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Phytochemical screening, antioxidant and antimicrobial activity of three medicinal plants from Libya

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Abstract

This study is to perceive the most effective components such as the flavonoids, alkaloids and saponins and also the investigation of the biological effect of plant extracts and antioxidant effectiveness of the each one of them. Were carried out on three plants ("Palm-pollen, Anagallis-arvensis and Leopoldia-comosa") of different factions included, Whose soever the active ingredients of plant powder and its activities of qualitative and quantitative of chemical components for these medicinal plants from Libya. In addition polyphenols and antioxidants were evaluated with activities of antimicrobial for four types of negative and positive Gram of bacteria. These scattered natural plants from Libya and endured used in traditional folk medicine, These natural plants from Libya used in traditional folk medicine, this research aims to deviate from chemically produced and the trend to medicinal plants as a source of medical drugs.

Keywords: Effective Components, Biological Effect, Antioxidant Effectiveness

1. Introduction

Herbal medication ancient method of remedying human diseases, this manner returns the treating to strike distant times in the olden times, perhaps accompanied human history since the beginning of its existence on the ground. Medication conflict, contrary to infectious pathogens is cumulative suggestively universal. Usage is based on antibiotics, which are expensive and moreover reasons contrary side effects. Natural products and their components can be a decent optimal in the regulator of infection as they are low-cost, active without side effects. Furthermost plants and herbs in the Libyan wild are not efficiently considered to realize their chemical constituents and assessment for ingesting. A good considerate of these plant suggestions the potential of reducing the ended necessity of man on the few known harvests [1]. "Anagallis arvensis" was extensively dispersed through the world, presence originates in altogether temperate regions in both hemispheres [2-4]. Owing to the attendance of salicylic acid, cinnamic acid and caffeic acid "Anagallis arvensis" inhibited germination, a growth of six assessment species [5]. It was utilized for remediation of some illness, for instance, leprosy, epileptic attacks, gout, cerebral affections, dropsical affections and other complains of the nervous system. Also, "Anagallis arvensis" was correspondingly used as diuretic, expectorant for the treatment of rheumatism, diaphoretic, dropsy and hepatic and renal complaints [6-7]. "Palm-pollen", Glucan, (a polysaccharide) was insulated from the Libyan dates and its anticancer investigation revealed that glucan has potent antitumor activity may be due to (1-3) -β-D-glucan linkages. This was the first study attained on dates for its anticancer activity specified by (Ishurd and Kennedy, 2005) [8]. Natural products are a good remedy in

the suppression of NF-kB (Transcription factors) and performances as anti-inflammatory causes. Studies have shown that constituents of plants such as Phenolics and flavonoids act as excellent anti-inflammatory agents. The leaves of dates are good source of natural antioxidant and anti-inflammatory medicines induction of pollen postponement. Also, hot water of a date's fruit extract of Phoenix dactylifera can also stimulate cellular immune system in animal experimental [9]. The numerous parts of Phoenix dactylifera usually are used in traditional remedies for the treatment of many illnesses which include in Flammarion, fever, and loss of consciousness, memory disturbances, paralysis and nervous disorders [10-11]. In this study, phytochemical screening was carried out to accomplish the presence or absence of chemical constituents such as alkaloids, glycosides, flavonoids, terpene, saponins, carbohydrates, tannin, cardio glycosides, coumarins, and phenolic compounds.

2. Material and Methods

2.1. Plants Sample Collection

"Anagallis-arvensis, Leopoldia-comosa leaves, and Palm-pollen", were collected from Zliten region in Libya during the period of flowering in the spring of the year 2015. They were authenticated in Biology Department College Science Mergeb University, Khums Libya. And the voucher specimens were deposited in the herbarium.

2.2. Extraction of Plant Material

Twenty grams from the required fine powdered plant parts were extracted by Soxhlet Apparatus with 500 ml of appropriate solvents (aqueous and ethanol separately) at a 40% (w/v) concentration (20g leaves powder in 500 ml solvent), and then each extract was filtered and kept in a refrigerator at 4°C until use [12-13].

2.3. Qualitative Analysis

2.3.1. Qualitative Phytochemical Analysis

Aqueous and ethanolic extracts were qualitatively examined for phytochemicals constituent's according to standard procedures to identify the constituents [12, 14-20].

Flavonoids: Ammonium Solution: 20 ml of extract was boiled in distilled water and 1ml of dilute ammonia solution was added then addition concentrated H2SO4 (1ml). An appearance of yellow colour confirmed the presence of flavonoids.

Alkaline Reagent Test: 10 ml of the extracts was treated with a few drops of sodium hydroxide solution, the formation of intense yellow color, which becomes colorless on the addition of diluted hydrochloric acid, indicates the presence of flavonoids.

Alkaloids: Dragendroff's Reagent: 20 ml extract was boiled in methanol, 2 ml and filtered. 1 % HCl was added, followed by a few drops of Dragendroff's reagent. After addition prescribed reagents a brownish red precipitate was shown the presence of alkaloids.
Mayer's Reagent: 20 ml extract was boiled in methanol, 2 ml and filtered. 1 % HCl was added, followed by a few drops of Mayer's reagent. After addition, a white precipitate was shown the presence of alkaloids.
Wagner's Reagent: 20 ml extract was boiled in methanol, 2 ml and filtered. 1 % HCl was added, followed by a few drops of Wagner's. After addition, a brownish precipitate was shown the presence of alkaloids.

Carbohydrates: Fehling's Test: 5ml of distilled water was added to 3ml of extract, then filtered after that a few drops of Molisch's reagent, an appearance of violet ring evidence presence of carbohydrates.
Molluscs Test: a few drops of Fehling reagent were added to 20ml of extract then boiled for 10 minutes. After addition a red precipitate was showed the presence of alkaloids.

Saponins: Olive's oil: 5ml distilled water was added to 20 ml of extract was then a few drops of olive's oil was added then shake for 3 minutes. Appearance froth and stable foam were indicated the presence of saponins.
Mercuric Chloride ($HgCL_2$): 3ml of Mercuric Chloride ($HgCL_2$) solution was added to 5 ml of extract. Appearance white precipitate indicated the presence of saponins.

Cardiac Glycosides: Keller-Killani Test: 20 ml of each extract (separately) was treated with 8 ml of glacial acetic acid containing one drop of ferric chloride solution then shake. This was under layer with 1 ml of concentrated Sulphuric acid. A brown ring of the interface indicates a deoxy sugar characteristic of cardenolides. A violet ring

may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout the thin layer.

Coumarins: 5ml of extract placed in a test tube, then covered by wet filter paper with sodium hydroxide solution (NaOH) then heated in a water bath for a few minutes after that exposed to the ultraviolet light, greenish yellow color proof of the existence Coumarins in the crude extract.

Tannins & Phenols: Tannins: Ferric Chloride (FeCl3): 10 ml of extract was boiled and add a few drops of Ferric Chloride solution to the filtrate. Bluish green color indicated the presence of Tannins.
Lead Acetate Pb $(C_2H_3O_2)_2$: 10 ml of Lead acetate solution added to 3ml of the extract and shake for a 2-minute appearance of gel precipitate indicated the presence of tannins.
Phenols:10ml of extract were treated with 4-5 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

Proteins and Amino Acids: Biuret's Reagent: few drops of biuret's reagent were added to 10 ml of the extract, appearance of pink color in the ethanolic layer indicated the presence of proteins.
With Nitric Acid (HNO_3): few drops of concentrated Nitric acid were added to 10 ml of the extract, appearance of yellow color indicated the presence of proteins.

Terpenoids: Salkowski's test: 5ml of chloroform was added to 10ml of extract, then added 2ml of concentrated Sulphuric acid (H_2SO_4) with care, then heated for 2 minutes; a reddish brown coloration of the interface was formed to show the presence of terpenoids.

Resins: 10ml of ethyl alcohol were added to 20ml of the extract, then heated and filtered, after that 10ml of diluted hydrochloric acid (HCl) were added, the appearance of turbidity indicated to a presence of resins.

2.4. Quantitative Phytochemical Analysis

2.4.1. Extraction of Plant Materials

The quantities of the phytochemical components were determined via the procedures of Harborne J.B., 1973 and Obadoni B.O., Ochuko B.O, 2001 and D. Krishnaiah 2009. [21-23] as shown below.

2.4.2. Plant Yield Determination

The extracts were weighed after separating the solvents by evaporated under reduced pressure and dried using a rotary evaporator at 40 °C then a percentage yield for each extract was obtained using this formula $W_2 - W_1/W_0 \times 100$. Where W2 is the weight of the extract and the container, W1 the weight of the container alone and W0 the weight of the initially dried sample:

$$Yield = \frac{W_2 - W_1}{W_0} \times 100 \qquad (2.1)$$

2.4.3. Flavonoids Determination

20 g of finely powdered leaves was frequently extracted with 200ml of 80% aqueous methanol at room temperature. The mixture was then filtered through a filter paper into a reweighed 500ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed. The percentage flavonoid was calculated by difference [20].

2.4.4. Saponins Determination

50g of fine powdered leaves were separate in 500ml of 25% ethanol. The suspension was heated over a hot water bath for 4 hrs with continuous stirring at 60 °C. The mixture was filtered and the residue re-extracted with another 400ml of 20% ethanol. The combined extracts were reduced to 80ml over a water bath at 90 °C. The concentrate was transferred into a 250ml separator funnel and 40ml of diethyl ether was added and shaken vigorously. The aqueous fraction was recovered while the ether layer was discarded. The purification process was repeated thrice. 120ml of n-butanol extracts were washed twice with 20ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The Saponin content was calculated in percentage [21].

2.4.5. Alkaloid Determination

10g of the sample were weighed into a 500 ml beaker and 500 ml 25% acetic acid in ethanol was added and covered to stand for 4hrs.This was filtered and the extract was concentrated using a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was

added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration, dried and weighed [20, 22-24].

2.4.6. Determination of Antibacterial Effectiveness

Bacterial Strains and Culturing: The concentrated extracts of the plants were transported from Department of Chemistry, Faculty of Science, Alasmareia University Zliten Libya to the Department of Microbiology Laboratory at Zliten Teaching Hospital, Zliten, Libya. Were four pathogenic bacterial strains used in this study (Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumonia, these strains were cultured at 37°C.

Determination of Minimum Inhibitory Concentration (MIC): 50 µl of bacterial culture (OD600 = 1.0) was inoculated in each LB media tubes containing 10 to 150 mg/ml sample. Two negative controls were employed, one was LB broth only and the second one was LB broth with extract (100 mg/ml). Positive control was LB broth and a test organism. After 24 h incubation at 37°C, observance of a suspension was measured, using a spectrophotometer at a wavelength (λ) = 600 nm. The concentration of test sample at which growth of bacterial culture was inhibited was considered as the MIC (Kumar et al., 2011; Ettebong and Nwafor, 2009) [24-33].

Disc-Diffusion Assay: The disc - diffusion assay was sorted out to conclude the activity against bacterial strains. Lysogenic Broth (LB) agar plates were planted with bacterial culture (250 µl bacterial culture with OD600 of about 1 per 100 ml LB media). Four discs at the distance of approximately 6mm were used. Consistent broth inoculums were wiped onto sterile Lysogenic Broth (LB) agar plates by swabbing with sterile cotton swabs. 6 mm, sterile paper discs was saturated with aqueous and Ethanolic leaf extracts distinctly (pre-dissolved in 10% dimethyl sulphoxide (DMSO)) to attain ultimate concentrations of extract per disc, 2 discs sterile paper have 6 mm overloaded with Solvents (Aqueous and ethanolic solvent) was used as the growth control. The discs were placed aseptically and individually onto the inoculated (LB) agar plates. Agar plates were incubated at 37°C for 16-18 hours and inhibition zones formed were measured and compared with those of antibiotics (Nitrofurantoin). The test was performed in triplicates.

Agar-Well Diffusion Assay: Agar-well diffusion assay was sorted out to conclude the activity against bacterial strains. Lysogenic Broth (LB) agar plates were planted with bacterial culture (250 µl bacterial culture with OD600 of about 1 per 100 ml LB media). Four wells at the distance of approximately 6mm were made by using 6 mm cork borer. First and second wells were four samples (Aqueous and ethanolic extracts), 50 µl of dilution (100 mg/ml), was poured into the wells separately. Third and fourth wells were loaded with controls (as negative control 50 µl of solvents (water and ethanolic extract) and as a positive control 50 µl Nitrofurantoin (40 mg/ml) separately). The plate was then incubated at 37°C overnight. The diameter of the zone of inhibition was measured in millimeters. This procedure is followed to all extracts. The experiment was replicated thrice.

2.4.7. Antioxidant Activity

Determination of Total Phenolics (TP): Total Phenolics content was considered consistent with Singleton and Rossi [1965] method. An aliquot of a slurry was extracted with buffer, including water, acetic acid and acetone (29.5:0.5:70 v/v) for 2 h in the dark. Three parallel extracts were attained from each genotype. Then, extracted, Folin-Ciocalteu's reagent and water were incubated for 8 min, followed by adding 7% sodium carbonate. Subsequently 2 h, the absorbance was measured by an automated UV–VIS spectrophotometer at 750 nm. Gallic acid was used as a standard. The results were expressed as µg Gallic acid equivalent in one gram of fresh weight (GAE g-1 FW) [34].

Determination of total antioxidant activity: Free radical scavenging activity: Free radical scavenging activity The free radical scavenging capability of several extracts of "Anagallis-arvensis leaves, Leopoldia-comosa leaves and Palm pollen" was measured via α, α- diphenyl-β-picrilhydrazyl (DPPH) assay (Juntachote and Berghofer) [34]. The absorbance of the samples was measured at 517 nm. The radical-scavenging activity was conceded as the percentage of inhibition and calculated with the following formula:

Table 2.1: Results of Qualitative analysis

Plant's Name		Anagallis-arvensis		Palm-pollen		Leopoldia-comosa	
Chemical Constituents		Aqua. Extra.	EtOH Extra.	Aqua. Extra.	EtOH Extra.	Aqua. Extra.	EtOH Extra.
Flavonoids	Ammonium Solution	+++	+++	++	+	++	+
	Alkaline Reagent	+++	+++	++	++	+	+
Alkaloids	Dragendroff's	++	++	+++	+++	+++	++
	Mayer's	++	++	+++	+++	+++	++
	Wagner's	+	++	+++	+++	+++	++
Carbohydrates	Fehling's	+++	+++	+++	+++	+	++
	Molisch's	++	+++	+++	+++	++	+++
Saponin	Olive oil	+++	+++	++	+++	++	++
	Mercury (II) Chloride	+++	+++	+++	+++	++	++
Cardiac Glycosides (Keller-Killani)		+++	++	+++	+++	+++	++
Coumarins		++	++	++	+++	++	+
Tannins & Phenols	Ferric Chloride	+++	+++	++	+++	+++	++
	Lead Acetate	+++	+++	+++	+++	++	++
Proteins	Biuret	+++	+++	+++	+++	+++	++
	ConcentratedNitric acid	+++	++	+++	++	++	+
Terpenoids (Salkowski's)		+++	+++	++	+++	+++	+
Resins		+++	+++	++	+++	+++	+

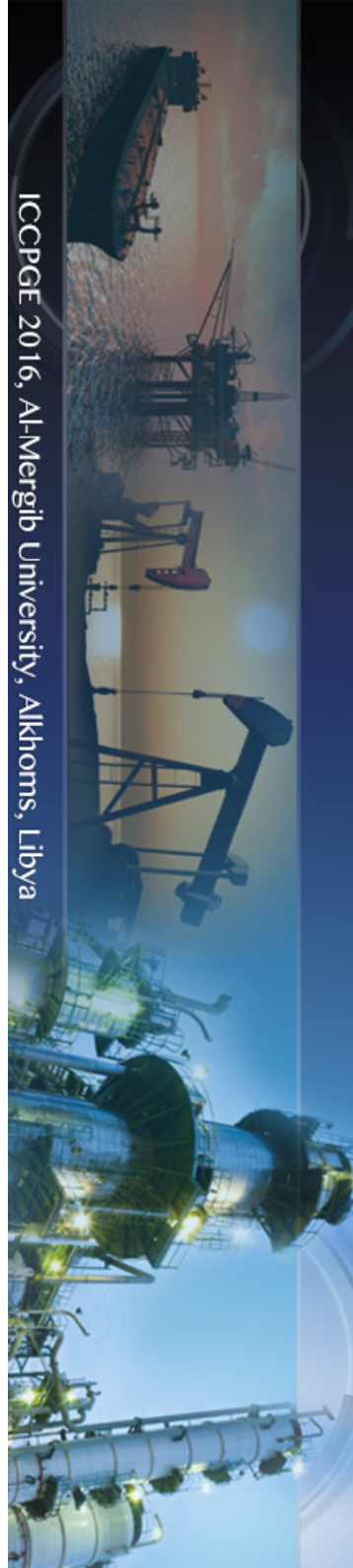

Table 2.2: Results of percent yield %

Name of Plants	Percentage yield (%)				
	EtOH Extra. %	Aqua. Extra %	Alkaloids %	Saponins %	Flavonoids %
Palm-pollen	12	17	13	80	93
Anagallis-arvensis	10	13	11	86	90
Leopoldia-comosa	11	15	15	79	88

Table 2.3: Results of Total Phenols

Plant's Name	Total Phenols (mg/g)
Palm-pollen	2.9
Anagallis-arvensis	25.3
Leopoldia-comosa	11.9

Table 2.4: Results of Antioxidant Activity

Plant's Name	Sample concentration (5g/25ml)	DPPH%
Palm-pollen	40µl	32.04
	60µl	45.7
	80µl	50.7
	10µl	62.6
Anagallis-arvensis	40µl	69.8
	60µl	71.8
	80µl	75.6
	100µl	80.5
Leopoldia-comosa	40µl	71.8
	60µl	73.5
	80µl	74.03
	100µl	75.1

% inhibition = [(A control – A sample) / (A control)] × 100. Where A is an absorbance at 517nm [35].

3. Results and Discussion

The Phytochemical screening of crude extracts of three plants (aqueous and ethanolic) as showed in Table 2.1 revealed presence of Flavonoids, Alkaloids, Carbohydrates, Saponin, Cardiac Glycosides, Coumarins, Tannins &phenols, Proteins, Terpenoids and Resins, Wherever the Biochemical analysis of pollen grains has exposed the attendance of varied sorts of biochemically and nutritionally important constituents such as carbohydrates and proteins, were the olive pollen extract contained antimicrobial activity and highest concentrations of phenolic content with antioxidant. "Pseudomonas aeruginosa" and "staphylococcus aureus" bacteria were inhibited by extracts of pollen of ethanol solution. These different type of sensitively are due to different phenolic compounds in pollen. [36] And as showed in Tables 2.2 and 2.3 the total phenolic and Antioxidant activity Flavonoids present in plants possess various health welfares, which include antioxidant and radical scavenging activities, a concession of convinced chronic diseases, inhibition of some cardiovascular complaints and definite varieties of cancerous developments [37].The effect of water extracts the pathogenic bacteria as showed in Table 2.5 propose that the antibacterial capacity needed, as compared to antioxidant activity, which has a respectable effectiveness with crude extracts of "Anagallis arvensis" leaves, "Leopoldia-comosa" leaves and "Palm-pollen", more concentration. As well, the effect of crude extracts (Aqueous and Ethanolic) on pathogenic bacteria were the growth inhibited, Ethanolic and aqueous extracts of "Leopoldia-comosa" and "Anagallis-arvensis" were 18 and 15mm were the uppermost inhibition's against Pseudomonas aeruginosa, "Escherichia coli" and "Klebsiella pneumonia" subsequently. Further the lowermost inhibited were aqueous extracts of "Anagallis-arvensis" leaves and Ethanolic extract of "Palm pollen" 7mm for both plants. Likewise, our finding shows crude extracts of plants were active against both gram-positive and gram-negative organisms assorted from 7mm to 18mm. with MIC (Minimum Inhibitory Concentration) values of 495and 435 µg/mL, respectively *55+. As well the ethanolic extract of "Leopoldia-comosa" the most favourable and provide a great result against the pathogenic bacteria's in comparing to the antibiotic consequences. Moreover, the antibacterial activity revealed by the methanolic extract may well support the traditional usage of "Anagallis-arvensis" as a folk med-

Table 2.5: Diameters inhibiting antibiotic evidence on the types of bacteria

Bacterial Types	Aqua. Extra.	EtOH Extra.	Nitrofu-rantoin	Anagallis-arvensis		Palm-pollen		Leopoldia-comosa	
				Aqua. Extra.	EtOH Extra.	Aqua. Extra.	EtOH Extra.	Aqua. Extra.	EtOH Extra.
Klebsiella Pneumonia	-	7	24	13	15	11	13	8	13
Escherichia coli	-	-	20	10	13	12	9	15	12
Pseudomonas aeruginosa	-	-	13	7	9	-	7	8	18
Staphylococcus aureus	-	-	10	9	8	-	9	-	11

ication universal. The source of a variable degree of sensitivity of verified revealing of methicillin-resistant "Staphylococcus aureus"to plant extract could be in arrears to the natural easiness of it and the nature and combinations of phytochemical existing in the plant extract. This effect can be associated with uppermost concentrations of saponins and alkaloids in the plant extract [38]. The mechanisms conception which accountable for the activity of these phytochemicals against "Staphylococcus aureus" may comprise enzyme inhibition by the oxidized compounds which act as a derivation of unchanging free radical and frequently attain to deactivation of the protein and damage of function. They have the facility to the compound not only with extracellular and soluble proteins but also bacterial cell walls and disturb microbial membranes [39]. "Leopoldia-comosa" used in the Basilicata region of southern Italy to treat a toothache and headache and were used correspondingly to treat skin and soft tissue infections [39-40]. "Leopoldia-comosa" is found mostly in the Mediterranean region and the bulbs were cast off as a nutrition basis for generations [41].

4. Conclusion

In conclusion, this study requests the consideration to the necessity of additional biochemical study of the plant's components (isolation and identification) and applications suggestion in the enhancement of scientific field reviews to estimate the efficiency of the herbalist use of medicinal plants in a usage of diabetes and other diseases. Also, the present study suggested that "Leopoldia-comosa, Palm-pollen", and"Anagallis-arvensis" could be a potential source of natural antioxidant and thus could be useful as remedial.

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