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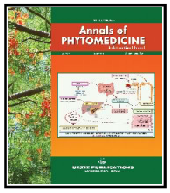

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Direct bioautography and gas-chromatography-mass spectrometry profiling of ethanolic extracts of leaves of *Ipomoea marginata* (Desr.) Verdc. against select oral microorganisms

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Abstract

Antimicrobial resistance is a global concern in management of bacterial infections. Plant based therapeutics have immense potential in combating bacterial infections without causing antimicrobial resistance. The oral cavity harbours a diverse and well-structured microbiome. *Streptococcus mutans* (*S. mutans*) is a highly virulent microorganism causing tooth decay and *Treponema denticola* (*T. denticola*) is known to cause inflammation of the gums termed gingivitis. Though, mouth washes such as chlorhexidine (CHX) are having proven antibacterial action, recent literature has reported oral dysbiosis and development of bacterial resistance on long term use of these mouth washes. Identification of the bioactive compounds of herbal extracts and proving their antibacterial action against pathogenic oral microorganisms can pave way for novel therapeutic oral formulations. This study was designed to identify the bioactive compounds of ethanolic extract of leaves of *Ipomea marginata* (Desr.) Verdc. by gas chromatography-mass spectrometry (GC-MS) profiling and antibacterial screening of the bioactive fraction present by direct bioautography. GC-MS analysis of the leaves of ethanolic extract of *I. marginata* was performed on a GC-MS equipment. Direct bioautography of the bioactive compounds was carried out using Mueller Hinton agar (MH agar) plate covered with sterile lens paper and the dried TLC plates with matching spots placed aseptically on it. Two compounds; namely, triethyl citrate and phenol 3,5 -bis (1,1- dimethylethyl) were eluted using GC-MS. On performing direct bioautography, *S. mutans* and *T. denticola* showed antibacterial activity and zone of inhibition similar to that of standard CHX. The results of the study indicated the two bioactive compounds present in ethanolic extract of leaves of *I. marginata* exhibited antibacterial property against *S. mutans* and *T. denticola*.

1. Introduction

Antibiotic resistant pathogens are threatening human mankind and pose a threat to management of various infections (Poorniammal *et al.,* 2022). Recent studies have reported evidence of antimicrobial resistance to antiseptic mouth washes such as CHX which suggests increased vigilance is required to monitor the presence of multidrug resistant bacteria (Saleem *et al.,* 2014).

Indian folk medicine has been practiced since time immemorial, a frequently used plant in folk medicine belongs to genus Ipomea, family Convolvulaceae commonly known as 'Morning glory' (Deena *et al.,* 2021). The most common biologically active constituents from plants of genus Ipomoea are alkaloids, phenolics compounds, and triterpenes compounds (Meira *et al.,* 2012). These secondary metabolites are responsible for the remedial effects of these plants (Zaynab *et al.,* 2018; Gorlenko *et al.,* 2020).

GC-MS is a well proven platform for secondary metabolite profiling of volatile compounds in both plant and non-plant species (Robertson *et al.,* 2005; Fernie *et al.,* 2004; Kell *et al.,* 2005). Direct bioautography is a rapid and sensitive tool used for screening of antibacterial activity of the compounds eluted by GC-MS. To the best of our knowledge till date, there are no published reports related to the possible bioactive compounds isolated from the leaves of *I. marginata.*

S. mutans is a Gram-positive bacillus majorly responsible for causing demineralization of teeth leading to an infectious disease of dental hard tissue structures known as dental caries (Lemas *et al.,* 2019). The bacterium is highly virulent and forms a biofilm on the tooth surface known as bacterial plaque (Matsumoto *et al.,* 2018). Though, the tooth is the hardest structure in the human body *S. mutans* dissolves the tooth structure by converting sucrose to lactic acid resulting in dental caries (Zore *et al.,* 2024).

T. denticola is a Gram-negative spirochete that is responsible for causing bleeding and inflamed gums leading to a condition called

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gingivitis. Chronic inflammation of the gums can lead to loosening of teeth and ultimately tooth loss (Sela *et al*., 2001).

CHX is a bisbiguanide antimicrobial mouthwash effective against oral microorganisms such as *S. mutans* and *T. denticola*; however, developing bacterial resistance, altered taste sensation, change in oral microbiome are some of its inherent side effects (Cieplik *et al.,* 2019). Literature evidence suggests phytochemicals that contribute to the rich medicinal property of this plant such as alkaloids, saponins, phenolic compounds, and flavonoids were observed in family Convovulacea (Masacerenhas *et al.,* 2017).

In the present study, we aimed to identify the bioactive semi-volatile and volatile compounds of ethanolic extract of *I. marginata* and demonstrate the antibacterial action of these compounds against *S. mutans* and *T. denticola.*

2. Materials and Methods

2.1 Sample collection and authentication

With the guidance of local traditional healers, the plant was identified and the leaves were collected from Auroville forest (12.0052° N, 79.8069° E) Pondicherry, South India. The plant was authenticated by a Botanist, Professor Ayappan from French Institute of Pondicherry, Pondicherry, India. Voucher specimen was deposited in the Herbarium of French Institute of Pondicherry (Voucher specimen code: V.S. 001).

2.2 Extraction

The leaves of the plant were carefully cleaned, dried in the shade and ground into a powder using a blender. The extraction process employed a Soxhlet extraction equipment. Within the apparatus's upper chamber, a porous thimble held 50 g of powdered leaf material. The lower boiling flask was filled with 200 ml of ethanol as a solvent. A heating mantle managed by a thermostat was used to raise the temperature of the flask to above 78^0C. After heating the solvent to reflux, it was extracted. Following collection, the solvent extract was concentrated independently under low pressure. Following total evaporation, the residue's weight was recorded and saved. Complete extraction was ensured until a colourless liquid was collected at the top.

2.3 GC-MS analysis

A Perkin-Elmer GC Clarus 500 system, consisting of an AOC-20i auto-sampler and interfaced to a mass spectrometer fitted with an Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused with a capillary column (30 × 0.25 ìm × 0.25 ìm df), which was used to analyse the ethanolic extract of leaves of *I. marginata*. Helium gas (99.999%) used as the carrier gas at a constant flow rate of 1 ml/min. The eluted constituents were compared to the National Institutes of Standard and Technology (NIST) library and data tabulated.

2.4 Direct bioautography

2.4.1 Test pathogens

The microbial strain used in the study was *S. mutans* (ATCC 700610), *T. denticola* (ATCC 35405) procured from Hi Media, Mumbai, India

2.4.2 Preparation of inoculum

The inoculum was prepared by growing an overnight culture of the test bacteria and the next day the turbidity was matched to McFarland 0.5 standard. The inoculum was spread onto MH agar plates.

MH agar plates were swabbed with the inoculum of representative bacterial strains in the direct bioautography procedure. Sterile lens paper was used to cover the seeded MH agar plate, aseptically and the dried TLC plates with corresponding spots were layered on top of it. The TLC plate was placed face down with the silica-coated side evenly in contact with the lens paper, and it was incubated for 24 h at 35 ± 2°C. The TLC plate readings were compared and the zone of inhibition was observed. The fractions were gathered, placed on sterile discs, and utilized to confirm the active fraction.

3. Results

Figure 1 demonstrates the chromatogram of the eluted compounds while Table 1 shows the retention time, molecular formula and molecular weight of the eluted compounds. Two peaks were observed in the chromatogram first peak corresponded to phenol, 3, 5-bis (1,1- dimethylethyl) and second peak triethyl citrate.

Table 1: Compounds identified in the ethanolic extract of *I. marginata* in GC-MS with biologic activity

S.No.	Retention time	Compound name	Molecular weight	Molecular formula
1	15.910	Phenol, 3,5-bis (1,1- dimethylethyl	206.3239	$C_{14}H_{22}O$
2	19.351	Triethyl citrate	276.2830	$C_{12}H_{20}O_7$

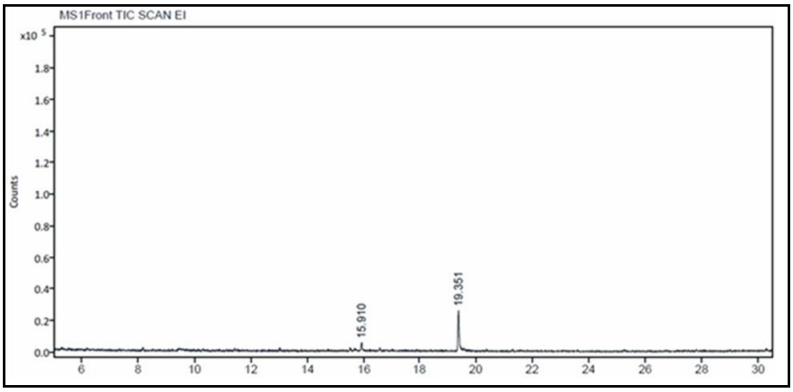
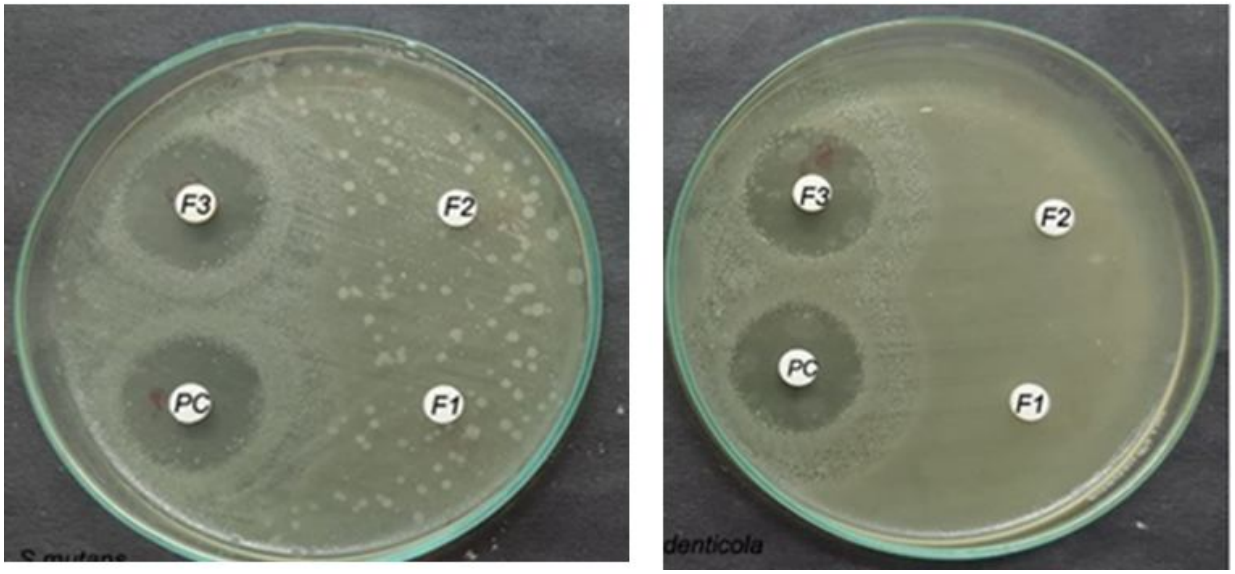


Figure 1: Chromatogram of compounds eluted by GC-MS.

Direct contact bioautography demonstrated 3 fractions; namely, Fraction 1, Fraction 2 and Fraction 3 corresponding to R_f values 0.71, 0.79, 0.85. Bioassay results revealed *S. mutans* and *T. denticola* were resistant to Fractions 1 and 2; however, Fraction 3 demonstrated sensitivity against both the organisms. Figure 2 showed the zone of inhibition for *S. mutans* and *T. denticola* was 16 mm. 0.2% CHX was used as positive control and the zones of inhibition for positive control for *S. mutans* was 16 mm (p=0.013) and for *T. denticola* was 17 mm (p=0.001), respectively. Table 2 demonstrated the bioactive compounds from ethanolic extract of *I. marginata* showed significant antibacterial property ($p \leq 0.05$) comparable to positive control CHX.

Table 2: Zone of inhibition (mm) of three fractions and chlorhexidine by direct bioautography

Pathogens	Fraction 1	Fraction 2	Fraction 3 (mm)			Ethanolic extract (100 µg/ml) (Mean ± SD)	Chlorhexidine (Mean ± SD) (100µg/ml)	*p* value
S. mutans	0	0	16	15	15	15.6	16	0.013
T. denticola	0	0	16	16	17	16.3	17	0.001



(A) (B)

Figure 2: Zone of inhibition (in mm) of ethanolic extract of leaves of *I. marginata* and CHX with respect to *S. mutans* (A) and *T. denticola* (B).

4. Discussion

Phenol 3,5-bis (1,1- dimethylethyl) is a phenolic compound present in plants to protect it from stress and pathogenic attacks. Previous studies have shown that they are important secondary metabolites that contribute to its antimicrobial, and antioxidant property (Muzahid *et al.,* 2022).

In an ethnopharmacological study conducted to study the anti-inflammatory property of *Woodfordia floribunda,* it was found the petroleum ether extract demonstrated significant anti-inflammatory activity by reducing rat paw edema (Pankaj Naikwadi *et al.,* 2022). In another study to evaluate the antibacterial effect of *Aerva lanata* (L) against *Escherichia coli* and *Staphylococcus aureus*, it was observed *A. lanata* can be an effective antibacterial agent (Shafqat Qamer *et al.,* 2023). Literature also has shown *in silico* analysis of phytochemical compounds present in *T. arjuna* and *A. paniculata* against drug resistant pathogens (Vidya Devanathadesikan Seshadri, 2021).

One such plant having widespread medicinal value is *I. marginata.* The plant has been used from time immemorial as an antidote to snake poisoning and treatment of skin infections (Santosh Kumar *et al.,* 2019). In another study, phytochemical screening and antibacterial activity of the whole plant and the leaves of *I. marginata* was evaluated against *S. aureus, Shigella sonnei, Enterobacter faecalis, Salmonella typhimurium, Micrococcus luteus, Vibrio cholerae, Klebsiella pneumoniae, Streptococcus pyogenes, E. coli* and *Bacillus subtilis* (Sukitha *et al.,* 2016).The observations of the study indicated methanol, acetone, benzene, water, and ethanol extracts of *I. marginata* exhibited antibacterial activity and further research can prove the extract from the plant to be a potent antibacterial agent. The observations of the present study also validated the antibacterial activity of ethanolic extract of *I. marginata* against two virulent oral microorganisms *S. mutans and T. denticola.* However, to the best of our knowledge this is the first study to have evaluated the antibacterial action against two virulent oral pathogens namely *S. mutans* and *T. denticola* which are known to cause dental caries and inflammation of gums leading to tooth loss.

5. Conclusion

Nowadays, there is a paradigm shift towards plant-based biologics in the manufacture of oral formulations such as tooth pastes, mouthwashes and oral gels, as they do not cause bacterial resistance and have very minimal side effects. The observations of the present study indicated ethanolic extract of leaves of *I. marginata* possessed significant antibacterial bioactive compounds. These compounds exhibited antibacterial activity comparable to standard antimicrobial 0.2% CHX against most common oral microorganisms such as *S. mutans* and *T. denticola*.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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