Research Article

Antibacterial activity of *Cassia alata* Linn (Acapulco) roots and barks crude extracts

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Abstract

Objective: Present study was aimed to determine antibacterial activity of *Cassia alata* Linn (Acapulco) roots and bark extract against *Staphylococcus aureus* and *Escherichia coli*. **Material and methods:** Two hundred fifty (250) grams of roots and three hundred (300) grams of bark were collected, washed and pounded in a pestle. The pounded mass was then filtered with a cheese cloth to extract the juice which was stored for future use. Commercially prepared antibacterial medicine (Chloramphenicol and Tetracycline) served as the positive control. Testing for the antibacterial activity of the crude extract was done using Whattman filter paper discs soaked in the test substances and impregnated into the plates streaked with *S. aureus* and *E. coli*. Observation for the presence or absence of an inhibition zone (clear area around the disc) was done after an incubation period of 24 hours at room temperature. Zones of inhibition were then measured with a plastic ruler. **Results and conclusion:** Results reveal that the root and bark crude extracts of *Cassia alata* (Linn) had antibacterial activity against the test organisms as indicated by the zones of inhibition around the filter and paper discs. Acapulco root crude extract had an average zone of inhibition of 0.3 mm on S. *aureus* and 3.0 mm on *E. coli*. The bark crude extract was more effective than the root extracts in as much as the former had a zone of inhibition of 1.3 mm on *S. aureus* and 6.3 mm on *E. coli*. Statistical analysis of the results show a significant difference on the activities of the root and bark crude extracts and the commercial antibiotic drugs. This implies that the extracts are not comparable in their effect on the test organisms with the commercial antibacterial drug.

Keywords: Antibacterial, acapulco, *S. aureus*, *E. coli*, *Cassia alata*

Introduction

Akapulko, or Acapulco in English, is a shrub found throughout the Philippines. It is known under various names in different regions of the country. Locals call the plant "katanda", "andadasi", and "palochina" in Tagalog, Ilocos and in the Visayas regions, respectively. The shrub belongs to the plant family Leguminosae, and grows to about one to two meters tall. It has thick branches and the leaves are embraced with 8 to 20 leaflets that are oblong-elliptical in shape. The flowers of the Akapulko have oblong sepals, and its fruits are tetragonal, which are also winged and glabrous. It is a medicinal herb that contains chrysophanic acid, a fungicide used to treat fungal infections, like ringworms, scabies, and eczema. Akapulko also contains saponin, a laxative that is useful in expelling intestinal parasites (Philippine Herbal Medicine © 2005-2014).

The primary part used for herbal purposes are the leaves, although the roots and flowers are also used for certain preparations with medicinal value. The extract from the Akapulko plant is commonly used as an ingredient for lotions, soaps, and shampoos (Philippine Herbal Medicine © 2005-2014).

The leaf extracts exhibit various pharmacological properties: antimicrobial and anti-fungal activities as well as anti-inflammatory effects. The therapeutic efficacy of *Cassia alata* Linn leaf extract against *Pityriasis versicolori* has been reported and finally the anti-aging effect of *Cassia*

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alata was demonstrated allowing the use of extracts of *Cassia alata* in cosmetic and/or dermatological skin care products. The main medicinal uses of *Cassia alata* are as a laxative or purgative and in the treatment of skin problems. For laxative purposes, usually a decoction of the leaves is drunk, and less often the flowers, roots or the stem are used. Skin problems treated with *Cassia alata* include ringworm, favus and other mycoses, impetigo, syphilis sores, psoriasis, herpes, chronic lichen planus, scabies, shingles, eczema, rash, and itching. Skin problems are most often treated by applying leaf sap or by rubbing fresh leaves on the skin. Other ailments treated in tropical Africa with *Cassia alata* Linn include stomach pain during pregnancy, dysentery, hemorrhoids, blood in the urine, schistosomiasis, gonorrhoea, convulsions, heart failure, edema, jaundice, headache, hernia, and one-sided weakness or paralysis. Decoctions of the wood are used to treat liver problems (jaundice), urticaria, rhinitis, and loss of appetite caused by gastro-intestinal problems (Bosch, 2007).

In this study, the researcher tested the effectiveness of Acapulco roots and bark crude extract against *Escherichia coli* and *Staphylococcus aureus*. The researcher was also interested to know if Acapulco roots and bark crude extract are comparable enough to that of commercial antibacterial drugs.

Materials and methods

This study was conducted at the Research Laboratory of the College of Science, University of Eastern Philippines, University Town, Northern Samar. Experimental method was used by the researcher as its research design.

The antibacterial activity of *Cassia alata* Linn roots and bark crude extract focused on their bacteriostatic activity. Bacteriostatic activity of *Cassia alata* Linn roots and bark crude extract were tested on Gram-positive bacteria *S. aureus* and Gram negative bacteria *E. coli*. Phytochemical screening of the extract to determine the active chemical responsible for its bacteriostatic activity was not included in the study due to lack of chemicals needed to perform the procedure.

Preparation of sample

The roots and bark of Acapulco were collected from Barangay Polangi, Catarman, Northern Samar. These were brought to the Research Laboratory Room of the College of Science, University of Eastern Philippines, University Town, Northern Samar for proper identification and to prepare them for extraction.

About 250 grams of roots and 300 grams bark of *Cassia alata* Linn were segregated and washed with distilled water. These were chopped into small pieces using a clean, sterile knife. It was then ground using a mortar and pestle in order to extract the juice. To separate the solid part from the liquid extracts, the roots and bark crude extracts were filtered first with the use of cheese cloth. After filtration, the extract was placed in a beaker with cover using Aluminum foil and it was labeled for easy identification.

Antibacterial activity

To prepare the sensitivity disc a filter paper disc from a Whattman assay disc was prepared. A metal puncher was used in cutting the paper discs. These were put in a sterile Petri dish and were wrapped with an aluminum foil and sterilized in the oven for 12 hours at 37° C. With sterile forceps, sterile filter paper discs were picked and separately immersed in plant extracts and in the positive controls (Chloramphenicol and Tetracycline) and then placed in sterile Petri dishes which were inoculated with the test organisms.

In preparing for positive control, five hundred milligrams (500 mg) of commercially obtained Chloramphenicol and Tetracycline in powdered form was used as positive control, each of which was placed in a beaker separately, added with one (1) mL of water and stirred until totally dissolved. Chloramphenicol for *Escherichia coli* and Tetracycline for *Staphylococcus aureus* was used as positive controls to compare the antibacterial effect of *Cassia alata* Linn roots and bark crude extracts.

In preparing culture media, nineteen (19) grams of Mueller-Hinton Agar for assaying *S. aureus* and *E. coli* (BioTest) was weighed, suspended in five hundred (500) mL distilled water and stirred. The media was completely dissolved by boiling and was sterilized in a pressure cooker at 121°C for 15 minutes at 15 pounds pressure. The media was allowed to cool to about 50 °C and was aseptically poured into individual Petri dishes and allowed to solidify.

Pure cultures of *Escherichia coli* and *Staphylococcus aureus* were bought from the Philippine National Collection of Microorganisms at the University of the Philippines at Los Baños (UPLB). The test microorganisms were transported to the College of Science, University of Eastern Philippines and was incubated for 24 hours at a temperature of 32°C to keep the microorganisms alive until it was used for the experimental procedure.

The 500 mL of prepared Mueller-Hinton nutrient medium was divided into two, each about 250 mL. The first 250 mL of nutrient medium was inoculated with *Staphylococcus aureus* and the other 250 mL of nutrient medium was for *Escherichia coli.* During the inoculation procedure, a sterile inoculating loop was dipped into the pure culture of

microorganism and transferred to 250 mL of liquid nutrient agar in a sterile Erlenmeyer flask. The inoculated nutrient agar was poured into eighteen (18) sterile Petri dishes and allowed to solidify. Paper discs soaked in *Cassia alata* Linn roots and bark crude extracts and in the positive control (Chloramphenicol and Tetracycline) were placed in the center of each Petri plate with inoculated nutrient agar. The eighteen (18) Petri dishes were wrapped in a piece of paper in an inverted position and was incubated for 24 hours at 37° C.

To determine bacteriostatic property, twenty four (24) hours after inoculation, the Petri plates were examined for the bacteriostatic property of *Cassia alata* Linn roots and bark crude extracts. The clear zone of inhibition was measured using a Vernier caliper to determine the antibacterial effect of *Cassia alata* Linn roots and bark crude extracts and the positive controls (Chloramphenicol and Tetracycline) on the microorganism used in the study.

Statistical analysis

F-test was used to determine the level of significance of Antibacterial effect of *Cassia alata* Linn roots and bark crude extracts and the positive control, whose formula is presented below (Broto, 2003):

Results and discussion

Effectiveness and significant difference of the *Cassia alata* Linn roots and bark crude extract and commercial antibacterial drug (Chloramphenicol and Tetracyline)

Table 1 shows the result of the test of the antibacterial activity of *Cassia alata* Linn roots and bark crude in inhibiting the growth of *Staphylococcus aureus*. The procedure was repeated three (3) times. As shown in the table, Acapulco roots extract has a mean zone of inhibition of 0.3 mm, 1.3 mm for the barks extract, and a 16 mm mean zone of inhibition of inhibition for Tetracycline (positive control), which means that Acapulco roots extract were less effective than bark extract, although as expected, it was not comparable to the effect of the positive control.

Table 1. Effectiveness of the antibacterial activity of *Cassia alata* Linn roots and barks crude extract and Tetracycline (positive control) on *Staphylococcus aureus.*

***Staphylococcus aureus* (Gram positive)**	***Cassia alata* Linn roots crude extract**	***Cassia alata* Linn bark crude extract**	**Tetracycline (positive control)**
Trial 1	0	1	17
Trial 2	0	1	14
Trial 3	1	2	17
Total	**1**	**4**	**48**
Mean	**0.3**	**1.3**	**16**

Table 2. Effectiveness of the antibacterial activity of *Cassia alata* Linn roots and barks crude extract and Chloramphenicol (positive control) on *Escherichia coli*

***Escherichia coli* (Gram-negative)**	***Cassia alata* Linn roots crude extract**	***Cassia alata* Linn bark crude extract**	**Chloramphenicol (positive control)**
Trial 1	0	6	12
Trial 2	4	9	17
Trial 3	5	4	16
Total	**9**	**19**	**45**
Mean	**3**	**6.3**	**15**

Therefore, the result implies that Acapulco root and bark crude extract has antibacterial activity on *Staphylococcus aureus* which means that Acapulco root and barks crude extract can be an antibacterial agent against *Staphylococcus aureus*, although as expected, it was not comparable with the effects of the positive control.

Effectiveness of the antibacterial activity of *Cassia alata* Linn roots and barks crude extract and Chloramphenicol (positive control) on *Escherichia coli*

Table 2 shows the result of *Cassia alata* Linn roots and bark crude extract in inhibiting the growth of *Escherichia coli*. The procedure was also repeated three (3) times. As shown in the table, Acapulco roots extract had a mean zone of inhibition of 3 mm, 6.3 mm mean zone of inhibition of Acapulco bark extract, and 15 mm mean zone of inhibition of Chloramphenicol (positive control). This means Acapulco bark extract were more effective than the roots extract.

Therefore, the result implies that Acapulco roots and barks crude extract also has antibacterial activity on *Escherichia coli* which means that Acapulco roots and barks crude extract can also be an antibacterial agent against *Escherichia coli*.

Effect of *Cassia alata* Linn roots and bark crude extract on *Staphylococcus aureus*

Result on the determination of the significant difference of antibacterial property of roots and bark crude extract on *Staphylococcus aureus* shows that, since the F-computed value of 4.1 is within the critical F-tabular value of 7.71 at 0.05 level of significance with 1 and 4 degrees of freedom, the null hypothesis is accepted that there is no significance difference between the root and bark crude extracts in terms of their antibacterial potential against *S. aureus*. Therefore, the

Table 3. Effect of *Cassia alata* Linn roots and bark crude extract on *Staphylococcus aureus*

Sources of Variation	Degrees of Freedom	Sum of Squares	Mean of Squares	F – value		Interpretation
				Comp.	Tabular	
Between Groups K-1	1	1.43	1.43	4.1	7.71	NS
Within Groups (N-1) – (K – 1)	4	1.4	0.35			
TOTAL	5	2.83				

result implies that both the root and bark extract of Acapulco is effective on *Staphylococcus aureus* as to its antibacterial activity. Thus, it is included that both Acapulco roots and bark crude extract were effective as antibacterial agent against *S. aureus*.

Effect of *Cassia alata* Linn roots and crude extract on *Escherichia coli*

Since the F-computed value of 2.5 is within the critical value of 7.71 for F at 0.05 level of significance with 1 and 4 degrees of freedom, the null hypothesis is also accepted that there is no significant difference between the effects of *Cassia alata* roots and bark crude extract in terms of its activity against *Escherichia coli* when tested for its antibacterial activity, which further implies the effectiveness of both extracts as antibacterial agents against *Escherichia coli*.

Significant Difference between *Cassia alata* Linn roots and bark crude extract and commercial antibacterial drug (Tetracycline for *S. aureus* and Chloramphenicol for *E. coli*)

Results showed that the F-tabular value of 5.44 is lesser than the F-computed value of 169.72, the null hypothesis is rejected. This means that there is a very high significant difference between *Cassia alata* Linn roots and bark crude extract as compared with commercial antibacterial drug (Tetracycline) on *S. aureus* in terms of its antibacterial activity.

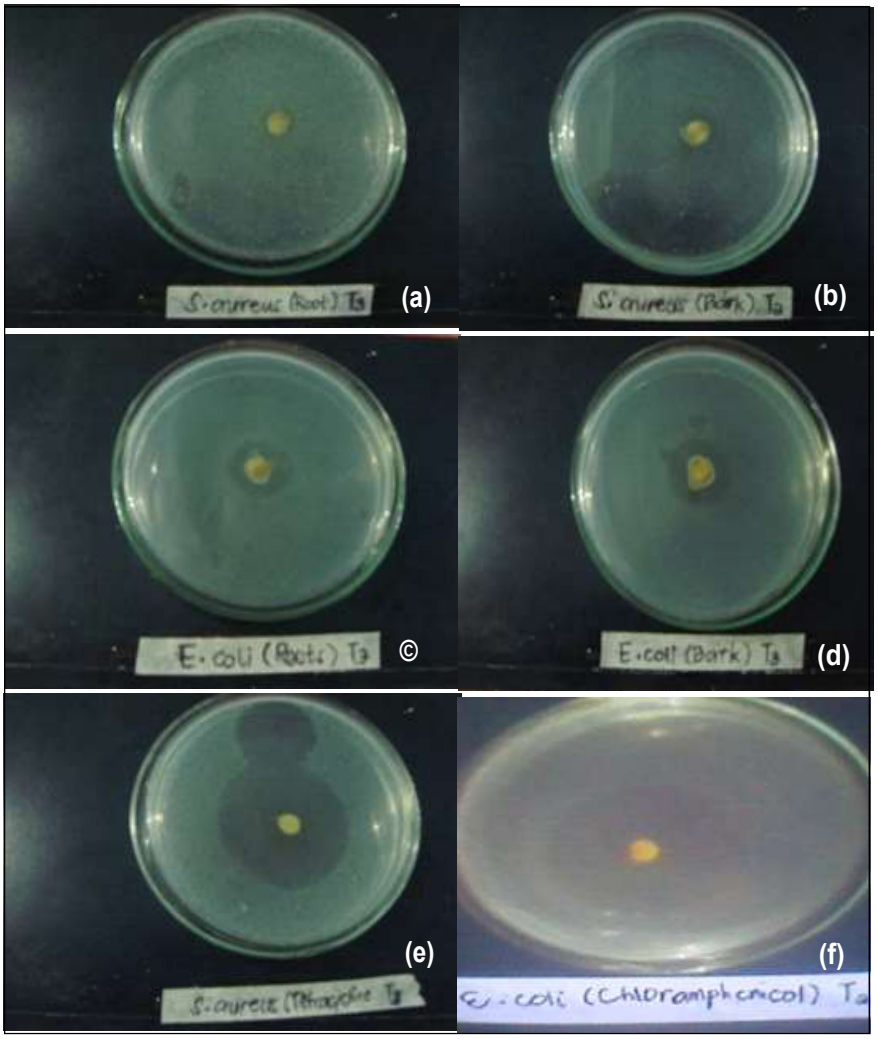

Figure 1. Zones of Inhibition Test of the **(a)** root extract on *S. aureus*;**(b)** bark extract on *S. aureus* ;**(c)** root extract on *E. coli*; **(d)** bark extract on *E. coli* ; **(e)**Tetracycline on *S. aureus, and* ; **(f)** Chloramphenicol on *E. coli.*

Table 4. Effect of *Cassia alata* Linn roots and crude extract on *Escherichia coli*

Sources of Variation	Degrees of Freedom	Sum of Squares	Mean of Squares	F – value		Interpretation
				Comp.	Tabular	
Between Groups K-1	1	16.7	16.7	2.5	7.71	NS
Within Groups (N-1) – (K – 1)	4	26.7	6.7			
TOTAL	5	43.4				

Table 5. Significant difference between *Cassia alata* Linn roots and crude bark extract and commercial antibacterial drug (Tetracycline) on *S. aureus*

Sources of Variation	Degrees of Freedom	Sum of Squares	Mean of Squares	F – value		Interpretation
				Comp.	Tabular	
Between Groups K-1	2	461.5	230.75	187.6	5.44	HS
Within Groups (N-1) –(K –1)	6	7.4	1.23			
TOTAL	8	468.9				

Table 6. Significant difference between *Cassia alata* Linn roots and crude extract and bark commercial antibacterial drug (Chloramphenicol) on *E. coli*

Sources of Variation	Degrees of Freedom	Sum of Squares	Mean of Squares	F – value		Interpretation
				Comp.	Tabular	
Between Groups K-1	2	230.2	115.05	16.97	5.44	HS
Within Groups (N-1) –(K– 1)	6	40.7	6.78			
TOTAL	8	270.9				

Therefore, the result implies that *Cassia alata* Linn roots and bark crude extract is not comparable to commercial antibacterial drug (Tetracycline) on *S. aureus* in terms of its antibacterial activity. So, Acapulco roots and bark extract were less effective than Tetracycline on *S. aureus.*

Significant Difference between *Cassia alata* Linn roots and crude extract and bark commercial antibacterial drug (Chloramphenicol) on *E. coli*

F-tabular value of 5.44 is lesser than the F-computed value of 16.97, the null hypothesis is therefore rejected. This means that there is a very highly significant difference between *Cassia alata* Linn roots and bark crude extract when compared with the commercial antibacterial drug (Chloramphenicol) on *E. coli* in terms of its antibacterial activity.

Therefore, the result implies that *Cassia alata* Linn roots and bark crude extract is not comparable to the commercial antibacterial drug (Chloramphenicol) on *E. coli* as far as its antibacterial activity is concerned. Thus, the crude extract could not be more effective than Tetracycline against *E. coli*.

Conclusions

Based on the results of this study, the researcher arrived at the following conclusions:

Cassia alata Linn roots and bark crude extract is effective as an antibacterial agent against *S. aureus* and *E. coli.* There is no significant difference in the antibacterial activity between *Cassia alata* Linn roots and bark crude extract on *S. aureus* and *E. coli.* There is a very high significant difference of antibacterial activity between *Cassia alata* Linn roots and bark crude extract and the commercial antibacterial drugs on *S. aureus* and *E. coli.*

Conflicts of interest: Not declared.

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