Research Article

Phytochemical screening and allelopathic evaluation of aqueous and methanolic leaf extracts of *Populus nigra* L.

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Abstract

Allelopathic compounds are secondary metabolites which are produced during different metabolic pathways and apparently they have no prominent role in plants’ growth and development. However, these compounds are supposed to play an important role in defense and interactions of producers with receivers. In this study, aqueous and methanolic leaf extracts of *Populus nigra* were screened for the presence of secondary metabolites and tested for the allelopathic effects on seed germination of four wheat cultivars (Ghazanvi, Siran, Atta Habib and Janbaz). Phytochemical screening revealed the presence of alkaloids, flavonoids, steroids, terpenoids, saponins, tannins, phlobatanins, and glycoside, reducing sugars, triterpenes, phytosterols and proteins in leaf extracts. Allelopathic bioassay demonstrated that leaf extracts had detrimental influence on the seed germination of four cultivars of wheat, which depended on the amount of powder dissolved in extracts (10, 20, 40 and 80 g). Methanolic extracts exhibited greater degree of phytotoxicity than aqueous leaf extracts. Among the tested cultivars, Janbaz and Ghaznawi were more sensitive to aqueous and methanolic extracts particularly at the highest dose of concentration (80 g). The study suggests the presence of wide range of bioactive compounds in *P. nigra* leaves with phytotoxic potentials on wheat germination.

Keywords: Allelopathy; Alkaloids; Inhibitory effect; Secondary metabolites; Wheat

Introduction

Allelopathy is an important biological phenomenon which is directed by the releases of biological molecules from different plant parts in the form of root-exudates, leachates from above ground parts, volatilization or decomposed residues to interact with other plants, microbes and other organisms for either beneficial or negative association [1, 2]. The process is intricate in nature which may be due to the interaction of various groups of chemical compounds like alkaloids, flavonoids, phenolics, glycosides, lactones, quinines, volatile terpenes, organic acids compounds and other secondary metabolites (allelochemicals) —which are produced during secondary metabolic pathways and apparently have no active role in growth and development of plants but are concerned with defense and interaction with other organisms [3, 4]. Usually, the allelopathic effect of one plant on the other is...
perceived to be due to the synergetic or antagonistic effects of different compounds in order to make the environment conducive for allelopathic plants [5].

In many countries including Pakistan, wheat is grown close to poplar (Populus nigra) – a multipurpose tree of significant economic importance. However, the leaves of these trees might release toxic compounds when they fall and undergo decay process in the soil. This is a common problem but the underline mechanism is not fully understood. A number of researchers assessed the allelopathic effects of different Poplar species on agricultural crops which includePopulus deltoides [6-8], Populus tomentosa [9], and Populus euphratica [10]. However, research is lacking in review on phytotochemical screening and allelopathic effects of P. nigra on wheat. Thus understanding of the allelopathic nature of Populus leaves, their phytotochemical screening and effects on wheat are crucially needed for possible crop modification pattern in an agro-farming system where populous trees are cultivated. The aim of the present work was therefore to carryout phytotochemical screening and allelopathic effects of P. nigra on wheat. Thus understanding of the allelopathic nature of Populus leaves, their phytotochemical screening and effects on wheat are crucially needed for possible crop modification pattern in an agro-farming system where populous trees are cultivated. The aim of the present work was therefore to carryout phytotochemical screening and allelopathic effects of P. nigra on wheat. Thus understanding of the allelopathic nature of Populus leaves, their phytotochemical screening and effects on wheat are crucially needed for possible crop modification pattern in an agro-farming system where populous trees are cultivated.

**Materials and methods**

**Extraction and fractionation**
Leaves of P. nigra were collected from District Charsadda in October 2014; washed with running tap water for removing the surface contaminants and dust, then dried at room temperature for two weeks in shade, and was crushed using electric blender. The leaf powder (800 g) was extracted by soaking it in fresh water and methanol at room temperature separately. The extracts were then filtered and concentrated by Rotavapor under reduced pressure at temperature below 50°C. The final residue obtained was weighed (15.2 g) in fresh water and (21.1g) in methanol. Each of the extract was screened for the presence of phytotochemicals as per the standard procedures [11]. The aqueous extract (210 g) was subjected to fractionation and was partitioned successively with n-hexane, ethyl acetate, chloroform and methanol. Each extract was evaporated to dryness under reduced pressure that yielded ethyl acetate (7 g), chloroform (5 g) and methanol fraction (20 g) respectively. However, no n-hexane fraction was obtained.

**Phytochemical Screening**
Crude extracts of P. nigra including hot water and tap water and methanol as well as their sub-fractions were screened for qualitative determination of various secondary metabolites according to standard analytical methods [12].

**Test for reducing sugars**
To the extracts in separate test tubes 3-4 drops Fehling’s solution was added and heated. The appearance of red color revealed reducing sugar was present.

**Steroids**
2 ml of acetic anhydride and 2 ml concentrated H₂SO₄ were added to 5 ml of the extracts in separate test tubes. Change of color from violet to blue confirmed the presence of steroids.

**Glycosides**
2 ml of glacial acetic acid containing 1 drop of ferric chloride solution and 1 ml of concentrated H₂SO₄ were added to 4 ml of extracts. Appearance of a brown ring indicated the presence of glycosides.

**Tannins test**
Crude extracts (0.5 g) were suspended in water and heated on water bath and filtered, followed by adding few drops of ferric chloride to the filtrates. The appearance of green color indicated the presence of tannins.

**Alkaloids test**
Crude extracts (0.5 g) were warmed with 5 ml of 2% H₂SO₄ for 2 minutes, filtered and 2-3 drops of Dragendorf’s reagent was added to each filtrate and observed for red precipitation.
Saponins test
Crude extracts (0.5 g) were shaken with 5 ml of distilled water and was heated to boiling. Formation of froth with persistence time of 5 minutes or more represent saponins.

Triterpenes and phytosterols test
To 5 ml extracts 2 ml chloroform was added and filtered. To this filtrate 3 ml concentrated H$_2$SO$_4$ was added, shaken and allowed to stand and observed for golden yellow color that’s indicate triterpenes, while red color in the lower layer represent phytosterols.

Flavonoids test
Crude extracts (0.5 g) were dissolved in 1ml diluted NaOH and 3-4 drops HCl was added. Yellow color that turns colorless indicates the presence of flavonoids.

Anthraquinones test
Crude extracts (0.5 g) were boiled with 5 ml 10% HCl for few minutes in water bath, filtered and allowed to cool. To the filtrate 3 ml CHCl$_3$ was added. 2-3 drops of 10% ammonia was added to the mixtures and heated. Formation of rose-pink color indicated the presence of anthraquinones.

Phlobatanins test
Crude extracts (0.5 g) were dissolved in 5 ml distilled water and filtered. The filtrate was boiled with 1ml of 2% HCl solution. Formation of precipitation shows the presence of phlobatanins.

Terpenoids test
Crude extracts (0.5 g) were mixed with 2 ml of chloroform and concentrated H$_2$SO$_4$ (3 ml) was carefully added to form a layer. The formation of a reddish brown coloration at the interface indicated positive results for the presence of terpenoids.

Seed bioassay
Allelopathic bioassay for seed germination of four wheat cultivars was performed at Botany Department. 10 seeds of each wheat cultivar were placed on filter paper in sterilized petri dishes. 5 ml of each aqueous and methanolic extracts at 10, 20, 40 and 80 g were applied to petri dishes which were arranged in a completely randomized design. Distilled water was used as control treatment. For each treatment, five replicates of petri dishes were used. Germination % was calculated in each treatment at 10th day of the onset of germination bioassay. Least significant difference test was used to record the effect of extracts on germination % of wheat cultivars.

Results
Phytochemical evaluation of the aqueous leaf extracts of P. nigra revealed that secondary metabolites such as phenols, alkaloids, flavonoids, steroids, terpenoids, saponins, tannins, glycoside, reducing sugars, triterpenes and phytosterols were present in the samples. In methanolic extracts glycoside, reducing sugars and phytosterols were not detected while other compounds were present (Table 1). Furthermore, the organic fractions such as chloroform, ethyl acetate and methanol showed differential indication for the tested phytochemicals (Table 2). Germination bioassay demonstrated that four varieties of wheat differed in their response to the applied extracts; showing decline at increasing concentration of extracts (Fig. 1). In aqueous extract treatments, lowest germination percentage (28%) in cultivars Siran and Atahabib respectively at 80 g extract concentration was observed, which was significantly lower than control i.e., 68 and 70% respectively. In cultivar Ghaznavi, 34% germination was observed at 80 g extract against 64% in control. Cultivar Janbaz revealed 31% germination at 80 g when compared to control where it was 64%. All the extract concentrations showed inhibitory effects on germination of four wheat varieties; however, highest concentration (80 g) was more phytotoxic.
Table 1. Phytochemical screening of the crude extracts *Populus nigra* leaves; present (+), absent (-)

| Chemical components | Methanol extracts | Aqueous extracts |
|---------------------|-------------------|------------------|
| Alkaloids           | +                 | +                |
| Steroids            | +                 | +                |
| Terpenoids          | +                 | +                |
| Flavonides          | +                 | +                |
| Anthraquinones      | _                 | _                |
| Tannins             | +                 | +                |
| Phlobatanins        | +                 | +                |
| Saponins            | +                 | +                |
| Glycoside           | _                 | +                |
| Reducing sugars     | _                 | +                |
| Triterpenes         | +                 | +                |
| Phytosterols        | _                 | +                |
| Phenol              | +                 | +                |
| Proteins            | +                 | +                |

Table 2. Phytochemical screening of aqueous extract organic fractions of *Populus nigra* leaves; present (+), absent (-)

| Chemical components | Chloroform (2 g) | Ethyl acetate (3 g) | Methanol (10 g) |
|---------------------|------------------|---------------------|-----------------|
| Alkaloids           | _                | _                   | +               |
| Steroids            | +                | +                   | +               |
| Terpenoids          | +                | +                   | +               |
| Flavonides          | +                | _                   | +               |
| Anthraquinones      | _                | _                   | _               |
| Tannins             | +                | _                   | +               |
| Phlobatanins        | _                | _                   | _               |
| Saponins            | _                | _                   | +               |
| Glycoside           | +                | +                   | +               |
| Reducing sugars     | +                | +                   | +               |
| Triterpenes         | +                | +                   | +               |
| Phytosterols        | +                | +                   | +               |
| Phenol              | +                | +                   | +               |
| Proteins            | _                | _                   | +               |
Effect of methanolic extracts on seed germination of four cultivars of wheat is presented in (Fig. 2). Results revealed that all the extract concentrations were strongly phytotoxic which significantly lowered germination parentage in all the cultivars (or varieties?); however, unlike to aqueous extracts, 40 g extract concentration was more drastic in effects than other concentrations in methanolic extract. In cultivar Ghaznavi, lowest germination (12%) was observed at 40 g when compared to control (64%). Germination was 18% at 80 g extracts. Similar tendency was recorded in cultivar Janbaz where germination was significantly lowered to 14% by 40 g extract concentration. Cultivar Siran was found most susceptible to the allelopathic stress at 40 g where only 10% seeds germinated while in Atahabib germination percentage was 20%. In general, methanolic extracts were found to be more inhibitory than aqueous extracts towards seed germination of wheat.

![Figure 1](image-url)  
**Figure 1.** Effect of different concentrations of aqueous leaf extracts of *P. nigra* on seed germination (%) of four wheat varieties
Discussion
Our results regarding phytochemical screening accede with findings of those reported by Kaur and Arora [12] and Yadav and Agarwala [13] which documented different phyto-constituents in aqueous and organic-fractions extracts of different plants. Moreover, significantly lower germination of wheat cultivars in both aqueous and methanolic extracts may be attributed to the presence of phytotoxic chemicals as we identified the presence of phenols, alkaloids, tannins, glycosides, reducing sugar, saponins, carbohydrates, steroids, terpenoids, reducing sugars, triterpenes, phytosterols, and proteins in sample extracts. The differences in phytotoxicity under aqueous and methanolic extracts might be due to the release of more quantity or more types of phytotoxins or both. Alleopathy (inhibitory) potential may vary in relation to the change in polarity index of solvents i.e. methanol fraction was more phytotoxic as compared to chloroform and ethyl acetate fraction. This might be due to the nature of the organic chemical used accounting for qualitative and quantitative differences in extracted phytotoxins in different fractions. The allelochemicals investigated in the extracts can affect physiological functions such as seed germination, respiration, photosynthesis, ion uptake, enzyme activity, water status, transpiration, stomatal opening, hormone levels [2, 14, 15]. Furthermore, they can affect cell division and differentiation, signal transduction, gene expression and cell membrane and wall structure and permeability [1]. Allelochemicals can also provoke the production of reactive oxygen species which can disturb the metabolic activities of targeted plants [16]. Presence of phenolic compounds might interfere with the
activities of respiratory enzymes in seed germination thus causing inhibitory effect on its germination. Enzymes that mostly affected by phenolic compounds are aldolase and glucosephosphate isomerase, involved in glycolysis and glucose 6-phosphate dehydrogenase, an enzyme involved in the first step of oxidative pentose phosphate pathway [17]. Our results generally agree with previous studies conducted on the allelopathic activities of several plants on wheat germination and growth [18, 19].

Conclusion
The present study suggests that *P. nigra* is an allelopathic plant, which is capable of suppressing the germination of tested wheat cultivars. *P. nigra* leaves crude extracts contain different bioactive compounds e.g., alkaloids, flavonoids, steroids, terpenoids, saponins, tannins, phlobatanins, and glycoside, reducing sugars, triterpenes, phytosterols and proteins which might be responsible for its allelopathy effects.

Authors’ contributions
Conceived and designed the experiments: N Inayat & Z Muhammad, Performed the experiments: N Inayat, Analyzed the data: Rehmanullah & A Majeed, Contributed reagents/ materials/ analysis tools: Z Muhammad, Wrote the paper: N Inayat & Z Muhammad.

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