PRELIMINARY PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF BIOACTIVE CONSTITUENTS IN THE ETHANOLIC EXTRACT OF EMPTY FRUIT BUNCHES


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ARTICLE DETAILS

ABSTRACT

For thousands of years plants have been an essential medicinal source with qualities. Empty fruit bunches (EFB) have medicinal values. Ten grams of powdered sample was extracted with 50 mL ethanol overnight and filtered through ash less filter paper, this plant’s ethanol extract has been analyzed using Gas Chromatography–Mass Spectrometry (GC-MS), while the compound mass spectra contained in the extract has been matched with the National Institute of Standards and Technology (NIST) library. The GC-MS analysis provided different peaks determining the presence of eight different phytochemical compounds namely 10-Undecenoic acid, Propanoic acid, Cyclopentane acetic acid hydrazide, 1,4-Cyclohexanedicarbonitrile. The compounds were identified by comparing their retention time and peak area with literature and by interpreting the mass spectra. Many of them have antioxidant, anti-inflammatory, antimicrobial, antifungal, antitumor, antiviral, antiinflamamtory, analgesic and anti diabetic properties.

KEYWORDS

EFB, Phytochemistry, GC-MS, 10-Undecenoic acid, Propanoic acid.

1. INTRODUCTION

The source of many plants (herbs and spices) can often be identified from the peak pattern of the chromatograms obtained directly from headspace analysis. Similarly, unique qualitative and quantitative patterns from a GC analysis will often help identify the source of many alcoholic beverages. The technique of fingerprint could really identify the false herbal products. The construction of chromatographic fingerprints aims at evaluating the quality of Herbal Medicines (Sermakkan and Thangapiran, 2012). Phoenix dactylifera L. (palm of date) is a monocotyledonous perennial, flowering and woody fruit species included in the Arecaceae family. Dates are a concentrated source of essential nutrients, vitamins, minerals, and carbohydrates, which are necessary for the maintenance of optimum health. Dates may be considered as an ideal food to provide a wide range of essential nutrients and potential health benefits. Because of its high nutritional value and its long life the date palm has been mentioned as the ‘tree of life’ (Medicine of family and Community, 2016). It’s one of the oldest known fruit crops and has been cultivated in North Africa and the Middle East for including many states of the Arabian Gulf countries especially in Iraq (El-far et al., 2018).

Date palm (Fig 1a) is the most successful and important subsistence crop in most of the hot arid desert regions. Generally, whole dates are harvested and marketed at three stages of development: mature firm (Khalal), full ripe (Rutab) and dry (Tamar). The decision for harvesting at one or other stage depends on cultivar characteristics, especially soluble tannins levels, climatic conditions and market demand (Awad, 2007). The date palm over the centuries has also provided a large number of other products which have been extensively used by man in all aspects of daily life. Because of the biology of the date palm, its cultivation has a number of unusual features that are not common in other perennial crops. The tree are pinnate and divided, each having a length varying between 4 m and 7 m (Amroune et al., 2015; Al-Msaaleem, 2020).

Empty fruit bunches (EFB) (Figure 1b) are an organic substrate; a by-product of palm oil mill processing, it was Remained after removal of palm fruits. There are small mill plantations with integrated facilities that utilize shredded EFB. However, the mills that utilize EFB are limited since the upfront investment cost for shredding and pressing facilities outweigh the benefits. Therefore, most of the EFBs are simply burned in the incinerators to produce fertilizer (Afizam et al., 2015). The fruit bunches are connected from bottom in the heart of palm (palmite) which is a vegetable harvested from the inner core and growing bud of certain palm trees. Harvesting of many uncultivated or wild single-stemmed palms results in palm tree death. When harvesting the cultivated young palm, the tree is cut down and the bark is removed, leaving layers of white fibers around the center core (Al-Abachi, 2019).

After pollination, bunches are often tied to the leaf stalks to support the weight of the fruit. Fruit thinning is sometimes practiced in date cultivation. Fruit thinning is used to decrease alternate bearing, increase fruit size, improve fruit quality, advance fruit ripening, and facilitate bunch management. Fruit thinning can be carried out through three ways: removal of entire bunches reduction in the number of fruits per bunch, and reduction in the number of fruit per strand. Cultivar, climate, and cultural practices influence the appropriate levels of fruit thinning. Bunches of dates are usually covered (bagged) with brown craft paper, white paper, or cotton or nylon mesh bags (Chao and Krueger, 2007). The refuse after stripping the bunches is used for mulching and manuring; the ash after burning is sometimes used in soap making (Atimmo and Bakre, 2003).

The composition of nutrients in Empty Fruit Bunch (EFB) contains 6.3% hydrogen, 0.2% sulfur, 48.8% carbon, 36.7% oxygen, 0.2% nitrogen, and 7.3% ash. So, the empty fruit bunches show potential as viable substrates for mushroom cultivation either alone or in combinations of both as substrate for the cultivation of Pleurotus ostreatus. Both substrates produce Pleurotusostreatusfruit bodies (Naïsaa et al., 2008).

2. SUBJECTS AND METHODS

2.1 Plant sample

Empty fruit bunches EFB were collected from households in Baghdad Province, Al-Tarmia area prior to production of empty fruit bunch. These empty fruit bunches are washed and dried under shade. The dried EFBs are manually chopped and cut into smaller pieces and later grinded to be used in the extraction methods.

2.2 Extraction Method

Required powder quantity (10 gm approximately) was weighed and transferred to a stopped flask and treated with ethanol (50 ml). Until the powder was fully immersed for the first 6 hours, the flask was shaken every hour and then put aside and shaken again after 24 hours. Repeat this process for 3 days, and then filter the extract. By using a vacuum distillation machine, the extract was collected and evaporated to dryness. The extract contains both polar and nonpolar components of the plant material and 2 μl of the sample solution was employed in GC-MS for analysis of different compounds (Gopinath et al., 2013; Ezhilan and Neelamegam, 2011).

2.3 Preliminary phytochemical screening

A chemical test is carried out on the powdered of Empty fruit bunches EFB and their extracts by using standard procedures to preliminary phytochemical screening, as follows (Yaseen et al., 2019; Richardson, 1985; Sofowora, 1993; Louis et al., 2018; Vaghasiya et al., 2011):

2.3.1 Test for Carbohydrate

Fehling’s and Benedict’s tests: were screened contents of carbohydrates. The protocol of Fehling’s test was carried out by adding 5 ml of Fehling’s A reagent to 5 ml of Fehling’s B reagent along with well mixing, then 2 ml of Fehling’s mixture was added to the same volume of crude extract sample and heated to boiling. Brick red deposits appear at the bottom of the test tube to confirm the presence of reducing sugars. In Benedict’s test when 2 ml of Benedict’s reagent mixed with crude extract sample and boiled, the reddish-brown precipitates denoted the presence of carbohydrates.

2.3.2 Test for Alkaloids

Wagner’s test: was used to check the presence of alkaloids. Two drops of reagent (Wagner’s reagent) should be added to 1 ml of aqueous extract. A yellow or brown precipitate indicates the presence of alkaloids.

2.3.3 Test for Steroids

Salkowski’s test: two drops of concentrated H₂SO₄ to adding to 1 ml of the tested solution (EFB) gradually on the test tube side. The development of red color confirms the existence of the steroids.

2.3.4 Test for Flavanoids

Ferric chloride test: 1 ml of the test solution mixed with few drops of neutral ferric chloride solution, formation of blackish red color indicates the presence of flavonoids.

2.3.5 Test for Tannin and phenolic compounds

Ferric chloride test: To 1 ml of extract, a few drops of 0.1% ferric chloride solution were added. Dark blue or greenish black color solution indicates the presence of tannins or phenolic compounds. While brown color indicates the presence of pseudotannins.

Dichromate test: to 1 ml of the extract solution, add 2 ml of 20% aqueous potassium dichromate solution, a yellow color precipitate indicates the presence of tannins and phenolic compounds.

2.3.6 Test for Saponins

Foam’s test: 2 ml of the alcoholic extract was shaken strongly for 10 seconds and permitted to stand. The formation of determined honeycomb like froth is the positive examination for the presence of saponin.

2.3.7 Test for Amino acids and proteins

Biuret test: It was used in qualitative testing of proteins and amino acids. This test protocol, two to three drops of 1% CuSO₄ (copper sulfate solution) were added to 1 ml of 40% NaOH solution till a blue color has appeared, then 1 ml of extract was added. The presence of protein (and amino acids) is demonstrated when a pink to purple color rose.

2.3.8 Test for Glicosides

Liebermann’s test: Crude extract was mixed with each of 2 ml of chloroform and 2 ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H₂SO₄ was added. A color change from violet to blue to green indicated the presence of steroidal nucleus, i.e., glycone portion of glycoside.

Sodium hydroxide test: The extract solution was mixed with equal amount of aqueous solution of 5% sodium hydroxide; formation of yellow color indicates the presence of glycosides.

2.3.9 Test for Lignin

Labat test: The test solution was mixed with gallic acid; it developed olive green color indicating the positive reaction for lignins (Joshi et al., 2013).

2.3.10 Test for terpenoids

Chloroform test: Crude extract was dissolved in 2 ml of chloroform and evaporated to dryness. To this, 2 ml of concentrated H₂SO₄ was added and heated for about 2 minutes. A grayish color indicated the presence of terpenoids.

2.4 GC-MS Analysis

The sample was analyzed in Ministry of Science and Technology/Department of Water & Ecology/Ecology research center. Interpretation of mass spectrum was conducted using the database of National Institute of Standards and Technology (NIST, USA). The database consists of more than 62,000 patterns of known compounds. The spectrum of the extract was matched with the spectrum of the known components stored in the NIST library. Empty fruit bunch GC-MS analysis was carried out in a GC system (Agilent 7890A series, USA). The flow rate of the carrier gas, helium (He) was set to be 1 ml min⁻¹, split ratio was 1:50. The injector temperature was adjusted at 250°C, while the detector temperature was fixed to 280°C. The column temperature was kept at 40°C for 1 min followed by linear programming to raise the temperature from 40 to 120°C (at 4°C min⁻² with 2 min hold time), 120°C to 170°C (at 6°C min⁻² with 1
min hold time) and 170 °C to 200 °C (at10 °C min⁻¹ with 1 min hold time). The transfer line was heated at 280 °C. Two microliter of FAME sample was injected for analysis. Mass spectra were acquired in scan mode (70 eV); in the range of 50–550 m/z (Hussien et al., 2017).

3. RESULTS

3.1 Chemical Investigation Results

Phytochemicals analysis of samples results displayed that the extract of Empty Fruit Bunch EFB contains carbohydrate, saponins, tannin and phenolic compounds (Table 1).

<table>
<thead>
<tr>
<th>Bioactive constituent</th>
<th>Test/s used for screening</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>Fehling’s test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Biuret’s test</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Ferric chloride test</td>
<td>–</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Wagner’s test</td>
<td>–</td>
</tr>
<tr>
<td>Steroids</td>
<td>Sallowki’s test</td>
<td>–</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam’s test</td>
<td>+</td>
</tr>
<tr>
<td>Tannin and phenolic compounds</td>
<td>Dichromate test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Ferric chloride test</td>
<td>+</td>
</tr>
<tr>
<td>Amino acids and proteins</td>
<td>Biuret test</td>
<td>–</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Liebermann’s test</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Sodium hydroxide test</td>
<td>–</td>
</tr>
<tr>
<td>Lignin</td>
<td>Labat test</td>
<td>–</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Chloroform test</td>
<td>–</td>
</tr>
</tbody>
</table>

The active principles are presented in (Table 2) and calculate with their retention time (RT), molecular formula (MF), molecular weight (MW), and concentration (%).

The Figure 2 the more detailed knowledge can be provided by gas chromatography combined with mass spectrometry (GC-MS) in qualitative research. The GC-MS analysis of Empty fruit bunch revealed the presence of eight compounds. (Table 1) showed the identified compounds possess many biological properties as shown in (Table 3). For instance, Formamide (R/T 2.053) Figure 3 and Succinaldehyde (R/T 13.970) Figure 4, they are organic compound that have on biological effectiveness.

**Table 2**: Components identified in the ethanol extract of empty fruit bunch EFB By GC-MS

<table>
<thead>
<tr>
<th>S. NO.</th>
<th>RT</th>
<th>Name of the compound</th>
<th>Molecular Formula MF</th>
<th>Molecular Weight MW</th>
<th>Peak area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.053</td>
<td>Formamide$S$</td>
<td>CH$_3$NO</td>
<td>45</td>
<td>1.20</td>
</tr>
<tr>
<td>2</td>
<td>13.970</td>
<td>SuccinylaldehydeButanediol</td>
<td>C$<em>6$H$</em>{12}$O$_2$</td>
<td>86</td>
<td>11.70</td>
</tr>
<tr>
<td>3</td>
<td>17.629</td>
<td>10-Underenoic acid, 2- (acetoxy), methyl ester</td>
<td>C$<em>6$H$</em>{12}$O$_4$</td>
<td>256</td>
<td>0.32</td>
</tr>
<tr>
<td>4</td>
<td>19.429</td>
<td>Propanoic acid, 2- methyl- $S$ Isobutyric acid $S$</td>
<td>C$<em>6$H$</em>{12}$O$_2$</td>
<td>88</td>
<td>1.67</td>
</tr>
<tr>
<td>5</td>
<td>19.562</td>
<td>Phosphorus trifluoride$S$</td>
<td>F$_3$P</td>
<td>88</td>
<td>0.38</td>
</tr>
<tr>
<td>6</td>
<td>20.651</td>
<td>Cyclopentane acetic acid</td>
<td>C$<em>6$H$</em>{12}$N$_2$O</td>
<td>142</td>
<td>6.71</td>
</tr>
<tr>
<td>7</td>
<td>20.874</td>
<td>1,4-Cyclohexanedicarboximide</td>
<td>C$<em>6$H$</em>{12}$N$_2$</td>
<td>134</td>
<td>0.70</td>
</tr>
<tr>
<td>8</td>
<td>21.302</td>
<td>(Z)-6-Pentadecen-1-ol$S$</td>
<td>C$<em>{16}$H$</em>{32}$O</td>
<td>226</td>
<td>77.33</td>
</tr>
</tbody>
</table>

**Table 3**: Activity of components identified in the ethanol extract of empty fruit bunch EFB By GC-MS

<table>
<thead>
<tr>
<th>S. NO.</th>
<th>Name of the compound</th>
<th>Molecular formula</th>
<th>Nature of compound</th>
<th>*Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Formamide</td>
<td>CH$_3$NO</td>
<td>Amide compound</td>
<td>No activity reported</td>
</tr>
<tr>
<td>2</td>
<td>Succinaldehyde</td>
<td>C$<em>6$H$</em>{12}$O$_2$</td>
<td>Aldehyde compound</td>
<td>No activity reported</td>
</tr>
<tr>
<td>3</td>
<td>10-Underenoic acid</td>
<td>C$<em>6$H$</em>{12}$O$_4$</td>
<td>unsaturated fatty acid</td>
<td>Antimicrobial, antifungal and antioxidant</td>
</tr>
<tr>
<td>4</td>
<td>Propanoic acid</td>
<td>C$<em>6$H$</em>{12}$O$_2$</td>
<td>carboxylic acid compound</td>
<td>Arachidonic acid-Inhibitor, Antioxidant,antibacterial,antitumour,antimicrobial, analgesic and antiinflammatory.</td>
</tr>
<tr>
<td>5</td>
<td>Phosphorus trifluoride</td>
<td>F,P</td>
<td>Phosphorus halide compound</td>
<td>Gas It is highly toxic</td>
</tr>
<tr>
<td>6</td>
<td>Cyclopetanone acetic acid hydrazide</td>
<td>C$<em>6$H$</em>{12}$N$_2$O</td>
<td>Nitrogen compound</td>
<td>Antimicrobial, antitumor, antiviral,Araachidonic Acid-Inhibitor,Inhibit Production of lactic Acid</td>
</tr>
<tr>
<td>7</td>
<td>1,4-Cyclohexanedicarboximide</td>
<td>C$<em>6$H$</em>{12}$N$_2$</td>
<td>heterocyclic compounds</td>
<td>Antibacterial, antifungal, anticancer,anti-inflammatory,anticonvulsant and antitubercular</td>
</tr>
<tr>
<td>8</td>
<td>(Z)-6-Pentadecien-1-ol</td>
<td>C$<em>{16}$H$</em>{32}$O</td>
<td>sesquiterpenes</td>
<td>Increase Zinc Bioavailability,OligoSporic charide Provider</td>
</tr>
</tbody>
</table>

*CActivity source: Dr. Duke’s Phytochemical and Ethnobotanical Database*
The researchers indicated that the oil palm wastes are found to contain phytochemicals which have anti-cancer, antioxidants and other vital biological activities. About 17–65 kg of carotenoids, 0.1–60 kg phenolic compounds, 0.6–39 kg sterols and 4.0–62 kg tocols could be extracted from these wastes which would not only boost the economy but also help improve human health and promote clean environments (Olori-Boateng, 2013). The study was assessing the phytochemistry of oil palm wastes and their pharmacological activities beneficial to the nutraceutical industry with the view of utilizing oil palm wastes for sustainable development.

The results of this study were in agreement with in which they analyze the effect of different extraction solvents in cellulose nanofibers (CNFs) isolation and properties (Sollikhin et al., 2019). FTIR spectra showed that hot water extraction for CNFs isolation was able to remove low-molecular weight carbohydrates (hemicellulose and pectin), whereas ethanol and ethanol/benzene extraction for CNFs isolation was able to remove tannin, fatty acids, and waxes. However, amorphous lignin was still present indicated with IR transmission peak at 1558 cm⁻¹. Carboxylic acids, esters, ketones, and benzyl units were the chemical compounds of CNFs, indicating the presence of cellulose, hemicellulose, and lignin in which long-chain fatty acids were the most dominant compounds. There were five thermal degradation peaks for ethanol- and hot water-pretreated CNFs thermal stability, whereas ethanol/benzene- and non-extraction-pretreated CNFs had four thermal degradation peaks. Solvent-pretreated CNFs had better thermal stability and higher char residue obtained above 85.1% than that of non-extraction-pretreated CNFs.

5. CONCLUSION

The GC-MS method is a direct and fast analytical approach for identification of terpenoids and steroids and only few grams of plant material is required. The importance of the study is due to the biological activity of some of these compounds. From the results obtained in this study, it could be concluded that empty fruit bunch possesses compounds have been identified by gas chromatography-mass spectrometry (GC-MS analysis). Thus, GC-MS analysis is the first step towards understanding the nature of active principles in this plant. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

REFERENCES


Awad, M.A., 2007. Increasing the rate of ripening of date palm fruit


