International Journal of Basic and Applied Biology
p-ISSN: 2349-5820, e-ISSN: 2349-5839, Volume 6, Issue 2; April-June, 2019, pp. 83-85

http://www.krishisanskriti.org/Publication.html

Identification and Evaluation of Anti-microbial Activity of Selected Medicinal Plants of Kashmir Himalayas

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Abstract—*The anti-microbial activity of Thymus linearis, Artemisia absinthium, Aconitum heterophyllum and Digitalis purpurea were examined using methanol as solvent and tested against E.coli (DH5α) strain, using agar well diffusion method and minimum inhibitory concentration (MIC). All the plants showed significant activity against this pathogen, and the alcoholic extract of Artemisia absinthium showed maximum zone of inhibition and MIC against this bacterium and Aconitum showed the lowest activity.*

1. INTRODUCTION

Plants are an important source of traditional medicine for the treatment of various diseases. It has been estimated that herbal medicines are used by more than 80% of the world's population in developing countries to meet the primary health care needs (WHO). Medicinal plants are a valuable natural resource and are regarded as potentially safe drugs. They have been playing an important role in alleviating human sufferings by contributing herbal medicines in the primary health care systems of rural and remote areas where more than 70% of the population depends on folklore and traditional system of medicine[1].In the contemporary global milieu, the documentation of the biological resources and the associated indigenous knowledge existing within a country has assumed priority. Medicinal plants have played a vital role in the treatment of diseases since pre-historic times and are one of the most important areas of research in the world today. India and China are two of the largest countries in Asia which have the richest arrays of registered and relatively well known medicinal plants. India enjoys the privilege of having time tested traditional system of medicines based on the natural products. Plant based products have been in use for medicinal, therapeutic or other purposes right from the dawn of history. The knowledge of medicinal plants have been accumulated in the course of many centuries based on different Indian systems of medicine such as Ayurveda, Unani, Siddha, Rigveda and Atharvaveda which dates back to 2000-1000BC are the important ancient sources of information on medicinal plants. Recent past has witnessed an upsurge in the popularity of the herbal medicine. In the developing countries about 80% of the people depend upon the traditional system of medicine and 95% of the industrial need of this is met through indiscriminate collection from wild so much so that over half a million tones of dry raw material. Medicinal plants as a group comprise approximately 8000 species and account for around 50% of all higher flowering plant species of India. India possesses almost 8% of the estimated biodiversity of the world with around 0.126 million species. There are about 400 families in world of the flowering plants; at least 315 are represented in India. India is one of the 12 mega biodiversity centres with 4 biodiversity hotspots such as; Western Ghats, Himalaya, Indo-Burma and Sundaland[2].Kashmir Himalaya, often referred to as "Terrestrial Paradise on Earth", is located at the North-Western tip of the Himalayan biodiversity hotspot[3]. The region supports a rich and spectacular biodiversity of great scientific curiosity and promising economic benefits; chiefly owing to its topographic variations spanning from valley floor through the terraced tablelands (Karewas) and dense forests, elevating up to snow-capped alpine peaks. Since ages, through trial and error, people in the Himalayan region have learned and practiced the medicinal usage of plants growing in their close vicinity for treating various ailments[3]. It is interesting to note that the ethno medicinal uses of plants is one of the most successful criteria used by the pharmaceutical industry in finding new therapeutic agents for the various fields of biomedicine. Forexample some outstanding medicinal drugs which have been developed from the ethno medicinal uses of plants include Vinblastineand Vincristine from *Catharanthus roseus* (Periwinkle) used for treating acute lymphoma, acute leukaemiasetc. Reserpine from the roots of *Rauwolfia serpentine Benth. ex Kurz*(IndianSarpaghanda) used for treating hypertension, Aspirin from *Salix Alba* L. (Willow) used for treating inflammation, pain and thrombosis and Quinine from *ChichonapubescensVahl* (Chinchona) used for treating malaria[4]. Thus, medicinal plants are used in crude or purified form in the preparation of drugs in different Indian systems of medicine.The main objective of our project is to survey and document the knowledgeof medicinal plants for

future phytochemical and pharmacological studies and also the conservation of endemic and threatened medicinal plants of Jammu and Kashmir.

2. MATERIAL AND METHODS

The present study was carried out from April 2015-April 2016 in order to study the various specific biological activities of selected medicinal plants of Jammu and Kashmir. For the sake of clarity and better understanding the methods are further divided into the following sub sections.

2.1 Study area

The present study was conducted in Gulmarg areas of district Baramulla (34˚17′ 04' N 75˚35′ 46' E altitude 12,068 ft) of Kashmir valley.The plant materials were collected during May- August. Baramulla has temperate type climate with four distinct seasons The total area of district Baramulla is 4,588sq km, rainfall 1270mm, temperate range is -8 to 280 C.

2.2 Collection of plant material

Efforts were made to collect the medicinal plants (*Artemisia absinthium, Aconitum heterophyllum, Digitalis purpurea, Thymus linearis*) from the natural habitats in their flowering and fruiting stages, (May- August, 2015). The mature plants were collected in polythene bags and processed by a standard technique adopted by Kashmir University Herbarium(KASH). The labelled fresh specimens of these plants were identified and authenticated by a plant taxonomist, The various population of these species such as, *Artemisia absinthium, Aconitum heterophyllum, Digitalispurpurea and Thymus linearis*, are frequently seen throughout Kashmir valley in higher altitudes.

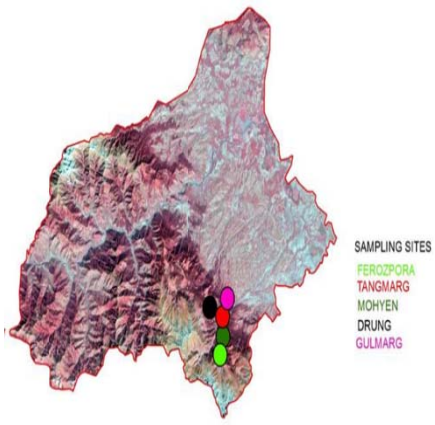


Figure 1: Map showing different sites of collection

2.3 Procedure

The collected plant materials were sorted and sun shade dried in a room free from moisture for 15-20 days. After 15-20 days, the sun shade dried plant materials were further dried in an oven, which were crushed and the plant powder was weighed. The respective weighed plant material powder was dissolved in 40 ml of methanol solvent in a falcon tube (closed vessel) and continually shaked on vortex.Shaking was done occasionally for about 7 days. The methanolic liquid was strained-off in a beaker. The liquid extract was then filtered using wattman filter paper no.1 in a beaker. The liquid plant extract was evaporated and condensed in a beaker on a heating.

Figure 2: Plant extracts of Artemisia, Aconitum, Digitalis and Thymus

2.4 Antimicrobial activity

Broad spectrum antibiotics, ampicillin was used as a control(positive).Commonly used bacterial strain Escherichia coli (DH5α) was used, obtained from Biotechnology department of Kashmir University.

2.5 Determination of anti-microbial activity

Anti-microbial activity was performed by standard method, "well diffusion on agar" and the zone of inhibition was calculated usingMueller-Hinton Agar Medium, Mueller-Hinton agar was prepared from a commercially available dehydrated mediumaccording to the manufacturer's instructions.

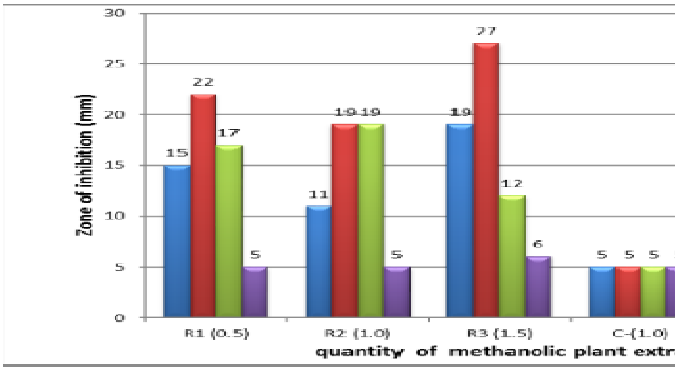
Figure3: Preparation and inoculation of medium (MHA)

2.6 Preparation of antibiotic stock solutions

Powder of antibiotic ampicillin were accurately weighed and dissolved in sterile distilled water (1.0mg/10ml of dd water), sterile five petriplates containing MHA medium were taken. Using sterile swab (spreader), inoculation of the MHA medium was done by spreading the microbial colony all over the surface.Then five wells in each petriplate were made and loaded with 0.5 (R1), 1.0 (R2), 1.5 (R3)µı of methanolic plant extract in any of the three wells and antibiotic solution was added in 4th (C^+) one as positive control and only solvent was added in 5th (C^-) one as negative control. In an incubator, incubate plates at 37° C for 24 hrs.Evaluate anti-microbial activity by measuring zones of inhibition by using scale.

3. RESULTS

The anti-microbial potential of the experimental plants was evaluated according to their zones of inhibition against E.coli and the results (zone of inhibition) were compared with the activity of the standards Ampicillin (1.0 mg/10ml of dd H_20) (Figure 4). The results revealed that all the extracts except aconitum were having potent antibacterial activity against *E.coli* strain. Methanol extract showed maximum inhibition zone with diameter of: 22.6mm (*Artemisia absinthium*), 16mm (*Thymuslinearis),* 15mm *(Digitalis purpurea)* and 5.3mm *(Aconitum heterophyllum)* in *E.coli*



Fiureg 4: Comparison of zone of inhibition of different methanolic plant extract and antibiotic solution.

R1, R2, R3: Different concentration replicates of methanolic plant extracts

C^-: Negative control (solvent only)

C^+: Positive control antibiotic solution

ZOI: Zone of inhibition

Dg:*Digitalis purpurea*
Ar: *Artemisia absinthium*
Th: *Thymuslinearis*
Ac: *Aconitum heterophyllum*

Table 1: Anti-microbial activity (zone of inhibition, mm) of plant extracts and Antibiotics on resistant bacteria (*E.coli*)

Plant extract	R1(ZOI) (mm)	R2 (ZOI) (mm)	R3(ZOI) (mm)	Mean (ZOI) (mm)	C^- (ZOI) (mm)	C^+ (ZOI) (mm)	Result(Anti-microbial activity)
Aconitum heterophyllum	5	5	6	5.3	5	23	Negligible
Artemisia absinthium	22	19	27	22.66	5	24	Positive
Digitalis purpurea	15	11	19	15	5	23	Positive
Thymus linearis	17	19	12	16	5	23	Positive

4. DISCUSSION

Present study provides a comprehensive report on vast wealth of traditional medicinal plant knowledge possessed by the local population of Kashmir Himalaya, India. It is well known fact that indigenous/traditional uses of plants is one of the most successful criteria used by pharmaceutical industries in finding new therapeutic agents for the various fields of biomedicine. The same holds true when we talk about some outstanding drugs such as Vinblastine, vincristine, reserpine, Aspirin and Quinine that have been discovered from various plants keeping in the view their traditional uses. Likewise, present study could also serve an important tool for the discovery of new compounds against several diseases because if the documented plants are subjected to thorough phytochemical and pharmacological investigations, new patent leads against various pharmacological targets could be definitely discovered. In this study, the anti-microbial activity of Methanolic extracts of *Artemisia absinthium ,Thymus linearis, Digitalipurpureaand Aconitum heteropyllum*and their MIC was calculated and compared, which depicted to what extent these medicinal plants can prove to be potential drugs against pathogenic bacteria, and make further efforts to explore and conserve these rich resources.

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