CUKUROVA UNIVERSITY

INSTITUTE OF NATURAL AND APPLIED SCIENCES

PhD THESIS

Research on the Antibacterial and Cytotoxic Effects of Four Plants Used for Medicinal Purposes in the Central African Region

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Department of Biology

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This Doctorate Thesis was evaluated by the following Jury Members on 24/03/2025 and was approved by unanimity / majority of votes.

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ABSTRACT

This study investigates the secondary metabolites of the root and stem bark of *Crossopteryx febrifuga benth*, *Khaya anthotheca*, *Gardenia ternifolia*, and *Terminalia glaucescens*, obtained through maceration, using tub reactions. Antimicrobial and cytotoxic activities were assessed using antibiogram disc tests on *Pseudomonas aeruginosa* (ATCC 27853), *Enterobacter hormaechei* (ATCC 700323), *Bacillus spizizenii* (ATCC 6633), *Enterococcus casseliflavus* (ATCC 700327), *Staphylococcus aureus* subsp. *Rosenbach* (ATCC 6538), and *Salmonella Typhi* (ATCC 6539). Cytotoxicity was evaluated using the MTT assay on NIH3T3 mouse fibroblast cells. Extracts from the bark of *Terminalia glaucescens* and *Crossopteryx febrifuga* Benth exhibited antimicrobial activity against four bacterial strains, notably *Enterobacter hormaechei* (3 ± 2 cm), *Bacillus spizizenii* (5 ± 2 cm), *Staphylococcus aureus* (5 ± 2 cm), and *Salmonella Typhi* (3 ± 2 cm). Extracts from *Khaya anthotheca* and *Gardenia ternifolia* demonstrated activity against only two strains: *Enterobacter hormaechei* (4 ± 2 cm) and *Bacillus spizizenii* (5 ± 2 cm). The most potent extracts were obtained via maceration (hydromethanol 90%), inhibiting cell growth by 19%, whereas supercritical water extraction (125°C, 60 ATM) resulted in the lowest cytotoxicity. Higher extract concentrations correlated with increased inhibition, with plant E extracts exhibiting the strongest effect at 1 g/ml.

Keywords: Antimicrobial Activity, Cytotoxic Effect, Phytochemical Activities and Maceration.

ÇUKUROVA ÜNİVERSİTESİ
FEN BİLİMLERİ ENSTİTÜSÜ

DOKTORA TEZİ

Orta Afrika Bölgesinde Tedavi Amaçlı Kullanılan Dört Bitkinin Antibakteriyel ve Sitotoksik Etkilerinin Araştırılması

DAHIROU AMADOU

Danışman: Prof. Dr. Hatice KORKMAZ GÜVENMEZ

Biyoloji Anabilim Dalı

ÖZ

Bu çalışma, *Crossopteryx febrifuga benth*, *Khaya anthotheca*, *Gardenia ternifolia* ve *Terminalia glaucescens*'in kök ve gövde kabuğundan makerasyon yöntemiyle elde edilen sekonder metabolitlerini, tüp reaksiyonları kullanarak incelemektedir. Antimikrobiyal ve sitotoksik aktiviteler, *Pseudomonas aeruginosa* (ATCC 27853), *Enterobacter hormaechei* (ATCC 700323), *Bacillus spizizenii* (ATCC 6633), *Enterococcus casseliflavus* (ATCC 700327), *Staphylococcus aureus* subsp. *Rosenbach* (ATCC 6538) ve *Salmonella Typhi* (ATCC 6539) üzerinde antibiogram disk testi ile değerlendirilmiştir. Sitotoksisite, NIH3T3 fare fibroblast hücrelerinde MTT testi kullanılarak analiz edilmiştir. *Terminalia glaucescens* ve *Crossopteryx febrifuga* Benth kabuk ekstreleri, dört bakteri türüne karşı antimikrobiyal aktivite göstermiştir: *Enterobacter hormaechei* (3 ± 2 cm), *Bacillus spizizenii* (5 ± 2 cm), *Staphylococcus aureus* (5 ± 2 cm) ve *Salmonella Typhi* (3 ± 2 cm). *Khaya anthotheca* ve *Gardenia ternifolia* ekstreleri ise yalnızca iki bakteri türüne karşı aktivite göstermiştir: *Enterobacter hormaechei* (4 ± 2 cm) ve *Bacillus spizizenii* (5 ± 2 cm). En güçlü ekstreler, makerasyon (hidro-metanol %90) yöntemiyle elde edilmiş ve hücre büyümesini %19 oranında inhibe etmiştir. Süperkritik su ekstraksiyonu (125°C, 60 ATM) ise en düşük sitotoksisiteyi göstermiştir. Daha yüksek ekstrakt konsantrasyonları, artan inhibisyon ile korelasyon göstermiş olup, bitki E ekstreleri 1 g/ml konsantrasyonda en güçlü etkiyi göstermiştir.

Anahtar Kelimeler: Antimikrobiyal Aktivite, Sitotoksik Etki, Fitokimyasal Aktiviteler ve Makerasyon.

GENİŞLETİLMİŞ ÖZET

Orta Afrika Cumhuriyeti, Afrika kıtasının tam ortasında yer alan, 623.000 kilometrekarelik bir yüz ölçümüne sahip, denize kıyısı olmayan bir ülkedir. Kuzeyde Çad, doğuda Sudan ve Güney Sudan, batıda Kamerun, güneyde ise Demokratik Kongo Cumhuriyeti ve Kongo Cumhuriyeti ile sınır komşusudur. Tropikal nemli iklim kuşağında bulunan ülkede, güneybatı ve güneydoğuda dokuz aya kadar süren yağışlar, merkezde yedi ay süren yağışlar ve kuzeyde beş ila altı ay süren yağışlar görülmektedir. Bu coğrafi yapı, güneyde yaklaşık 3,5 milyon hektarlık ormanlık alanlar ile kuzeyde ağaçlık savanların bulunduğu zengin bir bitki örtüsü oluşturur. Ülke genelinde deniz seviyesinden ortalama 650 ila 850 metre yükseklikte bulunan nispeten düz bir arazi yapısı mevcuttur. Bu fiziksel ortam, bulaşıcı hastalıkların yayılmasını teşvik eden faktörlerden biri olup, ulaşım yollarının yetersizliği ve izole bölgelerin varlığı nedeniyle sağlık hizmetlerine erişimi de zorlaştırmaktadır.

Orta Afrika'da birçok yerel bitki, halk arasında yaygın olarak tıbbi/terapötik etkileri olduğuna inanılan ve nesiller boyunca aktarılan geleneksel ilaçlar olarak kullanılmaktadır. Özellikle Orta Afrika Cumhuriyeti ve Kamerun'daki kırsal bölgelerde farmasötik ilaçlara erişimin sınırlı olması nedeniyle, bu çalışma, bu bölgede binlerce yıldır kullanılan tıbbi bitkilerin bilimsel olarak değerlendirilmesini amaçlamaktadır. Tıbbi bitkiler üzerine kapsamlı araştırmalar yürütmek, toksisite riski olmadan etkili doğal ilaçlar geliştirilmesine ve bu ilaçların herkes, özellikle kırsal kesimde yaşayanlar için erişilebilir olmasına katkıda bulunacaktır.

Bu koşullara rağmen, nüfusun %70'inden fazlası düzenli olarak bitkisel ilaçları kullanmaktadır. Ancak, çok az sayıda tıbbi bitki, etkinliklerini, güvenilirliklerini veya dozajlarını bilimsel olarak kanıtlamak amacıyla kapsamlı araştırmalara tabi tutulmuştur. Geleneksel tedavi yöntemleri genellikle şifacılar veya nesiller boyunca sözlü olarak aktarılan kültürel pratikler aracılığıyla belirlenmektedir. Göçebe çoban topluluklarında, aynı tıbbi bitkiler hem insanlar hem de hayvanlar için tedavi amaçlı kullanılmaktadır.

Bu çalışmada, halk tarafından hem kendi sağlıklarını hem de çiftlik hayvanlarını tedavi etmek için sıkça kullanılan bazı bitkiler, fitokimyasal bileşenlerini, antimikrobiyal aktivitelerini ve sitotoksik özelliklerini belirlemek amacıyla seçilmiştir. Elde edilen sonuçlar, kullanıcıların tıbbi bitkilerini daha iyi anlamalarına yardımcı olacağı gibi, biyoteknoloji ve farmasötik endüstrileri için yeni biyoaktif bileşik kaynaklarının keşfedilmesine de olanak sağlayacaktır. Orta Afrika Cumhuriyeti'nde ve Orta Afrika'nın büyük bir kısmında bitkisel ilaçlar, geleneksel tıbbın temelini oluşturmakta olup, nüfusun %85'inden fazlası birincil sağlık hizmetlerinde bitkisel tedavilere güvenmektedir. Bununla birlikte, modern tıbbın gelişmesine rağmen, birçok kırsal topluluk hala geleneksel tıbbı kullanmakta ve bitkisel tedavileri günlük sağlık uygulamalarının ayrılmaz bir parçası olarak görmektedir. Bu durum, tıbbi bitkilerin daha ayrıntılı bir şekilde araştırılmasını ve biyoaktif bileşiklerinin belirlenmesini gerekli kılmaktadır.

Bu çalışmanın temel amacı, Orta Afrika'daki nüfusun ve geleneksel şifacıların tıbbi bitkilerini daha iyi anlamalarına katkıda bulunmak ve bu bitkilerin biyoaktif bileşikler açısından zenginliğini belirlemektir. Çalışma, bölgedeki en yaygın kullanılan dört tıbbi bitkinin fitokimyasal, antibakteriyel ve sitotoksik potansiyellerini incelemeye odaklanmaktadır. Bu dört bitki olan *Crossopteryx febrifuga benth, Khaya anthotheca, Gardenia ternifolia ve Terminalia glaucescens*, Google Forms platformunda gerçekleştirilen bir anket aracılığıyla geleneksel şifacılar ve tıbbi bitki kullanıcıları tarafından seçilmiştir. Anket, Orta Afrika Cumhuriyeti ve Kamerun'da yerleşik göçebe Fulani çoban toplulukları arasında yürütülmüş ve bu bitkilerin hem insan hem de hayvanlarda mikrobiyal enfeksiyonlara bağlı hastalıkların tedavisinde en çok tercih edilenler olduğu belirlenmiştir.

Bu çalışmanın temel amacı, Orta Afrika'daki yerel halkın ve geleneksel şifacıların tıbbi bitkiler hakkındaki bilgi birikimlerini daha iyi anlamalarına katkıda bulunmak ve bu bitkilerin biyoaktif bileşikler açısından sahip oldukları potansiyeli bilimsel olarak ortaya koymaktır. Orta Afrika Cumhuriyeti ve Kamerun gibi ülkelerde, halkın önemli bir kısmı sağlık sorunlarını tedavi etmek için modern tıbbın yanı sıra geleneksel şifa yöntemlerine de başvurmaktadır. Ancak, bu bitkilerin etkinliği ve güvenilirliği üzerine yapılan bilimsel çalışmalar oldukça sınırlıdır. Bu nedenle, araştırmamız hem geleneksel tıbbın bilimsel temellere dayandırılmasını sağlamak hem de tıbbi bitkilerin olası farmakolojik etkilerini belirlemek açısından büyük bir önem taşımaktadır. Çalışmamız, bölgede yaygın olarak kullanılan ve halk arasında güçlü iyileştirici özelliklere sahip olduğuna inanılan dört farklı tıbbi bitkiyi ele almaktadır.

Bu araştırma kapsamında incelenen dört tıbbi bitki *Crossopteryx febrifuga Benth, Khaya anthotheca, Gardenia ternifolia ve Terminalia glaucescens* olup, bunlar yerel halk ve geleneksel şifacılar tarafından en çok kullanılan türler arasında yer almaktadır. Bitkilerin seçim süreci, Orta Afrika Cumhuriyeti ve Kamerun'da yaşayan göçebe Fulani çoban toplulukları arasında yapılan kapsamlı bir anket çalışmasına dayanmaktadır. Bu anket, Google Forms platformu kullanılarak yürütülmüş ve farklı bölgelerdeki geleneksel şifacıların ve tıbbi bitki kullanıcılarının görüşleri toplanmıştır. Katılımcılara, en sık kullandıkları tıbbi bitkiler, bunların hangi hastalıkların tedavisinde kullanıldığı ve hangi dozlarda uygulandığı gibi sorular yöneltilmiştir. Toplanan veriler analiz edildiğinde, bu dört bitkinin hem insanlarda hem de hayvanlarda yaygın olarak görülen mikrobiyal enfeksiyonlara bağlı hastalıkların tedavisinde en çok tercih edilen türler olduğu tespit edilmiştir.

Çalışmada, söz konusu bitkilerin fitokimyasal profillerinin ayrıntılı olarak incelenmesi hedeflenmiş, aynı zamanda antibakteriyel ve sitotoksik potansiyelleri de laboratuvar ortamında test edilmiştir. Böylece, geleneksel kullanımlarının bilimsel temellere dayandırılması ve gelecekte olası farmasötik uygulamalar açısından değerlendirilmesi amaçlanmaktadır. Bölgedeki geleneksel tıp uygulamalarının bilimsel olarak desteklenmesi, modern tıp ile geleneksel tıp arasındaki köprünün kurulmasına yardımcı olabilir ve tıbbi bitkilerin sürdürülebilir kullanımını teşvik edebilir. Çalışmamız, yalnızca bu bitkilerin biyolojik etkilerini anlamakla kalmayıp, aynı zamanda Orta

Afrika'daki doğal bitki örtüsünün korunmasına ve geleneksel bilgi sistemlerinin sürdürülebilir şekilde gelecek nesillere aktarılmasına da katkı sağlamayı hedeflemektedir.

Araştırma kapsamında, bu bitkilerin kök ve gövde kabuklarından macerasyon yöntemi ile elde edilen ikincil metabolitler tüp reaksiyonları kullanılarak analiz edilmiştir. Bu analizler, fitokimyasal bileşenlerin varlığını belirlemek amacıyla gerçekleştirilen temel laboratuvar testlerini içermektedir. Antimikrobiyal aktiviteleri, *Pseudomonas aeruginosa* (ATCC 27853), *Enterobacter hormaechei* (ATCC 700323), *Bacillus spizizenii* (ATCC 6633), *Enterococcus casseliflavus* (ATCC 700327), *Staphylococcus aureus subsp. Rosenbach* (ATCC 6538) ve *Salmonella Typhi* (ATCC 6539) üzerinde antibiyogram disk testi kullanılarak değerlendirilmiştir. Bu yöntem, bakteri türlerinin test edilen bitki ekstrelerine karşı duyarlılıklarını belirlemek için yaygın olarak kullanılan güvenilir bir tekniktir. Ek olarak, farklı ekstraksiyon yöntemleri kullanılarak elde edilen ekstrelerin minimum inhibitör konsantrasyon (MIC) değerleri ölçülerek, hangi ekstrelerin en etkili olduğu belirlenmiştir.

Araştırma sonuçları, *Terminalia glaucescens* ve *Crossopteryx febrifuga Benth* kabuğundan elde edilen ekstrelerin *Enterobacter hormaechei* (3 ± 2 cm), *Bacillus spizizenii* (5 ± 2 cm), *Staphylococcus aureus* (5 ± 2 cm) ve *Salmonella Typhi* (3 ± 2 cm) bakterilerine karşı belirgin antimikrobiyal aktivite gösterdiğini ortaya koymuştur. *Khaya anthotheca* ve *Gardenia ternifolia* kabuk ekstreleri ise yalnızca *Enterobacter hormaechei* (4 ± 2 cm) ve *Bacillus spizizenii* (5 ± 2 cm) bakterilerine karşı etkinlik göstermiştir. En güçlü ekstreler macerasyon yöntemi (hidrometanol %90) ile elde edilirken, superkritik su ekstraksiyonu (125°C, 60 ATM) yöntemiyle elde edilen ekstrelerin en düşük sitotoksisiteyi gösterdiği belirlenmiştir. Daha yüksek konsantrasyona sahip ekstreler, hücre büyümesi üzerinde daha yüksek inhibisyon etkisi göstermiştir. Bitki E ekstreleri, 1 g/ml konsantrasyonda en güçlü inhibisyon etkisini sergilemiştir. Ayrıca, bu ekstrelerin gram pozitif bakterilere karşı daha güçlü bir inhibitör etki gösterdiği, gram negatif bakterilere karşı ise daha düşük bir etki sunduğu belirlenmiştir. Çalışmada kullanılan farklı ekstraksiyon yöntemlerinin antimikrobiyal aktivite ve sitotoksisite üzerindeki etkileri detaylı olarak değerlendirilmiştir.

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This research is dedicated to my beloved parents, my wife, and my children. May the strong ties between Turkey and the Central African Republic continue to flourish.

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SYMBOLS AND ABBREVIATIONS

CCE : Counter-Current Extraction

CAR : Central African Republic

DMSO : Dimethyl Sulfoxide

MTT : (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) Assay

NaOCl : Sodium Hypochlorite

NIH3T3 : Fibroblast Cell Line Derived from a Mouse NIH/Swiss Embryo

WHA : World Health Assembly

WHO : World Health Organization

1. INTRODUCTION

To enhance the understanding of bioactive molecules in medicinal plants among the population and traditional healers of Central Africa, a study was conducted to evaluate the phytochemical composition, antibacterial properties, and cytotoxic potential of four widely used medicinal plants: *Crossopteryx febrifuga benth, Khaya anthotheca, Gardenia ternifolia, and Terminalia glaucescens*. These plants were selected based on a survey carried out using the Google Forms platform, involving six traditional healers and three regular medicinal plant users from the nomadic Fulani herder communities in the Central African Republic and Cameroon. The results of this survey revealed that these four plants were among the most frequently cited for treating microbial infections in both humans and livestock across the region.

The selected plants were collected from western Cameroon and subsequently transported to the molecular biology laboratory at the Department of Biology, Çukurova University, for further analysis. The plant extracts used in this study originate from species that thrive in tropical and subtropical climates and have been documented in the pharmacopoeias of several African, South American, and Asian countries. In the Central African Republic, these plants are widely utilized in traditional medicine for both human and veterinary applications. However, despite their extensive use, scientific studies assessing their therapeutic efficacy, potential toxicity, and overall safety remain scarce. This research aims to bridge that knowledge gap by providing empirical data on their pharmacological potential, which could contribute to their validation in modern medical applications.

1.1. Crossopteryx febrifuga benth

Crossopteryx febrifuga benth is a deciduous tree from the *Rubiaceae* family, commonly found in the wooded savannas of Africa. It can grow up to six meters in height, with opposite, oval-shaped leaves that are often reddish at the base. The tree produces highly fragrant white flowers, which are clustered at the tops of the branches and eventually develop into round green fruits that turn black when ripe. Its dry seeds can remain attached to the tree for one to two years before dispersing (Jardin-du-Monde, 2017).

This species plays a significant role in African traditional medicine, where it is widely used for treating ailments such as dysentery, diarrhea, malaria, fever, and inflammatory disorders. Previous research has demonstrated that *C. febrifuga* extracts possess analgesic, anti-inflammatory, antipyretic, antimicrobial, hypoglycemic, cytotoxic, antioxidant, anti-plasmodia, and anti-trypanosome properties.

In the Central African Republic, the powdered bark of *C. febrifuga* is traditionally mixed with salt and given to cattle as a remedy. However, the true health effects of this practice remain scientifically unverified, highlighting the need for further research to determine its actual medicinal benefits and potential risks (Kayangar et al., 2019).

Table 1.1. Botanical Classification of *Crossopteryx Febrifuga Benth* (Balde ve ark., 2016)

Group	*Dicotyledons*
Family	*Rubiaceae*
Genus	*Crossopteryx*
Species	*Crossopteryx febrifuga (Afzel. ex G.Don) Benth.*

Figure 1.1. *Crossopteryx febrifuga benth* Branch (Philippe Birnbaum, 2003)

1.2. Khaya anthotheca

Khaya anthotheca is a tree known for its reddish-brown wood, which is highly valued in carpentry, sculpture, and the construction of traditional canoes. The *Khaya genus* comprises four species native to the African continent and belongs to the *Swietenoideae* subfamily within the *Meliaceae* family. It is closely related to *Carapa* and *Swietenia.* While species of *Khaya* share strong similarities in their flowers and fruits, they can be distinguished by differences in their leaflet structures (Obboa et al 2013).

Due to selective logging, many species within the *Khaya* genus, including others species, are now classified as endangered on the International Union for Conservation of Nature (IUCN) Red List, particularly in the tropical forests of Africa (Maroyi, 2008).

The bark of Khaya anthotheca has a bitter taste and is widely used in traditional medicine for both humans and animals. In folk medicine, its decoctions and infusions are commonly used to treat fever, cough, colds, pneumonia, abdominal pain, vomiting, and gonorrhea. Additionally, its external application is believed to help heal wounds, abrasions, and ulcers (Obboa et al 2013).

In Democratic Republic of Congo (DRC), the leaves of K. anthotheca are traditionally used to prepare arrow poison (Maroyi, 2008). In Tanzania, a root decoction is consumed to treat anemia, dysentery, and rectal prolapse. Historically, the *Shamba* people used the bark to produce reddish brown dye (Obboa et al 2013). In Central Africa, bark is also used in veterinary medicine, particularly as an anthelmintic (deworming agent) for calves and cattle. Despite its extensive traditional applications, more scientific studies are needed to validate its medicinal properties and assess its safety for both humans and animals (Obboa et al 2013).

Table 1.2. Botanical Classification of *Khaya anthotheca* (Chauvet, 2015)

Group	*Dicotyledons*
Family	*Meliaceae*
Genus	*Khaya*
Species	*Khaya anthotheca* (Welw.) C.DC.

Figure 1.2. Indicates Two *Khaya anthothica* Trees from different positions in its Habitat in Central African Savannah

1.3. Gardenia ternifolia

It is a flowering plant in the family *Rubiaceae* native to tropical, subtropical regions of Africa, Australasia and Oceania. Gardenia T. is an evergreen shrub, 1 to 6 meters high. Its short stems (or trunks) support an irregular and open crown. The bark greenish-yellow flaking off in fine irregular grayscales after the passage of fires. Their leaves are grouped in tufts at the end of thick, very short, rigid branches. They are 2 to 10 cm long. The pericarp is thick and fibrous. The fruits remain on the shrubs for much of the year. An ethno pharmacological study reveals that Gardenia T. is mainly used in the treatment of malaria, hypertension, diabetes, cough, asthma, rheumatism, diarrhoea, dental caries, leprosy, hernia, haemorrhoid and cancer (Agbodjento, et al., 2019). In the Central African Republic, the powder of its bark is mixed with salt to be used as cattle feed. It has a antimicrobial properties (Magassouba et al., 2007) and used again haemorrhoid lesions, malaria in Ethiopia (Nureye et al., 2018), again diabetes in Cameroon (Njomen G. et al.; 2008).

Table 1.3. Botanical Classification of *Gardenia Ternifolia* (Agbodjento et al., 2019)

Group	*Dicotyledons*
Family	*Rubiaceae*
Genus	*Gardenia*
Specie	*Gardenia Ternifolia*

Figure 1.3. Shows *Gardenia ternifolia* Tree in its Habitat in Central African Savannah

1.4. Terminalia glaucescens

Terminalia glaucescens is a flowering plant in the *Combretaceas* family and is commonly found in tropical and subtropical regions. The plant is locally abundant, and its common names are: *bawshe (Hausa), bawshihi (Fulani), Idi Odan (Yoruba), Edo (Igbo).* The plant is also reported to have traditional medicinal uses such as antimalarial, treatment of diarrhoea and tooth decay. The potential of aqueous extracts of *T. glaucescens* stem bark against some pathogenic organisms have been extensively investigated (Adebayo, 2009). It has proven antimicrobial properties and zero cytotoxic effects (Konan K., 2016; Magassoubave ark., 2007).

Figure 1.4. Botanical Classification of *Terminalia glaucescens* (Pakull et al., 2019)

2. PRELIMINARY WORK

2.1. Traditional Medicine:

Traditional medicine has been practiced since ancient times, encompassing the knowledge, skills, and practices rooted in theories, beliefs, and experiences unique to specific cultures. It is used for maintaining health as well as for the prevention, diagnosis, treatment, and cure of both physical and mental ailments. In some countries, traditional medicine is synonymous with complementary, alternative, or gentle medicine (WHO, 2014).

While traditional medicine has been in use for centuries and has gained increasing popularity in recent years, it remains unrecognized in many countries. The practices associated with traditional medicine vary significantly across different countries and regions, shaped by cultural, environmental, and religious factors. This diversity has contributed to a lack of proper attention and support for training and research in the field. There is insufficient data, both in quantity and quality, on the safety and effectiveness of traditional medicine, making it difficult to recommend its global adoption. The absence of comprehensive data is linked to health policies and the lack of established research methodologies for evaluating traditional medicine. Although some countries have published research on the subject, there is a clear need to foster further research into its safety and effectiveness, as well as to enhance the overall quality of studies (WHO, 2014; Mahomoodally et al., 2012).

2.1.1. Some Definitions in Traditional Medicine

Complementary Medicine

The terms "complementary medicine" or "alternative medicine" refer to a wide range of healthcare practices that are not part of a country's conventional or traditional medical system, nor fully integrated into the mainstream healthcare system. In some countries, these terms are used interchangeably with traditional medicine (WHO, 2014).

Herbal Medicines

Herbal medicines encompass herbs, herbal materials, herbal preparations, and finished products that contain plant parts, plant materials, or their combinations as active ingredients (WHO, 2014).

Characteristic Compound

A characteristic compound is a natural component found in a plant that helps to establish the identity or quality of herbal preparation. However, its biological or therapeutic activity is not necessarily reliant on this compound (WHO, 2000).

2.1.2. Biological Activities:

Biological activities refer to the alteration of an animal's basic functions or parts of an animal due to the administration of a test substance (WHO, 2000).

Therapeutic Activity

Therapeutic activity is an intervention that improves the symptoms of human diseases (WHO, 2000).

Traditional Therapy

Traditional therapies use various techniques that do not involve pharmaceuticals. These include acupuncture, chiropractic, osteopathy, manual therapies, qi gong, tai chi, yoga, naturopathy, thermal therapy, and other practices that address physical, mental, and spiritual health, as well as those that involve both the mind and body (WHO, 2000).

2.1.3. Phytotherapies

Plant-based medicines are defined as completed medicinal products that are labeled and consist solely of plant materials-either from the aerial or underground parts of plants, other plant substances, or combinations of plants-either in their raw form or as processed preparations. These plant products may include juices, gums, fatty oils, essential oils, and similar substances. In addition to the active ingredients, herbal medicines may also contain excipients. Products that combine plant materials with chemically defined active ingredients isolated from plants are not classified as herbal medicines. In some countries, however, herbal medicines may traditionally include natural, organic, or inorganic active ingredients that are not derived from plants (WHO, 2000).

2.2. WHO Recommendations on the Regulation of the Use of Medicinal Plants

The use of herbal medicines has experienced significant growth in recent years. As part of its initiative to promote traditional medicine, the WHO has been approached by member countries for assistance in regulating and encouraging the safe use of phototherapy within national healthcare systems.

Since 1991, in a report to the Forty-Fourth World Health Assembly, the WHO Director-General has emphasized the importance of herbal medicines in community health systems. Earlier, in 1978, the Thirty-First World Health Assembly passed a resolution (WHA 31.33) directing the Director-General to create and periodically update a therapeutic classification of medicinal plants alongside the classification of all medicines. Following this, the WHA 40.33 resolution, adopted in 1987, urged Member States to ensure quality control of medicines derived from traditional herbal remedies by employing modern methods, applying appropriate standards, and following good manufacturing practices. In 1989, *Resolution 42.43* called on Member States to regulate and control

herbal medicinal products and develop and enforce proper standards. Furthermore, the International Conference on Primary Health Care in Alma-Ata (USSR) in 1978 recommended incorporating traditional remedies with proven efficacy into national pharmaceutical policies and regulatory measures.

The goal of these guidelines is to establish essential criteria for evaluating the quality, safety, and efficacy of herbal medicines, thereby assisting national regulatory bodies, scientific organizations, and manufacturers in assessing the documentation related to these products. Generally, this evaluation should consider traditional use, which refers to prolonged use along with the medical, historical, and ethnological origins of these products. The definition of "prolonged use" may vary by country but should span at least several decades. Therefore, the evaluation should include descriptions found in medical or pharmaceutical literature or similar sources, as well as documentation regarding the use of a preparation with no specific time limit. Market authorizations for similar products should also be considered (WHO, 2000).

2.3. The Methods for Extracting Secondary Metabolites

There are various methods employed to extract secondary metabolites from medicinal plants. Extracting secondary metabolites involves isolating the medicinally active components of plant (and animal) tissues by using selective solvents through standardized procedures. The resulting products from plants are typically complex mixtures of metabolites, which may be in a liquid or semisolid state, or in dry powder form after the solvent is removed. These products are intended for both oral and external use (Sukhdev H., 2008).

2.4. General Techniques of Medicinal Plant Extraction

2.4.1. Maceration

Maceration is a process where the whole or coarsely powdered crude drug is placed in a stoppered container with a solvent and left to stand at room temperature for a period of at least 3 days, with occasional agitation, until the soluble materials have dissolved. After this, the mixture is strained, and the marc (the damp solid residue) is pressed. The combined liquids are then clarified through filtration or decantation after standing (Presta, 2014).

2.4.2. Infusion

Infusions are prepared by macerating the crude drug for a short period, either with cold or boiling water. Fresh infusions create dilute solutions of the readily soluble constituents of the crude drugs (Rakesh, 2008).

2.4.3. Percolation

Percolation is one of the most used methods for extracting active compounds, particularly in the preparation of tinctures and fluid extracts. The process typically involves a percolator, which is a narrow, cone-shaped vessel open at both ends. The solid materials are first moistened with an appropriate amount of men strum and left to stand for about four hours in a well-sealed container. After this, the mass is packed, and the top of the percolator is sealed. Additional men strum is then added to create a thin layer above the solid mass, allowing it to macerate in the closed percolator for 24 hours. Once this period has elapsed, the percolator's outlet is opened, allowing the liquid to drip out gradually. More men strum is added as needed until the percolate reaches approximately three-quarters of the desired final volume. The remaining solid residue (marc) is then pressed, and the extracted liquid is combined with the percolate. Sufficient men strum is added to achieve the required volume, and the resulting mixture is clarified by either filtration or by letting it stand before decanting (Sukhdev H. et al, 2008).

2.4.4. Digestion

Digestion is a modified form of maceration in which mild heat is applied during the extraction process. This method is employed when a slightly elevated temperature is acceptable, as it enhances the solvent's extraction efficiency (Hanan B. et al, 2013).

2.4.5. Decoction

Decoction involves boiling the crude drug in a specified amount of water for a predetermined period, followed by cooling and straining or filtering. This technique is suitable for extracting water-soluble and heat-stable components. It is widely used in Ayurveda medicine for preparing extracts. The initial crude drug-to-water ratio is standardized, such as 1:4 or 1:16, and the volume is reduced to one-fourth of the original by boiling during extraction. The concentrated extract is then filtered and either used directly or subjected to further processing (Hanan B. et al, 2013).

2.4.6. Hot Continuous Extraction (Soxhlet extractor)

In this technique, finely powdered crude drug material is placed inside a porous bag or "thimble" made of durable filter paper, which is then inserted into chamber E of the Soxhlet apparatus. The extracting solvent, contained in flask A, is heated, causing its vapors to rise and condense in condenser D. The condensed solvent drips into the thimble, where it encounters the crude drug and dissolves its active compounds. Once the liquid level in chamber E reaches the top of siphon tube C, the extracted solution is siphoned back into flask A. This cycle continues continuously until a drop of solvent from the siphon tube leaves no residue upon evaporation. Compared to previously mentioned extraction techniques, the Soxhlet method enables the extraction of larger quantities of plant material while using a significantly smaller volume of solvent (Konan K., et al 2016; Sukhdev

H., 2008). This results in considerable savings in time, energy, and financial costs. On a small scale, it is used as a batch process, but it becomes more cost-effective and efficient when adapted into a continuous extraction method for medium- to large-scale operations (Memet I., 2014).

2.4.7. Aqueous (K.)-Alcoholic Extraction by Fermentation

Some *Ayurveda* medicinal preparations, such as cassava and arista, employ fermentation as a means of extracting active components. In this method, the crude drug, either in powdered form or as a decoction is soaked for a specific duration, during which it ferments and produces alcohol naturally. This alcohol acts as both an extracting, aiding in the dissolution of active compounds, and a preservative. If the fermentation process is conducted in an earthen vessel, the vessel should not be newly made; it must first be treated by boiling water in it. For large-scale production, alternative containers such as wooden vats, porcelain jars, or metal vessels are used instead of earthenware. Examples of traditional preparations obtained through this method include *karpurasava, kanakasava*, and *dasmularista*. While this approach has not yet been standardized in Ayurveda, advancements in fermentation technology provide the potential for refining and regulating this technique to produce herbal drug extracts (Memet I., 2014).

2.4.8. Counter-Current Extraction (CCE)

In counter-current extraction (CCE), the raw material is first moistened and ground using toothed disc disintegrators to create a fine slurry. This slurry is then introduced into a cylindrical extractor where it is subjected to the extraction solvent. As the material progresses through the extractor, it becomes increasingly concentrated with the desired extract. Optimal extraction is achieved by fine-tuning the solvent and material quantities, as well as their respective flow rates. The process is highly efficient, taking little time and posing no risk of damage due to high temperatures. The final product is a concentrated extract that exits from one end of the extractor, while the residual marc, which contains minimal solvent, is expelled from the other end.

The counter-current extraction method offers several notable advantages:

1. Efficient Solvent Usage – A smaller volume of solvent is required to extract the same amount of plant material compared to other methods such as maceration, decoction, and percolation.
2. Room Temperature Process – Since CCE is typically carried out at room temperature, heat-sensitive compounds are preserved, unlike in methods that use elevated temperatures.
3. Prevention of Heat Damage – During the pulverization step, the heat generated is absorbed by water, protecting heat-sensitive constituents from thermal degradation.

4. Enhanced Extraction Efficiency – CCE has been shown to be more effective and efficient than traditional continuous hot extraction methods (Nureye et al., 2018).

2.4.9. Ultrasound Extraction (Sonication)

This method utilizes ultrasound waves with frequencies between 20 kHz and 2000 kHz to enhance cell wall permeability and induce cavitation. While it proves beneficial in specific cases, such as extracting compounds from rauwolfia root, its large-scale use remains restricted due to high operational costs. A notable drawback of this technique is the potential negative impact of ultrasound energy exceeding 20 kHz, which may lead to the generation of free radicals. This, in turn, can cause unwanted alterations in the molecular structure of active constituents in medicinal plants (Hanan B., 2013)**.**

2.4.10. Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction (SFE) is an advanced sample preparation technique designed to minimize the use of organic solvents while enhancing sample throughput. Key factors influencing the process include temperature, pressure, sample volume, analytic collection, co solvent addition, flow regulation, pressure control, and the use of restrictors. Typically, cylindrical extraction vessels are employed in SFE, demonstrating high performance and efficiency. However, the proper collection of extracted analytes is crucial, as losses at this stage can give a false impression of poor extraction efficiency (Sukhdev H., 2008).

Carbon dioxide (CO_2) is the most used supercritical fluid in SFE due to its favorable physical properties, affordability, safety, and abundance. However, CO_2 has polarity limitations, which can hinder the extraction of polar solutes or substances with strong analytic-matrix interactions. To address this, organic solvents are often introduced as co solvents. Recently, argon has been explored as an alternative due to its cost-effectiveness and greater inertness. Component recovery rates tend to improve with increasing pressure and temperature, with argon achieving optimal recovery at 500 ATM and 150°C (Sukhdev H., 2008).

The extraction procedure possesses the following distinct advantages.

i. The extraction of constituents at low temperature, which strictly avoids damage from heat and some organic sol vents
ii. No solvent residues.
iii. Environmentally friendly extraction procedure. The largest area of growth in the development of SFE has been the rapid expansion of its applications. SFE finds extensive application in the extraction of pesticides, environmental samples, foods and fragrances, essential oils, polymers and natural products. The major deterrent

in the commercial application of the extraction process is its prohibitive capital investment.

2.4.11. Phytonics Extractions (With Hydro fluorocarbon Solvents)

The photonic extraction method utilizes a hydrofluorocarbon-134a solvent and innovative technology to enhance its exceptional properties in extracting plant materials. This approach offers significant environmental benefits and health, and safety advantages compared to traditional extraction methods, particularly producing high-quality natural fragrant oils, flavors, and biological extracts. Developed by Advanced Phytonic Limited (Manchester, UK), this patented technology, known as the "Phytonics process," is primarily used to extract aromatic components from essential oils and biological or phytopharmacological extracts. These products can typically be used directly without the need for further physical or chemical treatments (Sukhdev H., 2008).

2.5. Quality Control Parameters for Medicinal Plants

Medicinal plants consist of various molecules in different proportions, with each molecule potentially having a specific effect on the physiological system of an organism. These effects may be either beneficial or harmful depending on the intended use. The concentrations of these molecules must be within tolerable limits for the organism; otherwise, they can cause toxicity, leading to cell death, organ dysfunction, or even death in extreme cases due to the accumulation of harmful molecules.

2.5.1. Safety Evaluation

Traditional therapies are generally considered safe when administered correctly by qualified practitioners, though accidents can occur, particularly when those providing the therapy lack proper training. To ensure safety, therapies should follow established regulations, and therapeutic indications should be based on verified evidence wherever possible. While these therapies rarely result in severe adverse effects, obtaining comprehensive data on their safety can be challenging. Therefore, a systematic safety evaluation of herbal medicines is crucial and can be conducted through a series of tests, such as:

- Genotoxicity tests (in vitro); Haemolysis tests (in vitro); Systemic toxicity tests (in vivo) and cytotoxicity tests (in vitro)

And Clinical trials in humans and animals.

2.5.2. Evaluation of Cytotoxicity

Cytotoxicity of plant extracts can be evaluated using two primary methods. The first is a basal toxicity test, which is applicable to all cell types. An example of this test is the trypan blue assay, which determines cell viability by assessing whether the cell is alive or dead. The second method is a specific toxicity test, which is designed for primary cultures and aims to assess whether the cell performs its intended function. For instance, the MTT test can be used to study mitochondrial function, providing insights into the cell's ability to carry out its normal activities (WHO, 2022).

2.5.3. Evaluation of Effectiveness

The evaluation of effectiveness should encompass all critical aspects. This includes reviewing relevant documentation and providing references or copies of original articles. If previous studies have been conducted, they should be considered in the evaluation. It is important to specify or describe the pharmacological and clinical effects of the active ingredients, and if available, those of their therapeutically active constituents. Several traditional therapies, such as acupuncture and manual therapies, are already integrated into the healthcare systems of various countries. However, there is a growing demand for studies to assess their effectiveness. The efficacy of many traditional therapies is largely dependent on the practitioner's expertise, particularly their skills and experience (OMS, 2022).

2.6. The Health System in the Central African Republic

In terms of socio-health conditions, the Central African Republic has experienced a continuous deterioration of all health indicators over the past fifteen years, reflecting the decline in living conditions of the population and the failure of the health system. The geographical accessibility to health services within a radius of 5 km increased from 45% in 1995 (EDS 1994/95) to 65.2% in 2000 (MICS 2000). However, this improvement conceals disparities based on residency: 98% in urban areas compared to 47% in rural settings. It should be noted that at least 25% of the population in rural areas travel more than 10 km on foot or using makeshift means to reach a health facility. The analysis of the distribution of public health infrastructure carried out in the Second National Health Development Plan (PNDS II) highlights an unequal distribution across regions.

Infectious and parasitic diseases are the primary cause of current health expenditure. In 2015 and 2016, respectively, 73.3% and 72.8% of current health expenditures were related to these diseases. The share of current health expenditure attributed to them slightly decreased to 68.9% in 2017 and 68.3% in 2018. Among these diseases, malaria alone accounted for between 30.8% (2016) and 35.4% (2018) of current health expenditures. HIV/AIDS and other sexually transmitted diseases accounted for between 6.2% (2018) and 18.1% (2016) of current health expenses. Respiratory infections are increasingly responsible for significant portions of current health expenditures, with proportions gradually increasing from 5.3% in 2015 to 6.4% in 2018. Current health expenditure

related to reproductive health has shown constant growth during the period 2015-2018, rising from 6.8 billion CFA francs to 27 billion CFA francs(HeRAMS, 2023).

2.6.1. Access to Health System Data in the Central African Republic

Infrastructure density per 1000 km² categorizes the regions into three groups:

- Group 1: Health Regions 5 and 6 have less than one infrastructure per 1000 km²;
- Group 2: Health Regions 1, 2, 3, and 4 have approximately 1 to 2 infrastructures per 1000 km²;
- Group 3: Health Region 7, which stands out with 567 infrastructures per 1000 km². The analysis of services per ten thousand inhabitants reveals that regions with larger areas and lower population densities typically have the highest number of services relative to their population: Health Region 6 has 2.74 services, Health Region 5 has 3.57, Health Region 3 has 2.54, Health Region 2 has 1.75, Health Region 1 has 2.24, Health Region 4 has 2.41, and Health Region 7 has 0.71 services. In 2012, the country had a total of 780 health facilities, including 667 in the public sector and 113 in the private sector (WHO, 2022).

2.6.2. The Most Common Diseases in Central Africa

The most prevalent diseases in Central Africa include infectious and parasitic diseases, HIV/AIDS, other sexually transmitted diseases (STDs), tuberculosis, malaria, respiratory infections, diarrheal diseases, neglected tropical diseases, vaccine-preventable diseases, hepatitis, Ebola Virus Disease (EVD), other unspecified infectious and parasitic diseases, as well as maternal and perinatal conditions (WHO, 2022).

3. MATERIAL AND METHOD

3.1. Simple Collection of Plants

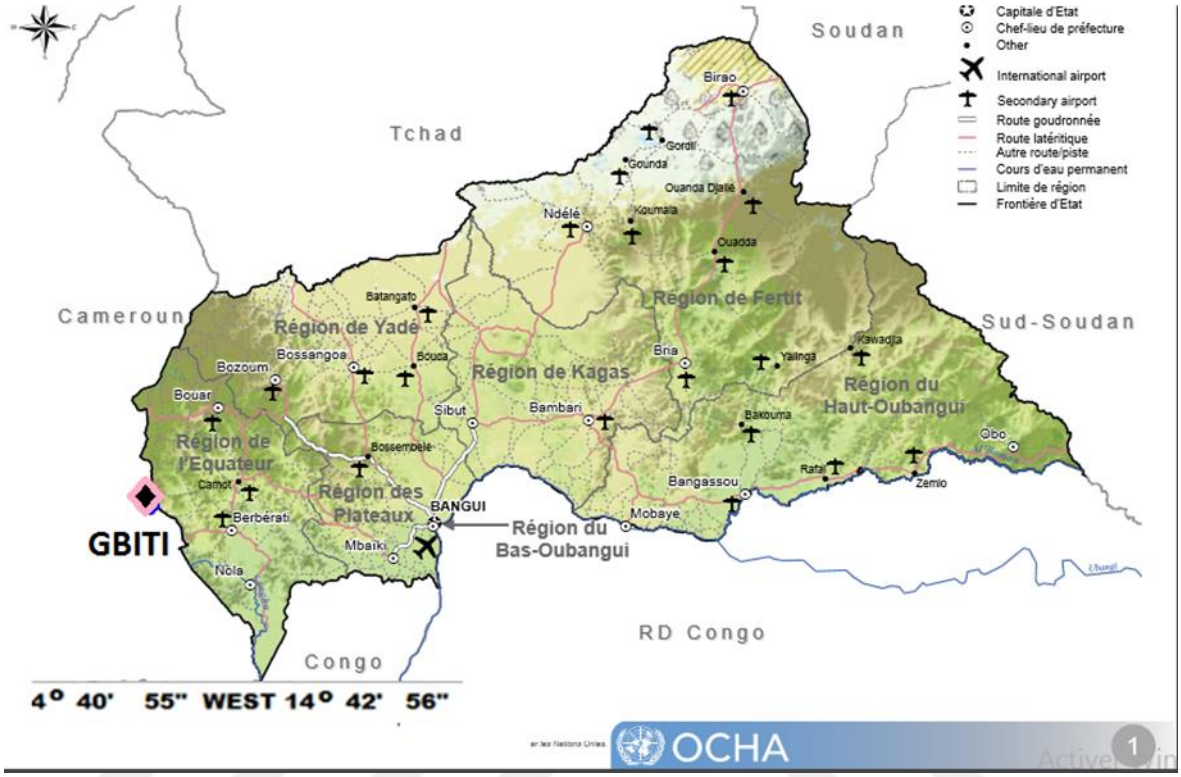


Figure 3.1. Geographical Coordinates of the Plant Sampling Area

The collection of plants was performed following the method outlined by (Hanan B., 2013). Barks from *Crossopteryx febrifuga* Benth, *Khaya anthotheca* bark and roots, *Gardenia ternifolia* bark and roots, and *Terminalia glaucescens* bark were gathered in the village of Gbiti (4° 40′ 55″ W, 14° 42′ 56″) in the western part of the Central African Republic (CAR), near the Cameroon border, in November 2019. The collected plant materials were packed in opaque plastic containers and transported to Turkey. Although the plants were harvested in November 2019, extraction was performed seven months later in July 2020. The plants were thoroughly cleaned and washed with water, then dried in the shade at room temperature. The roots and stems were cut into small pieces and powdered using an electric mixer. The resulting powders were stored in clean plastic containers, kept at -80°C away from light, heat, and moisture, until further use in July 2020. Maceration and subcritical water extraction methods were employed for the extraction process.

Figure 3.1. Geographical Coordinates of the Plant Sampling Area

3.2. Plant Extraction

Various secondary metabolites can be identified through reactions in test tubes or using thin-layer chromatography (TLC).

3.2.1. Maceration Extraction (Methanol Extraction Preparation)

For maceration, 1g of powdered plant leaves and stems were mixed with 5 ml of 90% hydro-methanol for 24 hours while agitating at room temperature. Afterward, the extracts were filtered using a 0.6 Millipore Watt man filter paper (Salawu ve ark., 2009). The extracts were then concentrated using a rotary evaporator at 40°C under reduced pressure. The concentrated extracts were weighed and stored at -20°C for future use in various tests (Hanan Bandar, 2013). The extraction was performed by macerating bark or root powder in 90% hydro-methanol. One gram of plant material was soaked in 5ml of 90% hydro-methanol (40g/200ml), left to stir for two days at room temperature to allow the soluble compounds to dissolve. The mixture was filtered through a 6mm number Watt man filter (Salawu ve ark., 2009). The solvent was then evaporated using a rotary evaporator at 40-60°C, and the remaining solid was dissolved in Dimethyl Sulfoxide (DMSO) at concentrations of 0.2g/ml and 0.5g/ml. These extracts were tested for antimicrobial effects and cytotoxicity using the MTT assay (Yeo et al., 2014).

3.2.2. Subcritical Water Extraction

Subcritical fluids are liquids kept below their critical temperature by compression and are used above their boiling point under pressure. Also known as hot liquid solvents or pressurized liquid solvents, subcritical fluids are employed in a process called accelerated solvent extraction. The solvent power of subcritical fluids is influenced by the temperature, with pressure helping maintain the liquid state while temperature enhances the solvent's effectiveness. Subcritical extractions at lower temperatures and pressures take longer but allow essential oils in the plant to retain terpenes and other sensitive compounds (Giray, 2008). For the extraction, 1g of herbal material was placed in an aluminum tube. Wool and sea sand plugs were inserted at both ends to serve as filters. The extraction column was then mounted in a furnace, and the inlet valve was opened to pressure the column with water. Once the valve was closed, the oven was quickly heated to the desired temperature. The extraction process occurred in two stages: the hydrostatic phase, which lasts 30 minutes at 125°C and 60/80 ATM (boiling phase), and the ethanol-dynamic phase, lasting 20 minutes (collection phase), during which water is pumped from the column at a specific flow rate, and the extracts are collected in a bottle (Giray, 2008; Memet I., 2014).

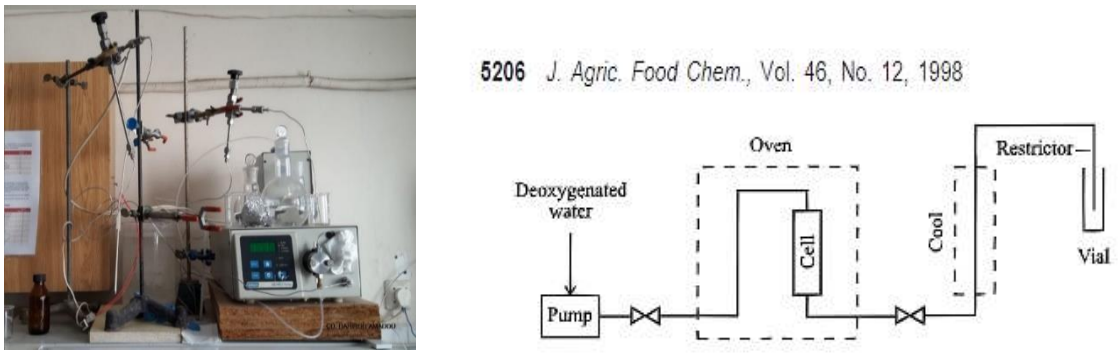


Figure 3.2. Superheated Water Extraction Device and Schematic Diagram

3.3. Phytochemical Composition Analysis Using the Tube Reaction Method (Prashant T., et al 2011)

3.3.1. Detection of Alkaloids

Principle

The identification of alkaloids is based on precipitation reactions using general alkaloid reagents, including Mayer's reagent and Dragendorff's reagent.

Procedure

A sulfuric extract is prepared by macerating 10 grams of powdered plant material in 50 ml of 10 % diluted sulfuric acid (H_2SO_4) for one hour. The mixture is then filtered and washed with distilled water to obtain a final volume of 50 ml filtration.

- In two separate test tubes, 1ml of the filtrate is introduced.
- To the first tube, 5 drops of Mayer's reagent are added, while 5 drops of Dragendorff's reagent are added to the second tube.
- A third tube containing 1ml of caffeine solution serves as a control, with 5 drops of Dragendorff's reagent added.
- The presence of alkaloids is confirmed by the formation of a precipitate.

3.3.2. Identification of Polyphenolic Compounds

This test is aimed at detecting secondary metabolites such as tannins, flavonoids, and anthraquinones (Kaloustian et al., 2008).

3.3.3. Detection of Tannins

Tannins are classified into two categories: Catechic tannins and Gallic tannins, each identified by distinct chemical tests.

Procedure

A 5% aqueous infusion (5 ml) is prepared by infusing the plant material for 15 minutes. To this, 1ml of an aqueous iron (III) chloride ($FeCl_3$) solution is added. If tannins are present, a greenish or blue-black coloration appears (Kaloustian et al., 2008).

3.3.4. Detection of Catechic Tannins

A 5% infusion (5 ml) is mixed with 1ml of concentrated hydrochloric acid (HCl). The mixture is then boiled for 15 minutes and filtered. The formation of a red precipitate, which is soluble in iso-amyl alcohol, confirms the presence of Catechic tannins (Prashant T., et al 2011).

3.3.5. Detection of Anthocyanins

A 5% infusion (5ml) is mixed with 5ml of 10% sulfuric acid (H_2SO_4) followed by the addition of 5ml of diluted ammonium hydroxide (NH_4OH). If anthocyanins are present, the color intensifies with acidification and subsequently turns blue violet into a basic medium (Prashant T., et al 2011).

3.3.6. Detection of Sterols and Triterpenes using the Liebermann-Bur chard Reaction

Procedure

One gram of plant material is mixed with 20 ml of diethyl ether in a sealed container. The mixture is stirred and left to macerate for 24 hours before being filtered. The final volume is adjusted to 20 ml to ensure consistency in the analysis.

Principle

The dry ether extract (10 ml) obtained from the previous procedure is dissolved in 1 ml of acetic anhydride and supplemented with 1 ml of chloroform. The resulting solution is divided into two test tubes, with one serving as a control. A pipette is used to carefully introduce 1 ml of sulfuric acid at the bottom of the experimental tube. The presence of sterols and triterpenes is indicated by the formation of a brownish-red or purple ring at the interface of the two liquid layers, while the supernatant turns green or purple (Kaloustian et al., 2008).

3.3.7. Search for Saponosides

Principle

The presence of saponins is determined quantitatively by measuring the foam index, which represents the dilution level of an aqueous decoction that generates persistent foam under controlled conditions.

Procedure (Prashant T., et al. 2011).

- Prepare a 1% decoction of the plant material by boiling for 30 minutes.
- Allow the decoction to cool and filter it, then adjust the final volume to 100 ml.
- Distribute increasing volumes of the decoction (from 1 ml to 10 ml) into a series of 10 numbered test tubes.
- Adjust the total volume in each tube to 10 ml using distilled water.
- Shake each test tube horizontally for 15 seconds.
- Allow the tubes to rest in a vertical position for 15 minutes and record the foam height in centimeters.
- If the foam height approaches 1 cm in tube X, the foam index is calculated using the appropriate formula.

$$I = \frac{H\ x\ X\ 5}{X}\ x\ 100$$

- *I: Foam Index.*
- *Hx: Height of foam in cm in the X^{th} Tube.*
- *X: Number of the tube where the foam height is equal to 1cm.*

3.4. Determination of Antimicrobial Susceptibility (Mosmann, 1983).

The antibacterial activity of the extracts was evaluated using the agar well diffusion method. Each experiment was conducted in triplicate. Bacterial strains were prepared in physiological saline solution, adjusted to a turbidity of 0.5 McFarland standard.

3.4.1. Preparation of Bacterial Cultures

Bacterial strains were reactivated 24 hours before inoculation. Each species was cultured in an appropriate medium suited to its physiological requirements. The bacteria were spread uniformly onto solid media. Using a blue tip, wells with a diameter of approximately 6 mm were created in the agar medium. To prevent extract diffusion between the Petri dish and the agar, 100 microliters of Mueller-Hinton Agar were added to the base of each well.

In each well, 200 µL of the plant extract, at concentrations of either 20 mg/ml or 50 mg/ml, was introduced for testing. The plates were then left at room temperature for 30 minutes to allow diffusion. Subsequently, they were incubated for 24 hours at the optimal growth temperature for each bacterial strain. The presence or absence of an inhibition zone was recorded to assess antimicrobial activity (Aladag, 2016).

Table 3.1. List of Bacterial Strains Used for Antibiogram Testing

Code	Bacterial species	Medium	(^{O}C)
I	*Pseudomonas aeruginosa* (ATCC 27853),	Tryptic soy agar	37
II	*Enterobacter hormaechei* (ATCC700323),	Tryptic soy agar	30
III	*Bacillus spizizenii* (ATCC6633),	Brain Heart infusion agar	30
IV	*Enterococcus casseliflavus* (ATCC700327),	Brain Heart infusion agar	37
V	*Staphylococcus aureus subsp. rosenbach* (ATCC 6538),	Muller Hilton Agar	37
VI	*Salmonella Typhi* (ATCC 6539)	Muller Hilton Agar	30

3.4.2. Antibiogram Disc Preparation

The sensitivity of four bacterial strains to plant extracts was assessed using the Kirby-Bauer disc diffusion method, following the protocol outlined by (Hudzicki., 2009)This method is designed to evaluate the susceptibility or resistance of pathogenic aerobic and facultative anaerobic bacteria to different antimicrobial agents. In this assay, the target bacterial strains are cultured on Mueller-Hinton agar in the presence of filter paper discs impregnated with antimicrobial compounds. The presence or absence of bacterial growth around these discs serves as an indirect indicator of the antimicrobial compound's effectiveness in inhibiting the pathogen (Hudzicki., 2009).

3.5. Cell Proliferation Assay

Several techniques can be employed to evaluate the cytotoxicity of plant extracts, including the XTT staining method, MTT staining method, and neutral red absorption staining method (Tokur & Aksoy, 2017). To determine the cytotoxic potential of the extracts, the MTT colorimetric assay was utilized, as described by (Gonzalez et al., 2018).

3.5.1. MTT Test

MTT Test Principle

The MTT assay is based on the conversion of tetrazolium salt into a colored formazan compound through mitochondrial activity in viable cells. In this process, MTT is reduced to purple formazan by NADH. Since dead cells lack mitochondrial activity, they do not form formazan crystals. Various tetrazolium compounds can be utilized to detect viable cells, with MTT being a commonly used compound due to its positive charge, allowing it to easily penetrate living eukaryotic cells (Mosmann, 1983).

Objective:

The primary objective of the MTT assay is to assess cytotoxicity by measuring metabolic activity, specifically mitochondrial function, to distinguish between viable and non-viable cells.

Currently, the MTT assay is widely recognized as one of the most used cell viability tests in biomedical research (Hart, 2012).

3.5.2. Preparation of Plant Extracts

Two distinct methods were employed for extraction. Initially, maceration was conducted using 5 ml of 90 % methanol per gram of plant material. Subsequently, the subcritical water extraction method was applied, wherein 1 g of dried plant material was subjected to 125 °C at either 60 atm or 80 atm pressure.

The resulting extracts were filtered using a 0.6 mm Watt man filter and then concentrated at 60 °C using a rotary evaporator. The remaining residue was dissolved in DMSO at a concentration of 0.1 g/ml. The extracts were stored at 6 °C for 24 hours, after which they were centrifuged at 2000 rpm for 10 minutes. Following this, 2 ml of supernatant was collected and centrifuged at 130 rpm for another 10 minutes. The extracts were then filtered using a sterile 0.20 µm Watt man Nano filter.

To evaluate the cytotoxic effects of the plant extracts using the MTT method, sequential dilutions were prepared according to the protocol described by Mossman (1983). The initial concentration (C0) was set at 1 g/ml, followed by two sequential dilutions: C1 at 0.1 g/ml and C2 at 0.01 g/ml

3.5.3. In-Vitro Cell Culture

NIH3T3 mouse embryonic fibroblast cells were used for the cytotoxicity assays, obtained from the Department of Medical Biology, Faculty of Medicine, Çukurova University.

Cell Culture

- Cells were cultured in T75 flask.
- Removed media.
- Added 4 ml trypsin.
- Centrifuge and refill the supernatant.
- Cell counts in THOMA cell.
- 100µl added to each well of 96 palates.
- In each well, 10,000 cells are grown two days before treatment.
- Incubation at 37ºC and 5% carbon dioxide for up to 24hours.
- Change culture medium / 100µl of medium added per well.
- Incubated for 4 hours in a 37ºC incubator.
- Added 100µl of DMSO per well after removing the culture medium.
- Added 100 µl of plant extracts to each well.
- Incubation at 24 h to room T°.

Following the removal of the medium using a micropipette, 20 µl of MTT solution (5 mg/ml) was added to each well and mixed thoroughly. The medium was carefully removed, and 100 µL of DMSO was added. After the treatment and incubation period with the DMSO solution, absorbance readings were taken at 630 nm using the Biochrome EZ READ 4000 Microplate Reader, with a reference wavelength of 560 nm.

- Control (+): 4 % in 2 µl NAOCL 0.04 isopropanol was used
- Control (-): Empty culture medium used
- K (+): Positive control
- K (-): Negative control.

10×10^3 mouse embryonic fibroblast cells (NIH3T3), suspended in 100 μl of growth medium, were seeded in 96-wellplates containing100 μl of medium in the presence of 1 μl of each tested plant extract, previously dissolved in DMSO, final concentration 1% v/v since no adverse effects on cell growth were observed at this concentration (María L. et al., 2018).

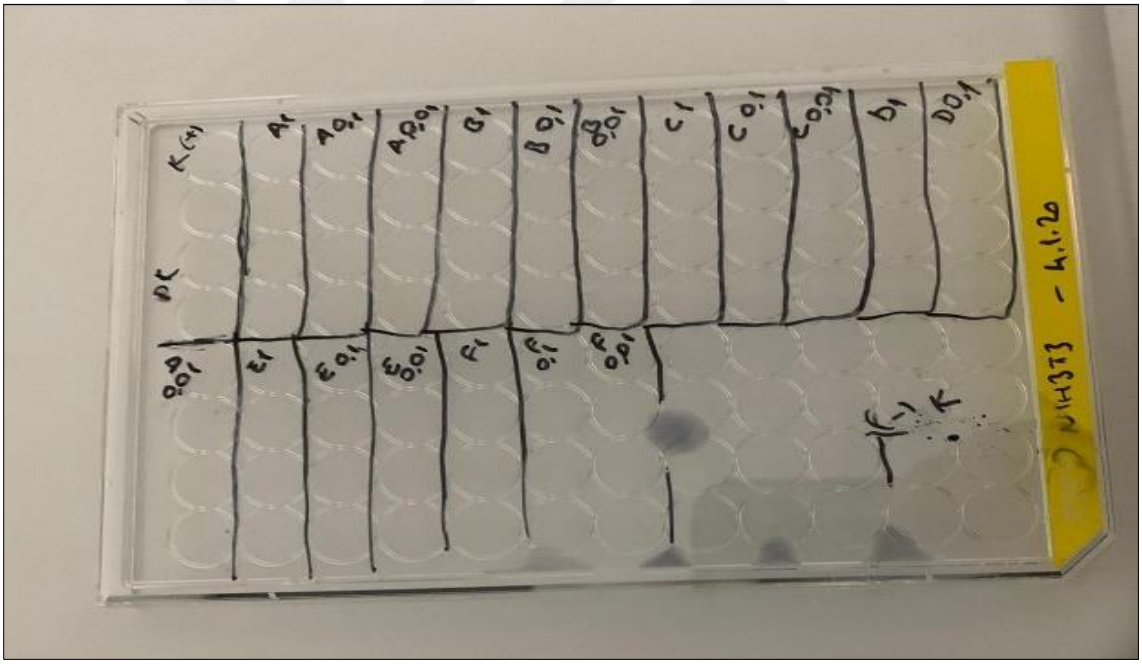


Figure 3.3. Each Sample was Diluted in 3 Concentrations (1; 0.1 and 0.01 µg/µl) in 96 Micro Pallets and Repeated in 4 Parallels

The extracts were evaluated at a final concentration of 0.1mg/ml. Following the primary screening, those extracts with promising activity on all studied cells were tested at serial dilutions ranging from 0.1 to 0.001 mg/ml. The isolated compound was tested at 0.1, 0.01, and 0.001 mg/ml. After 24h for, respectively, 20 μl of 5mg/ml solution of MTT in sterile PBS was added to each well and further incubated for 2h. Then, the supernatants were removed and replaced with 100 μl DMSO to solubilize the resulting purple formazan crystals produced from metabolically viable cells.

Absorbance was measured with Bio chrome EZ READ mark 4000 Micro plate Reader at 560 nm. Four wells were used for each sample assayed and two independent experiments were performed. Untreated and DMSO (1%)-treated cells were used as controls, while DOX (added to reach final concentrations of 0.003 to 40 μg/ml) was used as reference. And 2 µl of NAOCL 0.04 isopropanol 4% was used like positive control.

3.5.4. Interpretation of Results

The percentage of cytotoxic activity was calculated using the following formula (Barnabe, 2017):

$$\text{Cytotoxicity } (\%) = 1\frac{(\text{optical density of treated cells} - \text{optical density DMSO})}{(\text{opticaldensity of control cells} - \text{opticaldensity DMSO})} \times 100.$$

A cytotoxicity value of 120% suggests that cell growth in the sample is 20% higher compared to the control. In contrast, a cytotoxicity value of 75% indicates a 25% reduction in cell proliferation. This implies that when the cytotoxicity percentage exceeds the control value (100%), the sample does not inhibit cell growth and is considered less toxic. Conversely, if the sample's value falls below 100%, it proportionally inhibits cell proliferation and growth, indicating higher toxicity (Lindhagen et al., 2008; Pomothy et al., 2016; Yilmaz et al., 2020). The data was analysed by using Microsoft Office excel.

4. RESULTS AND DISCUSSIONS

Table 4.1. Resume of the results

Samples	Alkaloids	Tannins	Anthocyanin	Saponosides	Sterols and tri-terpenes	Cytotoxic effects	Antimicrobial activities (X Positive/X Tested)
Crossopteryx febrifuga Benth (Stem bark)	+	++	+	+++	-	13%	3.5/6
Khaya anthotheca (Stem bark)	+++	++++	+++	+++	++	19%	3/6
Khaya anthotheca (Root)	+++	+++	+++	+++	++	50%	2/6
Gardenia ternifolia (Stem bark)	+	+	+	+++	-	68%	2/6
Gardenia ternifolia (Root)	+	++	++	+++	++	95%	1/6
Terminalia glaucescens (Stem bark)	+++	+++	+	+++	+	30%	4/6

Indications: *+++: Very abundant; +: Presence -: Absence*

4.1. Phytochemical Screening Results

The extracts from plants B, C, and F exhibit similar chemical compositions, with a high concentration of alkaloids, tannins, anthocyanins, and saponosides, along with a moderate presence of sterols and triterpenes. Notably, saponins are highly abundant across all plant extracts. However, the extracts from plants A and D tested negative for sterols and triterpenes, which are recognized for their antioxidant, antiviral, and antifungal properties.

4.1.1. Result of Alkaloids and Poly-Phenolic Compounds

Table 4.2. Alkaloids and Poly-Phenolic Compounds

Samples	Alkaloids	Tannins	Anthocyanin	Saponosides		Sterols and tri-terpenes
				Presence	Foam index	
A	+	++	+	+++	487,71	-
B	+++	++++	+++	+++	1116,68	++
C	+++	+++	+++	+++	1152,77	++
D	+	+	+	+++	508,29	-
E	+	++	++	+++	2215	++
F	+++	+++	+	+++	1108,33	+

Indication: *+++: Very abundant; +: Presence -: Absence*

The table above provides an overview of the phytochemical composition of different plant extracts (A to F), specifically assessing the presence of alkaloids, Polyphenolic compounds (tannins and anthocyanins), saponins, sterols, and Triterpenes. The concentration of each compound is represented on a graded scale (+ to ++++).

The bark of *Khaya anthotheca* (B), the roots of *Khaya anthotheca* (C), and the bark of *Ternifolia glaucescens* (F) exhibit a high abundance (+++) of alkaloids. In contrast, the bark of *Crossopteryx febrifuga benth* (A), *Gardenia ternifolia* (D), and the roots of *Gardenia ternifolia* (E) display low to moderate levels (+ to ++).

With respect to tannins, extracts from the bark and roots of *Khaya anthotheca* (B, C) demonstrate a very high abundance (++++), whereas the bark of *Crossopteryx febrifuga Benth* (A) and *Gardenia ternifolia* (D) contain only low levels (+).

For anthocyanins, the bark of *Khaya anthotheca* (B), the roots of *Khaya anthotheca* (C), and the roots of *Gardenia ternifolia* (E) exhibit a strong presence (+++), while the bark of *Crossopteryx febrifuga Benth* (A) and *Gardenia ternifolia* (D) contain low to moderate levels (+).

Saponins are highly abundant (+++) in all extracts, except for the bark of *Crossopteryx febrifuga Benth* (A), which exhibits a lower presence.

Lastly, sterols and Triterpenes are detected in all tested samples, with concentrations ranging from + to +++.

4.1.2. Calculation Method (Prashant T. et al., 2011)

Formula

$$I = \left(\frac{5.Hx}{X}\right).100$$

I: Foam Index; Hx: Height of foam in cm in the X^{th} Tube.

X: Number of the tube where the foam height is equal to 1cm

4.1.3. Foam height

Table 4.3. Saponosides Extract Foam Level (cm)

Samples	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10
A	-	-	-	1,8	2,7	3,8	4	4,7	5	5,2
B	-	1,5	2,1	3,3	4	4,7	5,1	5,2	6	6,3
C		1	2,3	3,6	4,7	5,1	5,5	6	6,3	7
D	-	-	1,7	2,2	2,4	2,8	3	3,5	3,9	4,9
E	2,2	2,8	3,3	4	4,5	5	5,2	5,9	4,8	6,6
F	-	2	2,5	3,2	4	4,5	5,1	5,6	5,8	6,8

4.1.4. Foam Index

Table 4.4. Saponosides Foam Index (cm)

Samples	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	Foam Index (Median)
A	-	-	-	225	337,5	475	500	587	625	650	487,71
B	-	875	525	825	1000	1175	1275	1300	1500	1575	1116,68
C	-	250	575	900	1175	1275	1375	1500	1575	1750	1152,77
D	-	-	283	367	400	467	500	583	650	817	508,29
E	1100	1400	1650	2000	2250	2500	2600	2950	2400	3300	2215
F	-	500	625	800	1000	1225	1275	1400	1450	1700	1108,33

The Foam Index, which measures the foaming height produced by saponins, serves as an indicator of their surfactant activity. The root extracts of *Gardenia ternifolia* (E) exhibit the highest Foam Index (2215), indicating a strong foaming capacity, followed closely by the bark extracts of *Khaya anthoteca* (B: 1116.68), the root extracts of *Khaya anthoteca* (C: 1152.77), and the bark extracts of *Ternifolia glaucescens* (F: 1108.33). This suggests that these samples contain higher concentrations of active saponins, making them effective surfactant agents. In contrast, the bark extracts of *Gardenia ternifolia* (D) exhibit a relatively low Foam Index (508.29), indicating reduced surfactant activity. Similarly, the bark extracts of *Crossopteryx febrifuga Benth* (A) display a low Foam Index (487.71), consistent with their low saponin content.

4.1.5. Comparison of Samples

The bark extracts of *Khaya anthoteca* (B) and the root extracts of *Khaya anthoteca* (C) are particularly rich in alkaloids, tannins, anthocyanins, and saponins, as well as possessing strong foaming capacity. These characteristics suggest their potential applications in pharmaceutical or cosmetic formulations.

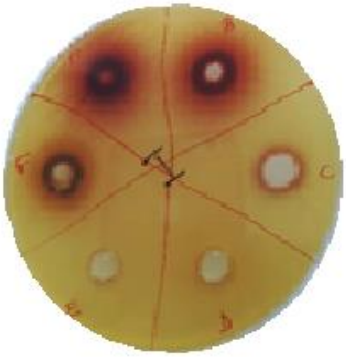
Despite the high Foam Index of the root extracts of *Gardenia ternifolia* (E), their moderate alkaloid content may limit their suitability for applications where alkaloid presence is essential. Meanwhile, the bark extracts of *Gardenia ternifolia* (D) contain low levels of most bioactive compounds and have a low Foam Index, suggesting their limited potential in formulations requiring significant bioactive properties.

Table 4.5. Results of Antimicrobial Tests Performed with the Dark Diffusion Method at 20mg/ml and 50mg/ml

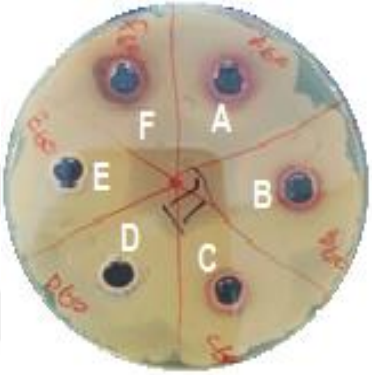
Plants→		*Crossopteryx febrifuga Benth* (Stem bark)		*Khaya anthotheca* (Stem bark)		*Khaya anthotheca* (Root)		*Gardenia ternifolia* (Stem bark)		*Gardenia ternifolia* (Root)		*Terminalia glaucescens* (Stem bark)		Control CN 10
Bacteria's Cod↓		20 mg/ml	50 mg/ml	20 mg/ml	50 mg/ml	20 mg/ml	50 mg/ml	20mg/ml	50 mg/ml	20 mg/ml	50 mg/ml	20 mg/ml	50 mg/ml	10 µg
*Maceration	I	0	0	0	0	0	0	0	0	0	0	0	0	9±2
	II	5±2	7±2	4±2	5±2	1±1	2±1	5±2	6±2	2±1	3±2	3±2	3±2	5±2
	III	8±2	6±2	0	0	2±1	4±2	2±1	5±2	0	0	5±2	6±2	10±2
	IV	0	0	0	0	0	0	0	0	0	0	0	0	3±1
	V	5±2	3±2	0	4±2	0	0	0	0	0	0	5±2	5±2	7±2
	VI	5±2	0	2±2		0	0	0	0	0	0	3±2	0	5±2
** 125^{O} C and 60 ATM	I	0		0		0		0		0		0		9±2
	II	1±1		1±1		0		1±1		0		1±1		4±2
	III	3±2		2±2		0		0		1±1		4±2		10±2
	IV	0		0		0		0		0		0		8±2
	V	5±2		0		0		0		0		0		5±2
	VI	0		0		0		0		0		0		5±2
***Sub critic water 125^{O} C and 80 ATM	I	0		0		0		0		0		0		9±2
	II	1±1		1±1		0		2±1		0		1±1		4±2
	III	2±1		1±1		1±1		2±1		1±1		4±2		10±2
	IV	0		0		0		0		0		0		6±3
	V	0		0		0		0		0		5±2		5±2
	VI	3±2		0		0		0		0		2±2		5±2

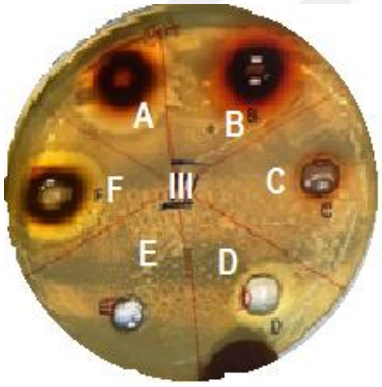
* First extraction: It was carried out by softening the plants in H2O-Met solution (90%) with stirring for 48 hours at an amount of 5g/ml.
** The second extraction was obtained by sub critic water extraction at 125ºC-60 ATM;
*** The second extraction was obtained by sub critic water extraction at 125ºC 80ATM.
Code I: Pseudomonas aeruginosa (ATCC 27853);
*Code II: Enterobacter hormaechei (*ATCC700323)*;*
Code III: Bacillus spizizenii (ATCC6633);
Code IV: Enterococcus casseliflavus (ATCC700327);
Code V: Staphylococcus aureus subsp.Rosenbach (ATCC 6538);
Code IV: Salmonella Typhi (ATCC 6539);
CN10: Gentamicin 10mg (Bio analysis)

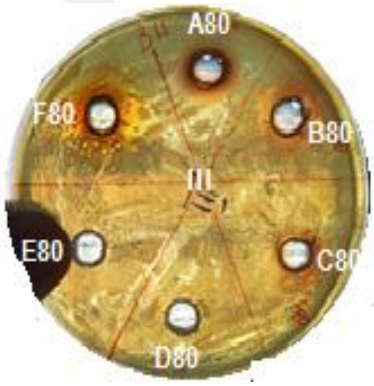
Table 4.6. Results of antimicrobial tests performed with dark diffusion method at 20mg/ml (200µL) and 50mg/m (500µL)



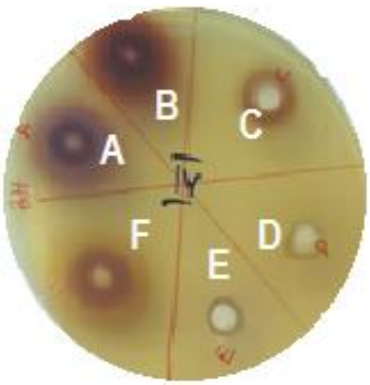
(I) **Maceration** **A-250OC60ATM** **A-250OC80ATM**

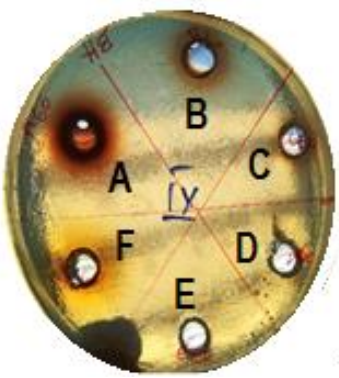




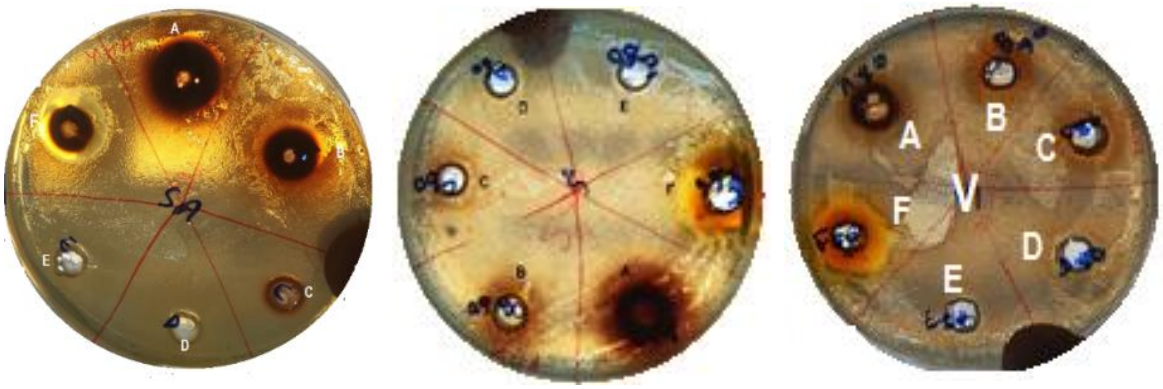


(III) **Maceration** **A-250OC60ATM** **A-250OC80ATM**

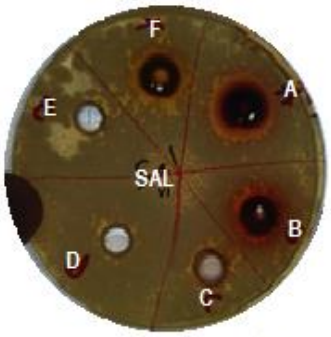




(IV) **Maceration** **A-250OC60ATM** **A-250OC80ATM**



(V) **Maceration** **A-250OC60ATM** **A-250OC80ATM**



(VI) Maceration

(I): Crossopteryx febrifuga Benth (Stem bark);

(II): Khaya anthotheca (Stem bark);

(III): Khaya anthotheca (Root);

(IV): Gardenia ternifolia (Stem bark);

(V): Gardenia ternifolia (Root);

(VI): Terminalia glaucescens (Stem bark)

Antibacterial activity varies among different plant extracts and extraction techniques. The control consistently demonstrates a certain degree of antibacterial activity (9 ± 2 to 10 ± 2), indicating that the control solution inherently possesses antibacterial properties against the tested bacterial strains. However, most extracts exhibit limited antibacterial effects, with several trials showing either no activity (0) or only minimal inhibition across different methods.

The maceration method generally results in low antibacterial activity, particularly against *Pseudomonas aeruginosa* and *Enterococcus casseliflavus*, where most extracts show no activity (0). However, *Khaya anthotheca* (stem bark) exhibits some antibacterial effects in trials against *Enterobacter hormaechei* and *Bacillus spizizenii* (5 ± 2, 6 ± 2, 4 ± 2), while *Gardenia ternifolia* (stem

bark) demonstrates activity against *Enterobacter hormaechei* (5 ± 2). In *Salmonella Typhi*, *Khaya anthotheca* (root) and *Gardenia ternifolia* (stem bark) show some response, both recording values of 4 ± 2.

Subcritical water extraction yields slightly better results than maceration. The extraction method at 125 °C and 60 ATM demonstrates some antibacterial activity, particularly against *Bacillus spizizenii* (3 ± 2 for *Khaya anthotheca* stem bark and 4 ± 2 for *Terminalia glaucescens*). The extraction process at 125 °C and 80 ATM also shows positive effects, notably in *Bacillus spizizenii*, where several extracts (*Khaya anthotheca* root, *Gardenia ternifolia* stem bark) display activity (1±1).

Overall, antibacterial activity remains low across all trials, but certain extracts display notable effects. *Khaya anthotheca* (stem bark and root) exhibits relatively stronger antibacterial properties compared to other extracts, especially using the maceration method. *Gardenia ternifolia* shows occasional activity, whereas *Terminalia glaucescens* do not demonstrate consistent antibacterial effects.

Extracts derived from the barks of *Terminalia glaucescens* and *Crossopteryx febrifuga Benth* exhibit moderate cytotoxicity alongside notable antibacterial potential. All tested extracts show relatively high sensitivity towards *Enterobacter hormaechei* (ATCC700323, Code II) and *Bacillus spizizenii* (ATCC6633, Code III). The extracts from *Crossopteryx febrifuga Benth* and *Terminalia glaucescens* also demonstrate sensitivity toward *Bacillus spizizenii* (ATCC6633, Code III) and *Salmonella* (Code IV). Conversely, *Pseudomonas aeruginosa* (ATCC27853, Code I) and *Enterococcus casseliflavus* (ATCC700327, Code IV) show no sensitivity to any of the tested extracts. Notably, only the extracts obtained from the barks of *Crossopteryx febrifuga Benth* (A) and *Terminalia glaucescens* (F) exhibit sensitivity against five of the six tested bacterial strains, including *Salmonella typhi*.

4.2. MTT TESTI

Table 4.7. Inhibition Value in % of the Different Extracts at Concentrations 1; 0.1 and 0.01 µg/ µL on the Proliferation of Fibroblast Cells in the Embryo of an NIH3T3 Mouse

Samples	**A1**	**A0,1**	**A0,0**	**B1**	**B0,1**	**B0,0**	**C1**	**C0,1**	**C0,0**	**D1**	**D0,1**	**D0,0**	**E1**	**E0,1**	**E0,0**	**F1**	**F0,1**	**F0,0**	**Cont + (NaOCl)**	**Cont - (Medıum)**
Maceration	112	62	76	71	94	88	28	101	62	3	118	113	7	61	72	49	78	73	93	0.35
125c /60ATM(%)	106	143	152	87	96	93	86	96	163	37	176	138	8	92	113	138	104	156	158	
125C 80ATM(%)	134	106	125	77	61	100	29	63	75	55	64	108	6	128	98	76	55	133	134	
Average %	117	104	118	78	84	94	48	87	100	32	119	120	7	94	94	88	79	121	128	100

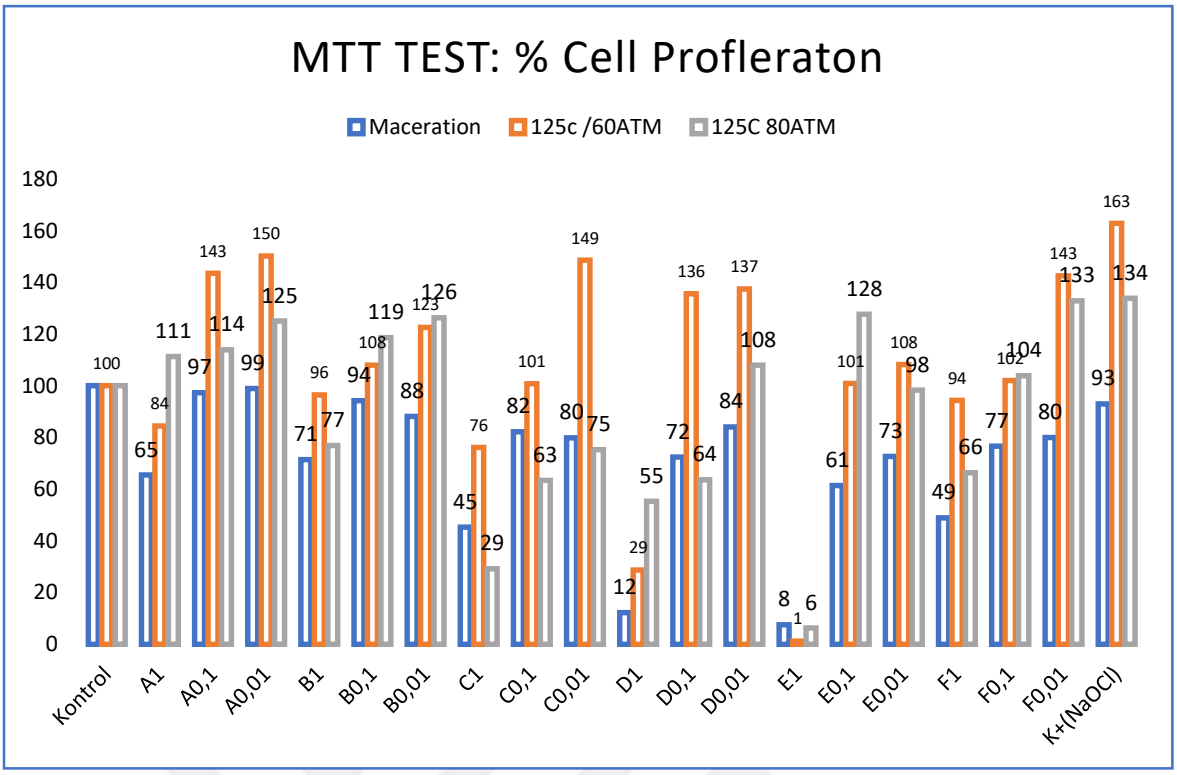


Figure 4.1. Inhibition in % of the Different Extracts on the Proliferation of Fibroblast Cells From the Embryo of an NIH3T3 Mouse

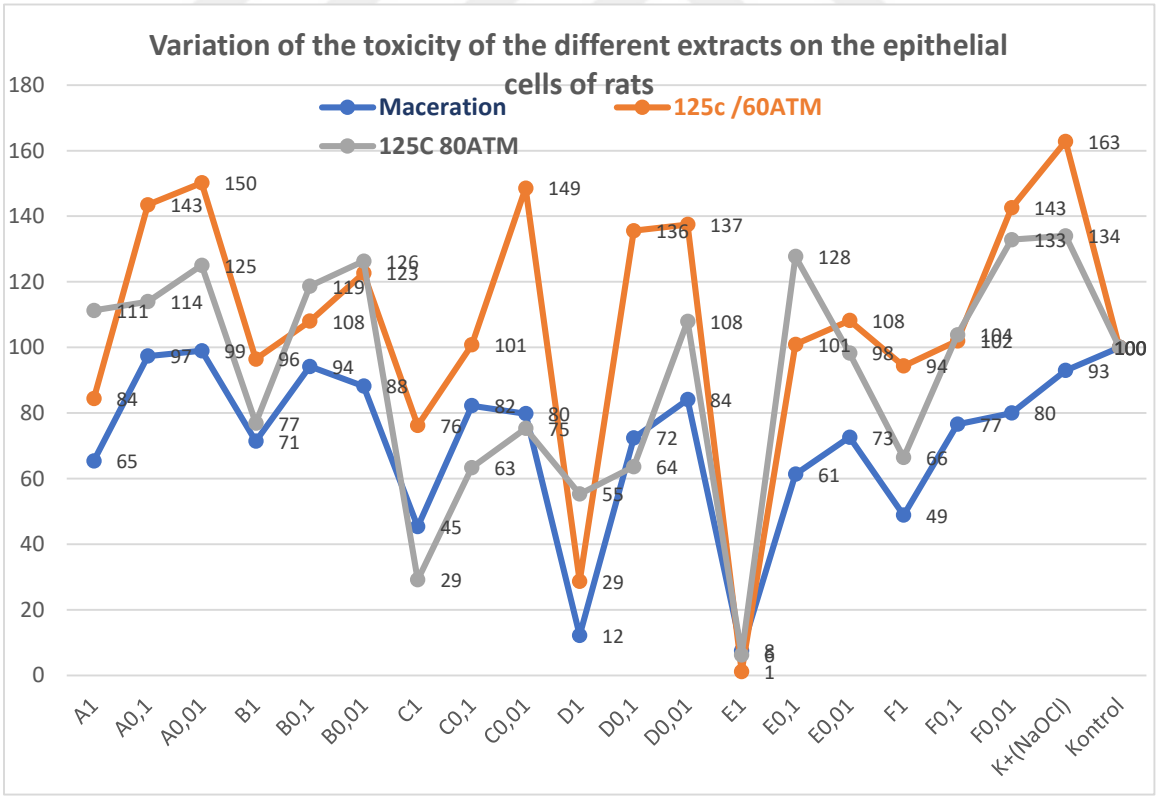


Figure 4.2. Inhibition in % of the Different Extracts on the Proliferation of Fibroblast Cells from the Embryo of an NIH3T3 Mouse Treated with Tetrazolium by the MTT Method

A): *Crossopteryx febrifuga benth (Stem bark);* (B): *Khaya anthotheca (Stem bark);* (C): *Khaya anthotheca (Root);* (D): *Gardenia ternifolia (Stem bark);* (E): *Gardenia ternifolia (Root);* (F): *Terminalia glaucescens (Stem bark).*

The table presents the results of an MTT assay evaluating the viability and proliferation of fibroblast cells (NIH3T3) following exposure to various plant extracts at different concentrations (1, 0.1, and 0.01 µg/µl). The control group, representing untreated cells, exhibits 100% viability, serving as the reference for comparison. Experimental groups (A1, A0.1, A0.01, etc.) correspond to different extracts and concentrations. Inhibition values, derived from the percentage of the control, indicate the extent to which each extract affects cell proliferation. Positive values suggest enhanced viability, whereas negative values denote reduced viability.

Extract of *Crossopteryx febrifuga benth* (Stem Bark): At 1 µg/µl (A1), cell viability is 65%, indicating a significant inhibition of 35%. Lower concentrations (A0.1 and A0.01) yield better viability (97% and 99%, respectively), demonstrating a dose-dependent response.

Extract of *Khaya anthotheca* (Stem Bark): At 1 µg/µl (B1), viability is 71%, with lower concentrations showing improved viability (94% at B0.1 and 88% at B0.01).

Extract of *Khaya anthotheca* (Root): Like the stem bark, the root extract at 1 µg/µl (C1) results in 45% viability, while lower concentrations exhibit improved viability.

Extract of *Gardenia ternifolia* (Stem Bark): At 1 µg/µl, viability is notably low (12 %), indicating strong cytotoxicity. While lower concentrations show slight improvement, overall viability remains below 80 %.

Extract of *Gardenia ternifolia* (Root): Exhibits highly variable effects, with 1 µg/µl (8% viability) being particularly detrimental.

Extract of *Terminalia glaucescens* (Stem Bark): This extract exerts a moderate effect on cell viability, with the highest viability observed at lower concentrations.

The positive control (K+; NaOCl) demonstrates a strong inhibitory effect, with a total inhibition value of 1239 and an average viability of 65%, indicating significant cytotoxicity. Across all extracts, higher concentrations generally result in greater inhibition of cell viability, suggesting a dose-dependent response where lower concentrations exert less toxicity and, in some cases, may even promote cell viability.

The average inhibition percentages vary across extracts and concentrations, with lower concentrations often corresponding to increased viability. Extracts of *Crossopteryx febrifuga benth* (Stem Bark), *Khaya anthotheca* (Stem Bark), and *Khaya anthotheca* (Root) show promising effects at lower concentrations, whereas *Gardenia ternifolia* (Stem Bark) and *Gardenia ternifolia* (Root) display pronounced cytotoxicity. The "Sum" and "Average" columns provide an overall assessment of each extract's impact across all tested concentrations. Notably, *Gardenia ternifolia* (Stem Bark) emerges as the inhibitoriest extract, while *Khaya anthotheca* (Stem Bark) and *Khaya anthotheca* (Root) exhibit more favorable cytotoxic profiles at lower concentrations.

Extracts obtained through the maceration method (Hydro Methanol 90%) induce significant inhibition of cell growth (-36 %) compared to those obtained via subcritical water extraction at 125°C and 80atm or 125°C and 60atm. Extracts obtained through the 125°C 60 atm supercritical water

method exhibit the lowest impact on cell proliferation. Higher extract concentrations generally correlate with stronger inhibitory effects. At 1 µg/µl, extracts from the roots of *Gardenia ternifolia* (E) show the most pronounced inhibition among all tested extracts. Conversely, the bark extract of *Khaya anthotheca* (B) demonstrates relatively low cytotoxicity, with a 27% lower inhibition rate compared to the control.

5. CONCLUSIONS

From a biochemical standpoint, the findings of this study emphasize that the extracts derived from the bark of *Khaya anthotheca* (B), the roots of *Khaya anthotheca* (C), and the roots of *Gardenia ternifolia* (E) show the most promising characteristics. These extracts are distinguished by their significant concentrations of bioactive compounds and their ability to produce foam. Such characteristics suggest that these plant materials may have a wide range of applications in various industries, including food, pharmaceuticals, and cosmetics. The foaming capacity of these extracts, along with their richness in bioactive compounds, presents them as ideal candidates for formulations that require surfactant-like properties, such as in personal care products or medicinal formulations. In contrast, the extracts obtained from *Gardenia ternifolia* (D) exhibit relatively low levels of bioactive compounds and foam production, thus indicating their limited potential in similar industrial applications. Despite their lesser efficacy, further research could explore how these extracts may still contribute to niche applications or complement other plant-based compounds in formulations.

In line with the work of Diarra and Traoré (2022), this study further reinforces the notion that extracts from both the bark and roots of *Khaya anthotheca* contain a significant concentration of bioactive compounds, which contribute not only to their foaming ability but also their potential as pharmaceutical agents. The presence of high levels of alkaloids and saponins in these extracts is consistent with prior research, suggesting that *Khaya anthotheca* possesses notable therapeutic properties. Alkaloids are known for their antimicrobial, analgesic, and anticancer properties, while saponins are valued for their ability to enhance the absorption of nutrients and their surfactant properties, which makes these extracts particularly suitable for pharmaceutical applications. This biochemical profile strengthens the argument that *Khaya anthotheca* could be utilized in the development of new drug formulations or as a natural additive in various healthcare products.

Regarding *Gardenia ternifolia*, the findings corroborate those of Nwosu and Okeke (2021), who highlighted the significant variation in surfactant properties among different *Gardenia* species. Specifically, the relatively low foam Index observed for the bark of *Gardenia ternifolia* (D) aligns with their previous findings, indicating that this extract may not be as effective in applications requiring significant foaming capacity. Although this extract's low foam production suggests limitations for certain uses, further exploration of its other properties, such as its potential as an antimicrobial or antioxidant, could yield valuable insights into its utility in other sectors, including food preservation or natural cosmetics.

The results for *Crossopteryx febrifuga* Benth align with the work of Okwu et al. (2020), which highlighted the plant's limited potential in some industrial applications due to its relatively low content of bioactive compounds, such as saponins, and its correspondingly low Foam Index. These findings are consistent with the conclusion that the plant's application in certain fields may be restricted unless specific bioactive compounds can be isolated or enhanced through further

processing. However, the presence of some bioactive compounds in *Crossopteryx febrifuga* Benth suggests that it may still have applications in areas where less foaming and lower bioactivity are required, such as in traditional medicine or as a supplementary ingredient in cosmetic products. These results suggest the need for continued investigation into methods that might increase the bioactivity and yield of *Crossopteryx febrifuga* extracts, thereby expanding its potential uses.

The present study also supports existing literature regarding the antibacterial properties of *Crossopteryx febrifuga* Benth and *Terminalia glaucescens*. Both plants show moderate cytotoxicity but also exhibit significant antibacterial potential, particularly against *Bacillus spizizenii* and *Salmonella Typhi*. These findings corroborate those of Okwu et al. (2022), who noted the antibacterial effectiveness of *Crossopteryx febrifuga* against several bacterial strains. This highlights the importance of optimizing the concentration of extracts to maximize their antibacterial efficacy while minimizing toxicity. Such findings open up new avenues for further research aimed at isolating specific antibacterial compounds within *Crossopteryx febrifuga* and *Terminalia glaucescens*, which could be harnessed in the development of novel antimicrobial agents.

Among the studied extracts, *Khaya anthotheca* consistently demonstrates stronger antibacterial properties. This supports the findings of Ogunbanwo et al. (2018), who reported significant antibacterial effects of *Khaya anthotheca* against common pathogens like *Escherichia coli* and *Staphylococcus aureus*. These results suggest that *Khaya anthotheca* may be particularly effective for addressing bacterial infections, making it a potential candidate for use in antibiotic development or in treatments for bacterial infections. On the other hand, while the extracts from *Gardenia ternifolia* (bark and root) show considerable cytotoxic effects, earlier studies, including those by Ajibesin et al. (2013), have identified promising antibacterial properties, particularly against certain bacterial strains, despite the notable toxicity. This suggests that *Gardenia ternifolia* may require further investigation to explore its antibacterial potential while minimizing harmful effects on cellular growth, potentially through dosage adjustments or formulation modifications.

In terms of extraction methods, this study confirms that subcritical water extraction is more efficient than maceration in terms of both the yield and the antibacterial activity of the extracted compounds. This finding agrees with the work of Essien et al. (2021), who demonstrated that the choice of extraction method significantly influences the quantity and quality of bioactive compounds extracted from medicinal plants. The superior performance of subcritical water extraction suggests that it could be a more effective method for obtaining compounds from plants for use in pharmaceutical and industrial applications. This technique may also allow for the extraction of compounds that are not readily extracted by traditional methods, thus broadening the potential uses of plant-based extracts in various sectors.

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