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SWEET BAY (*LAURUS NOBILIS* L.) ESSENTIAL OIL: A STUDY ON ITS APPLICATION IN DENTISTRY.

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ABSTRACT

Dental plaque is an oral biofilm that adheres to teeth, comprising of more than 700 species of bacteria and fungi. It is one of the etiological factor leading to oral related diseases namely gingivitis, periodontitis and dental caries. Therefore its treatment becomes a crucial factor in maintaining oral health. Use of plant essential oils in the treatment of dental plaque can be beneficial due to the antimicrobial and non-toxic properties making them ideal for their use in oral care products. Since ages, essential oils have been researched and there is evidence for their long term effects in controlling dental plaque. The present study aims to screen the efficacy of *Laurus nobilis* essential oil in treating dental plaque. The essential oil was tested against three dental pathogens –*S.mutans, L.acidophilus and C.albicans*. The results demonstrated that *L.nobilis* essential oil showed inhibition against the three dental pathogens and proves its efficacy to be used as an antimicrobial agent to control dental plaque.

KEYWORDS: Dental plaque, essential oil, *L.nobilis*, disc diffusion.

INTRODUCTION

Aromatic plants are those that have the property of enhancing aroma and flavour of food and are used as spices. Along with this property, they also have medicinal uses and can be used as food preservative. The extracts and essential oils extracted from these plants are used in therapeutics.[1] *Laurus nobilis* is one such aromatic shrub or small evergreen ornamental tree belonging to family Lauraceae, commonly called as sweet bay, bay laurel, Grecian laurel, true bay and bay. Leaves of *Laurus nobilis* L have been used in the past in European folk medicine and is an evergreen tree native to the Mediterranean region. Traditionally the leaves have been used in flavouring and increasing the storage life of foods.[2]

Our oral cavity is a busy place with millions of microorganisms on the move, among which bacteria predominates the oral microbial population. Some of these bacteria cause harm directly to the teeth and gums, residing as a yellowish or white sticky film on the teeth called as plaque. Dental plaque is the community of microorganisms found on a tooth surface as a biofilm, embedded in a matrix of polymers of host and bacterial origin. It is natural and contributes to the normal development of the physiology and defences of the host like any other resident microflora present in the other parts of the body.[3] Since our oral cavity is under constant bombardment with food and drinks, the homeostasis between the oral microflora is disturbed causing a shift from a healthy to diseased oral condition . This shift in turn leads to the most common oral diseases – gingivitis, periodontitis and dental caries suffered by most of the people worldwide due to improper oral habits. Of clinical relevance, biofilms are less susceptible to anti-microbial agents. Along with the conventional oral hygiene techniques, a number of mouth rinses have known to subside the growth of oral microbes accompanying many side effects. This scenario has necessitated the search for an alternative to incorporate in oral hygiene products. Particularly, there has been an increased research in the effectiveness of essential oils of aromatic plants on human health. Essential oils a mixture of terpenoids (monoterpenes, sesqueterpenes and diterpenes) which contribute to its anti-microbial and antioxidant properties, serve as a potential alternative to synthetic anti-microbial agents. Despite advances in the application of essential oils on human health, there are very less studies on the application of *L.nobilis* essential oil in the field of dentistry.[4] Therefore, the aim of the present study was to analyze the chemical composition of the bay essential oil using Gas Chromatography – Mass Spectroscopy and investigate the growth inhibitory effects of the essential oil extracted from *Laurus nobilis* against a panel of microbial strains (bacterial and fungal) associated with dental plaque.

MATERIALS AND METHODS

Collection and Extraction of the essential oil.

Fresh leaves of Sweet bay (*L.nobilis*) used for the study were harvested from the Aromatic garden at The Department of Horticulture, UAS (B), GKVK, Bangalore. The leaves were dried for one week at room temperature prior to hydrodistillation. A known weight of dried leaves was loaded in the Clevenger's apparatus for the extraction of essential oil for three hours. The yield (%) of the essential oil was tabulated.

Quality analysis of the essential oil

Physical and Chemical Parameters

The quality of sweet bay essential oil was analyzed through physiochemical parameters namely Refractive Index(25°C), Color, Acid value, Ester value and Saponification value.

Gas Chromatography- Mass Spectrometry analysis

The chemical composition of the sweet bay essential oil was determined using GC-MS. The oil was analyzed by Agilent equipment, model GC-7890 with FID, MS- 5975 C with inert MSD. Equipped with a DB -17 MS J & W 122-4762, Silica Capillary column of 60 m*0.25 mm* 0.25 um. The carrier gas used was helium at a flow rate of 1 mL/min, detector and injector temperatures were 280 °C and 250 °C, respectively, and the injection volume was 0.2 µL and the split ratio was 30:1. The oven temperature was programmed from 70^0C at 2^0C/min upto 100^0C at 2.6^0C/min and upto 200^0C at 5^0C/min till 270^0C. Identification of compounds was done with multi databases including Wiley/Nist/ SOM.

Antimicrobial Analysis of the essential oil

The organisms selected for the study were Gram positive bacteria – *Streptococcus mutans* (MTCC 497) and *Lactobacillus acidophilus* (MTCC 10307) and fungi *Candida albicans* (MTCC 7315) procured from MTCC, Institute of Microbial Technology, Chandigarh.

S.mutans was propagated for 24 hours on Brain and Heart Infusion Agar at 37°C under anaerobic condition ; *L.acidophilus* was grown on Lactobacilli MRS agar plates incubated at 37°C for 48 hours and *C.albicans* propagated on Yeast Mannitol agar for 48 hours at 25°C. Assessment of the growth inhibitory effect of sweet bay essential oil was done by disc diffusion method.

S.mutans was resuspended in peptone water (10%), *L.acidophilus* was resuspended in Lactobacilli MRS broth and *C.albicans* was resuspended in Yeast Mannitol broth and incubated for 24 hours. The 24 hour microbial cultures were used for the antimicrobial assay and were spread over their respective culture media agar plates. Sterile paper disc of 6*mm* diameter was aseptically saturated with 30µl of the respective volatile oil obtained from *L.nobilis* for an hour and were placed on the respective agar plates and incubated at their respective growth conditions.

The results were recorded as three independent observations by measuring the zone of growth inhibition (*mm*) around the disc. The recorded inhibition zone (*mm*) of the sample were compared with the inhibition zone of the standard antibiotics (Ampicillin/Sulbactum (A/S $^{10/10}$), Streptomycin (S ^{10} 10mcg/disc) procured from HiMedia an Flucanazole(100mg).

RESULTS AND DISCUSSION

Microflora accumulated in the oral cavity are responsible for dental caries. It has been reported that essential oils are capable of inhibiting the growth of these microorganisms and formation of the biofilm.[5] Several antibiotics such as ampicillin and cholorhexidine have been very effective in preventing oral related problems but adverse effects such as vomiting, diarrhea, tooth staining have been associated with the use of these chemicals.[6] In the present study, the yield of the essential oil extracted from *L.nobilis* was 0.9% (v/w)(on dry weight basis) after three hours of hydro distillation. The oil yield reported was comparable with the essential oil extracted in various parts of the world, which is in the range of 0.59-4.3%(v/w).[7,8,9]

Quality Assessment of the essential oil

Physical and Chemical Characteristics of the oil

Physicochemical properties of the essential oil can be used as a criterion to evaluate the purity of oil.[10] The essential oil of *L.nobilis* was analyzed for physicochemical parameters like color, Refractive Index, acid value, ester value and saponification value. The results obtained were tabulated (Table 1) with values within the range of previous studies. [11,12]

Table 1 – Physiochemical characteristics of *Laurus nobilis*.L essential oil.

Parameter	*L.nobilis* essential oil
Oil yield (%)	0.9 %
Color	Pale yellow
Refractive Index	1.4700
Acid value	2.8055
Saponification value	194.51
Ester value	191.7

Gas Chromatography-Mass Spectroscopy Analysis(GC-MS)

The GC-MS analysis of sweet bay essential oil (Fig 1) reported 37 compounds representing 98.25% of the total composition (Table 2). The prominent constituents characterized were 1,8 cineole(32.97%), linalool(11.41%) and α-terpinyl acetate(12.56%).

Table 2: Chemical composition of *L.nobilis* essential oil using GC-MS

Sl.No.	Component	AREA %	20	Nerol	0.19
1	Alpha Thujene	0.35	22	Bornyl Acetate	0.26
2	Alpha pinene	3.06	23	Alpha Terpinyl Acetate	12.56
3	Camphene	0.21	24	Eugenol	3.53
4	Sabinene	5.94	25	Beta Elemene	0.43
5	Beta Pinene	2.39	26	Methyl Eugenol	5.91
6	Beta myrcene	0.31	27	Caryophyllene	1.06
7	Alpha Terpinene	0.28	28	Alpha Humulene	0.18
8	P Cymene	0.34	29	Bicyclogermacrene	0.19
9	Limonene	1.18	30	Delta Cadinene	0.18
10	1,8 Cineole	32.97	31	Elemicin	0.25
11	Gamma Terpinene	0.62	32	Nerolidol Isomer	3.04
12	Trans Sabinene Hydrate	0.51	33	Spathulenol	0.66
13	Alpha Terpinolene	0.20	34	Caryophyllene oxide	1.70
14	Linalool	11.41	35	Alpha Cadinol	0.11
15	Cis Sabinene Hydrate	0.46	36	Tau Muurolol	0.30
16	Camphor	1.28	37	Alpha Eudesmol	0.53
17	Terpineol	0.33	38	other	1.75
18	Terpin 4 ol	2.38			
19	Alpha Terpineol	2.95			

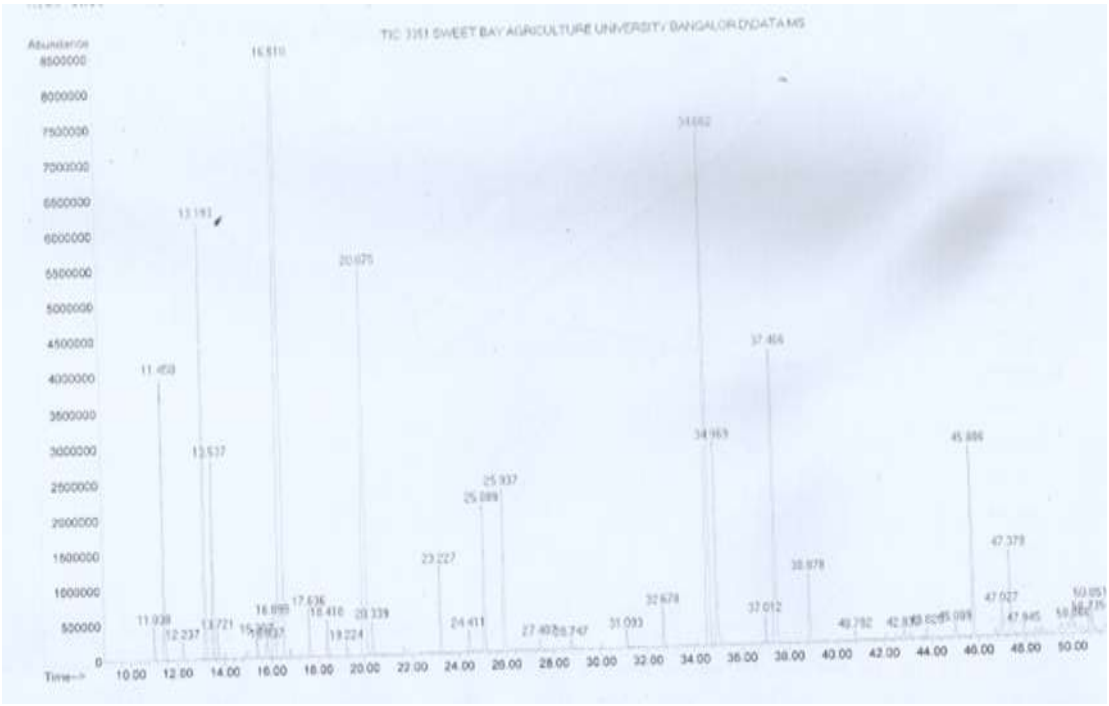

Figure 1: GC-MS Chromatogram of *L.nobilis* essential oil

The characteristic odor can be attributed to the composition of the essential oil. It has previously been reported that 1,8 cineole, linalool and α-terpinyl acetate are known to possess various pharmacological properties like anti-cancerous, anti-inflammatory and antimicrobial

property.[7,8,13] Thus sweet bay can be considered as an oil with high therapeutic value. From the chemical composition of *L.nobilis* essential oil it is evident that 1,8-cineole is the major component present in the oil and can be considered as a sustainable source of 1,8 cineole as compared to other aromatic plants such as *Ocimum sanctum* and *Artemesia sieberi* having 20.78% and 21.1% of 1,8 cineole content respectively.[5]

Assessment of Growth Inhibitory Effects of the Essential Oil

Anti-microbial activity of the essential oil against common oral pathogens was tested. The results were tabulated in terms of inhibition zone measured in mm. *L.nobilis* oil showed inhibition against all the three pathogens tested. Complete inhibition was seen for *Streptococcus mutans* (90±0mm) and *Lactobacillus acidophilus* showed inhibition zone of 11.67 ±0.41mm comparable with the standard antibiotics. While the oil showed inhibition against *Candida albicans* with an inhibition zone of 24.61±1.47mm as compared with Flucanazole standard (Table 3; Fig.2a,2b).

Table 3: Assessment of Growth Inhibitory effect of *L.nobilis* essential oil.

Oral pathogen	Zone of Inhibition (mm)			
	L.nobilis	**Ampicillin**	**Streptomycin**	**Flucanazole***
Streptococcus mutans	Complete Inhibition (90±0)	13.67±0.41	28.67±0.82	NA
Lactobacillus acidophilus	11.67±0.41	29.00±0.0	13.33±0.41	NA
Candida albicans	24.61±1.47	NA	NA	29.33±0.82

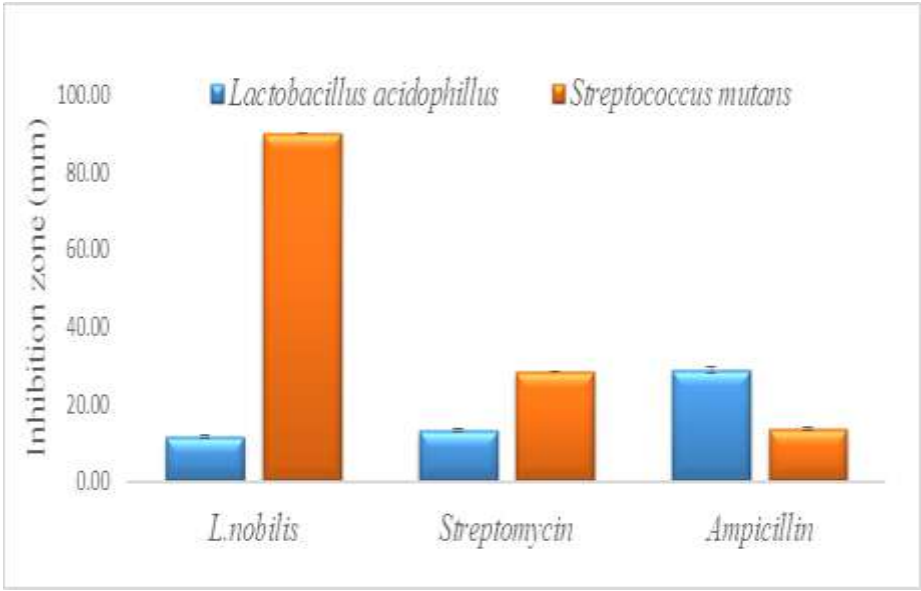

Figure 2a: Antibacterial activity of *L.nobilis* essential oil.

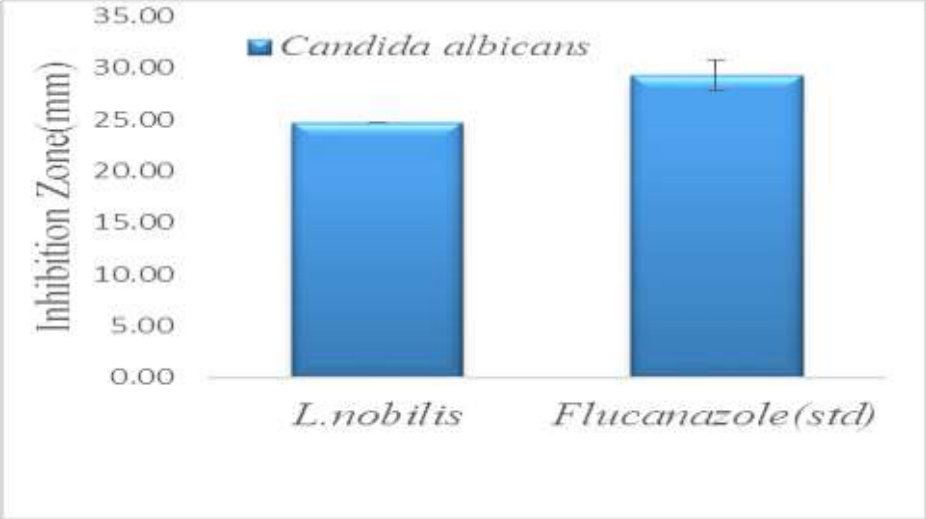

Figure 2b: Antifungal activity of *L.nobilis* essential oil

L.nobilis essential oil was effective against the 3 dental pathogens tested – *Streptococcus mutans*, *Candida albicans* and *Lactobacillus acidophilus*. *S.mutans*, one of the most important oral bacteria and also an initiator of dental biofilm formation having cariogenic property. Therefore, it becomes important to control mouth flora especially *S.mutans*. *Lactobacillus acidophilus* was considered as a major etiological agent of dental caries due to its profile as a acidogenic and aciduric Gram positive bacteria.[14] *Candida albicans*, a fungus, is an opportunistic human pathogen that colonizes in the oral cavity of large proportion of population and can cause a number of mucosal infections including oral candidiasis.[14]

Bay leaf oil has shown complete inhibition and performed better than the standard antibiotics Ampicillin and Streptomycin in case of *S.mutans* and has shown inhibition against *Candida albicans* and *L.acidophilus* comparable with the standards, proving its efficacy as an anti-microbial agent. Collectively, these observations provide evidence that bay essential oil/components has the capability to alter the cell membrane of the microorganisms which eventually result in complete loss of integrity. It is most likely that the activity can be attributed to the synergistic effect of all the components of the oil, mainly 1,8-cineole having significant antimicrobial properties either alone or in combination with other monoterpenes or drugs.[5]

CONCLUSION

In conclusion, these results support the idea of usage of essential oils in denture cleansers and mouth rinses due to its high efficacy in anti-microbial activity. Furthermore, pleasant odor and flavor of the bay leaf oil are additional characteristics to their antimicrobial activity to be

used as a mouth rinse and in other oral hygiene products. Further studies using isolated compounds and combinations will give insights into the anti-microbial mechanism of the essential oil.

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