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COMPOSITION OF ESSENTIAL OIL OF HEALTHY AND DISEASED INDIAN ROSEWOOD TREES (*DALBARGIA SISSOO* DC.)

SABA IDREES, MUHAMMAD ASIF HANIF*, MIRZA MUHAMMAD IKRAM, MUHAMMAD ADNAN AYUB[1], ASMA HANIF[2] AND AHMAD IRFAN[3]

Nano and Biomaterial Lab, Department of Chemistry, University of Agriculture, Faisalabad-38040-Pakistan

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Abstract

The evergreen rosewood tree (*Dalbargia sissoo* or Indian rose wood tree), was once a major source of fuel wood and later used in the furniture industry, is now dying in a slow and mysterious death across Pakistan, India and other South Asian countries. In the present study chemical changes produced in essential oil of diseased *Dalbergia sisso* and chemical derivatization of essential oil were investigated to determine antioxidant and antitermitic activities. The potential ability of secondary metabolites present in disease affected trees was much lower to act as effective antioxidants to fight against oxidative damage. Results obtained showed that Tetradecane, Tertadecene and their derivatives present in *Dalbergia sissoo* trees play very vital role for keeping trees healthy. The Tukey HSD test, Scheffé, Bonferroni and Holm multiple comparison post-hoc tests clearly showed that Tetradecane and its derivative are significantly different in healthy and disease affected trees from each other.

Introduction

Dalbergia (*Dalbargia sissoo*) belonging to *Fabaceae*, has more than 300 species which grow in many tropical and sub-tropical areas of Asia, Africa, South Africa, Central America, South America. *D. sisoso* DC. is known by different common names of Indian rosewood (English), Shisham (Urdu), Sisso (Spanish), Agaru (Hindi) and Tali (Punjabi). It is mostly present in Pakistan, Afghanistan, India, Bangladesh, Bhutan, and Malaysia. Indian rosewood is medium to large size tree. Its height is 25 meters with grey to yellow trunk. It can grow in somewhat saline soils. Seedlings cannot grow in shade (Bhattacharya *et al.* 2014). Both sapwood and heartwood have different color. It has ability to tolerate a temperature range of 4 - 49ºC. It is one of the most important forest trees because of its many uses such as furniture wood, agricultural implements, building timber, fuel purposes and plywood industries. It is cultivated in forest plantations as well as along the roadsides, canals, water channels and railway lines (Khan *et al.* 2004).

Dieback disease caused up to 55% mortality of the shisham population in various regions of subcontinent and caused up to 55% mortality of the shisham population in different regions of subcontinent (Shah *et al.* 2021). In Pakistan, *Dalbergia sissoo* 5% mortality occurred through 1990 - 1991, 43% in 2001 and 25% during 1999 - 2000 in different districts of Punjab province (Gill *et al.* 2001). Though in another detailed survey of Punjab, mortality of shisham has been recorded 20 - 40% beside highways and roadsides, 70 - 80% beside the canal. Though the main reasons for mortality of shisham are wilting and dieback, the underlying causes are various and interrelated. Almost 8.94 to 20.80% variation in mortality was reported in Shisham due to dieback in Eastern Utter Pradesh. According to previous studies, fungal pathogens were found as main

*Author for correspondence: <drmuhammadasifhanif@gmail.com>. 1Department of Chemistry, University of Sahiwal, Sahiwal, Pakistan. 2Department of Chemistry, Government College Women University Faisalabad (GCWUF), Faisalabad, Pakistan. 3Department of Chemistry, College of Science, King Khalid University, P.O. Box 9004, Abha 61413, Saudi Arabia..

cause of *D. sissoo* decline (Zakaullah 1999, Gill *et al.* 2001, Khan 2002, Idrees *et al.* 2006, Poussio *et al.* 2010). Khan (2000) reported that root rot fungus, *Ganoderma lucidum*, is a primary pathogen of dieback.

Secondary metabolites of low volume, highly concentrated, high valuable products and are manufactured and stored in glands which are present in several parts of plant like roots, seeds, fruits, flowers, barks and leaves (Perrino *et al.* 2021). There are many chemical and physical methods which are used for extraction of essential oil. The simple and traditional method which is used for extraction of essential oil is hydrodistillation. In agriculture essential oil has different applications like anti feedants, growth boosters, natural herbicides, repellents and botanical insecticides (Usman *et al.* 2013, Arshad *et al.* 2014, Rehman *et al.* 2016, Morah *et al.* 2017, Nadeem *et al.* 2017). The present study first time reports the essential oil composition of diseased *D. sissoo* trees.

Materials and Methods

Samples of healthy and diseased *Dalbergia sissoo* mature trees were collected from central Punjab, Pakistan using the Randomized Complete Block Design (RCBD) and wood were cut into pieces and then further processed through pilot scale hydrodistillation to obtain essential oil.

GC-MS analysis of the essential oils was done using Agilent-Technologies gas chromatographic (GC) system 7890A equipped with MS model 5975C using column HP-5MS (30 m × 250 µm × 0.25 µm) using helium (1.5 ml/min) as a carrier gas at 220 and 290°C, injector and detector temperatures, respectively in a column that was temperature programmed from 50 to 300 °C as follows: 50°C for 0 min then 10°C/min to 120°C for 2 min, then 5°C/min to 200°C for 4 min, then 10°C/min to 300°C for 5 min. The total run time was 44 min. At split ratio 1: 100, 1.0 µl of sample was injected. For MS detection was performed using ionization energy of 70 eV and quantification was made using built-in data-handling program.

Indian rosewood oil is a natural source of antioxidants. Its antioxidant activity was tested using standard methods of determining total phenolics contents, flavonoids contents, DPPH and reducing power ability assays used by Khan *et al.* (2012). Antifungal activity of *D. sisso* was determined against using *Aspergillus niger* by disc diffusion method. The zone of Inhibition was measured using digital Vernier caliper in mm. The no-choice bioassay method was used to assess the anti termitic activity of plant essential oil. Essential oil (0.01 g/ml) was dissolved in ethanol and sprayed to filter paper (Whatman 3, 8.5 cm diameter) placed in a Petri dish. Filter paper sprayed with only solvent was used as a test control. Moist sand (5g) was placed in bottom of Petri dish to control starvation. After the solvent was removed from treated filter paper through air drying at ambient temperature, active termites were put on filter paper in Petri dishes. The dishes were covered and placed in incubator at 25°C and 80% relative humidity. A few drops of water were periodically dripped onto the bottom edge of Petri dish. The mortality of termites was counted manually.

Results and Discussion

The essential oils of healthy and diseased *Dalbergia sissoo* mature trees extracted using hydrodistillation and yields were 0.20 and 0.01%, respectively. The steam and oil mixture were condensed in their liquid form, essential oil was lighter than water. It was easily separated using separating funnel (Ali *et al.* 2019). Table 1 showed a comprehensive comparison between the composition of secondary metabolites of healthy and disease affected trees. GC-MS analysis confirmed the presence of 26 compounds with a total percentage up to 99.96 and 99.99%, respectively in essential oils of healthy and disease affected *Dalbergia sissoo* trees. It is evident

from results that major components (>10%) present in the essential oil of healthy *D. sissoo* trees were Tetradecane, 2,6,10-trimethyl- (26.57%), Tetradecane (10.12%), and 7-methyl-Z-tertadecene-1 ol acetate (11.11%). Disease affected *D. sissoo* trees were found to contain Tetradecane, 2,6,10-trimethyl-, Tetradecane, and 7-methyl-Z-tertadecene-1 ol acetate 17.30, 5.36 and 7.47, respectively. The percentage of major components present in healthy *D. sissoo* trees essential oil found to decrease significantly in essential oil of disease affected trees. It was also found that percentage of lower molecular compounds was higher in disease affected trees. It is

Table 1. Chemical Components present in essential oils of healthy and disease affected *Dalbergia sissoo* trees.

Sl. No.	Retention time (min)	Compound name	% in healthy trees oil	% in diseased trees oil
1.	5.80	Cyclohexanol,2-methyl-5-(1-methylethenyl)-	0.11	1.63
2.	6.73	Undecane	0.21	2.73
3.	7.80	1,7,7-trimethyl-2-vinylbicyclo[2.2.1]hept-2-ene	0.35	1.81
4.	8.35	Dodecane	0.86	3.77
5.	8.59	Octadecane,6-methyl	0.21	1.25
6.	9.43	1,7,7-trimethyl-2- vinylbicyclo[2.2.1]hept-2-ene	0.43	1.70
7.	10.55	Tridecane	5.54	5.20
8.	11.30	(3S,9Z)-Heptadeca-1,9-diene-4,6-diyn-3-ol	0.97	1.69
9.	11.68	2,9-heptadecadiene-4,6-diyn-8-ol,(Z,E)	0.23	3.12
10.	12.61	2,9-heptadecadiene-4,6-diyn-8-ol,	0.54	1.59
11.	12.92	Tetradecane	10.12	5.36
12.	13.65	8-isopropyl-1,3-dimethyltricyclo[4.4.0.02,7]dec-3-ene	5.54	5.27
13.	14.36	(3S,9Z)-Heptadeca-1,9-diene-4,6-diyn-3-ol	3.12	3.61
14.	15.31	Tetradecane,2,6,10-trimethyl-	26.57	17.30
15.	16.53	1-heptatriacotanol	2.60	2.67
16.	16.98	1-methyl-6-methylidene-4-propan-2-yl-3,4,5,7,8,8*a*-hexahydro-2*H*-naphthalene-1,4*a*-diol	3.60	3.66
17.	17.66	Hexadecane	6.11	5.76
18.	18.21	3H-cyclodeca[b]furan-2-one,4,9-dihydroxy-6-methyl-3,10dimethylene-3a,4,7,8,9,10,11,11a-oct	2.61	2.50
19.	18.71	7-methyl-Z-tetradecen-1-ol acetate	3.11	3.14
20.	20.79	7-methyl-Z-tetradecene-1-ol acetate	2.21	3.29
21.	22.00	1-hexadecanol,2-methyl	1.97	2.00
22.	22.16	Octadecane,3-ethyl-5-(2-ethylbutyl)	1.24	2.80
23.	24.00	7-methyl-Z-tertadecene-1 ol acetate	11.11	7.47
24.	25.98	Octadecane,3-ethyl-5-(2-ethylbutyl)	6.68	6.57
25.	29.12	Dibenz[a,c]cyclohexane,2,4,7-trimethoxy-	1.12	1.10
26.	30.70	3-(Adamantan-2-yliden-methoxymethyl)-phenol	2.80	3.00
Total			99.96	99.99

well known that higher molecular weight compounds have higher ability to act as antioxidants. The potential ability of secondary metabolites present in disease affected trees was much lower to act as effective antioxidants to fight against oxidative damage. Results obtained also showed that Tetradecane, Tertadecene and their derivatives present in *D. sissoo* trees play very vital role for

keeping trees healthy. However, more studies are required to understand the role of Tetradecane, Tertadecene and their derivatives in *D. sissoo* trees disease protection. Results of statistical comparisons presented in Table 2 showed p-value corresponding to the F-statistic of one-way ANOVA was lower than 0.05, suggesting that percentages of Tetradecane and its derivative are significantly different in healthy and disease affected *Dalbergia sissoo* trees. The Tukey HSD test, Scheffé, Bonferroni and Holm multiple comparison post-hoc tests clearly identify that Tetradecane and its derivative are significantly different in healthy and disease affected trees from each other.

Table 2. Statistical comparison of Tetradecane, and its derivatives present in healthy and disease affected *Dalbergia sissoo* trees.

One-way ANOVA of your kk=2 independent treatments					
Source	Sum of squares SS	Degrees of freedom νν	Mean square MS	F statistic	p-value
Treatment	281.5350	1	281.5350	Inf	0.0000e + 00
Error	0.0000	4	0.0000		
Total	281.5350	5			
Tukey HSD results					
Treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inferfence		
A vs B	inf	0.0010053	** p<0.01		
Scheffé results					
Treatments pair	Scheffé TT-statistic	Scheffé p-value	Scheffé inferfence		
A vs B	inf	0.0000e+00	** p<0.01		
Bonferroni and Holm results: all pairs simultaneously compared					
Treatments pair	Bonferroni and Holm TT-statistic	Bonferroni p-value	Bonferroni inferfence	Holm p-value	Holm inferfence
A vs B	inf	0.0000e+00	** p<0.01	0.0000e+00	** p<0.01
Bonferroni and Holm results: only pairs relative to A simultaneously compared					
Treatments pair	Bonferroni and Holm TT-statistic	Bonferroni p-value	Bonferroni inferfence	Holm p-value	Holm inferfence
A vs B	inf	0.0000e+00	** p<0.01	0.0000e+00	** p<0.01

Total Phenolic Contents (TPC) in healthy and diseased *Dalbergia sissoo* mature trees analysed through Folin-Ciocalteu method were 672.28 ± 2.23 and 363.32 ± 1.23 µg GAE/ml of essential oil, respectively. Total Flavonoid Contents (TFC) in healthy and diseased *Dalbergia sissoo* mature trees were 215.14 ± 1.03 and 113.12 ± 1.20 µg QE /ml of essential oil, respectively. 2,2-Diphenyl-2-Picrylhydrazyl (DPPH) is a commercially available free radical generating compound and is widely used to determine the free radical scavenging capacity of plant (Khan *et al.* 2012). DPPH scavenging activities of healthy and diseased *Dalbergia sissoo* mature trees were 71.67 ± 0.87 and 51.67 ± 0.65 µg /ml of essential oil, respectively (Table 3). Compounds with high reducing power are strong antioxidants (Lakshmi *et al.* 2014, Yasmeen and Gupta 2021). Reducing power ability of healthy and diseased *D. sissoo* mature trees were 360.15 ± 2.10 and 140.11 ± 8.20 µg /ml of essential oil.

Table 3. Antioxidant activities of healthy and disease affected *D. sissoo* trees essential oil.

Essential oil/ Standard	Total phenolics (µg/ml)[A]	Total flavonoid contents (µg/ml)[B]	DPPH free radical scavenging activity (%)	Total antioxidant contents/FRAP[C]
Healthy trees oil	672.28 ± 2.23[a]	215.14 ± 1.03[b]	71.67 ± 0.87[b]	360.15 ± 2.10[a]
Diseased trees oil	363.32 ± 1.23[b]	113.12 ± 1.20[a]	51.67 ± 0.65[c]	140.11 ± 8.20[b]
BHT	-	-	98.21 ± 0.40[a]	-

Values are mean ± Standard Deviations of three separate determinations. Different letter in superscripts represent significant difference among healthy and disease affected *Dalbergia sissoo* trees essential oil. A=Total phenolic contents (µg/ml of essential oil, measured as Gallic acid equivalent). B = Total flavonol contents (µg/ml of essential oil, measured as Catechin equivalent). C = Total antioxidant contents/FRAP (µg/ml of essential oil, measured as Gallic acid equivalent).

Table 4. Anti-fungal activities of healthy and disease affected *Dalbergia sissoo* trees essential oil.

Sl. No.	Essential oil (µl)	Zone of inhibition (mm)	
		Healthy trees	Diseases Trees
1	2.5	8.04	3.27
2	5	12.03	5.54
3	10	16.37	7.32
4	Positive control	18.67	18.67

Table 5. Anti-termite activities of healthy and disease affected *Dalbergia sissoo* trees essential oil.

Sl. No	Essential oil conc. (µl)	Dead termite									
		1h		2h		3h		4h		5h	
		A	B	A	B	A	B	A	B	A	B
1	Blank	3	3	1	1	1	1	9	9	6	6
2	6.5	4	3	3	2	3	2	7	9	3	4
3	12.5	10	4	1	3	3	3	4	9	2	1
4	25	10	4	2	3	2	3	6	9	-	1
5	50	10	4	-	3	10	3	-	9	-	1

A = Healthy *Dalbergia sissoo* trees essential oil, B = Diseased *Dalbergia sissoo* trees essential oil.

Dalbergia sissoo essential oil have significant antifungal activity (Majeed *et al.* 2019). Essential oil of healthy and diseased *Dalbergia sissoo* mature trees method at different concentrations caused high mortality in the feeding tests, in short time exposure when no choice bioassay (Table 4). At various concentrations, essential oil of healthy trees showed higher anti-termite activities and much higher antifungal activities against *Aspergillus niger* as compared to disease affected trees (Table 5). These results clearly suggest that fungal fighting ability of disease affected tree was much affective. From the results it may be concluded that healthy trees were more resistant to fungus and termite attacks. It is further can be said that the Tukey HSD test, Scheffé, Bonferroni and Holm multiple comparison post-hoc tests clearly identify Tetradecane and its derivative are significantly different in healthy and disease affected trees from each other. Essential oil of healthy *Dalbergia sissoo* trees was found to contain higher antioxidant levels and Tetradecane, Tertadecene and their derivatives present in *D. sissoo* trees play very vital role for keeping trees healthy. However, more studies are required to completely understand the role of Tetradecane, Tertadecene and their derivatives in *D. sissoo* trees disease protection.

References

Ali I, Rizwani GH, Rasheed M, Ali M, Hassan A, Hassan S, Ishrat G, Zaheer E, Hussain K and Rehman S. 2019. Chemical analysis of *Dalbergia sissoo* (Roxb.) pod oil by (GC-MS)/GC-FID and evaluation of antioxidant potential. Pakistan J. Pharm. Sci. 32.

Arshad Z, Hanif MA, Qadri RWK and Khan MM. 2014. Role of essential oils in plant diseases protection: a review. Int. J. Chem. Biochem. Sci. **6**: 11-17.

Bhattacharya M, Singh A and Ramrakhyani C. 2014. *Dalbergia sissoo* - An important medical plant. J. Med. Plants 2.

Gill M, Ahmed I, Khan A, Aslam M, Ali S, Rafique M and Khan M. 2001. *Phytophthora cinnamomi* – a cause of Shisham decline in Punjab, Pakistan. *Proc. 3rd Nat. Conf. Plant Pathol. (Oct)*, 1-3.

Idrees M, Zaidi S, Khan A and Akhtar A. 2006. Studies on mycoflora of Shisham and development of dieback disease. *Third Nat. Sem. Shish. Dieback, May 11, 2006, Faisalabad, Pakistan*: PFRI.

Khan M. 2000. Shisham die-back in Pakistan and remedial measures. Shisham die-back in Pakistan and remedial measures. 45-49.

Khan MM, Hanif MA and Abraham AS. 2012. Variations in basil antioxidant contents in relation to deficit irrigation. J. Med. Plants Res. **6**: 2220-2223.

Khan S. 2002. Tree decline in Pakistan. Integrated plant disease management". *Proc. 3rd Nat. Conf. Plant Pathol.* 15-16.

Khan SH, Idrees M, Muhammad F, Mahmood A and Zaidi SH. 2004. Incidence of shisham (*Dalbergia sissoo* Roxb.) decline and *in vitro* response of isolated fungus spp. to various fungicides. Int. J. Agril. Biol. **6**: 611-614.

Lakshmi TM, Radha R and Jayshree N. 2014. *In vitro* antioxidant activity, total phenolic and total flavonoid content in extracts from the bark of *Dalbergia sissoo* Roxb. Int. J. Pharma Sci. Res. **5**: 226-231.

Majeed FA, Munir H, Rashid R and Zubair MT. 2019. Antimicrobial, cytotoxicity, mutagenicity and anti-epileptic potential of ethanol extracts of a multipurpose medicinal plant *Dalbergia sissoo.* Biocata. Agricul. Biotechnol. 19: 101155.

Morah FN, Emehige EP and Mowang MM. 2017. Chemical composition and antimicrobial activity of *Nauclea latifolia* leaf essential oil. International J. Chem. Biochem. Sci. **11**: 44-50.

Nadeem F, Azeem MW and Jilani MI. 2017. Isolation of bioactive compounds from essential oils–A comprehensive review. Int. J. Chem. Biochem. Sci. **12**: 75-85.

Perrino EV, Valerio F, Gannouchi A, Trani A and Mezzapesa G. 2021. Ecological and plant community implication on essential oils composition in useful wild officinal species: A pilot case study in Apulia (Italy). Plants **10**: 574.

Poussio GB, Kazmi MR, Akem C and Fateh FS. 2010. First record of Ceratocystis fimbriata associated with shisham (*Dalbergia sissoo*) decline in Pakistan. Australasian Plant Disease Notes **5**: 63-65.

Rehman R, Hanif MA, Mushtaq Z, Mochona B and Qi X. 2016. Biosynthetic factories of essential oils: The aromatic plants. Nat. Prod. Chem. Res. **4**: 227-238.

Shah KK, Tiwari I, Modi B, Pandey HP, Subedi S and Shrestha J. 2021. Shisham (*Dalbergia sissoo*) decline by dieback disease, root pathogens and their management: A review. J. Agric. Nat. Reso. **4**: 255-272.

Usman L, Ismaeel R, Zubair M, Saliu B, Olawore N and Elelu N. 2013. Comparative studies of constituents and antibacterial activities of leaf and fruit essential oils of *Ocimium basilicum* grown in north central Nigeria. Int. J. Chem. Biochem. Sci. **3**: 47-52.

Yasmeen S and Gupta P. 2021. Cosmeceutical and anticancer potential of aqueous extracts of *Dalbergia sissoo* Roxb. aerial parts. J. Herb. Med. 100456.

Zakaullah C. 1999. Shisham decline in Pakistan. *Proc. Nat. Conf. Plant Path. Sep*. 27-29.

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