Introduction

1.1 General Introduction

Nepal, predominantly Himalayan country, is situated between latitude of $26^0$22' N to 30^0 27' N and longitude of $80^0$4' E to 88^0 12'E. The average east-west length in about 885km and north-south width varies from 145-241 km that makes a total area of 1, 47, 181sq.km (Anonymous 1998).

It has been estimated that approximately 250,000 to 750,000 species of higher plants exist on the earth but less than 5 % have been scientifically investigated for their medicinal efficacy (Handa 2000). Nepal has a gift of over 7000 species of vascular plants among them 1463 species of medicinal plants have been reported, representing about 20% of the total flora (Tiwari 1999).

It is estimated that various communities in Nepal use approximately 1000 species of wild plants in traditional medicinal practice (Chaudhary 1998). However, there are approximately 1600-1900 species commonly used for medicinal purposes (Shrestha *et al*. 2000; Baral and Kurmi 2006; Ghimire 2008).

The history of medicine and medicinal plants in Nepal can be traced back to the Vedic period, where Nepal-Himalaya was mentioned as a sacred heaven of potent medicinal and aromatic plants (Baral and Kurmi 2006).

The major medicinal systems being practiced in Nepal are Allopathic, Homeopathic, Ayurvedic, Tibetan, Unani, and traditional faith healing. On the rural area different ethnic groups use a large number of plants and plant products for the treatment of many diseases. Despite the reputation earned by medicinal and aromatic plants of Nepal, very little work has been reported for their chemical constituents. Therefore isolation of the active constituents from such medicinal plants and study of their biological activity may provide a scientific support for their use.

Medicinal plants are not only used in traditional medicine but also have great demand in research institution involving in investigation and exploring active pharmaceutical compound found in them. Thus medicinal plant can be considered as one of the important natural resources for raising the economic status of the country. Therefore, research on the medicinal plants for least developed countries like Nepal is of utmost importance.

Nardostachys grandiflora DC.

Family- Valerianaceae.

Common name: Spikenard (Eng). Jatamansi (Nepali, Sanskrit, and Hindi), Gansong or Gansongxiang (Chinese), Pampe (Bhutan).

Vernacular names: Tapaswini, Bhutle, Hanswa, Naswa, Balchad, Masijara (Nepali), Panbu (Sherpa). Pangpoe (Gurung). Drakpoo (Tibetan).

Nardostachys grandiflora DC. is an erect, perennial, rhizomatous, growing 10-60 cm height with long stout woody rootstock found in between 3500-5000 m altitude. The woody rhizome (about 8mm thick) is covered with reddish brown fibers of petioles of old leaves and flowering stem. Leaves are entire, radical, elongate, spathulate, cauline few, longitudinally nerved, glabrous or slightly pubescent. The flowers are capitates, heads in cyme. The seeds are obovate, compressed about 4mm long, covered with ascending white hairs, crowned by the ovate, acute and often dentate calyx teeth.

The rootstock is woody, long, stout and covered with fibers from the petiole of withered leaves. The stem is about 10-60cm long, more or less pubescent, upward, often glabrate below and subscapose. The radical leaves are 15-20/2.5cm long, longitudinally nerved, glabrous or slightly pubescent and narrowed into the petiole. One or two pairs of cauline leaves are about 2.5-7.5 cm long, sessile oblong or sub ovate.

The plant is mostly found growing in steep areas with 25^0-45^0 slopes. It grows well on open, stony and grassy slopes and on the turf of glacial flats. The flowering takes place during June to July and fruiting in August to October. In the beginning of October, all leaves turn yellow. During the winter, the herb sheds all leaves, gets buried under the snow and remains dormant. In the beginning of summer, Jatamansi starts growing with the melting of snow.

Distribution

Nardostachys grandiflora DC. is distributed in the northern part of alpine to sub alpine Himalayan region from Panjab to Sikkim, Bhutan to Unan, Sejwan to Tibet and West China at attitude of 3000-5000m (Polunin *et al.* 1984).

Nardostaclys grandiflora DC. is a perennial aromatic rhizome bearing herb with height of 10-60 cm. It grows in dry to moist open forest or rocky slope. In Nepal it is found mostly in the northern most Nepal. It bears aromatic rhizomes which on hydro-distillation yield an essential oil with characteristic odors.

Mostly, *Nardostachys gradiflora* DC. is distributed in Jumla, Humla, Kalikoat, Dolpa, Lamjung,Gorkha and other districts of Nepal. The herb (up to 60cm tall) is collected **SPNP**: Chahkong – Suibutong (Kunasa valley) 4000m-4300m, Pungphoo (head of Pungphoo valley) 4200-4500m, Dokpa-Mukroman 3800-4200m, Jagdulla 3800-4300m, (Ghimire *et al.* 2001) . The plant species is present in all mountain districts, though commercially it is collected from following region (Das 2004).

Eastern Nepal - Taplejung and Terathum

Central Nepal - Dolakha and Rasuwa

Western Nepal - Manang and Gorkha

Mid western Nepal - Humla, Jumla, Dolpa and Mugu

Far western Nepal – Darchula and Bajhang

1.2 Importance

1.2.1 Rhizome of plant

The rhizome of Jatamansi is aromatic, tonic, stimulant, deobstruent, emmenagogue, antiseptic and antipyretic (Anonymous 1985). In Nepal, it is used as tonic, stimulants, as an antiseptic for the treatment of epilepsy hysteria, convolutions, heart palpitation, intestinal colic (Anonymous 1993), pain relief and treating a turgid chest (Zang *et al*. 1994).

It is also useful as antispasmodic, diuretic, carminative, stomachic and laxative in hysteria and cholera. Jatamansi remains an active component of many, Ayurvedic medicines including 'Tapaswiniwati', 'Jestalabangadi' 'Chandanadi churana' and 'Rachhogna ghrit' etc.

A tincture of rhizome is given in intestinal colic and flatulence. The rhizome is used as an aromatic adjunct in the preparation of medicinal oil (Anonymous 1985).

Rhizomes are used on incense in magical-religious rites known as 'Jagar' and to promote hair growth and also blackness (Anonymous 1985).

In China it is traditionally used for treating pain in the chest and abdomen that results from stagnation associated with common cold (*Hsu* 1986).

1.2.2 Application of the essential oil

The oil, commonly called spikenard oil, possesses antiarrhythmic activity with possible therapeutically usefulness in cases of auricular flutter. It has been found to be less effective than the drug used in auricular flutter such as quinidine but is less toxic. Jatamansone (one of the

constituent of Jatamansi oil) is more potent than the oil and is more active than quinidine in ventricular tachycardia that results from acute myocardial infarction. Jatamansone possesses anticonvulsant action as well. The oil exerts a hypotensive effect and in moderate dose it has a distinct depressant action on the central nervous system, higher doses cause deep narcosis and ultimately death within a few hours (Anonymous 1985).

Jatamansone shows marked tranquilizing activity in mice and it has antiemetic activity in dogs and reduces aggressiveness in monkeys (Parajuli *et al*. 1998).

Researches so far done on *Nardostachys* have focused on its antioxidant (Salim *et al*. 2003; Joshi and Parle 2006; Rajakannu *et al*. 2006, 2007), cytoprotective (Rajakannu *et al*. 2006), hepatoprotective (Ali *et al*. 2000), nerve growth promoting (Li *et al*. 1999a, 1999b) tranquilizing and memory enhancing (Joshi and Parle 2006) and fungi-toxic (Sarbhoy *et al*. 1978); (Mishra *et al*. 1995) activities. Methanolic extract of rhizome has been reported to exhibit antimicrobial activities against *Bacillus subtilis* (MBC value being 3.12 mg), *Salmonella paratyphi* (25 mg ml^{-1}) *Salmonella typhi* (25 mg ml^{-1}) (Timsina 2003).

The essential oil content of Jatamansi ranged 0.40-1.66% of dry weight in Manang, central Nepal (Chhetri 1999), 0.5-1.8% covering different parts of Nepal (Acharya *et al*. 1999), 0.98-1.7% in Garhwal, India (Bhojvaid *et al*. 2000), and 0.25-1.0% in Uttaranchal India (Nautiyal *et al*. 2003). Similarly, wild plant showed higher essential oil content in comparison to cultivated plants (Bhojvaid *et al*. 2000).

1.2.3 Harvesting and Cultivation

The appropriate time for harvesting Jatamansi is October- December. But the early snowfall in some years disturbs the harvesting during this period making the harvesting job difficult and sometimes impossible due to thick layer of snow in this season. Because of this, it is sometimes harvested during May or June. But the collection in May and June affects the herb's regeneration as the fruits mature only after October.

To maintain the quality and demand for the plant cultivation is essential. Such a practice is the only way for sustainable utilization of this highly valuable medicinal plant. Natural regeneration of *Nardostachys* takes by rhizome and seeds. Plants from rhizomes grow faster than from seeds but there is high risk of underground rhizome decay. Seeds hardly germinate in soil and it may take long period to give a better yield.

1.3 Trade of *Nardostachys*

Nepal is a primary exporter of this species exporting large amounts of unprocessed rhizomes. *Nardostachys* was the second highest export earning Jadibuti of Nepal next to Chiraito (*Swertia chirayita*) before its ban on export by government of Nepal in 1993(Parajuli *et al*.1998). Rhizomes were exported in India without processed. But now a day, government makes a policy to export after processing from Nepal. Jatamansi is collected and traded from all over Nepal Himalayas. However Gorkha and Manang district of western development region, Jumla, Humla, Dolpa, Mugu districts of Mid-western development region and Bajhang district of Far-western Nepal are some of the major Jatamansi collected areas.

Olsen (1997) has estimated that about 54,500kg of Jatamansi is exported annually from Gorkha district alone. Similarly in Dolpa about 20,000kg of Jatamansi has been estimated to be harvested annually for trade (Shrestha *et al*. 1998). The Marc (the residue after essential oil has been extracted) is also exported (Bhattarai 1999).

1.3.1 Trade Channel

At the village level, the local villagers harvest Jatamansi in its wild state and sell them to the primary traders. They later gathers the herb collected by villagers and sells them to the secondary traders at district level. The secondary traders get permission from district forest office (DFO) to take the herb to other parts of the districts. Then they carry the herb to the road head cities and sell to the wholesalers or to the retailers. The herbs from the wholesalers are exported to India either in crude form or after processing.

This trade channel is shown in figure as:

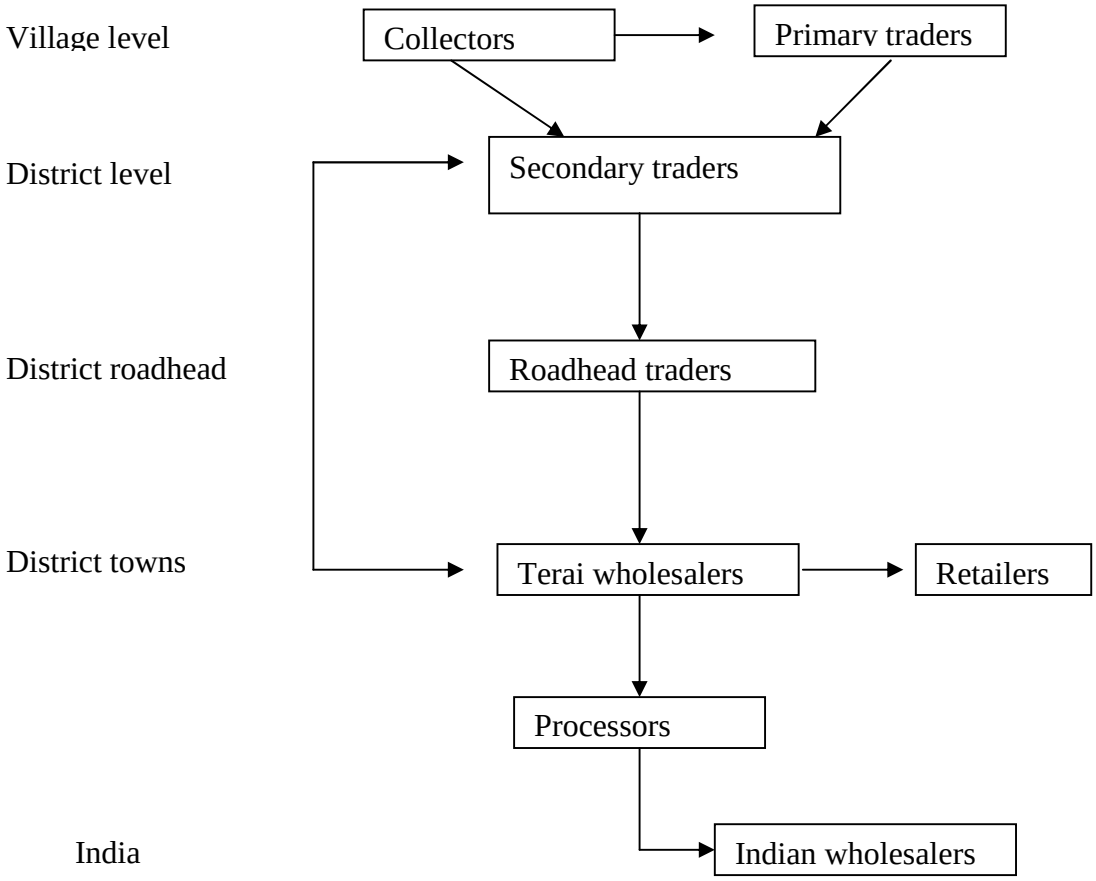


Fig.: Trade channel of medicinal and aromatic plants (Chhetri 1999).

1.4 Conservation Status

Jatamansi is in high demand for trade to export in other countries on one hand and on other hand local people use it as a medicine. Collectors collect rhizome without knowing or caring how to protect it in nature. They only want to sell it and earn money. Currently there is no practice for the sustainable harvesting of this plant.

The conservation status of *Nardostachys grandiflora* DC. is highly increased that it was vulnerable (CAMP 2001, UNEP 2003) then it is increased to endangered (Ved and Tandon 1998), and then highly threatened including the appendix II of CITES in 1997.

1.5 Market Value

Price of *Nardostachys* increases as it moves from its harvesting place to the major cities of Nepal and India. The increasing demand of commercial value of NRs 7000-9000/kg (ANSAB 2003, Shrestha and Shrestha 1999), indicate that it is an exportable commodity of our country. For example in Dolpa, the primary collectors sell the rhizome to the local traders at the rate of NRS 28/kg and local traders sell to the main traders in Nepalgunj and other roadheads of Nepal at the rate of NRs 60-70/kg. When the herbs enter in to Indian market, its price hikes up to NRS 140/kg (Aryal 1993).

The price of the spikenard oil ranges from Rs 5500-7800 per kg in local market and US$ 150-225 per kg overseas depending on the quality. The oil is exported to India, Europe, Japan and USA (Subedi and Shrestha 1999). Across Nepal's border in Nepal, Jatamansi has spawned a thriving black market.

1.6 Chemical Constituents

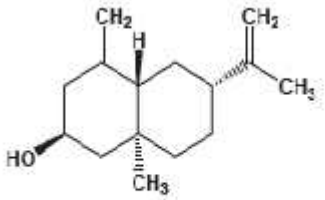
Extensive research has been done on the plant including essential oil extracted from the plant. A number of compounds have been isolated. A brief review of *Nardostachys grandiflora* DC. has been described below:

Rhizomes yield volatile essential oil known as spikenard oil or Jatamansi oil, which mainly contains mono and sesquiterpenoids. Other compounds isolated from its rhizome are alkaloid, actinidine, several aliphatic compounds, β – sitosterol, valepotreiates, phenolic compounds (such as Jatamansin, Jatamansinol, ligans and neoligans) and other substances (such as seychellene, seychelane, nor seychelanone nardostachnol, Jatamansic acid, bitter extractive matters and gum), (Sastry *et al.* 1967, Rucker *et al.* 1978, Bagchi *et al.* 1990 and Bagchi *et al.* 1991).

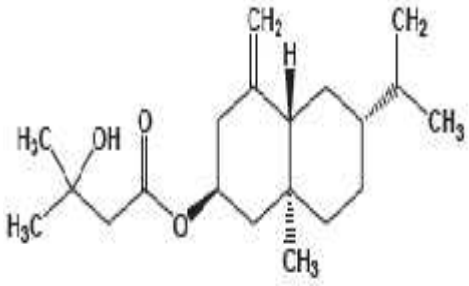
The essential oil of *Nardostachys grandiflora* was found to contain nardostachnol (9-aristolene 1-α-ol) (I), 69.4% as a major compound and Δ9,10 –aristolene (II), 0.2%; Δ1,10-aristolen (III), 2%; β-maaliene (IV), 2.9%; 1,2,9,10-tetradehydroaristelen (V), as minor compounds (Sun *et al.* 1980).

Nardostachol acetate and nardostachone (both prepared from nardostachnol, I) were especially suitable for compounding perfumes of rose and rose-sandal types and had a very good fixating property. They might be regarded as valuable perfumery materials (Sun *et al.* 1980).

From the roots and rhizomes of *Nardostachys grandiflora* two new eudesmane Jatamols A and B (VI) were isolated (Bagchi *et al*. 1991).



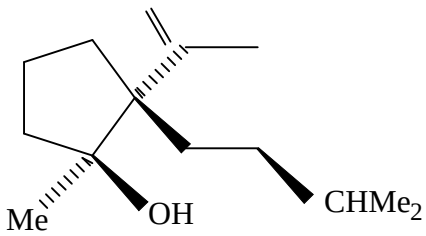
jatamol A



jatamol B

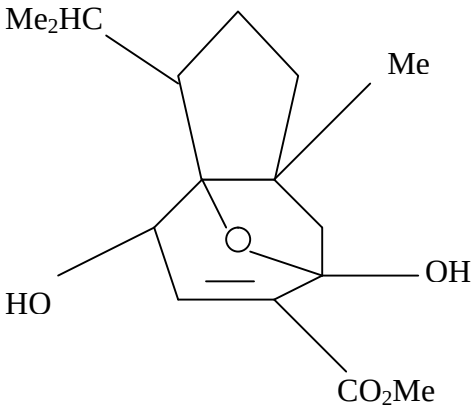
One new neolignan, erthyro - (3,4-dimethoxyphenyl) - 2-(2- methoxy -4-E propenlphenoxy)-propan -1- ol together with 2 known neolignans viroline and erythro-1-(4 hydroxy-3 methoxyphenyl)-2-(2-methoxy-4-E propenylphenoxy)-propan-1-ol and 2-ligans(+)-1-hydroxypinoresinol were isolated from the dichloromethane extract of *Nardostachys grandiflora* DC. roots (Bagchi *et al*.1991).

Spirojatamol(VII) a sesquiterpenoid, was isolated from the roots of *Nardostachys jatamansi*, which has a novel spirane sesquiterpenoid skeleton (Bagchi *et al*.1990).



(VII)

A novel compound BR, 606(VIII) was extracted and purified from Jatamansi roots as bone sorption inhibitor useful for the treatment of osteoporosis and hypocalcaemia. (VIII) Showed bone sorption inhibitor activity in isolated rabbit bone tissue culture with LC_{50} =24 µg/ml (Kawashima *et al*. 1996).



(VIII)

The structure of a new terpenoid ester nardostachysin, isolated from the rhizomes of *Nardostachys grandiflora* was established as 7'8'- dihydroxy-4' methyl-2-enehexahydrocyclo-pentapyran-1'-one-8' methyl ester of 7, 9-quaina-diene-14-oic-acid (Chaterjee *et al*. 2002).

Volatile components in 13 crude drug samples derived from *Nardostachys grandiflora* DC. and *Nardostachys chinensis* were studied by Solid Phase Micro Extn. (SPME), GC and SPME-GC-MS. Twenty components in newly collected samples of two species were identified.β-maalinene, 9-aristolene, calarene and patchauriale were identified as the major volatile constituent of *Nardostachys chinensis*, where as aromadendrene, spirojatamol and valeranone were identified as these of *Nardostachys grandiflora*, using the peaks of β-maalinene and 9-aristolene in GC profiles as the markers. Two *Nardostachys* species were clearly distinguished among the sample examined (Tanaka *et al*. 2008).

Pethkar *et al.* 2001 carried out the biosorptive removal of lead and cadmium from aqueous extracts of *Nardostachys grandiflora* DC. and *vinifera* with high efficiency. Different properties of the extract such as p^H, UV- visible spectra and total dissolved solids were unaltered after biosorption indicating that none of the components of extracts were removed. The findings opened up new advances for the application of metal biosorption technology.

An herbal preparation consisting of alcoholic extract of *Rauwolfia serpentiana* roots *Nardostachys grandiflora* rhizome powder together with aqueous extract of *Tinospora cardifolia* root, stem and leaf was tested as soporific in patients suffering from insomnia. The sleep latency and the number of total duration of nocturnal awakenings were also found to be decreased (Rani and Naidu 1998).

1.7 Research Questions

Owing to great economic potential and high medicinal properties of Jatamansi oil of sample from Humla and Jumla, following research questions have been presented in this research work:

- Do the essential oil of Jatamansi sample from Humla and Jumla contain high number of bioactive chemical constituents?
- Do the essential oil of Jatamansi sample from Humla and Jumla possess effective antibacterial properties or not?

1.8 Objectives

1.8.1 General Objective

- To evaluate the quality of the essential oil from rhizome of *Nardostachys grandiflora* collected from Humla and Jumla.

1.8.2 Specific Objectives

- To compare the percentage of essential oil of *Nardostachys grandiflora* DC. samples from Humla and Jumla.
- To examine and analyse the physical and chemical parameter of essential oil extract.
- To analyse and compare the chemical constituent of essential oil extracted from sample of Humla and Jumla districts using GC/MS.
- To screen the antibacterial activity of essential oil against selected gram positive and gram negative bacterial strains.

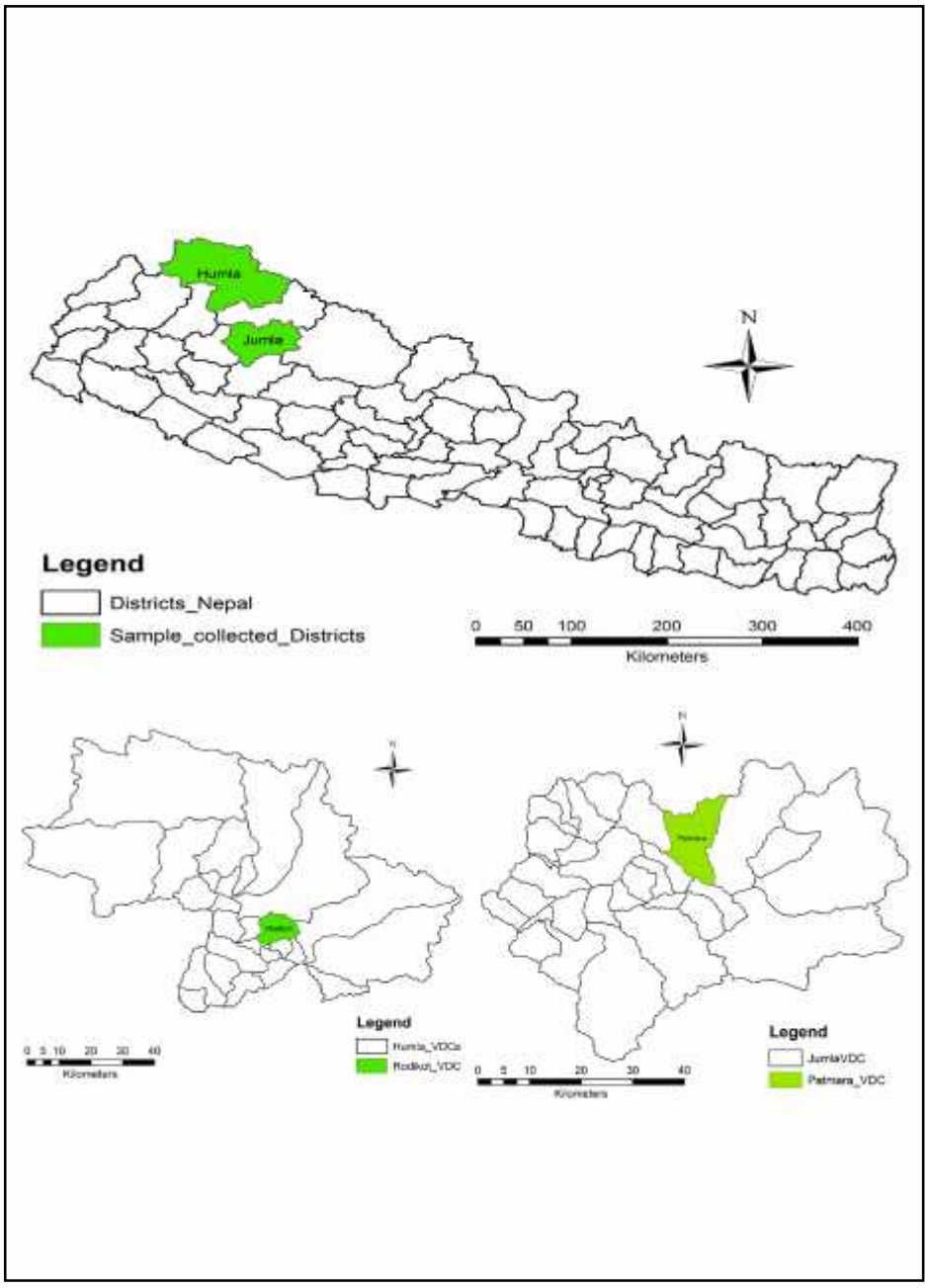
1.9 Rationale

Owing to great economic potency of *Nardostachys grandiflora* oil, there is high difference in commercial value with sample from Humla and Jumla. By estimating percentage of essential oil (*Nardostachys grandiflora*), chemical, physical and instrumental analysis were done to access purity and quality containing its quantities and structural compounds. With its high medicinal value, antibacterial activities of essential oil with gram-ive and gram+ve human pathogenic bacterial strains were done.

Materials and Methods

2.1 Specimen collection

Plant specimens were collected from Humla and Jumla district in October 2010. In physical appearance, rhizome collected from Humla is more shiny and attractive than rhizome collected from Jumla. They were compared with other rhizomes collected from Humla and Jumla which in used by traders also and identified in Central Department of Botany T. U., Kathmandu, Nepal.



Map of Nepal sample collected from Humla(Rodikoat VDC) and Jumla(Patmara VDC) districts.

2.2 Extraction of essential oil (Guenther 1972).

The essential oil content in the crude material was extracted by hydro distillation method using the Clevenger apparatus. The powdered material (100 gm) together with water (5-10 times) was taken in volumetric flask. The content of the flask was heated in heating mantle at 140^0C to boiling. The heating was continued boiling for 6 hours allowed to stand for some time and the stopper of the Clevenger apparatus was opened. The water was drawn off slowly until the surface of the oil layer corresponds to the preparation line and allowed to stand for more than one hour at room temperature. Then the surface of the oil layer was lowered to zero line or the volume (ml) of the oil was read at room temperature. This process was repeated three times for each sample variety of Jatamansi.

2.3 Determination of Physical Parameters (Guenther 1972).

2.3.1 Specific gravity

An ignition tube previously cleaned and dried was weighed and its weight was determined to be W. The tube was filled with the oil and was weighed as W_1. The same procedure was performed using the same tube containing water and its weight was noted as W_2. Then specific gravity was calculated using following equation.

$$dt = \frac{w_1 \, Zw}{w_2 \, Zw}$$

3.2.2 Refractive index

The refractive index of the oil was measured using Abbe's refractometer.

2.3.3 Optical rotation

Oil solution (1%) was prepared by dissolving 1 gm of the sample in 100 ml methyl alcohol in volumetric flask. From this solution, 0.5% and 0.25% oil solutions were prepared by proper dilution. The Polarimeter was switched on and left for five minutes. Then the reading of Polarimeter was set to zero using distilled water in the Polarimeter tube. The Polarimeter tube was rinsed with the oil solution and filled with the oil solution fixing the bubble at the center i.e. opposite of the hole. Then the reading was noted as angle of rotation in degree by adjusting the

point at equilibrium. Same procedure was repeated for other solution also. Then the specific rotation was calculated using the formula.

$$[\sim]^{t}_{D} \; X \frac{\sim}{l \mid c}$$

Where,

t = the temperature at the time of measurement

D = D line of sodium as light source.

$\sim$ = the angle of rotation of plane of plane polarized light.

l = the length of Polarimeter tube (mm).

c = the concentration of oil solution.

2.3.4 Solubility

The solubility was determined to describe the appearance of the solution are used in the laboratories of Fritzsche Brothers, Inc.

Clearly soluble	opalescent
Slightly hazy	slightly turbid
Hazy	cloudy

Slightly opalescent

2.4 Determination of Chemical Parameter (Guenther 1972).

2.4.1 Acid value

Oil (0.5gm) was accurately weighed into a 250ml conical flask. To this 15ml of neutral 95% alcohol and 2-3 drops of 1% phenolphthalein solution were added. The free acid was then titrated with a standard 0.1N aqueous sodium hydroxide (NaOH) solution adding the alkali drop wise at a uniform rate of about 30 drop per minute. The content of the flask was continuously agitated. The first appearance of the red coloration that did not fade within 10 seconds was considered the end point. Then the acid value (AV) was calculated using the following equation.

$$\text{A.V.} = \frac{5.61 \text{ (Number of ml of 0.1N NaOH)}}{\text{Weight of Sample in Gram}}$$

2.4.2 Saponification value

Oil (0.5 gm) was accurately weighted in a 250ml conical flask. The oil was dissolved in 10ml of absolute alcohol and then 10 ml 2.5N potassium hydroxide (KOH) solution was added. This procedure was performed in duplicate and blank experiment was also performed omitting the oil. The flask was refluxed on a sand bath for about two hours. It was cooled and then a few drops phenolphthalein indicator was added. The unreacted KOH was titrated standard N/2 oxalic acid until the pink color disappeared. Then, saponification value was determined using the following equation,

$$\text{Saponification value (S.V.)} = \frac{56 \times (V_1 - V_2) \times 1000}{2 \times 1000 \times w}$$

Where,

W= weight of oil taken

V_1 = Volume of N/2 oxalic acid for blank.

V_2 =Volume of N/2 oxalic acid for sample.

2.4.3 Iodine number

Oil (0.25gm) was accurately weighted and introduced into a conical flask of 250ml capacity and it was dissolved in 10 ml of chloroform. About 25ml of iodobromide solution was added to it accurately measured from burette and was allowed to stand for 30 minute protected from light.

The 30 ml of 1N potassium iodide and 100ml of distilled water were added and the liberated iodine was titrated with N/10 solution of sodium thiosulphate shaking thoroughly after each addition of thiosulphate. When iodine color became quite pale, 1ml of 1% starch until the blue color was discharged.

A blank set was carried out at the same time with the same quantities of chloroform and iodobromide solution allowing it to stand for the same length of time and titrating as above.

$$\text{Iodine number (I.N.)} = \frac{1.269(V_1 - V_2)}{w}$$

Where, W= Weight of sample taken

$V_{1=}$ Number of ml of thiosulphate consumed by the blank test.

V_2 = Number of ml of thiosulphate consumed by the actual test.

Preparation of Iodobromide Solution

Reagent iodine (13.2 gm) was dissolved in 1000 ml of glacial acetic acid with aid of gentle heating. The solution was cooled to 25^0C and the iodine content in 20 ml was determined by titration with N/10 sodium thiosulphate. The remainder of the solution a quantity of bromine molecularly equivalent to that of iodine present was added.

2.4.4 Phenol content

A well cleaned 250 ml cassia flask, having a long thin neck graduated in 0.l ml division was taken, and then introduced l0 ml of oil, measured from the pipette and 75 ml of an aqueous 1N potassium hydroxide solution measured from a graduated cylinder. Stopper and shake thoroughly for 1 hours. After then the undissolved oil is forced into the neck by the addition of most potassium hydroxide solution. The alkali solution was added carefully to avoid disturbing the layers of separated oil. In order to make any droplets of oil adhering to the sides of the flask rise into the neck, revolve the flask rapidly between the palms of the hands. Then, the quality of oil that does not dissolve in alkali was measured. The phenol content was calculated from the following formula.

Percentage of phenol =10 (10 - no of ml of undissolved oil)

2.5 Thin Layer Chromatography (TLC) (Chatwal and Ananda 2005).

TLC was carried out on pre coated silica gel (60F254 percolated TLC plate from Merck Darmstadt).

Solvent system: Toluene: ethyl acetate (93:7)

Spraying reagent: Vanillin-sulphuric acid reagent (VS)

0.5% ethanolic sulphuric acid (Solution I) and 1% ethanolic vanillin (Solution II). The plates was sprayed out vigorously with 10 ml solution I followed immediately by 5-10 ml solution II. After heating 110^0C for 5-10 min. under observation, the spot was evaluated in visualized.

2.6 Analytical condition for GC/MS

GC/MS oil sample was perfumed using GCMS QP2010 plus Shimadzu, Japan fitted MS detector applying Helium (He) was used as carrier gas at the rate of 0.96 ml/min. One percent solution was prepared in acetone and was used for the analysis.

Initial oven temperature was maintained at 40^0C. Sample was injected in split mode where 95% of the sample was split and only 5% of the total was passed for dilution in column. Oven temperature was hold for 40^0C for 30 sec. and was increased at the rate of 7^0C/min. up to 250^0C and final hold for 5 minute.

Injection temperature was 280^0C. Ion source temperature in MS was maintained at 200^0C with maintaining interface temperature as 280^0C. Total duration of the detection was 30 minute, 30 second and was run in scan mode. Obtained peaks were analyzed using NIST 05, NIST 05s, NIST 08, WILLEY 7 and SZTERP libraries. Capillary column (30m, 0.25mm id, 0.25µm df.) for Restek, U.S.A. was used.

2.7 Antibacterial Screening

Inhibition of bacterial growth was tested by using the paper disc diffusion method (Bauer *et al.* 1966, Parekh and Chanda 2007) with slight modification.

2.7.1 Collection of Test Organisms

The microbial strains employed were identified strains that were obtained from Central Department of Microbiology, T.U.. The studied strains include four different bacteria two gram-positive (*Klebsicela pneumoniae* and *Staphylococcus aureus*) and two gram-negative (*Escherichia coli* and *Proteus vulgaris*). They were taken on slants and later cultured on Petri plates having nutrient agar.

2.7.2 Preparation of the Test Discs

Sterile test discs were prepared by dipping and saturating sterilized filter paper discs in plant oil. Same sized filter paper discs (6 mm diameter), by cutting the Whatman no. 1 filter paper and absorbed the same volume of oil. For negative control petroleum ether discs were used, prepared by dipping the disc into the petroleum ether, while tetracycline paper discs were used as positive control. For tetracycline paper discs 10, ml solution was prepared mixing 0.8 ml tetracycline solution (prepared by dissolving 500 mg tablets of tetracycline in 20ml ethanol) with 9.2 ml of ethanol. The final concentration of tetracycline was 0.25 mg/ml.

2.7.3 Nutrient Agar

Nutrient agar was prepared with the help of manufactures (Hi-media) recommendations. 28 gm of nutrient agar was weighted and dissolved in distilled water to make final volume of 1000 ml. It was sterilized by autoclaving the media inside the round bottomed flask at 15 lb pressure and 121^0C for 15 minutes. It was then cooled to 50^0C. About 20 ml media was poured to sterilized Petri plates aseptically and labeled properly. For the slant preparation, the required amount of media was poured in appropriate sized screw capped bottle, autoclaved and cooled in tilted position to make slant.

2.7.4 Nutrient Broth

Nutrient broth was also prepared with the help of manufactures (Hi-media) recommendations. 13 gm of powder was weighted and dissolved in distilled water to make final volume of 1000 ml. It was sterilized by autoclaving at 15 lb pressure and 121^0C for 15 minutes inside the conical flask. It was cooled and 10 ml of it was poured inside the sterilized suitable sized, screw capped bottle.

2.7.5 Preparation of Culture Inoculums

Three to five colonies of similar appearance of the organism to be tested were aseptically touched with the help of inoculating loop from primary culture plate. It was transferred to a tube containing 10 ml sterile liquid media of nutrient broth. The tube was incubated overnight inside the incubator at 37 ± 1^0C.

2.7.6 Transfer of Bacteria on Petri Plates

The agar plates for the assay were prepared by labeling them with the date, the name of bacteria and the name code of the discs. The inoculums of bacteria were transferred into petri plates containing solid nutrient media of agar using sterile swab. The sterile cotton swab was dipped into a well mixed saline test culture and removed excess inoculums by pressing the saturated swab against the inner wall of the culture tube. The swab was used to spread the bacteria on the media in a confluent lawn. It was done by rotating the Petri plates at 90^0 and continuing the spread of bacteria. One swab was used for one species of bacteria and was left to dry.

2.7.7 Placing Test Discs

Dried test discs were transferred on bacterial lawn under aseptic condition using flame sterilized forceps each time. Each disc was placed gently on the agar surface on equidistance and patted with the forceps ensuring the disc to adhere on to the surface of agar, followed by incubation overnight at $37\pm1^{o}C$.

2.7.8 Observation of Result

After overnight incubation at $37\pm1^{o}C$, results were recorded as the presence or absence of inhibition zones. Resulting zones of inhibition (ZOI) were measured. The diameter of zone of inhibition produced by plant oil concentration on particular bacteria was also measured with the help of millimeter ruler. The inhibitory zone around test paper discs indicates absence of bacterial growth and that was recorded as positive and absence of zone as negative. For the reliability of the results, every experiment was done in triplicates.

Results

3.1 Extraction and quantification of the Essential oil

The essential oil present in the dried rhizome of *Nardostachys grandiflora* DC. from Jumla and Humla were obtained by hydro distillation method using Clevenger apparatus. The color of essential oil extracted from Humla was "Royal green" and from Jumla was "Greenish brown". The percentage amount of oil obtained from *Nardostachys grandiflora* DC. from Jumla and Humla are depicted below in table 3.1:

Table: 3.1 Volatile oil content samples from Humla and Jumla

S.N	Samples	Color	Percentage of volatile oil content
1	Humla (3500-3600m.)	Royal green	1.9
2	Jumla (3500-3600m.)	Greenish brown	1.52

Extraction of oil using Clevenger apparatus

3.2 Physio-chemical constants of volatile oil

In physico-chemical parameters sample from Humla, high saponification value (28.4), solubility, refractive index (1.561), and specific rotation were obtained. Like this sample from Jumla, high specific gravity (0.989), acid value (6.2), phenol content (2%), and Iodine number (152) were obtained.

Table: 3.2 Physico-chemical parameters

S.N.	Parameters	Jumla	Humla
1.	Specific gravity at 13^0 C	0.989	0.965
2.	Refractive Index at 13^0C	1.487	1.516
3	Specific rotation	-35.88^0	-39.9^0
4.	Solubility at 15^0C	Acetone 1 vol. in 1 vol.	1vol.in 1 vol. acetone.
		1 vol. in 3 vol. absolute alcohol.	1 vol. in 1 vol. absolute alcohol.
		Turbidity up to 10 vol. of 95% alcohol	Turbidity up to 3 vol. of 95% alcohol.
5.	Acid value at 15^0C	6.2	5.8
6.	Saponification value at 15^0C	20.81	28.4
7.	Phenol content	2%	< 2%
8.	Iodine number	152	148.5

3.3 Thin layer chromatography

Thin layer chromatography revealed the following spots with varying Rf values, which are given in table 3.3.

Table: 3.3 Sample from Jumla

S.N	Spot no.	Color	Rf value
1	I	Orange	0.27
2	II	Violet	0.31
3	III	Red	0.39
4	IV	Blue	0.45
5	V	Bluish- Black	0.59`
6	VI	Green	0.70
7	VII	Grey-Black	0.81

Table: 3.4 Sample from Humla

S.N	Spot no	Color	Rf value
1	I	Orange	0.23
2	II	Light violet	0.31
3	III	Light violet	0.36
4	IV	Deep violet	0.42
5	V	Pinkish red	0.45`
6	VI	violet	0.46
7	VII	yellow	0.54
8	VIII	Green	0.61
9	IX	Grey-red	0.70
10	X	Green	0.76
11	XI	Violet	0.81
12	XII	Reddish grey	0.92

Solvent system: Toluene: ethyl acetate (93:7)

3.4 Constituents Present in the Essential Oil

GC/MS of *Nardostachys grandiflora* DC. sample from Humla and Jumla coupled with mass library search, sixty four peaks were seen through gas chromatography all were identified, of which most were sesquiterpenes, monoterpenes and some were aromatic and coumarin derivatives.

Major constituents in sample from Humla were Bicyclo [3.1.1] heptanes, β-pinene (11.3%), diacetone alcohol, 2-pentanone, 4-hydroxy, 4-methyl (7.07%), α- pinene (6.53%), ristolene (5.95%), alloaromadendrene (4.46%). Which are given in table 3.6.

Major constituents in sample from Jumla were β-gurjunen (12%), Spathulenol (7.66%), thujopsadiene (6.92%), jatamanson (6.78%), and β-caryophyllene (5.8%). Which are given in table 3.5.

Table: 3.5 Identified Compound from *Nardostachys grandiflora* DC. from Jumla

S.N.	Compound	m/z	Retention Time	Area %	SI	Mol. wt.
1	Butanoic acid; Acetic acid, isopropyl-; Isovaleric acid.	60	5.608	0.13	92	102
2	2-Pentanone, 4-hydroxy-4-methyl- (CAS) Diacetone alcohol	43	5.725	3.71	95	116
3	Alpha-pinene, 2-Pinene.	93	7.7	0.38	94	136
4	6,6-dimethyl -2-methylene,beta-pinene	93	8.675	0.96	95	136
5	Cajeputene, dl-Limonene	68	9.8	0.08	86	136
6	Eucalyptol, Cineole.	43	9.892	0.21	92	154
7	d-2-Camphanone, Camphor, (1R,4R)-(+)-	95	12.45	0.19	94	152
8	Alpha-D2-3-phenylnitrite, alpha.-Terpinene	105	15.542	0.27	75	136
9	Myrtenyl acetate, 2-Pinen-10-ol, acetate	91	16.075	0.14	90	194
10	Napthalene, gamma.-Cadinene	161	17.175	0.21	83	204
11	Patchoulene, beta.-Patchoulene	119	17.3	1.28	92	204
12	Azulene, beta.-elemene	93	17.383	0.52	89	204
13	Alpha Gurjunene,	105	17.558	0.78	91	204

S.N.	Compound	m/z	Retention Time	Area %	SI	Mol. wt.
14	Aromadendrene, Alloaromadendrene	67	17.717	0.55	84	204
15	Dewar benzene, Hexamethyl Dewar benzene	147	17.783	1.04	83	162
16	Beta-Cadinene, Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,	161	17.833	3.44	93	204
17	Aristolene	105	17.992	1.85	93	204
18	Vetivenene, Aromadendrene, dehydro-	117	18.167	3.19	81	202
19	CALARENE OR (+)-.BETA.-GURJUNENE	161	18.242	12	94	204
20	Thujopsadiene, Aromadendrene, dehydro-	131	18.325	6.92	79	202
21	Seychellene, (-)-.alpha.-Panasinsen	122	18.492	3.25	93	204
22	Beta Caryophyllene, alpha.-Gurjunene	105	18.6	5.8	88	204
23	Humulen, :Humulen-(v1)	107	18.758	2.8	91	204
24	Anthracene, Benzene, 1,2-bis(1-buten-3-yl)-	143	18.833	1.61	80	186
25	Alpha- Gurjunene,(-)-Sinularene	133	18.967	0.49	81	204
26	Beta –Ionone, 3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	177	19.092	0.44	85	192
27	Delta Cadinene, Cadina-1(10),4-diene	161	19.142	0.7	86	204
28	Alpha-Guaiene; Alloaromadendrene.	105	19.217	1.08	91	204
29	Valencene;Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]-	161	19.308	1.27	91	204
30	Alpha-Guaien; alpha.-selinene	105	19.35	2.43	87	204
31	Delta Guaiene	107	19.5	0.32	91	204
32	Pentalene; OCTAHYDRO-1,4-DIIODO-	139	19.575	0.14	66	220
33	Alpha-Amorphene; :GERMACRENE-D	161	19.65	0.24	87	204
34	Alpha-Panasinsen; SELINENE <7-EPI-ALPHA-> DB5-1685	161	19.775	2.23	87	204
35	Benzol; 1-(1-BUTEN-3-YL)-4-PENTYL-	131	19.85	0.41	83	202
36	Isolongifolol; Palustrol	110	20.125	0.12	78	222
37	1-ethyl-4,4-dimethylcyclohexa-2-en-1ol	125	20.358	0.49	80	154
38	Patchouli alcohol; 1,3-Hexadiene, 3-ethyl-2,5-dimethyl-	123	20.55	0.39	78	138
39	Alpha-Selinene; .gamma.-Gurjunene	105	20.65	1.04	85	204

S.N.	Compound	m/z	Retention Time	Area %	SI	Mol. wt.
40	Epiglobulol; tau.-Cadinol	121	20.817	5.6	81	222
41	Neoalloocimene; :Alloaromadendrene	105	20.925	0.98	86	204
42	Guaiol; :Aromadendrene;	107	21.075	0.38	85	204
43	Carotol	161	21.142	0.78	80	222
44	Rosifoliol; beta.-Eudesmol	149	21.208	0.37	77	222
45	Veridiflorol; Palustrol	109	21.275	0.17	82	222
46	Benzene, 2-(2-butunyl)-1,3,5-trimethyl	159	21.342	0.23	74	174
47	Elixene; bicyclogermacrene	121	21.692	1.01	82	204
48	Beta-Guaiene	161	21.817	0.82	73	204
49	1,1,6,6-Tetramethylspirol[4.4]nonan-2one	95	21.95	0.11	64	194
50	Kuromatsuen; (-)-Isolongifolol, acetate	95	22.042	0.62	85	204
51	Spathulenol; :6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	159	22.283	7.66	76	220
52	Jatamansone; Valeranone, (+)-	98	22.425	6.78	89	222
53	Valerenal	91	23.083	4.15	92	218
54	Alpha-Terpinolene; .alpha.-Chamigren	93	23.192	0.37	67	136
55	Methyl phenyl Pentenal; (2,3-Diethoxy-cyclopropylethynyl)-benzene	159	23.25	0.22	74	230
56	Humulen; :Valencene	91	23.517	0.15	82	204
57	Cyclohexane; 1,3-diisopropenyl-6-methyl-	119	23.792	1.06	83	176
58	Aristolene	105	23.85	0.65	83	218
59	Biphenylene,1,2,3,6,7,8,8a,8boctahydro-4,5dimethyl	145	23.933	0.15	73	188
60	Veridiflorol; Illudol	109	24.033	0.58	79	222
61	10,10-dimethyl-2,6-dimethylenebicyclo[7.2.0]undecane	95	24.242	0.06	72	204
62	Valerenic acid	105	24.508	3.77	77	234
63	Aromadendrene,dehydro-	117	27.667	0.09	79	202
64	Vetivenene;Aromadendrene, dehydro-	145	27.808	0.11	81	202

Table: 3.6 Identified Compound from *Nardostachys grandiflora* DC. From Humla

S.N.	Compound	m/z	Retention Time	Area %	SI	Mol. Wt.
1	Diacetone alcohol; 2-Pentanone, 4-hydroxy-4-methyl-	43	5.733	7.07	94	116
2	Alpha-Phellandrene; .alpha.-Thujene	93	7.533	0.18	92	136
3	Alpha-Pinene	93	7.708	6.53	96	136
4	Beta-pinene	93	8.675	11.3	95	136
5	p-Cimene; Benzene, 1-methyl-4-(1-methylethyl)-	119	9.717	0.23	94	134
6	dl-limonene; l-Limonene	68	9.808	0.57	95	136
7	Eucalyptol; Cineole	43	9.9	0.34	95	154
8	Gama-terpinen;	93	10.475	0.18	92	136
9	1-terpinen-4-ol; 4-Carvomenthenol	71	13.108	0.83	91	154
10	Beta fenchyl alcohol; ALPHA. TERPINEOL	59	13.375	0.93	95	154
11	Isobutylisovalerate; Pentanoic acid, 2-pentyl ester	85	14.692	0.84	84	172
12	Alpha-terpinene	105	15.542	2.39	79	136
13	Myrtenyl acetate	91	16.083	0.59	94	194
14	Octahydro-Pentalen -1-ol	67	16.508	0.4	81	124
15	Zingibebene	119	17.558	1.32	93	204
16	Styrlyl Propionate; Benzenemethanol, .alpha.-methyl-, propanoate	105	17.733	0.87	73	178
17	Longipinene; alpha.-Bergamotene	119	17.792	1.7	86	204
18	Cadinene; Delta-Selinene	133	17.85	0.51	73	204
19	Aristolene	105	18	0.6	83	204
20	trans-alpha-Bergamotene; .alpha.-Bergamotene	93	18.167	0.82	84	204
21	Calarene; Isoledene	161	18.25	2.99	91	204
22	Aristolene; Aristol-9-ene	105	18.367	5.95	90	204
23	Beta-Caryophylene	69	18.508	2.3	89	204
24	Alloaromadendrene; NEOALLOOCIMENE	105	18.608	4.46	91	204
25	Veridiflorol; EPIGLOBULOL	127	18.8	2.24	80	222
26	Gamma-curcumene; beta.-Himachalene	119	18.933	0.58	90	204
27	Alpha-curcumene; Curcumene	132	18.992	0.7	90	202
28	trans-Caryophyllene; trans-.beta.-Farnesene	69	19.092	0.57	84	204
29	Muurola-3,5-diene; GERMACRENE-D	161	19.15	0.4	84	204
30	Neoalloocimene; Alloaromadendrene	105	19.233	0.56	89	204
31	Valencene	105	19.317	3.76	95	204
32	Cyclohexane,2a,3e-dimethyl-1e,5a-divinyl	107	19.433	0.3	80	164

S.N.	Compound	m/z	Retention Time	Area %	SI	Mol. wt.
33	Di-epi-alpha-cedrene; Cedr-8-ene #	119	19.492	0.96	89	204
34	Veridiflorol;a lpha.-Bisabolol	139	19.583	0.28	77	222
35	Beta-Sesquiphellandrene	69	19.717	0.28	85	204
36	Eudesma-3,7(11)-diene; Selina-3,7(11)-diene	107	19.783	0.62	86	204
37	5-n-butyltetralin; Naphthalene, 5-butyl-1,2,3,4-tetrahydro-	131	19.85	0.53	82	188
38	Longipinocarveol	123	20.158	0.3	73	236
39	Alpha-Cedrol; Farnesol	119	20.242	0.24	86	222
40	Myrtenyl acetate	91	20.325	0.66	90	194
41	Valerenol; 3-Ethyl-3-hydroxyandrostan-17-one #	121	20.817	1.67	80	318
42	Methyl ionone	43	20.883	0.64	77	206
43	Gurjunene-gamma;(+)-Cycloisosativene	105	20.95	0.37	83	204
44	Nuciferol	119	21.142	1.77	78	204
45	(+-)-E-Nuciferol	119	21.617	1.63	87	218
46	Thujopsene; Acoradiene	121	21.692	2.09	83	204
47	Zingiberene	93	22.017	0.99	80	204
48	Bisabolol beta; .alpha.-Bergamotene	93	22.2	1.56	83	204
49	Patchouli alcolol	138	22.292	1.81	82	222
50	Longiborneol	98	22.245	1.99	77	222
51	Bisabolol beta->db5-2045	72	22.517	1.04	71	222
52	Bisabolol	72	22.708	1.18	72	222
53	3- Cyclohexene-1-ol ,2-(1,5-dimethyl-4-hexnyl)-4-methyl	72	22.867	0.42	71	222
54	Santalol,cis,alpha; Bergamotol, Z-.alpha.-trans-	93	22.975	1.03	76	220
55	Benzenebutanal,gamma,4-dimethyl	119	23.142	1.77	79	176
56	2,5,8-Trimethyltetralin; METHYL PHENYL PENTENAL	159	23.258	2.18	77	174
57	(+-)-E- Nuciferol	119	23.658	1.81	83	218
58	Beta Ionol	119	23.792	3.75	85	194
59	Valeranal	91	23.875	0.35	73	218
60	Biphenylene,1,2,3,6,7,8,8a,8b-octahdro-4,5-dimethyl	91	23.942	0.27	78	188
61	Veridiflorol	109	24.042	3.37	80	222
62	Thujopsadiene DB5-1547	91	24.517	0.55	75	188
63	Benzenebutanal, gamma,4-dimethyl	119	24.775	0.52	71	218
64	(+-)-(E)-Nuciferol	119	25.183	0.39	80	218

Table: 3.7 Comparison of identified compounds found in the essential oil of *Nardostachys grandiflora* DC. Sample from Humla and Jumla(area% >1).

Compound name	Area %	
	Jumla	**Humla**
2-pentanone 4- hydroxyl-4-methyl;Diacetone alcohol	3.71	7.07
Alpha- Pinene		6.53
Beta- pinene		11.3
Patchoulene, beta.-Patchoulene	1.28	
Zingiberene		1.32
Dewar benzene; Hexamethyl Dewar benzene	1.04	
Beta-Cadinene, Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,	3.44	
Aristolene, Aristolene; Aristol-9-ene	1.85	5.95
Longipinene; alpha.-Bergamotene		1.7
Vetivenene; Aromadendrene, dehydro	3.19	
Calarene; Isoledene		2.99
CALARENE OR (+)-.BETA.-GURJUNENE	12	
Thujopsadiene, Aromadendrene, dehydro-,	6.92	2.09
Seychellene, (-)-.alpha.-Panasinsen	3.25	
Beta Caryophyllene, alpha.-Gurjunene	5.8	2.3
Alloaromadendrene; NEOALLOOCIMENE, Alpha-Guaiene; Alloaromadendrene.	1.08	4.46
Humulen, :Humulen-(v1)	2.8	
Veridiflorol; EPIGLOBULOL Epiglobulol; tau.-Cadinol,	5.6	2.24
Anthracene, Benzene, 1,2-bis(1-buten-3-yl)-	1.61	
Valencene;Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]-	1.27	3.76
Alpha-Guaien; alpha.-selinene	2.43	
Alpha-Panasinsen; SELINENE <7-EPI-ALPHA-> DB5-1685	2.23	
Valeronol		1.67
Alpha-Selinene; .gamma.-Gurjunene	1.04	
Nuciferol		1.77
(+-)-E-Nuciferol		1.63
Elixene; bicyclogermacrene	1.01	
Bisabolol beta; .alpha.-Bergamotene		1.56
Spathulenol; :6-Isopropenyl-4,8a- dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	7.66	
Patchouli Alcohol		1.81
Jatamansone; Valeranone, (+)-	6.78	
Longiborneol		1.99
Bisabolol –beta		1.04
Bisabolol –beta, 3-Cyclohexen-1-ol, 2-(1,5-dimethyl-4-hexenyl)-4-methyl-		1.18
Santalol,cis,alpha; Bergamotol, Z-.alpha.-trans-		1.03
Valerenal	4.15	

Compound name	Area %	
	Jumla	Humla
Benzenebutanal, .gama.,4-Dimethy		1.77
Trimethyltetralin; METHYL PHENYL PENTENAL		2.18
(+-) - (E) –Nuciferol, 6-(p-Tolyl)-2-methyl-2-heptenol		1.81
Beta-Ionol		3.75
Veridiflorol	3.77	3.37
Cyclohexane; 1,3-diisopropenyl-6-methyl	1.06	
Valerenic acid	3.77	

In comparison there are 26 major compounds in sample Humla and 24 major compounds in sample Jumla were obtained with more than 1% area.

3.5 Antibacterial Screening of essential oil samples from Humla and Jumla

In comparative study of antibacterial activity from sample Humla and Jumla, there is high zone of inhibition (13.5mm) in sample Jumla with gram negative bacteria *proteus vulgaris*.

Table 3.5.1 Zone of Inhibition (mm) in sample from Jumla.

Test organism\ concentration	Zone of Inhibition(ZOI in mm)*			
	0.25mg/ml	0.5mg/ml	0.75mg/ml	1mg/ml
E. coli	8±0.76	8±0	8±0.57	9±0.57
K. pneumoniae	8.5±0.76	8.5±0.76	9±0.57	10±0.57
Proteus vulgaris	9.5±0.28	10±1	11.5±0.5	13.5±0.76
Staphylococcus aureus	9±0.57	9±0.57	10±1	10.5±0.28

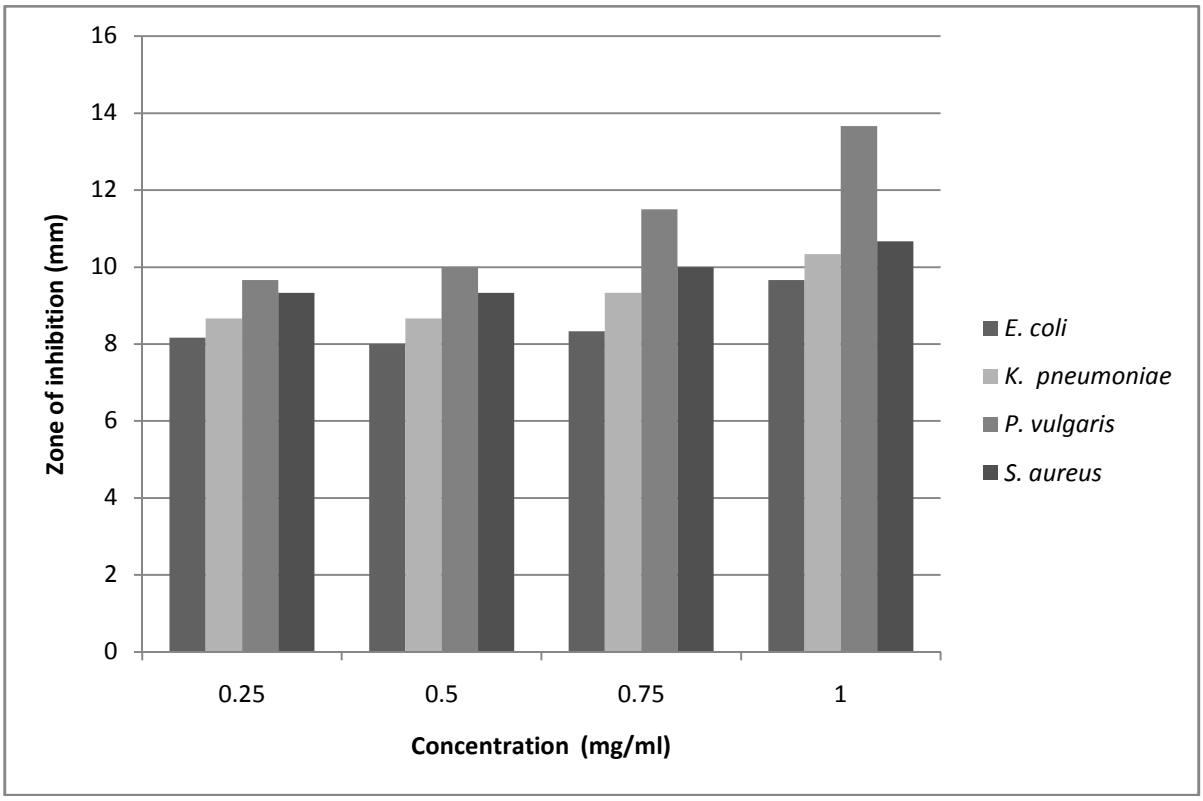
**Mean ±SD (n=3)*

Table 3.5.2 Zone of Inhibition (mm) in sample from Humla.

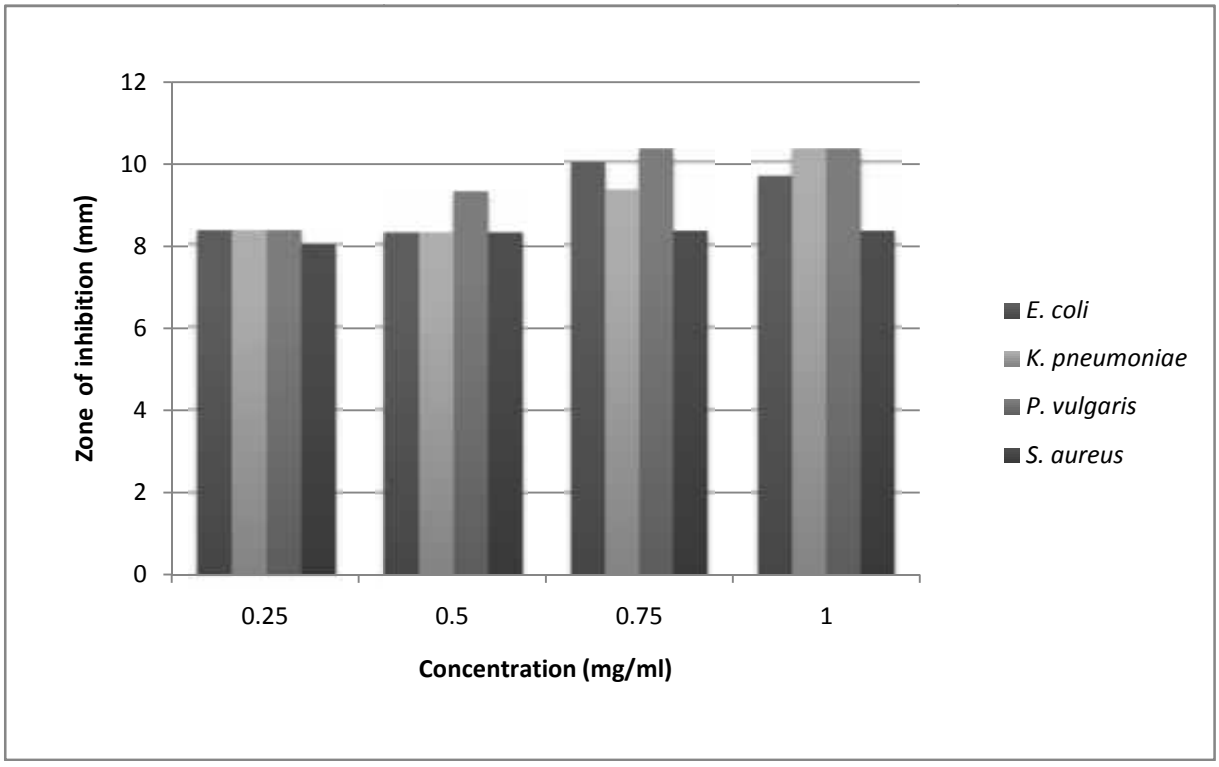
Test organism\ concentration	Zone of Inhibition(ZOI in mm)*			
	0.25mg/ml	0.5mg/ml	0.75mg/ml	1mg/ml
E. coli	8±0.57	8±0.57	10±1	10±0.57
K. pneumoniae	8±0.57	8±0.57	9±0.57	10±0.57
Proteus vulgaris	8±0.57	9±0.57	10±0.57	10±0.57
Staphylococcus aureus	8±0	8±0.57	8±0.57	8±0.57

**Mean ±SD (n=3)*

Note: *E. - Escherichia, K. - Klebsicela*



Measurement of Zone of Inhibition in (mm) sample from Jumla.



Measurement of Zone of Inhibition in (mm) sample from Humla.

Discussion

The essential oil extracted from rhizome samples harvested from Jumla and Humla showed slight variation in percentage with the yield of 1.9% with "Royal green" color from Humla and was 1.52% with "Greenish brown" color from the sample of Jumla. The variation in oil percentage may be due to the age difference of plant sample and their quality (Anonymous 1962). The sample collected from Humla was shiny and brighter than sample from Jumla during plant harvest.

Flavonoid is a pigment that absorbs light within the range of wavelength 210-536 nm. The Rf value of both samples have been found almost similar. Less variation in Rf value between sample from two place may be due to differential phenological state of the plant and their environment (Paudel 2003).

Essential oil from rhizome of *Nardostachys jatamans*i from the Indian Himalayas contained nine monoterpenes (1.7%), 25 sesquiterpenes (43.9%) and 7 non-terpenic components (24.4%). The major sesquiterpenes include nardol (10.1%), α-selinene (9.2%), β-caryophyllene (3.3%), cubebol (2.9%), α-gurjunene (2.5%), γ-gurjunene (2.3%) and α-humulene (2.3%) (Mahawal *et al*. 2002). In present study, the compounds present in Jumla contained 12 monoterpenes (13.81%), 30 sesquiterpene (67.43%), aromatic hydrocarbons, aliphatic unsaturated hydrocarbon, flavonoid and so on. Out of them, 3 monoterpenes (11.21%) and 13 sesquiterpenes (63.02%) were found with more than 1% area. Similarly, compounds present in Humla were 12 monoterpenes (25.85%), 19 sesquiterpenes (33.07%), one coumarin, ketones and so on. Out of them, 4 monoterpenes (22.31%), 12 sesquiterpenes (29.1%) were with more than 1% area. However in present study, most of the detected compounds were sesquiterpenes which is higher than previous research in both number of compounds and percentage of compounds. So from this research work prove that samples from Humla and Jumla of western Nepal contained higher number and percentage of chemical constituents than previous researches conducted in India, which might be due to the differential geographical gradients with different physiological stresses.

Monoterpenes such as α- pinene, β- pinene, camphene, limonene, 1:8-cineole and sesquiterpenes - α-gurjunene, β-gurjunene (calarene) , aristolene, α-patchoulene, patchouli alcohol(Bruns, *et al.* 1980), sesquiterpene such as Jatamansone (Valeronone), nardostachone, nardol are reported in *Nardostachys jatamansi* (Sastry *et al.*1967).

Essential oil of *Nardostachys jatamansi* possesses sesquiterpene such as β- maaliene (Sun *et al*. 1980), eudesmane jatamols (Bagchi *et al*. 1991), and spirojatamol (Bagchi *et al.* 1990).

Sesquiterpene has several compounds such as α- cadinene; γ – cadinene; carotol,α-cadinol; 1,2,3,4,4a,5,6,7,8,8a –decahydro – 1,4a – dimethyl -7-(1-methylethylidene) -1-napthalenol; 1,2,3,4,5,6,7,8a- octahydro – 3,6,8,8 – tetramethyl-1H-3a,7-methanoazulene; 4, 4a,5,6,7,8-hexahydro -4, 4a-dimethyl-6- (1-methylethylidene) -2(3H) –naphthalenone; 1a,2,3,3a,4,5,6,7b, -octahydro – 1,1,3a,7 – tetramethyl -1H- cyclopropanephthalene, the aromatic compound and coumarin derivative are 4-(1, 1-dimethylethyl) – benzene methanol and 4 –mathoxy -7H-furo 3, 2-g 1-benzopyran -7-one respectively as their compounds are reported in *Nardostachys grandiflora* sample from Kathmandu market (Paudyal 2004). Large number of sesquiterpenes is presented in present research which is similar with previous research (Mahawal *et al*. 2002; Paudyal 2004).

From comparative study of essential oil in present study between Jumla and Humla samples, found 24 and 26 compounds to be more than 1% area respectively. Among them, β-gurjunene (12% area), spathulenol (7.66 % area), thujopsadiene (6.92 % area), jatamansone (6.78% area) and β-caryophyllene (5.8% area) are major compounds in Jumla in retention time as 18.242, 22.283, 18.325, 22.425 and 18.6, respectively. Likewise, β- pinene (11.3% area), pentanone (7.07% area), α-pinene (6.53% area), aristolene (5.95% area) and alloaromadendrene (4.46% area) were major compounds in Humla in retention time as 8.675, 5.733, 7.708, 18.367 and 18.608, respectively.

It was analyzed that α- pinene (6.53% area) and β-pinene (11.3% area) were major compounds present in the essential oil of sample collected from Humla but sample from Jumla were in trace amount (0.38 and 0.96% area). Similarly β-gurjunene (12% area) was major compound present in sample from Jumla whereas, it was very less (2.99% area) in sample from Humla. Another major compound β- caryophyllene was 5.8% area in sample from Jumla but which was 2.3% area in sample from Humla.

Compounds, detected in sample of both from Humla and Jumla were 64, recorded highest number for the first time in *Nardostachys grandiflora*, which was carried out with the help of GC/MS co-operating with five libraries - NIST05, NIST05S, NIST08, and WILLEY7 and SZTERP. Only 15 compounds were identified using only one searching library (public/NIST) (Paudyal 2004). In present study the number of compounds are larger number than previous research in which the sample was collected from Kathmandu market. That sample might be stored for a long time, the collection of sample from different area, ageing of sample was also variable, slope of aspect of collection might be different, and so other technical method of GC/MS may also different.

In present research *Nardostachys grandiflora* from Jumla has contained 13.81% monoterpenes, 67.43% sesquiterpenes and other compounds. Similarly, sample from Humla has contained 25.85% monoterpenes, 33.07% sesquiterpenes and other compounds. From above result, we can conclude that sample from Jumla contains higher amount of sesquiterpenes than sample from Humla but amount of monoterpenes is higher in Humla than Jumla.

In this study the essential oil of sample from Humla and Jumla, some differences in compound identification, physical, chemical and other organoleptic characters are observed. That may be attributed due to difference in climatic and topographic factors. One of the important causes might be maturity of sample, one rhizome sample is highly matured and another rhizome sample is less matured. The reason is not fully known but it might be due to the genetic character which guides the character of the sample and constituents present in the sample.

The aroma improves in ageing also showed higher percentage in matured plants than in immature sample which are more likely due to the variation in quality and quantity of essential oil because of the seasonal factors and possibly culture conditions *(Anonymous* 1962).

A comparative study of the compounds detected in the essential oil of *Nardostachys grandiflora* DC. was taken from Humla and Jumla proves that they possess similar compounds but not identical components. This finding is not surprising because it has been widely believed that a number of technological and agrobiological factors, weather and geographical conditions of cultivation and harvesting, different conditions of storage and other several factors determine the quality of essential oil (Alexandrov and Zinchenko 2003) and the facts of research in compounds.

4.1 Antibacterial Screening of essential oil samples from Humla and Jumla

In comparison to the gram-positive and gram-negative bacteria, gram- positive bacterial strains were more susceptible to the concentration of oil as compared to gram- negative bacteria suggesting that plant extracts are more active against gram-positive bacteria (Vlietinck *et al*. 1995, Rabe and Van Staden 1997, Lin *et al*. 1999, Parekh and Chanda 2007). These differences may be attributed to the fact that the cell wall in gram-positive bacteria are of a single layered where as that of gram-negative are multilayered (Yao *et al.* 1995). So the passage of the active compound through the gram-negative cell wall may be inhibited. In addition, the microorganism

show the variable sensitivity to chemical substances related to different resistant levels between strains (Cetin and Gurler 1989).

But the result of present research work shows differential result, which may be due to chemical constituents present in the essential oil sample from Jumla.

Several sesquiterpens were present at appreciable level, aristolene (8.5%), (E, E)-farnesol (4.3%), gurjunene (1.4%), 4-hydroxy coumarins were shown to have some antibacterial activity (IUCN 2005).

The fruit of *Iryanthera ulei* W. containing spathulenol (12.1%), muurolol (13.2%), showed antibacterial activity against the gram- positive and gram-negative bacteria. *S. aureus* strain was found to be the most sensitive microorganism (Luis 2009) which corresponds with our present research work. Aromadrendrene present major constituent in essential oil of *Eucalyptus globulus* showed the antibacterial activity against multidrug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (Mulyaningsih *et al.* 2010) which is in agreement with present research.

Screening process only indicates whether or not any compound inhibits or kills particular bacteria which may not suggest about definite potency of antibacterial substance. Plant essential oil having antibacterial activity against large numbers of bacterial strains may have little or no importance, the potency of essential oil possessed by the medicinal plants is also crucial step during new drug manufacturing from natural products.

Evaluation of antibacterial activity was the main aspect of this study as compound having broad spectrum may have less potency and less value in development of new drugs. The evaluation of antibacterial substance becomes the essential step during new drug research from natural product.

In disc diffusion technique, the antibacterial substances diffusing in the media kill or inhibit the bacteria and thus zone of inhibition appears around the disc in agar surface. There is gradual decrease in the concentration of antibacterial substance as the distance from disc is increased. A critical point arises after certain distance. After this point there will be growth of bacteria, the concentration of antibacterial substance at that critical point is actually minimum inhibitory concentration. By measuring the diameter of zone of inhibition we can simply evaluate the potency of the antibacterial drugs. Among the tested bacteria, *Proteus vulgaris* showed high value of ZOI (8-13.5 mm) where as bacteria *Escherichia coli* showed lowest value of ZOI (8-9 mm).

Conclusion

A comparative study of the essential oil samples of Jatamansi (*Nardostachys grandiflora* DC.) obtained from Humla and Jumla districts of Nepal was performed.

In this research work, sample from Humla has comparatively high oil content than that from Jumla. Color of the oil is “Royal green” and "Greenish brown” in sample from Humla and Jumla respectively. By comparing the oil content in two samples, we can conclude that sample from Humla has more commercial value than sample from Jumla because sample from Humla has 1.9% oil content.

The majority of components present were Sesquiterpenes in both sample of the essential oil and 64 components in both sample were identified. Beta- Gurjunene and Spathulenol were found to be major in Jumla. Beta–Pinene and 2- Pentanone were found to be major in Humla. Organoleptic and Physicochemical properties of both samples of Jatamansi oil were similar. However it should be noted that some structural variance and difference in the percentage amount (GC %) with in those two samples of essential oil exist.

Jatamansi samples from Jumla and Humla were selected for screening purpose against four different bacteria among them two are gram-positive (*Stahylococcus aureus* and *Klebsicella pneumoniae*) and two are gram-negative (*Escherichia coli* and *Proteus vulgaris*). Among these bacterial strains gram- positive bacteria were more resistant as compared to gram- negative bacteria. Both sample of Jatamasi from Jumla and Humla showed activity against all tested bacteria. The ZOI value ranges up to 13.5 mm. The maximum value of ZOI (13.5 mm) was shown by sample from Jumla on bacteria *Proteus vulgaris*.

RECOMMENDATION

Following recommendation has been outlined based on the present research:

- Isolation of bioactive compounds to identify the high medicinal properties of the essential oil.
- Biological screening of essential oil to ascertain the biological activity of Jatamansi against different organisms such as, fungi, bacteria, and virus.

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Abstract

Nardostachys grandiflora DC. is commonly known as “Jatamansi” belongs to the family Valerianaceae is an important medicinal plant and major source of terpenoids (sesquiterpene) and flavonoid. The essential oil from this plant was extracted with hydrodistillation process using Clevenger apparatus. The physical and chemical parameters of essential oil were examined.

The essential oil of rhizomes of *Nardostachys grandiflora* DC. sample from Humla and Jumla were examined by GC and GC/MS. Four major compounds were found in both sample of essential oil were bicyclo [3.1.1] heptanes, β-pinene (11.3%); diacetone, 4-hydroxy, 4- methyl, 2- pentanone(7.07%); α-pinene(6.53%); aristolene(5.95%) in Humla and β-gurjunen(12%); spathulenol(7.66%); thujopsadiene (6.92%); jatamanson(6.78%); β-Caryophyllene (5.8%) in Jumla. Furthermore the physicochemical properties of both samples were similar.

The essential oil of both sample from Jumla and Humla showed the antibacterial activity which was identified with measurement of zone of inhibition (ZOI in mm). The antibacterial study was performed in both gram-positive and gram-negative bacteria with *Proteus vulgaris*, *Escherichia coli*, *Staphylococcus aureus* and *Klebsicela pneumoniae*.

Higher value of ZOI was observed in sample from Jumla with bacteria *Proteus vulgaris* (13.5 mm) and lowest value of ZOI was observed in sample from Humla with bacteria *Staphylococcus aureus* (8 mm).

The medicinal plant *Nardostachys grandiflora* DC., sample collected from Humla and Jumla contain large number of compounds (64 in both sample) with high value of antibacterial activity on gram negative bacteria *(Proteus vulgaris*).

CONTENTS

ANNEX

Photographs of rhizomes of plant sample and its research works.

Structure of some major identified compounds.

Spectra of major identification compounds (sample from Humla and Jumla).

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Finally, my most sincere appreciation goes to my parents Mr. Chhabi Lal Kadel and Mrs. Mun Sara Kadel, and other family members for their encouragement, assistance and inspiration in my whole academic years.

Khem Lal Kadel
khemlalkadel@yahoo.com
Central Department of Botany
T.U. Kirtipur, Kathmandu

ABBREVIATIONS

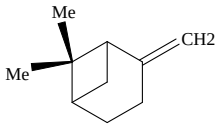
ANSAB	Asia Network for Sustainable Agriculture and Bio-resources.
CITIES	Conservation on International Trade in Endangered Species and Wild Fauna and Flora
DPR	Department of Plant Resources
TLC	Thin Layer Chromatography
MBC	Minimum Bacterial Concentration
GC	Gas Chromatography
GCMS	Gas Chromatography Mass Spectrometer
SPNP	Shey- Phoksundo National Park.
CAMP	Conservation assessment management plan
UNEP	United Nation Environment Protection.
WWF	World Wide Fund for Nature/World Wildlife Fund
VDC	Village Development Committee
NRs	Nepali Rupees
NTFP	Non Timber Forest Product
mg	Milligram
ml	Milliliter
mgl^{-1}	Milligram per liter.
TU	Tribhuvan University
UV	Ultra Violet
µg	Microgram

LC	Lethal Concentration
SPME	Solid Phase Micro Extraction
SPME-GC-MS	Solid Phase Micro extraction-Gas Chromatography-Mass Spectrometer
mm	Millimeter
µl	Micro liter
Rf	Retention factor.
m/z	Mass/charge ratio
ZOI	Zone of Inhibition

Structure of the major identified compounds

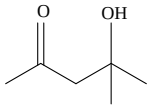
Humla

1.



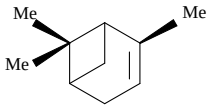
(I.) 2-beta-Pinene

2.



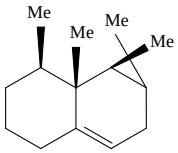
(II) 2-Pentanone 4- methyl 4-hydroxy, Diacetone alcohol.

3.



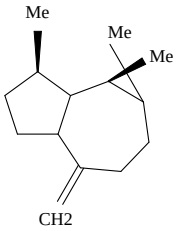
(III) Alpha - Pinene

4.



(IV) Aristolene

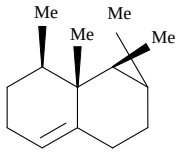
5.



(V) Alloaromadendrene

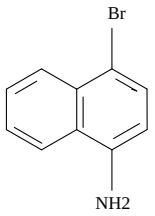
Jumla:

1.



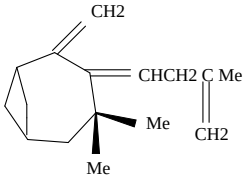
(VI) (Calarene) Beta-Gurjunene

2.



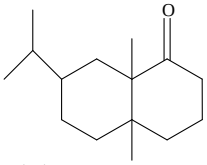
(VII) Napthalenamine,4 Bromo

3.



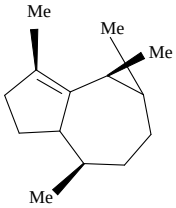
(VIII) 4,4-Dimethyl-3-(3-methylbut-3-enylidene)-2-methylenebicyclo[4.1
] heptane ,(Thujopsadiene)

4.



(IX) Jatamansone

5.



(X) Alpha-Gurjunene,Beta- Caryophyllene

Sample from Humla

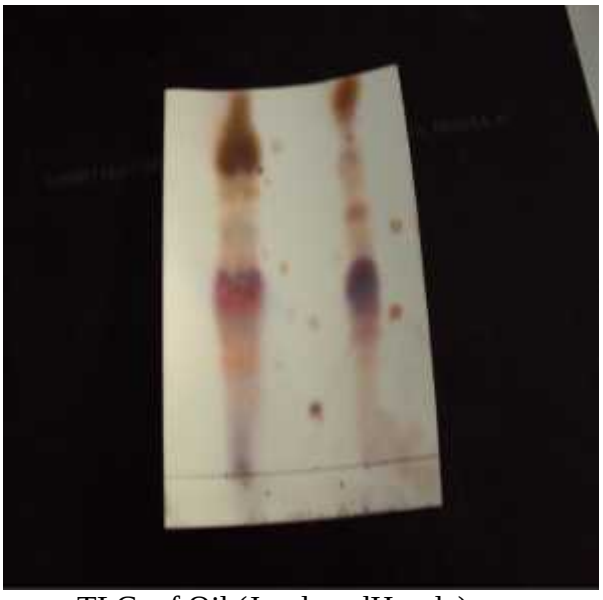
Extraction of oil using Clevenger apparatus

Marc of Jatamansi

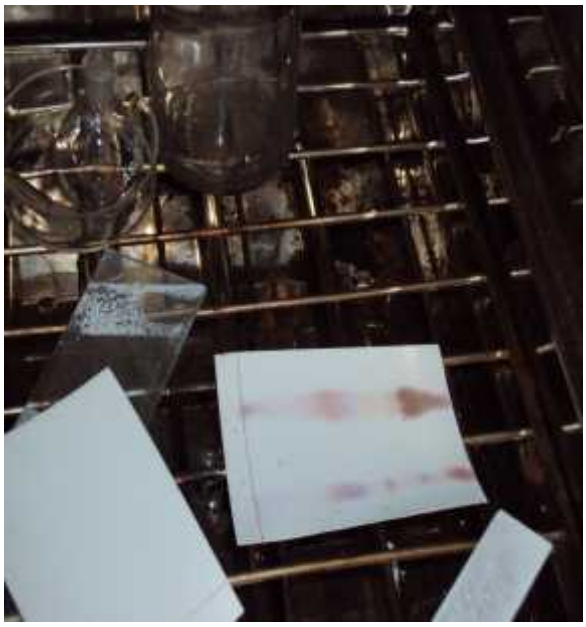
Oil sample of Jatamansi
(Humla,Jumla,andChinese)

Sample from Jumla

Abbe's Refractometer.



TLC of Oil (JumlaandHumla)



Heating of TLC in hot air oven

Seedling of Jatamansi

GCMS machine (QP2010)

Taking sample in the starting process

Running process of sample

Column of GCMS

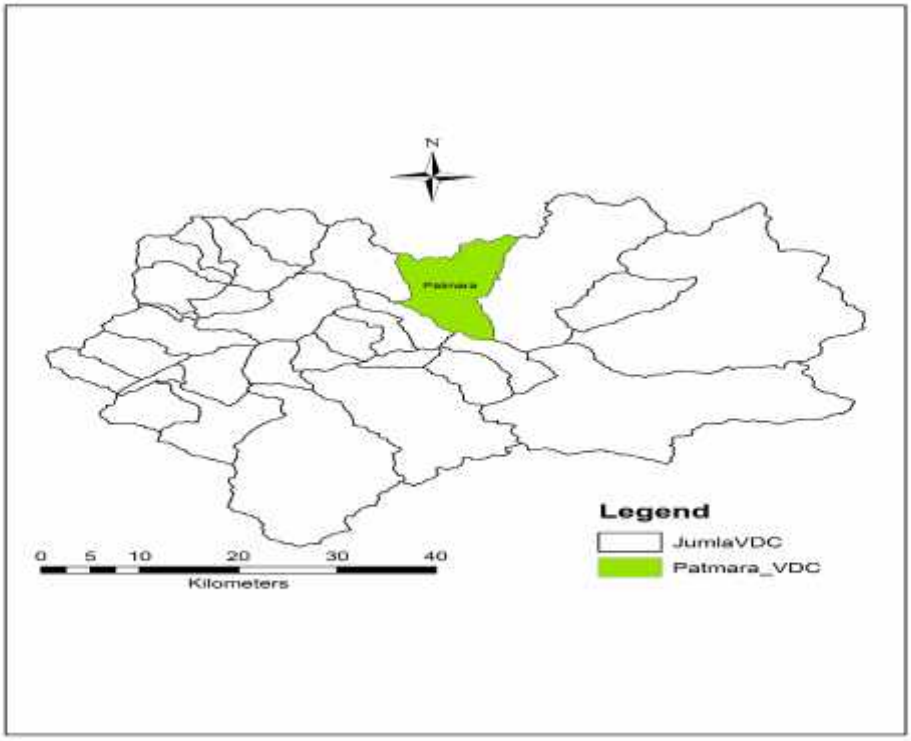
GCMS peak of sample

GCMS peak of sample

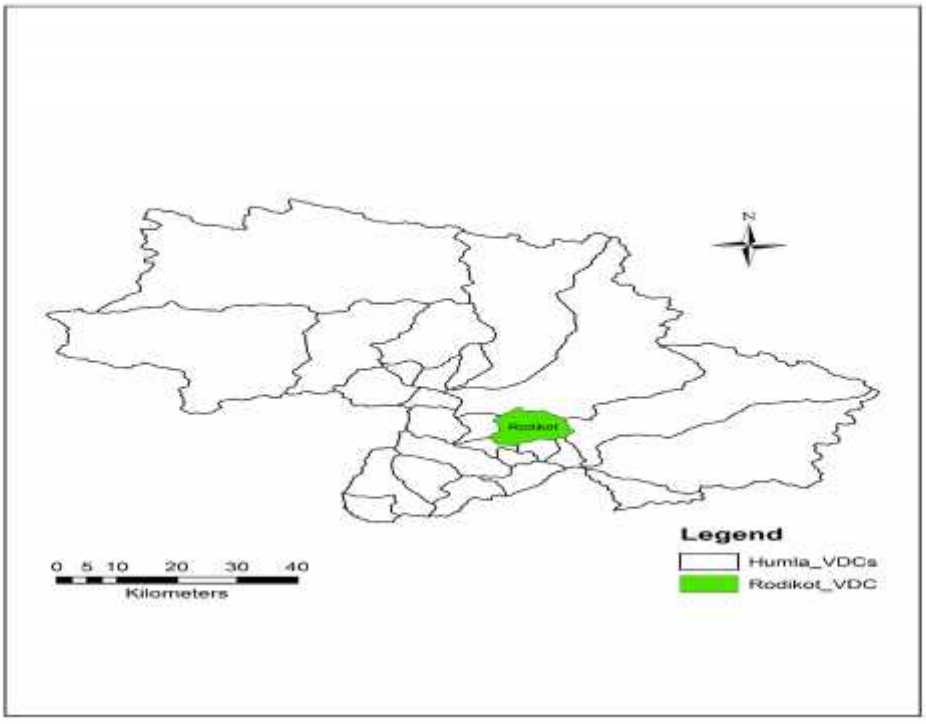





Photo plate: antibacterial activity of essential oil (HumlaandJumla) in different bacteria.



Sample collected from Patmara VDC of Jumla.



Sample collected from Rodikot VDC of Humla.

C:\GCMSsolution\Data\Project1\2067-68\Khem Kandel\171 Jatamansi-Jumla.qgd

Method

Analytical Line 1

[GCMS-QP2010 Plus]

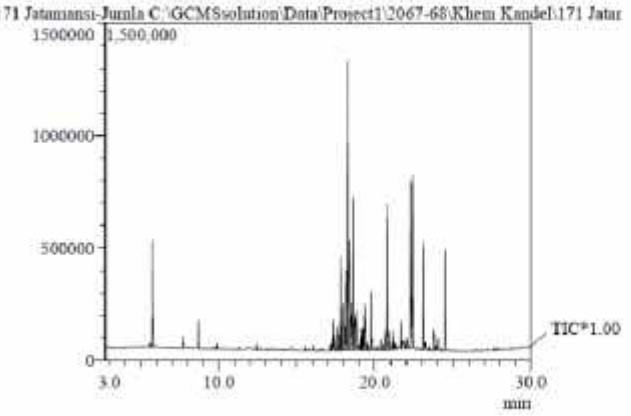
IonSourceTemp	200.00 °C
Interface Temp	280.00 °C
Solvent Cut Time	2.50 min
Detector Gain Mode	Relative
Detector Gain	0.00 kV
Threshold	0

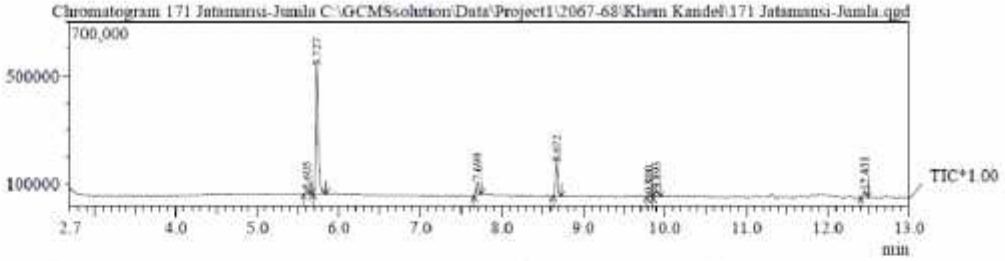
[MS Table]

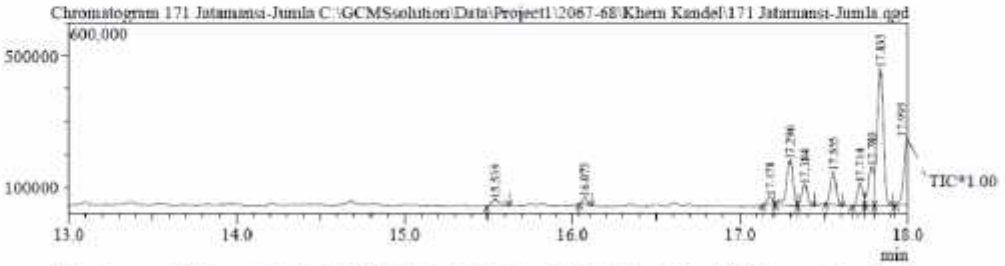
-Group 1 - Event 1-	
Start Time	2.50min
End Time	30.50min
ACQ Mode	Scan
Event Time	0.50sec
Scan Speed	1000
Start m/z	35.00
End m/z	500.00
Sample Inlet Unit	GC

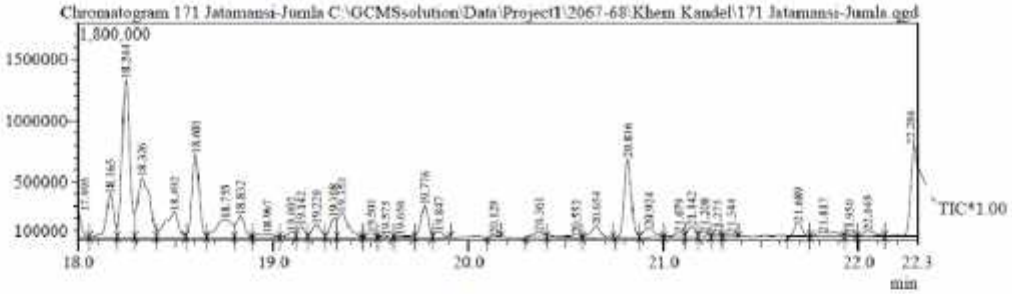
[MS Program]

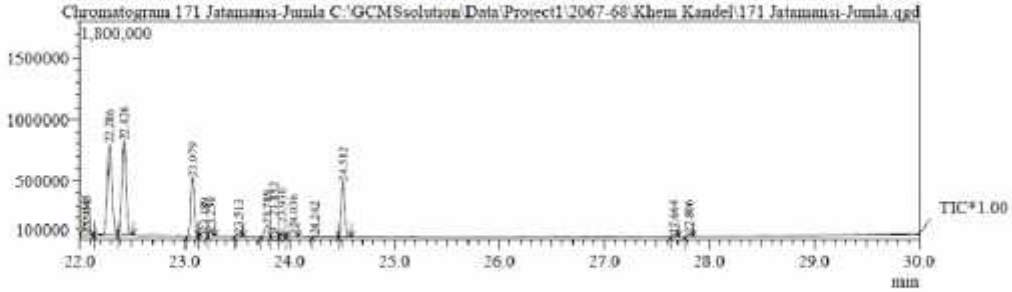
Use MS Program	OFF

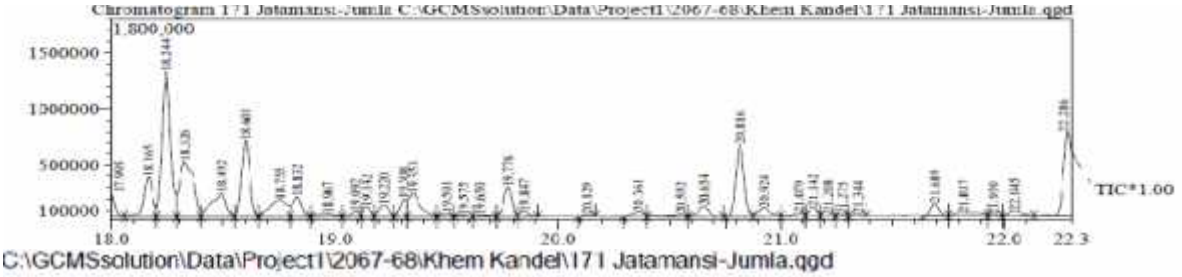












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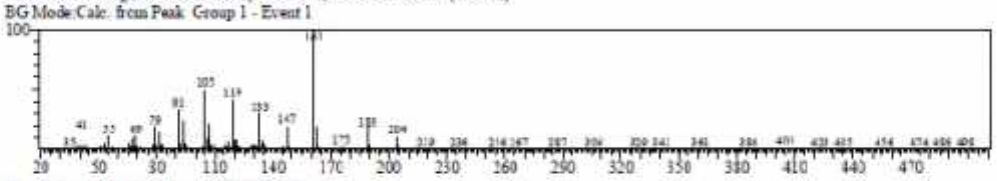

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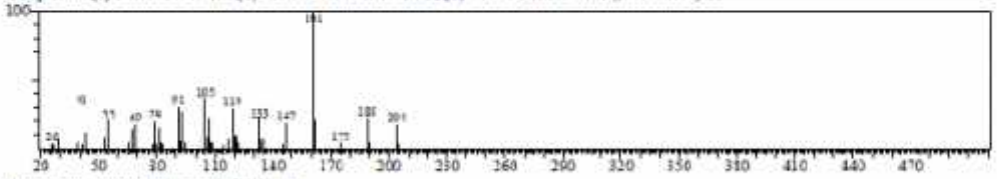

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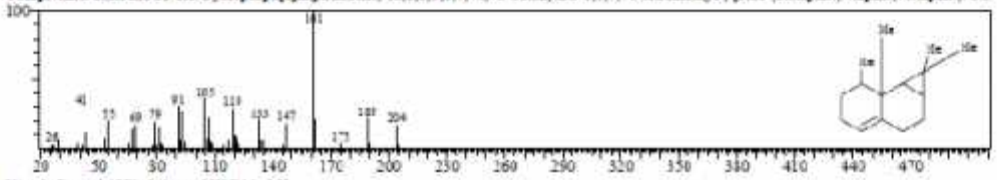

Hit#:3 Entry:16772 Library:NIST05s.LIB
SI:94 Formula:C15H24 CAS:17334-55-3 MolWeight:204 RetIndex:1403
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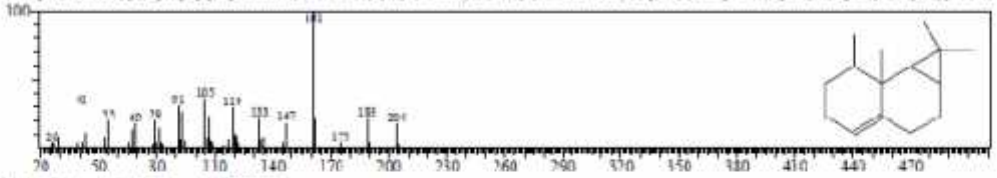

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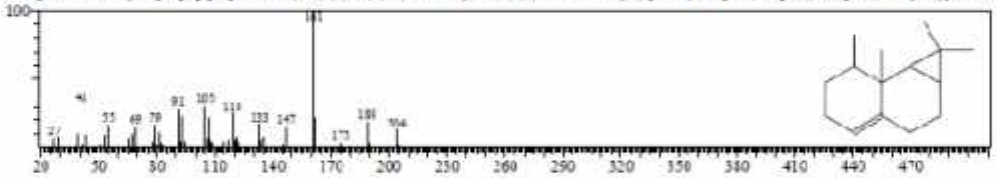
CompName:1H-Cycloprop[a]naphthalene, 1a,2,3,3,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)]- $$ 1



Hit#:5 Entry:101027 Library:WILEY7.LIB
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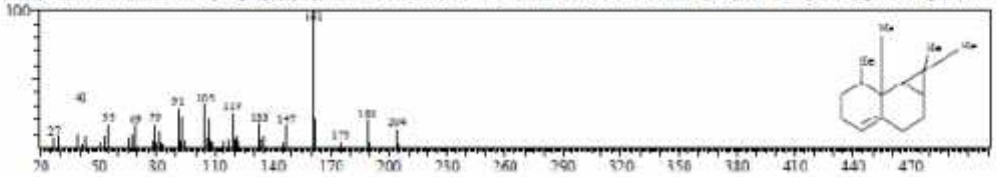
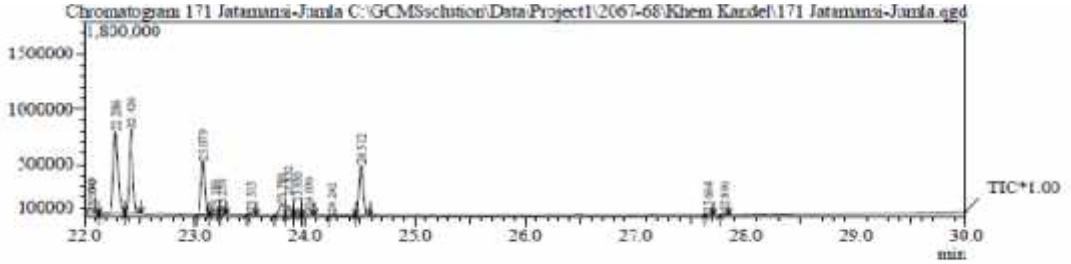
CompName:Calarene $$ 1H-Cycloprop[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.alpha.,7b.a





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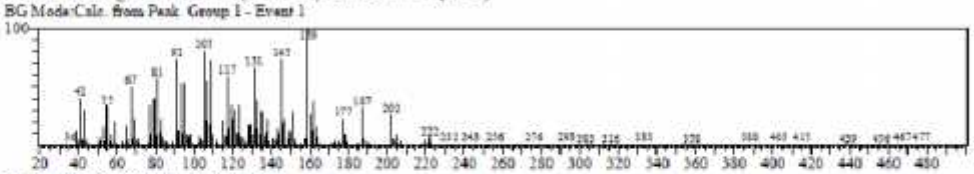

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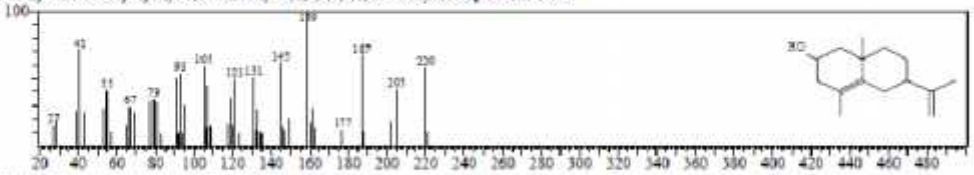

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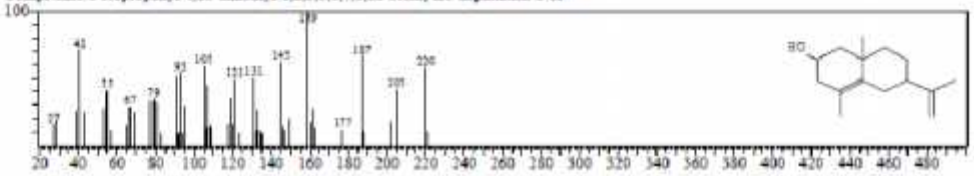
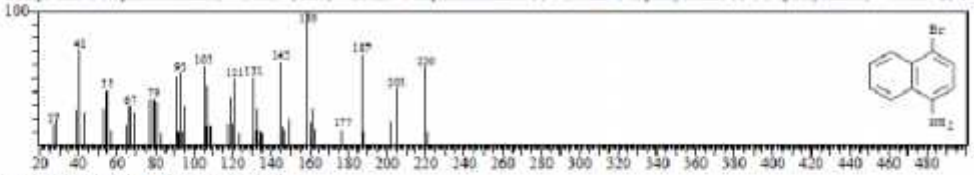
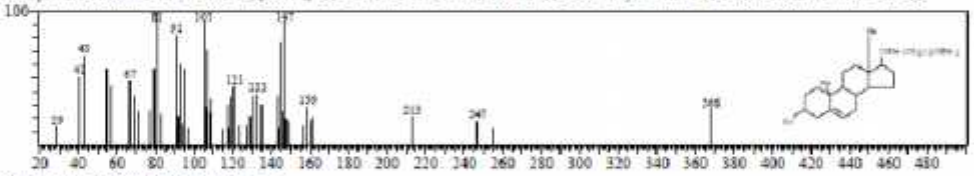


Hit#:3 Entry:121122 Library:WILEY7.LIB
SI:80 Formula:C15 H24 O CAS:2298-07-9 MolWeight:220 RetIndex:0
CompName:1-Naphthalenamine, 4-bromo- (CAS) 4-Bromo-1-naphthalenamine $$ 4-Bromo-1-naphthylamine $$ 1-Naphthylamine, 4-bromo- $$ 1



Hit#:4 Entry:303859 Library:WILEY7.LIB
SI:78 Formula:C27 H45 BR CAS:516-91-6 MolWeight:448 RetIndex:0
CompName:Cholest-5-ene, 3-bromo-, (3.beta.)- $$ Cholest-5-ene, 3.beta.-bromo- $$ Cholesteryl bromide $$ 3.beta.-Bromocholest-5-ene $$



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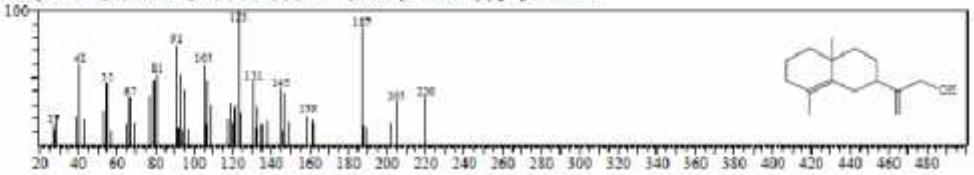
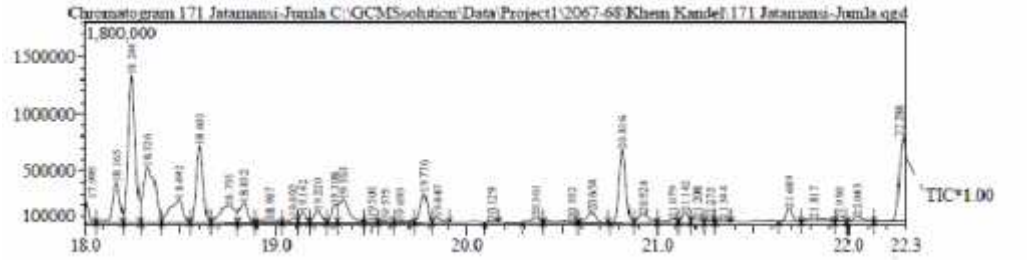
CompName:2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol





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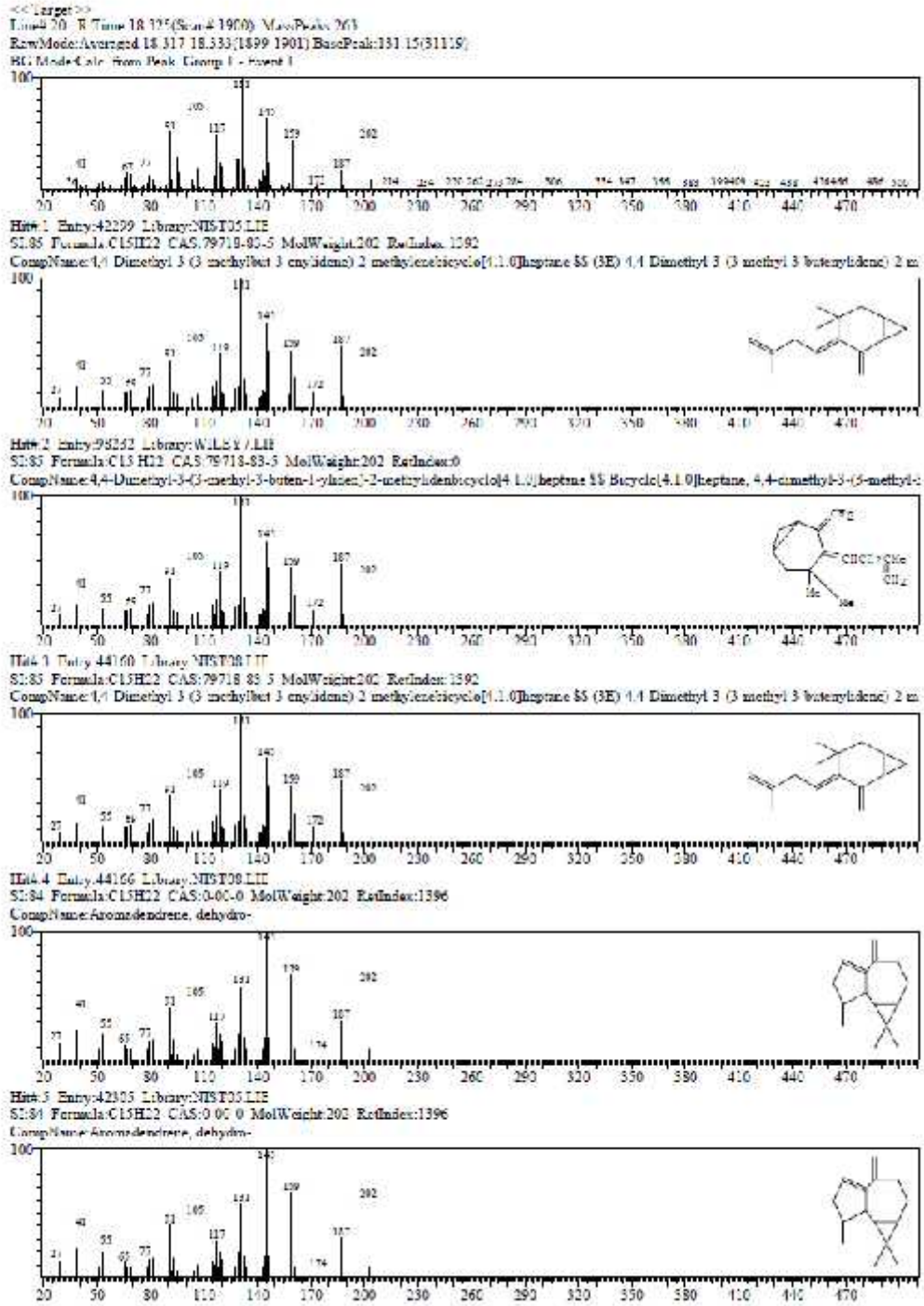

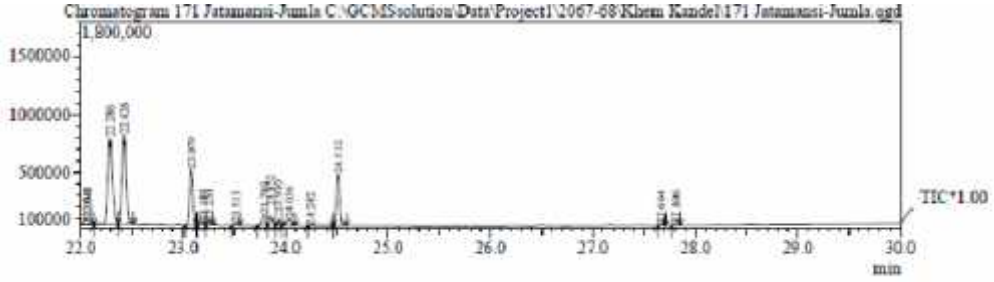

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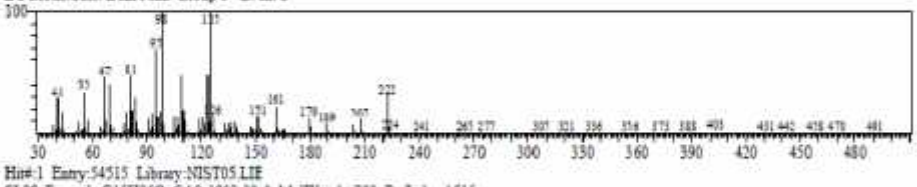
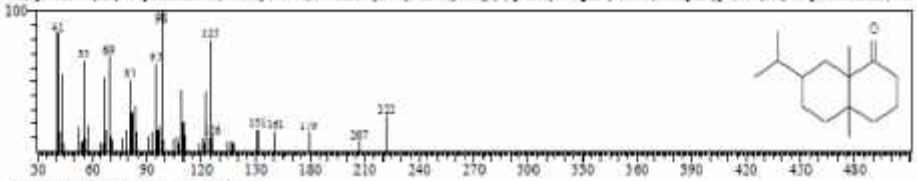
BG Mode:Calc. from Peak Group 1 - Event 1



Hit#:1 Entry:54515 Library:NIST05.LIB
SI:89 Formula:C15H26O CAS:1803-39-0 MolWeight:222 RetIndex:1615
CompName:1(2H)-Naphthalenone, octahydro-4a,8a-dimethyl-7-(1-methylethyl)-, [4aR-(4a.alpha.,7.beta.,8a.alpha.)]- $$ 1(2H)-Naphthalenone, oct



Hit#:2 Entry:37480 Library:NIST08.LIB
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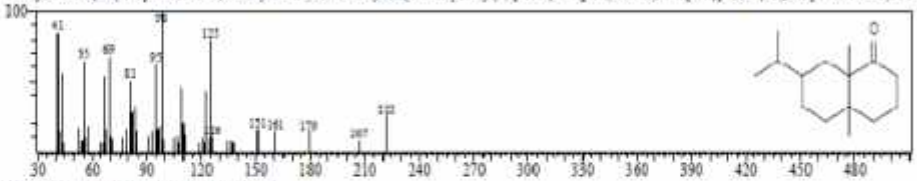
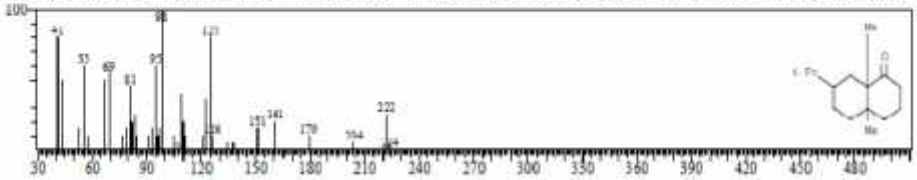
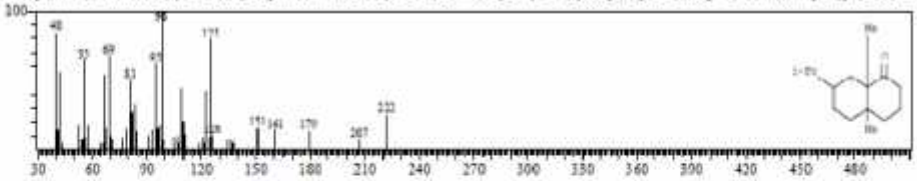
CompName:1(2H)-Naphthalenone, octahydro-4a,8a-dimethyl-7-(1-methylethyl)-, [4aR-(4a.alpha.,7.beta.,8a.alpha.)]- $$ 1(2H)-Naphthalenone, oct



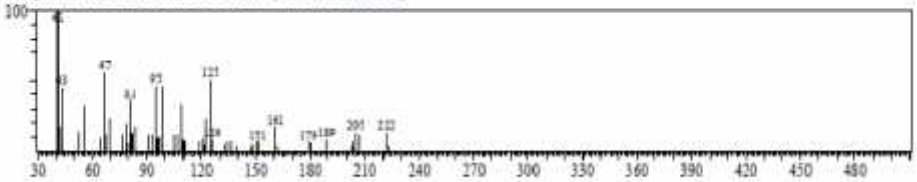
Hit#:3 Entry:124010 Library:WILEY7.LIB
SI:89 Formula:C15 H26 O CAS:1803-39-0 MolWeight:222 RetIndex:0
CompName:Valeranone, (+)- $$ 1(2H)-Naphthalenone, octahydro-4a,8a-dimethyl-7-(1-methylethyl)-, [4aR-(4a.alpha.,7.beta.,8a.alpha.)]- (CAS) Ja



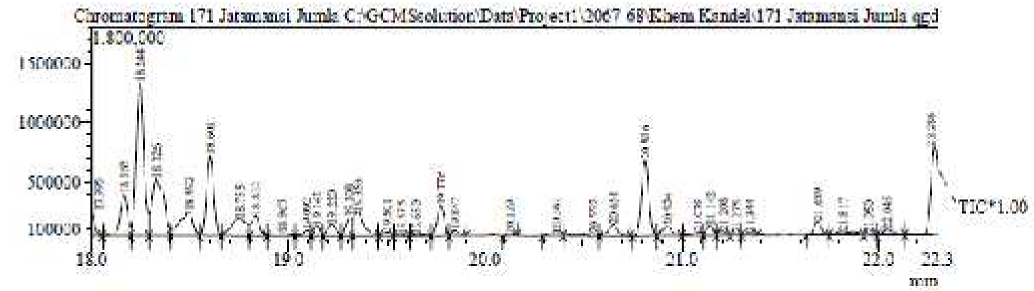
Hit#:4 Entry:124009 Library:WILEY7.LIB
SI:85 Formula:C15 H26 O CAS:1803-39-0 MolWeight:222 RetIndex:0
CompName:Valeranone, (+)- $$ 1(2H)-Naphthalenone, octahydro-4a,8a-dimethyl-7-(1-methylethyl)-, [4aR-(4a.alpha.,7.beta.,8a.alpha.)]- (CAS) Ja



Hit#:5 Entry:785 Library:SZTERP.LIB
SI:82 Formula:C15 H26 O CAS:0-00-0 MolWeight:222 RetIndex:0
CompName:VALERANONE DB5-2047 (=JATAMANSONE)

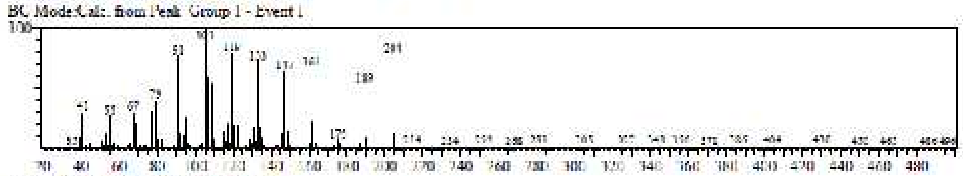


Chromatogram 171 Jatamansi Jumla C:\GCMSsolution\Data\Project1\2067 68\Khem Kandel\171 Jatamansi Jumla.qgd

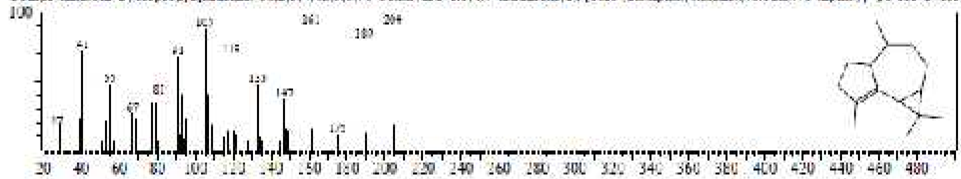


C:\GCMSsolution\Data\Project1\2067 68\Khem Kandel\171 Jatamansi Jumla.qgd

<<Target>>
Line#:72 R.Time:18.600(Scan#:1933) MassPeaks:251
RawMode:Averaged 18.592-18.608(1932-1934) BasePeak:105.10(41412)
BG Mode:Calc. from Peak Group 1 - Event 1

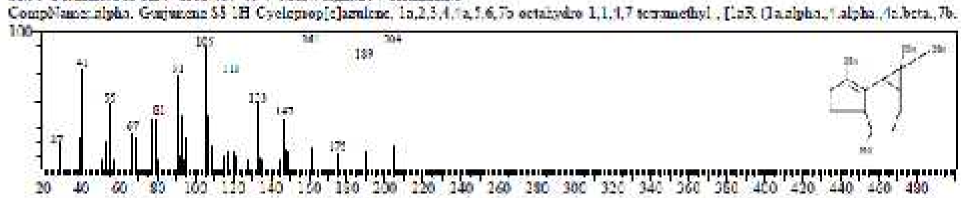


Hit#:1 Entry:45554 Library:NIST05.LIB
SI:90 Formula:C15H24 CAS:489-40-7 MolWeight:204 RetIndex:1419
CompName:1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]- $$ 1H-Cyclo



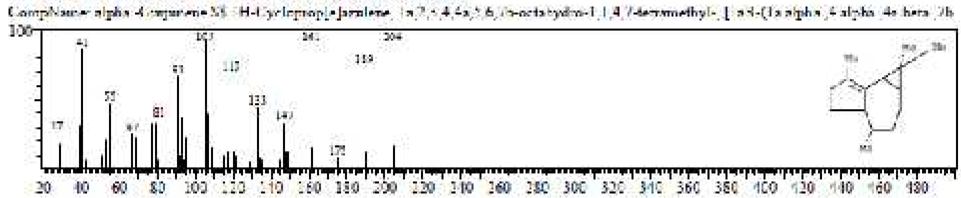
Hit#:2 Entry:45587 Library:NIST05.LIB
SI:90 Formula:C15H24 CAS:489-40-7 MolWeight:204 RetIndex:1419
CompName:1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]- $$ 1H-Cyclo

Hit#:3 Entry:100994 Library:WILEY7.LIB
SI:90 Formula:C15 H24 CAS:489-40-7 MolWeight:204 RetIndex:0
CompName:.alpha.-Gurjunene $$ 1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7b.



Hit#:4 Entry:101001 Library:WILEY7.LIB
SI:90 Formula:C15 H24 CAS:489-40-7 MolWeight:204 RetIndex:0
CompName:.alpha.-Gurjunene $$ 1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7b.

Hit#:5 Entry:100997 Library:WILEY7.LIB
SI:89 Formula:C15 H24 CAS:489-40-7 MolWeight:204 RetIndex:0
CompName:.alpha.-Gurjunene $$ 1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7b.



Method

Analytical Line 1

[GCMS-QP2010 Plus]

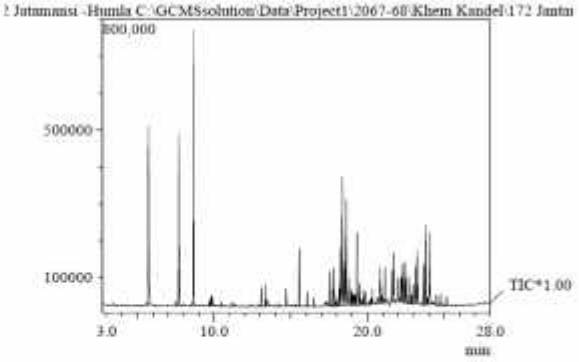
IonSourceTemp	200.00 °C
Interface Temp	280.00 °C
Solvent Cut Time	2.50 min
Detector Gain Mode	Relative
Detector Gain	0.00 kV
Threshold	0

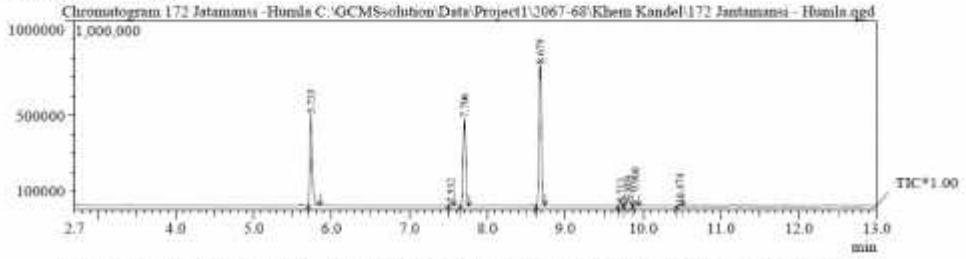
[MS Table]
--Group 1 - Event 1--

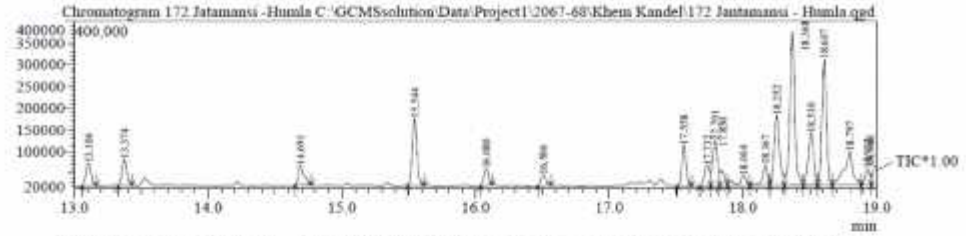
Start Time	2.50min
End Time	30.50min
ACQ Mode	Scan
Event Time	0.50sec
Scan Speed	1000
Start m/z	35.00
End m/z	500.00
Sample Inlet Unit	GC

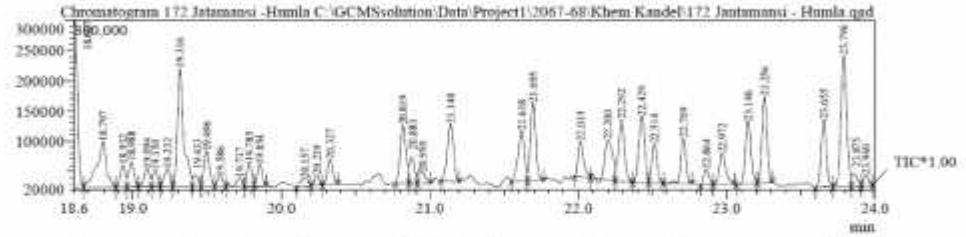
[MS Program]

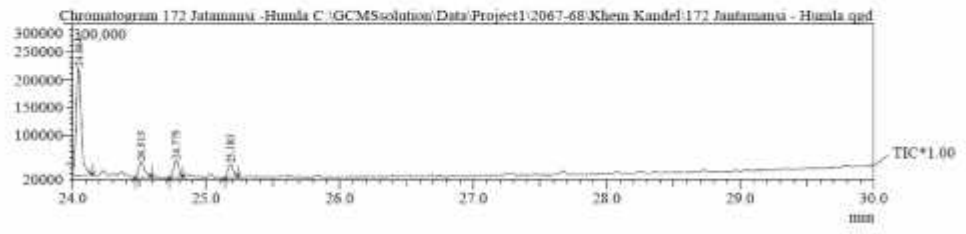
Use MS Program	OFF

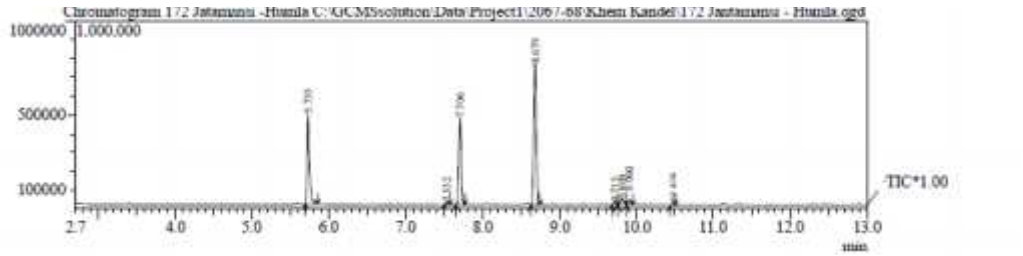












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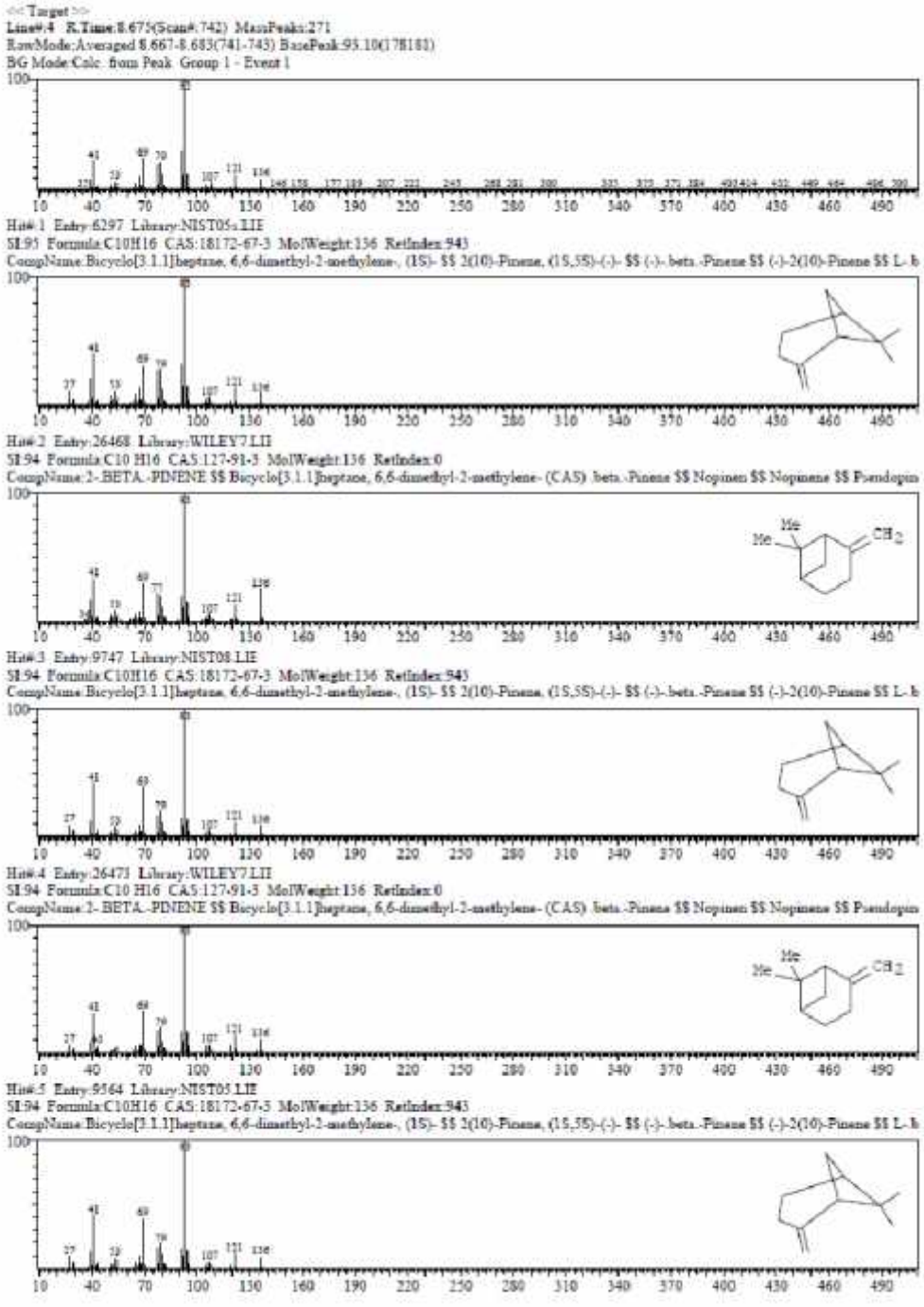
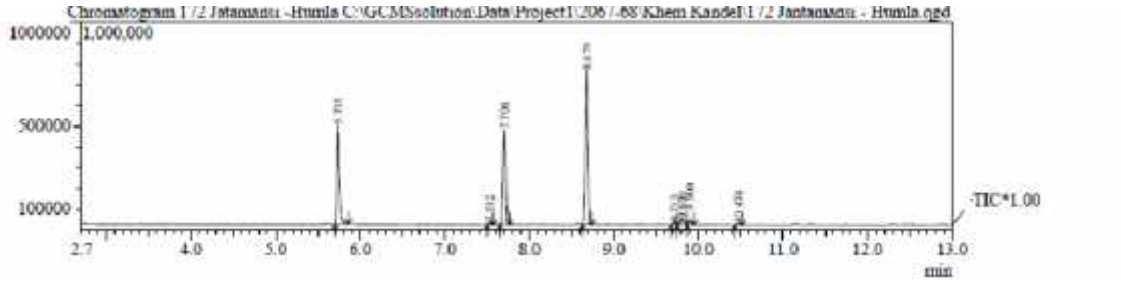




C:\GCMSsolution\Data\Project1\2067-68\Khem Kandel\172 Jantamansi - Humla.qgd

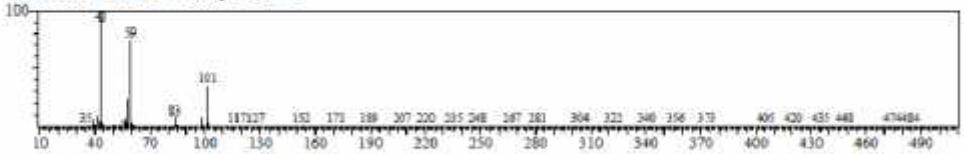
Library

<< Target >>

Line#:1 R.Time:5.753(Scan#:589) MassPeaks:254

RawMode:Averaged 5.725-5.742(588-590) BasePeak:43.00(146936)

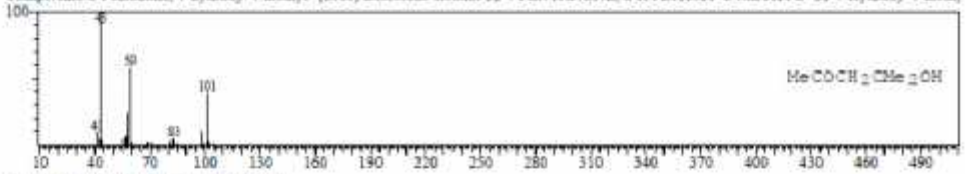
BG Mode:Calc. from Peak Group 1 - Event 1



Hit#:1 Entry:15776 Library:WILEY7.LIB

SI:94 Formula:C6 H12 O2 CAS:123-42-2 MolWeight:116 RetIndex:0

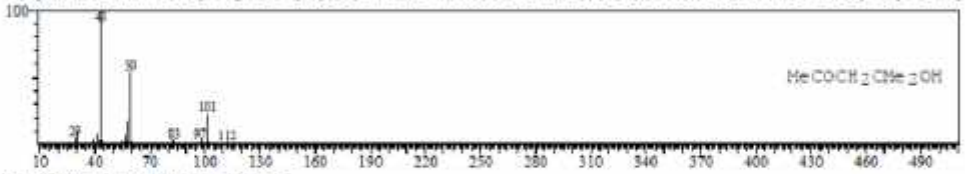
CompName:2-Pentanone, 4-hydroxy-4-methyl- (CAS) Diacetone alcohol $$ 4-PENTANONE, 2-HYDROXY-2-METHYL- $$ 4-Hydroxy-4-methy



Hit#:2 Entry:13765 Library:WILEY7.LIB

SI:94 Formula:C6 H12 O2 CAS:123-42-2 MolWeight:116 RetIndex:0

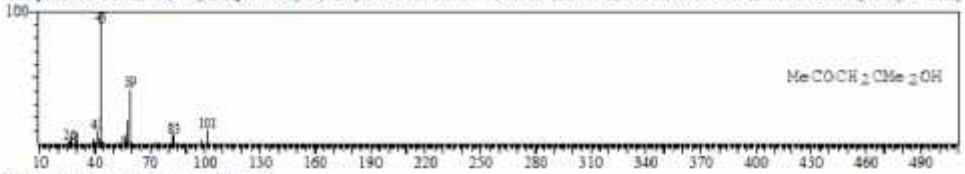
CompName:2-Pentanone, 4-hydroxy-4-methyl- (CAS) Diacetone alcohol $$ 4-PENTANONE, 2-HYDROXY-2-METHYL- $$ 4-Hydroxy-4-methy



Hit#:3 Entry:13759 Library:WILEY7.LIB

SI:92 Formula:C6 H12 O2 CAS:123-42-2 MolWeight:116 RetIndex:0

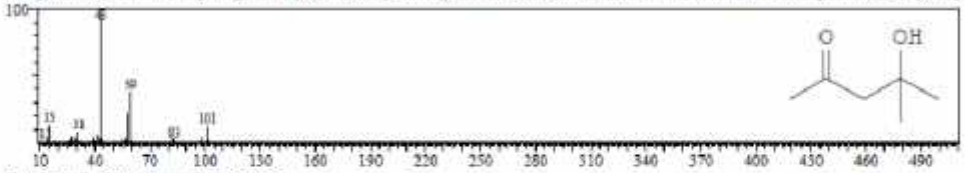
CompName:2-Pentanone, 4-hydroxy-4-methyl- (CAS) Diacetone alcohol $$ 4-PENTANONE, 2-HYDROXY-2-METHYL- $$ 4-Hydroxy-4-methy



Hit#:4 Entry:3443 Library:NIST05.LIB

SI:91 Formula:C6H12O2 CAS:123-42-2 MolWeight:116 RetIndex:845

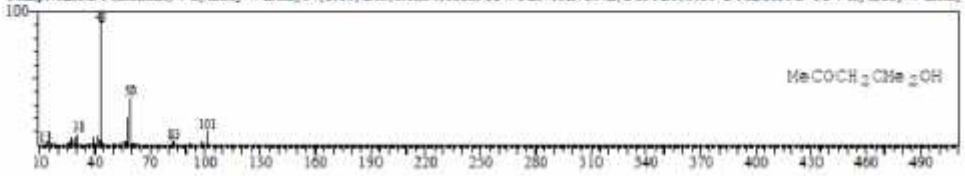
CompName:2-Pentanone, 4-hydroxy-4-methyl- $$ Acetonylidimethylcarbinol $$ Diacetone alcohol $$ Diketone alcohol $$ Tyrantone $$ 4-Hydroxy

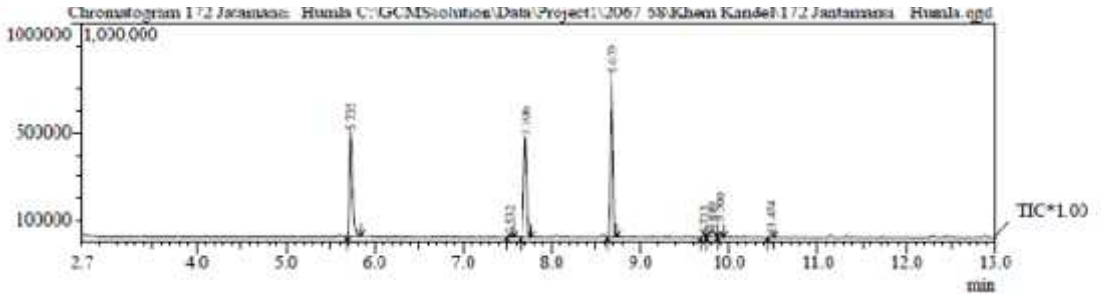


Hit#:5 Entry:13768 Library:WILEY7.LIB

SI:90 Formula:C6 H12 O2 CAS:123-42-2 MolWeight:116 RetIndex:0

CompName:2-Pentanone, 4-hydroxy-4-methyl- (CAS) Diacetone alcohol $$ 4-PENTANONE, 2-HYDROXY-2-METHYL- $$ 4-Hydroxy-4-methy





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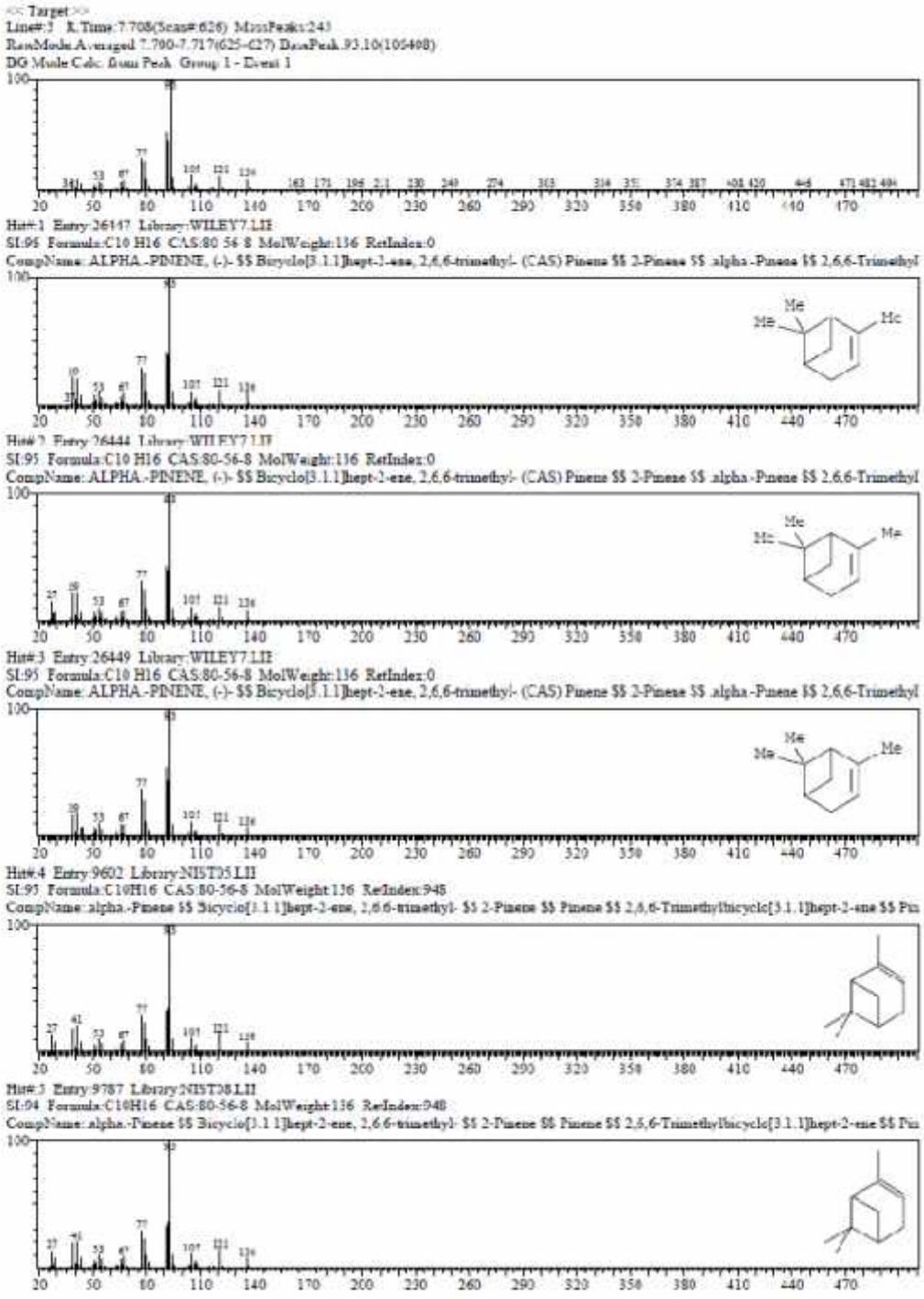
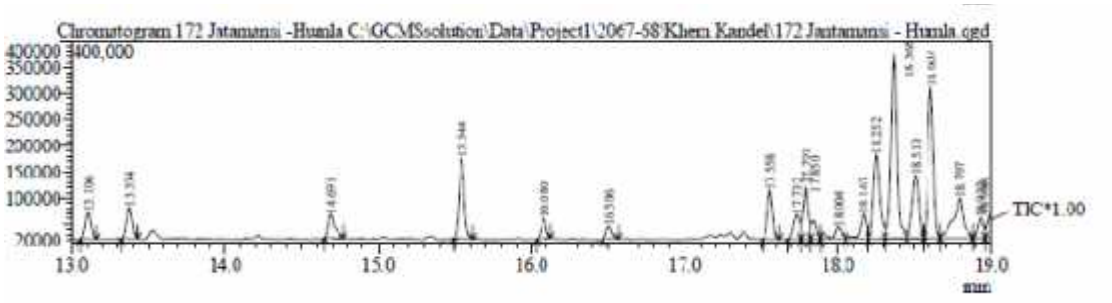
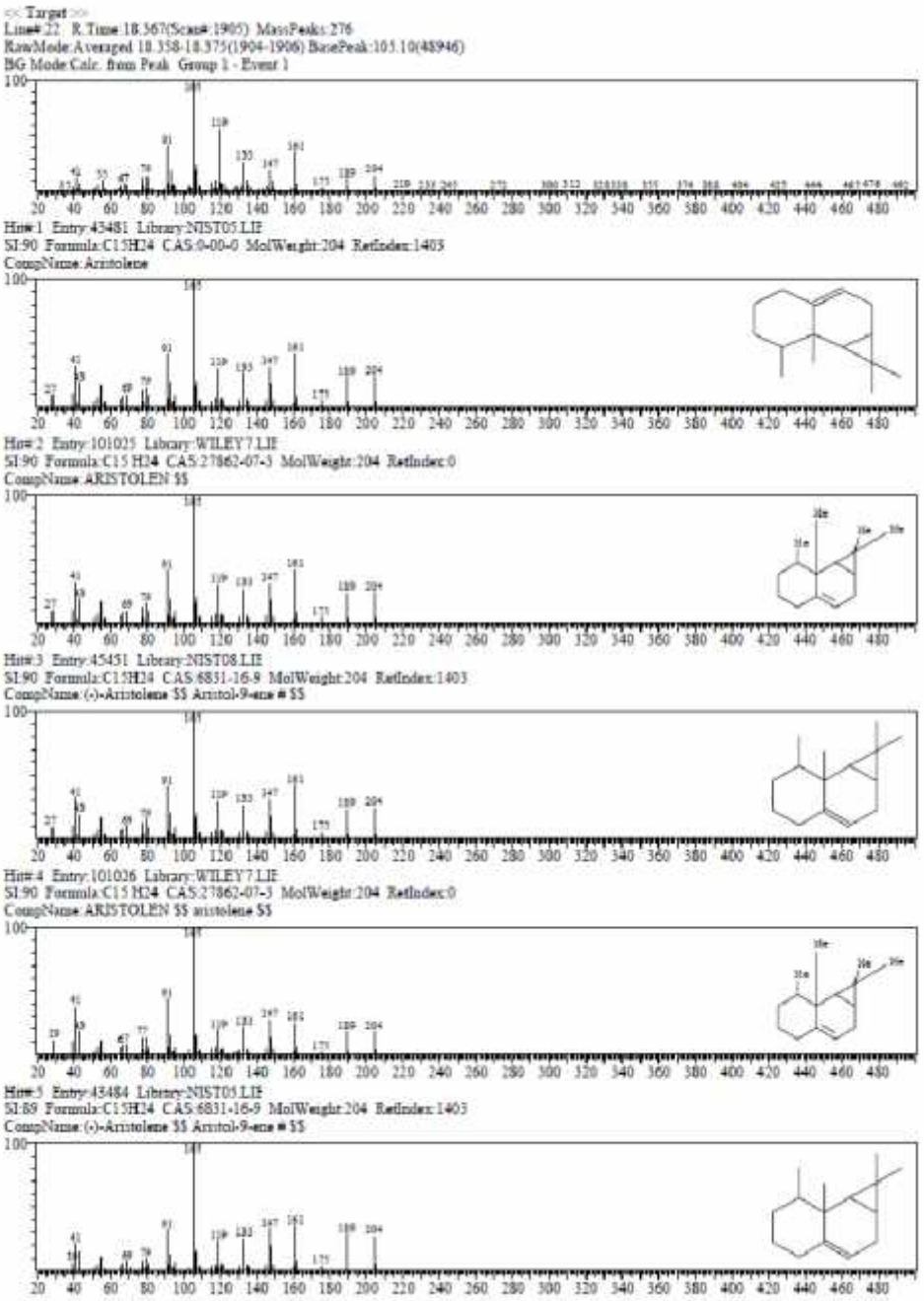
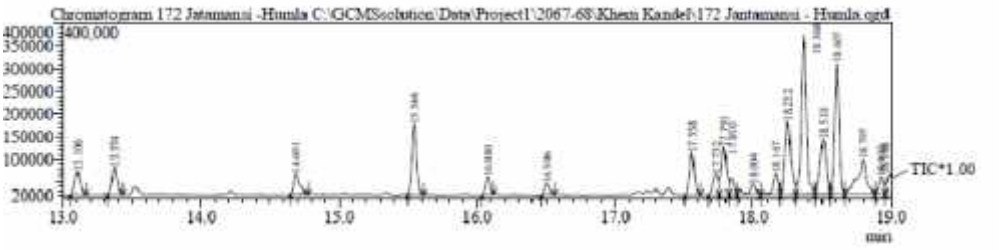




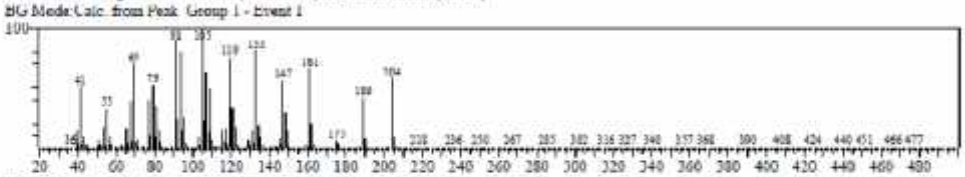
C:\GCMSsolution\Data\Project1\2067-68\Khem Kandel\172 Jantamansi - Humla.qgd



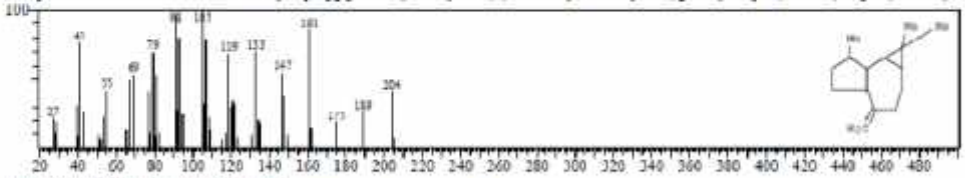


C:\GCMSsolution\Data\Project1\2067-68\Khem Kandel\172 Jantamansi - Humla.qgd

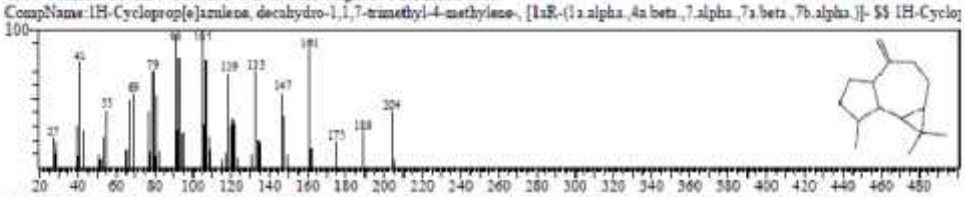
<< Target >>
Line#:24 R.Time:18.608(Scan#:1934) MassPeaks:308
RawMode:Averaged 18.600-18.617(1933-1935) BasePeak:105.10(15411)
BG Mode:Calc. from Peak Group 1 - Event 1



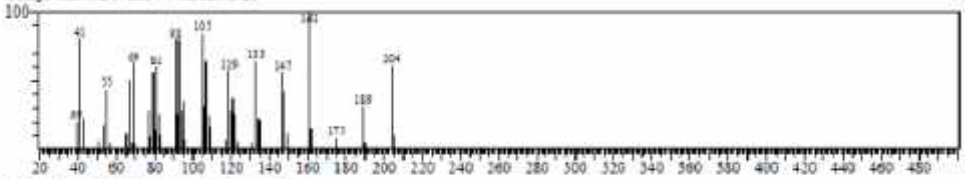
Hit#:1 Entry:101014 Library:WILEY7.LIB
SI:91 Formula:C15 H24 CAS:25246-27-9 MolWeight:204 RetIndex:0
CompName:Alloaromadendrene $$ 1H-Cycloprop[e]azulene, decahydro-1,1,/-trimethyl-4-methylene-, [1aR-(1a.alpha.,4a.beta.,/.alpha.,/a.beta.,/t



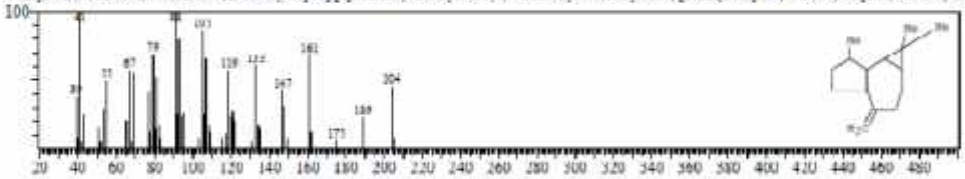
Hit#:2 Entry:16 /2/ Library:NIST05s.LIB
SI:91 Formula:C15H24 CAS:25246-27-9 MolWeight:204 RetIndex:1386
CompName:1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1a.alpha.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]- $$ 1H-Cyclo



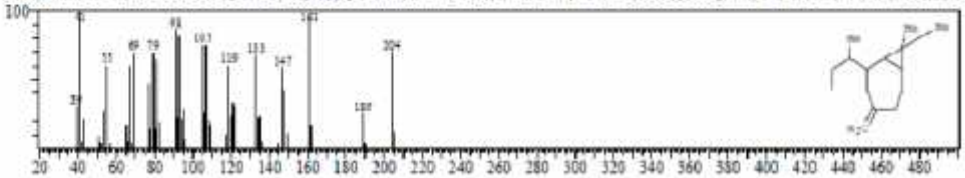
Hit#:3 Entry:100331 Library:WILEY7.LIB
SI:91 Formula:C15 H24 CAS:0-00-0 MolWeight:204 RetIndex:0
CompName:NEOALLOOCIMENE $$

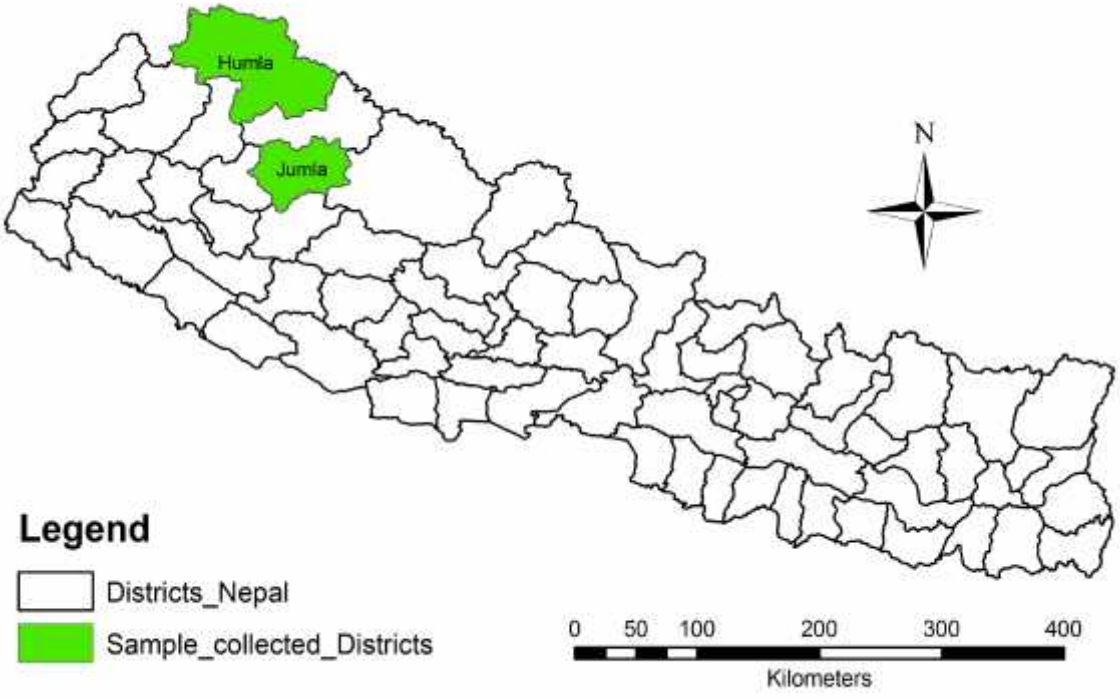


Hit#:4 Entry:101016 Library:WILEY7.LIB
SI:91 Formula:C15 H24 CAS:25246-27-9 MolWeight:204 RetIndex:0
CompName:Alloaromadendrene $$ 1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1a.alpha.,4a.beta.,7.alpha.,7a.beta.,7t



Hit#:5 Entry:101015 Library:WILEY7.LIB
SI:90 Formula:C15 H24 CAS:25246-27-9 MolWeight:204 RetIndex:0
CompName:Alloaromadendrene $$ 1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1a.alpha.,4a.beta.,7.alpha.,7a.beta.,7t




Humla
Jumla
N
Legend
Districts_Nepal
Sample_collected_Districts
0 50 100 200 300 400
Kilometers

Chemical constituents

In the essential oil of the roots of *N. grandiflora*, five compounds, nardostachnol (9-aristolen-1α-ol), Δ9,10-aristolene, Δ1,10-aristolene, β-maaliene and 1,2,9,10-tetradehydroaristolene were identified (Sun *et al*.)). In the essential oil of the rhizome of *N. jatamansi* purchased in Kathmandu market, fifteen compounds were identified of which thirteen were sesquiterpenes and one each aromatic and coumarin derivatives. Two major constituents in this essential oil were β-gurjunene (29.1%) and jatamansone (9.7%) (Paudyal 2004). *N. jataman*si rhizome essential oil from the Indian Himalayas contained nine monoterpenes (1.7%), 25 sesquiterpenes (43.9%) and 7 non-terpenic components (24.4%). The major sesquiterpenes include nardol (10.1%), α-selinene (9.2%), β-caryophyllene (3.3%), cubebol (2.9%), α-gurjunene (2.5%), γ-gurjunene (2.3%) and α-humulene (2.3%) (Mahawal *et al*.2002). Sesquiterpenes such as jatamols A and B (Bagchi *et al*. 1991), nardin (Chatterjee *et al*. 2005) terpenoid ester, nardostachysin (Chatterjee *et.al.* 2000), spirojatomol (Bagchi *et al.* 1990), seychellene and seychelane (Mahewshwori et al.) and norseychelanone, α - and β -patchoulenes and patchouli alcohol (Ruecker *et al*. 1976) were also obtained from its rhizome. Furthermore, neolignans and lignans have also been reported from *N. jatamansi* roots (Bagchi *et al.* 1991). The structures of jatamansone, jatamol A, jatamol B and nardin are presented below. But our research there some difference that ……. Area % sesquiterpene, ….. monoterpene ,….% coumarine and …% aromatic compound that is due to different causes.

……..

……

……..

jatamansone

jatamol A OCH3CH3CH3CH3CH2CH CH3HHOCH2CH3CH3HCH3COOH

Sun H., Ding J., Lin Z., Che F., Yunnan Zhiwu Yanjiu, 1980, 2, 213−223.

Mahalwal V. S., Ali M., J Essent Oil-Bearing Plants, 2002, 5, 83−89.

Bagchi A., Oshima Y., Hikino H., Planta Med., 1991, 57, 282−283.

Chatterjee A., Basak B., Datta U., Banerji J., Neuman A., Prange T., Ind J Chem., 2005, 44, 430−433.