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RESEARCH ARTICLE

EXTRACTION AND CHARACTERIZATION OF CRUDE FIBER FROM KHIMP (LEPTADENIA PYROTECHNICA) AND DATE PALM TREE (PHOENIX DACTYLIFERAL.) AND SCREENING THEIR PHYTOCHEMICALS

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Abstract

Three samples of the plant (khimp, date palm tree leaves, and fiber) were studied to extract the crude fiber and phytochemicals. The study employed ordinary laboratory equipment together with Fourier Transformation Infrared (FTIR) spectroscopy and Ultra Violate –visible(UV-Vis) spectrophotometer. The results obtained showed that the fiber contents were 57.8%, 73.6 %, and 83.3 % for date palm tree leave, khimp, and date palm tree fiber respectively. 12 phytochemicals in all extracts were screened. The structure showed that the three samples contain cellulose, hemicelluloses, and lignin. It is concluded that the differential extraction method is valuable for the extraction of plant fiber and phytochemicals simultaneously.

Key Words: khimp, date palm, phytochemicals, differential, extraction.

1.Introduction

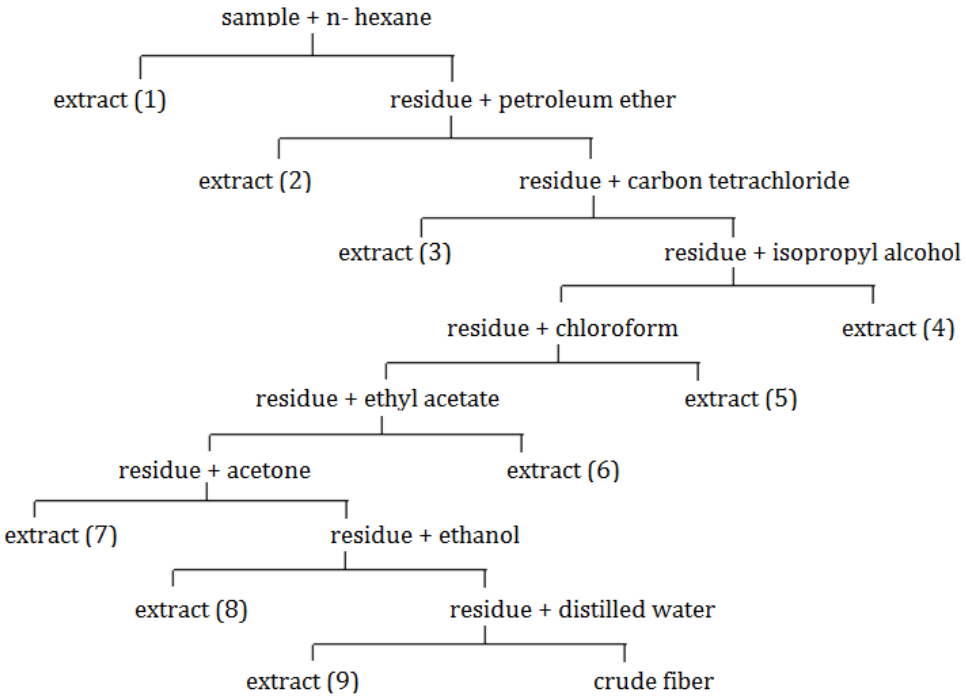
Lignocellulosic biomass is a valuable renewable resource that is primarily composed of cellulose, hemicellulose, and lignin (1). Cellulose is a linear homopolymer composed of D-glucose units linked by β-1, 4-glycosidic bonds. Hemicellulose is a polysaccharide that includes a basic chain containing residues of D-xylose, D-mannose, D-glucose, or D-galactose and other glycosyls as branched chains linked to this basic chain. Lignin is the exclusive chemical composition of gymnosperm and angiosperm(2). The utilization of biomass has gained increased importance due to threats of uncertain petroleum supply shortly and concerns about environmental pollution(3). Natural fibers possess several advantages over synthetic fibers such as low density, low cost, low tool wear, appropriate stiffness mechanical properties, and also high disposability and renewability. Also, they are recyclable and biodegradable(4,5). The future need for natural fibers of all kinds is projected due to increase uses in both historic and new applications, possibly replacing and reducing the use of petroleum-based fibers(6). Depending on their properties; natural fibers such as cellulose nanoparticles have been extensively used to reinforce biopolymers to produce biodegradable composites(7-9). This study is focusing on the extraction and characterization of crude fiber from khimp and date palm trees. Date palm tree (Phoenix dactyliferaL.) is one of the oldest, main, and ancient crops in Southwest Asia and North Africa. It is also can be grown in Australia, Mexico, South America, southern Africa, and the United States(10). The main purpose of planting dates is its fruit. The leaves of date palm trees were used in several applications such as making baskets, ropes, and mats, etc. Unfortunately, the huge amount of the non-food products from the date palm remains as landfill materials without specific usage(11). Recently the date palm tree fiber was used as a reinforcement agent in polymeric materials(12). Leptadenia pyrotechnica (Forsk.) commonly known as Khimp, is a leafless, erect, and evergreen shrub. According to the literature review, all parts of khimp are used in traditional medicines and phytochemical analyses showed the presence of many interesting pharmacological compounds. Khimp has furthermore potential to be developed as a fiber plant for industrial use(13). There are many procedures to extract fiber from specific plant tissues; mechanical, chemical and biological(14,15). According to the literature review, the extraction of plant fiber was carried out by alkali or acidic treatment and these procedures destroyed the extracted fiber. To avoid this drawback this study is focused on the extraction of crude fiber by using the differential extraction method; in which many organic solvents were used. Besides the extraction of plant fiber; this method is suitable for the extraction of phytochemicals; biologically active compounds present in plants and used as sources of direct medicinal agents(16). Phytochemicals can be extracted by conventional technique (using hexane, acetone, methanol, ethanol. etc.) and carried out generally at atmospheric pressure while new techniques using pressure and/or elevated temperatures(17-19). The extracted crude fiber can be characterized by FTIR(20,21).

2.Experimental

The plant's samples were collected from their local areas. All chemicals used are of analytical grade. Sample (g) to solvent (cm3) ratio is 1: 10. Infrared spectra were recorded on A Shimadzu 8400S FTIR spectrophotometer calibrated with polystyrene

film. The UV -visible spectrophotometer has been used for the determination of soluble lignin.

Extraction of fiber (general procedure):



2.1 Qualitative analysis of the extracted phytochemicals:

Test for Saponins: 5ml of the extract and distilled water (20 ml) were heated in a water bath and filtered, 10ml of the filtrate was mixed with 5ml distilled water and shaken vigorously. The frothing was mixed with 3 drops of olive oil and shaken vigorously, the formation of emulsion indicates the presence of saponins.

Test for Flavonoids:3ml of 1% (w/v) Aluminium chloride solution were added to 5ml of the extract. A yellow coloration indicates the presence of flavonoids. 5ml of dilute ammonia solution was added to the above mixture followed by the addition of concentrated H_2SO_4. The yellow coloration disappeared on standing which is a positive test for flavonoids.

Test for Steroids: 2ml acetic anhydride were added to 2ml extract followed by careful addition of 2ml concentrated H_2SO_4. The color changed from violet to blue or green indicates the presence of steroids.

Test for Terpenoids (Salkowski test): 5 ml of the extract were mixed with 2ml chloroform and 3ml concentrated H_2SO_4were added carefully to form a layer. The reddish-brown coloration of the interface indicates the presence of terpenoids.

Test for Cardiac Glycosides and Cardenolides (Keller – Killani test):

5 ml of the extract were treated with 2ml glacial acetic acid containing one drop of ferric chloride solution followed by careful addition of 1ml concentrated H_2SO_4 acid. The formation of the brown ring at the interface indicates deoxysugar characteristics of cardenolides which confirms the presence of cardenolides. A violet-green ring appearing below the brown ring, in the acetic acid layer, indicates the presence of glycoside.

Test for chalcones:2ml ammonia solution was added to 5ml of extract. The formation of reddish coloration indicates the presence of chalcones.

Test for Alkaloids: 1ml of the extract was stirred and heated with 5ml of HCl (0.03 M); then filtered while hot. 10 ml Distilled water were added to the residue and 1ml of the filtrate was treated with a few drops of Wagner's reagent (solution of iodine in Potassium iodide). The formation of reddish-brown precipitate was giving a positive test for alkaloids.

Test for Tannins:1ml of the extract was boiled in 20 ml Distilled water and then filtered; a few drops of 0.1% (w/v) $FeCl_3$ was added; observation of green or blue-black color confirms the presence of tannins.

Test for Phlobatannins: Deposition of a red precipitate when 2ml of the extract was boiled with 5ml HCl (0.03 M) was taken as evidence for the presence of phlobatannins.

Test for Phenols: 5ml of the extract was pipetted into a 30ml test tube, then 10ml distilled water was added. 2ml of ammonium hydroxide solution and 5 ml of amyl alcohol were added; the mixture was left to stand for 30min. The development of bluish-green color confirms the presence of phenols.

Test for Anthraquinone:5ml of the extract was mixed with 10ml benzene, filtered and 5ml of 10% (v/v) NH_3 solution was added to the filtrate. The
mixture was shaken and the presence of violet color in the ammoniac (lower) phase indicates the presence of anthraquinones(22).

Analysis of raw lignocellulosic material:2.5 g of raw biomass was weighed, extracted by Soxhlet at 70 ^{oC} with 150 ml acetone(24201, Sigma-Aldrich) for 4 h, after that dried in the air for few minutes and then in a convection oven at 105 ^{oC}. The difference in weight before and after is the extractives content.

To determine hemicellulose content1 g of dried extractive-free biomass was transferred into a 250 ml Erlenmeyer flask, 150 ml of 0.5 M NaOH (06203, Sigma-Aldrich) was added, boiled for 3.5 h with distilled water; filtered then washed to pH 7. The residue was dried to a constant weight at 105 ^{oC} in a convection oven. The difference between the sample weight before and after this treatment is the hemicellulose content. Lignin content: 0.3 g of dried extracted raw biomass was weighed in glass test tubes and 3 mL of 72% H_2SO_4(07208, Sigma-Aldrich) was added. The sample was kept at room temperature for 2 h with carefully shaking at 30 min intervals. After that, 84 ml of distilled water was added. The sample was then treated into an autoclave for 1 h at 121 °C. The slurry was then cooled at room temperature, filtered through a vacuum using a filtering crucible. The acid-insoluble lignin was determined by drying the residues at 105 oC and accounting for ash by incinerating the hydrolyzed samples at 575 oC in a muffle furnace. The acid-soluble lignin fraction was determined by measuring the absorbance of the acid hydrolyzed samples at 320 nm (by using a UV-visible spectrophotometer). The lignin content was calculated as the summation of acid-insoluble lignin and acid-soluble lignin.

The cellulose content: was calculated by difference, assuming that extractives, hemicellulose, lignin, ash, and cellulose are the only components of the entire biomass(23).

3.Results and discussion:

After differential extraction of the three samples; the crude fiber percentages were calculated as follow:

Sample one (khimp) = 73.6%

Sample two (date palm tree leave) = 57.8 %

Sample three (date palm tree fiber) = 83.3 %

The extraction was conducted using 9 solvents, the polarities increased gradually from nonpolar (n-hexane) to most polar (water). 12 phytochemicals (saponins, flavonoids, steroids, terpenoids, glycosides, cardinolides, chalcones, alkaloids, tannins, phlobatannins, phenols, and anthraquinone) were screened for all extracts. Saponins consist of both hydrophobic aglycone (a sapogenin) and hydrophilic glycosidic moiety (sugar). They exhibit foaming characteristics due to the combination of those two components (sapogenin and sugar). To determine the real composition of saponins, cold extractions with ethanol-water solutions would be better(24), and in agreement with this published works; saponins were isolated in ethanol (sample one and sample two) and water (sample one only). More polar flavonoids dissolve in polar solvents such as water and ethanol(25). According to the obtained results, acetone is also suitable for the extraction of flavonoids (less polar). Steroids are bioactive compounds; many of them behave like hormones, prohormones, drugs, prodrugs and control many important physiological actions(26). This study showed that steroids can be isolated by nonpolar solvents such as n-hexane, petroleum ether, and carbon tetrachloride. Phytochemicals like Terpenoids have much attention because of their important physiological, ecological roles, extensive pharmaceutical and industrial applications(27). According to the literature review terpenoids are classified as polar and nonpolar; as the result of this work; terpenoids can be extracted by different solvents with different polarities. Glycosides and Cardinolides have a structural resemblance to the steroid saponins and have the same solubility and foaming characteristics. Depending on their structure; these compounds were isolated by polar and nonpolar solvents as mentioned above. Chalcones are a class of natural products; generally obtained from edible plants that belong to the flavonoid family and display several pharmacological activities which are very important but soluble in ethanol and ethyl acetate than flovanones (28.29). As this study depends on the differential extraction method; chalcones are isolated in solvents with moderate polarities (isopropyl alcohol, chloroform, and ethyl acetate). Alkaloids are a group of complex heterocyclic nitrogen compounds, which have strong physiological activity, are often toxic, and retain their basic chemical properties(30). Solvents such as isopropyl alcohol, ethanol, and water are suitable for the extraction of these compounds. Tannins are a range of natural polyphenols; their biological role in the plant is related to protection against infection, insects, or animal herbivory(31). Tannins and Phlobatannins were generally soluble in isopropyl alcohol, ethyl acetate, acetone, ethanol, and water. The terms ‘phenolic compounds’, ‘phenolics' or 'polyphenolics' refer to more than 8,000 compounds found in the plant kingdom and possessing at least an aromatic ring with one or more hydroxyl substituents, including functional derivatives like esters, methyl ethers, glycosides, etc. they regulate the various metabolic functions including structure and growth, pigmentation and are resistant to different pathogens in plants Chemical test for these compounds showed that they can

be isolated by carbon tetrachloride, isopropyl alcohol, chloroform, and ethyl acetate. Except for the sample, three phenols appear in n-hexane extract. Isopropyl alcohol is a very suitable solvent to extract anthraquinone from the three tested samples.

3.1 Phytochemical screening

Table (1) Phytochemicals present in the extract of sample one(leptadeniapyrotechnica):

Solvent	phytochemical					
	Saponins	Flavonoids	steroids	terpenoids	glycosides	Cardin- olides
n- hexane	-	-	-	+	-	+
Petroleum ether	-	-	+	+	+	+
Carbon tetrachloride	-	-	+	+	+	+
Isopropyl alcohol	-	+	-	+	-	+
Chloroform	-	-	-	+	+	+
Ethyl acetate	-	-	-	+	-	+
Acetone	-	+	-	+	+	+
Ethanol	+	+	-	+	+	-
Water	+	-	-	-	+	-

Solvent	Phytochemical					
	chalcone	Alkaloids	Tannins	Phlobatannins	Phenols	Anthra-quinone
n- hexane	-	-	-	-	-	-
Petroleum ether	-	-	-	-	-	-
Carbon tetrachloride		-	-	-	+	-
Isopropyl alcohol	-	+	+	+	+	+
Chloroform	+	-	-	-	+	-
Ethyl acetate	+	-	+	-	+	-
Acetone	-	-	+	-	-	-
Ethanol	-	+	+	+	-	-
Water	-	+	-	-	-	-

Table (2) Phytochemicals present in the extract of sample two(date palm tree leave):

Solvent	Phytochemical					
	Saponins	Flavonoids	Steroids	Terpenoids	Glycosides	Cardeno-lides
n- hexane	-	-	+	-	+	-
Petroleum ether	-	-	+	+	-	+
Carbon tetrachloride	-	-	+	+	+	-
Isopropyl alcohol	-	-	-	+	-	+
Chloroform	-	-	-	+	+	+
Ethyl acetate	-	-	-	+	+	+
Acetone	-	+	-	-	-	+
Ethanol	+	-	-	+	-	+
Water	-	+	-	+	-	+

Solvent	Phytochemical					
	Chalcone	Alkaloids	Tannins	Phloba-tannins	Phenols	Anthra-quinone
n- hexane	-	-	-	-	-	-
Petroleum ether	-	-	-	-	-	-
Carbon tetrachloride	-	-	-	-	+	-
Isopropyl alcohol	+	-	+	+	+	+
Chloroform	+	-	-	-	+	-
Ethyl acetate	-	-	+	-	+	-
Acetone	-	-	+	+	-	-
Ethanol	-	+	+	+	-	+
Water	-	-	+	-	-	-

Table (3) Phytochemicals present in the extract of sample three(date palm tree fiber)

Solvent	phytochemical					
	Saponins	Flavonoids	Steroids	Terpenoids	Glycosides	Carden-olides
n- hexane	-	-	-	-	+	-
Petroleum ether	-	-	-	+	+	-
Carbon tetrachloride	-	-	-	+	-	+
Isopropyl alcohol	-	-	-	+	-	+
Chloroform	-	-	-	+	+	+
Ethyl acetate	-	-	-	+	-	+
Acetone	-	-	-	-	+	+
Ethanol	-	-	-	+	-	+
Water	-	-	-	+	-	-

Solvent	Phytochemical					
	Chalcone	Alkaloids	Tannins	Phloba-tannins	Phenols	Anthra-quinone
n- hexane	-	-	-	-	+	-
Petroleum ether	-	-	-	-	-	-
Carbon tetrachloride	-	-	-	-	-	-
Isopropyl alcohol	+	-	-	-	+	+
Chloroform	+	-	-	-	+	-
Ethyl acetate	+	-	+	-	+	-
Acetone	-	-	-	-	-	-
Ethanol	-	-	+	+	-	-
Water	-	-	-	-	-	-

3.2Analysis of raw lignocellulosic material

The samples were treated with acetone as a solvent in the soxhlet extraction technique. Acetone is a moderately polar solvent so it is not expected to dissolve all phytochemicals that existed in the plant sample and this is why the extractive free biomass percentage differs from that of crude fiber (which is extracted by 9 solvents).

Table (4) Components of lignocellulosic materials under investigation.

Component	Entity		
	Sample one	Sample two	Sample three
Extractive	16.4 %	17 %	5.6 %
Hemicelluloses	12.5 %	12.2 %	18.8 %
cellulose	55.376 %	56.8 %	62.95 %
Lignin	15 %	11%	10.94 %
Ash	0.724 %	3. %	1.71 %
total	100 %	100 %	100 %

Sample three has higher cellulose content with lower lignin content and vice versa in sample one. The lignin content was determined as insoluble lignin (by difference) and soluble lignin by the UV-Visible spectrophotometer, the λmaxfor soluble lignin was 320 nm.

Characterization of the Extracted Fiber:

The chemical compositions of the extracted fibers were determined using FTIR as a powerful analytical technique. The FTIR spectra in this work are confirmed with studies accomplished by other researchers; because the chemical compositions of all biomass are considered to be the same (cellulose, hemicellulose, and lignin) concerning the plant type, species, and the conditions at which the plant was cultivated. The three components of the extracted fiber (cellulose, hemicellulose and lignin) are consist of ester, alcohol, aldehyde, ketone with different oxygenated functional groups e.g. OH (3649cm-1 - 3032 cm-1), C = O (1734cm-1 –1653 cm-1), C–O–C (1111cm-1 -), and C–O–(H) (1033 cm-1), etc. the fingerprint region of lignin in 1791cm-1 – 781cm-1 corresponds with 1830cm-1 - 730cm-1 stated according to literature review[21].The absorption band at the range (1260 – 1234 cm-1) is due to the phenolic hydroxyl of lignin(32). Absorption bands located at 1558cm-1 and 1506cm-1 are related to vibrations of the aromatic rings present in lignin(20).The peaks at2902 cm-1 and 3032 cm-1 attributed to stretching vibration of SP3C-H and SP2C-H (respectively) and the bending vibration of these groups observed at (1448cm-1 and 617cm-1 respectively) of all hydrocarbon constituents in polysaccharides. The crystallinity region of cellulose appears at 1445cm-1 and the amorphous region observed(19) at 896cm^{-1}

Table (5) FTIR spectrum of crude fiber extracted from leptadeniapyrotechnica.

Reference band cm-1	Observed band cm-1	The functional group
3650 – 3200	3446	-OH (stretching)
3100 – 3020	3032	SP2 C - H (stretching)
2970 – 2860	2902	SP3 C - H (stretching)
1780 – 1650	1734, 1683, 1653	- C = O (stretching)
1600 – 1500	1558 - 1506	Benzene (from lignin)
1450 – 1420	1448	SP3 C - H (bending)
1430 – 1420	1425	Crystallinity region of cellulose
1380 – 1340	1373	C-H cellulose, hemicellulose
1260 – 1234	1246	Phenolic hydroxyl
1250 – 1050	1161, 1111	- C – O – C (stretching)

1040	1033	- C – O(H) stretching
897	896	Amorphous region of cellulose
669	663	- OH (out of plane bending)
1000 – 600	617	SP2 C- H (bending)

Table(6) FTIR spectrum of crude fiber extracted from date palm tree leaves.

Reference band cm-1	Observed band cm-1	The functional group
3650 - 3200	3354	-OH (stretching)
3100 - 3020	3026	SP2 C - H (stretching)
2970 - 2860	2931	SP3 C - H (stretching)
1780 - 1650	1734	- C = O (stretching)
1600 - 1500	1610, 1521	Benzene (from lignin)
1430 - 1420	1419	Crystallinity region of cellulose
1380 – 1340	1375, 1363	C-H cellulose, hemicellulose
1260 - 1234	1230	Phenolic hydroxyl
1250 - 1050	1109	- C – O – C (stretching)
669	661	- OH (out of plane bending)
1000 – 600	900	SP2 C- H (bending)

Table (7) FTIR spectrum of crude fiber extracted from date palm tree fiber.

Reference band cm-1	Observed band cm-1	The functional group
3650 - 3200	3336, 3275	-OH (stretching)
3100 - 3020	3024	SP2 C - H (stretching)
2970 - 2860	2901	SP3 C - H (stretching)
1780 - 1650	1734 - 1683	- C = O (stretching)
1600 - 1500	1558 - 1506	Benzene (from lignin)
1450 – 1420	1448	SP3 C - H (bending)
1430 - 1420	1425	Crystallinity region of cellulose
1384 – 1346	1375, 1338	C- H cellulose, hemicellulose
1260 - 1234	1244	Phenolic hydroxyl
1250 - 1050	1161, 1107	- C – O – C (stretching)
1040	1040	- C – O(H) stretching
897	896	Amorphous region of cellulose
669	665	- OH (out of plane bending)
1000 – 600	771, 696,600	SP2 C- H (bending)

4.Conclusions & Recommendations

1.The modified differential extraction procedure can be suitable for extraction of crude fiber from plants other than studied.

2.Fibers extracted by this method are ready for esterification without further purification.

3.The extracted phytochemicals during the differential extraction process need to be investigated quantitatively.

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