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Proximate and Phytochemical Analysis of Crude Powder and Different Extracts of *Zaleya pentandra*

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ABSTRACT

Zaleya pentandra, a plant species found in disturbed soils and roads across Asia, Africa, and Australia, has been traditionally used for treating various ailments such as coughs, malaria, kidney stones, ulcers, and jaundice. **Objectives:** To conduct a quantitative examination of *Z. pentandra*, focusing on proximate analysis and metabolite composition and to better understand its features. **Methods:** Crude plant powder underwent analysis for total moisture, total ash, water-soluble ash, acid-insoluble ash, sulfated ash, water-soluble extractive, and alcohol-soluble extractive. Quantification (mg/gm) of principal metabolites i.e., carbohydrates, proteins, and lipids in the crude plant powder was done. Dry plant powder was subjected to counter-current extraction using n-hexane, petroleum ether, and chloroform. UV-visible and FTIR spectra were examined to determine the chemical composition. **Results:** Our findings showed total moisture (9.306%), total ash (21.73%), water-soluble ash (12.75%), acid-insoluble ash (0.35%), sulfated ash (29.75%), water-soluble extractive (6.23%), alcohol-soluble extractive (5.7%). Principal metabolites included high quantities of carbs (65.34), proteins (15.29), and lipids (30.90) in the crude plant powder. n-hexane (3.073%), petroleum ether (4.45%) and chloroform (7.47%) were extracted. UV-visible and FTIR spectra revealed a variety of chemicals, indicating both polar and non-polar molecules with possible oxidative characteristics. **Conclusions:** *Z. pentandra* exhibits high carbohydrate, protein, and fat content. The diverse chemical composition suggests a high oxidative potential, supporting its traditional therapeutic uses. Further research, especially in identifying and isolating bioactive components, is warranted. *Z. pentandra* holds promise for traditional medicine and broader applications based on its nutritional and bioactive potential.

INTRODUCTION

Zaleya pentandra, a perennial prostrate herb, is found in Africa, Asia, Australia, Egypt, Senegal, Iran, Madagascar, Palestine, Qatar, Saudi Arabia, West Pakistan at 200–1600 m, Gawadar, Jiwni, Miri Kalat, Farasan islands, Mozambique, Malawi, Zambia, and Zimbabwe (July–August) [1]. Punjabi, an indigenous language of Pakistan, calls it “itsit”. It flowers from December to June in Africa and April to August in Pakistan [2]. The Aizoaceae family has 1170 species and 128 genera. Six *Zaleya* species are randomly

scattered across Africa, Asia, and Australia. Pakistan has only one species, *Z. pentandra* L. (*Trianthema pentandra* L.). Studies suggest that several *Zaleya* species are effective folk medicine for bronchial disorders, cathartic, irritating, anti-pyretic, anti-constipation, urinary tract infections, and kidney and bladder stones [3]. This genus is pharmacologically rich. *T. decandra* is antibacterial, antidiabetic, hepatoprotective, analgesic, anti-inflammatory, antimicrobial, and antioxidant [4]. *T.*

portulacastrum is used to treat cancer, hypoglycemic, anti-hyperglycemic, hypolipidemic, hepatoprotective, anthelmintic, and renal disorders [5]. Trianethenol, trianthemine, ecdysteroid, flavonoids, phytoserolines, and ketones are found in this genus [6]. Snake bite and kidney stones have been treated using *Z. pentandra* juice [7]. To treat influenza, phlegmatic cough, stomach problems, hematuria, and uncomfortable urination, root powder has been utilised [8]. In Azad Kashmir, Pakistan, its roots are draped around the neck to cure jaundice [9]. Afzal *et al.*, and Ahmed *et al.*, studied this herb's antifungal, antibacterial, and antioxidant properties [3, 10]. Medicinal plants and herbs contain phytochemicals that can scavenge free radicals and treat chronic diseases like ageing, cancer, diabetes, obesity, cardiovascular disease, and neurodegenerative disorders [11]. Understanding phytoconstituents helps synthesise complicated biomolecules like anthocyanins, steroids, and tannins. Secondary metabolites, chemically complicated substances with a large taxonomy, are employed in human illness treatment, agricultural sciences, and scientific research [12]. Secondary metabolites exhibit anticancer, antidiabetic, antifungal, antibacterial, and antioxidant properties [13]. Polyphenols-hydroxycinnamic acids, are found in fruits and vegetables [14]. The most frequent flavonoids in fruits and vegetables include apigenin, kaempferol, luteolin, and myricetin [15]. Frauendorfer and Schieberle revealed that edible macromycetes' polysaccharides such lectins and proteases are anticancer, hypocholesterolemic, anti-inflammatory, antioxidant, and prebiotic [16]. *Z. pentandra* contains alkaloids, anthraquinones, cardio-active glycosides, saponins, and steroids [10, 17]. Our research focused on phytochemicals' qualitative and quantitative assessment for treating numerous human illnesses due to their high biological potential.

METHODS

The present study was conducted in the Pharmaceutical Chemistry Laboratory at University College of Pharmacy, University of the Punjab, Lahore, Pakistan, from September 2016 to September 2017. The chemicals used included n-hexane, petroleum ether, chloroform, concentrated sulfuric acid, sodium carbonate, copper sulfate, potassium sodium tartrate, quercetin, aluminum nitrate, bovine serum albumin, gallic acid, potassium acetate, and anhydrous glucose. All chemicals used were of analytical grade and were supplied by Sigma Merck, Germany. The laboratory equipment and instruments used were a rotary evaporator (Hei Dolph, Model 2002, Germany), an oven (Dawlance, Pakistan), an ultra-sonicator (Sonics & Materials, USA), a flask shaker (OHAUS, USA), a muffle

furnace (Carbolite Gero, Nabertherm, Germany), a vortex mixer (Scilogex, Korea), an incubator (Thermo Fisher Scientific, USA), a UV-visible spectrophotometer (Shimadzu, Japan), an atomic absorption spectrophotometer (Hitachi, Japan), and a Fourier Transform Infrared spectrophotometer (PerkinElmer, America).

Proximate Analysis of the Crude Powder of *Z. Pentandra*

Proximate analysis of the crude powder of *Z. pentandra* was determined with reference to the USP (2009). Percentages of proximate analysis were calculated using equation given as;

% moisture content = Total weight reduced/weight of total powder taken x 100

% Total ash = (wt. of ash/ total wt. of powder) x 100

% acid insoluble ash = (wt. of acid insoluble ash/wt. of powder) x 100

% water soluble ash = (wt. of water-soluble ash/ wt. of sample) x 100

% sulphated ash = (wt. of ignited sample/ wt. of total plant powder) x 100

% alcohol-soluble extractive value = (wt. of dried filtrate/ wt. of total plant powder used) x 100

% of water-soluble extractive value = (wt. of dried filtrate/ wt. of powder taken) x 100

Estimation of Primary Metabolites of *Z. pentandra*

According to Bhattacharya and Chatterjee, plant powder (60 g) was extracted with solvent n-hexane (1.5 L) using Soxhlet apparatus [18]. It was dried in the rotary evaporator and weighed to find out the total lipids expressed as mg/g. Following the method of Sagar *et al.*, 1g of plant powder was mixed with 10 mL of distilled water and 5 drops of Triton-X [19]. After shaking for 30 min and centrifuging at 2700 rpm for 10 min, 100 μ L of the supernatant was diluted to 1 mL with distilled water. Next, 3 mL of reagent C (prepared from 2% sodium carbonate solution and 0.5% copper sulfate with 1% sodium potassium tartrate), and 200 μ L of Folin Ciocalteu's reagent (FC) were added. The mixture was incubated at room temperature for 30 min, and the absorbance was measured at 600 nm using a UV-Visible spectrophotometer. Bovine serum albumin (BSA) was used as a standard in concentrations of 20, 40, 60, 80, and 100 μ g/mL, following the same procedure. A calibration curve of BSA was used to estimate the protein content in the plant powder. Al-Mamary *et al.*, devised an equation for determining percentage total carbohydrates given as [20]; Total carbohydrates (%) = 100 - (total moisture contents + total ash + total fat + total proteins).

Determination of Secondary Metabolites

The central idea is that the study involved the determination of polyphenolic content using the Slinkard and Singleton method with gallic acid as a standard,

quantification of flavonoids using the method proposed by Chang *et al.*, with quercetin as the standard, and assessment of total polysaccharides based on method proposed by Jaganathan *et al* [21, 22]. Additionally, the study involved the extraction and weighing of precipitates after refluxing the plant extracts with methanol and acetone.

Glycosaponins (%) = (pwt. of dry precipitates/ wt. of total extract taken) x 100

Alkaloids were determined following the method of Savithramma *et al* [23].

Characterization of Extracts using Analytical Techniques

Ultra-Violet Visible Spectra

Methanolic solution of each extract with 50 µg/mL concentration was prepared and scanned in the spectrum mode of the UV-Visible spectrophotometer after baseline correction. Spectrum was measured between 800-200 nm ranges against methanol taken as a blank [24].

Fourier Transform Infra-Red (FTIR) Profiling

Fine powder of *Z. pentandra* (5 mg) was ground with potassium bromide (100 mg) and then its transmittance was measured in the middle infra-red region (4000-400 cm⁻¹) using FTIR spectrophotometer.

Determination of Metals

Plant powder was examined for metal content following the method proposed by Ahmed *et al.*, with slight adjustments [10]. A gram of fine powder underwent digestion with a nitric acid and HCl mixture (1:3, v/v) until fumes ceased. After filtration, the filtrate was diluted to 100 mL with distilled water. An atomic absorption spectrophotometer analyzed the solution for metals (Zn, Cu, Mn, Pb, Cr, and Mg). Test solutions were prepared from 1000 ppm standard stock solutions to auto-calibrate metal ion concentrations.

Statistical Analysis

All the samples were prepared and tested in triplicates and results were enlisted as mean ± SD.

RESULTS

Proximate Analysis

Findings of proximate analysis of the plant powder are given in Table 1.

Table 1: Proximate Analysis of Fine Powder of *Z. pentandra* (n=3)

Physicochemical Tests	Mean ± SD
Moisture content	9.306 ± 0.011
Total ash	21.733 ± 0.305
Acid insoluble ash	0.356 ± 0.011
Water soluble ash	12.753 ± 0.005
Sulphated ash	29.756 ± 0.005
Alcohol soluble extractive value	5.7 ± 0.01
Water soluble extractive value	6.23 ± 0.01

Estimation of Primary Metabolites

Primary metabolites were found in the plant powder and are enlisted in Table 2.

Table 2: Primary Metabolites (mg/g) in *Z. pentandra* Powder, (n=3)

Primary metabolites	Mean ± SD
Total lipids	30.906 ± 0.005
Total proteins	5.296 ± 0.005
Total carbohydrates	65.340 ± 0.312

After analysing, plant powder was found rich in carbohydrates. It has also manifested considerable amount of lipids and least percentage of proteins.

Estimation of Secondary Metabolites

Secondary metabolites were found using plant extracts and are given in Table 3.

Table 3: Secondary Metabolites (mg/g) in Different Extracts of *Z. pentandra*

Secondary Metabolites	Petroleum Ether	n-Hexane	Chloroform
Total polyphenols	199.74 ± 0.54	216.72 ± 1.08	227.42 ± 0.54
Total flavonoids	73.83 ± 0.5	67.16 ± 0.5	88.83 ± 0.5
Total polysaccharides	11.14 ± 0.72	17.14 ± 0.74	42.85 ± 0.74
Total glycosaponins	41.07 ± 0.001	27.03 ± 0.005	86.10 ± 0.003
Total alkaloids	25.36 ± 0.5	139.03 ± 0.5	226.36 ± 0.5

The analysis of secondary metabolites in various extracts of *Z. pentandra* revealed intriguing patterns, shedding light on the plant's chemical composition. Notably, the chloroform extract exhibited a relatively higher concentration of secondary metabolites compared to the petroleum ether and n-hexane extracts. This disparity among the extracts underscores the significance of solvent choice in extracting specific bioactive compounds from the plant material [25].

UV-Visible Profile of Different Extracts of *Z. pentandra*

Ultraviolet spectra of *Z. pentandra* extracts were taken and their overlay was constructed to compare and evaluate the peaks. Results are given in Figure 1. Overlay of all the extracts reveal almost similar peaks that is an indication of almost same number of compounds. The peaks that fall in the Ultra-Violet region, those reflect the compounds which exhibit sigma-sigma star electronic transitions requiring high energy and high frequency waves for excitation of electrons. The compounds showing spectrum in the visible region give an insight of the coloured compounds and non-bonding to pi-star or pi-pi star electronic transitions. Which require low amount of energy for electronic excitations, thereby, fall in the low frequency region indicating unsaturated nuclei present in the centre of molecules.

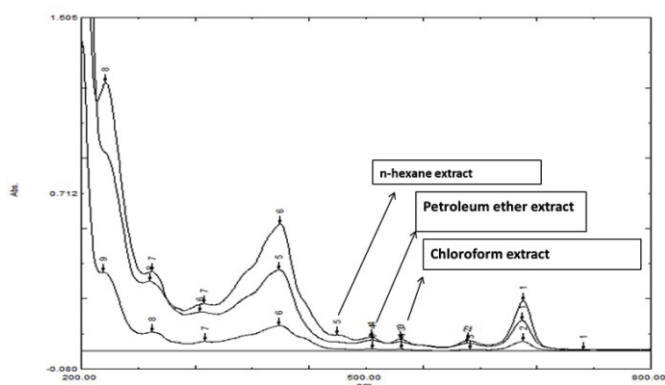


Figure 1: Overlay of UV-Visible Spectra of Different Extracts of *Z. pentandra*

Fourier Transform Infra-Red Spectroscopy (FTIR) Analysis of Fine Powder of *Z. pentandra*

FTIR analysis of fine powder of *Z. pentandra* gives information of some main functional groups reflecting a particular class of compounds. Each peak fall at a particular wavenumber (Figure 2), that reflects a specific functional group or chemical bond.

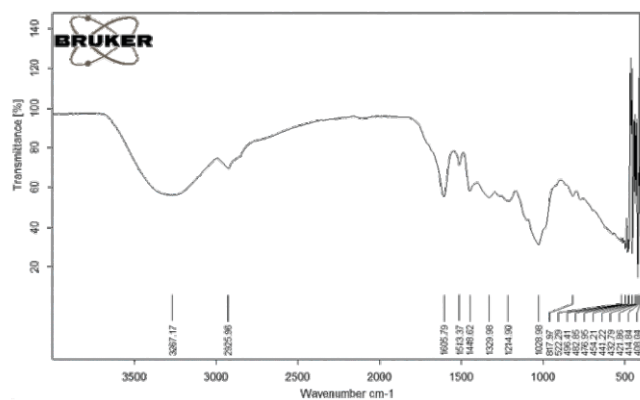


Figure 2: FTIR Spectroscopic Image of Fine Powder of *Z. pentandra*

Determination of Metals

The measurement of metals (ppm) in powder plant material using atomic absorption spectrophotometer were found as zinc (19.5), copper (5.0), iron (2.25), manganese (12.25), lead (2.0), chromium (3.75) and magnesium (79.0). Zinc is an essential element for normal growth and reproduction. It also plays a pivotal role in tissue repairing and wound healing. Iron is involved in redox reactions in all living bodies and is a necessary constituent of several protein molecules such as haemoglobin, myoglobin, cytochrome c, metallo-flavoproteins and enzymes like catalase and peroxidase. Manganese is also required for biosynthesis of haemoglobin. Magnesium is required for protein synthesis and its deficiency brings some serious physiological abnormalities such as irritability of the nervous system, peripheral vasodilation and cardiac arrhythmias [26].

DISCUSSION

Any sample's volatile matter and fixed carbon concentrations may be estimated using approximate analysis [27]. High moisture content indicates low dry matter and increases plant vulnerability to insect attack and fungal degradation, requiring tight storage conditions. Total ash indicates medication purity, while acid insoluble ash indicates calcium oxalate concentration [28, 29]. Polar solvent extractive values reveal tannins, glycosides, and phenols [30]. Polyphenols and flavonoids, antioxidants, were found in all plant extracts (n-hexane, petroleum ether, and chloroform extracts) demonstrating a homogenous distribution in the plant material [31]. The extracts have different polysaccharide, glycosaponin, and alkaloids concentrations. The chloroform extract has a high polysaccharide content, suggesting it might provide complex carbs [32]. The chloroform extract also included glycosaponins, which have several pharmacological uses, emphasising its importance in herbal therapy [25]. The chloroform extract had a greater concentration of bioactive alkaloids, suggesting it might be a source [31]. Due to its selective extraction, petroleum ether extract has less polysaccharides and alkaloids. The extracts' metabolite content showed the relevance of using various solvents in phytochemical studies to acquire a complete profile of secondary metabolites in a plant species [33]. In conclusion, solvent selection in phytochemical research is important since *Z. pentandra* extracts vary in secondary metabolite concentration. Polysaccharides, glycosaponins, and alkaloids were abundant in the chloroform extract, suggesting therapeutic uses. These discoveries help us comprehend the plant's chemical richness and promise in traditional medicine and pharmaceutical research. About 88-95% of plant, *Z. pentandra* material was approximated [34]. Plant extracts had significant polyphenol content, whereas plant powder had high carbs. Polyphenols and flavonoids were detected in the plant, and the FTIR spectrum showed hydroxyl groups. Polyphenols, natural antioxidants, repair coronary artery disease, ischemic heart disease, cancer, and asthma [35]. Flavonoids has antioxidant capability that can treat coronary heart disease and cancer [36]. Plant extract UV spectra show polar and non-polar molecules. Plant powder contains diterpenes and triterpenes according to FTIR spectra. The visible region contains conjugation systems and unsaturated double or triple bonds, while the UV region contains chromophore groups like nitro, nitroso, diazo, acetonitrile, and auxo-chromic groups like sulfhydryl, hydroxyl, and amines that shift the wavelength from UV to visible.

CONCLUSIONS

In conclusion, this study provides valuable insights into the physicochemical properties of powdered *Z. pentandra* plant material and its various extracts. These findings serve as a comprehensive reference for the standardization of extracts. Notably, the plant exhibits a rich composition, including significant levels of carbohydrates, lipids, carotenes, phenolic compounds, flavonoids, glycosaponins, polysaccharides, alkaloids, and metal ions. These diverse components collectively contribute to the plant's substantial nutritional value.

Authors Contribution

Conceptualization: AF

Methodology: AF, MR, SR

Formal analysis: AF, MR, TR

Writing-review and editing: AF, TR, SR

All authors have read and agreed to the published version of the manuscript

Conflicts of Interest

The authors declare no conflict of interest.

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