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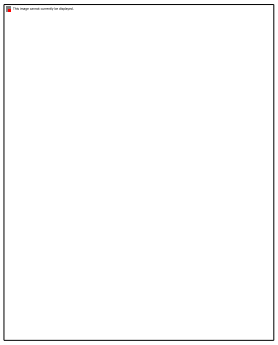

FACULTY OF AGRICULTURE AND ENVIRONMENTAL SCIENCE

TOPIC: Qualitative And Quantitative Phytochemical Analysis Of Four Plants Used To Treat *Theileriosis* In Cattle.

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS OF THE BACHELOR OF SCIENCE HONOURS DEGREE IN AGRICULTURE IN ANIMAL HEALTH PRODUCTION AND EXTENSION.

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APPROVAL FORM

The undersigned certify that they have supervised, read and recommend to Bindura University of Science Education for the acceptance of a research dissertation entitled: **QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF FOUR PLANTS USED TO TREAT *THEILERIOSIS* IN CATTLE submitted by Juliana Madzinga** in partial fulfilment of the Bachelors Degree of Agriculture Science Honours in Animal Health and Production Extension (BSCHAHPE) with the approval of the following Supervisor:

Signature of student……Date.....

Signature of supervisor……Date.....

Signature of chairperson……Date.....

Declaration

I Juliana Madzinga hereby declare that I am the sole author of this dissertation. I authorise Bindura University to lend this dissertation to other institutions or individuals for the purpose of scholarly research

Sign……. Date…….

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ABSTRACT

Extracts of various parts of Erythina abbssinicia, Cissus quadrangularis, Cassia abbreviata and Brachylaena rotundata are claimed to have potent bioactivity. Traditional practice and use of the plants is based on guesswork and experience. In this research the phytochemical composition of hydroethanolic stem and bark extracts of the plants was evaluated. Maceration of powdered plant material in 60 % ethanol was employed to extract phytochemicals. Presence of alkaloids was confirmed using Mayer's test, foaming test was applied for sapponins. To test for phenolics, flavonoids, glycosides and tannins, lead acetate, alkaline, Bontager and the ferric chloride tests were used respectively. The acid test was done for quinones. To test for terpenoids and steroids, chloroform/sulphuric acid and alcohol/sulphuric acid test were used respectively. Three phytochemicals namely tannins, phenolics and alkaloids were quantified. The dry extrant yields obtained for each plant are as follows: C. abbreviata 19.9 %, B. rotundata 16.6 %, C. quadrangularis 15.5 % and lastly E. abbyssinica 13.8 %. All the plants had positive results for the tested phyto-compoundas except C. abbreviata which tested negative for saponins and steroids. In quantitative phytochemical analysis, C. quadrangularis had the highest content of tannins (476 mg/mL), followed by E. abbyssinica (400 mg/mL), C. abbreviata (180 mg/mL) and B. rotundata (123 mg/mL). The findings of this research provide an insight into the phytochemical composition of E. abbyssinica, C. quadrangularis, C. abbreviata and B. rotundata providing a baseline for further exploration of the plants' phytochemicals in managing livestock diseases.

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CHAPTER 1

1.0 INTRODUCTION

1.1 BACKGROUND

Livestock has a significant contribution towards the livelihood of bulk of the rural population. Mostly, communal farmers in Africa face constraints in terms of resources, (Sungirai et al., 2016). Consequently, their livestock is under frequent attack and threat from tick-borne diseases. Livestock productivity is severely affected by parasites and diseases. This continues to hinder growth of the livestock sector in Zimbabwe. A 9% mortality rate was recorded in the national beef herd in the year 2019, (Nyahangare, 2019). In 2018 alone, more than 50 000 cattle perished due to tick-borne diseases. An estimated figure of 5.5 million heads accounts for the Zimbabwean national beef cattle herd, (Bennett et al., 2019). About 90% of that figure is in the hands of small-scale farmers. Basically, control of vectors (ticks) through dipping and acaricides is the main tool in controlling tick-borne diseases in Zimbabwe. However, there is widespread emergence of tick species that are resistant to organophosphates, an acaricide group mostly used in the control of ticks. The increase in the number and incidence of the *Rhipicephalus appendiculatus* ticks and *theileriosis* cases is mainly attributed to inconsistent cattle dipping routines as well as shortage of acaricides and drugs,(Muvhuringi et al., 2022). Another crippling factor is high cost of the drugs which falls beyond the affordability range to most rural farmers, (Sungirai et al., 2016). Medicinal plants are endowed with diverse phytochemicals that have diverse bioactivity. Phytocompounds have remained a major source of new drugs. In the past decades, research has been focused on scientific evaluation of traditional drugs of plant origin. Ethno-veterinary medicine or traditional animal health care is based on folk beliefs, traditional knowledge skills and methods for curing diseases and maintaining healthy animals, (Maroyi, 2012). Ethno-veterinary medicine is gaining popularity in developing countries because it is readily accessible, easy to prepare and administer. Moreover, the plants are available at little cost to the farmer. In many economically challenged rural areas ethno-veterinary medicines play an important role in animal production and livelihood development, (Munodawafa, 2012) and often becomes the available means for farmers to treat ill animals, *E. abbssinicia, C. quadrangulaeis, C. abbreviata* and *B.rotundata* have wide application in managing tick-borne diseases. Despite the increasing acceptance of

traditional veterinary medicine in Zimbabwe, the rich indigenous knowledge on traditional veterinary remedies is not adequately documented. The growing scientific evidence suggests that this ethnic knowledge supplemented with the new scientific insights can offer socially acceptable and eco-friendly approaches vital for the sustainable development of the local communities, (Khan et al., 2021) Therefore, the need to document knowledge of ethno-veterinary information cannot be overemphasised. Documentation of plants used in ethno-veterinary practices so that it can be preserved, (Kirika et al., 2015) plants conserved and sustainably managed and utilised for control of livestock diseases. This thesis therefore seeks to investigate the phytochemical composition of *E. abbssinicia, C. quadrangulaeis, C. abbreviata* and *B.rotundata* and lay a background in the use of indigenous plants that to treat theilleriosis thus emphasizing plant roles in basic livestock health care.

1.2 PROBLEM STATEMENT

Government veterinary services department is providing preventive measures against diseases by vaccination, tick control, veterinary surveillance, drugs quality assurance and extension services. The national herd has been affected by deaths due to outbreak of diseases in the period 2018 to 2022. Deaths due to *Theilleriosis* shot to the peak resulting in a sharp decrease in the national herd. Theilleriosis has resulted in a decrease by 65 % of the national herd since the period 2018 to 2022. The most common animal diseases in Zimbabwe are tick-borne diseases, eye problems, retained afterbirth, fleas, lice and diarrhoea. Most of the ethno-veterinary remedies (78%) are collected from the wild. The remedies are mainly administered as decoctions or infusions of single plants. The plants are used not only as alternatives to conventional veterinary drugs but also because in certain diseases they are thought to be potent. In view of increased uses of wild plants as ethno- veterinary remedies, further research into their pharmacological activities and composition cannot be overemphasised, (Maroyi, 2012)Economic challenges have forced farmers to resort to ethno-medicines to manage livestock diseases. Farmers are using ethno-medicines without sufficient knowledge information. There is need to document the phytochemical constituents of the plants used in

folklore treatment methods. This work seeks to analyse the phytochemical constituents of *Erythina abbyssinica, Cissus quadrangularis, Cassia abbreviata* and *Brachylaena rotundata.*

1.3 OBJECTIVES

1.3.1 MAIN OBJECTIVE

To deterrmine the phytochemical composition of plants used to treat *Theilleriosis* in cattle.

1.3.2 SPECIFIC OBJECTIVES

The objectives of this research are:

1. To identify ethnobotanical plants used to treat *Theilleriosis.*
2. To extract the phytochemicals in *Erythina abbyssinica, Cissus quadrangularis, Cassia abbreviata* and *Brachylaena rotundata.*
3. To analyse phytochemical components of the 4 plants used in the treatment of *theilleriosis*.

1.4 SIGNIFICANCE OF THE STUDY

The communities have no capacity to develop and produce comparative products for formal markets even though they might know that certain plants treat livestock ailments. The study is going to benefit farmers who use ethnovet plants The research is done to analyse the phytochemical constituent of plants that can be used to treat *theilleriosis.* Identification of bioactive constituents is important and can be used as a basis for discovery of alternatives for the conventional drugs as well as potent leads in discovery of new drugs that are affordable, have wide spectrum of activity, are able to curb rampant resistance and have lesser negative impacts on the environment.

1.5 DELIMITATIONS OF THE STUDY

In this study plant species were identified in Sadza, Chikomba district in Zimbabwe, ward 21. Four plants *Erythina abbssinicia species, Cassius quadranguraliris, Cassia abbreviata and Brachlaena rotundata* were used in this study.

1.6 LIMITATIONS

1.6.1: Research finances

Travelling throughout Chikomba district ward 21 as well as to and from Bindura University required financial support. The chemicals used in phytochemical analysis are very expensive.

1.6.2 Intellectual rights

Patent rights have always been a thorny issue associated with plant based product development largely because a lot of information about abuse of these plants is based on indigenous knowledge platforms with knowledge emanating from farmers and rural communities, (Nyahangari et al 2015). However these communities have no capacity to develop and produce competitive products for formal markets which is where scientist and private sector play an important role. There is mistrust between researchers and farmers, mostly from farmers side because they think when researchers conduct ethno-surveys it is to milk them of information while farmers get nothing but more drugs on market.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 LIVESTOCK PRODUCTION OVERVVIEW

Livestock play a crucial livelihood role for 70% of the world's resource-poor population are living in rural areas. The importance of livestock is even greater in drier agro-ecological zones where crop production is restricted by low and poorly distributed rainfall coupled with recurrent droughts. Mostly, these areas are only ideal for extensive livestock husbandry (especially cattle and small ruminants), wild animals and drought resistant crops. While these areas are suitable for livestock, animal performance still remains subdued due to numerous interacting factors key amongst them, diseases and poor health management across all species. One of the major health concerns affecting livestock are ecto-parasites, particularly ticks and tick-borne diseases (TTBD). At least 80% of the world's cattle population are at risk from tick born disease such as *theilleriosis* among others.

2.2: OVERVIEW OF THEILLERIOSIS

Protozoa in the genus *Theileria* are tick-borne parasites that have been found in many species of mammals. More than a dozen species of *Theileria* occur in cattle, water buffalo, sheep and goats. Some tend to circulate with few or no clinical signs, but others can cause serious illnesses with high morbidity and mortality rates, especially in naive animals, (Nyahangare, 2019). The two organisms with the greatest economic impact in cattle are *Theileria parva* and *Theileria annulata*, which cause East Coast fever or corridor disease and tropical *Theilleriosis* respectively. *Theileria lestoquardi, Theileria uilenbergi* and *Theileria luwenshuni* are the most virulent species in sheep and goats, (Sungirai et al., 2016). In addition to causing direct losses, highly virulent species of *Theileria* can limit the movement of livestock between countries and are a constraint on the importation of new breeds or improved stock, (Muvhuringi et al., 2022).

2.2.1: TRANSIMISION

Theileria are transmitted by ticks acting as biological vectors, and can be transmitted transstadially. Transovarial transmission is not thought to occur. Genera of ticks reported to act

as vectors include *Rhipicephalus* (*T. parva, T. taurotragi, T. ovis, T. lestoquardi*), *Hyalomma* (*T. annulata, T lestoquardi, T. separata*), *Haemaphysalis* (*T. orientalis, T. uilenbergi, T. luwenshuni*) and *Amblyomma* (*T. Mutants, T. velifera*). *Theileria* spp. enter the body as sporozoites in the saliva of a feeding tick, (Sungirai et al., 2016). Ordinarily, *T. parva* and *T.annulata* only mature after an infected tick attaches to a host, and the tick must be attached for a few days before these organisms are transmitted, (Nene et al., 2016). However, *T. parva* can develop to the infectious stage in ticks on the ground if environmental temperatures are high. These organisms may enter the host within hours of attachment, (Muvhuringi et al., 2022). Inside the mammalian host, *Theileria* undergoes a complex life cycle involving the replication of schizonts in leukocytes and development of piroplasms in erythrocytes. Piroplasms infect ticks when they feed on the animal's blood,

2.2.2: CLINICAL SIGNS OF THEILLERIOSIS

East Coast fever or corridor disease (*T. parva*) is characterized by fever, generalized lymphadenopathy, anorexia, loss of condition and, in some animals, nasal-discharge and/or diarrhea, (Perry, 2016). *Petechiae* and *ecchymoses* may be found on the conjunctiva and oral mucous membranes, and milk yield usually decreases in lactating animals.Corneal opacity, exophthalmia and skin lesions (nodules, hemorrhagic or ulcerative lesions, and erythematous popular rashes) have been reported occasionally, (OIE, 2019). Terminally ill animals often develop pulmonary oedema, with severe Dyspnoea and a frothy nasal discharge. Some animals have poor productivity after recovery and their growth is stunted,(Silatsa et al., 2020). Although ocular signs including corneal opacity sometimes resolve with treatment, permanent loss of eyesight is possible. *T. parva* can also cause a fatal condition called "turning sickness," where infected cells block capillaries inthe central nervous system and cause neurological signs, (Silatsa et al., 2020).

2.3: ANTHOLOGY OF PLANTS

2.3.1: OVERVIEW OF *C. ABBREVIATTA* (MURUMANYAMA)

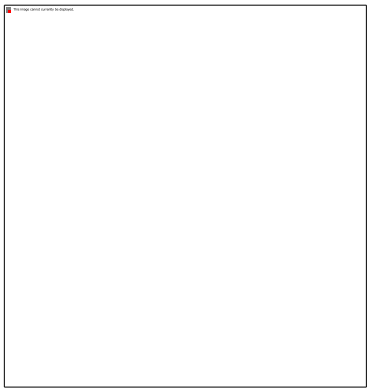

Figure 1: ***C. abbreviata***

C. abbrreviata is a shrub or small rounded tree which is dark greenish brown, longitudinally farrowed. Leaves form without any glands on the ranches or petiole. There are leaflets in 7-12 pairs, hairy on the underside particularly when young. It has ovate-elliptic flowers with rounded apex in large lax terminal sprays yellow appearing before leaves. Fruit is a long cylindrical brown pod up to 90 cm in length which remains on the tree for many months, (Hikaambo, 2022). Plant leaves, stem bark, and roots are the widely used parts the management of varied ailments like malaria, stomach-ache, diarrhoea as well as skin lesions in African traditional medicines, (Kabuka et al., 2022). The roots are dried, macerated in water and then orally. The stem bark is also used in the same way after crushing and soaking in water. Phytochemistry includes terpenes, anthocyanins, flavonoids, phenolics alkaloids and steroids,Yang et al., (2021) ; I., (2013).

2.3.2: REVIEW OF *E. ABYSSINICA* (MUTITI)

E. abyssinica is an important ethno-medicinal plant in Africa that harbours useful bioactive phytochemicals against various diseases exhibiting significant potency coupled with minimal toxicity to mammalian cells. Therefore, this plant has potential to provide novel molecules against diseases for further exploration. Field trials evaluating the efficacy and safety of compounds isolated from *E. abyssinica* are recommended, (Kirika et al., 2015). *E. abyssinica* is use by traditional herbalists in East, Central, and Southern Africa to prepare herbal remedies for various human and livestock ailments including bacterial and fungal infections, diarrhoea, inflammatory diseases and skin and soft tissue injuries. Different extracts and phytochemicals

from parts of *E. abyssinica* have been scientifically This versatile pharmacological activity is due to the abundant flavonoids, Obakiro et al., (2021);Kirika et al., (2015).

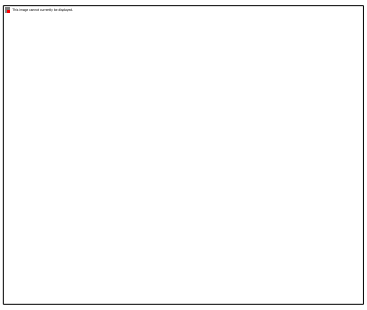

Figure 2.3.2: *E. abyssinica*

It is a deciduous shrub or tree up to 12 m in height. It has orbicular leaves and long seeds which poisonous. On blooming, it has some unusual bright red flowers with thread like filaments. The leaves are edible and are crowded towards the ends of the branches with long petioles. The root is large and succulent and on drying forms an extremely light corky mass. The bark is grey, corky and rough, (Nyahangare, 2019).Young branches have dense brown hairs. Its light wood is used in fishermen's floats. It is one of the several plants producing red and black seeds called lucky bean which are sewn into necklaces. The tree is well distributed in southern Africa and in Zimbabwe it is mostly common in the north, east, south and central parts. The tree is common to higher altitudes in wooded grassland. It can be propagated from the seed or truncheons, (Obakiro et al., 2021).

2.3.3: REVIEW OF *C. QUADRANGULARIS* (MUVENGAHONYE)

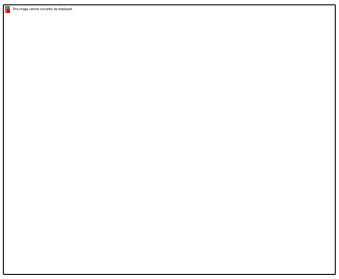

Figure 2.3.3: *C. quadranguralis*

C. quadranguralis is a shrub or small tree up to 6 m high. Its leaves are pinnately compound with 10-17 alternate or sub-opposite leaflets and a terminal leaflet. The leaves are densely dotted with glands and have a strong scent when crushed, (Sen & Dash, 2012). The scent has been likened to aniseed and opinions vary on its pleasantness. The flowers are small but attractive, white or cream in colour with orange-yellow stamens. The fruits are small, ellipsoid, about 13 mm in diameter, shining blue-black drupes. Locally it is common in high rainfall areas and grows well on the margins of, evergreen forest, (Teware et al., 2011). This plant is commonly hung in houses or put on fires to keep away mosquitoes and evil spirits. The powdered roots, with lime and guinea grains are applied to rheumatic and other pains in Nigeria where also the leaves are considered anthelmintic. In East Africa *C. quadrilanguralis* is used for its odoriferous properties, especially under beds and toothbrushes are made from the twigs, (Camil Rex & Ravi, 2020). It is well known for its anti-diabetic properties and is therefore widely used by traditional healers. It is also used for treating epilepsy and cancer. The leaves of the plant are normally used and methods of administration differ according to the type of sickness, (Teware et al., 2011). Phytochemically, the plant is endowed with active constituents such as steroids, flavonoids and triterpenoids which are responsible for its pharmacological activities, (Teware et al., 2011).

2.3.4: REVIEW OF *B. ROTUNDATA* OR B. DISCOLOR (MUPASA)

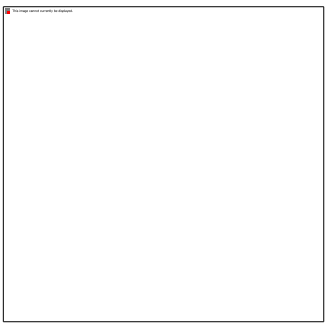

Figure 2.3.4: ***B. rondutata***

It is a deciduous shrub or small tree. Its leaves alternate oblong to broad oblanceolate up to 15 cm long, thinly white woolly above only when young and more densely greyish or pale brownish woolly with veins prominent, (Maroyi, 2020b) margin entire or dentate towards the apex. The petiole is 2-7 mm long. Florets are dull yellow without rays, (Mellem, 2013). Pharmacologically, the aerial parts and leaf extracts of B. discolor and compounds isolated from the species are anthelmintic, antibacterial, antifungal and anticancer, (Maroyi, 2020a).

Phytochemical such as alkaloids, fllavonoids, phenolics, phlobatannins, saponins, sesquiterpenes, steroids, tannins and terpenoids have been isolated from *B. rotundata*. These are responsible for the bioactivity associated with the plant, (Maroyi, 2020b).

2.4: REVIEW OF PHYTOCHEMICALS

The phytochemical composition of a plant is determined by the presence of the following; alkaloids, flavanoids, saponins, glycosides, tannins, terpenoids, quinones, peptosteroids, terpenes and phenolic compounds.

2.4.1: Alkaloids

They are one of the chemical groups that are derived from natural products and have had so many drugs that are derived from their synthesis. Alkaloids are basic substances that contain one or more nitrogen atoms, usually in combination or as part of a cyclic system. Over 5 500 alkaloids are known and they comprise the largest single class of secondary metabolites. Many of the earliest isolated pure compounds with biological activity were alkaloids. This was due to the ease of isolation. The nitrogen generally makes the compound basic and the compound exists in the plant as a salt. Thus, alkaloids are often extracted with water or mild acid and then recovered as crystalline material by treatment with base.Many alkaloids are terpenoid in nature whilst others are mainly aromatic compounds, for example colchicines. In plants they may exist in free-state or as salts. Alkaloids show great variety in structure and virtually all the known nitrogen ring systems occur. Previously carried research has shown that alkaloids are antibacterial, antiprotozoal, anti-fungal and have anaelgestic effects, Cahlíková et al., (2021), Silatsa et al., (2020), Zorrilla & Evidente, (2022).

2.4.2: Quinones

They are derived from *cinchona* species. The *cinchona* bark has been used against fever since 1960. Colhicine; an amorphous yellowish alkaloid is currently used in the treatment of acute gout and arthritis. It is found in *Colhicum autumnale* which has been used since 1952 by Arabian physicians in the treatment of gout and rheumatism. They are antinflamatory, antibiotic and antiprotozoal, (Babotă et al., 2022).

2.4.3: Flavonoids

Flavonoids are water soluble polyphenolic molecules containing 15 carbon atoms. Flavonoids belong to the polyphenol family. Flavonoids can be visualized as two benzene rings which are joined together with a short three carbon chain. One of the carbons of the short chain is always connected to a carbon of one of the benzene rings, either directly or through an oxygen bridge, thereby forming a third middle ring, which can be five or six-membered. The flavonoids consist of 6 major subgroups: chalcone, flavone, flavonol, flavanone, anthocyanins and isoflavonoids. Together with carotenes, flavanoids are also responsible for the coloring of fruits, vegetables and herbs. Flavonoids have antioxidant activity. They are becoming very popular because they have many health promoting effects. Some of the activities attributed to flavonoids include: anti-allergic, anti-cancer, antioxidant, anti-inflammatory, anti-viral, antithrombotic and vaso-protective effects, Ahmad & Ahamad, (2020), Ognyanov et al., (2022).

2.4.4: Tannins

Tannins are generally defined as naturally occurring polyphenolic compounds of high molecular weight. They are able to form complexes with proteins of animal hides and prevent their decaying, thus converts hides to leather. Many types of tannin are glycosides, for instance glucogallin. Tannins act as a defense mechanism in plants against pathogens, herbivores and hostile environmental conditions. Generally, tannins induce a negative response when consumed. These effects can be instantaneous like astringency or a bitter or unpleasant taste or can have a delayed response related to anti-nutritional or toxic effects. Tannins have been traditionally used for protection of inflamed surfaces of the mouth and treatment of wounds, haemorrhoids, and diarrhoea. They can be used as an antidote in heavy metal poisoning. *Rubus villosus* (Blackberry root bark) is an example of a herb that contains tannins and is used as an astringent tonic for diarrhoea and dysentery. Tannins have an antiinflamatory effect, antibiotic, antiprotozoal and antioxidant potency, Kumar et al., (2022), Ahmad & Ahamad, (2020).

2.4.5 Saponins

Saponins are phytochemicals which are natural detergents found in many plants, especially certain desert plants. They are also present in small amounts in some foods, such as soybeans and peas. Saponins consist of polycyclic aglycones attached to one or more sugar side chains. The aglycone part, which is also called sapogenin, is either steroid (C27) or a triterpene (C30).

Saponins have detergent or surfactant properties because they contain both water-soluble and fat soluble components. They consist of a fat-soluble nucleus, having either a steroid or triterpenoid structure, with one or more side chains of water-soluble carbohydrates (sugars). Because of their surfactant properties, they are used industrially in mining and ore separation, in preparation of emulsions for photographic films, and extensively in cosmetics, such as lipstick and shampoo. Saponins have a bitter taste. Some saponins are toxic and are known as sapotoxins. Saponins have an antinflamatory, antifungal and antibiotic effects, Altemimi et al., (2017), Ognyanov et al., (2022).

2.4.6: Glycosides

Cardiac glycosides are drugs used in the treatment of congestive heart failure and cardiac arrhythmia. These glycosides are found as secondary metabolites. Cardiac glycosides are found in a diverse group of plants including *Digitalis purpurea* and *Digitalis lanata* (foxgloves), Cardiac glycosides most often are used to treat severe heart failure and arterial fibrillation that can occur with congenital heart defects. They also represent an important class of useful, albeit somewhat dangerous, steroids. These compounds are characterized by the steroidal cardenolide aglycone bonded at the C-3 position to a sugar moiety which can range from a monosaccharide to a trisaccharide. The wellknown use of these compounds is in the preparation of digitalis, a drug used to treat congestive heart failure, super ventricular tachycardia, and several other heart conditions. It is a cardio-tonic agent which increases the tone of the heart muscle causing more effective emptying of the heart chambers, Kabuka et al., (2022), Ahmad & Ahamad, (2020), Babotă et al., (2022).

CHAPTER 3

3.0 METHODOLOGY

3.1. STUDY SITE

The study was carried out in Sadza, Mashonaland East Province in region II Zimbabwe. Rainfall received typically is 133.31 mm and has 122.6 days annually. Maximum temperature is 25 ℃ and minimum temperature is15 ℃. Trees found are the musasa, muchakata, mutsubvu, mubvamaropa and mutondo, mupasa, muvengahonye, mutiti. The grasses found are savanna grasses. The agricultural activities found are crop farming, poultry, fishery and livestock farming. Brick moulding constitutes some economic activities.

3.2: MATERIALS AND METHODS

The research instrument employed was of initially a focus group of 28 farmers called to come out with key informants. During discussions of plants used ethno-medicinally, the key informants named 20 plants. Of the twenty plants mentioned, the researcher took those mentioned more frequently. These were *E. abbsinnicia, C. quadrilangularis, C. abbreviata* and *B. rotundata*.

3.3: COLLECTION AND PULVERISATION

The bark of the four plants were collected and dried separately under a shade with free air circulation. The dried plant material was pulverised into powders and stored in packs until time of use.

3.4: EXTRACTION OF PHYTOCHEMICALS

Maceration in hydroethanolic solution (40% distilled water and 60% ethanol) was used to extract the phytochemicals. The flasks were stoppered tightly with cotton plug and kept at room temperature for 48 hours with intermittent shaking. The contents of each flask were filtered through a muslin cloth to remove plant residue. The filtrate was passed through a Whatman No. 1 filter paper. The final supernatant liquids were transferred to a rotary evaporator for concentration. The concentrated extracts were placed in labelled pre-weighed petri dishes.

They were left to evaporate to dryness after which the mass of each of the extracts was measured. The dried extracts were stored in air tight screw cap amber vials and kept in a fridge until further use.

3.5: EXPERIMENTAL DESGN

The researcher employed a completely randomized design. Four plants were tested in three experimental treatments and the factor under study was phytochemical constituents namely: alkaloids, saponins, phenolic compounds, tannins, steroids, glycosides, flavonoids, quinones and terpenoids .Three replications were made (table 3.5.1) in the in the lab to avoid biased results.

Key for the treatments

MUT –mutiti

MUR – murumanyama

MUV-muvengahonye

MUP –mupasa

Table 3.5.1: Allocation of treatments

REP 1		REP 2		REP 3	
1 MUT	3 MUP	5 MUR	6 MUT	9 MUV	10 MUT
2 MUV	4 MUR	7 MUV	8 MUP	11 MUR	12 MUP

3.6: QUALITATIVE PHYTOCHEMICAL TESTS

3.6.1: Alkaloids

Mayer's test

I ml of the extract was added to 1ml of potassium mercuric iodide solution (Mayer's reagent) in a test tube. The contents were shaken gently. Presence of the alkaloids is proved by formation of a white precipitate.

3.6.2: Saponins

Froth forming test

5ml of plant extract was shaken with 5mls distilled water in a test tube for 15 minutes. A positive response shows stable foam in the test tube.

3.6.3: Phenolic compounds

Lead acetate test

1mI of plant extract was mixed with 1ml of 10% lead acetate solution. A white precipitate shows positive results.

3.6.4 Tannins

Ferric chloride test

1ml of the extract was diluted with distilled water. 2 drops of ferric chloride were added. A transient green or black colour indicates the presence of tannins.

3.6.5: Flavonoids

Alkaline test

Drops of sodium hydroxide are added to the plant extract followed by drops of sulphuric acid. If flavonoids are present, the solution turns colourless after adding sulphuric acid.

3.6.6: Quinones

Add 1ml of hydrochloric acid to 1ml plant extract. A pink or red colour indicates the presents of quinones.

3.6.7: Terpernoids

Add 1ml of chloroform to 1ml plant extract, swirl to mix and add 3ml of sulphuric acid. A reddish brown colouration at the interface shows the presence of terpenoids.

3.6.8 Glycosides

Bontagers test

Add 1gm of the extract sample to 5-10ml of HCl. Add 10 ml boiling water and filter extract with benzene. Appearance of a pink to red colour indicates the presence of of glycosides.

3.6.9 Steroids

Plant extract is mixed with alcohol and a concentrated acid and the product is a coloured substance if steroids are present.

3.7: QUANTITATIVE PHYTOCHEMICAL TESTS

Determination of Total Tannin Content

Modified Folin Ciocalteu method was used to quantify tannins. In brief, 1 ml of plant extract was added to 5 ml of distilled water followed by 0.3 ml of Folin's phenol reagent, 1 ml of 35 % sodium carbonate in aqueous. Absorbance was measured at 725 nm. Calibration was done with tannic acid dilutions as standard solutions. The results were expressed in terms of tannic acid in mg/ml of extract.

Determination of Total Phenolic Content

Modified Folin Ciocalteu method was used to determine phenolic content of the extracts. 100µL of extract were added to 400 µL of Folin Ciocalteu reagent mixture followed by 1 ml of 7.5 % sodium carbonate. Resulting solution was diluted by a factor of 7 with distilled water and incubated for 1 hour in the dark. Absorbance was measured at 765 nm. Gallic acid was used as a standard. The results were expressed in terms of Gallic acid in mg/ml of extract.

Determination of Total Alkaloid Content

50 ml of 10 % acetic acid in ethanol were added to 1.5 g of extract powder, covered and left for 4 hours. The supernatant was concentrated on a water bath to reduce volume by 4 times. Ammonium hydroxide was added drop wise until precipitation was complete. The solution was allowed to settle. The precipitate was collected and washed with dilute ammonium hydroxide, then filtered. The residue was dried and weighed.

CHAPTER 4

4.0.DATA PRESANTATION AND ANALYSIS

4.1: EXTRACTION YIELDS

The dry yields obtained for each plant sample are recorded in Table 4.0. The highest yield was obtained from Murumanyama (19.9 %), followed by Mupasa (16.6 %), Muvengahonye (15.5 %) and lastly Mutiti (13.8 %)

Table 4.0: The yields of hydroethanolic extracts in grams and percentages

	Mutiti	Mupasa	Muvengahonye	Murumanyama
Mass yield/g	1.38	1.66	1.55	1.99
% yield	13.8	16.6	15.5	19.9

4.2: QUALITATIVE PHYTOCHEMICAL TESTS RESULTS

The plant extracts were subjected to qualitative phytochemical analysis in a randomised fashion. The results obtained are summarised in Table 4.1. The results were consistent throughout. All the plants had all the tested phyto-compoundas except Murumanyama which tested negative for saponins and steroids.

Table 4.2: Qualitative results for phytochemical analysis

Sample	Alkaloids	Saponins	Phenolics	Tannins	Glycosides	Steroids	Flavonoids	Quinones	Terpenoids
1	+	+	+	++	+	+	+	+	+
2	+	+	+	++	+	++	+	+	+
3	++	+	+	+	+	+	+	+	+
4	+	-	+	+	+	-	+	+	+
5	+	-	+	+	+	-	+	+	+

6	+	+	+	++	+	+	+	+	+
7	+	+	+	++	+	++	+	+	+
8	++	+	+	+	+	+	+	+	+
9	+	+	+	++	+	++	+	+	+
10	+	+	+	++	+	+	+	+	+
11	+	-	+	+	+	-	+	+	+
12	++	+	+	+	+	+	+	+	+

Key: ++ excess amount of the constituent

+ average amount of the constituent

- constituent absent

4.3: QUANTITATIVE PHYTOCHEMICAL ANALYSIS RESULTS

All the extracts of *E. abbyssinica, C. quadrangularis, C. abbreviata* and *B. rotundata* were shown to contain considerable amounts of tannins, phenolics and alkaloids in varying proportions (Figure 4.2 a-c respectively). *C. quadrangularis* had the highest content of tannins (476 mg/mL), followed by *E. abbyssinica* (400 mg/mL), *C. abbreviata* (180 mg/mL) and *B. rotundata* (123 mg/mL).

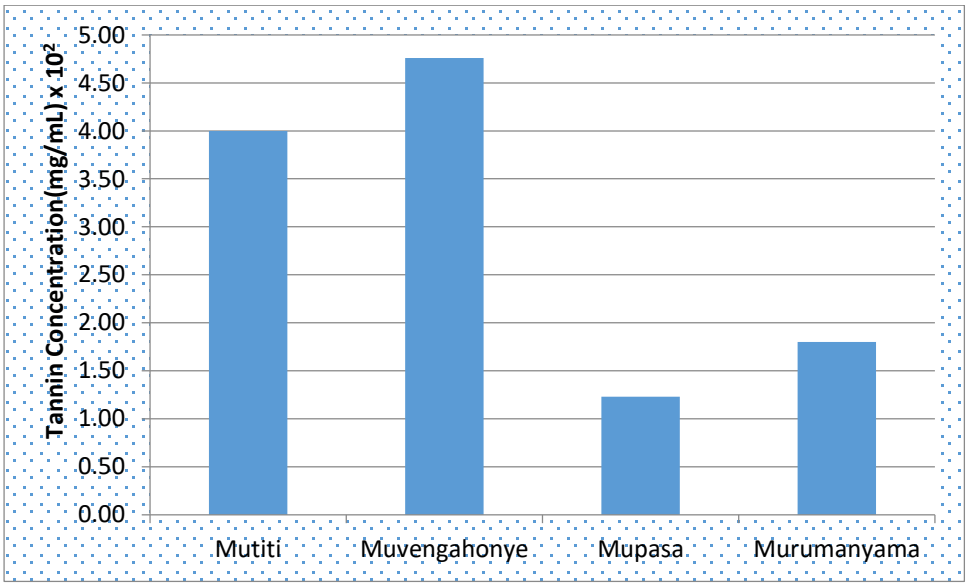

Figure 4.2a: Total tannin content of the plant extracts under study

C. abbreviata had the highest content of tannins (166 mg/mL), followed by *B. rotundata* (132 mg/mL), *E. abbyssinica* (118 mg/mL) and *C. quadrangularis* (98 mg/mL).

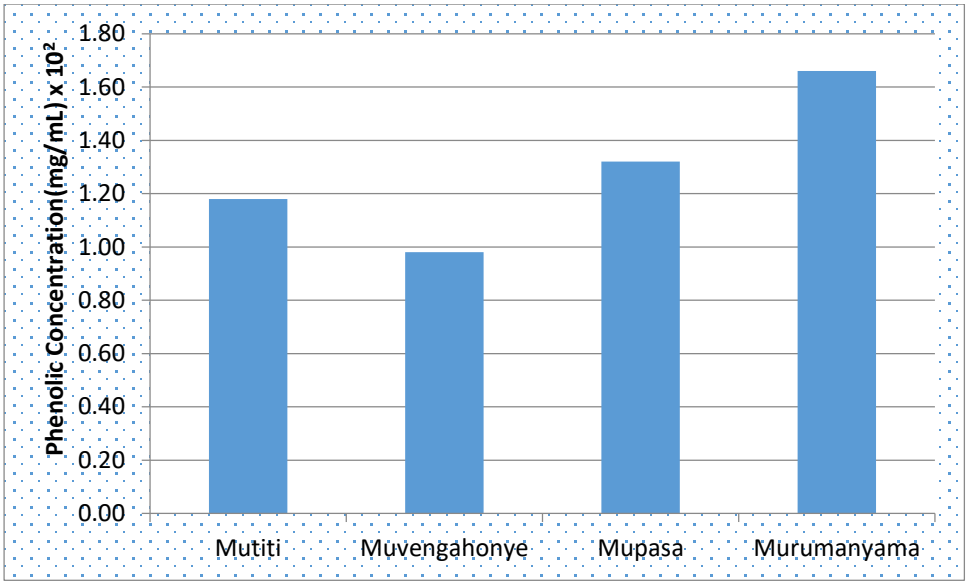

Figure 4.2b: Total phenolic content of the plant extracts under study

The estimated alkaloid content in the hydroethanolic extracts of *E. abbyssinica*, *C. quadrangularis*, *C. abbreviata* and *B. rotundata* 89, 92, 44 and 55 mg respectively. The highest amount was observed in *C. quadrangularis.*

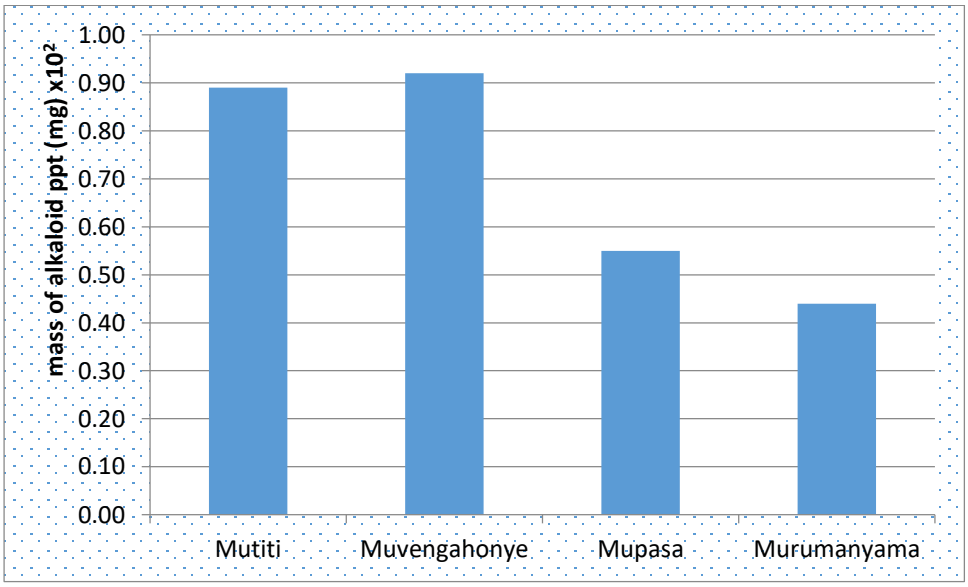

Figure 4.2c: Total alkaloid content of the plant extracts under study

CHAPTER 5

5.0 DISCUSSION, CONCLUSION AND RECOMENDATIONS

5 .1.DISCUSSION

Phytochemical analysis, (both qualitative and quantitative) of *E. abbyssinica, C. quadrangularis, C. abbreviata* and *B. rotundata* was carried out to determine the constituents present in the 4 plants (Table 4.1). Alkaloids, flavonoids, saponins, tannins, steroids, glycosides and phenols were found in the 4 plants save for *C. abbreviata* which tested negative for saponins and steroids. There are studies that have reported several compounds of confirmed biological activity such as alkaloids, flavonoids, saponins, tannins, steroids, glycosides and phenols from *E. abbyssinica, C. quadrangularis, C. abbreviata* and *B. rotundata* which agree with the results obtained herein. Amongst the important discoveries in the medicinal research, close to 25% of the medicines are plant derived and quite a substantial amount of them are currently in use, (Siddiqui et al., 2017). Standardisation of the crude extracts for *C. quadrangularis* is essential because it has great potency against many diseases including *Theileriosis*. It is important to note that side-effects have been proved to be minimum-to-nil in dosage of 5000 mg/kg weight in animal model, (Camil Rex & Ravi, 2020). In another study, alkaloids, saponins, phytosterols, phenols and tannins were reported in petroleum ether, alchohol and ethyl. The bioactive constituents were phytosterols, flavonoids and triterpenoids of stem of *C. quadrangularis*, Teware et al., (2011) and Anuradha., (2017).Phytochemical studies done on methanol extract of *C. quadrangularis* revealed the presence of triterpenes including α and β amyrin, β- sitosterol, ketosteroid, phenol and tannins, (Shukla et al., 2015). The stem of the plant contains two asymmetric bioactive triterpenoids, onocer -7-ene-3-α-2,1-β-diol and onocer -7-ene -3-β-2,1-α-diol, (M & P, 2016).

Phytochemical screening of *C. abbreviata* showed that the extracts contained different classes of compounds which included alkaloids, tannins, flavonoids, saponins, terpenoids, steroids, and phenols, while the ethanolic extract contained the same except alkaloids and saponins, Hikaambo, (2022) and Yang et al., (2021). The variation from the results obtained in this study could be attributed to the plant's exposure to different geographical and environmental conditions as well the extraction methods. The variation from the results obtained in this study

could be attributed to the plant's exposure to different geographical and environmental conditions. Mongalo & Mafoko, (2013) confirm similar results in phytochemical analysis of *C. abbreviata*. Tannins were found high concentrations in the root and the stem bark of both the aqueous and ethanolic extracts of *C. abbreviata* mainly due to the fact that tannins are soluble in both water and ethanol, (Kabuka et al., 2022). Figure 4.2a and b in this study have the same results obtained from quantification of tannins and phenolics. The term phenolic actually refers to classes of compounds that include flavonoids, tannins and anthocyanins among others. Quantifiable alkaloids were found to be 44 mg (Figure 4.2c).

Some researchers have investigated the phytochemical composition of *B. rotundata* aerial parts and leaves and the compounds found were alkaloids, flavonoids, phenolics, phlobatannins, saponins, sesquiterpene lactones, steroids, tannins and terpenoids. Qualitative screening for phytochemicals in the leaves of *B. rotundata* methanolic extract confirmed the presence of tannins, saponins, flavonoids, terpenoids, alkaloids and phlobatannins, (Mellem, 2013). These chemical compounds account for the pharmacological properties of this plant species, (Maroyi, 2020a). Mtshali, (2019) also analysed bioactive terpenes in *B.rotundata*. In this study terpenes were found in the plant's hydroethanolic extract. Quantifiable alkaloids were found to be 55 mg (figure 4.2c).

There studies confirming alkaloids, benzofurans, flavonoids, chalcone, and other pterocarpans which exhibit a wide spectrum of biological activity of constituents of *E. abyssinica,* (Obakiro et al., 2021). This versatility in pharmacological activity comes from the phytochemicals such as flavonoids, alkaloids and terpenoids present various parts of the plant, (Kirika et al., 2015). The results of this study agree with the results of other researchers with quantifiable alkaloids being 89 mg and tannins and phenolic being 400 and 118 mg/ml respectively (figure 4.2a-c). Field trials evaluating the efficacy and safety of the extracts and isolated compounds of *E. abyssinica* are therefore recommended.

5.2: CONCLUSION

The research has proved scientific evidence to the phytochemical constituents of *E. abbyssinica, C. quadrangularis, C. abbreviata* and *B. rotundata*. Hydroethanolic extracts of the plants' stem bark were found to be rich in flavonoids, steroids, terpenes, phenolics and glycosides. Findings of this research authenticated the phytochemical constiuents of *E. abbyssinica, C. quadrangularis, C. abbreviata* and *B. rotundata*. Quantification of tannins, phenolics and alkaloids proved that the plants contain substantial amounts of these compounds.

The identified phytochemicals could be the reason for the diverse ethno-veterinary uses of the plants studied in this work.

5. 3: RECOMMENDATIONS

This work has established a strong foundation for the phyto-chemistry of *E. abbyssinica, C. quadrangularis, C. abbreviata* and *B. rotundata*. However, to advance the understanding of their bioactivity in ethno-veterinary medicine and possibly formulate commercial products, isolation of the phytochemicals using chromatographic techniques is a requirement. Moreover, thorough bioactivity and efficacy of the active compounds need to be researched. Apart from carrying out efficacy and efficiency studies, studies to establish long-term safety toxicity issues are necessary. This is important since some plant extracts work in synergy and may lose functionality properties after isolation or incorporation with some excipients. Therefore, the synergistic effect of the combined plants extracts is another area of study.

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