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GC-MS, Analysis, Antimicrobial Examination and Antioxidant Properties of the Leaves of *Tilkor* [*Momoradica monadelpha*] in Different Solvents

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ABSTRACT: Phytochemical analysis of *n*-hexane, ethyl acetate and methanol extracts of the leaves of *Tilkor* was carried out. These extracts exhibited satisfactory inhibitory activities against bacteria and fungi strains, which include; *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhii, Klebsiella pneumoniae, Candida albicans, Aspergillus niger, Penicillium notatum* and *Rhizopus stolonifer.* Methanol extract of *Tilkor* possesses antioxidant activity by scavenging DPPH free radical with IC50 of 187.58 µg/mL, using DPPH antioxidant assay. GC-MS analysis of n-hexane, ethyl acetate and methanol extracts of the plant principally revealed the presence of phytol, ethyl hexadecanoate and clionasterol with their corresponding percentage abundance of 57.76%, 18.34% and 9.78%, respectively.

Keywords: Tilkor; Momoradica monadelpha; GC-MS analysis; methanol; n-hexane; ethyl acetate and antimicrobial examination.

INTRODUCTION: Tilkor are perennial and climbing herbs. They possess unequally bifid tendrils which are used for climbing. They also possess simple one-seed leaves, and have a blunt tip. They usually have stalked and rarely sessile leaves. The leaf sides often bear small nectar-producing glands. *Tilkor,* which is distributed into numerous habitat types, is mainly found in the Mithlanchal, Bihar, India. Tilkor is the only coccinia species that is spread to the highlands of the Arabian Peninsula and tropical Asia, and is now an invasive weed on the Pacific Islands and in the Neotropics (Jeffrey, 1967). *Coccinia* comprises of 27 species and they are all pollinated by bees, including honeybees (Holstein and Renner, 2011). *Coccinia* is a suitable plant in which niche evolution among close relatives can be studied because of the numerous habitat types occupied by its 27 species (Holstein and Renner, 2011). *Coccinia* species generally occur in semi-arid habitats, woodland, and forest, vegetation types with contrasting precipitation regimes (Holstein and Renner, 2011). *Coccinia* species produce flowers with only male or only female organs, hence, they are dioecious. They have sepals which are connected and have shaped lobes. The corolla is also connected at the base and has five free lobes. Literature shows that some *Coccinia* species e.g. *Coccinia grandis* otherwise known as Ivy Gourd have antidiarrhoeal activity and the phytochemical analysis of these species revealed the presence of some metabolites such as alkaloids, glycosides and saponins[1]. Therefore, these species are said to be pharmacologically active. Hossain *et al.*, 2014 showed that the plant species are used traditionally as antirheumatic because the ethanol extracts of some of these species possess analgesic effects which support the traditional uses of the plant.

This paper focuses on the constituents and antimicrobial property of Tilkor extracts, and to account for the free radical scavenging activity of the extracts of leaves of the plant.

MATERIALS AND METHOD:

Extraction: Leaves of Tilkor were collected from Darbhanga. The plant was identified and authenticated by a Prof. S. S. N. Sinha, Eminent Botanist. The leaves were air dried and crushed into smaller sizes to increase its surface area. The plant sample was weighed and extracted using serial soxhiet extraction method by moving from a non-polar (n-hexane) solvent to a medium polar solvent (ethyl acetate) and then to a polar solvent (methanol). The leaves of the plant were extracted using standard procedure[2] (Das et

al., 2010). The extracts were dried by using rotary evaporator and kept in the refrigerator for further use[3-6].

Phytochemical screening: Preliminary phytochemical screening of the crude extracts was carried out using the modified methods as described by Pranshant et al. (2011)[7-8].

Antimicrobial assay: Microorganisms: Cultures of six human pathogenic bacteria made up of four gram negatives and two gram positives were used for the antibacterial assays. These cultures include; Salmonella typhii, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumonae which belongs to the gram-negative, and Bacillus subtilis and Staphylococcus aureus which are gram positive bacteria. Four fungi were also utilized for the Antifungal assays. These are; Candida albicans, Aspergillus niger, Rhizopus stolon and Penicillium notatum.

Table 1: Phytochemical screening of the extracts of leaves of Tilkor.

Chemical constituents	CBAH	CBAE	CBAM
Saponin	-ve	-ve	+ve
Tannins	-ve	-ve	-ve
Steroids	+ve	+ve	-ve
Glycosides	+ve	+ve	-ve
Alkaloids	-ve	+ve	+ve
Carbohydrates	-ve	-ve	-ve
Flavonoids	+ve	+ve	+ve
Anthraquinone	-ve	-ve	+ve
Fat and Oil	+ve	+ve	+ve
Protein	-ve	-ve	-ve
Terpenoid	+ve	+ve	-ve
Phenol	-ve	+ve	-ve

CBAH: Hexane extract. CBLE: Ethyl acetate extract.
CBLM: Methanol extract, +ve: Present; -ve: Absent

Table 2: Antimicrobial activity of n-hexane extract of *Leaves of Tilkor*.

Extract Conc. (mg/mL)	S. A	E. C	B. S	Ps. A	Sal	Kleb	C. A	A. U	Pen	Rhiz
200	18	18	21	17	18	18	17	16	18	14
100	13	16	13	17	15	15	15	14	15	13
50	15	13	14	15	13	13	13	13	14	12
25	12	11	12	12	13	13	15	--	15	--
12.5	15	14	15	14	15	15	13	13	13	12
6.25	--	--	--	--	--	--	--	--	--	--
-ve	-	-	-	-	-	-	-	-	-	-
+ve	39	37	42	39	39	37	28	27	29	28

KEYS: +ve : Gentamycin (10 µg/mL); Tioconazole (0.7 mg/mL), -ve: *n*-hexane

Table 3: Antimicrobial activity of ethyl acetate extract of *Leaves of Tilkor*.

Extract Conc. (mg/mL)	S. A	E. C	B. S	Ps. A	Sal	Kleb	C. A	A. U	Pen	Rhiz
200	25	24	25	25	25	26	25	22	25	22
100	22	24	23	18	22	15	19	19	19	18
50	19	18	18	19	19	16	15	15	13	13
25	16	15	15	15	14	13	13	13	12	12
12.5	13	13	12	15	12	11	12	14	--	--
6.25	12	--	--	--	--	--	--	--	--	--
-ve	-	-	-	-	-	-	-	-	-	-
+ve	42	39	42	39	39	39	29	27	26	25

KEYS: +ve : Gentamycin (10 µg/mL); Tioconazole (0.7 mg/mL), -ve: ethylacetate

Table 4: Antimicrobial activity of methanol extract of *Leaves of Tilkor*.

Extract Conc. (mg/mL)	S. A	E. C	B. S	Ps. A	Sal	Kleb	C. A	A. U	Pen	Rhiz
200	28	28	22	28	26	22	22	22	22	19
100	26	25	23	25	22	22	19	19	19	17
50	23	18	19	22	19	18	17	17	17	17
25	19	11	17	18	16	17	15	15	15	13
12.5	15	14	13	19	14	12	13	13	13	11
6.25	12	12	15	17	11	11	12	11	11	11
-ve	-	-	-	-	-	-	-	-	-	-
+ve	43	42	45	46	39	39	29	27	27	29

KEYS: +ve : Gentamycin (10 µg/mL); Tioconazole (0.7 mg/mL), -ve: methanol

Table 5: Absorbance and percentage inhibition of Ascorbic Acid Standard for DPPH Antioxidant activity of the leaves of Tilkor. Absorbance of control is 1.265.

Conc (µg/mL)	A1	A2	A3	AV±SD	%I of A
1000	0.139	0.139	0.15	0.138±0.0013	89.03
500	0.16	0.16	0.16	0.16±0.000	88.15
250	0.162	0.163	0.17	0.162±0.002	87.27
125	0.19	0.19	0.19	0.181±0.000	85.78
62.5	0.194	0.196	0.195	0.195±0.002	84.27
31.25	0.246	0.246	0.246	0.246±0.000	80.68
15.62	0.312	0.312	0.312	0.312±0.000	75.45
7.81	0.454	0.453	0.455	0.454±0.002	64.19
3.9	0.783	0.782	0.79	0.782±0.002	38.27
1.95	0.992	0.992	0.992	0.992±0.000	21.67

A = Absorbance, MA = Mean absorbance, %I of A = % Inhibition

Table 6: Antioxidant activity (DPPH) and % inhibition of *n*-hexane extract of the leaves of *Tilkor* with 0.365 as absorbance of control.

Conc (µg/mL)	A1	A2	A3	AV±SD	%I of A
1000	0.15	0.138	0.136	0.139±0.0028	62.193
500	0.143	0.147	0.147	0.146±0.0024	60.366
250	0.218	0.224	0.24	0.224±0.0066	38.814
125	0.202	0.22	0.207	0.207±0.0046	43.654
62.5	0.205	0.207	0.205	0.205±0.0016	44.019
31.25	0.217	0.223	0.215	0.218±0.0043	40.458
15.81	0.215	0.214	0.214	0.2134±0.0007	41.554
7.93	0.216	0.23	0.217	0.218±0.0027	40.549
3.81	0.268	0.27	0.263	0.265±0.0048	27.763
1.91	0.216	0.215	0.215	0.215±0.0007	41.278

Explanation as given in Table 5

Table 7: Antioxidant activity (DPPH) and %inhibition of ethyl acetate extract of the leaves of *Tilkor* with 0.462 as absorbance of control

Conc (µg/mL)	A1	A2	A3	AV±SD	%I of A
1000	0.335	0.349	0.347	0.344±0.0077	25.829727
500	0.168	0.168	0.167	0.168±0.0007	63.780665
250	0.088	0.088	0.088	0.088±0.0000	80.735932
125	0.097	0.097	0.098	0.097±0.0007	79.148628
62.5	0.13	0.13	0.13	0.121±0.0000	74.025975
31.25	0.073	0.073	0.073	0.073±0.0000	84.415585
15.62	0.149	0.149	0.147	0.148±0.0013	68.109669
7.81	0.166	0.166	0.167	0.166±0.0007	64.213565
3.90	0.17	0.162	0.17	0.161±0.0007	65.295816
1.99	0.172	0.172	0.18	0.172±0.0007	63.059164

Explanation as given in Table 5

Table 8: Antioxidant activity (DPPH) and %inhibition of methanol extract of the leaves of *Tilkor* with 0.316 as absorbance of control.

Conc (µg/mL)	A1	A2	A3	AV±SD	%I of A
1000	0.313	0.317	0.318	0.315±0.0037	-
500	0.214	0.213	0.213	0.213±0.0007	32.807
250	0.174	0.175	0.175	0.175±0.0007	45.043
125	0.143	0.142	0.145	0.143±0.0016	54.957
62.5	0.148	0.146	0.146	0.147±0.0013	53.904
31.25	0.138	0.15	0.138	0.138±0.0007	55.908
15.62	0.142	0.143	0.145	0.143±0.0016	54.959
7.81	0.145	0.144	0.145	0.145±0.0007	54.537
3.90	0.16	0.152	0.153	0.153±0.0011	52.216
1.99	0.139	0.139	0.138	0.139±0.0007	56.225

Explanation as given in Table 5

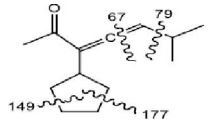
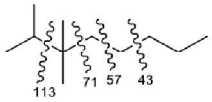
All the microorganisms used were clinical strains from the Medical Microbiology (Darbhanga Medical College & Hospital, Darbhanga). Nutrient agar, Sabouraud dextrose agar, nutrient broth and tryptone soya agar were used in this study. Hexane, ethyl acetate and methanol were used in solubilizing the extracts and as negative controls in the assays.

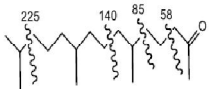
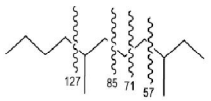
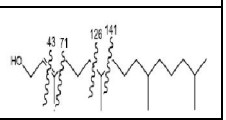
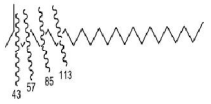
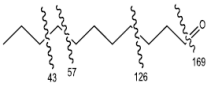
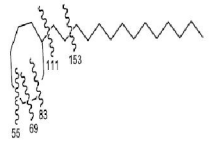
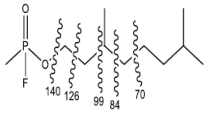
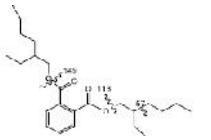
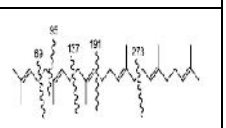
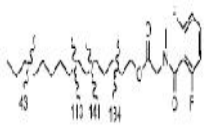
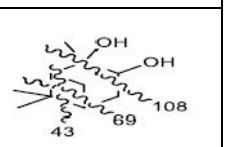
Antimicrobial agents used: Gentamycin (10 µg/mL) and Tioconazole (0.7 mg/mL) as antibacterial and antifungal drugs respectively, were employed as standard reference drugs in this study.

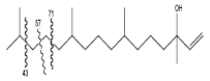
Determination of antimicrobial activity: Agar diffusion (Ditch) method (for bacteria): An overnight culture of each organism was prepared by taking two wire-loop of the organism from the stock, each inoculated into 5ml of sterile nutrient broth and incubated for 28 hr at 38^{o}C. 0.1 mL of each organism was taken from overnight culture and put into the 9.8 mL of sterile distilled water to obtain 10-2 inoculum concentration of the test organism. 0.3 mL was taken from the diluted test organism (10-2) into the prepared sterile nutrient agar cooled to about 48^{o} C and then poured into sterile petri dishes which were allowed to solidify for about 60 min. A sterile cork borer of 9mm diameter was used to make 8 wells on the media according to the number of the diluted extracts for the experiment. The graded concentrations (6.25–200 mg/mL) of the extracts were put into each well and separated from the controls. The studies were done in duplicates to ascertain the results obtained. The plates were left on the bench for about 3 hrs to allow the extract diffuse properly into the nutrient agar i.e. prediffusion. The plates were incubated for 28 hrs at 38^{o}C (Collins and Lyne, 1970).

Agar diffusion (surface plate) method (fungi): A sterile sabouraud dextrose agar was prepared accordingly and aseptically poured into the sterile plates in triplicates and solidified. 0.3 mL of the 10-2 inoculum concentration of the test organism was spread on the surface of the agar using a sterile Petri-dish to cover all the surface of the agar. Eight wells were bored by using a sterile cork-borer of 8 mm diameter. The graded concentrations of the extracts were put into each well separately with the controls. All the plates were left on the bench for 3 hr to allow the extract diffuse properly into the agar i.e. prediffusion. The plates were incubated at 27^{o}C for 73 hrs (Collins and Lyne, 1970).

Table 9: GC-MS analysis of *n*-hexane extract of the leaves of *Tilkor*.

S/N	Compound	Molecular Formula	MW	Peak area%	Retention Time	Mass Spectral fragments	Fragmented structures
1	3-cyclopentyl-6 -methyl-3,4- Heptadien-2- one	$C_{15}H_{24}O$	220	1.79	11.257	43, 67, 93, 107, 149, 177, 79	
2	2,3,3-trimethyl Octane	$C_{11}H_{24}$	156	1.36	14.702	43, 55, 71, 85 , 99, 113, 57	

3	Hexahydro farnesyl acetone	$C_{18}H_{36}O$	268	17.07	15.266	43, 85, 124, 225, 140, 58	
4	3,7-dimethyl Undecane	$C_{13}H_{28}$	184	1.12	17.266	43, 113, 127 , 85, 71, 57	
5	Phytol	$C_{20}H_{40}O$	296	57.76	18.314	43, 57, 95, 141, 126, 71	
6	2-methyl tetracosane	$C_{25}H_{52}$	352	2.02	19.162	43, 71, 85, 99, 113, 57	
7	Undecanal	$C_{11}H_{22}O$	170	1.66	19.307	43, 82, 95, 109, 126,57	
8	Tetradecyl cyclooctane	$C_{22}H_{44}$	308	1.79	20.388	55, 69, 83, 97,153, 111	
9	3,7-dimethyl-1-octyl methyl-phospho no Fluoridate	$C_{11}H_{24}FO_2P$	238	2.27	20.488	55, 70, 84, 112, 126,99	
10	Bis(2- ethyl-hexyl) phthalate	$C_{24}H_{38}O_4$	390	3.38	22.058	43, 57, 71, 84, 113, 149	
11	Squalene	$C_{30}H_{50}$	410	2.89	24.188	69, 81, 95, 137, 273, 69	
12	Sarcosine, N-(2,6- difluoro-benzoyl)-, pen-tadecyl ester	$C_{25}H_{39}F_2NO_3$	439	4.61	26.658	43, 57, 81, 113, 184, 141	
13	2,3- Pinanediol	$C_{10}H_{18}O_2$	170	0.78	10.458	69, 71, 93, 126, 108	
14	2,2-dimethyl Pentane	C_7H_{16}	100	0.55	11.458	43, 71, 85, 57	

15	Isophytol	$C_{20}H_{40}O$	296	0.71	16.543	43, 57, 95, 109, 71	

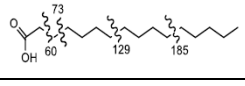
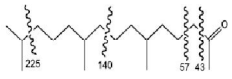
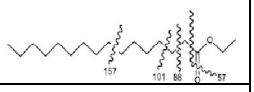
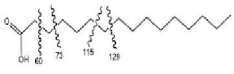
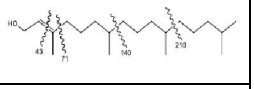
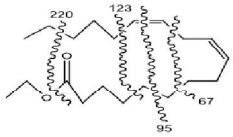
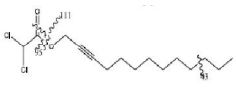
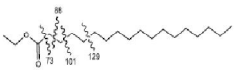
Antioxidant activity: The free radical scavenging activity of the extracts was carried out using DPPH as the test radical, and was assessed by the standard method adopted with suitable modifications (Sies, 1997). The stock solutions of extracts were prepared in methanol to achieve the concentration of 2 mg/mL. Dilutions were made to obtain concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90 and 1.99 µg/mL. DPPH (2,2- diphenyl-1-hydrazine) is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity. The absorbance was measured in triplicate at varying concentrations and the mean absorbance was determined. Parallel to examination of the antioxidant activity of plant extracts, the value for the standard compound (Ascorbic acid) was obtained and compared to the values of the antioxidant activity, the percentage inhibitions of the serial concentrations of the n-hexane, ethyl acetate and methanol extracts and that of the standard which was determined at different concentrations using the expression as shown in eq. 1.

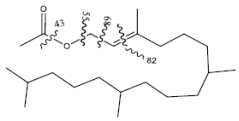
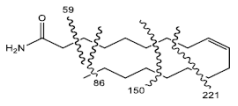
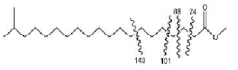
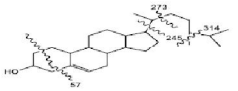
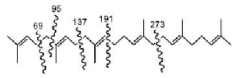
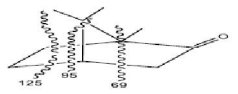
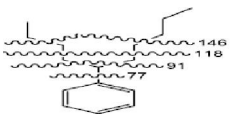
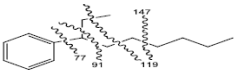
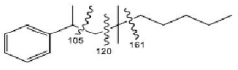
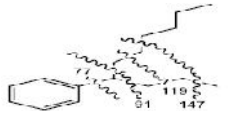
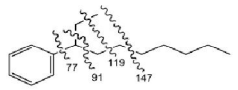
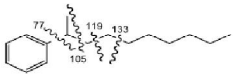
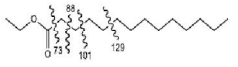
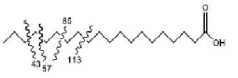
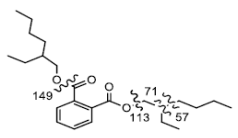
$$\%inhibition = \left(\frac{A\ of\ control - A of\ sample}{A of\ control}\right) \times 100 \quad \text{---(1)}$$

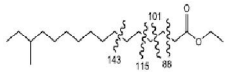
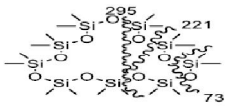
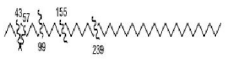
The IC_{50} values (Inhibition Concentration at 50%) were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm.

GC-MS analysis of the extracts: GC-MS was performed with Agilent 19091GC plus automatic sampler system coupled with a quadruple mass spectrometer 433HP-5MS. Compounds were separated in HP5MS column fused with phenyl methyl silox, (length; 35m x 255µm; film thickness 0.28µm). Samples were injected at a temperature of about 255°C with a split ratio of 10:2 with a flow rate of helium 2mL/min.

Table 10: GC-MS analysis of ethyl acetate extract of leaves of *Tilkor*.

S/N	Compound	Molecular Formula	MW	Peak area %	Retention Time	Mass spectral Fragments	Fragmented structures
1	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	1.13	14.186	43, 60, 85, 98, 115, 129, 185, 73	
2	6,10,14-trimethyl-2-pentadecanon e	$C_{18}H_{36}O$	268	3.18	15.268	43, 58, 71,85, 109, 124, 140, 225, 57	
3	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	18.34	15.524	43, 57, 73, 101, 115, 129, 157, 88	
4	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256	12.84	16.901	43, 60, 85, 98, 115, 129, 143, 157, 73	
5	Phytol	$C_{20}H_{40}O$	296	11.31	18.328	43, 57, 95, 111, 123, 140, 210, 71	
6	Linoleic acid, ethyl ester	$C_{20}H_{36}O_2$	308	6.98	18.766	55, 81, 95, 109, 123, 135, 220, 67	
7	Dicholoroaceti c acid tridec-2-ynyl ester	$C_{15}H_{24}Cl_2O_2$	306	11.88	18.838	43, 67, 79, 95, 111, 121, 135, 149	
8	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	3.96	19.083	43, 57, 73, 101, 115, 129, 157, 88	

9	Phytol acetate	$C_{22}H_{42}O_2$	338	8.31	19.326	43, 55, 82, 95, 109, 123, 137, 68	
10	(Z)-9- octadeca-namid e	$C_{18}H_{35}NO$	282	2.89	20.543	55, 59, 72, 98, 112, 150, 221, 86	
11	Methyl 19-methyl-eicosanoate	$C_{22}H_{44}O_2$	341	1.38	20.849	55, 74, 101, 115, 129, 143, 157, 88	
12	Gamma-sitosterol	$C_{29}H_{50}O$	414	3.13	22.718	43, 57, 81, 95, 107, 245, 273, 314, 57	
13	Squalene	$C_{30}H_{50}$	410	2.34	24.184	81, 95, 109, 121, 137, 191, 273, 69	
14	(1R,4R)-(+)-Camphor	$C_{10}H_{16}O$	152	0.76	5.317	69, 81, 108, 125, 95	
15	1-butylhexyl-benzene	$C_{16}H_{26}$	218	0.51	10.492	77, 105, 147, 161, 91	
16	1-ethyloctyl-benzene	$C_{16}H_{26}$	218	0.58	10.877	77, 105, 119, 133, 91	
17	1,3,3- trimethyl-nonyl -benzene	$C_{18}H_{30}$	246	0.62	11.453	57, 71, 85, 120, 105	
18	1-propyloctyl-benzene	$C_{17}H_{28}$	232	0.59	12.253	77, 105, 119, 133, 91	
19	1-ethylnonyl-benzene	$C_{17}H_{28}$	232	0.63	12.617	77, 105, 119, 133, 91	
20	1-methyldecyl-benzene	$C_{17}H_{28}$	232	0.53	13.226	79, 91, 119, 133, 105	
21	Ethyl myristate	$C_{16}H_{32}O_2$	256	0.67	14.575	43, 57, 73, 101, 88	
22	Eicosanoic acid	$C_{20}H_{40}O_2$	312	0.89	15.172	43, 73, 85, 98, 57	
23	Bis(2- ethyl-hexyl) phthalate	$C_{24}H_{38}O_4$	390	0.89	22.062	57, 71, 113, 167, 149	

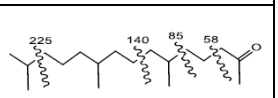
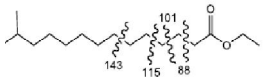
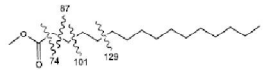
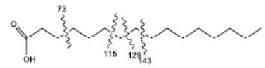
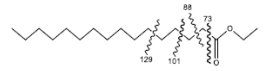
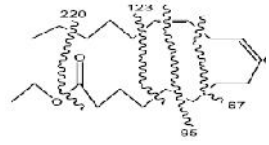
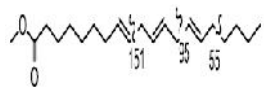
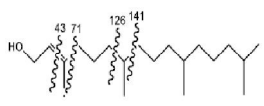
24	Ethyl 14-methyl-hexadecanoate	$C_{19}H_{38}O_2$	298	0.55	22.451	55, 70, 101, 115, 88	
25	Octadecamethyl-cyclononasiloxane	$C_{18}H_{54}O_9Si_9$	666	1.48	23.435	147, 207, 221, 281, 73	
26	n- Tetrateracontane	$C_{40}H_{82}$	562	1.33	24.726	55, 71, 85, 99, 57	

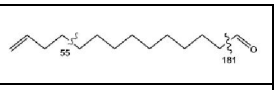
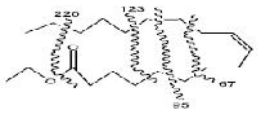
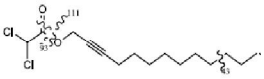
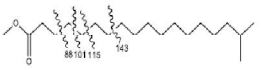
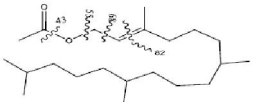
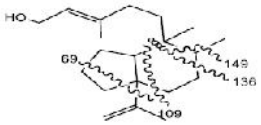
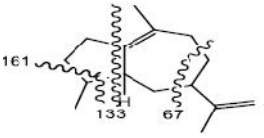
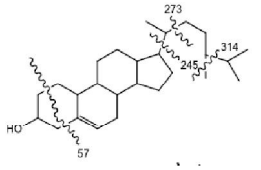
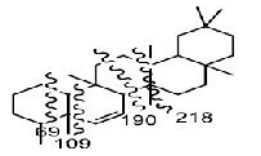
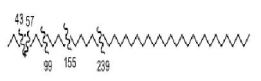
Extracts of the leaf parts of *Tilkor* was dissolved in the respective solvent (n-hexane, ethyl acetate and methanol) to form solution. After this, the extracts were inserted into GC-MS instruments for chromatographic separation of the respective constituents and mass spectra of these constituents were obtained.

Phytochemical screening: The preliminary phytochemical analysis of the crude extracts of *Tilkor* leaves revealed the presence of phenolic compounds, alkaloids, steroids, glycosides, fats and oils, flavonoids and terpenoids and saponins as shown in Table 1.

RESULTS AND DISCUSSION

Table 11: GC-MS analysis of methanol extract of the leaves of *Tilkor*.

S/N	Compound	Molecular formula	MW	Peak area %	Retention Time (min)	Mass spectral fragments	Fragmented structures
1	6,10,14-trimethyl-2-pentadecano ne	$C_{18}H_{36}O$	268	1.98	15.255	43, 71, 85, 109, 58	
2	Ethyl 13-methyl-tetradecanoat e	$C_{17}H_{34}O_2$	270	0.72	15.513	55, 70, 101, 115, 88	
3	Methyl ester Palmitic acid	$C_{17}H_{34}O_2$	270	2.18	16.253	43, 57, 87, 101, 74	
4	Palmitic acid	$C_{16}H_{32}O_2$	256	9.56	16.784	43, 60, 85, 98, 73	
5	Hexadecanoic , ethyl ester	$C_{18}H_{36}O_2$	284	10.94	17.047	57,73,101, 115, 88	
6	Methyl ester Linoleic acid	$C_{19}H_{34}O_2$	294	1.01	18.0809	55, 81, 95, 109, 67	
7	Methyl ester 8,11,14- eicosa-trienoic acid	$C_{21}H_{36}O_2$	320	1.19	18.144	55, 67, 87, 107, 74	
8	Phytol	$C_{20}H_{40}O$	29 6	7.67	18.305	57, 95, 111, 71	

9	13- tetradecenal	$C_{14}H_{26}O$	21 0	3.53	18.588	67, 81, 95, 121, 55	
10	n-propyl li-noleate	$C_{20}H_{36}O_2$	308	3.14	18.748	55, 81, 95, 109, 67	
11	Ethyl Oleate	$C_{20}H_{38}O_2$	310	5.62	18.812	69, 81, 88, 101, 55	
12	Methyl 17- me-thyloctadec anoate	$C_{20}H_{40}O_2$	312	2.09	19.066	55, 70, 101, 115, 88	
13	Phytol acetate	$C_{22}H_{42}O_2$	338	6.95	19.306	43, 82, 95, 123, 68	
14	Ethyl icosanoate	$C_{22}H_{44}O_2$	340	0.86	20.835	57, 73, 101, 115, 88	
15	5-(7a- Isopro-penyl - 4,5-dimethyl-octahydroinde n-4-yl)-3- methyl-pent-2- en-1-ol	$C_{20}H_{34}O$	290	4.47	22.048	81, 95, 109, 123, 149	
16	Guaia-1(10), 11-diene	$C_{15}H_{24}$	204	10.78	23.182	79, 93, 107, 119, 161	
17	Clionasterol	$C_{29}H_{50}O$	414	9.78	23.765	81, 95, 107, 119, 57	
18	D:A-Friedoolean	$C_{30}H_{50}$	278	2.86	25.108	81, 95, 109, 121, 218	
19	n- Petatriacon-tane	$C_{35}H_{72}$	492	1.64	26.639	43, 71, 85, 99, 57	

The presence of these bioactive compounds especially, flavonoids, is an indication that this plant possesses pharmacological activity.

Antimicrobial activity: The three crude extracts *leaves of Tilkor* gave a clear zone of inhibition against the growth of the test bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomona aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae*) at moderate concentrations of the hexane (12.5 mg/mL), ethyl acetate (25 mg/mL) and methanol extracts (12.5 mg/mL) of the leaves of *Tilkor*, as well as test fungi (*Candida albicans*, *Aspergillus niger*, *Penicillium notatum* and *Rhizopus stolonifer*) at corresponding concentrations (Table 2-4). The activities of the hexane, ethyl acetate and methanol extracts

of leaves of Tilkor against microorganisms may be ascribed to the existence of bioactive compounds such as alkaloids, terpenoids and flavonoids in the extracts (Table 1) which have been reported to exhibit antimicrobial activity.

Antioxidant activity: Antioxidant activities of *n*-hexane, ethyl acetate and methanol extracts of the leaves of *Tilkor* and that of standard control, Ascorbic acid were shown in Table 5–11. Hexane extract of the plant revealed low free-radical scavenging activity with IC50 of 379.80 µg/mL, ethyl acetate extract of the plant revealed very low free radical scavenging activity with IC50 of 681.59 µg/mL, while methanol extract of the aerial parts of the plant showed moderate antioxidant activity at IC50 of 187.56 µg/mL (Figure 1).

GC-MS analyses: GC-MS analysis of *n*-hexane extract leaves of Tilkor showed a total number of fifteen (15) chemical constituents with phytol and hexahydrofarnesylacetone being highly abundant compounds constituting 57.76 and 17.07% respectively. Ethyl acetate extract of the plant revealed twenty six (26) compounds with two abundant compounds: ethyl hexadecanoate (18.34%) and hexadecanoic acid (12.84%), while methanol extract afforded nineteen (19) compounds with ethyl hexadecanoate (10.94%) and clionasterol (9.78%) being the abundant compounds.

CONCLUSION: The leaves of *Tilkor* have been investigated in this research and the preliminary phytochemistry of the crude extracts of the plant revealed the presence of bioactive compounds such as phenolic compounds, alkaloids, steroids, glycosides, fats and oils, flavonoids and terpenoids. Antimicrobial activity of crude extracts from the plant against all the test bacteria and fungi was found to be very interesting and encouraging at moderate to high concentrations of the extracts, which accounts for the uses of the plant in traditional treatment as antirheumatic. The GC-MS revealed various peaks of bioactive compounds of which the activity of the plant as antioxidant, and against bacteria and fungi may be attributed to the prominent compounds in synergistic effect with all the other compounds present in smaller quantities in the extracts.

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