Biochemical Composition, Antioxidant and Biological Activities of the Essential Oil and Fruit Extract of *Xanthium strumarium* Linn. From Northern Iran

S. Ghahari\(^1\), H. Alinezhad\(^1\), Gh. A. Nematazedeh\(^2\), M. Tajbakhsh\(^1\), and R. Baharfar \(^1\)

**ABSTRACT**

This study examines the chemical composition of the essential oil of *Xanthium strumarium* fruits, and evaluates its antioxidant and antimicrobial activities on various plant pathogens that commonly cause irreparable damages to agricultural crops. The essential oil of *X. strumarium* fruits was analyzed by Gas Chromatography coupled to Mass Spectrometry (GC/MS). Antimicrobial activity was tested against 14 microorganisms, including three gram-positive, five gram-negative bacteria and six fungi, using disk diffusion method and the Minimum Inhibitory Concentration (MIC) technique. The *X. strumarium* fruits were also subjected to screening for possible antioxidant activity by using catalase, guaiacol peroxidase, superoxide dismutase enzymes and 2, 2-DiPhenyl-1-Picryl Hydrazyl (DPPH) assay. Thirty six components were identified, representing 97.89% of the total oil, with methyl linoleate (40.64%), methyl oleate (13.12%), and methyl palmitate (12.43%) being the major ingredients. The essential oil showed significant activity against *Rathayibacter toxicus* (MIC= 25 µg mL\(^{-1}\)), and *Pyricularia oryzae* (MIC= 12.5 µg mL\(^{-1}\)). In addition, the analysis of free radical scavenging activities of the *X. strumarium* fruits revealed antiradical activity of 138.87 µg mL\(^{-1}\) in DPPH, 32.766 µmole activity/mg protein in catalase, 5.567 mmol activity/mg protein in guaiacol peroxidase and 1.714 U mg\(^{-1}\) protein in superoxide dismutase. Furthermore, the phytochemical analysis showed moderate to good amounts of alkaloid (0.54 mg g\(^{-1}\)), phenolic (54.44 mg g\(^{-1}\)) and flavonoid (20.11 mg g\(^{-1}\)) compounds in *X. strumarium* fruits. Our results suggest that this plant may be a potential source of biocide, for economical and environmentally friendly disease control strategies. It may also be a good candidate for further biological and pharmacological investigations.

**Keywords:** Antibacterial activity, Antifungal effect, Antioxidant activity, Plant pathogens, *Xanthium strumarium* Linn.

**INTRODUCTION**

Herbs and medicinal plants have played an important role in pharmaceutical, cosmetics, and food industries, due to their antimicrobial, medicinal and agricultural properties (Joshi, 2014; Gomathi *et al*., 2015). Therefore, much work are carried out these days with the purpose of utilizing medicinal and aromatic plants as a source of new therapeutic agents, in order to control diseases or induction of resistance (Hanif *et al*., 2015). Essential oils are valuable natural products, used as raw materials in many fields such as perfumes, cosmetics, aromatherapy, spices, and nutrition (Hammami *et al*., 2015). Several studies have reported the biocide activity of essential oils against many different...
pathogens (Kchaou et al., 2016; Mazidi et al., 2012; Sharafati Chaleshtori et al., 2015; Jabri Karoui et al., 2016; Elsherbiny et al., 2016).

The oxygen consumption inherent in cell growth leads to the generation of a series of Reactive Oxygen Species (ROS) (Borra et al., 2013). Normally, ROS are responsible to help several signal transduction and intercellular communications (Kumar et al., 2011). ROS cause cellular damage by reacting with the various biomolecules of body such as membrane lipids, nucleic acid, proteins and enzymes (Kumar et al., 2012) leading to cardiovascular disease, ageing, cancer, inflammatory diseases and a variety of other disorders (Kumar et al., 2011). Antioxidants can act as scavengers of free radicals and can neutralize potentially harmful reactive free radicals in body cells before they cause lipid and protein oxidation and may reduce potential mutation and therefore, help prevent cancer or heart diseases (Borra et al., 2013). Plants contain substantial amounts of phytochemical antioxidants such as phenolics, carotenoids, flavonoids and tannins which can be used to scavenge the excess free radicals present in the body. The antioxidant potential of phenolics is because of their redox properties which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Kumar et al., 2012).

Plant pests and diseases cause considerable damage to agricultural crops. Also, they have a huge impact on quality of crops. Therefore, crop protection against plant pests and diseases is essential in modern agriculture (Martins et al., 2012). The use of chemical pesticides to control plant pests and diseases has been very common during recent decades, but the vast use of such chemical pesticides has considerable drawbacks, such as the increase of strain resistance to pesticides, increasing the cost of production, transportation risks, concerns about chemical residues, toxic effects of synthetic chemicals on non-target organisms and great threats to both human and environmental health (Dalvie et al., 2014). The awareness of these problems has encouraged the researchers to find novel resources for biocides of plant origin. Natural plant products are an important source of new chemicals in agricultural scopes used in the control of plant diseases and pests (Amadioha, 2000). Recently researches have shown that natural chemicals can act as a potential source of non-phytotoxic, systemic and comfortably biodegradable replace pesticides (Amadioha, 2000).

*Xanthium strumarium* Linn. belonging to the family Asteraceae, is commonly called as common cocklebur, hedgehog bur weed, sea burdock, sheep bur, clot bur, button bur and ditch bur. It is distributed all over the world but is most common in the moderate zone and is a main weed in India, Australia, America, and South Africa (Sarwade et al., 2012). This plant possesses antitussive, cytotoxic, hypoglycemic, antibacterial, antifungal, antimalarial, sinustis, urticaria, constipation, diarrhoea, lumbago, leprosy, stomachic, tonic, diuretic, sedative, allergic rhinitis, antirheumatic, antispasmodic, pruritis and antidiabetic properties (Khuda et al., 2012). *X. strumarium* was screened for antifungal activity against economically important phytopathogenic fungi, *Pythium aphanidermatum* (Bahraminejad, 2012). Adult leaves of *X. strumarium* can be applied as a source of botanical fungicidal preparation for controlling foliar fungal diseases of tea (Saha et al., 2012). Antimicrobial activity of *X. strumarium* Linn. leaf extracts were evaluated against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Clostridium perfringens*. *X. strumarium* leaf extracts were obtained using 3 different extraction methods and 4 different solvents, in triplicate. The employed methods were maceration, dynamic maceration and soxhlet. Solvents were 80% ethanol, 80% methanol, ethyl acetate and dichloromethane/chloroform (1:1). All extracts had strong antimicrobial activity against the evaluated microorganisms (Scherer et al., 2009). Antifungal activity of the 70% ethanolic extracts obtained from rough cocklebur was studied against *Aspergillus ochraceus* and *Acromonium chrysospermum*. In order to determine the minimum concentration of extract that inhibits the growth of fungi, the following concentrations were used: 0.3, 0.15 and 0.075 g mL\(^{-1}\). Fungi were completely inhibited in the concentration of 0.3 g extract mL\(^{-1}\) 70% ethanol. When used concentration of 0.075 g extract mL\(^{-1}\) 70% ethanol, the
development of fungi was significantly inhibited (Butu et al., 2013). The antimicrobial and antioxidant effects and the compounds of the essential oil and supercritical extracts of X. strumarium leaves were studied. The oil and the extracts were diluted in DMSO. The final concentrations of the extracts were 1.8, 1.5, 1.2, 1.0, 0.8, 0.6, 0.4 and 0.2 mg mL\(^{-1}\). All the X. strumarium extracts showed strong antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Clostridium perfringens*, however no differences were observed between the extracts. Poor antioxidant activity was found for all the X. strumarium extracts (Scherer et al., 2010). The chemical composition and antimicrobial activity of the essential oil from fresh cocklebur leaves was investigated. The essential oil was tested against three gram-negative bacteria: *Klebsiella pneumonia*, *Escherichia coli* and *Pseudomonas aeruginosa*; three gram-positive bacteria *Staphylococcus aureus*, *Staphylococcus epidermis* and *Bacillus subtilis*; and two fungi: *Aspergillus niger* and *Candida albicans*. Different concentrations of essential oil (10, 20, 40, 60, 80 and 100 μg mL\(^{-1}\)) were evaluated against bacteria and fungi by disc diffusion method. X. strumarium essential oil significantly inhibited the growth of Gram-positive (*S. aureus* and *B. subtilis*) and Gram-negative (*K. pneumoniae*) bacteria (P< 0.05). MIC for *S. aureus*, *B. subtilis* and *K. pneumoniae* were 0.5±0.1, 1.3±0.0 and 4.8±0.0 μg mL\(^{-1}\) of essential oil, respectively. *S. aureus* was the most sensitive microorganism, because of its very low MIC. *P. aeruginosa* was slightly inhibited in the disc diffusion assay, and its MIC was 20.5±0.3 μg mL\(^{-1}\) of essential oil in the broth dilution assay. In addition, the essential oil significantly inhibited *C. albicans* and *A. niger* (P< 0.05), at all the assayed concentrations. MIC for *C. albicans* and *A. niger* were 55.2±0.0 and 34.3±0.0 μg mL\(^{-1}\) of essential oil, respectively (Sharifi-Rad et al., 2015).

This study research the chemical composition, antioxidant effect, antimicrobial activities and phytochemical analysis of X. strumarium fruits.

**MATERIALS AND METHODS**

**Plant Material**

Fresh fruits of X. strumarium were collected on December 2\(^{nd}\) 2015 from research field of Sari Agricultural and Natural Resources University (SANRU), located at 53° 04’ E and 36° 39’ N, and identified from flora resources and a voucher specimen (946510601), was deposited at the Herbarium of the Botany Department of SANRU.

**Isolation of the Essential Oil**

Air-dried fruits (75 g) were subjected to hydro-distillation for 3 hours, utilizing a Clevenger-type apparatus. The essential oil was dried over anhydrous sodium sulphate and stored in Eppendorf tubes, packed by aluminum foil in the dark, and stored at 4°C before gas chromatography, coupled to mass spectrometry (Ghahari et al., 2017).

**Gas Chromatography-Mass Spectrometry (GC/MS) Analysis**

GC/MS analysis was performed on a Thermoquest-Finnigan Trace GC/MS instrument, equipped with a fused silica capillary DB-5 column (30 m×0.25 mm iD, film thickness 0.25 μm). The oven temperature was raised from 60 to 250°C at a rate of 5 °C min\(^{-1}\), and then held at 250°C for 10 minutes; transfer line temperature was 250°C. Helium was employed as the carrier gas, at a flow rate of 1.1 mL min\(^{-1}\); split ratio was, 1/10. The quadrupole mass spectrometer was scanned over the 40-460 amu, with an ionizing voltage of 70 eV, as well as an ionization current of 150 μA, Inlet temperature was 250°C.

**Antioxidant Assays**

The 0.5 g dry fruits were homogenized at 4°C in 1 mL of extraction buffer [50 mM sodium phosphate buffer (pH= 7.0), 1 mM EDTA and 1% PVP] with mortar and pestle.
The homogenate was then centrifuged (Eppendorf centrifuge 5430R) at 10,000×g for 15 minutes and the supernatant fraction was utilized for the determination of enzyme activities and protein contents (Gapińska et al., 2008).

**Determination of Catalase (CAT)**

Catalase was assayed by measuring the initial rate of disappearance of hydrogen peroxide, according to the method of Chance and Maehly (1955). The reaction mixture contained 2.5 mL of 50 mM phosphate buffer (pH= 7.0), 0.1 mL of 1% H₂O₂ and 50 µL of enzyme extract, diluted in order to keep the measurements within the linear range of the analysis. The absorbance of the reaction mixture was measured at 240 nm by spectrophotometer (Biochrom WPA Biowave II UV/Visible), and the decrease of H₂O₂ was due to decline in absorbance at 240 nm. The activity was expressed as µmole activity mg⁻¹ protein.

**Determination of Guaiacol Peroxidase (GPX)**

Guaiacol Peroxidase (GPX) activity was determined according to the method of Upadhyaya et al. (1985). The reaction mixture contained 2.5 mL of 50 mM phosphate buffer (pH= 7.0), 1 mL of 1% H₂O₂, 1 mL of 1% guaiacol and 20 µL of enzyme extract. The absorbance of the reaction mixture was measured at 470 nm by spectrophotometer (Biochrom WPA Biowave II UV/Visible), and the increase in absorbance at 470 nm was followed for 1 min. The activity was expressed as mmol activity mg⁻¹ protein.

**Determination of SuperOxide Dismutase (SOD)**

The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of Nitro Blue Tetrazolium (NBT), according to the method of Giannopolitis and Ries (1997). The 3 mL reaction mixture, contained 50 mM phosphate buffer (pH= 7.8), 13 mM methionine, 75 µM NBT, 2 µM riboflavin, 0.1 mM EDTA, and 50 µL enzyme extract. Riboflavin was added last, and the tubes were shaken and placed 30 cm beneath a light bank, comprising two 15W fluorescent lamps for 15 minutes. The absorbance of the reaction mixture was read at 560 nm by spectrophotometer (Biochrom WPA Biowave II UV/Visible). A non-irradiated reaction mixture did not develop color, and served as control. The activity was expressed as U mg⁻¹ protein.

**Protein Determination**

Protein concentration was determined based on the method proposed by Bradford (1976), using Bovine Serum Albumin (BSA) as standard protein.

**2, 2-Di-Phenyl-1-Picryl Hydrazyl (DPPH) Scavenging Assay**

The antiradical activity of the essential oil was determined by spectrophotometer, employing the method of Liyana-Pathirana and Shahidi (2005). A solution of 0.135 mM DPPH in methanol was prepared, and 1.0 mL of this solution was mixed with 1.0 mL of essential oil in methanol containing 40-270 µg of the essential oil. The reaction mixture was vortexed thoroughly, and left in the dark at room temperature for 30 minutes. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was utilized as reference. The ability to scavenge DPPH radical was calculated using the following equation:

\[
\text{DPPH}^\circ \text{scavenging assay (\%)} = \frac{[\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}]}{\text{Abs}_{\text{control}}} \times 100
\]

Where \(\text{Abs}_{\text{control}}\) is the absorbance of DPPH radical+methanol; and \(\text{Abs}_{\text{sample}}\) is the absorbance of DPPH radical+sample essential oil.

The radical scavenger activity was expressed in terms of the amount of antioxidants necessary to decrease the initial DPPH absorbance by 50% (IC₅₀). The IC₅₀ value for
each sample was determined graphically by plotting the percentage disappearance of DPPH as a function of the sample concentration.

**Phytochemical Analysis**

Total Alkaloid Content (TAC) of the test sample was determined by combining methods of Kaufman et al. (1999) and Lucio et al. (1997). Atropine was used as standard. Extraction of the alkaloid was carried out using the Soxhlet apparatus. The sample was moistened with sufficient amount of 50% ethanol for 24 hours. The ethanol extract was filtered, and then evaporated under vacuum evaporator. The residue was dissolved in 5% (w/v) sulfuric acid aqueous solution and was made colorless by adding diethyl ether. The solution was alkalinized to a pH higher than 9 with sodium hydroxide (10N), and was extracted twice with chloroform. The chloroform phase was separated and completely evaporated under vacuum at a temperature lower than 50°C. The residue obtained in this way, was dissolved in methanol and the absorbance of the reaction mixture was measured at 258 nm by spectrophotometer (Biochrom WPA Biowave II UV/Visible). The calibration curve was primed by preparing different concentrations of atropine standard solution at 258 nm. Based on the measured absorbance, the concentration of alkaloid content of extract was expressed as mg of atropine equivalents g⁻¹ dry matter.

Total Phenolic Content (TPC) of the test sample was determined employing the Folin-Ciocalteu method of Yu et al. (2002), using gallic acid as standard. Briefly, 900 µL of double distilled water was added to 100 µL of methanolic solution of test sample (100 µg mL⁻¹). To this, 500 µL of Folin-Ciocalteu reagent was added. This was followed by the addition of 1.5 mL of 20% sodium carbonate. The volume of mixture was made up to 10 mL with distilled water. The mixture was thereafter incubated for 2 hours at room temperature. After 2 hours of reaction, the absorbance was determined using spectrophotometer (Biochrom WPA Biowave II UV/Visible) at 725 nm. The same procedure was repeated for the standard solutions of gallic acid. Based on the measured absorbance, the concentration of phenolic content was read from the calibration line. Finally, the total phenolic content of methanolic extract was expressed as mg Gallic Acid Equivalents (GAE) g⁻¹ dry matter.

Colorimetric aluminum chloride method was employed for the determination of flavonoid (Chang et al. 2002). About 0.5 mL of X. strumarium fruits extract (1:10 g mL⁻¹) in methanol was separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate, and 2.8 mL of distilled water. The extract was kept at room temperature for 30 minutes; thereafter, the absorbance of the reaction was measured at 415 nm with a Biochrom WPA Biowave II UV/Visible spectrophotometer with Life Science Methods. The calibration curve was prepared by preparing quercetin solutions at concentrations of 12.5 to 100 µg mL⁻¹ in methanol. Then, the total flavonoid content was expressed as mg of quercetin equivalents g⁻¹ of dry sample.

**Antibacterial Screening**

The following microorganisms were utilized: Bacillus subtilis PTCC 1023, Staphylococcus aureus PTCC 1431, Rathayibacter toxicus ICMP 9525, Escherichia coli PTCC 1330, Pseudomonas aeruginosa PTCC 1074, Pseudomonas syringae subs. syringae ICMP 5089, Pseudomonas viridiflava ICMP 2848 and Xanthomonas campestris pv. campestris ICMP 13.

**Disc Diffusion Method**

The antibacterial activity of the essential oil was evaluated by disc diffusion method using Mueller-Hinton agar (Ghahari et al., 2017), and determination of inhibition zones of the essential oil of X. strumarium fruits. The filter paper discs of 6 mm diameter (Padtan, Iran) were sterilized then impregnated
with 20 µL of essential oil. The sterile impregnated discs were placed on the agar surface with flamed forceps and gently pressed down to ensure complete contact of the disc with the agar surface. The incubation condition was 37°C for quality control strains and 27°C for plant bacteria for 24 hours. Gentamicin was used as a positive standard control and clean disk (without any compound) was used as negative control. All experiments were tested in triplicate and the results were reported as mean±SD.

The antibacterial activity was studied by determining the Minimum Inhibitory Concentration (MIC), employing the broth dilution method (Barry, 1976). Each strain was tested with an essential oil serially diluted in Luria broth, to obtain concentrations ranging from 100 to 0.8 µg mL⁻¹. The samples were thereafter stirred, inoculated with 50 µg mL⁻¹ of physiologic solution containing 5×10⁸ microbial cells, and incubated at 37°C for quality control strains, and 27°C for plant bacteria for 24 hours.

A number of wells were reserved on each plate for sterility control (no inoculum), inoculum viability (no essential oil added), and the positive control (Gentamicin). The MIC was defined as the lowest concentration of essential oil that visibly inhibited the growth of bacterial spots. The assays were carried out in triplicate.

To determine the MBC, 10 µL of aliquot broth were taken from each well, and plated in Mueller–Hinton agar for 24 hours at 37°C for quality control strains, and 27°C for plant bacteria. The MBC represents the concentration required to kill 99.9% or more of the initial inoculum (Bosio et al., 2000). The assays were carried out in triplicate.

**Antifungal Activity**

The following microorganisms were used: *Pyricularia oryzae*, *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, *Alternaria alternate*, *Botrytis cinerea* and *Rhizoctonia solani*.

The antifungal property of the essential oil was tested by agar-well diffusion method (Ghahari et al., 2015). Potato Dextrose Agar (PDA) was seeded with tested fungus. Sterile paper discs (6 mm diameter, Padtan, Iran) were impregnated with 25 µL of the essential oil of *X. strumarium* fruits. The sterile impregnated discs were placed on the surface of the seeded agar plate. The incubation conditions used were 28°C and 70% RH for 12-14 days for *Pyricularia oryzae* and 7-9 days for *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, *Alternaria alternate*, *Botrytis cinerea* and *Rhizoctonia solani*. Antifungal activity was visualized as a zone of inhibition of fungal growth around the paper disc and the results were reported as mean±SD after three repeats. Pathogen grown on PDA without plant oil was used as control.

**Minimum Inhibitory Concentration (MIC)**

In order to investigate the antifungal activity, a modified micro dilution technique was utilized. The fungal spores were washed from the surface of agar plates with sterile saline containing 0.1% Tween 20 (v/v). The spore suspension was adjusted with sterile saline to a concentration of approximately 1×10⁵ spores per mL. The inoculums were stored at 4°C for further use. Dilutions of the inoculums were cultured on solid PDA, in order to verify the absence of contamination and also check the validity of the inoculums. The Minimum Inhibitory Concentration (MIC) was performed by an essential oil serially diluted in potato dextrose broth, to obtain concentrations ranging from 100 to 0.8 µg mL⁻¹. The sample was previously sterilized with a 0.22 µm Ministart (Sartorius). The sample was inoculated with 100 µL of fungal spores containing 1×10⁵ microbial cells, and incubated for 7 days for *P. oryzae* and 3 days for other fungus at 28°C. Pathogen growth on potato dextrose broth without plant essential oil was used as control (Ghahari et al., 2017).

**Statistical Analysis**

Essential oil was extracted and tested in triplicate for chemical analysis and bioassays. Data were subjected to Analysis Of Variance (ANOVA), following an entirely randomized
design to determine the Least Significant Difference (LSD) at P< 0.05 using SPSS statistical software package (SPSS v. 11.5, IBM Corporation, Armonk, NY, USA). All results were expressed as mean±SD.

RESULTS AND DISCUSSION

Essential Oil Compositions

The hydro-distillation of X. strumarium fruits provided yellow essential oil in 1.02% (w/w) yield, based on the dry weight of the plant fruits. Thirty six components were identified representing 97.89% of the total essential oil. The essential oil compositions are shown in Table 1, where compounds are listed in order of their elution on the DB-5 column. GC-MS analysis revealed that the major constituents of the essential oil were methyl linoleate (40.64%), methyl oleate (13.12%), methyl palmitate (12.43%), and intermedeol<neo-> (9.68%). Sharifi-Rad et al. (2015) reported the main components including cis-β-guaiene (34.2%), limonene (20.3%), borneol (11.6%) and bornyl acetate (4.5%) from the essential oil of fresh X. strumarium Linn. leaves grown in Iran. Scherer et al. (2010) studied the X. strumarium leaf essential oil from São Paulo, Brazil. Among the 24 components identified in that work, β-guaiene was the most abundant (79.6%). Esmaeil et al. (2006) collected X. strumarium plants at full flowering stage, from Khoramabad, Lurestan Province, Iran, and obtained the essential oil from stems and leaves. They reported that 22 compounds (86.4%) were identified in the stem essential oil, among which bornyl acetate (19.5%), limonene (15.0%) and β-selinene (10.1%) were the most abundant. In the leaf essential oil, 28 components were identified (85.2%) and then characterized by higher amounts of limonene (24.7%) and borneol (10.6%). Although the above compounds have already been reported from X. strumarium leaf essential oil, methyl linoleate (40.64%), methyl oleate (13.12%), methyl palmitate (12.43%), and intermedeol< neo-> (9.68%) compounds are introduced for the first time in this study from X. strumarium fruit essential oil.

<p>| Table 1. Essential oil compositions of X. strumarium fruits. |
|---------------------------------|--------|--------|--------|</p>
<table>
<thead>
<tr>
<th>Compound</th>
<th>Rt&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Ri&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-n-Pentylfuran</td>
<td>5.09</td>
<td>993.41</td>
<td>0.12</td>
</tr>
<tr>
<td>Mesitylene</td>
<td>5.78</td>
<td>1026.4</td>
<td>0.04</td>
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<tr>
<td>(E)-2-Nonenal</td>
<td>8.92</td>
<td>1169.1</td>
<td>0.07</td>
</tr>
<tr>
<td>(E)-Cinnamaldehyde</td>
<td>11.93</td>
<td>1278.8</td>
<td>0.11</td>
</tr>
<tr>
<td>Elemene&lt;beta-&gt;</td>
<td>14.86</td>
<td>1394.1</td>
<td>0.12</td>
</tr>
<tr>
<td>n-Tetradecane</td>
<td>14.96</td>
<td>1398</td>
<td>0.12</td>
</tr>
<tr>
<td>n-Pentadecane</td>
<td>17.31</td>
<td>1494.3</td>
<td>0.16</td>
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<tr>
<td>Phenol, 2,4-di-tert-butyl-</td>
<td>17.89</td>
<td>1518.8</td>
<td>0.5</td>
</tr>
<tr>
<td>Elemol</td>
<td>18.7</td>
<td>1553.4</td>
<td>0.39</td>
</tr>
<tr>
<td>Caryophyllene oxide</td>
<td>19.49</td>
<td>1587.2</td>
<td>0.34</td>
</tr>
<tr>
<td>Viridiflorol</td>
<td>19.73</td>
<td>1597.4</td>
<td>0.38</td>
</tr>
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<td>Isoaromadendrene oxide</td>
<td>20.1</td>
<td>1613.9</td>
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<tr>
<td>Diapicidrène-1-oxide</td>
<td>20.66</td>
<td>1639</td>
<td>1.92</td>
</tr>
<tr>
<td>Selina,3,11-dien-6-alpha-o</td>
<td>20.79</td>
<td>1644.8</td>
<td>1.61</td>
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<tr>
<td>β-Eudesmol</td>
<td>21.05</td>
<td>1656.5</td>
<td>1.35</td>
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<td>Intermedeol&lt;neo-&gt;</td>
<td>21.17</td>
<td>1661.9</td>
<td>9.68</td>
</tr>
<tr>
<td>Elemol acetate</td>
<td>21.55</td>
<td>1678.9</td>
<td>1.2</td>
</tr>
<tr>
<td>Eudesma-4,11-dien-2-ol</td>
<td>22.31</td>
<td>1714</td>
<td>1.25</td>
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<tr>
<td>Methyl tetradecanoate</td>
<td>22.55</td>
<td>1725.6</td>
<td>0.58</td>
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<tr>
<td>trans-Z-α-Bisabolene epoxide</td>
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<td>1730.4</td>
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<td>1792.8</td>
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<td>Hexahydrofarnesyl acetone</td>
<td>25.04</td>
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<td>Methyl palmitoleate</td>
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<td>1906.2</td>
<td>0.59</td>
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<tr>
<td>Methyl palmitate</td>
<td>26.69</td>
<td>1929</td>
<td>12.43</td>
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<td>27.45</td>
<td>1968.4</td>
<td>2.99</td>
</tr>
<tr>
<td>Ethyl palmitate</td>
<td>27.98</td>
<td>1995.9</td>
<td>0.36</td>
</tr>
<tr>
<td>Manoyl oxide</td>
<td>28.05</td>
<td>1999.5</td>
<td>0.98</td>
</tr>
<tr>
<td>Methyl linoleate</td>
<td>29.91</td>
<td>2099.5</td>
<td>40.64</td>
</tr>
<tr>
<td>Methyl oleate</td>
<td>30</td>
<td>2104.5</td>
<td>13.12</td>
</tr>
<tr>
<td>10-Octadecenoic acid, methyl ester</td>
<td>30.08</td>
<td>2109</td>
<td>1.35</td>
</tr>
<tr>
<td>Methyl octadecanoate</td>
<td>30.44</td>
<td>2129.2</td>
<td>1.03</td>
</tr>
<tr>
<td>Ethyl linoleate</td>
<td>31.06</td>
<td>2164</td>
<td>0.7</td>
</tr>
<tr>
<td>Ethyl Oleate</td>
<td>31.17</td>
<td>2170.2</td>
<td>0.36</td>
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<tr>
<td>n-Tricosane</td>
<td>33.41</td>
<td>2300</td>
<td>0.3</td>
</tr>
<tr>
<td>n-Tetracosane</td>
<td>35.06</td>
<td>2340</td>
<td>0.29</td>
</tr>
<tr>
<td>n-Pentacosane</td>
<td>36.64</td>
<td>2380</td>
<td>0.34</td>
</tr>
<tr>
<td>Total identified</td>
<td></td>
<td></td>
<td>97.89</td>
</tr>
</tbody>
</table>

<sup>a</sup>Retention rate, <sup>b</sup>Retention Index.

Enzymes Activity and Protein Content

Plants have evolved antioxidant pathways that are usually sufficient to protect them from oxidative damage during periods of normal growth and moderate stress. Both enzymatic and non-enzymatic systems protect tissue from activated oxygen species, generated as the result of external environmental stresses, such as chilling, drought and air pollution. Some of
the enzymatic antioxidant defense systems include SuperOxide Dismutase (SOD), Catalase (CAT), and Guaiacol Peroxidase (GPX) (Kang and Saltveit, 2001). In this study, the activity of 3 enzymes (CAT, GPX and SOD) was assayed. In addition, protein content was determined utilizing bovine serum albumin as a standard. The results are shown in Table 2. As shown, the analysis of free radical scavenging activities of the X. strumarium fruits revealed antiradical activity of 32.766 µmole activity/mg protein in catalase, 5.567 mmol activity mg⁻¹ protein in guaiacol peroxidase and 1.714 U mg⁻¹ protein in superoxide dismutase. Investigation of the activity of these 3 enzymes (CAT, GPX and SOD) in X. strumarium plant was done for the first time in this research.

**DPPH-Radical Scavenging Assay**

The effect of antioxidants on DPPH• is thought to be due to their hydrogen-donating ability (Brighente et al., 2007). Figure 1 illustrates the dose-response curve of DPPH radical scavenging activity of the different concentrations of the essential oil of X. strumarium fruits, compared with ascorbic acid. As shown, it was evident that the essential oil did show proton-donating ability, and could serve as a free radical inhibitor or scavenger, acting possibly as primary antioxidant. The free radical scavenging capacity of the essential oil was determined with an IC₅₀ value of 138.87 µg mL⁻¹.

**Phytochemical Analysis**

The alkaloid extracts gained from medicinal plants have biological activities, comprising antimalarial, antimicrobial, anti-hyperglycemic, anti-inflammatory and pharmacological effects (Tackie et al., 1993). The determination of total alkaloid content was based on the absorbance value of X. strumarium fruits solution, followed by comparing with the standard solution of atropine equivalents. The standard curve of

**Table 2. Determination of Catalase, Guaiacol Peroxidase, Superoxide Dismutase and Protein content of X. strumarium fruits.**

<table>
<thead>
<tr>
<th>Xanthium Strumarium Linn. fruits</th>
<th>Content</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>32.766</td>
<td>µmole Activity mg⁻¹ protein</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>5.567</td>
<td>mmole Activity mg⁻¹ protein</td>
</tr>
<tr>
<td>Superoxide Dismutase</td>
<td>1.714</td>
<td>U mg⁻¹ protein</td>
</tr>
<tr>
<td>Protein</td>
<td>3.034</td>
<td>mg g⁻¹ dry weight</td>
</tr>
</tbody>
</table>

**Figure 1.** DPPH radical scavenging activity of fruits essential oil of X. Strumarium.
atropine was done by using atropine concentration ranging from 12.5 to 100 μg mL\textsuperscript{-1}. The following equation expressed the absorbance of atropine standard solution as a function of concentration:

\[ Y = 0.143x - 0.037, \quad R^2 = 0.9865 \]

Where, \( x \) is the absorbance and \( Y \) is the quercetin equivalent (mg g\textsuperscript{-1}). The total alkaloid content of X. strumarium fruits was 0.54 mg atropine equivalents g\textsuperscript{-1} dry matter.

**Total Flavonoid Content of the Extract**

It has been identified that flavonoids display antioxidant effect and their efficacy on human health and nutrition are remarkable. The mechanisms of action of flavonoids are via scavenging or chelating process (Pourmorad et al., 2006). The determination of total flavonoid content was based on the absorbance value of X. strumarium fruits solution that react with aluminum chloride reagent, followed by comparing with the standard solution of quercetin equivalents. The standard curve of quercetin was done by using quercetin concentration ranging from 12.5 to 100 μg mL\textsuperscript{-1}. The following equation expressed the absorbance of quercetin standard solution as a function of concentration:

\[ Y = 0.0056x + 0.1764, \quad R^2 = 0.9878 \]

Where, \( x \) is the absorbance and \( Y \) is the quercetin equivalent (mg g\textsuperscript{-1}). The flavonoid content of the extract was 20.11 mg of quercetin equivalents g\textsuperscript{-1} of dry sample.

Phenolic compounds obtained from plants are a class of secondary metabolites which act as antioxidant or free radical terminators. So, it is required to specify the total amount of phenols in the plant under study (Kumar et al., 2011). The determination of total phenolic content was based on the absorbance value of X. strumarium fruits solution (100 μg mL\textsuperscript{-1}) that react with Folin-Ciocalteu reagent, followed by comparing with the standard solution of gallic acid equivalents. The standard curve of gallic acid was done by using gallic acid concentration ranging from

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Table 3. Minimal Inhibitory Concentration (MIC, μg mL\textsuperscript{-1}) and Minimum Microbicidal Concentration (MBC, μg mL\textsuperscript{-1}) of X. Strumarium fruits essential oil against bacteria.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth inhibition zone (mm) ( a )</th>
<th>Gentamicin</th>
<th>MIC (MBC) ( b ) (μg mL\textsuperscript{-1})</th>
<th>Gentamicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis</td>
<td>12.90±0.10</td>
<td>26.00±1.70</td>
<td>100 (&gt; 100)</td>
<td>6.24</td>
</tr>
<tr>
<td>S. aureus</td>
<td>13.10±0.15</td>
<td>20.30±1.50</td>
<td>50 (100)</td>
<td>3.12</td>
</tr>
<tr>
<td>R. toxicus</td>
<td>14.40±0.10</td>
<td>NS</td>
<td>25 (50)</td>
<td>NS*</td>
</tr>
<tr>
<td>E. coli</td>
<td>11.60±0.15</td>
<td>19.60±1.10</td>
<td>&gt;100</td>
<td>1.56</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>12.00±0.00</td>
<td>15.60±0.50</td>
<td>100 (&gt; 100)</td>
<td>12.48</td>
</tr>
<tr>
<td>P. syringae</td>
<td>13.30±0.20</td>
<td>NS</td>
<td>50 (100)</td>
<td>NS</td>
</tr>
<tr>
<td>P. viridiflava</td>
<td>11.50±0.50</td>
<td>NS</td>
<td>100 (&gt; 100)</td>
<td>NS</td>
</tr>
<tr>
<td>X. campestris pv. Campestris</td>
<td>9.50±0.05</td>
<td>NS</td>
<td>&gt; 100</td>
<td>NS</td>
</tr>
</tbody>
</table>

\( a \) Tested at 20 μg disc\textsuperscript{-1}; \( b \) MBC is reported in brackets when different from MIC, c Not Specified.

Table 4. Disc diffusion method and Minimal Inhibitory Concentration (MIC, μg mL\textsuperscript{-1}) of X. strumarium fruits essential oil against fungi.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Growth inhibition zone (mm) ( a )</th>
<th>MIC (μg mL\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyricularia oryzae</td>
<td>20.30±0.15</td>
<td>12.5</td>
</tr>
<tr>
<td>Fusarium oxysporum</td>
<td>15.75±0.25</td>
<td>50</td>
</tr>
<tr>
<td>Alternaria alternata</td>
<td>-</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Sclerotinia sclerotiorum</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>Botrytis cinerea</td>
<td>-</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Rhizoctonia solani</td>
<td>-</td>
<td>&gt; 100</td>
</tr>
</tbody>
</table>

- : Not inhibitory effect, \( a \) Tested at 25 μg disc\textsuperscript{-1}.  

---

...
12.5 to 100 µg mL\(^{-1}\). The following equation expressed the absorbance of gallic acid standard solution as a function of concentration:
\[ Y = 0.0954x + 0.196, R^2 = 0.9973 \]
Where \( x \) is the absorbance and \( Y \) is the gallic acid equivalent (mg g\(^{-1}\)). The Total Phenolic Content (TPC) of \( X. \) strumarium fruits was 54.44 mg Gallic Acid Equivalents (GAE) g\(^{-1}\) dry matter. These results are in agreement with the outcome of the studies done by Rad et al. (2013), Rajashekar et al. (2011) and Eftekhar et al. (2010).

**Antibacterial Screening**

Table 3 illustrates the disk diffusion method, \( MIC \) and \( MBC \) values of the essential oil of \( X. \) strumarium fruits against tested bacteria. The essential oil of \( X. \) strumarium fruits showed maximum activity against \( R. \) toxicus with \( MIC= 25 \) µg mL\(^{-1}\), followed by \( S. \) aureus and \( P. \) syringae subsp. syringae (MIC= 50 µg mL\(^{-1}\)), \( B. \) subtilis, \( P. \) viridiflava and \( P. \) aeruginosa (MIC= 100 µg mL\(^{-1}\)), \( E. \) coli and \( X. \) campestris pv. Campestris (MIC= > 100 µg mL\(^{-1}\)), respectively. The lower susceptibility of gram-negative bacteria to the essential oil may be explained in terms of diffusion limitations of essential compounds, through their external membrane, owing to the presence of a hydrophilic barrier. Although this barrier is not totally impermeable, it however hinders the transport of macromolecules and hydrophobic components (Ghahari et al., 2017; Pierozan et al., 2009).

**Antifungal Activity**

Disk Diffusion Method and Minimum Inhibitory Concentration (MIC)

The antifungal activity of the essential oil against six pathogenic fungi is shown in Table 4. The maximum antifungal activity was observed against \( P. \) oryzae (MIC= 12.5 µg mL\(^{-1}\)), followed by \( F. \) oxysporum (MIC= 50 µg mL\(^{-1}\)), \( S. \) sclerotiorum (MIC= 100 µg mL\(^{-1}\)), \( B. \) cinerea, \( A. \) alternata and \( R. \) solani (MIC= > 100 µg mL\(^{-1}\)), respectively. The results of the bioassays (Tables 3 and 4) showed that the essential oil exhibited moderate to strong antibacterial and antifungal activities against all tested pathogens.

**CONCLUSIONS**

Following and confirming the work of other researchers on the efficacy of \( X. \) strumarium extract on various pathogens (Khuda et al., 2012; Bahraminejad, 2012; Saha et al., 2012; Scherer et al., 2009; Butu et al., 2013; Scherer et al., 2010; Sharifi-Rad et al., 2015), in this study, the antifungal activities of \( X. \) strumarium fruits essential oil on Pyricularia oryzae (rice blast pathogen), Fusarium oxysporum (fungal pathogen of tomato at the worldwide green houses and open fields) and Sclerotinia sclerotiorum (an important fungal disease of sunflower, cucumber and so on) were evaluated for the first time. Furthermore, enzymes activity and phytochemical analysis of \( X. \) strumarium fruits were done in this research. Results indicated that \( X. \) strumarium fruits possessed good antioxidant activity which may be due to the presence of polyphenolic compounds in fruits. In general, our results suggest that this plant may be a potential source of biocide for economical and environmentally friendly disease control strategies. Furthermore, this plant may be a good candidate for further biological and pharmacological investigations.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


فول (44/34 میلی گرم/گرم) و فلاورونید (11/20 میلی گرم/گرم) در میوه‌های گیاه توق را مورد تایید قرار دادند. نتایج نشان داد، این گیاه می‌تواند بصورت بالقوه به عنوان یک منبع زیست کننده برای کنترل بیماری‌های گیاهی مورد استفاده قرار گیرد که علاوه بر سازگاری با محیط زیست بودن، مقرن به صرفه نیز خواهد بود. همچنین ممکن است موضوع مناسب برای تحقیقات بیولوژیکی و دارویی باشد.