WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.632
Volume 9, Issue 3, 1091-1099 Research Article ISSN 2278 – 4357

EVALUATION OF ANTI-MICROBIAL (IN-VITRO) ACTIVITY OF AMARANTHA CURQMINT TABLET

[1]*Dr. Sanjay Nipanikar, [2]Dr. Snehal Agnihotri, [2]Dr. Suneeta Panicker, [2]Nivedita Shelke

[1]Head - Research and Development, Ari Healthcare Pvt. Ltd. Office at No. 107 1st Floor, S.No.1, World Trade Center, Tower one, Opp. EON SEZ, Kharadi, Pune-411014, Maharashtra, India.

[2]Principal and HOD, Department of Microbiology, Department of Microbiology, Dr. D.Y. Patil Arts, Commerce and Science College, Pimpri, Pune- 411018.

[3]Assistant Professor, Department of Microbiology, Dr. D. Y. Patil ACS College Pimpri, Pune 411018.

Article Received on
10 Jan. 2020,

Revised on 31 Jan. 2020,
Accepted on 21 Feb. 2020

DOI: 10.20959/wjpps20203-15543

***Corresponding Author**
Dr. Sanjay Nipanikar
Head - Research and Development, Ari Healthcare Pvt. Ltd. Office at No. 107 1st Floor, S.No.1, World Trade Center, Tower one, Opp. EON SEZ, Kharadi, Pune-411014, Maharashtra, India.

ABSTRACT

In modern medicine, adverse effects, resistance and expensiveness associated with the use of antibiotics are of a great concern. There is an urge for development of prevention and treatment options that are natural, safe, effective and economical. Ari Healthcare Pvt. Ltd. has conceptualized and developed a formulation called "Amarantha CurQmint Tablet" for treatment of halitosis, cough, common cold and sore throat. In the present study, Amarantha CurQmint Tablet was tested for anti-microbial activity against oral cavity pathogens such as *E. coli*, *Staph. auerus* and *Candida albicans* in comparison with marketed product, methanol and standard drug i.e. Chlorhexidine. Various concentrations of Amarantha CurQmint Tablet i.e. 2%, 3% and 4% showed more zone of inhibition (of 25 mm each) against *Staph. auerus* than Methanol (11 mm) and Chlorhexidine (19 mm). Also, 3% and 4% concentrations of Amarantha CurQmint Tablet showed more zone of inhibition (of 23 mm and 25 mm respectively) against *E. coli* than Methanol (11 mm) and Chlorhexidine (19 mm). All concentrations of Amarantha CurQmint Tablet i.e. concentration of 1%, 2%, 3% and 4% showed more zone of inhibition (of 20 mm, 25 mm, 25 mm, and 25 mm, respectively) against *Candida albicans* than Methanol (11 mm) and standard drug Chlorhexidine (19 mm). The zones of inhibitions of Marketed Product

group against all three strains were 0, which was suggestive that Marketed Product has no antimicrobial activity against all three microorganisms. The study concluded that Amarantha CurQmint Tablet possesses antibacterial and antifungal activities and can be used in halitosis, cough, common cold and sore throat.

KEYWORDS: Amarantha CurQmint Tablet, Anti-microbial, *E. coli*, *Staph. auerus*, *Candida albicans.*

INTRODUCTION

Plant extracts are used in different systems of medicine for the treatment of various human ailments, and for treatment of viral and fungal infections since ancient times. Plant-derived medicines have been a part of our traditional health care system. In various research studies, the antimicrobial properties of plant derived compounds are well documented. Also, the side effects, expensiveness and resistance of modern medicines are tending to move patients and physicians toward the use of alternative treatment options.[1] Herbal medicines are one of effective and less harmful treatment options available with negligible side effects to treat various diseases. So, in the past few decades the use of herbal medicine has gained importance to counteract the potential demerits of the allopathic system.[2] In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions.[3]

Keeping in mind the basic concept of Ayurveda, Ari Healthcare Pvt. Ltd., has developed 'AHPL/AYTAB/1514' tablet i.e. marketed as Amarantha CurQmint Tablet for effective management of halitosis, cough, common cold and sore throat.

S. aureus is found to be present in substantial numbers on teeth in geriatric and intensive care patients. The colonization of oral *S. aureus* is one of the major oral and dental risk factor for aspiration pneumonia in veteran residents[15] (Terpenning *et al.*, 2001). The emergence of Methicillin Resistant *Staphylococcus aureus* (MRSA) in oral cavity[16] (Crusta *et al.*, 2010), pose a new threat to oral health. Facultative microorganisms such as *Enterococcus faecalis*, aerobes like *Staphylococcus aureus*, and even *Candida albicans* are considered to be the most resistant species, and one of the possible causes of root canal treatment failure. *Candida albicans* is the most common yeast isolated from the oral cavity, and is associated with oral fungal infections, endocarditis and septicaemia.[17] *Escherichia coli* is known opportunistic pathogen.

There are several anti-caries agents available commercially, however due to side effects and resistance, the search for an effective natural agent still continues. Natural products have shown to be good alternative to synthetic chemical substances for caries prevention.[18-20] Thus considering the above mentioned factors, the present study was conducted to evaluate antimicrobial activity of Tablet CurQmint on oral pathogens like *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* in comparison with standard Chlorhexidine.

MATERIAL AND METHODS

Preparation of stock solutions

Powders of extracts were weighed accordingly and dissolved in appropriate solvent to yield the required concentration, using sterile glassware and was stored at 4^{o}C until further use.

Inoculum preparation

Test organisms were grown in respective media to get approximately 10^{6} cfu per ml and 100µl of this was used for the antimicrobial assay.

PROCEDURE

Inoculation of Test Plates: The dried surface of a Müeller-Hinton agar plate was inoculated by spreading culture suspension (100 µl) on agar surface.

The wells were bored into the surface of the inoculated agar plate and the known concentration of extracts (100 µl) was added to the wells in triplicates. Plates were kept in the freeze for pre-diffusion for 30 minutes and then placed in an incubator set to 37°C for 24 hours.

- Amarantha CurQmint Tablets (55 mg active ingredient per tablet) were dissolved in methanol to obtain different concentrations (1% to 5%).
- Muller Hinton agar (Hi Media) was used for bacterial cultures and PDA was used for fungal culture.
- The cups were bored in agar medium spread with the test organism, using a sterile cork borer with 8 mm inner diameter.
- These cups were filled with 100 µl test solutions and the plates were incubated at 37^{0}C for bacteria and 30^{0}C for fungus.
- Marketed product, Methanol and standard Chlorhexidine were used as control.
- All experiments were performed in triplicate.

- The assessment of the antimicrobial activity was based on the measurement of the diameter of the zone of inhibition.

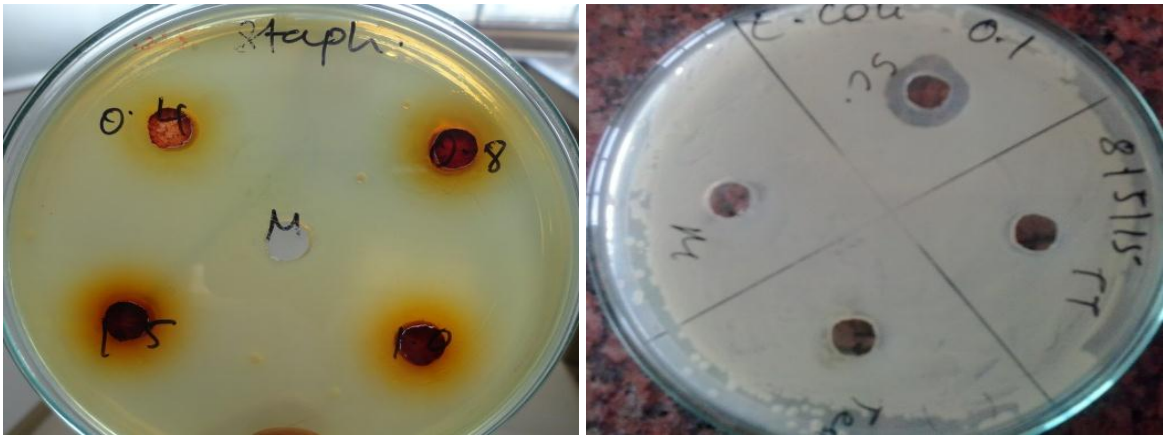
Reading Plates and Interpreting Results

After incubation, each plate was examined for presence or absence of the antimicrobial activity. Zones of inhibition were measured, including the diameter of the well.

RESULTS

Antibacterial activity of Amarantha CurQmint Tablet

Various concentrations of Amarantha CurQmint Tablet i.e. 2%, 3% and 4% showed more zone of inhibition (of 25 mm each) against *Staph. auerus* than Methanol (11 mm) and standard drug Chlorhexidine (19 mm). One percentage concentration of Amarantha CurQmint Tablet showed more zone of inhibition (of 16 mm) against *Staph. auerus* than Methanol (11 mm). Also, 3% and 4% concentrations of Amarantha CurQmint Tablet showed more zone of inhibition (of 23 mm and 25 mm respectively) against *E. coli* than Methanol (11 mm) and standard drug Chlorhexidine (19 mm). One percentage and 2% concentrations of Amarantha CurQmint Tablet showed more zone of inhibition (of 14 mm & 16 mm respectively) against *E. coli* than Methanol (11 mm). The details are presented in Table 1 and Figure 1.

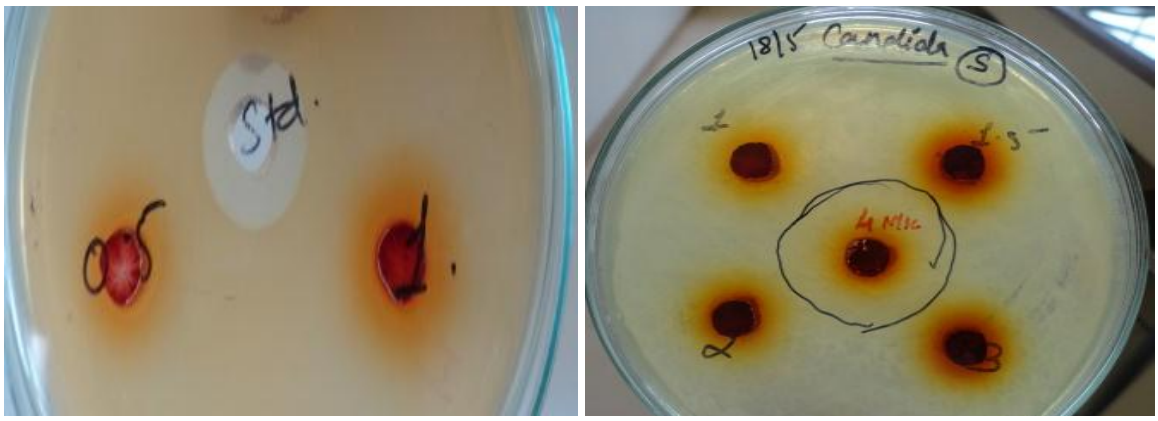


Staph. Auerus ***E. coli***

Fig. 1: Antimicrobial activity of different concentrations of Amarantha CurQmint Tablet against *Staph aureus* and *E. coli.*

Antifungal activity of Amarantha CurQmint Tablet

Amarantha CurQmint Tablet was active against *Candida albicans* even at a lower concentration of 1% showing a significant zone of inhibition of 20 mm as compared to the standard drug Chorhexidine. All concentrations of Amarantha CurQmint Tablet i.e. concentration of 1%, 2%, 3% and 4% showed more zone of inhibition (of 20 mm, 25 mm, 25 mm, and 25 mm, respectively) against *Candida albicans* than Methanol (11 mm) and standard drug Chlorhexidine (19 mm). This showed that Amarantha CurQmint Tablet possesses better anti-fungal activity against *Candida albicans* than Methanol and Chlorhexidine. The details are presented in Table 1 and Figure 2.



Candida albicans for STD ***Candida albicans for test***

Fig. 2: Antimicrobial activity of different concentrations of CurQmint formulation against *Candida albicans.*

Table 1: Antimicrobial activity of different concentrations of CurQmint formulation against *Staph aureus, E. coli* and *Candida albicans.*

	Zone of inhibition (mm)Dilutions						
Name of the organism	**1%**	**2%**	**3%**	**4%**	**Marketed Product**	**Methanol**	**Chlorhexidine**
E.coli	14	16	23	25	00	11	15
Staph. auerus	16	25	25	25	00	11	18
Candida albicans	20	25	25	25	00	11	19

DISCUSSION

In the present study, antimicrobial (*in-vitro*) activity of Amarantha CurQmint Tablet against *E. coli, Staph auerus* and *Candida albicans* was investigated. The study results indicated that Amarantha CurQmint Tablet possesses strong antimicrobial activity against all the three tested strains i.e. *E. coli, Staph auerus* and *Candida albicans*.

All concentrations of Amarantha CurQmint Tablet showed more zone of inhibition against *Candida albicans* than Methanol and Chlorhexidine. This showed that Amarantha CurQmint Tablet possesses better anti-fungal activity against *Candida albicans* than Methanol and Chlorhexidine. The 2%, 3% and 4% concentrations of Amarantha CurQmint Tablet showed more zone of inhibition against *Staph. auerus* than Methanol and Chlorhexidine. This showed that 2%, 3% and 4% concentrations of Amarantha CurQmint Tablet possess better anti-bacterial activity against *Staph. auerus* than Methanol and Chlorhexidine. The 1% concentration of Amarantha CurQmint Tablet has more zone of inhibition against *Staph. auerus* than Methanol which is suggestive of better anti-bacterial activity of Amarantha CurQmint Tablet than methanol. Three percentage and 4% concentrations of Amarantha CurQmint Tablet showed more zone of inhibition against *E. coli* than Methanol and Chlorhexidine. This indicated that 3% and 4% concentrations of Amarantha CurQmint Tablet possess better anti-bacterial activity against *E. coli* than Methanol and Chlorhexidine. The 1% and 2% concentrations of Amarantha CurQmint Tablet showed more zone of inhibition against *E. coli* than Methanol which is suggestive of better anti-bacterial activity of Amarantha CurQmint Tablet than methanol. It has also been observed that as the concentration of the Amarantha CurQmint Tablet was increased the zone of inhibition increased against all three microorganism.

The zones of inhibitions of Marketed Product group against all three strains were 0, which was suggestive that Marketed Product has no antimicrobial activity against all three microorganisms.

Amarantha CurQmint Tablet contains Haridra (*Curcuma longa*), Pippali (*Piper longum Linn*.) and *Putiha* (*Mentha arvensis*). Haridra (*Curcuma longa*), which is associated to the *Zingberiaceae* family[4], is a rich source of beneficial phenolic compounds known as the turmeric[5] and is widely used as a spice, food preservative and colouring agent, and is also known for its medicinal properties. Various *Sesquiterpenes* and *Curcuminoids* have been isolated from the rhizome of *C. longa*, attributing a wide array of biological activities such as antioxidant, anti-inflammatory, wound healing, anticancer and anti-proliferative, antifungal[6] and antibacterial[7]. *Curcumin* [1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione; Diferuloylmethane], a yellow bioactive pigment is the major component of turmeric.

Pippali (*Piper longum Linn*.), (Javanese, Indian long pepper, pippali), is a small shrub characterized by fruits called berries borne in spikes, oblong, blunt and blackish green in

colour[8]. The fruits contain 1% volatile oil, resin, a waxy alkaloid, a terpenoid substance and alkaloids piperine and piperlongumine.[9] Pippali which was mostly used for household cooking purposes as a spice and as seasoning now is a component of medicine as attested by several studies. It is reported as good remedy for treating gonorrhoea, menstrual pain, tuberculosis, sleeping problems, respiratory tract infections, chronic gut-related pain and arthritic conditions.[10] Since long time, *P. longum* has been used to possess immunomodulatory and antitumor activities.[11] *P. longum* is also used as an antibacterial[12], and as a gastroprotective.[13] In addition, Piperine has also shown to enhance the bioavailability of several drugs, for example sulfadiazine, tetracycline, streptomycin, rifampicin, pyrazinamide etc.

Putiha (*Mentha arvensis*) or Mint is an aromatic perennial herb commercially cultivated for its oil popularly known as *Pudina* in India, is an important medicinal plant of widespread utility both in crude and extracted form. Many studies have established that *M. arvensis* leaves extracts have potent anti-inflammatory, anti-microbial, neuroprotective, ulcer protective, and hepatoprotective properties.[14] The literature survey of the folklore medicine reveals the use of *Mentha arvensis* leaves to the treatment of tooth.

It has been reported in various experiments that all the ingredients of Amarantha CurQmint Tablet i.e. Haridra (*Curcuma longa*), Putiha (*Mentha arvensis*) and Pippali (*Piper longum*) possess anti-microbial activity against various pathogens. Several reports have shown that the flavonoids in *C. longa* may, in part, be related to the antimicrobial effects.[25] The leaves of *M. arvensis* are also a rich source of flavonoids which have been shown to possess several biological properties related to antimicrobial and antioxidant mechanism. *Piperine*, an alkaloid in the fruits of *P. longum* is responsible for anti-inflammatory, anti-amoebic, antifungal and antibacterial activities.[21] Also, alkaloid has ability to link with bacterial DNA leading to kill it.[22] While phenol compound has precipitative activity on microbial enzyme and leads to inhibit and loss of their function[23-24] reported that hydroxyl group in flavonoids have ability to composite with cell wall proteins and break down the cell membrane of bacteria. The anti-microbial activity of Amarantha CurQmint Tablet against *E. coli, Staph auerus* and *Candida albicans* could be because of the synergistic action of proportionally perfect combination of various extracts present in the formulation.

CONCLUSION

The study results showed that Amarantha CurQmint Tablet possesses antimicrobial activity against all the three oral cavity microbial strains (*E. coli, Staph auerus* and *Candida albicans*). Thus, Amarantha CurQmint Tablet may be used to treat halitosis, cough, common cold and sore throat.

REFERENCES

1. Shahi SK, Shukla AC, Bajaj AK, Banerjee U, Rimek D, Midgely G, Dikshit A. Broad spectrum herbal therapy against superficial fungal infections. Skin Pharmacol Appl Skin Physiol, 2000; 13(1): 60-64.
2. Alviano DS, Alviano CS. Plant extracts: search for new alternatives to treat microbial diseases. Curr. Pharm. Biotechnol, 2009; 10: 106-112.
3. Chen YR, Tan TH. Inhibition of the c-Jun N terminal kinase (JNK) signaling pathway by curcumin. Oncogene, 1998; 17: 173-178.
4. Chattopadhyay I, Biswas K, Bandyopadhyay U, Banerjee RK. Turmeric and curcumin: Biological actions and medicinal applications. Curr. Sci., 2004; 87: 44-53.
5. Menon VP, Sudheer AR. Antioxidant and anti-inflammatory properties of curcumin. Adv. Exp. Med. Biol., 2007; 595: 105-125.
6. Apisariyakul, A, Vamittanakom N, Buddhasukh D, Antifungal activity of turmeric oil extracted from Curcuma longa (Zingiberaceae). *J.* Ethnopharmacol, 1995; 49: 163–169.
7. Sankaranarayanan J, Jolly CI. Phytochemical, antibacterial, and pharmacological investigations on Momordica charantia Linn., Emblicaoffidnalis Gaertn. and Curcuma longa Linn. Indian Journal of Pharmaceutical Science, 1993; 55(1): 6.
8. Ali MA, Alam NM, Yeasmin MS, Khan AM, Sayeed MA. Antimicrobial screening of different extracts of *Piper longum* Linn. Res J Agri Biol Sci., 2007; 3: 852-7.
9. Wu S, Sun C, Pei S, Lu Y, Pan Y. Preparative isolation and purification of amides from the fruits of *Piper longum* L. By upright counter-current chromatography and reversed phase liquid chromatography. J Chromatogr, 2004; 1040: 193-204.
10. Mehta A, Zitzmann, N, Rudd PM. Alphaglucosidase inhibitors as potential broad based anti-viral agents. FEBS Letters, 1998; 430: 17-20.
11. Courageot MP, Frenkiel MP, Dos Santos CD. Alpha-glucosidase inhibitors reduce dengue virus production by affecting the initial steps of virion morphogenesis in the endoplasmic reticulum. Journal of Virology, 2000; 74: 564–572.

12. Ratner L, Vander Heyden N, Dedera D. Inhibition of HIV and SIV infectivity by blockade of alpha-glucosidase activity. Journal of Virology, 1991; 181: 180–192.
13. Wakade AS, Shah AS, Kulkarni MP. Protective effect of Piper longum L. on oxidative stress induced injury and cellular abnormality in adriamycin induced cardiotoxicity in rats. Indian J Exp Biol., 2008; 46(7): 528-33.
14. Lund RG, Serpa R, Nascente PD, Ribeiro GA, Freitag RA, Pino FA. In-vitro study on the antimicrobial effect of hydroalcoholic extracts from Mentha arvensis L. (Lamiaceae) against oral pathogens. Acta Sci Biol Sci., 2012; 34(4): 437-42.
15. Terpenning MS, Taylor GW, Lopatis DE, Kerr CK, Dominguez BL, Loesche WJ. Aspiration Pneumonia: dental and oral risk factors in an older veteran population. JAGS, 2001; 49(5): 557-563.
16. Crusta AL, Jewtuchowicz V, Brusca MI. Prevalence of Staphylococcus Spp and Candida spp in the oral cavity and periodontal pockets of periodontal disease patients. Acta Odontol Latinoam, 2010; 23(1): 20-26.
17. Belazi M, Velegraki A, Koussidou-Eremondi T, Andrealis D, Hini S, Arsenis G, Eliopoulou C, Destouni E, Antoniades D. Oral Candida isolates in patients undergoing radiotherapy for head and neck cancer: prevalence, azole susceptibility profiles and response to antifungal treatment. Oral Microbiol Immunol, 2004; 19: 347
18. Beck J, Garcia R, Heiss G, Vokonas PS. Offenbacher S. Periodontal Disease and Cardiovascular Disease. Journal of Periodontology, 67: 1123-1137.
19. Scannapieco FA. Role of Oral Bacteria in Respiratory Infection. Journal of Periodontology, 70: 793-802.
20. Flemingson, Pamela E, Ambalavanan, Ramakrishna T, Vijayalakshmi R. Effect of three commercial mouth rinses on cultured human gingival fibroblast: An in vitro study. Indian J Dent Res., 2008; 19(1): 29-35.
21. Mishra P. Isolation, spectroscopic characterization and computational modelling of chemical constituents of Piper longum natural product. Int J Pharma Sci Rev Res., 2010; 2: 78-86.
22. Marrtt, W, Tan GT, Gordell GA, Pezzutuo JM. Biological activity of novel macrocylic alkaloids from Albizia amara detected on the basis of interaction with DNA. J.Nat. Prod, 1991; 54(6): 1531-1542.
23. Farag RS, Daw ZY, Hewed FM, Elbaraty GS. Antimicrobial activiy of some Egyption Spice essential oil. J. Food Pro, 1989; 52: 665-670.

24. Reed JD. Nutritional toxicology of tannins and related polyphenols in Forage legnmes. J. Animal Soc., 1995; 73: 1516-1528.
25. Niamsa N, Sittiwe C. Antimicrobial activity of curcuma longa aqueous extract. J of Pharmacology and Toxicology, 2009; 4(4): 173-77.