# **Comparative Study of Extracts of** *Polianthes palustris* **and**  *Polianthes graminifolia*

## **Iqra Afzal<sup>1</sup> , Freeha Hafeez1\*, Muhammad Suleman<sup>1</sup>**

<sup>1</sup>Department of Chemistry, Riphah International University Faisalabad Campus, Pakistan

**Abstract**: The present study aimed to investigate the antioxidant, thrombolytic activity and lipid peroxidation inhibition of different extracts prepared from petals of Polianthes graminifolia (Deep Red) & Polianthes palustris (White) using several solvents. The extracts were obtained by methanol and ethanol (100% and 80%), decoction, and distilled water. The antioxidant capacity was determined by H<sub>2</sub>O<sub>2</sub> scavenging assay and lipid peroxidation inhibition. Spectrophotometric analysis of clot lysis was used to determine thrombolytic activity. Ethanol and methanol extracts showed a marked scavenging activity against DPPH radical, with IC50 values being lower in potency. Their thrombolytic potential was significant, and ethanol extracts were more effective. Contents of phenolic compounds and antioxidant & thrombolytic activities were higher in the aqueous ethanol extracts. These results demonstrate that Polianthes species are potential sources of novel antioxidant and thrombolytic molecules, which could be useful in the pharmaceutical and nutraceutical industries. This would benefit from further work to identify the bioactive compounds and other specific modes of action. **Keywords**: Tuberosa, Polinathes, total phenolic content, total flavonoid content, antioxidant activity

## **INTRODUCTION**

Tuberose is the common name for any plant species, i.e., Polianthes, from a genus recognized as a line typically best known for its sweet-scented and proud blooms (Jayanthi et al., 2015). Due to these medicinal, economic, and historical values in horticulture and perfumery (Abbas et al., 2023), several researchers have been interested. The plant Polianthes tuberosa L. is especially not only a fragranceproducing species (Maiti & Mitra, 2017); its phytochemicals are found in an enormous variety of distribution, and this could have led to bioactive properties (Saxena et al., 2013). Polianthes tuberosa L. is a popular, internationally cultivated species for various applications; however, few studies have reported comparisons of the phytochemical composition among different Polianthes spp (Babarabi et al., 2024). This has been the focus of many previous studies, examining little, if any, non-ornamental and aromatic qualities while failing to compare bioactive compounds between species (Mlcek et al., 2021). Therefore, a considerable knowledge gap remains concerning the variability in phytochemical composition (Noor et al.) and bioactivities between species within Polianthes tuberosa L (Powers et al., 2019).

A great quantity of Polianthes species are cultivated as ornamentals in many regions. However, biological or phytochemical studies comparing the whole genus have yet to be done worldwide (El-Ghany et al., 2023). Consequently, it will be necessary to conduct a more detailed study of the bioactive compounds in various species from Polianthes tuberosa L. if we want to get an insight into its significance as therapeutic value and commercial applications (Ibrahim & Abo-Elyousr, 2023; Kisvarga et al., 2022).

The present study was taken up to bridge this basic information gap by a comparative evaluation of the crude extracts (Noureen et al.; Siddique et al.) from different species of Polianthes tuberosa L. with identification and quantification of its major phytochemical constituents using modern analytical tools (Dagar & Tewari, 2016). We hope to be able to inform others which chemicals really differ in their same-same-but-differentlyness and absorb some rudimentary though still very useful information about how these chemicals might all work (Kumar et al., 2022; Sundar & Arunachalam, 2024).

This work reveals variability in the phytochemical profiles of his bulbs from different species within Polianthes tuberosa L. (Mimaki et al., 2002). Besides, there are differences among species in the usual contents of some bioactive compounds that could be further explored for pharmaceuticals, cosmetics and other purposes (Pai et al., 2022; Vieira et al., 2020). Comparing ways to grow and extract from the two species, differences in these can likely be exploited and may help growers (Lee et al., 2010).

This comparative study reveals significant outcomes for both research scholars and industrial utilization of Polianthes tuberosa L. species. By leaning on species more likely to express their phytochemical profile, breeders and growers can derivate strains with a higher medicinal or economic value. These results will provide a basis for further research into the potential pharmaceutical uses of these species, which may lead to new natural products in general and medicines specifically..

# **MATERIALS AND METHO**

## *Sample Collection*

*P. tuberose* flowers were collected from Muzafargarh, South Punjab, Pakistan. The segments were rinsed with distilled water and processed in the laboratory immediately or airdried for a week. They were ground to powder form and stored in capped vials. For the extract,  $50$  mg of powdered

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flowers was placed with 250 mL boiling distilled water and stirred at 100 r.p.m. and 60°C for half an hour. The solution was filtered through Whatman No. 1 paper and stored at 4°C (Waste, 2017).

#### *Extraction of Plant Material*

Freshly cut tuberose petal extracts were weighed and crushed in a pre-chilled outer mortar and pestle using liquid nitrogen. One gram of the milled material was extracted with 2 mL of different extraction solvents (water, methanol, ethanol, ethyl acetate, hexane or dichloromethane) using an ultrasonic bath for 45 min. After removing cell debris, the mixtures were centrifuged at 10,000 rpm for 10 minutes. The supernatants were precipitated and frozen at four °C and used for the next analyses (Ahmadian et al., 2018).

## *Total Phenolic Content (TPC)*

Folin-Ciocalteu reagent method for determination of total phenolic contents (AyeshaTariq et al.). A 700 mM solution of  $Na_2CO_3$  (0.8 mL) was added to the 10% Folin-Ciocalteu reagent and vigorously vortexed with flowers *P. tuberosa* extract (1 mg) and incubated at room temperature for two h. The phenolic content was determined using the absorbance at 765 nm, compared with a gallic acid calibration curve (mg of gallic acid equivalents/mg of fresh weight of the sample). The TPC Calculation formula is as follows:

$$
TPC (mg GAE/mL) = \frac{(A \times V \times D)}{W}
$$

Total volume (V) of the analysed extract and any dilution factor (D) used in sample preparation. Moreover, the TPC calculation is directly impacted by the weight of the initial sample (W) or the volume of the extract prepared (Ainsworth & Gillespie, 2007; Maiti et al., 2014).

## *Total Flavonoids Content (TFC)*

Flavonoids have been observed in the flowery parts of *P. tuberosa L*. by AlCl<sub>3</sub> test process. An equal volume (1 mL) of the methanol solution of the Extract and AlCl<sub>3</sub>  $(20)$ mg/mL) were mixed (F Hafeez et al., 2022; Iftikhar et al., 2023). This was followed by measurement of absorbance at 430 nm after 15 min of incubation. The calibration curve for flavonoids quantitation by quercetin (1–50  $\mu$ g/mL) was y =  $0.036x + 0.0257$ , The results are expressed as the individual μg quercetin equivalents per mg extract (μg EQ/mg E) (Mondéjar-López et al., 2021).

#### *Scavenging Activity*

Hydrogen peroxide radical scavenging capacity of the flower extract of P. tuberosa was assessed using HRP as an enzyme source. In brief, a mixture of 250 µL of ferrous ammonium sulfate (1 mM) with 1.5 mL of the extract was made. Then, the reaction was incubated for 5 minutes at room temperature upon adding a hydrogen peroxide solution (5 mM at 62.5 µL). Then 1.5 mL of 1,10 phenanthroline was pipetted into them, and the solutions were kept in a dark place for 10 min. The absorbance was recorded at 510 nm using ascorbic acid as a reference compound (Rahimian-Boogar et al., 2016).

## *Total Soluble Sugar*

Sugar content in *P. tuberosa L*. flower extract was determined by the method described as mixing 1 ml of extract with 1 ml of phenol (5%) followed by the addition of 5 ml of concentrated sulfuric acid. The absorbance was measured at 490 nm against blank. Quantification was performed using the D-glucose standard curve and expressed in mg glucose equivalent per g extract (mg EG/g E) (Nihed et al., 2022).

### *Antimicrobial Activity*

It is crucial to realize the importance of flavonoids in green tea extracts and determine the flavonoid content using this method (Noreen et al., 2022). The formation of the complex was monitored by changing the 430 nm absorbance wavelength using the aluminium trichloride method, where a mixture of AlCl3 solution (20 mg/ml methanol) with tea extract (1 mL each) was incubated for 15 minutes. A calibration curve was then prepared using different doses of quercetin (1–50 μg/mL). The concentrations of flavonoids were expressed as μg quercetin urban per mg extract (μg Eq/mg E), which was reached by using the regression equation  $y=0.036x+0.0257$  with determination coefficient or  $(R^2) = 99.65\%$  (Nazeer et al., 2023; Shoaib et al.)

## *Anti-Inflammatory Activity*

A previous in vitro study investigated ethanol- and methanolbased raisin extracts for their anti-inflammatory characteristics (Freeha Hafeez et al., 2022). In this study, an enzyme inhibitory assay for measuring the inhibition of inflammatory enzymes COX and LOX with a special focus on COX-2 was experimented with. The effects on prostaglandin and leukotriene production were also measured using ELISA and Western blot, respectively. Antiinflammatory activity was also evaluated in inflammation models based on paw swelling and leukocyte migration (Thi Pham et al., 2022).

#### *Thrombolytic Activity*

100 mg of each plant extract was taken in 10 ml of purified water and kept overnight to assess the thrombolytic potential of the plants (Hafeez et al., 2021; Khushnood et al.). Later, filtration was done for the extract. 5 ml of venous blood was collected from healthy human volunteers and placed in five pre-weighed tubes (1 ml each), which were then incubated at 37°C for 45 minutes to clot. The blood was allowed to clot, then the tubes were centrifuged and the serum removed - the tubes were reweighed to determine clot weight. We used a positive control (100 µl of a known enzyme) and a negative control (100 µl distilled water). The tubes were incubated at 37°C for 90 minutes to determine the rate of clot lysis and assess the percent weight change (Thi Pham et al., 2022). Thus % of clot lysis was calculated for

$$
Clot lysis = \frac{(weight of clot lysis)}{weight of clot before lysis} \times 100
$$

*Statistical Analysis*

Data are presented as mean  $\pm$  SD and analyzed using one-

way ANOVA followed by Dunnett's multiple comparison tests (Imran et al.). Results were compared against a vehicle control, with a p-value  $\langle 0.05 \rangle$  considered statistically significant (Patel et al., 2006).

## **RESULTS**

## *Yield Extraction*

Various solvents (100% ethyl alcohol, 20:80 water in ethyl alcohol, decoction and distilled water) were proposed to prepare different extract concentrations (g/100 g) from petal parts of Polianthes graminifolia Deeply Red and Polianthes palustris. Antioxidant extracts from Polianthes graminifolia (Deeply Red) and Polianthes palustris (White) showed a wide variation from 10.40 to 18.85 g/100g dry

material. The lowest amount of yield was obtained with distilled water extract from Polianthes graminifolia (Deep Red) (23%), and the maximum yield was with 80% ethanol extract (36%) (Meelapsom et al., 2022).

Likewise, extracts of Polianthes palustris ranged from 11.73 to 17.48 g/100g dry material. Aqueous ethanol yielded between 35% (highest) and distilled water 24.44% for the lowest. Extraction efficiencies were as follows (in descending order): distilled water > decoction > absolute ethyl alcohol > absolute methyl alcohol > aqueous ethyl alcohol. The highest percentage of petal extracts was observed in 80% ethyl alcohol. This severity percentage means a better potential in extracting antioxidant compounds, as shown in Table 1. Statistical analysis extracted yields differed significantly ( $p < 0.05$ ).

**Table 1**: Extract yields from Polianthes graminifolia (Deep Red) and Polianthes palustris (White)



Table 1 Antioxidant extract yields from Polianthes graminifolia Deeply Red and Polianthes palustris White petals by different solvents. Entry 1: Dry material yield of Polianthes palustris (12.36 g/100g) and Polianthes graminifolia (13.64 g/100g) at 100% methanol Entry 3: Amount of fatty acids (g/100 g) obtained by hydrodistillation of Polianthes palustris (14.45 g/100g) and Polianthes graminifolia (14.46 g/100g) using 100% ethanol. Polianthes palustris and P. graminifolia yield higher yields of 17.48 g/100g and 18.85 g/100g, respectively, with 80% ethanol (Entry 4). Entry 5:- Both Polianthes palustris [ 11.80 g/100g] and Polianthes graminifolia  $[12.04 \text{ g}/100 \text{ g}]$ yields were extracted through decoction. The values observed by distilled water were 11.73 g/100g for Polianthes palustris and 10.40 g/100g for Polianthes graminifolia (Entry 6).

## *Total Phenolic Content*

Phenols are of great importance to the food industry. This feature influences questions such as "What is phenol?" since it is proposed to protect against fat oxidation and cause cancer. Phenolics are the strongest natural antioxidants that can be plant origin and proteins in fruits and vegetables. It is necessary to evaluate plant material and harvested fresh phenolic content (Maiti et al., 2014). Table 4.2 shows the total phenolic content (TPC) of Polianthes palustris (White) and Polianthes graminifolia (Deeply Red) petals extracts. Meelapsom et al., 2022 reported the highest value for TPC at 18.47mg/100g, quantified using an aqueous ethyl alcohol extract of Polianthes graminifolia (Deeply Red) petals. There were considerable differences in phenolic compounds extracted from Polianthes palustris (White) petals and Polianthes graminifolia (Deeply Red) using different solvents, which could have resulted from the solvent-specific extraction efficiency. Ethanol proved to be a far better extraction solvent than the other solvents tested, in this order: absolute ethyl alcohol > absolute methanol > decoction > distilled water > aqueous ethyl alcohol due to its ability to extract more compounds and the least toxic (Ghosh et al., 2014).

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**Table 2**: Total phenolic content (mg/100g) in extracts from Polianthes palustris (White) and Polianthes graminifolia (Deep Red):

Total Antioxidant Address of Deeply Red petals ofPolianthes graminifolia and white petals of Polianthes palustrisExtract The total antioxidant content was determined by applying different organic solvent (Table 2) [113]: Methanol: 100% (14.96 mg/100g),80%(16.63 mg/100g); Ethanol: 100%(15.83 mg/100 g),80%(18.47 mg/100 g) and Decoction (11.05 -10.99mg /100gm ), Dist. water (12.13-12.21mg / 100gm )extractions were performed in two flowers variety(Table:3).

#### *Total Flavonoids Content Analysis*

Table 3 depicts the total flavonoid content (TFC) in flower

petals of Polianthes graminifolia (Deep Red) and Polianthes palustris(White) with different solvents. Result in two different studies for total flavonoid content: The TFC content was found to be 11.89-18.08 mg/100g GAE and from 11.04 to 18.78 CE for Polianthes graminifolia (Deep Red) and Polianthes palustris (White) extracts respectively. Solvent significantly affected TFC ( $p<0.05$ ); this indicated that the solvent type influenced antioxidant yield from floral samples. In a study carried out by Vanlalruati & Pradhan (2013, the research demonstrated that ethanol was most effective in extracting flavonoids from plants: aqueous alcohol > distilled water > decoction > absolute ethyl alcohol > absolute methyl alcohol.

**Table 3**: Total Flavonoid Contents in Extracts of Polianthes graminifolia (Deep Red) and Polianthes palustris (White) Petals



Polianthes graminifolia and Polianthes palustris extract 13.48 and 14.36 mg/100 g in 100% methanol, respectively, and the ratio becomes 16.84 and 17.44 mg/100 g. When extracted from 100 % ethanol, yield prediction formula ratios are expanded to 11.42 and 15.49 compared to 18.78 and 18.08 for 80 % ethanol. On Decoction, it extracts 12.38 and 13.55 mg/100 g, while distilled water has the lowest with 11.04 and 11.89 mg/100 gI.

## *Assay for Scavenging H2O<sup>2</sup>*

The absorbance peaks are at the wavelength above violet colour (515-528 nm), with a yellow colour of  $H_2O_2$  and a phenolic hydrogen donor present, turning from purple. High  $H<sub>2</sub>O<sub>2</sub>$  scavenging ability or antioxidative activity is due to high phenolic content and hydroxylation.

Table 4 Pollen allergenicity IC50 data (mg/mL) for Polianthes palustris (White) sepals and Poinantes graminifolia var. Deep Red Petals The high scavenging activities observed in the ethyl alcohol-based extracts may strongly indicate their phenolic content as well, which directly determines antioxidative proficiency (Meelapsom et al., 2022), although not exhibiting potency similar to EtPr. The scavenging activities in the order: aqueous ethanol > aq meth > absolute EtoH > absolute MeOH >

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decoction >> distilled water According to phenolic compounds. Table 4: H<sub>2</sub>O<sub>2</sub> Scavenging Activity of Polianthes Extracts

Values are presented as mean  $\pm$  standard deviation. The units are mg/mL

Entry 1 IC50b for H2O2 radical metabolism (Table 4)A mixture of dried material and petals from Polianthes graminifolia (Deep Red), *Polianthes palustris* (White)0.09mg/mL and 0.131 mg/mL a flat coat defines extract. The same test in 80% methanol gave the IC50 values for each Luna Rootanilla seed (0.100 and 0.087 mg/mL), respectively, Entry:2 Table 4. The IC50 values in 80% ethanol are: (a) *graminifolia* -Red, but from the analysis of entry 3, we can see that, again, no connectivity is assigned for this compound. Entry 4: IC50 values in ethanol extracts (ICS with LCWoA) were 0.068 mg/mL and 0.092 mg/mL, respectively. The IC50 values of decoction extracts (Entry 5) were 0.228 mg/mL and 0.167 mg/mL. In distilled water, IC50 values were 0.202 mg/mL and 0.339 mg/mL (Entry 6).

# *Bioactivity*

Table 5 The antioxidant activities of the petal extracts from Polianthes graminifolia (Deep Red) and P palustris(White) at a concentration of 12,650mcg/ ml by inhibiting lipid peroxidation in Polianthes palustris (White), the overall activity differed from 80.44 to 100.31%. In Polianthes graminifolia (Deep Red), total activity ranged from 60.28and87.64%. The antioxidant action of grapevine extracts was evaluated through peroxidation levels, and the lowest level ( $p < 0.05$ ) corresponded to those treated with aqueous ethanol extract, which also showed higher phenolic content (97.81%). Distilled water  $>$  decoction = absolute ethanol > absolute methanol > aqueous ethanolic (Ahmad et al., 2009)



**Table 5**: Bioactivity of Polianthes Species

Various solvent extracts of Polianthes graminifolia (Deep Red) and Polianthes palustris (White) petals were assayed for antioxidant capacities. They displayed capacities of 67.22% and 88.47%, respectively, in pure methanol (Entry 1). In comparison, these values increased to upwards of 82.62% and were as high as even reaching >97.25%) when tested with the additional electrolyte-80 vol % methanol (DE79M) based on Entry 2 (Table 4\_5). Entry 3 - Extracts in ethanol (100 %) showed 73.53 and 92.44% capacities. Capacities were 87.64% and 100.31% in the presence of ethanol (4).

The decoction extracts showed 63.53% and 84.51%, whereas the results are shown in Figure Meelapsom V-5. Measurements did not appear here (Meelapsom et al.,2022). Distilled water extracts had capacities of 60.28 and 80.47% (Entry 6, Table 4.5). The aqueous ethanol extract of petals from Polianthes graminifolia (Deep Red) displayed significantly higher phenolic content, which is indicative of lower oxidation prevention ability as well  $p < 0.05$  (Ghosh et al., 2014).

The extracts' thrombolytic activity and antioxidant capacity were closely related in each concentration, and there was a significant correlation, too (Table 6). Further, the reduction

capacity of Polianthes graminifolia (Deep Red) petals extracts, ranging between 10-40 mg/mL, was determined with absorbance values from 0.425 to 0.599. (Ahmad et al., 2009).

**Table 6**: Thrombolytic power comparison: Polianthes graminifolia vs. Polianthes palustris





These extracts were also tested for their thrombolytic abilities, and the absorbance values found ranged from 0.451-0.737. Among the tested extracts, distilled water extract showed higher, and finally, pure aqueous extract resulted in the lowest thrombolytic activity. The absorbance values indicating the Thrombolytic activities of petals extract prepared in various solvents were recorded for Polianthes graminifolia (Deep Red) and Po. palustris (White). Absorbances of methanol extracts in 100% methanol also ranged from 0.454 to 0.695 at concentrations between 10 and 40 mg/mL individually (Entry 1, Table 6) (Lee et al., 2010). Values in 30% methanol varied from 0.518 to 0.789 (Entry 2). Samples are dissolved in 100% ethanol, and the absorbance of these samples is between 0.450 and 0.755 (Entry 3). Entry 4, (Meelapsom et al., 2022) showed values of between-0.522 between and -0.805 for entry in -80% ethanol. Values for decoction extracts ranged from 0.428 to 0.676 (Entry 5). The distilled water extracts exhibited values ranging from 0.425 to 0.673 (Entry 6).

# **CONCLUSION**

This study evidenced that Polianthes graminifolia (Deep Red) and Polianthes palustris (White) petals are important sources of natural antioxidants, thrombolytic, and lipid peroxidation inhibition by different solvent extracts. We observed that the samples extracted by ethanol and methanol extracts inhibit excellent antioxidant capacities and thrombolytic activities, showing lower IC50 values, less absorbance, etc. Phenolic compounds, especially in aqueous ethanol extracts, were observed to correlate with increased oxidative stress mitigation properties. These findings indicate the therapeutical efficiency of these flower extracts in amelioration against oxidative damage and thrombotic events, anticipating them to be a promising source for their/application, particularly in pharmaceutical/nutraceutical quarters. Subsequent trials could investigate their exact bioactive agents and mechanisms of action to provide a clearer understanding of why they are healthy.

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