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Research Article

Preliminary Phytochemical Screening, Morphological Evaluation And Physicochemical Analysis Of *Ajuga Bracteosa*

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ABSTRACT

Ajuga bracteosa is a medicinal plant belonging to the family Lamiaceae and this is a perennial herb. Ajuga bracteosa also known as neelkhanti in local language. It is found in Himalayan area. Ajuga bracteosa possessed many kinds of pharmacological properties like antioxidant, hepatoprotective, antimicrobial, antidiabetic, gastroprotective and other biological activities. The purpose of this study is to perform its morphological study, its phytochemical screening and also its physicochemical analysis to understand the plant more.

INTRODUCTION

From the ancient time the people are depend on medicinal Plant for curing their complaint. History of medicinal plant is as old as mortal history. From centuries the history of drugstore and pharmacognosy is connected. Herbal medicine is used worldwide for treatment of wide ranges of conditions, so medicinal factory plays a vital part in world health. Despite of great advancement in ultramodern drugs, people are still dependent on shops for health care. It's approached that directly or laterally nearly 25of entire ultramodern drugs are deduced from shops. Medicinal shops show distribution worldwide but they're more abundant in tropics. According to WHO, 60- 80population of developing countries depends on plant for their primary health care. From the last decades the use of medicinal shops come so popular that numerous important shops are at threat of destruction due to over exploitation. Genus Ajuga of family Lamiaceae has multitudinous pharmacologically vital groups of unfolding shops. These species are rich in the home of western Himalaya and upper Gangetic plant. [1,2] Gastric ulcers are one of the major gastrointestinal diseases that do due to an imbalance between the descent (gastric acid stashing) and protective (gastric mucosal integrity) factors [3]. Currently, there are two main

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approaches for curing peptic ulcers the first approach is to reduce the gastric acid stashing and another approach is to support the gastric mucosal protection [4]. shops have been a precious foundation of new medicines and considered as an indispensable strategy in hunt for new medicines. There's a rich extravagancy of shops used in traditional drug known to retain antiulcer properties [5]. Ajuga bracteosa is to be a highly important medicinal plant the majority of the natural population of the plant is currently under severe pressure due to high demand. This species is rapidly declining as a result of overexploitation. This herb is in high demand in the pharmaceutical industry at both the local and international levels. But the fact is that it is extremely endangered and, if it continues to be exploited at the current rate, will go extinct within the next few years [6] Therefore, long-term use of this incredibly healing species is required to preserve for its numerous known uses. This species has received a lot of attention in the last decade. A multifaceted strategy is necessary for maintenance, which could offer a solution to the current issue. This strategy comprises the selection of higher-quality genotypes, as well as ex-situ and in-situ conservation, followed by multiplication utilizing both conventional and biotechnology means [7].

Any medicinal plant's worth is based on the active components that are present in that species. Elite clone development would be desirable. Chemo-profiling and different molecular marker approaches can be used to find superior clones. Commercial plantations can be multiplied and grown for conservation using conventional propagation techniques as well as plant tissue culture procedures. To speed up the creation of favoured genotypes and commercial micro propagation, tissue culture can be employed as an alternative to traditional in vitro propagation techniques. Plant tissue culture techniques are now used for gene transfer, selection, and regeneration of transformants [8]. The Cell suspension culture, in addition to in vitro propagation, is useful for large-scale secondary metabolite production. Another factor that influences plant quality is post-harvest handling. Herbal material collectors pay less attention to material quality during harvesting, handling, and storage. Mycotoxin-producing fungi have been discovered in herbal drug samples that have been stored. Cultivation practices must be addressed as well. Wild harvested plants vary in consistency and quality due to genetic and environmental differences [9]. The efficacy of medicinal plants is also influenced by regional environmental conditions. Temperature, photoperiod, soil characteristics, and rainfall all have a significant impact on the production of active constituents. As a result, consistent efforts should be made at the community level to ensure the long-term management of medicinal plants. Shivanee et al reported that MS medium supplemented with IAA (2 mg/L) and BA (5 mg/L) induced 100 % shoot regeneration [10]. In this experiment leaf, petiole and root as explants were selected. Leaf displayed quickest response followed by petiole while root was shown the slowest response. It was further experimentally proved that shoot induction is predominantly dependent on plant growth regulators added to the culture medium. Full- or half-strength Murashige and Skoog medium with or without auxin is used for in vitro rooting. An estimated survival rate of 82-100% was achieved when rooted shoots are acclimatized in the greenhouse [11]. Micropropagation is a key technique used in our previous work to conserve the plant. Thus, in this study, aim to perform a phytochemical investigation of leaves of Ajuga bracteosa plant to isolate its major compounds. Preliminary Qualitative Analysis is performed to determine different chemicals like alkaloids, carbohydrates, glycosides, proteins, flavonoids, terpinoides, etc.

Material and Method

Collection and extraction of Plant material

This work was carried out in Department of Pharmacognosy, Abhilashi University,Mandi. Plant was collected from allied hills of Himachal during the month of July 2023. The plant was preserved in the advanced pharmacognosy laboratory of Abhilashi University. The plant was thoroughly washed with water and dried under shade for about 10-15 days. This powdered herb was then extracted by using a soxhlet extraction method and ethanol is used as solvent. 100gm of powder was extracted with 700 ml of ethanol for 6-8hr. The ethanolic extract of Ajuga bracteosa was concentrated with distillation method and evaporate excessive solvent.

Morphological Evaluation

The macroscopic evaluation of the plant Ajuga bracteosa is carried out to check its taste, odor, color shape apex, nature, length, width, thickness, etc. [12]

Phytochemical analysis

Phytochemical analysis is performed to identify different phytochemical present in the leaf of Ajuga bracteosa by using different tests.

Test	Procedure	Observation	Reference
Alkaloids			
Dragendroff's/ Kraut's test	Few mL of plant extract + 1-2 mL Dragendorff's reagents	A reddish-brown precipitate	[13]
Hager's test	Few mL Plant Extract + 1-2 mL Hager's reagents	A creamy white precipitate	[13]
Mayer's/ Bertrand's/ Valser's test	Few mL Plant Extract + 1-2 drops of Mayer's reagent (Along the sides of test tube)	Creamy white/yellow precipitate	[13]
Carbohydrates			
Barfoed's test	1mL Extract + 1mL Barfoed's reagent + Heated for 2 min.	A red precipitate	[13]
Molish's test	2mL Extract + 2 drops of alcoholic α–naphthol + 1mL conc.H2SO4 (along the sides of test tube)	A violet ring	[13]
Seliwanoff's Test	1mL extract solution + 3mL seliwanoff's reagent + heated on water bath for 1 min.	A rose red colour	[13]
Cardiac Glycosides			
Keller-Killani test	1mL extract + 1.5mL glacial acetic acid + 1 drop of 5% ferric chloride + conc. H2SO4 (along the side of test tube)	A blue coloured solution	[13]
Kedee's test	4mL extract evaporated to dryness + 1-2 mL methanol + 1-2 mL alcoholic KOH + 3-4 drops of 1% alcoholic 3,5-dinitrobenzene + heated.	A disappearing violet colour	[13]
Bromine water test	Plant extract + few mL of bromine water	A yellow precipitate	[13]
Proteins and Amino Acids			

Biuret test	2mL filtrate + 1 drop of 2% copper sulphate sol. + 1mL of 95% ethanol + KOH pellets	A pink-coloured sol. (in ethanolic layer)	[13]
Millon's test	2mL filtrate + few drops of Millon's reagent	A white precipitate	[13]
Ninhydrin test	2mL filtrate + 2 drops of Ninhydrin solution (10mg ninhydrin + 200mL acetone)	A purple-coloured sol.	[13]
Flavonoids			
Alkaline reagent test	1mL extract + 2mL of 2% NaOH solution (+ few drops dil. HCl)	An intense yellow colour, becomes colourless on addition of diluted acid	[13]
Lead acetate test	1mL plant extract + few drops of 10% lead acetate solution	A yellow precipitate	[13]
Tannins			
Gelatin test	Plant extract is dissolved in 5mL distilled water + 1% gelatin solution + 10% NaCl	A white precipitate	[13]
10% NaOH test	0.4mL plant extract + 4mL 10% NaOH + shaken well	Formation of emulsion	[13]
Sterols			
Salkowaski test	In 1 mL of extract added with 1 mL chloroform then by addition of 1 mL of concentrated H2SO4 along with walls.	Red tint indicated the presence of sterol.	[13]
Liebermann's test	About 2 mL of extract added in 2 mL of chloroform, 1 mL of glacial acetic acid with the length of walls after that added 2 mL conc. H2So4.	Green shade layer Indicated occurrence of sterol.	[13]
Terpinoides			
	2ml chloroform + 5mL plant extract, (evaporated on water bath) + 3mL conc. H2SO4 (boiled on water bath)	A grey coloured solution	[13]
Saponin			
Foam examination	In 1 mL extract and added 2-3 mL of water, on strongly shaking foam formation occurred.	If foam remains stable for 10 minutes it point out saponin occurrence	[13]
Bromine water experiment	In 2 mL of ethanolic extract in it add some bromine's water drop.	Golden precipitate point out saponin occurrence.	[13]

Phytoconstituents [14,15]

Phytochemically, A. bracteosa contains various compounds such as neo-clerodane diterpenoids, flavonol glycosides, iridoid glycosides, ergosterol-5,8-endoperoxide and phytoecdysones[2,9-11]. These chemical compounds were either synthesized or isolated from the plant. Cytotoxicity level was evaluated using skin carcinoma cell line and it was found that ergosterol-5,8-endoperoxide and neo-clerodane diterpenoids were not cytotoxic at higher concentration used for antiplasmodial activity. Phytochemically, A. bracteosa contains various compounds such as neo-clerodane diterpenoids, flavonol glycosides, iridoidglycosides, ergosterol-5,8-endoperoxide and phytoecdysones[2,9- 11]. These chemical compounds were either synthesized or isolated from the plant. Cytotoxicity level was evaluated using skin carcinoma cell line and it was found that ergosterol-5,8-endoperoxide and neo-clerodane diterpenoids were not cytotoxic at higher concentration used for antiplasmodial activity. Phytochemically, A. bracteosa contains various compounds such as neo-clerodane diterpenoids, flavonol glycosides, iridoid glycosides, ergosterol-5,8-endoperoxide and phytoecdysones. These chemical compounds were either synthesized or isolated from the plant. Cytotoxicity level was evaluated using skin carcinoma cell line and it was found that ergosterol-5,8-endoperoxide and neo-clerodane diterpenoids were not cytotoxic at higher concentration used for anti- plasmodial activity. Compounds produced by A. bracteosa have a variety of medicinal effects. Flavonoids, saponins, phenols, tannins, terpenoids, xanthoproteins, glycosides, and other compounds are among them. According to Zahra et al., ethanol extract had the highest level of flavonoid concentration while chloroform-methanol extract had the highest level of radical scavenging ability. Polyphenols like pyrocatechol, gallic acid, resorcinol, catechin, chlorogenic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, vanillic acid, coumarin, sinapinic acid, trans-cinnamic acid, rutin, and kaempferol were confirmed using RP-HPLC-based quantification. 6-deoxyharpagide and raptoside are iridoid glycosides present in the plant. These compounds are optically active cyclopentonoids monoterpenes and could be used for defence action. Ajuga bracteosa has a rich phenolic content and is hence a superior choice for phenolic-guided pharmacological activities. According to studies, the constituent 20-hydroxyecdysone is present but its concentration varies depending on where it is found due to the action of various exogenous factors. One such exogenous factor, cold temperature, is ideally suited for consistent 20-hydroxyecdysone synthesis. Studies have also suggested that this steroid might also have therapeutic benefits for a number of respiratory illnesses as well as cardiometabolic and neuromuscular problems. Lactone steroids withanoloide, which serve as cholinesterase inhibitors, is also present in the plant. Dichloromethane extract of whole plant of A. bracteosa produced a variety of clerodane and neoclerodane diterpenoids. Neoclerodane diterpenoids have been shown to be effective as an anti-bacterial in tests. As per report analysis the antimicrobial activity and insect anti-feedent activity can also be correlate Ajuga bracteosa. There are several other biologically active compounds were isolated and identified from the methanol extract of aerial part of Ajuga which are showing anti-mutagenic activity.

Phytoconstituents present in species Ajuga [16,17]

Ajugapitin	Ajugamarin
Dihydroajugapitin Chamaepitys	Deacetylajugarin
Ajugamarin	Ajugamarin
Dihydroajugamarin	Ajugacumbin
Ajugamarin chlorohydrin	Ajugachin A

Desrhamnosylverbascoside	Ajugachin B
2-O-(p-Coumaroyl)-	Ajugacumbin E
Decumbeside	Ajugavensin
Ajugavensin B	3β-Hydroxyajugavensin B
Ajugavensin C	3α-Hydroxyajugamerin
Ajureptoside C	Areptin B
Ajugapitin	Ajugamarin
Dihydroajugapitin Chamaepitys	Deacetylajugarin
Ajugamarin	Ajugamarin
Dihydroajugamarin	Ajugacumbin
Ajugamarin chlorohydrin	Ajugachin A
Desrhamnosylverbascoside	Ajugachin B
2-O-(p-Coumaroyl)-	Ajugacumbin E
Decumbeside	Ajugavensin

Physicochemical analysis [18]

This analysis included the total ash, water soluble ash, corrosive- insoluble ash and sulphated as value and these tests were carried out according to standard procedure. Ash values the ash esteems for air dried powdered leaves of A. bracteosa had been chosen as indicated by true procedure. The resolve of debris is valuable for distinguishing poor-quality items, depleted medication and extra of sandy or natural matter. Various types of debris esteems are utilized in identification of rough medications like, general debris, corrosive insoluble debris, water dissolvable debris and sulphated debris. All out ash is valuable in distinguishing the rough medications that are blended in with different mineral substances like sand, soil, calcium oxalate, chalk powder or other medication with various inorganic substances to work on their appearance.

Method:

2 to 3g of the air-dried unrefined medication was weighed precisely and taken in a tared platinum or silica dish and burned at a temperature not surpassing 450oC until liberated from carbon then, at that point cooled and gauged. The level of debris regarding the air-dried medication was determined.

corrosive insoluble ash- Corrosive insoluble debris is the build-up gotten in the wake of heating up the complete debris with weaken hydrochloric corrosive and touching off the leftover insoluble matter. This actions the measure of silica present, particularly as sand and siliceous earth.

Method:

The insoluble matter was collected in a Gooch cauldron or on ashless channel paper and washed with warm water and touched off, then chilled in a dessicator and gauged. The amount of corrosive insoluble debris in the air-dried medicine was measured.

water-soluble ash-

Water solvent ash is utilized to distinguish the presence of material depleted by water. In case carbon is as yet present subsequent to warming at a moderate temperature, the water-dissolvable debris might be isolated and the buildup again touched off.

Method:

The ash from the entire debris was bubbled for 5 minutes with 25ml of water, and the insoluble residue was collected in a Gooch pot or on ashless channel paper, cleaned with warm water, and lighted for 15 minutes at a temperature exceeding 450oC. The weight of the insoluble matter was subtracted from the weight of the general debris, resulting in a weight qualification for the water solvent debris. With the help of the air-dried medication, the level of water solvent debris was.

RESULT

Morphological Evaluation

The plant Ajuga bracteosa is also commonly known by the local people Neelkanthi which is 10-15 cm tall and leaves are ovate and 3.5cm long. Flowers are yellowish in color.

Morphological Study of leaf of Ajuga bracteosa

Parameters	Leaf
Taste	Bitter
Odor	Pungent
Color	Green
Texture of powder	Fine
Shape	Ovate
Margin	Linear
Apex	Obtuse

Phytochemical Analysis

The qualitative phytochemical investigation of ethanolic extract of leaves extract of the Ajuga bracteosa.

Tests		Hydroethanolic Extract
Test for alkaloids	Wagner's	+
	Dragendrofs test	+
	Mayers Reagent	+
Flavonoids	Shinoda test	-
Tannins	Ferric Chloride test	+
Saponin	Foam test	+
Triterpenoids	Salkowasky test	-
	Hishron test	+
Amino acids	Ninhydrin Test	+
Test for proteins	Millon's Test	-
	Biuret Test	-
Test for glycosides	Keller Killani Test	+
	Legal take a look at	+
	Baljet test	-
Test for alkaloids	Wagner's	+

Physicochemical analysis

The ash value of the herb showed high content of total ash value, corrosive-insoluble ash followed by water insoluble ash.

Table 3. Ash Values of Ajuga Bracteosa

Parameter	Value % (w/w)
Total ash	28.06 ± 0.80
Acid insoluble ash	15.02± 0.063
Water soluble ash	3.04± 0.072

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