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## Chemical investigation of medicinal plant *Ajuga bracteosa*

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### ABSTRACT

*In the present work, a comprehensive study was made on the analysis of chemical composition of essential oil and antibacterial activity from the leaves of *Ajuga bracteosa*. The essential oil was extracted by Cleavenger type apparatus and analyzed by GC-MS. The main components present in the essential oil of leaves are Limonene,  $\alpha$ -humulene,  $\beta$ -Myrcene, Elemol, Camphene,  $\beta$ -Caryophellene,  $\alpha$ -phellendrene. The antibacterial activity of the essential oil was evaluated. The essential oil was active against *Staphylococcus*. The plant extracts of *Ajuga bracteosa* was evaluated for antimicrobial activity. The methanol extract is acting against *Staphylococcus aureus* and acetone extract also active against *E. coli*. The plant extract showed minimum inhibiting concentration against the bacterial strain e.g. *E. coli*, *Bacillus*, *Pseudomonas* and *Staphylococcus*.*

**Keyword:** *Ajuga bracteosa*, column chromatography, GC-MS, *E. coli*, Cleavenger type apparatus.

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### INTRODUCTION

Ayurveda is derived from word 'Ayur' (Life) and 'Veda' (Science), hence means the science of life. In India, the sacred Vedas, dating back 3500 B.C- 800 B.C give many references of medicinal plants [1]. An estimate of the World Health Organization (WHO) states that around 85–90% of the world's population consumes traditional herbal medicines. Use of herbal remedies is on the rise in developing and developed countries [2]. The healing properties of many herbal medicines have been recognized in many ancient cultures. Early herbalists believed that the plant part resembling any part of the human body was considered useful for the ailment of those parts and there is no part of the body without its corresponding herb, a hypothesis known as the "Doctrine of Signature" [3]. In modern medicines, plants occupy a significant place as raw material for some important drugs, although synthetic drugs and biotechnology have brought about a revolution in controlling different diseases [4]. Also there is a growing tendency all over the world, to shift from synthetic to natural based products including medicinal and

aromatic plants. Less than 5% of the plants have so far been analyzed as potential medicine and still there is a great scope of research in this field. There is a need to develop strong linkages between growers, collectors, health experts and pharmaceutical industries for developing scientific basis on which these systems of medicine are working [5]. Thus, natural products remain as important sources of new drugs. Statistics show that in the 10 years from 1983 to 1994, while only about 5% of newly approved drugs were natural products [6]. Primary metabolism refers to the processes producing the carboxylic acids of the Krebs cycle,  $\alpha$ -amino acids, carbohydrates, fats, proteins and nucleic acids, all essential for the survival and well-being of the organism. Secondary metabolites, on the other hand, are non-essential to life but contribute to the species' fitness for survival [7]. Plants are capable of synthesizing an overwhelming variety of low-molecular-weight organic compounds called secondary metabolites, usually with unique and complex structures. Presently 100,000 such compounds have been isolated from higher plants [8]. Numerous plant secondary metabolites possess interesting biological activities and find applications, such as pharmaceuticals, insecticides, dyes, flavors, and fragrances. Many metabolites have been found to protect plants against viruses, bacteria, fungi, and most importantly against herbivores. Many secondary metabolites such as cyanogenic glycosides, glucosinolates, terpenes, saponins, tannins, anthraquinones, and polyacetylenes also act as allelochemicals, influencing the growth and development of neighbouring plants [9]. For example, monoterpene limonene has shown deterrent and insecticide properties and carvone is used as sprouting inhibitors [10,11]. Although it has become clear in the last decade that jasmonic acid (JA) is a key regulator in the development, physiology, and defense of plants [12]. There is also strong evidence supporting a central role of JA in plant defense [13-21].

Labiatae, a plant family is playing a very important role in the medicinal and Ayurvedic world. Many plants of the family have medicinal properties. We will focus on "Neelkanth" a medicinal plant of this family, botanic name of this plant is *Ajuga bracteosa*.

A very little work has been done on the natural products or medicinal plants of Chamba region, it is hilly. It has been observed that plant growing in this region should be highlighted due to their chemical aspects.

The family Labiatae is a large family of order Lamiales [22]. It comprises about 170 genera and 3000 species of worldwide distribution, growing under great variety of soils and climate but more abundant in Mediterranean and hilly region [23]. Several genera of the family "Labiatae" contain biologically active compounds [24]. The family consists of aromatic annual or short lived glandular and hairy herbs but rarely shrubs. Leaves are nearly alternate, simple ex-stipulate with hairs. Flowers are in verticillaster inflorescence [25]. *Ajuga* is one of the important genera of the family "Labiatae" [26]. The genus *Ajuga* has great medicinal and economic importance. In north area of India *Ajuga bracteosa* it is given in the treatment of fevers, neuro diseases. *Ajuga bracteosa* (Labiatae) is a perennial herb occurring in Western Himalayas from Kashmir to Nepal [27,28]. The leaves are diuretic, stimulant [27] and used as a substitute for cinchona [29]. The plant is also reported to possess cardiostimulant action in animals [30] and anticancer activity in rats and mice [31]. Previous work on this plant includes isolation of saturated and unsaturated acids [32], insect antifeedant diterpenes [33,34] and insecticidal diterpene [35]. Other species have been used as astringent [36] and in swollen wounds, also used as febrifuge in stomach ache, diarrhoea, rheumatic fevers, burns, tumours, bites of insects, eyes trouble as well as diseases of

bladder [37]. The plants of this genus have also been used in female's menstrual disorders urinary obstructions. *Ajuga spectabalis* exhibit stimulating activity on smooth and cardiac muscles [38]. *Ajuga reptans* has been considered good for the bad effect of excessive drinking for lowering the pulse and lessening its frequency. It is also employed for cough and allays irritation of the throat [39].

## MATERIALS AND METHODS

### 2.1. Plant Material:

The fresh leaves of the plant *Ajuga bracteosa* was collected from Chamba region of Himachal Pradesh. The plant was taxonomically identified by Dr. Suman Arora, D.A.V Ayurvedic College Jalandhar, Punjab. The leaves of the fresh plant was studied for essential oil composition.

### 2.2. Extraction of essential oil:

1kg of fresh leaves of *Ajuga bracteosa* collected from Chamba region Himachal Pradesh was subjected to hydro distillation using Clevenger type apparatus [40]. The oil was extracted by n-hexane (HPLC grade) [41]. The oil was dried over anhydrous sodium sulphate. The yield of oil was 0.2 ml. The essential oil so obtained was confirmed by thin layer chromatography. The oil was stored in sealed vial at low temperature before analysis [42].

### 2.3. Analysis of essential oil:

GC was carried out on Perkin Elmer Auto System XL fitted with a PE-5 (5% phenyl, 95% dimethyl polysiloxane), capillary column (50 mm x 0.32 mm); film thickness 0.20 µm; carrier gas H<sub>2</sub>. Oven temperature 100° C for 2 min and then programmed from 100-280° C at 3° C/min. injector and detector temperature 220° C and 300° C respectively.

### 2.4: GC/GCMS Fragmentation:

GC-MS analysis was carried out on a Perkin Elmer Turbomass coupled with GC-Auto- XL, MS at 70 eV; column and tempewrature programme same as above using carrier gas Helium. Inlet pressure 10 psi. The constituents were identified by comparing their retention indices with those of authentic samples or identified in essential oils of known compounds. The mass spectra were compared with those stored in spectrometer database and built in libraries [43,44].

### 2.5. Preparation of extracts:

For extraction; pet.ether, methanol and acetone were used as solvents. Hot extraction with Soxhlet apparatus was carried out. 1000 g of leaves were extracted with pet ether (1000ml) for 18-20 h. The extract was concentrated under reduced pressure using rotavapour. The marc so obtained was subsequently extracted with acetone (1000 ml) for 18-20 h. The extract so obtained was concentrated using rotavapour. The marc left behind was than extracted with methanol (1000 ml) for 6-8 h. The extract was then concentrated.

### 2.6. Antimicrobial activity:

#### *Bacterial Strains:*

Two gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*; and two gram negative bacteria *Escherichia coli* and *Pseudomonas aerogenosa* were used in the study (Table I).

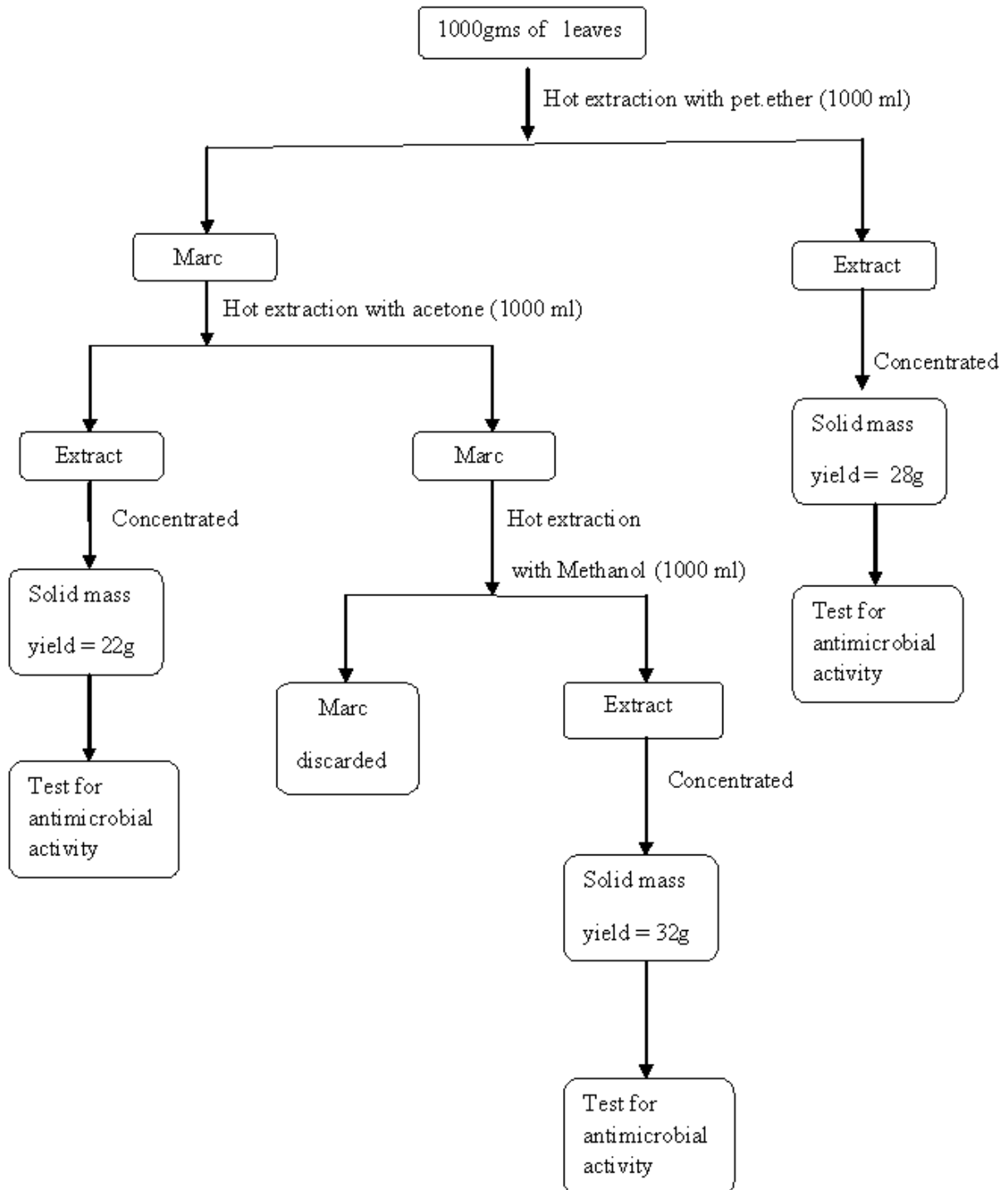


Fig.1 Flow chart showing the plant extraction

**Table I: Pathogenic microorganisms used for antimicrobial activity tests**

Group	Strain	Cultivation condition
Gram (+)	<i>Bacillus subtilis</i>	Nutrient agar/37°C
Gram (+)	<i>Staphylococcus aureus</i>	MacConkey agar/37°C
Gram (-)	<i>Escherichia coli</i>	Nutrient agar/37°C
Gram (-)	<i>Pseudomonas aerogenosa</i>	MacConkey agar/37°C

### 2.6.1. Agar disc diffusion method:

The essential oil and plant extracts of *Ajuga bracteosa* was tested for antimicrobial activity using agar disc diffusion method on solid media [45]. Luria agar was used as basal medium for *E. coli* and *B. subtilis*; and nutrient agar was used as basal medium for *P. aeroginosa*, *S.aureus*. Luria agar and nutrient agar was poured in the sterile Petri plates. Mother culture of each organism was set up 24 h before the assays in order to reach stationary phase of growth [46]. The tests were assessed by inoculating Petri dishes from the mother cultures which had been surface spread with 0.1 ml of each bacteria, with the aim of obtaining microorganism concentration of 10<sup>5</sup> colony forming units (CFU/ml) [47]. 0.5 ml of hexane was added to the 0.2 ml of *Ajuga bracteosa* 1 ml of DMSO was added to the extract in order to obtain 10 mg/ml concentration range. The stock solution of above concentration was absorbed on the sterile Whatmann filter paper No.1 discs (5mm disc diameter), which were subsequently placed in inoculated Petri plates. Discs with only hexane and DMSO were used as control. Therefore the Petri plates were than incubated at 37° C for 24 h. The antibacterial activity was determined by measuring the diameter of zone of inhibition surrounding bacterial growth [48].

### 2.6.2. Minimum inhibitory concentration of plant extracts:

The test bacteria used to determine MIC involves *E. coli*, *B. subtilis*, *P.aeroginosa* and *S. aureus*. The broth dilution method was used to measure MIC in order to determine the antibacterial effect of plant extracts. Two fold serial dilutions were prepared in broth media to obtain a concentration range of 0.142 mg/ml to 4.571 mg/ml using sterile screw bottles [49].

Bacterial colonies (mentioned above) were suspended in saline solution (0.85%) and turbidity of the saline solution was adjusted to 0.5 Mc Farland standards [50]. To each test tube 100 µl of standardized suspension of test bacteria were added and incubated at 37° C for 24 h. The end result of the test was the minimum concentration of the plant extract which gave clear solution i.e. no visual growth [51].

## RESULTS

The composition and identification of the main components present in the essential oil of *Ajuga bracteosa* are shown in (Table: II). The presence of various constituents and their percentage were examined by GC and GC-MS. The fragmentation patterns of various components of oil were correlated with that reported earlier. The antimicrobial activity of essential oil against four human pathogenic bacteria (*P. aerogenosa*, *B. subtilis*, *S. aureus*, *E.coli*) was determined. The essential oil obtained from the leaves possessed activity against the bacteria (*Staphylococcus aureus*) (Table: III). The antimicrobial activity of plant extracts was determined by agar disc diffusion method. The microorganisms that were used for the tests were sensitive to the all three plant extracts (Table: IV). It was found that the Acetone extract showed maximum activity

against *E. coli*. However the methanol extract is acting against *Staphylococcus aureus*. The minimum inhibitory concentration was also evaluated for these three plant extracts by broth dilution assay. The minimum inhibitory dilution for the plant obtained from the leaves varied between 0.142 mg/ml to 4.571 mg/ml for the entire microorganisms that were tested. The minimum inhibitory concentration for disappearance of visible microbial growth was observed against *Bacillus subtilis* by the acetone extract of *Ajuga bracteosa* (Table: V).

**Table II: The chemical composition of essential oil of *Ajuga bracteosa***

Sr.No	Components	Retention Index	Percentage
1	Limonene	1012	18.40
2	$\alpha$ -humulene	1462	15.54
3	$\beta$ - myrcene	992	14.60
4	Elemol	1557	12.96
5	Camphene	926	12.86
6	$\beta$ -Caryophyllene	1383	8.16
7	$\alpha$ -phellendrene	1007	15.54
	Total Percentage		98.06

**Table III: Antibacterial activity of essential oil of *Ajuga bracteosa* leaves.**

S.N.	Bacterial strain	Group	Concentration(ml)	Zone of inhibition
1.	<i>Bacillus subtilis</i>	Gram(+)	0.1ml	-
2.	<i>Staphylococcus aureus</i>	Gram(+)	0.1ml	0.1
3.	<i>Escherichia coli</i>	Gram(-)	0.1ml	-
4.	<i>Pseudomonas aeruginosa</i>	Gram(-)	0.1ml	-

**Table IV: Antibacterial activity of plant extracts using Agar disc diffusion method:**

S. N.	Bacterial strain	Group	Concentration (ml)	Zone of inhibition		
				Pet. Ether	Ac.	MeOH
1.	<i>Bacillus subtilis</i>	Gram(+)	0.1ml	-	-	-
2.	<i>Staphylococcus aureus</i>	Gram(+)	0.1ml	-	-	6mm
3.	<i>Escherichia coli</i>	Gram(-)	0.1ml	-	8mm	
4.	<i>Pseudomonas aeruginosa</i>	Gram(-)	0.1ml	-	-	4-ve

Z.O.I: Zone of inhibition in diameter mm.

**Table V: MIC for Plant extracts at particular concentration**

Bacterial strain	Conc. mg/ml		
	Pet. Ether.	Acetone	Methanol
<i>Bacillus subtilis</i>	1.286	0.857	1.142
<i>Staphylococcus aureus</i>	1.143	1.286	1.00
<i>Escherichia coli</i>	1.00	1.429	1.142
<i>Pseudomonas aeruginosa</i>	1.286	1.142	1.00

## DISCUSSION

The various constituents and their percentage were examined by GC and GC-MS studies. It was found that the main constituents of essential oil are Limonene 18.40%;  $\alpha$ -humulene 15.54%;  $\alpha$ -

phellendrene 15.54%;  $\beta$  - myrcene 14.60%; Elemol 12.96%; Camphene 12.86% and  $\beta$ -Caryophyllene 8.16%. The essential oil of *Ajuga bracteosa* was found to very less active against *Staphylococcus aureus* with zone of inhibition 1mm ; while no activity was against *P. aurogenosa* and *B.subtilis*. The plant extracts of *Ajuga bracteosa* were tested for antimicrobial activity. The plant extracts showed significant activity against the following test organisms.

The acetone extract showed maximum zone of inhibition of 8 mm against *E.coli*.where as methanol extract showed minimum zone of inhibition of 6 mm against *Staphylococcus aureus*.The minimum inhibitory concentration was also observed for the these three plant extracts. The acetone extract showed minimum inhibitory concentration of 0.857 mg/ml against *B.subtilis*.

It is quite evident from the above results that the plant extracts are very active against most of the tested microorganisms and the minimum inhibitory concentration is quite low which indicated that very small volume of the extract is needed to kill microorganisms.

### CONCLUSION

The objective of the present work was to find the medicinal importance of the plant *Ajuga bracteosa*.The essential oil of *Ajuga bracteosa* was studied as no work on its essential oil was done earlier. The antibacterial activity of essential oil was also carried out and essential oil was found to be potent against *Staphylococcus aureus*. Apart from this extraction of leaves of *Ajuga bracteosa* with different solvents was also carried out. The antibacterial activity of plant extracts was evaluated. It was found that acetone extract of *Ajuga bracteosa* was most active against *E.coli*.

Thus the results obtained confirm the therapeutic potency of *Ajuga bracteosa* used in traditional medicine. This forms a good basis for the selection of plant for further phytochemical and pharmacological investigation. So present work gives a direction for future investigators to carry out research on the extracts and oil of the plant so that they could get some medicinally important drugs.

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