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# GCMS Qualitative Analysis and Antimicrobial Activity of Essential Oils of *Cinnamomum tamala* (Buch.-Ham.) Nees and Eberm. (Tejpat) Leaves collected from Different Parts of Makwanpur District, Nepal

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**Abstract**

**GCMS technique was employed for the qualitative analysis of essential oil obtained from *Cinnamomum tamala* (Tejpat) leaves by hydrodistillation process using Clevenger type apparatus. The major components of the essential oil include á-Pinene, â-Pinene, Myrcene, á-Phellandrene, p-Cymene, Limonene, Eucalyptol, Linalool, á-Terpineol, E-Cinnamaldehyde, Bornyl acetate, E-Caryophyllene, E-Cinnamyl acetate and Caryophyllene oxide. In our work, essential oils obtained from the leaves collected from three different places of Makwanpur district, Nepal were analyzed by GCMS technique. The comparison was made between the chemical constituents and antimicrobial activity of these oil samples. It was found that the chemical constituents present in the Tejpat oil samples of the plant collected from different places varied, attributing probably to the variation in environment and topography. The throughput from the research suggested that extraction of Tejpat oil from the plants found in Makwanpur district is economically viable due to high content of constituents such as: linalool, eucalyptol and cinnamaldehyde, (which is good for isolation of large quantity of these constituents) and has very effective therapeutic uses because of their high antimicrobial activity.**

***Keywords:*** *Antimicrobial activity, Cinnamomum tamala (Tejpat), Essential oil, GCMS, Qualitative analysis*

## Introduction

*Cinnamomum tamala* belongs to genus *Cinnamomum* under *Lauraceae* family that encompasses 270 of naturally found species in Asia and Australia (Sharma & Nautiyal, 2011). *Cinnamomum tamala*, commonly called Indian Bay Leaf and locally known as ***Tejpat***, is a moderate sized evergreen tree found in the forests and farmlands in the Chure and Mid-Hill of Nepal from west to east at an elevation of 450-2000m (Shrestha & Joshi, 2015). *C. tamala* (Tejpat) leaves are widely used as spices and also yield essential oil, the oil extracted from the leaves is called Tejpat oil (Sharma & Nautiyal, 2011). In Nepal mostly dried or fresh leaves are consumed as spices, the plant is chiefly cultivated in Palpa and Udayapur districts of Nepal for commercial purpose (Shrestha & Joshi, 2015). Tejpat is frequently mentioned in various Ayurvedic literatures for its various medicinal values. Leaves and bark have aromatic, astringent, stimulant and carminative qualities and used in rheumatism, colic, diarrhea, nausea and vomiting. Ancient literature has revealed that in the first century A.D., dried leaves and bark of this plant were prescribed for fever, anemia and body odor (Shah & Panchal, 2010; Pravin et al., 2013).

The essential oil (EO) is extracted by techniques such as steamdistillation, hydrodistillation, solvent extraction, soxhlet extraction, etc. and in our study EO from Tejpat leaves is extracted by hydrodistillation method. It was found in previous studies that the major constituents of Tejpat oil are eugenol, methyl eugenol, cinnamaldehyde, E-cinnamyl acetate, linalool, eucalyptol, spathulenol, viridiflorene, aromadendrene, E-sabinene hydrate, (*Z*)-*â*-ocimene, myrcene, *á*-pinene, *â*-sabinene, germacrene A and *á*-gurjunene (Kumar et al., 2012; Lohani et al., 2012; Kapoor et al., 2009; Mir et al.,

2004). By far the research conducted on major chemical constituents of an essential oil have shown that the several factors such as nutrients, environmental conditions, extraction processes, drying methods, soil conditions, climatic conditions, etc. affects the components in essential oils (Rajeswara et al., 1990; Mejdoub et al., 1998; Aminzadeh et al., 2010; Paudel et al., 2016). The GCMS technique provides a means to analyze the chemical constituents present in the oil (Paudel et al., 2016).

The EOs of several species of *Cinnamomum* were already investigated before in order to study their chemical constituents (Kumar et al., 2012; Abdelwahab et al., 2017; Boniface et al., 2012; Hammid et al., 2016; Mohan et al., 2012), antioxidant activities (Kapoor et al., 2009; Abdelwahab et al., 2017) and antimicrobial properties (Pravin et al., 2013; Kapoor et al., 2009; Boniface et al., 2012; Mohan et al., 2012; Hassan et al., 2016). The EOs have very potent antimicrobial activities and are also used in many therapeutic purpose such as aromatherapy. In our work, the chemical constituents and antimicrobial properties of the *C. tamala* (Tejpat) oil are studied using GCMS technique and Agar well diffusion method.

It is stated that about 900 tons of bay leaves are produced in Udayapur district only and exports of these products to India and other neighboring countries is increasing even more today, indicating that the species have great potential for income generation for poor and disadvantaged people (Shrestha & Joshi, 2015). Hence, this research also intends to assess the variation in chemical composition and antimicrobial potential of Tejpat oil collected from different places of Makwanpur district, Nepal and we expect the results would favor to increase the market value of Tejpat leaves of Makwanpur district in Nepal and India.

## Materials and Methods

### *Plant specimen collection and extraction of Essential Oils*

At first, Tejpat leaves were collected from three different places of Makwanpur District, Nepal in spring season (April – May). The first plant sample was collected from Brindawan Botanical Garden (BBG), Hetauda of Makwanpur District. The other two places were collected from Tistung Botanical Garden (TBG), Tistung & District Plant Resources Office (DPRO), Hetauda of Makwanpur district, Nepal. These plant samples were then identified at Department of Plant Resources (DPR), Kathmandu, Nepal. 100gm fresh leaves of each sample of *C. tamala* Tejpat collected from all three different places were subjected to the hydrodistillation process using Clevenger apparatus to extract the essential oil for about 5 hours at 45°C. The quantity of essential oils obtained from each 100gm sample i.e. the Oil % was also noted. The oils from each place thus obtained were separated from the hydrosol, tagged and then stored at 4°C for further analysis.

### GCMS qualitative analysis

The chemical constituents in the essential oils were separated using a Shimadzu gas chromatograph (GC 2010) with Rtx-5MS column (25m×0.25mm× 0.25µm). 1 µL of the essential oil diluted with spectroscopic grade acetone (1:100) was injected into the GC inlet maintaining column flow rate of 1mL/min and purge flow 3 mL/min after fixing the split ratio at 150. The initial column oven temperature was set at 50°C and the injection temperature was 200°C. The qualitative analysis of the essential oil was further continued in a Shimadzu GCMS-QP2010 Plus. During the analysis, the ion source temperature and the interface temperature was set at 200°C and 250°C respectively. The detector scanning start time was 2.00 min and end time was 22.50 min; event time was 0.50 sec with scanning range of m/z 40-550. The MS library used in the analysis process was NIST 11 and FFNSC 1.3.

**Table 1:** Oven Temperature Program.

| Rate | Temperature (°C) | Hold Time (min) |
|---|---|---|
| - | 50.0 | 0.00 |
| 8.00 | 80.0 | 0.00 |
| 4.00 | 100.0 | 0.00 |
| 10.00 | 140.0 | 0.00 |
| 15.00 | 206.0 | 0.00 |
| 20.00 | 250.0 | 3.00 |

### *Antimicrobial activity by Agar well diffusion method*

Agar well diffusion method (Perez et al., 1990) was employed in the study of screening and evaluation of antimicrobial activity of the Tejpat oils. Inhibition of the microorganism growth was measured in the form of zone of inhibition (ZOI).

**Test species**

- Five species of bacteria viz. *Bacillus subtilis* (Ehrenberg) Cohn, *Proteus vulgaris* Hauser, *Salmonella enteric* subsp. *enterica*(ex Kauffmann and Edwards) Le Minor and Popoffserovar *Typhi*, *Shigella dysenteriae* (Shiga) Castellani and Chalmers and *Staphylococcous aureus* Rosenbach ATCC 6538P, and two species of fungi viz. *Candida albicans* (Robin) Berkhout ATCC 2091 and *Saccharomyces cerevisiae* Hansen were used as the test organisms.

**Preparation of test solutions**

- Test solutions of Tejpat oils of concentration 100mg.ml$^{-1}$ was prepared by dissolving it in 10% aqueous DMSO with 5% v/v polysorbate 80 (Prabuseenivasan et al., 2006).

**Preparation of inoculums**

- Direct colony suspension method was used to prepare the inoculum of each test organism. Isolated colonies of test organisms were transferred from 18 to 24-hrs culture (nutrient agar culture for bacterium and potato dextrose agar culture for fungi) into normal saline solution using sterilized loops. The suspension was homogenized by vortexing. The turbidity of the resulting suspension was adjusted to achieve a turbidity equivalent to that of 0.5 McFarland Standard (CLSI, 2012).

**Antimicrobial screening**

- The oil samples were screen for antimicrobial activity using agar well diffusion as described by Perez et al. (1990). A sterile swab was used to evenly distribute bacterial or fungal culture drawn from respective inoculums over the appropriate medium (Muller-Hinton agar for bacteria; potato dextrose agar for fungi). The plates were allowed to dry for 15 minutes before use in the test. Four wells (three wells for test samples and one well for solvent as negative control) of 6 mm diameter were then created in the inoculated plates using a sterile cork borer. Micropipettes were used to place 50 µl of the test solutions of the oil samples and solvent as negative control into each of the four wells. The plates were left in upright condition with lids closed for half an hour so that the test solutions diffused into the media. The inoculated plates were then incubated in inverted position at suitable temperature (37°C for bacteria; 25°C for fungi) after which they were examined for zone of inhibition (ZOI) around the well with no growth of microorganisms. Diameter of each ZOI was measured using digital venier caliper to the nearest whole millimeter.

## Results and Discussion

Altogether three samples of the plant under study were collected from three different places of Makwanpur District, Nepal. The hydrodistillation of the Tejpat leaves yield yellow colored oils. The oil percentages of *C. tamala* (Tejpat) leaves obtained during hydrodistillation are shown below in Table 2.

**Table 2:** Oil % of Tepat samples collected from three different places of Makwanpur.

| S. No. | Place of sample collection | Oil % |
|---|---|---|
| 1 | District Plant Resources Office (DPRO), Hetauda | 1.0 |
| 2 | Brindawan Botanical Garden (BBG), Hetauda | 1.2 |
| 3 | Tistung Botanical Garden (TBG), Tistung | 1.0 |

### *Chemical Constituents identified by GCMS qualitative analysis*

The MS data obtained for each peak during GC data analysis were compared with the NIST 11 and FFNSC 1.3 library and the components were identified for each chromatogram of Tejpat oil samples. The chromatograms obtained for each of the oil sample are shown in figure 1.

The constituents identified by GCMS analysis along

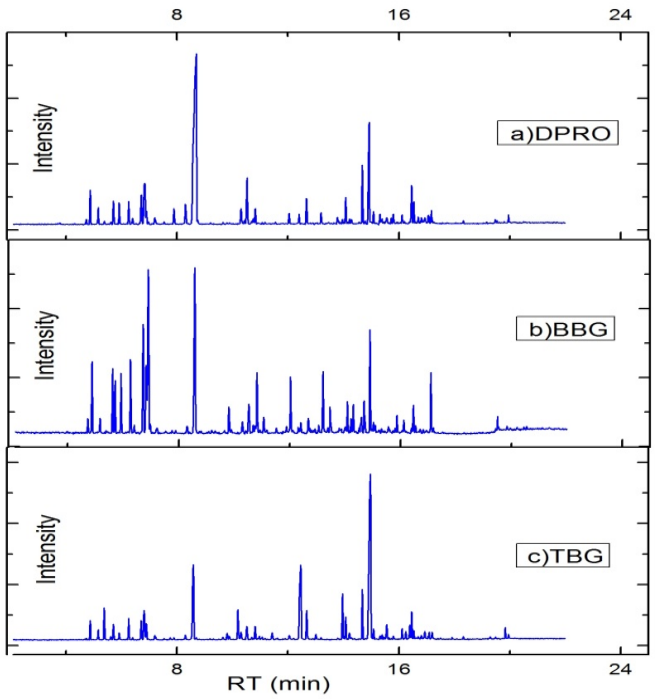


**Figure1:** Chromatogram of Tejpat oils of different places of Makwanpur District.

with their relative % are tabulated in table 3 below.

Here in table 3, the numerical values of the constituents are the relative peak area % in chromatogram without considering correction factors. The peak area % corresponds to the composition % of the constituents present in the oils. Table 3 indicates that altogether 56 total constituents were identified during the data analysis of all three Tejpat oils and separately, 36 constituents in Tejpat oil from District Plant Resources Office (DPRO), Hetauda that represents 98.16% of total oil constituents, 25 constituents in Tejpat oil from Brindawan Botanical Garden (BBG), Hetauda that represents 90.93% of total oil constituents and 32 constituents in Tejpat oil from Tistung Botanical Garden (TBG), Tistung that represents 97.37% of total oil were identified. From above table the major constituents of the Tejpat oils were found to be á-pinene, â-pinene, myrcene, á-phellandrene, p-cymene, limonene, eucalyptol, linalool, á-terpineol, E-cinnamaldehyde, bornyl acetate, E-caryophyllene, E-cinnamyl acetate and caryophyllene oxide. Similarly, camphene, camphor, borneol, nerol, copaene and á-humulene were found in minor % and some traces of other volatile constituents in the oil samples.

Tejpat oil from DPRO had linalool (41.39%) as the most significant component which is 15.34% and 10.31% in oil from BBG and TBG tejpat sample respectively. Eucalyptol encompassed 12.08% composition in Tejpat oil from BBG and only 0.85% and 1.49% composition in oil samples from DPRO and TBG respectively. Tejpat oil from TBG has (E)-Cinnamyl acetate (30.45%) as highest constituent which is only 8.92% and 5.53% in DPRO and BBG samples respectively. Moreover, Tejpat oil from TBG also contains (E)-Cinnamaldehyde about 12.42% which is below 1% in oils form DPRO and BBG. Apart from these major variations, the three oil samples had more or less similar % of other major and minor constituents. Some constituents present in one or more oil samples were absent in other samples. These variations indicated topography and environment are major factors affecting the chemical constituents of the essential oil (Paudel et al., 2016). These topographical and environmental differences can be considered to be the differences in soil conditions, altitudes and atmosphere; however no detail studies were carried out on these factors in our study.

### *Antimicrobial activities of the oil samples*

The diameters of Zone of Inhibition (ZOI) produced by Tejpat oils of study on particular microorganisms were measured for the estimation of their antimicrobial activity. The results obtained from the experiments are tabulated in Table 4.

**Table 3:** Chemical composition of Tejpat oils collected from three different places of Makwanpur District.

| S. No. | Name of Constituents | Retention Time (RT)/min | Area % (from MS) | | |
|---|---|---|---|---|---|
| | | | DPRO | BBG | TBG |
| 1 | α-Thujene | 4.73 | - | 0.83 | - |
| 2 | α-Pinene | 4.87 | 2.32 | 4.19 | 1.59 |
| 3 | Camphene | 5.16 | 1.07 | - | 0.83 |
| 4 | Benzaldehyde | 5.38 | - | - | 2.75 |
| 5 | Sabinene | 5.62 | - | 3.76 | - |
| 6 | β-Pinene | 5.71 | 1.66 | 3.06 | 1.26 |
| 7 | Myrcene | 5.92 | 1.49 | 3.56 | 0.58 |
| 8 | α-Phellandrene | 6.26 | 1.84 | 4.64 | 1.83 |
| 9 | Carene | 6.40 | 0.37 | - | - |
| 10 | p-Cymene | 6.71 | 2.22 | 7.61 | 1.72 |
| 11 | Limonene | 6.83 | 4.91 | 6.08 | 3.09 |
| 12 | Eucalyptol | 6.90 | 0.85 | 12.08 | 1.49 |
| 13 | Salicylaldehyde | 7.20 | 0.68 | - | 0.55 |
| 14 | Z-Linalool oxide | 7.89 | 1.39 | - | - |
| 15 | E-Linalool oxide | 8.30 | 2.05 | - | - |
| 16 | Linalool | 8.69 | 41.39 | 15.34 | 10.31 |
| 17 | Camphor | 9.81 | - | 1.84 | 0.57 |
| 18 | Hydrocinnamaldehyde | 10.20 | - | - | 2.97 |
| 19 | Isoborneol | 10.30 | - | 0.64 | - |
| 20 | Borneol | 10.30 | 1.37 | - | 0.86 |
| 21 | Benzofuran, 2-methyl- | 10.53 | 4.14 | - | 1.75 |
| 22 | Terpinen-4-ol | 10.53 | - | 2.62 | - |
| 23 | Cryptone | 10.75 | 0.70 | - | - |
| 24 | α-Terpineol | 10.82 | 1.12 | 3.69 | 1.29 |
| 25 | (E)-Sabinol | 11.07 | - | 0.98 | - |
| 26 | (Z)-Cinnamaldehyde | 11.43 | - | - | 0.80 |
| 27 | Nerol | 12.04 | 0.73 | - | 0.58 |
| 28 | (E)-Cinnamaldehyde | 12.40 | 0.62 | 0.66 | 12.42 |
| 29 | Bornyl acetate | 12.67 | 1.93 | 0.84 | 2.44 |
| 30 | 2-Hydroxycineol | 13.19 | 0.74 | 3.75 | - |
| 31 | Isoascaridole | 13.46 | - | 1.56 | - |
| 32 | Hydrocinnamyl acetate | 13.97 | - | - | 3.76 |
| 33 | Lavandulyl acetate | 14.08 | - | 1.47 | - |
| 34 | Copaene | 14.09 | 1.62 | - | 1.70 |
| 35 | (E)-Isocarveol | 14.29 | - | 1.44 | - |
| 36 | (E)-Caryophyllene | 14.68 | 3.82 | 2.16 | 4.04 |
| 37 | (E)-Cinnamyl acetate | 14.92 | 8.92 | 5.53 | 30.45 |
| 38 | α-Humulene | 15.09 | 0.94 | - | 0.79 |
| 39 | Bicyclogermacrene | 15.56 | - | - | 1.31 |
| 40 | α-Amorphene | 15.56 | 0.88 | - | - |
| 41 | γ-Muurolene | 15.72 | 0.46 | - | - |
| 42 | δ-Cadinene | 15.80 | 0.60 | - | - |
| 43 | o-methoxy-Cinnamaldehyde | 15.87 | - | 0.83 | - |
| 44 | Caryophyllene oxide | 16.46 | - | 1.76 | - |
| 45 | (E)-Nerolidol | 16.12 | 0.38 | - | 0.69 |
| 46 | Spathulenol | 16.39 | - | - | 0.96 |
| 47 | Caryophyllene oxide | 16.46 | 2.56 | - | 2.11 |
| 48 | Viridiflorol | 16.54 | - | - | 0.53 |
| 49 | Guaiol | 16.54 | 1.21 | - | - |
| 50 | Humulene epoxide II | 16.70 | 0.45 | - | - |
| 51 | delta cadinol | 16.81 | 0.48 | - | - |
| 52 | Cadin-4-en-10-ol | 17.06 | 0.92 | - | - |
| 53 | Bulnesol | 17.17 | 0.87 | - | - |
| 54 | Patchouli alcohol | 17.20 | - | - | 0.60 |
| 55 | Kaur-16-ene | 19.83 | - | - | 0.72 |
| 56 | Phytol | 19.95 | 0.47 | - | - |
| | **Total identified constituents** | | **98.16** | **90.93** | **97.37** |

**Table 4:** Zone of Inhibition (ZOI) of Tejpat oils from DPRO, BBG and TBG.

| Microorganisms | Zone of Inhibition, ZOI (mm) of Tejpat Oils from: | | | |
|---|---|---|---|---|
| | DPRO | BBG | TBG | Negative control (Solvent) |
| ***S. aureus*** | 15 | 16 | 23 | - |
| ***B. subtilis*** | 10 | 11 | 15 | - |
| ***S. typhi*** | 12 | 9 | 10 | - |
| ***P. vulgaris*** | 8 | 8 | 10 | - |
| ***S. dysenteriae*** | 8 | 9 | 11 | - |
| ***C. albicans*** | 10 | - | 8 | - |
| ***S. cerevisiae*** | 13 | 13 | 29 | - |

Note: (-) = No effective antimicrobial activity

Table 4 indicates that Tejpat oils collected from DPRO, BBG and TBG have shown effective inhibitive property for both bacteria and fungi of our interest with zones of inhibition ranging from 8–29 mm except for oil from BBG in *C. albicans* which is nil. It is clear from the table that Tejpat oil from TBG has the most significant antimicrobial activity in almost all test organisms since its ZOI is widest compared to other two oil samples. The highest inhibition shown by all three oils were against *S. aureus* (bacterium) and *S. cerevisiae (*fungus) and widest ZOI was shown by oil from TBG for *S. aureus* (ZOI= 23mm) and *S. cerevisiae* (ZOI= 29mm). The significant antimicrobial activity of Tejpat oil from TBG can be attributed for high content of constituents such as (E)-Cinnamyl acetate (30.45%) and (E)-Cinnamaldehyde (12.42%) known during GCMS analysis (Boniface et al., 2012). Table 4 also indicated the oil from DPRO has more potent antimicrobial activity compared to the oil from BBG which can be accounted by its high content of Linalool (Herman et al., 2016) and (E)-Cinnamyl acetate. Moreover, Tejpat oil from DPRO showed inhibition for two pathogenic microorganisms (*S. typhi* and *C. albicans*) more than by oils from TBG and BBG.

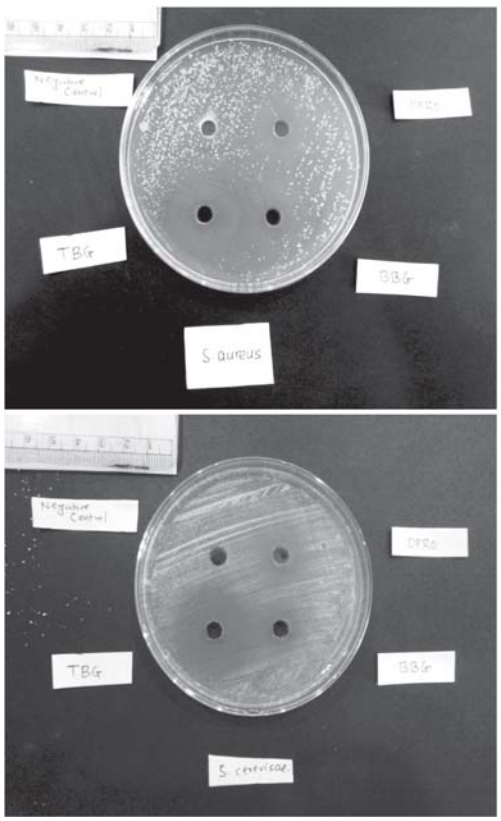


**Figure 2:** Widest ZOI shown by Tejpat oils from DPRO, BBG and TBG in inoculated plate of *S. aureus* (a bacterium) in left side and *S. cerevisiae* (a fungus) in right side.

Figures 2 and 3 depict some significant antimicrobial activities shown by Tejpat oils observed during our study.


**Figure 3:** Plates of *B. Subtilis, S. aureus and S. cerevisiae* showing significant antimicrobial activity by Tejpat oils of study.

## Conclusion

We conclude that Oil% is slightly high in Tejpat samples collected from Brindawan Botanical Garden (BBG), Hetauda than from other places during our study. The major chemical constituents of the oils under study were á-Pinene, â-Pinene, Myrcene, á-Phellandrene, p-Cymene, Limonene, Eucalyptol, Linalool, á-Terpineol, E-Cinnamaldehyde, Bornyl acetate, E-aryophyllene, E-Cinnamyl acetate and Caryophyllene oxide. The presence of significant % of linalool (41.39%) in DPRO sample, Eucalyptol (12.08%) in BBG sample, (E)-Cinnamyl acetate

(30.45%) and (E)-Cinnamaldehyde (12.42%) in TBG sample and variation of other constituents from GCMS analysis provides an evidence that the chemical constituents of essential oil of *C. tamala* (Tejpat) leaves vary due to the topography and environment. And these constituents are responsible for the effective antimicrobial activities of Tejpat oils from Makwanpur district among which the oil from Tistung Botanical Garden (TBG) has the most significant antimicrobial activity. Based on these findings we can infer that cultivation of *C. tamala* (Tejpat) in Makwanpur district is quite encouraging for the farmers. However, since this finding is only based on single year data and sample collections were carried out in the same season limited to only three places and of random plants without considering studies on soil nutrients, physiochemical parameters, etc., so there are some limitations in our study and further research seems necessary.

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