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Research

Qualitative and Quantitative Analysis of Photochemical and Study of the Effect of Phytoconstituents in Seed Germination

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Abstract: Ten distinct locally accessible plant sections were subjected to photochemical screening in methanol extract. Plants include a variety of phytoconstituents, including quinine, terpenoid, flavonoid, steroid, alkaloid, cardiac glycoside, glycoside, volatile oils, etc. The investigation into the role of phytoconstituents in the germination of Pisum sativum seeds indicated that these components had a cytotoxic impact on live cells, or the *Pisum sativum* seeds' ability to germinate. The plant extract's phytoconstituents had an impact on the growth and multiplication of cells. Therefore, these plants may be useful in the development of medications that target cancer cells as well as germs and other microorganisms.

Keywords: Cytotoxicity, Methanol extract, Photochemical screening, Phytoconstituents, Seed germination

Introduction

Medicinal plants are important species of plants that according to traditional medicinal practices and also from modern scientific studies are useful for medicinal purposes to alleviate diseases and make human health more invigorating. These plants are contemplated as rich sources of ingredients that can be used in the synthesis and production of drugs (Oladeji et al. 2019). Plants consist of various kinds of chemical constituents known as phytoconstituents (Mercy et al. 2017). Phytoconstituents Serve the plants by contributing some secondary functions like; helping in plant growth, safeguarding the plants by activating defense mechanisms, and imparting color, odor, and flavor to the plants (Molyneux et al. 2007). Natural products and their derivatives exhibit minimal side effects and improved efficacy than other synthetic counterparts (Batiha *et al*. 2020).

These plant-derived components like flavonoids, quinine, terpenoids, etc conduct certain biological functions that enhance therapeutic activities such as anti-carcinogenic, anti-mutagenic, anti-inflammatory, and antioxidant properties (Batiha et al. 2020). Photochemical screening is the scientific process of analyzing, examining, extracting, experimenting, and thus identifying different classes of phytoconstituents present in various parts of the base for the discovery of drugs, the active components could be further taken for investigation and research.

The process was qualitative which is termed phytochemical screening. The outcome of the research could be fruitful in developing potent drugs against various diseases.

Table 1. List of medicinal plants and uses.

Name	Part taken	Local name	Local uses
Allium cepa (ACB)	Bulb	Onion	Vegetable.
Curcuma longa (CLR)	Rhizome	Turmeric	Antiseptic, anti-diabetic and antibacterial agent (Maithalikarpagaselvi *et al*. 2020)
Ocimum sanctum (OSL)	Leaves	Tulsi	Antioxidant, Anti-inflammatory (Chaudhary *et al.* 2020)
Mentha arvensis (MAL*)*	Leaves	Mint	Antibacterial and antiseptic agent (Patil *et al.* 2016)
Allium sativum (ASB)	Bulb	Garlic	Antioxidant (Melania *et al*. 2019)
Zingiber officinale (ZOR)	Rhi-zome	Ginger	Treats cold, cough, in gastric problems (Arwande *et al*. 2018)
Acorus calamus (ACR)	Rhi-zome	Calamus	Treats throat and stomach problems (Nath & Yadav, 2016)
Zanthoxyllum armatum (ZAS)	Seeds	Timur	Used in intestinal problems (Bharti & Bhushan 2015)
Nyctanthes arbortristis (NAL)	Leaves	Parijat	Anti-diabetic (Haque *et al*. 2015)
Nyctanthes arbortristis (NAF)	Flowers	Parijat	Anti-diabetic, treats hypertension (Haque *et al*. 2015)

The aim of this study is the phytochemical screening of plants and the cytotoxic activity of phytoconstituents in living cells. Plants used for the study along with their local name, parts taken, and local use are shown in Table 1. The study is important because plants showing cytotoxic effects in living cells could be further investigated and specifically studied for developing drugs against cancer and also against microbes and bacteria. Curcumin present in turmeric has been reported anticancer properties (Carroll et al. 2011). Fruits and vegetables containing flavonoids showed cancer chemo-preventive activity (Mishra et al. 2013). The presence of classes of phytochemicals as such; flavonoid, alkaloid, and tannin showed cytotoxic effects (Chaudhary et al. 2017).

Materials and Methods

Ten different plant samples were identified and collected. Collection of plants for the study purpose which is located in Bhopal areas and is situated at an altitude of 1412 m height with latitude 85°27' 32"east and longitude 27°38' 42" north. The climate of the village is moderate with deciduous vegetation. The plants were collected in October.

Collected plant parts were washed with distilled water, cut into small pieces, and dried in shade for 4 weeks. Dried plant parts were ground into fine powder using an electric grinder. 100 g of each powdered sample was soaked in 100-150 mL methanol in a conical flask, shaken occasionally to mix, and macerated for 72 hours at room temperature. Maceration intends to soften and break the plant's cell wall to release the soluble phytoconstituents (Handa et al. 2008). All the laboratory activities were performed in the laboratory of the Department of Chemistry, SAM Global University. An electronic analytical balance was used for weighing the powdered sample and chemicals in the experiments. Then the solution was percolated through cotton. Filtrate and marc were obtained.

Phytochemical Screening: The prepared extract of all ten plants was used to test various phytoconstituents present in them. Different chemical reagents were prepared and specific tests, for specific phytochemicals were done. These various tests were qualitative and hence termed phytochemical screening. All chemicals and solvents were procured from Fisher Scientific, India, and were used without further purification. The tests were done by following standard procedures based on journal articles. (Alamzed et al. 2013), (Thusa and Mulmi 2017), and (Talukdar & Chaudhary, 2010).

Test for Tannin/Polyphenol (Talukdar & Chaudhary, 2010): To the diluted extract, 3-4 drops of 10% $FeCl_3$ were added, a blue color was seen for gallic tannins and the presence of catechol tannin turned the solution green.

Test for Reducing Sugar (Thusa and Mulmi 2017): To 0.5 mL of plant extract, add 1mL of water, and 5-8 drops of Fehling's solution were added and heated. The presence of reducing sugar was indicated by the appearance of brick-red precipitation.

Test for Quinine (Thusa and Mulmi 2017): To the extract, freshly prepared $FeSO_4$ solution (1mL) and ammonium thiocyanate were added then conc. H_2SO_4 was added drop by drop. The deep red color indicated the presence of quinine.

Test for Glycosides (Alamzed et al. 2013): Molisch's Reagent Test: To the extract, 5 mL of Molisch's reagent and concentrated H_2SO_4 were added. The Violet color indicated glycosides.

Test for Flavonoids (Talukdar and Chaudhary 2010): Shinoda test: 4 mL of extract solution, 1.5 mL of 50% methanol solution a small magnesium chunk were warmed. 5-6 drops of concentrated HCl were added, red color was observed for flavonoids.

Dil. NH_3 Test: 5 mL of dilute NH_3 solution in the extract was taken with the addition of conc. H_2SO_4. The appearance of yellow-colored precipitation indicated flavonoids.

Test for Terpenoids (Alamzed et al. 2013): 0.2 g of each sample was mixed with 2 mL chloroform, 3 mL conc. H_2SO_4. Reddish-brown coloration indicated the presence of terpenoids.

Test for alkaloids: Meyer's Test (Talukdar and Chaudhary 2010): To 2 mL of extract, 1 mL of Meyer's reagent was added. The presence of a pale yellow precipitate indicated the presence of alkaloids.

Dragendroff's Reagent Test (Alamzed et al. 2013): 2 mL of extract was warmed with 2% H_2SO_4. A few drops of Dragendroff's reagent were added. Orange-red precipitate indicated the presence of alkaloids.

Test for Saponins (Alamzed et al. 2013): 2 g of powdered sample was boiled in 20 mL of distilled water. 10 mL of filtrate and 5 mL of

distilled water were quivered vigorously. The appearance of frothing indicated the presence of saponins.

Test for Volatile Oils (Talukdar and Chaudhary 2010): 2 mL extract was shaken with 0.1 mL of NaOH and a small quantity of dilute HCl. White precipitate indicated the presence of volatile oil.

Test for Cardiac Glycosides (Talukdar and Chaudhary 2010): 5 mL of plant extract was treated with 2 mL of glacial acetic acid with one drop of $FeCl_3$ solution. A violet ring may appear or a greenish ring may form just which shows the presence of cardiac glycosides.

Test for Steroids (Talukdar and Chaudhary 2010): 1 g of plant extract was dissolved in a few drops of acetic acid and a drop of conc. H_2SO_4 was added. The appearance of green color indicated the presence of steroids.

Study of Effect in Seed Germination

Germinating *Pisum* seeds were taken as the representative of living cells which was the basis to study the cytotoxic activity of phytoconstituents in living cells. The study of the effect of phytoconstituents in the germination of *Pisum* seed was done in aqueous extract, methanol extract, and methanol by soaking in the solutions for five days. The method implied was based on procedures given by Radwan et al. (2019), Hassan and Samy (2007) and Chekuboyina and Rao (2015).

Results and Discussion

The results of the various phytochemical screening tests obtained during the experiment are shown in Table 2. Tannin, quinine, terpenoid, flavonoid, steroid, alkaloid, cardiac glycoside, glycoside, volatile oils, etc were the phytoconstituents found in plants. According to the literature and the tally done with the obtained result, Curcumin present in turmeric has reported improved insulin resistance, and glucose uptake, affected blood pressure, and reduced inflammation (Azhdari et al. 2019). Flavonoids cause risk reduction mainly from cardiovascular diseases and cancer (Ballard and Marostica 2019).

Table 2. Photochemical screening of different medicinal plants.

	Phytochemical Screening													
			Flavonoid					Alkaloid						
Plant extract	Tannin	Reducing	Quinine	Glycoside	Shinoda	Dil. NH_3	Terpenoid	Meyer's	Dragendroff	Saponin	Volatile oil	Cardiac Glycoside	Steroids
ACB	–	–	+	+	+	+	+	–	–	+	+	+	+
			+	+	+	+	+				+		
			+	+	+	+	+				+		
CLR	+	–	+	+	+	+	+	+	+	+	+	+++	+
	+		+	+	+	+	+	+	+	+	+		+
	+		+	+	+	+	+	+	+		+		
OSL	+	–	+	+	+	+	+	+	+	+	+	+++	+
	+		+	+	+	+	+	+	+	+	+		+
	+		+	+			+	+	+		+		+
MAL	+	–	+	+	+	+	+	+	+	+	+	+++	+
	+		+	+	+	+	+	+	+	+	+		+
	+		+	+	+	+	+	+	+		+		+
ASB	–	–	+	+	+	+	+	–	–	+	+	+	+
			+	+			+				+		
			+	+			+				+		
ZOR	+	–	+	+	+	+	+	+	+	+	+	+++	-
	+		+	+	+	+	+	+	+	+	+		
	+		+	+	+	+	+	+	+	+	+		
ACR	–	–	+	+	+	+	+	+	+	+	+	+++	+
			+	+		+	+	+	+	+	+		
			+	+			+	+	+	+	+		
ZAS	+	+	+	+	+	+	+	+	+	–	+	++	+
	+		+	+	+	+	+	+	+		+		+
	+		+		+	+	+	+	+		+		
NAL	+	–	+	+	+	+	+	+	+	+	+	+++	+
	+		+	+	+	+	+	+	+	+	+		+
	+		+	+			+	+	+	+			
NAF	+	–	+	+	+	+	+	+	+	+	+	+++	–
	+		+	+	+	+	+	+	+	+			
	+		+	+	+	+	+	+	+	+			

Source – Experimental results, - indicated absent, + indicated present, ++ indicated moderate present, +++ indicated high presence.

The presence of classes of phytochemicals as such; flavonoid, alkaloid, and tannin showed cytotoxic effects (Chowdhury et al. 2017). The color and aroma imparting flavonoids were stated to show anti-cancer properties. Additionally, cholesterol-lowering, as well as cytotoxic qualities, anti-bacterial, and anti-viral properties, are credited to the presence of saponin (Bailly and Vergote 2020). Tannin shows an anticancer property that is perceptible from its inhibitory activity toward growth (Mazni, ho Yin, Azizul, & Nurdin, 2016). Plants containing a high amount of flavonoids could be useful as anti-bacterial (Ballard and Marostica 2019). So plants like *Zingiber, Curcuma,* and *Acorus* could be used as antibacterial, and antiseptic agents.

Plants containing phenolic compounds could be useful as antioxidants. Quinine showed antipyretic properties so plants containing quinine like *Ocimum, Nyctanthes*, *Mentha,* etc could be used to reduce fever. *Mentha* is also used as a soothing agent, for relieving toothache, and also as an anti-bacterial anti-helminthic agent (Patil and Godghate 2016). *Nyctanthes and zingiber* also play a role in maintaining blood sugar. *Zingiber, Acorus, and Curcuma* consist more amount of cardiac

glycoside which is beneficial for the heart. The phenolic compound, tannin, terpenoid, and flavonoids possess an anti-helmintic property so the plant *Zanthoxylum*, *Acorus* could be used to treat stomach problems (Nath and Yadav 2016). The polyphenolic compounds, flavonoids, terpenoids found in *Allium cepa,* and *Allium sativum* are useful as antioxidant, anti-inflammatory, and antibacterial agents. Likewise, they play an important role in reducing blood pressure, in preventing heart disease.

Study of the Effect of Plant Extracts in the Germination of *Pisum sativum*

Seeds:

According to the observation germination of seed in water was with a short length of 0.9 cm. Water and methanol were used as positive control and negative control respectively. The observed results in the aqueous extract and methanol extract are shown in Table 3. The inhibition in growth in the aqueous extract may be due to the presence of phytoconstituents. In the methanol extract of the plant sample, the seeds did not germinate. The table below indicates the length of shoot of the seeds in aqueous and methanol extract which was the obtained result for determining the cytotoxic effect of the extracts.

Table 3. Shoot growth in the extracts.

Plant Extract	ACB	CLR	OSL	MAL	ASB	ZOR	ACR	ZAS	NAL	NAF
Shoot growth in Aq. Ext. (cm)	0.2	0.2	0.3	0.2	0.2	0.2	0.3	0.3	0.3	0.3

Source – Experimental Result, Shoot growth in methanol extract: - ve Shoot growth in water: 0.9 cm.

This result revealed that the phytoconstituents in plant extracts showed a cytotoxic effect in the germinating *Pisum sativum* seeds. Hence these plants can be further studied and experimented with to develop drugs against cancer cells and also against microbes and bacteria.

Conclusions

Hence, the phytochemical screening of the selected plant sample was done. From the study, it could be concluded that plants are a great source of phytochemicals that could be utilized in curing various ailments. Tannin, quinine, terpenoid, flavonoid, steroid, alkaloid, cardiac glycoside, glycoside, volatile oils, etc were the phytoconstituents present abundantly in plants.

Phytochemical screening played an important role in identifying various phytoconstituents present in plant extracts. Phytochemicals in the aqueous extract slightly inhibited the growth. This study helped to know the cytotoxic effect of the phytoconstituents present in plant extracts on the living cells.

The study provided an important basis for further investigation into the isolation and characterization of phytoconstituents from the selected plants for the development of drugs. The study was only based on qualitative analysis and screening. It would be better if a quantitative detection, their bioactivity, and IR spectra of the various phytochemicals could be performed. The study would be more beneficial if the detection, analysis, and separation of the phytoconstituents could be done.

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