ejpmr, 2019,6(11), 286-290

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

ANTIBACTERIAL EFFICACY OF HERBAL EXTRACTS & ESSENTIAL OILS FROM TRADITIONAL MEDICINAL PLANTS AGAINST BACTEROIDES FRAGILIS GROUP ISOLATED FROM CLINICAL SPECIMEN

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Article Received on 03/09/2019 **Article Revised on 24/09/2019** **Article Accepted on 14/10/2019**

ABSTRACT

Introduction: *B fragilis* group is one of the most frequently isolated anaerobic pathogens with the highest antibiotic resistance. They also exist as normal flora in the human gut and female genital tract. Due to empirical treatment and lack of monitoring, the incidence of drug resistant strains has increased and treatment has become challenging. The emergence of these resistant strains has created the demand for alternative drugs. **Aim and objectives**: To evaluate the antimicrobial efficacy of various medicinal herbal extracts against *B fragilis* group. **Materials and Methods**: A total of 58 strains of *B fragilis* group were isolated from various clinical samples and identified according to standard procedures. The essential oils from medicinal plants were extracted using Neo-Clevenger apparatus and alcohol extracts by Soxhlet apparatus. Screening of the antimicrobial activity of herbal products was performed by employing disc diffusion technique. Minimum inhibitory concentration was determined by agar dilution method. **Results**: Essential oils (lemongrass, cinnamon, nutmeg, clove bud, ginger, garlic) and Kokum extract (aqueous and alcohol) were tested for their antibacterial efficacy. Essential oils of Cinnamon, clove and lemongrass were very active to these organisms with a percentage of 98.2%, 96.5%, 89.6% respectively. The MIC ranges from 5µg/ml to 0.156µg/ml for cinnamon oil. **Conclusion**: The present study has demonstrated that herbal products exhibit good in vitro antimicrobial action against *B fragilis* group. The results suggest that they have high potential to open new avenues in the development of alternate therapeutic options.

KEYWORD: *B fragilis* group, antimicrobial susceptibility testing, essential oils, herbal extract.

INTRODUCTION

Members of *Bacteroides fragilis* group consists of non-sporing, non-motile, bile resistant, gram negative bacilli. They are commensals of the normal human flora in the gastrointestinal tract and female genital tract and also occurs as opportunistic pathogen.[1,2]

Review of literature documents their ability to cause serious infections such as intra-abdominal infections, deep- seated infections, diabetic foot ulcer. Among the members of the group, *B fragilis* predominates the scenario, accounting for 80% of infections with the high virulence potential.[3-5]

Emergence of multi drug resistance has been reported globally, especially to drugs like metronidazole, carbapenem, clindamycin which is a matter of concern[6-10] However, occurrence of drug resistant anaerobic strains is under reported because of the lack of anaerobic isolation facility in routine lab settings. Therefore, in this context, researchers are prompted to find alternative and novel antimicrobial options with lesser side effects for treatment.

For decades, traditional herbal remedies have contributed in treating and preventing many harmful infections. India is well-known for its unique indigenous system of medicine which offers a vital role towards global health care. The antimicrobial efficacy of essential oils has been documented in literature.[11] Components of essential oil such as terpenes, terpenoids, phenol- derived aromatic and aliphatic compounds attributes to their bactericidal/ bacteriostatic, fungicidal and virucidal properties.[12]

Demonstration of antimicrobial efficacy of herbal extracts against *B fragilis* group is scanty in the literature.[13] Results of our investigations underscores the invitro antibacterial efficacy of essential oils and

extracts of herbal origin. The minimum inhibitory concentration (MIC) of the more effective essential oils were selected and tested by agar dilution method against *B fragilis* group.

MATERIALS AND METHOD

The study was conducted in a tertiary care hospital at Mangalore, Karnataka, South India. A total of 58 *B fragilis* group strains isolated from various clinical samples such as pus drainage, high vaginal swab, wound swab, blood and body fluids from January – December ,2018 was included in the study. The study was approved by Institutional Ethics Committee (FMMC/FMIEC/4229/2017). The samples were collected in Robertson Cooked Meat Medium and transferred to the lab for further investigations. The samples were cultured on Anaerobic sheep blood agar and a selective media such as *Bacteroides* Bile Esculin Agar and the isolated strains were identified using standard protocol.[14]

A) Preparation of Herbal Extracts: The fresh leaves of lemongrass was acquired from a local nursery; cinnamon bark, nutmeg seed, clove bud, ginger rhizome, garlic clove and dry kokum rinds were bought from a reputed local retail store. The taxonomic identification and authentication were done in the Department of Botany, University College Mangalore. Extracts were prepared at the Shree Devi College of Pharmaceutical Sciences Mangalore. The plant materials were dried under the sun/ oven and homogenized into coarse powder and stored with sterile precautions.

Table 1: Authentication of Plant materials that were collected and used for the investigation.

Common name	Scientific Name	Family	Part of herb used
Nutmeg	*(Myristica fragrans)*	*Myristicaceae*	Seed
Cloves	*(Syzygium aromaticum)*	*Myrtaceae*	Bud
Ginger	*(Zingiber officinale)*	*Zingiberaceae*	Dry roots
Garlic	*(Allium sativum)*	*Amaryllidaceae*	Cloves
Cinnamon	*(Cinnamomum verum)*	*Lauraceae*	Bark
Lemon grass	*(Cymbopogan citratus)*	*Poaceae*	Leaves
Kokum	*(Garcinia indica)*	*Clusiaceae*	Dried rind

a. Preparation of Essential oils

The essential oils of seven herbal plants were extracted using Neo-Clevenger's method. Briefly, 100 grams of coarse powder of the plant materials was added to 500 ml of distilled water and was heated at a mantle temperature set at 90℃ for 8-12 hours. After distillation, lower part of the condenser was cooled and the volume of oil collected was withdrawn in a sterile vial.

b. Preparation of alcohol and aqueous extract of dry kokum

The alcohol extract of kokum was done by ethanol using Soxhlet apparatus. 100 grams of coarse powder of Kokum rind was placed in the Soxhlet apparatus. 500ml of alcohol was added and heated at a mantle temperature of 90℃ for 8-12 hours. The final product was collected after the maceration process. Aqueous extract was prepared by soaking 100 gms of the plant material in 500ml water and 2ml of chloroform for 3-5 days by Maceration process.

B) Demonstration of Antimicrobial efficacy.

a. Preparation of inoculum:

A loopful of fresh *B fragilis* group isolates were inoculated in Vande Levure (VL)broth and incubated at 37℃ for 24 hrs under anaerobic condition. Turbidity of the suspension was adjusted to 0.5 McFarland Standard (1.5×10^8 colony-forming unit /ml) by mixing with sterile broth.

b. Screening by disc diffusion method

Bacterial suspension of *B fragilis* group adjusted to 0.5 McFarland opacity was inoculated as lawn culture on the surface of Brucella Blood agar supplemented with hemin and vitamin K. The in-house sterile filter paper discs (Whatmann filter paper 1 -6mm) was impregnated with the appropriate undiluted essential oil and plant extracts. The impregnated disc was placed on the lawn cultured Brucella blood agar plate (5-6 disc/plate). The plates were incubated at 37℃ for 48 hours anaerobically in BD Gaspak Jar. Bacterial growth showing clear zone of inhibition range were measured and graded according to Alves et al.[15]

c. Agar dilution method for determination of MIC

MIC was determined for the essential oils by the agar dilution method according to Wadsworth-KTL anaerobic bacteriology manual and Clinical and Laboratory Standards Institute (CLSI), M100-S23 document.[14,16] Essential oils were diluted using DMSO and the concentration in the Brucella agar plates was prepared ranging from 10µl/ml to 0.3125 µl/ml of media.

The bacterial suspensions adjusted to 0.5 McFarland Standard were spot inoculated onto the marked area in the plates. Two Brucella blood agar plates without essential oils were also inoculated, out of which one was growth control incubated anaerobically along with the test plates and an aerobic control to rule out aerobic contamination. MIC was interpreted as the lowest concentration of essential oil yielding no growth. MIC of the essential oils was also determined with ATCC 25285 *B fragilis*.

RESULT

Among the 58 strains of *B fragilis* group isolated from clinical samples were subjected to the susceptibility testing, the essential oils of cinnamon, clove and lemongrass were found to be very active i.e. 98.2%, 96.5%, 89.6% respectively. The results of the sensitivity pattern of herbal extracts varied between 1+ to 4+ grades as demonstrated in **table 2.**

Table 2: Results of disc diffusion assay. (Total number of strains =58)

Essenital oil/ plant extract	Grading					
	+1	+2	+3	+4	Total sensitive	Resistant
Clove	4 (6.8%)	14 (24.1%)	18 (31%)	20 (34.4%)	56 (96.5%)	2(3.4%)
Lemongrass	13 (22.4%)	9 (15.5%)	14 (24.1%)	16 (27.5%)	52(89.6%)	6 (10.3%)
Cinnamon	2 (3.4%)	0	0	55 (94.8%)	57(98.2%)	1(1.7%)
Nutmeg	11 (18.9%)	2 (3.4%)	2(3.4%)	5 (8.6%)	20(34.4%)	38 (65.5%)
Ginger	10 (17.2%)	5 (8.6%)	1(1.7%)	1(1.7%)	17(29.3%)	41 (70.6%)
Garlic	6 (10.3%)	2(3.4%)	0	0	8(13.7%)	50 (86%)
Kokum Alcohol extract	2(3.4%)	1(1.7%)	0	0	3 (5.17%)	55 (94%)
Kokum Aqueous extract	2(3.4%)	0	0	0	2(3.4%)	56 (96.5%)
Key: *<9mm→ Resistant, 9mm-12mm →+1, 13mm-16mm→ +2, 17mm-20mm → +3, >20mm →+4						

Four essential oils with maximum activity were selected for determination of minimum inhibitory concentration by agar dilution method. 58 strains were screened for antimicrobial activity and subjected to determine the MIC. All the strains were inhibited at concentration of 10 µl/ml by clove oil, lemongrass oil and nutmeg oil. Cinnamon oil was the most active and inhibited all the tested strains at a concentration of 5µl/ml. (Table 3)

Table 3: Details of number of isolates of *B fragilis* group inhibited at various concentration of selected essential oil (Total Number tested 58).

Essential oils	Various No. of strains inhibited at dilutions					
	0.3125µl/ml	0.625µl/ml	1.25 µl/ml	2.5 µl/ml	5µl/ml	10 µl/ml
Clove oil	1	12	26	32	48	58
Lemongrass	6	8	12	19	29	58
Cinnamon	20	36	43	49	58	58
Nutmeg	3	5	7	17	19	58

DISCUSSION

The emergence of resistance to several antibiotics in *B fragilis* group has become a challenge to the clinicians, hence development of new and effective alternative treatment is the need of the hour. Reports regarding the antimicrobial potentials of natural products against *B fragilis* group are scanty from our country. India has practiced natural herbal medicine since antiquity. Natural products have exhibited antimicrobial efficacy acknowledged by other researchers.[11,17,18] These phytochemical components were known to have antimicrobial, antifungal, antioxidant, anti-inflammatory and analgesic properties.[11,12]

Lemongrass oil extracted from the leaves of *Cymbopogon citratus* and *Cymbopogon flexuosus* has been documented as a remedy for various ailments. Researchers had reported the anti- inflammatory and antimicrobial activity of lemongrass oil against resistant strains.[19] One of the investigators reported better results on low concentration of lemongrass oil in broth dilution compared to the agar dilutions.[20]

Clove oil has the highest antioxidant property due to its component eugenol up to 95%.[21] Studies demonstrated its effectiveness and high potency as an antimicrobial agent which also agrees with the present study.[22,23] It also serves as a remedy for toothache associated with periodontitis[17] while aromatic therapy employing clove oil could be used to eliminate halitosis.[24]

Among all essential oils used in the present study, cinnamon oil has shown the highest activity against *B fragilis* group both by disc diffusion and agar dilution which is in accordance with other investigators.[18,23] The component cinnamaldehyde was the main factor and major active compound responsible for its antioxidant, anti diabetic, antifungal and antibacterial properties.[25,26] It has been documented in the literature that the major constituent in the essential oil of nutmeg contain myristicin, elemicin, safrole and sabenene which comprise 80% of the oils. Nutmeg exhibits a good potential against oral infections.[27]

Garlic and ginger have been the most common and popular spice, traditionally used as a dietary supplement that helps in digestion and gastrointestinal disorders. The medicinal properties of garlic and ginger have been well documented in literature.[28,29] Based on the evaluation of the present study garlic and ginger oil had comparatively low antibacterial effect on the tested strains which is in agreement with other studies.[29]

Kokum (Garcinia indica) rind consist of a phenolic compound called garcinol, responsible for its antibacterial properties. It has been used to treat gastrointestinal problems[30] and recent study has also reported a significant antidepressant and anxiolytic effect.[31]

CONCLUSION

As reflected in the documents, natural products can be used as an alternative treatment options to synthetic drugs. Our study has demonstrated and evaluated the effectiveness of the essential oils & herbal extracts of various plants that has shown promising results. The findings may conclude that these results can open up new ventures for therapeutic options.

ACKNOWLEDGEMENT

The authors are thankful to Dr J.V Kamath, Principal of Shree Devi College of Pharmacy, Kenjar, Mangalore, India for providing and supporting necessary facilities for the extraction of essential oils and plant extracts. We also acknowledge Dr Siddha Raju MN, Assistant Professor, Department of Botany, Mangalore University, Mangalore for the authentication of the herbal plants.

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