

Screening of Phytoconstituents in Medicinal Plants and Study of Their Effect in Seed Germination

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Abstract

Due to the existence of bioactive phytochemicals in plants, they have been traditionally utilized as medicine for a very long time. The present study was aimed to determine the phytoconstituents present in ten different plants collected from Katunje-Bhaktapur, Nepal. The methanol extract of the sample was used for analyzing the phytoconstituents present in the plant sample. The result revealed the existence of several phytoconstituents like alkaloid terpenoid, quinine, tannin, saponin and flavonoid in these plants. Similarly, the impact of phytoconstituents on germination of *Pisum sativum* seeds in water, aqueous extract, methanol, and methanol extract were analyzed. Shoot germination was retarded on all extracts.

Keywords: *Phytochemical Screening, Phytoconstituents, Cytotoxicity, Methanol Extract, Seed Germination*

1. Introduction

Plants used as medicine to treat certain ailments are called medicinal plants. These comprise whole plant or plant components including leaves, flowers, fruits, seeds, stems, woods, barks, roots, rhizomes, that may be whole, broken up or powdered. Plants produce hundreds of chemical compounds for purposes like protection against insects, fungus, and illnesses. Globally, medicinally significant plants and the biologically active phytoconstituents contained in them are utilized to treat a wide range of human illnesses, including respiratory infections, cancer, heart disease, and gastrointestinal infections (Mahmood et. al, 2019). The nutritive values that they have

are highly recommended for their healing powers with no side effects. Medicinal plants are still used in non-industrialized societies because they are readily available, cheaper than modern medicines, inadequate supply of drugs, the side effects of synthetic drugs, etc. Since medicinal plants have been harvested, used, and managed for a long period using regional customs and knowledge, they are inextricably linked to local livelihoods.

Higher plants have been used as sources of therapeutic chemicals and as major contributors to the preservation of human health since antiquity (Farombi, 2003). The biological potency of secondary metabolites is one explanation for the use of plants as food and medicine (Giri and Rajbhandari, 2020). There is enough proof that man and his search for medicine in nature have a long history together, including written records, preserved monuments, and even the original plant medicines. After battling ailments for a long time, man learnt to look for pharmaceuticals in barks, seeds, and other plant parts. As a result, there is now an awareness of the usage of therapeutic plants. Through trial and error, people gradually learned how to use plants to make food and medicine, and they were able to meet their requirements by adapting to their surroundings.

The continuous and perpetual people's interest in medicinal plants has caused today's modern and complicated fashion of their processing and usage. With the development of civilizations and the creation of new facilities, human knowledge has gradually come to an end (Kia et al., 2018). More than half of all current clinical medications come from natural sources (Sumathi and Krishnaveni, 2012). According to WHO statistics, 80% of people worldwide currently utilize herbal medications for some purposes related to basic healthcare. Nepal is blessed with numerous medicinal plants. Being benefited from the geographical structure, these plants grow wild without human interference which makes it even more effective. Most of the herbs found in Nepal are utilized in traditional healing systems like Homoeopathy, Ayurveda, Amchi, etc. For example, the ayurvedic usage of *Bergenia ciliata* as an antidiarrheal and antidysenteric (Bajracharya, 1979; Dey, 1998); fruits of *Zanthoxylum armatum* to treat toothache and as anti-inflammatory (Kumar et al., 2000). Some plants are even used for allopathic medicine; for instance, the terpene paclitaxel from *Taxus baccata*, and the vinca alkaloids from *Catharanthus roseus*, are effective anticancer drugs first derived from plants (Newman et. at., 2003; Li-Weber, 2009). An effort with effective coordination and cooperation is important for the preservation, promotion, and development of various systems of traditional medicine and related resources.

Phytochemical screening is the basis for the discovery of such phytoconstituents which can be utilized to make medicine to cure various diseases. Study of phytoconstituent is significant because both research institutes and pharmaceutical corporations have a commercial stake in the development of novel medications for the treatment of various diseases. Medicinal plants and herbal preparations are garnering widespread interest in scientific circles due to their consistent pharmacological activities and low cost to the general public, making them effective in the treatment of a variety of disorders. The main purpose of the present study is phytochemical screening in the methanolic extract of ten different medicinal plants of Katunje-Bhaktapur, Nepal.

2. Materials and methods

Plant collection

Table 1: Plants collected for the study

Plants	Parts	Local Name	Local uses
<i>Urtica dioica</i> (UDS)	Shoot	Sisno	It lowers blood sugar level, relieves joint and body pain.
<i>Hibiscus rosa sinensis</i> (HRSF)	Flower	Ghantiful	For hair growth, treatment of piles.
<i>Cuscuta reflexa</i> (CRV)	Vine	Aakashbeli	Treatment of jaundice (yellow fever).
<i>Tagetes patula</i> (TPF)	Flower	Kukurful	Ornamental
<i>Tagetes erecta</i> (TEF)	Flower	Sayapatri	Ornamental
<i>Drymaria cordata</i> (DCS)	Shoot	Abijalo	Treat common cold, wounds.
<i>Artemisia vulgaris</i> (AVS)	Shoot	Titepati	Antiseptic, antibacterial, antimalarial, heal the wound faster, stop bleeding from cuts.
<i>Aloe vera</i> (AVL)	Leaf	Ghiukumari	Antioxidant, skin hydration, burns lower high blood pressure.
<i>Cynodon dactylon</i> (CDWp)	Whole plant	Dubo	Treatment of headache, diarrhea.
<i>Jasminum mesnyi</i> (JML)	Leaf	Jaiful	Treatment of sore throat, oral sores.

Ten different plants were collected from Katunje, Suryabinayak Municipality, Bhaktapur, Nepal in September, October, and November. The place is located at 1,339 m (4393 ft.) above sea level, latitude 27° 39' 59.4" north and longitude 85° 24' 26.8" east. Plant samples were collected, cleaned, and dried in the shade. Dried plants were ground into powder. Plants collected for the study are listed in Table 1.

Extraction

Plant parts were collected, cut into small pieces, dried in shade and finally ground with electric grinder. Plant extract was prepared in methanol by maceration process. In a conical flask, 50 g of powdered plant parts were weighted and 100-150 mL of methanol was added and left for 3 days. It was shaken occasionally. In the experiment, powdered samples and chemicals were weighed using an Ohaus AR3130 electronic analytical balance. The mixture was subsequently filtered with cotton, and resulting extract was employed in a variety of assays. Preserved extract was later used to assess growth inhibition induced by phytoconstituents in pea seed. For the

preparation of aqueous extract, 20 mL of water was mixed with 10 g of powdered material and left for 3 days.

Phytochemical screening

A little amount of methanol extract was subjected to phytochemical screening. Various chemical reagents were prepared and tests for specific phytochemicals were performed. All required solvents and chemicals were obtained from Fisher Scientific, India and utilized without any additional processing. The process employed was given by Alamzed *et al.* (2013; Talukdar and Chaudhary, 2010; Thusa and Mulmi, 2017). Procedures used are as follows:

Preparation of reagents

Meyer's reagent

Mercuric chloride (0.679 g) was weighed in a 50 mL volumetric flask and dissolved in distilled water. To this solution, Potassium iodide (25 g) was added. The scarlet red precipitate was dissolved by shaking and diluted with distilled water up to the mark of volumetric flask.

Dragendroff's reagent

Bismuth Nitrate (4 g) was dissolved in 5 N Nitric acid (10 mL) to make a solution. To prepare solution B, first, in 20 mL distilled water, Potassium iodide (13.5 g) was dissolved. Then, in 50 mL volumetric flask, solutions A and B were mixed.

Sodium picrate

Picric acid (0.25 g) was dissolved in distilled water (50 mL) to make an aqueous picric acid solution. The solution was neutralized with sodium bicarbonate. A strip of filter paper was dipped in the prepared solution. The paper was dried completely and protected from external contamination. Thus-prepared sodium picrate paper was used for cyanogenic glycoside detection.

Molisch's reagent: α -Naphthol (5 g) was dissolved in methanol (50 mL).

Phytochemical tests

Test for tannin / polyphenol (Talukdar and Chaudhary, 2010): 3-4 drops of 10% FeCl_3 were added to a portion of extract diluted with water; blue hue was observed for gallic tannins and for catholic tannins, green hue.

Test for reducing sugar (Thusa, and Mulmi, 2017): 0.5 mL of plant extract with 1 mL of water and 5–8 drops of Fehling's solution was heated over water bath. Development of brick-red precipitate suggested the presence of reducing sugar.

Test for quinines (Thusa, and Mulmi, 2017): A freshly produced FeSO_4 solution (1 mL) and Ammonium Thiocyanate (few crystals) were added to the extract. Upon dropwise addition of conc. H_2SO_4 , an intense red color formed, indicating the presence of quinines.

Test for glycosides (Alamzeb, *et al.*, 2013).

Molisch's reagent test: The extract was mixed with Molisch's reagent (5 mL) and then conc. H_2SO_4 was added drop by drop without causing any disturbance to the solution. At the junction of

two liquids, a violet ring was observed which upon shaking turned the solution entirely violet, suggesting the presence of glycosides.

Test for flavonoids (Talukdar and Chaudhary, 2010).

i. Shinoda test: 4 mL of the extract was combined with 1.5 mL of a 50% methanol solution. After warming the solution, metal magnesium was added. The solution was colored red for flavonoids and orange for flavones after 5–6 drops of strong HCl were added.

ii. 5 mL of diluted NH₃ solution and then conc. H₂SO₄ were added to the aqueous filtered solutions of each fraction. Flavonoid content was determined by the appearance of a yellow color. The golden tint vanished after a while (Alamzeb, Khan, Ali, Shah and Mamoon, 2013).

Test for terpenoids (Alamzeb *et al.*, 2013): About 0.2 g of sample received a mixture of 2 mL of chloroform and 3 mL of concentrated H₂SO₄. At the interface, a reddish-brown hue formed, which indicated presence of terpenoid.

Test for alkaloids

i. Meyer's test (Talukdar and Chaudhary, 2010): Meyer's reagent (1 mL) was added to plant extract (2 mL). Pale yellow or white precipitate indicated presence of alkaloid.

ii. Dragendroff's reagent test (Alamzeb *et al.*, 2013): For about 2 minutes, 2 mL of extract was warmed with 2% H₂SO₄. It was then filtered. Then, to each filtrate, few drops of Dragendroff's reagent were added. Orange-red precipitate indicates the presence of alkaloids.

Test for saponins (Alamzeb *et al.*, 2013): About 2 g of pulverized sample was boiled in 20 mL of distilled water over a water bath and subsequently filtered. Distilled water (5 mL) was added to 10 mL of the filtrate and shaken vigorously. Frothing indicated the existence of saponin.

Test for volatile oils (Talukdar and Chaudhary, 2010): 2 mL of extract, 0.1 mL of NaOH, and a little amount of dilute HCl were mixed together. In the presence of volatile oils, a white precipitate forms.

Test for cardiac glycosides (Talukdar and Chaudhary, 2010): Plant extract (5 mL) was treated with glacial acetic acid (2 mL) that also contained one drop of FeCl₃ solution. A brown ring at the interface suggested the deoxy-sugar property of cardenolides. Beneath the brown ring, a violet ring may appear. Greenish ring may slowly emerge in acetic acid indicating presence of cardiac glycosides.

Test for steroids (Talukdar and Chaudhary, 2010): A few drops of acetic acid were used to dissolve 1 gram of plant extract. A drop of conc. H₂SO₄ was put alongside a tube after it was gradually warmed and cooled under a tap. Green colour suggested existence of steroid.

Study of effect of phytoconstituents in seed germination

Effect of phytoconstituents on seed germination was performed on *Pisum sativum* seeds in water, aqueous extract, methanol, and methanol extract. For five days, the seeds were soaked in the methanol extract of the plant sample, aqueous extract of the plant, and methanol separately. The approach used was based on methods given by (Chekuboyina et al., 2015; Khatri *et al.*, 2020; Hassan and Samy, 2007; Radwan *et al.*, 2019; Sharma *et al.*, 2020).

3. Results and discussion

Table 2: Table showing the phytochemical screening of methanol extract of plants

S.N.	Phytochemicals	Plant extract										
		UDS	HRSF	CRF	TPF	TEF	DCS	AVS	AVL	CDWp	JML	
1	Tannin/ Polyphenol	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	
2	Reducing sugar	-	+	-	-	+++	-	-	-	-	-	
3	Quinine	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	
4	Glycoside	+++	+	-	+++	+++	+++	-	+++	+++	+++	
5	Flavonoid	Shinoda test	+	+++	+	++	++	++	++	+	+	+++
		NH ₃ + H ₂ SO ₄	+	+++	+	++	++	++	++	+	+	+++
6	Terpenoid	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	
7	Alkaloid	Meyer-s test	+	+	+	+++	++	+++	++	+	+	+++
		Dragendroff's test	++	+	+	+++	+	++	+++	++	+	+
8	Saponin	+++	+++	+++	+++	+++	+++	+++	+++	+	+	
9	Volatile oil	-	-	+	+	-	+	-	-	+	-	
10	Cardiac Glycoside	++	++	+	++	+++	++	-	++	++	-	
11	Steroid	-	+++	+++	++	-	++	+++	+	+++	+	

+++ = High presence ++ = Moderate presence + = Low presence - = Absent

The result of preliminary phytochemical tests are shown in the Table 2. Phytochemicals like alkaloid, terpenoid, tannin, flavonoid, saponin, quinine are present in all plants. Many pharmacological properties of alkaloids have been studied, including antidiabetic, antiprotozoal, (Cherian and Augusti, 1995) and anti-inflammatory (Liu, 2003; Adamski *et al.*, 2020) antiviral, anticancer and antibacterial (Adamski *et al.*, 2020), anti-helminthic (Zaman *et al.*, 2020)

properties. So, all the ten plants especially TPF, DCS, AVS and JML can be used for these properties as they show high presence of alkaloids. HRSF, JML shows high presence of flavonoid, which have been shown to have several health advantages, including antioxidant, anti-inflammatory, anti-cancer, and anti-helminthic properties (Zaman *et al.*, 2020), anti-bacterial, antiseptic properties (Kassa and Mesay, 2014). Saponin and terpenoids also have anticarcinogenic properties. Thus, UDS, HRSF, CRF, JML etc can be used as anticarcinogenic agents. Saponin possesses antioxidant, anti-inflammatory, anti-apoptosis, immunostimulant and anti-helminthic properties (Zaman *et al.*, 2020). Quinine possesses antipyretic properties (Kassa and Mesay, 2014). Since quinine is present in all the ten plants, all of them can be used to reduce fever. Alkaloids and flavonoids, in addition to tannin and phenolic compounds, are water-soluble antioxidants and free radical scavengers that prevent oxidative cell damage and have significant anticancer activity (Del-Rio *et al.*, 1997). Cardiac glycoside is beneficial for the heart. In nutshell, all these plants have better therapeutic efficacy.

Study of the effect of plant extracts in the germination of *Pisum sativum* seeds:

Table 3: Shoot length of *Pisum sativum* seed in aqueous and methanol extract of various plants.

Plants	UDS	HRSF	CRV	TPF	TEF	DCS	AVS	AVL	CDWp	JML
Shoot length in aqueous extract (cm)	0.4	0.5	0.4	0.4	0.4	0.4	0.5	0.4	0.4	0.4

Shoot length in water = 0.9cm

Shoot length in methanol = - ve

Table 3 shows the shoot length in various solutions. Shoot length in water was observed to be 0.9cm, in aqueous extract of *Urtica dioica*, *Cuscuta reflexa*, *Aloe vera*, *Tagetes erecta*, *Tagetes patula*, *Cynodon dactylon*, *Jasminum mesnyi*, and *Drymaria cordata* was 0.4 cm and that in *Hibiscus rosa sinensis*, and *Artemisia vulgaris* was 0.5 cm. Seed germination was inhibited more by the methanol extract than the aqueous extract. The reduction of shoot lengths on both aqueous extract and methanol extract was dramatic in comparison to water but more in methanol extract showing no-shoot formation. Seed germination was slightly inhibited in the aqueous extract, which is due to the phytoconstituent present. It was completely inhibited in methanol and methanol extracts which is due to methanol and the phytoconstituents present in the methanol extract. From the result, it can be said that phytoconstituents showed a cytotoxic effect on the germinating seeds. They are able to show effect in cell growth and can show similar effects in bacteria, microbes; hence can be further studied for drug development. Chemotherapy as a treatment of cancer relies on the effect of cytotoxic agents to kill or damage cancer cells.

4. Conclusion

The phytochemical screening of various extracts showed the presence of secondary metabolites, which are gaining popularity owing to their functional potential in boosting human health. The preliminary screening assays could help detect bioactive principles and lead to drug discovery and development. As phytoconstituents retarded seed germination, it might inhibit the growth of disease-causing pathogens but further studies are required. The plant extract studied might be an alternative for people searching for better therapeutic pharmaceuticals derived from natural sources, which are regarded to be more effective and have less side effects than commonly used synthetic chemotherapy treatments.

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