

Research Paper

**PHYTOCHEMICAL SCREENING AND ASSESSMENT OF BIOMOLECULES
COMPOUNDS IN *SCILLA HYACINTHINA* (Roth) Macbr. BULB**

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Abstract

In most of the developing countries drugs of herbal origin have been used in traditional systems of medicines since ancient times. Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. Ethno medicine represents one of the best avenues in searching new economic plants for medicine. Thus herbs are staging a comeback and herbal 'renaissance' is happening all over the Globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. The drugs are derived either from the whole plant or from different organs, like leaves, stem, bark, root, flower, seed, etc. Some drugs are prepared from excretory plant product such as gum, resins and latex. It is very much evident that plants have bio-active chemicals called Phytochemicals which have physiological effect on human body; these phytochemicals have formed the bases of modern drug industries. Hence, present investigation is carried out to establish the preliminary phytochemical screening of petroleum ether, chloroform, acetone and aqueous extracts of bulb of *Scilla hyacinthina* (Roth) Macbr. The phytochemical evaluation and quantification of various extracts revealed the presence of high carbohydrate (42.95 %), protein (5.5%) and secondary metabolites such as alkaloid (13.4gm), flavonoids (9.9gm) and saponin (23.2gm). qualitatively in aqueous and acetone extract as compared to other solvent. These studies will be helpful in developing standards for quality, purity and secondary metabolites in preparations of herbal medicine.

Key words: *Scilla hyacinthina*, Herbal medicine, Phytochemicals, Secondary metabolites.

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body and these chemical substances are called as phytochemicals. Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The constituent presents in the plant play a significant role in the identification of crude drug. Phytochemical screening is very important in identifying the new sources of therapeutically and industrially important compounds like alkaloids, flavonoids, phenolic compounds, saponin, steroids, tannins, terpenoids etc (Akindele and Adeyemi, 2007). Previously the drugs were identified by comparison only with the standard description available but recently due to the advancement in the field of pharmacognosy various techniques have been following for the standardization of crude drugs (Savithramma *et al*, 2010).



Scilla hyacinthina(Roth) Macbr Family- Liliaceae common among grasses on the floor of scrub, found in India and some Asian countries (Mathew,1983). With the onset of pre-monsoon, clouds, the plant *Scilla hyacinthina* raise their heads above the ground and produce first flush of white -pink inflorescence. 10-15 cm herb with a perennial tunicated bulb. The largest size of bulb ranges from 10-14gm. Leaves are simple, oblong, succulent with purplish blotches on the upper surface of leaf is an identification feature. The leaves are acts as vegetative prapogative, the apex of leaf on touch with soil produces new plant from adventitious bud. Scape 5-10 cm tall, flowers in terminal raceme, white-pink colour, perianth 6 lobes, persistent, stamens 6, anthers versatile, ovary sessile, three celled. Fruit is capsule, globose, thin walled. Seeds ovoid and black. Squill contains mucilage, calcium oxalate, dextrose, starch, albuminous bodies and volatile oil. It is medicinally important, since it is used as an expectorant, diuretic and cardiac stimulant. The present study deals with the preliminary phytochemical screening of Scilla bulb, by studying the presence of phytochemicals, the uses of this plant in traditional treatment can be explained scientifically.

MATERIALS AND METHODS

The fully matured healthy plant material (Bulb) of *Scilla hyacinthina* (Roth) Macbr. was collected from GVISH Campus, Amravati during the rainy season. The material was thoroughly washed; air dried under shade and powdered by the help of mechanical process. The coarse powder of bulb was stored in airtight container for further studies.

Preliminary Phytochemical Analysis: Qualitative screening of bulb was performed for the identification of various classes of active chemical constituents using the methods described by Kokate 2005, Raman, 2006.

Quantitative analysis:

Total Carbohydrate and Protein of bulb was measured by the methods described by Sadashivam and Manickam, 1997. The crude quantification of major phytochemicals was done by using precipitation method. Each sample was analyzed in triplicates. Only alkaloids, flavonoids and saponin from the plant under study were quantified.

RESULTS AND DISCUSSION

Qualitative Analysis:

Qualitative phytochemical studies of bulb was performed on its water, acetone, petroleum ether and chloroform extracts (1:10) to identify its carbohydrate and glycosides, protein and amino acids, alkaloids and other secondary metabolites like phenolic compounds and flavonoids, phytosterol and terpenoid by using suitable chemicals and reagents (Table 1). The color intensity was shown as '+', '++', '+++ and '++++' for low/slight, moderate, good and high means positive tests respectively and '-' for no change means negative test. The Test for all the phytoconstituents were mostly observed to be positive with different intensity (+, ++, +++, +++++). The result showed that the carbohydrates which constitute the major edible part of the plants were found to present in all the four extract but higher percentage were found to be in aqueous extract. Among all the test Fehling's test was found to be sensitive for the presence of the carbohydrate. Acetone extract showed negative test for Molisch, Seliwanoff's and Bradfords test. This may be due to insolubility of carbohydrates. Proteins which form the structural and functional basis of the cell were found to be present in all the four extract i.e. aqueous, acetone, petroleum ether and chloroform. Except ninhydrin test rests of the three tests viz Biuret, Millons and Xanthophorin test were showed the positive results in all the three extract except acetone extract. Amongst the above three tests Xanthophorin test showed good results, this indicated the presence of aromatic amino acids such as phenyl alanine, tyrosine and tryptophan in the extract. Ninhydrin is an oxidizing agent which reacts only with alpha amino group. As there was no change in the Ninhydrin test might be due to the absence of alpha amino group in the extract. However acetone extract of bulb showed negative in Biuret, Ninhydrin and Millons test. Petroleum ether extract showed slight present in Millon test, this might be due to insolubility of protein in ether and alcohol. The test for alkaloids showed their strong presence in aqueous extract for Dragendorff's test, Mayer's test and Wagner's test. The test showed good results in acetone extract also. Amongst all the test Dragendorff's test showed strong positivity in all four extract, this indicated the strong presence of tertiary amines in the extract or this may be due to the coupling of heavy metals in the reagent with the nitrogen in the alkaloid to form ion pairs that form an insoluble ppt, whereas Mayer and Wagner's test showed slight presence of alkaloid in Petroleum and chloroform extract. Flavonoids showed good response for the entire test in all the four extract except alkaline test showed slight presence in acetone extract and lead acetate showed negative test for petroleum ether and chloroform. Amongst all the test shinoda test showed abundant presence in all extract this indicate the presence of flavones in the test sample then they are reduced to anthocyanin under Shinoda test, dramatic change in colour indicate the presence of flavonoid. Glycosides and cardiac glycosides showed good characteristic colour and precipitate in all the tested extract. The presence of phenolic compounds and tannins were confirmed in aqueous, acetone and petroleum

ether extract and absent in chloroform extract. Gelatin test, Lead acetate, acetic acid test and ferric chloride test showed moderate to strong presence in aqueous extract. Acetone extract showed strong response to ferric chloride and gelatin test and good for lead acetate test, negative in acetic acid test. However ferric chloride and gelatin test showed good results in petroleum ether extract, whereas lead acetate and acetic acid test showed no change for the same. Phenolic compounds and tannins were found absent in chloroform extract. Salkowaski test showed strong presence of phytosterols. The strong presence of Phytosterols, triterpenoids and terpenoids has been seen in aqueous, acetone and chloroform extracts and found to be absent in petroleum ether extract.

Table 1: Qualitative Phytochemical analysis of *Scilla hycinthiana* bulb

Chemical constituent	Tests	Bulb			
		AQ	AC	PE	CH
Carbohydrates and Glycosides	Molisch's Test	+++	-	+++	+++
	Benedict's Test	+++	+	+++	++
	Fehlings test	++	++	+++	++++
	Seliwanoff's tests	+++	-	+++	+
	Bradford's tests	+++	-	-	-
	Borntrager's test	+++	+++	+++	+++
Proteins & amino acid	Biuret Test	+++	-	+++	+
	Ninhydrin Test	-	-	-	-
	Millon's Test	+++	-	+	++
	Xanthophorin Test	+++	+++	+++	+++
Alkaloids	Dragendorff's Test	++++	++	+++	++++
	Mayer's Test	+++	++	+	+
	Wagners Test	+++	++++	+	-
Cardiac Glycosides	Killer-Killiani Test	+++	+++	+++	+++
Flavonoids	Shinoda Test	++++	++++	++++	++++
	Alkaline reagent (NaOH)	+++	+	+++	+++
	Lead Acetate Test	+++	+++	-	-
Phenolic compounds & Tannins	Ferric Chloride Test	++++	++++	+++	-
	Lead Acetate Test	+++	+++	-	-
	Acetic Acid Test	+++	-	-	-
	Gelatin Test	++	++++	+++	-
Phytosterols & Triterpenoids	Salkowaski Test	++++	++++	-	++++
	Libermann's Burchard T	+	++++	-	++++
	Libermann's test	++++	++++	-	-
Terpenoids		+++++ yellow orange color + ppt			

-, Negative; +, Slight; ++, Good; +++, Moderate; +++++, Strong

The above qualitative phytochemical screening showed the aqueous extract of *Scilla* bulb is rich source glycosides, proteins, alkaloids, flavonoids, phenolic compounds and tannins, phytosterols and terpenoid and triterpenoids. However, presence of all these compounds is limited in other extracts.

Quantitative analysis-

Carbohydrate, protein and the secondary metabolites in plant under study were found to present in appreciable concentration.

Table 2- Quantitative analysis of Phytochemical constituents

Plant Extract	Phytocostituents				
	Carbohydrate (%)	Proteins (%)	Alkaloids (g/100 gm)	Flavonoids (g/100gm)	Saponin (g/100gm)
Scilla Bulb	42.97	05.5	13.4± 0.05	9.9±0.04	23.2±0.04

Where, results are depicted as mean ± SD of three determinants

The quantitative findings showed bulb possesses higher amount of carbohydrates (42.97%), Protein content of (5.5%), and the other secondary metabolites like alkaloids, flavonoids and saponin in Scilla bulb was showed the percentage of alkaloids 13.4± 0.05g/100g, flavonoids 09.9±0.04g/100g and saponin 23.2±0.04g/100g. the quantitative analysis showed maximum quantity of saponin in bulb.

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities (Yadav & Agrawal 2011). Analysis of the plant extracts revealed the presence of phytochemicals such as alkaloids, flavonoids, saponins ,phenols, tannins, , glycosides, terpenoids, Alkaloids, have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity (Nobori,*et.al.*,1994). Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms in vitro. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall. They also are effective antioxidant and show strong anticancer activities (Salah, *et.al.*, 1995 and Del-Rio, *et.al.*, 1997).The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation (Just, *et.al.*, 1998), also has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo,*et.al.*,2000). Glycosides are known to lower the blood pressure according to many reports(Nyarko, and Addy, 1990). The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and these plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

CONCLUSION

The results revealed the presence of medicinally important constituents in the plant studied. Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive. Several studies confirmed the presence of these phytochemicals contribute medicinal as well as physiological properties to the plants studied. Therefore the extract from the bulb could be seen as a good source for useful drugs. Further work should be carried out to isolate, purify and characterize the active constituents responsible for the activity of this plant.

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