Journal of Applied Biosciences 105:10055 –10063

ISSN 1997–5902

Oxalis corniculata L. in Tanzania: traditional use, cytotoxicity and antimicrobial activities

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Original submitted in on 23rd June 2016. Published online at www.m.elewa.org on 30th September 2016
http://dx.doi.org/10.4314/jab.v105i1.2

ABSTRACT

Objectives: Oxalis corniculata Linn (creeping wood sorrel) is a weed plant species traditionally widely used as a raw vegetable and in folk medicine to treat different human ailments by different Tanzanian ethnic groups. However, not many reports are available on the pharmacological rationale for its wide application in the country. This study presents the traditional use, cytotoxicity and antimicrobial activities of *Oxalis corniculata* indigenous from Tanzania.

Methodology and results: Extracts were made from whole plant using water, methanol and ethanol solvents. Traditional uses were collected from interviewing 25 ethnic groups each represented by at least five members using a guided questionnaire. The extracts were tested for antifungal, antibacterial activities and brine shrimp cytotoxicity. Results showed relatively mild cytotoxic activity against brine shrimp larvae LC_{50} value of 156 µg/ml. The antimicrobial activities were positive against standard fungal and both Gram-positive and Gram-negative bacteria strains of medical importance with the highest inhibition zone in a Gram-negative bacteria *Escherichia coli* (7.5 mm/48 hrs) and a fungi *Candida albicans* (4 mm/48 hrs).

Conclusion and application of findings: The traditional uses of *Oxalis corniculata* in treating tonsillitis, toothaches, flue, diarrhea and antirust were recorded for the first time. Besides, the plant extracts showed strong antimicrobial activities against pathogens of human importance and less toxic to cells, which support its use as a cure for some human ailments. The study findings thus support its traditional medicinal use and envisage a purposeful thorough study for isolating the bioactive compounds, up scaling for possible developments into nutraceutics and drugs.

Key words: *Oxalis corniculata*, cytotoxicity, folk medicine, *Escherichia coli, Candida albicans, S. aureus*, antimicrobial activity.

INTRODUCTION

Plants are well known valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies (e.g. Fagbohun *et al*., 2012; Sofowora, 2008). Tropical eco-region is well known for harboring majority of plants that provide raw material for addressing a range of medical disorders and pharmaceutical requirements (Kubmarawa *et al*., 2008). Fatope *et al*. (2001) noted that, the secondary metabolites harbored by these plants are due to the presence of chemical substances that produce a definite physiological

action on the human body. The most important of these include alkaloids, glucosides, glycosides, steroids, flavonoids, fatty oils, phenols, resins, phosphorus and calcium for cell growth, replacement, and bodybuilding (Chidambara *et al*., 2003). About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants, which should be investigated to better understand their properties, safety and efficiency (Ellof, 1998). Many Tanzanians who reside in rural areas and per-urban areas rely on plants for their primary health care needs, a creeping wood sorrel *Oxalis corniculata*, also known as creeping wood sorrel being one of them. It is a perennial, low-growing, herbaceous plant in the family Oxalidaceae. It spreads by seed, rhizome, or stolons and is commonly found as a weed of lawns, gardens, and pathways. This species is cosmopolitan in its distribution, and its place of origin is not well known although some propose it to be indigenous to tropical and subtropical regions of the world (Kumar *et al*., 2012). The leaves of *Oxalis corniculata* are edible, usually eaten raw and most liked due to its tangy taste of lemon. In some parts, it is made into a drink by infusing the leaves in hot water for about 5-15 minutes, sweetening and then chilling. In spite of its wide application in folk medicine in the country, its therapeutic rationale is not well established. This study thus presents the traditional uses of this plant, its antimicrobial activities and cytotoxicity assay in order to establish its pharmacological rationale in folk medicine in the country.

MATERIALS AND METHODS

Collection of plant samples: *Oxalis corniculata* whole plant (Figure 1) was collected from Dar es Salaam region and Tanga in three consecutive years 2014-2016. Plants were identified by a plant taxonomist from the Botany Department of the University of Dar es Salaam.

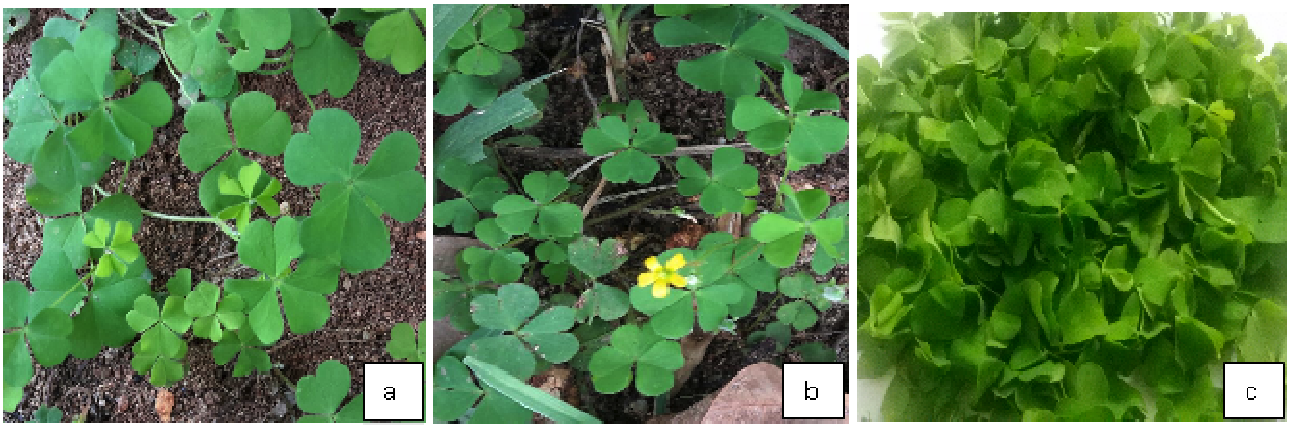

Figure 1: *Oxalis corniculata* (a&b) growing in garden (c) Fresh leaves collected for experimentation

Cytotoxicity assay: This was done using the Brine shrimp lethality test (BST). The Brine shrimp eggs were bought from Dohse Acquaristic, Bonn (Aus Dem Hause Dohse Acquaristik, Germany). Sea salt was prepared locally by evaporating water collected from the Indian Ocean, along the Dar es Salaam Coast.

Extracts preparation and Serial dilution: Powdering of 120 gram of fresh leaves of *Oxalis corniculata* was done by grinding it with a mortar and pestle. It was then incubated for extraction using methanol and water for 48 hours. After incubation period, the mixture was filtered using cheesecloth and separating funnel to obtain the filtrate. The filtrate was then allowed to evaporate to dryness at 40ºC by aid of rotary evaporator with 90 rpm (Labrota 4001, Heidolph® Essex Scientific Laboratory Supplies LTD) under reduced pressure to obtain the crude extract. The crude extract was weighed using weighing analytical balance and found to be 0.5 gram. This was followed with serial dilutions preparations whereby 0.3 g of crude extract was dissolved in 10 ml of dimethylsulfoxide (DMSO) to obtain stock solution of 0.03 g/ml, which was equivalent

to 30 mg/ml. Serial dilutions were then made from the stock solution to obtain a concentration of 15 mg/ml, 7.5 mg/ml, 3.75 mg/ml, 1.875 mg/ml and 0.9375 mg/ml by taking 1ml from the stock solution and diluted in DMSO of equivalent volume deduced from the dilution formula, CiVi=CfVf

Whereby: C= concentration of the solute (*C. corniculata*); V= Volume of solvent; i= Initial; f= Final

BST assay: Cytotoxic activity in the crude extract of the *Oxalis corniculata* was carried out using Brine Shrimp Test (BST). As detailed in Sosovele *et al*. (2014), the procedures included dissolving the crude extracts in DMSO to make a concentration of 4 mg/ml (stock solution) for each sample. One teaspoon of brine shrimp eggs (Great Salt Lake, USA) was gently poured in a 300 ml of conical flasks full of filtered seawater. It was then allowed to shake for 48 hours while illuminated using an electric bulb, which helped attracting the hatched shrimps. Using 100 µl pipette, 10 hatched shrimp larvae were selected and transferred into different sample wells, which contained 1m of each dilution of extract with one control with only DMSO. The mixture were mixed gently to allow good mixing and incubated at room temperature for about 24 hours. The number of survivor and dead nauplii were counted and recorded directly on the compound microscope (OLYMPUS BX50 PHASE POL DARKFIELD MICROSCOPE, JAPAN) whereby the percentage mortality was then calculated logarithmically. Based on the concentration in relation to the number of dead nauplii the graph was plotted and regression equation obtained which was used to calculate the LC_{50}. The percentage mortality were obtained by using the following formula

$$\% \text{ Mortality} = \frac{\text{number of dead nauplii} \times 100\%}{\text{Total number of nauplii}}$$

Antimicrobial activity test: A Gram-positive bacteria, *Bacillus subtilis* (DSM 347), a yeast *Candida albicans* (ATCC 90028) which were both obtained from the Department of Molecular Biology and Biotechnology (MBB), University of Dar es Salaam and a clinical strain *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) from the Muhimbili University of Health and Allied Sciences (MUHAS) were used. The bacteria were grown in Nutrient broth and incubated at 37°C for 24 h. A standard concentration of 20 mg /ml of the filtrate was prepared using sterile distilled water as a diluent at a dilution rate of 0.04 g of extract in 2 ml of sterile distilled water. Ten mils of the pure culture were then centrifuged. Using sterile physiological saline, they were washed twice and the suspension adjusted to optical density of 0.1 at 600 nm, equivalent to a cell population of 106 cells/ml based on McFarland (1907) standard. The suspension was kept in the test tube and stored at 4°C until used. The assay for antibacterial activities of each extract was determined by agar well diffusion method of Stoke and Ridgway (1980). Bacterial and fungal cultures were seeded into Mueller Hinton agar and Sabourad agar plates. Well measuring 7mm diameter was made on each plate using sterile cork borer. About 30 microliter of the extract was introduced into bore agar wells using sterile micropipette. The plates were kept inside the refrigerator at 4ºC for 3 hours to allow proper diffusion of the extracts into the medium. Extracts with activities at this concentration were regarded as having antimicrobial properties while other with no activity at this concentration were disregarded as explained in Hirasawa *et al*. (1999). All the experiments were carried out in duplicates while distilled water was used as negative control. The plates were incubated at 37 ºC for 24 hours. The zones of inhibition produced were measured in millimeters (mm), while those in which no zone of inhibition were regarded as negative (Table 1).

Oxalis corniculata **and Tanzanian folk medicine:** Guided and focused face to face interview was used to collect the information from twenty-five different ethnic groups. Each group was represented by at least five persons and thus more than 125 individuals were interviewed. Information was solicited in an open-ended fashion with a guiding question form prepared by the author as detailed in Tibuhwa (2012, 2013). Through this questionnaire, the interviewees were asked if they knew the plant, its local name, where does it grow mostly? Any use of the plant they know, and what pretreatment (if any) are carried out before the use of the plant.

Data analysis: Data analysis for the BST whereby a concentration that killed 50% of the brine shrimp nauplii (LC_{50} in µg/ml and Confidence Intervals 95%) was determined using a computer programme for Probit analysis according to Finney (1971) and Throne *et al*. (1995). The graph of percentage mortality of brine shrimp larvae against logarithmic Concentrations was used where by LC_{50} values were directly determined from probit analysis or calculated by substituting 50% for "y" into the curve equation in the graph.

RESULTS AND DISCUSSION

The results of this study found *Oxalis corniculata* Linn from Tanzania (Figure 1) with active antimicrobial activity against pathogen of medical importance (Table 1), less toxic to cells and playing a significant role in folk medicine system. The plant from Tanzania is similar to other species described elsewhere in the world. The plant was found growing under the tree shade, in banana plantation, cultivated trees and beside the house, a place usually dominated with shades. It was distinctively characterized morphologically by having a tangy taste of lemon. It posses 3 heart-shaped leaflets, yellow flowers with 5 petals occurring in an umbel (Figure 1). Its cotyledons are small and round while its seed are long, thin, angular, and pubescent. It is further characterized by well-developed deep thick taproots, which make it difficult to hand pull from the substrate. Molecular characterization are underway to supplement conventional taxonomy.

Antimicrobial activity: The antimicrobial assay carried in this study established high antimicrobial activities of *Oxalis corniculata* extracts on both Gram-positive and Gram-negative pathogen (Table 1). Recently, there is an increased trend of human pathogens to develop resistance against available antibiotics (Mitsuyama *et al*. 1987; Mathias *et al*. 2000; Martino *et al*. 2002). This study used both water and methanol which is a polar solvent thus expected to generate high extraction yield as it has been reported previously to be effective in several studies such as Sachin *et al*. (2014) and Banerjee (2012). No obvious reasons can be given as to why methanol extracts gave negative results in this study (Table 1). It was however interesting to get positive results in water extracts for both Gram-positive and Gram-negative bacteria as well as antifungal activity. The minimum inhibition zone for a Gram-negative *E. coli* was higher up to 7.5 mm/48hrs (Figure 2).

Table 1: Antimicrobial activities of the *Oxalis corniculata* extracts against pathogen of medical importance after 48 hrs of incubation

S/N	Tested microbe	Gram stain	Water extracts	Methanol Extracts	Average inhibition zones in mm
1	*Escherichia coli*	Gram-negative	+ + +	-	< 7.5
2	*Bacillus subtilis*	Gram-positive	+ +	-	< 3.0
3	*Staphylococcus aureus*	Gram-positive	-	-	-
4	*Candida albicans*	N/A	++ +	-	< 4

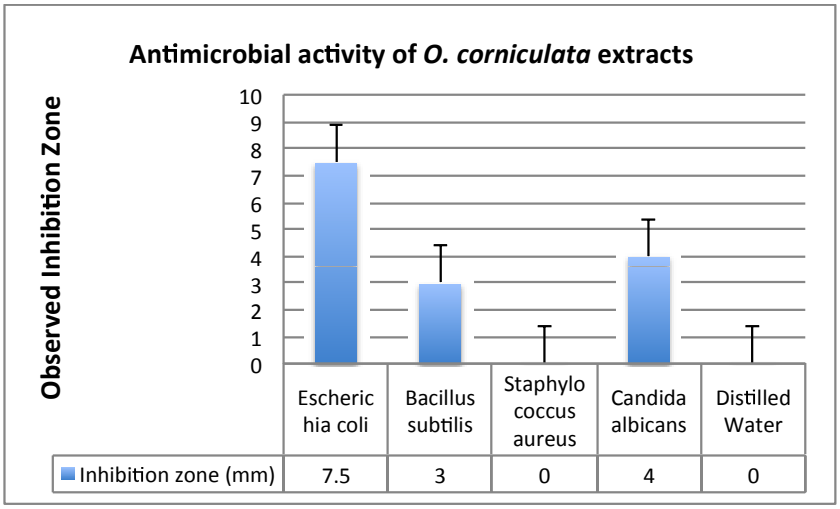

Figure 2: Antimicrobial activity of *O. corniculata* water extracts against four pathogens of medical importance. Error bars show standard errors of mean. (Bar standard deviation).

Gram-negative bacteria are always difficult to respond to antimicrobial chemicals because they possess thick lipid bilayer on the outside, which is selectively permeable thus make it less susceptible to antibiotics than Gram-positive bacteria. Results in this study reveal antimicrobial activity from *C. corniculata* extracts being active against both Gram-positive and Gram-negative bacteria. Besides, it also portrayed antifungal activities against *Candida albicans* with a minimum inhibition zone of up to 4 mm/48hrs (Table 1). The broad antimicrobial activities in both Gram-positive and Gram-negative bacteria, apart from the portrayed antifungal activity, show that *O. corniculata* herbal remedies contains several constituents which work together synergistically. This result thus shows that this studied plant is a potential source of broad-spectrum antibiotics as well as alternative source of antifungal.

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3	*Staphylococcus aureus*	Gram-positive	-	-	-
4	*Candida albicans*	N/A	++ +	-	< 4

Cytotoxicity assay of *Oxalis corniculata:* Cytotoxicity test on the *C. corniculata* extracts using brine shrimp lethality bioassay, portrayed a significant cytotoxic activities of LC_{50} 156 µg /ml, which is greater than100 µg/ml, thus making it non-toxic and safe. The LC_{50} of the control was 256 µg/ml. The extract is said to be highly toxic if it portray LC_{50} <1.0 µg/ml, between 1.0 - 10.0 µg/ml is toxic, 10.0 - 30.0 µg/ml toxic to moderately toxic while LC_{50}>30 <100 µg/ml and > 100 µg/ml are referred to as mildly toxic and non-toxic, respectively (Sosovele *et al*., 2014; Moshi *et al*., 2010). It was interesting to note that mortality rate of the brine shrimp increased relatively with the increase in the concentration of the sample as it was also reported by Sosovele *et al* (2014) but in *Actinomycetes* species isolated along the coast of Indian Ocean. The same was also reported in Krishnaraju (2005) during assessment of medicinal plants from India.

Oxalis corniculata **and Tanzanian folk medicine:** Synthetic drugs are alleged to be more damaging and less safe to the human body than natural herbal remedies (Tibuhwa *et al*. 2012, Kim 2013, Martin 2013). This has envisaged a purposeful proliferation of scientific research toward screening of plants for biological activities with therapeutic potential. Indigenous knowledge on uses and folk medicine is among the vital criteria for a plant to be considered from screening for herbal remedies.This study interviewed local people from twenty-five different ethnic groups for the local names, uses, its habitat, any use of the plant and accompanying pre treatment for medicinal applications (Table 2). It was established that *Oxalis corniculata* plays a big role in Tanzanian folk medicine. It was noted that different ethnic groups use it for treating different human ailment including dysentery, wounds, fever, gastrointestinal disorders, anti-inflammatory agent, good appetizer and some using it as a raw vegetable and in preparing tangy juice, prevent vomiting, cleaning rusts and cleaning sticky plant exudates (Figure 3). Out of the 25 ethnic groups interviewed in this study, 24 were reported to know the plant and use it as food especially raw. Only the Maasai who naturally do not eat vegetable reported not to know or use it at all. Traditional uses that are least reported in the explored ethnic groups are use as a spice, treating teeth ache and antirust. The last two, together with treating tonsillitis, flue and diarrhea that had ethnic frequency of 7, 7 and 12 respectively are reported for the first time in this study. It was interesting to note that the number of respondents irrespective of the ethnic groups relatively portrayed the same ranking with 'eaten raw as vegetable' being reported by 114 respondents followed by 'treating stomach with 102 while the least was 'treat heartburn reported by only 4 respondent out of 125 interviewees (Figure 3 b).

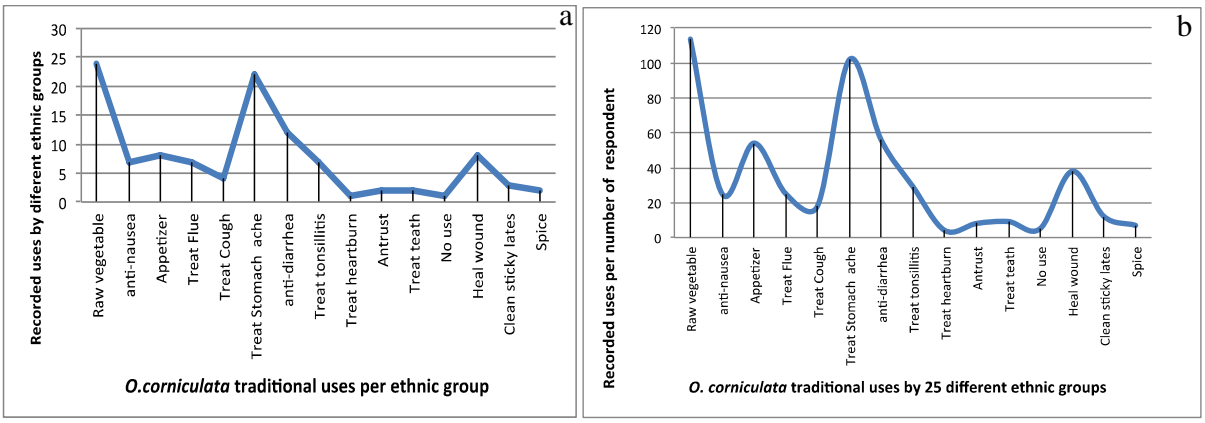

Figure 3: Traditional uses frequency of *O. corniculata* reported from: (a) twenty-five ethnic groups (b) Based on number of respondents in both cases dominated by eating at as raw food

Besides, more interesting economical uses were noted as it has been also established in other parts of the world (PFAF, 2013). It was revealed that the leaves of *O. corniculata* is used raw by adding them into salads which give them the tangy taste or cooked with other vegetables to give them sour flavor the practices which has been also recorded into other studies. The PFAF (2013) also presents other uses of *Oxalic corniculata* in folk medicine including use of the whole plant as anthelmintic, antiphlogistic, astringent, depurative, diuretic, emmenagogue, febrifuge, lithontripic, stomachic and styptic. It further denoted that it is used in the treatment of influenza, fever, urinary tract infections, enteritis, diarrhea, traumatic injuries, sprains as well as poisonous snakebites. Besides, its juice mixed with butter have been applied to muscular swellings, boils and pimples, its infusion used as a wash to rid children of hookworms, source of vitamin C, leaves used as an antidote to poisoning by the seeds of *Datura* spp, arsenic, mercury, insect bites, burns and skin eruptions and antibacterial activities. This study among other things established five new traditional uses of *C. corniculata* including treating tonsillitis, toothaches, flue, diarrhea and antirust from the 25 ethnic groups examined. It was interesting to correlate some traditional uses of the plant with the scientific explanation, especially the obtained antimicrobial activities results (Tables 1 and 2). It goes without doubt that probably its use in treating tonsillitis, flue and diarrhea might be associated with the portrayed strong antimicrobial activity against Gram-negative and Gram-positive bacteria species as well as a representative fungal species *Candida*. It is thus important to explore more of the medicinal application of this plant for further development into potential drugs.

Table 2: Indigenous knowledge on *Oxalis corniculata* and its utilization from different 25 ethnic groups in Tanzania.

S/No	Ethnic group	Local name	Traditional uses	Local preparation and administration
1	Sambaa	Kidadaishi	Food, appetizer, Medicinal	Eaten raw or added to stew to make it taste, juice taken by sick people to improve their healthy appetite and stop stomach upset including diarrhea /vomiting/anti nausea, wound healing
2	Chaga-Machame	Manyonyo	Vegetable, Medicinal	Eaten raw especially by children, Treat children first milk tooth irritation and aching, remove 'sticky plant latex, nausea and brings appetite, stop stomach aching and anti rusts.
3	Kurya	Mnyonyo	Vegetable	Eaten raw, tonic for various gastrointestinal ailments in infants and diarrhea
4	Nyambo	Bunyunyambuzi	Vegetable and	Eaten raw, few know it as tonic for various gastrointestinal ailments (e.g., abdominal pain, diarrhea and stomach

			medicinal	ache), wound healing
5	Kerewe	Bukanoro	Food	Eaten raw or boiled and taken as a juice The juice used to infants in treating stomach aching and severe diarrhea, treat flue, tonsillitis and cough, brings appetite
6	Jita	NK		Eaten as raw vegetable, treat stomach upset, diarrhea, tonsillitis, flue and cough
7	Masaai	NK	None	Do not eat vegetables like cows!
8	Nyakyusa	Mwakatilolo	Food and Medicinal	Eaten as raw vegetable, treat flue, tonsillitis, its juice used as medicine stop stomach upset, diarrhea, increase appetite, remove ' sticky banana latex,
9	Haya	Kanywambuzi	Food	Eaten raw, stop stomach upset, diarrhea, anti nausea in pregnant mothers and give them appetite, remove ' sticky banana latex and healing wounds
10	Lugulu	Kikwadangu	Vegetable	Eaten raw and treat stomach aching and diarrhea in infants
11	Hangaza	Umunyuwanyamanza	Food, medicinal	Eaten as raw vegetable, its juice used as medicine to treat, wound, stomach upset, flue in infants and spice to make the stew tasty
12	Ha	NK	Food and medical	Eaten raw and treat stomach upsets
13	Zinza	NK	Food and medicinal	Eaten raw, treat wound, stop stomach upset and nausea especially for pregnant mothers and give them appetite
14	Hehe	Linyanyinyanyi	Food Good appetizer	Eaten raw, mixed with salads to make it sour for increasing appetite Stop nausea, stomach upset, diarrhea and heart burn
15	Simbiti	NK	Food and medicinal	Eaten raw, treat wound, flue, tonsillitis and cough
16	Gweno	Uchecheri	Medicinal Food	Eaten raw, treat tonsillitis and flue when chewed, stomach upsets, diarrhea and nausea,
17	Chaga-Rombo	Vedivedi	Food, medicinal	Eaten as raw vegetable, its juice used as medicine to treat stomach upset in infants Cleaning rusts
18	Sukuma	Buseli	Food, medicinal	Eaten raw, treat stomach upsets and diarrhea especially in infants
19	Pare	Ushesheri	Food, medicinal	Eaten raw, treat wounds, tonsillitis, flue when chewed and stomach upsets
20	Zaramo	NK	Food, medicinal	Eaten raw, treat stomach aching and diarrhea
21	Hehe	NK	Food, medicinal	Eaten raw, treat stomach upsets diarrhea especially in children
22	Bena	NK	Food, medicinal	Eaten raw and treat stomach upsets
23	Machame	Misii	Food, medicinal	Eaten raw and treat stomach upset, nausea and brings appetite
24	Chaga-oldmoshi	Kisinga mana-heho	Food, medicinal	Eaten raw, treat tooth ache and stomach upset in infants
25	Wakibosho	Ndela	Food, Medicinal	Eaten raw and used in treatment of wound, cough, flue and tonsillitis

* NK= Not known

CONCLUSION

In this study, *Oxalic corniculata* Linn from Tanzania showed strong antimicrobial activities against pathogens of human importance and less toxic to cells, which support its folkloric use as a cure for some human ailments. The literature for this study revealed its enormous global consumption. It is high time more researches be conducted that will further elucidate and characterize the active components found in the plant, their pharmacological actions and possible development into drugs as well as its inclusion in pharmacovigilance systems.

ACKNOWLEDGEMENTS

The Department of Molecular Biology and Biotechnology University of Dar es Salaam is acknowledged for providing venue and facilities during the study. The author is also indebted to Mr. Kimati F of Department of Molecular Biology and Biotechnology University of Dar salaam for helping with Laboratory work.

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