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Antimicrobial Efficacy of Carabao Grass *(Paspalum conjugatum)* leaves on *Staphylococcus aureus*

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ABSTRACT

In the Philippines, the *Paspalum conjugatum* or known by its common name as "carabao grass" is usually consumed for livestock as well as for landscaping. *Paspalum conjugatum* has been known to have phytochemicals that kill microorganisms. This experimental research determined the phytochemicals present and antimicrobial efficacy of *Paspalum conjugatum* against *Staphylococcus aureus.* Samples of the carabao grass was purchased, washed and then dried. An extract was obtained from the sample. The extract was then sent to an accredited testing center for phytochemical test to identify the different components of the plant extract. The antimicrobial activity of the *paspalum conjugatum* extract was then determined through the use of the disc diffusion method. The zone of inhibition of *Paspalum Conjugatum* was also compared to a control standard such as oxacillin.
The results of the study revealed to have abundant constituents of sterols, flavonoids and tannins; moderate levels of triterpenes, saponins and glycosides; and traces of alkaloids. The antimicrobial testing showed results that *Paspalum Conjugatum* had 10mm zone of inhibition which means that it has a complete inhibitory activity with severe reactivity against the tested organism, *staphylococcus aureus*.
The study concludes that *Paspalum conjugatum* can be used to eliminate staphylococcus aureus. It is recommended that further studies be conducted for the use of *Paspalum conjugatum* in the treatment and prevention of diseases caused by *staphylococcus aureus*.

Keywords: *Paspalum conjugatum, Carabao grass, Staphylococcus aureus*

INTRODUCTION

In many parts of the world, grass is the most common plant. Grass grows in both wild areas to within city limits and can grow in various biomes that allow it to grow. In the Philippines, this variation of grass is *Paspalum conjugatum*, known by its common name as "carabao grass". Just like the other types of grass in other countries, carabao grass is mainly used as forage for livestock. The name "carabao" grass is after the word carabao, which is a type of buffalo from the Philippines. Goats, horses, sheep, cows and even carabaos graze on this type of grass.

Carabao grass is important for plantations as well, specifically in the rice and coconut plantations. This is due to the allelochemicals in the carabao grass, which produces biochemicals that positively affect the growth and reproduction of other bio organisms (Kobayashi, 2004). For other uses, carabao grass is used by landscaping companies as landscape material for malls, parks, and other recreational areas. So far, there is little evidence that carabao grass is used as an antimicrobial agent in the Philippines.

In other parts of the world, carabao grass is used for medicinal purposes. In Ecuador, native people used carabao grass as a remedy for headaches (Russo, 2000)In West Papua, carabao grass is used to treat wounds (Lense, 2012) Inhabitants of Morocco use carabao grass as an antifungal application. Like all other plants, carabao grass has phytochemicals that can influence various microorganisms (Bnouham, 2010)

However, there is little to no research in the Philippines regarding the use of carabao grass for medicinal purposes. In this study, phytochemicals were extracted and the antimicrobial efficacy of carabao grass (*Paspalum conjugatum)* leaves were determined against *Staphylococcus aureus.*

LITERATURE REVIEW

This chapter presents different literatures and studies related and relevant to the study. Related literatures are discussed regarding its relationship to this study. Overview of theses literature provided information and basis for this research.

In the Philippines, grass specifically Carabao grass (Paspalum Conjugatum) is widely observed in abundance in open places and settled areas, about towns, along trails, and streams. Carabao grass is a vigorous, creeping perennial with long stolons rooting at nodes. This plant has various vernacular names in other countries: China (Shuang sui que bai, Liang er cao, Cha zi cao); Danish (Hirse); French (Herbe sure, Herbe créole); German (Dallisgras); Italian (Paspalo dilatato); Japanese (Shima suzume no hie); snd Spanish (Grama de agua, Hierba dallis, Grama de agua, Pasto dallis, Zacate dallis). Indonesia, Malaysia, Thailand and the Philippines have varying terms for it. In Indonesia, they call it either jampang pahit or paitan. In Malaysia, its name is rumput ala negri or rumput kerbau, while in Thailand, it is known as ya-hep ya-nomnon, and in the Philippines, the Tagalogs call it kulape, the Ilocanos call it kalo-kawayan, and the Visayans refer to it as kauat-kauat (Jr Stuart, 2015).

Paspalum Conjugatum

Carabao grass is *(Paspalum Conjugatum*) classified as a stoloniferous grass. It vigorously crawls on the soil with elongated stolon. Roots will develop at the nodes as it multiplies through the soil. This lets the grass to diverge out quickly. It has flat, thin, and narrow leaves. Stems are expanding and diverging below, with the flowering branches 20 to 70 centimeters high. Leaves are slender lanceolate, flat and thin, 8 to 20 centimeters long, 5 to 15 millimeters wide. Two slim terminal spikes sprout from the center of the plant and are 6 to 12 centimeters long. The spikelet are enveloped, 1.2 to 1.4 millimeters long, dull green, plano-convex, and the empty pods have long, supple, white bordering hairs (Jr Stuart, 2015).

Leaves

The leaves of the carabao grass are evergreen. The dark-green, simple leaves are alternate. They are lanceolate with entire margins and parallel venation. The benefits of the leaves are such as its usage for fever, stomach problems, weakness, and pulmonary diseases are highlighted. Decoction of leaves is also beneficial for dysentery. It can also be used for treating wounds and cuts. An ointment can be made out of the leaves and used for contusions, sprains, and dislocations (Jr Stuart, 2015).

Roots

The roots of the carabao grass embedded in the ground help keep and reconstruct the soil. The grass roots exist in the soil and they are certainly available to the organisms that disrupt biological materials such as bacteria, fungi, worms, and insects. The roots of the carabao grass are also used for medicinal purposes. Decoction of the roots are taken orally for diarrhea and dysentery (Jr Stuart, 2015).

Phytochemical Constituents of *Paspalum conjugatum* (carabao grass) leaves

The carabao grass leaves have been found out to have biologically active compounds. Phytochemicals were found to have variety of plant ingredients with different structures that were capable of health-promoting effects. These Phytochemicals have medicinal and pharmacological properties.

1. Glycosides. Glycosides play abundant vital roles in organic organisms. Many plants supply chemicals in the system of inactive glycosides. These can be stimulated by enzyme hydrolysis, which breaks down the sugar, allowing the chemical obtainable for use. Glycosides showed various significant therapeutic activities such as anti-

inflammatory activity, antioxidant activity, anti-diabetes activity, hepatoprotective activity, antibacterial, antiviral, antifungal activities, anticancer and antitumor activity, and other biological effects. Glycosides are widespread in plants and are extremely varied in action and effect. Glycosides are easily extracted and thus are ideal for medicinal application (Capanoglu, E., Jassbi, A. R., Miron, A., & Xiao, J., 2016)

2. Saponins. Plants Have the ability to synthesize almost unlimited number of substances which serves as the plant's defense mechanism against microorganism, insects and herbivores. One of the active compounds present in the plant is Saponin. It is often used as natural detergents because of its foaming and emulsifying properties. Steroidal and tripernoid saponins can even be used in many industrial applications, from the preparation of steroids hormones in pharmaceutical company ton utilization as food preservatives (Kregiel D, et al., 2017)
3. Steroids. A steroid is a biological compound commonly found in animals, plants, and fungi. All steroids are assembled in cells from the sterols lanosterol (animals and fungi) or cycloartenol (plants). They are necessary for plant development, reproduction, and responses to several biological and external stressors (Shah, 2013)
4. Alkaloids. Alkaloids are structurally diverse compounds generally classified as such due to the basic character of the molecule and a presence of at least one nitrogen atom, preferably in a heterocycle. Many of the naturally occurring alkaloids have biological activity. Some of them are used as drugs in modern medicine (e.g. morphine, codeine, reserpine, etc.). Although originally discovered in plants and then in marine sponges, many alkaloids have recently been identified in cultivable microorganisms, which provide opportunities for their sustainable production. Alkaloids are found primarily in plants and are especially common in certain flowering plants. These phytoconstituents help protect the plant from destruction caused by insects and herbivores. Alkaloids are also known for their action as analgesics, anesthetics, treatment for fibrillation, anti-tumor agents, anti-malarial, anti-arrhythmic, anti-bacterial, and painkillers (Hélio Nitta Matsuura, Arthur Germano Fett-Neto, 2017)
5. Flavonoids. According to a study by (Gregory, H., Robin R., & Steven, S. , 2017), flavonoids were found out to have biological activities in vitro and in vivo. Flavonoids can act as natural pesticide in plants, providing defense against fungal ailments and insects. Flavonoids also work as signaling particles for plants and promoting expansion of roots by nitrogen-fixing bacteria and mycorrhizal fungi. Flavonoids can be found in

dried leaves, herbs, juice, wine, fruits, vegetables, olive oil, honey, cereals, and legumes. It was found out that flavonoids have high blood antioxidant enzyme activities and they differ in their effect on biomarkers for stroke, heart disease, and stroke. Flavonoids lower total cholesterol. Flavonoids have organic activities in vitro and in vivo. Flavonoids can be used as a natural pesticide to promote colonization of roots of plants, to lower cholesterol, and to prevent heart disease and stroke. Flavonoids have anti-protozoal, anti-inflammatory and antioxidant effect (Jorge, T.S. et al. , 2017)

6. Tannins**.** Tannins are a heterogeneous group of high molecular weight, soluble in water, polyphenolic compounds, naturally present in cereals, legumes, primarily, in various fruits and vegetables, where they deliver protection against a wide range of living and non-living stressors. Fruits and tannin-rich extracts of fruits have shown anti-diabetic, anti-microbial, anti-inflammatory, anti-oxidant, and biological activity as well as anti-cancer, anti-nutritional properties, protecting the heart from any diseases and immune-regulating activities (Baoru, Y. & Pengzhan, L. . , 2013)
7. Triterpenoids. Triterpenoid saponins are glycosides with notable diversities in physical and having biological effects. They are turning out progressively important in cancer treatment because of their efficacy and safety. It is shown to have anti-cancer properties, anti-inflammatory, anti-oxidative, anti-viral, anti-bacterial, and anti-fungal activities (Smeriglio, A., Davide, B., Ersilia, B and Thrombeta, D, 2016)

The Test Organism: *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive, round-shaped bacterium found in the normal flora of the body. It is frequently found in the skin, respiratory tract, and nose. This bacterium is a non-compulsory anaerobe that can grow and develop without the need for oxygen. Although *S. aureus* is not always pathogenic, it is a common cause of skin infections, respiratory infections such as sinusitis, abscesses and (Sunhyo Ryu ,Peter I. Song ,Chang Ho Seo ,Hyeonsook Cheong and Yoonkyung Park, 2014)

Staphylococcus aureus is a major bacterial human pathogen that causes a wide variety of clinical manifestations. Infections are common both in community-acquired as well as hospital-acquired settings and treatment remains challenging to manage due to the emergence of multi-drug resistant strains such as MRSA (Methicillin-Resistant *Staphylococcus aureus*). This microorganism is also found in the environment and in natural human flora in the skin, mucus membranes (common in the nasal area). *S. aureus* does not normally cause infection on healthy

skin; however, if it is allowed to enter the bloodstream or internal tissues, these bacteria may cause a variety of potentially serious infections. Transmission is typically from direct contact. However, some infections involve other transmission (Taylor TA, Unakal CG. , 2019). The history of S. aureus therapy is marked by the development of resistance to each new class of anti-staphylococcal antimicrobial drugs, including the penicillins, sulfonamides, tetracyclines, glycopeptides, and others, complicating therapy. S. aureus isolates identified in the 1960s were sometimes resistant to methicillin, a ß-lactam antimicrobial active initially against a majority S. aureus strains according to (David, MZ and Daum RS, 2017)

METHODS

The researchers purchased 3 kilograms of Carabao grass leaves that were already cut from the roots from a Landscaping Garden Shop at Bagong Kalsada, Calamba, Laguna. It was then sent to the Department of Science and Technology and were washed thoroughly with distilled water to retain the properties of the leaves and remove any other microorganisms. The carabao grass leaves were dried through the Multi-Commodity Heat- Pump Dryer and the dried carabao grass leaves were then pulverized using mortar and pestle. Pulverized carabao grass leaves were sent to the Chemical and Testing Division of the Department of Science and Technology.

Preparation for Phytochemical Analysis

Material: Pulverized Carabao Grass Leaves

Methods

The pulverized Carabao grass leaves were sent to the Organic Chemistry Laboratory Standards and Testing Division, Department of Science and Technology in Bicutan, Taguig, Metro Manila for qualitative phytochemical analysis. The succeeding procedures were used for Phytochemical Analysis

Test for Glycosides

Fehling's Test - The plant extract was dissolved in hot water. After the dissolution of the extract, the solution was filtered. The resulting filtrate was utilized for examination. Two ml of the filtrate was placed in two test tubes for a total of 4 ml of the filtrate. One ml dilute HCL was added in one of the test tubes, while the other test tube did not have any other additives. The test tube that only had the filtrate was the control tube. The test tubes were

left in boiling water for 5 minutes, after which the test tubes were cooled down. Anhydrous sodium carbonate was added to neutralize the samples, until the samples stopped producing gas or bubbles. Fifteen ml of Fehling's A and 15.0 ml of Fehling's B was mixed to create Fehling's solution. One ml of Fehling's solution is then added to both of the sample tubes. The sample tubes were then placed in a hot water bath for 2 minutes.
If the extract was positive for glycosides, then the hydrolyzed solution produced a red-brown precipitate with a green suspension.

Test for Saponins
Froth Test – The extract was dissolved in hot water; after which the solution was filtered. Two ml of the filtrate in water was placed in a test tube. The test produced a positive result when a froth was present after shaking the test tube.

Test for Steroids and Triterpenoids
Liebermann-Burchard's Test – The dried extract was treated with a couple of drops of acetic anhydride in a test tube. The solution was then boiled and allowed to cool. When the solution was cooled, the solution was decanted to leave the solute. One to 2 drops of concentrated sulfuric acid were added by dropping the sulfuric acid down the side of the test tube. A brown ring was formed in between two formed layers. When the top layer turns green, steroids were present. When the bottom layer formed a dark red color, then the test showed that triterpenoids were present.
Salkowski's Test – One to 2 drops of concentrated sulfuric acid were added to the extract. One to 2 drops of acetic anhydride were also added to the solution in chloroform. The formation of a red color at the lower layer identified the presence of steroids, whereas if a yellow color was formed, then there was the presence of triterpenoids.

Test for Alkaloids
Mayer's Test – 3.0 ml of the extract is treated with 1.0% HCL and then filtered. One to 2 drops of Mayer's reagent were added to the filtrate. A positive result was observed if a cream-colored filtrate was formed.
Wagner's Test - 1.0 ml of dilute acetic acid is used to treat the sample extract. One to 2 drops of Wagner's reagent were dropped onto the extract. A positive result indicated a brownish or cream-like precipitate.

Test for Flavonoids

Shinoda Test – The extract was dissolved in ethanol and then filtered. The filtrate was treated with some drops of 10% concentrated HCl. A positive result of a pink red color indicated the presence of flavonoids when magnesium turnings were added to the solution.

Test for Tannins

Ferric Chloride Test – The extract was cleaned and filtered through ethanol. One ml of the resulting extract was combined with 2.0 ml water in a sample tube. One to 2 drops of diluted ferric chloride were placed into the test tube. A positive result of a blue-green or blue-black color showed the presence of tannins.

Antimicrobial Assays

1. Preparation of Test Organisms

Transfer bacterial isolate to 5.0 mL of Tryptic Soy Broth (TSB) in a test tube. Incubate the tube overnight at 35 °C. After preparation of the bacterial sample, use for Step B.

2. Preparation of Antimicrobial Assay Plates

Adjust the turbidity of the overnight culture with a solution of sterile saline or broth to 0.5 McFarland standard. Use a sterile non-toxic swab to transfer the adjusted bacterial suspension onto a petri dish.

Add about 15ml-20ml of Mueller-Hinton Agar, swirl, and allow plates to form and dry, ready for the bacterial swab. Incubate bacterial plates at 35°C for 30 minutes to an hour depending on the colony. Use filter paper discs containing samples. Refer to Step C.

3. Addition of Extract/Control Filter Paper Discs
    1) Pipette 10uL of the sample into 10mm sterile paper discs, then use sterile forceps to transfer the discs onto the petri dish containing the colony from Step B.
    2) Dip the 10mm paper discs in the sample extract and allow the discs to be marinated by the sample. Remove excess extracts pressing the discs on the side of a container using sterile forceps. Transfer to the petri dish in Step B. 1c. For dry extracts and solid samples, take some of the extract and add 1.0 mL of water. The 10 mm filter paper discs will then be processed in a similar manner to 1b.

4. Use of Positive Controls

Commercially available antibiotic discs are used as positive controls. Should the customer require to use another set of positive controls, the addition of positive control to the plate will be similar to the procedure on Step C (depending on the form of the positive control provided), unless the customer has another procedure on the preparation of positive control. This is upon approval of the laboratory.

1. Incubation and Interpretation of the Results Incubate the plates inverted overnight at 35°C.
2. Observe for zones of inhibition after incubation.
3. Measure the zones of inhibition using caliper. If there are no zones surrounding the paper discs, aseptically lift the paper and observe the area under the sample

RESULTS AND DISCUSSION

The 33-gram black crude extract of *Paspalum conjugatum* (carabao grass) was examined through a phytochemical test for its plant constituents with the results of its composition shown below.

Tabel 1. **Phytochemical Test Result**

Constituents	Results
Sterols	(+++)
Triterpenes	(++)
Flavonoids	(+++)
Alkaloids	(+)
Saponins	(++)
Glycosides	(++)
Tannins	(+++)

Note: (+) Traces, (++) Moderate, (+++) Abundant, (-) Absence of constituent

According to the phytochemical test results described above, the carabao grass leaves extract was abundant in sterols, flavonoids, and tannins. The carabao grass leaves extract also had a moderate number of triterpenes, saponins, and glycosides while there are trace amounts of alkaloids.

Sterols in the carabao grass leaves are structural components of its cell walls, while also serving as hormones which give the carabao grass the ability to grow and develop in the field (Valitova, JN, Sulkarnayeva, JV, Minibayeva, FV, 2016)). Flavonoids are plant pigment compounds that are known to have antibacterial activities (Babii, C., Bahrin, L. G., Gostin, I., Mihai, C. T., Mihalache, G., Neagu, A., & Stefan, M, 2018)Tannins are also polyphenolic compounds that have antimicrobial properties while reducing oxidative stressors (Barreca, D, Bellocco, E, Smeriglio, A, & Trombetta, D, 2017)Plants with triterpenoids contain compounds that have various wound-healing properties as well as having anti-inflammatory effects (Agra, L.C., Barbosa, F.T., Barreto, E., & Ferro, J. S. , 2015)Saponins are phytochemical compounds known to have triterpenoids that are classified as under the same group of compounds, which also have antimicrobial properties (Acharya, C., & Khan, N. , 2013)Glycosides are sugar molecules bonded through a glycosidic bond. Many medicines are derived from glycosides when the sugar group is removed (Kadu, S. S., & Korpe, G. V. , 2016)

Alkaloids are part of the group of polyphenolic compounds that highly affect the rate at which bacteria grows. (Ou, Z. M., Wu, C. D., & Xu, X., 2017) By combining the results of various studies and comparing the presence of the reported phytochemicals, one can conclude that carabao grass leaves can be used to achieve the same effects if treated and produced properly. Since the carabao grass leaves have a high content of sterols, flavonoids, and tannins, then it can be used to treat various infections.

Antimicrobial Assay Test Result

The 33g black crude extract of Carabao grass (*Paspalum conjugatum)* leaves was put through an antimicrobial assay. The test method was done through disc diffusion. The extract was set against *Staphylococcus aureus* with the results of the antimicrobial essay shown below.

Table 2. **Antimicrobial Assay Test**

Antimicrobial Assay						
	Staphylococcus aureus					
Sample/Control	Replicate 1	Replicate 2	Replicate 3	Total mean zone of inhibition (mm)	Reactivity	Inhibitory activity
Paspalum						

conjugatum (Carabao Grass) Extract (10 mm)	10	10	10	10	2	(+++)
Positive Control: *Oxacillin* 1 ug (6 mm)	20.54	-	-	20.54	4	(+++)
Negative Control: Sample-free disc (10 mm)	0	-	-	0	0	(-)

Reactivity Rating:	0 - None (No detectable zone around or under specimen)
	1 - Slight (Some malformed or degenerated cells under the specimen)
	2 - Mild (zone limited under the specimen)
	3 - Moderate (zone extends 5 to 10 beyond specimen)
	4 - Severe (zone extends greater than 10 mm beyond specimen)
Inhibitory Rating	(+++) complete; (++) partial; (+) slight, and (-) negative

Table 2 showed the antimicrobial assay results of the carabao grass leaves extract against *Staphylococcus aureus.* The results of the antimicrobial assay results of the carabao grass leaves extract against *Staphylococcus aureus*, the sample produced complete inhibitory activity with mild reactivity against the test organism, *Staphylococcus aureus*. Oxacillin (1 ug) served as the positive control for *Staphylococcus aureus,* which produced complete inhibitory activity with severe reactivity against the test organism, *Staphylococcus aureus*. The sample-free disc, which served as negative control, had no inhibitory activity and no reactivity against the test organism. These results overall showed the effects of the phytochemicals found within the carabao grass leaves extract against *Staphylococcus aureus*.

A study was done using the extract of Paspalum Conjugatum (Carabao Grass) as bio reductant and was evaluated for green synthesis of silver nanoparticles. Result showed that there is an antibacterial activity against E. coli (MTCC 40), Pseudomonas aeruginosa (MTCC 424) gram negative and Bacillus subtilis (MTCC 619), Staphylococcus aureus (MTCC 96) gram positive bacteria. Green synthesized silver nanoparticles may play a vital role in the field of biocontrol of plant diseases. (Debnath, G., Saha, AK., Dutta, S., Das, P., , January 2016).

CONCLUSION

After a period of evaluation, the researchers were able to determine the phytochemical constituents found in carabao grass leaves and its efficacy against Staphylococcus Aureus. There were abundant traces of sterols, which have positive effects in the body such as increased muscle mass, tissue repair, endurance, and fat loss. Flavonoids, which have anti-protozoal, anti-inflammatory and antioxidant effects are beneficial to persons with heart disease and stroke. They can also lower the total cholesterol found in the blood. Tannins have shown anti-diabetic, antimicrobial, anti-inflammatory, antioxidant and free radical scavenging activity as well as anti-cancer, anti-nutritional, cardio-protective properties and immune-regulating activities. There were also a few traces of alkaloids found in the sample known for their action as analgesics, anesthetics, fibrinolytics, anti-tumor agents, antimalarials, anti-arrhythmic, anti-bacterial, and as pain killers. Phytochemical Constituents which are moderately abundant were triterpenes, saponins, and glycosides. Just like the other phytochemicals these three constituents have their own actions and benefits, particularly in immune-regulating activities. The results have shown that the carabao grass leaves extract have complete inhibitory activity with mild reactivity against the test organism Staphylococcus aureus, which is a bacterium causing skin infections.

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