African Journal of Pharmacology and Therapeutics Vol. 2 No. 4 Pages 130-133, 2013
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Research Article

An Investigation of the Antimicrobial Activity of *Acmella caulirhiza*

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Background: *Acmella caulirhiza* is a plant that is used traditionally to treat several disorders such as mouth ulcers, sore throat, toothache and earache, among others. It is a small annual or perennial herb whose location is widespread worldwide.

Objectives: The objective of the study was to determine whether the leaves, stem and the flowers extract of the plant possess antibacterial and antifungal activity and to find out which part of the plant is the most active, if any.

Methodology: *Acmella caulirhiza* was collected from the wild in Kericho County. The flower heads, the leaves and the stems were dried separately, ground into a powder and extracted with chloroform. The plant extracts were tested for activity against *Escherichia Coli, Staphylococcus aureus, Candida albicans and Bacillus pumilus.*

Results and Discussion: The plant extracts inhibited the growth of *Escherichia Coli, Staphylococcus aureus and Bacillus pumilus.* The activity was highest in the stems extracts. The extracts, however, did not have any anti-fungal activity when tested against *Candida albicans*. It was concluded from these results that the anti-bacterial activity may aid in the efficacy when the plant is used to treat mouth ulcers or sore throat.

Key words: *Acmella caulirhiza*, anti-fungal activity, anti-bacterial activity, traditional medicine

Received: September, 2013
Published: December, 2013

1. Introduction

Acmella Caulirhiza, also known as *Spilanthes acmella,* is a flowering herb in the plant family Asteraceae. It is also often referred to as toothache plant. It is a small creeping and ascending plant which grows quickly and sends up gold and yellowish flowers. It is annual or perennial. *Acmella Caulirhiza* is used traditionally to treat various medical conditions by different communities in Kenya and the rest of Africa. Its use seems to be widespread. Just to mention a few, flowers or sometimes stems or leaves are chewed or a decoction is made with water then used as mouth wash to treat decayed teeth, gingivitis or wounds in the mouth, toothache and sore throat. Sap from the pounded leaves is applied to the tongues of children suffering from sores in the tongue. Among other uses, the plant is also claimed to treat stomachache and earaches. An infusion from the flower heads is used as ear-drop to treat ear problems. Fresh flowers and leaves are crushed, mixed with water and the juice drunk for stomachache and diarrhea (Chhabra et al, 1989; Giday et al, 2010; Kokwaro, 2009; Njoroge and Bussmann, 2006).

Literature survey reveals that only few pharmacological and phytochemical studies have been undertaken. The leaves and flower heads have been shown to contain spilanthol, a fatty acid amide, which is believed to be responsible for the local anesthetic properties (Ramsewak et al, 1999). This is thought to numb the pain when used to treat toothache. Extracts from the plant have also been shown to be active against

ISSN 2303-9841

mosquito larvae (Ramsewak, et al, 1999; Spelman, et al, 2011).

The plant is used to treat wounds in the mouth, sores on the tongue and sore throat, which suggests that it may have anti-bacterial and anti-fungal activities. This study therefore aimed at investigating its effect on common bacterial and fungal pathogens. Secondly, from the literature survey, it appears that various parts of the plant are used and it is not clear which part is most active, if any. This study thus also sought to compare the activities of the flower heads, the stem and the leaves. It is also evident from the literature that no pharmacological studies have been undertaken on the Kenyan varieties. It was considered of interest therefore to undertake studies on the local plant and compare the results with those reported elsewhere. These types of basic research are justified since they may lead to discovery of novel compounds which could be exploited for clinical, pharmacological or chemical/industrial application.

2. Methods

2.1 Collection and processing of plant material

The plant material was collected from the wild in Kericho County and its identity confirmed by the East African Herbarium, where a voucher specimen (Number KER/1/2011) has been deposited.

2.2 Extraction procedure

The flowers, leaves and the stems were separated and dried in the shade for several days. It was ground into powder and 100 gm each macerated separately in 1L of chloroform for 48 hours. It was then filtered through filter paper and evaporated to dryness. The residue was dissolved in DMSO to make a concentration of 50 mg/ml and its anti-fungal and anti-bacterial effect determined.

2.4 Preparation of the media

Bacterial culture Media: 40 mg of Tryptone Soya Agar (TSA, Himedia Laboratory PVT Ltd, India) was suspended in 1L of distilled water; 100 ml of resultant suspension was boiled to dissolve completely and sterilized by autoclaving at 121 °C for 15 min. It was then cooled to about 50 °C. 20 ml of sterilized media was put into sterilized Petri dishes and allowed to set overnight in a refrigerator.

***Fungal culture Media*:** 65 gm of Sabouraud Dextose Agar (SDA, Himedia Laboratory PVT Ltd, India) was suspended in 1L of distilled water; 100 ml of resultant suspension was boiled to dissolve completely and sterilized by autoclaving at 121 °C for 15 min. It was then cooled to about 50 °C. 20 ml of sterilized media was put into sterilized Petri dishes, transferred into a refrigerator and allowed to set overnight.

2.5 Microorganisms and preparation of subcultures

Staphylococcus aureus (NC 07447), *Bacillus pumilus* (NC 08241), *Escherichia coli* (ATCC 35218) and *Candida albicans* (NC PF 2275) were kind donations from the National Quality Control Laboratories (NQCL, Kenya).

Master cultures of *S. aureus, E. coli* and *B. pumulus* were stored refrigerated at 4 °C. A loop was sterilized using a foot operated burner and used to scoop samples from the master cultures. Nutrient media, which was sterilized and cooled as explained above (TSA for bacteria and SDA for fungi), was seeded with an appropriate micro-organism culture using the loop. These were incubated over night (bacteria at 37°C and fungi at 25°C).

2.6 Determination of antimicrobial activity

Nystatin (Universal Corporation Ltd, Kenya; 0.118 mg/ml) and Erythromycin: (Colpaldas Visram and Co. Ltd, India; 0.125 mg/ml) were the positive controls used for the anti-fungal and antibacterial assays, respectively. Dimethyl suphoxide (DMSO, Fisher Scientific, UK) was used as the negative control.

50µl each of plant extracts and the controls were introduced into separate wells in the relevant media in triplicate and left to incubate overnight after which the zones of inhibitions of each were measured.

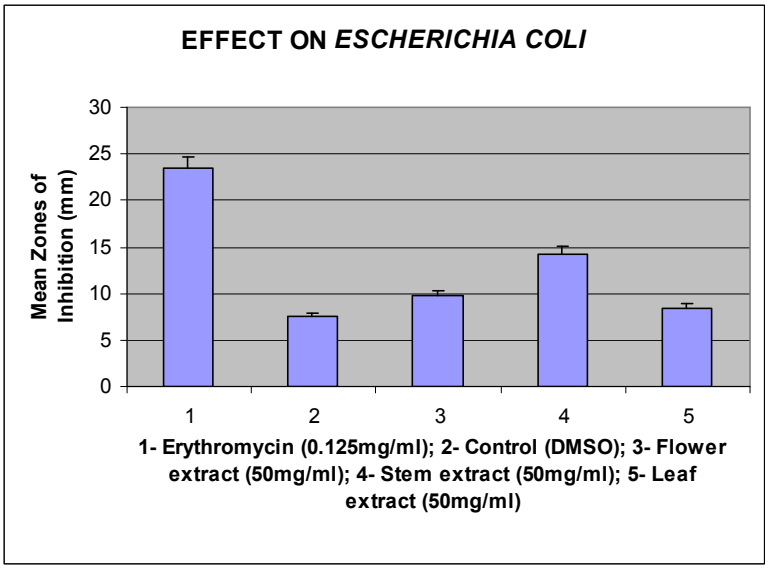

Figure 1: Effects of various extracts on the growth of *Escherichia coli*

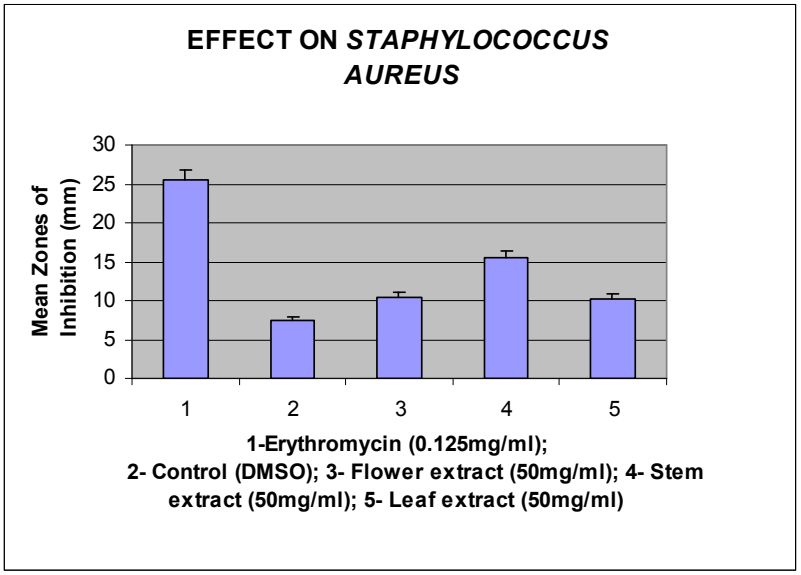

Figure 2: Effects of various extracts on the growth of *Staphylococcus aureus*

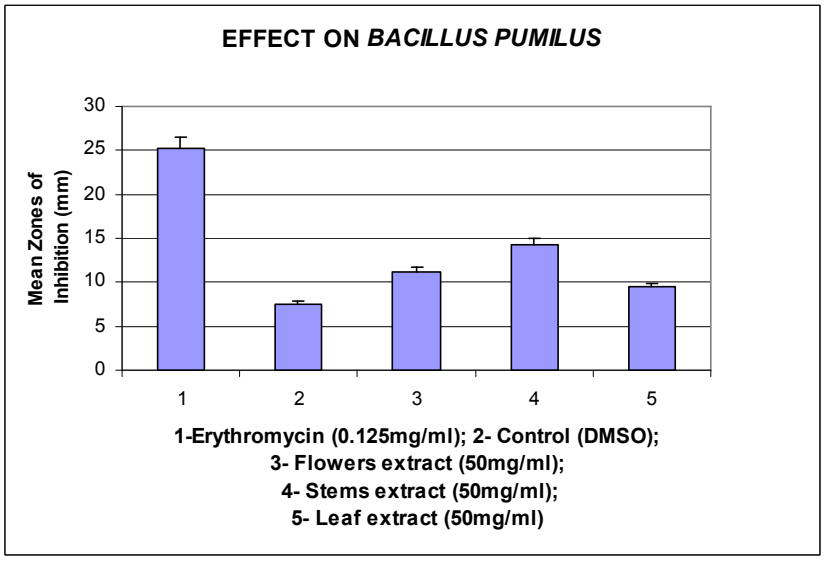

Figure 3: Effects of various extracts on the growth of *Bacillus pumilus*

3. Results

Each reported result was a mean of three determinations. Student's t-test was used to assess whether the difference between two means of interest was statistically significant. The vertical bars represent standard errors of the mean (SEM).

Effect on *Escherichia coli*

As seen in **Figure 1**, the flowers and the stems extract (each at concentration of 50 mg/ml) had statistically significant anti-bacterial effect on *Escherichia coli* when compared to controls ($p<0.001$).Leaves extract on the other hand did not have a statistically significant effect when compared to controls even at a higher concentration of 100 mg/ml. The effect of the stems extract was much higher when compared to that by the flower extract ($p<0.001$) but less than that of erythromycin (0.125 mg/ml), the positive standard.

Effect on *Staphylococcus aureus*

The flowers, the stems and the leaves extracts (each 50 mg/ml) had statistically significant effect on *Staphylococcus aureus* when compared to controls ($p<0.001$ respectively). The stems extracts had the highest activity ($p< 0.01$) when compared to the leaves and the flower extracts respectively (**Figure 2**).

Effect on *Bacillus pumilus*

The flowers, the stems and the leaves extracts at a concentration of 50 mg/ml each had statistically significant anti-bacterial effect on *Bacillus pumilus* when compared to controls: $p<0.05$, $p<0.02$ and $p<0.02$ respectively. Though it might appear from the histograms that the activity was higher in the stems extract, the difference was not statistically significant from those of the other extracts (**Figure 3**).

 ISSN 2303-9841

Effect on *Candida albicans*

The effect of extracts (50 mg/ml) of the stem, the flowers and the leaves had no statistically significant antifungal effect on *Candida albicans* when compared to controls even at higher concentrations of 100 mg/ml (results not shown).

4. Discussion

The results presented above have clearly demonstrated that the extracts of the flowers, the stems and the leaves of *Acmella caulirhiza* posses antibacterial activity against *Escherichia coli, Staphylococcus aureus and Bacillus pimulus.* The stems extract had a higher activity than the leaves and the flower extracts. The antibacterial activity of the flower head extract was comparable to that of the leave extract since there was no statistical difference between the two on all the bacterial species examined. *Bacillus pimulus* appeared least sensitive to the plant extracts than either *Staphylococcus aureus or Escherichia coli.* However there was no demonstrable antifungal activity against *Candida albicans.*

As cited in the introduction, among the documented uses of *Acmella caulirhiza* are decayed teeth, gingivitis, tonsillitis, wounds in the mouth and sores on the tongue; the latter use being more common especially in young children. The most common cause of sores in the mouth and tongue is infection of the oral cavity by the fungus *Candida albicans,* a normal flora which can turn pathogenic under certain conditions. One would therefore have expected the plant extracts to have antifungal activity; but surprisingly, this effect could not be demonstrated in the present study. Nevertheless, whatever the cause, wounds in the mouth and tonsillitis would most likely be accompanied by bacterial infections which would be expected to exacerbate the condition. The antibacterial effect against the common pathogens demonstrated in this study would therefore aid in the healing process. This might thus partly explain the use of this plant in treatment of these conditions.

In conclusion, the present study has clearly demonstrated that *Acmella caulirhiza* possess antibacterial activity against three common bacterial species and that the stem extracts have greater activity than the flowers or the leaves extracts. It is appreciated that antibacterial activity recorded in this study is modest. This could be because crude extracts were used in this preliminary work. Fractional purification and extraction in future work is expected to improve the said antibacterial activity. It is therefore recommended that further work be undertaken to determine the chemical identity of the active ingredient(s) and explore their potential for clinical, pharmacological or chemical/ industrial application.

Conflict of Interest declaration

The authors declare no conflict of interest

Acknowledgement

The authors acknowledge the National Quality Control Laboratory (NQCL, Kenya) for the kind donation of the microorganisms used in this study.

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 ISSN 2303-9841