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RESEARCH ARTICLE

# Determination of Phytoconstituents of *Dolichandrone Falcata* Seem. Flower through GC-MS

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## ABSTRACT

Objective: Investigation of biochemical components presents in the *Dolichandrone falcata* flowers by using GC-MS (Gas Chromatography – Mass Spectroscopy). Methods: The flowers of *Dolichandrone falcata* were collected, dried in shade and converted to the powder form. This powder is then extracted through Soxhlation method using methanol and chloroform solvents differently for GC-MS analysis. The concentrated extract is then analysed by GC-MS technique and the various secondary metabolites were identified using NIST library search. Results: The result shows the presence of different phytoconstituents which includes fatty acids, phenols, glycosides, ketones etc. having the properties such as allelochemic, anti-mutagenic, anti-septic, antibacterial etc. Conclusion: *Dolichandrone falcata* flowers contain the various phytoconstituent that can be useful in different fields like pharmaceuticals; perfume, drug development etc. and plant can be recommended as a plant of phytopharmaceutical importance.

## KEYWORDS

*Dolichandrone falcata*, GC-MS, Phytoconstituents, Secondary Metabolites

## INTRODUCTION

Nowadays medicinal plants are increasingly gaining acceptance even in literates of urban settlements. Probably this may be due to the inefficient response of modern drugs used for the control of many infections such as typhoid fever, gonorrhoea and tuberculosis, also the increasing resistance of several bacteria towards the antibiotics and increasing cost of prescription of drugs for personal health care purposes. Different plant species contains different bioactive compounds responsible for particular activity. This is may be due the presence of bioactive compound in them responsible for specific activity.

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If a single plant is used for various diseases it may contain more than one bioactive compound(s). If used in mixture they may show the synergic effect of two or more bioactive compounds. The use of different parts of plant for particular activity gives the idea that these bioactive compounds may be restricted to the particular part of plant body.[1,2,3]

Gas chromatography and mass spectroscopy technique is compatible in many ways. GC can separate the compounds of volatile and semi volatile nature with great efficiency but cannot identify them. On the other hand MS can identify the compounds with the great efficiency but cannot separate them. This technology provides its application in identification as well as quantification of organic compound which are volatile and semi volatile in nature present in complex biological mixture. It can determine

the molecular weights of compounds and elemental composition of unknown organic compounds. It can also elucidate the structure of unknown organic compounds in mixture by matching their spectra with reference spectra.[4] Combination of these powerful separation and detection techniques like gas chromatography and mass spectroscopy (GC-MS) provides the non-biased, large scale analysis of known and unknown metabolites present in the complex mixtures.[5]

*Dolichandrone falcata* Seem. Belonging to family Bignoniaceae is a small to medium size tree distributed in the central and southern parts of India. Flowers are terminal, few flowers are recemes, pedicel is 1.3-2 cm long, pubescent, with short stout mucro at apex. Corolla is white in colour, 2-5 cm long. Capsules are flat, much falcately curved, 25-45 cm long and 2 cm wide.[6] *Dolichandrone falcata*, an aboriginal plant has been a very fundamental part of the life of tribal in Northeast Maharashtra to cure stomach problems.[7] It is a traditional medicinal plant of Ayurveda used for the purpose of abortion and fish poisoning.[8] The plant leaves are reported to have anxiolytic effect on rats.[9] The bark paste of *Dolichandrone falcata* is applied in case of fractures.[10] The bark juice is used for menorrhagia and leucorrhoea.[11] The leaves of this plant are used as antioxidant, antiestrogenic and antidiabetic.[12,13] The leaves of this plant were found to possess higher content of Vitamin E and display the presence of other important secondary metabolites.[14] Despite of these applications this plant is yet to be worked out for its chemical composition. As there are no reports on phytoconstituents in the flowers of this plant, the present study aims at the identification of phytoconstituents from *Dolichandrone falcata* flowers.

## MATERIALS AND METHOD

### Collection of Plant Material

The flowers of *Dolichandrone falcata* were collected from Melghat forest of Amravati district, Maharashtra, India. The collected flowers were carefully examined for infected parts and were removed accordingly. Only fresh flowers were taken for the analysis. These flowers were dried in the shade till all its moisture gets evaporated. These dried leaves then pulverized to the powder form for further analysis.

### Extraction

5 gram of flower powder was extracted using Soxhlet apparatus for 24 hours in methanol and chloroform solvents separately. These extract then evaporated to dryness. At the time of analysis dried extract was dissolved in same solvent and these samples taken for GC – MS analysis.

### GC – MS Analysis

The analysis was carried out using gas chromatography – high resolution mass spectrophotometer. Dried extract were dissolved in the 5 ml of respective solvents. 2 ul of this solution is employed for GC – MS analysis. The GC-MS analysis was carried out using Alegent Hp 7880 with column of 30 meter length, 0.25 mm ID, and 0.32 thicknesses. Helium gas is used as carrier gas at constant flow rate of 1ml/ minute. Injector temperature was set at $100^0$C. The oven temperature were programmed from $50^0$C to $280^0$ C at $10^0$ C/ minute to $200^0$C then $10^0$C/3 minutes to $250^0$C ending with a 5 minutes isothermal at $280^0$C. The sample was injected in split mode as 50:1. Identification of the compounds was done by comparing the spectral data of sample compound with the compound spectra present in spectral libraries (NIST).

## RESULTS

The flowers extracted in methanol shows the presence of sixteen phytoconstituents. Figure 1 represents the chromatogram of methanol extract and table 1 represents the phytoconstituents identified in the methanol extract with their retention times, relative percentage, molecular weight and molecular formula of metabolites. Figure 2 displays the structure of identified compounds. The flowers extracted in chloroform solvent shows the presence of six compounds. Figure 3 explore the chromatogram of chloroform extract and table 2

Table 1: Phytocomponents identified in methanol extract

| Sr. No. | R.T. | Name of Compound | Rel. % | MF | MW |
|---|---|---|---|---|---|
| 1 | 4.0 | Isobutaric acid | 8.88 | $C_4H_8O_2$ | 88.05 |
| 2 | 4.3 | 2-Propanone 1,1-dimethoxy | 2.98 | $C_5H_{10}O_3$ | 118.13 |
| 3 | 6.0 | Propanal, 2,3-dihydroxy | 2.76 | $C_3H_6O_3$ | 90.08 |
| 4 | 9.9 | Amino ethyl phosphonic acid | 1.90 | $C_2H_8NO_3P$ | 125.06 |
| 5 | 11.3 | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 2.53 | $C_6H_8O_4$ | 144.13 |
| 6 | 12.4 | 2-Furancarboxaldehyde, 5-(hydroxymethyl)- | 4.95 | $C_6H_6O_3$ | 126.11 |
| 7 | 14.1 | 2,3 Pinanediol | 1.92 | $C_{10}H_{18}O_2$ | 170.25 |
| 8 | 15.2 | 1'-Hydroxy-4,3'-dimethyl-bicyclohexyl-3,3'-dien-2-one | 18.68 | $C_{14}H_{20}O_2$ | 220.31 |
| 9 | 15.2 | 2-Cyclohexen-1-one, 3-methyl- | 21.26 | $C_7H_{10}O$ | 110.15 |
| 10 | 15.4 | Dodecanoic acid, 3-Hydroxy | 4.13 | $C_{12}H_{24}O_3$ | 216.32 |
| 11 | 16.2 | Methyl 3,4-ethyliden-.alpha.-D-galactopyranoside | 8.21 | $C_9H_{16}O_6$ | 220.22 |
| 12 | 16.6 | Coumarine, 3,4,5,6-tetrahydro-6,8a-epidioxy-4a-methyl- | 4.26 | $C_{10}H_{12}O_4$ | 196.20 |
| 13 | 16.7 | 6-dedecanol | 4.53 | $C_{12}H_{26}O$ | 186.33 |
| 14 | 17.0 | Decane, 1,1'-oxybis | 3.29 | $C_{20}H_{42}O_2$ | 314.55 |
| 15 | 17.3 | p-Cresol, 2-ehtyl | 1.45 | $C_9H_{12}O$ | 136.19 |
| 16 | 23.7 | 10-Undecencic acid, octyl ester | 3.28 | $C_{19}H_{36}O_2$ | 296.48 |

Table 2: Phytocomponents identified in chloroform extract

| Sr. No. | R.T. | Name of Compound | Rel. % | MF | MW |
|---|---|---|---|---|---|
| 1 | 4.2 | 1,2,3,4-Pentatetraene | 4.22 | $C_5H_4$ | 64.08 |
| 2 | 4.6 | Benzothiazole, 2,6-dichloro | 84.74 | $C_7H_3Cl_2NS$ | 204.07 |
| 3 | 15.2 | 2-Cyclohexen-1-one,6-(1-hydroxy-1-methylethyl)-3-methyl | 4.29 | $C_{10}H_{16}O_2$ | 168.23 |
| 4 | 15.4 | Dodecanoic acid, 3-Hydroxy | 1.04 | $C_{12}H_{24}O_3$ | 216.32 |
| 5 | 17.0 | 2-Octene, 2,6-dimethyl | 1.22 | $C_{10}H_{20}$ | 140.27 |
| 6 | 23.7 | n-Heneicosane | 1.50 | $C_{21}H_{44}$ | 296.57 |

Demonstrate the identified metabolites in chloroform extract with their retention times, relative percentage, molecular weight and molecular formula of metabolites. Figure 4 represents the structures of identified metabolites.

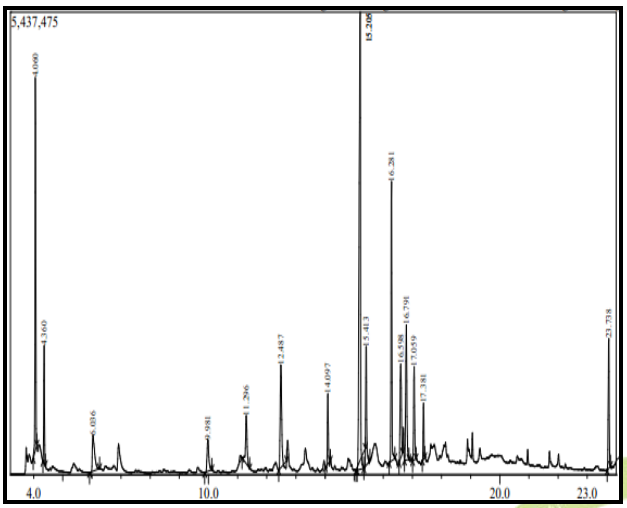
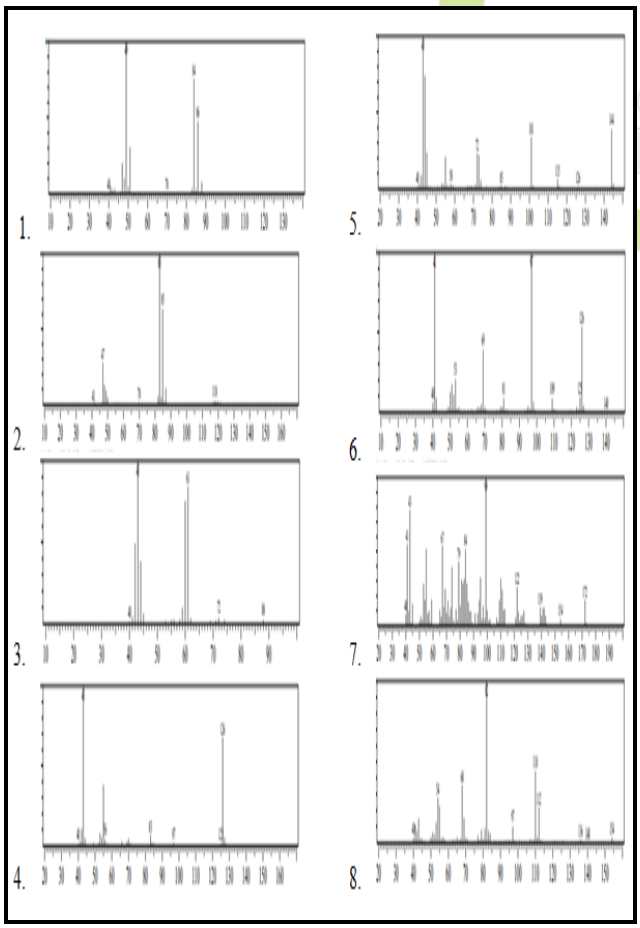
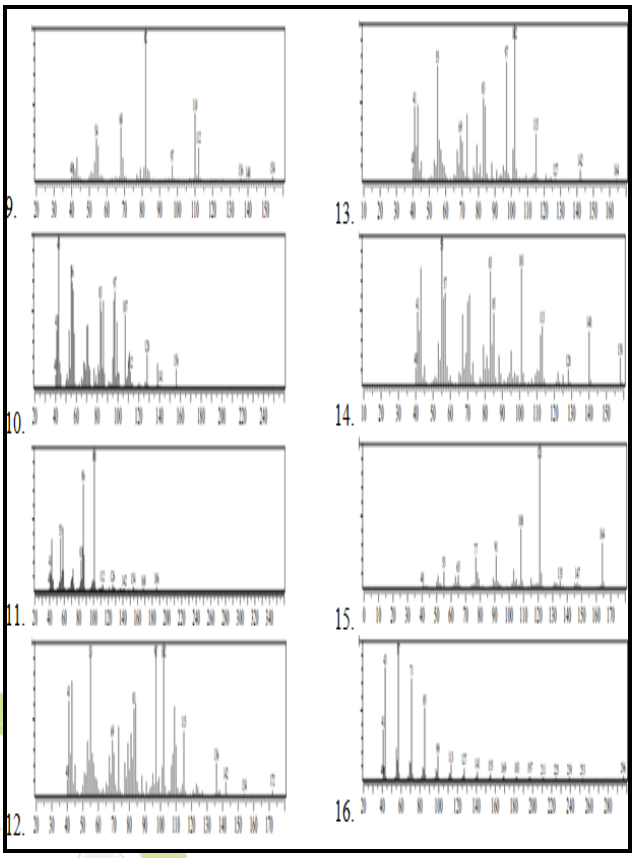



Figure 1: The total ion chromatogram of methanol extract showing Peaks with retention times




Fragmentation patterns of identified compounds correspond to Sr. No. shown in table 1.

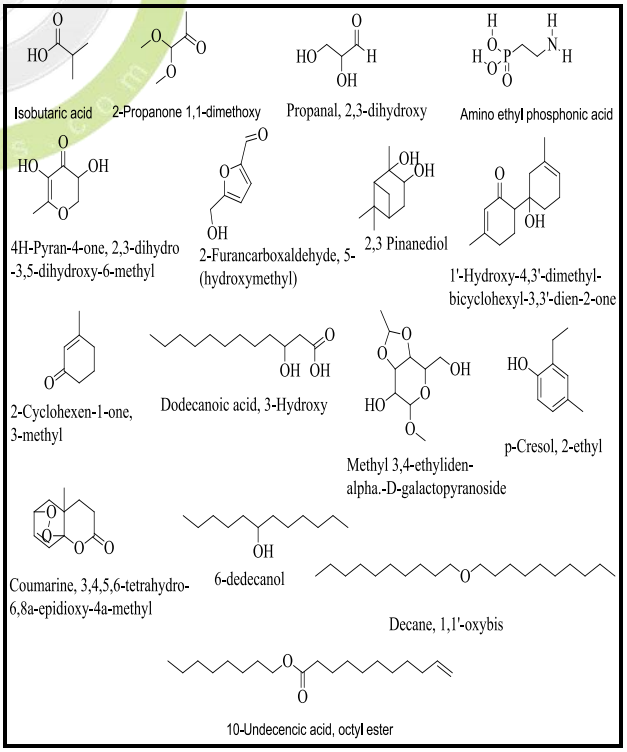



Figure 2: Structures of the compounds identified in above sample

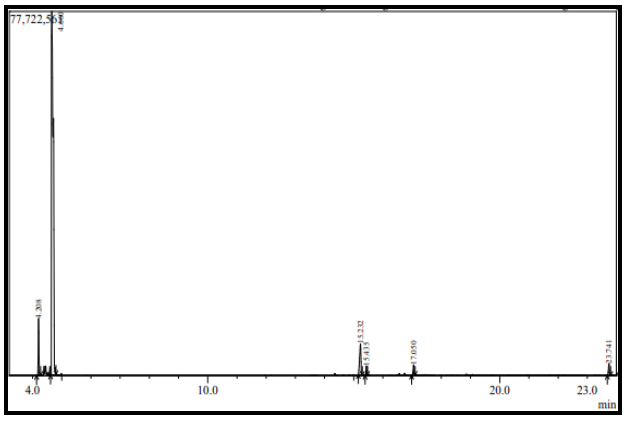
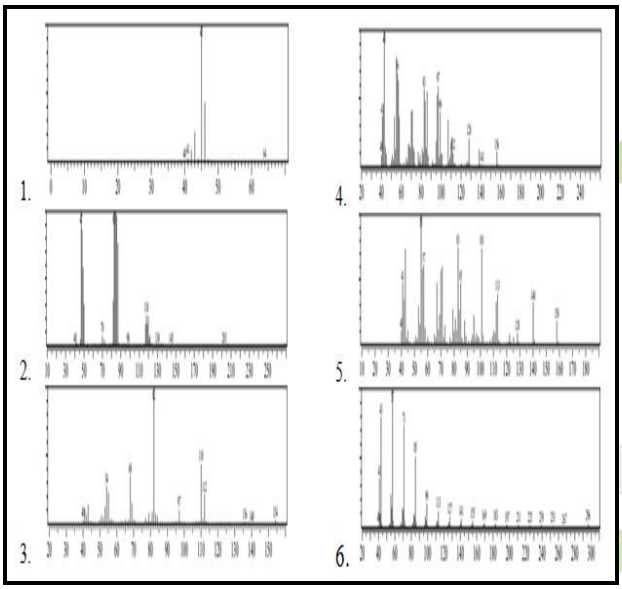



Figure 3: The total ion chromatogram of chloroform extract showing peaks with retention times




Fragmentation patterns of identified compounds correspond to Sr. No. shown in table 2.

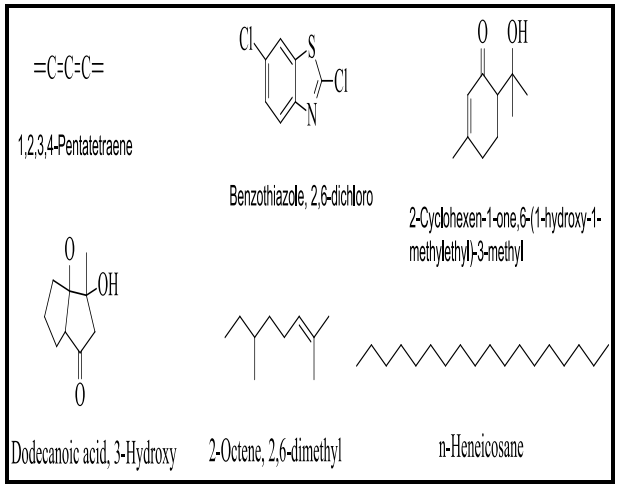



Figure 4: Structures of the compounds identified in above sample

## DISCUSSION

In the present investigation flowers of *Dolichandrone falcata* were extracted using methanol and chloroform solvent followed by the GC – MS analysis which authenticates the sixteen and six compounds in each respective sample. Methanol extract observed the presence of some fatty acids such as Isobutaric acid, Dodecanoic acid, 3-Hydroxy and 10-Undecencic acid, octyl ester. Flower extracted in methanol solvent show the presence of 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 2-Cyclohexen-1-one, 3-methyl, 2-Furancarboxaldehyde, 5-(hydroxymethyl)-, 2,3 Pinanediol, 1'-Hydroxy-4,3'-dimethyl-bicyclohexyl-3,3'-dien-2-one, Methyl 3,4-ethyliden-.alpha.-D-galactopyranoside, Coumarine, 3,4,5,6-tetrahydro-6,8a-epidioxy-4a-methyl-, Amino ethyl phosphonic acid and p-Cresol, 2-ethyl 2-Furancarboxaldehyde, 5-(hydroxymethyl), 2-Cyclohexen-1-one, 3-methyl and 1'-Hydroxy-4,3'-dimethyl-bicyclohexyl-3,3'-dien-2-one as secondary compounds.

Methyl 3,4-ethyliden-.alpha.-D-galactopyranoside mass spectrum shows the base peak at 101 and other fragments as 186, 168, 154, 126, 112, 86, 55, 41 etc. 4H-Pyran-4-one, 2,3-dihydro-3,,5-dihydroxy-6-methyl mass spectrum shows the mass peak at 144 and base peak at 43. Other fragments includes the 126, 115, 101, 97, 82, 68, 54, 43 etc. furancarboxaldehyde, 5-(hydroxymethyl)- mass spectrum shows the base peak at m/z 97 and mass peak at 126 and other major fragments at m/z 123, 109, 95, 81, 69, 55 and 41. Also known as hydroxymethylfurfural is an organic compound found as result of dehydration of certain sugars. This compound is yellow in colour and highly soluble in water. Its structure contains the furan ring, aldehyde and alcohol functional groups. It is observed in variety of heat processed foods such as milk, fruit juice, spirits, honey etc. Hydroxymethylfurfural is obtained from cellulose without the use of fermentation and is a potential source of carbon neutral feedstock for fuels and chemicals. Its derivative 5-hydroxy methyl-2-furfural has been

found to bind specifically with intracellular sickled haemoglobin and inhibits the formation of sickle cells in blood. Higher quantities of hydroxymethylfurfural can be found naturally in coffee and dried fruits.[15,16,17,18,19,20] P-Cresol, 2-ethyl mass spectrum shows the mass peak at 136 and base peak at 121 with fragments like 108, 91, 77, 65, 55, 40 etc, p-Cresol, 2-ethyl is a derivative of p-cresol. P-cresol is a natural phenolic compound reported from many plants and shown to have properties like allelochemic, anti-mutagenic, anti-septic, cancer preventive, flavour, parasiticide and rodenticide.[21]

2, 3 Pinanediol mass spectrum shows the base peak at 99 and mass peak 170 with other fragments at 154, 139, 122, 110, 84, 79, 67, 55, 43 etc. 2,3 Pinanediol is an alcoholic derivative of pinene; a monoterpine found in pine tree. This monoterpine commonly found in the essential oils and has economic importance in flavour and fragrance industries. The parent compound pinene is a major ingredient in turpentine and may have played significant role in the activity against the hydrocarbon degrading bacteria in nature.[22,23,24] Amino ethyl phosphonic acid mass spectrum shows the mass peak at 125 and base peak 43 with other fragments such as 97, 83, 70, 55, 43 etc. Amino ethyl phosphonic acid is an organophosphorous compound also known as ciliatine. Initially it is isolated from plant *Tetrahymena pyraformis* and in other ciliated organisms that's why the name cillatane is given to this compound. It is not only found in plants but also in animals including humans.[25,26] Coumarine, 3,4,5,6-tetrahydro-6,8a-epidioxy-4a-methyl mass spectrum shows the base peak at 102 with other fragments like 172, 154, 142, 136, 124, 115, 97, 83, 69, 55, 43 etc. Coumarine, 3, 4, 5, 6-tetrahydro-6,8a-epidioxy-4a-methyl- is cuamarin derivative compound. Coumarins constitute one of the major classes of naturally occurring compounds. There are four main classes of coumarins; Coumarin comes under the class of simple coumarins. Coumarin is the natural volatile active compound found in many plants.[27,28,29,20]

Flower extracted in chloroform shows the presence of Benzothiazole, 2,6-dichloro and 2-Cyclohexen-1-one,6-(1-hydroxy-1-methylethyl)-3-methyl as secondary metabolites. Benzothiazole, 2,6-dichloro mass spectrum shows the mass peak at 204 and base peak at 87 with fragments like 145, 130, 118, 98, 70, 47 etc. which matches accurately with standard NIST mass spectra of Benzothiazole, 2,6-dichloro (NIST Web book). 2-Cyclohexen-1-one,6-(1-hydroxy-1-methylethyl)-3-methyl mass spectrum shows the base peak at 92 with other fragments like 154, 140, 136, 112, 97, 86, 54, 40 etc. 2-Cyclohexen-1-one,6-(1-hydroxy-1-methylethyl)-3-methyl is also known as diosphenol, it is naturally occurring monoterpine kitone reported from plants like *Menth longifolia*, *Agathosma betaline* etc. This compound can acts as an antibacterial, antiseptic and diuretic. This compound is also reported from invertebrates like species of Diplosama.[30,20,31,32]

## CONCLUSION

The flowers of *Dolichandrone falcata* observed for the presence of important secondary metabolites like p-Cresol, 2-ethyl which possesses activities such as allelochemic, anti-mutagenic, anti-septic, cancer preventive, flavour, parasiticide and rodenticide. 2, 3 Pinanediol, a monoterpine has economic importance in flavour and fragrance industries. Diosphenol, a naturally occurring monoterpine kitone can acts as an antibacterial, antiseptic and diuretic. This shows the importance *Dolichandrone falcata* flowers; which can be utilized in various fields of research in near future.

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