to *Cryptococcus neoformans* infection and those caused by *Pseudomonas aeruginosa* and *Streptococcus pyogenes* in COVID-19 co-infected patients of great concern in management of Chronic respiratory diseases [12, 29, 38]. Management of chronic respiratory diseases is challenging, and the most common medications are inhalation of bronchodilator, theophylline, and cessation from smoking and vaccination to reduce impact of pneumonia and influenza infection. However, the effectiveness of these therapies depends on stage and rate of infection where in most cases it is suggested to combine more than two therapies [35]. Studies show that in most cases COPD are associated with asthma although it is difficult to distinguish between the two but in most cases minor infections are mostly ignored during diagnostic and treatment. Recently, studies showed the failure of these therapies including corticosteroids which calls for alternative medicines particularly herbal drug with unique mode of treating inflammation and microbial infections in respiratory system.

As the world is shifting from reliance on synthetic drug to herbal medicine as studies revealed the potential of natural compounds in treatment of chronic diseases that uncured by conventional drugs. Despite the phytochemical and pharmacological studies on herbal medicine, the applicability of these products is limited due to uncovered facts on cytotoxicity, pesticide residue, aflatoxin and other microbial contaminants that have health implications [34, 2]. Screening for effectiveness of herbal medicines and quality assessment would improve the Herbal Medicine industry [21, 24]. This study was conducted to reveal the phytochemical constituent, efficiency and assessment of heavy metal and toxic metabolite in Care Spiradul used for validating its efficiency and safety for treatment of chronic respiratory diseases in Tanzania.

2. Material and Methods

2.1. Collection of Plant Materials

The stem of *Bersama abyssinica* and *Ocimum gratisimum* leaves were collected in dry season in 2021 from Ilolo village in Mbeya region with the voucher specimens number ND. Zekeya (Nos. 01 and 02) respectively and were deposited in the National Herbarium. Plant materials were collected through collection permit number FMM 4052 from the University of Dar es Salaam. Following collecting, the leaves and stem bark were dried in the shade at room temperature before being crushed into small pieces before extraction. The plants are no longer considered to be of conservation concern, according to national and international rules.

2.2. Reagents and Microbial Isolates

Methanol and Dimethyl sulfoxide (DMSO) were purchased from RFCL Limited, Hayana, India while microbial culture media for mycobacteria, bacteria and fungus namely; Middlebrook broth, nutrient broth, Saboraud dextrose broth respectively were bought from HIMEDIA®, India. Standard drugs, Rifampicin, Azithromycin (Azuma), fluconazole, ciprofloxacin and iodonitrotetrazolium chloride were bought from SIGMA® (Sigma- UK). RIDASCREEN Aflatoxin Kit was purchased from RIDACOM, Bulgaria. Bacteria isolates; *Klebsiella pneumonia Mycobacteria tuberculosis*, Staphylococcus aureus, Streptococcus pyogenes (clinical isolate), *Escherichia coli*, *Pseudomonas aeruginosa*, *Vibrio cholera* and Fungal isolates; *Cryptococcus neoformans* (clinical isolate) was obtained from department of Microbiology and Immunology, School of Medicine at Muhimbili Institute of Health and Allied Sciences.

2.3. Extraction of Active Metabolites

Bersama abyssinica stem bark and *Ocimum gratissimum* leaves extracted by decoction and infusion in water method respectively. About 100g of *B. abyssinica* stem barks were chopped into small pieces and dried under the shade followed boiling into 1 L of water for 20 minutes. The concoction was left for cooling for 4 hr followed by double filtration using cotton wool followed ant thereafter whatman® filter paper number 1 and the filtrates. The concoction was ready for use and labeled as BASA. On other hand, *O. gratissimum* leaves were extracted by infusing at 80°C into boiled water during cooling process until when become warm. This was followed by double filtration using cotton wool followed ant thereafter whatman® filter paper number 1 and the filtrates followed by double filtration using cotton wool followed ant thereafter whatman® filter paper number 1 and the filtrates was packed and ready for use and labeled as OGLA. After separate extraction of both extracts were mixed in ratio of 60:40 BASA and OGLA and the mixture was labeled as BASLA which is used as herbal medicine (Care Spiradul) while a portion of each extract was oven dried at 50-60°C to obtain dry extracts for further analysis.

2.4. Qualitative Test for Secondary Metabolites

All extracts were subjected to the qualitative test for Tannin, Saponin, Antioxidant, Phenol and Flavonoid and the extracts that showed positive results were further subjected for quantitative test for the evaluation of the exactly amount of particular group of secondary metabolites present. The Qualitative analysis were performed by using the standard procedures previous described by “Trees and Evance” before subjected to the Quantitative analysis. The concentrations of 100mg/ml were prepared in each sample for the analysis of Phenol, Saponin, Antioxidant, Flavonoid and Tannin accordingly. Test for Phenol involved addition of about 2ml of Iron III chloride solution to the 2ml of 100mg/ml of each extract; the appearance of deep bluish-green solution indicates the presence of phenolic compounds. Flavonoid was tested by diluting 5ml of ammonia solution by adding into 2ml of 100mg/ml of extracts followed by addition of few drops of concentrated Sulphuric acid, a yellow coloration

indicates the positive result for the presence of flavonoid compounds. Test for Antioxidant was done by dissolving 100mL of each extract in the 1ml of water in test tube, followed by the addition of 0.5ml of pre-prepared 0.1mM DPPH in the one of the test tubes and the second test tube act as control of the reaction, the mixture was shaken and allowed to stand for the 1minutes. The formation of de-coloration in comparison with control indicate the present of antioxidant compounds in the extract. Saponin was tested by dissolving 100ml of each extract into 2ml of distilled water in the test tube and warmed; this is followed by vigorously shaken. The formation of froth for at least a minute indicated the present of saponin compounds.

2.5. LC-MS/MS Analysis for Polyphenolic Compounds

Liquid chromatography Mass Spectrometer (LC-MS) method was used to separate compounds from a liquid sample prior to mass spectrometry analysis. This technique is very sensitive separate compounds within the mobile and stationary phases. Thereafter chromatographic separations, compounds eluted off of the column into the gas phase and ionized for the mass spectrometer for mass analysis as per GCLA procedures.

2.6. Analysis for Heavy Metal and Pesticide Residue for Quality Assurance

All three extracts were subjected to test for presence of heavy metal that would hinders utilization of herbal medicine where the presence of Lead, Asernic, cardimium and chromium were tested using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) analysis Technology at the Government Chemist Laboratory Authority (GCLA). All samples were subjected to this instrument to trace a level element including heavy metal if present in the sample and quantified them. Finally, samples were tested for pesticide residue to ensure safety of the concoction which was performed using Gas Chromatography- Mass Spectrometer (GC-MS/MS).

Working standard solutions were prepared at concentrations ranging from 0.5 to 2 μg/mL and were stored in a freezer. Blank and recovery tests were done to check the performance of the procedures and instruments. All sample types were analyzed concurrently with matrix blanks; 6 blanks were analyzed. A known volume of a mixture of pesticide standards solution was spiked into blank samples for recovery tests. Each spiked sample was homogenized, extracted, cleaned up, concentrated, and analyzed just like the ordinary samples. Six recovery tests were done for the matrix blank samples.

2.6.1. Aflatoxin Test

RIDASCREEN Aflatoxin total kit containing (100%-aflatoxin B_1, 48%-B_2, 78%-G_1 18%-G_2) was used to test the quality of extracts according to manufactures instructions. Tests were conducted using three extracts OGLA, BASA and BASLA. Six standards (STD1, STD2, STD3, SD4, STD5 and STD6) with known concentration of aflatoxins: 0.00, 0.05, 0.15, 0.45, 1.35 and 4.05 µg/L, respectively were used together to compare aflatoxin content with extracts. Samples were prepared as per manufacturer's instructions with minor modification; extraction done in 70% methanol and centrifuged at 3500 g at room temperature (23°C) and the filtrate was diluted six times for the test. The optical densities (OD) were measured at 450 nm using SYNERGY HTX Multi-mode reader (BioTek Digital, USA). Extracts and standards were analyzed together in duplicate wells on plate and the experiment repeated.

2.6.2. Determination of Minimum Inhibitory Concentrations on Pathogenic Microbes

All plant extracts namely; BASA, OGLA and BASLA were evaluated for antimicrobial activity against the selected clinical microbial isolates by determining the minimum inhibitory concentrations (MICs). The selection of microbes was based on those that affect respiratory system and those having immune compromise with COVID-19 patients. Both fungal and bacterial isolates were assayed by serial micro dilution method using 96-wellmicrotitre plates. The places were initially preloaded with 50 µL of the nutrient broth for bacteria and potato dextrose broth fungi respectively followed by addition of 50 µL of 100 mg/mL in each of the first row to make a total volume of 100 µL per well. The content was though mixed and thereafter, 50 µL were drawn from the well and added into the subsequent rows using 12 channel micropipettes. The same was repeated into subsequent columns to the last row where 50 µL were withdrawn and discarded to reach the required volume. Thereafter, 50 µL of the pathogenic microbes' suspensions were added in each well to make the final volume of 100 µL. Where in positive controls, Rifampicin, Azithromycin (Azuma) for respiratory pathogens, ciprofloxacin for antibacterial activity and fluconazole for antifungal were added as positive while DMSO was employed as negative control. Rows with nutrient broth, Middlebrook broth and Saboraud's dextrose broth were used to monitor bacterial and fungal growth, respectively. Plates were incubated at 35°C for 24 hr. From each plate the MICs for both bacteria and fungi were determined by adding 20µL of 0.02% p-iodonitrotetrazolium (INT) chloride dye followed by incubation for 1hr at 35°C. Growth and contamination of bacteria and fungi was indicated by color change. The lowest concentration which showed no growth was considered as the MIC of the extract.

2.6.3. Assessment of Use Care Spiradul (BASLA) for Treatment of Respiratory Diseases in 2021

Care Spiradul was supplied from May to December 2021 to hospitalized COVID 19 and out patients who were confirmed to have infection. Each patient was supplied with two bottles measuring 500 mls each where 240mls was considered as dose according to prescribed instructions. The administration of Care Spiradul to patients was taking 40mls orally thrice a day in adults whereas 20 mls twice a day for children between 5-10 years. Data of all patients using herbal medicine dose and their progress was recorded in each disease and per region for eight months consecutively.

3. Results

The phytochemical screening of *B. abyssinica* stem bark (BALA) and *O. gratissimum* leaves (OGLA) which formed BASLA (Care Spiradul) revealed the five active compounds in groups of coumarin, flavonoid, glucose, acid and vitamin namely; 3-acetamido coumarin, 3-hydroxy -3-4-5 trimethoxyflavone, B-D glucopyranose, 1,6-anhydro, citric acid and vitamin C respectively. The results revealed the absence of alkaloids, saponin, aflatoxin and pesticide residue in the concoction (Figure 1). The qualitative analysis revealed the presence possessed two polyphenols; coumarin and flavonoid in Care Spiradul all with antioxidant activity (Table 1). Quantitative analysis of heavy metals showed that Care Spiradul contain insignificant number of heavy metals where the amount of chromium, arsenic, cadmium measured was <0.0001 ppm and lead was <0.0005 ppm compared to control where the amount of heavy metal ranged from 2 in chromium and 10 ppm in lead (Table 2). The presence of pesticide residue in extracts was detected by the established limits of the analytes that was based on the lowest injected amounts in samples that resulted in peak heights three times higher than the baseline noise level. Every signal below the limit was treated as no pesticide detected where all extracts were below the limit (Table 2). However, the presence of aflatoxins was indicated by high level of the optical density (OD) values in aflatoxin containing standards. Extracts OGLA, BASA and BASLA exhibited optical densities of 3.01nm, 3.09 nm and 3.17 nm respectively with 0.00 µg of aflatoxin metabolites that was similar to control (STD 1) with aflatoxin level of 0.00 µg which was regarded as undetectable. Contrary, standard 2 up to 6 had aflatoxin concentration of 0.05 µg 0.15 µg, 0.45 µg, 1.35 µg and 4.05µg respectively, with inverse optical densities ranging from 3.4 nm to 0.5 nm.

The antimicrobial results showed that the water extract of *B. abyssinica* was efficacy against seven pathogenic microbes with BASLA exhibiting the lowest MIC of 0.19 compared to BASA and OGLA where the extract exerted high effect in comparison to pure compounds (standard synthetic drug) (Table 3). BASLA extract exhibited high antimicrobial activity that was similar to control. The concoction supplied to patients during the third wave of COVID-19 pandemic from May 2021 reached more than 4000 patients who were relieved from COVID-19 symptoms, asthma, pharyngitis, tonsillitis, bronchitis, pneumonia and TB (Figure 2). The region with high number of COVID-19 patients who used herbal medicine were Dar es salaam, Arusha, Kilimanjaro and Dodoma whereas Mara, Kigoma, Simiyu and Unguja experienced a lower number of patients as depicted in Figure 3. The concoction was also found effective in treatment of rheumatism. Thereafter, the safe dosage for children and adult were revealed to be 500 mls and 1000mls while lung cancer patients used up to 2000Mls respectively for patients with COVID-19 symptoms.

Table 1. *Qualitative Analysis active secondary metabolites and toxic residue in extracts.*

Extract	Group of Secondary Metabolites Tested						
	Phenol	Flavonoid	Antioxidant	Saponin	Alkaloid	Aflatoxin	Pesticides
BASA	√	X	√	X	X	X	X
OGLA	√	√	√	X	X	X	X
BASLA	√	√	√	X	X	X	X
Control	√	√	√	√	√	√	√

KEY: - √-Present, X-absent

Table 2. *Analysis for heavy metal and pesticide residue for determination of safety of herbal medicine.*

Extract	Group of Chemical subjected for analysis						
	Heavy metals (ppm)				Pesticide residue		
	Chromium	Arsenic	Lead	Cadmium	Organo-phos Phate	Organo-Chlorine	Pyrethroid
BASA	<0.0001	<0.0001	<0.0005	<0.0001	-	-	-
OGLA	<0.0001	<0.0001	<0.0005	<0.0001	-	-	-
BASLA	<0.0001	<0.0001	<0.0005	<0.0001	-	-	-
Control	2	2	10	0.3	>2.0 µg/ml	>2.0 µg/ml	>2.0µg/ml

Table 3. *Invitro antimicrobial activity of extracts against selected pathogens.*

Treatment	Pathogenic Isolates						
	M. tuberculosis	*K. pneumoniae*	*P. aeruginosa*	*S. pyrogens*	*C. neoformans*	*E. coli*	*V. cholerae*
OGLA	1.56	0.78	0.39	0.78	0.78	0.78	0.78
BASA	0.78	0.78	0.39	0.39	0.78	0.78	0.78
BASLA	0.19	0.19	0.39	0.19	0.19	0.19	0.19
Rifampicin	0.19	0.19	0.19	0.19	0.39	0.39	0.78
Azuma	0.39	0.19	0.19	0.19	0.39	0.39	0.78
Fluconazole	0.78	0.39	0.39	0.39	0.19	0.39	0.39
Ciprofloxacin	0.78	0.39	0.39	0.39	0.19	0.19	0.19

KEY: OGLA-*Ocimum gratissimum* Leaves Aqueous extract; BASA- *Bersama abyssinica* Stem bark aqueous extract; BASLA- *Bersama abyssinica* Stem bark and leaves aqueous extract

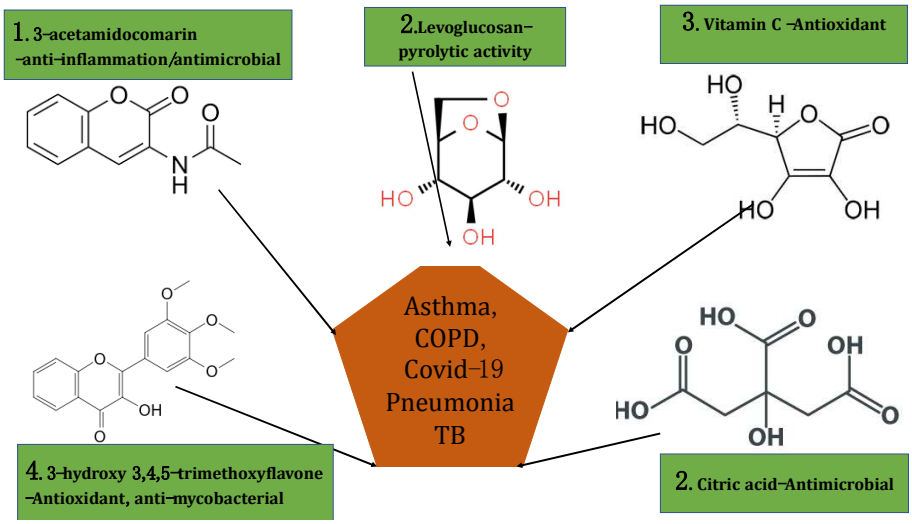

Figure 1. *Graphical presentation of five active compounds from water extracts (Care Spiradul) exhibiting synergetic activity against chronic respiratory diseases.*

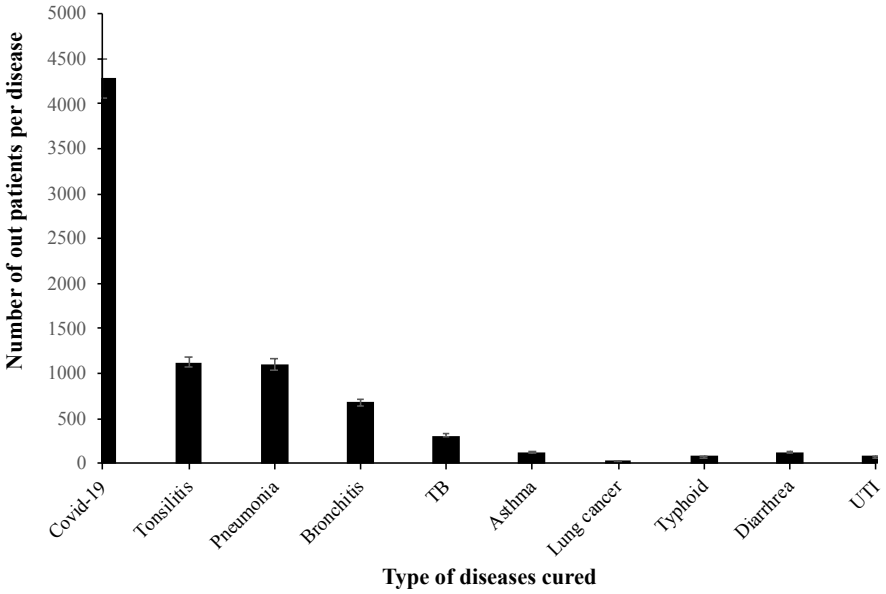

Figure 2. *A cumulative number of patients in 13 regions of Tanzania used Care Spiradul for treatment of diseases from May to June 2021.*

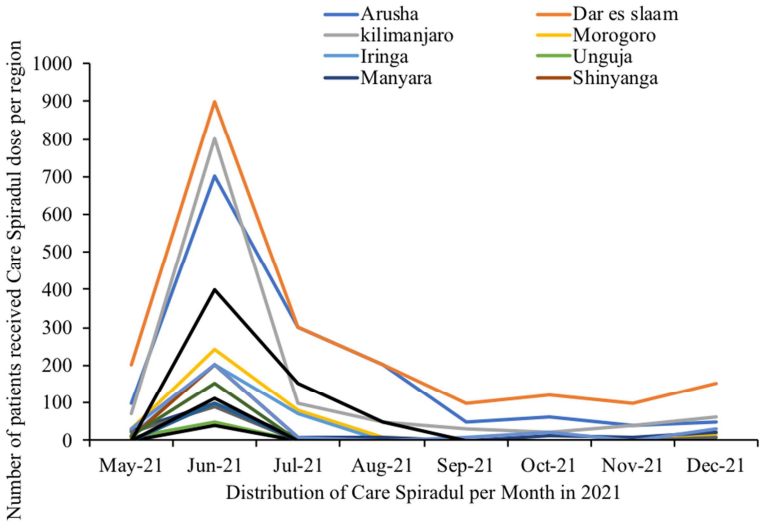

Figure 3. *A graph showing uses of Care Spiradul by region for treatment of COVID-19 during third wave in 2021.*

4. Discussion

Herbal medicines have for long time been used in treatment of several chronic diseases and recently gained popularity against COVID-19, despite of lack of scientific approval and safety for human. Despite the effectiveness of herbal medicine, quality control for insuring user's safety is critical in traditional medicine [21, 8, 33]. Heavy metal and microbial contaminants are reported to affect the quality and consumption of traditional medicine in health care canters [36]. This study revealed the presence of active metabolite that is not contaminating with aflatoxin, heavy metal and pesticides. This reflects an ideal herbal medicine which should be efficacy, readily available, free from toxic metabolites like heavy metal that would hinder utilization of herbal medicines. The effectiveness of herbal medicine on COVID-19 is undoubtful due to diverse of active metabolites most of which exert an antioxidant activity suppressing oxidative stress which causes several pathogenic and chronic diseases in human body. Following the rapid eruption of COVID-19, it is obvious almost all the world was unprepared for the disease as result most African country relied even on unregistered herbal medicines to rescue the situation. In Tanzania, several herbal concoctions including Care Spiradul were prepared and taken as remedies against COVID-19. Studies revealed the effectiveness of herbal medicine for fighting respiratory diseases including COVID 19 [20] and against lung cancer [22]. The phytochemical analysis of Care Spiradul revealed the presence of phenolic, coumarin and flavonoid compounds with that are good antioxidants. Antimicrobial screening revealed that the concoction is effective against several pathogens some of which coinfect COVID-19 patients especially those causing breathing problem. This study revealed that the presence of a 3-acetamido coumarin an active metabolite for antimicrobial and antioxidant with anticancer activities [13, 1, 23]. This study revealed the existence of 3-hydroxy -3-4-5 trimethoxyflavone in herbal concoction which is report to treat pulmonary tuberculosis [41], and the similar result is revealed by another study that showed the presence and effectiveness of flavonoids from medicinal plants with antioxidant and antimicrobial [18]. Studies revealed the antimycobacterial activity of flavone from natural products [32, 40]. This study revealed the presence of vitamin C which could be associated with prevention and treatment of respiratory pathways and studies showed that it is a source of antioxidant with high ability to treat chronic respiratory diseases [9, 14]. Martin-Lorenzo, M. et al. [26] revealed the anti-inflammatory and antioxidant activity of citric acid which its presence in Care Spiradul would have influence in the treatment of inflammation and pathogens in COVID-19 patients. Recently, it has also been reported that this flavonoid has a wide range of pharmacological applications hence potential for drug development [28]. Levoglucosan (1,6-anhydrous d-glucopyranose) revealed as an important component in as anti-HIV, blood coagulant and pyrolysis and act as initial material in formation of polysaccharides help in controlling diabetes mellitus [5, 19]. The effectiveness of herbal medicine on respiratory pathogens would prevent COVID-19 patient from serious condition resulted from severe pneumonia and bronchitis that are reported to be resistant in some COVID-19 patient [4, 27, 29] which increases cost for hospitalization and mortality due to limited breathing. Studies show that treating infectious diseases relieves COVID-19 patient and prevent getting into chronic conditions [25, 37]. Further, analysis revealed that Care Spiradul possessed no heavy metal and aflatoxin that would induce other health problems. Heavy metals, aflatoxin and pesticide residue are among factors hindering the commercialization of herbal medicine. Preparation of safe and high-quality herbal medicine is required to improve the stand of herbal medicine as they are mostly available and used by high population in rural areas in Africa. Since February 2021, Care Spiradul has been used for treatment of respiratory diseases particularly COVID-19. However, high demand for Care Spiradul was recorded in July especially in highly populated cities of Tanzania particularly in Dar es salaam, Arusha, Moshi and Dodoma. This could be due to rapid increase in COVID-19 cases in June and July reaching 26, 154 patients by October 2021 while deaths are reported to reach 725 [42]. The findings on the demand of Care Spiradul is positively corelated to reemergence of the disease where in June and July 2021 more cases were reported compared to other months.

5. Conclusion

This study revealed the active and safe phytochemical composition of aqueous extract of *Bersama abyssinica* and *Ocimum gratissimum* for treatment of respiratory diseases. However, this study recommends future studies to focus on isolation of active compound and improvement of formulation to enhance efficacy and commercialization of the herbal drug for wide application on treatment of chronic diseases across the world.

Declaration

All authors declared that there is no competing interest.

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