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Antibacterial Effects of Tagetes Minuta Plant Extracts Against Escherichia Coli and Staphylococcus Aureus in Rwanda

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Abstract: **Antibacteria potential of extracts from fresh and dried *Tagetes minuta* plant was investigated against *Escherichia coli* and *Staphylococcus aureus* by using disc diffusion method. Extracts from both dried and fresh plant materials was prepared using two different solvent such as distilled water and methanol. The efficacy of crude extract was compared to that of standard antibiotic penicillin. The *Staphylococcus aureus* was isolated from row milk and was identified by biochemical test and *Escherichia coli* was isolated from patient's sample at Centre Hospitalier Universitaire de Kigali (CHUK). The susceptibility of tested bacteria to the extracts was obtained by measuring the diameter of zones provided by growth inhibition of bacteria inoculated on nutrient agar plates. The solid and liquid extracts obtained were later prepared into different concentration. The results revealed that the methanol extracts has higher inhibitory effects on both bacteria while the fresh and dried extracts showed less effect on both bacteria. The most susceptible bacteria in this study were *Staphylococcus aureus* than *Escherichia coli*. The extracts from dried plant showed more antibacterial activity than that from fresh plants and the standard antibiotic showed greater inhibition zone than that of plant extracts. This study has confirmed and justified the use of *Tagetes minuta plants* amongst the people especially those in rural communities where the practice has become prevalent owing to easy accessibility to the plant and relatively low cost of herbal preparation.**

Keywords: Tagetes minuta, **plant extracts, antibacterial activity, Standard antibiotic.**

I. INTRODUCTİON

Tagetes minuta is an erect annual herb reaching 1 to 2 m and is very strong and sharp smelling herb and with pale yellow flowers. Leaves are slightly glossy green, and are pinnately dissected into 4 to 6 pairs of pinnate and the Leaf margins are finely serrate and although the genus is known as marigold it must not be confused with Pot Marigold which is *Calendula officinalis*. It has very strong insecticidal property and the whole part of this plant and oil are used (Drewes *et al*., 1991).

The *Tagetes minuta* plant has been traditionally used by Rwandan people especially those of rural area since pre-colonial period to treat gastritis, indigestion, intestinal worms, skin infections and haemorrhoids. The plants have provided a source of inspiration for novel drug compounds and the preparation of herbal medicines remains an important part of healthcare for both humans and livestock, especially in rural area (Nahayo *et al*., 2010).

Traditional medicine is the sum of total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illnesses. Many people believe that because medicines are herbal (natural) or traditional they are safe (or carry no risk for harm). However, traditional medicines can cause harmful and adverse reactions if the product or therapy is of poor quality, or it is taken inappropriately or in conjunction with other medicine (Azaizeh *et al*., 2003).

Today according to the World Health Organization (WHO) estimates that 80% of the world's population uses herbal medicine for some aspect of primary health care. İn Asian and African countries 80% of the population depends on

traditional medicine for primary health care. İn many developed countries 70% to 80% of the population has used some form of alternative or complementary medicine (WHO, 2010).

The reason why the people in Rwanda use medicinal plants is that; these are widely available; the forest is the pharmacy and those who know the medicinal uses of the plants can get their roots, stems or leaves and make their own preparation. The preparation made from those plants work for certain illness. The current and most effective antibiotics are expensive because of life condition of Rwandan people especially in rural area. The medicinal plants, such as *Tagetes minuta*, is readily available and affordable in Rwanda.

This study was therefore aimed at providing scientific evidence to the acclaimed antibacterial potentials of *Tagetes minuta* for being used to treat bacterial diseases for severe skin, abdominal and many other types of infections caused by *Staphylococcus aureus* and *Escherichia coli*.

II. MATERIALS AND METHOD

Plants collection:

The fresh *Tagetes minuta* plant were collected from Mount Kigali during May 2012. Taxonomic identification of the plant materials was confirmed by comparison with plant specimens located at herbarium department in Institute of Scientific and Technological Research (IRST).

Bacterial culture:

The tested bacteria *Escherichia coli* were isolated from patient's sample in microbiology laboratory of Centre Hospitalier Universitaire de Kigali (CHUK) and identified following standard procedures as described by Shrotrie *et al.*, (2012). The *Staphylococcus aureus* was isolated from raw milk and identified by biochemical test in Kigali İnstitute of Science and Technology (KIST) Biotechnology laboratory.

Preparation of extracts:

Preparation of extracts from fresh plant:

The extract from fresh *Tagetes minuta* plant was prepared directly after collection. The *Tagetes minuta* plant were grounded by using Mortar and pestle. The 1000g of grounded fresh plant material were weighed out and soaked in 250ml of distilled water in 500ml Conical flask. After 24 hours the mixture was filtered by using filter paper to separate the neat extract from residue. After filtration was brought to Rotary evaporator for evaporation of water. Then, the 75ml of obtained extracts form fresh plant were labeled and stored in refrigerator at 5^{o}C till use.

Preparation of extracts from dried plant:

The 1000g of fresh plant material were dried in an Oven at 37^{o}C for three days. Then the dried plant materials were grounded by using blender. The 75g of obtained powder was soaked in 250 ml of distilled water in 500ml Conical flask and covered with cotton wool. The mixture was boiled for 15minutes in Conical flask. After boiling the mixture was allowed to cool for 1 hour. The mixture was filtered by using filter paper. The filtrate was then evaporated to get final extract using a rotary evaporator. The standard extracts obtained was stored in a refrigerator at 5°C till use.

Preparation of Methanol extracts:

The 75gr of powder from dried plant material were soaked in 250 ml methanol in 500ml Conical flask. The mixture was allowed to dry at room temperature until dry methanol extracts was obtained. The extracts was filtered by using filter paper and dried under vacuum using rotary evaporator. Extracts obtained was stored in a refrigerator at 5°C till use.

Agar diffusion for antibacterial susceptibility testing:

This method was used to investigate the efficacy of antibacterial activity of *Tagetes minuta* plant extracts and antibiotic Penecellin against *Escherichia coli* and *Staphylococcus aureus* bacteria. Filter paper discs of 10mm diameter were prepared and sterilized till uses. The identified bacteria *Escherichia coli* and *Staphylococcus aureus* was tested for their sensitivity against both extracts and standard antibiotic Penicillin.

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The colony of each bacterium (*E. coli* from Mc Conkey agar and *S. aureus* from Monitol salt agar) were emulsified in 0.85gl^{-1} normal saline by using sterilized loop, then mix thoroughly to make sure that there is no solid material from the colony was visible. Then, the swab was dipped into the broth culture of the organisms (normal saline) repeated for each. Then, the swab was gently squeezed against the inside of the tube to remove excess fluid. After that, the swabs were used to streak a Mueller-Hinton agar plate for a lawn of growth. Then all plates were allowed to dry for about 5 minutes in laminar flow hood.

After that the disc soaked in each concentration of extracts and standard antibiotic penicillin and placed in the center of each culture media. Then, each plate was labeled with the organism, concentration of each extract and antibiotic used. After that, all labeled plates were incubated in the standard upside down position at 37^{o}C for 24 hours. After that, the diameter of inhibition zone for each plate was measured by using ruler. Then the plates were discarded in the biohazard container in order to avoid any further contamination.

Preparation of concentration:

Table 1: The concentration of extracts from Tagetes minuta plant by different solvent.

Plant material	Amount of plant material	Volume	Extracts obtained
Fresh	1000gr	250ml of water	75ml
Dried	75gr	250ml of water	25ml
Methanol	75gr	250ml of Methanol	5mg/ml

III. RESULTS AND DISCUSSION

The results of the evaluation of *Tagetes minuta* plant extracts against *E.coli* and *S. aureus* showed significant antibacteria potantial to each tested bacteria. The inhibition zones provided by extract from dried *extract* were greater than those provided by fresh extracts.

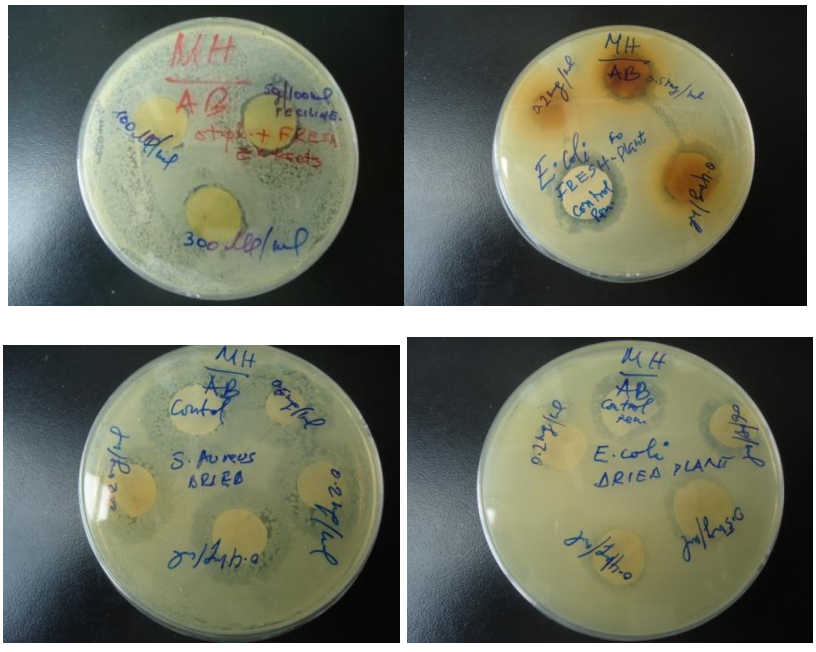

Figure 1: Inhibition zones of extracts and Standard antibiotic against *E. coli* and *S. aureus*.

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Table 2: Antibacteria potential of various concentation of *Tagetes minuta* plant extracts against *E.coli* and *S. Aureus*

Name of Organism	Diameter of inhibition zone			
	Fresh extracts (mm)	Dried Extracts (mm)	Methanol Extracts (mm)	Penicillin (mm)
Escherichia coli	12 - 14	13 - 15	16 - 18	22 -23
Staphilococus aureus	13 -15	18 - 19	21- 23	24 - 25

The yield of extracts from fresh and dried extracts obtained was greater than that from the methanol extracts (Table1). It indicated that, water is a good solvent when the objective is to obtain the large amount of extracts but high extractives doesn't mean high antibacterial potential. These results are in conformity with the findings of Parekh *et al.* (2005) who reported that the aqueous extract provide the highest yield of extracts.

However, the extracts from Fresh and dried plant material was in high amount than methanol extracts, they showed less antibacterial activity than methanol extracts according to the zone of inhibition (Table: 2 and Figure 1), this fact is similar to the findings of Alalor *et al*. (2012) who noticed that the aqueous extracts haven't enough efficacy of killing some microorganisms. This was mainly due to the chemical compound or bioactives of *Tagetes minuta* plant being organic in nature which have the effects on *E. coli* and *S. aureus* are not enough soluble in water but are soluble in methanol. This is similar to the report of Parekh *et al*. (2005).

The current study was shown that, the *S. aureus* was more susceptible to the methanol extracts than *E. coli* (Table: 2 and Figure 1). This was correlated with the observations of Ergene *et al.* (2006) who showed that the *S. aureus* are more susceptible than other tested microorganisms. The lowest inhibition zone was exhibited by *E. coli* that may possess a mechanism of tolerant or detoxifying the chemical compound that inhibit their growth to non toxic compound.

This study was showed that an increase of concentration of extracts corresponded to the increase of diameter of inhibition zone (figure 1). This was similar to the findings of Esimone *et al.* (1998) for which extracts of plants inhibit the growth of various microorganisms at different concentrations.

The current study was indicated that the antibiotic penicillin have greater inhibition zone than *Tagetes minuta* plant extracts (Table 2 and figure 1).It may be caused by the fact that the synthetic antibiotics are in pure form but the crude extracts may contain some impurity compound that may be inert and do not have any antibacterial effects. This was confirmed by George *et al*. (2002) who reported that the synthetic antibacterial are more trustily usable than plant extracts.

The results obtained are in line with the traditional uses of the *Tagetes minuta* plant as antibacterial drugs. In many cases, the therapeutic benefits are attributed to the consumption of plant mixtures in which different plant parts are prepared and/or consumed in combination or in sequence *(*Alalor *et al*. (2012*).* In this study, *Tagetes minuta* plant was found to have antibacterial properties that can be useful to treat infectious diseases caused by some gram-negative and gram-positive bacteria.

IV. CONCLUSION

The extracts of *Tagetes minuta* plant have demonstrated significant antibacteria potential against the tested pathogenic bacteria. This has confirmed and justified the use of *Tagetes minuta* plant amongst the people especially those in rural communities. However, further research is required to determine the active chemical components present in *Tagetes minuta* plan, responsible to control the studied pathogenic bacteria.

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