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ANTIMICROBIAL ACTIVITY OF *MENTHA ARVENSIS* AGAINST CLINICAL ISOLATES OF HUMAN CARIOGENIC PATHOGENS- AN *IN-VITRO* STUDY

Deepak Dwivedi [1], Gaurav Khandelwal [2], Rakesh Kumar Patidar [1] and Vinod Singh*[1]

Department of Microbiology, Barkatullah University [1], Bhopal- 462 003, Madhya Pradesh, India
Department of Botany and Microbiology, Gurukula Kangri University [2], Haridwar- 249 404, Uttarakhand, , India

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Correspondence to Author:

Dr. Vinod Singh

Associate Professor & Head,
Department of Microbiology,
Barkatullah University, Hoshangabad Road, Bhopal, Madhya Pradesh, India

ABSTRACT

Patients with chronic dental infection are usually treated with antibiotics. However, the value of antibiotics was decreasing because increased resistance in bacteria. The objective of this study is to evaluate the efficacy of herbal crude extract of *Mentha arvensis* in human Cariogenic pathogens. In this study we obtained crud extract of *Mentha arvensis* in different solvent 50% and 10% methanol, ethyl acetate, chloroform and was tested against human Cariogenic pathogens *Streptococcus mutans, Streptococcus sangunis, Staphylococcus aurues, Lactobacillus casei* were isolated from patients having dental disease. The crude extracts activity were studied by disc diffusion and both dilution methods in different concentration. Studies were also undertaken to assess the phytochemical composition of the *Mentha arvensis* extract. 50% methanolic extract at 2.5mg/ml and 5mg/ml concentration shows slightly bigher zone of inhibition (ranging from 26 to 30 mm and 28 to 32 mm), and 10% methanolic 2.5mg/ml and 5mg/ml extract shows slightly small zone (ranging from 20 to 24 mm and 22 to 27 mm) and comparison with ethyl acetate and chloroform shows small zone at 5mg/ml ranging from 15 to 18 mm and 13 to 17 mm and in 2.5gm/ml ranging from 14 to 15mm and 09 to 16 mm or to be moderately sensitive. MIC results exhibit the profound and promising activity of *Mentha arvensis* on BHI 0.090 mg/ml. The secondary metabolites commonly present in the test leaves are Alkaloids, Tannins, Flavonols, Steroids, Xantones and glycosides, The GCMS analysis of revealed, the presence of Eucalyptol, Isomethone, Linalool, methnol, 4-Terpineol, OleicAcid, Tetradecanoic acid, 12-methyl-, methyl ester, Hexadecanoic acid, (Palmitic acid) methyl ester. These data suggest that extracts of *Mentha arvensis* contain significant amounts of phytochemicals with antioxidative properties which could serve antimicrobial property of the *Mentha arvensis* and it is exploited as a potential source for plant-based pharmaceutical products. These results could form a sound basis for further investigation in the potential discovery of new natural bioactive compound.

INTRODUCTION: Dental caries is an infectious microbial disease that results in localized dissolution and destruction of the calcified tissues of the teeth [1]. Streptococcus mutans is known as the causative bacteria in the formation of dental plaque and dental caries. The acid producing *S. mutans* inhabiting the mouth causes damage by dissolving tooth structures in the presence of fermentable carbohydrates such as sucrose, fructose, and glucose [2]. The food debris, acid, bacteria, and saliva combine in the mouth to form a sticky substance called “plaque” that adheres to the teeth. If plaque is not removed thoroughly and routinely, tooth decay will not only begin but flourish [3].

Persistent dental disease is painful, and most importantly, it has also been suggestively linked to diabetes, high blood pressure, heart disease, and multiple sclerosis later in life [4, 5]. Treatment often prevents further infection of the tooth structure. Early treatment is less painful than treatment of extensive decay. Dental caries can also cause bad breath and foul tastes. In highly progressed cases, infection can spread from the tooth to surrounding soft tissues which may lead to an edentulous mouth.

Recent natural remedies with the use of medicinal plants, which are good reservoirs of chemo-therapeutants can be, contributed as an alternative for antibiotic effects such as hypersensitivity reaction, supra infections, and teeth stainings. It has been well documented that medicinal plants confer antimicrobial activity towards oral bacteria [6].

The literature survey of the folklore medicine reveals the use of Mentha arvensis leaves to the treatment of tooth. Despite several anticaries agents being available commercially but the search for an effective natural agent still continues. Natural products have shown to be good alternative to synthetic chemical substances for caries prevention [7, 8, 9, 10].

Indian medicine has a long history, and is one of the oldest organized systems of medicine. Its earliest concepts are set out in the sacred writings called the Vedas, especially in the metrical passages of the Atharvaveda, which may possibly date as far back as the 2nd millennium BC. Knowing the fact that little literature is available on the use of *Mentha arvensis* in oral infection, the study is focused on assessing the *Mentha arvensis* extracts with different solvents.

Hence, for the present investigation, *S. mutans*, *S. sangunis, S. aurues*, *Lactobacillus casei* are the bacterial strains selected as target organisms from patients and screened using ethyl acetate, chloroform and methanol extracts of leaves of Mentha arvensis.

Once the antimicrobial property of the plant extracts is screened under in vitro condition against oral pathogens, *in vivo* trials can be carried out for the treatment of dental caries by external application on the caries tooth or as a preventive mouth rinse.

MATERIALS AND METHODS:

Microorganisms. The human dental caries pathogens, *Streptococcus mutans, Streptococcus sangunis, Staphylococcus aurues, Lactobacillus casei* used in this study, were isolated from patients of the OPD's of Peoples Dental Academy, Bhopal, M.P., India.

Media Used. Thioglycolate broth (TGB) and brain heart infusion broth (BHI) (Himedia laboratories, Mumbai India) are the transport media used to maintain clinical dental caries sample in viable condition. Thioglycolate broth (TGB) (Himedia laboratories, Mumbai India) Growth media used in examining the samples at aerobic and anaerobic condition includes, nutrient agar (NA), blood agar (BA), Mutant Sanguis Agar, Manitol Salt Agar, de Man, Rogosa Sharpe Agar (MRS) (Himedia laboratories, Mumbai India) and Brain Heart Infusion broth (BHI) are used for the antimicrobial susceptibility testing.

Collection and Recovery of Caries Sample. The samples from patients were collected with strict aseptic condition. Patient was made to rinse the tooth with water, and it was isolated with a rubber dam. The tooth and the surrounding field were cleaned with 3% hydrogen peroxide and then decontaminated with a 2.5% sodium hypochlorite solution. The food debris on the chewing surface was removed using a dental excavating instrument. The dental caries sample was collected from the patient using an excavator under aseptic conditions by a clinician and was introduced into the 2ml broth of TGB or BHI in appropriate sterile screw cap bottles. The clinical samples were mixed well using a magnetic stirrer before incubation. Then samples were inoculated on specific media, Mutans Sangunis Agar for *S. mutans* and *S. Sangunis*, Manitaol Salt Agar for *S. aureus* and MRS modified Agar for *L. casei* under various culture conditions. These isolates were confirmed by their specific biochemical tests.

Plant Materials Collection. We selected Mentha arvensis plant for antimicrobial assay, based on their ethno medicinal and traditional uses against infectious diseases based on literature survey and interaction with herbal healers.

Preparation of Crude Extracts. The leaves were shade-dried and powdered and used for extraction, 100 g of dry powder was taken in an aspirator bottle, 300mL 10% methanol (1: 3 W/V) was used and the mixture was shaken occasionally for 48 hour. Then, the extract was filtered. This procedure was repeated three times and all extracts were decanted and combined. The extracts were filtered before drying using Whatman filter paper no. 2 on a Buchner funnel, and the solvent was removed by vacuum distillation in a rotary evaporator at 40◦C for quantitative determination; the extracts were placed in pre weighed flasks before drying. The remaining plant residue was extracted with 50% methanolo, ethyl acetate and chloroform sequentially [11].

Phytochemical analyses: The presence of phytochemicals such as alkaloids, flavonol, xantones, tannins, terpenoids (2,4-dintrophenyl hydrazine) and steroids (Liebermann–Burchard test) were evaluated according to the methods described by Edeoga et al. [11].

Antimicrobioal Susceptibility Assay:

a. **Disc Diffusion Method-** Antimicrobial activity was carried out using disc-diffusion method, Petri plates were prepared with 20mL of sterile brain heart infusion agar (BHI) for (Himedia laboratories, Mumbai India) the test cultures (100 µL of suspension containing 10^8 CFU/mL bacteria) were swabbed on the top of the solidified media and allowed to dry for 10min. The tests were conducted at three different concentrations of the crude extract (200 mg crude extract dissolved in 5% dimethyl sulfoxide (DMSO) (Mark Chemicals), respectively, 5mg and 2.5mg per disc). The sterile 6mm disc (Himedia laboratories, Mumbai India) impregnated with different concentrations of extracts. The loaded discs were placed on the surface of the medium and left for 30min at room temperature for compound diffusion. Negative control was prepared using respective solvent. Amoxicilline were used as positive control. The plates were incubated for 24 h at 37^{o}C. Zone of inhibition was recorded in millimeters and the experiment was repeated twice.

b. **Minimum Inhibitory Concentration-** The extracts were dissolved in water +2% dimethyl sulfoxide (DMSO). The initial concentration of extract was 5 mg/ml to 0.090 mg/ml. The initial test concentration was serially diluted two-fold. Each well was inoculated with 5 µL of suspension containing 10^8CFU/mL of bacteria. The antibacterial agent Amoxicilline include in the assays as positive controls. The plates with bacteria were incubated 24 h at 37◦C. After incubation, 5 µL of tested broth was placed on the sterile BHI plates and incubated at respective temperature. The MIC for bacteria was determined as the lowest concentration of the extracts inhibiting the visual growth of the test cultures on the agar plate. Triplicates were maintained.

Gas Chromatography-Mass Spectrometry (GC-MS). The active extracts were quantified using gas chromatograph (GC-MS-Shimadzu) equipped with a CPB-capillary column (mm inner diameter × 50m length) mass spectrometer (ion source 200◦C, RI 70 eV) programmed at 40◦C–280◦C with a rate of 4◦C/min. Injector temperature was 280◦C; carrier gas was He (20 psi).

RESULT: The antibacterial activity of Mentha arvensis is shown in **Table 1**.

TABLE 1: ANTIBACTERIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF *MENTHA ARVENSIS*

Name of oral pathogens	Mentha arvensis extract (Zone of Inhibition of 10µl extract in mm)								
	Amoxicilline	50% Methenolic		10% methenolic		Ethyl acetate		Chloroform	
	5mg/ml	2.5mg/ml	5mg/ml	2.5mg/ml	5mg/ml	2.5mg/ml	5mg/ml	2.5mg/ml	5mg/ml
Streptococcus mutans	12	30	31	24	27	14	15	09	13
Streptococcus sanguinis	13	26	28.5	20	22	15	17	12	14
Staphylococcus aurues	12	29	32	22	23	15	18	14	15
Lactobacillus acidophilus	14	26	28	24	26	15	17	16	17
Lactobacillus casi	11	28	30	21	24	15	16	13	13

The antibacterial efficacy of various solvent extracts namely 50% Methanol, 10% Methanol, ethyl acetate chloroform, of the *Mentha arvensis* plants against the human cariogenic bacteria showed varied level of inhibition. The activity of the different extracts of all the screened plants were compared with standard drug Amoxicillin. Activity of different solvent extract of the *Mentha arvensis* was investigated by disc Diffusion method and well diffusion method. As per result, methanolic extract showed a broad spectrum of very significant antibacterial activity of producing a clear zone of inhibition against *Streptococcus mutans, Streptococcus sanguis, Stephylococcus aureus* and *Lactobacillus acidophilus* and *Lactobacillus casei*.

50% methanolic extract at 2.5mg/ml and 5mg/ml concentration shows slightly bigher zone of inhibition (ranging from 26 to 30 mm and 28 to 32 mm), and 10% methanolic 2.5mg/ml and 5mg/ml extract shows slightly small zone (ranging from 20 to 24 mm and 22 to 27 mm) and comparison with ethyl acetate and chloroform shows small zone at 5mg/ml ranging from 15 to 18 mm and 13 to 17 mm and in 2.5gm/ml ranging from 14 to 15mm and 09 to 16 mm or to be moderately sensitive.

Based on the preliminary screening assay, the *Mentha arvensis* extracts were further evaluated to determine the minimum inhibitory concentration (MIC). MIC was determined as the lowest concentration of the extract, which inhibited the growth of the tested micro-organisms. Results exhibit the profound and promising activity of *Mentha arvensis* on BHI 0.090 mg/ml (**Table 2**). The secondary metabolites commonly present in the test leaves are Alkaloids, Tannins, Flavonols, Steroids, Xantones and glycosides (**Table 3**).

TABLE 2: MINIMUM INHIBITORY CONCENTRATIONS (MIC) OF DIFFERENT SOLVENT EXTRACTS OF *MENTHA ARVENSIS* BY MICROBROTH DILUTION METHOD

Name of oral pathogens	Mentha arvensis extract (Minimum inhibitory concentration mg/mL)			
	50% Methenolic	10% methenolic	Ethyl acetate	Chloroform
Streptococcus mutans	0.090	0.090	0.72	0.360
Streptococcus sanguinis	0.090	0.090	0.360	1.50
Staphylococcus aurues	0.360	0.72	0.72	0.72
Lactobacillus acidophilus	0.090	0.72	0.72	0.72
Lactobacillus casei	0.090	0.360	1.50	1.50

TABLE 3: PHYTOCHEMICAL ANALYSIS OF *MENTHA ARVENSIS* PLANT EXTRACT ON DIFFERENT SOLVENT

Plant Extract of	Alkaloids	Tannins	Flavonols	Steroids	Xantones	Carbohydrate	Proteins
Methanol	+	+	+	+	+	+	+
Ethyl acetate	-	+	+	+	+	+	+
Chloroform	+	-	-	-	+	-	+

In the present study, the Mentha arvensis have been subjected GCMS analysis. The GCMS analysis revealed, the presence of Eucalyptol, Isomethone, Linalool, methnol, 4-Terpineol, Oleic acid, Tetradecanoic acid, 12-methyl-, methyl ester, Hexadecanoic acid, (Palmitic acid) methyl ester have been shown to possess antimicrobial, activities (**Table 4**).

TABLE 4: SHOWING IDENTIFIED COMPONENT IN THE METHANOLIC EXTRACT OF *MENTHA ARVENSIS* BY GC-MS

RT	Name of Molecule	RI b	RI c	RI d
3.871	dl- Limonene	-	1154	1.47
4.048	Eucalyptol	-	1206	6.91
4.458	Alfa-pinene	-	1039	1.13
8.367	3-Octanol	1340	1382	1.82
10.962	Isomethone	1426	1452	3.82
2.916	Linalool	1487	1538	2.20
13.134	Neo-Methol acetate	1494	-	1.29
14.210	methnol	1529	1599	19.70
14.342	4-Terpineol	1533	1551	4.29
14.32	Octadecanoic acid, methyl ester (Stearic acid methyl ester)	1602	1655	1.37
15.59	9-Heneicosanone	1653	--	0.41
18.688	D-Xylose, tetrakis(trimethylsilyl	1661	-	0.34
17.29	Myo-Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl	1669	-	0.41

23.33	OleicAcid	1875	1956	1.34
24.26	Tetradecanoic acid, 12-methyl-, methyl ester	1883	1927	1.79
26.50	-Methyl-6-(5-methyl-2-thiazolin-2-ylamino)pyridine	2001	1979	0.12
27.15	Hexadecanoic acid, (Palmitic acid) methyl ester	2053	2003	3.76
29.46	Tetrasiloxane, decamethyl-	2127	2100	0.53
30.52	cis-2-Hexen-1-ol, trimethylsilyl ether	2190	2108	0.21

DISCUSSION: Oral cavity is the manifested for microorganism. Specific diseases are produced by a specific microorganism while others are clinically specific and may be caused by any of a broad group of microorganisms. This microbial specificity or non specificity is characteristic of all pathogens isolated during present disease. *Streptococcus mutans, Streptococcus sanguis, Staphylococcus aureus* and *Lactobacillus acidophilus* and *Lactobacillus casei* play a major role in dental caries formation [12].

Antibiotics (antimicrobials) are often prescribed for the adjunctive treatment of dental caries and large use of these antibiotics, antibiotics resistance capacity are incrassating significantly natural remedies are the strongest tool for the for the treatment of this infection causative agents of dental caries and dental plaque, isolated pathogens were tested for morphological and biochemical's and compared with ATCC cultures [13].

The present study has shown that *Mentha arvensis* is potentially a rich source of antibacterial agent [8]. This demonstrates their importance in traditional remedies. *Mentha arvensis* leaves extracts tested, inhibited the growth of all pathogens and very effective as compare with standard antibiotic Amoxicillin. The methanolic extract is highly effective against all pathogens because more organic compounds were leached in this solvent [14, 15, 16, 17]. Screened extract to detect antimicrobial activity and clearly demonstrated that alcohol is a better solvent as compared to ethyl acetate and chloroform.

Mentha arvensis have the potential to generate herbal metabolites. The crude extracts demonstrating anti dental caries activity could result in the discovery of new chemical classes of antibiotics that could serve as selective agents for the maintenance of human health and provide biochemical tools for the study of infectious diseases.

CONCLUSION: This study is a preliminary evaluation of antimicrobial activity against dental caries causing pathogens. *Mentha arvensis* have herbal metabolites which could serve as selective agents for the anti dental caries property. The potential for developing antimicrobials from plants appears rewarding as it leads to the development of new drugs which is needed today. Further research is necessary to find the active compounds within and their full spectrum of efficacy. However, the present study of in vitro antibacterial activity of *Mentha arvensis* forms primary platform for further phytochemical and pharma-cological studies in cariogenic pathogens.

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REFERENCES:

1. Loesche W. J: Role of Streptococcusmutans in human dental decay. Microbiological Reviews 1986; 50: 53–380.
2. Kohler B, Andreen I, Jonsson B: The effect of cariespreventive measures in mothers on dental caries and the oral presence of the bacteria *Streptococcus mutans* and *lactobacilli* in their children. Archives of Oral Biology 1984; 29: 879–883.
3. Holloway PJ, Moore WJ: The role of sugar in the etiology of dental caries. Journal of Dental Research 1983; 3:189–213.
4. Marsh PD: Microbial ecology of dental plaque and its significance in health and disease. Advance in Dental Research 1994; 8:263-271.
5. Roda RP, Bagán JV, Bielsa JMS, Pastor EC: Antibiotic use in dental practice: A review. MedIcine Oral Patologia Oral Cirugia Bucal 2007; 12:186-92.
6. Smullen J, Koutsou GA, Foster HA, Zumbe A, and Storey DM: The antibacterial activity of plant extracts containing polyphenols against *Streptococcus mutans*. Caries Research 2007; 41: 342–349.
7. Carounanidy U, Satyanarayanan R and Velmurugan A: Use of an aqueous extract of *Terminalia chebula* as an anticaries agent. Indian Journal of Dental Research. 2007; 18:152–156.
8. Akram M, Uzair M, Malik NS, Mahmood A, Sarwer N, Madni A and Asif HM : Mentha arvensis Linn.: A review article. Journal of Medicinal Plants Research 2011; 5:18-4499-4503.
9. Ahmad I, Mehmood Z & Mohammad F: Screening of some Indian medicinal plants for their antimicrobial properties. J of Ethnopharmaco. 1998; 62:183-193.
10. Edeogal HO, Okwu DE and Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology 2005; 7: 685-68

11. National Committee for Clinical Laboratory Standards, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi. Approved Standard M38-A, National Committee for Clinical Laboratory Standards, Wayne, Pa, USA, 2002.
12. Aas JA, Paster BJ, Stokes LN: Defining the normal bacterial flora of the oral cavity. Journal of Clinical Microbiology 2005. 43:5721-5732
13. Ahmad I & Beg AZ: Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. Journal of Ethnopharmacology. 2001; 74: 113-123.
14. Vigil GV, Wayman BE, Dazey SE, Fowler CB, Bradley DV: Identification and antibiotic sensitivity of bacteria isolated from periapical lesions. Journal of Endodontic 1997; 23:110–4.
15. Jaju S, Pahwa S, Kumari S, Fuloria N: Pharmacognostical studies and antibacterial activity of the leaves of *Murraya koenigii*. Pharmacogonosy Journal 2009; 1; 210-214.
16. Mahesh B. and Satish S: Antimicrobial Activity of Some Important Medicinal Plant Against Plant and Human Pathogens. World Journal of Agriculture Science 2008; 4: 839-843.
17. Sharma S, Khan I, Ali I, Ali F, Kumar M, Kumar A, Johri R. K, Abdullah S T, Bani S, Pandey A, Suri K. A, Gupta BD, Satti N K, Dutt P, and Qazi G N: Evaluation of the Antimicrobial, Antioxidant, and Anti-Inflammatory Activities of Hydroxychavicol for Its Potential Use as an Oral Care Agent. Antimicrobial and chemotherapy 2009; 53:216–222.

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