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Original article

Potential of natural phenolic antioxidant compounds from *Bersama abyssinica* (Meliathacea) for treatment of chronic diseases

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ABSTRACT

Chronic diseases including cardiovascular, diabetes and cancer persist for a long time in the course of treatment affecting health and are currently the cause of many deaths. In most cases, the treatment of chronic infectious diseases especially Tuberculosis relies on conventional drugs which are currently becoming fruitless due to drug resistance and unpredicted complications in course of treatment. However, herbal medicines have for a long time been used in prevention and treatment of chronic diseases including asthma and heart diseases in Africa. In this study, we extracted metabolites and screened for active compounds with potential free radical scavenging and pharmacological activities from *Bersama abyssinica,* the plant commonly used in traditional medicine in Tanzania. *B. abyssinica* root, stembark and leaf were air dried, sequentially extracted in various solvents including petroleum ether, dichloromethane, ethylacetate and methanol to yield extracts and fractions. The extracts and fractions were tested for the presence of several metabolites and antioxidant activity. The analysis of chemical compounds from resultant extracts was done by GC–MS for non-polar factions and LC-MS/MC for moderate polar extracts.High amount of phenolic acid, flavonoids and tannin were identified in ethylacetate fraction compared to ethanol, dichloromethane and petroleum ether. The GC–MS analysis of petroleum ether extract of *B. abyssinica* stem back yielded twelve (12) compounds with varying composition. The most abundant compounds were 2-Butenoic acid, 3-methyl-, ethyl ester comprising 33.8%, n-Hexadecanoic acid comprising 16.7% and Ethanolpentamethyl- yielded in 16.7%.The LC-MS/MS analysis of Ethyl acetate fractions yielded 20 compounds including; Mangiferin and Isoquercitin were abundant in leaves, stembark and roots. Lastly, ethyl vanillate was identified in both roots and leaves whereas Quercitrin and 7,8-Dimethoxycoumarin were found in stembark and root.These findings indicated that *B. abyssinica* is rich in phenolic compounds ranging from phenolic acids, flavonoids and coumarin that possess high antioxidant and pharmacological properties potential for treatment of chronic diseases.

1. Introduction

Oxidative stress occurring due to continuous production of reactive oxygen species that react with metals or other molecules is a major cause of cell and organ damage (Sies et al., 2017). The most serious oxidative stress diseases affecting the world include inflammation, cancers, diabetes, rheumatism, aging problem and asthma (Lee et al., 2011). However, the situation is reported to occur in immune compromised people complicating health and treatment. Infectious diseases including; Tuberculosis, Human Immunodeficiency Virus (HIV) disease, ulcers and other viral dis-

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eases are increasingly reported to be associated with reactive oxygen species (Rajopadhye et al., 2017).

Recent studies showed failure of synthetic drugs in preventing or treating the oxidative stress linked diseases (Hanafy et al., 2020). There is a high need for bioprospecting on natural sources with antioxidant compounds for combating these chronic diseases which threaten health systems. Antioxidants are well known for treatment of chronic diseases caused by oxidative stress by scavenging on the free radicals (Sochocka et al., 2013). Antioxidants play a vital role as anti-inflammatory, anticancer, antidiabetic, anti-aging, and protect human against cardiovascular diseases and obesity (Barteková et al., 2021).

Over the periods since ancient time, traditional medicines have been proven to be effective in treatment and overcoming challenges of drug failure in a wide range of chronic diseases (El Hachlafi et al., 2020; Li et al., 2015). Plants are good source of antioxidant and people consuming many vegetables and fruits are at low risk of getting chronic diseases (Boeing et al., 2012). Plant compounds including phenolic compounds such as phenolic acids, tannins, flavonoids and others have antioxidant activity to prevent overproduction of reactive oxygen species and reactive nitrogen species in the human body (Sarrafchi et al., 2016). The oxidants block pathogens is of many chronic diseases and non-pathogenic diseases. Polyphenols are among the main source of antioxidant (Ciampi et al., 2020). Organic polyphenols from plants are the most important and safe antioxidants for prevention and treatment of respiratory diseases including pneumonia (Zhou et al., 2019). The mode of action of natural phenols in oxidants is by substitution of hydroxyl groups in the aromatic rings of phenolics (Mathew et al., 2015).

Bersama abyssinica (Melianthaceae) possesses high phenolic content, mostly the polyphenols (Sinan et al., 2020). This plant is widely used for treatment of several diseases in Africa (Sinan et al., 2020). This plant is known to possess combinations of wide range of phenolic compounds providing a synergic activity against chronic diseases (Sinan et al., 2020). However, there is scarce information regarding identified secondary metabolites and pharmacological activity of each fraction including antioxidant activity from *B. abyssinica* stembark which is widely used for medicinal purpose in Tanzania. In this study we screened and compared the phytochemical activity of secondary metabolites extracted from root, stembark and leaf in petroleum ether, dichloromethane, ethyl acetate and methanol respectively for determining metabolites abundance along with their biological activities for recommending usage by the local community.

2. Materials and methods

2.1. Plant materials collection

The leaves, roots and stem of winged bersama were collected in January 2021 from Idweli village in Mbeya region. The plant was identified by a Botanist Dr. Ester Mvungi from University of Dar es salaam in which the voucher specimen number ND.Zekeya Nos.01 was deposited in the herbarium at University of Dar es salaam Herbarium.

2.1.1. Chemicals and reagents

Methanol (absolute) was bought from FlukaChemie GmbH (Sigma-Aldrich®, Zwijndrecht, Netherlands) and Dimethyl sulfoxide (DMSO) was purchased from RFCL Limited, Hayana, India. Dicholoro methane, ethyl acetate and Ethanol were purchased from LobaChemiePvt Ltd, Mumbai, India). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was bought from Sigma-Aldrich®.

Reagent for determination of active metabolites namely; Vanillin, Ascorbic acid, Gallic acid, Ammonium solution Concentrated sulphuric acid, Aluminium chloride, Catechin, Folin-Ciocateu'phenol reagent, Ferric chloride, Mayer's reagent and Qualija were purchased from Sigma-Aldrich®.

2.1.2. Chromatographic materials and chemicals

TLC foils (precoated) Silica gel 60 GF254, 0.2 mm and TLC foils (precoated) Cellulose F, 0.2 mm, were purchased from Merck, Darmstadt, Germany. Sephadex LH-20 was purchased from Pharmacia Biotech, Uppsala, Sweden. Gallic acid was purchased from Merck, Germany and DPPH from Sigma Chemical Company, USA.

2.2. Extraction of active metabolites

This study was conducted from February to October 2021 at the department of Microbiology, Biochemistry and Immunology, Institute of Traditional medicine at Muhimbili Institute of Health and Allied Sciences, Dar es salaam, Tanzania. The plant materials (leaves, roots and stem bark) were chopped in small size and drying under the shade followed by grinding into fine particles by using heavy duty blender, Ken Wood, Japan. The sequential extractions were performed by increasing polarity starting from Petroleum ether to Dichloromethane, Ethyl acetate and finally absolute Ethanol. The soaking period of each solvent was 24 h repeated twice to maximize the extraction process.This was followed by double filtration using cotton wool, whatman® filter paper number 1 and the filtrates were then evaporated to dryness under reducing pressure by using Buchirotating evaporator machine in order to obtained extracts.

2.2.1. Determination of percentage yield

The extract yields percentage of each plant part with respect to the extractor solvent and the total percentage yield of plant parts were calculated by the following formulas;

$$\text{percentage yield of each extaract} = \frac{\text{weight of each extract}}{\text{Weight of soaked plant materilal}} \times 100(\text{i})$$

$$\text{The total percentage yield} = \frac{\text{Total weight of all extract}}{\text{Total Weight of all soaked plant materilal}} \times 100$$

2.3. Determination of secondary metabolites

The qualitative analysis was performed by using the standard procedures previous described by (Sinan et al., 2020; Tyagi, 2017) with some modification. The concentrations of 100 mg/ml were prepared in each sample for the analysis of phenol, saponin, antioxidant, flavonoid and tannin.

2.3.1. Determination of phenol

Two (2) ml of Iron III chloride solution were added to the 2 ml of 100 mg/ml of each extracts, the appearance of deep bluish-green solutionindicated the presence of phenolic compounds.

2.3.2. Determination of flavonoid

5 ml of dilute ammonia solution were added to the 2 ml of 100 mg/ml of extracts followed by addition of few drops of concentrated Sulphuric acid, a yellow coloration indicated the positive result for the presence of flavonoid compound/s.

2.3.3. Test for tannin

100 mg of each extracts were boiled in 2 ml of water in a test tube and then filter which was followed by addition of few drops of 0.1% Ferric chloride solution. A brownish green, blue black coloration indicated the present of Tannin compound/s.

2.3.4. Determination of alkaloid

Mayer's reagent was used to test for presence of alkaloid in extracts adding 2mls of reagent into 5 ml of extract. The solution was shaken for 30 s then left for observation. The presence of alkaloid was indicated by the formation of creamy precipitate.

2.3.5. Determination of saponin

100 mg of each extracts were dissolved into 2 ml of distilled water in the test tube and warmed; this was followed by vigorously shaken. The formation of froth for at least a minute indicated the present of Saponin compounds.

2.3.6. Determination for antioxidant

100 mg of each sample were dissolved in the 1 ml of extractor solvents, filter and divide equally into two different test tubes. This was followed by the addition of 0.5 ml of pre prepared from 0.1 mM DPPH in one of the test tube and the second test tube as control and the mixture was shaken and allowed to stand for 1 min. The formation of discoloration in comparison to control indicated the present of antioxidant compounds in the extract.

2.4. Phytochemical constituent's analysis

The chemical constituents were evaluated by using Gas Chromatography-Mass Spectrometry (GC–MS) and Liquid Chromatography Mass Spectrometer (LC-MS/MS) for petroleum ether fractions and ethyl acetate fractions respectively. Both methodological conditions and results are shown below;

2.4.1. GC–MS analysis of B. Abyssinica stem bark petroleum ether fraction (BASP)

The GC–MS is capable of analyzing volatile extract with low quantity and uses inert gas the same capacity. The low yield of petroleum ether stem bark extract which showed antioxidant than the rest was solely analysed by this method. Analysis of the sample was done by Gas Chromatography- Mass Spectrometry. GC–MS were recorded in a GCMS-QP 2010 Ultra (Shimadzu instrument) operating in Electron Ionization (EI) mode (MS) at 70ev, and Flame Ionization Detector (FID) for GC. A Restek-5MS column (30 m × 0. 25 mm × 0.25 μm) was used. The oven temperature program was 90 °C to 280 °C and held at 90 °C for two minutes which was then increased to 280 ^{0}C for ten minutes (hold time) at the rate of 15 °C per minute. The injection temperature was 250 °C with split injection mode with the flow rate of a carrier gas Helium was 1.21 ml min-^{1}. The ion source temperature and interface temperature in MS were 230 °C and 300 °C respectively. The identification of compounds in the sample was done by scan method which involved the use of Mass Spectral Library and Search Software. Quantification of compounds in the extract was done using Peak Integration method whereby ion allowance was 20%, target ion and other five quantification ions were used on quantitative analyses.

The obtained sample (1.3 mg) was dissolved in 1 ml of dichloromethane which was injected in GC MS and calculation was done based on percentage composition of each scanned compounds using peak areas obtained from peak integration (Area normalization method).

2.4.2. LC-MS/MS analysis of ethyl acetate fractions from root bark, stembark and leaf

Analysis of the ethyl acetate fractions was done by LC-MS/MS (Q-orbitrap-Ultra High Performance Thermofisher Company). The extracts were re-dried by using Rota evaporator under reducing pressure with flowing of Nitrogen gas 15psi at 45℃ then the Liquid Chromatography was eluted by mobile phases of ((A)0.1% formic acid in water followed by (B)0.1% Formic acid in Acetonitrile). The column conditions are 35℃ and 1.9μ of oven temperature and particle size respectively. The coupled MS was scanned in range of 150–2000 *m*/*z* with resolution 140,000, AGC Target1e6. The maximum IT setting was 200 ms with ionization mode (HESI) collision Energy 45v.

3. Results

This study revealed high composition of petroleum ether compounds of which about 80% were extracted from stembark whereas roots released 20%. The dichloromethane extraction yielded 20%, 40%, 20% in roots, stembark and leaves respectively (Table 1). The ethyl acetate extraction yielded 80% fraction in all parts while the ethanolic fractionswere yielded in 80%, 40% and 80% in roots, stembark and leaves respectively (Table 1 and 2).

Furthermore, the study revealed that *B. abyssinica* possessed high amount of phenol acids compared to the flavonoids and coumarin. However, high number of flavonoids and tannin were identified in ethylacetate fraction compared to ethanol, dichloromethane and petroleum ether. Additionally, this study revealed the absence of alkaloid and saponin in all tested extracts. Interestingly, antioxidant activity was revealed in all parts of plant tested in all solvent extractors as shown in Table 2.

The GC–MS analysis of petroleum ether extract of *B. abyssinica* stembark yielded twelve (12) compounds with varying composition. The most abundant compounds were 2-Butenoic acid, 3-methyl-, ethyl ester comprising 33.8%, n-Hexadecanoic acid comprising 16.7%, Ethanol, pentamethyl- whichyielded16.7%, Eicosanoic acid, ethyl ester 7.15% and the rest observed low than 7% composition (Table 3). This analysis depicted mostly acidic compounds with various biological activities including n-Hexadecanoic acid and Eicosanoic acid, ethyl ester with essential pharmacological activities as indicated in Table 4.

The LC-MS/MS analysis of ethyl acetate fractions yielded twenty compoundsof which roots exhibiting 12 followed by leaves bearing 8 and stembark exhibiting 7 compounds. Out of 20 identified metabolites some were present in all or two parts while others were identified solely from one part (Table 5). Mangiferin and Isoquercitin were abundant in leaves, stembark and roots. Other

Table 1
The Percentage yields of leaf, stembark and root of *B. abyssinica* obtained from different extractor solvents.

Plant parts (Plant material)	Extractor Solvents	(Amount) Percentage yield	(Total amount) Total percentage yield
Stem bark (1000 g)	Petroleum ether	(1.7 g) 0.17%	(79.3 g) 7.93%
	Dichloromethane	(3.8 g) 0.38%	
	Ethyl acetate	(3.7 g) 0.37%	
	Ethanol	(70.1 g) 7.01%	
Root bark (1455 g)	Petroleum ether	(1.2 g) 0.082%	(88.8 g) 6.103%
	Dichloromethane	(2.5 g) 0.171%	
	Ethyl acetate	(6.8 g) 0.467%	
	Ethanol	(78.3 g) 5.381%	
Leaf (420 g)	Petroleum ether	(4.3 g) 1.023%	(28.9 g) 6.880%
	Dichloromethane	(4.1 g) 0.976%	
	Ethyl acetate	(2.7 g) 0.642%	
	Ethanol	(17.8 g) 4.238%	

Table 2
The scoring index of group of *B. abyssinica* secondary metabolites.

Extractor solvents	Scoring Index					
	Ratio			Percentage		
	Root	Stem	Leaf	Root	Stembark	Leaf
Petroleum ether	1/5	4/5	0/5	20%	80%	0%
Dichloromethane	1/5	2/5	1/5	20%	40%	20%
Ethyl acetate	4/5	4/5	4/5	80%	80%	80%
Ethanol	4/5	2/5	4/5	80%	40%	80%

Table 3
Qualitative results of Selected Group of Secondary metabolites present in *B. abyssinica* extracts and fractions.

Extractor Solvents	Plant parts	Group of Secondary Metabolites Tested					
		Tannin	Phenol	Flavonoid	Alkaloid	Saponin	Antioxidant
Petroleum ether	Stem bark	+	+	–	–	+	+
	Root	–	–	–	–	–	+
	Leaf	–	–	–	–	–	+
Dichloromethane	Stem bark	+	–	–	–	–	+
	Root	–	–	–	–	–	+
	Leaf	–	–	–	–	–	+
Ethyl acetate	Stem bark	+	+	+	–	+	+
	Root	+	+	+	–	+	+
	Leaf	+	+	+	–	+	+
Ethanol	Stem bark	+	+	+	–	–	+
	Root	+	+	–	–	–	+
	Leaf	+	+	+	–	+	+

Key: + = indicates presence of secondary metabolites and - = indicates absence of secondary metabolites.

Table 4
GC–MS analysis of *B. abyssinica* stem bark petroleum ether fraction constituents.

SN	Compound name	% Composition	R. Time	Target ion	P.Area
1	2-Butenoic acid, 3-methyl-, ethyl ester	33.869	10.013	83.1	423,679
2	Ethanol, pentamethyl-	14.633	11.287	59.05	240,839
3	Dodecanoic acid	1.396	21.072	60.05	30,905
4	Tetradecanoic acid	1.061	25.351	60.05	29,604
5	Perhydrofarnesyl acetone	1.476	26.849	58.05	19,670
6	n-Hexadecanoic acid	16.758	29.385	60.05	447,724
7	Eicosanoic acid, ethyl ester	7.193	29.773	88.1	86,325
8	9-Octadecegnoic acid, (E)-	6.181	32.519	55.05	99,919
9	Oleic Acid	5.038	32.47	55.05	101,281
10	9-Octadecenoic acid, (E)-	6.181	32.519	55.05	99,919
11	Eicosanoic acid	3.360	32.924	57.1	41,094
12	4,8,12,16-Tetramethylheptadecan-4-olide	2.854	35.967	99.1	39,122

Table 5
Analysis of *B. abyssinica* Petroleum ether extract compounds and biological activities.

SN	Compound name	Group	Biological activities	Reference
1	2-Butenoic acid, 3-methyl-, ethyl ester	Fatty acid	Aroma in breweries	(Lee et al. 2008)
2	Ethanol, pentamethyl-	Alcohol	Pyrolysis	(Jayaveeran and Pugazhvadivu, 2016)
3	Dodecanoic acid	Fatty acid	Potential compound for energy production	(Desgrosseilliers et al. 2013)
4	Tetradecanoic acid	Fatty acid	Larvicidal and Repellent activities	(Sivakumar et al. 2011)
5	Perhydrofarnesyl acetone	Terpenes		
6	n-Hexadecanoic acid	Fatty acid	Antiiflamatory and antirheumatism	(Aparna et al. 2012)
7	Eicosanoic acid, ethyl ester	Fatty acid	Antimicrobial activity	(Igwe and Okwu 2013)
8	9-Octadecenoic acid, (E)-	Fatty acid	Antimicrobial activity	(Ghavam et al. 2021)
9	Oleic Acid		Lubricant	(Zhang et al. 2011)
10	9-Octadecenoic acid, (E)-	Fatty acid	Quenching and anti-biofilm	(Singh et al. 2013)
11	Eicosanoic acid	Fatty acid	Antiurease and antioxidant	(Sokmen et al. 2012)
12	4,8,12,16-Tetramethylheptadecan-4-olide	Fatty acid	Antimicrobial activity	(Behera et al. 2020)

mostly compounds recorded were ethyl vanillate in both roots and leaves whereas Quercitrin and 7, 8-Dimethoxycoumarin were found in stembark and root (Table 5). However, 6 compounds namely 7-Diethylamino-3-formycoumarin, Dipicolinic acid, O-Desmethylnaproxen, 3,4-Dimethoxy-5,7,3-trihydroxyflavone, Triamterene, 5,7,3,4-Tetramethoxyisoflavone 5,7,3,4-Tetramethoxy isoflavone were solely identified from root fraction whereas four unique compounds specifically; 3-Hydroxy-6,3,4-trimethoxyflavone, L-Carnitine, 7,8-Dihydroxy-4-methylcoumarin-3-acetic acid and jasmonic acid were from leaves. Three unique metabolites namely; 3-Hydroxy-7, 2, 3-trimethoxyflavone, 5, 7, 4-Trimethoxyisoflavone and syringaldehyde were also identified in

stembark (Table 5). The GC-MS analysis of ethyl acetate fractions revealed the occurence of mangiferin, quercetrin, coumarin and more active compounds as depicted in Table 6, all with recognizable pharmacological activity against chronic diseases (Table 7).

4. Discussion

Plant parts possess a wide variety of active metabolites with unique mode of action. Each group of plant metabolite requires different solvent and extraction process such as infusion, digestion in order to obtain the desired amount for bioactivity (Abubakar and Haque, 2020). The Sequential extraction of extracts of *Bersama abyssinica* root, stembark and leaf showed a diverse range of metabolites with potential pharmacological functions. The petroleum ether and ethyl acetate fractions yielded more compounds compared to methanolic fractions. The findings infer that plant is rich in non-polar, polar compounds and polar compounds as per previous study by (Sinan et al., 2020). This study revealed the presence of some active non polar compounds such as Oleanolic acid which is reported to be essential in treatment of chronic diseases (Coimbra et al., 2011). This study revealed that the ethyl acetate possessed high amount of active compound which was similarly reported by (Sinan et al., 2020). In most cases the polar compounds are recommended for pharmacological use due to high solubility power in water compared to non-polar compounds. However, less polar compounds should be administered in other methods such as inhalation to increase their pharmacological activity (Recharla et al., 2017). On the other hand, moderate polar compounds were abundant and previous studies revealed their anticancer, anti-inflammatory antioxidant and antimicrobial properties (Sinan et al., 2020). Therefore, solubility of non-polar and medium polar compounds could be improved by incorporating into lipoisomer and other current technology for pharmacological application Coimbra et al., 2011; Recharla et al., 2017).

The most dominant class of metabolites from this study was phenol in stem bark. The class expresses a wide range of polyphenolic compounds with high antioxidant activity. The isoquercitin and syringaldehyde demonstrated antimicrobial, antiviral, antioxidant and hypoglycemic activities (Kalyani and Jamunarani, 2015). This study also revealed the presence of Mangiferin, and Isoquercitin from leaves which have been reported by other studies for the treatment of insulin resistance, kidney and cardiovascular diseases (Du et al., 2018; Kshirsagar et al., 2016), which were also found in *B. abyssinica* stem bark (Sinan et al., 2020). Some compounds including Jasmonic acid, 3-Hydroxy-6,3,4-trimethoxyfla vone, Triamterene and L-carnitine were only found in ethlyacetate of *B. abyssinica* leaves. This provides an opportunity for harvesting leaves for medicinal purposes rather than stembark and roots for sustainable utilization of medicinal plants. This study is in consistent with other studies that revealed that polyphenols were essential remedy for treatment of chronic diseases including cardiovascular diseases, diabetics, viral and hypertension (García-Sánchez et al., 2020; Shahidi and Yeo, 2018). However, this study reported for the first time the presence of three important polyphenols; 3-Hydroxy-7, 2, 3-trimethoxyflavone, 5, 7, 4-Trimethoxyisoflavone and 7, 8-Dimethoxycoumarin in Ethylacetate fraction of Tanzania *B. abyssinica* with antioxidant, anticancer and anti-inflammatory activity (Xu et al., 2019; Mehrbod et al., 2019). Most studies show that polyphenols from plant are greater source of antioxidants potential for suppressing effects of oxidative stress in animals (Gessner et al., 2017). For instance, plant extract from olive oil presents a high amount of phenolic compounds for prevention of overproduction of reactive oxygen species which have health effects includingmembrane damage in human body (Rodríguez-Ramiro et al., 2011). Although the traditional medicine industry is gradually growing, the use of herbal medicine has for long time improving livelihood of most rural people relying on services from local health practitioners. Hence researches and clinical trials on herbal medicine are inevitable to improve health and prevent the increasing number of chronic diseases particularly cancers.

5. Conclusion

This study revealed the presence of wide range of phenolic compounds from nonpolar and moderate polar extracts of *B. abyssinica* root, stembark and leaf. The study showed that the active compounds have high antioxidant activity that could be potential for treatment of chronic diseases. However, further studies are recommended on isolation and testing of pure compounds against

Table 6
LC-MS/MS Analysis of ethyl acetate fractions of constituents.

SN	Mass to Charge Ratio(m/z)	Fractions codes and names of compounds eluted		
		BAREt	BASEt	BALEt
1	422.08491	Mangiferin	Mangiferin	Mangiferin
2	196.073559	Ethyl Vanillate	–	Ethyl Vanillate
3	328.094688	–	3-Hydroxy-7,2,3-trimethoxyflavone	–
4	448.100561	Quercitrin	Quercitrin	–
5	250.047738	–	–	7,8-Dihydroxy-4-methylcoumarin-3-acetic acid
6	245.105193	7-Diethylamino-3-formycoumarin	–	–
7	210.125594	–	-g	Jasmonic acid
8	182.057909	–	Syringaldehyde	–
9	464.095476	Isoquercitin	Isoquercitin	Isoquercitin
10	167.021857	Dipicolinic acid	–	–
11	216.078644	O-Desmethylnaproxen	–	–
12	312.099773	–	5,7,4-Trimethoxyisoflavone	–
13	206.057909	7,8-Dimethoxycoumarin	7,8-Dimethoxycoumarin	–
14	158.084398	–	–	1,4-Diaminonaphthalene
15	342.110338	5,7,3,4-Tetramethoxyisoflavone	–	–
16	328.094688	–	–	3-Hydroxy-6,3,4-trimethoxyflavone
17	253.107594	Triamterene	–	–
18	161.105193	–	–	L-Carnitine
19	282.089209	6,7-Dimethoxyisoflavone	–	–
20	262.012209	3,4-Dimethoxy-5,7,3-trihydroxyflavone	–	–

Key: -Not detected.
BaSP = *Bersamaabysinica* Stem petroleum ether, BaSEt = *Bersama abysinica* stembark ethyl acetate, BaLP = *Bersama abysinica* leaves petroleum ether, BaLEt = *Bersama abysinica* leaves ethyl acetate, BaRP = *Bersamaabysinica*rootpetroleum ether and BaREt = *Bersamaabysinica* root ethyl acetate.

Table 7
Analysis of *B. abyssinica* Ethylacetate fractionsand their biological activities.

S/N	Compound Name	Group	Pharmacological activities	Reference
1	Mangiferin	Phenol	Anti-oxidant, antitumor, antidiabetic, Anti-inflammation, anti-viral (HIV), Enhancing immune response, treatment of liver	(Du et al., 2018; Kshirsagar et al., 2016; Matkowski et al., 2013; Telang et al., 2013)
2	Ethyl Vanillate	Phenol	Antimicrobial(histoplasmosis)	(Pendota et al., 2013)
3	3-Hydroxy-7,2,3-trimethoxyflavone	Flavonoid	Antioxidant	(Menezes et al., 2016)
4	Quercitrin	Flavonoid	Antioxidant, anti-inflammatory, antiviral Immune modulatory activities	(Xu et al., 2019; Mehrbod et al., 2019)
5	7,8-Dihydroxy-4-methylcoumarin-3-acetic acid	Coumarin	Anticancer, protect against leukemia	(Vasques et al., 2013)
6	7-Diethylamino-3-formycoumarin	Coumarin	Not known	–
7	Jasmonic acid	Acetic acid	anti-cancer, anti-inflammatory	(Jarocka-Karpowicz and Markowska, 2021)
8	Syringaldehyde	Phenol	Antihyperglycemic, antimicrobial	Musthafa et al., 2021; Huang et al., 2012
9	Isoquercitin	Flavonoid	Antiviral, antioxidant, anticardiovascular	(Deng et al., 2020; Yang et al., 2020)
10	Dipicolinic acid	Acid	Mediate the resistance of Metallo- β-lactamase bacteria	(Chen et al., 2017)
11	O-Desmethylnap Roxen	Acid	Anti-inflammatory	(Dionísio et al., 2020)
12	5,7,4Trimethoxyisoflavone	Flavonoid	Intermediate for drug synthesis	(Matsjeh et al., 2017)
13	7,8Dimethoxycoumarin	Coumarin	Antioxidant, anticancer, anti-inflammatory	(Lee et al., 2019; Lee et al., 2020)
14	1,4Diaminonaphthalene	Protein	Anti-inflamatory	(Lu et al., 2020)
15	5,7,3,4Tetramethoxyisoflavone	Flavonoid	Antimicrobial activity	(de Oliveira et al., 2017)
16	3-Hydroxy-6,3,4-trimethoxyflavone	Flavonoid	Antimicrobial	(Han et al., 2008)
17	Triamterene	Phenol	potassium-sparing diuretic, Lowering hypertension	(Tu et al., 2016; Friedman et al., 2012)
18	L-Carnitine	Protein	Treatment of kidney and insulin	(Xu et al., 2017)
19	6,7-Dimethoxyisoflavone	Flavonoid	Anticancer	(Bae et al., 2012)
20	3,4-Dimethoxy-5,7,3-trihydroxyflavone	Flavonoid	Inhibit human colon cancer	(Ren et al., 2012)

selected chronic diseases particularly cancer to validate for their-pharmacological uses.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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