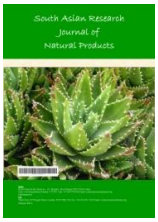

South Asian Research Journal of Natural Products

4(3): 44-58, 2021; Article no.SARJNP.67716

Characterization of Phenolic Compounds from Leaf Extract of *Bidens Pilosa* Linn. Var. Radiata

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MLN, FK and GKM designed the study. Author MLN collected the samples and performed laboratory analyses. Authors FK and GKM supervised the work and provided technical support. Authors MLN, FK and GKM performed a literature search and analyzed the collected data. Author MLN wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. V. Y. Atsu Barku, University of Cape Coast, Ghana.
(2) Dr. Prasong Srihanam, Mahasarakham University, Thailand.
Reviewers:
(1) Jaiber Humberto Rodriguez Llanos, São Paulo University, Brazil.
(2) Rehab Fikry Taher, National Research Centre, Egypt.
(3) Luana Beatriz dos Santos Nascimento, University of Florence, Italy.
Complete Peer review History: http://www.sdiarticle4.com/review-history/67716

Original Research Article

Received 05 March 2021
Accepted 11 May 2021
Published 22 May 2021

ABSTRACT

Aim: To extract and characterize phenolic compounds in extract of *Bidens pilosa* Linn. Var. Radiata leaves.
Study Design: Experimental.
Place and Duration of Study: The study was carried out in the Chemistry Laboratory, Department of chemistry and biochemistry, School of Sciences and Aerospace Studies, Moi University (Kenya) between January 2020 and March 2021.
Methodology: Extraction of *Bidens pilosa* leaves was done using the Soxhlet method, with dichloromethane/ methanol (1:1) as the solvents used for extraction. The analysis of phenolic

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compounds present in the *B. pilosa* extract employed a combination of spectroscopic and chromatographic techniques including Fourier Transform Infra-Red spectroscopy and gas chromatography-tandem mass spectrometry (GC-MS/MS).
Results: Functional groups identified by FT-IR ranged from 995-665cm^{-1} related to C=C bending for alkenes (flavonoids); 1470–1150 cm^{-1} could be due to C-H stretching vibration for phenols; 1250-1020 cm^{-1} related to C-N stretching vibration for amines; 1275-1200 cm^{-1} due to C-O stretching vibration for vinyl ether. GC-MS/MS analysis afforded the identification and quantification of phenolic compounds, with 2,4-Di-tert-butyl phenol, 2,6-Bis (tert-butyl)phenol, 3,5-ditert-butyl phenol, and 2,5-bis(1,1-Dimethylethyl)phenol being the major components of the leaf extract.
Conclusion: The data derived from the characterization of *B. Pilosa* is a clear indication that the plant leaves are rich in bioactive compounds, verifying its claimed traditional use for treating various diseases This study recommends further studies on the identified bioactive compounds. It also recommends isolation of the identified compounds and testing for their pharmacological activities such as antibacterial, antifungal, and antiulcer.

Keywords: Bidens pilosa; characterization; natural products; phenolic compounds.

1. INTRODUCTION

Natural products play an important role in drug development programs in the pharmaceutical industry [1]. However, the World Health Organization (WHO) also has recognized the importance of traditional medicine and has been active in creating strategies, guidelines, and standard for botanical medicines i.e., Soy, Echinacea and Cranberry [2]. Therefore, there is a need to find alternative therapies chemical medicines, which have negligible adverse impacts on the patient, for the cure of diseases. Chemical medicines have been reported to possess side effects including; headache, diarrhea and skin rash or dermatitis. This can be made possible by the use of traditional medicines and herbal remedies, given their low incidences of side effects.

Medicinal plants contain numerous biologically active compounds which help improve life and in the treatment of disease: These include compounds such as carbohydrates, proteins, enzymes, fats, oils, terpenoids, flavonoids, sterols, simple phenolic compounds, and many more [3]. The presence of various life-sustaining constituents in plants has made scientists investigate many plants for their uses in treating certain infective diseases and management of diseases as well [4]. Medicinal plants are cheaper and more accessible to most of the population in the world. Thus, there is a need to encourage the use of medicinal plants as potential sources of new drugs. Indeed, there has been a heightened interest in herbal remedies in several parts of the world [5].

In recent years, secondary plant metabolites have been investigated as a source of medicinal agents [6]. Herbal medicines have great importance in maintaining the health of every person [7]. Plants have several pharmacological roles i.e., the phytochemicals with adequate antibacterial activity are used for the treatment of bacterial infections Plants have also been used as antioxidant, antiviral, anticancer, antimicrobial, antifungal, and antiparasitic agents [8]. Plants have free radical scavenging molecules, including flavonoids, phenols, anthocyanins, and vitamins, which show antioxidant-like antioxidant-like activity [4)]. Demands of Herbal medicines are increasing in both developed and developing countries due to the growing recognition of natural plants having lesser side effects, are easily available in the immediate environment, and are of low cost [9].

Bidens pilosa is an important traditional medicine in South Africa that has been used by various cultural groups for a wide range of treatments [10]. The whole plant including the root, stem, leaf, and flower is used in various folk medicines and as a popular herbal tea ingredient [11]. For instance, a leaf decoction is used to treat headaches, ear infections, kidney problems, and flatulence [12]. It has been proven to be effective for curing infectious hepatitis [13], and diabetes mellitus [14,15]. The leaf extract is also used to cure malaria [16], stomach and mouth ulcers [17] , diarrhea [18], hangover [19], and the whole plant is also used as a poison antidote [20]. In sub-Sahara Africa, both fresh and dry shoots, as well as young leaves of *B. pilosa,* are sometimes used as human food, although they are believed to contribute to the etiology of human esophageal cancer [21]. Despite the medicinal applications of *Bidens pilosa*, by traditional healers, there is no scientific basis for using *Bidens pilosa* as a medicinal plant. Hence, this

study mainly aims at the extraction and characterization of medicinal phenolic compounds from *Bidens pilosa*.

2. MATERIALS AND METHODS

2.1 Plant Collection and Preparation

Fresh *Bidens pilosa* leaves were harvested from naturally growing *B. pilosa* plants in Moi University farm, Eldoret, Kenya ($0^0$17'22.2"N $35^0$17'50.5"E). The samples were identified by Mr. Ali Rono a botanist at the Department of Biological Sciences, Moi University. The samples were washed under running tap water to remove dust particles, air-dried at room temperature under the shade for two weeks. The dry leaves were ground into powder using an electric grinder. Measured 10 grams of *B. pilosa* leaves powder was Soxhlet extracted with 200 ml of hexane for 8 hours (Mogana et al, 2011), followed by extraction with dichloromethane: methanol (1:1). The extract was decanted and then filtered using Whatman No. 1 Filter Paper. The extract was concentrated on a rotary evaporator at 60^0C to remove volatile solvent from the nonvolatile solvent of interest, followed by drying under vacuum.

2.2 Identification and Characterization

2.2.1 FT-IR analysis

Potassium bromide (KBr) based pellets was prepared by establishing a pressure of 10 kg/cm^2 for about 30 seconds. A pure KBr tablet was used as a blank for background subtraction. Loopful of the extract was placed on the KBr disk and read at wavenumbers ranging between 4000-400cm^{-1}. Polystyrene infrared was used as the standard.

2.2.2 GC-MS/MS analysis

The 0.2g dichloromethane: methanol (1:1) sample was diluted in dichloromethane: methanol (1:1) mixture of solvents, then filtered through 0.22 µm PTFE syringe filters and transferred to 2 ml vials for GC-MS/MS analysis. A Shimadzu QP 2010-SE GC-MS coupled to an autosampler was used for the analysis. Ultrapure Helium was used as the carrier gas at a flow rate of 1ml / minute. A BPX5 nonpolar column, 30m; 0.25 mm ID; 0.25 µm film thickness, was used for separation. The GC temperature program was: 50 ^{0}C (1 minute); 10 ^{0}C/min to 180 ^{0}C (1 min); 3 ^{0}C/min to 250 ^{0}C (22 min). The total run time was 60 minutes. Only 1 µL of the sample was injected at 200 ^{0}C in split mode (10:1). The interface temperature was 250 ^{0}C and the EI ion source temperature was 200 ^{0}C. Mass analysis was done in full scan mode, 50-700 nm (to accommodate high molecular-weight compounds), with an initial solvent-delay time of 3 minutes. Raw mass spectra was matched against the NIST 2014 library of mass spectra for possible identification of compounds.

3. RESULTS AND DISCUSSION

3.1 FT-IR Spectra

The extract gave bands with noticeable peaks that are characteristic of phenolic compound structures with ranges from 995-665cm^{-1} related to C=C bending for alkenes (flavonoids); 1470–1150 cm^{-1} could be due to C-H stretching vibration for phenols [22]; 1250-1020 cm^{-1} related to C-N stretching vibration for amines; 1275-1200 cm^{-1} due to C-O stretching vibration for vinyl ether [23]; 1440 - 1395 cm^{-1} related to O-H bending for alcohol and carboxylic acid [23]; 1650 - 1580cm^{-1} related to N-H bending for amines which shows the presence of alkaloids [24]; 3500-3200cm^{-1} attributed to O-H stretching vibration for carboxylic acid and alcohols; 1070-1030cm^{-1} could be due to S=O stretching vibration [25] and around 3700cm^{-1} which can be attributed to the polysaccharides and/or lignins as reported by [26] (Figure and Table 1). The identified functional groups are similar to those in a study on characterization and quantification of phenolic compounds from the leaf of *Agarista salicifolia* [27]. Hence, the functional groups identified by the FT-IR analysis are found in phenolic compounds.

3.2 GC-MS/MS Results

The GC-MS results are consistent with earlier indications - from FTIR analysis - that phenolic compounds are the major constituents in *B. pilosa* leaf extracts [28]. These phenolic compounds have been associated with bioactive activities including; 2,4-ditert-butylphenol, 4,6-di-tert-Butyl-m-cresol and 2,2'-Methylenebis(6-tert-butyl-4-ethylphenol) as antibacterial [29], 3,5-ditert-butylphenol and 2,5-bis(1,1-Dimethylethyl)phenol as antioxidant and antimicrobial [30,31], 2,6-Bis(tert-butyl)phenol [8,32] and 2,2'-methanediylbis(6-tert-butyl-4,5-dimethylphenol) and 2-tert-Butyl-6-(3-tert-butyl-2-methoxy-5-methylbenzyl)-4-methylphenol which has not been reported (Figure and Table 2).

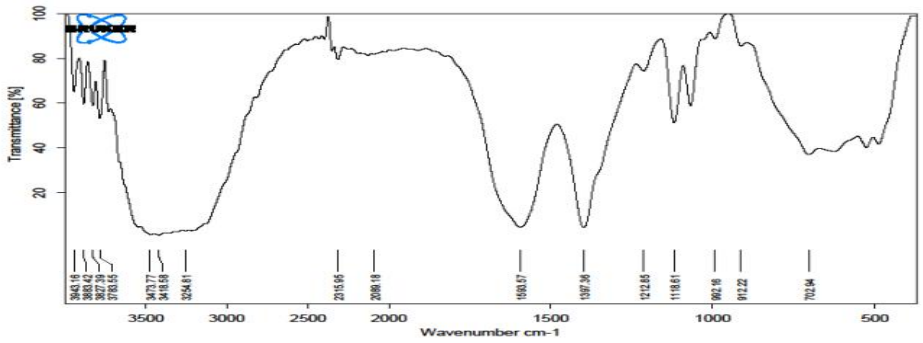


Fig. 1. FTIR spectra for Dichloromethane: Methanol leaf extract of *Bidens pilosa*

Table 1. FTIR peak values of extract

S. No.	Peak value	Possible bond	Functional group
1	3943.16		Unknown
2	3883.42		Unknown
3	3827.39		Unknown
4	3783.55		Unknown
5	3473.77-3418.58	O-H	Alcohol
6	3254.81	O-H	Carboxylic acid
7	2315.95		Unknown
8	2089.18		Unknown
9	1593.57	N-H	Amines
10	1397.36	O-H	Carboxylic acid
11	1212.85	C=O	Acids(acyl)
12	1118.61	C-O	Ethers
13	992.16	C=C	Alkenes
14	912.22	C=C	Alkenes
15	702.94	C=C	Alkenes

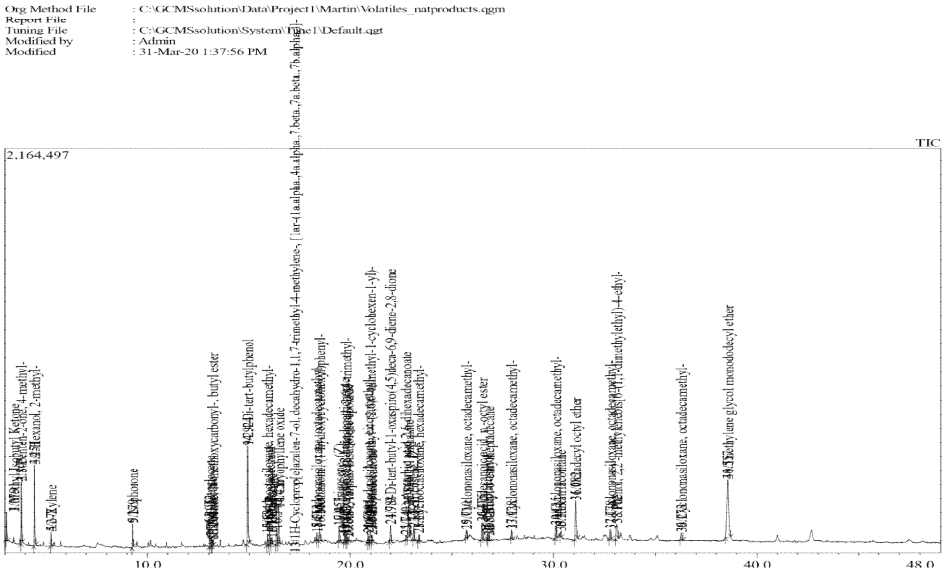
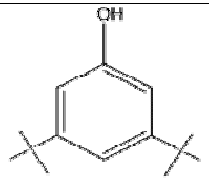
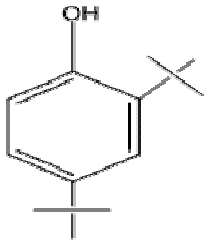
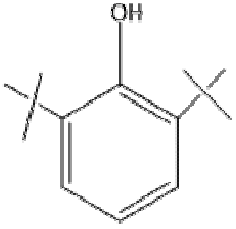
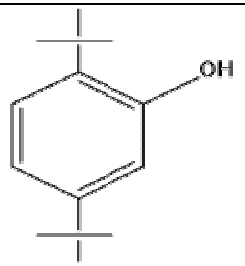
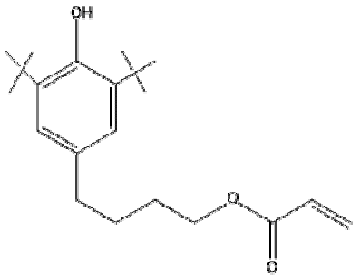
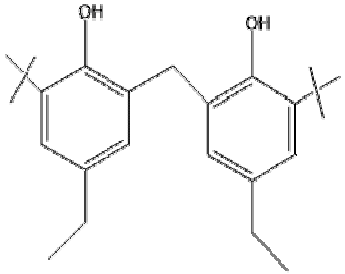
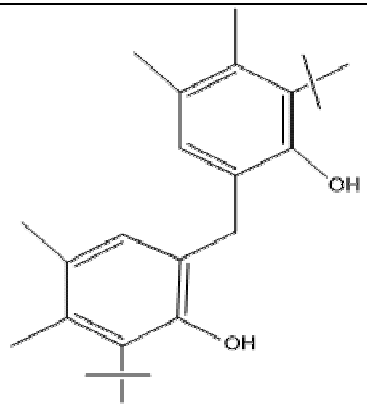
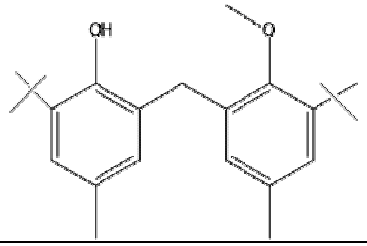


Fig. 2. GC-MS/MS chromatogram of leaf extract

Table 2. Phenolic compounds identified in the leaf extract of *B. pilosa* by GC-MS

Peak no.	Retention time	Molecular weight	Formula	Structure	IUPAC name	Class of the compound	Activity	Reference
9	14.923	206	$C_{14}H_{22}O$		3,5-ditert-butylphenol	Phenols	Antioxidant and pro-oxidant	[31,33]
9	14.923	206	$C_{14}H_{22}O$		2,4-Di-tert-butylphenol	Phenols	Antibacterial, Antioxidant	[34,35]
9	14.923	206	$C_{14}H_{22}O$		2,6-Bis(tert-butyl)phenol	Phenols	Antifungal	[36]

Peak no.	Retention time	Molecular weight	Formula	Structure	IUPAC name	Class of the compound	Activity	Reference
9	14.923	206	$C_{14}H_{22}O$		2,5-bis(1,1-Dimethylethyl)phenol	Phenols	Antioxidant	[30]
24	21.988	332	$C_{21}H_{32}O_3$		4-(3,5-Di-tert-butyl-4-hydroxyphenyl)butyl acrylate	Phenols	Antimicrobial	[37]
38	33.100	368	$C_{25}H_{36}O_2$		2,2'-Methylenebis(6-tert-butyl-4-ethylphenol)	Phenols	Antibacterial	[38]

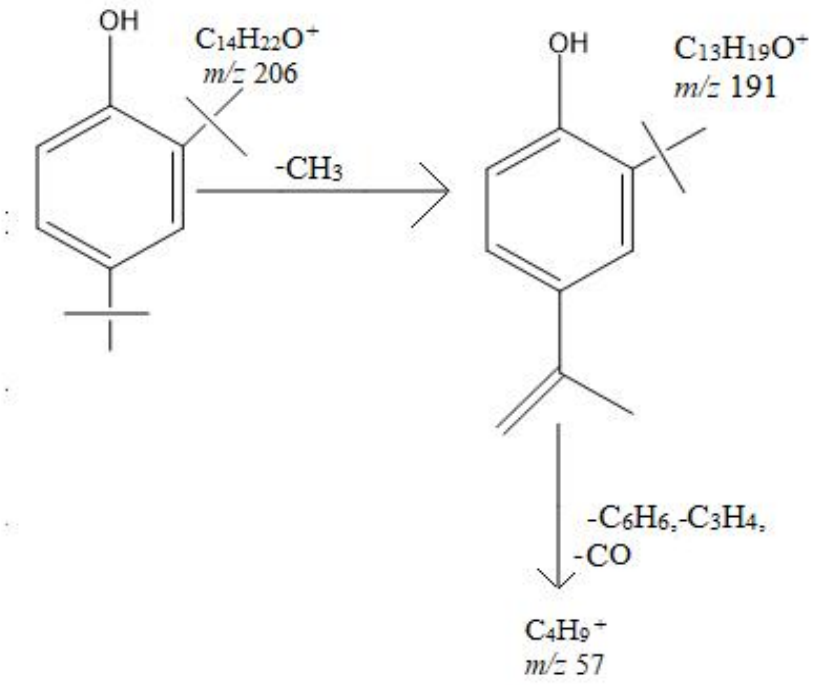
Peak no.	Retention time	Molecular weight	Formula	Structure	IUPAC name	Class of the compound	Activity	Reference
38	33.100	368	$C_{25}H_{36}O_2$		2,2'-methanediylbis(6-tert-butyl-4,5-dimethylphenol)	Phenols	Not reports	
38	33.100	354	$C_{24}H_{34}O_2$		2-tert-Butyl-6-(3-tert-butyl-2-methoxy-5-methylbenzyl)-4-methylphenol	Phenols	Not reports	

40 peak numbers were identified, the phenolic compounds identified were; 3,5-ditert-butylphenol (m/z 57, 191, 206) entry 51536, 2,4-ditert-butylphenol (m/z 41,57, 191, 206) entry 51535, 2,6-Bis(tert-butyl)phenol (m/z 41, 57, 131, 191, 206) entry 51537, 2,5-bis(1,1-Dimethylethyl)phenol (m/z 57, 191, 192, 206) entry 51541, 4-(3,5-Di-tert-butyl-4-hydroxyphenyl)butyl acrylate (m/z 41, 55, 57, 147, 189, 203, 219, 332) entry 160098, 2,2'-Methylenebis(6-tert-butyl-4-ethylphenol) (m/z 57, 91, 119, 135, 141, 163, 175, 191, 368) entry 187878, 2,2'-methanediylbis(6-tert-butyl-4,5-dimethylphenol) (m/z 57, 135, 163, 178, 191, 312, 368) entry 187879 and 2-tert-Butyl-6-(3-tert-butyl-2-methoxy-5-methylbenzyl)-4-methylphenol (m/z 41, 57, 119, 163, 178, 354) entry 178091 (Table 3)

Table 3. Precursor ions and main productions obtained by GCMS/MS for the phenolic compounds

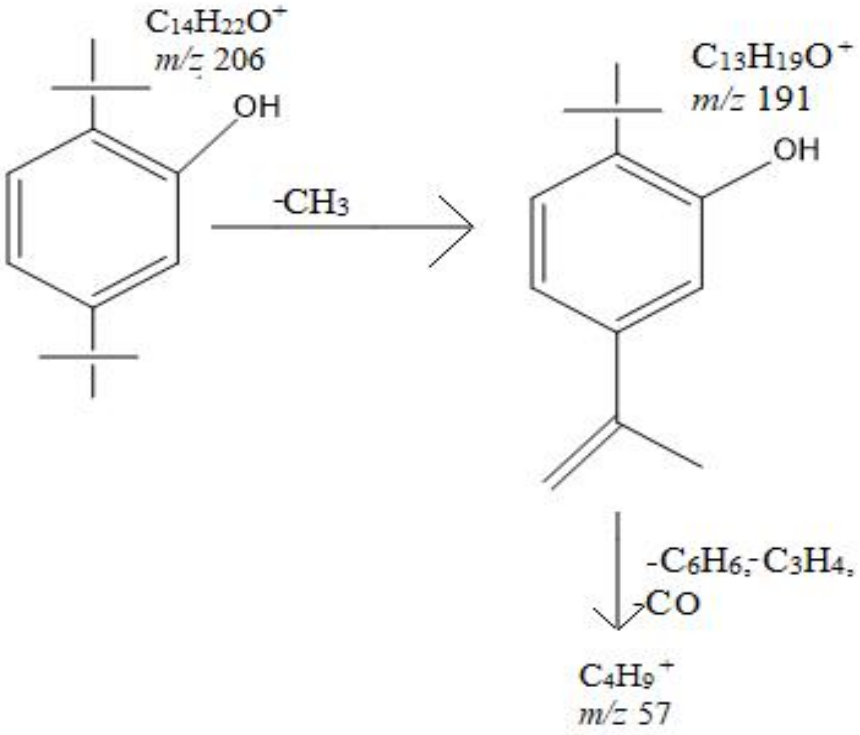
Name	Formula	$M^{+\cdot}$	Fragmentation ions
2,4-ditert-butylphenol	$C_{14}H_{22}O^{+\cdot}$ *m/z* 206	206	191$[M^{+\cdot}-(\cdot CH_3)]^+$ 57$[M^{+\cdot}-(\cdot C_5H_5)-(\cdot C_3H_5)-(\cdot CH_3)-CO]^+$
2,6-Bis(tert-butyl)phenol	$C_{14}H_{22}O^{+\cdot}$ *m/z* 206	206	191$[M^{+\cdot}-(\cdot CH_3)]^+$ 57$[M^{+\cdot}-(\cdot C_5H_5)-(\cdot C_3H_5)-(\cdot CH_3)-CO]^+$
2,5-bis(1,1-Dimethylethyl)phenol	$C_{14}H_{22}O^{+\cdot}$ *m/z* 206	206	191$[M^{+\cdot}-(\cdot CH_3)]^+$ 57$[M^{+\cdot}-(\cdot C_5H_5)-(\cdot C_3H_5)-(\cdot CH_3)-CO]^+$
3,5-ditert-butylphenol	$C_{14}H_{22}O^{+\cdot}$ *m/z* 206	206	191$[M^{+\cdot}-(\cdot CH_3)]^+$ 57$[M^{+\cdot}-(\cdot C_5H_5)-(\cdot C_3H_5)-(\cdot CH_3)-CO]^+$
2,2'-Methylenebis(6-tert-butyl-4-ethylphenol)	$C_{25}H_{36}O_2^{+\cdot}$ *m/z* 368	368	191$[M^{+\cdot}-(\cdot C_5H_7)-(\cdot C_3H_5)_2-(\cdot CO)]^+$ 175$[M^{+\cdot}-(\cdot C_5H_7)-(\cdot C_3H_5)_2-(\cdot CH_3)-(\cdot HCO)]^+$ 163$[M^{+\cdot}-(\cdot C_6H_7)-(\cdot C_3H_5)_2-(\cdot CH_3)-(\cdot HCO)]^+$ 57$[M^{+\cdot}-(\cdot C_6H_6)-(\cdot C_3H_5)_2-(\cdot CH_3)-(\cdot HCO)_2]^+$
2,2'-methanediylbis(6-tert-butyl-4,5-dimethylphenol)	$C_{25}H_{36}O_2^{+\cdot}$ *m/z* 368	368	312$[M^{+\cdot}-(\cdot CH_3CCH_2)-(\cdot CH_3)]^+$ 191$[M^{+\cdot}-(\cdot C_5H_7)-(\cdot C_3H_5)_2-(\cdot CO)]^+$ 178$[M^{+\cdot}-(\cdot C_6H_7)-(\cdot C_3H_5)_2-(\cdot HCO)]^+$ 163$[M^{+\cdot}-(\cdot C_6H_7)-(\cdot C_3H_5)_2-(\cdot CH_3)-(\cdot HCO)]^+$ 57$[M^{+\cdot}-(\cdot C_6H_6)-(\cdot C_3H_5)_2-(\cdot CH_3)-(\cdot HCO)_2]^+$
2-tert-Butyl-6-(3-tert-butyl-2-methoxy-5-methylbenzyl)-4-methylphenol	$C_{24}H_{34}O_2^{+\cdot}$ *m/z* 354	354	178$[M^{+\cdot}-(\cdot C_5H_5)-(\cdot C_3H_5)_2-(\cdot HCO)]^+$ 163$[M^{+\cdot}-(\cdot C_5H_5)-(\cdot C_3H_5)_2-(\cdot CH_3)-(\cdot HCO)]^+$ 57$[M^{+\cdot}-(\cdot C_6H_6)_2-(\cdot C_3H_5)_2-(\cdot HCO)_2]^+$
4-(3,5-Di-tert-butyl-4-hydroxyphenyl)butyl acrylate	$C_{21}H_{32}O_3^{+\cdot}$ *m/z* 332	332	219 $[M^{+\cdot}-(\cdot CH_3CH_3CCH_2)-HCO-CO]^+$ 203 $[M^{+\cdot}-(\cdot CH_3CH_3CCH_2)-(\cdot CH_3)-(\cdot HCO)_2]^+$ 189 $[M^{+\cdot}-(\cdot C_2H_5)-(\cdot CH_3CH_3CCH_2)-(\cdot HCO)_2]^+$

2,4-Di-tert-butylphenol



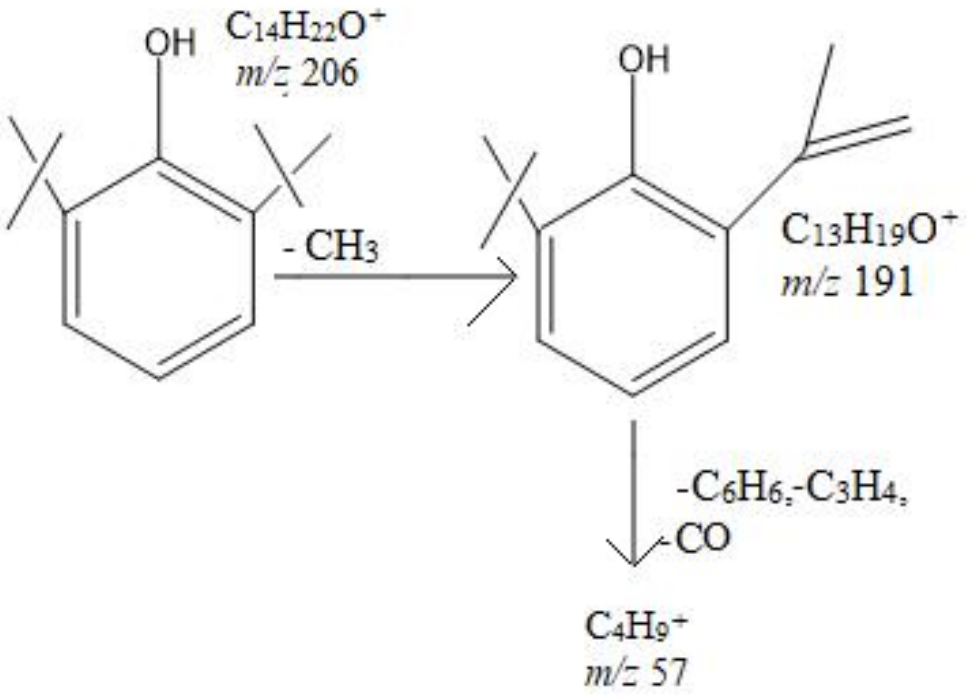
i)

2,5-bis(1,1-Dimethylethyl)phenol



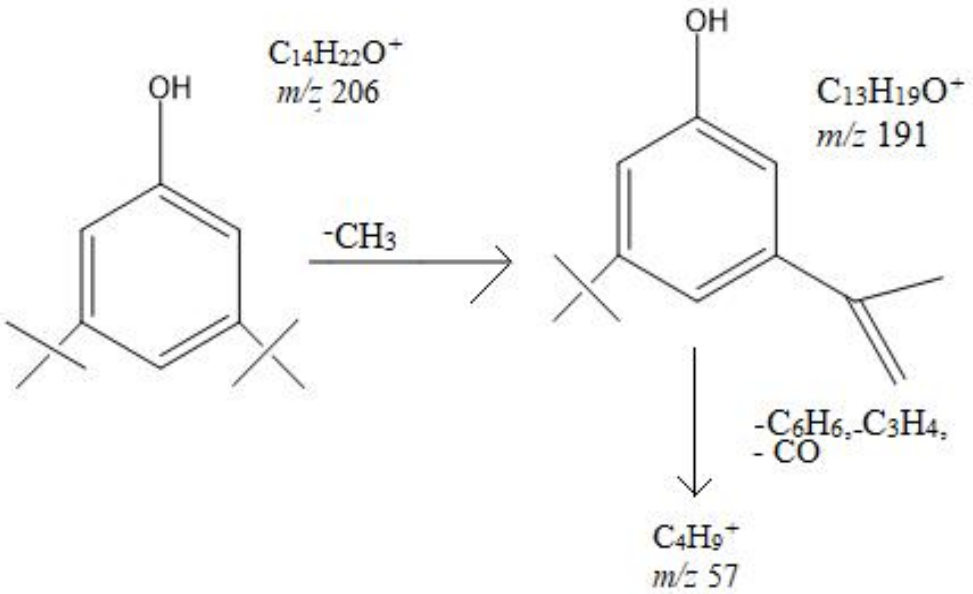
ii)

2,6-Bis(tert-butyl)phenol



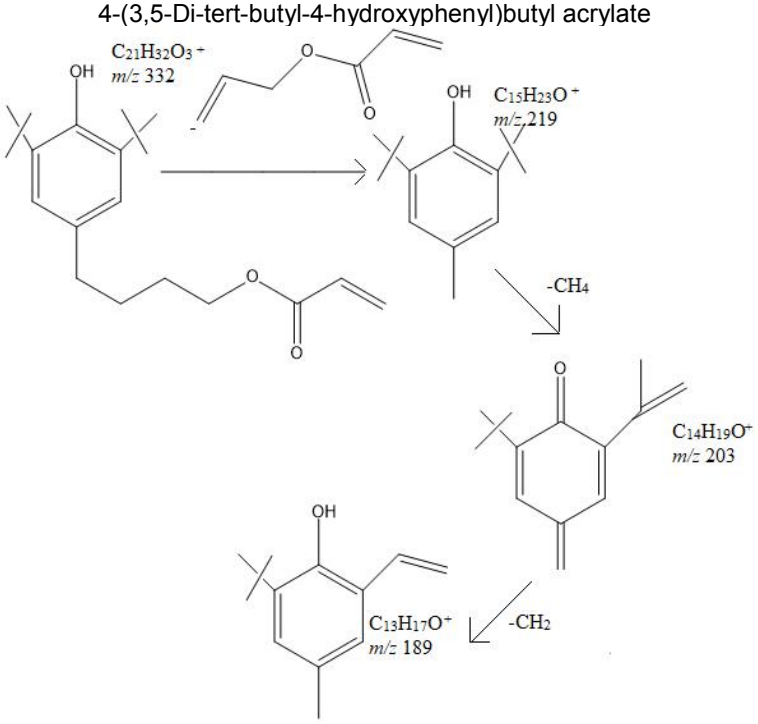
iii)

3,5-ditert-butylphenol



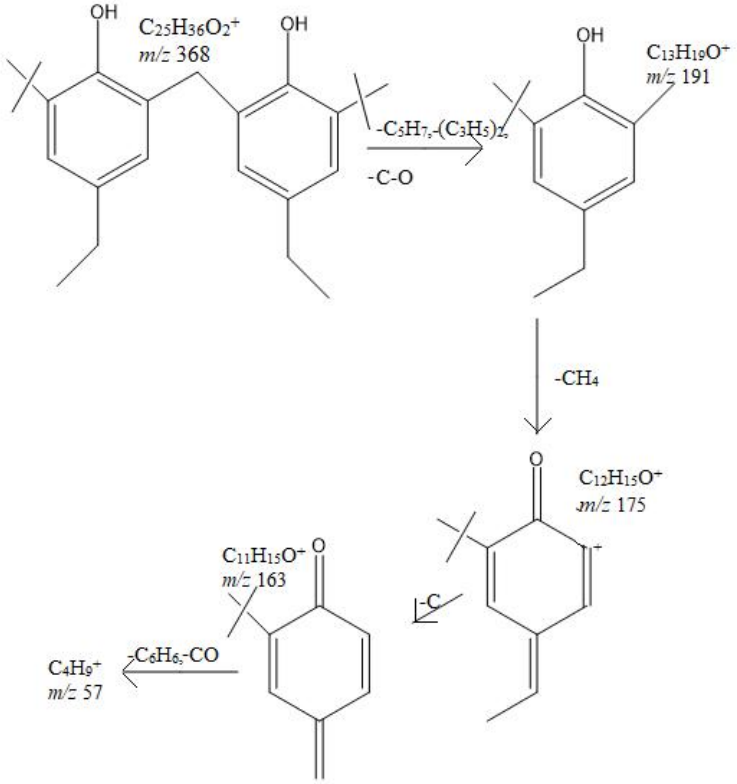
iv)

4-(3,5-Di-tert-butyl-4-hydroxyphenyl)butyl acrylate



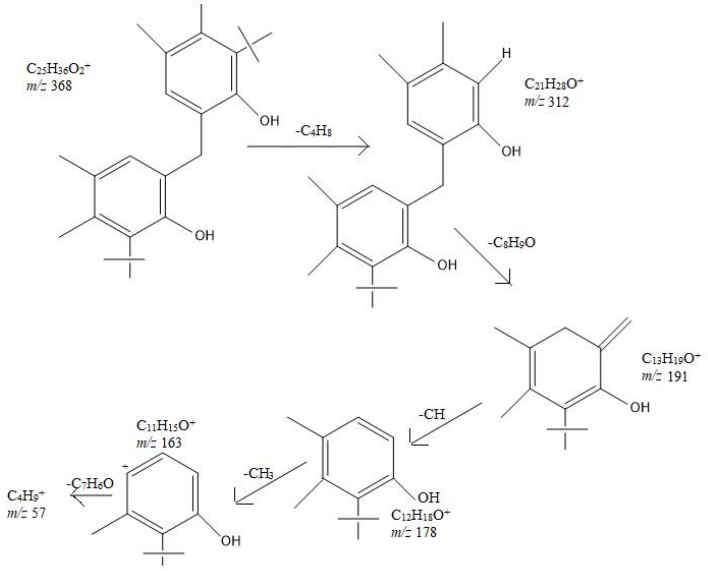
v)

2,2'-Methylenebis(6-tert-butyl-4-ethylphenol)



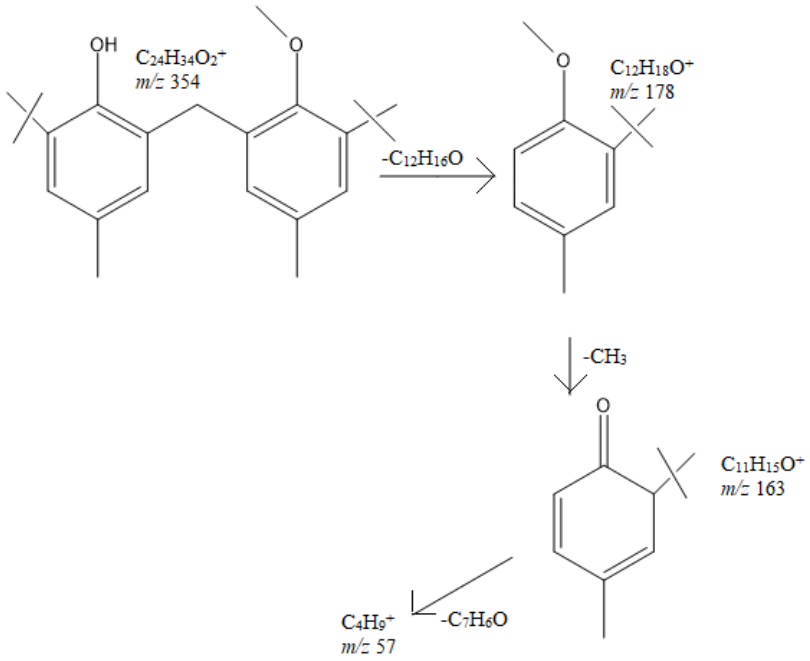
vi)

2,2'-methanediylbis(6-tert-butyl-4,5-dimethylphenol)



vii)

2-tert-Butyl-6-(3-tert-butyl-2-methoxy-5-methylbenzyl)-4-methylphenol



viii)

Fig. 3. (i-viii). Fragmentation patterns of the phenolic compounds of *Bidens pilosa leave* studied by GC/MS-MS

4. CONCLUSION

Previous studies have shown that *B. pilosa* contains compounds like flavonoids, phenylacetylenes, alkaloids, sterols, triterpenoids and tannins [39] that are responsible for different ailments. Other compounds such as phytosterols (β-sitosterol), triterpenes (friedelin and friedelan-

3β-ol) and caffeic acid(s) are also reported from *B. pilosa*. Its roots suppressed the growth of both gram-positive and gram-negative bacteria as reported in a study done by [40].

This study reveals the phenolic compounds ; 2,4-ditert-butylphenol, 4,6-di-tert-butyl-m-cresol and 2,2'-methylenebis(6-tert-butyl-4-ethylphenol) as antibacterial , 3,5-ditert-butylphenol and 2,5-bis(1,1-dimethylethyl)phenol as antioxidant and antimicrobial, 2,6-bis(tert-butyl)phenol and 2,2'-methanediylbis(6-tert-butyl-4,5-dimethylphenol) and 2-tert-butyl-6-(3-tert-butyl-2-methoxy-5-methylbenzyl)-4-methylphenol that were characterized by GC-MS/MS which have also been associated with ailment treatments. The study recommends further studies on the isolation of these compounds and tested against the treatments associated.

ACKNOWLEDGEMENTS

The authors are grateful to the World Bank scholarship awarded to Merab Lilian Ndiege through the Africa Center of Excellence II in Phytochemicals, Textiles and Renewable Energy (ACE II PTRE) at Moi University, Kenya which led to this communication.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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http://www.sdiarticle4.com/review-history/67716