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Phytochemical and Antibacterial Screening of *Euphorbia thymifolia* Linn and *Cassia alata* Linn Species in the Province of Abra, Philippines: An Alternative Source of Antibiotics

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ABSTRACT

Plants have been aiding humanity around the world by serving as an abundant source of valuable drugs, antibiotics, nourishment, spices, seasonings, dyes, and other commercial and industrial products. The study investigated the antibacterial activity and phytochemical screening of *Cassia alata* Linn and *Euphorbia thymifolia* Linn ethanolic extracts against *Staphylococcus aureus* and *Escherichia coli*. The research was laid out using Complete Randomized Design (CRD) which was replicated three times. Varying concentrations of *Cassia alata* Linn and *Euphorbia thymifolia* Linn ethanolic extracts at 75%, 50% and 25% concentrations were utilized for the antimicrobial assay using the Kirby Bauer method. Phytochemical analysis revealed the presence of phenolics in both plants and some other bioactive components that have been found in vitro to have antibacterial properties. Statistical analysis using ANOVA and DMRT

at .05 level of significance shows that among the two plants tested, *Euphorbia thymifolia* Linn ethanolic extract at 75% concentration exhibited a significant mean diameter of zone of inhibition against *Staphylococcus aureus and Escherichia coli*. Further laboratory analysis and testing should be conducted through the Bureau of Food and Drugs (BFAD) for further confirmatory results.

Keywords **—** Clinical laboratory, phytochemical, secondary metabolites, experimental method, Philippines

INTRODUCTION

The therapeutic importance of plants is coupled with some biochemical substances also known as phytochemicals that give a definite biological action on the human body (Amer, Abouwarda, El Garf, Dawoud & Abdelmohsen, 2013). An essential form of bioactive components that originate from plants are flavonoids, terpenoids, alkaloids, glycosides, essential oil, saponins, tannins, steroids, resins, proteins, and others. The most effective method in extracting plant bioactive compounds is solely dependent on the type of solvent used. According to Eloff (1998), ethanol, acetone, and methanol are commonly utilized in extracting bioactive compounds. But herbal medicine manufacturers much prefer to use ethanol because it can be safely utilized by herbal clients (Dog, 2009).

Most of these bioactive components are valuable sources of natural antimicrobial compounds (Mahady, 2005). Nowadays, a growing concern about antibiotic resistance is expanding worldwide (Gardam, 2000). This encourages the resurgence of research on the role of plants against resistant strains of disease-causing bacteria which reveal that pharmaceutical plant extract has the potential to treat incurable diseases (Alviano & Alviano, 2009: Iwu, Duncan, & Okunji, 1999).

Indigenous people particularly in the upland municipalities of Abra are still practicing traditional methods of medications. Herbs are utilized to treat various ailments by using prepared concoctions, poultice or ointments either for oral or external applications. *Cassia Alata* Linn and *Euphorbia thymifolia* Linn are common plant species in Abra that are being utilized as a medicinal plant. However, their phytochemical component as well as their antibacterial

activity is not yet fully evaluated and studied in the province. Hence, the study was conceived. The result of the investigation can be utilized to provide a better comparison of activities of the two local plant species with the results obtained in other countries.

OBJECTIVE OF THE STUDY

The study aimed to determine the presence of secondary metabolites through phytochemical screening and the potential antibacterial activity of Andadasi (*Cassia alata* Linn) and Tartaristis (*Euphorbia thymifolia* Linn*)* against *Staphylococcus aureus and Escherichia coli*.

Specifically, it aimed to determine: 1) the bioactive components of Andadasi leaf (Cassia *alata* Linn) and Tartaristis (*Euphorbia thymifolia* Linn) plant; 2) the antibacterial activity of Andadasi and Tartaristis ethanolic extract at 75%, 50%, and 25% concentration towards *Staphylococcus aureus* and *Escherichia coli* regarding the diameters of growth inhibition; 3) if there is a significant difference between the zone of inhibition of Staphylococcus Aureus and Escherichia Coli subjected to Andadasi (*Cassia alata* Linn) and Tartaristis (*Euphorbia thymifolia* Linn) ethanolic extracts at 75%, 50%, and 25% concentrations and; 4) which plant and which of the three dilutions has the best potential antibacterial effect against the pathogenic microorganisms tested.

MATERIALS AND METHODS

Collection, identification and preparation of plant material

Cassia alata Linn and *Euphorbia thymifolia* Linn samples were harvested in the early morning from various places in Abra, Philippines on January to March 2015. It was authenticated by Professor Liezel Molina Magtoto from the Department of Biology College of Science University of the Philippines, Baguio City. Plant samples were oven-dried for 4 hrs (GEN LAB, Germany) for not more than 40°C, grind using an electrical grinder and immediately proceeded for extraction.

Extraction

Grind plant parts (200 g/plant sample) were placed in sterile glass bottles and 500 ml of ethanol was added and soaked for five days. The separated extracts (500 mL) were filtered using Whatman's no. 1 filter paper and were placed

in an Erlenmeyer flask. The ethanol filtrate was evaporated into a grease-like consistency using an evaporating dish heated in a magnetic stirrer hotplate. The separated plant extracts were individually concentrated to 50 mL and stored in a vial at 4°C for further experimental procedures in accordance with the procedures set by Nwachukwu and Uzoeto (2010).

Test organisms

The microorganisms used were obtained from the Department of Science and Technology Microbiology Laboratory (DOST) at Taguig City, Metro Manila. All bacterial cultures were checked for purity and maintained in a nutrient agar slants. The organisms tested were *Staphylococcus aureus*, and *Escherichia coli.*

Bioassay studies

The test was done by using the Kirby-Bauer disc diffusion (Bauer, Kirby, Sherris, & Turck, 1996). This method was performed by soaking filter paper discs having the same sizes into the different concentrations (75%, 50%, and 25%) of plant extracts. Seventy-five (75) mL of Mueller-Hinton Agar was poured into petri plates and uniformly inoculated with the sub cultured test organisms using a sterile inoculating loop using the streak plate method. Filter paper discs soaked in plant extracts together with the filter paper disc soaked in Penicillin (positive control) and the filter paper disc soaked in sterile water (negative control) were placed on the surface of the seeded Mueller-Hinton agar using a sterilized forceps. The plates were incubated overnight at 35°C. The growth of inhibition measured in millimeter using a digital Vernier caliper and the effects of the different treatments were compared with that of the positive and negative control. The antimicrobial activity testing was done in three replications. Interpretation for the range of inhibition was adopted from Ontengco (2005).

Table 1. Range of Standard Zone of Inhibition

Zone of Inhibition	Inhibitory Activity
> 17	$^{+++}$, strong
12 – 16	$^{++}$, moderate
7 – 11	$^{+}$, weak
0-6	$^{-}$, negative

Phytochemical Analysis

Fresh and clean two kilograms of *Cassia alata* Linn and *Euphorbia thymifolia* Linn were placed in sterile containers and sent to SLU Natural Science Research Unit Baguio City for Phytochemical analysis (Tiwari, Kumar, M. Kaur, G. Kaur, & H. Kaur, 2011; Soni, Sharma, Patel, Mishra, & Singhai, 2011). By this analysis, the presence of several phytochemicals like alkaloids, flavonoids, glycosides, phenolics, saponins, steroids, tannins, and triterpenes was tested.

Statistical Analysis of Data

Statistical analysis was conducted using the SPSS software on the antibacterial assay. It was further subjected to ANOVA and DMRT at .05 level of significance.

Table 2. Comparative phytochemical results of *Cassia alata* Linn. and *Euphorbia thymifolia* Linn

Name of samples	Alkaloid	Anthraquinone glycosides	saponins	Phytosterol	Phenolics	Tannins	Flavonoid	Triterpenes
C. Alata Linn.	-	+	-	-	+	-	+	-
E. thymifolia L.	-	-	-	+	+	-	-	+

RESULTS AND DISCUSSION

Phytochemical Screening

Anthraquinone glycoside and flavonoids are found in *Cassia alata* Linn while phytosterol and triterpenes are present in *Euphorbia thymifolia* Linn These secondary metabolites found in both plants have also been found in vitro to have antibacterial properties.

According to Geissman (1963), phenolics are bioactive phytochemicals that are toxic and inhibitory to organisms such as viruses, bacteria, and fungi.

Anthraquinone has a strong antimicrobial effect. Its probable targets in the microbial cell are surfaced exposed adhesions, cell wall polypeptides and membrane-bound enzymes. It may also render substrates unavailable to microorganisms. A study conducted by Kazmi, Malik, Hameed, Akhtar, & Ali, 1994) described an anthraquinone from Cassia italica, a Pakistan tree, which was bacteriostatic for Bacillus anthracis, corynebacterium pseudodiphtherium, and

Pseudomonas aeroginosa and bactericidal for Pseudomonas pseudomalliae.

Flavonoids have also been found in vitro to be effective antimicrobial substances against a wide array of microorganisms (Dixon, Dey, Lamb, 1983) while triterpenes or terpenoids are active against bacteria, fungi, viruses and protozoa (Taylor, Edel, Manandhar & Towers, 1996).

A study that was conducted by Batista *et al.* (1994), isolated two diterpenes and found out to work well against Staphylococcus aureus, V. cholera, P. aeroginosa, and Candida species.

Thus, the extracts from *Cassia alata* Linn and *Euphorbia thymifolia* Linn can be possibly be used as a source of antibacterial drugs if the specific compounds can be isolated and purified as to their contents and origin.

Antibacterial Activity

Table 3. The Antibacterial Activity of *Euphorbia thymifolia* Linn Ethanolic extracts at 75%, 50% and 25% concentrations against *Staphylococcus aureus* and *Escherichia coli* in terms of Mean Diameter of Zone of Inhibition (mm)

Plant	Concentrations %	*S. Aureus*	*Eschericha coli.*
E. thymifolia Linn.	75	16++	14++
	50	10+	9.67+
	25	11+	11.67+

Legend: +++ (strong), ++(moderate) and + (weak)

Table 3 shows that *Euphorbia* thymifolia Linn at 75% concentration exhibits moderate antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* with 16mm and 14mm in mean diameter of the zone of inhibition, while at 50%, and 25% concentrations exhibit weak antibacterial activity. This means that *Euphorbia thymifolia* Linn ethanolic extract works best at higher concentration in inhibiting the growth of *Staphylococcus aureus* and *Escherichia coli*.

Similar antibacterial activity of the plant extracts has been reported by Muthumani et.al (2013), and their work reveals that *Euphorbia thymifolia* Linn shows both antioxidant and antihelminthic and antibacterial property. Their result further revealed that the growth of all bacteria was inhibited through varying degrees, thus, substantiating the utilization of plant extract as an alternative form

of medication to treat enteric infections such as E.coli and S. typhii. The strength of the extract can be further enhanced under acidic conditions at elevated temperatures (Muthumani *et al.*, 2013).

Table 4. Antibacterial Activity of *Cassia alata* Linn Extracts at 75%, 50% and 25% concentrations against Staphylococcus aureus and Escherichia coli in terms of Mean Diameter of Zone of Inhibition (mm)

Plant	Concentrations	*S. Aureus*	*Eschericha coli.*
Cassia alata Linn.	75%	13.33++	12++
	50%	9+	9 +
	25%	9.3+	7.3+

Legend: +++ (strong), ++(moderate) and + (weak)

Table 4 shows the effect of *Cassia alata* Linn ethanolic extracts at different concentrations of both *Staphylococcus aureus* and *Escherichia coli*. Results show that 75% concentration of the plant extract also exhibits moderate anti-staphylococcus aureus and *Escherichia coli* properties with 13.33 mm and 12 mm mean diameter of zone of inhibition while at lower concentrations both plant extracts manifested weak effects towards the two tested organisms.

Table 5. Duncan's Multiple Range Tests of the Mean Zones of Inhibition of the Different Extract Concentrations of *Euphorbia thymifolia* Linn and *Cassia alata* Linn Against *Staphylococcus aureus* and *Escherichia coli*

Concentrations	***Euphorbia thymifolia* L.** (*S. aureus*)	***Euphorbia thymifolia* L.** (*E.coli*)	***Cassia alata* Linn.** (*S.aureus*)	***Cassia alata* Linn.** (*E. coli*)
75%	16^{a}	14^{a}	13.33^{a}	12^{a}
50%	10^{b}	9.67^{a}	9^{b}	9^{ab}
25%	11^{b}	11.67^{a}	9.3^{b}	7.3^{b}

* Means with the same letter are not significant

Duncan's Multiple Range Test reveals that *Euphorbia thymifolia* Linn and *Cassia alata* Linn ethanolic extract have significant effect on *Staphylococcus aureus* at 75% concentration. This shows that both plant extracts are effective against *Staphylococcus aureus* at higher concentrations.

CONCLUSIONS

Both plants contain phenolics. Anthraquinone glycoside, flavonoids are found in *Cassia alata Linn.* while phytosterols and triterpenes are present in *Euphorbia thymifolia* Linn. These secondary metabolites which are identified in both plants have been found in vitro to have antibacterial properties. *Euphorbia thymifolia* Linn ethanolic extract works best at higher concentration in inhibiting the growth of *Staphylococcus aureus* and *Escherichia coli*.

Analysis of variance (ANOVA) has shown that *Euphorbia thymifolia* Linn and *Cassia alata* Linn extracts have a significant effect towards *Staphylococcus aureus.* This shows that the organism is more susceptible to the effect of both plant extracts. The sensitivity of *Staphylococcus aureus* can be attributed to its bacterial thin cell membrane that can be easily affected by the secondary metabolites present.

Euphorbia thymifolia Linn has the best potential antibacterial effect against the pathogenic microorganisms tested at 75% concentration. It signifies that the higher the concentration, the stronger the antibacterial property.

TRANSLATIONAL RESEARCH

The extracts from *Euphorbia thymifolia* Linn and *Cassia alata* Linn can be used as a source of antibacterial drugs if the specific compounds are isolated and purified as to their contents and origin. *Euphorbia thymifolia* Linn plant is not well known by the natives of Abra especially its medical importance. Therefore, the researcher recommends further study especially on the exact part of the plant where the antibacterial properties are concentrated. Folks should also be informed or oriented on the benefits they can enjoy using the said herbal plants.

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Appendix

Table 6. Summary Table on the Mean Difference of the Zone of Inhibition of *Cassia alata* Linn and *Euphorbia thymifolia* Linn Ethanolic Extracts Towards *Staphylococcus aureus* at 75%, 50% and 25% concentrations in millimeters(mm)

% Concentration	Zone of Inhibition for *Euphorbia thymifolia L* (mm)	Zone Of Inhibition for *Euphorbia thymifolia L.* (mm)	Mean Difference	t Value	t Probability
75%	16	13.33	2.66	1.6	.185
50%	10	9	1.0	1.73	.158
25%	11	9.33	1.66	1.89	.132

The table shows that there is no significant difference between the effects of *Cassia alata* Linn and *Euphorbia thymifolia* Linn ethanolic extracts at different concentrations in terms of the mean diameter of zone of inhibition against *Staphylococcus aureus*. This shows that both plants have similar antibacterial potency in preventing the growth of the test organism. It also validates the presence of bioactive components or secondary metabolites that are proven to have antimicrobial properties as shown in the phytochemical analysis conducted on both plants (Cowan, 1999).

This signifies that *Cassia alata* Linn and *Euphorbia thymifolia* Linn ethanolic extracts are good sources of anti-staphylococcus aureus drugs when properly isolated and refined to cure staphylococcal skin diseases and would infections.

Table 7. Summary Table on the Mean Difference of the Zone of Inhibition of *Cassia alata* Linn and *Euphorbia thymifolia* Linn Ethanolic Extracts Towards Escherichia Coli at 75%, 50% and 25% concentrations in millimeters (mm)

% Concentration	Zone of Inhibition for Tartaristis(mm)	Zone of Inhibition for Andadasi(mm)	Mean Difference	t Value	t Probability
75%	14	12	2	.926	.407
50%	9.67	9.0	.66	.277	.795
25%	11.67	7.33	4.33	9.19	.001

The table shows that there is a significant difference between the effects of *Cassia alata* Linn and *Euphorbia thymifolia* Linn ethanolic extract towards *Escherichia coli* at 25% concentration having a mean diameter of zone of inhibition of 11.67mm in diameter and 7.33mm diameter with a mean difference of 4.33mm. This indicates that at lower strength, the two test plants differ in their antimicrobial action towards *E. coli* where *Euphorbia thymifolia* Linn is more potent than *Cassia alata* Linn. This result is due to the presence of phytosterol, a steroid compound similar to cholesterol. The same compound was derived by Sharma (1993) using petroleum ether extract from the leaves of *Annona squamosa*, *Adenocalymna alliceum* and *Amaranthus tricolor* which exhibited potent antibacterial properties towards gram positive and gram negative bacteria. Thus, phytosterols, are considered as wide-spectrum antibacterial agent. As to 75%, 50%, and 25% concentrations, both test plants have no significant difference as to zone of inhibition, meaning, both plants have a similar antibacterial effect towards E.coli at higher concentrations.

Table 8. One Way Analysis of Variance of the Mean Zone of Inhibitions of the different Ethanolic Extract Concentrations of *Cassia alata* Linn and *Euphorbia thymifolia* Linn against *S. aureus and E.coli*

	Sum of Squares	Df	Mean square	F	Sig.
Tartaristis Between Groups	62 .000	2	31.000	23.250	.001
(S. Aureus) Within Groups	8.000	6	1.333		
Total	70.000	8			
Tartaristis Between Groups	28.222	2	14.111	2.396	.172
(E. Coli) Within Groups	35.333	6	5.889		
Total	63.556	8			
Andadasi Between Groups	34.889	2	17.444	6.826	.028
(S.aureus) Within Groups	15.333	6	2.556		
Total	50.222	8			
Andadasi Between Groups	33.556	2	16.778	3.512	.098
(E. Coli) Within Groups	28.667	6	4.778		
Total	62.222	8			

Analysis of variance shows that *Euphorbia thymifolia* Linn and *Cassia alata* Linn ethanolic extracts have a significant effect towards *Staphylococcus aureus* with a level of significance of .001 and 0.28, respectively. It points that *Staphylococcus aureus* is more susceptible to the effect of both plant extracts. The sensitivity of *Staphylococcus aureus* can be attributed to its bacterial thin cell membrane that can be easily affected by the secondary metabolites present.