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Research

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

Sarvar Jahan
Department of Chemistry, School of Sciences, SAM Global University, Raisen-464551,Madhya Pradesh, India
CorrespondingEmail:sarvarjahan92@gmail.com
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Abstract: Ten distinct locally accessible plant sections were subjected to photochemical screening in methanol extract. Plants contain a range of phytoconstituents such as quinine, terpenoids, flavonoids, steroids, alkaloids, cardiac glycosides, glycosides, and volatile oils. Research on the influence of phytoconstituents on the germination of Pisum sativum seeds showed that these substances exerted a cytotoxic effect on living cells or impacted the seeds' germination ability. The phytoconstituents in the plant extract influenced cell growth and proliferation. Therefore, these plants may be helpful 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 etal.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, safe guarding 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).

Natural compounds from plants, including flavonoids, quinine, and terpenoids, perform specific biological roles that boost therapeutic effects such as anti-carcinogenic, anti-mutagenic, anti-inflammatory, and antioxidant properties (Batiha et al. 2020). Photochemical screening is a scientific approach that involves analyzing, examining, extracting, and experimenting to identify various classes of phytoconstituents found in different parts ofthe base for drug discovery. The active components identified can then be used for further 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.

Table1. List of medicinal plants and uses.

Name	Part taken	Local name	Localuses
Alliumcepa (ACB)	Bulb	Onion	Vegetable.
Curcuma longa (CLR)	Rhizome	Turmeric	Antiseptic, anti-diabetic and antibacterial agent(Maithalikarpagaselvietal.2020)
Ocimum sanctum (OSL)	Leaves	Tulsi	Antioxidant,Anti-inflammatory(Chaudharyetal.2020)Antibacterialandantisepticagent(Patiletal.2016)
Mentha arvensis (MAL)	Leaves	Mint	
Alliums ativum(ASB)	Bulb	Garlic	Antioxidant (Melaniaetal.2019)
Zingiber officinale (ZOR)	Rhi-zome	Ginger	Treats cold, cough, in Gastric problems (Arwandeetal.2018)
Acorus calamus (ACR)	Rhi- zome	Calamus	Treats throat and Stomach problems (Nath&Yadav,2016)
Zanthoxyllumarmatum(ZAS)	Seeds	Timur	Used in intestinal problems (Bharti &Bhushan2015)
Nyctanthes arbortristis(NAL)	Leaves	Parijat	Anti-diabetic (Haque *et al*.2015)
Nyctanthes arbortristis (NAF)	FlowersParijat		Anti-diabetic, treats hypertension(Haqueetal.2015)

The objective of this research is to conduct phytochemical analysis of plants and evaluate the cytotoxic effects of phytochemicals on living cells. Plants used for the study along with their local name, parts taken, and localuse 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 existence of various classes of phytochemicals, including flavonoids, alkaloids, and tannins, demonstrated 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 4weeks. 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 maceratedfor72hours at room temperature. Maceration intends to soften and break the plant's cell wall to release the soluble phyto-constituents (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 assessments were conducted in accordance with established protocols derived from published research 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% FeCl3 were incorporated, resulting in a blue color indicative of gallic tannins, while the presence of catechol tannin altered the solution to a green.

Test for Reducing Sugar (Thusa and Mulmi 2017): To 0.5ml of plant extract add 1mL of water, and 5-8 drops of Fehling's solution were added and heated. The emergence of brick-red precipitate signifies the presence of reducing sugars.

Test for Quinine (Thusa and Mulmi 2017): To the extract, freshly prepared $FeSO_4$ solution (1mL) and ammoniumthiocyanate 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 along with concentrated H2SO4 were introduced. The appearance of a violet color suggested the presence of glycosides.

Test for Flavonoids (Talukdar and Chaudhary 2010): Shinoda test involved mixing 4 mL of the extract solution with 1.5 mL of 50% methanol solution and a small piece of magnesium, then warming the mixture. Subsequently, 5-6 drops of concentrated HCl were introduced, resulting in a red color that indicated the presence of flavonoids.

Dil. NH_3 Test: 5 ml sample of diluted NH3 solution from the extract was mixed with concentrated H2SO4. The formation of yellow precipitation indicated the presence of flavonoids.

Test for Terpenoids:(According to Alamzed et al. (2013), 0.2 g of each sample was combined with 2 mL of chloroform and 3 mL of concentrated H2SO4. The formation of a reddish-brown color suggested the presence of terpenoids.

Test for alkaloids: Meyer's Test (Talukdar and Chaudhary 2010): 1 ml of Meyer's reagent was combined with 2 ml of the extract. The appearance of a pale yellow precipitate

suggested the existence 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 suggested the existence of alkaloids.

Test for Saponins (Alamzed *et al.* 2013):2g of powdered sample was boiled in 20mL of distilled water were quivered vigorously. The appearance of frothing indicated the presence of saponins.

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

Test for Cardiac Glycosides 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 : 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.

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, volatileoils, etcwerethe 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 (Ballardand Marostica 2019).

Table2. Photochemical screening of different medicinal plants

	Phytochemical Screening													
				Flavonoid				Alkaloid						
Plant Extract	Tanin	Reducing	Quinine	Glycoside	Shinoda	Dil NH3	Terpinoid	Meyer's	Dragendroff'	Saponins	Volatile Oils	Caruuac Glycosides	Steroids
ACB			+	+	+	+	+	+	+	_	+	+ +	+
			+	+	+	+	+	+	+		+		+
	+	_	+	+	+	+	+	+	+		+		
CLR	+		+	+	+	+	+	+	+	+	+	+ + +	+
	+		+	+	+	+	+	+	+	+	+		+
	+	_	+	+	+	+	+	+	+	+			
OSL	+		+	+	+	+	+	+	+	+	+	+ + +	_
	+		+	+			+	+	+	+			
	+	_	+	+	+	+	+	+					
MA L	+		+	+	+	+	+	+					
	+		+	+	+	+	+	+	+	+	+	+ + +	+
	_	_	+	+	+	+	+	_	+	+	+		+
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, The presence of saponin is recognized for its cholesterol-lowering effects, along with its cytotoxic, antibacterial, and

antiviral properties (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 exhibits fever- reducing properties, indicating that plants with quinine, such as Ocimum, Nyctanthes, and Mentha, could be utilized to alleviate fever. Additionally, Mentha is known for its calming effects, its ability to ease tooth pain, and its functions as an antibacterial and anti- helminthic agent (Patil and Godghate 2016).*Nyctanthes and zingiber* also play a role in maintaining blood sugar, glycoside which is beneficial for the heart. The plant Zanthoxylum and Acorus contain phenolic compounds, tannins, terpenoids, and flavonoids, which have anti-helminthic properties, suggesting their potential use in treating stomach issues. (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 0.9cm. 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. 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. This result revealed that the phytoconstituents 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.

Table3. 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.

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. The aqueous abstract of phytochemicals demonstrated a mild inhibition of growth. This research contributed to understanding the cytotoxic impact of the phytoconstituents found in plant extracts on 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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