Tanzania Journal of Health Research
Volume 25: Number 3, July 2024
https://dx.doi.org/10.4314/thrb.v25i3.11

Phytochemical screening of selected medicinal plants of the West Usambara Mountains in Tanzania

Elias N. Mussa[1*], Nyatwere D. Mganga[2], Hamisi D. Nchimbi[3]

[1]*Department of Biology, College of Natural and Mathematical Sciences, The University of Dodoma, Tanzania,*
[2]*Department Biology, College of Natural and Mathematical Sciences, the University of Dodoma, Tanzania,*
[3]*Department of Biology, College of Natural and Mathematical Sciences, The University of Dodoma, Tanzania,*

Abstract

Background: Medicinal plants have been vital for human health for many years due to their restorative properties. Phytochemicals contribute to medicinal plants' healing power, including flavonoids, tannins, glycosides, exudates, terpenes, alkaloids, and phenolics. Several medicinal herbs used by the local community have unknown phytochemical compositions. Knowing the phytochemical composition helps identify bioactive compounds that can be further developed into pharmaceutical drugs. Hence, this study aims to look into the phytochemical composition of *Vernonia iodocalyx* and *Myrica. salicifolia,* which is extensively used in Tanzanian traditional medicine.

Methodology: The extracts from methanol and petroleum ether were obtained through the Soxhlet extraction technique. Preliminary phytochemical screening was conducted using standard methods, while GC-MS analysis was used for in-depth analysis of the identified phytochemicals.

Results: According to the test tube reaction method data, methanolic extract outperformed petroleum ether extracts regarding phytochemical compositions. GC-MS analysis revealed that *Vernonia iodocalyx* and *Myrica salicifolia* contained 21 and 22 phytochemicals, respectively. Out of the identified phytochemicals, 7 from *Vernonia iodocalyx* and 5 from *Myrica salicifolia* have been reported to possess different bioactive compounds essential for drug synthesis.

Conclusion: Methanolic leaf extract of both *Vernonia iodocalyx* and *Myricasalicifolia* has the satisfactory number of phytochemicals. Hence, these plants are recommended for the isolation of active compounds for pharmacological studies.

Keywords: Medicinal plants, Phytochemical, GC-MS, leaf extract

Introduction

In various cultures all over the world, plants are used as medicine and as a source of many potent drugs due to the presence of certain phytochemicals (Anand, Jacobo-Herrera, & Altemimi, 2019). Phytochemicals present in plants are useful in treating certain disorders through individual effects, synergic action, or additives to improve human health(Olivia, Goodness, & Obinna, 2021). Phytochemical analysis involves the quantification and determination of bioactive compounds within the extracts of the plant (Manandhar, Luitel, & Dahal, 2019).

Several medicinal plants in Tanzania have had a fair number of phytochemicals where new drugs have been approved from them (Kilonzo, Rubanza, & Richard, 2019). Due to chemotherapy

[*] **Corresponding author**: Elias Nuru Mussa; **Email:** mussaelias94@yahoo.com; **Phone:** 255754830856

failure in recent years, an increasing occurrence of antibiotic-resistant organisms has been recorded (Arulmozhi, Vijayakumar, & Kumar, 2018). Hence it is important to improve the method of treatment and prevent the spreading of these organisms (Arulmozhi et al., 2018). Hence searching for not only new drug targets but also for improved versions of available drugs has been an urgent need. (The World Health Organization (WHO), 2013). Natural product-derived compounds are still proving to be an invaluable source of medicine for humans regardless of the recent drug discovery research using computational chemistry, molecular modelling and other synthetic chemical methods (Lahlou, 2013). Recently several studies have reported medicinal plants having diverse phytochemicals essential for drug discovery, for example, terpenoids, phenolics and alkaloids are known to be the source of antibacterial and antifungal drugs (Kurmukov, 2013). However, these phytochemicals cannot be generalized to be found in a wide range of plants. This is because the phytochemical composition of medicinal plants can be affected by several factors. including climate, soil quality and altitude. In this respect, two plant species (*V. iodocalyx* and *M. salicifolia*) were selected based on ethnobotanical information from local people of Lushoto and previous literature.

V. iodocalyx O. Hoffm belong to the Compositae family. The plant is an erect shrub with coarsely dentate leaves and spread over East Africa (Amri & Kisangau, 2012). Traditionally this plant has been employed for numerous pharmaceutical applications to treat various ailments. The roots and back serve as antibacterial agents for the treatment of diarrhea and stomach, they are also used as painkillers to treat headaches in eastern Tanzania (Amri & Kisangau, 2012).

Myrica salicifolia Hochst. is an aromatic and resinous medicinal shrub(Silva, Seca, Barreto, & Pinto, 2015) normally grown in humid lower highlands and is found in Ethiopia, South Africa, Kenya, Malawi, Zambia, Saudi Arabia and many mountain ranges in Tanzania that are above 1200 m. It grows well in shallow soils, heath and rocky areas (Silva et al., 2015). This plant has been traditionally utilized for numerous pharmaceutical applications to treat various ailments. Leaf extracts of this plant have been tested for mutagenicity, toxicity and antimicrobial activities. A study by Amri and Kisangau, (2012) based on the local community knowledge revealed that leaves and backs of *V. iodocalyx* have been used to treat stomach ache, diarrhoea and headache. On the other hand, other studies on *M. salicifolia* indicated the presence of steroids, terpenoids, tannins, phlobatannins, saponins and phenolics (Emiru, Periasamy, Karim, Ur Rehman, & Ansari, 2020)The study of the phytochemical constituents of V. iodocalyx and M. salicifolia leaf extracts, especially the GC-MS analysis to determine these constituents, is scarce.

Therefore, the current study aimed to evaluate the phytochemical composition of the methanolic leaf extracts of V. iodocalyx and M. salicifolia.

Materials and Methods

Preparation and extraction of plant extracts

Plant materials of *V. iodocalyx* and *M. salicifolia* were collected from Vitti and Irente villages respectively in Lushoto District. Identification of plant species was done by a botanist and Voucher specimens coded VI-0001 for V. iodocalyx and MS-0002 for *M. salicifolia* were collected and transported to the Department of Biology of the University of Dodoma. In the laboratory, the plant materials were air-dried and then ground into powder using an electric blender. Pulverized materials (250g roots & leaves) were macerated in petroleum ether sequentially for medium-polar and non-polar extraction (Arulmozhi et al., 2018). The extracts were filtered using Whatman No 1 filter paper on a plug of glass wool in a column of glass and a rotary evaporator was used to evaporate solvents

 https://dx.doi.org/10.4314/thrb.v25i3.11

through the vacuum. 250g of roots and leaves of the same pulverized materials were added to 1 L of pure water at 70°C and left to cool in a water bath until reaching 40°C. The extracted samples were decanted and subjected to centrifugation at 5000 rpm for 10 minutes (Zhang, Lin, & Ye, 2018). The supernatant was gathered and passed through filter paper (Whatman No. 1) before undergoing freezing to remove water through sublimation. All samples were preserved in a deep freezer set at -20°C for more procedures. Each solution underwent shaking every half-hour to ensure thorough extraction for six hours and then was left to settle for 48 hours as explained by Nugraha et al., (2020). The obtained extracts were sieved using Whatman No. 1 filter paper; also, the solvent was removed via a rotary evaporator under the vacuum. The resulting residues underwent preliminary phytochemical screening and GC-MS analysis.

Calculation of yield percentage

Percentage yield which is the amount of the extract in percentage obtained after the extraction and evaporation process was obtained by taking the weight of crude extract (dry) divided by the dry weight of plant material as follows:

$$\text{Percentage Yield } (\%) = \frac{\text{Dry weigh of extract}}{\text{Dry weight of plant material}} \times 100$$ (Manandhar et al., 2019)

Initial phytochemical screening

Screening of Alkaloids

Three drops of Wagner's reagent (solution of iodine in potassium iodide) were separately added into three drops of methanolic and petroleum ether plant extracts and the formation of a reddish brown precipitate signalled the presence of alkaloids(Gupta, Thakur, Sharma, & Gupta, 2013; Karmakar et al., 2020)

Screening of Flavonoids

To test the flavonoid presence an alkaline reagent test was used. (Oshadie, Silva, Abeysundara, Minoli, & Aponso, 2017). One ml of sodium hydroxide 20% was added to the two ml of the extracts. The presence of flavonoids was signalled by a yellow colour which disappeared after the addition of dilute sulphuric acid (Selvakumar et al., 2019; Karmakar et al., 2020)

Screening of Saponins

The 50 mg of extract was diluted using distilled water to make 20 ml of the suspension. The suspension was shaken in a graduated cylinder for 15 minutes. The formation of a 2 cm layer of permanent foam indicated the presence of saponins (Banu & Cathrine, 2015; Karmakar et al., 2020; Oshadie et al., 2017; Selvakumar et al., 2019).

Screening of Tannins

Two ml of 5% ferric chloride was added into one ml of plant extract. The appearance of dark blue/green/black colour indicated the presence of tannins. Hydrolysable tannins were indicated by the formation of a dark blue colour while the presence of condensed tannin was indicated by the formation of a green colour (Selvakumar et al., 2019; Karmakar et al., 2020)

 https://dx.doi.org/10.4314/thrb.v25i3.11

Screening of phenols

A ferric chloride test was used to test the presence of phenols. Adding 2 ml of distilled water to 1 ml of plant extract was followed by two drops of 10% ferric chloride. The formation of blue/green colour indicated the presence of phenols (Selvakumar et al., 2019; Karmakar et al., 2020).

Screening of terpenoids

The terpenoids were tested by mixing 0.5 ml of the plant extract with 2 ml of chloroform and concentrated sulphuric acid. The presence of terpenoids was signalled by the formation of a reddish-brown colour at the interface (Wadood, 2013).

GC-MS analysis

The GC-MS analysis was done using Agilent 5975C (Agilent technologies, USA) with a capillary column (HP-5) of a length of 30 meters, diameter of 0.25 mm and thickness of 0.25 µm film connected to the Agilent 6890N GC. The gas used as a carrier was helium gas (99.999%) at 1Ml/minute constant flow and the employed volume of injection was 1 µL. The injector temperature was constant at 250°C and the ion source temperature was 280°C, the oven temperature being programmed starting from 110°C to 200°C with an increase of 10°C/min, then 5°C/minutes to 280°C ending with 9 minutes isothermal at 280°C. The mass spectrometer operated in electron ionisation mode with an ionising energy of 20eV and the ion source temperature was 230°C. The inlet line temperature was 200°C and the running time of the GC-MS was 45 minutes. Mass spectrum interpretation was done through the database of the National Institute of Standards and Technology (NIST) with 62,000 patterns and more. Mass spectra of plant extracts from two selected medicinal plants, namely, *Myrica salicifolia and Vernonia iodocalyx* were compared with spectra compounds stored in the NIT 05 L mass spectra library which are known and published literature. Finally, the percentage of each constituent was determined based on the relative peak area observed in a chromatogram.

Results

Extraction Yield of the Plant Extracts

Through the cold percolation technique, the highest yield was from *M. salicifolia* methanolic extracts at 10.83% while the lowest yield was petroleum ether extracts of *V. iodocalyx* with 4.73% (Table 1)

Table 1: Percentage yield of selected medicinal plant samples

Plants	Solvent used	Part used	% Yield
Vernonia iodocalyx	Methanol	Leaf	10.35
	Petroleum ether	Leaf	4.73
Myrica salicifolia	Methanol	Leaf	10.83
	Petroleum ether	Leaf	7.22

The Preliminary phytochemical screening of *V. iodocalyx* and *M. salicifolia* methanolic leaf extracts showed the presence of phytochemical compounds which are terpenoids, alkaloids, flavonoids, phenols, tannins and saponins (Table 2). Moreover, the GC-MS technique was employed to identify the volatile phytochemical compound within the extract. Peak areas, time of retention, molecular formulas, and the molecular weight of these compounds are presented in Table 3 and Table 4 for *V. iodocalyx* and *Myrica salicifolia* leaf extracts respectively.

https://dx.doi.org/10.4314/thrb.v25i3.11

Table 2: Examination of phytochemicals in selected medicinal plants from the West Usambara Mountains

Plant	Solvent	Phytochemicals					
		Alkaloids	Terpenoids	Flavonoids	Phenols	Tannins	Saponins
Vernonia iodocalyx	Methanol	+	+	-	+	+	-
	P. Ether	-	-	+		-	-
Myrica salicifolia	Methanol	+	-	-	+	+	+
	P. Ether	-	+	+		+	-

Key: +=Presence of the compound, - =Absence of the compound

https://dx.doi.org/10.4314/thrb.v25i3.11

Table 3: Bioactive constituents found in the methanolic extract of *M. salicifolia*

S/N	Peak name	Peak no	RT (min.	Peak area %	Molecular Formula	Similarity %	MW(g mol.$^{-}$)
1	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	1	4.248	0.25	$C_{10}H_{18}O$	93	154.246
2	Alpha-terpinyl isovalerate	4	5.301	0.41	$C_{15}H_{26}O_2$	94	238.37
3	Phenol, 2-methoxy-3-(2-Propenyl)-	5	5.347	0.32	$C_{10}H_{12}O_2$	98	164.2
4	Trans-Cinnamic acid(Ruwizhi & Aderibigbe, 2020)	6	5.410	0.33	$C_9H_8O_2$	97	148.16
5	Caryophyllene	8	5.931	0.36	$C_{15}H_{24}$	99	204.35
5	Aromadendrene	9	6.062	0.24	$C_{15}H_{24}$	95	204.35
6	.Beta.-Guaiene	13	6.537	0.72	$C_{15}H_{24}$	94	204.35
7	.Alpha.-Calacorene		7.001	0.30	$C_{15}H_{20}$	98	200.32
8	Bicyclo[4.1.0]heptan-3-ol,4,7,7-trimethl-, [1R-(1.alpha.,3.alpha.,4.alpha.,6.alpha.)]-	21	7.395	0.79	$C_{10}H_{16}$	70	152.2334
9	Caryopyllene oxide	23	7.544	1.37	$C_{15}H_{24}O$	92	220.35
10	Cyclohexene, 1-methyl-4-(11-methylethylidene)-	26	7.996	0.92	$C_{10}H_{18}$	91	138.25
11	Epizonarene	27	8.065	3.87	$C_{15}H_{24}$	94	204.35
12	Azulene, 1,4-dimethyl-7-(1-methyllethyl)-	32	8.740	0.64	$C_{15}H_{18}$	95	198.3034
13	Isolongifolene, 9,10-dehydro-	39	11.916	0.51	$C_{15}H_{22}$	92	202.33
14	9-Octadecen-1-ol, (E)-	40	12.242	2.41	$C_{18}H_{36}O$	70	268.4778
15	1-Hexadecyne	42	13.037	0.61	$C_{16}H_{30}$	70	222.41
16	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	44	13.684	0.99	$C_{20}H_{40}O$	80	296.5
17	n-Hexadecanoic acid	48	17.014	2.33	$C_{16}H_{32}O_2$	99	256.4241
18	9-Octadecenoic acid, (E)-	51	22.158	0.27	$C_{18}H_{34}O_2$	96	282.46
19	Di-n-octyl phthalate	55	30.249	39.60	$C_{24}H_{38}O_4$	91	390.55
20	Isolongifolene, 9,10-dehydro-	58	32.469	0.22	$C_{15}H_{22}$	83	202.33
20	Squalene	59	32.561	0.80	$C_{30}H_{50}$	99	410.7
21	Pregn-5-en-3-ol, 21-bromo-20-	62	39.393	2.09	$C_{22}H_{35}BrO$	93	395.4

https://dx.doi.org/10.4314/thrb.v25i3.11

	methyl-, (3.beta.)-						
22	Olean-12-ene	63	40.205	2.22	$C_{30}H_{50}$	83	410.7

Table 4: Bioactive compounds found in the methanolic extract of V. *iodocalyx*

S/N	Peak name	Peak no	RT (min)	Peak area %	Molecular formula	Similarity %	MW (g mol.$^{-}$)
1	2(3H)-Furan one	1	3.407	0.02	$C_4H_4O_2$	46	84.07
2	Silane, ethenyldiethylmethyl-	2	3.962	0.03	$C_7H_{16}Si$	52	128
3	Phytol	3	4.060	0.04	$C_{20}H_{40}O$	38	296.5
3	Trans-2-Decen-1-ol, methyl ether	3	4.060	0.04	$C_{11}H_{22}O$	38	170.29
3	Isophytol	3	4.060	0.04	$C_{20}H_{40}O$	38	296.5
4	Butanoic acid, ethyl ester	4	4.180	0.02	$C_6H_{12}O_2$	72	116.16
5	9-Tricosene	5	4.260	0.03	$C_{23}H_{46}$	83	322.6
5	Eugenol	5	4.260	0.03	$C_{10}H_{12}O_2$	98	164.20
6	Benzhydrazide	13	5.482	0.02	$C_7H_8N_2O$	79	136.15
7	Longipinene epoxide	14	5.565	0.02	$C_{15}H_{24}O$	60	220.35
8	Benzene	15	5.633	0.17	C_6H_6	98	78.11
9	Vanillin	16	5.690	0.05	$C_8H_8O_3$	95	152.15
10	Benzhydrazide	13	6.120	0.04	$C_7H_8N_2O$	42	136.15
11	Silane, trichlorooctadecyl-	25	7.487	0.01	$C_{18}H_{37}Cl_3Si$	93	387.94
12	Bicyclo(6,1,0)non-1-ene	26	7.539		C_9H_{14}	86	122.21
13	3-Eicosyne	38	13.02	0.05	$C_{20}H_{38}$	76	178.5
14	Hexadecanoic acid, 14-methyl-, methyl ester	40	15.49	0.06	$C_{18}H_{36}O_2$	97	284.47
15	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	42	20.92	0.01	$C_{19}H_{34}O_2$	94	294.47
16	Methyl 7,10,13-hexadecatrienoate	43	21.077	0.02	$C_{17}H_{28}O_2$	93	264.4
17	(3-Methylbenzoyl) carbamic acid	51	25.088	0.09	$C_6H_{11}NO_2$	91	165.19
18	Urs-12-en-3-ol, acetate, (3, beta.)	52	25.408	0.30	$C_{32}H_{52}O_2$	70	468.8
19	8-Isopropenyl-1,3,3,7-tetramethyl-	53	22.041		$C_{15}H_{12}O$	82	218.33
20	Farnesyl bromide	74	32.206	2.07	$C_{15}H_{25}Br$	86	285.26
21	4,4,6a,6b,8a,11,12,14b-octamethyl-	95	40.451	0.47	$C_{30}H_{48}O$	99	424.7

https://dx.doi.org/10.4314/thrb.v25i3.11

1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a ,14,14a,14b-octadecahydro-2H-picen-3-one

Discussion

Screening the phytochemicals in methanolic extracts of *M. salicifolia* and *V. iodocalyx* revealed the existence of various phytochemicals which are recognized for their medicinal and physiological effects.

Using gas chromatography linked with a mass spectrometer, secondary metabolites belonging to Phenolic compounds, Sesquiterpenoids, Fat acids, triterpenoids, Monoterpenoids, and Acyclic-diterpenoid were identified. Most of the phytochemicals identified have been documented as the source of drugs against non-communicable and infectious diseases of humans.

Among the identified bioactive components, Caryopyllene oxide and Aromadendrene from *Aquilaria rassna* in Malaysia have been documented to exhibit antifungal and antibacterial activities respectively (Dahham et al., 2015; Sobrinho et al., 2020). Additionally, Trans-Cinnamic acid has been reported to exhibit anticancer, neuroprotective and antidiabetic (Ruwizhi & Aderibigbe, 2020). Furthermore, Squalene has been reported to exhibit skin dehydration and antioxidant properties (Lozano-grande, Gorinstein, Espitia-rangel, Gloria, & Mart, 2018). On the other hand, aromadendrene and beta.-Guaiene have been respectively documented to exhibit antidepressant and anti-inflammatory activity (Hordyjewska, Ostapiuk, Horecka, & Kurzepa, 2019; Sahi, 2016). Also, n-hexadecanoic acid has been documented to exhibit antipsychotic and ant-androgenic activities (Tyagi & Agarwal, 2017). Eugenol and Vanillin have been reported to exhibit antiviral and cardioprotective respectively (Derong, Xiao, Zhao, Li, & Xing, 2016; Olatunde, Mohammed, Ibrahim, Tajuddeen, & Shuaibu, 2022).

The findings of the study on the phytochemical composition of selected medicinal plants resemble those of Ndanyi, et al., (2021) The study on phytochemical screening and acute oral toxicity of *Myrica salicifolia* (Bayberry) extracts done in Kenya, showed the presence of flavonoids, alkaloids, saponins, steroids, tannin and phenolics from methanolic extract of Myrica salicifolia.On the other hand, the study from Kenya showed the presence of only alkaloids from petroleum ether extract contrary to our study which revealed the absence of alkaloids but the presence of terpenoids, flavonoids and tannins. Differences in phytochemical composition between these two studies may be influenced by geographical area and seasons of harvesting which influence the content of plant extracts,. Temperature, humidity, sunlight and precipitation can all impact the synthesis of phytochemicals in plants(Pant, Pandey, & Dall'Acqua, 2021).

Most of the phytochemical constituents identified from *M. salicifolia* and *V. iodocalyx* are sources of drug synthesis. Some sesquiterpenoids show potential as lead compounds for the development of drugs for conditions including diabetes, Alzheimer's disease and cardiovascular diseases (Adelusi et al., 2022). Fatty acids are known as a source of drugs for skin disorders, neurological disorders and skin diseases (Taylor, 2009)

Possession of Sesquiterpenoids, Fat acid, triterpenoids, Monoterpenoids, and Acyclic-diterpenoid by *M. salicifolia* and *V. iodocalyx* which have diverse pharmacological activities make them valuable sources for drug synthesis and development

Conclusion

In this study, the methanolic leaf extract of *M. salicifolia* and *V. iodocalyx* demonstrated a range of secondary metabolites, exhibiting numerous pharmacological properties. The GC-MS analysis revealed the presence of 22 and 21 phytochemical constituents from *M. salicifolia* and *V. Iodocalyx, respectively,* which contribute to drug synthesis and development. Hence, the presence of phytochemicals is responsible for their therapeutic effects. However, the characterization and

isolation of phytochemical compounds from these plants need further investigation since they could contribute to drug discovery.

Competing interests: The authors declare that there are no competing interests

Ethical approval: Ethical clearance for conducting this study was obtained from the University of Dodoma

Consent for publication: Not applicable

Availability of data and materials: The data sets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Funding: The study was self-funded by the First author.

Contributions of the authors: This study was conducted in collaboration between all authors out in collaboration between all authors. EM and NM were responsible for designing the study. Author HN managed the searches of the literature and analysis of the study. EM authored the initial draft of the manuscript. All authors reviewed and endorsed the final manuscript

Acknowledgement: The authors express their gratitude to the Herbalists from Irente and Vitti villages for providing ethnobotanical information on medicinal plants.

References

Adelusi, T. I., Oyedele, A. Q. K., Boyenle, I. D., Ogunlana, A. T., Adeyemi, R. O., Ukachi, C. D., … Abdul-Hammed, M. (2022). Molecular modelling in drug discovery. *Informatics in Medicine Unlocked*, *29*(February), 100880. https://doi.org/10.1016/j.imu.2022.100880

Amri, E., & Kisangau, D. P. (2012). Ethnomedicinal study of plants used in villages around Kimboza forest reserve in Morogoro, Tanzania. *Journal of Ethnobiology and Ethnomedicine*, *8*(1), 1. https://doi.org/10.1186/1746-4269-8-1

Anand, U., Jacobo-herrera, N., & Altemimi, A. (2019). *A Comprehensive Review on Medicinal Plants as Antimicrobial Therapeutics : Potential Avenues of Biocompatible Drug Discovery*. 1–13.

Arulmozhi, P., Vijayakumar, S., & Kumar, T. (2018). Phytochemical analysis and antimicrobial activity of some medicinal plants against selected pathogenic microorganisms. *Microbial Pathogenesis*, *123*(January), 219–226. https://doi.org/10.1016/j.micpath.2018.07.009

Banu, K. S., & Cathrine, L. (2015). *General Techniques Involved in Phytochemical Analysis*. *2*(4), 25–32.

Dahham, S. S., Tabana, Y. M., Iqbal, M. A., Ahamed, M. B. K., Ezzat, M. O., Majid, A. S. A., & Majid, A. M. S. A. (2015). *The Anticancer, Antioxidant and Antimicrobial Properties of the Sesquiterpene β-Caryophyllene from the Essential Oil of Aquilaria crassna*. 11808–11829. https://doi.org/10.3390/molecules200711808

Derong, Xiao, M., Zhao, J., Li, Z., & Xing, B. (2016). *An Overview of Plant Phenolic Compounds and Their Importance in Human Nutrition and Management of Type 2 Diabetes*. (Figure 2). https://doi.org/10.3390/molecules21101374

Emiru, Y. K., Periasamy, G., Karim, A., Ur Rehman, N., & Ansari, M. N. (2020). Evaluation of in vitro α-amylase inhibitory activity and antidiabetic effect of Myrica salicifolia in streptozotocin-induced diabetic mice. *Pakistan Journal of Pharmaceutical Sciences*, *33*(4), 1917–1926. https://doi.org/10.36721/PJPS.2020.33.4.SUP.1917-1926.1

Gupta, M., Thakur, S., Sharma, A., & Gupta, S. (2013). Qualitative and quantitative analysis of phytochemicals and pharmacological value of some dye-yielding medicinal plants. *Oriental Journal of Chemistry*, *29*(2), 475–481. https://doi.org/10.13005/ojc/290211

Hordyjewska, A., Ostapiuk, A., Horecka, A., & Kurzepa, J. (2019). Betulin and betulinic acid : triterpenoids derivatives with a powerful biological potential. *Phytochemistry Reviews*, *18*(3), 929–951. https://doi.org/10.1007/s11101-019-09623-1

Karmakar, S., Ghosh, P., Das, C., Biswas, S., Nag, S. K., Dutta, A., … Chatterjee, S. (2020). Phytochemical composition analysis and evaluation of in vitro medicinal properties and cytotoxicity of five wild weeds: A comparative study. *F1000Research*, *9*(July). https://doi.org/10.12688/f1000research.22966.1

Kilonzo, M., Rubanza, C., & Richard, U. (2019). *Antimicrobial activities and phytochemical analysis of extracts from Ormocarpum trichocarpum (Taub .) and Euclea divinorum (Hiern) used as traditional medicine in Tanzania Antimicrobial activities and phytochemical analysis of extracts from Ormocarpum* . (August 2022). https://doi.org/10.4314/thrb.v21i2.6

Kurmukov, A. G. (2013). Phytochemistry of medicinal plants. *Medicinal Plants of Central Asia: Uzbekistan and Kyrgyzstan*, *1*(6), 13–14. https://doi.org/10.1007/978-1-4614-3912-7_4

Lahlou, M. (2013). The Success of Natural Products in Drug Discovery. *Pharmacology & Pharmacy*, *04*(03), 17–31. https://doi.org/10.4236/pp.2013.43a003

Lozano-grande, M. A., Gorinstein, S., Espitia-rangel, E., Gloria, D., & Mart, A. L. (2018). *Plant Sources, Extraction Methods, and Uses of Squalene*. *2018*.

Manandhar, S., Luitel, S., & Dahal, R. K. (2019). In Vitro Antimicrobial Activity of Some Medicinal Plants against Human Pathogenic Bacteria. *Journal of Tropical Medicine*, *2019*. https://doi.org/10.1155/2019/1895340

Ndanyi, M. K., Kamau, D., & Karanja, S. (2021). Phytochemical screening and acute oral toxicity study of Myrica Salicifolia (Bayberry) root extracts. *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, *16*(March), 1–5. https://doi.org/10.9790/3008-1602010105

Nugraha, A. S., Permatasari, A. E. N., Kadarwenny, C. P., Pratoko, D. K., Triatmoko, B., Rosyidi, V. A., … Wangchuk, P. (2020). Phytochemical Screening and the Antimicrobial and Antioxidant Activities of Medicinal Plants of Meru Betiri National Park–Indonesia. *Journal of Herbs, Spices and Medicinal Plants*, *26*(3), 303–314. https://doi.org/10.1080/10496475.2020.1734136

Olatunde, A., Mohammed, A., Ibrahim, M. A., Tajuddeen, N., & Shuaibu, M. N. (2022). Vanillin: A food additive with multiple biological activities. *European Journal of Medicinal Chemistry Reports*, *5*(May), 100055. https://doi.org/10.1016/j.ejmcr.2022.100055

Olivia, N. U., Goodness, U. C., & Obinna, O. M. (2021). Phytochemical profiling and GC-MS analysis of aqueous methanol fraction of Hibiscus asper leaves. *Future Journal of Pharmaceutical Sciences*, *7*(1). https://doi.org/10.1186/s43094-021-00208-4

Oshadie, G., Silva, D., Abeysundara, A. T., Minoli, M., & Aponso, W. (2017). Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. *~ 29 ~ American Journal of Essential Oils and Natural Products*, *5*(2), 29–32.

Pant, P., Pandey, S., & Dall'Acqua, S. (2021). The Influence of Environmental Conditions on Secondary Metabolites in Medicinal Plants: A Literature Review. *Chemistry and Biodiversity*, *18*(11).

https://doi.org/10.1002/CBDV.202100345

Ruwizhi, N., & Aderibigbe, B. A. (2020). *Molecular Sciences Cinnamic Acid Derivatives and Their Biological Efficacy*. https://doi.org/10.3390/ijms21165712

Sahi, N. M. (2016). Evaluation of the insecticidal activity of bioactive compounds from eucalyptus citriodora against Tribolium castaneum. *International Journal of Pharmacognosy and Phytochemical Research*, *8*(8), 1256–1270.

Selvakumar, S., Vimalanban, S., & Balakrishnan, G. (2019). *Quantitative determination of phytochemical constituents from Anisomeles malabarica*. 19–21. https://doi.org/10.15406/mojbb.2019.06.00130

Silva, B. J. C., Seca, A. M. L., Barreto, M. do C., & Pinto, D. C. G. A. (2015). Recent breakthroughs in the antioxidant and anti-inflammatory effects of Morella and Myrica species. *International Journal of Molecular Sciences*, *16*(8), 17160–17180. https://doi.org/10.3390/ijms160817160

Sobrinho, A. C. N., de Morais, S. M., de Souza, E. B., Albuquerque, M. R. J. R., dos Santos, H. S., de Paula Cavalcante, C. S., … dos Santos Fontenelle, R. O. (2020). Antifungal and Antioxidant Activities of Vernonia Chalybaea Mart. ex DC. Essential Oil and their Major Constituent β-caryophyllene. *Brazilian Archives of Biology and Technology*, *63*. https://doi.org/10.1590/1678-4324-2020190177

Taylor, B. L. (2009). Plant-Based Drugs and Medicines. *Drugs*, 1–8.

Tyagi, T., & Agarwal, M. (2017). Phytochemical screening and GC-MS analysis of ethanol ACN extract. *Journal of Pharmacognosy and Phytochemistry*, *6*(1), 195–206.

Wadood, A. (2013). Phytochemical Analysis of Medicinal Plants Occurring in Local Area of Mardan. *Biochemistry & Analytical Biochemistry*, *02*(04), 2–5. https://doi.org/10.4172/2161-1009.1000144

World Health Organization (WHO). (2013). WHO Traditional Medicine Strategy 2014-2023. *World Health Organization (WHO)*, 1–76. https://doi.org/2013

Zhang, Q. W., Lin, L. G., & Ye, W. C. (2018). Techniques for extraction and isolation of natural products : a comprehensive review. *Chinese Medicine*, 1–26. https://doi.org/10.1186/s13020-018-0177-x