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Comparative LC TOF/MS instrument analysis of *Olea dioica* Roxb. Infected with the rust fungus *Zaghouania oleae* (E.J. Butler) Cummins and non-infected plants

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Abstract
Fungus infection on plants induces the production of an array of secondary metabolites, and these have proved to be a gold mine to isolate new and novel molecules, useful for the treatment of various ailments. Once the properties of the secondary metabolites are revealed, it would replace conventional molecules and ultimately enhance the health industry. *Olea dioica* Roxb., belonging to the family 'Oleaceae' is a tree species seen to be infected with the rust fungus *Zaghouania oleae* (E.J. Butler) Cummins. The infection of *Z. oleae* is restricted to leaves and tender shoots, and it causes blisters on leaves, hypertrophy, Littling, thickening and unusual elongation of the infected shoot. The hypertrophied tender twigs are being cooked and eaten by Chenchu tribal ladies in Andhra Pradesh to get rid of infertility problems in women. In the present investigation, a comparative LC TOF/MS instrument analysis of leaves of *O. dioica* Roxb infected with *Z. oleae* (E.J. Butler) Cummins and non-infected plants has been carried out. The results confirmed the abundance of secondary metabolites in *O. dioica* Roxb infected with fungus in comparison with the non-infected leaves.

Keywords: *Olea dioica* Roxb. *Zaghouania oleae* (E.J. Butler) Cummins, infection, Phytochemistry, LC TOF/MS instrument analysis

1. Introduction
All plants in natural ecosystems are thought to be symbiotic with mycorrhizal and/or endophytic fungi [1], and the very existence of the fungi infected plants has been known for over a hundred years. The association between fungi and plants represents a unique balanced multipartite ecological relationship, which along with other abiotic components of nature forms a complex interplay of the components. The fungus infected plants, especially the ethnomedicinally important plants have been considered to be unique, and are viewed as an outstanding source of bioactive natural products [2]. The plants infected by fungi also provide an array of bioactive secondary metabolites with unique structures which include alkaloids, Benzopyranones, flavonoids, phenolic acids, quinones, steroids, terpenoids, tetralones, Xanthones, Chinones, phenols, Isocoumarins, Benzopyranones, Cytochalasins, enniatines and others [3, 4]. Most of the secondary metabolites are very good natural antioxidants [5] having a wide array of medicinal properties like Hepatoprotection, anti-inflammation, immunomodulation, Anti carcinogenicity, anti-infertility, etc. Thus the secondary metabolites produced upon fungus infection have proved to be a gold mine to isolate new and novel molecules, useful for the treatment of various ailments [6, 7]. Once the properties of the secondary metabolites are revealed, it would replace conventional molecules and ultimately enhance the health industry.

Olea dioica Roxb., belonging to the family 'Oleaceae' is a tree species commonly found distributed in the semi-evergreen and moist deciduous forests in diverse geographical niches of India. In Kerala, the plant is locally known as 'Edana'. Previously, the plant is known for its sporadic use as a medicinal plant. Often, during the winter season, the plant is seen to be infected with the fungus *Zaghouania oleae* (E.J. Butler) Cummins, belonging to the family Pucciniaceae [8]. The infection of *Z. oleae*is restricted to leaves and tender shoots, and it causes blisters on leaves, hypertrophy, littling, thickening and unusual elongation of the infected shoot [9]. These hypertrophied tender twigs are being cooked and eaten by Chenchu tribal ladies in Andhra Pradesh to get rid of female infertility disorders. The present study was undertaken to investigate comparative LCTOF/MS instrument analysis of fungus infected and non-infected leaves of *O. dioica*

2. Materials and Methods

Olea dioica Roxb, infected with the fungus *Zaghouania oleae* (E.J. Butler) Cummins and non-infected plants were collected from Wayanad district of Kerala and dried under shade for about three weeks. The dried material was powdered, 100g of the powdered material was extracted with methanol in a soxhlet for 72h. The extract was filtered and the filtrate was concentrated under reduced pressure in rotavapor R-215. The fungus infected plant extract is referred to as INF and the non-infected plant extract is referred to as NON. The extract was further subjected to comparative LCTOF/MS instrument analysis as per the instrumentation analysis conditions furnished below.

Instrument name - Xevo G2 Q-ToF
Ionization - ESI (electrospray ionization) negative Mode/positivemode

Analysis Conditions

Positive mode

Capilary voltage	-	3 KV
Sampling cone	-	30 V
Extraction Cone	-	2 V
Source temperature	-	135^0C
Dessolvation temp	-	350^0C
Cone gas Flow	-	50 L/h
Dessolvation gas	-	900L/h

Negative mode

Capilary voltage	-	2.5 KV
Sampling cone	-	30 V
Extraction Cone	-	3V
Source temperature	-	1350C
Dessolvation temp	-	3500C
Cone gas Flow	-	50 L/h
Dessolvation gas	-	900L/h

LC conditions

Name of instrument	-	Waters ACQUITY UPLC
Flow rate	-	0.25 ml/min
Injection volume	-	5 Micrelitter
Total run time	-	10 min
Solvent system	-	Water + 0.1% HCOOCH (A) and Methanol (C) gradient elution

Time	**A**	**C**
0 min	95	5
0.1 min	95	5
7 min	5	95
7.5 min	5	95
9 min	95	5

one min for stabilizatin

Column conditions; Aquity Uplc Beh C18, 1.7 micrometer particle size, 2.1X50mm

3. Results and Discussion

The fungus infected plants, especially ethnomedicinally important ones have been considered unique, and are viewed as an outstanding source of bioactive natural products. Upon infection with fungi, the plants synthesise an array of metabolites, many of which are bioactive. These compounds referred to as phytoalexins help the host plants overcome adverse effects. This forms a complex system with different classes of secondary metabolites that have immense utility, particulary from the therapeutic perspective. Since herbal drugs are a storehouse of an array of phytoconstituents, it has become essential to single out those concerned with therapeutic effects. The present study refers to comparative phytochemical evaluation of methanolic extract of *O. dioca* on the one hand and the fungus, *Z. oleae* infected extract of the plant on the other. Previously, such studies involving the screening of fungus infected ethnobotanical plants for their therapeutic value, particularly those involving phytochemical analysis is unique and rare. In the present investigation, LC/TOF-MS profile of both the extracts had been carried out. On LC/TOF-MS the fungus infected leaf extract showed M+ H+ major signals at 443.116, 471.147, 595.166, 693.218, 121.065, 357.1307, 323.111, 271.061, 307.152, 339.251 and 367.282. The non-infected leaf extract at M+ H+ showed major signals at 443.116, 471.146, 579.166, 623.195, 609.279, 725.206, 709.211 and 593 .276. Briefly, a difference in the major signals between the two has been observed.

The results from the study have shown that both the infected and non infected plants of *O. dioica* displayed more or less similar qualitative phytochemical profiles. However, distinction in some of the secondary metabolites has been observed between the two. Fungus infected plants exhibited a higher amount of these classes of compounds in comparison with the non infected. Similarly, LC/TOF-MS profile of the extracts also displayed significant differences with respect to the bands/signals. A difference in the major signals between the two has been observed in LC TOF/MS analysis. Obviously, this has been seen reflected in the efficacy of the drug to combat infertility disorders and the same substantiate the tribal claim. Probably, the differences visualized between the two extracts might be due to the variation of the phenolics including flavonoids. This is because, the phenolics including flavonoids have shown differences in their concentration in the previous preliminary qualitative phytochemical analysis studies [10]. However, it needs further corroboration by undertaking detailed studies.

4. Conclusion.

The study assumes importance from diverse perspectives. Primarily, this is the first report of a comparative preliminary qualitative phytochemical screening of fungus infected and non-infected leaf extracts of *O.dioica*. Secondly, the plants infected with fungi producing flavonones and phenols have been known to be combating various infertility disorders [11, 12]. Thirdly, this is an attempt for validation of the tribal claims concerning the evaluation of the medical efficacy of the fungus infected plants of *O. dioica.* The findings gleaned out of the present investigation points to the fact that significant production of an additional class of secondary metabolites in fungus infected plants and the same in turn substantiates the tribal claim. Hence these potential bioactive compounds can further be characterized and can also be used in the formulation of pharmaceutical drugs and other herbal products.

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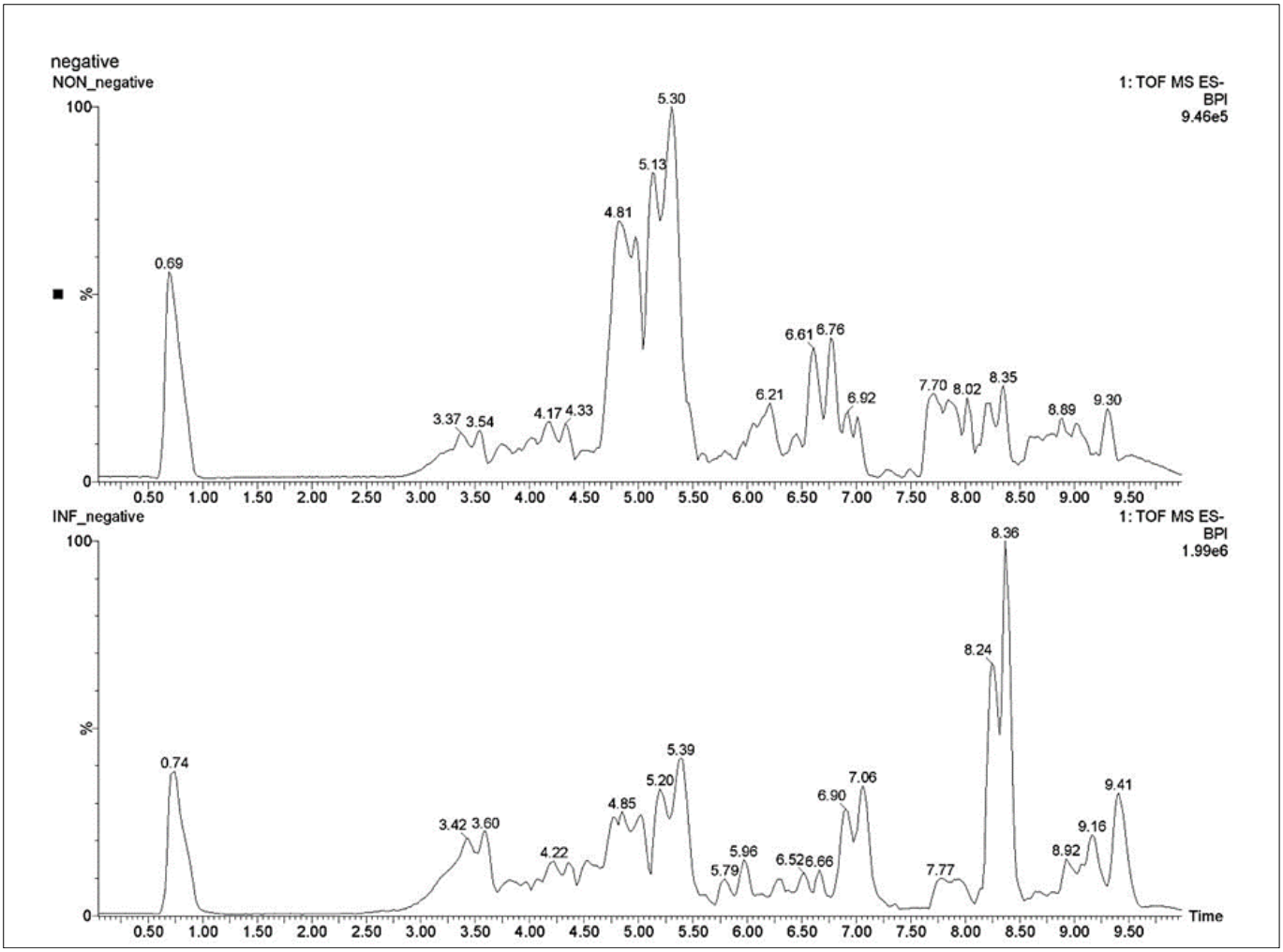
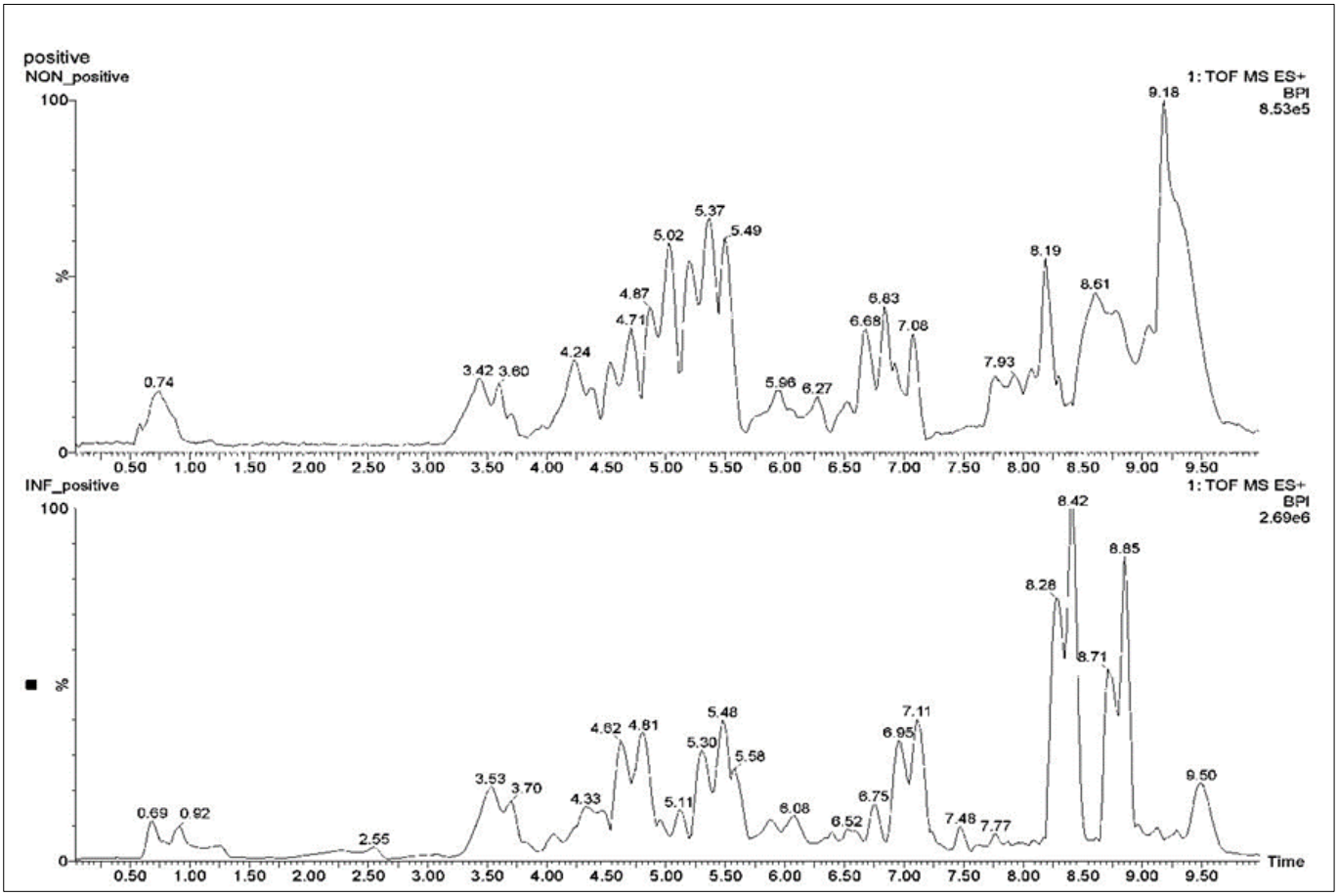
Table 1: Results of the LC TOF/MS analysis data of both fungus infected and non-infected extracts of *O. dioica*

Retention Time (minute)	Molecular weight of the compounds showed 00 % abundance		Molecular weight of the compounds showed showed less than 100 % abundance	
	INF	NON	INF	NON
3.701	443.1162	443.116	181.05	181.0498
4.24		595.1665		317.0592
4.36	595.1669		663.1898	
4.745		471.1467		237.0754
4.814	471.1474		181.0498	
5.039		579.1689		137.0601
5.37		623.1957		181.0499
5.458	121.0651		563.1741	
5.858	357.1307		434.1455	
5.998		323.1101		461.1417
6.032	323.1111		401.1213	
6.275		725.2069		523.1605
6.66		271.0599		745.2672
6.78	271.0612			
6.834		709.2117		507.1653
7.076	693.218		491.1713	
7.476	307.1525		193.0862	
8.417	339.2515		281.2485	
8.8		593.2769		319.221
8.851	367.2823		327.2897	
9.182		609.2711		331.2611



Graph 1: Results of the LC TOF/MS analysis data of both fungus infected (INF) and non-infected (NON) extracts of *O. dioica* in negative mode



Graph 2: Results of the LC TOF/MS analysis data of both fungus infected (INF) and non-infected (NON) extracts of *O. dioica* in positive mode.

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