MAKERERE UNIVERSITY

COLLEGE OF NATURAL SCIENCES
SCHOOL OF BIOSCIENCES
DEPARTMENT OF BIOCHEMISTRY AND SPORTS SCIENCE

PHYTOCHEMICAL SCREENING, *IN VITRO* ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF EXTRACTS OF ROOTS *OF Euclea mayottensis* H. perrier AND FRUITS OF *Tambourissa comorensis* D.H. Lorence

By

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Declaration

I, Idaroussi Faida Khadidja, hereby declare that this work has never been submitted to any other University or Academic Institution for purposes of academic award. This work is original and all the information in this dissertation is based on my research findings.

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Approval

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Dedication

I dedicate this work to my parents Fatima Binti Mohamed and Idaroussi Aboudou Mohamed for their unconditional support throughout my studies in Uganda and in my entire life. I also dedicate it to my little sister Idaroussi Fairouze and to my special person for the love they showed me. May Allah bless them all!

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List of abbreviations

D.	Diospyros
DPPH	2,2-Diphenyl-1-picrylhydrazyl
E.	*Euclea*
EBL+	Extended Beta Lactamase positive
Fig.	Figure
FRAP	Ferric reducing antioxidant power assay
g	Gram
GAE	Gallic acid equivalent
MBC	Minimum bactericidal concentration
mg	Milligram
MIC	Minimum inhibitory concentration
Min.	Minute
ml	Milliliter
MP	Medicinal plants
MRSA	Methicillin Resistant *Staphylococcus aureus*
P.	*Pseudomonas*
S.	*Staphylococcus*
S.	*Streptococcus*
T.	*Tambourissa*
TB	Tuberculosis
WHO	World Health Organization

Abstract

There is increased antimicrobial drug resistance as common antibiotics are no longer effective. In Africa and Comoros in particular the use of medicinal plants as a remedy for the common bacterial infections is on the rise. *Euclea mayottensis* and *Tambourissa comorensis* are two endemic medicinal plants from the Archipelago of Comoros. They are used to treat different ailments including bacterial infections. However, the scientific basis of their use as medicinal remedies is still lacking. This study, therefore, aimed at identifying the phytochemicals present in *E. mayottensis* and *T. comorensis* as well as determining their antimicrobial and antioxidant activities.

Maceration method was used for the extraction process using distilled water, ethanol and ethyl acetate as solvents. The qualitative phytochemical screening was done using standard procedures. Total phenolic content (TPC) was determined using the Folin-Ciocalteu method. The antibacterial activity was determined using the agar well diffusion method, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was established using the broth microdilution assay. The antioxidant activity was determined using the DPPH radical scavenging activity and the ferric reducing antioxidant power (FRAP) methods.

The screening of the root extracts of *E. mayottensis* and fruit extracts of *T. comorensis* revealed that both plants are rich in phytochemicals. The highest TPC value was seen in the ethanolic extracts of both *E. mayottensis* (1.22 mg GAE/g dry extracts) and *T. comorensis* (0.89 mg GAE/g dry extracts). The antibacterial activity showed that both plants have a promising inhibitory effect on all the strains used with diameter of inhibitions varying from 6.6 to 20 mm. The highest MIC value was observed in the ethanolic extracts of both plants (0.16 mg/ml). The DPPH assay showed that the water extracts had the highest scavenging effect (51.42% for *T. comorensis* and 44.6% for *E. mayottensis*). The FRAP assay showed a promising reducing power of all the extracts. *E. mayottensis* ethanolic extract showed the highest reducing power (0.543-0.908) while for *T. comorensis* the highest value was observed in the ethyl acetate extract (0.329-0.773).

In conclusion, the study showed that the presence of the different phytochemicals as well as their great antibacterial and antioxidant activities may explain the therapeutic effects exhibited by *E. mayottensis* and *T. comorensis* and gives a scientific basis for their use in the community. Further studies to isolate and identify the bioactive compounds and assess the bioactivities of the extracts in vivo is recommended.

Chapter One: Introduction

1.1 Background

Bacteria are universal and play an important role in maintaining the environment in which we live. From food industry to biotechnology and medical industry, they are important and help us improve our lives in a daily basis. However, there is a small percentage of the world's bacteria that can cause infections and diseases (Rene, 2016). These infections have a large impact on public health (Doron & Gorbach, 2008). Emerging and reemerging infectious diseases have been posing a challenge to medical communities since the 1950s. More than 700,000 people worldwide die annually from drug resistant strains of common bacterial infection (O'Neill, 2016). In Africa, the probability of a child's acquiring invasive bacterial infection during the first five years of life is estimated to be 2.5% (Mulholland & Adegbola, 2005). Emerging pathogens are now considered to be a major microbiological public health threat (Vouga *et al.,* 2016). Increasing global antimicrobial resistance (AMR) is a major threat to human and animal endangering decades of improvements in health-care outcomes (Gay et al., 2017). It is well known that bacteria have found a way of evolving and are, for some or even most of them, able to 'fight' antibiotics and have become resistant to conventional drugs. Misuse and overuse of antimicrobials are the main drivers in the development of drug-resistant pathogens (WHO, 2021).

Antibiotics remain the main remedy for preventing and treating microbial infections. Nevertheless, by their synthetic nature, most antibiotics are known to have numerous side effects including diarrhea, nausea, vomiting, etc. (Leigh Ann, 2021). Furthermore, due to the increase of AMR around the world, common antibiotics are not effective against common bacteria, and more expensive antibiotics have to be used. For the average person, use of antibiotics in the management of microbial infections is simply burdensome. Due to the high cost of treatment and the adverse effects associated with several synthetic antibiotics, populations in Comoros and other nations have resorted to use of medicinal plants, as they offer a cheaper and easily accessible alternative option.

Medicinal plants can be defined as plants possessing therapeutic properties or exert beneficial pharmacological effect on the human or animal body (''Pharmacognosy'', 2019). Plants have been used for medicinal purposes long before prehistoric period. Worldwide it is expected that 80% of the population uses medicinal herbs, and in the developing countries rates could be as high as 95% (Khan & Ahmad, 2019). In the Comoros, population relies mostly on medicinal plants in their primary health care (Saive *et al.,* 2020). Due to poverty and poor access to modern health care system, the Comorians have developed their own health system based on natural products and this knowledge is passed down from generation to generation (Kaou *et al.,* 2008; Soidrou *et al.,* 2013). With their extensive worldwide utilization, WHO regulates the use of medicinal plants by facilitating the integration of traditional medicines into national health cares, producing guidelines, stimulating strategic research as well as advocating the rational and managing information of medicinal plants (WHO, 2002).

The Comoros archipelago is located in the South East coast of Africa between Madagascar and the Mozambique Chanel. The archipelago is composed of 4 islands: Ngazidja, Ndzuani, Mwali that form the Comoros and Maore (under French authority). The country is known for its panoply of medicinal and aromatic plants; the Comorian population has formed their health system based on natural products from plant origin. Its blend of African-Bantu and Arab-Muslim gave it a traditional medicine specifically rich and well diversified. This knowledge is usually passed down orally from one generation to the other (Kaou *et al.,* 2008). In their study, Adjnohoun and his colleagues (1982) estimated that vegetal biodiversity of Comoros was more than 2000 species with approximately 500 that are endemic to the country. Among the endemic plants are *Euclea mayottensis* and *Tambourissa comorensis* which are known locally as 'Mlala' and 'Mbossa or Mledjeza' respectively.

Euclea mayottensis belongs to the Ebenaceae family. The species under this family are widely used in Africa for their medicinal properties: *Diospyros mespiliformis* is commonly used for the treatment of malaria and pneumonia (Ebbo *et al.,* 2019); *Euclea undulata* is known to be used to treat body pains, diabetes and toothache in South Africa (Maroyi, 2017), *E. divinorum* and *D. lycioides* are used to treat toothache in Zimbabwe (Joshua *et al*., 2013). *E. mayottensis* is notably an endemic plant of the Comoros archipelago. In the Islands, the dried roots of the plant are mainly used as tooth stick in the prevention and/or treatment of dental diseases.

On the other hand, *T. comorensis* belongs to the Monimiaceae family in the major group of Angiosperms- the flowering plants. It is a well-known medicinal plant family used to treat several diseases: skin diseases, gastrointestinal disorders, in the therapy of cold, fevers and rheumatism (Leitão *et al.*, 1999). Currently in the Comoros, the genus is represented by several species with 50% endemicity (Soule et al., 2017): *T. leptophylla*, *T. paradoxa*, *T. moheliensis* and *T. comorensis.* An ethnobotanical interview with local people in the country showed that *T. comorensis* is widely used to treat stomachache, headache, dysmenorrhea, malaria, and other sicknesses (Asma-Ilhousna, 2012). This plant is also used as a cosmetic product locally called 'Msindzanu' which is a beauty mask known to protect the skin from the sun, remove pimples and dark spots as well as to prevent them. In this case, the grounded fruit powder is mixed with water and applied directly to the face.

Like all plants, *T. comorensis* and *E. mayottensis* have compounds responsible for their bioactivities. A preliminary phytochemical screening of *T. comorensis* showed the presence of flavonoids and tannins as well as phenolic compounds (Soule *et al*., 2017). In the case of *E. mayottensis*, no phytochemical screening has been reported at least in the current literature yet. Although these plants are used in the treatment of microbial infections by Comorians, their efficacy has not been experimentally validated. The World Health Organization recommends that all local traditional medicines used for treatment and management of diseases by communities be scientifically validated. This study therefore sought to scientifically validate the antibacterial efficacy of *T. comorensis* and *E. mayottensis.*

1.2 Problem statement

The Comoros Islands, known for their vegetation diversity, have plenty of medicinal plants; some of them are actually indigenous. However, for most of them, no studies have been conducted to authenticate their medicinal properties and ensure the safety of the populations who use these plants. The Comorians use *T. comorensis* as a sun screen as well as in the treatment of skin infections (acnes, pimples, and dark spot). *E. mayottensis* is used to treat toothache. However, in spite of these benefits, there is no scientific study evidence to support the use of these plants as herbal remedies. This study, therefore, was designed to determine antibacterial

and antioxidant activities as well as identifying the phytochemicals in these plants as readily available remedies.

1.3 Objectives

1.3.1 General objective

To determine the bioactivities exhibited by the phytochemicals presents in the roots of *Euclea mayottensis* and fruits of *Tambourissa comorensis*.

1.3.2 Specific objectives

(i) To identify the phytochemicals present in *E. mayottensis* and *T. comorensis*

(ii) To determine the antibacterial activity of E. *mayottensis* and *T. comorensis*

(iii) To determine the antioxidant activity of *E. mayottensis* and *T. comorensis*

1.4 Research questions

1. What are the phytochemical compounds present in *Euclea mayottensis* and *Tambourissa comorensis*?
2. Do *E. mayottensis* and *T. comorensis* exhibit antibacterial activity?
3. Do *E. mayottensis* and *T. comorensis* have an antioxidant potential?

1.5 Justification

Common bacterial infections are becoming increasingly resistant to treatments. In 2020, WHO report an increase of over 20% of *E. coli* isolates that are resistant to both first line drugs and second line treatment. According to that report 4.95 million deaths were associated with bacterial antibiotic resistance worldwide. Medicinal plants are one way population worldwide use to stem the recurrence of antibacterial drug resistance. *Tambourissa comorensis* and *Euclea mayottensis* are medicinal plants which are commonly used in the Comoros folk medicine, albeit lack a strong scientific basis.

This study screened for the presence of active chemicals in the roots of *E. mayottensis* and fruits of *T. comorensis* and any therapeutic activity exhibited. Screening for the presence of

phytochemicals was to provide more information about the plants and the compounds responsible for their bioactivities. Assaying their antimicrobial activity was to help establish if they are effective against the common pathogens known to be responsible for the widespread bacterial infections. Proof of antimicrobial effect of *T. comorensis* and *E. mayottensis* was to promote their use as traditional medicine in the Comoros. It was also important to know if they have a potential antioxidant activity and thus if they could be used to treat diseases caused by oxidative stress. In general, this study helped to know more about the medicinal utility of *E. mayottensis* and *T. comorensis* which provided the scientific basis for their medicinal use.

1.6 Significance

In the Comoros, traditional medicine has always been used for treating different kind of diseases from a simple headache, stomachache, toothache to diseases like malaria, inflammatory disease, ulcers, cancers, etc. The archipelago has panoply of plants known as medicinal plants however, many of them have not yet been studied, particularly the endogenous and endemic species of the archipelago. It is the case of *T. comorensis* and *E. mayottensis*. Given the increase of bacterial infections and their resistance to common antibiotics, medicinal plants could offer the cheapest and easiest way to overcome this problem. Furthermore, plant secondary metabolites are known to be unique resources for pharmaceuticals, food additives, and fine chemicals. They offer a good alternative as they have minimum negative effects and are cost friendly and effective. *E. mayottensis* and *T. comorensis* have been reported to have antimicrobial properties which needed to be clarified, thus studying the phytochemical compounds present in these plants and their bioactivities was of interest. By studying the plants and bioactivities they exhibited was important to improve the knowledge on the plants and give an insight on their medicinal properties. Plants being used as food or raw material in traditional medicine are more likely to yield pharmacologically active compounds. Studying the plants could then pave the way for the isolation of pure bioactive compounds from the plants and the design of pharmaceutical drugs from them in future studies. After evaluation and dissemination of the study on these two medicinal plants, local people will be better aware and satisfied regarding efficacious drug treatment and improved health status.

1.7 Conceptual framework

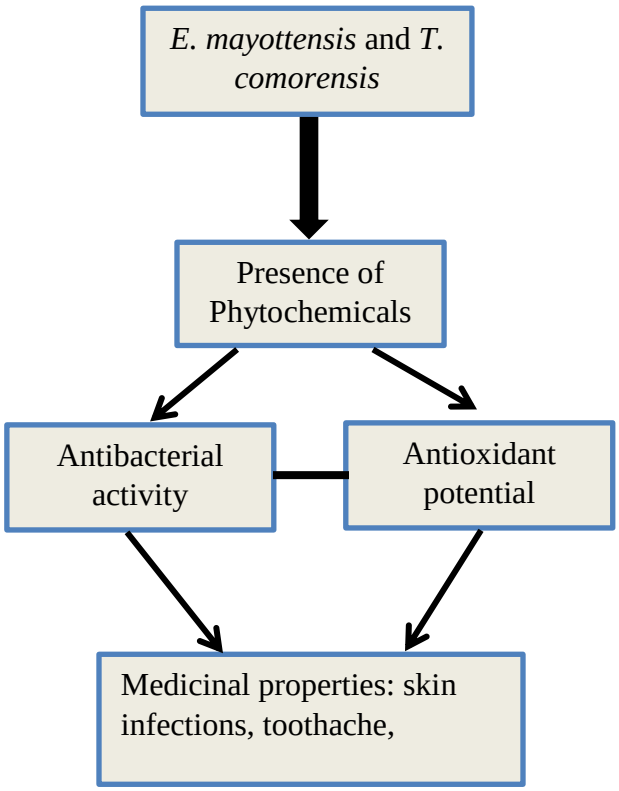


Fig 1.1: Conceptual framework

Due to an increase of microbial resistance, populations are now using plants as an alternative way of treating infections. Medicinal plants examined in this study are *E. mayottensis* and *T. comorensis*. Plant secondary metabolites or phytochemicals are mainly synthesized when the plants is facing stressful conditions such as viral and microbial infections, depletion of water and nutrients etc.

Chapter Two: Literature Review

2.1 Bacterial infections

Due to the evolution and the emergence of new resistant strains of bacteria that have now acquired new resistant mechanisms against the common antibiotics, bacterial infections have become a burden worldwide. In contrast to some other health challenges, antimicrobial resistance (AMR) is a problem that concerns every country irrespective of its level of economy and development as resistant pathogens do not respect borders (O'Neill, 2016). According to a report published in 2016 by the independent Review on Antimicrobial Resistance, more than 700,000 people die every year globally from drug resistant strains of common bacterial infections. This number is likely to be an underestimate due to poor reporting and surveillance (O'Neill, 2016).

In Africa, infectious diseases are a challenge for both its economic development and human health (Rweyemamu, 2006). Bacterial infections are one of the major causes of diseases in the continent with the annual overall risk of bacterial diseases for children being 505 per 100,000 under the age of five years (risk equivalent to a 2.5% probability of a child's acquiring invasive bacterial infection during the first five years of life (Mulholland & Adegbola, 2005). Common bacterial infections have become the second main cause of deaths and were related to one out of every 8 deaths in the world in 2019 while in Sub-Saharan Africa, in the same year deaths due to bacterial infections were per 100,000 people (Ikuta et al., 2022). The Comoros, like many other Sub-Saharan African countries, suffers from high burden of infectious diseases (Deng et al., 2018; WHO, 2018).

2.1.1 Antimicrobial drugs

The aim of antimicrobial therapy is to kill or inhibit the infecting organism without damaging the host (Pursell, 2019). This process is known as selective toxicity and is commonly accomplished by the use of antimicrobial drugs. Antimicrobial drugs including antibiotics, antiviral, antifungal and antimalarial drugs, are medicines that are active against a range of infections providing the first weapon used against microbial infections. Antibiotics are generally classified according to their molecular structure and their antimicrobial mechanisms (Becker, 2013): targeting bacterial

cell (beta-lactam anbiotics and glycopetides), targeting protein biosynthesis (aminoglycosides, macrolides, etc.), inhibiting DNA replication (quinolones), etc.

Resistance to antimicrobials is a natural process that has been observed since the first antibiotics were discovered, however due to their misuses and overuses, it has increased the rate at which resistance is developing and spreading (O'Neill, 2016). Due to the increase of antimicrobial resistant diseases, populations in the Comoros and in other nations, have resorted to medicinal plants as their main way of treating diseases.

2.2 Medicinal plants

The use of medicinal plants for the treatment of diseases dates back to the history of human life. Since human beings have sought a tool in their environment to recover from a disease, the use of plants was their only choice of treatment (Halberstein, 2005). More than a tenth of the plant species (over 50 000 species) are used in pharmaceutical and cosmetic products (Jamshidi-Kia *et al.,* 2018). Several plants contain biodynamic ingredients that have been verified as medicinally beneficial through repeated field/clinical testing and laboratory analyses (Halberstein, 2005). Medicinal plants (MP) are known to exhibit wide range of bioactivities such as antidiabetic, antifungal, antimalarial, anti-inflammatory, antipyretic, gastroprotective and hepatoprotective effects (Gupta et al., 2016). They have also been shown to display antioxidant activity: Phyllanthus fischeri (Odongo et al., 2017), Eugenia caryophyllus (Khalaf et al., 2008), Asparagus acutifolius (Adawia et al., 2016). These antioxidants help prevent oxidative stress which has been linked to chronic and degenerative disorders. MPs also exhibit antimicrobial activity against numerous microorganisms: Newbouldia laevis extracts showed to be effective against Escherichia coli, and Staphylococcus aureus (Oloyede et al., 2010) while Anabasis aphylla exhibit an antimicrobial effect against Proteus miribilis, Candida albicans and Aspergillus niger (Shakeri et al., 2012). *Euclea mayottensis* is used in the Comoros in the treatment of dental caries. The local people use the dried roots as tooth stick to brush their tooth. *Tambourissa comorensis* is used to treat several diseases including bacterial infections. The fruits are the main part used for treatment of skin diseases. A powdered dried fruits is mixed with water and applied directly in the face. The mask is also used to protect the face against sun light.

2.2.1 Antibacterial activity of medicinal plants

For ages nature has gifted humans with plenty of herbs and plants which form the main source of traditional medicines used to help in relief from illness and are still widely used all over the world (Al-Daihan *et al.,* 2012). The rise of antibiotic-resistant microorganisms has given extra impetus in the search for novel antibacterial compounds (Tan & Lim, 2015). Medicinal plants are known to possess antibacterial effect against a large number of pathogens (Table 2.1): Oils from *Cinnamomum osmophloeum* have been shown to possess antibacterial activity against *Escherichia*

Table 2.1: Antibacterial range of some phytochemicals present in other medicinal plants

Compound	Classification	Plants species	Antibacterial range	Reference
1,8-cineol (Eucalyptol)	Terpenoids	*Eucalyptus astrengens*	*B. cereus, E. coli, S. aureus*	Sebei *et al,* 2015
Chrysoeriol-7-O-β-D-xyloside	Flavonoid glycosides	*Graptophyllum grandulosum*	*S. aureus, V. cholerae*	Tagousop *et al.,* 2018
Artemisin	Terpenoids	*Artemisia annua*	*S. aureus, B. subtilis, Salmonella sp*	Appalasemy *et al., 2014*
Tomato glycosides	Glycosides	*Solanum lycopersicum*	*E. coli*	Li & Zhang, 2014
Luteolin-7-O-glucoside	Flavonoids	*Mentha longifolia*	*S. aureus, B. cereus, B. subtilis*	Akroum *et al.,* 2009
Quercetin-3-O-glycosid	Flavonoids	*Mentha longifolia*	*S. aureus, P. aeruginosa, E.coli*	Akroum *et al.,* 2009
Vitaflavan (dietary complement)	Polyphenols	*Vitis vinifera*	*E. faecalis, S. aureus, S. pneumoniae*	Cueva *et al.,* 2012
3-O-α-L-arabinopyranosyl-phytolaccagenic acid	Saponins	*Chenopodium quinoa*	*S. aureus, S. epidermis*	Dong *et al.,* 2020
Sanguinarine	Alkaloids	*Chelidonium majus*	*S. aureus*	Zielińska *et al.,* 2019
Ellagitannin	Tannins	*Nuphar variegata*	*P. vulgare*	Nishizawa *et al.,* 1990

coli, *Enterococcus faecalis*, *Staphylococcus aureus* (Ncube *et al.,* 2008); *Terminalia arjuna* chloroform extract exhibit activity against *E.coli* and *Candidas albican* (Gupta *et al.,* 2016). Methanolic extract of *Zingiber officinale* in the study of Al-Daihan *et al.,* (2013) showed activity against *Staphylococcus pyogenes*. The methanol extract of the stem bark of *W. ugandensis* showed antibacterial effect against a multi-drug resistant strain of *E. coli* (Njiire *et al.,* 2014). *C. albicans* showed a susceptibility against *Bidens pilosa, Senna didymobotrya* (Maobe *et al.,* 2013), and *W. ugandensis* (Olila *et al.,* 2001). *E. natalensis* root bark extract showed a potent antibacterial effect against gram-positive bacterial strains (Weigenand *et al.,* 2004) while compounds isolated from *E. crispa* leaves showed antimicrobial activity with inhibition zone against *S. aureus* and *E.coli* (Palanisamy *et al.,* 2019).

2.2.2 Antioxidant activity of medicinal plants

Antioxidant phytochemicals are present widely in fruits, vegetables and medicinal plants (Zangh *et al.,* 2015). Research has proved that foods with antioxidant phytochemicals help to provide protection against diseases such as cancer, diabetes, heart disease, and other type of chronic diseases (Patil *et al.,* 2016). Natural antioxidants mainly come from plants in the form of phenolic compounds (flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, tocotrienols) ascorbic acid and carotenoids (Adawia *et al.,* 2016). The antioxidant activity of medicinal plants has been shown in numerous studies: Butanol extracts of *Andosonia digitata* and *Alchornea laxiflora* showed 70-78% antioxidant activity using the ferric thiocyanate method (Oloyede *et al.,* 2010); Methanolic extracts of *Punica granatum* peel exhibited 70.8% antioxidant activity using the DPPH method (Hamadnala, 2020). Ethyl acetate extract of *Anabasis anaphyla* exhibited 81.8% antioxidant activity using the β carotene/ linoleic acid assay while the methanolic extract showed 79.3% (Shakeri *et al.,* 2012). The essential oils of *Vetivera zizanoids* showed a 60.43% antioxidant activity using the DPPH method (Soidrou *et al.,* 2020). Using the free radical scavenging method, a preliminary study of *T. comorensis* showed a potential antioxidant activity of 78% (Soule *et al*., 2017). E. schimperi leaf extracts showed a significant amount of antioxidant activity using the DPPH radical scavenging effect assay and FRAP (Mekonnen *et al.,* 2018). E. natalensis and E.crispa are also known to exhibit potent antioxidant effect (Maroyi et al., 2017; Palinasamy *et al.,* 2019).

2.3 *Tambourissa comorensis*

2.3.1 Monimiaceae family

T. comorensis belongs to the family Monimiaceae which comprises about 34 genera. The representatives of the family are mostly occurring in humid evergreen tropical and subtropical regions up to 3000 m above sea level. The trees grow as the forest subcanopy and lower woody level (Bobrov *et al*., 2017). According to the post molecular botanical classification, Monimiaceae are dicotyledons belonging to the Laurales order (*Table 2.2*), including Amborellaceae, Trimeniaceae, Gomortegaceae, Calycanthaceae and Hernandiaceae (Lhuilier, 2007).

Table 2.2: Taxonomy of Monimiaceae according to Cronquist (Lhuilier, 2007)

Divison	Spermatophyta
Subdivision	Angiospermeae
Class	Magnoliopsida
Subclass	Magnoliidae
Order	Laurales
Family	Monimiaceae

Monimiaceae family is used in numerous ways. The genus Hedycarya, Doryphora, Peumus are used as fire wood; some species producing essential oils, are used locally in perfume industry (Lhuilier, 2007). Some species are also used as medicinal plants. In the Comoros archipelago, they are used to treat malaria, headache, stomachache and sterility (Mohamed, 2012).

2.3.2 Tambourissa genus

The Tambourissa genus is represented by around 43 species exclusively distributed in the islands of the Indian Ocean which comprises 26 species(spp) in Madagascar, 5 spp in Comoros, 10 spp in Mauritius and 2 spp in La Reunion (Lhuilier, 2007; Mohamed, 2012). Those species show a high degree of endemicity, each being restricted to a given island. In the Comoros, Tambourissa genus is known to be used to treat dermal disease and malaria. It is also used as a painkiller. *T. comorensis* is an endemic species of the Comoros locally known as ‘Mbosa or Mledjeza’.

Fig 2.1: Tree (*Left*), Fruit (*Right*) and Leaves (*Below*) of *T. comorensis*

It is a tree with a rigorous bark (Fig 2.1). Its leaves are ovals with an indented apex and around 13 to 14 cm long and 5.5 cm wide. The fruits are brown with a large orifice and are normally found in the tree trunk (Mohamed, 2012). *T. comorensis* is an endemic species of the Comoros archipelago. Different part of the plants are used to treat different type of ailment. In the islands of Ngazidja, the fruits are the main part used. Fresh fruits are used to treat stomachache and ulcers while its dried fruit is mainly used in the treatment of skin problems (acnes, dark spot, etc.). The dried fruit is ground to a fine powder and mixed with water to make a mask. This mask is applied directly in the whole face or where the acnes or dark spot is for a few hours. The mask is also to protect from the direct sun light. A preliminary screening has shown the presence of flavonoids and tannins (Soule *et al*., 2017).

2.4 Euclea mayottensis

2.4.1 Ebenaceae family

The Ebenaceae (ebony family) (Table 2.3.) are pantropical in distribution and encompass the genera *Diospyros* and *Euclea* with approximately 500 to 600 species. They are trees, shrubs or subshrubs with simple leaves. The bark of the roots are black for many species and the leaves are simple, usually alternate (Wallnöfer, 2001). Ebenaceae family has species used as a tooth stick and in the treatment of toothache for instance *E. undulata*, *E. divinorum*, and *D.lycioides* (Joshua *et al*., 2013). *D. mespilifprmis* is used to treat certain diseases including wounds (Ebbo *et al.*, 2019). *E. shimperi* is used in the treatment of headache, pain and spasm (Mekonnen *et al*., 2018).

Table 2.3: Taxonomy of Ebenaceae family

Kingdom	Plantae
Subkingdom	Virdiplantae
Infrakingdom	Streptophyta
Superdivision	Embryophyta
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Superorder	Asteranae
Order	Ericales
Family	Ebenaceae

2.4.2 Euclea genus

Euclea genus is represented by 18 species including *Euclea mayottensis.* The main centers of diversity of the plants are in South East Asia, Madagascar and tropical Africa (Wallnöfer, 2001). Numerous phytochemical compounds have been observed from Euclea genus : flavonoids, glycosides and phenols have been observed in *E. racemosa* root (Teklay *et al*., 2015). Compounds belonging to naphthoquinone and pentacyclic terpenoids classes have been isolated from *E. natalensis* leaves (Maroyi, 2017a). Phytochemical analysis of the methanol leaf and stem extracts *E. undulata* showed the presence of alkaloids, diterpenes, glycosides, saponins and tannins (Maroyi, 2017b).

Fig 2.2: Leaves, stem and roots of *E. mayottensis*

Euclea mayottensis (Fig 2.2) is located in the Comoros archipelago. Although most people uses the leaves in the preparation of herbal medicine, Comorians use mainly the roots of E. mayottensis. They are first dried then wash with water. After that they are used firectly as toothbrush for the treatment and prevention of dental caries. However, no scientific study has been done to validate its medicinal properties.

2.5 Plant secondary metabolites

In addition to the primary metabolites (proteins, carbohydrates, fats), that play a major role in maintenance of plant viability, a series of compounds which belong to the secondary metabolism, are also synthesized (Ferdes, 2018). They include terpenes, polyphenols, quinones, alkaloids, and peptides. Secondary metabolites are presents in some species and often exhibit an organ or tissue specificity. Many of the plant secondary metabolites are constitutive, existing in healthy plants in their biologically active forms, but others occur as inactive precursors. They are activated in response to tissue damage or pathogen attack. The bioactivity exhibited by the plants is because of the plant secondary metabolites, and has increased the interest in the pharmaceutical design of new drugs of plant origin.

Plant secondary metabolites, or ‘‘phytochemicals’’, are produced by plants for a myriad of functions, from UV protection, protection against pathogens and herbivores, to other means of

improving the plant's survivability and health. They are not being directly involved in crucial functions of the plant like the growth and reproduction and are produced through various pathways (*Fig 2.3*). In the past few decades, a great increase in scientific interest around these compounds and their benefit to human health has been noticed, as many of them exhibit considerable antioxidant and antibacterial activity (Tan & Lim, 2015). Numerous alkaloids, flavonoids, glycosides, terpenes, tannins and polyphenols from plant origins have been shown to have antibacterial, antifungal and antioxidant properties.

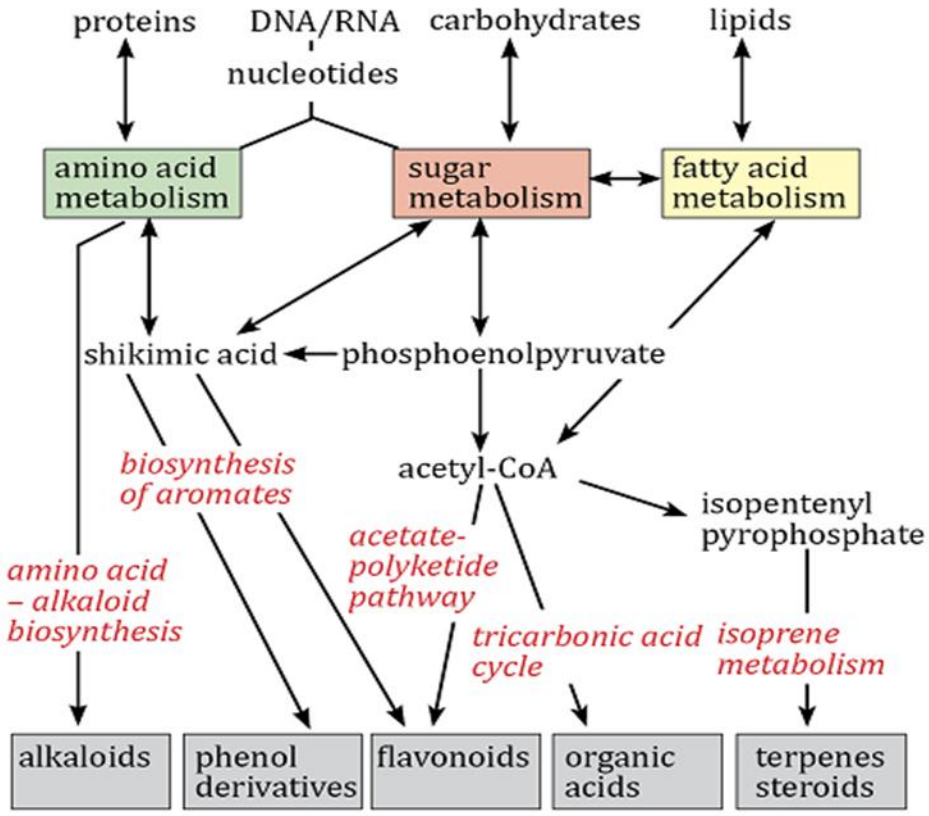


Fig 2.3: Biosynthetic pathways and precursors for major classes of secondary metabolites (Ferdes, 2018).

2.5.1 Alkaloids

The alkaloids are a large family of more than 15,000 nitrogen-containing secondary metabolites. They are found in approximately 20% of vascular plant species (Norton & Karczub, 2015).

Many of the earliest isolated pure compounds with biological activity were alkaloids (Ncube *et al.,* 2008). Alkaloids are divided into 3 sub-major classes depending on the precursors and the final structure. They are usually synthesized from one of a few common amino acids—in particular, lysine, tyrosine, or tryptophan (Norton & Karczub, 2015). Generally, a plant contains several alkaloids. The alkaloid content depends on the age of the plant, region, climate and season. Alkaloids exert considerable physiological effects on humans and animals and are used in therapeutics as narcotics and have a calming effect (Ferdes, 2018). Nearly all alkaloids are toxic to humans when taken in large quantities. For example, strychnine, atropine, and coniine are classic alkaloid poisons At lower doses, however, many are useful pharmacologically, for instance morphine, codeine, and scopolamine are just a few of the plant alkaloids currently used in medicine (Norton & Karczub, 2015). Other alkaloids, including cocaine, nicotine, and caffeine have widespread nonmedical uses as stimulants or sedatives (Norton & Karczub, 2015). Alkaloids also serves as scaffolds for important antibacterial drugs such as metronidazole and the quinolones (Cushnie *et al.,* 2014). As a group of promising natural antibiotics, the alkaloids can be recovered from many natural sources, and they have a wide antibacterial spectrum with a good antibacterial effect on common clinical strains, including drug-resistant bacteria (Yan *et al.,* 2021). In an antibacterial activity test using the broth dilution method, thalicfoetine (isoquinoline alkaloid) significantly inhibited *Bacillus subtilis* with an MIC of 3.12 µg/mL while isoquinoline alkaloids isolated from *Chelidonium majus* showed that chelerythrine was the most effective against *Pseudomonas. aeruginosa* (MIC, 1.9 µg/mL) (Zielińska *et al.,* 2019; Ding *et al.,* 2019).

2.5.2 Polyphenols

Polyphenols are one of the most important and at the same time the most numerous of the secondary metabolite groups, omnipresent in the plant kingdom (Ferdes, 2018). Plant phenolics are synthesized by several different routes (*Fig 2.4*) and thus constitute a heterogeneous group from a metabolic point of view (Norton & Karczub, 2015). Depending on the number of phenolic rings they contain and the structural elements linking those rings, polyphenols are classified as phenolic acids, flavonoids, lignans, stilbens and other polyphenols with non-flavonoids structure (Ferdes, 2018). They are generally involved in the defense against ultraviolet radiation, oxidizing agents or the aggression of some phytopathogenic agents (Ferdes,

2018). Polyphenols have many health beneficial properties: studies have shown that they decrease the risk of cardiovascular diseases, improve dental health by protecting against bacterial induced dental caries and were also found to have anti-ageing properties (Badyal *et al.*, 2020). The phenolic content of *T. comorensis* was estimated to be 0.63 g/l GAE (Gallic Acid Equivalent) in its methanolic extract (Soule *et al.*, 2017). The antimicrobial activity of polyphenols occurring in medicinal plants has been investigated against a wide range of microorganisms: phenolic acids (non-flavonoids polyphenols) have been shown to have an antibacterial effect against *S. aureus*, *E. coli* and *P. aeruginosa* (Daglia, 2012). The ability to inhibit the growth of respiratory pathogenic bacteria was reported for several wine phenolic compounds, in particular gallic acid and ethyl gallate (Cueva *et al.*, 2015).

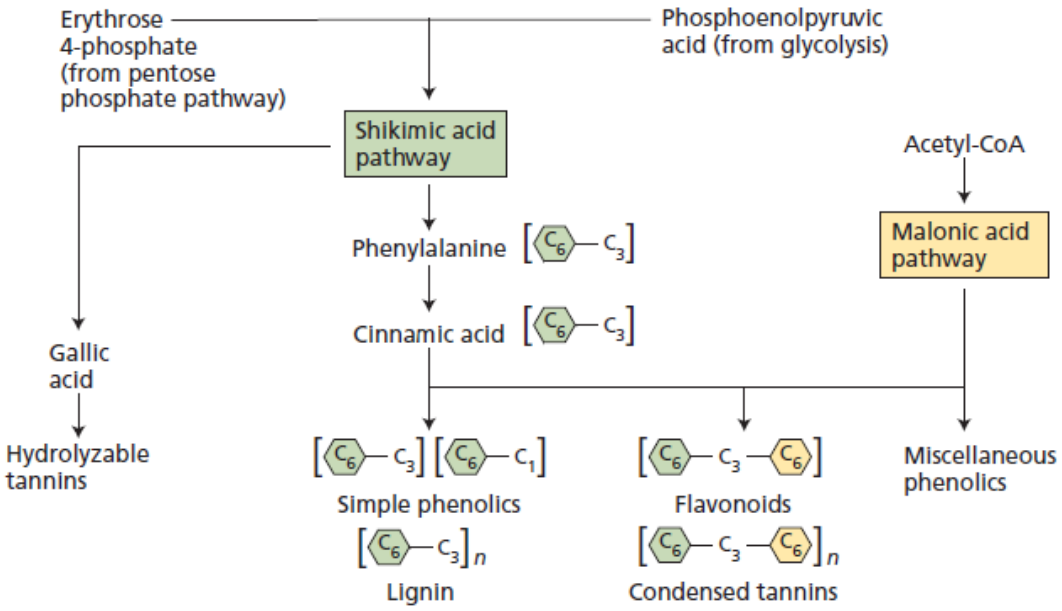


Fig 2.4: Biosynthesis pathway of plant phenols (Norton & Karczub, 2015)
Formulas in brackets indicate the basic arrangement of carbon skeletons: C_6 indicates a benzene ring, and C_3 is a three-carbon chain.

2.5.3 Terpenes/Terpenoids

Terpenes are the organic, chemical components of essential oils and one of the most diverse plant natural products as far as their structure is concerned (Badyal *et al.*, 2020). They are among the largest and most diverse groups of plant secondary metabolites and they include sterols and

triterpenes (*Fig 2.5*) (Ncube *et al*., 2008). Another sub-class of compounds under the terpenes is essential oils. They are aromatic compounds insoluble in water but soluble in organic solvents (Ferdes, 2018). Terpenes are synthesized from acetyl-CoA or its glycolytic intermediates. 'Terpenoids' are similar to the terpene with a slight difference; terpenoids are the derivatives of terpenes which have been denatured by oxidation, that is, they possess an extra oxygen atom in their chemical structure (Badyal *et al.,* 2020). They represent the most important group of active compounds in plants with more than 23,000 known structures (Kabera *et al.,* 2014). Studies have demonstrated a broad spectrum of pharmacological and physiological properties of terpenes. They possess antihypertensive, antimicrobial and insecticidal properties (Kabera *et al.,* 2014). Essential oils possess biological activity including antibacterial, antiviral, antifungal and anti-inflammatory effects (Ncube *et al.,* 2008). 3-acetyl aleuritolic acid, a triterpenoid isolated from *Spirostachys africana* exhibited a minimum inhibitory concentration of 50 µg/ml against *E. coli* and *S. aureus* (Mathabe *et al.,* 2008). Eugenol and terpineol, two terpenoids found in essential oils, exhibited rapid bactericidal effect against *Salmonella enterica* and *S. aureus* respectively (Guimarães *et al.,* 2019).

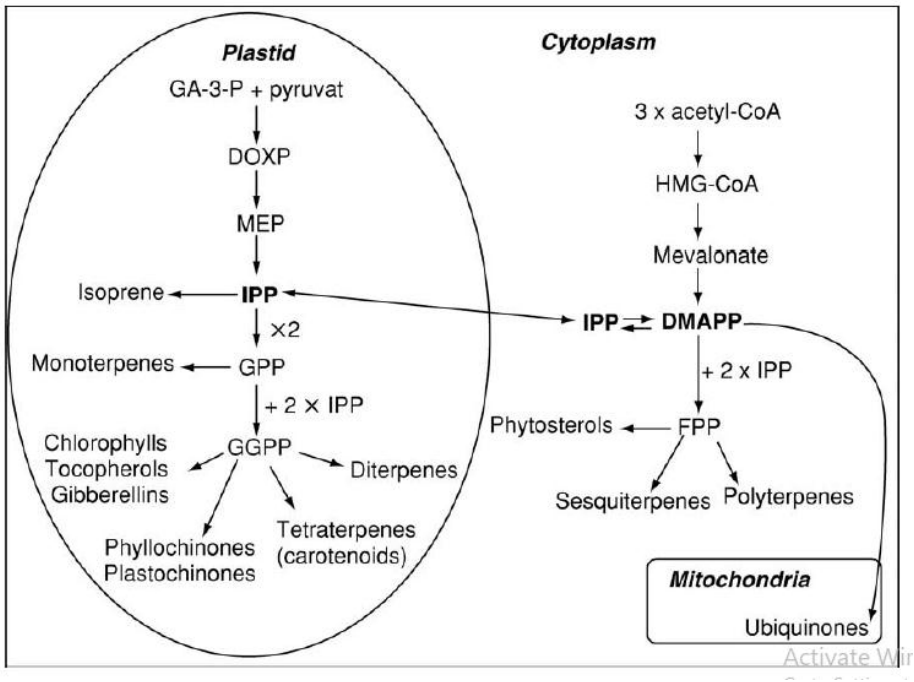


Fig 2.5: Basic reactions during terpenoids synthesis (Grassman, 2005)

[GA- 3- P: D-glyceraldehyde- 3- phosphate; DOXP: 1- deoxy- D- xylulose- 5- phosphate; MEP: methylerythritol-

4- phosphate; IPP: isopentenyl diphosphate; GPP: geranyl diphosphate; GGPP: geranylgeranyl

diphosphate; HMG: hydroxymethylglutaryl; DMAPP: dimethylallyl diphosphate; FPP: farnesyl diphosphate]

2.5.4 Tannins

Tannins are polymeric phenolic compounds with different biological activities; they are synthesized in all organs of the plant, especially in leaves, roots and stems (Ferdes, 2018). Tannins have been found in a variety of plants utilized as food and feed which include food grains such as sorghum, millets, barley, dry beans, etc. and fruits such as apples, bananas, blackberries, dates, grapes, peaches, pears, raspberries, and strawberries (Chung *et al.,* 1998). There are two categories of tannins: condensed and hydrolyzable. Condensed tannins are compounds formed by the polymerization of flavonoid units while hydrolyzable tannins are heterogeneous polymers containing phenolic acids, especially gallic acid, and simple sugars (Norton & Karczub, 2015). Tannins play an important biochemical role for the plants: they increase the resistance of plants to viruses and microorganisms; they stimulate phagocytic cell activity; and they have antibacterial and antifungal activities (Ferdes, 2018). A preliminary phytochemical screening of *T. comorensis* showed the presence of tannins in its methanolic extracts (Soule *et al.,* 2017). Tannins also constitute the active ingredients of plant-based medicines (Chung *et al.,* 1998; Kabera *et al.,* 2014). They have been reported to be bacteriostatic and/or bactericidal for *Staphylococcus aureus, Streptococcus pneumonia, Bacillus anthracis, Shigella dysenteriae,* and *Salmonella senftenberg* (Chung *et al.,* 1998). Tannins extracted from *Solanum trilobatum* showed a great antibacterial activity against *S. aureus* and *Streptococcus pyrogens* with MIC value of 1 and 2 mg/ml respectively (Doss *et al.,* 2008). Hydrolyzable tannins extracted from different medicinal plants showed a promising antibacterial activity against *H. pylori* and *E. coli* (Funatogawa *et al.,* 2004).

2.5.5 Flavonoids

Flavonoids are phenolic compounds synthesized in the cytoplasm of the plant cell and then accumulated in vacuoles. The flavonoids are one of the largest classes of plant phenolics (*Fig 2.6*), classified primarily on the basis of the degree of oxidation of the three-carbon bridge (Norton & Karczub, 2015). Whereas both hydroxyl groups and sugars increase the water solubility of flavonoids, other substituents, such as methyl ethers or modified isopentyl units, make flavonoids lipophilic (hydrophobic) (Norton & Karczub, 2015). They have a defensive

function against insects, fungi and viruses, as well as against invading invertebrates (Ferdes, 2018). Flavonoids protect plants from different biotic and abiotic stresses and act as unique UV filters, function as signal molecules, allopathic compounds, phytoalexins, detoxifying agents and antimicrobial defensive compounds (Panche *et al.,* 2016). The phytochemical screening of T comorensis showed the presence of flavonoids and has been estimated to be 0.47 µg/ml QE (Quercentin equivalent) in the methanolic extract and 0.14 µg/ml QE for its ethyl acetate extracts (Soule *et al*., 2017).

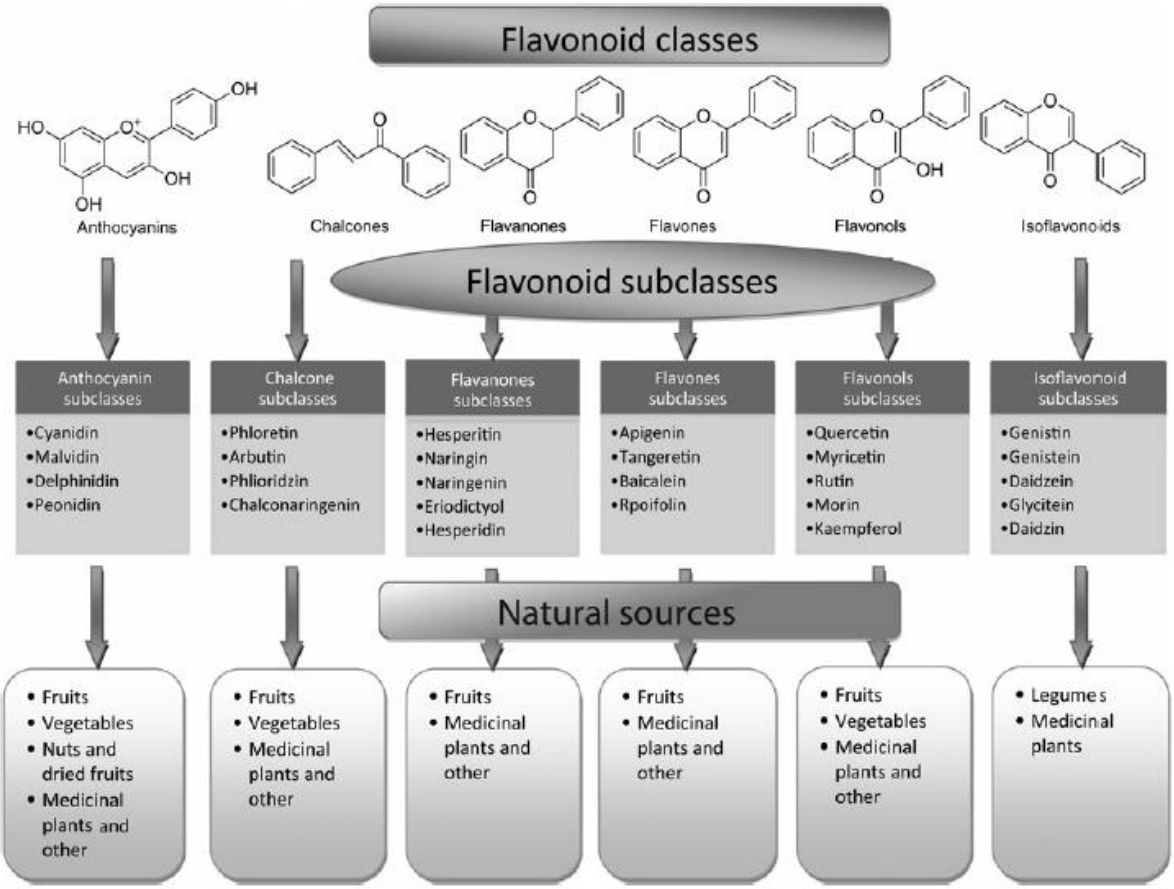


Fig 2.6: Flavonoids classes, subclasses and natural sources (Panche *et al.*, 2016)

2.5.6 Saponins

Saponins are compounds derived from steroids or triterpenoid glycosides, which occur in many plants and act on microbial cells by permeabilization of the membrane. The presence of both

lipid-soluble (the steroid or triterpene) and water-soluble (the sugar) elements in one molecule gives saponins detergent properties, and they form a soapy lather when shaken with water (Norton & Karczub, 2015). The main function of saponins in plant is antimicrobial activity and to protect plants from the attack of insects (Badyal *et al.,* 2020). In their study, Avato *et al* (2016) reported the antibacterial effect of the sapogenins from *Medicago arborea* against *Bacillus subtilis* (MIC= 125 µg/ml) and *Bacillus cereus* (MIC= 42.5 µg/ml). Moreover, medicagenic acid also acted against *S. aureus* (MIC= 52.5 µg/Ml). Oleanolic acid and glucopyranosyl ester, two saponins extracted from *Melanthera elliptica* showed a definite antimicrobial effect against *S. aureus*, *E. coli* and *C. neoformans* (Tagousop *et al.,* 2018). Saponins are widely distributed in the plant kingdom and have many physiochemical (foaming, emulsification, sweetness, bitterness, solubilization, etc.) and biological properties (haemolytic, antimicrobial, antioxidant, insecticidal, etc.) exploited in many applications in food, cosmetics, pharmaceutical industries and soil bioremediation (Kabera *et al.,* 2014). Some saponins may enhance nutrient absorption and aid in animal digestion.

2.5.7 Glycosides

Glycosides may be phenol, alcohol or sulfur compounds and they are characterized by a sugar portion or moiety attached by a special bond to one or more non-sugar portions (Kabera *et al.,* 2020). Many plants store chemicals in the form of inactive glycosides. These can be activated by enzyme hydrolysis, which causes the sugar part to break, making the chemical available for use. Glycosides are reported to have antifungal, antimicrobial, anticancer, antioxidant and anti-inflammatory activities (Ferdes, 2018). 11-hydroxystearic acid 11-O-(6-O-acetyl-β-D-glucopyranoside, a glycoside isolated from *Ibicella lutea* showed a great antibacterial activity against *S. aureus* and MRSA clinical isolates (Rosa *et al.,* 2010). In their study, Pattananandecha et al (2021), revealed that cyanidin 3-O-glucoside, the major anthocyanin glycoside present in purple rice had a high antibacterial activity against foodborne pathogens including *S. aureus, E. coli, S. enteritidis*, and *Vibrio parahaemolyticus.*

2.6 Phytochemical screening of plant secondary metabolites

Plant secondary metabolites usually accumulate in specific parts. A typical extraction method require consideration of the following 3 steps (Sticher, 2008):

- Preparation of plant materials
- Choice of solvents
- Choice of extraction methods

2.6.1 Preparation of plant materials

The easiest and efficient way to prepare the plants is to dry and grind the plant material (Sticher, 2008). Drying is an important process which reduces the moisture content of fresh materials for long storage and minimizes the costs of transportation and preservation. There are different methods of drying such as air-drying, microwave-drying, oven-drying and freeze-drying. Air-drying doesn't force dried plant materials using high temperature thus allowing preservation of heat-labile compounds such as polyphenols (Sticher et al, 2008).

2.6.2 Choice of solvents

The choice of the solvents is an important step of the extraction. Depending on the phytochemical, one solvent would give a high yield compared to others. Factors that should be considered when choosing a solvent or solvent system for extracting plant material include solubility of the target constituents, safety, ease of working with the solvent, potential for artifact formation, and the grade and purity of the solvent (Sticher, 2008). The solvents are classified depending on their polarity:

- Polar: water, ethanol, methanol, etc.
- Medium polar: ethyl acetate, dichloromethane, etc.
- Non polar: n-hexane, etc.

Depending on the phytochemical, one solvent would give a high yield compared to others: The ethyl acetate extracts of *T. comorensis* tested negative for the presence of tannins while they were presents in its methanolic extracts (Soule *et al.,* 2017). *Punica granatum* showed a higher amount of tannins extracted in its chloroformic extracts than in its ethyl acetate extracts (Hamadnalla *et al.,* 2020).

2.6.3 Choice of extraction methods

The aim of every extraction is to separate the soluble plant metabolites from the insoluble ones. A crude extract contain a mixture of plant secondary metabolites such as alkaloids, flavonoids, phenolics, terpenoids and glycosides (Non, 2015). To separate these metabolites, different extraction methods can be used such us maceration, percolation, Soxhlet extraction, microwave-assisted extraction and ultra sound-assisted extraction each varying in cost and level of complexity.

Percolation is useful and efficient for medium and large sample size. Maceration is mainly used for small sample. Maceration is useful in preserving thermolabile phytochemical such as polyphenols as no heat is needed during extraction (Nn, 2015). Moreover maceration is cost friendly and a simple and quick process that can be repeated fairly easily (‘‘Extraction Magazine’’, 2022).

Soxhlet extraction, is a convenient method for extraction of small to moderate volumes of plant material and also use small amounts of solvent compared to percolation and maceration, however the heat needed may cause thermolabile constituents to form artifacts or decomposition products (Sticher, 2008).

Microwave and ultra sound assisted extractions both don’t need a large amount of solvent. However these techniques are expensive to set up (‘‘Extraction Magazine’’, 2018).

Chapter Three: Materials and Methods

3.1 Overview of the methodology

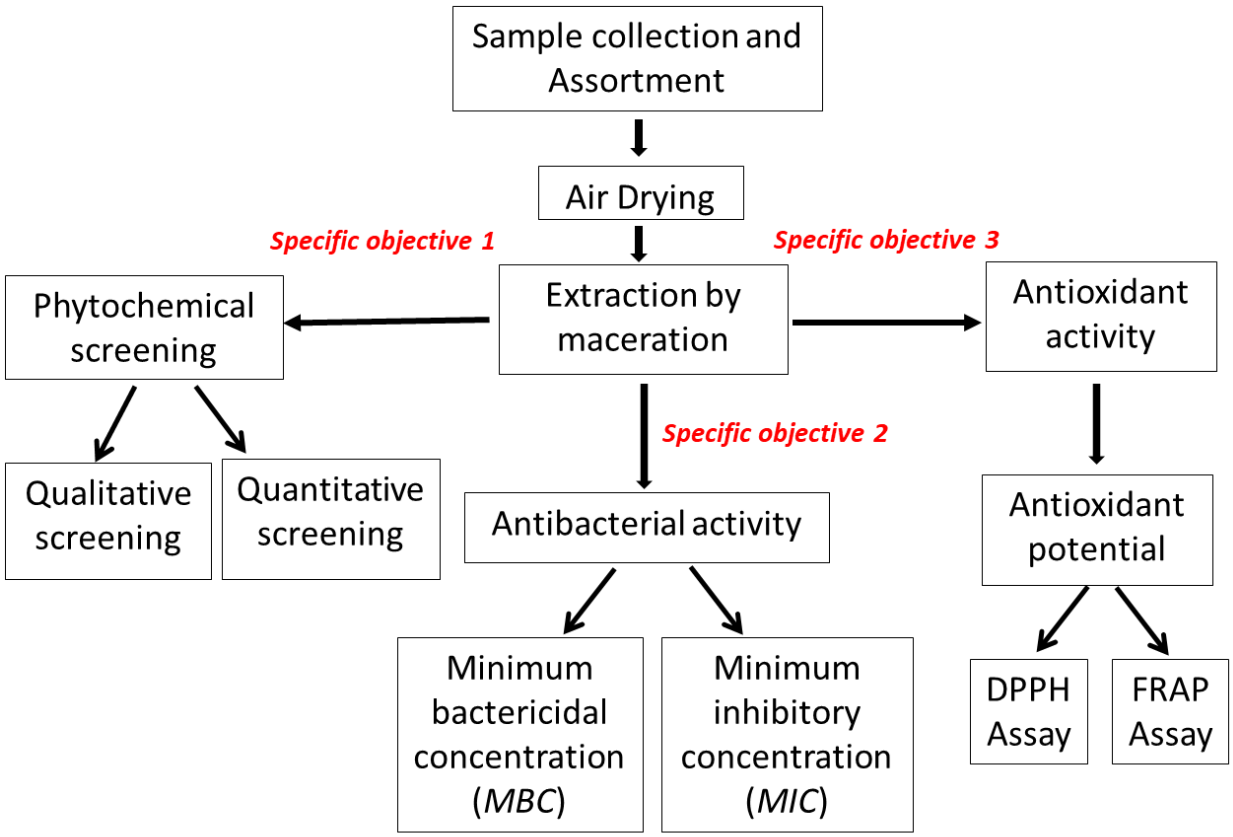


Fig 3.1: Flow chart of the methods.

3.2 Collection of plant materials, drying and storage

The plant materials were collected from the Comoros, specifically in the Islands of Ngazidja. *Tambourissa comorensis* fruits were harvested in Maoueni in the region of Mboude (11° 27' 48" South, 43° 18' 58" East). *Euclea mayottensis* roots were collected from Diboini Plateau (11° 33' 25" South, 43° 18' 52" East). Each parts of the collected plants weighed 250g. The two plants were identified at the National Herbarium of Comoros where voucher specimens have been deposited. After collection, the plants were dried using air drying method (*Fig 3.1*). They were kept in a room where air could circulate freely without the presence of the sun until they were

completely dried. After drying, the plant materials weighed approximately 200 g each. The dried plant materials were then airlifted to Uganda where the grinding to fine particles took place. Analysis of the fine plant powders took place at the Departments of Biochemistry and Sports Science and Medical Microbiology, Makerere University.

3.3 Plant extractions

The plants were extracted using the maceration method as described by Gberikon *et al.,* (2015) with slight modifications. Briefly, 20 g of powdered plant materials were weighed and put in sterile bottles. Three different solvents were used: water, ethyl acetate and ethanol (70%). Each weighed-out plant powders was macerated in 100 ml of each solvent separately in tightly covered bottles. They were left to macerate for 72 hours and were shaken every 24 hours. The resultant suspensions were filtered into sterile beakers, and filtrates collected were re-filtered using Whatman No. 1 filter paper into sterile sample bottles. The solvents were evaporated and the extracts were dried using an oven at 40° C. They were then labelled appropriately and stored in a fridge at 3° C for further analyses.

3.4 Qualitative phytochemical screening

Plant extracts were screened for the presence of alkaloids, flavonoids, glycosides, tannins, saponins, terpenoids, and polyphenols. The liquid form of the extracts for the phytochemical screening was obtained by preparing solution of 0.01 mg/ml of the extracts.

3.4.1 Alkaloids

The presence of alkaloids was determined using Wagner reagent as described by Shah & Hossain (2014): To a 2 ml of the extract, 2 ml of Wagner reagent was added by the side of the test tube. A prominent brown or yellow precipitate indicated the test was positive.

3.4.2 Flavonoids

As described by Shah & Hossain (2014), few drops of dilute sodium hydroxide were added to one milliliter of the extract. An intense yellow color which became colorless on the addition of a few drops of dilute acid indicated the presence of flavonoids.

3.4.3 Polyphenols

Using the ferric chloride acid method (Sticher, 2008), 5% (w/v) of ferric chloride (FeCl3) were dissolved in water. 10 drops of the solution were then added to 3 ml the extract which produced a blue, blue-black, or blue-green color reaction in the presence of polyphenols.

3.4.4 Tannins

Three milliliters of the extract and 5 drops of 1% lead acetate were mixed in a test tube. A yellow precipitate indicated the presence of tannins (Shah & Hossain, 2014).

3.4.5 Terpenoids

Few milliliters of the extracts were suspended in 2 ml of chloroform in a test tube. Then 5 ml of concentrated sulphuric acid was added carefully to form a layer. A reddish-brown color interface indicated a positive result for the presence of terpenoids.

3.4.6 Saponins

Following the method of Shah & Hossain (2014), the crude extract solutions were diluted with 20 ml of distilled water and were agitated in a graduated cylinder for 15 min. The formation of 1cm foam layer showed the presence of saponins.

3.4.7 Glycosides

To 2 ml of extract, 1 ml of glacial acetic acid, 3 drops of 5% Ferric chloride and concentrated drop-wise suphuric acid were added. A reddish brown color at the junction of two layers and bluish green in upper layer indicated the presence of glycosides (Hamadnalla, 2020).

3.5 Quantitative screening

3.5.1 Determination of total phenolic content

Total phenolic content was determined spectrophotometrically using the Folin Ciocalteu method (Sahu & Saxena, 2013) with slight modifications: A 100ml stock solution of concentration

1mg/ml of gallic acid was prepared in methanol (100%). Standard solutions of concentration varying from 0.01 to 0.05 mg/ml were prepared from the stock solution and used to construct a calibration curve. The extract solutions of concentration 0.6 mg/ml were also prepared in methanol (100%). Then 0.5 ml of each sample was introduced into test tubes and mixed with 2 ml of a 10-fold dilute Folin-Ciocalteu reagent. After 6 min, 2 ml of 7.5% sodium carbonate was added in the test tubes. They were then covered with parafilm and allowed to stand at room temperature for 30 min. The absorbance was read at 750 nm using a UV spectrophotometer. Gallic acid was used a standard and the total phenolic content was expressed as mg GAE/g dry extracts. This was done in triplicates.

3.6 Antibacterial assay

The different plant extracts were tested against 4 different microorganisms: *Esherichia coli* (ATCC 25922), *Staphilococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and *Viridae streptococci*. Resistant isolates of *E. coli* (Extended Beta Lactamase positive 8403), *S. aureus* (Methicillin Resistant 8372) and *P. aeruginosa* (Carbapenem resistant 7106) were also used.

3.6.1 Zones of inhibition

The microbial growth inhibitory potential of the plant extracts were determined using the agar well diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS, 1993) with slight modifications: The microorganisms were first spread and incubated overnight in MacConckey agar for the gram negative and blood agar for gram positive. Then pure colonies were emulsified in sterile normal physiological saline to 0.5 Mc Farland (107 CFU/ml) of turbidity. After that, inoculum of each bacterial culture to be tested was spread on Mueller Hinton agar plates with a sterile swab moistened with the bacterial suspension. Subsequently, wells of 7 mm diameter were punched into the agar medium and filled with approximately 100 µl of different concentrations of plant extracts; stock solutions, 40 mg/ml, 20 mg/ml and 1mg/ml. The concentration of the stock solutions was: *E. mayottensis* water 256 mg/ml; *E. mayottensis* ethanol 54.5 mg/ml; *E. mayottensis* ethyl acetate 173 mg/ml; *T. comorensis* water 193 mg/ml; *T. comorensis* ethanol 88 mg/ml and *T. comorensis* ethyl acetate

87.5 mg/ml. The extracts were allowed to diffuse at room temperature for 2 hours. This was done in triplicates. The plates were then incubated in the upright position at 37° C for 24 hours.

Wells containing approximately the same volume of ethanol, ethyl-acetate, and distilled water served as negative controls while standard antibiotic discs of ampicillin, erythromycin, piperacillin and penicillin were used as the positive controls respectively against *E. coli, S. aureus, P. aeruginosa* and *V. streptococci*. After incubation, the diameters of the growth inhibition zones were measured in mm using a ruler.

3.6.2 MIC/MBC determination

The minimum inhibitory concentration (MIC) and the minimum bacteriocidal concentration (MBC) were determined using the broth micro-dilution assay as described by (Mbanga *et al.*, 2013) with slight modifications. The inocula of the different microorganism was adjusted to the 0.5 McFarland standard. A dilution of 1/100 of the standardized bacteria was performed. Using sterile broth as diluent, a dilution series for each assayed extract was made in a total volume of 100 ml giving final concentrations ranging from 0.16 mg to 256 mg/ml. A 96 well plate was used and each well was inoculated with 50 µl of extracts and 20 µl of the test bacterial suspension. The wells were then incubated aerobically at 37° C overnight. After the overnight incubation, 20 µl of 0.0015% resuzirin dye was added in each well and they were incubated for 2 hours. A purple to blue color indicated inhibition of the tested strains while a pink color indicated growth in the plate. The MIC was recorded as the lowest concentration inhibiting growth. Positive control consisted of a well having 50 µl of the diluent and 20 µl of the tested strains. Negative control well contained only the broth. A culture suspension of 50 µl was taken from each plate that showed no growth, and sub-cultured onto Mueller Hinton agar. The plates were then incubated aerobically at 37°C for 48 h. The lowest concentration of the extract which completely inhibited growth was expressed as the MBC. These were done in triplicates.

3.7 Antioxidant assay

3.7.1 Free radical scavenging activity by DPPH method

The effect of the extracts on the scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined according to the method described by Adawia *et al.* (2016) with slight modifications. DPPH is a free stable radical with purple color. When DPPH is mixed with a substrate that can donate a hydrogen atom (antioxidants), the odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from the antioxidant which results in the loss of the violet color. A solution of 0.135 mM DPPH in methanol was prepared and 3.0 ml of this solution was mixed with 1.0 ml of extract in methanol of different concentration varying from 0.2 mg/ml to 1 mg/ml. The reaction mixture was left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Ascorbic acid and Gallic acid were used as standards and the same concentrations were prepared as the plant extracts. 3 ml of DPPH reagent put in 1 ml of methanol was used as a control. Inhibition of DPPH free radical in percentage was calculated by the formula:

Percentage (%) of DPPH radical scavenging = [(A0 - A1) /A0 x 100)

Where: A0 is the absorbance of the control,

A1 is the absorbance of the plant species or standards.

This was done in duplicates.

3.7.2 Ferric reducing antioxidant power (FRAP)

The ferric ions reducing power was measured according to the method of Oyaizu (1986) with slight modifications. FRAP method is based on electron transfer and it involves the ability of the antioxidant to reduce Fe^{3+} to Fe^{2+}. Different concentrations of the extracts ranging from 0.2 to 1 mg/ml were prepared. 1 ml of the extracts was mixed with 2.5 ml of 20 mM phosphate buffer and 2.5 m of 1% w/v potassium ferricyanide and then the mixture was incubated at 50 ºC for 30 min. Afterwards, 2.5 ml of 10% w/v trichloroacetic acid and 0.5 ml of 0.1% w/v ferric chloride was added to the mixture which were kept aside for 10 min. Finally, the absorbance was

measured at 700 nm. Ascorbic acid was used a positive reference standard. This was done in duplicates.

3.8 Regulatory approval

Approval to obtain and transport the plant materials to Uganda for the purpose of this research was obtained from INRAPE (National Research Institute on Agriculture, Fisheries and Environment). Letter that shows the process to obtain approval is attached in the Apendix.

3.9 Statistical analysis

Data from each objective were directly entered into Microsoft excel. MINITAB 16 and Graphpad Prism were used for statistical analysis. P level less than 0.05 was considered significant.

Objective 1

Results were presented in a table, and Chi square analysis for categorical data was used to statistically compare the different extracts. For the TPC determination, One Way ANOVA and Tukey Honest Significant Difference were used to compare the results of the different extracts. The appropriate graphs were drawn using Microsoft Excel.

Objective 2

Statistical comparisons between the different extracts were performed using one-way analysis of variance (One way ANOVA). Results was expressed as Means ± SD. The appropriate graphs were drawn using Microsoft Excel.

Objective 3

One way ANOVA was used to test if there is a statistical difference between the extracts. All results were expressed as Mean ± SD. All graphs were drawn using Microsoft Excel.

Chapter Four: Results

Objective 1: To identify the phytochemicals present in E. mayottensis and T. comorensis.

4.1 Qualitative phytochemical screening

4.1.1 *Tambourissa comorensis*

The fruit extracts of *T. comorensis* showed the presence of numerous phytochemicals. The ethanolic extracts revealed the presence of 6 phytochemicals while the ethyl-acetate and aqueous extracts each showed the presence of 4 phytochemicals (*Table 4.1*). Flavonoids, glycosides and terpenoids were present in all the extracts. The presence of tannins was observed in the ethanolic extracts. Saponins were present in high quantity in the ethanolic extracts. Polyphenols were present in the alcoholic and ethyl acetate extracts. Moreover, Chi-square statistical test showed there might not be any significant difference in chemical composition of *T. comorensis* fruit extracts prepared by different solvents (p=0.159). From the qualitative results, ethanol is more effective in extracting the different phytochemicals of the fruits of *T. comorensis*.

Table 4.1: Phytochemical contents of *T. comorensis fruits and E. mayottensis roots*

Plants	Extracts	Flavonoids	Tannins	Saponins	Terpenoids	Glycosides	Polyphenols	Alkaloids
T. comorensis fruits	Water	+	-	+	+	+	-	-
	Ethyl-acetate	+	-	-	+	+	-	+
	Ethanol (70%)	+	+	++	+	+	-	+
E. mayottensis roots	Water	-	-	+++	+	+	-	-
	Ethyl-acetate	-	+	+	+	+	-	+
	Ethanol (70%)	-	-	++	+	+	-	+

Key: (+): Present, (++): Present in high quantity, (-): Absent.

4.1.2 *Euclea mayottensis*

The phytochemical screening of the *E. mayottensis* roots extracts revealed the presence of tannins, saponins, terpenoids, glycosides and alkaloids (*Table 4.1*). Among the three solvents, ethyl acetate was able to extract 5 phytochemicals while ethanol extracted 4 and water 3 phytochemicals among the 7 phytochemicals that were screened. Saponins were present in greater amount in the ethanolic and aqueous extracts. The ethyl acetate extracts indicated the presence of tannins, saponins, terpenoids, glycosides and alkaloids. Saponins, terpenoids and glycosides were present in all extracts while flavonoids were unexpectedly absent in all. Polyphenols were only absent in the aqueous extract. And, there was no significant difference between *E. mayottensis* root extracts prepared by different solvents (p=0.497). Moreover, from the qualitative results, ethyl acetate was more effective in extracting the different phytochemicals of the roots of *E. mayottensis* (*Fig 4.1*).

4.2 Quantitative phytochemical screening

4.2.1 Total phenolic contents

The total phenolic content (TPC) of *E. mayottensis* and *T. comorensis* are displayed in *Table 4.2*. The quantitative screening of both plants showed that they are rich in phenolic compounds. The ethanolic extracts had the highest amount of phenols with 1.228 mg GAE/g and 0.896 mg GAE/g for *E. mayottensis* and *T. comorensis* respectively. And for both plants, the aqueous extracts showed a TPC value less than 0.6 mg GAE/g dry extracts (0.58 for *E. mayottensis* and 0.538 for *T. comorensis*). However, there was no significant difference in total phenolic content between the different extracts of *T. comorensis*. For *E. mayottensis*, there was a significant difference between the extract.

Table 4.2: Total phenolic contents of the extracts of *E. mayottensis* and *T. comorensis*

TPC (mg GAE/g dry extracts	Ethanol	Ethyl acetate	Water	*P value*
E. mayottensis	1.228 ± 0.02^{a}	0.831 ± 0.06^{b}	0.580 ± 0.08^{c}	*0.000*
T. comorensis	0.896 ± 0.06^{a}	0.683 ± 0.40^{a}	0.538 ± 0.17^{a}	*0.304*

Note: Values indicate Mean ± SD.
Values with different superscripts in the same row are significantly different ($p<.05$).

The results in *Table 4.2* clearly indicate that ethanol is more effective in extracting polyphenols in the root of *E. mayottensis* and the fruits of *T. comorensis*.

Objective 2: *To determine the antibacterial activity of E. mayottensis and T. comorensis*

4.4 Antibacterial activity of the roots of E. mayottensis

The diameters of inhibition of *E. mayottensis* root extracts against the microorganisms are shown in *Table 4.3*. In general, the ethanol and ethyl acetate extracts exerted a great inhibition effect against test isolates.

Table 4.3: Diameters of inhibition of *E. mayottensis* root extracts

Extracts	Concentration (mg/ml)	Diameters of inhibition (mm) (P value < 0.05)						
		ATCC25922	8403	ATCC27853	7106	ATCC25923	8372	8860
Water	Stock	-	-	-	-	-	12.6±0.57	-
	40	-	-	-	-	-	11.6±0.57	-
	20	-	-	-	-	-	8±0	-
	1	-	-	-	-	-	-	-
Ethanol	Stock	13.3±1.15	12.3±0.57	13.3±1.15	12.3±0.57	13±1	13.3±0.57	9±0
	40	11±1	10.6±0.57	10.6±0.57	11±1	13±1	11.3±0.57	8±0
	20	11.6±0.57	10.3±0.57	11±1	11.3±1.15	11±0.57	11±0	7±0
	1	10.6±0.57	9.6±1.52	10.3±0.57	10.6±0.57	10.6±0.57	11±0	6.6±0.57
Ethyl acetate	Stock	18±1	15.5±0.70	20±5.29	14.3±2.08	10.5±0.70	10.5±0.70	11±0
	40	14.6±1.52	12.5±0.70	18±4.35	12±1	9.3±0.57	9.3±0.57	10.3±0.57
	20	11.3±1.52	11.3±1.15	10.3±0.57	11.5±.70	8.6±0.57	8.6±0.57	8.3±0.57
	1	10±1	10.6±0.57	8.6±0.57	7.6±1.15	7.3±0.57	6.6±0.57	7.6±0.57

(-): No inhibition observed.

[ATCC25922: Susceptible *E. coli*, 8403: EBL+ *E. coli*, ATCC27853: Susceptible *P. aeruginosa*, 7106: Carbapenem resistant *P. aeruginosa*, ATCC25923: Susceptible *S. aureus*, 8372: MR *S. aureus*; 8860: Susceptible *V. streptococci*]

For the alcoholic stock extract, the strongest inhibition was observed against the susceptible *E. coli* isolate (ATCC 25922), the susceptible *P. aeruginosa* isolate (ATCC 27853) and the resistant

S. aureus isolate (8372) with a diameter of inhibition of 13.3 mm. The ethyl acetate extract showed the highest inhibitory effect with a diameter of inhibition of 20 mm observed against the susceptible strain of *P. aeruginosa* followed by 18 mm against the susceptible isolate of *E. coli*. Surprisingly, the aqueous extracts were not effective against almost all isolates. The inhibition was only observed against the resistant isolates of *S. aureus* with diameters of inhibition of 12.6 mm, 11.6 mm and 8 mm for a concentration of 256 mg/ml (stock solution), 40 mg/ml and 20 mg/ml respectively. At a concentration of 1 mg/ml, the aqueous extracts didn't inhibit the growth of the microorganisms. Nonetheless, there was a significant difference in the growth inhibition between the extract.

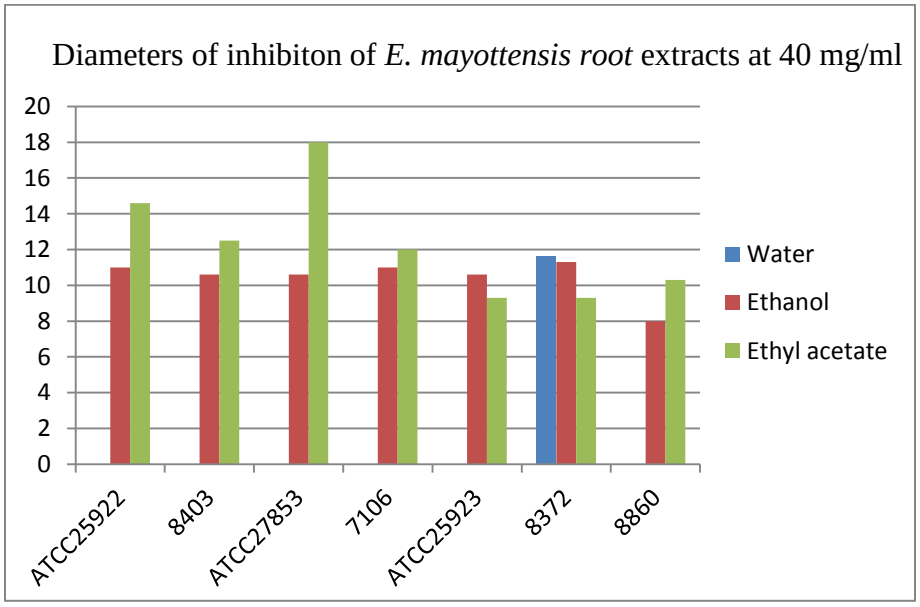


Fig 4.1: Diameters of inhibition of *E. mayottensis* root extracts at 40 mg/ml.

[ATCC25922: Susceptible *E. coli*; 8403: EBL+ *E. coli*; ATCC27853: Susceptible *P. aeruginosa*; 7106: Carbapenem resistant *P. aeruginosa*; ATCC25923: Susceptible *S. aureus*; 8372: MR *S. aureus*; 8860: Susceptible *V. streptococci*]

4.5 Antibacterial activity of *T. comorensis* fruits

The antibacterial activity of the fruit extracts of *T. comorensis* are summarized in *Table 4.4*. The alcoholic and ethyl acetate extracts were efficient against all tested organisms at all concentration. However, the aqueous fruit extract had no inhibitory effect against all isolates

tested. The highest inhibition was seen in the ethyl acetate extracts. The stock solution showed an inhibition of 19 mm against the susceptible strain of *E. coli* and 14 mm against the resistant strain. At 1 mg/ml of concentration of the ethyl acetate extracts, the highest inhibition was seen against the resistant strain of *P. aeruginosa* (11 mm) and the lowest was recorded against the susceptible strain of *S. aureus* (7.6 mm). For the ethanolic extract, the stock solution exhibited a diameter of inhibition of 18.6 mm against the resistant strain of *S. aureus* followed by 15.6 mm against the resistant strains of *P. aeruginosa.* At a concentration of 1 mg/ml, the highest zone of inhibition was seen against the resistant strain of *P. aeruginosa* (12 mm) while the lowest was against the susceptible strain of *S. aureus*. Moreover, there was a significant difference in the growth's inhibition of the bacterial strains between the extracts.

Table 4.4: Diameters of inhibition (mm) of *T. comorensis* fruit extracts

Extracts	Concentration (mg/ml)	Diameters of inhibition (mm) (P value < 0.05)						
		ATCC25922	8403	ATCC27853	7106	ATCC25923	8372	8860
Water	Stock	-	-	-	-	-	-	-
	40	-	-	-	-	-	-	-
	20	-	-	-	-	-	-	-
	1	-	-	-	-	-	-	-
Ethanol	Stock	12.3±0.57	12.6±1.15	13.3±0.57	15.6±2.08	14±1	18.6±0.57	15±1
	40	12.3±0.57	11.6±0.57	11.3±0.57	14±2	12.6±0.57	16±1	13.3±1.15
	20	11±0	11.3±0.57	11.3±1.15	13±0	11.6±1.15	15±1	11.6±0.57
	1	10±0	9.6±.57	10.6±0.57	12±1	9.33±0.57	10±1	10±1
Ethyl acetate	Stock	19±2.64	14±1	11±1	14.3±1.52	10.3±0.57	12.3±0.57	14.5±0.70
	40	11.3±1.15	11±1	10.3±0.57	14±1	9.3±0.57	10.6±0.57	13.5±0.70
	20	10±0	11±0	10.3±0.57	12.3±0.57	9.6±0.57	11±1.73	9.6±1.15
	1	8.3±2.08	10.6±0.57	8.3±0.57	11±1	7.6±0.57	8.3±0.57	8.3±0.57

(-): No inhibition observed.

[ATCC25922: Susceptible *E. coli*; 8403: EBL+ *E. coli*; ATCC27853: Susceptible *P. aeruginosa*; 7106: Carbapenem resistant *P. aeruginosa*; ATCC25923: Susceptible *S. aureus*; 8372: Methicillin resistant *S. aureus*; 8860: Susceptible *V. streptococci*]

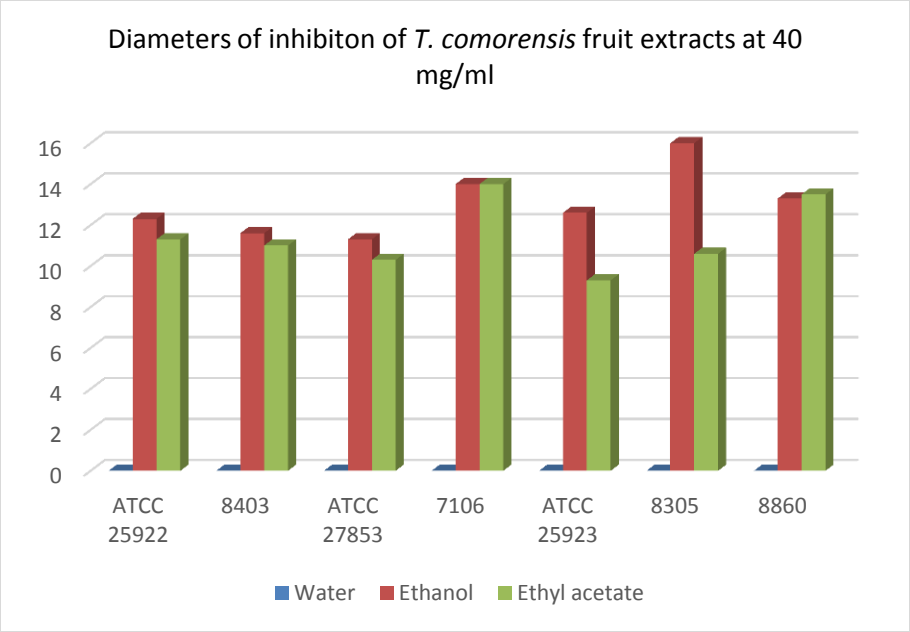


Fig 4.2: Diameters of inhibition of *T. comorensis* fruit extracts at 40 mg/ml.

[ATCC25922: Susceptible *E. coli*; 8403: EBL+ *E. coli*; ATCC27853: Susceptible *P. aeruginosa*; 7106: Carbapenem resistant *P. aeruginosa*; ATCC25923: Susceptible *S. aureus*; 8372: Methicillin resistant *S. aureus*; 8860: Susceptible *V. streptococci*].

4.6 Diameters of inhibition of the controls

The negative controls (distilled water, ethanol and ethyl acetate) did not show any inhibition against all microorganisms. The positive controls all had a great inhibitory effect against all the isolates with diameters of inhibition varying from 9 mm to 25.33 mm (*Table 4.5*). Erythromycin was more effective against the susceptible isolate of *S. aureus* (25.33 mm) compared to the different plant extracts. However, for the resistant isolate, the extracts worked better compared to the positive control (10.6 mm). For both isolates of *E. coli*, the extracts were more efficient than ampicillin (12.33 mm for the susceptible isolate and 9 mm for the resistant isolate). Penicillin which was used against *V. streptococci* exhibited a diameter of inhibition of 14.66 mm which was slightly lower than the one of the extracts of the stock solution of *T. comorensis* (15 mm) but higher compared to *E. mayottensis* (9 mm). Piperacillin which was used against *P. aeruginosa* had a greater inhibition effect against both the susceptible and resistant isolates of *P. aeruginosa* (18.66 mm and 15.66 mm respectively) compared to the extracts.

Table 4.5: Diameter of inhibition of the controls (Positive and negative)

Microorganisms	Diameters of inhibition (mm)						
	Positive controls				Negative Controls		
	Ampicillin	Erythromycin	Piperacillin	Penicillin	Distilled Water	Ethanol	Ethyl acetate
ATCC 25922	12.33±2.51	NA	NA	NA	-	-	-
8403	9±1.73	NA	NA	NA	-	-	-
ATCC 27853	NA	NA	18.66±2.51	NA	-	-	-
7106	NA	NA	15.66±2.08	NA	-	-	-
ATCC 25923	NA	25.33±6.50	NA	NA	-	-	-
8372	NA	10.66±2.51	NA	NA	-	-	-
8860	NA	NA	NA	14.66±1.15	-	-	-

NA: Not applicable; (-): no inhibition observed.

[ATCC25922: Susceptible *E. coli*; 8403: EBL+ *E. coli*; ATCC27853: Susceptible *P. aeruginosa*; 7106: Carbapenem resistant *P. aeruginosa*; ATCC25923: Susceptible *S. aureus*; 8372: MR *S. aureus*; 8860: Susceptible *V. streptococci*].

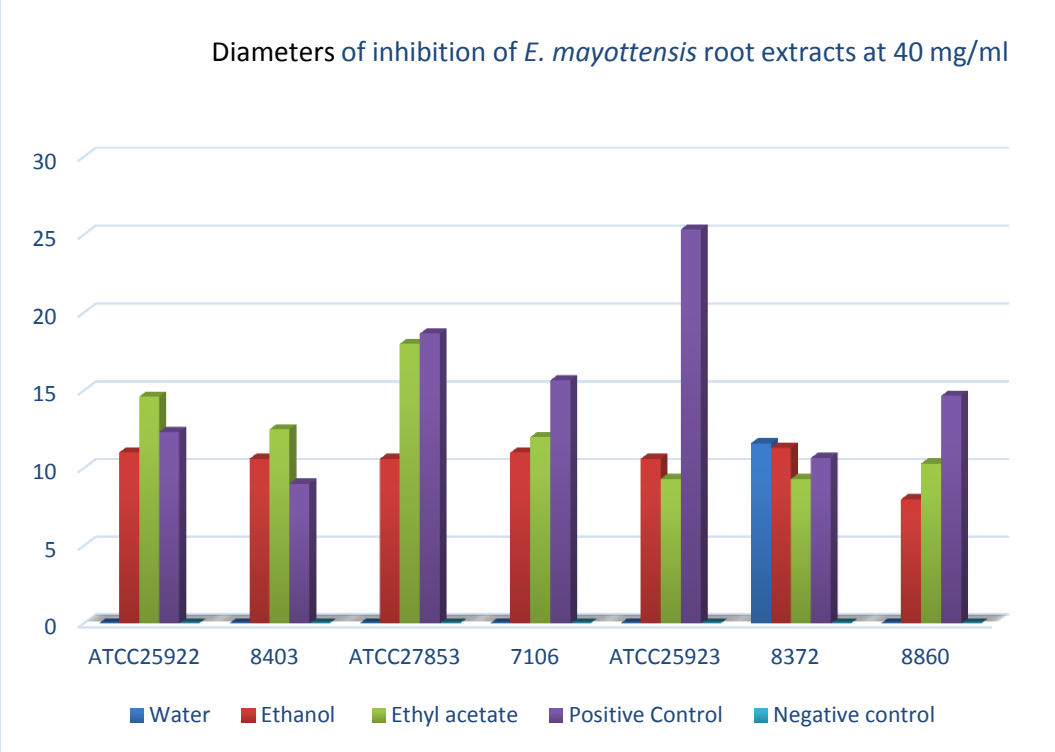


Fig 4.3: Diameters of inhibition of E. mayottensis extracts and the controls at 40 mg/ ml.

[ATCC25922: Susceptible *E. coli*; 8403: EBL+ *E. coli*; ATCC27853: Susceptible *P. aeruginosa*; 7106: Carbapenem resistant *P. aeruginosa*; ATCC25923: Susceptible *S. aureus*; 8372: Methicillin resistant *S. aureus*; 8860: Susceptible *V. streptococci*].

4.7 Determination of MIC and MBC

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts against the tested microorganisms are presented in table 4.6 and 4.7. The MIC values varied from 0.16 mg/ml to 16.40 mg/ml for *E. mayottensis* ethanol and 0.16 mg/ml to 11 mg/ml for *T. comorensis* ethanol (*Table 4.6*). For both plants, the ethanolic extracts indicated an MIC value of 0.16 mg.ml against both susceptible and resistant isolates of *P. aeruginosa*. The highest MIC value for the ethanolic extract was recorded for the resistant isolate of S. aureus (16.40 mg/ml) for *E. mayottensis* and susceptible isolate of *E. coli* (11 mg/ml) for *T. comorensis*. For the ethanolic extracts, the MBC value was only recorded for *T.comorensis* with the susceptible isolate of *E. coli* (22 mg/ml). For the other isolates, no bactericidal effect was recorded (*Fig 4.8*).

Table 4.6: MIC and MBC of the ethanolic extracts of *E. mayottensis* and *T. comorensis*

Microorganisms	*E. mayottensis* ethanol extract		*T. comorensis* ethanol extract	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
ATCC 25922	5.46±0	-	11±0	22±0
8403	8.19±3.86	-	8.25±3.88	-
ATCC 27853	0.16±0	-	0.16±0	-
7106	0.16±0	-	0.16±0	-
ATCC 25923	4.09±1.93	-	2.06±0.97	-
8372	16.40±7.73	-	2.75±0	-

(-): No value recorded.

[ATCC25922: Susceptible *E. coli*; 8403: EBL+ *E. coli*; ATCC27853: Susceptible *P. aeruginosa*; 7106: Carbapenem resistant *P. aeruginosa*; ATCC25923: Susceptible *S. aureus*; 8372: Methicillin resistant *S. aureus*]

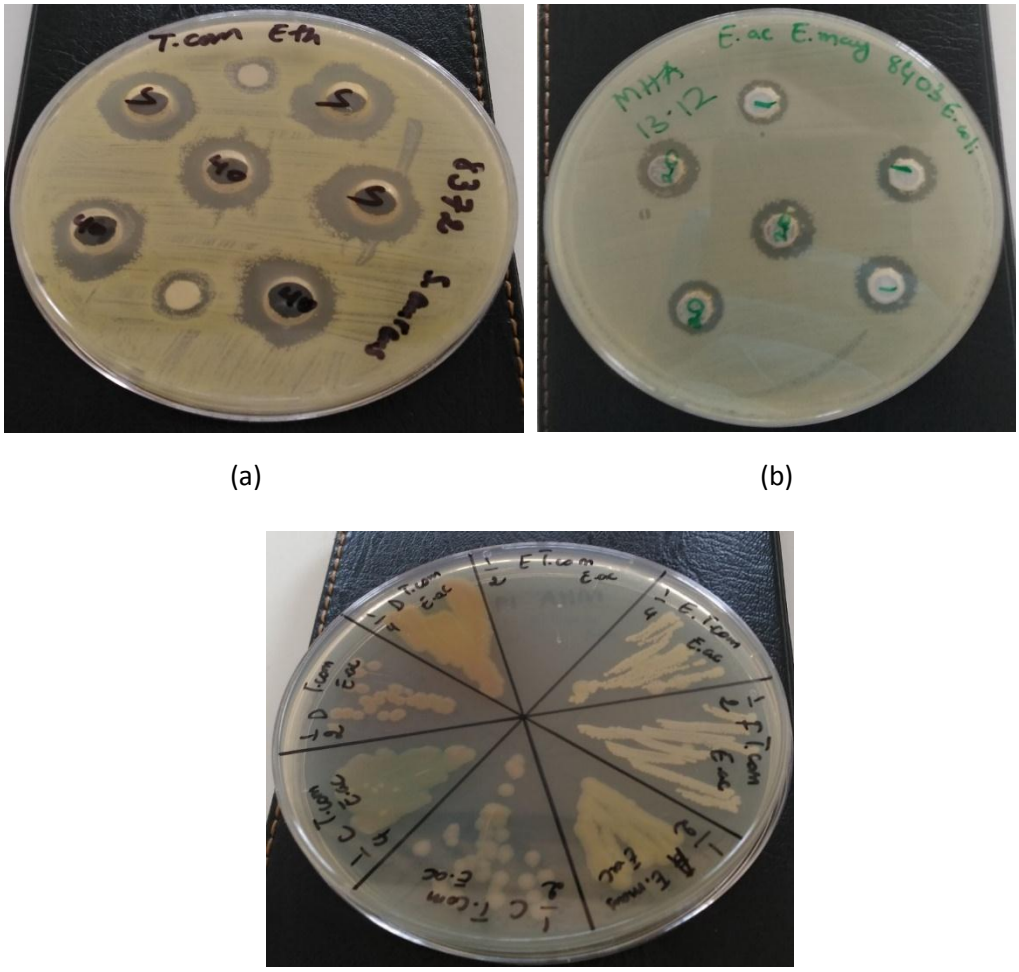
For the ethyl acetate extracts, the MIC values for *E. mayottensis* varied from 16.21 mg/ml to a concentration of 86.5 mg/ml while for *T. comorensis*, they varied from 16.40 mg/ml to 43.75 mg/ml (*Table 4.7*). *E. mayottensis* extracts against the resistant isolates of *P. aeruginosa* and *S. aureus* had the same MIC value of 64.87 mg/ml whereas against the resistant isolate of E. coli, the MIC was 86.5 mg/ml. For *T. comorensis*, the smallest MIC value was recorded against the susceptible isolate of *P. aeruginosa* (16.40 mg/ml) while the highest was against the resistant isolates of *E. coli* and *S. aureus. T. comorensis* extract had a bactericidal effect only against the susceptible isolate of *S. aureus* with a MBC value of 43.75 mg/ml.

Table 4.7: MIC and MBC of the ethyl acetate extracts of *E. mayottensis* and *T. comorensis*

Microorganisms	*E. mayottensis* Ethyl acetate		*T. comorensis* Ethyl acetate	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
ATCC 25922	21.62±0	-	32.80±15.48	-
8403	86.5±0	-	43.75±0	-
ATCC 27853	16.21±7.64	-	16.40±7.73	-
7106	64.87±30.58	-	21.87±0	-
ATCC 25923	43.25±0	-	32.81±15.47	43.75±0
8305	64.87±30.58	-	43.75±0	-

(-): No value recorded.

[ATCC25922: Susceptible *E. coli*; 8403: EBL+ *E. coli*; ATCC27853: Susceptible *P. aeruginosa*; 7106: Carbapenem resistant *P. aeruginosa*; ATCC25923: Susceptible *S. aureus*; 8372: Methicillin resistant *S. aureus*]



(a) (b)

(c)

Fig. 4.4: Zones of inhibition of *T. comorensis* and *E. mayottensis* and their MBC determination.

The inhibition of the bacterial strains by the plant extracts are as shown in figure 4.8. (a): T. comorensis ethanolic extract against MRSA; (b): E. mayottensis ethyl acetate extract against ESBL+ *E. coli*; (c): MBC determination.

Objective 3: *To determine the antioxidant activity of E. mayottensis and T. comorensis*

4.8 DPPP radical scavenging assay

The DPPH radical scavenging assay is based on the ability of the antioxidants present in the extract to scavenge the radical present in the DPPH molecule. The percentages of inhibition of the radical by the extracts are presented in *Table 4.8.* The plant extracts exhibited a concentration dependent scavenging effect on the radical of DPPH but lower than Gallic acid and ascorbic acid which were used as standards. Among the plant extracts, the aqueous extracts for both plants showed the highest values of inhibition: *T. comorensis* aqueous fruit extracts showed an inhibition varying from 42.89% (0.2 mg/ml) to 51.42% (1 mg/ml), whereas for *E. mayottensis* roots the inhibition varied from 21.71% (0.4 mg/ml) to 45.17%/. The aqueous extract of *E. mayottensis* showed no inhibition on the radical of DPPH at a concentration of 0.2 mg/ml, while the alcoholic and ethyl acetate extracts had no effect on the radical scavenging of DPPH at a concentration of 0.2 mg/ml and 0.4 mg/ml.

Table 4.8: Radical scavenging activity of *T. comorensis* and *E. mayottensis*

	Radical scavenging activity (%)							
Concentration (mg/ml)	Ascorbic Acid	Gallic Acid	*T.comorensis* Water	*T.comorensis* Ethanol	*T.comorensis* Ethyl acetate	*E.mayottensis* Water	*E.mayottensis* Ethanol	*E.mayottensis* Ethyl acetate
0.2	81.81±1.60	54.54±1.60	42.89±0.40	31.25±0.40	34.09±1.60	0	0	0
0.4	84.65±3.21	55.96±3.61	47.15±2.41	31.53±6.02	34.37±5.22	24.71±2.81	0	0
0.6	86.93±4.01	56.53±1.20	48.01±0.40	34.37±2.81	36.07±2.81	28.69±3.61	12.78±1.20	8.80±2.81
0.8	87.21±2	56.81±0.80	48.57±2	40.90±0.80	36.64±1.20	34.94±3.61	23.86±3.21	28.40±1.60
1	89.22±0.40	57.67±1.20	51.42±5.22	45.17±2	46.30±1.20	44.60±3.61	42.04±0.80	38.63±2.41

Percent inhibition Values: Mean ± SD

For *T. comorensis* the lowest inhibition was recorded with the alcoholic extract and for *E. mayottensis*, the ethyl acetate extract had the least effect on DPPH radical (*Fig 4.9*). Moreover, there was a significant difference on the radical scavenging activity of *T. comorensis* extracts at

concentration 0.8 mg/ml, 0.6 mg/ml and 0.2 mg/ml with *p value* of 0.008, 0.018 and 0.003 respectively. At concentration 1 mg/ml and 0.4 mg/ml there was no significant difference between *T. comorensis* extracts (p value=0.216 and 0.09 respectively). For *E. mayottensis* at low concentration (0.6 mg/ml and 0.4 mg/ml) there was a significant difference between the extracts (*p value* = 0.01 and 0.001 respectively) while at high concentration (0.8 mg/ml and 1 mg/ml), no significant difference on the radical scavenging activity of *E. mayottensis* extracts was observed *(p value*= 0.07 and 0.193 respectively).

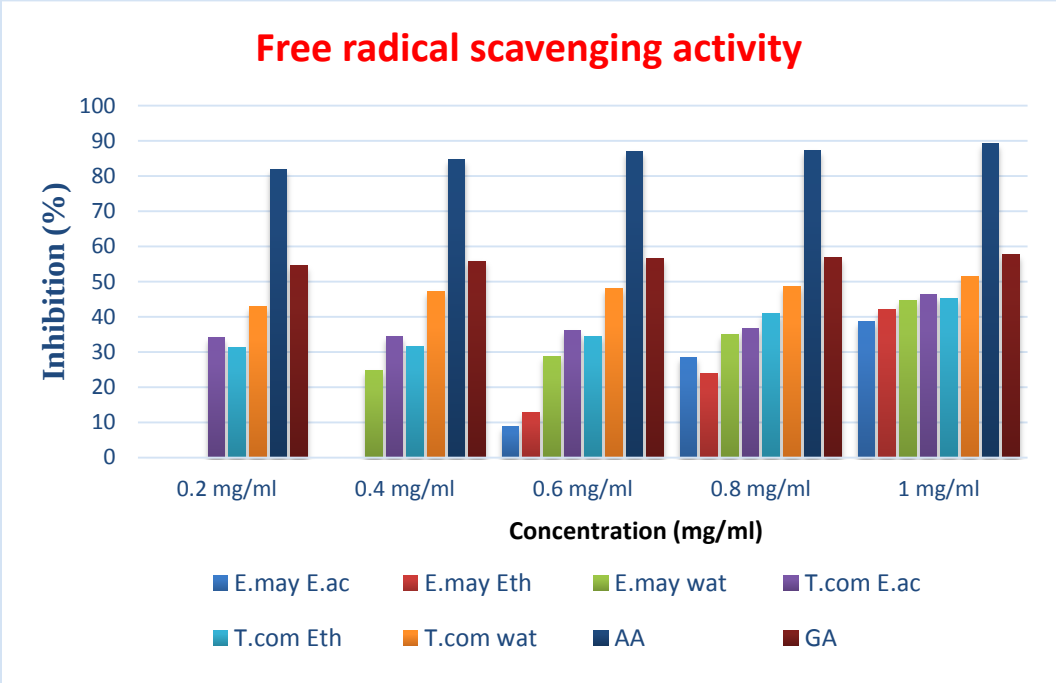


Fig 4.5: Free radical scavenging activity of *T. comorensis* and *E. mayottensis* extracts.

Among the extracts, the aqueous extracts of *T. comorensis* had the highest inhibition effect on the radical of DPPH. **Key**: E. may E.ac: *E. mayottensis* Ethyl acetate; E. may Eth: *E. mayottensis* Ethanol; E. may Wat: *E. mayottensis* Water; T. com E. ac: *T. comorensis* Ethyl acetate; T.com Eth: *T. comorensis* Ethanol; T. com Wat: *T. comorensis* Water; AA: Ascorbic acid; GA: Gallic acid

4.9 Ferric reducing antioxidant power assay (FRAP)

The plant extracts exhibited a promising high reducing power but lower than that of ascorbic acid (*Fig 4.10*). At concentration 1 mg/ml, the alcoholic extract of *E. mayottensis* showed the highest reducing power (0.908) followed by the ethyl acetate extract (0.897). For *T. comorensis*, the ethyl acetate extract showed the highest reducing power (0.773) followed by the ethanolic extract (0.680). For both *T. comorensis* and *E. mayottensis*, the aqueous extracts showed the lowest reducing power (0.666 and 0.449 respectively) (*Table 4.9*). Further still, at all concentration, there was no significant difference between the extracts of *T. comorensis* (*p value* ranging from 0.06 to 0.32). For *E. mayottensis*, there was a significant difference between the extracts (*p value* ranging from 0.005 to 0.03).

Table 4.9: Reducing power activity of *E. mayottensis* and *T. comorensis*

Concentration (mg/ml)	**Absorbance at 700 nm**						
	Ascorbic acid	*T.comorensis* ethanol	*T.comorensis* ethyl acetate	*T.comorensis* water	*E.mayottensis* ethanol	*E.mayottensis* ethyl acetate	*E.mayottensis* water
0.2	0.267±0.01	0.357±0.007	0.329±0.02	0.264±0.03	0.543±0.05	0.368±0.01	0.278±0.002
0.4	0.490±0.03	0.475±0.07	0.429±0.03	0.382±0.02	0.610±0.009	0.525±0.04	0.337±0.002
0.6	0.739±0.01	0.542±0.02	0.513±0.02	0.447±0.01	0.666±0.01	0.627±0.02	0.357±0.02
0.8	1.004±0.07	0.603±0.04	0.577±0.03	0.543±0.07	0.735±0.01	0.663±0.15	0.424±0.004
1	1.534±0.003	0.68±0.03	0.773±0.02	0.666±0.01	0.908±0.004	0.897±0.03	0.449±0.17

Absorbance values: Mean ± SD

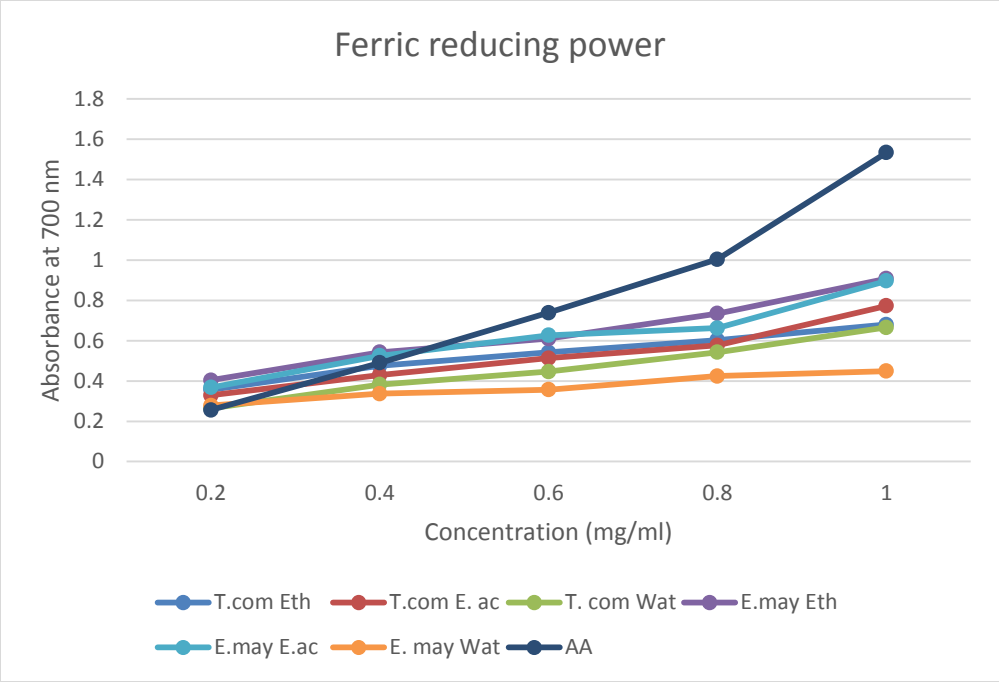


Fig 4.6: Ferric reducing power of *T. comorensis* and *E. mayottensis* extracts

Among the extracts, the ethanolic extract of *E. mayottensis* and the ethyl acetate extracts of *T. comorensis* showed the highest reducing power potential. **Key**: E. may E.ac: *E. mayottensis* Ethyl acetate; E. may Eth: *E. mayottensis* Ethanol; E. may Wat: *E. mayottensis* Water; T. com E. ac: *T. comorensis* Ethyl acetate; T.com Eth: *T. comorensis* Ethanol; T. com Wat: *T. comorensis* Water; AA: Ascorbic acid.

Chapter Five: Discussion

The present study revealed a range of different phytochemicals in the extracts of the roots of E. mayottensis and fruits of T. comorensis. The results further suggest that the identified phytochemical compounds may be the bioactive constituents of the plants and thus making a valuable reservoir of bioactive compounds of substantial medicinal merit.

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The current study is the first of kind that carried out co-current phytochemical screening and the associated biological activities of *E. mayottensis*. The screening showed that *E. mayottensis* plant has phyto-compounds that exhibit anti-microbial activities. Although polyphenols are believed to be common phytochemicals found in medicinal plants, their presence was not qualitatively detected when *E. mayottensis* aqueous extracts were screened, but these were quantitatively identified in small amounts (0.538 mg GAE/g). This could be due to the fact that when performing the qualitative screening, very small amounts of the extracts were pipetted. However, for the quantitative assay, a substantial concentration of 0.6 mg/ml of the same extracts was needed. The presence of various phytochemicals was shown in other species of the Ebenaceae family that includes terpenes in *E. natalensis* (Maroyi, 2017), alkaloids, glycosides, saponins and tannins in *E. unduluta* (Joshua *et al.,* 2013). *E. crispa* and *E. natalensis* extracts also showed the presence of flavonoids, polyphenols, saponins and terpenes (Nkala *et al.*, 2022).

T. comorensis screening also revealed the presence of a range of phyto-components of medicinal importance. The presence of tannins and flavonoids in *T. comorensis* in the current study agrees with the study of Soule *et al* (2017) which also reported their presence in the methanolic and ethyl acetate extract of the plant. Alkaloids and phenolic compounds (flavonoids and phenolic acid) were reported in *Peumeus boldus*, a species from the Monimiaceae family (Oteru *et al., 2022*; Da Cruz *et al.,* 2019).

The total phenolic content of *T. comorensis* fruit extract was estimated to be between 0.538 - 0.896 mg GAE/g dry extracts for a concentration of 0.6 mg/ml of extracts (*Table 4.2*). And the alcoholic extract had the highest value. This could mean that ethanol was more efficient in extracting the polyphenolic compounds of the plant. In the study by Soule et al (2017), the alcoholic extract of *T. comorensis* also had the highest content of polyphenols (0.63 g/L GAE)

which agrees with our study. However, they did not mention precisely the concentration of the extract they used in the total phenolic content assay. *E. mayottensis* root extracts showed a TPC value ranging from 0.580 to 1.228 mg GAE/g of dry extracts (*Table 4.3*). The ethanolic extract had the highest amount of phenolic content followed by ethyl acetate and aqueous extract. Study in other species of Euclea genus showed a TPC value of 36.60 mg GAE/ g in the methanolic extracts of *E. crispa* and 13.51 mg GAE/g for *E. natalensis* (Nkala *et al.*, 2021).

The antimicrobial activity of *T. comorensis* and *E. mayottensis* showed that the extracts had a promising antimicrobial effect on the different strains used. The zones of inhibition reported varied from 6.6 to 20 mm (*Table 4.3* & *Table 4.4*). These extracts had a high antimicrobial activity against the resistant strains (extended beta lactamase (+) *E. coli*, methicillin resistant *S. aureus* and carbapenem resistant *P. aeruginosa*) used.

T. comorensis extracts showed a high antibacterial activity against *E. coli*, *P. aeruginosa* and *S. aureus* with diameter of inhibition observed for both the susceptible and resistant strains used. The screening of *T. comorensis* showed the presence of different phytochemicals that are known to act as antibacterial agents which includes alkaloids, saponins, polyphenols, tannins, glycosides, flavonoids and terpenoids (Ferdes, 2018). Their presence in the plant would explain the high antibacterial observed. Furthermore, aqueous extract of *T. comorensis* was not effective against any of the microorganisms used where the diameters of inhibition were too low to be recorded at concentration 40 mg/ml and below. This could be explained by the fact that the aqueous extract only revealed the presence of few phytochemicals and thus high concentration of the extract is needed to give better antibacterial activity. In contrast with the results obtained from this study, Soule *et al* (2017) showed that *T. comorensis* methanolic extract had a very low antibacterial effect on the same strains used (*E. coli*, *P. aeruginosa* and *S. aureus*). The diameters of inhibition were only 8 mm and 9 mm against *E. coli* and *S. aureus* respectively while for *P. aeruginosa*, no inhibition was recorded. However, in their study, Soule et al used Soxhlet method to extract the phytochemicals. The high temperature used during the Soxhlet extraction and the time of passage in the Soxhlet apparatus may have destroyed some of the phytochemicals that would have exhibited an antibacterial effect. On the other hand, a study using another species of the Monimiaceae family showed a very strong antibacterial activity against *S. aureus* (19mm) and MRSA (23mm) (Wendakoon *et al.*, 2012). The extracts of *E. mayottensis* showed a

great antibacterial effect against the test organisms used (*S. aureus*, *E. coli*, *P. aeruginosa* and *V. streptococci*) both the susceptible and resistant strains. This could be explained by the rich presence of phytochemicals (saponins, alkaloids, terpenoids and tannins) that are reported to be effective against bacteria (Ferdes, 2018). Additionally, the aqueous extract of the plant was only effective against the methicillin resistant *S. aureus* while against other microorganisms, the inhibition was too low. This could mean that when the strain of *S. aureus* gained its resistance against methicillin, it might have lost a property that now make it susceptible to the compounds (saponins, terpenoids, glycosides) present in the aqueous extract of *E. mayottensis*. Moreover, a consequent amount of extract is also needed to give a better inhibition effect. These results are in accordance with studies done in other species of Euclea where the methanolic extracts showed a very strong antibacterial activity against *Streptoccus mutans*, *S. aureus* and *E.coli* (Mbanga *et al.*, 2013; Mbabazi *et al.*, 2020).

The ethyl acetate extract of *E mayottensis* showed the highest antibacterial effect among the different extracts and its phytochemical screening showed the presence of alkaloids, tannins, terpenoids, polyphenols, glycosides and saponins. For *T. comorensis*, the ethanolic extract showed the highest inhibition effect and the phytochemical screening showed the presence of alkaloids, terpenoids, saponins, polyphenols, glycosides and flavonoids. The rich presence of those phytochemicals in the extracts could explain the great antibacterial activity observed as these compounds are known to exhibit antibacterial activities (Ferdes, 2018).

This study also investigated the antioxidant activity potential of *T. comorensis* and *E. mayottensis* using two different methods: the DPPH radical scavenging activity and the ferric reducing antioxidant power assay (FRAP). The DPPH radical scavenging activity showed that both plant had a low to moderate radical scavenging effect on the DPPH molecule for the range of extracts concentration used (0.2-1 mg/ml) as compared to the standards gallic acid and ascorbic acid (*Fig 4.5*). The aqueous fruit extract of *T. comorensis* had the highest scavenging effect with an inhibition varying from 42.89 to 51.42% while its ethanolic extract had the lowest with only 31.25 to 45.17% of inhibition (*Table 4.8*). The ethyl acetate fruit extract of *T. comorensis* had a scavenging effect varying from 34.09 to 46.30%. The aqueous extract of *T. comorensis* which had the highest radical scavenging power revealed the presence of flavonoids, saponins, terpenoids and glycosides that exhibit potent radical scavenging activity. This agrees

with the study of Soule et al (2017), where the ethyl acetate extract of *T. comorensis* had a higher scavenging power compared to the methanolic extract on the range of concentration used. A study done in *Peumus boldus,* a species from the Monimiaceae family, showed that the plant had a great DPPH radical scavenging effect for the ethyl acetate and water soluble fractions (Schmeda-Hirschmann *et al.,* 2003).

On the other hand, *E. mayottensis* had a low radical scavenging effect for the range of concentration used with a percentage inhibition wavering from 8.80% to 44.6% (*Table 4.8*). The aqueous extracts had an inhibition effect varying from 24.71% to 44.6% which was the highest for the plant, followed by the ethanolic extract (12.78% to 42.04%) and ethyl acetate extract (8.80% to 38.63%) (*Table 4.9*). Saponins, which were present in great amount in the aqueous extract as well as glycosides and terpenoids are known to exhibit potent radical scavenging activity against DPPH (Gao *et al.,* 1999; Wang *et al.,* 2019, Nwaehujor *et al.,* 2017). These results disagree with the study done using another Euclea species, where the methanolic extrat of *E. schimperi* were able to scavenge the DPPH radical with a percentage scavenging activity above 70% for a concentration of 1 mg/ml (Mekonnen *et al.,* 2018).

Both plants (for the range of concentration of 0.2-1 mg/ml) using FRAP assay, had a great reducing power although not as much as ascorbic acid, the standard used (*Fig 4.10*). For *T. comorensis,* the highest reducing power was seen in the ethyl acetate fruit extract (0.777 absorbance at 700nm for 1mg/ml) while the aqueous extract showed the lowest reducing power (0.666) (Table 4.9). Tannins, saponins, terpenoids, glycosides and alkaloids that were observed in the ethyl acetate and ethanolic extracts are all known to possess antioxidant potential. These results disagree with those of Soule *et al.,* (2017) where the ethyl acetate *T. comorensis* extract showed the highest reducing power for the range of concentration used. In the case of *E. mayottensis*, the ethanolic roots extract demonstrated the highest reducing power (0.908) while its aqueous extract showed the lowest (0.449) (*Table 4.10*). In the study of Mekonnen *et al* (2018), the methanolic extract of *E. schimperii* demonstrated the highest reducing power (0.832 at 1 mg/ml).

Medicinal plants, generally, are known to possess various bioactivities, including the antioxidant activity (Othman et al., 2017). These bioactivities are attributed to the presence of plant

secondary metabolites or phytochemicals. In the incessant search for new bioactive natural products against oxidative damage and inflammation, terpenes are emerging as a rich source of such compounds (Baccouri & Rajhi, 2012). Monoterpenes and sesquiterpenes (major components of essential oils) show strong antioxidant activities. Using different antioxidant activity assays, essential oils have confirmed their hydrogen- donating capabilities (Grassman, 2005), with apparent effective counteraction of free radicals and reactive oxygen species. It is, thus plausible to state that *Euclea mayottensis* and *Tambourissa comorensis*, the two medicinal plants investigated, contain some essential oils in the form of terpenoids that may explain, at least in part, the plants' antioxidant properties observed in this study.

Chapter 6

Conclusions, Limitations and Recommendations

6.1 Conclusions

1. This study has shown that fruits of *Tambourissa comorensis* and roots of *Euclea mayottensis* have a wide range of phytochemicals of medicinal importance.

2. The roots of *E. mayottensis* and fruits of *T. comorensis* showed potential antibacterial activities against susceptible and resistant strains of different bacteria. However, this antibacterial activity varied tremendously, depending on the solvent used in extraction.

3. The DPPH radical scavenging activity and ferric reducing power assays of the crude plant extracts showed moderate to high antioxidant activities, illustrating the fact that these plants possess different antioxidant mechanisms of action.

4. Overall, the findings of this study validate the medicinal use of the fruits of *T. comorensis* and roots of *E. mayottensis* by local people in the Comoros.

6.2 Limitations

1. Difficulty in accessing chemicals and reagents attributed to lack of sufficient funds. Certain key reagents/standards could not be bought because they were too expensive.

2. Much of the precious time was lost due to Covid-19 lockdowns and counter-lockdowns. Samples could not be shipped to Uganda in time from the Comoros.

6.3 Recommendations

1. Different phytochemicals were detected in both *E. mayottensis* and *T. comorensis* crude extracts. So isolation and identification is recommended to confirm the bioactive compounds presents in the plants.

2. This study only looked at the fruits of *T. comorensis* and roots of *E. mayottensis*. Thus use of other parts of the plants such as leaves, stem would be better to identify which phytochemicals are presents in each part of the plants and how active those parts are.

3. The study determined the total phenolic content of the extracts and used a light spectrophotometer to quantify them. Therefore use of other quantification methods such mass spectroscopy, HPLC, etc. would yield better results. Quantifications of other phytochemicals is also recommended.

4. During the MIC determination, the solvents used for preparation of the extract tended to volatize along the way, which may have resulted in high values of MIC. So use of different solvents initially that would be less volatile is recommended.

5. There is need to study possible mechanisms of antibacterial action of the extracts.

6. Study on the toxicity of the plants is also highly recommended.

References

Adawia, K., Rawaa, A.-K., & Ghalia, S. (2016). Phytochemical Screening and Antioxidant Activity of Selected Wild Plants in Liliaceae Family Growing Syria. *International Journal of Pharmacognosy and Phytochemical Research*, *8*(12), 2025–2032.

Adjanohoun, E. J., Aké Assi, L., Ahmed, A., Eymê, J., Guinko, S., Kayonga, A., ... & Lebras, M. (1982). Contribution aux études ethnobotaniques et floristiques aux Comores. Rapport présenté à l'ACCT.

Akroum, S., Bendjeddou, D., Satta, D., & Lalaoui, K. (2009). Antibacterial activity and acute toxicity effect of flavonoids extracted from Mentha longifolia. *American-Eurasian Journal of Sustainable Agriculture, 4*, 93-96.

Al-Daihan, S., Al-Faham, M., Al-shawi, N., Almayman, R., Brnawi, A., Zargar, S., & Bhat, R. shafi. (2013). Antibacterial activity and phytochemical screening of some medicinal plants commonly used in Saudi Arabia against selected pathogenic microorganisms. *Journal of King Saud University - Science*, 25(2), 115–120. https://doi.org/10.1016/j.jksus.2012.11.003

Avato, P., Bucci, R., Tava, A., Vitali, C., Rosato, A., Bialy, Z., & Jurzysta, M. (2006). Antimicrobial activity of saponins from Medicago sp.: structure-activity relationship. *Phytotherapy Research* : PTR, 20(6), 454–457. https://doi.org/10.1002/ptr.1876

Badyal, S., Singh, H., Yadav, A. K., Sharma, S., & Bhushan, I. (2020). Plant secondary metabolites and their uses. *Plant Archives* Vol. 20, Supplement 2, 2020. 3336-3340 e-ISSN:2581-6063 (online), ISSN:0972-5210

Baccouri, B., & Rajhi, I. (2021). Potential Antioxidant Activity of Terpenes. In S. Perveen, & A. M. Al-Taweel (Eds.), Terpenes and Terpenoids - Recent Advances. *IntechOpen.* https://doi.org/10.5772/intechopen.96638

Becker D. E. (2013). Antimicrobial drugs. *Anesthesia progress*, 60(3), 111–123. https://doi.org/10.2344/0003-3006-60.3.111

Bobrov, A. V., Iurmanov, A. A., & Lorence, D. H. (2017). Phylogenetic biogeography of the family Monimiaceae. *Geography, Environment, Sustainability*, 10(4), 16–26. https://doi.org/10.24057/2071-9388-2017-10-4-16-26

Chung, K. T., Wong, T. Y., Wei, C. I., Huang, Y. W., & Lin, Y. (1998). Tannins and human health: A review. *Critical Reviews in Food Science and Nutrition*, 38(6), 421–464. https://doi.org/10.1080/10408699891274273

Cushnie, T. P. T., Cushnie, B., & Lamb, A. J. (2014). Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *International Journal of Antimicrobial Agents*, 44(5), 377–386. https://doi.org/10.1016/j.ijantimicag.2014.06.001

Cueva, C., Mingo, S., Muñoz-González, I., Bustos, I., Requena, T., del Campo, R., Martín-Álvarez, P.J., Bartolomé, B., & Moreno-Arribas, M.V. (2012). Antibacterial activity of wine phenolic compounds and oenological extracts against potential respiratory pathogens. *Letters in Applied Microbiology*, *54*.

Da Cruz, J., da Costa Guerra, J. F., de Souza Gomes, M., Freitas, G., & Morais, E. R. (2019). Phytochemical Analysis and Evaluation of Antimicrobial Activity of Peumus boldus, Psidium guajava, Vernonia polysphaera, Persea Americana, Eucalyptus citriodora Leaf Extracts and Jatropha multifida Raw Sap. *Current Pharmaceutical Biotechnology*, *20*(5), 433–444. https://doi.org/10.2174/1389201020666190409104910

Daglia M. (2012). Polyphenols as antimicrobial agents. *Current Opinion in Biotechnology*, *23*(2), 174–181. https://doi.org/10.1016/j.copbio.2011.08.007

Deng, C., Huang, B., Wang, Q., Wu, W., Zheng, S., Zhang, H., Li, D., Feng, D., Li, G., Xue, L., Yang, T., Tuo, F., Mohadji, F., Su, X. Z., Xu, Q., Wu, Z., Lin, L., Zhou, J., Yan, H., Bacar, A., Song, J. (2018). Large-scale Artemisinin-Piperaquine Mass Drug Administration With or Without Primaquine Dramatically Reduces Malaria in a Highly Endemic Region of Africa. Clinical infectious diseases : an official publication of the *Infectious Diseases Society of America*, 67(11), 1670–1676. https://doi.org/10.1093/cid/ciy364

Ding, C., Qin, X., Yu, H., Liu, Y., Wang, X., & Luo, X. (2019). Thalicfoetine, a novel isoquinoline alkaloid with antibacterial activity from Thalictrum foetidum. *Tetrahedron Letters*. doi: 10.1016/j.tetlet.2019.151135

Doron, S., & Gorbach, S. L. (2008). Bacterial Infections: Overview. 11. *International Encyclopedia of Public Health*. 2008: 273–282. Published online 2008 Aug 26. https://doi:10.1016/B978-012373960-5.00596-7. PMCID: PMC7149789

Doss, A & Mubarack, H. & Rangasamy, D. (2008). Antibacterial activity of tannins from the leaves of Solanum trilobatum Linn. *Indian J. Sci. Technol*. 2. 10.17485/ijst/2009/v2i2/29391.

Ebbo, A. A., Sani, D., Suleiman, M. M., Ahmed, A., & Hassan, A. Z. (2019). Phytochemical composition, proximate analysis and antimicrobial screening of the methanolic extract of diospyros mespiliformis Hochst ex a. DC (Ebenaceae). *Pharmacognosy Journal*, 11(2), 362–368. https://doi.org/10.5530/pj.2019.11.55

Extraction Magazine. (2018). Ultrasonic and Microwave Assisted Cannabis Extraction Techniques – Extraction Magazine https://extractionmagazine.com/category/applied-technology/ultrasonic-microwave-assisted-cannabis-extraction/ Opened on 16/01/2023

Extraction Magazine (2022). The Differences Between Maceration, Percolation and Soxhlet Extraction - Extraction Magazine https://www.bing.com/search?q=+is+maceration+and+percolation+expensive&qs=n&form=QBRE&sp=-1&pq=is+maceration+and+percolation+expensive&sc=7-39&sk=&cvid=5AD715AF3B11400CB5AD8E81F554E0CC&ghsh=0&ghacc=0&ghpl= Opened on 16/01/2023

Ferdes, M. (2018). Antimicrobial compounds from plants. In Fighting Antimicrobial Resistance (Issue July, 243–271). https://doi.org/10.5599/obp.15.15

Funatogawa, K., Hayashi, S., Shimomura, H., Yoshida, T., Hatano, T., Ito, H., & Hirai, Y. (2004). Antibacterial activity of hydrolyzable tannins derived from medicinal plants against Helicobacter pylori. *Microbiology and immunology*, 48(4), 251–261. https://doi.org/10.1111/j.1348-0421.2004.tb03521.x

Gao, J. J., Igalashi, K., & Nukina, M. (1999). Radical scavenging activity of phenylpropanoid glycosides in Caryopteris incana. *Bioscience, Biotechnology, and Biochemistry*, 63(6), 983–988. https://doi.org/10.1271/bbb.63.983

Gay, N., Belmonte, O., Collard, J.-M., Halifa, M., Issack, M. I., Mindjae, S., Palmyre, P., Ibrahim, A. A., Rasamoelina, H., Flachet, L., Filleul, L., & Cardinale, E. (2017). Review of Antibiotic Resistance in the Indian Ocean Commission: A Human and Animal Health Issue. *Frontiers in Public Health*, 5, 162. https://doi.org/10.3389/fpubh.2017.00162

Gberikon, G.M., Agbulu, C.O. and Adeoti, I.I. (2016) Single and Combined Effects of Antibacterial Activities of Fruit and Stem Bark Extracts of Tetrapleura tetraptera on Streptococcus mutans and Pseudomonas aeruginosa. *International Journal of Sciences*, 4, 46-49. https://doi.org/10.9734/EJMP/2016/29585

Guimarães, A. C., Meireles, L. M., Lemos, M. F., Guimarães, M., Endringer, D. C., Fronza, M., & Scherer, R. (2019). Antibacterial Activity of Terpenes and Terpenoids Present in Essential Oils. *Molecules (Basel, Switzerland)*, *24*(13), 2471. https://doi.org/10.3390/molecules24132471

Gupta, D., Dubey, J., & Kumar, M. (2016). Phytochemical analysis and antimicrobial activity of some medicinal plants against selected common human pathogenic microorganisms. *Asian Pacific Journal of Tropical Disease*, 6, 15-20. https://doi.org/10.1016/S2222-1808(15)60978-1

Halberstein R. A. (2005). Medicinal plants: historical and cross-cultural usage patterns. *Annals of Epidemiology*, 15(9), 686–699. https://doi.org/10.1016/j.annepidem.2005.02.004

Hamadnalla, H. M., Hamas, M. A. B., & Adam, A. A. I. (2020 Phytochemical Screening, Antimicrobial and Antioxidant Activities of Punica Grantum Peel.Peel. Open Access *Journal of Biogeneric Science and Research*. 1. 10.46718/JBGSR.2020.01.000020.

Ikuta, K. S., Swetschinski, L. R., Aguilar, G. R., Sharara, F., Mestrovic, T., Gray, A. P., ... & Dhingra, S. (2022). Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019. The Lancet, 400(10369), 2221-2248.

Jamshidi-Kia, F., Lorigooini, Z., & Amini-Khoei, H. (2018). Medicinal plants: Past history and future perspective. *Journal of Herbmed Pharmacology*, 7(1), 1–7. https://doi.org/10.15171/jhp.2018.01

Joshua, M., lenkosi, N., & Albert, M. (2013). Antimicrobial activity of Euclea undulata, Euclea divinorum and Diospyros lycioides extracts on multi-drug resistant Streptococcus mutans. Journal of Medicinal Plants Research, 7(37), 2741-2746. https://doi.org/10.5897/JMPR12.1180

Kabera, J. N., Semana, E., Mussa, A. R., & He, X. (2014). Plant Secondary Metabolites: Biosynthesis, Classification, Function and Pharmacological Properties. *Journal of Pharmacy and Pharmacology*, 2(January), 377–392.

Kaou, A. M., Mahiou-Leddet, V., Hutter, S., Aïnouddine, S., Hassani, S., Yahaya, I., Azas, N., & Ollivier, E. (2008). Antimalarial activity of crude extracts from nine African medicinal plants. *Journal of Ethnopharmacology*, 116(1), 74–83. https://doi.org/10.1016/j.jep.2007.11.001

Khalaf, N. A., Shakya, A. K., Al-Othman, A., El-Agbar, Z., & Farah, H. (2008). Antioxidant activity of some common plants. *Turkish Journal of Biology*, 32(1), 51–55.

Khan, M. S. A., & Ahmad, I. (2019). Herbal medicine: current trends and future prospects. In New look to phytomedicine (pp. 3-13). Academic Press.

Leigh Ann, A. (2021). Antibiotics - Common Side Effects, Allergies and Reactions (drugs.com) https://www.drugs.com/article/antibiotic-sideeffects-allergies-reactions.html#:~:text=When%20used%20appropriately%2C%20most%20antibiotics%20are%20relatively%20safe,or%20other%20allergic%20reactions.%20Soft%20stools%2C%20short-term%20diarrhea. Opened on 16/01/2023

Leitão, G. G., Simas, N. K., Soares, S. S. V., De Brito, A. P. P., Claros, B. M. G., Brito, T. B. M., & Delle Monache, F. (1999). Chemistry and pharmacology of Monimiaceae: A special focus on Siparuna and Mollinedia. *Journal of Ethnopharmacology*, 65(2), 87–102. https://doi.org/10.1016/S0378-8741(98)00233-5

Lhuilier, A. (2007). Contribution à l'étude phytochimique de quatre plantes malgaches : Agauria salicifolia Hook.f ex Oliver, Agauria polyphylla Baker (Ericaceae), Tambourissa trichophylla Baker (Monimiaceae) et Embelia concinna Baker (Myrsinaceae). PhD, *Institut National Polytechnique de Toulouse*, 214.

Rosa, M., Olivaro, C., Cerdeiras, M.P., & Vazquez, A. (2010). Antibacterial Activity of Ibicella lutea glycosides. *Revista Latinoamericana de Química, 38*, 98-102.

Maobe, M. A. G., Gitu, L., Gatebe, E., Rotich, H., Karanja, P. N., Votha, D. M., Nderitu, I. W., & Kungu, W. (2013). Antifungal activity of eight selected medicinal herbs used for the treatment of diabetes, malaria and pneumonia in Kisii Region, Southwest Kenya. *World Journal of Medical Sciences*, 8(1), 74–78. https://doi.org/10.5829/idosi.wjms.2013.8.1.6662

Maroyi, A. (2017). Euclea undulata Thunb.: Review of its botany, ethnomedicinal uses, phytochemistry and biological activities. *Asian Pacific Journal of Tropical Medicine*, 10(11), 1030–1036. https://doi.org/10.1016/j.apjtm.2017.10.005

Maroyi, A. (2017). Review of ethnomedicinal uses, phytochemistry and pharmacological properties of euclea natalensis A.DC. *Molecules*, 22(12). https://doi.org/10.3390/molecules22122128

Mathabe, M. C., Hussein, A. A., Nikolova, R. V., Basson, A. E., Meyer, J. J., & Lall, N. (2008). Antibacterial activities and cytotoxicity of terpenoids isolated from Spirostachys africana. *Journal of Ethnopharmacology*, *116*(1), 194–197. https://doi.org/10.1016/j.jep.2007.11.017

Mbabazi, I., Fedha, P., & Kowino, I. (2020). Antimicrobial Activity of Euclea divinorum Hern (Ebenaceae) Leaves, Tender Stems, Root Bark and an Herbal Toothpaste Formulated from Its Ethanolic Root Bark Extract. 3. 8-16.

Mekonnen, A., Atlabachew, M., & Kassie, B. (2018). Investigation of antioxidant and antimicrobial activities of Euclea schimperi leaf extracts. *Chemical and Biological Technologies in Agriculture*, 5(1), 1–12. https://doi.org/10.1186/s40538-018-0128-x

Mohamed, A.-I. (2012). Approches ecologique et descriptive du genre Tambourissa de la famille Moniamiaceae a Ngazidja et Ndzouani (Comores).

Mulholland, E. K., & Adegbola, R. A. (2005). Bacterial Infections — A Major Cause of Death among Children in Africa. *New England Journal of Medicine*, 352(1), 75–77. https://doi.org/10.1056/nejme048306

National Committee for Clinical Laboratory Standard (1993). Performance Standards for Antimicrobial Disc Suspectibility Tests. Approved Standard NCCLS Publication M2-A5, Villanova, PA, USA

Ncube, N. S., Afolayan, A. J., & Okoh, A. I. (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. *In African Journal of Biotechnology* (Vol. 7, Issue 12, pp. 1797–1806). https://doi.org/10.5897/AJB07.613

Njiire, M. M., Mbudambula, N. L. M., & Kiiru, J. (2014). Antimicrobial activity of Warbugia ugandensis against gram- negative multi- drug resistant bacteria. *The Journal of Agriculture, Science and Technology*, 16(2), 45–57.

Nkala, B.A, Mbongwa, H.P, Qwebani-Ogunleye, T (2021); Estimation Of The Preliminary Screening Of Phytochemicals, Total Phenolic Content, Total Flavonoid Content, Antioxidant And Alpha-Amylase Inhibitory Activities Of The Selected South African Plant Extracts For Medicinal Purposes; *International Journal of Scientific and Research Publications (IJSRP)* 11(10) (ISSN: 2250-3153), DOI: http://dx.doi.org/10.29322/IJSRP.11.10.2021.p11822

Nn, A. (2015). A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. *Medicinal & Aromatic Plants*, 04(03), 3–8. https://doi.org/10.4172/2167-0412.1000196

Norton, M. P., & Karczub, D. G. (2015). Appendix 4: Secondary Metabolites. *Plant Physiology and Development*, 605–606. http://6e.plantphys.net/appendices.html#

Nwaehujor, C., Onyenweaku, E. O., Mgbang, J. E., & E., E. C. (2017). In vitro Free Radical Scavenging Activities of the Saponin-rich Fractions from Vernonia amygdalina Del. (Compositae). *American Journal of Pharmacological Sciences*, 5(1), 11-17.

Odongo, E., Mungai, N., Mutai, P., Karumi, E., Mwangi, J., Okalebo, F., & Simiyu, J. (2017). Antioxidant and anti-inflammatory activities of selected medicinal plants from western Kenya. *African Journal of Pharmacology and Therapeutics*, 6(4), 178–182.

Olila, D., Olwa-Odyek, & Opuda-Asibo, J. (2001). Antibacterial and antifungal activities of extracts of Zanthoxylum chalybeum and Warburgia ugandensis, Ugandan medicinal plants. *African Health Sciences*, 1(2), 66–72.

Oloyede, G. K., Onocha, P. A., & Soyinka, J. (2010). Phytochemical screening, antimicrobial and antioxidant activities of four Nigerian medicinal plants. 8.

O'Neill J (2016). Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. *The Review on Antimicrobial Resistance* (http://amrreview.org/sites/default/files/160525_Final%20paper_with%20cover.pdf)

Otero, C., Miranda-Rojas, S., Llancalahuén, F. M., Fuentes, J. A., Atala, C., González-Silva, G., Verdugo, D., Sierra-Rosales, P., Moreno, A., & Gordillo-Fuenzalida, F. (2022). Biochemical characterization of Peumus boldus fruits: Insights of its antioxidant properties through a theoretical approach. *Food chemistry*, *370*, 131012. https://doi.org/10.1016/j.foodchem.2021.131012

Othman, A., Ismail, A., Ghani, N.A., & Adenan, I. (2007). Antioxidant capacity and phenolic content of cocoa beans. *Food Chemistry, 100*, 1523-1530.

Palanisamy, C. Palanisamy, R. Selvarajan, R. Selvarajan, F.O. Balogun, F. Balogun, D. Kanakasabapathy, D. Kanakasabapathy, & A.O.T. Ashafa, A. Ashafa. (2019). Antioxidant and antimicrobial activities of (6E, 10E)-2, 6, 24-trimethyl pentacosa-2, 6, 10-triene from Euclea crispa leaves. South African journal of botany, 124, 311-319. doi: 10.1016/j.sajb.2019.03.019

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. *Journal of Nutritional Science*, 5. https://doi.org/10.1017/jns.2016.41

Patil, P. S., Patil, K. N., Dudhgaonkar, T. D., Mohite, S. K., & Magdum, C. S. (2016). A Review On : Phytochemicals as Nutraceuticals. 2(2), 254–260.

Pattananandecha, T., Apichai, S., Sirilun, S., Julsrigival, J., Sawangrat, K., Ogata, F., Kawasaki, N., Sirithunyalug, B., & Saenjum, C. (2021). Anthocyanin Profile, Antioxidant, Anti-Inflammatory, and Antimicrobial against Foodborne Pathogens Activities of Purple Rice Cultivars in Northern Thailand. *Molecules (Basel, Switzerland)*, *26*(17), 5234. https://doi.org/10.3390/molecules26175234

Rene. F.K. (2016). Microbes and Pathogens - dummies https://www.dummies.com/article/academics-the-arts/science/biology/microbes-and-pathogens-169109/ Opened on 16/01/2023

Rweyemamu, M., Otim-Nape, W., Serwadda, D. (2006) Foresight. Infectious Diseases :preparing for the future. Africa. *Office of Science and Innovation*, London. Available at: https://library.au.int/foresight-infections-diseases-preparing-future-africa-3

Sahu, R., & Saxena, J. (2013). Screening of total phenolic and flavonoid content in conventional and non conventional species of curcuma. *International Journal of Pharmaceutical Sciences Review and Research*, 21(2), 24–26.

Saive, M., Frederich, M., & Fauconnier, M. L. (2020). Plants used in traditional medicine in the Comoros archipelago: a review. Biotechnologie, Agronomie, Société et Environnement, 24(2).URL : https://popups.uliege.be/1780-4507/index.php?id=18553

Schmeda-Hirschmann, G., Rodriguez, J. A., Theoduloz, C., Astudillo, S. L., Feresin, G. E., & Tapia, A. (2003). Free-radical scavengers and antioxidants from Peumus boldus Mol. ("Boldo"). *Free radical research*, *37*(4), 447–452. https://doi.org/10.1080/1071576031000090000

Sebei, K., Sakouhi, F., Herchi, W., Khouja, M. L., & Boukhchina, S. (2015). Chemical composition and antibacterial activities of seven Eucalyptus species essential oils leaves. *Biological research*, *48*(1), 7. https://doi.org/10.1186/0717-6287-48-7

Shah, M. D., & Hossain, M. A. (2014). Total flavonoids content and biochemical screening of the leaves of tropical endemic medicinal plant Merremia borneensis. *Arabian Journal of Chemistry*, 7(6), 1034–1038. https://doi.org/10.1016/j.arabjc.2010.12.033

Shakeri, A., Hazeri, N., Vlizadeh, J., Ghasemi, A., & Tavallaie, F.Z. (2012). Phytochemical screening, antimicrobial and antioxidant activities of Anabasis aphylla extracts. *Kragujevac Journal of Science*, 34.

Soidrou, S. H., Soule, H. H., Farah, A., Hassane, S. O. S., Chaouch, A., & Lachkar, M. (2014).

Ethnopharmacological investigation of four plants used as medicinal in Ngazidja island. *International Journal of Phytopharmacology*, 5(6), 416-422

Soidrou, S. H., Farah, A., Satrani, B., Ghanmi, M., Lachkar, M., & Mohamed, A. S. (2020). Chemical composition, antioxidant and antimicrobial activity of Vetiveria zizanioides roots essential oil harvested in Ndzuwani, Comoros. *World Journal of Pharmacy and Pharmaceutical Sciences*, 1(10), 82–95. https://doi.org/10.20959/wjpps202010-17288

Soule, H. H., Mrani, D., Hajaji, H. El, Elkhamlichi, A., Said Hassane Soidrou, A. F., & Lachkar, M. (2017). Screening , Total Phenols and Flavonoids Contents , Antioxidant and Antibacterial Activities of Tambourissa comorensis ... *Der Pharma Chemica*, 11(9), 56–61.

Sticher, O. (2008). Natural product isolation. *In Natural Product Reports* (Vol. 25, Issue 3). https://doi.org/10.1039/b700306b

Tagousop, C. N., Tamokou, J. D., Kengne, I. C., Ngnokam, D., & Voutquenne-Nazabadioko, L. (2018). Antimicrobial activities of saponins from Melanthera elliptica and their synergistic effects with antibiotics against pathogenic phenotypes. *Chemistry Central journal*, 12(1), 97. https://doi.org/10.1186/s13065-018-0466-6

Tagousop, C. N., Tamokou, J. D., Ekom, S. E., Ngnokam, D., & Voutquenne-Nazabadioko, L. (2018). Antimicrobial activities of flavonoid glycosides from Graptophyllum grandulosum and their mechanism of antibacterial action. *BMC complementary and alternative medicine*, 18(1), 252. https://doi.org/10.1186/s12906-018-2321-7

Tan, J. B. L., & Lim, Y. Y. (2015). Critical analysis of current methods for assessing the in vitro antioxidant and antibacterial activity of plant extracts. *Food Chemistry*, 172, 814–822. https://doi.org/10.1016/j.foodchem.2014.09.141

Teklay, G., Abula, T., & Gebrelibanos, M. (2015). Antibacterial and phytochemical screening of root extracts of Euclea racemosa subsp. Schimperi. 2(2), 66–70. https://doi.org/10.13040/IJPSR.0975-8232.IJP.2(2).66-70

The Pharmacognosy (2019). Medicinal Plants and Traditional Medicine (thepharmacognosy.com). https://thepharmacognosy.com/medicinal-plants/#:~:text=Accordingly%2C%20the%20WHO%20consultative%20group%20on%20medicinal%20plants,are%20precursors%20for%20the%20synthesis%20of%20useful%20drugs.%E2%80%9D. Opened in 12/01/2022

Vouga, M., & Greub, G. (2016). Emerging bacterial pathogens: the past and beyond. Clinical microbiology and infection : the official publication of the *European Society of Clinical Microbiology and Infectious Diseases*, 22(1), 12–21. https://doi.org/10.1016/j.cmi.2015.10.010

Wallnöfer, B. (2001). The biology and systematics of Ebenaceae: a review. Annalen Des Naturhistorischen Museums in Wien. *Serie B Für Botanik Und Zoologie*, 485–512.

Wang, C., Chen, Y., & Hou, C. (2019). Antioxidant and antibacterial activity of seven predominant terpenoids. *International Journal of Food Properties*, 22, 230 - 238.

Wang, J., Liu, L., Zhang, Q., Zhang, Z., Qi, H., & Li, P. (2009). Synthesized oversulphated, acetylated and benzoylated derivatives of fucoidan extracted from Laminaria japonica and their potential antioxidant activity in vitro. *Food Chemistry*, 114, 1285-1290.

Weigenand, O., Hussein, A. A., Lall, N., & Meyer, J. J. (2004). Antibacterial activity of naphthoquinones and triterpenoids from Euclea natalensis root bark. Journal of natural products, 67(11), 1936–1938. https://doi.org/10.1021/np030465d

Wendakoon, C; Peter C; & Daniel G. (2012). Evaluation of Selected Medicinal Plants Extracted in Different Ethanol Concentrations for Antibacterial Activity against Human Pathogens. *Journal of Medicinally Active Plants* 1, (2):60-68.

World Health Organization. Programme on Traditional Medicine. (2002). WHO traditional medicine strategy 2002-2005. *World Health Organization*. https://apps.who.int/iris/handle/10665/67163

World Health Organization (2018). World health statistics 2018: monitoring health for the SDGs, sustainable development goals. Geneva: *World Health Organization*; 2018. Licence: CC BY-NC-SA 3.0 IGO (https://www.who.int/gho/publications/world_health_statistics/2018/EN_WHS2018_TOC.pdf?ua=1)

World Health Organization. (2020). Global antimicrobial resistance surveillance system (GLASS) report: early implementation 2020. World Health Organization. https://apps.who.int/iris/handle/10665/332081. License: CC BY-NC-SA 3.0 IGO

World Health Organization, (2021). Antimicrobial resistance (who.int), https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance. Opened in 13/12/2021

Yan, Y., Li, X., Zhang, C., Lv, L., Gao, B., & Li, M. (2021). Research Progress on Antibacterial Activities and Mechanisms of Natural Alkaloids: A Review. *Antibiotics*, 10(3), 318. MDPI AG. http://dx.doi.org/10.3390/antibiotics10030318

Zielińska, S., Wójciak-Kosior, M., Dziągwa-Becker, M., Gleńsk, M., Sowa, I., Fijałkowski, K.J., Rurańska-Smutnicka, D., Matkowski, A., & Junka, A.F. (2019). The Activity of Isoquinoline Alkaloids and Extracts from Chelidonium majus against Pathogenic Bacteria and Candida sp. *Toxins*, 11.

APPENDIX

Approval letter request for the transportation from the Comoros of *Euclea mayottensis* and *Tambourissa comorensis*

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Mardi, le 9 Mars 2021

Ministère de l'environnement
Comores, Moroni

Objet: Demande pour obtenir des échantillons de Tambourissa comorensis et Euclea mayottensis pour mes recherches de fin de cycle

Mr/Mme,

Je me nomme Idaroussi Faida Khadidja, étudiante comorienne actuellement en Ouganda. Je suis en 2eme année de master en biochimie à l'Université Makerere.

Mon projet de fin de cycle consiste à un screening phyto-chimique, antimicrobienne et antioxydante activités de deux plantes médicinales communément utilisées aux Comores: Euclea mayottensis et Tambourissa comorensis.

L'étude consistera à apporter de nouvelles données concernant les composés phyto-chimiques de ces deux endémiques plantes des Comores ainsi que de leur potentielle bioactivités.

De ce fait, pour mener à bien mon projet de fin d'étude, je sollicite la permission pour pouvoir obtenir les échantillons de ces deux plantes

En attente d'une réponse favorable de votre part, veuillez accepter mes salutations les plus distinguées.

Cordiallement,

Idaroussi Faida Khadidja