BIBLIOGRAPHY

MALEL, JOHN K. APRIL 2013. Antibacterial activity of *Bidens pilosa*, *Tithonia diversofolia*, *Coix lacryma* and *Crassocephalum crepidioides.* Benguet State University, La Trinidad, Benguet.

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ABSTRACT

The study was conducted at the College of Veterinary Medicine, Benguet State University, La Trinidad, Benguet to determine the antibacterial effects of *Bidens pilosa*, *Tithonia diversofolia*, *Coix lacryma* and *Crassocephalum crepidioides.* Specifically it aimed to show the positive and negative zone of inhibition of the four medicinal plants.

A total of 4 treatments were observed. Each treatment against the bacteria *Escherichia coli* and *Staphylococcus aureus*.

The result of the study showed that there was no significant zone of inhibition recorded on the gram positive bacteria *Staphylococcus aureus*. However, *Tithonia diversofolia* recorded a positive zone of inhibition of 10mm. This indicated that the medicinal plant *Tithonia diversofolia* water extract had antibacterial activity.

INTRODUCTION

Many infectious microorganisms are resistant to synthetic drugs; hence an alternative therapy is much needed. Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating diseases because they contain numerous active constituents of immense therapeutic value.

Bidens pilosa locally known as *burburtak* is a wild crafted plant and is treated as weeds in vegetable gardens in Benguet. Due to their abundance and reported biological uses the researcher intends to test the antibacterial activity of water extracts of the plant mentioned above against bacterial organism commonly found animals that can cause diseases in man.

Crassocephalum crepidioides, also called "*Ebolo"* (in Yoruba land in Nigeria) is an erect annual slightly succulent herb growing up to 180 cm tall. Its use is widespread in many tropical and subtropical regions, but is especially prominent in tropical Africa where the fleshy mucilaginous leaves and stems are eaten as vegetable and many parts of the plant use for medical purposes. Medicinal folklore use of *C. crepidioides* include treatment of indigestion (in Southern Nigeria), treatment of stomach upset (in DR Congo), treatment for fresh wound (in Uganda), the decoction of the leaf is used in Nigeria for the treatment of headache, in Tanzania a mixture of the leaf sap of C. *crepidioides* and *Cymbopogon giganteus* is used orally and externally for the treatment of epilepsy. Also in Tanzania, the dried leaf powder is applied as a snuff to stop nose bleeding and smoked to treat sleeping sickness. Tannin found in the roots of the plant is used to treat swollen lips. The plant is known to contain a large number of phytochemical compounds which include: tannin,

dihydroisocoumarins, pyrrolizidine alkaloids such as jacobine and jacoline and monoterpenes such as myrcene, limonene and α-copaene.

Tithonia diversifolia, also called wild sunflower is a small to medium-sized annual shrub with rather stout, almost glabrous branches. Leaves are alternate, petioled, membranaceous, ovate to orbicular, entire or 3- to 5-lobed, with toothed margins. It was introduce to the Philippines as an ornamental, and has escaped cultivation to become a weed in waste places.

In Central America, leaf extracts are used externally for the treatment of wounds and hematomas. A methanol extract of the dried leaves of *Tithonia diversifolia* was investigated for anti-inflammatory and analgesic activities. The extract produced dose-related inhibition of carrageenan-induced paw oedema and cotton pellet-induced granuloma in rats. At the same doses, analgesic effect was also observed with hot plate latency assays maintained at (55 °C) as well as on the early and late phases of formalin-induced paw licking in rats. The results of the present study further confirm the use of *Tithonia diversifolia* traditionally for the treatment of painful inflammatory conditions (Wuraola *et al.*, 2004).

Job's Tears also known as *Coix lachrymal* has anti-inflammatory effects. Results showed the methanol seed extract of seeds of CL showed anti-inflammatory properties which may involved the inhibition of NO and O_2 production by activated macrophages. Study on the anti-inflammatory activities of six benzoxazinoids from roots of *Coix lachryma*-jobi var. Ma-yuen showed the free hydroxyl group in the benzoxazinone skeleton involved in the expression of inhibitory activity

The main objective of the study is focused on the antibacterial activity of *Bidens ilosa*, *Crassocephalum crepiodioes* jobs tears and *Tithonia diversifolia* extracts against bacterial organism.

Specifically, it aimed to:

1. To determine the antibacterial activity of *Bidens pilosa,Tithonia diversifolia, Coix lachryma* and *Crassocephalum crepidioides*, water extracts against *Staphylococcus aureus*, and *E.coli*

2. To compare the antibacterial activity of water extracts on the inhibition of bacterial organisms; and

3. To determine the best among the various treatments, *Bidens Pilosa, Crassocephalum crepidioides, Coix lachryma* and *Tithonia diversifolia,* water extracts and the positive control (Anpicillin) in the inhibition of bacterial organism.

Farmers will mostly benefit from the use of the above plants as an antibacterial control for animal organism. It can sustain the productivity of the livestock industry in the Cordillera through the promotion of good health and cheap alternative medicines for animal diseases. Because of the abundance of this plant in the locality, they can be easily utilized by farmers and other locals.

The study was conducted at the BSU College of Veterinary Medicine laboratory, Balili, La Trinidad Benguet in February, 2013.

REVIEW OF LITERATURE

Bidens pilosa

Bidens pilosa is called by various names, such as Spanish needle, Black jack or Beggar ticks in English and "burburtak" in Ilokano, Kalinga and Bontok Mt. Province ; "furfurtak" in Botbot and Kalinga; "Silew" in Bauko Mt. Province- Kankanai; "Monmonot" in Sagada Mt. Province-kankanai; Onward or engguad in Ifugao; Nuad or Ang-anguad in North Benguet or Puriket in Tuba- ibaloi.

It is an erect, branched, less hairy herb, about 0.2 to 1.5 m high. The leaves are two pinnafid and 15 cm more or less, with the upper one being usually much smaller. The leaves are ovate-lanceolate about 2-5 cm long and toothed. The flowers discs are brown, yellowish or nearly whit flowering head about 8 mm long. The fruits achenes are black, long and slender, linear, 1.0-1.5 cm long and characterized by four projections at the apex (Mvere, 2004).

The entire plant is common in waste places, from Batanes and Babuyan Islands and Northern Luzon to Mindanao. The entire plant utilized usually as a decoction or poultice in folkloric medicine against inflammation and as an external wash.

Bidens pilosa Folkloric Uses

In the Cordillera, the hot water decoction is used for the treatment of diarrhea, prophylactic for gastrointestinal disorder, while it is usually taken with ginger decoction in the treatment of gastric pains. The hot water decoction is also used as a foot bath for the treatment of wound, inflammation. The sap from fresh leaves is mixed with honey for the treatment of painful urination and acute nephritis.

In many regions of Africa and tropical America, the roots, leaves and seed of *Bidens pilosa* have been reported to possess antibacterial, antidysenteric, anti-inflammatory, antimicrobial, antimalarial, diuretic, hepayo-protective and hypotensive activities (Mvere, 2004).

In Africa, the sap from crushed leaves is used to treat ear infection, speed up clotting of blood in fresh wounds (Uganda); applied to burns (Tanzania). While the leaf decoction is used for treating kidney problems (Uganda); cure stomach and mouth ulcers (Zimbabwe) and treating arthritis (South Africa) and for treating jaundice (Cote d 'Ivoire) and suspension of powdered leaves as an enema for abdominal by Zulu in South Africa and leaf extracts are used to treat swollen spleen of children by the Giriama tribe of Kenya. The Giriama tribe also use a mixture of ground dried *Bidens pilosa* leaves, soap and hot pepper for the control of leaf miners in plants (Mvere, 2004).

Bidens pilosa,Antimicrobial Properties

A number of studies have demonstrated the antibacterial activity of *Bidens pilosa* against *Staphylococcus aureus* (Mvere, 2004). *Klebsiella pneumonia, Bacillus*, *neisseria gonorrhea*, *pseudomonas*, and *salmonella*, *Mycobacterium tuberculosis* and *M. smegmatis* (Mvere, 2004). The plant extracts is also effective against Candida and herpes virus (Chiang *et al*., 2003).

The plant sap is bacteriostatic to *Staphylococcus aureus* but not to *Escherichia coli* and the water extracts exhibit activity against intestinal pathogenic bacteria herpes virus (Chiang *et al*., 2003). Ethanol and methanol extracts of roots of *Bidens pilosa* exhibit

bacteriostatic effect against *Staphylococcus aureus*, *Staphylococcus epidermidus* *Escherichia coli* and *Kebsella pneumonia* (Ashafa and Afolayayn, 2009).

However, water extracts of *Bidens pilosa* have no inhibitory property against Staphylococcus aureus beta hemolytic streptoccus, *Bacillus cereus* and *E. coli* and *Pseudomonas* and *Candida* (Rojas *et al.*, 2006).

Bidens pilosa Phytochemical Analysis

Studies have shown that the plant contains iodine; the leaves, tannin and saponin; the flowers, sulfur polyacetylene and flavonoid, and acetylene have been identified from the plant extracts (Mvere, 2004). The entire plant contains Anthraquininone.

Phytochemical analysis of the plant showed that it is highly positive for gums, mucilages and glycosides; carbohydrates and reducing sugars; tannin and tannin derivatives and proteins and proteins derivatives. It is also positive for secondary metabolites as steroids (unsaturated sterols) and deoxysugars; unsaturated sterols and triterpenes and polyphenolic compounds

NSRU (2009) as cited by Battad (2009) reported that the phytochemical analysis of the leaves of *Coix lachrymal-jobi* showed that it is highly positive for gums, mucilages and glycosides; moderately positive carbohydrates and reducing sugars; highly positive for tannin and tannin derivatives and proteins and protein derivatives. It is also positive for secondary metabolites such as steroids (unsaturated sterols) and deoxysugars; unsaturated sterols and titerpenes and polyphenolic compounds.

Tithonia diversifolia Analgesic/Anti-Inflammatory

Study of methanol extract of dried leaves of *Tithonia diversifolia* produced dose-related inhibition of carrageenan-induced paw edema and cotton pellet-induced granuloma in rats. The analgesic effect was observed with hot plate latency assays. Results confirm the traditional use of *Tithonia diversifolia* for the treatment of painful inflammatory conditions (Wuraola *et al.,* 2003).

Staphylococcus aureus

Staphylococcus aureus is a Gram – Positive spherical bacteria that occur in clusters resembling grapes. It colonizes mainly the nasal passages, but may be found regularly in the skin, oral cavity and gastrointestinal tract of man and animals. It cause superficial skin lesions such as boils, stys and furuncles. It also causes food poisoning by releasing anterotoxins into food and toxic shock syndrome by release of super antigens into the blood stream (Todar, 2008). This bacterium has the ability to develop resistance against antibiotics. MRSA are stains of the *Staphylococcus* that are resistant to the action of methicillin and related beta-lactam antibiotics like penicillin, oxacillin and amoxicillin.

Battad (2009) reported that leaves of *Bidens pilosa* and *Coix lachrymal* in ethanol extract have very active antibacterial effect on *Staphylococcus aureus* and *E. coli*.

Cogasi (2006) mentioned that the “ange” or the camote leaves and vines is the main feed given to pigs by the people in the municipality of Besao. When the time comes that it becomes scarce, most especially during summer time or during the dry season, the swine raisers would use the other indigenous feeds, taro of which are the *Bidens pilosa* and *Crasophalum crepedioides* or the Japanese weed.

METHODOLOGY

Materials

The materials used in the study include leaves of *Bidens pilosa*, or "burburtak, *Crassocephalum crepidioides* or Japanese weeds, *Tithonia diversifolia* or wild sunflower, and *Coix lachryma* or Jobs tears. The test organisms were gram positive bacteria: *Staphylococcus aureus* and *Escherichia coli*. The media that was used in the study was Muller Hinton agar (MHA and sterile distilled water). Ampicillin disc was the positive control against gram positive. The equipments that were used in the study were Ohaus digital weighing scale, hot oven sterilizer biological incubator, auto clave, rotary vaporizer, hot plate and laminar flow.

The glass wares used in the study include sterile beakers, graduated cylinder, Erlenmeyer flask, stirring rod and vials. Other materials used in the study were sterile cotton balls and swabs, sterile rubber gloves, denatured alcohol, alcohol lamp, match, inoculating loop, wire basket, aluminum foil, scissors, ball pen, ruler, marker, notebook, camera, and computer.

Methods

Experimental treatments. There were four treatments involved in the study. Each treatment was replicated three times. The different treatments were as follows:

T_0 – Antibiotics disc (Ampicillin)

T_1 - *Bidens pilosa* leaves

T_2 – *Crassocephalum crepidioides* leaves

T_3 – *Tithonia diversifolia* leaves

T_4 – *Coix lachryma* leaves

Preparation of plant extract. Fifty grams (50g) leaves of each of the plants namely *Bidens pilosa*, *Crassocephalum crepidioides*, *Tithonia diversifoli*a and *Coix lachryma* were weighed separately. These plant leaves were gathered at Balili, La Trinidad, Benguet. These were washed and air dried. The leaves were cut using a sterile knife on a chopping board and placed in the beaker. One hundred ml distilled water was added then heated and was allowed to simmer for 10 minutes (Figure 1) The decoction was then decanted and the liquid was transferred to another sterile container and was set aside (Figure 2). A sterile filter paper disc (Whatman 10 ash less" filter paper) was soaked into the decoction overnight. The Kirby-Bauer technique for determining antibacterial activity of the plants used in the study was used.

Figure 1. The researcher heating the plants

Figure 2. The decanted decoctions

Inoculation of Mueller Hinton agar plate. Using a sterile cotton swab, the tip was slightly moisten with 0.5 McFarland standard turbidity of test organism was used in nutrient broth culture to Muller-Hinton agar plates. Then using sterile forceps, the disc soaked in the plants' extracts were placed on the agar plates (Figure 3). The same was done with the antibiotic disc. The inoculated agar plates were wrapped with paper and incubated at 35^{o}C for 24 hours.

Preparation of filter paper. The filter paper discs were made by cutting Whatman Ash-less filter paper No. 42 with a puncher to make 6mm diameter discs. The filter paper discs was wrapped in a clean aluminum foil and sterilized in a hot oven sterilizer for an hour in 100^{o}C (Figure 4) The sterile filter paper discs were then soaked overnight into the plant decoctions.

Figure 3. Inoculation of Mueller Hinton agar plate

Figure 4. The researcher sterilizing the filter paper discs

Preparation of assay plates. Mueller-Hinton agar was prepared following the manufacturer's recommendations based on weight that was 3.07 grams per 100 ml of distilled water. This was sterilized for 15 minutes the agar was then dissolved in 100 ml distilled water.

Definition of Terms

1. Incubation. This is the maintenance of temperature, humidity and other conditions while microbiological culture is being grown to multiply.

2. Zone of inhibition. This is the area where the growth of the bacteria is restricted. The interpretation of the zone of inhibition measurement, as cited by Battad (2009), is as follows:

Zone of Inhibition (mm)	Interpretation
Less than 10	Inactive
10-13	Partially active
14-19	Active
Above 19	Very active

Data Gathered

1. Zone of inhibition of the water extracts or decoctions derived from the plants and the positive and negative control on the gram positive bacteria. This was measured at the widest zone of inhibition using a ruler or caliper.

RESULTS AND DISCUSSION

Zone of Inhibition

Staphylococcus aureus. Results of the study revealed no recordings on the zone of inhibition were obtained in all the plant extracts against *Staphylococcus aureus* (Table 1). This means that the water extracts of the four plants namely *Bidens pilosa* or "burburtak", *Crassocephalum crepidioides* or Japanese weed, *Tithonia diversifolia* or sunflower and *Coix lachrymal* or Jobs tears have no inhibitory properties against *Staphylococcus aureus*. Ampicillin, the control, had also a zero zone of inhibition.

The above result is similar to the findings of Rojas *et al* (2006) who also found out that water extract of *Bidens pilosa* has inhibitory properties against *Staphyloccus aureus*. However, the result is in contrast to the findings of both Chang (2003) and Mvere (2004) who both reported that the plant extract or plant sap of *Bidens pilosa* is bacteriostatic or has antibacterial activity against *Staphylococcus aureus*.

Battad in 2009 also found out that both the *Bidens pilosa* and even *Coix lachryma* are very active (23.33 and 25.33mm zones of inhibition, respectively) in inhibiting *Staphylococcus aureus* but this was using ethanol extract. Ahmad and Ismail (2003) reported that the water extract of *Coix lachryma* is used to treat fever and cough but unfortunately it did not come out to have antibacterial effect with both *Staphylococcus aureus* and *Escherichia coli*.

According to Cogasi (2006), *Bidens pilosa* and *Crassocephalum crepidioides* are two of the common garden weeds fed by the swine raisers in Besao, Mountain Province to their animals. These are normally cooked before feeding to the pigs. The pigs are

Table 1. The zone of inhibition of the water extracts of the plants used in the different treatments

TREATMENT	ZONE OF INHIBITION	
	Staphylococcus aureus	*Escherichia coli*
Ampicilin (Control)	0	0
Bidens pilosa	0	0
Tithonia diversofolia	0	10 mm
Crassocephalum crepidioides	0	0
Coix lachrymal	0	0

Water extract of *Tithonia diversofolia* (sunflower) has pronounced inhibitory against *E coli*.

usually fed with camote leaves and vines (the most commonly used feedstuffs for swine) and this constitute the major part of the ration. However, in times when there is scarcity of this camote leaves and vines, *Bidens pilosa* and *Crassocephalum crepidioides* can serve as substitutes. While there is no scientific basis, it could be possible that the giving of boiled *Bidens pilosa* and *Crassocephalum crepidioides* in greater amounts could have helped in preventing illness to the pigs caused by *Staphylococcus aureus*.

Escherichia coli. Results of the study also showed that the water extracts obtained from the leaves of *Bidens pilosa*, *Crassocephalum crepidioide*s, and *Coix lachryma* had no antibacterial activity against *Escherichia coli* as revealed by the zero zone of inhibition. However, for the *Tithonia diversofolia* or wild sunflower, results of the study gave a reading of 10 mm on zone of inhibition against *Escherichia coli*. Though the 10mm zone of inhibition obtained was considered the minimum to have an antibacterial effect as cited by Battad (2009), perhaps when the concentration or level of the wild sunflower leaves is

increased in producing the water extract, its antibacterial activity is increased also. Among the leaves used in the study, the wild sunflower is very bitter in taste. This means that it has a component not present in the other leaves used which has an antibacterial activity.

The above result is similar to the findings of Chiang (2003) and Rojas *et al* (2006) who both found out that the plant sap or water extract of the leaves of *Bidens pilosa* is not bacteriostatic or has no inhibitory properties to *Escherichia coli*. On the other hand, Battad (2009), in her study, found out that both the *Bidens pilosa* and *Coix lachryma* are very active on inhibiting *Escherichia coli* but she made used of the ethanol extracts of both.

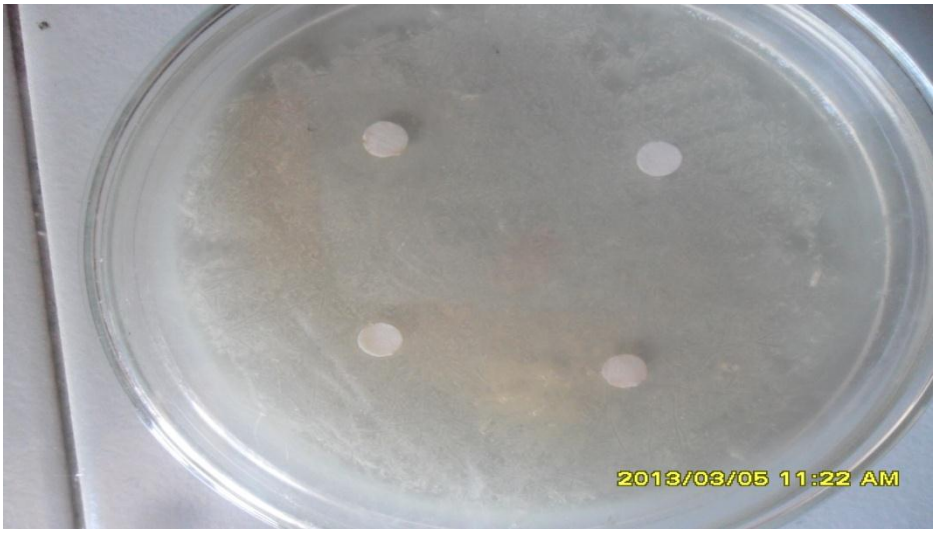

Figure 5. Zone of inhibition

The *Crasocephalum crepidioides*, *Coix lacrymai* and the *Bidens pilosa* water extracts does not have any antibacterial activity against *Escherichia. coli* and *S. Aureus*, it enhances the ability of the immune system.

SUMMARY, CONCLUSION AND RECOMMENDATION

Summary

This study was conducted to evaluate the antibacterial properties of plants namely *Bidens pilosa* or "burburtak", *Crassocephalum crepidioides* or Japanese weed, *Titonia diversofolia* or wild sunflower and *Coix lachrymal* or Jobs tears agains against *Staphylococcus aureus* and *Escherichia. coli.*

The plants used in the study were collected at Balili, La Trinidad, Benguet. The plants composed the treatments as follows: T_0 – Ampicillin; T_1 – *Bidens pilosa;* T_2 – *Crassocephalum crepidioides*; T_3 – *Tithonia diversifolia* and T_4 – *Coix lachrymal*.

Fifty grams of each of the plants in the treatments were chopped and used in the study. This was diluted with 50 ml distilled water to produce the water extract to be tested.

Results of the study revealed that there were no significant antibacterial properties of all the plants used in the study except the *Tithonia diversifolia* or wild sunflower. There was a positive zone of inhibition of the wild sunflower against *Esherichia coli*.

Conclusion

Based on the results of the study, it can be concluded that the *Bidens pilosa*, *Crassocephalum crepidioides*, and *Coix lachrymal* water extracts do not have any antibacterial effects against *Staphylococcus aureus* and *Escherichia coli*. The *Tithonia diversifolia* water extract has no antibacterial effect against *Staphylococcus aureus* also but has an antibacterial effect against *Escherichia coli*.

Recommendation

It is recommended that further studies should be conducted to include higher levels of the plant leaves in the water extract; their antibacterial effects on other bacterial pathogens aside from *Staphylococcus aureus* and *Escherichia coli* and to include other ways of extraction.

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