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 typhi, 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. 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 soxhlet 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. The

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**Phytochemical screening:** Preliminary phytochemical screening of the crude extracts was carried out using the modified methods as described by Pranshant et al. (2011). The extracts were dried by using rotary evaporator and kept in the refrigerator for further use.

**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 pneumoniae 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. CBAE: Ethyl acetate extract. CBAM: 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   | 14  | 15  | 15  | 13   |
| 50                   | 15  | 13  | 14  | 15   | 13  | 13   | 13  | 14  | 13  | 12   |
| 25                   | 12  | 11  | 12  | 13   | 13  | 15   | --  | 15  | --  | --   |
| 12.5                 | 15  | 14  | 15  | 15   | 15  | 13   | 13  | 13  | 12  | 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  | 15  | 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: ethyl acetate

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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  | 11   |
| 25                    | 19   | 11   | 17   | 18    | 16  | 17   | 15   | 15   | 13  | 11   |
| 12.5                  | 15   | 14   | 13   | 19    | 14  | 12   | 13   | 13   | 11  | 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±0.0000  | 88.15   |
| 250         | 0.162| 0.163| 0.17 | 0.162±0.0002 | 87.27   |
| 125         | 0.19 | 0.19 | 0.19 | 0.181±0.0000 | 85.78   |
| 62.5        | 0.194| 0.196| 0.195| 0.195±0.0002 | 84.27   |
| 31.25       | 0.246| 0.246| 0.246| 0.246±0.0000 | 80.68   |
| 15.62       | 0.312| 0.312| 0.312| 0.312±0.0000 | 75.45   |
| 7.81        | 0.454| 0.453| 0.455| 0.454±0.0002 | 64.19   |
| 3.9         | 0.783| 0.782| 0.79 | 0.782±0.0002 | 38.27   |
| 1.95        | 0.992| 0.992| 0.992| 0.992±0.0000 | 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 | 63.193  |
| 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| 0.213±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        | 0.172| 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| 54.337  |
| 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°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°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. pre-diffusion. The plates were incubated for 28 hrs at 38°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°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-cyclohexyl-6-methyl-3,4-heptadien-2-one | C_{15}H_{24}O | 220 | 1.79       | 11.257        | 43, 67, 93, 107, 149, 177, 79 | ![Fragmented structures](image1) |
| 2   | 2,3,3-trimethyl Octane | C_{11}H_{24} | 156 | 1.36       | 14.702        | 43, 55, 71, 85, 99, 113, 57 | ![Fragmented structures](image2) |
|   |   |   |   |   |
|---|---|---|---|---|
| 3 | Hexahydrofarnesyl acetone | C₁₈H₃₆O₂ | 268 | 17.07 | 15.266 | 43, 85, 124, 225, 140, 58 |
| 4 | 3,7-dimethyl Undecane | C₁₃H₂₈ | 184 | 1.12 | 17.266 | 43, 113, 127, 85, 71, 57 |
| 5 | Phytol | C₂₀H₄₀O | 296 | 57.76 | 18.314 | 43, 57, 95, 141, 126, 71 |
| 6 | 2-methyl tetracosane | C₂₅H₅₂ | 352 | 2.02 | 19.162 | 43, 71, 85, 99, 113, 57 |
| 7 | Undecanal | C₁₁H₂₂O | 170 | 1.66 | 19.307 | 43, 82, 95, 109, 126, 57 |
| 8 | Tetradecyl cyclooctane | C₂₂H₄₄ | 308 | 1.79 | 20.388 | 55, 69, 83, 97, 153, 111 |
| 9 | 3,7-dimethyl-1-octyl methylphosphono fluoride | C₁₁H₂₄FO₂P | 238 | 2.27 | 20.488 | 55, 70, 84, 112, 126, 99 |
| 10 | Bis(2-ethylhexyl) phthalate | C₂₄H₃₈O₄ | 390 | 3.38 | 22.058 | 43, 57, 71, 84, 113, 149 |
| 11 | Squalene | C₃₀H₅₀ | 410 | 2.89 | 24.188 | 69, 81, 95, 137, 273, 69 |
| 12 | Sarcosine, N-(2,6-difluorobenzoyl)-pentadecyl ester | C₂₅H₃₉F₂N₂O₃ | 439 | 4.61 | 26.658 | 43, 57, 81, 113, 184, 141 |
| 13 | 2,3-Pinanediol | C₁₀H₁₉O₂ | 170 | 0.78 | 10.458 | 69, 71, 93, 126, 108 |
| 14 | 2,2-dimethyl Pentane | C₃H₁₀ | 100 | 0.55 | 11.458 | 43, 71, 85, 57 |
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.

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

The IC\textsubscript{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\textsubscript{14}H\textsubscript{28}O\textsubscript{2} | 228 | 1.13        | 14.186         | 43, 60, 85, 98, 115, 129, 185, 73 |
| 2   | 6,10,14-trimethyl-2-pentadecanone | C\textsubscript{18}H\textsubscript{36}O | 268 | 3.18        | 15.268         | 43, 58, 71, 85, 109, 124, 140, 225, 57 |
| 3   | Hexadecanoic acid, ethyl ester    | C\textsubscript{18}H\textsubscript{36}O\textsubscript{2} | 284 | 18.34       | 15.524         | 43, 57, 73, 101, 115, 129, 157, 88 |
| 4   | n-hexadecanoic acid               | C\textsubscript{16}H\textsubscript{32}O\textsubscript{2} | 256 | 12.84       | 16.901         | 43, 60, 85, 98, 115, 129, 143, 157, 73 |
| 5   | Phytol                            | C\textsubscript{20}H\textsubscript{40}O | 296 | 11.31       | 18.328         | 43, 57, 95, 111, 123, 140, 210, 71 |
| 6   | Linoleic acid, ethyl ester        | C\textsubscript{20}H\textsubscript{40}O\textsubscript{2} | 308 | 6.98        | 18.766         | 55, 81, 95, 109, 123, 135, 220, 67 |
| 7   | Dichlorooctadecanoic acid tridec-2-ynyl ester | C\textsubscript{18}H\textsubscript{26}Cl\textsubscript{2}O\textsubscript{2} | 306 | 11.88       | 18.838         | 43, 67, 79, 95, 111, 121, 135, 149 |
| 8   | Octadecanoic acid, ethyl ester    | C\textsubscript{20}H\textsubscript{40}O\textsubscript{2} | 312 | 3.96        | 19.083         | 43, 57, 73, 101, 115, 129, 157, 88 |
| No. | Compound                              | Formula   | Retention Time | Library Match Factor | Mass Elution (m/z) | Diagram |
|-----|---------------------------------------|-----------|---------------|----------------------|-------------------|---------|
| 9   | Phytol acetate                        | C_{22}H_{42}O_{2} | 338           | 8.31                | 19.326            | ![Diagram](image) |
| 10  | (Z)-9- octadecanamide                 | C_{24}H_{35}NO | 282           | 2.89                | 20.543            | ![Diagram](image) |
| 11  | Methyl 19-methyl-eicosanoate          | C_{22}H_{44}O_{2} | 341           | 1.38                | 20.849            | ![Diagram](image) |
| 12  | Gamma-sitosterol                      | C_{29}H_{55}O | 414           | 3.13                | 22.718            | ![Diagram](image) |
| 13  | Squalene                              | C_{30}H_{50} | 410           | 2.34                | 24.184            | ![Diagram](image) |
| 14  | (1R,4R)-(+)-Camphor                   | C_{10}H_{16}O | 152           | 0.76                | 5.317             | ![Diagram](image) |
| 15  | 1-butylhexyl-benzene                  | C_{16}H_{26} | 218           | 0.51                | 10.492            | ![Diagram](image) |
| 16  | 1-ethyloctyl-benzene                  | C_{16}H_{26} | 218           | 0.58                | 10.877            | ![Diagram](image) |
| 17  | 1,3,3- trimethyl-nonyl-benzene        | C_{14}H_{30} | 246           | 0.62                | 11.453            | ![Diagram](image) |
| 18  | 1-propyloctyl-benzene                 | C_{17}H_{28} | 232           | 0.59                | 12.253            | ![Diagram](image) |
| 19  | 1-ethylnonyl-benzene                  | C_{17}H_{28} | 232           | 0.63                | 12.617            | ![Diagram](image) |
| 20  | 1-methyldecyl-benzene                 | C_{17}H_{28} | 232           | 0.53                | 13.226            | ![Diagram](image) |
| 21  | Ethyl myristate                       | C_{16}H_{32}O_{2} | 256           | 0.67                | 14.575            | ![Diagram](image) |
| 22  | Eicosanoic acid                       | C_{20}H_{40}O_{2} | 312           | 0.89                | 15.172            | ![Diagram](image) |
| 23  | Bis(2-ethylhexyl) phthalate           | C_{23}H_{38}O_{4} | 390           | 0.89                | 22.062            | ![Diagram](image) |
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.

**RESULTS AND DISCUSSION**

**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.

**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-pentadecanoate | C_{18}H_{36}O_{2}  | 268  | 1.98        | 15.255               | 43, 71, 85, 109, 58    |                       |
| 2   | Ethyl 13-methyl-tetradecanoate   | 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_{16}H_{30}O_{2}  | 284  | 10.94       | 17.047               | 57,73,101, 115, 88     |                       |
| 6   | Methyl ester Linoleic acid       | C_{19}H_{36}O_{2}  | 294  | 1.01        | 18.0809              | 55, 81, 95, 109, 67    |                       |
| 7   | Methyl ester 8,11,14-eicosatrienoic acid | C_{21}H_{36}O_{2}  | 320  | 1.19        | 18.144               | 55, 67, 87, 107, 74    |                       |
| 8   | Phytol                          | C_{20}H_{40}O      | 296  | 7.67        | 18.305               | 57, 95, 111, 71        |                       |
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*, *Pseudomonas 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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