MJSAT

Malaysian Journal of Science and Advanced Technology

journal homepage: https://mjsat.com.my/

Warburgia Salutaris Metabolites of Medicinal Value – A Review

Vuyiseka Nkqenkqa *[1], and Richard Mundembe [1]

[1]*Department of Biotechnology and Consumer Science, Faculty of Applied Sciences, Cape Peninsula University of Technology, Cape Town 8000, South Africa*

KEYWORDS

Warburgia salutaris
Extraction
Solvent
Phytochemical analysis
Secondary metabolites

ARTICLE HISTORY

Received 16 August 2023
Received in revised form 31 October 2023
Accepted 1 November 2023
Available online 10 November 2023

ABSTRACT

Warburgia salutaris is a medicinal plant that occurs in central and southern Africa and has been investigated by many natural and social scientists in a somewhat fragmented and uncoordinated way. Many phytochemical studies on medicinal plants are motivated by traditional knowledge and medicinal use of the plant. Phytochemical investigations on *W. salutaris* were based on a targeted approach, focusing on individual compounds or subclasses of metabolites but not the entire metabolome. However, in this era of omics and ready access to databases, there is a need for comprehensive information on the metabolic profiles of individual medicinal plants. This paper defines the term metabolome as it applies to plants with bioactive compounds and attempts to define a metabolomic approach for such investigations. The paper proceeds to review how the metabolites of *W. salutaris* were isolated, including the plant parts used, extraction methods and solvents used, and analytical instrumentations employed for identification and quantification. It further explores whether it is possible to construct a "compendium of metabolites" or subclass of *Warburgia* spp. metabolome based on published phytochemical studies and concludes by making recommendations for an online researcher-updated resource that lists all reported metabolites for individual medicinal plants.

1. INTRODUCTION

The genus *Warburgia* is a member of the Canellaceae family, which consists of four genera, namely *Warburgia elongata* Verdc., *Warburgia salutaris* (G. Bertol.) Chior., *Warburgia stuhlmannii* Engl., *Warburgia ugandensis* Sprague (with two subspecies, *W. ugandensis* and *W. longifolia* Verdc) [1,2]. Indigenous people have used this genus for generations, hence its long history of traditional medicinal use in the African continent. It is widely distributed in the southern African countries, including the Democratic Republic of Congo (DRC), Ethiopia, Kenya, Malawi, Mozambique, South Africa, Eswatini, Tanzania, Uganda, Zambia, and Zimbabwe [1]; but has not been reported elsewhere in the world. The genus *Warburgia* is characterised by its aromatic trees with pungent bark and alternate leaves with glands containing volatile compounds [3].

The species that is of particular interest in this study is *W. salutaris*, found in Mozambique, South Africa, Eswatini, and Zimbabwe [4]. The International Union for Conservation of Nature (IUCN) categorised the plant species under the Red List as endangered facing a high risk of global extinction [4]. In South Africa, the plant is considered endangered, and in Zimbabwe, it is facing extinction in the wild [5]. In Eswatini, the plant is categorised as critically endangered [6]. However, Dludlu et al. [7] analysed more data and suggested that the status of the plant be considered endangered. South Africa is, therefore, the only country where a natural population of *W. salutaris* can be found.

Warburgia salutaris has been used for the treatment of human and animal ailments. Some traditional healers mix the plant parts, mostly bark, in traditional medicine they give to their clients [8]. Due to its potency in treating human ailments [8], *W. salutaris* is in high demand as it is only available in Kwazulu Natal, Limpopo, and Mpumalanga provinces of South Africa, where it also exists as a cultivated population.

*Corresponding author:
E-mail address: Vuyiseka Nkqenkqa <nkqenkqav@gmail.com>.
https://doi.org/10.56532/mjsat.v3i4.182

Maroyi [1] has extensively reviewed the traditional uses of *W. salutaris* in African countries. The author discussed and listed the widespread use of the genus *Warburgia* both for medicinal (human diseases, ailments, as well as veterinary ailments or uses) and non-medicinal purposes [1,9]. The traditional and modern-day use of the genus for human ailments, preparation, and administration has been summarised by Leonard and Viljoen [2]. Thus, the current manuscript focused on the *W. salutaris* metabolites with medicinal properties. In most instances (79%), the bark is used to treat these ailments compared to other plant parts [1]; the human ailments reported include colds, fever, gastrointestinal diseases, headaches, respiratory systems, and sexual dysfunction. Maroyi [1] also reported on the pharmacological properties of the species. It exhibits a wide range of antimicrobial activities including antibacterial, antifungal, antimycobacterial, anti-inflammatory, and antiplasmodial activities. In addition to its medicinal properties, its bark has valuable use as it is used in ritual functions such as cleansing to prevent bad luck, used as an aphrodisiac in humans, charm for good luck, and used to chase away evil spirits when burnt just outside one's residential premises [8]; this therefore, makes the use of the plant species substantial.

The efficacy and safety of using *W. salutaris* have not been clinically studied [10]. However, many investigators have conducted bioassays on microorganisms to evaluate the biological potency of the plant. Phytochemical investigations on the plant species have also been conducted. These investigations used hypothesis-based approaches, that target certain classes or individual metabolites in the plant. Additionally, the extraction methods and procedures used vary based on the compounds or class of secondary metabolites targeted. Different studies detected various chemical compounds in the plant. The genetic variation among the plants, their developmental stages, and the environmental conditions under which the plants were grown are sources of variation. It is also essential to note the organs or tissues used and whether fresh or dry material was employed, as well as the conditions for storage of the plant material, as these lead to varying chemical profiles. Finally, the extraction procedures and the methods of detection and identification of the metabolites must also be considered.

In that respect, it is imperative to introduce a metabolomics perspective to consider all the qualitative and quantitative analyses of all metabolites reported to be present within this plant by conducting a literature search. Metabolomics forms part of the -omics technologies and is a relatively new approach in natural products research [11]. The word metabolomics was introduced in 2002 by Oliver Fiehn [11] and is defined as the qualitative and quantitative study of the entire range of metabolites present within an organism [11,12]. The term metabolome is defined as the complete set of primary and secondary metabolites of living tissues [13]. Already, there is an initiative to develop standards for metabolomics experiments and reporting [14], and this was reviewed by Spicer et al. [15]. The genome of a plant does not depend on environmental factors; the transcriptome, proteome, and metabolome, on the other hand, are influenced by environmental conditions. The different methods used in metabolomics involve target and non-target approaches [16]. The targeted approach focuses on the analysis of certain specified metabolites, while the non-targeted approach is more comprehensive and global, covering both primary and secondary metabolites. Recently, Deda et al. [17] reported metabolomic analyses of medicinal plants on *Ceratonia siliqua*. The authors performed a targeted metabolomic analysis using LC-MS/MS on syrup and powder matrices to optimise various factors to isolate and identify the largest number of metabolites per analysis. Also, recent attempts at comprehensive isolation and characterisation of secondary metabolites include the report by Faleva et al. [18] in *Rubus chamaemorus* lipophilic and hydrophilic extracts using a combination of 2D nuclear magnetic resonance (NMR) spectroscopy and liquid chromatography–high-resolution mass spectrometry (LC-HRMS). They reported on a total of 68 compounds from their analyses. Plants consist of approximately 200,000 secondary metabolites [19] which are complex and diverse in structures [13]. Most of the investigations on phytochemical studies are motivated and informed by local traditional uses of the plant and are designed to give a small subset or individual compound for use in specific applications such as bioassays-guided isolation and identification of bioactive compounds. They were, therefore, never meant to provide comprehensive metabolic profiles. We propose that for each species a comprehensive list of these metabolites be compiled into a compendium.

The aim of this review is to analyse the literature on phytochemical studies of *W. salutaris* to devise a "compendium of metabolites". There is minimal information on phytochemical studies on *W. salutaris*, so other *Warburgia* species will be discussed to give a complete understanding. The "compendium of metabolites" is intended to be a resource for use by researchers working with *Warburgia* spp. Other omics studies, such as transcriptomics may then be informed by the compendium. In other species, such studies have been done in *Withinia somnifera* [20], *Litchi chinensis* [21], *Hydrangea arborescens* [22], and *Triticum aestivum* L. [23]. The review will first describe the biological activities and different classes of secondary metabolites found in *W. salutaris* organs and tissues, and subsequently, consider extraction methods and solvents.

2. PLANT SECONDARY METABOLITES

Plants are exposed to attack by herbivores and other plants as well as microbes. They have developed a defence mechanism based on chemical compounds (secondary metabolites) that have biologically active properties, including antibacterial, antifungal, antifeedant, plant-growth regulators, antioxidants, cytotoxic, phytotoxic, piscicidal and molluscicidal, and ultraviolet (UV) protectants effects to defend themselves [24,25]. This, therefore, explains how humans and animals fight ailments and diseases by treatment with medicinal plants such as *W. salutaris* [1,9].

Secondary metabolites are organic compounds with a low molecular weight of $\leq$ 1.5 kD, which have great structural diversity and differ in polarity [13,19]. Plants produce a wide range of secondary metabolites, including alkaloids, flavonoids, terpenoids, and phenolic compounds [19]. There is a wide range of publications that report on secondary metabolites isolated from *W. salutaris*; alkaloids, terpenoids, saponins, tannins, flavonoids, and steroids extracted from the bark and leaves of the plant [2,26]. The metabolites from *Warburgia* species have been investigated to validate their ethnopharmacological use by Rabe and van Staden [27]; Olila and Opuda-Asibo, [28]; Frum et al. [29]; Mohanlall and Odhav,

[30]; Muthaura et al. [31]; Abuto et al. [32]; Chauke et al. [33]; Nyaba et al. [34]; Soyingbe et al. [35]; Khumalo et al. [36].

3. Extraction Methods, Solvents and Analytical Techniques

Several methods are used to extract metabolites from plants. These methods include solvent extraction (maceration, percolation, soxhlet extraction, pressurised solvent extraction, steam distillation), supercritical fluid extraction, and solid-phase extraction [11,37] with solvent extraction being the most popular [38]. Even though there are several extraction methods, no single experiment can be used to extract, detect, and identify all natural products in a plant 'in one stroke'. However, experiments using different analytical techniques may be used in combination to comprehensively identify the broad spectrum of metabolites in crude extracts [11,39] to provide robust information concerning a particular study.

The choice of method of extraction depends on many factors, including the target of the extraction [37,38], whether the target is a known or unknown compound(s), a structurally related group of compound(s) within an organism, diversity of compound(s) in the population of the same species or species of the same genus or to identify all secondary metabolites present in an organism for metabolomics study [40].

The most common method of extraction used by traditional healers and herbalists is solvent extraction, using water as their extractant. Since like dissolves like, water will, therefore, dissolve polar compounds [41]. Traditionally, the medicinal plant material is brewed or decocted in hot or cold water (aqueous - polar). In a laboratory setting, other extraction methods that use organic solvents with varying polarities have been previously employed to explore metabolite constituents found in plants with different solubilities when not subjected to aqueous extractants. There are common solvents used to extract compounds and other metabolites in plants (Table 1). These solvents are polar-, medium polar- and nonpolar extractants with varying physicochemical properties, as shown in Table 1. Other studies have used various solvents, including dichloromethane (DCM), different methanol: dichloromethane (MeOH:DCM) ratios, and water or steam for extracting bioactive compounds in *W. salutaris* for further analysis [10,42– 45].

Table 1. Physicochemical properties of some common solvents used in natural product extraction

Solvent	Type of polarity	Polarity index	Boiling point (°C)	Viscosity (cPoise)	Solubility in water (% w/w)
n-Hexane	Nonpolar	0.0	69	0.33	0.001
Dichloromethane (DCM)	Medium polar	3.1	41	0.44	1.6
Iso-propanol	Polar	3.9	82	2.30	100
n-Butanol	Polar	3.9	118	2.98	7.81
n-Propanol	Polar	4.0	92	2.27	100
Chloroform	Nonpolar	4.1	61	0.57	0.815
Ethyl acetate (EtOAc)	Medium polar	4.4	77	0.45	8.7
Methanol (MeOH)	Polar	5.1	65	0.60	100
Acetone	Polar	5.1	56	0.32	100
Ethanol (EtOH)	Polar	5.2	78	1.20	100
Water (Aqueous)	Polar	9.0	100	1.00	100

Table 2 presents a "compendium of metabolites" of *Warburgia* species based on a literature search to establish extraction methods, solvents, and identification techniques. The genus *Warburgia* is rich in drimane sesquiterpenes [1] and comprises other classes of sesquiterpenes, including essential oils that are volatile organic hydrocarbons known to exhibit biological activities. The comprehensive list of drimane sesquiterpenoids that have been found in phytochemical studies of the plant species using various methods are warburganal, mukaadial, polygodial, isopolygodial, salutarisolide, muzigadial, ugandensidial, iso-mukaadial acetate and 2α-acetal-polygodial [10,42–45], as reported in Table 2.

Table 2. Extraction process, isolation, identification, and characterisation of compounds in *Warburgia* plant organs

Plant part/ organ	Sample pre-processing	Solvent	Extraction procedure	Separating procedures	Fractions	ID and Structural elucidation, Spec. analysis	Metabolites	*Warburgia* species	References
		Aqueous							
Leaves	Air dried, ground	Water	3hr, Hydrodistillation			GC-FID, GC-MS, Retention indices (RI), MS	α-Pinene, Myrcene, α-Phellandrene, Limonene, (*Z*)-β-Ocimene, (*E*)-β-Ocimene, α-Terpinolene, Linalool, *allo*-Ocimene, α-Terpineol, α-Copaene, β-Elemene, β-Caryophyllene, α-Humulene, (*E*)-β-Farnesene, Germacrene D, (*E,E*)-α-Farnesene, δ-Cadinene, (*E*)-Nerolidol, Caryophyllene oxide, Humulene epoxide II, Drimenol	*W. salutaris*	[43]
Bark/ Stem bark		Water	24hr Hydrodistillation	Column chromatography		GC-FID, ^{1}H, ^{13}C NMR	Dremenol, *E*-nerolidol	*W. salutaris*	[36]
		Organic							
		DCM		column chromatography – silica gel	TLC	^{1}H NMR, ^{13}C NMR, Mass spec	Warburganal, Polygodial	*W. salutaris*	[10]
Leaves	Air-dried 2-3 weeks at 4°C	DCM				GC-MS	Copaene-15-ol, Epizonarene, α-bergamotene, Himachala-2,4-diene, Calamenene, (-)-zingiberene, Hexadecane, 7-epi-α-selinene, Drimenin, D-mannitol, β-cadinene, Ar-himachalen-2-ol, α-terpineol, Berkhey aradulene, Nerolidol 2 γ-costol, Germacrene B, (-)-isolongifolol, β-bisabolene, Caryophyllene, Linoleic acid, Oleic acid, Selina-3,7-(11)-diene, γ-cadinene, α-muurolene, α-cubebene, β-humulene, Longifolenaldehyde, Geranyl linalool, Geranylgeraniol, Manool, Phytol, Driminol, Palmitic acid, n-decanoic acid, 1-decanol, 2-hexyl-Octadecanoic acid, Schottenol, Pollinastanol, B-tocopherol, Guaiacol, Stigmast-5-en-3-ol, oleate, Stigmasterol acetate, Squalene, (-)-β-elemene, β-patchoulene, Isolongifolene oxide, Alloaromadendrene, α-santalene, Bakkenolide A, γ-himachalene, cis-muurola-3,5-diene	*W. ugandensis*	[44]
		MeOH				GC-MS	Copaene-15-ol, Epizonarene, α-bergamotene, Himachala-2,4-diene, Calamenene, (-)-zingiberene, 7-epi-α-selinene, Drimenin, D-mannitol, β-cadinene,	*W. ugandensis*	[44]

Plant part/ organ	Sample pre-processing	Solvent	Extraction procedure	Separating procedures	Fractions	ID and Structural elucidation, Spec. analysis	Metabolites	*Warburgia* species	References
							Ar-himachalen-2-ol, α-terpineol, Berkhey, adulene, Nerolidol 2, γ-costol, Germacrene B, (-)-isolongifolol, β-bisabolene, Caryophyllene, Linoleic acid, Oleic acid, Selina-3,7-(11)-diene, γ-cadinene, α-muurolene, α-cubebene, β-humulene, Longifolenaldehyde, Geranyl linalool, Geranylgeraniol, Manool, Phytol, Driminol, Palmitic acid, n-decanoic acid, 1-decanol, 2-hexyl-Octadecanoic acid, Schottenol, Pollinastanol, B-tocopherol, Guaiacol, Stigmast-5-en-3-ol, oleate, Stigmasterol acetate, Squalene, (-)-β-elemene, β-patchoulene, Isolongifolene oxide, Alloaromadendrene, α-santalene, Bakkenolide A, γ-himachalene, cis-muurola-3,5-diene		
Bark/ Stem bark	Obtained dry	MeOH: DCM		Flask column chromatography – silica gel		^{1}H, ^{13}C NMR	12α-acetyl polygodial, polygodial, ugandensial, warburganal	*W. salutaris*	[36]
		DCM		FCC – silica gel	TLC	^{1}H NMR, ^{13}C NMR, Mass spec	Warburganal, Polygodial, Muzigadial, Ugandensial	*W. salutaris*	[10]
	Sun-dried, 5 days	DCM		Column chromatography – silica gel	TLC	FT-IR, NMR (^{1}H, ^{13}C, COSY, DEPT, NOESY, HMBC, HSQC, HREI-MS), X-ray crystallographic	Iso-mukaadial acetate	*W. salutaris*	[34]
	Air-dried 2-3 weeks at 4°C	DCM				GC-MS	Copaene-15-ol, Epizonarene, α-bergamotene, Himachala-2,4-diene, Calamenene, (-)-zingiberene, Hexadecane, 7-epi-α-selinene, Drimenin , D-mannitol, β-cadinene, Ar-himachalen-2-ol, α-terpineol, Nerolidol 2, γ-costol, Germacrene B, (-)-isolongifolol, β-bisabolene, Caryophyllene, Linoleic acid, Oleic acid, Selina-3,7-(11)-diene, γ-cadinene, α-muurolene, α-cubebene, β-humulene, Longifolenaldehyde, Geranyl linalool, Geranylgeraniol, Manool, Phytol, Driminol, Palmitic acid, n-decanoic acid, 1-decanol, 2-hexyl-Octadecanoic acid, Schottenol, Pollinastanol, B-	*W. ugandensis*	[44]

Plant part/ organ	Sample pre-processing	Solvent	Extraction procedure	Separating procedures	Fractions	ID and Structural elucidation, Spec. analysis	Metabolites	*Warburgia* species	References
							tocopherol, Guaiacol, Stigmast-5-en-3-ol, oleate, Stigmasterol acetate, Squalene, β-patchoulene, Isolongifolene oxide, Alloaromadendrene, α-santalene, Bakkenolide A, γ-himachalene, cis-muurola-3,5-diene		
		MeOH				GC-MS	Copaene-15-ol, Epizonarene, α-bergamotene, Himachala-2,4-diene, Calamenene, (-)-zingiberene, 7-epi-α-selinene, Drimenin, D-mannitol, β-cadinene, Ar-himachalen-2-ol, α-terpineol, Nerolidol 2, γ-costol, Germacrene B, (-)-isolongifolol, β-bisabolene, Caryophyllene, Linoleic acid, Oleic acid, Selina-3,7-(11)-diene, γ-cadinene, α-muurolene, α-cubebene, β-humulene, Longifolenaldehyde, Geranyl linalool, Geranylgeraniol, Manool, Phytol, Driminol, Palmitic acid, n-decanoic acid, 1-decanol, 2-hexyl-Octadecanoic acid, Schottenol, Pollinastanol, B-tocopherol, Guaiacol, Stigmast-5-en-3-ol, oleate, Stigmasterol acetate, Squalene, (-)-β-elemene, β-patchoulene, Isolongifolene oxide, Alloaromadendrene, α-santalene, Bakkenolide A, γ-himachalene, cis-muurola-3,5-diene	*W. ugandensis*	[44]
	Oven-dried, 50°C, powdered	EtOAc	8hr Soxhlet	Vacuum liquid chromatography – silica gel	TLC	^{1}H, ^{13}C NMR	Muzigadial	*W. salutaris*	[27]
		Petrol-EtOAc-MeOH		Column – silica gel	TLC	^{1}H NMR, IR	Warburganal, polygodial, mukaadial, ugandensial, cinnamolide-3β-acetate, muzigadial	*W. ugandensis*	[51]
				VLC – silica gel	TLC	HPLC, 1H NMR, IR	Warburganal, polygodial, mukaadial, ugandensial, cinnamolide, cinnamolide-3β-ol, cinnamolide, cinnamolide-3β-acetate, ugandensiolide, deacylugandensiolide, muzigadial, muzigadiolode	*W. stuhlmannii*	[51]

Most of the published research on *W. salutaris* utilised the targeted method for the isolation of bioactive compounds. Nevertheless, the specifics of the methods used also differed based on the research question to be answered. The following investigations, [10,42] focused on targeted metabolites based on interest in bioactive compounds in medicinal plants and whether leaf constituents provide the basis for bark substitution, respectively. The investigation on phytochemical constituents by Mashimbye et al. [42] was conducted on *W. salutaris* plant material collected in the Zoutpansberg mountains near Louis Trichardt, SA, which was dried and subjected to eight days of extraction with DCM at room temperature. The authors employed a separating technique such as thin-layer chromatography (TLC) on silica gel plates eluting with different ratios of hexane-ethyl acetate, which resulted in the drimane sesquiterpenoid lactone, salutarisolide, an amorphous compound mukaadial, drimane sesquiterpenes including, polygodial and isopolygodial as well as a warburganal compound.

On the other hand, Drewes et al. [10], dried bark and leaves of *W. salutaris* collected from 15-year-old trees in Durban Silverglen Medicinal Plant Nursery for three days at room temperature and further dried for another three days in a fume hood after grounding. The ground leaves and powdered bark were extracted with DCM choice of solvent for four days at 25°C and five days at room temperature. The bark extract was separated using flash column chromatography (FCC) on a silica gel column and eluent chloroform afforded the drimane sesquiterpene compounds warburganal, polygodial, muzigadial and ugandensidial, which were further purified by chromatotron. Furthermore, the concentrate of the leaf sample was separated by column chromatography on silica gel with hexane-ethyl acetate (7:3). This resulted in 17 fractions containing a mixture of compounds. The further separation was conducted by TLC and yielded factions with mainly warbuganal and polygodial. This investigation was probably sparked by the fact that leaves and bark treat different ailments. So, this was to check the constituents of each plant part based on what each organ treats.

The other perspective of interest to targeted research methodology includes the use of bioassay-guided fractionation, where the plant constituents to be analysed are selected based on the bioactivity of the separated fractions. In *W. salutaris*, that approach has been used by Rabe and van Staden [27], where the authors used ethyl acetate (EtOAc) for extracting oven-dried (50°C) and powdered bark, which was collected from Silverglen Nature Reserve, Durban, in July 1996. The extract was concentrated at 40°C under reduced pressure and fractionated by vacuum liquid chromatography (VLC) over silica gel using solvents with increased polarities. The fractions of interest based on the bioassay antimicrobial activities were eluted with 20% EtOAc in hexane and subjected to further separation by TLC on silica gel plates with 4:1 hexane:EtOAc which were further elucidated by one-dimensional NMR (1H, 13C). This way, Rabe and van Staden [27] isolated and identified the sesquiterpene compound muzigadial for the first time in *W. salutaris*. This compound has been reported in other *Warburgia* species (*W. ugandensis* and *W. stuhlmannii*).

A similar approach was followed by Nyaba et al. [34]. They collected the bark of *W. salutaris* from the University of KwaZulu Natal's Botanical Gardens, Pietermaritzburg Campus, KwaZulu-Natal (SA), sun-dried it for five days, and milled. The powdered bark was extracted for three days with DCM, and the mixture was filtered through a Whatman (No. 1) paper which was then concentrated at 45°C under reduced pressure. The DCM extract was separated through a column chromatography with silica gel which resulted in 35 fractions which were then eluted with 8:2 hexane:ethyl acetate. Further analyses on TLC plates grouped the fractions into seven combined fractions using 20% H_2SO_4 in the methanol mixture, resulting in an amorphous compound, which was further characterised using spectroscopic techniques including, Fourier transform infra-red (FT-IR), both dimensional methods of NMR and X-ray crystallography. After structural determination, the compound isolated was found to be iso-mukaadial acetate.

Also, Khumalo et al. [36] employed the same approach where dried bark of *W. salutaris* purchased from the muthi market in Johannesburg (SA) was extracted with different ratios of MeOH:DCM. After evaluating antimicrobial activities in different ratios, a 1:1 MeOH:DCM ratio was selected for further analysis. In addition, essential oils were isolated from the bark of a cultivated tree by hydrodistillation for 24 hours. The bark extract and essential oils were separated by flash column chromatography (FCC) with silica gel and eluted with 1:9 cyclohexane (Cyclohex):ethyl acetate (EtOAc) which was increased gradually to 2:8 Cyclohex:EtOAc for bark extract. The constituents of the essential oils were identified using GC-FID. Different fractions from the dried bark were also obtained and identified using 1H, 13C NMR spectra and detected for the first time the presence of 12α-acetal-polygodial and ugandensial in *W. salutaris* together with the known compounds such as polygodial and warburganal from the bark extract. Furthermore, drimenol and volatile sesquiterpene *E*-nerolidol were the two major components isolated from the bark essential oils [36].

In another investigation, leaves of *W. salutaris* were collected from the Botanical Garden of the University of KwaZulu-Natal, KwaDlangezwa Campus, KwaZulu-Natal (SA) by Lawal et al. [43]. They isolated oil from air-dried and powdered leaves using the hydrodistillation method for three hours. To identify the oils, gas chromatography (GC) based on retention indices and gas chromatography-mass spectrometry (GC-MS) by comparison of their mass spectra from those on the database were used. The composition of the oil consisted of 72.9% monoterpene hydrocarbons (majoring in limonene, (E)-β-ocimene and (Z)-β-ocimene) as the main classes of compounds, 15.6% sesquiterpene hydrocarbons (prominent α-humulene and β-caryophyllene) and oxygenated terpenes with 6.3% and 2.9% for monoterpene and sesquiterpene derivatives, respectively.

4. CHEMICAL COMPOUNDS FOUND IN OTHER SPECIES OF *WARBURGIA*

Ethnobotanical studies have been conducted in other *Warburgia* species in African countries, where the species is used as the primary source of traditional medicine. Phytochemical investigations revealed different chemical compositions in different species of *Warburgia* plants. Diverse terpenoids were found in the DCM and MeOH extracts of the bark and leaf of five populations of *W. ugandensis* [44]. The chemical composition of the extracts varied greatly based on classes of compounds with substantial variations in the levels of phytochemicals present in the leaf and stem bark extracts of

W. ugandensis. Nevertheless, sesquiterpenoids were the most dominant compounds found in all the plant populations.

Phytochemical studies of *Warburgia* species revealed the presence of several drimane sesquiterpenes. The most interesting compounds that are known to exhibit biological activities include warburganal, polygodial, mukaadial, ugandensidial, and muzigadial [24]. Well-known examples of drimane-sesquiterpenes in medicinal plants are dialdehyde warburganal, ugandensial, muzigadial, and polygodial [3]. These compounds have been reported to possess several pharmacological activities [2].

Phytochemical investigation of the bark extract of *W. ugandensis* led to the isolation of sesquiterpene dialdehydes, warburganal, muzigadial, polygodial, mukaadial, ugandensidial and epipolygodial which were all tested against bacterial, fungal, yeast and mould pathogens [45,46]. Opiyo et al. [47] isolated 7α-acetylugandensolide and thirteen known drimane-type sesquiterpenes (bemadienolide, drimenin, polygodial, warburganal, mukaadial, ugandensidial, muzigadial, 6α-hydroxymuzigadial, 9-deoxymuzigadial, ugandensolide, deacetoxyugandensolide, cinnamolide 3β-acetoxycinnamolide) from the bark of *W. ugandensis*. Known sesquiterpenoids and the first report of ugandensial A, a drimane-type sesquiterpenoid were isolated from the bark extract of *W. ugandensis* [48]. Through FT-IR spectrometry, phytochemical analysis of *W. ugandensis* revealed several functional groups represented by various secondary metabolites such as alkaloids, terpenoids, flavonoids, cardiac glycosides, and polyphenols [49]. A phytochemical study of two populations of *W. ugandensis* Sprague from the Great Rift Valley, Kenya showed the presence of 18 drimane sesquiterpenes, which were analysed by GC-MS [44]. These include drimendiol, isodrimeninol, warburganal, mukaadial, pereniporin A, 12-hydroxy-epi-albrassitriol, deacetyl ugandensiolide and the rest have not yet been identified. Also, Abuto et al. [44] identified a high number (32.50% - 58.56%) of sesquiterpenoids by GC-MS from *W. ugandensis* Sprague plant parts (bark and leaf) also collected from different populations across the Great Rift Valley, Kenya. Apart from drimanes, other sesquiterpene-type, including the volatile compounds copaene, caryophyllene, farnesene, humulene, and cubebene were isolated from *W. ugandensis* and identified using GC-MS [50].

In a comparative study of the stem bark of both *W. ugandensis* and *Warburgia stuhlmannii*, Kioy et al. [51] found the following known compounds: warburganal, polygodial, mukaadial, and ugandensidial. In addition to that, they found four novel compounds: cinnamolide-3β-acetate in both species, and cinnamolide-3β-ol, deacetylugandensolide, and muzigadiolide only in *W. stuhlmannii*. In the study, ugandensidial, ugandensolide and muzigadial were recorded for the first time in *W. stuhlmannii* [51]. No significant leaf volatile oil composition was found between the two genera [51]. In another study, an extract of *W. stuhlmannii* leaves showed the presence of glycosides in the form of drimane-type sesquiterpenes which were characterised to be mukaadial 6-O-β-D-glucopyranoside and mukaadial 6-O-α-L-rhamnopyranoside [52]. The authors isolated other compounds from the same extract, including mukaadial, deacetylugandensolide, quercetin, kaempferol, kaempferol 3-O-α-L-rhamnopyranoside, quercetin 3-O-β-D-glucopyranoside, kaempferol 7-O-β-D-glucopyranoside, myricetin 3-O-α-L-rhamnopyranoside, quercetin 3-O-α-L-rhamnopyranoside, quercetin 3-O-sophoroside and isorhamnetin 3-O-β-D-glucopyranoside [52].

The abundance of terpenoids, particularly sesquiterpenoids in this plant genera suggests responsibility for the various medicinal properties of the plant. Among the drimanes and colorotane-type sesquiterpenoids that are mostly found in *Warburgia* extracts, there are other sesquiterpene-type organic compounds known to have a beneficial influence on humans as health-promoting compounds, used as flavour enhancement, insect deters and plant defence against herbivores and pathogens.

5. TOWARDS A *WARBURGIA SALUTARIS* METABOLOME

Table 2 shows "stepwise" methods used, from extraction processes to the characterisation of chemical compounds detected and isolated from *W. salutaris*. Literature reports on these phytochemical studies showed several chemical compounds extracted with different choices of isolation methods, such as solvent extraction and hydrodistillation using a Clevenger-type apparatus. The reported chemical compounds were isolated from different plant parts (leaves and bark/stem bark) of this plant collected at different locations. In addition, different analytical techniques such as column chromatography, flash column chromatography, gas chromatography-mass spectrometry/flame ionisation detector, mass spectra, retention indices (RI), Fourier-transform infrared spectrophotometry, infrared spectrophotometry (IR), ultraviolet-visible spectroscopy (UV-vis), one-/two-dimensional techniques of nuclear magnetic resonance (NMR) and X-ray crystallographic have also been used in isolation, identification and characterisation of known and novel compounds from this plant.

The literature reviewed focused on different plant parts with most investigations focused on stem bark followed by leaves. This could be because traditionally, for *W. salutaris*, the commonly used plant part to treat ailments is bark followed by leaves, roots, and stalks [1]. The plant species reviewed revealed many sesquiterpenoids, including that from essential oils and a relative number of monoterpenes. This may be because these publications used a targeted-based approach in conducting the research, rather than isolating all compounds present in crude extracts. Even so, there are factors that affect the variability of detected compounds in natural products including biotic and abiotic, growth stages and genotype of the plant population used, amongst others [53]. The levels of chemical compounds vary even on similar plant parts of different plant specimens harvested from the same vicinity [37]. This shows that the chemical composition of a plant depends on many environmental factors, including temperature, nitrogen content in the soil, soil fertility, age of the plant, and plant part used amongst others [44]. Also, the chemical profile of compounds in plant material harvested from its wild native habitat may differ from that of cultivated plants [54]. A similar concern was raised by a community that claimed that cultivated *W. salutaris* might weaken the value of the tree, and only trees occurring naturally should be used in medicine [8].

In addition, the discrepancies in detected compounds could be from the variations in sample preparations and data mining, in which both are vital steps in compounds isolated and the

accuracy of the results [38,41]. The various procedures employed in the literature have many steps in common, such as sample collection, drying, extraction, and preparation for analysis. Furthermore, each step is reviewed and discussed in detail by Kim and Verpoorte [38]. The use of these many procedures could be due to no standardised preparatory method as some of these steps may be omitted depending on the analytical techniques to be used or the research question to be studied. Nonetheless, the preparatory processes in literature considered the thermolabile and volatile compounds depending on the targeted class of compounds or individual compound(s), while the traditional preparatory method is to air-dry or sun-dry the plant material for several days at room temperature.

Different organic solvents and the mixture of solvents used with ratios released fractions of the extracts on subjection to different separating techniques. Among the common organic solvents used include DCM, MeOH, EtOH, EtOAc, and petrol. The solvents used in extraction methods are also known to affect the release of chemical compounds from natural products due to various physicochemical properties, including the difference in polarity and selectivity [38]. Dhawan and Gupta [55] and Ngo et al. [56] studied the impact of different solvents on the extraction efficiency of bioactive components in plants with medicinal properties. Methanol was reported to be the best extraction solvent for many flavonoids and phenols from *Datura metel* plant leaves [55]. At the same time, 50% of acetone gave optimum results by yielding high contents of solids, phenolic compounds, flavonoids, and saponins from the root of *Salacia chinensis* L [56]. In both stem bark extracts of *W. ugandensis* and *W. stuhlmannii* by methanol resulted in rich condensed tannins [51]. This discrepancy shows that the type of plant extracted varies greatly based on secondary metabolites present from the extract of plant material. Also, the extracts of secondary metabolites of the same plant material obtained with solvents of different characteristics have distinct biological properties [19]. The literature reported research from an ethnobotanical perspective, hence using these different solvents to exploit the chemical constituent of this plant, especially with bioactive properties yielded different metabolites.

The detected chemical compounds were found using various analytical techniques at the end of the isolation- and purification process. The differences in the preparatory and extraction procedures may affect the yield of the target compound or constituents in plants. For structural elucidation, spectroscopic data were compared to literature data and or standard samples. Some used various spectroscopic techniques for structural determination, including NMR and IR.

All the strategies used were targeted approaches as the overall aims of the literature search involved analyses of known metabolites. Drewes et al. [10] and Mashimbye et al. [42] isolated and characterised sesquiterpenoids in plant parts (bark and/or leaf) of *W. salutaris* based on traditional ethnomedicinal uses and known compounds, respectively, whereas some authors focused on bioassay-guided fractionation where only fractions that have biological activities (antibacterial and antimalarial) were considered for further analyses (identification and characterisation) [27,34,36]. The latter approach limits the identification of many compounds in plants. Nonetheless, it improves scientifically, and the broad spectrum uses and validates the usage of the plant as it is known to have medicinal value. Also, with each study conducted in this manner, authors tend to record novel and other known compounds found in the plant at different concentrations.

The "compendium of metabolites" presented here consists of 89 metabolites. While this is far less than the natural products found in plants, which have approximately 200,000 metabolites, this is a good starting reference point. Only 24 compounds were obtained from the water-soluble fraction. The rest, 65, were characterised by organic solvents. A wide diversity of metabolome was registered such as monoterpenes, sesquiterpenes, fatty acid derivatives, phenolics, phytosterols, tocopherols, ketones, and aldehydes. The class monoterpene was the most dominant, followed by sesquiterpenoids and other classes.

Table 3 presents the compendium in an alternative format. This format will be further developed as an online resource and repository for medicinal plants. Links to various omics resources such as the genome, transcriptome, proteome, and metabolome will be provided in the compendium. The compendium is currently hosted at https://www.cput.ac.za/academic/faculties/appliedsciences/departments/biotechnology-consumer-studies/compendiumofmetabolites. Researchers are encouraged to register, add, and update pages of their species of interest.

Table 3. Compendium of metabolites with links to various omics and biomolecular resources

Name of species and Links to omics resources (Genome/Transcriptome/Proteome/Metabolome)	Metabolites	References	Links to biomolecular resources
	(-)-isolongifolol	[44]	https://webbook.nist-.gov/cgi/cbook.cgi?I-D=R185003
	(-)-zingiberene	[44]	https://www.genome-.jp/entry/C09750
Warburgia spp.	(-)-β-elemene	[44]	https://www.genome.-jp/entry/C17094
	(*E*)-Nerolidol	[43]	https://www.genome.-jp/entry/C09704
Transcriptome: https://www.ncbi.nlm.nih.gov/sra/SRX970743	(*E*)-β-Farnesene	[43]	https://www.genome.-jp/dbget-bin/www_bget?C09666
	(*E*)-β-Ocimene	[43]	https://www.genome.-jp/dbget-bin/www_bget?cpd:C09873
	…	…	…

6. CONCLUSION

Warburgia salutaris contains bioactive chemical compounds that have potent antimicrobial properties. The extensively studied plant part is bark compared to other parts in which drimane sesquiterpenes were mostly detected. The literature searched does not cover the entire metabolome but covers only the subclasses of the metabolome. Extraction, identification, and determination of bioactive compounds in plants depend on the extraction processes, data analyses, and data mining as these are factors affecting the process of obtaining a metabolome. With many other modes of preparation of the plant, traditionally the plant parts are suspended in either cold or boiling water, and the extract is taken orally for the treatment of various diseases. However, when this kind of procedure is used in plants for analysis, it releases fewer bioactive compounds than organic solvents. Therefore, it is essential to use as many extraction procedures as possible and various analytical techniques to comprehensively recover as many compounds as possible. The establishment of stepwise procedures for subclass metabolome for this medicinal plant is ideal such that there is no replication of metabolite extraction studies for already existing data.

ACKNOWLEDGEMENT

The authors would like to thank the Biotechnology programme, Faculty of Applied Sciences, CPUT, for their support. We would like to acknowledge support from CSIR and CPUT - URF. Lastly, we would also like to thank the SANBI – Kirstenbosch Botanical Garden for granting permission to collect *Warburgia salutaris* leaves.

REFERENCES

[1] A. Maroyi, The genus Warburgia: A review of its traditional uses and pharmacology. Pharm. Bio., Vol. 52, no.3, pp. 378-391, 2014.

[2] C.M. Leonard, and A.M. Viljoen, Warburgia: A comprehensive review of the botany, traditional uses and phytochemistry. J. Ethnopharmacol., Vol. 165, pp. 260-285, 2015.

[3] V.T. Amiguet, P. Petit, C.A. Ta, R. Nunez, P. Sanchez-Vindas, L.P. Alvarez, M.L. Smith, J.T. Arnason, and T. Durst, Phytochemistry and Antifungal Properties of the Newly Discovered Tree Pleodendron costaricense. J. Nat. Prod., Vol. 69, pp. 1005-1009, 2006.

[4] C., Hilton-Taylor, R., Scott-Shaw, J. Burrows, and N. Hahn, Warburgia salutaris. The IUCN Red List of Threatened Species 1998: e.T30364A9540142. 1998.

[5] A. Maroyi, Ethnobotanical study of two threatened medicinal plants in Zimbabwe. Int. J. Biodivers. Sci. Manag., Vol. 4, no. 3, pp.148-153, 2008.

[6] T.S. Dlamini, and G.M. Dlamini, Swaziland. Southern African Plant Red Data Lists. SABONET, Vol. 14, pp. 121-134, 2002.

[7] M.N. Dludlu, P.S. Dlamini, G.F., Sibandze, V.S. Vilane and C.S. Dlamini, Distribution and conservation status of the Endangered pepperbark tree Warburgia salutaris (Canellaceae) in Swaziland. Oryx, Vol. 51, no. 3, pp. 451-454, 2017.

[8] W. Kozanayi, and B. Mukamuri, Socio-Economic Issues Related to Warburgia salutaris: A Powerful Medicinal Plant in Zimbabwe. pp. 11-19, 1999.

[9] A. Maroyi, Warburgia salutaris (Bertol. F.) Chiov.: A multi-use ethnomedicinal plant species. J. Med. Plants Res., Vol. 7, no. 2, pp. 53–60, 2013.

[10] S.E. Drewes, N.R. Crouch, M.J. Mashimbye, B.M. de Leeuw, and M.M. Horn, A phytochemical basis for the potential use of Warburgia salutaris (pepper-bark tree) leaves in the place of bark. S. Afr. J. Sci.,Vol. 97, pp. 383-386, 2001.

[11] F., Tugizimana, L. Piater, and I. Dubery, Plant metabolomics: A new frontier in phytochemical analysis. S. Afr. J. Sci., Vol. 109, no. 5-6, pp. 01-11, 2013.

[12] S.D. Sarker, and L. Nahar, Hyphenated Techniques and Their Applications in Natural Products Analysis. In Natural Products Isolation. Methods in Molecular Biology (Methods and Protocols), S. Sarker, and L. Nahar Eds., Humana Press, 2012, Vol. 864, pp. 301-340.

[13] G. Barchet, A brief overview of metabolomics: What it means, how it is measured, and its utilisation. SCQ 2007. https://www.scq.ubc.ca/a-brief-overview-of-metabolomics-what-it-means-how-it-is-measured-and-its-utilization/ [30-01-2020].

[14] Fiehn, O., Robertson, D., Griffin, J., van der Werf, M., Nikolau, B., Morrison, N., Sumner, L.W., Goodacre, R., Hardy, N.W., Taylor, C. and Fostel, J. The metabolomics standards initiative (MSI). Metabolomics, Vol. 3, no. 3, pp. 175-178, 2007.

[15] R.A., Spicer, R. Salek, and C.A. Steinbeck, Decade after the metabolomics standards initiative it's time for a revision. Sci.Data, Vol. 4, 2017.

[16] A., Ribbenstedt, H. Ziarrusta, and J.P. Benskin, Development, characterisation and comparisons of targeted and non-targeted metabolomics methods. PLoS ONE, Vol. 13, no. 11, pp. 1-18, 2018.

[17] Deda, O., Begou, O., Gika, H., Theodoridis, G. and Agapiou, A. Optimization of Carob Products Preparation for Targeted LC-MS/MS Metabolomics Analysis. Metabolites, Vol. 13, no. 5, pp. 645, 2023.

[18] A.V. Faleva, N.V. Ul'yanovskii, A.A. Onuchina, D.I. Falev, and D.S. Kosyakov, Comprehensive Characterization of Secondary Metabolites in Fruits and Leaves of Cloudberry (Rubus chamaemorus L.). Metabolites, Vol. 13, no. 5, pp. 598, 2023.

[19] P.M.G. Paiva, F.S. Gomes, T.H. Napoleão, R.A. Sá, M.T.S. Correia, and L.C.B.B. Coelho, Antimicrobial activity of secondary metabolites and lectins from plants. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, Vol. 1, pp. 396-406, 2010.

[20] S. Tripathi, R.S. Sangwan, B. Mishra, J.S. Jadaun, and N.S. Sangwan, Berry transcriptome: insights into a novel resource to understand development dependent secondary metabolism in Withania somnifera (Ashwagandha). Physiologia plantarum, Vol. 168, no. 1, pp. 148-173, 2020.

[21] F. Abbas, S. Guo, Y. Zhou, J. Wu, S. Amanullah, H.C. Wang, and J. Shen, Metabolome and transcriptome analysis of terpene synthase genes and their putative role in floral aroma production in Litchi chinensis. Physiologia Plantarum, Vol. 174, no. 6, pp. 13796, 2022.

[22] Y. Ke, Y. Zhou, Y. Lv, Y. Qi, H. Wei, Y. Lei, F. Huang, and F. Abbas, Integrated metabolome and transcriptome analysis provides insights on the floral scent formation in Hydrangea arborescens. Physiologia Plantarum, pp. 13914, 2023.

[23] X. Liu, H. Liu, B. Tian, G. Shi, C. Liu, J. Guo, G. Cao, and F. Wei, Metabolome and Transcriptome Analyses of Anthocyanin Biosynthesis Reveal Key Metabolites and Candidate Genes in Purple Wheat (Triticum aestivum L.). Physiologia Plantarum, pp. 13921, 2023.

[24] B.J.M. Jansen, and A. De Groot, Occurrence, biological activity and synthesis of drimane sesquiterpenoids. Nat. Prod. Rep., Vol. 21, no. 4, pp. 449-477, 2004.

[25] M. Wink, Modes of Action of Herbal Medicines and Plant Secondary Metabolites. Medicines, Vol. 2, pp. 251-286, 2015.

[26] M.A.G. Maobe, E. Gatebe, L. Gitu, and H. Rotich, Preliminary Phytochemical Screening of Eight Selected Medicinal Herbs Used for the Treatment of Diabetes, Malaria and Pneumonia in Kisii Region, Southwest Kenya. EJAS, Vol. 5, pp. 01-06, 2013.

[27] T. Rabe, and J. van Staden, Isolation of an antibacterial sesquiterpenoid from Warburgia salutaris. J. Ethnopharmacol., Vol. 73, pp. 171–174, 2000.

[28] D. Olila, and J, Opuda-Asibo, Bioassay-guided studies on the cytotoxic and in vitro trypanocidal activities of a sesquiterpene (Muzigadial) derived from a Ugandan medicinal plant (Warburgia ugandensis). African Health Sci., Vol. 1, no. 1, pp. 12-15, 2001.

[29] Y. Frum, A.M. Viljoen, S.E. Drewes, and P.J, Houghton, In vitro 5-lipoxygenase and anti-oxidant activities of Warburgia salutaris and

drimane sesquiterpenoids. S. Afr. J. Bot., Vol. 71, no. 3-4, pp. 447-449, 2005.

[30] V. Mohanlall, and B. Odhav, Furans and furanones with antimycotoxigenic activity isolated from Warburgia salutaris (Canellaceae). J. Med. Plants Res., Vol. 3, no. 4, pp. 231-240, 2009.

[31] C.N. Muthaura, J.M. Keriko, C. Mutai, A. Yenesew, J.W. Gathirwa, B.N. Irungu, R. Nyangacha, G.M. Mungai, and S. Derese, Antiplasmodial potential of traditional antimalarial phytotherapy remedies used by the Kwale community of the Kenyan Coast. J. Ethnopharmacol., Vol. 170, pp. 148-157, 2015.

[32] J.O. Abuto, A. Muchugi, D. Mburu, A.K. Machocho, and G.M. Karau, Variation in antimicrobial activity of Warburgia ugandensis extracts from different populations across the Kenyan Rift Valley. J. Microbiol. Res., Vol. 6, no. 3, pp. 55-64, 2016.

[33] S.H. Chauke, N. Lall, and Q. Kritzinger, Antifungal activity of South African indigenous plants against aflatoxigenic Aspergillus species. S. Afr. J. Bot., Vol. 115, pp. 318, 2018.

[34] Z.N. Nyaba, P. Murambiwa, A.R. Opoku, S. Mukaratirwa, F.O. Shode, and M.B. Simelane, Isolation, characterisation, and biological evaluation of a potent antimalarial drimane sesquiterpene from Warburgia salutaris stem bark. Malaria J., Vol. 17, pp. 296, 2018.

[35] O.S. Soyingbe, N.I. Mongalo, and T.J. Makhafola, In vitro antibacterial and cytotoxic activity of leaf extracts of Centella asiatica (L.) Urb, Warburgia salutaris (Bertol. F.) Chiov and Curtisia dentata (Burm. F.) CA Sm-medicinal plants used in South Africa. BMC complementary and alternative medicine, Vol. 18, pp. 1-10, 2018.

[36] G.P. Khumalo, N.J. Sadgrove, S. Van Vuuren, and B.E. Van Wyk, Antimicrobial activity of volatile and non-volatile isolated compounds and extracts from the bark and leaves of Warburgia salutaris (Canellaceae) against skin and respiratory pathogens. S. Afr. J. Bot., Vol. 122, pp. 547-550, 2019.

[37] V. Seidel, Initial and Bulk Extraction of Natural Products Isolation. In Natural Products Isolation. Methods in Molecular Biology (Methods and Protocols), S. Sarker, L. Nahar, Eds., Humana Press, Vol. 864, pp. 27-41, 2012.

[38] H.K. Kim, and R. Verpoorte, Sample preparation for plant metabolomics. Phytochem. Analysis, Vol. 21, pp. 4–13, 2010.

[39] J.W. Allwood, D.I. Ellis, and R. Goodacre, Metabolomic technologies and their application to the study of plants and plant–host interactions. Physiol. Plant., Vol. 132, no. 2, pp. 117-135, 2008.

[40] S.D., Sarker, Z. Latif, and A.I. Gray, (red.). Methods in Biotechnology: Natural Products Isolation, 2nd Ed., Totowa, NJ, 2006, pp. 1–26.

[41] M.A. Salem, L. P. de Souza, A. Serag, A.R. Fernie, M.A. Farag, S.M. Ezzat, and S. Alseekh, Metabolomics in the Context of Plant Natural Products Research: From Sample Preparation to Metabolite Analysis. Metabolites, Vol. 10, no. 37, pp. 1–30, 2020.

[42] M.J. Mashimbye, M.C. Maumela, and S.E. Drewes, A drimane sesquiterpenoid lactone from Warburgia salutaris. Phytochemistry, Vol. 51, pp. 435-438, 1999.

[43] O.A. Lawal, I.A. Ogunwande, A.R. Opoku, A.A. Kasali, and A.O. Oyedeji, Chemical Composition and Antibacterial Activities of Essential Oil of Warburgia salutaris (Bertol. f.) Chiov. from South Africa. J. Biol. Activ. Prod. Nat., Vol. 4, no. 4, pp. 272-277, 2014.

[44] J.O. Abuto, A. Muchugi, and A.K.O. Machocho, Diversity in the Phytochemical Profiles of Warburgia ugandensis Sprague from Different Populations across the Kenyan Rift Valley. J. Pharm. Chem. Biol. Sci., Vol. 6, pp. 41-51, 2018.

[45] I. Kubo, and M. Taniguchi, Polygodial, an antifungal potentiator. J. Nat. Prod., Vol. 51, pp. 22-29, 1988.

[46] M. Taniguchi, and I. Kubo, Ethnobotanical Drug Discovery Based on Medicine Men's Trials in The African Savanna: Screening of East African Plants for Antimicrobial Activity II. J. Nat. Prod., Vol. 56, no. 9, pp. 1539–1546, 1993.

[47] S.A. Opiyo, L.O. Manguro, P. Okinda-Owuor, E.M. Ateka, and P. Lemmen, 7α-Acetylugandensolide and antimicrobial properties of Warburgia ugandensis extracts and isolates against sweet potato pathogens. Phytochem. Lett., Vol. 4, no. 2, 161-165, 2011.

[48] M. Xu, M. Litaudon, S. Krief, M.T. Martin, J. Kasenene, B. Kiremire, V. Dumontet, and F. Guéritte, Ugandenial A, a New Drimane-type Sesquiterpenoid from Warburgia ugandensis. Molecules, Vol. 14, pp. 3844–3850, 2009.

[49] P.S. Were, W. Waudo, H.S. Ozwara, and H.L. Kutima, Phytochemical Analysis of Warburgia ugandensis Sprague using Fourier Transform Infra-Red (FT-IR) Spectroscopy. Int. J. Pharmacogn. Phytochem. Res., Vol. 7, no. 2, pp. 201–205, 2015.

[50] S., Drage, B. Mitter, C. Tröls, A. Muchugi, R.H. Jamnadass, A. Sessitsch, and F. Hadacek, Antimicrobial drimane sesquiterpenes and their effect on endophyte communities in the medical tree Warburgia ugandensis. Front. Microbiol., Vol. 5, no. 13, pp. 1-17, 2014.

[51] D. Kioy, A.I. Gray, and P.G. Waterman, A comparative study of the stem-bark drimane sesquiterpenes and leaf volatile oils of Warburgia ugandensis and W. stuhlmannii. Phytochemistry, Vol, 29, no. 11, pp. 3535-3538, 1990.

[52] L.O.A. Manguro, I. Ugi, R. Hermann, and P. Lemmen, Flavonol and drimane-type sesquiterpene glycosides of Warburgia stuhlmannii leaves. Phytochemistry, Vol. 63, 497-502, 2003.

[53] A. Piasecka, P. Kachlicki, and M. Stobiecki, Analytical Methods for Detection of Plant Metabolomes Changes in Response to Biotic and Abiotic Stresses. Int. J. Mol. Sci., Vol. 20, no. 379, pp. 1-22, 2019.

[54] J., George, M.D. Laing, and S.E. Drewes, Phytochemical research in South Africa. S. Afr. J. Sci., 97, pp. 93-105, 2001.

[55] D. Dhawan, and J. Gupta, Comparison of Different Solvents for Phytochemical Extraction Potential from Datura metel Plant Leaves. Int. J. Biol. Chem., Vol. 11, pp. 17-22, 2017.

[56] T.V. Ngo, C.J. Scarlett, M.C. Bowyer, P.D. Ngo, and Q.V. Vuong, Impact of Different Extraction Solvents on Bioactive Compounds and Antioxidant Capacity from the Root of Salacia chinensis L. J. Food Qual., pp. 1-8. 2017.