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Antimicrobial activity of *Acmella caulirhiza* on *Candida albican* and *Escherichia coli*

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Abstract

Drug-resistant pathogens pose a big problem to the word today. Some infections resulting from drug-resistant pathogens do not respond to some of the drugs in the market today. This created a need for the development of drugs that are not resisted by the pathogens. This study was conceived with the aim of extracting antimicrobial compounds from *Acmella caulirhiza.* The *Acmella caulirhiza* plant was obtained from botanic garden in Egerton University. Plant extracts from leaves, stems, and flowers were obtained separately using methanol. The antimicrobial activity of the extracts against *Candida albicans* and *Escherichia coli* was carried out using Kirby Bauer's disc diffusion technique. Minimum Inhibitory Concentration (MIC) was carried out using the microliter technique. The data obtained were analyzed using the Statistical Package of Social Sciences (SPSS) version 22 software. The means were separated using one-way analysis of variance (ANOVA). A P value < 0.05 was considered as significant. *Acmella caulirhiza* leaf, stem and flowers leaf extracts had antimicrobial properties against *C. albicans* and to *E. coli*. There is a need to further purify the antimicrobial metabolites from *Acmella caulirhiza* and determine the mechanisms for their action against *E. coli* and *Candida albicans.*

Keywords: *Acmella caulirhiza. Candida albicans, Escherichia coli,* Drug resistant

1. Introduction

The increasing awareness of drug-resistant pathogens has drawn the attention of the pharmaceutical and scientific communities towards the studies of alternative sources of drugs (Borokini *et al*., 2012). Medicinal plants are known to contain physiologically active compounds that have been exploited for many years in traditional medicine for the treatment of various ailments (Bedi *et al*., 2017). These compounds are thought to contain antimicrobial properties. Antimicrobial of plant origin have enormous therapeutic potential and are known to mitigate several side effects that are often associated with synthetic antimicrobials (Paulraj *et al*., 2013).

Medicinal plants are known to be a great source of antimicrobial creating the need for screening more plants for their antimicrobial properties (Shakeri *et al*., 2013). Currently, about 30% of the modern pharmacological drugs are found either directly or indirectly from plants. Furthermore, plants have provided a rich source of new drugs and cure for diseases (Wendakoon *et al*., 2012). Many such medicines including strychnine, aspirin, and taxol which are made from plants. In addition, medicinal plants are used for primary healthcare by about 70 % - 90 % of populations in developing countries (Rosakutty *et al*., 2012). The use of herbal and other alternative therapies is popular in many developing countries because of the high cost of accessing conventional health care management systems and the high cost of developing a new pharmaceutical drug (Masoko *et al*., 2017).

According to WHO, traditional medicine is one of the ways to achieve total health care coverage of the world`s population (Vashist and Jindal, 2012). Drug inventions from ethnopharmacology and natural products are still an important aspect of in uplifting the deprived livelihoods of rural communities (Purushothaman *et al*., 2018). Nowadays, infections caused by bacteria and fungi have become difficult to eliminate due to the development of resistant strains of bacteria and fungi (Lagnika *et al*., 2016). The cost of treatment of bacterial infection is increasing and the time for elimination is long. Therefore there is a need to source novel antimicrobial compounds to address this emerging problem in health care (Sharma *et al*., 2012).

Acmella caulirhiza is one of the important medicinal plants with rich source of therapeutic constituents (Soares *et al*., 2014). The leaves and flower heads have been shown to contain spilanthol, a fatty acid amide, which is believed to be responsible for the local anaesthetic properties. This is thought to numb the pain when used to treat toothache. Extracts from the plant have also been shown to be active against mosquito larvae (Vibha *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 antibacterial and antifungal activities (Prachayasittikul *et al*., 2013). From the literature survey, it appears that various parts of the plant are used and it is not clear which part is most active. This creates the need for comparing the antimicrobial activities of the flower heads, the stem and the leaves. Sharma *et al*. (2013) maintain that very few pharmacological studies have been undertaken on antimicrobial properties of *Acmella caulirhiza* in developing countries. Studies on antimicrobial properties of plants extracts ate justified since they may lead to the discovery of novel compounds which could be exploited for clinical, pharmacological or chemical/industrial application (Arora *et al*., 2011).

2. Materials and methods

Sample collection

Acmella caulirhiza is a small creeping and ascending plant which grows quickly and sends up gold and yellowish flowers and is annual or perennial. The plant was collected from Botanical Garden of Egerton University.

Test pathogens

The antibacterial activity of *Acmella caulirhiza* was assessed against two pathogens.

Plant extract preparation

About 200g of fresh *Acmella caulirhiza* leaves, stem and flowers were chopped separately and crushed using pestle and mortar in 5ml of methanol and filtered using a Whatmann number 1 filter papers. The crude extracts were serially diluted up to 10^{-3} (Tambe *et al*., 2014).

Antimicrobial Activity of Fluconazole and Ciprofloxacin

The anticandida activity of fluconazole and anti *E. coli* activity of ciprofloxacin was evaluated by disc diffusion method on sabouraud dextrose agar (SDA) and nutrient agar (NA) respectively. Sterile media plates were allowed to gel then inoculated aseptically on the surface with a standard suspension of the test pathogenic microorganisms standardized using 0.5 McFarland turbidity standards. A 6mm disc in diameter impregnated with the diluted drug and air dried for 10 minutes were placed equidistant to each other on the plates. The plates were incubated at 37ºC for 24 h for *E. coli* and 28ºC for *C. albicans.* Zones of inhibitions were measured in mm using a ruler (Ahmed *et al*., 2012).

Antimicrobial Activity of *Acmella caulirhiza*

The antimicrobial activity of the extracts from *Acmella caulirhiza* was assayed by the disc diffusion method on sterile NA (*E. coli*) and SDA (*C. albicans*) agar plate according to NCCLS (Garcia *et al*., 2012). The media were inoculated aseptically with the standardized suspension of the test pathogenic microorganisms. The pathogens were separately spread on the media using an L-shaped glass. The extracts of *Acmella caulirhiza* were serially diluted up to 10^{-3}. A filter paper disc measuring 6mm in diameter was soaked in different dilutions of the extracts, air-dried for 10 minutes and placed on NA and SDA seeded with the test pathogens at equidistant points. The plates were incubated at 37ºC for 24 h for *E.coli* and 28ºC for 2d for *C. albicans*. The zones of inhibition were measured in mmm (Rao *et al*., 2012).

Minimum inhibitory concentration.

The minimum inhibitory concentration (MIC) value of *Acmella caulirhiza* crude extracts was according to a method given by Richards *et al.* (2014). Briefly, 25mL of 10mg/mL of the crude extracts were serially diluted using sterile distilled water in 96-well plates. The *C. albicans* and *E. coli* test pathogens were grown for 12h and the turbidity of the culture determined using McFarland standards. One hundred microliter of the test pathogens were separately transferred into each well and the microtiter plates incubated at 37ºC for bacteria and 25ºC for 24 h and 2d respectively. The wells were observed for the development of turbidity. The MIC was recorded as the lowest concentration of the extract that inhibited the growth of the test pathogens (Abeysinghe *et al*., 2014).

Phyto-constituents analysis of the crude extracts

The following photochemical analysis was performed according to a protocol given by Dubey *et al.* (2013): terpenoids, tannins, steroids, reducing sugar, saponins, phlobatannin, alkaloids, flavonoids and anthraquinones

Data analysis

The results are expressed as mean value, Standard Error of the Mean (SEM) of growth inhibition zones diameters obtained with the *Acmella caulirhiza.* Statistical differences between the two means of the data from the test microorganisms are analyzsed by analysis of variance (ANOVA). P values lower than 0.05($p < 0.05$) are considered.

3. Results

Antimicrobial activity of fluconazole and ciprofloxacin against *C. albicans* and *E.coli*

The zones of inhibition of *C. Albicans* by fluconazole varied from (19±0.1-23±0.2mm) (Table 1). However, the zone of inhibition of *E. coli* by ciprofloxacin ranged from 27±0.1mm to 31±0.3mm. The zones of inhibition of *C. albicans* by fluconazole and *E. coli* by ciprofloxacin varied significantly (P=2.1E-5).

Table 1: Zones of inhibition (mm) of *Candida albicans* and *Escherichia coli* when treated with fluconazole and ciprofloxacin respectively.

Replication	Zones of inhibition	
	Candida albicans	***Escherichia coli***
1	20±0.3	30±0.3
2	19±0.1	27±0.1
3	22±0.2	31±0.3
4	23±0.2	28±0.2
5	21±0.1	30±0.2

Antimicrobial activity of *Acmella caulirhiza* crude extracts against *C. albicans.*

The zones of inhibition of *C. albicans* by *Acmella caulirhiza* stems crude extract varied from 15±0.2 to 18±0.1mm. However, the zones of inhibition of *C.albicans* by the leaves extract ranged from 13±0.1 to 16±0.2mm and flowers (16±0.2-18±0.2mm). The zones of inhibition of *C. albicans* by stem, leaves, flowers of *Spilanthes acmella* crude extracts varied significantly (F=7.153846 P=0.013815).

Table 2: Zones of inhibition (mm) of *Candida albicans* by crude extracts of *Acmella caulirhiza*

Replication	Zone of inhibition		
	Stem	**leaves**	**Flowers**
1	15±0.2	14±0.2	18±0.2
2	16±0.3	13±0.1	17±0.3
3	17±0.1	13±0.2	16±0.2
4	18±0.1	16±0.2	17±0.2
5	16±0.2	15±0.1	18±0.1

Antimicrobial activity of *Acmella caulirhiza* crude extracts against *E.coli*

The zones of inhibition of *E. coli* by *Acmella caulirhiza* stems crude extract ranged from 14±0.2 to 17±0.1mm (Table 3). In addition, the zones of inhibition of *E. coli* by the leaves extract varied from 10±0.2 to 13±0.2mm. In crude extracts from flowers, the variation of the zones of inhibition was 12±0.2-15±0.2mm). The zones of inhibition of *E. coli* by stem, leaves, flowers of *Acmella caulirhiza* crude extracts varied significantly (F=16.93023 P=0.000321).

Table 3: Zones of inhibition of *E. coli* by crude extracts of *Acmella caulirhiza*

Replicate	Zones of inhibition		
	Stem	**leaves**	**Flowers**
1	14±0.2	10±0.2	14±0.2
2	16±0.3	11±0.1	13±0.1
3	15±0.2	12±0.1	15±0.2
4	17±0.1	13±0.2	14±0.2
5	16±0.2	10±0.2	12±0.2

Minimum Inhibition Concentration (MIC) of *C. albicans* and *E.coli* by crude extracts of *Acmella caulirhiza*

The minimum inhibition of C. albicans by the stem extracts of *Acmella caulirhiza* varied from 1.5±0.2 to 2.5±0.2mg/mL, leaves (1.5±0.1-2.5±0.2mg/mL) and flowers (1.5±0.1-2.5±0.2mg/mL) (Table 4). The MIC for the *Acmella caulirhiza* against *E. coli* in crude extracts from stems of *Spilanthes acmella* varied from 1.5±0.2 to 2.5±0.3 mg/mL, leaves (1.5±0.2-2.5±0.2 mg/mL) and flowers (1.5±0.1-2.5±0.3 mg/mL). However, MIC of stem, leaves and flowers crude extracts of *Acmella caulirhiza* against *C. albicans* and E.coli varied significantly ((F=0.222 P=0. 8039).

Table 4: Minimum inhibitory concentration (mg/mL) of *C. albicans* and *E. coli* by crude extracts of *Acmella caulirhiza*

Replication	*C. albicans*			*E. coli*		
	Stem	**leaves**	**Flowers**	**Stem**	**leaves**	**Flowers**
1	1.5±0.2	2.5±0.2	1.5±0.2	2.5±0.3	1.5±0.2	2.5±0.3
2	1.5±0.3	1.5±0.1	1.5±0.1	1.5±0.3	2.5±0.2	2.5±0.1
3	1.5±0.2	2.5±0.1	2.5±0.2	2.5±0.1	2.5±0.1	1.5±0.1
4	2.5±0.1	2.5±0.2	2.5±0.2	2.5±0.1	1.5±0.3	1.5±0.2
5	2.5±0.2	1.5±0.2	2.5±0.2	1.5±0.2	1.5±0.2	2.5±0.3

Phytochemical constituents of crude extracts of *Acmella caulirhiza*

The extracts from *Acmella caulirhiza* had terpenoids, tannins, steroids, reducing sugar, alkaloids and flavonoids (Table 5). However, the extracts lacked saponins, phlobatannin and anthraquinones.

Table 5: Phytochemical constituents of crude extracts of *Acmella caulirhiza*

Constituents	Occurrence
Terpenoids	+
Tannins	+
Steroids	+
Reducing sugar	+
Saponins	-
Phlobatannin	-
Alkaloids	+
Flavonoids	+
Anthraquinones	-

4. Discussion

In this study, fluconazole and ciprofloxacin were used as positive controls. The zones of inhibition shown by fluconazole and ciprofloxacin against *C. albicans* and *E.coli* respectively varied significantly (Table 1). This was consistent with a previous study by Mishra *et al*. (2015). This could be attributed to the use of the same strains of test pathogens in the two studies.

The result of this study indicated a significant difference in the zones of inhibition produced by the crude extracts of stem, leaves, and flowers form *Acmella caulirhiza* when tested against *C. albicans* (Table 2). In addition, the zones of inhibition given by the crude extracts from flowers were bigger than those obtained from crude extracts from stem and leaves. The same observation was made when the crude extracts were tested against *E.coli* (Table 3). This may be attributed to a higher accumulation of active compounds in flowers than in stems and leaves (Moreno *et al*., 2012). These results differed with those of a study carried on determination of antioxidant potential in *Acmella caulirhiza* by Sana *et al*. (2014). A possible reason could be environmental differences in which the plants were growing (Prachayasittukal *et al*., 2013).

The Minimum Inhibition Concentration of extracts obtained from the stems, leaves, and flowers of *Spilanthes acmella* did not vary significantly when tested against *C. albicans* and *E. coli* (Table 5). This disagreed with a study on spilanthol from *Spilanthes acmella* flowers, leaves and stems obtained by selective supercritical carbon dioxide extraction by Dias *et al*. (2012). According to Bae *et al*. (2010) the solvents used in extracting crude extracts from *Spilanthes acmella* MIC of the crude extracts since different solvents extracts varying active compounds.

However, the crude extracts obtained from *Acmella caulirhiza* in this study showed the presence of

terpenoids, tannins, steroids, reducing sugar, alkaloids and flavonoids (Table 5). However, they were lacking saponins, phlobatannin and anthraquinones. This disagreed with a study carried out by Abeysiri *et al.* (2013). Opoku and Osei *(2014)* maintained that the extraction of phytochemical compounds from medicinal plants is highly dependent on the type and polarity of solvent used.

Conclusion

Acmella caulirhiza leaf, stem and flower leaf extracts had antimicrobial properties against *C. albicans* and to *E. coli*.

Recommendation

There is a need to further purify the antimicrobial metabolites from *Acmella caulirhiza* and determine the mechanisms for their action against *E. coli* and *Candida albicans.*

Conflict of interest

The authors declare no conflict of interest.

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