Table 6.1: MIC values against the different bacterial and yeast strains

		B. montana			*S. aculeatissimum*			*A. grandidentata*			CHPL	AMPT B
Microbial species	**Gram (+ or -)**	***M**	***A**	***aq**	***M**	***A**	***aq**	***M**	***A**	***aq**		
B. cereus	+	2.5	2.5	-	2.5	1.25	-	2.5	1.25	-	<0.1	<0.1
C. perfringens	+	1.25	-	-	1.25	-	-	1.25	-	-	-	-
E. hormaechei	+	2.5		-	2.5	-	-	2.5	-	-	-	-
E. faecalis	+	2.5	1.25	-	2.5	2.5	-	1.25	1.25	-	-	-
E. coli	+	2.5	2.5	-	1.25	-	-	1.25	-	-	<0.1	<0.1
P. aeruginosa	+	1.25	-	-	0.625	-	-	0.625	-	-	<0.1	<0.1
S. epidermidis	-	1.25	-	-	2.5	-	-	2.5	-	-	<0.1	<0.1
S. pneumoniae	-	1.25	2.5	-	1.25	2.5	-	1.25	-	-	<0.1	<0.1
S. pyogenes	-	1.25	2.5	-	0.625	2.5	-	1.25	2.5	-	-	-

*aq=Aqueous extracts, *A= Acetone extracts, *M= Methanol extracts, *CHPL= Chloramphenicol, *AMPT B= Amphotericin B; All experiments were conducted in triplicates; (-) = No antimicrobial activity was found

Several studies corroborate the antibacterial activity observed in the methanolic and acetone extracts of *B. montana*, *S. aculeatissimum*, and *A. grandidentata* indicating that these plants have bioactive compounds with therapeutic potential. This is evident because from the current study, tannins were detected on the methanol and aqueous extracts derived from all the parts of *S. aculeatissimum* (leaves, stem, and roots), and acetone and methanol leaf extracts of *A. grandidentata*. In previous literature, methanolic extracts of *B. montana* roots have demonstrated inhibitory effects on *Staphylococcus aureus* and *Escherichia coli*, attributed to phenolic compounds like tannins and flavonoids, which disrupt bacterial cell walls and inhibit nucleic acid synthesis (Cowan, 1999; Eloff, 1998). Additionally, saponins and alkaloids in *B. montana* are known for their antimicrobial properties, particularly against Gram-positive bacteria, aligning with its effectiveness against *Bacillus cereus* and *Streptococcus species*.

For *S. aculeatissimum*, similar antibacterial effects have been reported, particularly against respiratory and gastrointestinal pathogens. Methanol extracts of this plant exhibit multiple biological activities, including anti-inflammatory, antibacterial, antioxidant, anti-obesity, and anti-cancer effects. They also contain the steroidal glycoalkaloids solasonine and solamargine, whose membrane-disrupting properties likely enhance the extract's broad antibacterial activity, particularly against S. *epidermidis* and *S. pneumoniae* (Burger et al., 2018; Yang et al.,2025). These glycoalkaloids have demonstrated bactericidal activity by compromising bacterial cell membranes and causing leakage of cellular contents.

Earlier research on *A. grandidentata* identified its methanol extracts as effective against *E. coli* and *P. aeruginosa*, similar to findings in this study (Ibrahim et al., 2013). This antibacterial activity may be linked to the plant's high content of terpenoids and flavonoids, which interfere with bacterial enzymes and DNA replication. Specifically, terpenoids like camphor and menthol are known to penetrate bacterial cell walls, while flavonoids inhibit DNA gyrase, leading to bacterial cell death (El-Saadony et al., 2025).

The findings of this study further the potential of *B. montana, S. aculeatissimum,* and *A. grandidentata* as natural sources of antibacterial agents. Their active compounds, including alkaloids, glycoalkaloids, saponins, terpenoids, and flavonoids, offer a multi-targeted approach to combating bacterial infections in childhood diseases (Smith et al., 2008). Further investigation into these compounds' specific mechanisms of action

and synergistic effects could enhance the therapeutic potential of these plants in paediatric antibacterial treatments.

6.3 Conclusions

In conclusion, the findings from this study highlight the promising antibacterial potential of *B. montana*, *S. aculeatissimum*, and *A. grandidentata*, particularly against bacterial strains associated with common childhood diseases. The inhibitory effects observed, with low MIC values across multiple bacterial species, suggest that these plants contain potent bioactive compounds, including alkaloids, glycoalkaloids, saponins, terpenoids, and flavonoids. Each compound class contributes to antibacterial action through mechanisms such as cell wall disruption, enzyme inhibition, and interference with DNA replication. Given the role of these pathogens in ailments such as gastrointestinal infections, respiratory conditions, skin infections, bloodstream infections, and meningitis in children, these plant extracts offer potential as alternative or complementary treatments for managing paediatric bacterial infections. To fully realise their therapeutic potential, further research is warranted to identify optimal extraction methods, examine specific mechanisms of action, and assess the synergistic effects of these compounds. Such studies could lead to the development of plant-based antibacterial therapies, addressing an urgent need for safer, effective alternatives in treating bacterial infections in children.

6.4 References

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CHAPTER 7
GENERAL DISCUSSION AND CONCLUSION

7.1 Introduction

In South Africa, medicinal plants are an important aspect of our lives. Medicinal plants are essential in managing and treating different illnesses in children in the Thaba 'Nchu area. There are over 45,000 plant species in Africa, and about 30,000 higher plant species, which contribute 10% of the world's flora, of which 3400 medicinal plant species belong to South Africa (Ndhlovu et al., 2019; Linder, 2014; Williams, 2013). Cape Floral Kingdom is the most diverse flora on earth, and Limpopo has a rich plant diversity in South Africa (Ndhlovu et al., 2019; Van Wyk et al., 2009). Free State has over 350 plant species used for medicinal purposes (Dingaan & Du Preez, 2017). According to Ndhlovu et al. (2021), there are 29 studies in the form of articles, books and dissertations with information of 194 plants used for treating ailments in children in nine provinces of South Africa. Despite these publications, there is still insufficient well-detailed documentation on the use of medicinal plants on children in the past years.

Children are highly vulnerable to a variety of illnesses and diseases, making them a major contributor to the global disease burden. This persists despite the widespread availability and use of conventional medications designed for prevention and treatment. However, these medications are frequently associated with adverse effects in children, which can complicate their management and lead to additional health concerns (Rieder, 2019). Medicinal plants are globally utilised because of their natural availability and the presence of secondary metabolites and beneficial biological properties that can help address the drawbacks of conventional drugs (Brown, 2017; Lopez et al., 2006).

As medicinal plants represent a good alternative, especially in South Africa where many of them are used for treatment of various diseases including those related to children. Although certain medicinal plants exhibit notable pharmacological properties, investigating their potential toxicity remains critically important (Somaida et al., 2020). Secondary metabolites such as alkaloids, saponins, glycosides and tannins found in S. aculeatissiumum extracts are known to exhibit toxic effects to humans and animals.

The toxic effects may range from neurotoxicity, hepatotoxicity, gastrointestinal irritation, hemolysis of red blood cells and reduced nutrient absorption when consumed in excessive amounts (Pan et al., 2014; Francis et al., 2002; Gleadow and Woodrow., 2002; Khanbabaee and Van Ree., 2011). Therefore, this study focused on documenting medicinal plants used for treating and managing childhood diseases in Thaba ‘Nchu.

7.2 Results

The current study findings showed that a smaller percentage of males (20%) participated in the ethnobotanical survey conducted on plants used to treat childhood diseases than females (80%) in Thaba ‘Nchu. The higher representation of women in the survey can be attributed to their primary caregiver role for children. Women are known to be experienced in addressing children's healthcare needs and therefore possess greater knowledge of using medicinal plants to manage childhood illnesses.

A total of sixty medicinal plants from 28 different families were recognised for their use in treating childhood diseases in Thaba ‘Nchu. The most frequently cited species were *Berkheya montana, Pachycarpus rigidus, Geranium caffrum, Dicoma anamala,* and *Solanum aculeatissimum*. Decoctions were the primary remedy preparation method, with remedies being administered orally (65%), topically (16%), rectally (7%), dermally (6%), and nasally (6%). These findings contribute to preserving this traditional knowledge for future generations and provide a valuable basis for selecting medicinal plants for further studies, including potential drug development.

Table 7.1 summarises the experimental results used to validate using the selected medicinal plants to treat childhood ailments documented in **Chapter 3**. *B. montana*, *S. aculeatissimum*, and *A. grandidentata* were selected for pharmacological investigation to validate their use in the treatment of childhood diseases.

Table 7.1: Summary of the experimental results obtained in the study

	Aloe grandidentata **(leaves)**	***Burkheya montana*** **(roots)**	***Solanum aculeatissimum*** **(whole plant)**
Phytochemical screening	Alkaloids, flavonoids, glycosides, phenols, steroids, tannins, terpenoids	Alkaloids, glycosides, phlobotannins, saponins, steroids, terpenoids	Alkaloids, flavonoids, glycosides, phenols, phlobotannins, saponins, steroids, tannins, terpenoids
Antibacterial activity (Minimum Inhibitory Concentration)	Methanol (9 microorganisms at 0.625 mg/ml - 2.5 mg/ml) Acetone (3 microorganisms at 1.25 mg/ml - 2.5 mg/ml)	Methanol (9 microorganisms at 1.25 mg/ml - 2.5 mg/ml) Acetone (5 microorganisms at 1.25 mg/ml - 2.5 mg/ml)	Methanol (9 microorganisms at 0.625 mg/ml-2.5 mg/ml) Acetone (4 microorganisms at 1.25 mg/ml - 2.5 mg/ml)
Cytotoxicity	Methanol: Non-toxic Acetone: Non-toxic Aqueous: Non-toxic	Methanol: Non-toxic Acetone: Non-toxic Aqueous: Non-toxic	Methanol: Toxic Acetone: Toxic Aqueous: Non-toxic

7.3 Discussion and Conclusion

The phytochemical constituents detected across *B. montana*, *S. aculeatissimum*, and *A. grandidentata* methanol, acetone and water extracts include alkaloids, flavonoids, glycosides, phenols, steroids, tannins, terpenoids, and phlobatannins. These secondary metabolites are recognised for their biological activity and are crucial to the therapeutic properties of medicinal plants (Hussein et al., 2019). The medicinal value of these plants is attributed to these phytochemical compounds, which exert distinct and targeted effects on the human body to treat various health ailments, including childhood diseases (Agidew, 2022). These secondary metabolites contribute to medicinal plants' pharmacological properties such as hypoglycemic, antidiabetic, antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic, and antimalarial activities (Yadav et al., 2014). Therefore, the presence of the identified compounds in *B. montana, S. aculeatissimum,* and *A. grandidentata* could explain why these plants are utilised in treating childhood diseases in Thaba ‘Nchu.

In the current study, the methanol and acetone extracts of *S. aculeatissiumum* were toxic against the Vero cell line. Secondary metabolites such as alkaloids, saponins, glycosides, and tannins found in *S. aculeatissiumum* extracts are known to exhibit toxic effects on humans and animals. Hence, the secondary metabolites identified in *S. aculeatissiumum* extracts could have played a role in the toxicity observed in this study. The toxicity demonstrated in this study does not indicate that *S. aculeatissiumum* extracts must be discarded; however, they must be used cautiously. Therefore, to fully understand the toxicity of *S. aculeatissiumum,* further invitro-cytotoxicity studies need to be conducted on isolated compounds.

Medicinal plants and natural remedies offer the potential to complement conventional treatments, reduce reliance on synthetic drugs, and mitigate the adverse effects of some pharmaceuticals. The current study revealed that *Aloe grandidentata, B. montana* and *S. aculeatissimum* extracts showed antibacterial activity against *Bacillus cereus, Clostridium perfringens, Enterobacter hormaechei, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae and Streptococcus pyogenes* at various MICs. Some of these microorganisms are known to be causatives for some of the childhood diseases such as pneumonia, diarrheal and emetic syndromes, urinary tract infections, wound, soft tissues and skin infections, neonatal meningitis, sepsis,

respiratory infections, pharyngitis and many other childhood diseases. Therefore, the observed activity of *Aloe grandidentata*, *B. montana*, and *S. aculeatissiumum* extracts against the aforementioned microorganisms provides strong evidence supporting the traditional use of these plants in treating childhood diseases caused by these pathogens. This validates their potential as alternative therapeutic agents, particularly in combating infections associated with these bacteria. The findings also underscore the importance of further exploring these plants to isolate and characterise the bioactive compounds responsible for their antimicrobial properties. Such research could lead to developing novel drug agents that address the growing challenges of antimicrobial resistance and enhance the list of available therapies for childhood and other infectious diseases.

7.4 Limitations of the Study

- The plants chosen for further investigation were determined by their availability in the study area, even if ethnobotanical surveys identified them as the most used plants for managing childhood diseases.
- Plant materials were collected from a single location, which may not represent the full chemical variability of the species.
- Seasonal and environmental differences might have affected the phytochemical composition of the plant, limiting generalizability.
- The selected plants were evaluated for their antimicrobial properties exclusively against bacterial species.
- The antimicrobial activity was perfomed only *in vitro*, this might not accurately be a reflection of how the compound on the plant would act on the living organism. The study could not be performed on human due to ethical approval processes.
- Resource constraints led to the screening of limited phytochemical groups leaving many compounds unidentified, this is because advanced analytical techniques such as LC-MS were not available to fully characterize active constituents of the plants.

7.5 Future Recommendations of the Study

Further analysis of the plant extracts/isolated compounds should be conducted.

- Identifying bioactive compounds using LC-MS should be conducted to increase the potential for developing new drugs from plants to treat childhood ailments and lead to the discovery of novel compounds.
- More research (Bio screening assay, hatching techniques and Sulfohodamine cytotoxicity assay) is needed to fully validate and understand the efficacy and safety of these plants for paediatric healthcare.
- Antimicrobial activity (antiviral, antibacterial and antifungal activity) of these plant extracts and their isolated compounds must be evaluated against other bacterial strains and fungi related to childhood ailments.

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APPENDICES

INFORMATION BROCHURE

PROJECT TITLE: An ethnopharmacological investigation of medicinal plants used for childhood ailments in Thaba 'Nchu, South Africa

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Introduction (purpose of study)

You are being asked to take part in a research study. In order to participate, you must be 18 years (older). Before you decide to participate in this study, it is important that you understand why the research is being done and what it will involve. Please read the following information carefully. Please ask the researcher if there is anything that is not clear or if you need more information. The decision to participate, or not, is up to you. The purpose of the study is investigate medicinal plants used to treat childhood infections in the Free State and determine their antimicrobial activity and to compile a catalogue of medicinal plants used to treat childhood infections for documentation purposes as well as a reference text for community members.

What is involved in the study?

If you decide to participate in the study you will be interviewed to share your knowledge and experience about childhood infections and the medicinal plants used to treat them. You will also be asked questions about how you prepare the plants for treatment as well as where you find or buy the plants. The researcher or assistants will be taking down notes on your responses or record you with your permission. Later on in follow-up visits you will be asked to identify the plants you mentioned in the interviews. You may also be asked to accompany the researcher on plant collection field trips to ensure the correct identification of the medicinal plants.

Time frame

This research is expected to last for two years so we expect to make frequent visits to see you especially during spring and summer and to let you know the outcomes of the study.

Benefits

We can't guarantee that you will personally experience benefits from participating in this study. It is reasonable to expect future benefits like the following from the information we find in this study:

- Help in creating an Ecostore (area for product development) for the medicinal plants used for infections in children.
- Fundamental knowledge on medicinal plants used for treatment of infections in children
- Full access to research processes and results
- Full access to the catalogue published in Sesotho and English
- Preserving the knowledge for future generations on the use of medicinal plants, their safety and efficacy
- Influence on planting medicinal plants in gardens

Risks

From this research, the risks or discomfort to the participant will be minimal or non- existent. The study is all about day to day, time to time experience you get when treating infections in children. You will never be put in a position where you may feel awkward, self-unconscious, or ashamed. There will be no damage to your financial standing, employability, or reputation.

Your rights as a research participant and Confidentiality

Participation in this study is completely voluntary and anonymous. Information gathered during the research will be used solely for the purpose of this study and all efforts will be made to ensure the confidentiality of participants' personal information. Indirect quotes will be used to avoid tracing it back to the individual if data will be published. Most of the data you provide will be grouped with other participants. Please note that while your name will be recorded with the data, it will not be used in the report. All identifiable data will be stored securely on a computer with password-restricted access and only the researcher (and supervisor if applicable), and ethics committee members will have access to it. All identifiable information will be destroyed at the end of the study or after 15 years, whichever comes first.

Please take time to read this entire form and ask questions before deciding whether to take part in this research project. If you decide not to participate there will not be any negative consequences. Please be aware that if you decide to participate, you may withdraw from the

study at any time and your data will be returned to you or destroyed. You may also decide not to answer any specific question.

Contacts for questions or problems

If you have questions about the study, any problems, or think that something unusual or unexpected is happening you can contact:

Dr IT Manduna
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If you have any questions or concerns about your rights as a research participant, contact:

Prof Samson Mashele Dean Faculty of Health and Environmental Sciences, Central University of Technology **Tel: (051) 5073111** Email: **smashele@cut.ac.za**	**Prof Carlu van der Westhuizen** Assistant Dean: Research, Innovation & Engagement Faculty of Health and Environmental Sciences, Central University of Technology Tel: **(051) 5073788** Email: **cvdwesth@cut.ac.za**

INFORMED CONSENT FORM

Thank you for your participation. By submitting this form you are indicating that you have read the description of the study, are over the age of 18, and that you agree to the terms as described in the short questionnaire that follows:

I have read and I understand the provided information and have had the opportunity to ask questions. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason and without cost. I understand that I will be given a copy of this consent form. I voluntarily agree to take part in this study. **I have had all my questions answered to my satisfaction**.

Yes ☐

No ☐

I grant permission for the research to be recorded and saved for purpose of review by the researcher, supervisor / principal investigator, and ethics committee.

Yes ☐

No ☐

I grant permission for the research recordings to be used in presentations or documentation of this study.

Yes ☐

No ☐

Participant's Name: _____

Address: _____

Contact details: _____

Signature _____ Date _____

Investigator's signature _____ Date _____

Verbal consent *(Applicable when participants cannot read or write)*

I hereby declare that I have read explained and clarify the contents of the data sheet to the research participant. The nature and purpose of the study were explained. The possible risks and benefits of the study were also explained. The research participant has undoubtedly showed that he/she is aware of the right to withdraw from the study at any time, for any reason and without threatening his/her relationship with the research team. I hereby certify that the research participant has verbally agreed to participate in this study.

Participant's Name: _____

Address: _____

Contact details: _____

Signature _____ Date _____

Investigator's signature _____ Date _____

Central University of Technology, Free State

INTERVIEW QUESTIONS FOR TRADITIONAL HEALERS

1. DEMOGRAPHIC INFORMATION

1.1 Name and Surname

1.2 Gender

1.3 Date of birth / Age

1.4 Location/Residence

1.5 What is your marital status? Single Married Divorced Widowed

---

1.6 How did you become a traditional healer?

1.7 What is your employment status/ occupation?

1.8 Religion

1.9 Ethnic group

---

2. KNOWLEDGE ATTRIBUTE

2.1 Do you have any knowledge on the use of medicinal plants?

2.2 Do you use medicinal plants to treat illnesses in children?

2.3 How long (time in years) have you been treating children with medicinal plants?

2.4 How many children have you treated so far?

---

3. PLANT KNOWLEDGE

3.1 Which illnesses do you treat in children?

3.2 Which plants can be used to treat illnesses mentioned in children?
(Local name in Sotho)

3.3 Do you use all the plants you mentioned above?

3.4 What is the age group of the children in which the plant is used for?

3.5 Basic description of the plant (Tree/ Herb/ Shrub/Climber)

3.6 Where is the plant collected) (Sources of the plant)

3.7 Are the plants abundant, rare, scarce or difficult to find in the place of collection? Compared to other years.

3.8 When is the plant harvested? (Season and time of the day)

3.9 Do you cultivate the plant?

3.10 What plant part(s) do you use?

3.11 Is the plant used individually or with a mixture of other plants? What other plants or items do they mix it with? Proportions

3.12 Do you use it fresh or dry?

If dry, how do you dry and store it after collection and for how long?

3.13 How do you prepare the plant for the remedies? 3.14 What is the mode of administration for children?

3.15 What dosage should they take, and for how long?

3.16 What are the Contraindications?

3.17 What are the side effects that can be observed?

3.18 What are the cultural observations?

The way the plant is supposed to be handled according to the culture.

Is it used for medicinal purposes only? If No, what other things is it used for?