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Comparative study of active constituents of some medicinal plants along altitudinal gradient

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Abstract
Plant-based medicine for curing diseases is as old as human civilization. Medicinal plants consist of an active constituents, which are used for treating various diseases and healing of wound in humans. This paper aims to identify bioactive phytochemical constituents of selected four medicinal plants viz. *Acorus calamus* L. (Bojho), *Eucalyptus camaldulensis* Dehnh. (Masala), *Artemisia indica* Willd. (Titepati) and *Cymbopogan winterianus* Jowitt ex Bor (Citronella) and compare their concentration along altitudinal gradient in Makawanpur district, central Nepal. The plant species were selected from different altitudinal regions and the phytochemical screening was done using Gas Chromatography-Mass Spectrometry (GC-MS) to identify major active constituents. The major active constituents in *Acorus calamus* L. of three altitudes were β-Asarone, Isoeugenol and α-Asarone and the β-Asarone was found to be present in highest concentration from temperate region. The major active constituents in *Eucalyptus camaldulensis* Dehnh. of tropical and temperate region were Eucalyptol, α-Pinene, β-Pinene, D-Limonene, α-Terpineol and Viridiflorol. In sub-tropical region, Viridiflorol was absent and among the major bioactive constituents, Eucalyptol was found in highest concentration in temperate region. The major active constituents in *Artemisia indica* Willd. of tropical and temperate region were 7-Oxabicyclol [2.2.1] heptane, 1-methyl-4-(1-methlethyl), β-Thujone, Sabinene, Borneol and Camphor. In *Artemisia indica* Willd. of sub-tropical region, β-Thujone was absent and the concentration of major active constituents, 7-Oxabicyclol [2.2.1] heptane, 1-methyl-4-(1-methlethyl), was found to be highest in sub-tropical region. The major active constituents in *Cymbopogan winterianus* Jowitt ex Bor of tropical and sub-tropical region were Geranial, Neral, (R) - Lavandulyl acetate, D-Limonene and β-Asarone and the concentration the major active constituents (R)- Lavandulyl was found to be highest in *Cymbopogan winterianus* Jowitt ex Bor from sub-tropical region. The findings of this study provide the empirical support for the cultivation of *Acorus calamus* L. and *Eucalyptus camaldulensis* Dehnh. mainly in temperate region, *Artemisia indica* Willd. in subtropical region whereas *Cymbopogan winterianus* Jowitt ex Bor in tropical region so as to obtain optimum yield of major active chemical constituents.

Keywords: medicinal plant, active constituent, gas chromatography-mass spectrometry (GC-MS), altitude

1. Introduction

A medicinal plant is the plant used in order to relieve, prevent or cure a disease or to alter physiological and pathological process or any plant employed as a source of medications or their precursors. Aromatic plant refers to a plant with elevated level of volatile oil or essential oils. In addition to therapeutic use, essential oils have wide applications in cosmetics and beauty products, flavors, fragrances and perfumery.

Medicinal & Aromatic Plants (MAPs) are botanical raw materials, also known as herbal drugs that are primarily used for therapeutic, aromatic and/or culinary purposes as components of cosmetics, medicinal products, health foods and other natural health products. They are also the starting materials for value-added processed natural ingredients such as essential oils, dry and liquid extracts and oleoresins. There is a clear industrial demand for MAPs due to the increased production of herbal health care formulations; herbal based cosmetic products and herbal nutritional supplements. In addition, traditional health care practitioners, traditional healers and consumption at the household level all contributed to the demand for herbal medicinal products (Ved & Goraya, 2007) [24]. Finished products made from medicinal and aromatic plants are increasingly prescribed and bought over the counter. According to a study of British Chamber of Commerce (BCC) the global demand for botanical and plant-derived drugs had increased by 11% from $19.5 billion in 2008 to $32.9 billion in 2013. Moreover, the recent trend shows that the global market for botanical and plant-derived drugs will grow from $29.4 billion in 2017 to around $39.6 billion by 2022 with a compound annual growth rate (CAGR) of 6.1% for the period of 2017-2022.

Since ancient time, traditional herbal medicine has been used in many parts of the world, where access to formal and modern healthcare is limited. Nepal is not exempt and in mid-hills, mountainous and rural areas of the country where access and services are limited, herbal medicine is the basis of treatment of most illness through traditional knowledge (Kunwar *et al*., 2010) [11]. It is estimated that approximately 90% of the Nepalese people reside in rural areas where access to government health care facilities is lacking (Manandhar, 2002) [13]. As a result, over 1800 medicinal and aromatic plants have been used indigenously in Nepal Himalaya (Shrestha *et al*., 2000; Baral & Kurmi, 2006) [20, 3].

There is an increased attention for medicinal plants than ever as they have potential of myriad benefits to society or indeed to all mankind, especially in the line of medicine and pharmacological. The medicinal value of these plants lies in the bioactive phytochemical constituents that produce definite physiological action on the human body (Akinmoladun *et al*., 2007) [2].

The discovery of new anti-infective agents from higher plants is possible because of the phytochemical research based on ethno-pharmacological information (Duraipandiyan *et al*., 2006) [5]. Knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents but also because such information may be of value in disclosing new sources of economic materials such as tannins, oil, gums, precursors for the synthesis of complex chemical substances. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Ghaderi *et al*., 2003) [6].

Essential oils are concentrated volatile aromatic compounds produced by plants - the easily evaporated essences that give plants their wonderful scents. Each of these complex precious liquids is extracted from a particular species of plant life. Each plant species originates in certain regions of the world, with particular environmental conditions and neighboring fauna and flora (Rao & Pandey, 2006) [18]. Essential oils are produced by plants following a complex physiological mechanism which, in turn, are extracted and used in a wide variety of consumer goods such as pharmaceuticals, perfumes, cosmetics, detergents, soaps, toilet products, confectionery food products, soft drinks, distilled alcoholic beverages and insecticides. The world production and consumption of essential oils and perfumes are increasing very fast. Production technology is an essential element to improve the overall yield and quality of essential oil. Essential oils are generally derived from one or more plant parts, such as flowers, leaves, bark, wood, roots, seeds, fruits, rhizomes and gums or oleoresin exudations.

Gas Chromatography-Mass Spectrometry (GC-MS) is an analytical method that combines the features of gas-chromatography and mass-spectrometry to identify different substances within a test sample (Sparkman *et al*., 2011) [19]. The gas chromatograph separates the components of a mixture in time and the mass spectrometer provides information that helps in the structural identification of each components (Kitson *et al*., 1996) [9]. It is useful for drug detection and identification of unknown samples.

Four medicinal plants had been selected in this study.viz. *Acorus calamus* L. (Bojho), *Eucalyptus camaldulensis* Dehnh. (Masala), *Artemisia indica* Willd. (Titepati) and *Cymbopogan winterianus* Jowitt ex Bor (Citronella).

Acorus calamus L. belongs to Acoraceae family. It has been an item of trade in many cultures and widely used medicinally for a wide variety of ailments for thousands of years. It is one of the highly valued herbal, medicinal and economically important plant species and used since the time of Ayurveda. It is commonly known as Bojho (Nepali) and Sweet flag (English). It is a native plant of Northern Asia from the Black sea to China and Japan. In Nepal, it is often cultivated in damp marshy places. There are many traditional uses such as clearing nasal congestion, improving memory, asthma, voice clarity, sedative, skin and hair care etc.

Eucalyptus camaldulensis Dehnh, locally known as Masala, belongs to Myrtaceae family. It is a common and widespread tree along watercourses. The essential oil found in the leaves is a powerful antiseptic and is used all over the world for relieving coughs and cold, sore throats and other infections.

Artemisia indica Willd. belongs to Asteraceae family and a very popular species in the study area in the name of Titepati. It is commonly known as Mugwort. It is useful in liver tonic; to promote circulation; and as a sedative. Traditional uses include treatment of hysteria, epilepsy, and convulsions in children. Women take Mugwort for irregular periods and other menstrual problems.

Cymbopogan winterianus Jowitt ex Bor belongs to Poaceae family. Essential oil is used in the manufacturing of soaps, perfumes, detergents, insecticides, soap flakes and household cleaners. It has been used in traditional medicine for treating menstrual problems, increased perspiration, mental fatigue, depression and as a safe repellent for pets. It is also used in varnishes, insecticides, and perfumery, for scenting low-priced technical products such as detergents, soaps, sprays and polishes (Uphof, 1959) [23].

Medicinal plants are useful for healing as well as for curing of human diseases only because of the presence of phytochemical constituents (Nostro et. al., 2000) [16]. Phytochemical occurs naturally in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases (Krishnaiah, *et al*., 2007) [10]. The qualitative analysis of active constituents is essential for the discovery of new drugs in curing various diseases prevailing in our community. The analysis of active constituents is important and carries commercial interest in both research institutes and pharmaceutical companies.

According to World Health Organization, approximately 80% of Nepalese population resides in the rural areas of Nepal where there is a lack of primary health care facilities. They depend on the traditional knowledge based medication system and practice medicinal plant based on their morphology rather than having knowledge of the active constituents which is responsible for the medicinal properties of the plants. Similarly, many of the studies have been focused only on documenting various medicinal and aromatic plants found in different regions of the country and still there is a gap of research regarding the phytochemical screening of medicinal and their effective mixing which is equally important. Phytochemical screening by Gas Chromatography-Mass Spectrometry (GC-MS) process is reliable for the identification of large number of active constituents within a very short period of time. This paper aims to identify bioactive phytochemical constituents of selected four medicinal plants and compare their concentration in different altitudinal regions of Makawanpur district, central Nepal.

2. Material and Methods

2.1 Study Area

Makawanpur district is a part of Province No. 3, central Nepal. The district comprises of diverse climatic conditions ranging from lower tropical (<300m), upper tropical (300-1000m), sub-tropical (1000-2000m) to temperate (2000-3000m). Tropical zone comprises Shorea and riverine forests at southern lower belts where *Shorea robusta* Gaertn., *Terminalia chebula* Retz., *Terminalia bellirica* (Gaertn.) Roxb., *Adina cordifolia* (Roxb.) Benth. & Hook. f. ex B. D. Jacks, *Acacia catechu* (L. f.) Willd. *Dalbergia sissoo* DC., *Bombax* ceiba L. etc. flourish very well. Subtropical region consists mainly *Schima-Castanopsis*, Chir pine and Alder forests comprising *Schima wallichii* Choisy, *Castanopsis indica* (Roxb. ex Lindl.) A. DC., *Castanopsis tribuloides* (Sm.) A. DC., *Pinus roxburghii* Sarg. as dominating species. Similarly, *Rhododendron arboretum* Sm., *Myrica esculenta* Buch.-Ham. ex D. Don, *Lyonia ovalifolia* (Wall.) Drude, *Quercus lanata* Sm. as dominate the temperate forests. Tamang (47.82%) is the largest ethnic group followed by Brahnin (14.9%), Chhetri (10.71%), Newar (6.21%), Chepang (4.57%), Magar (4.51%) and others (DDC, 2015). Usually, Tamang, Bankariya and Chapang communities are living very close to forest and practice a lot of traditional medication systems than other communities.

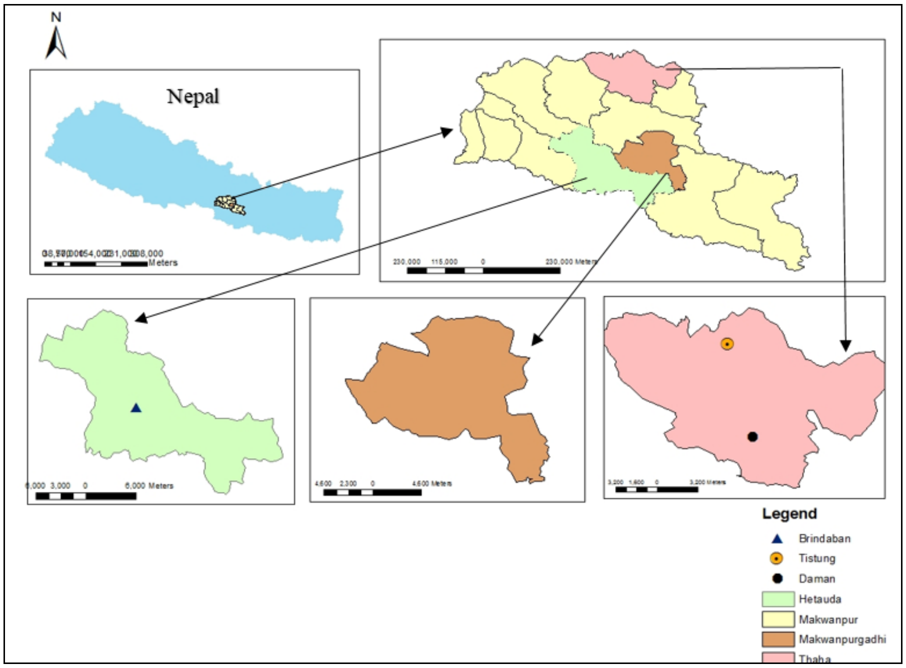

Fig 1: Map of Makawanpur District showing area of plant collection

2.2 Collection of Medicinal Plants

A total four medicinal plants were collected from different altitudinal regions viz. Tropical, Sub-Tropical and Temperate (Table - 1). Availability of medicinal plants in all three altitudinal regions and local use value were considered as selection parameter of the plants. The medicinal plants were mainly collected from Hetauda (350m), Makawanpurgadhi (1200m) and Daman (2320m).

Table 1: List of collected medicinal plants

S.N.	Botanical name	Local name	Collected part/s	Oil percentage (%)	Traditional Uses	Reference
1.	*Acorus calamus* L.	Bojho	Rhizome	0.5%	Cough, common cold,	Bhattarai et al. (2017) [4] Rana et al. (2015)
2.	*Eucalyptus camaldulensis* Dehnh.	Masala	Twigs	1%	Cough, cold and headache	Zhang et al (2010) [25]
3.	*Artemisia indica* Willd.	Titepati	Twigs	0.5%	Skin diseases, fever, anthelmintic, blood purification	Bhattarai et al. (2017) [4]; Hasan et al. (2013) [7]
4.	*Cymbopogan winterianus* Jowitt ex Bor	Citronella	Leaves, stem	1.%	Anti-inflammatory, mosquito repellent, to make soap	Bhattarai et al. (2017) [4]; Singh (2015) [21]

2.3 Extraction of Essential oil

The extraction of essential oil was conducted through hydro-distillation using Clevenger apparatus system (fig. 3). In this method, the plant was cut down into small pieces and placed in a around bottom flask and half filled with water which was placed in a heating mantle. The heat passed for 2-3 depending on the plant sample used at temperature of 80°C. The vapors was condensed in a condenser and this oil was collected in a bottle. This method was advantageous in extracting large amounts of essential oil with only small amount of plant.

Fig 3: Extraction of essential oil

2.4 Identification of active constituents

Identification of active phytochemical constituents was done by using Gas Chromatography-Mass Spectrometry (GC-MS). GC-MS was set for the whole process of identification with time of 60 min for each oil sample. The value of particular peak was shown in peak table through the use of GC-MS software which showed the total number of active constituents present and its respective concentration in oil sample through the area occupied by a particular peak in the chromatogram. Highest the peak of a particular active constituents, highest was the concentration of that active constituents in a particular oil sample.

2.5 Data Analysis

Quantitative data were analyzed by using appropriate statistical tools such as mean and percentage whereas qualitative data were analyzed in descriptive manner and presented in trend line and bar graph.

3. Results

All together 17 major active constituents were found in phytochemical screening of essential oils of four in four different medicinal plants through Gas Chromatography-Mass Spectrometry (GC-MS) technique (Table 2).

Table 2: Major active constituents present in selected species

S.N.	Major Chemicals	*Acorus calamus* L.			*Eucalyptus camaldulensis* Dehnh.			*Artemisia indica* Willd.			*Cymbopogan winterianus* Jowitt ex Bor	
		350 m	1200 m	2320 m	350 m	1200 m	2320 m	350 m	1200 m	2320 m	350 m	1200 m
1.	β-Asarone	+	+	+	+	+		+	+	+	+	+
2.	Isoeugenol <methyl-,(Z)->	+	+	+								
3.	α-Asarone	+	+	+								
4.	Eucalyptol	+		+	+	+	+					
5.	Pinene <alpha->				+	+	+	+	+	+		
6.	Pinene <beta->				+	+	+	+	+	+		
7.	D-Limonene				+	+	+	+		+	+	+
8.	Terpineol <alpha->				+	+	+	+	+	+		
9.	Viridiflorol				+		+					
10.	7-Oxabicyclol [2.2.1] heptane, 1-methyl-4-(1-methlethyl) and							+	+	+		
11.	Thujone <beta->	+						+		+		
12.	Sabinene							+	+	+		
13.	Borneol							+	+	+		
14.	Camphor							+	+	+		
15.	Geranial (R) -					+		+	+	+		
16.	Neral		+			+					+	+
17.	(R)Lavandulyl acetate										+	+

3.1 *Acorus calamus* L. (Bojho)

The chemical composition of essential oil extracted from rhizome of *Acorus calamus* L. collected from three different altitudinal zone was found as -

- **Tropical:** The analysis showed the presence of sixteen bioactive compounds that could contribute towards the medical properties of plant The major phytochemical constituent present were β-Asarone (71.02%), Isoeugenol <methyl-,(Z)-> (7.42%) and α-Asarone (4.03).
- **Sub-Tropical:** The analysis showed the presence of twenty-one bioactive compounds. The major phytochemical constituents present were β-Asarone (71.84%), Isoeugenol <methyl-,(Z)-> (5.31%) and α-Asarone (3.69%).
- **Temperate:** The analysis showed the presence of thirteen bioactive compounds. The major phytochemical constituents present were β-Asarone (81.82%), Isoeugenol <methyl-, (Z)-> (4.09%) and α-Asarone (2.33%).

The concentration of the major bioactive constituents, β-Asarone (81.82%) was found to be highest in *Acorus calamus* L. of temperate region (Figure-4). The highest quantity of phytochemicals constituents was found in sub-tropical region and were twenty-one (Figure-5).

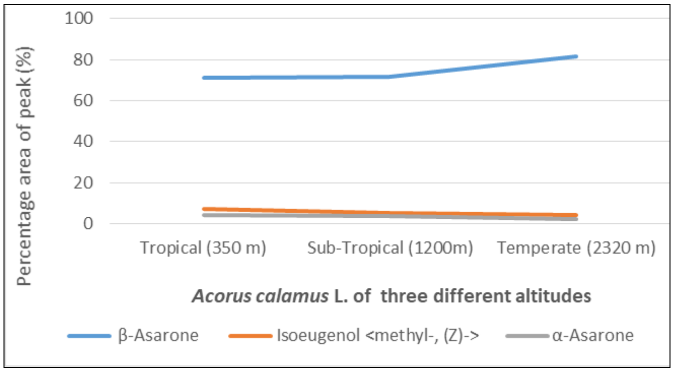

Fig 4: Major active constituents of *Acorus calamus L.*in tropical, sub-tropical and temperate region

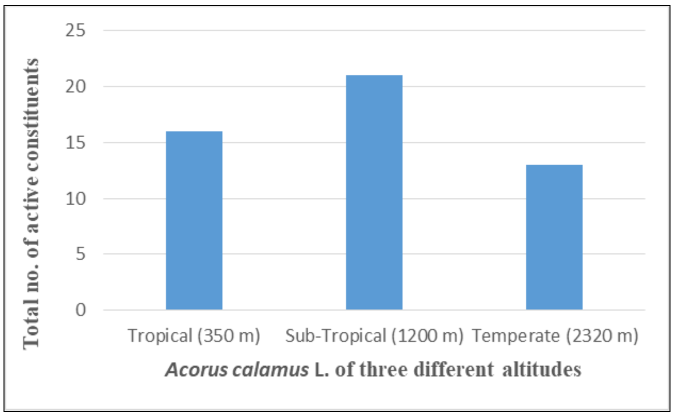

Fig 5: Total number of active constituents of *Acorus calamus L.*in tropical, sub-tropical and temperate region

3.2 Eucalyptus camaldulensis Dehnh. (Masala)

The chemical composition of essential oil extracted from rhizome of *Eucalyptus camaldulensis* Dehnh. collected from three different altitudinal zone was found as -

- **Topical:** The analysis showed the presence of twenty-five bioactive compounds that could contribute towards the medical properties of plant. The major phytochemical constituents present were Eucalyptol (41.56%), α-Pinene (12.20%), β-Pinene (10.10%), D-Limonene (6.26%), α-Terpineol (5.64%) and Viridiflorol (6.69%).
- **Sub-Tropical:** The analysis showed the presence of twenty-nine bioactive compounds. The major phytochemical constituents present were Eucalyptol (23.23%), α-Pinene (20.70%), β-Pinene (10.39%), D-Limonene (10.40%), α-Terpineol (4.87%) and Viridiflorol was not present.
- **Temperate:** The analysis showed the presence of nineteen bioactive compounds. The major phytochemical constituents present were Eucalyptol (51.13%), α-Pinene (8.80%), β-Pinene (0.56%), D-Limonene (17.16%), α-Terpineol (5.58%), Viridiflorol (4.12%).

The concentration of the major bioactive constituent, Eucalyptol (51.13%) was found to be highest in *Eucalyptus camaldulensis* Dehnh. of temperate region (Figure-6). The highest quantity of phytochemicals constituents were found in sub-tropical region and were twenty nine (Figure-7).

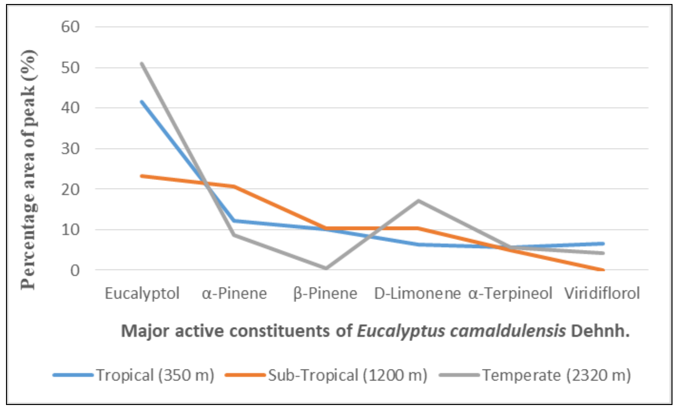

Fig 6: Major active constituents of *Eucalyptus camaldulensis* Dehnh. in tropical, sub-tropical and temperate region

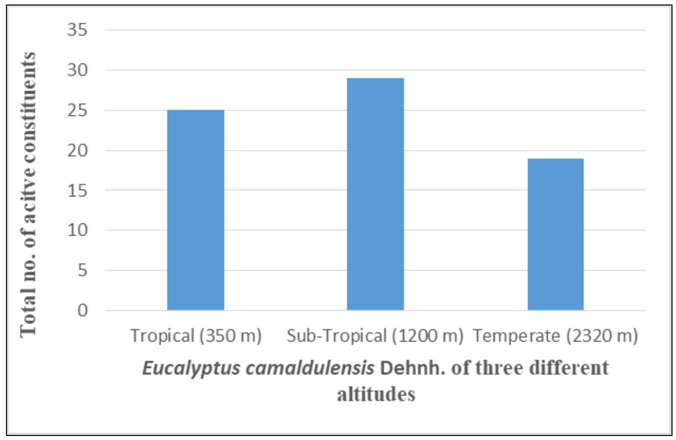

Fig 7: Total number of active constituents of *Eucalyptus camaldulensis* Dehnh. In tropical, sub-tropical and temperate region

3.3 Artemisia indica Willd. (Titepati)

The chemical composition of essential oil extracted from rhizome of *Artemisia indica* Willd. Collected from three different altitudinal zone was found as -

- **Tropical:** The analysis showed the presence of thirty-one bioactive compounds that could contribute towards the medical properties of plant. The major phytochemical constituents present were 7-Oxabicyclol [2.2.1] heptane, 1-methyl-4-(1-methlethyl) (17.32%), β-Thujone (15.84%), Sabinene (7.22%), Borneol (5.39%) and camphor (2.69%)
- **Sub- Tropical:** The analysis showed the presence of twenty bioactive compounds. The major phytochemical constituents present were 7-Oxabicyclol [2.2.1] heptane, 1-methyl-4-(1-methlethyl) (33.12%), Sabinene (6.42%), Borneol (6.59%) and Camphor (24.14%) and β-Thujone was absent.
- **Temperate:** The analysis showed the presence of forty-three bioactive compounds. The major phytochemical constituents present were 7-Oxabicyclol [2.2.1] heptane, 1-methyl-4-(1-methlethyl) (8.36%), Sabinene (3.81%), Borneol (2.87%) and Camphor (7.87%) and Thujone <beta-> (19.43)

The concentration of the major bioactive constituent, 7-Oxabicyclol [2.2.1] heptane, 1-methyl-4-(1-methlethyl) (33.12%) was found to be highest in *Artemisia indica* Willd. of sub-tropical region (Figure-8). The highest quantity of phytochemicals constituents were found in temperate region and were forty three (Figure-9).

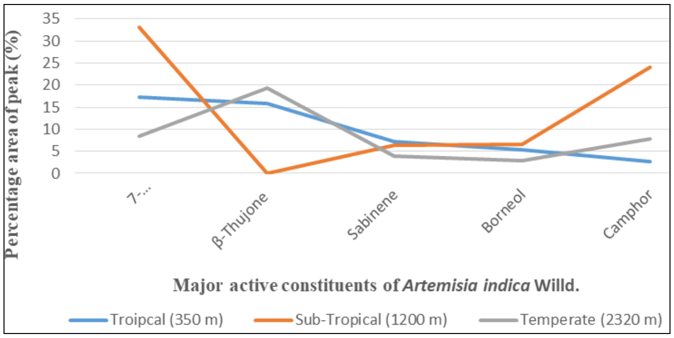

Fig 8: Major active constituents of *Artemisia* indica *Willd.* along altitudinal gradient

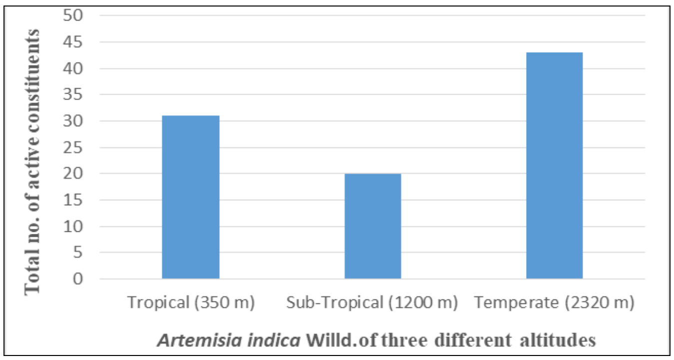

Fig 9: Total number of active constituents of *Artemisia indica* Willd. along altitudinal gradient

3.4 *Cymbopogan winterianus* Jowitt ex Bor (Citronella)

The chemical composition of essential oil extracted from rhizome of *Cymbopogan winterianus* Jowitt ex Bor collected from two different altitudinal zones was found as -

- **Tropical:** The analysis showed the presence of twenty-one bioactive compounds that could contribute towards the medical properties of plant. The major phytochemical constituents present were Geranial (29.83%), Neral (23.93%), (R) - Lavandulyl acetate (13.61%), D-Limonene (3.22%) and β-Asarone (10.58%)
- **Sub-Tropical:** The analysis showed the presence of fourteen bioactive compounds. The major phytochemical constituents present were Geranial (25.14%), Neral (20.33%), (R) - Lavandulyl acetate (33.45%), D-Limonene (4.59%) and β-Asarone (3.13%).

The concentration of the major bioactive constituent, (R) – Lavandulyl acetate (33.45%) was found to be highest in *Cymbopogan winterianus* Jowitt ex Bor of sub-tropical region (Figure-10). The highest quantity of phytochemicals constituents were found in tropical region and were twenty (Figure-11).

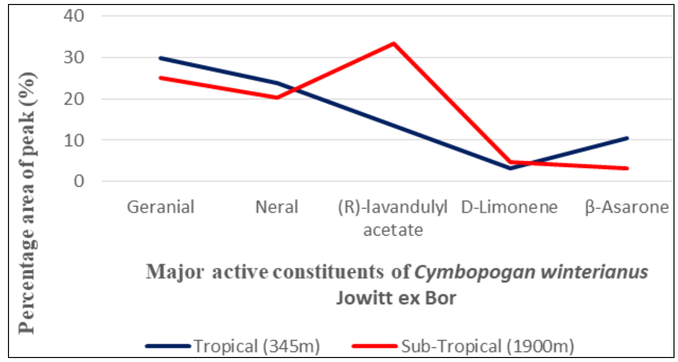

Fig 10: Major active constituents of *Cymbopogan winterianus* Jowitt ex Bor in tropical, sub-tropical and temperate region.

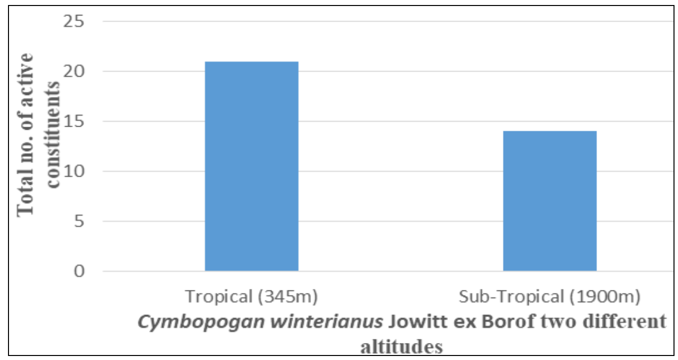

Fig 11: Total number of active constituents of *Cymbopogan winterianus* Jowitt ex Bor in tropical, sub-tropical and temperate

4. Discussion

GC-MS resulted in the identification of major components of essential oil in rhizome of *Acorus calamus* L. (Bojho) as β-Asarone and α-Asarone. This was similar to the study done by Mukherjee *et al.* (2007) [15] which also showed β-Asarone and α-Asarone as the main chemical constituents. Similar results were obtained in the research done by Raina *et al*. (2003) [17] in the lower region of the Himalayas.

The major components obtained from essential oil of *Eucalyptus camaldulensis* Dehnh. (Masala) were Eucalyptol, α-Pinene, β-Pinene, D-Limonene, α-Terpineol and Viridiflorol which was also supported from research of Akin *et al.* (2010) [1]. However, two different major components were present such as 1, 8-cineole and Spathulenol than the result of Tsiri *et al*. (2003) [22].

α-Thujone or Thujone isomer and camphor were determined as the main components in *Artemisia indica* Willd. (Titepati) from India (Misra & Singh, 1986) [14]. In North Lithuania, trans-thujone was determined as the first main component (Judzentien & Buzelyte, 2006) [8]. According to this research, the main components were 7-Oxabicyclol [2.2.1] heptane, 1-methyl-4-(1-methlethyl), β-Thujone, Sabinene, Borneol and camphor. However, α-Thujone was completely absent in all tropical, sub-tropical and temperate region.

Main phytochemical constituents of *Cymbopogan winterianus* Jowitt ex Bor (Citronella) identified in this research were Geranial, Neral, (R) - Lavandulyl acetate, D-Limonene and β-Asarone. The composition of essential oil in this study was significantly different from that previously reported by Mahalwal and Ali (2003) [12]. The major component of essential oil of Citronella in India were citronellal, Geranial, γ-Terpineol and cis-sabinene hydrate (Mahalwal & Ali, 2003) [12].

The total number of phytochemical constituents present in *Acorus calamus* L. of tropical region were sixteen, sub-tropical were twenty-one and temperate were thirteen. Therefore, highest chemical diversity was seen in *Acorus calamus* L. of sub-tropical region. The total number of phytochemical constituents present in *Eucalyptus camaldulensis* Dehnh. of tropical region were twenty-five, sub-tropical were twenty-nine and temperate were nineteen. Therefore, highest chemical diversity was seen in *Eucalyptus camaldulensis* Dehnh. of sub-tropical. The total number of phytochemical constituents present in *Artemisia indica* Willd. of tropical region were thirty-one, sub-tropical were twenty and temperate were forty-three. Therefore, highest chemical diversity was seen in *Artemisia indica* Willd. of temperate region. Likewise, the total number of phytochemical constituents present in *Cymbopogan winterianus* Jowitt ex Bor of tropical region were twenty-one and sub-tropical were fourteen. Therefore, highest chemical diversity was seen in *Cymbopogan winterianus* Jowitt ex Bor of tropical region.

5. Conclusion

Among the major chemical components found in *Acorus calamus* L. of tropical, sub-tropical and temperate region, β-Asarone was found to be in temperate region. Likewise, *Eucalyptus camaldulensis* Dehnh. was found to contain highest concentration of Eucalyptol as the major chemical component from the temperate region. Similarly, the highest concentration of 7-Oxabicyclol [2.2.1] heptane, 1-methyl-4-(1-methlethyl) was found to be in *Artemisia indica* Willd. of sub-tropical region as major active compounds whereas the highest concentration of (R) - Lavandulyl acetate was found to be in *Cymbopogan winterianus* Jowitt ex Bor of tropical region. The findings of this study provide the empirical support for the cultivation of *Acorus calamus* L.and *Eucalyptus camaldulensis* Dehnh.mainly in temperate region, *Artemisia indica* Willd. in subtropical region whereas *Cymbopogan winterianus* Jowitt ex Bor in tropical region so as to obtain optimum yield of major active chemical constituents.

6. Acknowledgement

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