THE EFFECT OF ETHANOLIC PLANT EXTRACT FROM *COSMOS CAUDATUS* AGAINST *SALMONELLA TYPHI* AND *STREPTOCOCCUS AGALACTIAE*

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CERTIFICATION

This is to certify that we have read this research paper entitled **'The Effects of Ethanolic Plant Extracts from *Cosmos caudatus* Against *Salmonella typhi* and *Streptococcus agalactiae'*** by Muhammad Alif Syazwan bin Mohd Basree; in our opinion, it is satisfactory in terms of scope, quality, and presentation as part of the requirement for the course DVT 5436 – Research Project.

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Thank You

DEDICATIONS

I dedicate my dissertation work to my family and friends. I would like to express my gratitude to my beloved parents, Mohd Basree and Madzenah, for supporting me and always praying for the best for me. Also, to my best and caring brothers, Mohd Tarmizee, Mohd Hafizuddin, and Mohd Shafeeq, who always stay with me. Not to forget my annoying little brother, Muhammad Arief Fahmi, who always support me.

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ABSTRACT

An abstract of the research paper was presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement for the course DVT 5436 – Research Project.

Cosmos caudatus or Ulam Raja have been used as a traditional medicine to treat various diseases. The emergence of disease resistance toward antibiotics has become a considerable problem and caused the need to develop new antimicrobials from other alternative sources. This study is conducted to investigate the antimicrobial activity of plant extract from *C. caudatus* against *Salmonella typhi* and *Streptococcus agalactiae*. The Agar diffusion method with ethanolic *C. caudatus* extract was used to test the antimicrobial activity of the plant toward *Salmonella typhi* and *Streptococcus agalactiae*. This study's results show no inhibition zone produced on the Mueller Hinton Agar (MHA) for both tested bacteria. In conclusion, there are no antimicrobial activities of ethanolic *C. caudatus* extract against *S. typhi* and *S. agalactiae*.

Keywords: *Cosmos caudatus, antimicrobial, Salmonella typhi, Streptococcus agalactiae*

ABSTRAK

Abstrak kertas penyelidikan yang dibentangkan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan, untuk memenuhi sebahagian daripada kursus DVT 5436 – Projek Penyelidikan.

Cosmos caudatus atau Ulam Raja telah digunakan sebagai ubat tradisional untuk merawat pelbagai penyakit. Kemunculan rintangan penyakit terhadap antibiotik menjadi masalah besar dan menyebabkan keperluan untuk membangunkan antimikrob baru daripada sumber alternatif lain. Kajian ini dijalankan untuk menyiasat aktiviti antimikrob ekstrak tumbuhan daripada *C. caudatus* terhadap *Salmonella typhi* dan *Streptococcus agalactiae*. Kaedah penyebaran agar dengan ekstrak etanol *C. caudatus* digunakan untuk menguji aktiviti antimikrob tumbuhan terhadap *Salmonella typhi* dan *Streptococcus agalactiae*. Hasil daripada kajian ini menunjukkan bahawa tiada zon perencatan yang dihasilkan pada Mueller Hinton Agar (MHA) untuk kedua-dua bakteria yang diuji. Kesimpulannya, tiada aktiviti antimikrob *C. caudatus* terhadap *S. typhi* dan *S. agalactiae*.

Kata kunci: *Cosmos caudatus, antimikrob, Salmonella typhi, Streptococcus agalactiae*

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1.0 INTRODUCTION

According to the World Health Organization, herbal remedies are used by 80% of the world's population in developing countries to cure ailments, while traditional medicine is still used by 42% of the population to manage vital health concerns (van Breemen et al., 2007). Natural and plant-based medicines abound in Malaysia's healthcare sector. Some of them are utilized as traditional folk remedies to treat conditions like high blood pressure, diabetes, arthritis, and fever, as well as health tonics (Bunawan et al., 2014).

Cosmos caudatus, commonly known as Ulam Raja, can be naturally found on grassy slopes, banks, and in monsoonal deciduous forests. This plant is a common weed in most countries such as Central America, the Caribbean, Fiji, and Australia. It can be found on the roadsides, on the agricultural land, or on pasture land (Puttock & Acevedo-Rodríguez, 2019). *C. caudatus* is edible and consumed by Malaysians as a salad vegetable. Besides that, C. caudatus also be used as a treatment in traditional medicine. In an animal model, *C. caudatus* was found to have a potential effect on improving plasma blood glucose levels (Cheng et al., 2015).

Staphylococcus aureus and *Streptococcus agalactiae* are the common bacteria found in cows in the case of mastitis, however, in a rare case Mycoplasma could also cause the disease. Intramammary bacterial infection is usually often the cause of clinical or subclinical mastitis. This infection may occur at any stage of the disease. The bacteria that most commonly cause mastitis can be categorized into two categories: contagious pathogens and environmental pathogens. Contagious pathogens can spread from one cow to another during the milking process via fomites, and environmental pathogens have their reservoir in the environment that the cows live in (Royster & Wagner, 2015).

Both typhoid and paratyphoid fever continue to be significant issues affecting public health across the globe and are big contributors to morbidity in parts of the developing world. Both typhoid and paratyphoid fever are acute and potentially fatal febrile illnesses caused by systemic infection with the bacterium *Salmonella enterica* serotype Typhi (*Salmonella typhi*) or Paratyphi, respectively. Typhoid and paratyphoid fever is caused by *Salmonella typhi* and *Salmonella enterica* serotypes Paratyphi; the common symptoms consist of pyrexia, hepatosplenomegaly, and stomach discomfort. Patients may occasionally report symptoms such as rash, nausea, anorexia, diarrhea or constipation, headache, relative bradycardia, and decreased degree of consciousness. Although both illnesses have similar clinical manifestations, the course of sickness associated with paratyphoid fever is often less severe. In the absence of appropriate treatment, the case-fatality rate for typhoid fever ranges from 10% to 30% (Buckle et al., 2012).

This study will be carried out to determine the antimicrobial effect of plant extract, which is *C. caudatus,* toward *Salmonella typhi* and *Streptococcus agalactiae*, as well as to find an alternative treatment for the disease caused by these bacteria.

2.0 PROBLEM STATEMENT

The spread of super bacteria is one of the most pressing concerns for human and animal wellbeing. Antibiotics are routinely given to commercial livestock as antimicrobial growth promoters, increasing the selection of resistant microorganisms, thus leading to the need to develop new antibacterial. In the laboratory, the plant extract for antimicrobial resistance of bacteria should be investigated as an alternative to standard antibiotics.

3.0 RESEARCH QUESTION

3.1 Does ethanolic *C. caudatus* extract have antimicrobial activity toward *Salmonella typhi* and *Streptococcus agalactiae*?

3.2 Which bacteria are more susceptible to ethanolic *C. caudatus* extract?

3.3 Does the different concentrations of plant extract affect the antimicrobial activity against the tested bacteria?

4.0 RESEARCH HYPOTHESIS

4.1 *Cosmos caudatus* does have antimicrobial activity toward *Salmonella typhi* and *Streptococcus agalactiae*.

4.2 *Salmonella typhi* and *Streptococcus agalactiae* are susceptible to ethanolic *C. caudatus* extract.

5.0 RESEARCH OBJECTIVE

5.1 To evaluate the antimicrobial activity of *C. caudatus* toward *Salmonella typhi* and *Streptococcus agalactiae.*

6.0 LITERATURE REVIEW

6.1 Experimental Plant Description

Cosmos caudatus, commonly known as Ulam Raja, can be naturally found on grassy slopes, banks, and in monsoonal deciduous forests. This plant is a common weed in most countries such as Central America, the Caribbean, Fiji, and Australia, which can be found on the roadsides, on the agricultural land, or on pasture land (Puttock & Acevedo-Rodríguez, 2019). *C. caudatus* is edible and consumed by Malaysians as a salad vegetable. Besides that, C. caudatus also be used as a treatment in traditional medicine. In an animal model, *C. caudatus* was found to have a potential effect on improving plasma blood glucose levels (Cheng et al., 2015).

Table 6.1: The use of *C. caudatus* in traditional medicine.

Scientific name	Family	Common name	Local name	Parts used	Traditional use
Cosmos caudatus	Asteraceae	Wild cosmos	Ulam Raja	Leaves	Promote the formation of healthy bone and help in blood circulation.

(Abas et al., 2003)

6.2 *Cosmos caudatus* as an alternative treatment

C. caudatus has been used in traditional medicine to improve blood circulation, reduce body heat, strengthen bone marrow due to high calcium content, promote fresh breath, and treat diseases caused by harmful bacteria (Rasdi et al., 2010). Several strains of pathogenic bacteria were shown to be susceptible to an ethanol extract of *C. caudatus,* such as *Salmonella* spp,

Proteus mirabilis, *Salmonella typhimurium, Listeria monocytogenes, Staphylococcus aureus* and *Vibrio cholera* (Bunawan et al., 2014).

6.3 Multiple drug resistance

Multiple drug resistance has hindered the development of new synthetic antimicrobial medications, forcing researchers to look for novel antimicrobials from other sources. Microbes, in general, have the genetic potential to transfer and acquire resistance to therapeutic medications. Using novel compounds that are not based on existing synthetic antimicrobial agents is one of the ways to prevent antibiotic resistance (Manandhar et al., 2019). Antibiotics can have adverse side effects on the host, such as hypersensitivity and immunological suppression, prompting the discovery of new antimicrobials, mainly from plant sources (Rasdi et al., 2010).

6.4 Streptococcal infection in cattle and human

Staphylococcus aureus and *Streptococcus agalactiae* are the most frequent pathogens that cause mastitis, and they are transferred among cows during milking, resulting in substantial economic losses. *S. agalactiae* has been found to be the most prevalent cause of invasive infection in neonates, however, it still can cause invasive and non-invasive infections in adult humans (Hameed et al., 2007). *S. agalactiae*, classified into Group B Streptococcus (GBS), is a Gram-positive bacteria that cause pneumonia, septicemia, and meningitis in newborns. *S. agalactiae* is a commensal microbe that can be found in humans' gastrointestinal and genitourinary tracts. *S. agalactiae* may also colonize the mammary glands of many ruminants, where it can live for long periods, producing clinical and sub-clinical mastitis and lowering milk quality. These bacteria have also been found in different species such as dogs, horses, guinea pigs, and fish. Various approaches have been used to investigate the diversity of *S.*

agalactiae strains. Based on variations in the capsular polysaccharide, *S. agalactiae* strains are divided into nine serotypes: Ia, Ib, and II to VIII (Brochet et al., 2006; Slotved et al., 2007).

6.5 Salmonellosis in human

Salmonella is a Gram-negative, flagellated bacilli and facultative anaerobic bacteria causing gastroenteritis in humans and animals. Pathogenic *Salmonella* usually enters the host body by ingesting contaminated feed, leading to gastrointestinal tract infection. Typhoid fever is a systemic febrile illness, a life-threatening disease caused by *Salmonella enterica* subspecies *serovar* Typhi *(Salmonella typhi)* and transmitted through fecal-oral route primarily from contaminated food and water in developing nations. The incubation period of *S. typhi* and *S. paratyphi* are 1-4 weeks, and human infected with these bacteria will show symptoms such as headaches, fever, body weakness and aches, constipation, or diarrhea (Wessel et al., 2021). Typhoid fever is a disease that has plagued people throughout history and remains a severe public health concern today. On a global scale, at least 16–20 million cases of typhoid fever are reported each year, with 600,000 fatalities (Kidgell et al., 2002). Typhoid fever is a life-threatening systemic disease, unlike most other Salmonella infections, which cause self-limiting gastroenteritis. Among the 2300 serovars of *S. enterica* subs. enterica, *S. typhi* is unusual because it is specifically suited to human disease. Our group discovered a hazardous activity linked with *S. typhi* that demonstrated many of the same characteristics as cells inebriated with an exotoxin known as a cytolethal distending toxin (CDT) (Galán, 2016).

7.0 METHODOLOGY

7.1 Sample Collection

Plant material which is *C. caudatus,* was purchased from the wet market in Kota Bahru. The plants were appropriately sampled into the ziplock plastic bag to prevent any form of contamination.

7.2 Preparation of Plant Extracts

The sample was cleaned under running tap water to remove dirt and allowed to air-dry for three days at constant room temperature (27℃). Using a mechanical grinder, the dried plant material was then ground to a fine powder. Then, the powder was weighed 10 g of ground powder from each plant, dissolved in 100 ml of ethanol, and allowed to sit at room temperature for one day. The extract was filtered using Whatman filter paper, and the filtrate was then concentrated under reduced pressure using a rotary evaporator at 44.9℃ to obtain crude extract. The crude extract was stored in the chiller at 4℃.

7.3 Microbial culture

In this study, pathogenic *Salmonella typhi* and *Streptococcus agalactiae* strains were obtained from the Bacteriology Laboratory, Faculty of Veterinary Medicine, UMK. Culturing on selective media, the microscopic examination was used to isolate and identify bacteria. Nutrient Agar and Blood Agar were used to culture the bacteria, which are *S. typhi* and *S. agalactiae*. Then, the bacteria were streaked onto the agar plate and incubated the agar at 37°C overnight.

7.4 Antimicrobial Assay of Plant Extracts

Disc diffusion method was used to perform an antimicrobial assay of plant extract against *Salmonella typhi* and *Streptococcus agalactiae*. After confirmation with 0.5 Mc Farland turbidity, the bacteria were swabbed onto the Mueller Hinton agar, MHA (Oxoid, England) using a sterile cotton swab. The plant extracts were diluted with 1 ml 10% Dimethylsulfoxide

(DMSO) and prepared for concentration of 62.5 mg/ml, 125 mg/ml, 250 mg/ml, 500 mg/ml and 1000 mg/ml. 10µl of was loaded onto the sterile filter paper and let dry at room temperature until the filter paper fully absorbed the extract. Then, the sterile paper was loaded with DMSO as a negative control, and the ampicillin-10 disc was used as a positive control for both bacteria. The plates were incubated at 35°C for 24h. The size of inhibition zone was measured from the disk to the border of the growth-free zone after 24 hours.

8.0 RESULT

8.1 Antimicrobial activities

There is no inhibition zone produced for both *S. typhi* and *S. agalactiae* on MHA agar with different concentration disc of ethanolic extract of *C. caudatus,* as shown in Table 8.1.

Table 8.1: Inhibition zone of *Cosmos caudatus* on *Salmonella typhi* and *Streptococcus agalactiae.*

Bacteria strains	Concentration of crude extract of *Cosmos caudatus* (mg/ml)				
	62.5	125	250	500	1000
S. typhi	-	-	-	-	-
S. agalactiae	-	-	-	-	-

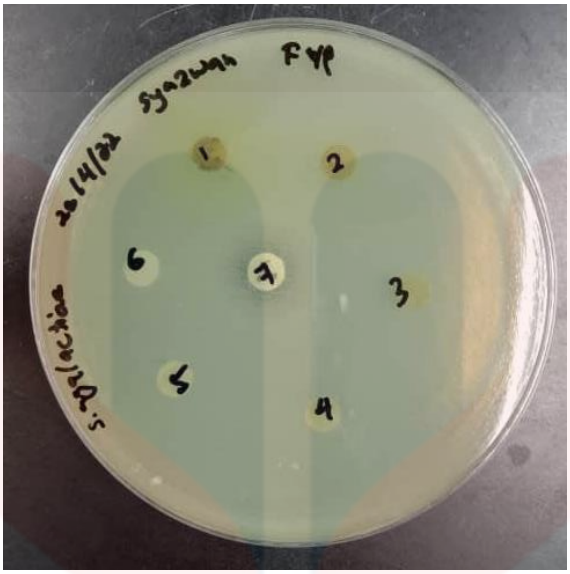


Figure 8.1: Ethanol extract concentration of *C. caudatus* shows no inhibition zone against *S. agalactiae.*

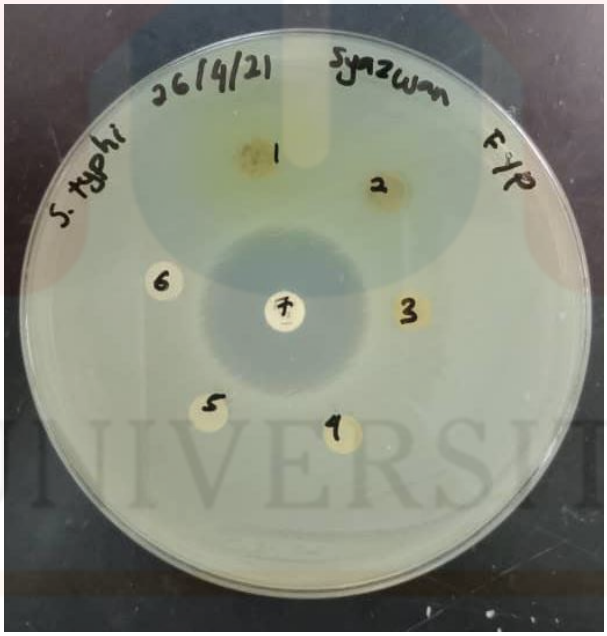


Figure 8.2: Ethanol extract concentration of *C. caudatus* shows no zone of inhibition against *S. typhi*.

9.0 DISCUSSION

The findings in Figure 8.1 and Figure 8.2 revealed that the bacteria samples tested with different concentrations of *C. caudatus* showed no inhibition zone in the MHA agar. Thus, there are no antimicrobial activities of *C. caudatus* extract toward *Salmonella typhi* and *Streptococcus agalactiae*. This is likely due to the microorganisms' structures because different microorganisms have different degrees of susceptibility toward plant extract. Based on a study by Tortora et al., (2001), gram-positive bacteria were more susceptible than gram-negative bacteria, and the bacterial strains were more susceptible than the fungal strain. These variations might be due to the type and amount of antimicrobial compounds present in the plant, their mode of action, and the strains' characteristic variances in microbial cell walls. Generally, the primary difference is due to gram-negative bacteria having an outer-membrane permeability barrier that prevents antimicrobial agents from entering their cytoplasmic region (Hendra et al., 2011).

Negative findings of the antimicrobial activity can be due to insufficient levels of active ingredients or elements in the extract to display antibacterial action (De Zoysa et al., 2019).

Extraction time is essential to ensure that the plant extracts exhibit antimicrobial activity. The total phenolic compound and tannins content decrease after 120 minutes. The longer extraction time causes exposure of oxygen which leads to the oxidation process. Other than that, there is presence of endogenous enzymes in the plant that may destroy the phenolic compound and reduce the phenolic compound in the *C. caudatus* (Naczk & Shahidi, 2004). Phenolic compounds have the capacity to link with proteins and bacterial membranes to form complexes.

A study conducted by Rasdi et al., (2010) showed that *Cosmos caudatus* extract with ethanol exhibits a dose-dependent relationship against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli and Candida albicans.* There is also a study by Lee

and Vairappan (2011) which found that ethanol extract of *C. caudatus* has antimicrobial activities on *Salmonella* spp*., Proteus mirabilis, Salmonella typhimurium, Listeria monocytogenes, Staphylococcus aureus,* and *Vibrio cholera.* This shows that *C. caudatus* has antimicrobial properties against some pathogenic microorganisms.

The type of solvent has a significant impact on the compounds extracted during the extraction procedure. Traditional medicine uses water as the principal solvent. However, substances extracted in organic solvents (ethanol and hexane) showed significantly higher antibacterial activity than those extracted in water in this investigation. Hexane extract had the most potent antibacterial activity of all the extracts tested, followed by ethanol extract. These findings could be explained by the polarity of the chemicals extracted by each solvent, as well as the capacity of extracts to diffuse and dissolve in the various growth mediums utilized in the experiment (De Zoysa et al., 2019).

Methanol can extract most polyphenolic components in plant-based extracts, such as flavonols and other bioactive compounds. Thus, it can act as an organic solvent for the extraction (Baravalia et al., 2009). The study by Abdullah et al., (2012) supported this as they found that when plant materials were extracted using methanol instead of hexane or water, there was a significant difference in antibacterial activity. Furthermore, the extraction yield can be increased by using methanol as a solvent (Caunii et al., 2012).

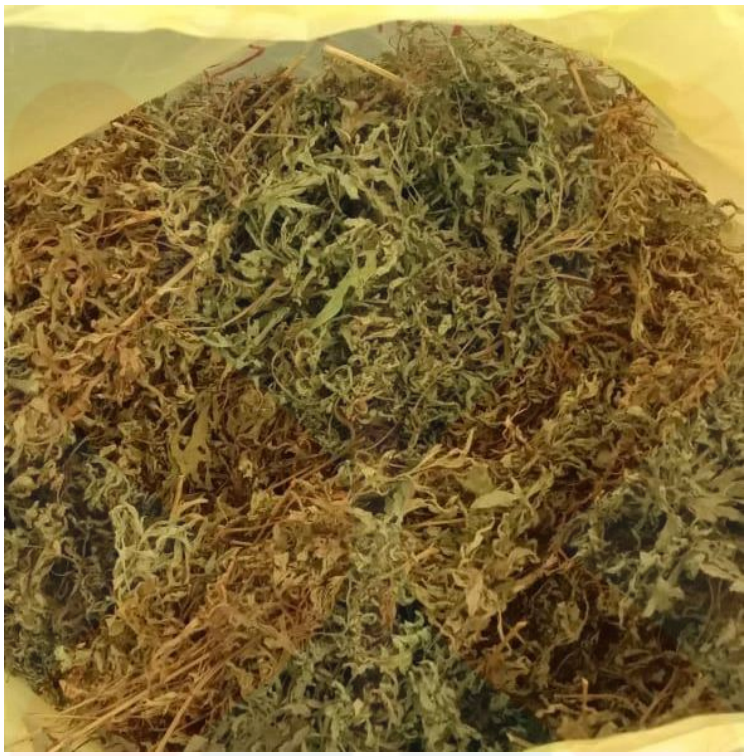
10.0 CONCLUSION

In a summary, *in vitro* antibacterial studies of *C. caudatus* demonstrated that ethanol extracts of the plant do not produce any inhibition zone despite the different concentrations of the extract. Thus, showing that *C. caudatus* were ineffective for bacterial growth suppression, specifically for *Salmonella typhi* and *Streptococcus agalactiae.*

11.0 RECOMMENDATION AND FUTURE WORK

From this study, a few flaws were discovered. For future reference, sequential solvent extraction is advised to avoid the antagonistic impact of additional secondary metabolites that might affect antibacterial effectiveness. It is necessary to perform experimental studies on the antagonistic effects of these secondary metabolites in plants. Thin Layer Chromatography (TLC) can be performed in future studies to establish which constituent contributes to antibacterial action. Other than that, the microdilution technique using 96 well micro-plates can also be done to determine the minimum inhibitory concentration in future work.

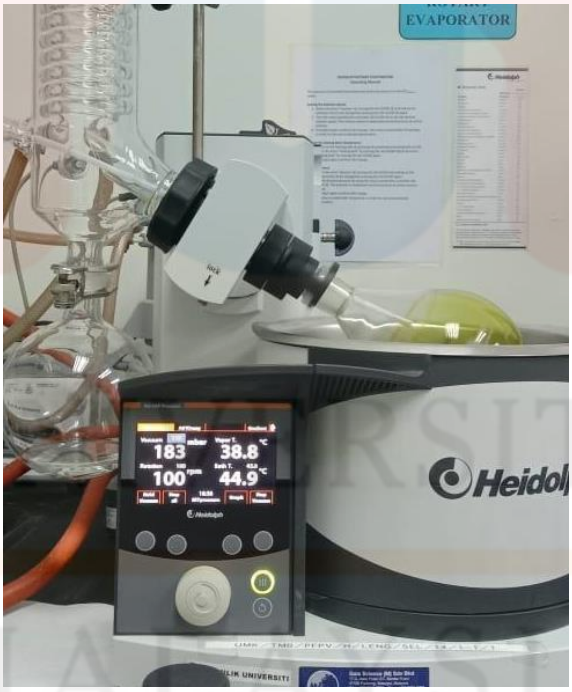
Appendix A



Appendix A.1: The dried plant material of *C. caudatus* (Ulam Raja).

Appendix A.2: The powder of plant material, *C. caudatus.*

Appendix A.3: *C. caudatus* soak in ethanol.



Appendix A.4: Rotatory evaporation process to obtain crude extract.

12.0 REFERENCES

Abas, F., Shaari, K., Lajis, N., Israf, D., & Kalsom, Y. (2003). Antioxidative and radical scavenging properties of the constituents isolated from *Cosmos caudatus* Kunth. Natural Product Sciences, *9*(4), 245-248.

Abdullah, E., Raus, R. A., & Jamal, P. (2012). Extraction and evaluation of antibacterial activity from selected flowering plants. American Medical Journal, 3(1), 27-32.

Baravalia, Y., Kaneria, M., Vaghasiya, Y., Parekh, J., & Chanda, S. (2009). Antioxidant and antibacterial activity of *Diospyros ebenum* Roxb. leaf extracts. Turkish Journal of Biology, 33(2), 159-164.

Brochet, M., Couvé, E., Zouine, M., Vallaeys, T., Rusniok, C., Lamy, M.-C., Buchrieser, C., Trieu-Cuot, P., Kunst, F., Poyart, C., Glaser, P. (2006). Genomic diversity and evolution within the species *Streptococcus agalactiae*. Microbes and Infection, 8(5), 1227-1243.

Buckle, G. C., Walker, C. L. F., & Black, R. E. (2012). Typhoid fever and paratyphoid fever: Systematic review to estimate global morbidity and mortality for 2010. Journal of global health, 2(1), 010401-010401.

Bunawan, H., Baharum, S. N., Bunawan, S. N., Amin, N. M., & Noor, N. M. (2014). *Cosmos caudatus* Kunth: A traditional medicinal herb. Global Journal of Pharmacology, 8(3), 420-426.

Caunii, A., Pribac, G., Grozea, I., Gaitin, D., & Samfira, I. (2012). Design of optimal solvent for extraction of bio–active ingredients from six varieties of *Medicago sativa*. Chemistry Central Journal, 6(1), 1-8.

Cheng, S.-H., Barakatun-Nisak, M. Y., Anthony, J., & Ismail, A. (2015). Potential medicinal benefits of *Cosmos caudatus* (Ulam Raja): A scoping review. Journal of research in

medical sciences: the official journal of Isfahan University of Medical Sciences, *20*(10), 1000-1006.

De Zoysa, M. H. N., Rathnayake, H., Hewawasam, R. P., & Wijayaratne, W. M. D. G. B. (2019). Determination of *In Vitro* Antimicrobial Activity of Five Sri Lankan Medicinal Plants against Selected Human Pathogenic Bacteria. International Journal of Microbiology, 2019, 7431439.

Galán, J. E. (2016). Typhoid toxin provides a window into typhoid fever and the biology of *Salmonella Typhi*. Proceedings of the National Academy of Sciences, 113(23), 6338-6344.

Hameed, K. G. A., Sender, G., & Korwin-Kossakowska, A. (2007). Public health hazard due to mastitis in dairy cows. Animal Science Papers and Reports, 25(2), 73-85.

Hendra, R., Ahmad, S., Sukari, A., Shukor, M. Y., & Oskoueian, E. (2011). Flavonoid Analyses and Antimicrobial Activity of Various Parts of *Phaleria macrocarpa* (Scheff.) Boerl Fruit. International Journal of Molecular Sciences, 12(6), 3422-3431. Retrieved from https://www.mdpi.com/1422-0067/12/6/3422

Kidgell, C., Reichard, U., Wain, J., Linz, B., Torpdahl, M., Dougan, G., & Achtman, M. (2002). *Salmonella typhi*, the causative agent of typhoid fever, is approximately 50,000 years old. Infection, Genetics and Evolution, 2(1), 39-45.

Lee, T. K., & Vairappan, C. S. (2011). Antioxidant, antibacterial and cytotoxic activities of essential oils and ethanol extracts of selected South East Asian herbs. Journal of Medicinal Plants Research, 5(21), 5284-5290.

Manandhar, S., Luitel, S., & Dahal, R. K. (2019). In Vitro Antimicrobial Activity of Some Medicinal Plants against Human Pathogenic Bacteria. Journal of Tropical Medicine, 2019, 1895340.

Naczk, M., & Shahidi, F. (2004). Extraction and analysis of phenolics in food. Journal of Chromatography A, 1054(1), 95-111.

Nor Hafipah Md Rasdi, Samah, O. A., Sule, A., & Ahmed, Q. U. (2010). Antimicrobial studies of *Cosmos caudatus* Kunth. (Compositae).

Puttock, C. F., & Acevedo-Rodríguez, P. (2019). *Cosmos caudatus* (wild cosmos).

Royster, E., & Wagner, S. (2015). Treatment of Mastitis in Cattle. Veterinary Clinics: Food Animal Practice, 31(1), 17-46.

Slotved, H.-C., Kong, F., Lambertsen, L., Sauer, S., & Gilbert, G. L. (2007). Serotype IX, a proposed new *Streptococcus agalactiae* serotype. Journal of clinical microbiology, 45(9), 2929-2936.

Tortora, G., Funke, B., & Case, C. (2001). Microbiology: An Introduction, 7th edition San Francisco, USA: Benjamin Cummings Publishing.

van Breemen, R. B., Fong, H. H. S., & Farnsworth, N. R. (2007). The role of quality assurance and standardization in the safety of botanical dietary supplements. Chemical research in toxicology, 20(4), 577-582.

Wessels, K., Rip, D., & Gouws, P. (2021). Salmonella in chicken meat: Consumption, outbreaks, characteristics, current control methods and the potential of bacteriophage use. Foods, 10(8), 1742.