ESSENTIAL OIL FROM THE AERIAL ROOTS OF FICUS ELASTICA AND THEIR ANTIOXIDANT ACTIVITY.

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Abstract

Essential oil of aerial roots of Ficus elastica was extracted through hydro-distillation followed by separation by diethyl ether and dried over anhydrous sodium sulfate. Gas chromatography-mass spectrometry study of essential oil was performed to determine its chemical constituents. Fifteen constituents were identified and major constituents were benzene, [1-methyldodecyl] (9.96%); benzene, [1-ethynundecyl] (14.14%); 3,9 β, 14,15-di-epoxypregn-16-en-20-one (15.72%); benzene,[1-propyldecyl] (5.24%); 9,12,15-octadecatrienoicacid (6.76%); 2,3 bis[trimethylsilyl] 1-oxylpropylester (12.44%); 9,12,15-octadecatrienoic acid,2,3-bis[trimethylsilyl-ox propylester (7.13%); octadec-9-enoi acid icosyl ester (7.07%) respectively. The antioxidant activity of the essential oil was measured by using DPPH and ascorbic acid as standard. The maximum antioxidant activity of the essential oil was 61.01% with 100 mg/ml while the antioxidant activity of ascorbic acid with similar concentration was 100%. Antioxidant prevents free radical induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition. Antioxidants obtained from natural resources are better for human health as they are less harmful.

Introduction:-

Ficus elastica belongs to the family Moraceae commonly known as the rubber fig, rubber bush, rubber tree, or Indian rubber bush. It is a widely-spread evergreen tree up to 30 m tall, having thick and dark green 7-20 cm long leaves with smooth edges and pointless tips.¹ Ficus elastica is belongs to genus ficus which has about 2000 varieties and 800 species.² In Egypt, many ficus species are found in streets, gardens, parks and outside the canal banks. The fruits of two ficus species are eatable by Egyptian peoples³. Rubber plants are not produce highly colorful or fragrant flowers for the attraction of other pollinators⁴. Ficus has most commonly species which is called ficus carica L has a fruit known as Fig which is a commercial fruit⁵.

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The white latex obtained from the bark of *ficus elastica* is used to prepare rubber. It is also used in medicine such as to cure the skin infection, allergies, anemia, and neurodegenerative disorder and hepatic problems; and also used as diuretic agent\(^\text{vi}\). Herbalism is a traditional or also known as folk medicine extract from plants\(^\text{vii}\). They have used in medicine as astringents, carminatives, stomachics, vermifuges, and anti-dysentry drugs\(^\text{viii}\). Ficus plants are also used not only in the treatment of cancer but in various diseases\(^\text{ix}\). *Ficus elastica* Roxb. and *Ficus bengalensis* Linn. are anti-inflammatory and analgesic\(^\text{x}\).

There is merely a report on *Ficus elastica* leaves, but not only a single report on the aerial roots in the literature. So, the current study was designed to investigate the chemical constituents of essential oil and its antioxidant activity. Most of the diseases are due to the oxidative stress, free radicals\(^\text{xi}\), these free radicals are the basic part of any biochemical process and shows an essential part if aerobic life and metabolism\(^\text{xii}\). Free radicals are adversely changed the lipids, proteins and DNA and large number of human diseases\(^\text{xiii}\). The essential oils are used as fragrance in perfumes and aromas in food industry\(^\text{xiv}\). These essential oils have number of biological activities, like antibacterial, antifungal and antioxidant properties\(^\text{xv}\).

**Materials and Methods:**

**Collection of Plant material:**
The plant sample (aerial roots) was collected from the PCSIR (Pakistan Council of Scientific and Industrial Research) on 20 December 2016 in Lahore Pakistan. The aerial roots were cut into small pieces, extraction of essential oil was carried out by hydro-distillation in the oil and fat research laboratory.

**Reagents:**
All the chemicals (diethyl ether, sodium sulfate anhydrous, DPPH, used were standard and of analytical grade.

**Extraction of essential oil:**
The aerial roots was collected and then cut into small pieces and essential oil was extracted through hydro-distillation by using Dean Stark apparatus. Top hazy layer was obtained and separation was carried out by using diethyl ether in a separating funnel. Organic combined layer was dried over sodium sulfate anhydrous and essential oil was stored in a well tight closed bottle in refrigerator for GC-MS and antioxidant studies.

**GC-MS analysis of essential oil of aerial roots of *ficus elastica***:
Essential oil extracted from the aerial roots of *ficus elastica* was analyzed for its chemical constituents by gas chromatograph coupled with mass spectrometry (GC-MS). Agilant 5973-6890 gas chromatograph–mass spectrometry system, operating in EI mode at 70 ev equipped with a split-splitless injector was used. Helium was used as a carrier gas at the flow rate of 1 ml/min, while HP-5MS (30 m, 0.25 mm, 0.25 um) capillary column was used. The initial temperature was programmed at 50-100 °C at the rate of 5 °C/min and then 100-250 °C at the rate of 3 °C/min followed by a constant temperature at 260 °C for a period of 20 minutes. Sample (2 µl) was injected to the column programmed at 200 °C and resolution of components was attained. Identification of components was performed by matching their retention indices and mass spectra with those obtained the NIST library.

**Antioxidant activity of essential oil of aerial roots of *Ficus elastica***:
Antioxidant activity of the essential oil of aerial roots of *ficus elastica* was evaluated by the scavenging activity of DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical. The DPPH assay was performed by following the method of Epsin et al. (2000)\(^\text{xvi}\). 0.1mM DPPH solution was prepared by dissolving 1mg of DPPH in 60 ml of methanol. Different concentration 20, 40, 60, 80 and 100 µL of plant extract took in test tubes; add 30ml of reagent in every test tube ranging R1, R2, R3, R4 and R5. The reaction mixture was incubated in dark condition at room temperature for about 30 minutes. For each samples three replicates were recorded. The disappearance of the DPPH was measured spectro-photometrically at 517 nm. The percentage scavenging of DPPH by the extract was calculated by the following equation.

\[
\text{% DPPH Radical scavenging effect} = \left[ \frac{(Ac - As)}{As} \right] \times 100
\]

Where

*Ac* is the absorption of the control at 30 minutes.

*As* is the absorbance of the sample at 30 minute
Results and Discussion:
The essential oil extracted from the aerial roots of Ficus elastica was slight yellowish in color and its yield was 0.02%. Their chemical constituents were analyzed by GC-MS. Agilent 5973-6890 gas chromatography-mass spectrometry system, operating in EI mode at 70 eV equipped with a split-split injector was used. Fifteen constituents were identified in the essential oil of Ficus elastica aerial roots (Table 1). Benzene, [1-propyldecyl], benzene, [1-propyldodecyl], 6-monooctacetophenone, benzene, [1-ethylundecyl], propanamide, N, N-diecyld-3-phenyl, benzene, [1-methylcyclohexyl], diethyl Phthalate, octadecane, 3-ethyl-5-[2-ethylbutyl], cyclopropanebutanoic acid, 2-[2-][2-pentylcyclopropyl]methylenecyclopropylmethylenecyclopropylmethyl-methyl ester, propanoic acid, 2-[3-acetoxy-4,4,1] 4-trimethylandrost-8-en-17-yl, 3,9 β,14,15-Diepoxypregn-16-en-20-one,3.1[1]β,18-triacetoxy, 9,12,15-octadecatrienoic acid, 2,3-bis[trimethylsilyl]oxypropylester, [Z,Z,Z], oleic acid, eicosyester, propanoic acid, 2-[3-acetoxy-4,4,1] 4-trimethylandrost-8-en-17-yl and D-glucose,6-α-D-galactopyranosyl, bis-o-[trimethylsilyl] derive..., cyclo tris[monoborane] with relative abundance 9.96%, 4.96%, 0.83%, 14.14%, 1.42%, 15.72%, 5.24%, 3.25%, 2.96%, 6.76%, 12.445, 7.13%, 7.07% and 4.42% respectively.

Table 1:- Chemical constituents of essential oil from the aerial roots of Ficus elastica.

| Peak # | Compound | Retention Time (Minute) | Relative abundance (%age) |
|-------|----------|-------------------------|--------------------------|
| 1     | Benzene,[1-propyldecyl] | 8.79 | 9.96 |
| 2     | Benzene,[1-propyldodecyl] | 9.01 | 4.96 |
| 3     | 6-Monoacetophenone | 9.58 | 0.83 |
| 4     | Benzene,[1-ethylundecyl] | 11.74 | 14.14 |
| 5     | Propanamide, N, N-diecyld-3-phenyl | 13.14 | 1.42 |
| 6     | Benzene,[1-methylcyclohexyl] | 14.09 | 15.72 |
| 7     | Diethyl Phthalate | 14.72 | 5.24 |
| 8     | Octadecane, 3-ethyl-5-[2-ethylbutyl] | 14.99 | 3.25 |
| 9     | Cyclopropanebutanoic acid, 2-[2-][2-pentylcyclopropyl]methylenecyclopropylmethylenecyclopropylmethyl-methyl ester | 15.20 | 2.96 |
| 10    | Propanoic acid, 2-[3-acetoxy-4,4,1] 4-trimethylandrost-8-en-17-yl | 15.86 | 6.76 |
| 11    | 3,9 β,14,15-Diepoxypregn-16-en-20-one,3.1[1]β,18-triacetoxy | 17.03 | 12.44 |
| 12    | 9,12,15-octadecatrienoic acid, 2,3-bis[trimethylsilyl] oxy propylester, | 18.09 | 7.13 |
| 13    | Octadec-9-enolic acid icosyl ester | 18.46 | 7.07 |
| 14    | Propanoic acid, 2-[3-acetoxy-4,4,1] 4-trimethylandrost-8-en-17-yl | 19.26 | 4.42 |
| 15    | D-Glucose,6-α-D-galactopyranosyl, bis-O-[trimethylsilyl] | 24.41 | 3.62 |

Antioxidant activity of the essential oil of aerial roots of Ficus elastica was evaluated by the scavenging activity of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical. The DPPH assay was performed by following the method of Epsin et al. (2000).

Table 2:- Antioxidant activity of essential oil from the aerial roots of Ficus elastica.

| Serial No. | Concentration (μL) | Absorbance (517 nm) | % Age Inhibition |
|------------|--------------------|---------------------|-----------------|
| 1          | 20                 | 1.104               | 6.44            |
| 2          | 40                 | 0.937               | 20.59           |
| 3          | 60                 | 0.687               | 41.77           |
| 4          | 80                 | 0.536               | 54.57           |
| 5          | 100                | 0.460               | 61.01           |

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Antioxidants can assist in this oxidative stress. At concentrations of 20 μL, 40 μL, 60 μL, 80 μL and 100 μL absorbencies were 1.104, 0.937, 0.687, 0.536 and 0.460 respectively, with percentage inhibition were 6.44 %, 20.59 %, 41.77 %, 54.57 %, and 61.01 % respectively at each concentration of extract and ascorbic acid considered as a standard with percentage inhibition was 92 % at concentration of 100 μL.
Conclusion:-
The essential oil from the aerial roots of *ficus elastica* was extracted through hydro-distillation and its GC-MS and antioxidant studied were carried out. Fifteen volatile constituents were identified and major constituents benzene, [1-methyldodecyl], 9.96%; benzene, [1-ethynundecyl] 14.14%; 3, β, 14,15-di-epoxypregn-16-en-20-one 15.72%; benzene,[1-propyldecyl] 5.24%; 9,12,15-octadecatrienoic acid 6.76%; 2,3 bis[trimethylsilyl] 1-oxypolyester 12.44%; 9,12,15-octadecatrienoic acid,2,3-bis(trimethylsilyl)-oxy propylester, 7.13%, octadec-9-enoic acid icosyl ester 7.07% were in the essential oil of *Ficus elastica* aerial roots respectively. The essential oil extracted from the aerial roots of *ficus elastica* was slight yellow in color with 0.02 % yield. Antioxidant activity of the essential oil was determined by using DPPH and ascorbic acid as standard. Maximum antioxidant activity 61.01% was observed at 100 μL. The antioxidant activity of the essential oil was concentration dependent.

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