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RESEARCH ARTICLE

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

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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 namely10-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, anticonvulsant, analgesic and antidiabetic properties.

KEYWORDS

Empty fruit bunches (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 (Sermakkani and Thangapandian, 2012). *Phoenix dactylifera L.* (palm of date) is a monocotyledonous perennial, flowering andwoody 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 cropsand has been cultivated in North Africa andthe Middle East for including many states of the Arabian Gulf countries especially in Iraq (El-far et al., 2018).

Date palm (Figure 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 (*Tamr*).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 datepalm, its cultivation has a number of unusualfeatures that are not common in other perennialcrops.The tree are pinnate and divided, each having a length varyingbetween 4 m and 7 m (Amroune et al., 2015; Al-Mssallem, 2020).

Empty fruit bunchesEFB (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 (Afnizam et al., 2015). The fruit bunches is connected from bottom in the heart of palm (palmito) 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).

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After pollination, bunches are often tied tothe leaf stalks to support the weight of thefruit. Fruit thinning is sometimes practicedin date cultivation. Fruit thinning is used todecrease alternate bearing, increase fruit size,improve fruit quality, advance fruit ripening,and facilitate bunch management. Fruit thinningcan be carried out three ways: removalof entire bunches reduction in the numberof strands per bunch, and reduction in thenumber of fruit per strand. Cultivar, climate, and cultural practices influence the appropriatelevels of fruit thinning. Bunches of datesare 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 formulching and manuring; the ash after burning is sometimesused in soap making (Atinmo and Bakre, 2003).

The composition of nutrients in Empty Fruit Bunch (EFB) contain6.3% Hydrogen,0.2% sulfer, 48.8% carbon, 36.7% Oxygen,0.2% nitrogen and 7.3% ash .So, the empty fruit bunch show potential as viable substrates for mushroomcultivation either alone or in combinations of both as substrate for the cultivation of*Pleurotus*as both substrates produce *Pleurotusostreatus*fruit bodies (Nafissa et al., 2008).

Figure 1: Photographs of (a) date palm tree and (b) fruit bunch branch

Taking into consideration of the medicinal importance of the plant, the ethanol extract of *Hypericummysorense*was analyzed for the GC-MS. This work will help to identify the compounds of therapeutic value.GC-MS is one of the technique to identify the bioactive constituents of long chain branched chainhydrocarbons, alcohols, acids, esters, etc.

2. Subjects and Methods

2.1 Plant sample

Empty fruit bunches EFB were collected from housegarden in Baghdad Province Al-Tarmia areaprior to productionof empty fruit bunch. These empty fruit bunches are washed and dried under shade.The dried EFB are manually chopped and cut into smaller pieces and latergrinded to be used in the extraction methods.

2.2 Extraction Method

Required powder quantity (10 gmApprox) was weighed and transferred to a stopped flask and treated with ethanol (50 ml)until the powder was fullyimmersed,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 nonpolarcomponents of the plant material and 2 µl of the sampleof the solutions was employed in GC-MS for analysis ofdifferent 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 carbohydrate. 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 (Wangers' 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 H_2SO_4 to adding to 1ml 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: 1ml of the test solution mixed with few drops of neutral ferric chloride solution, formation of blackish red colour indicates the presence of flavonoids.

2.3.5 Test for Tannin and phenolic compounds

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

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

2.3.6 Test for Saponins

Foam's test: 2ml 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 colour has appeared, then 1 ml of extract was added. The presence of protein (and amino acids) is demonstrated when a pink to purple colour rose.

2.3.8 Test for Glycosides

Liebermann's test: Crude extract was mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H_2SO_4 was added. A colour change from violet to blue to green indicated the presence of steroidal nucleus, i.e., glycine portion of glycoside.

Sodium hydroxide test: The extract solution was mixed with equal amount of aqueous solution of 5% sodium hydroxide; formation of a yellow colour 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 colour indicating the positive reaction for lignins (Joshi et al., 2013).

2.3.10 Test for terpenoids

Chloroform test: Crude extract was dissolved in 2ml of chloroform andevaporated to dryness. To this, 2ml of concentrated $H_2SO_4$was added and heated for about 2 minutes. A grayish colour 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 bunchGC–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 beat 1 mL min^{-1}, split ratio was 1:50. The injector temperature was adjusted at 250°C, while the detector temperature was fixed to280◦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° $min^{-1}$with 2 min hold time), 120 C° to 170 C° (at 6 C° $min^{-1}$with 1

min hold time) and 170 C° to 200 C° (at10°C min⁻¹with 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 1: The preliminary phytochemical screening of the ethanolic extract of Empty fruit bunch EFB

Bioactive constituent	Test/s used for screening	Result
Carbohydrate	Fehling's test	+
	Biuret's test	+
Flavonoids	Ferric chloride test	–
Alkaloids	Wagner's test	–
Steroids	Salkowski's test	–
Saponins	Foam's test	+
Tannin and phenolic compounds	Dichromate test	+
	Ferric chloride test	+
Amino acids and proteins	Biuret test	–
Glycosides	Liebermann's test	–
	Sodium hydroxide test	–
Lignin	Labat test	–
Terpenoids	Chloroform test	–

(+) present, (–) absent

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, Form amide (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 EFBby GC-MS

S. NO.	RT	Name of the compound	Molecular Formula MF	Molecular Weight MW	Peak area %
1	2.053	Formamide$$ Carbamaldehyde $$ Methanamide$$ Formimidic acid	CH_3NO	45	1.20
2	13.970	SuccinaldehydeButanedial $$ Succinaldehyde $$ Succinic aldehyde $$ Succinic dialdehyde	$C_4H_6O_2$	86	11.70
3	17.629	10-Undecenoic acid, 2-(acetyloxy)-, methyl ester $$ Methyl 2-(acetyloxy)-10-undecenoate	$C_{14}H_{24}O_4$	256	0.32
4	19.429	Propanoic acid, 2-methyl- $$ Isobutyric acid $$ .alpha.-Methylpropanoic acid $$ .alpha.-Methylpropionic acid	$C_4H_8O_2$	88	1.67
5	19.562	Phosphorus trifluoride$$ Phosphorus(III) fluoride $$ Phosphorous-trifluoride- $$ Phosphorus fluoride	F_3P	88	0.38
6	20.651	Cyclopentane acetic acid hydrazide $$ 2-Cyclopentylacetohydrazide	$C_7H_{14}N_2O$	142	6.71
7	20.874	1,4-Cyclohexanedicarbonitrile$$ 1,4-Cyclohexanedicarbonitrile, trans- $$ trans-1,4-Dicyanocyclohexane	$C_8H_{10}N_2$	134	0.70
8	21.302	(Z)6-Pentadecen-1-ol$$ (6Z)-6-Pentadecen-1-ol	$C_{15}H_{30}O$	226	77.33

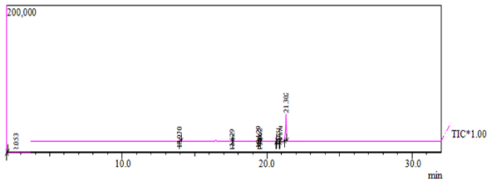

Figure 2: GC-MS chromatogram of the ethanol extract of empty fruit bunch EFB

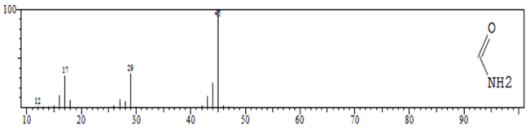

Figure 3: Mass spectrum of formamide

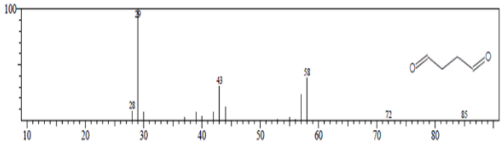

Figure 4: Mass spectrum of succinaldehyde

The 10-Undecenoic acid (R/T 17.629) Figure 5 unsaturated fatty acid owns several biological activities such as antibacterial, antifungal, antiviral (Herpes simplex virus) properties (Bourne et al., 1999). its derivatives were found to exhibit promising activity against both bacterial and fungal strains also affect cellular processes related to cancer (Oxime et al., 2016; Rahman et al., 2005). Propanoic acid (R/T 19.429) Figure 6 can be antioxidant, antibacterial, antitumour, antimicrobial, analgesic and antidiabetic properties (Dracheva et al., 2009). Phosphorus trifluoride (R/T 19.562) Figure 7. Gas it is colorless, slowly hydrolyzed by water, sensitive to air and moisture. Cyclopentane acetic acid hydrazide (R/T 20.651) Figure 8. The 1,4-Cyclohexanedicarbonitrile (R/T 20.874) Figure 9. (Z) 6-pentadecen-1-ol (R/T 21.302) Figure 10. It's an organic compound.

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

S. NO.	Name of the compound	Molecular formula	Nature of compound	*Activity
1	Formamide	CH_3NO	Amide compound	No activityreported
2	Succinaldehyde	$C_4H_6O_2$	Aldehyde compound	No activityreported
3	10-Undecenoic acid	$C_{14}H_{24}O_4$	unsaturated fatty acid	Antimicrobial, antifungal and antioxidant
4	Propanoic acid	$C_4H_8O_2$	carboxylic acid compound	Arachidonic acid-Inhibitor, Antioxidant,antibacterial, antitumour, antimicrobial, analgesic and antidiabetic.
5	Phosphorus trifluoride	F_3P	Phosphorus halide compound	Gas It is highly toxic
6	Cyclopentane acetic acid hydrazide	$C_7H_{14}N_2O$	Nitrogen compound	Antimicrobial, antitumor, antiviral,Arachidonic-Acid-Inhibitor,Inhibit Production of Uric Acid
7	1,4-Cyclohexanedicarbonitrile	$C_8H_{10}N_2$	heterocyclic compounds	Antibacterial, antifungal, anticancer,anti-inflammatory,anticonvulsant and antitubercular
8	(Z)6-Pentadecen-1-ol	$C_{15}H_{30}O$	sesquiterpenes	Increase Zinc Bioavailibility,Oligosaccharide Provider

*Activity source: Dr. Duke's Phytochemical and Ethnobotanical Database

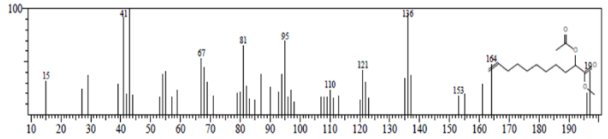

Figure 5: Mass spectrum of 10-undecenoic acid

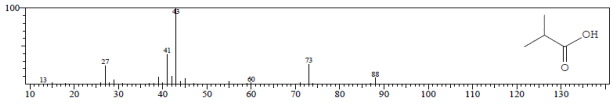

Figure 7: Mass spectrum of phosphorus trifluoride

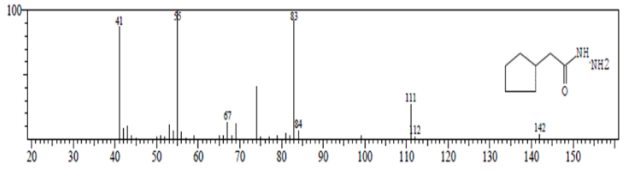

Figure 8: Mass spectrum ofcyclopentane acetic acid hydrazide

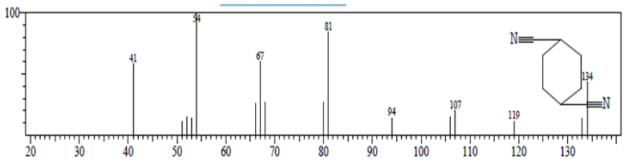

Figure 9: Mass spectrum of 1,4-cyclohexanedicarbonitrile

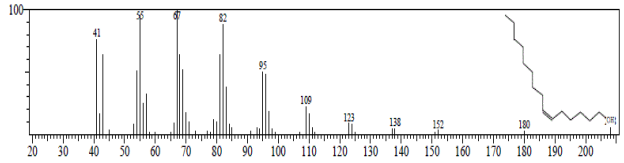

Figure 10: Mass spectrum of (Z)6-pentadecen

4. Discussion

The phenolic compounds are one of the largest most ubiquitous groups of plant metabolites. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds such as phenolic acid, tocopherols, flavonoid etc. (Maria et al., 2018). Tannins bind to proline rich protein and interfere with protein synthesis (Parbuntari et al., 2018). Saponins have the property of precipitating and coagulating red blood cell. Some of the characteristics of saponins include formation of foams in aqueous solutions, cholesterol binding properties, bitterness and hemolytic activity (Vaghasiya et al., 2011). Propanoic acid belong to Short-chain fatty acids is the principal metabolites produced. Potential effects of propionic acid on pathology and physiology have long been underestimated. It has been demonstrated that propionic acid lowers fatty acids content in liver and plasma, reduces food intake, exerts immunosuppressive actions and probably improves tissue insulin sensitivity (Bently, 2007). Synthesized various aroylpropionic acid derivatives containing 1,3,4-oxadiazole nucleus from its hydrazide derivatives. These compounds were tested *in vivo* for their anti-inflammatory activity and the compounds which showed activity comparable to the standard drug ibuprofen were screened for their analgesic, ulcerogenic and lipid peroxidation activities (Akhter et al., 2009).

Phosphorus trifluoride is safety profile it's considers moderately toxic by inhalation, a sever eye, skin and mucous membrane irritant (Phosphorus trifluoride, 2020). Many researchers studied the biological activity of hydrazide compounds and their derivatives, and many of them concluded that these compounds were effective against Antimycobacterial Agents such as, Evaluating a derivative in *vitro* antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv by broth dilution assay method (Joshi et al., 2008). As analgesic and anti-inflammatory agents (Manjunatha et al., 2010). As antiviral agents, human herpes viruses, including herpes simplex type 1 and 2 (HSV-1, HSV-2), *varicella zoster* virus (VZV), *Epstein-Barr virus* (EBV) and *human cytomegalovirus* (HCMV) are common and usually self-limiting in otherwise healthy individuals (Narang et al., 2012). Assasodilator agents (Silva et al., 2005). As antioxidant agents (Belkheiri et al., 2010). As hormone antagonist (LaFrate et al., 2008). As anticancer agent (Savini et al., 2004). The 1,4-Cyclohexanedicarbonitrile (R/T 20.874) Figure 9 possessantibacterial, antifungal, anticancer, anti-inflammatory, anticonvulsant and antitubercular properties (Faghih-mirzaei et al., 2018; Moustafa et al., 2014). (Z) 6-pentadecen-1-ol (R/T 21.302) Figure 10 it's an organic compound no biologicalactivity (Akilandeswari et al., 2015).

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 (Ofori-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 (Solikhin 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 benzoyl 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 8.51% than that of non-extracted-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 possesses8 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.

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