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PHYTOCHEMICAL SCREENING OF MEDICINAL PLANTS AND STUDY OF THE EFFECT OF PHYTOCONSTITUENTS IN SEED GERMINATION

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ABSTRACT

Phytochemical screening of ten different locally available plant parts was done in methanol extract. Tannin, quinine, terpenoid, flavonoid, steroid, alkaloid, cardiac glycoside, glycoside, volatile oils, etc were the phytoconstituents found in plants. The study of the effect of phytoconstituents in the germination of *Pisum sativum* seeds revealed that the phytoconstituents present in plant extracts showed the cytotoxic effect in living cells i.e. in germinating *Pisum sativum* seeds. The phytoconstituents present in the plant extract showed an effect on cell proliferation and growth. Hence these plants could be used to develop drugs against cancer cells and also may be effective against microbes and bacteria.

Keywords: phytochemical screening - phytoconstituents - cyto-toxicity - methanol extract - seed germination

INTRODUCTION

Medicinal plants are important species of plants that according to the traditional medicinal practices and also from modern scientific studies are useful for medicinal purposes to alleviate diseases, 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; helps in plant growth, safeguarding the plants by activating defense mechanism, 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, terpenoid, etc conduct certain biological functions that enhance therapeutic activities such as anti-carcinogenic, anti-mutagenic, anti-inflammatory, and antioxidant properties (Batiha *et al*. 2020). Phytochemical 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	As an antibacterial, and an antiseptic agent. (Patil *et al.* 2016)
Allium sativum (ASB)	Bulb	Garlic	Antioxidant. (Melania *et al*. 2019)
Zingiber officinale (ZOR)	Rhizome	Ginger	Treats cold, cough, in gastric problems (Arwande *et al*. 2018)
Acorus calamus (ACR)	Rhizome	Calamus	To treat throat problems 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 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 effect in living cells could be further investigated and specifically studied for developing drugs against cancer and also may be against microbes and bacteria. Curcumin present in turmeric has 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, tannin showed cytotoxic effect (Chaudhary, *et al.* 2017).

MATERIALS AND METHODS

Ten different plant samples were identified and collected. Palanshe, Bhaktapur was selected for the collection of plants for the study purpose which is located in Suryavinayak Municipality, Bhaktapur district, Bagmati province of Nepal 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 2017.

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, Bhaktapur Multiple Campus. Ohaus AR3130 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 the ten plants was used to test various phytoconstituents present in them. Different chemical reagents were prepared and specific test, for specific phytochemicals was 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 & 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, blue color was seen for gallic tannins and the presence of catechol tannin turned the solution green.

Test for reducing sugar (Thusa & Mulmi, 2017): To 0.5 mL of plant extract, 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 & 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 Molisch's reagent and concentrated H_2SO_4 were added. Violet color indicated glycosides.

Test for flavonoids (Talukdar & 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 & Chaudhary, 2010): To 2 mL of extract, 1 mL of Meyer's reagent was added. The presence of 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. 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, 5 mL of distilled water were quivered vigorously. The appearance of frothing indicated the presence of saponins.

Test for volatile oils (Talukdar & 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 & 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 showed the presence of cardiac glycosides.

Test for steroids (Talukdar & 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 & Samy, 2007), (Chekuboyina & Rao 2015).

RESULTS AND DISCUSSION

Table 2: Phytochemical screening of different medicinal plants

Plant extract	Phytochemical Screening												
					Flavonoid			Alkaloid					
	Tannin	Reducing sugar	Quinine	Glycoside	Shinoda Test	Dil. NH_3 Test	Terpenoid	Meyer'sTest	Dragendroff's Test	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 present

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, glucose uptake, effected in blood pressure, and reduced inflammation (Azhdari *et al*. 2019). Flavonoid caused risk reduction mainly from cardiovascular diseases and cancer (Ballard & Marostica, 2019). The presence of classes of phytochemicals as such; flavonoid, alkaloid, tannin showed cytotoxic effect (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, anti-viral properties, are credited to the presence of saponin (Bailly & Vergoten, 2020). Tannin shows an anticancer property that is perceptible from its inhibitory activity towards growth (Mazni, ho Yin, Azizul, & Nurdin, 2016). Plants containing a high amount of flavonoids could be useful as anti-bacterial (Ballard & Marostica, 2019). So the plants like *Zingiber, Curcuma,* and *Acorus* could be used as antibacterial, antiseptic agents. The plants containing phenolic compounds could be useful as an antioxidant. Quinine showed antipyretic property so the 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-helmintic agent (Patil & Godghate, 2016). *Nyctanthes, Zingiber* also plays a role in maintaining blood sugar. *Zingiber, Acorus, Curcuma* consisted more amount of more cardiac glycoside which is beneficial for the heart. The phenolic compound, tannin, terpenoid, flavonoids possess an anti-helmintic property so the plant *Zanthoxylum*, *Acorus* could be used to treat stomach problems (Nath & Yadav, 2016). The polyphenolic compounds, flavonoid, terpenoid found in *Allium cepa,* and *Allium sativum* is useful as an antioxidant, anti-inflammatory, antibacterial agent. Likewise, they play an important role in reducing blood pressure, in preventing heart diseases.

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 shoot length 0.9 cm. Water and methanol was used as positive control and negative control respectively. The observed results in the aqueous extract, 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 indicated 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 aqueous extract (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 may be 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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